MEDITERRANEAN AGRICULTURAL SCIENCES

Volume: 36
Number: 3
Year: December 2023

E-ISSN: 2528-9675

e-ISSN 2528-9675

MEDITERRANEAN AGRICULTURAL SCIENCES

Previous Name: Akdeniz University Journal of the Faculty of Agriculture

The peer reviewed scientific journal of Akdeniz University Faculty of Agriculture

Three issues are published per year in April, August and December

Abbreviation of the journal: Mediterr Agric Sci

Owner on behalf of Akdeniz University, Faculty of Agriculture
Prof. Dr. Mustafa ERKAN
Dean

Publishing Manager
Assoc. Prof. Dr. Demir OZDEMIR

Administration Address
Akdeniz University
Faculty of Agriculture
07058 Antalya, Türkiye
Phone: +90 242 310 2412
Fax: +90 242 310 2479
E-Mail: masjournal@akdeniz.edu.tr
Web site: www.dergipark.org.tr/en/pub/mediterranean

Publisher
Akdeniz University, Faculty of Agriculture
07058 Antalya, Türkiye

Phone: +90 242 310 2412
Fax: +90 242 310 2479

Subscription
Open access journal

Online access free of charge
www.dergipark.org.tr/en/pub/mediterranean

Cover design
Assoc. Prof. Dr. Suleyman OZDERIN

AIM AND SCOPE

MEDITERRANEAN AGRICULTURAL SCIENCES aims to share knowledge at both national and international levels by publishing the results of research in agriculture and life sciences in both Turkish and English. Consequently this journal is a multidisciplinary platform for related scientific areas. The journal primarily publishes original research articles and accepts a limited number of invited reviews in the areas of agricultural biotechnology, agricultural economics, agricultural machinery, animal husbandry, bioenergy, biostatistics and genetics, farm structure and irrigation, field crops, food science and technology, horticulture, landscape and nature conservation, natural resources, plant protection, soil science and plant nutrition.

ABSTRACTS AND INDEXING

MEDITERRANEAN AGRICULTURAL SCIENCES is indexed and abstracted in **CABI** data bases (**CAB Direct**), **TUBITAK-ULAKBIM** (National Data Bases-Data Base of Life Sciences), **CLARIVATE, WEB OF SCIENCE, MASTER JOURNAL LIST** (Zoological Records) and **DRJI** (Directory of Research Journals Indexing).

e-ISSN 2528-9675

www.dergipark.org.tr/en/pub/mediterranean

MEDITERRANEAN AGRICULTURAL SCIENCES

The journal was published under the name “Akdeniz University Journal of the Faculty of Agriculture” and with the number ISSN 1301-2215 until 2016.

Volume: **36** Number: **3** Year: December **2023**

e-ISSN 2528-9675

MEDITERRANEAN AGRICULTURAL SCIENCES

Volume: **36** Number: **3** Year: December **2023**

Contents

Research Article

www.dergipark.org.tr/en/pub/mediterranean

MEDITERRANEAN AGRICULTURAL SCIENCES
(2023) 36(3): 101-108
DOI: 10.29136/mediterranean.1300307

The effects of exogenous tyrosine supplement on spinach (*Spinacia oleracea* L.) cultivation under lithium stress

Nezahat TURFAN

Kastamonu University, Science Faculty, Biology Department, Kuzeykent, 37150, Kastamonu

Corresponding author: N. Turfan, e-mail: nturfan@kastamonu.edu.tr

ARTICLE INFO

Received: May 22, 2023
Received in revised form: September 20, 2023
Accepted: September 21, 2023

***Keywords*:**

Chemicals
Growth
Lithium
Spinach
Tyrosine

ABSTRACT

In this study, the effects of exogenous Tyrosine (Tyr: 2.5 mM) application on the variations of growth rate parameters, enzymatic and non-enzymatic constituents, oxidative stress, and mineral content under lithium-applied (Li1: 6.44 mM; Li2: 19.32 mM) seedlings of the Anlani F1 spinach cultivar were investigated. Results showed that a higher Li led to a significant reduction in the growth rate parameters including shoot, root, and leaf length, the fresh weight of shoot, root, and leaf, and leaf blade sizes, whereas a lower Li dose resulted in an increase in those parameters. In contrast, the Tyr supply to the Li-applied seedlings resulted in a rise in these measured parameters. Similarly, chlorophyll and polyphenol contents and PAL, APX, CAT, POD, and SOD activities were higher in all exogenous Tyr-treated groups, including lithium-treated groups. Whilst nitrate content was higher in the Li-applied seedlings, NR activity was lower. Also, MDA and H_2O_2 were found to be higher in the Li-applied group, but exogenous Tyr supplements reduced their levels in the seedlings. Li, Ca, Na, Cl, Mn, Fe, Ni, Cu, and Zn accumulation were induced by Li doses and Tyr applications together with Li, but Tyr applications alone reduced all of their levels. Also, exogenous Try supplementations to the Li-applied group caused an important decline in the Li accumulation. As a result, a higher Li dose exhibited a negative effect on the growth rate, chemical constituent, and antioxidant compounds of the Anlani F1 spinach cultivar, but exogenous Tyr supplement improved those examined traits in the Li-applied seedlings.

1. Introduction

Spinach (*Spinacia oleracea* L.) is a popular leafy nutrient source, which is considered one of the healthiest vegetables in the daily diet of humans. The health effect of spinach is due to antioxidant molecules including pigments, phenols, vitamins, amino acids, enzymes, and minerals. In addition, having a low calorie and high water content increases its importance in health (Bostancı and Ulger 2022). As it is a cheap and readily available vegetable in almost all seasons, thisis an important advantage for the consumer as industrial and commercial properties are important for spinach producers. Having cultivars that can be grown in every season, spinach is a vegetable that is economically important for Türkiye. However, the nutritional quality and yield of spinach differ depending on the growing conditions, and the dosage and type of the fertilizer used (Turfan 2023). In recent years, due to wrong irrigation, unconscious and excessive fertilization, and spraying for good-looking crops, the soil properties are slowly deteriorating and therefore there has been a high yield loss in spinach production as well as other crops (Bostancı and Ulger 2022; Saddıque et al. 2022). Spinach is moderately tolerant of alkalinity in winter conditions and moderately sensitive in spring and summer. Most of the studies in spinach agriculture in Türkiye have focused on NaCl and heavy metal toxicity and the effects of exogenous organic/inorganic fertilization. However, there are no studies on lithium-induced saline-alkaline stress in spinach. Li is an alkali metal used in almost 20 industries including ceramic, glass, aluminum, oil, battery, phones, tablets, computers, electric cars, and autonomous robots and it has gained more importance in recent years (Robinson et al. 2018). The excessive accumulation of lithium-containing wastes in the soil triggers saline-alkaline stress which limits plant growth by repressing root growth, water and mineral uptake from the roots (Mulkey 2005; Hawrylak-Nowak et al. 2012). In addition, since it is a mobile element, it is readily transported to the leaves after being taken up by the roots, causing many problems such as chlorosis, disruption of photosynthesis metabolism and prevention of other biochemical reactions (Bakhat et al. 2020; Wang et al. 2021). In recent years, to diminish, alkalinity stress damage, exogenous aromatic amino acids applications have been widely used (Zhang et al. 2021). Tyrosine (Tyr), one of the aromatic amino acids, is a precursor for pigments, phenolic compounds, vitamins, and enzymes specialized secondary metabolites that are vital for plant adaptation to environmental change (Feduraev et al. 2020). Also, it is a fundamental component of the complex producing oxygen in photosystem II, as an electron carrier. Moreover, the degradation of Tyr by the Krebs cycle can provide nitrogen, carbon, and ATP that are required for plant growth, especially during periods of carbohydrate shortage (Hildebrandt et al. 2015). The positive effects of exogenous L-tyrosine application on the plant growth rate, nutritional quality, and stress resistance were observed following the foliar application of it in beetroot (El-Sherbeny et al. 2012), arugula Al-Mohammad and Al-Taey

(2019), and *Hibiscus sabdariffa* (Helaly and Ibrahim 2019). Although Tyr is an important aromatic amino acid, little is known about its specific defining roles in plant growth and development, and how tyrosine levels are controlled in plant tissues. In addition, there are studies in the literature on the vegetative growth of tyrosine, accumulation of beneficial metals, and alkali metals in plants, whereas no study has been found on how exogenous tyrosine application affects the response of plants to lithium. Also, no such study carried out in Türkiye was found. Thus, the present study aimed to determine the effect of foliar tyrosine treatment on spinach cultivation treated with lithium. The effect of exogenous Tyr application on the growth rate and biochemical characteristics of the Anlani F1 spinach cultivar treated with Li was investigated by making use of morphological parameters, bioactive chemical constituents, and mineral status.

2. Materials and Methods

2.1. Experimental design

Anlani F1 spinach cultivar was used as a study material in the present research, which is a species tolerant of low temperatures, fragility, and diseases as well as suitable for greenhouse growing and open-area growing (Turfan 2023). The present study was carried out between 12 Nov 2022 and 14 March 2023 under greenhouse conditions with 14/22±2°C, relative humidity of 68±5%, and photoperiod of 16 h. First, balcony-type pots (80 x 23 x 24) were filled with 25 L turf and soil mixture (3:1) and spinach seeds were planted. The study was designed with a random parcel experimental design with 3 repeats. The six treatment patterns were as follows: 1) Control (0), 2) Tyr (2.5 mM L-tyrosine), 3) Li1 (6.44 mM $LiCl_2$), 4) Li2 (19.32 mM $LiCl_2$), 5) Tyr-Li1 (6.44 mM $LiCl_2$ + 2.5 mM L-tyrosine), and 6) Tyr-Li2 (19.32 mM $LiCl_2$ +2.5 mM L-tyrosine). Four pots were used in each group and 6x3= 18 pots in total. They were irrigated with a nutrient solution twice a week until lithium (Li) and tyrosine (Tyr) applications were started. After the 15^{th} day since germination, the number of plants in each pot were reduced to approximately 10 seedlings. Li applications ($LiCl_2$: Merck Lithium chloride CA 7447-41-8. EC: 231-213-3) were performed in the soil by dissolving in a nutrient solution (Hoagland and Arnon 1950) when seedlings had 4-5 leaves. L-tyrosine (Sigma-Aldrich, CAS 35424-81-8) application was performed on leaves with a tyrosine solution dissolved in a nutrient solution, while control plants were sprayed with nutrients. The applications were repeated twice a week for 8 weeks. Plants were harvested in the 9^{th} week and some of them were used in morphological measurements, some in chemical analyses, and nutrient assaying. The determination of the doses of the chemicals was carried out according to the preliminary study with the viol. The stimulant doses of lithium (Li1) and tyrosine were considered at the concentration that caused a considerable increase in shoot, root, and leaf lengths and fresh weight of seedlings. The toxic dose of lithium (Li2) was taken into account in the concentration that caused the greatest reduction in shoot, root, and leaf development and fresh weight of seedlings. The nutrient components were: available magnesium 24.65%, available potassium 14.80%, available calcium 9.66%, available sodium 7.92%, available phosphorus 6.53%, available sulphur 2.4%, available lithium 114.74 mg kg^{-1}, available manganese 980.8 mg kg^{-1}, available iron 44230 mg kg^{-1}, available nickel 140.8 mg kg^{-1}, available zinc 144.8 mg kg^{-1}, and available copper 46.8 mg kg^{-1}. The pH value of the soil was found to be 6.38, and also electrical conductivity (EC) of 6.53 dS m^{-1}.

2.2. Assay

2.2.2. Morphological measurement

After treatments, all spinach seedlings were harvested for shoot/root, leaf length, fresh weight of plant and leaf measurements. The parameters were examined in 10 plants for each application. Shoot, root, leaf and leaf blade length (cm) were measured by a ruler. Fresh weight (g) shoot and leaf were determined by weighing with a precision balance. All chemicals and mineral analyses were performed with three replications. Measurements for plant growth rate parameters were carried out with ten replications.

2.2.3. Chemical analyzes

The chlorophylls and lutein were homogenized using ethanol and the estimations were made using the methods described by Kukric et al. (2012). The amount of polyphenol was performed according to the method of Folin and Denis (1915). About 0.5 mL of an extract was introduced into test tubes followed by 2.5 mL of 10% Folin-Ciocalteu reagent and 2 mL of 7.5% Na_2CO_3. The mixture was allowed to stand for 30 minutes, and absorbance was recorded at 760 nm. The phenol content of the extract was determined using tannic acid standard curves. Phenylalanine ammonia-lyase (PAL) was assayed following the method of Dickerson et al. (1984). A 500 mg sample was homogenized with mortar and pestle in the extraction buffer (50 mM Tris-HCl buffer, pH 8.8, 5 mM EDTA, 5 mM ascorbic acid). The reaction mixture, containing 100 µl of crude enzyme extract, 500 µl of 50 mM Tris HCl (pH 8.8), and 600 µl of 1 mM L-phenylalanine, was incubated for 60 min at room temperature, and then the reaction ceased by the addition of 500 µl of 6 N HCl. The absorbance was recorded at 290 nm. The nitrate content was carried out by the salicylic acid method (Cataldo et al. 1975). The total amount of nitrate was calculated with the equation obtained from the NO_3 standard curve and the amount of nitrate in the samples was expressed as mg g^{-1}. Nitrate reductase (NR) activity was performed by following the method of Klepper et al. (1971). Antioxidant enzyme extraction was performed using a sodium phosphate buffer solution (50 mM, pH 7.6). 500 mg of fresh tissues were homogenized in a 5 Ml buffer containing 0.1 mM Na-EDTA (ethylenediamine tetraacetic acid disodium salt), then, were centrifuged for 15 min at 15000 rpm at 4°C. The resulting supernatant was used for the estimation of the activities of antioxidant enzymes such as ascorbate peroxidase (APX), catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) (Zhang et al. 2006). APX activity was determined by following the decrease in absorbance at 290 nm, as the ascorbate was oxidized. Enzyme activity was measured in a reaction mixture containing 0.5 mM ascorbate, 100 mM sodium phosphate buffer (pH 7.0), and enzyme extract. The reaction was started by adding 30 mM H_2O_2. APX activity was expressed as µmol H_2O_2 mg $protein^{-1}$ min^{-1}. The CAT activity of seedlings was determined using a mixture containing 100 mM sodium phosphate buffer (pH 7.0), 30 mM H_2O_2, and 100 µL of crude extract in a total volume of 3.0 mL. One unit (U) of CAT activity was expressed as the amount of enzyme that caused an absorbance change of 0.001 per minute under assay conditions. The activity of POD was calculated using 3 mL reaction mixtures containing 2 mL buffer (400 µl 8 mM guaiacol, 100 mM sodium phosphate pH 6.4), 1 mL of 24 mM H_2O_2 and 0.5 mL of enzyme extract. Absorbance values were recorded twice at 30-s intervals at 470 nm. POD activity was expressed as U $µl^{-1}$ min^{-1}. SOD activity was measured by inhibition of the photochemical reduction of NBT (nitroblue tetrazolium). A 3 ml reaction mixture was prepared which comprised crude extract, phosphate buffer (pH 7.0), riboflavin, methionine, NBT, and EDTA. Test tubes were irradiated by a lamp having white fluorescence for 15 min. The absorbance of solutions was recorded at 560 nm. One unit (U) of SOD activity was expressed as a U mg^{-1} protein. The Malondialdehyde (MDA) content of samples was measured according to the Lutts et al. (1996) method. 500 mg of fresh tissues were extracted in 10 mL of 0.25% thiobarbituric acid (TBA) in 10% trichloroacetic acid (TCA). The extracts were boiled at 95°C for 60 minutes and quickly cooled in an ice bath. After centrifuging at 5000 rpm for 10 minutes, the absorbance was read at 532 nm and 600 nm. The level of MDA was calculated as µmol g^{-1} of fresh weight using the extinction coefficient of 155 mM^{-1}. Hydrogen peroxide (H_2O_2) was determined by the method of Velikova et al. (2000). Fresh tissues (500 mg) were homogenized with 0.1% (w/v) TCA. After centrifuging at 12.000 rpm for 15 minutes, 0.5 ml phosphate buffer (pH 7.0) and 1 ml potassium iodide were added to the supernatant. Its absorbance was noted at 355nm and H_2O_2 concentration was estimated by using the H_2O_2 standard curve. All the chemical measurements were carried out directly with the fresh samples as triplicated.

To determine elemental analysis (Li, Mg, P, S, K, Ca, Na, Cl, Al, Mn, Co, Fe, Ni, Zn, Cu, Cr, Cd, Pb, and I), some soil samples were taken before the planting experiments. Then soil and leaf samples were dried at 65 °C and powdered. These samples were used to determine the elemental analysis in Kastamonu University's Central Research Laboratory using the ICP-OES (SpectroBlue II) device. Each sample was analyzed in triplicate. The pH values of the soil samples were determined using a digital pH meter (Gülçur 1974).

2.3. Statistical analysis

All experimental data obtained from the effects of tyrosine application to the Acosta spinach variety under lithium stress on growth rate parameters, bioactive chemicals, and mineral status were subjected to multiple analyses of variance (MANOVA) using SPSS statistical software (SPSS for Windows, Version 16). Following the results of ANOVAs, Tukey's honestly significance difference (HSD) test (α= 0.05) was also applied.

3. Results and Discussion

The effects of exogenous Tyr on the growth rate and leaf development of spinach seedlings treated with Li doses are presented in Table 1. All parameters significantly varied depending on the type and dose of application (*P*<0.0001).

As seen in Table 1, a higher Li dose caused a sharp decline in the shoot, root, and leaf development, but a slight increase occurred at the lower dose. The exogenous Tyr application to the non-stressed seedlings enhanced the length of the shoot, root, and leaf as well as their fresh weight. On the other hand, exogenous tyrosine supplement improved shoot, root, and leaf properties at low Li dose, but did not show the expected effect on the growth rate, except leaf weight and leaf length at a higher dose. Results showed that the growth rate traits were reduced significantly by the Li2 compared to the control. Also, the highest values of growth rate were obtained from the Tyr application followed by the Tyr-Li1 (Table 1). In a study examining the effects of lithium on the growth rate, Hawrylak-Nowak et al. (2012) exposed sunflower and corn plants to lithium stress and observed that lower Li doses increased the shoot and root development, and leaf development, whereas higher Li doses caused a significant decrease in shoot fresh weight, leaf surface area in both species. In another study, Kalinowska et al. (2013) showed that high Li concentrations suppressed the root growth of lettuce with metal toxicity from Li. On the other hand, the positive effects of Tyr application on the plant growth rate and leaf development are similar to the results obtained by Helaly and Ibrahim (2019) for okra, Feduraev et al. (2020) for wheat, and Tarasevičienė et al. (2021) for mints. The stimulatory effect of tyrosine on growth parameters may be due to increased pigment deposition in leaves, promoting increased photosynthetic gain (Perchlik and Tegeder 2018). Chlorophyll pigments, and lutein obtained from spinach seedlings are provided in Table 2, but secondary metabolism products are shown in Figures 1a, 1b. Compared to the control, the higher Li dose caused notable decrement in the chlorophyll a, chlorophyll b, and total chlorophyll content (Table 2). In contrast, there was a significant increase in the lower Li dose. Tyr alone enhanced all pigment content. Further, the Tyr supplement to the Li-applied group improved chlorophyll molecules, except chlorophyll a at the higher dose. Compared to the control, lutein content decreased in all applied groups, except the higher Li dose. Besides, there was an increase in the lutein in the Li-applied group upon the Tyr supply (Table 2). Similarly, while total phenolic content decreased in the Li-applied seedlings, it reached maximum levels by exogenous tyrosine supplementations to the Li-applied seedlings. In addition, the highest amount of TP was obtained from the tyrosine-applied group only (Figure 1a, 1b). Li applications caused a significant inhibition in the PAL activity, whereas its activity increased upon exogenous tyrosine supplement to Li-applied seedlings. The highest activity was recorded with the tyrosine only application followed by the Tyr-Li1, but the lowest activity was obtained from the Li2 group (Figure 1b).

Table 1. Effect of exogenous Tyr on the growth rate parameters of spinach seedlings under Li stress

Group	Shoot height (cm)	Root height (cm)	Fresh weight of plant (g)	Fresh weight of leaf (g)	Leaf height (cm)	Leaf blade width (cm)	Lead blade height (cm)	Leaf number per plan
0	10.89±0.01b*	9.31±0.07b	17.46±0.002b	1.87±0.05ab	8.60±0.06c	2.86±0.01b	3.80±0.02b	11.0±0.41b
Tyr	14.25±0.01c	12.90±0.14d	22.27±0.001d	3.48±0.10d	10.49±0.06d	4.40±0.06d	6.36±0.09d	12.5±0.29c
Li1	11.29±0.07b	9.99±0.06b	18.05±0.076bc	2.79±0.10bc	9.34±0.09cd	3.30±0.06c	4.28±0.07c	10.3±0.25ab
Li2	8.40±.06a	7.27±0.07a	13.95±0.002a	1.49±0.06a	5.84±0.01a	2.12±0.02a	2.71±0.06a	9.5±0.29a
Tyr-Li1	13.88±0.01c	11.47±0.08c	19.99±0.119c	3.27±0.07c	10.07±0.03d	3.85±0.07cd	4.91±0.06cd	11.3±0.25b
Tyr-Li2	11.87±0.10bc	9.81±0.09b	17.26±0.250b	2.51±0.06b	7.94±0.06b	2.77±0.01b	3.81±0.06b	10.5±0.29ab
F	1644.84	486.3	573.8	118.3	903.8	353.3	412.9	11.54
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

*: Means (±: n= 10) in the same column for each trait in each group with the same lower-case letter are not significantly different by ANOVA test at *P*≤0.05. 0: Control, Tyr: L-Tyrosine, Li1: 6.43 mM Lithium, Li2: 19.32 mM Lithium.

Table 2. Effect of exogenous Tyr on the chlorophyll pigments, carotenoids, and secondary metabolites in spinach seedlings under Li stress

Group	Chlorophyll a mg g^{-1}	Chlorophyll b mg g^{-1}	Total chlorophyll mg g^{-1}	Lutein mg g^{-1}
0	0.840±0.001bc*	1.436±0.001d	2.276±0.001c	1.42±0.002b
Tyr	0.978±0.001a	1.484±0.001c	2.462±0.001ab	0.73±0.002e
Li-1	0.781±0.001c	1.350±0.001e	2.126±0.002d	0.79±0.001d
Li-2	0.754±0.001d	1.241±0.001f	1.994±0.002e	1.52±0.002a
Tyr-Li1	0.857±0.001b	1.630±0.001a	2.486±0.001a	0.76±0.002d
Tyr-Li2	0.853±0.001b	1.509±0.001b	2.361±0.002b	0.87±0.001c
F	12503.9	8986.2	23092.8	91.65
P	<0.001	<0.001	<0.001	<0.001

*: Means (±: n= 3) in the same column for each trait in each group with the same lower-case letter are not significantly different by ANOVA test at $P \leq 0.05$. 0: Control, Tyr: L-Tyrosine, Li-1: 6.43 mM Lithium, Li-2: 19.32 mM Lithium.

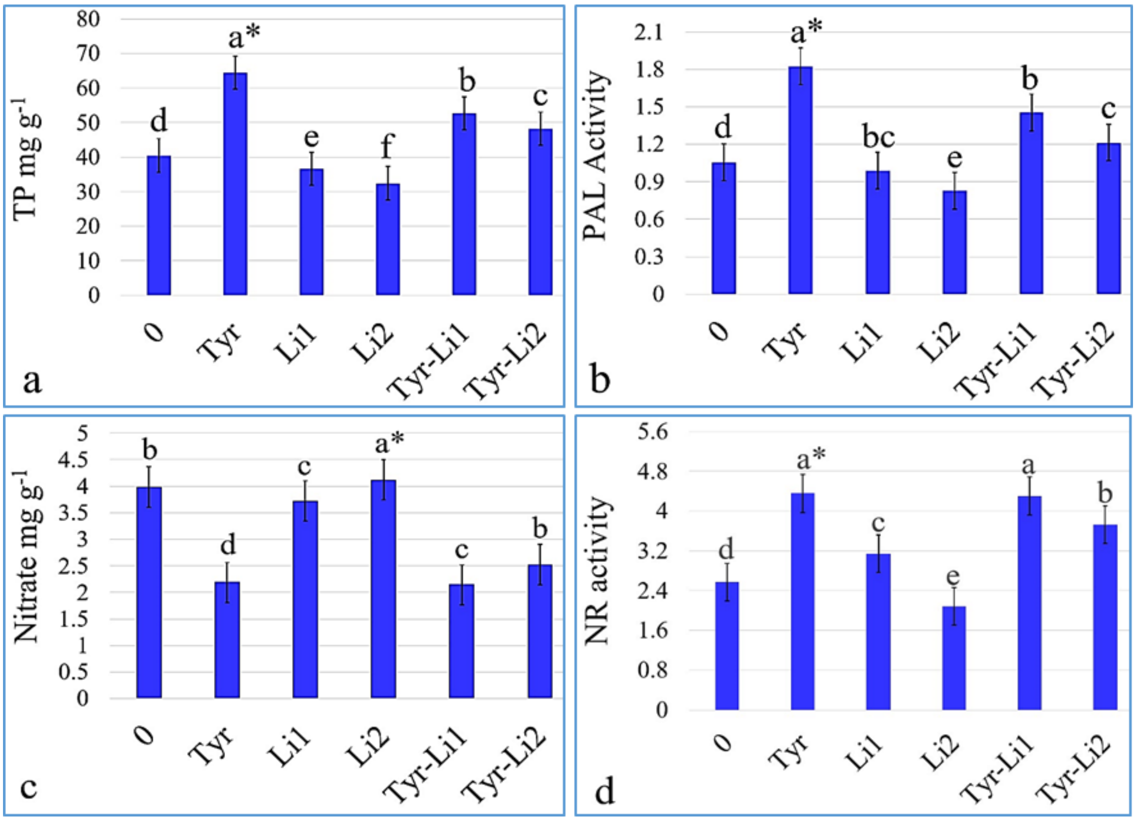

Figure 1. Variation of total phenolic (TP:1a), phenylalanine ammonia-lyase (PAL: 1b), nitrate (1c), and nitrate reductase (NR: 1d) levels in the seedlings. 0: Control, Tyr: 2.5 mM L-Tyrosine, Li1: 6.43 mM Lithium, Li2: 19.32 mM Lithium. Means (±: n= 3) in the same column for each trait in each group with the same lower-case letter are not significantly different by ANOVA test at $P \leq 0.05$.

The destructive influences of alkaline stress on leaf pigmentation, carotenoid, and secondary metabolite accumulation, in contrast, the ameliorative effect of amino acid supplements on stressed plants have been well-documented in many studies. Shams et al. (2016) in lettuce, Makus et al. (2006), and Bakhat et al. (2020) in spinach reported the pigment contents decreased at high lithium doses but increased at a lower dose. They suggested that a decline in pigment content may be connected with the degradation of chlorophyll caused by the replacement of Mg in chlorophyll molecules by Li. A high chlorophyll content in the spinach seedlings may be related to low NT content (Table 2, Figure 1c). It was reported that exogenous amino acids supply increases chlorophyll, carotenoid, and secondary compounds by adjusting nitrogen utilization (Al-Mohammad and Al-Taey 2019; Xu et al. 2019). The low level of lutein content of Li-applied seedling spinach was associated with low light intensity.

Similarly, Makus et al. (2006), and Verhoeven et al. (2018) recorded that low light intensity stimulates the carotenoid mechanism of plants, especially in greenhouse conditions. The decrease in polyphenol content in the Li-applied seedlings may be related to the inhibition of PAL activity by high Li doses. Likewise, Shams et al. (2016), Feduraev et al. (2020), and Tarasevičienė et al. (2021) observed that polyphenol accumulation decreased under alkaline stress conditions by the inhibition of PAL and PPO activities, which are responsible for the synthesis of secondary compounds. However, the same researchers reported that exogenous amino acid supplements to plants under stress mitigate stress damage by stimulating secondary metabolism. Nitrate (NO_3), is one of the principal nitrogen (N) sources for all plants. The utilization of it by plants is closely related to nitrate reductase enzyme (Citak and Sonmez 2010). NT content and NR activity of seedlings were influenced negatively by a higher Li level (Figure 1c, 1d). While the highest NT was observed in the Li2 group, the lowest level was recorded with the Tyr-Li1, followed by seedlings treated with justtyrosine. On the contrary, NR activity was at the lowest level at Li2, followed by the control seedlings. Also, the exogenous Tyr supplement to the Li-applied seedling exhibited a positive effect (Figure 1d). Similar results were achieved by Citak and Sonmez (2010), and Perchlik and Tegeder (2018), who observed that the higher NR activity lowered NT content in plants, especially in cold seasons. They also reported that exogenous amino acid application suppressed NT accumulation in leaves by replacing nitrate, resulting in NT decreasing. Saline-alkaline stress may provoke the production of oxidative stress agents such as MDA, H_2O_2, and other molecules, which have a destructive effect on the cell membrane and components (Shams et al. 2016).

The increase in MDA and H_2O_2 content, especially at high lithium doses, in seedlings applied with Li depicted oxidative stress induced by lithium (Figure 2a, 2b). Also, exogenous Tyr supplement to the stressed seedling led to a considerable decrement in those molecules (Figure 2a, 2b). This increase in MDA and H_2O_2 has been observed in sunflower (Hawrylak-Nowak et al. 2012), spinach (Bakhat et al. 2020), lettuce (Shams et al. 2016), and arugula (Kusvuran et al. 2019) under salt-alkaline stress. Likewise, the improvement effect of foliar amino acid applications under stress conditions was demonstrated by Al-Mohammad and Al-Taey (2019), Helaly and Ibrahim (2019), and Saddıque et al. (2022). The decline in MDA and H_2O_2 in the Tyr-Li group may have resulted from antioxidant enzyme activations induced by Tyr.

Upregulation of APX, CAT, POD, and SOD enzymes during stressful conditions strengthens stress resistance by scavenging toxic compounds, therefore, they help to improve increasing yield and quality (Kusvuran et al. 2019; Saddique et al. 2022). In the spinach seedlings APX, CAT, POD, and SOD activity were importantly inhibited by a higher lithium application (Figure 3a, 3d). Whereas exogenous tyrosine supplement to stressed seedlings enhanced APX, CAT, and SOD activity under both Li applications, it reduced POD activity at higher Li levels (Figure 3a, 3d). Among the applications, the highest APX, POD, and SOD were recorded with an application of only Tyr, but the highest CAT activity was observed in the Tyr-Li1 applied group (Figure 3b). Inhibition of antioxidants, under severe saline-alkaline stress, has been recorded in lettuce (Kalinowska et al. 2013), muskmelon (Xu et al. 2019), and spinach (Bakhat et al. 2020). Further, the upregulation of antioxidants was recorded under stress conditions by a foliar amino acid supplement in lettuce (Shams et al. 2016), spinach (Bakhat et al. 2020), mints (Tarasevičienė et al. 2021), and Brassica (Zhang et al. 2019). Results displayed that tyrosine ameliorated the negative effects of lithium by regulating the antioxidant defense of the seedlings. Variations of Li, Mg, P, S, K, and Ca concentrations in the seedlings are presented in Table 3

The variation of mineral status of seedlings is presented in Table 3 and Table 4. The Li content of seedlings was found to be lowest in the control, and it was the highest at Li2. Contrary, Mg and K contents were lower in all applications. Also, there was a rise in the P and S contents in the Li doses and the Tyr-Li1 group. While the Ca in the seedlings decreased only in the Tyr application compared to the control, it reached its highest level at Li_2 (Table 3). While Na content was lower in the control group, Cl content was lower in the group which was provided just with Tyr. On the other hand, Mg, P, S, K, Ca, and Cl accumulation was reduced by the application of just Tyr, but the Li and Na were enhanced. Variations of the trace elements of the seedlings are shown in Table 4.

Applications of just Tyr caused a remarkable reduction in the Mn, Fe, Ni, Cu, and Zn content in comparison to the control and other groups, but a lower Li led to the greatest rise in Ni, Cu, and Zn. Mn and Fe content increased with Li doses, but just exogenous Tyr applications caused a decline in both elements in a higher Li dose, compared to Li-applied groups (Table 4). A decrease in the Mg and K under applications was associated with Li-induced limitations and interactions of tyrosine with cations (Ruan and Rodgers 2004; Bakhat et al. 2020). It was thought that the synergistic effect of this element with lithium might be effective in increasing the Na content (Siu et al. 2004; Franzaring et al. 2016). The high Cl content in the seedlings may be due to the chlorine elements in the applied lithium salt. Differences in the interactions of foliar aromatic amino acid applications with cations and anions have been already reported by Bakhat et al. (2020). In addition, the antagonistic/synergistic interactions between the elements, the mobility of the elements, and their functions in the plants may have influenced the amounts of these elements in the spinach leaf tissue (Hawrylak-Nowak et al. 2012; Al-Mohammad and Al-Taey 2019).

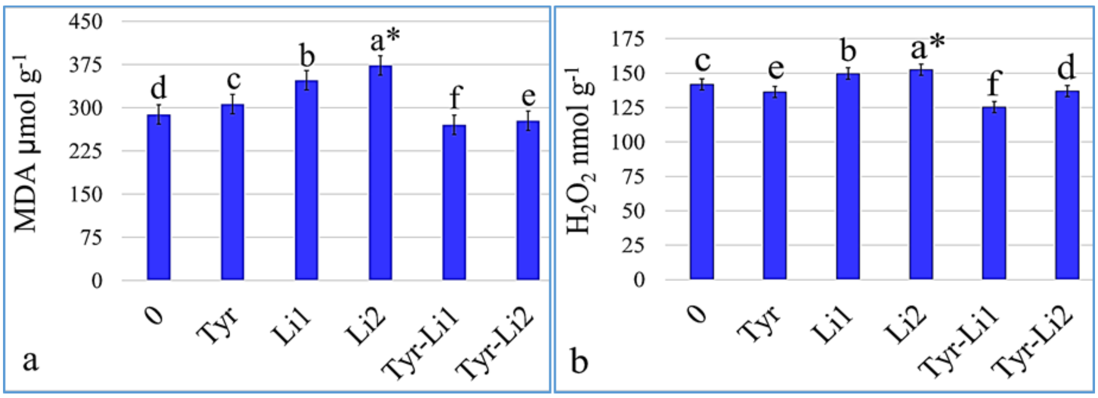

Figure 2. Variation of MDA (2a) and H_2O_2 (2b) levels in the spinach seedlings. 0: Control, Tyr: 2.5 mM L-Tyrosine, Li1: 6.43 mM Lithium, Li2: 19.32 mM Lithium. Means (±: n= 3) in the same column for each trait in each group with the same lower-case letter are not significantly different by ANOVA test at *P*≤0.05.

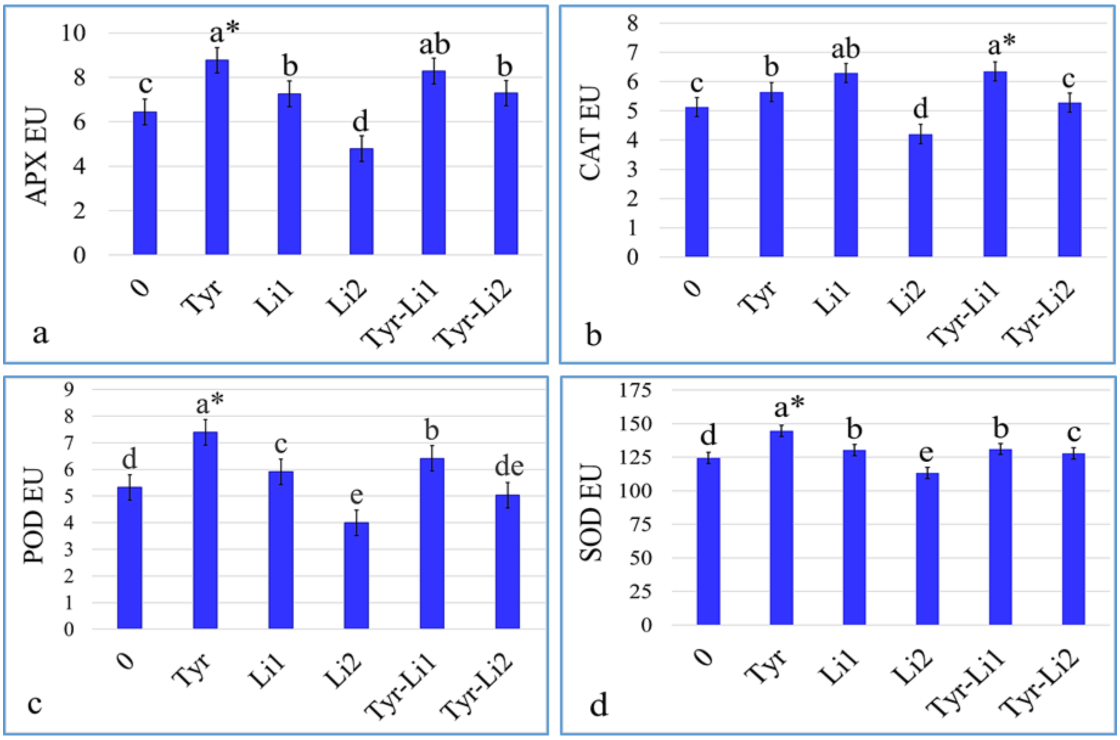

Figure 3. Variation of APX (3a), CAT (3b), POD (3c), and SOD (3d) activities in the spinach seedlings. 0: Control, Tyr: 2.5 mM L-Tyrosine, Li1: 6.43 mM Lithium, Li2: 19.32 mM Lithium. Means (±: n= 3) in the same column for each trait in each group with the same lower-case letter are not significantly different by ANOVA test at $P \leq 0.05$.

Table 3. Variation of Li, Mg, P, S, K, Ca, and Na concentrations (mg kg^{-1})

Group	Li	Mg	P	S	K	Ca	Na
0	21±2.5f*	12786±120a	5545±30.6c	6545±60.6c	86556±88a	15479±52c	5067±45d
Tyr	80.6±7.6e	10548±104d	4234±28.8e	5067±44.7d	66789±66e	12345±122d	12071±120b
Li1	845±70.6c	12456±120ab	5878±35.6b	7234±60.2b	83481±82b	18770±170b	8145±75c
Li2	1268±120ab	12430±120ab	6244±408a	7685±60.8a	81345±80bc	20356±202a	12456±125b
Tyr-Li1	612±60.8d	12054±120b	5566±33.2c	7066±55.6bc	82345±80b	17123±160bc	14223±140a
Tyr-Li2	1014±102	11884±115c	5346±304d	6495±40.4c	75669±76d	19245±190a	12355±122b
F	18232054	495646	689575	6059	30079386	7814416	13184637
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

*: Means (±: n= 3) in the same column for each trait in each group with the same lower-case letter are not significantly different by ANOVA test at $P \leq 0.05$. 0: Control, Tyr: L-Tyrosine, Li-1: 6.43 mM Lithium, Li-2: 19.32 mM Lithium.

Table 4. Variation of Cl, Mn, Fe, Ni, Cu, and Zn concentrations (mg kg^{-1})

Group	Cl	Mn	Fe	Ni	Cu	Zn
0	5678±45c*	96.7±5.6f	2545±20.6e	34.56±2.4c	13.57±0.8c	115.48±10.8b
Tyr	2880±23d	90.8±4.8g	2044±18.8f	30.35±2.2d	10.24±0.6d	80.68±2.4d
Li1	10566±104bc	175.5±12.6b	4456±36.6b	48.77±6.4a	18.80±1.2a	126.56±11.7a
Li2	13466±133a	150.4±10.5cd	3423±28.8c	40.56±5.4b	15.48±0.9b	107.46±9.8c
Tyr-Li1	11456±112b	125.5±8.8e	3054±25.7d	33.46±3.6c	12.99±0.6c	114.57±10.3b
Tyr-Li2	10567±104bc	180.3±13.6a	5026±38.8a	40.67±3.8b	15.47±0.8b	124.55±11.7a
F	21249159	8915	3093776	1103408	136044	7252572
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

*: Means (±: n= 3) in the same column for each trait in each group with the same lower-case letter are not significantly different by ANOVA test at $P \leq 0.05$. 0: Control, Tyr: L-Tyrosine, Li-1: 6.43 mM Lithium, Li-2: 19.32 mM Lithium.

4. Conclusion

In this study, it was concluded that higher lithium levels negatively affect spinach growth rate, accumulation of pigments, secondary metabolites, and activity of enzymes. The exogenous supplement of tyrosine had a positive effect in improving all these attributes in the seedlings, even in lithium-treated groups. Incremental values of growth parameters, including shoot, root, and leaves by Tyr supplements were parallel with the enhanced synthesis of chlorophylls. The amounts of chlorophyll, lutein, secondary constituents, and the activity of antioxidant enzymes were decreased by the higher lithium level, but MDA, H_2O_2, and nitrate content increased. However, an external supplement of tyrosine reduced these examined constituents by increasing the activity of enzymes, and secondary compounds. A Tyrosine supplement to seedlings inhibited nitrate accumulation by stimulating nitrogenous compounds but increased NR activity.

The Mg, K, P, S, and Ca contents were lower in the Tyr-applied group, but the Cl, Mn, Fe, Co, Ni, Cu, Zn, Cr, Si, Al, Ag, Cd, I, and P concentrations were reduced by the Tyr and the Li1-Tyr groups. On the other hand, while Li doses increased the Li contents in seedlings, exogenous Tyr supplements caused a notable decrement in Li accumulations. It seems that with the application of exogenous tyrosine to the plant, the synthesis of tyrosine-derived compounds that increase yield and quality, increase resistance to stress, and are also beneficial for human health, can be stimulated. In conclusion, it can be said that exogenous Tyr supplements showed a positive effect on the growth rate of the Anlani F1 spinach cultivar, therefore, exogenous Tyr applications in barren, calcareous, and alkaline soils can be an alternative in spinach cultivation.

References

Al-Mohammad MHS, Al-Taey DKA (2019) Effect of tyrosine and sulfur on growth, yield and antioxidant compounds in *Arugula* leaves and seeds. Research on Crops 20(1): 116-120.

Bakhat HF, Rasul K, Farooq ABU (2020) Growth and physiological response of spinach to various lithium concentrations in soil. Environmental Science and Pollution Research 27: 39717-39725.

Bostancı KB, Ulger S (2022) Comparison of spinach cultivation in floating hydroponic system and soil in a glasshouse and open field conditions. Medıterranean Agrıcultural Scıences 35(1): 7-14.

Cataldo DA, Haroon M, Schrader LE, Young VL (1975) Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. Communications in Soil Science and Plant Analysis 6(1): 71-80.

Citak S, Sonmez S (2010) Effects of conventional and organic fertilization on spinach (*Spinacea oleracea* L.) growth, yield, vitamin C and nitrate concentration during two successive seasons. Scientia Horticulturae 126(4): 415-420.

Dickerson DP, Pascholati SF, Hagerman AE, Butler LG, Nicholson RL (1984) Phenylalanine ammonia-lyase and hydroxycinnamate: CoA ligase in maize mesocotyls inoculated with *Helminthosporium maydis* or *Helminthosporium* carbonum. Physiological Plant Pathology 25(2): 111-123.

El-Sherbeny MR, Jaime A, Da Silva T (2012) Foliar treatment with proline and tyrosine affect the growth and yield of beetroot and some pigments in beetroot leaves. Journal of Horticultural Research 121(2):95-99.

Feduraev P, Skrypnik L, Riabova A, Pungin A, Tokupova T, Maslenikov P, Chupakhina G (2020) Phenylalanine and tyrosine as exogenous precursors of wheat (*Triticum aestivum* L.) secondary metabolism through PAL-associated pathways. Plants 9: 476.

Folin O, Denis W (1915) A colorimetric method for the determination of phenols (and phenol derivatives) in urine. Journal of Biological Chemistry 22(2): 305-308.

Franzaring J, Schlosser S, Damsohn W, Fangmeier A (2016) Regional differences in plant levels and investigations on the phytotoxicity of lithium. Environmental Pollution 6: 858-865.

Gülçur F (1974) Physical and chemical analysis methods of soil. Istanbul University Faculty of Forestry Publications No: 201, Istanbul.

Hawrylak-Nowak B, Kalinowska M, Szymańska M (2012) A study on selected physiological parameters of plants grown under lithium supplementation. Biological Trace Element Research 149(3): 425-30.

Helaly AAE, Ibrahim FR (2019) Influence of iron, zinc and tyrosine acid on growth, yield components and chemical constituents of *Hibiscus sabdariffa* L. plant. Current Science International 8(1): 128-139.

Hildebrandt TM, Nunes Nesi A, Araújo WL, Braun HP (2015) Amino acid catabolism in plants. Molecular Plant 8: 1563-79.

Hoagland DR, Arnon DI (1950) The water-culture method for growing plants without soil, California. Agriculture Experiment Station Circular 347: 1-32.

Kalinowska M, Hawrylak-Nowak B, Szymańska M (2013) The influence of two lithium forms on the growth, L-ascorbic acid content and lithium accumulation in lettuce plants. Biological Trace Element Research 15(2): 251-257.

Klepper L, Flesher D, Hageman RH (1971) Generation of reduced nicotinamide adenine dinucleotide for nitrate reduction in green leaves. Plant Physiology 48(5): 580-590.

Kukric ZZ, Topalic-Trivunovic LN, Kukavica BM, Matoš SB, Pavičic SS, Boroja MM, Savic AV (2012) Characterization of antioxidant and microbial activities of nettle leaves (*Urtica dioica* L.). Acta Periodica Technologica 43: 257-272.

Kusvuran A, Kaytz A, Yılmaz U, Kusvuran S (2019) The effects of exogenous amıno acıd on growth, ıonıc homeostasıs, bıochemıcal composıtıon and antıoxıdatıve actıvıty of guar (*Cyamopsıs tetragonoloba* (L.) taub.) seedlıngs. Applied Ecology and Envıronmental Research 17(6): 15519-15530.

Lutts S, Kinet JM, Bouharmont J (1996) Effects of various salts and of mannitol on ion and proline accumulation in relation to osmotic adjustment in rice (*Oryza sativa* L.) callus cultures. Journal of Plant Physiology 149: 86-195.

Makus DJ, Zibilske L, Lester G (2006) Effect of light intensity, soil type, and lithium addition on spinach and mustard greens leaf constituents. Subtropical Plant Science 58: 35-41.

Mulkey TJ (2005) Alteration of growth and gravitropic response of maize roots by lithium. Gravitational and Space Biology Bulletin 18(2): 119-120.

Perchlik M, Tegeder M (2018) Leaf amino acid supply affects photosynthetic and plant nitrogen use efficiency under nitrogen stress. Plant Physiology 178: 174-188.

Robinson BH, Yalamanchali R, Reiser R, Dickinson NM (2018) Lithium as an emerging environmental contaminant: Mobility in the soil-plant system. Chemosphere 197: 1-6.

Ruan C, Rodgers MT (2004) Cation−π interactions: structures and energetics of complexation of Na^+ and K^+ with the aromatic amino acids, phenylalanine, tyrosine, and tryptophan. Journal of the American Chemical Society 126: 14600-14610.

Saddıque M, Kausar A, Iqra I, Akhter N, Mujahıd N, Parveen A, Zaman Q, Hussaın S (2022) Amino acids application alleviated salinity stress in spinach (*Spinacia oleracea* L.) by improving oxidative defence, osmolyte accumulation, and nutrient balance. Turkish Journal of Agriculture and Forestry 46(6): 875-887.

Shams M, Yildirim E, Ekinci M, Turan M, Dursun A, Parlakova F (2016) Exogenously applied glycine betaine regulates some chemical characteristics and antioxidative defence systems in lettuce under salt stress. Horticulture, Environment, and Biotechnology 57: 225-231.

Siu FM, Ma NL, Tsang CW (2004) Competition between π and non-π cation-binding sites in aromatic amino acids: a theoretical study of alkali metal cation (Li^+, Na^+, K^+)–phenylalanine complexes. Chemistry Europe Journal 10: 1966-1976.

Tarasevičienė Ž, Velička A, Paulauskienė A (2021) Impact of foliar application of amino acids on total phenols, phenolic acids content of different mints varieties under the field condition. Plants 10(3): 599.

Turfan N (2023) The effects of exogenous melatonın applıcatıon on growth rate parameters and bıoactıve compounds of some spınach cultıvars (*Spınach oleracea* L.) grown under wınter condıtıons. Applied Ecology and Environmental Research 21(2): 1533-1547.

Velikova V, Yordanov I, Edreva A (2000) Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective role of exogenous polyamines. Plant Science 151(1): 59-66.

Verhoeven A, García-Plazaola JI, Fernández-Marín B (2018) Shared mechanisms of photoprotection in photosynthetic organisms tolerant to desiccation or low temperature. Environmental and Experimental Botany 154: 66-79.

Xu J, Liu T, Yang S, Jin X, Qu F, Huang N (2019) Polyamines are involved in GABA-regulated salinity-alkalinity stress tolerance in muskmelon. Environmental and Experimental Botany 164: 181-189.

Wang Y, Wang J, Guo D, Zhang H, Che Y, Li Y, Tian B, Wang Z, Sun G, Zhang H (2021) Physiological and comparative transcriptome analysis of leaf response and physiological adaption to saline-alkali stress across pH values in alfalfa (*Medicago sativa*). Plant Physiology and Biochemistry 167: 140-152.

Zhang LL, Han SC, Li ZG, Liu N, Li LY (2006) Effects of the infestation by *Actinote thalia pyrrha* (Fabricius) on the physiological indexes of *Mikania micrantha* leaves. Acta Ecologica Sinica 26(5): 1330-1336.

Zhang K, Wu Y, Hang H (2019) Differential contributions of NO_3^- /$NH4^+$ to nitrogen use in response to a variable inorganic nitrogen supply in plantlets of two Brassicaceae species in vitro. Plant Methods 15: 86.

Zhang TP, Zhang WX, Li DX, Zhou FL, Chen X, Li, CY, Yu S, Brestic M, Liu Y, Yang XH (2021) Glycinebetaine: a versatile protectant to improve rice performance against aluminium stress by regulating aluminium uptake and translocation. Plant Cell Reports 40: 2397-2407.

Research Article

www.dergipark.org.tr/en/pub/mediterranean

MEDITERRANEAN AGRICULTURAL SCIENCES
(2023) 36(3): 109-115
DOI: 10.29136/mediterranean.1327381

Biological control of sesame moth and tobacco whitefly in Antalya sesame fields

Hasan PART, Ismail KARACA

Isparta University of Applied Sciences, Faculty of Agriculture, Plant Protection Department, 32950, Isparta, Türkiye

Corresponding author: H. Part, e-mail: hasanpart.07@hotmail.com
Author(s) e-mail: ismailkaraca@isparta.edu.tr

ARTICLE INFO

Received: July 14, 2023
Received in revised form: October 6, 2023
Accepted: October 11, 2023

***Keywords*:**

Azadirachtin
Beauveria bassiana
Sesame moth
Tobacco whitefly
Trichogramma evanescens

ABSTRACT

Sesame moth and tobacco whitefly cause significant damage to sesame plants, especially in the second-period sesame cultivation areas, if early intervention is not implemented. This greatly reduces efficiency. Chemical control is generally preferred for these pests in Türkiye. Today, the use of chemical control creates many harmful results. Therefore, in our study, the aim is to use biological control agents and preparations as an alternative to chemical control. In this study, applications of Nostalgist Bl biological insecticide containing 1.5% *Beauveria bassiana* strain Bb-1 1x10^{8} CFU mil^{-1} spores, Nimbecidine biological insecticide containing 0.3 g L^{-1} Azadirachtin active ingredient, and *Trichogramma evanescens* Westwood (Hymenoptera: Trichogrammatidae) an egg parasitoid and activities were determined. The application area (2 da) was divided into 3 blocks; each block was named with the letters A, B, and C. Each block was divided into four parcels. The above applicatios and biological insecticides were applied separately in each parcel, including the control parcels in each block. In the counts made according to the application results, there was a significant decrease in whitefly eggs and nymphs on the leaf surface. A very significant decrease was observed in the populations per plant in the sesame moth larvae counts. The highest efficiency was obtained with Nimbecidine and Nostalgist applications. The yield was moderate in the plots with parasitoid application.

1. Introduction

Sesame is an important oil plant with high nutritional and economic value, and so it is used as a raw material in many sectors. Sesame [*Sesamum indicum* (Lamiales: Pedaliaceae)] is native to Africa and a secondary crop in Türkiye. It is grown in hot regions both around the world and in Türkiye. Sesame capsules contain a large number of seeds, which are used as a spice and as ingredients in products such as bagels, bread, cakes, buns, and pies. It is the raw material for the production of tahini, and helva. According to Atakisi (1991), after oil extraction from sesame, the pulp remains. Sesame meal is used as an important animal feed as it is also mixed with bread.

Sesame is cultivated as a second crop after cereal harvests in coastal areas of Türkiye (Tan 2015). Second sowing is done in June, July, and August.

In Türkiye, sesame was cultivated on an area of 254862 da in 2021, while this number was 53910 da in 2020, and 56598 da in 2021 just in Antalya. The yield of 84 kg da^{-1} was obtained in both years (TUIK 2022).

In Manavgat, 2400 tons of sesame are harvested on a production area of 41000 da. Approximately 20% of the sesame produced in Türkiye is produced in Antalya and approximately 80 % of the sesame produced in Antalya is produced in Manavgat district. Approximately 17% of the sesame grown in Türkiye is in Manavgat; in other words, three-quarters of the sesame cultivated in Antalya is grown in Manavgat. Manavgat sesame is known as Golden sesame and has a 60% oil content (MATSO 2020).

Since sesame is a plant that is used in many areas, it is necessary to increase the yield per unit area. The most important pests in sesame plants are whitefly and sesame moth. If they are not controlled, they cause high yield losses.

Bodenheimer (1958) stated in a study that the larvae of *Antigastra catalaunalis* Dup. (Lepidoptera: Pyralidae), which colonize sesame plants, cause significant damage during the seedling and flower phases. In a study carried out in the province of Antalya, it was reported that a 74.7 kg da^{-1} sesame yield was obtained in 1938, but then it decreased to 34.4 kg da^{-1} in 1939.

Larval damage usually occurs by twisting sesame leaves transversely, spinning webs, and feeding on leaves. In addition, at flower formation time, they weave the flower buds with nets and then eat them before the plant's entire flowering. For this reason, the sesame plant cannot develop and form capsules at all. At the time when sesame capsules emerge, the larvae can eat both the seeds by making holes in the capsules and the capsules as well by chewing them.

The whitefly, *Bemisia tabaci* Genn. (Homoptera: Aleyrodidae), another sesame pest causes damage by sucking and producing fumagine, especially during the flowering period. In addition, it facilitates fungal and viral diseases by vectoring them. Ashley (1993) mentioned that *Phyllody* virus and *Nicotiana* virus can be transmitted by whitefly in sesame.

In 1983-1984, 26 pest species were detected on sesame plants in the second growth of sesame in Aydın, İzmir, and Manisa provinces, and the most abundant species in terms of total number of individuals in all samples was *B. tabaci* (Genn.) (Hemiptera: Aleyrodidae) (Zümreoğlu and Akbulut 1988).

In this study, the aim was the control of pest populations by using biological control agents and bioinsecticides (*Beauveria bassiana*, *Trichogramma evanescens* and Azadiractin) without using chemical insecticides in the control of tobacco whitefly and sesame moth pests, which cause significant damage to sesame plants.

2. Materials and Method

2.1. The trial site

The trial was established in a field with an area of 2000 m^2 in the Çavuşköy zone of the Manavgat district in Antalya province. The heirloom seeds known as Manavgat sesame were sown in the field. The field was divided into three blocks as replications. The blocks were named A, B, and C. Each block was equally divided into four plots and were marked with numbers. The 4^{th} plots of each block were used as a control plots. In the 3^{rd} plot blocks, *Trichogramma evanescens*, the subject parasitoid wasp was released one by one. The 3^{rd} plot were isolated with 75% net curtains. Biological insecticides were used sequentially in the remaining blocks.

2.2. The origin of applied parasitoids and biological insecticides

Trichogramma evanescens as 20000 pieces were provided from Trimail Biological Agriculture of Ankara University's Teknokent. Nostalgist Bl biological insecticide containing 1.5% *B. bassiana* strain Bb-1 1×10^8 CFU mil^{-1} min spores and Nimbecidine biological insecticide containing 0.3 g L^{-1} Azadirachtin active ingredient were procured from Agrobest Agriculture Group. Yellow, blue, and black sticky traps were provided and then randomly hung in the plots according to the plant size in order to identify the adult individual's emergence of the subject pests and other pest types.

2.3. Identification of pest populations

The fight against the sesame moth, *Antigastra catalaunalis* Dup. (Lepidoptera: Pyralidae), was started with 50 sesame plants, which were randomly selected from each block based on the information stated on the page of the Ministry of Agriculture and Forestry that the application was started when the plant infestation rate was 20%. After an estimation of the infestation rate, the application was initiated.

For the tobacco whitefly population (Anonymous 2008), based on information data from the Ministry of Agriculture and Forestry stating that the control should be initiated when 60 leaves are counted, 1 each from the lower, common and upper parts of at least 20 randomly selected plants and when an average of 5 larvae or pupae per leaf are counted, the population densities were determined in the field as follows: 50 plants were randomly selected and tobacco whitefly eggs and nymphs were counted on the lower, middle, and upper leaves. Sesame moth larvae were counted in 50 randomly selected sesame plants, and infected plants were identified. In order to asses its effectiveness, the trial field scouting was carried out on 4 different dates (July 30, August 10, August 22, and August 31, 2021). taking into account the economic damage threshold.

2.4. Determination of trial plots and application

The 2000 m^2 trial field was divided into 3 blocks equally, and the blocks were lettered A, B, and C. Each block was equally divided into 4 plots marked with numbers. In each block plots, biological insecticide applications and parasitoid release were conducted in the 3 replications as shown in Figure 1. The 4^{th} plot was left as a control plot.

A1 Nimbecidine	B1 Nostalgist	C1 Nimbecidine
A2 Nostalgist	B2 Nimbecidine	C2 Nostalgist
A3 *Trichogramma evanescens*	B3 *Trichogramma evanescens*	C3 *Trichogramma evanescens*
A4 Control	B4 Control	C4 Control

Figure 1. Schematic of applications in the study plots.

Biological insecticides were applied by spraying on the plants with the help of a 16-liter knapsack sprayer, so the upper and underneath of the leaves were all well covered. Each plot was 165 m^2. Nimbecidine at a dosage of 40 mL and Nostalgist at a dosage of 42 mL (250 ml ha^{-1}) were applied to the plots except the 3^{rd} and 4^{th} plots of the blocks, which were treated with 8 L of water. The first application was conducted on September 1, 2021, and the second application was conducted on September 9, 2021.

On August 31, 2021, a total of 20000 *Trichogramma evanescens* parasitoids, 2000 in each box, were released separately within equal boxes in the 3^{rd} blocks by hanging the boxes on the plants.

2.5. Post-application counts

Similarly, for the counts carried out to determine population density, on September 1, 2021 (a week after the first application) 50 plants were randomly selected from each plot and counted for infested tobacco whitefly larvae and nymphs on the lower, middle and upper leaves. For sesame moth larvae, 50 plants that were randomly selected from each plot were observed and along with the previous. For sesame moth larvae, 50 plants that were randomly selected from each plot were observed, counted and then monitored by comparing with the previous situation. In a similar way, on September 9, 2021 (a week after the second application) counting was carried out again, and the effectivity graphs of all the pesticide types were drawn.

2.6. Evaluation of trials and statistical analysis

The effect of the treatments on yield as assigned in different plots was calculated after weighing the product obtained in the plots at harvest time. For this purpose, the plants in each treatment were harvested separately, and their biomass was weighed. Afterwards, the results were subjected to statistical analysis.

A one-way analysis of variance (One-Way ANOVA) was applied to the trial field that was designed as a completely randomized block. Then Tukey's multiple comparison test (Tukey 1949) was employed to determine the source of differences. Statistical analyses were carried out using IBM SPSS® Statistics (IBM SPSS 2008).

3. Results and Discussion

As a result of the experiment the number of insects caught in the traps before the treatment is shown in Table 1.

As can be seen in the table, varied numbers of insects were caught in differently colored traps in the two pre-treatment counts. The number of insects caught in the second count was higher than in the first count. Before application, thrips were not caught in the yellow sticky traps, and whitefly was not caught in the blue sticky traps and *Empoasca* sp. was caught in all three colored traps. *Macrolophus pygmaeus* Rambur (Hemiptera: Miridae), a polyphagous predatory species that feeds on pests such as whiteflies, mites, aphids, and gallery flies, was caught only in the black-colored sticky traps. As seen in Table 2, *Empoasca* sp. was caught equally in the yellow and black-colored traps, while it was caught less in blue traps. The sesame moth was not caught in any of the traps.

The number of insects caught in the traps after parceling and treatments is shown in Table 2.

As shown in Table 2, it was found that although there were numerical differences between the insects caught in traps, but for the post-treatment application, there was no statistical difference.

Although there was no difference between the treatments, there was a difference between the traps depending on the treatments. Whitefly individuals were not caught in the blue sticky traps but they were caught in the yellow traps in quite varied amounts as compared to the black traps. Thrips were caught only in the blue traps while *Empoasca* species were caught in all three colors of traps with the highest number caught in the yellow traps, followed by the black traps and the lowest number found in the blue traps. The predatory insect *M. phymaeus* was caught only in the black traps, although in small numbers.

A comparison between Table 1 and Table 2 leads to the conclusion that the yellow sticky traps can be used in sesame-growing areas for catching whiteflies, the blue sticky traps for thrips and the yellow and black sticky traps for *Empoasca* species.

The graphs showing the results of the direct counts conducted on 50 plants in the field but outside the traps are presented in Figures 2-6.

As can be seen in Figure 2, Nimbecidine was applied to the study plots on September 1 and 9, 2021, and it was determined that it was highly effective against whitefly in the subsequent monitoring. In a similar way to Nimbecidin, the applied Nostalgist was found to be highly effective against whitefly eggs and nymphs (Figure 3).

As presented in the Figures 4 and 5, Nimbecidine and Nostalgist were applied to the study plots on September 1 and 9, 2021. On the next counting dates, it was found highly effective against sesame and similarly for the whitefly.

Figure 6 shows the graph of sesame moth population change after the monitoring of sesame moth and the field releases of the parasitoid *Trichogramma evanescens* in the study plots. As can be seen in Figure 6, there was a significant decrease in the number of moths immediately after the release.

In this case, all figures are evaluated together, very significant effects of the biological insecticides on both whitefly and sesame moth were found.

It was also found that the parasiticide used was also effective in terms of reducing the population of Sesame moth.

The yield results obtained in the study plots in their treatments during the harvest period are presented in Table 3.

The presentation in Table 3 shows that the yield was also different in the plots with different treatments and the differences were statistically significant. However, all treatments were found statistically different from the control treatment. The highest yield was obtained in Nimbecidine and Nostalgist treatments and both treatments were statistically in the same group. The yield in plots treated with *Trichogramma evanescens* was in the moderate group and statistically different from the other groups.

Coşkuntuncel (1995), on the egg of *Ostrinia nubilalis* Hübner (Lepidoptera: Pyralidae), the maize worm, in the secondcrop corn production areas of Çukurova University, Faculty of Agriculture Research and Application Farm, on a 5 decare are a twice (40 thousand + 40 thousand) twice a week (40 thousand + 440 thousand) produced a mass release activity by releasing one thousand egg parasitoids *Trichogramma evanescens* Westwood (Hymenoptera: Trichogrammatidae). Success rates were 81.25% and 56.66% in 1993 and 1994, respectively.

Ferizli (1997) applied Azadirachtin to *Spodoptera littoralis* Boisd (Lepidoptera: Noctuidae) and stated that depending on the applied doses, it reduced the weight of the larvae, prolonged the larval period and caused larval death.

Çeribaşı (2001) investigated the effectiveness of azadirachtin at different concentrations on *Bemisia tabaci* and stated that azadirachtin was effective in the adult and pre-adult periods.

Kılıç (2006) applied the entomopathogenic fungus *Beauveria bassiana* to tobacco whiteflies. On the sixth day, 36% nymphal death and 48% adult death were observed.

Table 1. Number of insects caught in differently colored traps (number of insects/trap)

Date of Counting	Yellow sticky trap		Blue sticky trap		Black sticky trap		
	Whitefly	Cicadellid	Thrips	Cicadellid	Whitefly	Cicadellid	*Macrolophus pygmaeus*
25.7.2021	108.33	93.89	100.00	43.33	10.33	88.33	10.56
7.8.2021	213.89	201.11	193.89	51.67	5.00	173.89	10.89

Table 2. Number of insects caught in differently colored traps (number of insects/trap)

Insects	Whitefly		Thrips	Empoasca			*Macrolophus pygmaeus*
Trap	Yellow	Black	Blue	Yellow	Blue	Black	Black
Nimbecidine	134.0±24.58	16.6±1.66	131.6±10.92	180.00±21.24	19.00±3.78	116.67±41.76	9.33±3.84
Nostalgist	125.33±12.45	15.33±4.25	173.00±28.00	226.67±3.47	13.33±1.66	172.33±126.38	9.67±3.71
Trichogramma evanescens	110.67±9.82	27.33±2.84	260.67±57.52	153.33±23.95	4.33±4.33	95.00±22.81	6.00±0.57
Control	123.67±18.22	18.33±4.37	158.33±14.52	124.00±31.60	19.33±6.22	99.67±6.11	16.67±6.88

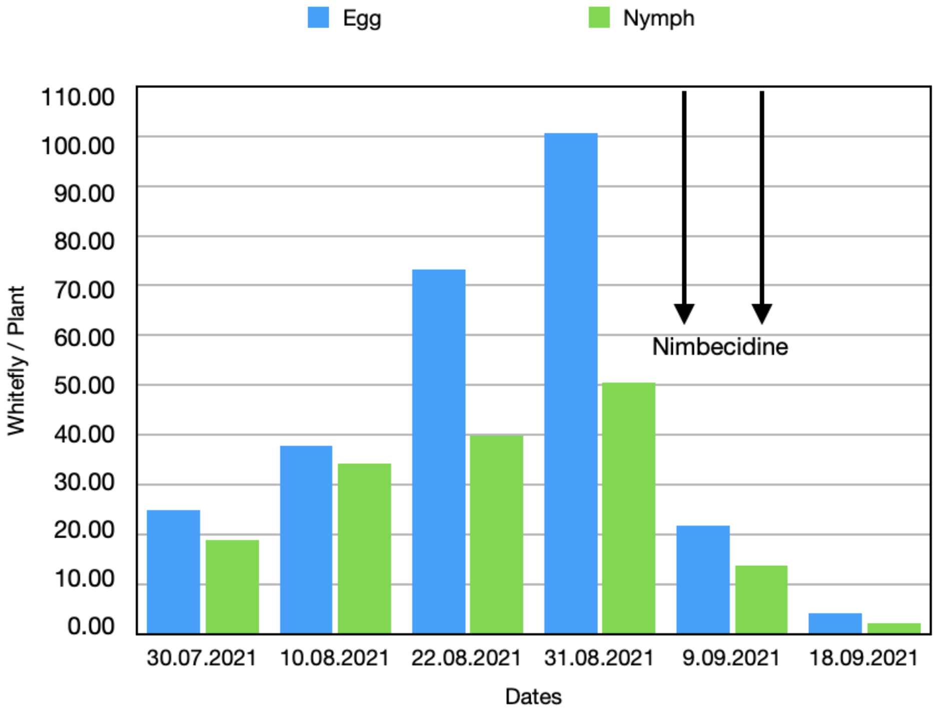

Figure 2. Whitefly numbers/plant.

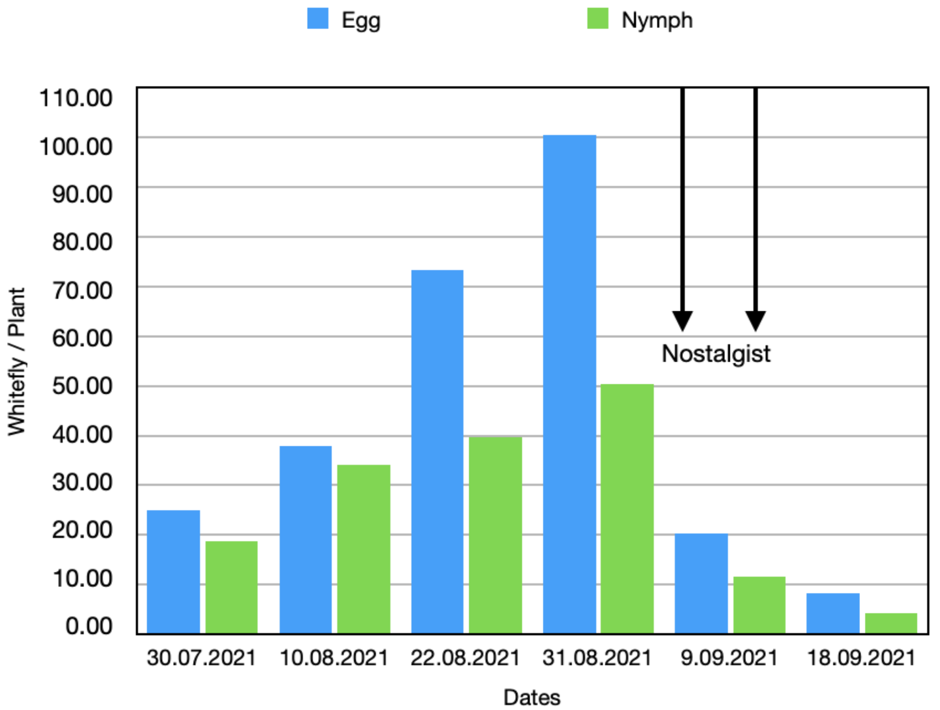

Figure 3. Whitefly numbers/plant.

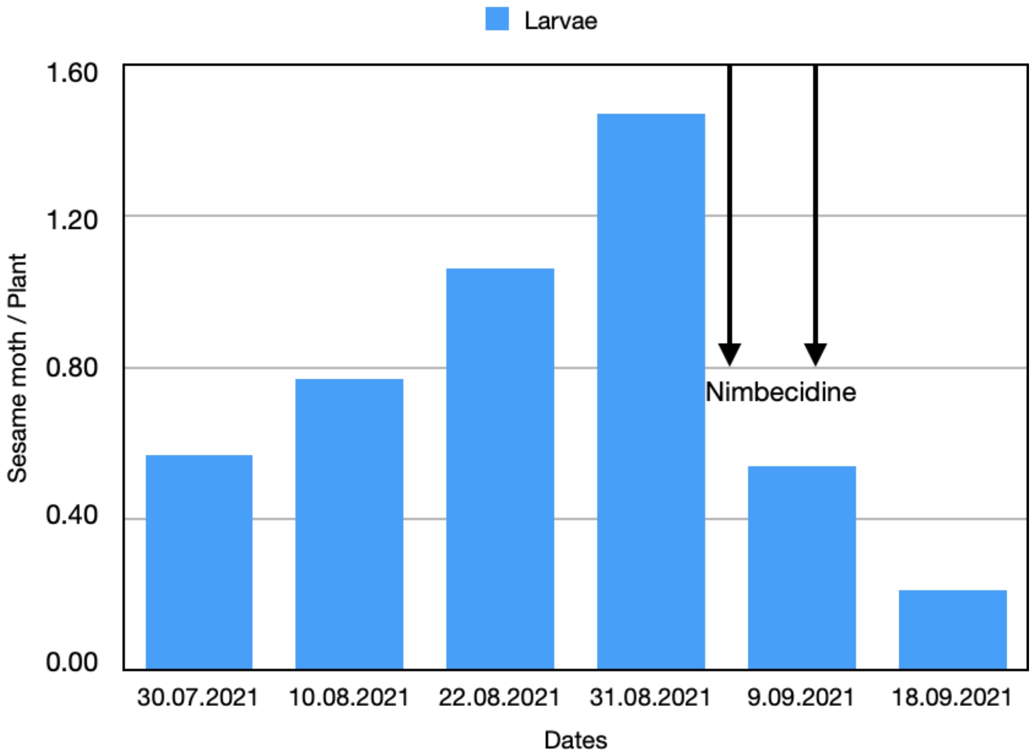

Figure 4. Sesame moths' number/plant.

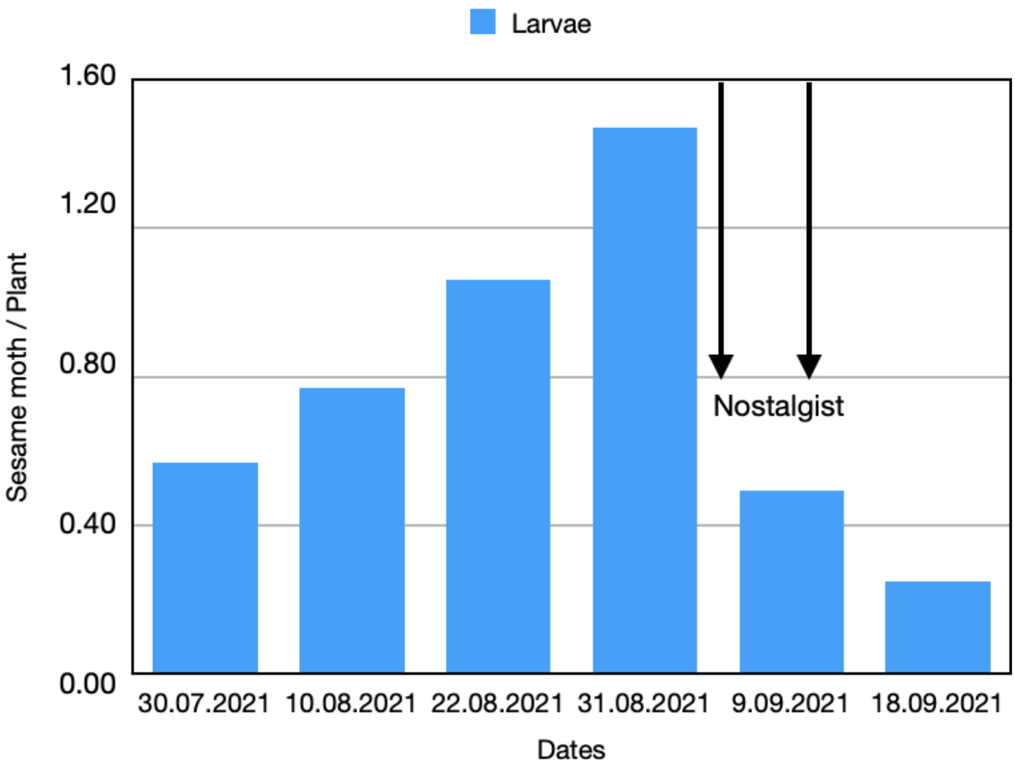

Figure 5. Sesame moths' number/plant.

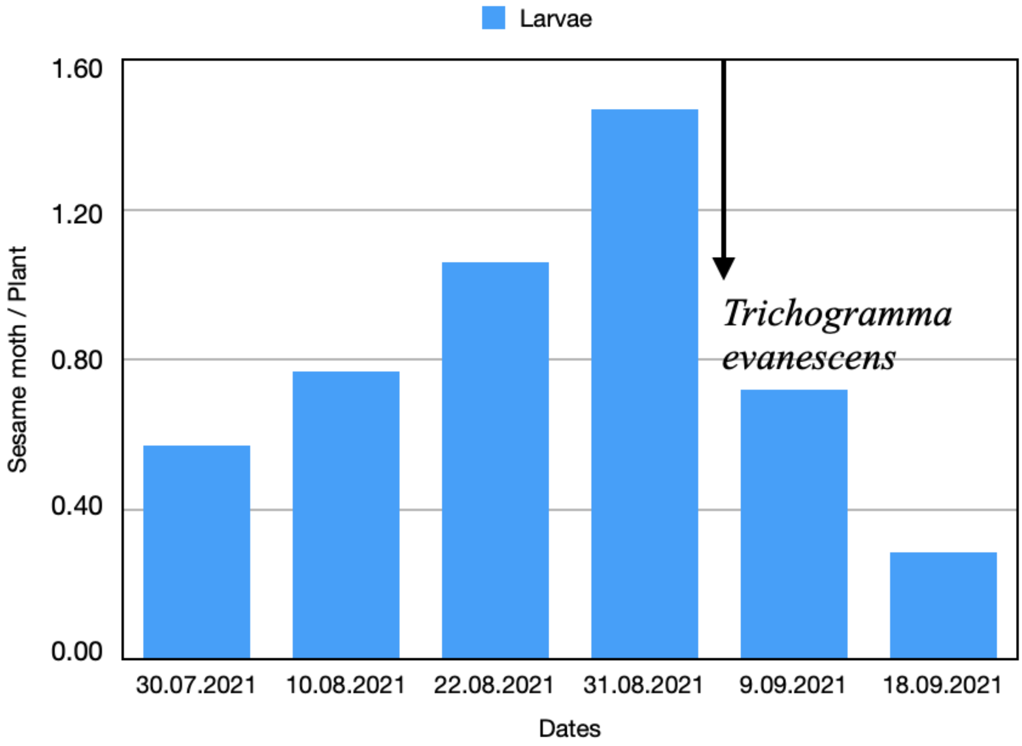

Figure 6. Sesame moths' number/plant.

Table 3. Effect of different treatments on yield (kg/treatment)

Treatments	Yield
Nimbecidine	15.88±0.44 a
Nostalgist	15.33±0.88 a
Trichogramma evanescens	12.50±0.28 b
Control	2.58±0.30 c

Ulusoy (2016) in his study, applied the entomopathogenic fungus containing *Beauveria bassiana* 1x107 spores to *Leptinotarsa decemlineta* larvae by the spraying method and observed 86.6% larval death on the 7th day.

Cırbın et al. (2017) applied *B. bassiana* isolates to *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) and found it quite effective. The LT_{50} values for BMAUM-E2003, BMAUM-E6001, and BMAUM-M6001 were calculated as 2.76, 3.97, and 4.23 days, respectively. He stated that BMAUM-E2003, and BMAUM-E6001 isolates have the potential to be used in future studies.

Akdaş et al. (2020) observed the activity of 12 subcultures of *Beauveria bassiana* BMAUM-E2003, and BMAUM-E6001 isolates, isolated from the soils of Isparta province and its districts in 2014 on mycelium development and *Spodoptera littoralis* larvae, which cause significant economic losses in agricultural products. It was determined that it caused 100% death on the 5th day.

4. Conclusion

So far, the biological pest control method has become very important. Based on the literature, it was found that there are very few biological control studies on the sesame plant. In fact, the results obtained from this study proved that biological control of the main pests in the sesame crop can be achieved effectively.

Parasitoid *Trichogramma evanescens* applied against sesame moth eggs in the study was effective and significantly reduced larval emergence.

An application of a bioinsecticide with an AZA active ingredient against sesame moth larvae resulted in a high efficacy.

The bioinsecticides AZA, and *B. bassiana*, that were applied to *B. tabaci*, decreased the population densities through out the successive post-treatment 7- days counts and it was later observed that those bioinsecticides were highly effective.

This study's data evaluation revealed that biological insecticides are effective on both whitefly and sesame moth and this effect is very important. Another remarkable result is that the parasiticide used was also effective in reducing the population of the sesame moth.

Moreover, when the results are evaluated in terms of the reflection of treatments on yield, it was found that higher yields were obtained from the two entomopathogens: Nimbecidine and Nostalgist. As a conclusion, the use of these agents for subject pests' control in sesame production is recommended.

References

Akdaş A, Kalkar Güven Ö, Karaca İ (2020) Efficacy of *Beauveria bassiana* Isolates Made from Subculture on *Spodoptera littoralis*. Turkish Journal of Biological Control 11(2): 175-187.

Anonymous (2008) Ministry of Agriculture and Forestry, Agricultural Struggle Technical Instructions. https://www.tarimorman.gov.tr/TAGEM/Belgeler/Teknik%20tal%C4%B1matlar%202008/C%C4%B0LT%202.pdf. 200-202 Accessed 07 December, 2022.

Ashley J (1993) Oil Seeds. In Dry Land Farming in Africa. J.R.J. Rowland (eds.), pp. 240-242.

Atakisi İK (1991) Oil Crops Cultivation and Breeding Trakya University. Tekirdağ Faculty of Agriculture. Lesson Book 10, Puplication 11, Publication No 148, pp. 181.

Bodenheimer FS (1958) A Study on Insects Harm ful to Agriculture and Trees in Türkiye and Combating Them. Bayur Printing House. https://kutuphane.tarimorman.gov.tr/pdf_goster?file=9d14b062069 bc02ddfafaa2889cb1511#book/217. Accessed 07 December, 2022.

Cırbın İ, Güven Ö, Karaca İ (2017) Some entomopathogenic fungi have *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) Determination of their effect on their larvae. Journal of Agricultural Faculty of Gaziosmanpasa University 34(3): 159-165.

Coşkuntuncel S (1995) Determination of Mass Release Activity and Natural Parasitization Rate of Egg parasitoid (*Trichogramma evanescens* Westwood, Hymenoptera: Trichogrammatidae) in Biological Control of Corn worm (*Ostrinia nubilalis* Hübner, Lepidoptera: Pyralidae) in Çukurova. Master Thesis, Çukurova University, Institute of Science and Technology, Adana.

Çeribaşı N (2001) Research on the effectiveness of Azadirachtin against some vegetable pests. Master's Thesis, Akdeniz University, Institute of Science and Technology, Department of Plant Protection, Antalya.

Ferizli AG (1997) *Azadirachta indica* A. Juss and *Melia azedarach* L. (Meliaceae) *Spodoptera littoralis* Boisd of Sdrains. (Lep: Noctuidae) Investigations on Some Plants. Ph.D. Thesis, Ankara University, Institute of Natural and Applied Sciences, Department of Plant Protection, Ankara.

Kılıç E (2006) Determination of the potentials of the isolates of *Beauveria bassiana* (Balsamo) Vuillemin, 1826 (Hyphomycetes: Deuteromycotina) in biological control with tobacco whitefly [*Bemisia tabaci* Gennedius, 1936 (Homoptera: Aleyrodidae)]. Ph.D. Thesis, Atatürk University, Graduate School of Natural and Applied Sciences, Erzurum.

MATSO (2020) Manavgat Golden Sesame is Registered With Geographical Indication. https://www.matso.org.tr/haberler/gungor-manavgat-altin-susami-cografi-isaret-ile-tescillendi.html. Accessed 07 December, 2022.

IBM SPSS (2008) Base 10.0 Application Guide. SPSS, Chicago, IL, USA.

Tan AŞ (2015) Sesame Farming. Republic of Türkiye Ministry of Food, Agriculture and Livestock. Aegean Agricultural Research Institute Publications.https://arastirma.tarimorman.gov.tr/etae/Belgeler/Egiti mBrosur/135-ciftcibro.pdf. Accessed 07 December, 2022.

TUIK (2022) Crop Production Statistics.https://biruni.tuik.gov.tr/medas/?kn=92&locale=tr. Cereals and Other Plant Products, Select Breakdown, Breakdowns, Sesame. Accessed 07 December, 2022.

Tukey JW (1949) Comparing Individual Means in the Analysis of Variance. Biometrics 5(2): 99-114.

Ulusoy M (2016) Investigation of the Effect of Entomopathogenic Fungus *Beauveria bassiana* (bals.-criv.) vuill spores on Some Harmful Insects. Master's Thesis, Hacettepe University, Department of Biology, Ankara.

Zümreoğlu S, Akbulut N (1988) Research on pests seen in the second crop sesame plantations of the Aegean Region. Turkish Journal of Entomology 12(1): 39-48.

Research Article

www.dergipark.org.tr/en/pub/mediterranean

MEDITERRANEAN AGRICULTURAL SCIENCES
(2023) 36(3): 117-122
DOI: 10.29136/mediterranean.1333554

Composition and distribution of Thysanoptera (Insecta) in the area of Balcalı, Adana Province, Türkiye

Ekrem ATAKAN

Çukurova University, Agricultural Faculty, Plant Protection Department, Adana, Türkiye

Corresponding author: E. Atakan, e-mail: eatakan@mail.cu.edu.tr

ARTICLE INFO

Received: July 27, 2023
Received in revised form: October 13, 2023
Accepted: October 30, 2023

***Keywords*:**

Balcalı
Distribution
Frankliniella occidentalis
Thrips tabaci
Thysanoptera

ABSTRACT

This study was conducted to identify thrips (Insecta: Thysanoptera) species in the Balcalı area of Adana Province, Türkiye, known as a polyculture area having diverse topographic features. Thrips were extensively collected by the tapping method from awide range of agricultural crop plants during 2019-2020. A total of 1150 adult thrips individuals were collected, representing 24 identified species. Among them, the most prevalent and abundant species were the cotton thrips, *Thrips tabaci* Lindeman and the western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae). These two species showed varying population dynamics based on the flowering patterns of the plants sampled. Thankfully, no thrips damage was observed on the sampled plants. Although invasive pest thrips species such as*Thrips hawaiiensis* (Morgan) and *Scirtothrips dorsalis* (Hood) (Thysanoptera: Thripidae) were found in low numbers, and both of them were collected mostly from flowers of sesame and green pepper plants. A low number of predatory thrips belonging to geniuses, *Aeolothrips* (Aeolothripidae) and *Scolothrips* (Thripidae) were detected on beans, potatoes and cotton after March.

1. Introduction

Thrips belonging to the order Thysanoptera are small and soft-bodied insects with a body size of 0.5-0.15 mm. Some of them are opportunistic and invasive pest species. There are approximately 6000 species defined in this order, and their feeding habits vary. Some species are phytophagous (plant-feeding), mycophagus (fungal-feeding) and predators (Morse and Hoodle 2006; Atakan et al. 2015). Thysanoptera species in Türkiye have mostly been studied in the Mediterranean, Aegean and some parts of Central Anatolia, and Türkiye Thysanoptera fauna has been published (Tunç and Hastenpflug-Vesmanıs 2016). Thysanoptera species have been detected in some temperate climate fruits (Atakan 2008), summer and winter vegetable species (Atakan 2007a, b) on stone and pome fruit trees in the Eastern Mediterranean Region, as well as ornamental plants grown both in greenhouses and outdoors(Atakan 2011). However, in recent years, due to climatic changes such as temperature increases, some tropical Thysanoptera species have spread over geographical areas due to global warming worldwide, and invasive pest insects including thrips have been causing serious damage to crop plants. For example, *Thrips hawaiiensis*, (Morgan) (Thysanoptera: Thripidae) known as Hawaiian flower thrips introduced to Spain (Goldaranzena 2011) and Türkiye (Atakan et al. 2021), seriously damaged, in particular,young lemon fruits. On the other hand, Chilli thrips, *Scirtothrips dorsalis* (Hood) (Thysanoptera: Thripidae) introduced into Türkiye in 2020, has caused serious damage on blueberries in Adana Province, Türkiye (Atakan and Pehlivan 2021).

Changes in crop plants diversity and climatic factors may affect the presence of Thysanoptera species and their distribution in species composition on arable crops (Lewis 1997). For example, the western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), which causes economic damage in many crops in our country, was reported for the first time in Türkiye in 1994, but it was revealed in the last published study that this species actually existed in the province of Antalya in 1993 (Tunç and Hastenpflug-Vesmanis 2016). For this purpose, Thysanoptera species were investigated according to arable crop plants in Balcalı (Adana, Türkiye) in a certain agro-ecosystem with different crop patterns. This article can provide information about the composition of thrips species, their abundance in certain crop plants or trees, and thus, it can provide useful some data in controlling efforts for the pest thrips.

2. Material and Methods

2.1. Description of sampling sites

Thrips (Thysanoptera) species, their seasonal densities and distributions on some crop plants were investigated in the research area of Balcalı, University of Çukurova in Adana Province, Türkiye in 2019-2020. In this polyculture area, different plant species such as field crops (cotton, soybean, sesame and peanut), citrus, temperate climate fruits (apple, nectarine and loquat), cereals, and winter and summer vegetables are grown for both production and research activities. This ecological area has a typical Mediterranean climate.

2.2. Sampling of thrips

In the sampling units, 20 plants or trees were selected randomly from annual herbaceous cultivars (vegetables and field crops) and perennial woody cultivars (fruit trees). For the sampling, the flowering periods of the plants were considered, and the sampling was started with the beginning of the flowering period of the plants in different plant groups, as thrips are mostly found on flowers (Funderburk et al. 2000, 2018). Flowering or flowering shoots 20-30 cm long in four different directions of the trees were randomly selected for sampling. In herbaceous plants, the upper halves of the plants were considered in the sample. In the surveyed areas, plants representing each plant group or flowering shoots were shaken for 5-10 seconds onto the white container with 34 × 23 × 7 cm, and then thrips individuals (adults and larvae) were collected with the help of a suction tube and/or a fine brush, and were transferred to the Eppendorf plastic tubes (50 cc) filled with 60% ethyl alcohol. In addition, label information such as the place of sampling, date and plant species were recorded. For this purpose, 47 surveys were conducted at regular 15-day intervals and a total of 28 cultivated plants, including 4 field crops, 8 fruit trees and 16 vegetables, were sampled for this purpose. In total, 197, 156, 147 and 74 samples were taken from winter, summer vegetables, fruit trees and field crops, respectively. Samplings were carried out on the same day between 08:00 and 12:00 am.

2.3. Thrips diagnosis

Microscopic preparations were made for the identification of the collected Thysanoptera species. The samples, which were kept in tubes containing ethyl alcohol (60%), were put into the AGA solution including 9-parts ethyl alcohol 1-part glacial acetic acid and 1-part glycerin, for 2 days in order to soften the body tissues and partially empty the body contents. In temporary preparations, samples that are not very dark were kept in 10% sodium hydroxide (NAOH) medium on the hot plate at 60ºC for about 30 minutes, and dark samples for about one hour until slight color changes occurred. The body contents of the samples were completely discharged by rubbing their bodies from the ventral or dorsal side in 96% alcohol. Individuals were placed in Hoyer medium and microscopic slides were made. The identification of the thrips specimens was done with the help of key identification guides referencing Priesner 1951, Yakhontov 1964 and zur Strassen 2003.

2.4. Evaluation of data

The total number of identified Thysanoptera species and their distribution on crop plant species is shown in the Tables 1 and 2. The seasonal averages of the main pest thrips species, according to the crop groups, were not statistically compared because the cultivated plants sampled from different plant groups were grown in different ecological areas and also due to the differences in their growing periods. The total number of individuals according to the plant species of the identified species whose numbers vary from 1 to 9 are not shown in Table 2. Population densities (20 plants or trees) of the two common thrips species by plant groups for the period October 2019 to October 2020, are shown as relevant.

Table 1. Thysanoptera species and their total numbers during 2019-2020

Thysanoptera species	2019	2020	Total (2019+2020)
Aeolothripidae			
Aeolothrips collaris Priesner	0	16	16
Aeolothrips ericae Bagnall	0	4	4
Aeolothrips gloriosus Bagnall	0	5	5
Aeolothrips fasciatus (Lin.)	0	1	1
Aeolothrips intermedius Bagnall	0	11	11
Thripidae			
Anophothrips sudanensis Trybom	0	2	2
Chirothrips aculeatus Bagnall	0	2	2
Frankliniella intonsa (Trybom)	0	5	5
Frankliniella occidentalis (Pergande)	73	152	225
Limothrips denticornis (Haliday)	0	1	1
Mycterothrips tschirkunae (Yakhontov)	7	1	8
Rubiothrips vitis Priesner	0	1	1
Thrips hawaiiensis (Morgan)	5	61	66
Thrips major Uzel	4	25	29
Thrips meridionalis (Priesner)	0	1	1
Thrips minutissimus	0	9	9
Thrips tabaci Lindeman	278	407	685
Scirtothrips dorsalis Hood	36	8	44
Scolothrips longicornis Priesner	0	1	1
Phlaeothripidae			
Haplothrips aculeatus (Fabricious)	2	18	20
Haplothrips distinguendus (Uzel)	0	2	2
Haplothrips ganglbaueri Schmutz	2	2	4
Haplothrips kurdjumovi Karny	2	1	3
Haplothips reuteri (Karny)	0	8	8
Total	406	744	1150

3. Results

3.1. Thrips composition

A total of 1150 adult thrips individuals were recorded in this study, with very few thrips larvae collected. In total, 24 Thysanoptera species were recorded, including 5 from the family Aeolothripidae, 14 from the family Thripidae, and 5 from the family Phlaeothripidae. Species from the family Aeolothripidae were found only in 2020. Depending on the number of samples, thrips individuals were mainly collected in 2020. The most common and abundant species were *Thrips tabaci* Lindeman, *Frankliniella occidentalis* (Pergande), and *Thrips hawaiiensis* (Morgan), respectively. *Scirtothrips dorsalis* (Hood), which was detected for the first time in Türkiye in 2021 was actually found to exist in the Adana Province in 2019.

3.2. List of plant species with some thrips species

The list of plant species that host some thrips species and the number of thrips individuals are shown in Table 2. Predatory thrips have been recorded in very small numbers (1-6 individuals). *Aeolothrips collaris* Priesner have been found in potatoes, *Aeolothrips intermedius* Bagnall in beans (4 individuals), cotton (3 individuals), and sesame (4 individuals), where they were relatively more abundant. Species from the family Aeolothripidae were recorded in very low numbers in both seasonal vegetables and field crops. The main thrips species, *T. tabaci*, was widely sampled and found in large numbers on winter vegetables such as cauliflower (113 individuals), cabbage (106 individuals), and summer vegetable beans (103 individuals). This harmful species is also abundant in potato plants cultivated as early crops in the Çukurova region. It is prevalent in soft-fleshed fruit trees as well. The individuals of *F. occidentalis,*

Table 2. A list of crop plant species with numbers of commonly found thrips species during 2019-2020

Plant species/English name (scientific name)	A.co	A.in	H.ac	F.oc	T.ha	T. ma	T.ta	S.do	Total
Amaranthaceae									
Spanich (*Spinacia oleracea*)	0	0	0	2	0	0	0	0	2
Asteraceae									
Lettuce (*Lactuca sativa*)	0	0	0	5	0	0	3	0	8
Brassicaceae									
Cauliflower *(Brassica oleraceavar. Botrytis*)	0	0	0	1	0	0	113	0	114
Red cabbage (*Brassica oleraceavar. capitata f. Rubra*)	0	0	0	0	1	0	106	0	107
Cabbage (*Brassica oleracea*)	0	0	0	0	0	0	42	0	42
Rocket (*Eruca vesicaria*)	0	0	0	0	0	0	1	0	1
Peppergrass (*Lepidium sativum*)	0	0	0	0	0	0	0	0	0
Radish (*Raphanus sativus*)	0	0	0	3	0	0	5	0	8
Cucurbitaceae									
Cucumber (*Cucumis sativus*)	0	0	0	0	0	0	6	0	6
Pumpkin (*Cucurbita moschata*)	0	0	0	1	0	0	4	0	5
Fabaceae									
Broad bean (*Vicia faba*)	0	0	0	4	7	3	6	3	23
Bean (*Phaseolus vulgaris*)	2	4	2	35	3		103	0	149
Soybean (*Glycine max*)	2	0	2	31	1	0	14	4	54
Groundnut *(Arachis hypogaea*)	2	0	3	9	0	2	0	0	16
Lythraceae									
Pomegranade (*Punica granatum*)	0	0	0	0	2	0	1	0	3
Malvaceae	0	0							
Cotton (*Gossypium hirsitum*)	0	3	0	41	6	0	0	0	50
Pedeliaceae	0								
Sesame (*Sesamum indicum*)	0	4	3	25	27	1	1	3	64
Rutaceae									
Citrus (*Citrus* spp.)	1	0	0	15	0	12	31	0	59
Rosaceae									
Pear (*Pyrus communis*)	0	0	0	0	0	0	1	0	1
Almond (*Prunus dulcis*)	0	0	0	1	0	0	2	0	3
Apple (*Malus domestica*)	1	0	9	1	1	7	23	0	42
Plum (*Prunus Domestica*)	0	0	0	0	0	0	4	0	4
Apricot (*Prunus persica*)	0	0	0	1	0		1	0	2
Loquat *(Eriobotrya japonica*)	0	0	0	0	1	4	29	0	34
Solanaceae									
Pepper *(Capsicum annuum*)	1	0	1	14	7	0	12	33	68
Tomato (*Solanum lycopersicum*)	1	0	0	9	5	0	3	0	18
Potato (*Solanum tuberosum*)	6	0	0	8	0	0	152	0	166
Eggplant (*Solanum melongena*)	0	0	0	16	5	0	22	1	44

A.co: *Aeolothrips collaris*, A.in: *Aeolothrips intermedius*, F. oc: *Frankliniella occidentalis*, T.ha: *Thrips hawaiiensis*, T.ma: *Thrips major*, T.ta: *Thrips tabaci*, S.do: *Scirtothrips dorsalis*, H. ac: *Haplothrips aculeatus*.

also known as western flower thrips, were mostly collected in higher numbers from field crops such as cotton (41 individuals), soybean (31 individuals), and beans (35 individuals) (Table 2). *Thrips hawaiiensis*, which was observed in Türkiye for the first time on lemon trees grown in the Erdemli district of Mersin Province, Türkiye, caused significant damage to flowers and fruits. The highest number of this species (27 individuals) was collected from sesame plants, while individual counts on other cultivated plants ranged between 1 and 7. *Scirtothrips dorsalis* was predominantly collected from pepper plants (33 individuals). *Thrips major* Uzel was mainly recorded on citrus trees (total of 12 individuals).

3.3. Population fluctuations of two common thrips species

The population fluctuations of two common thrips species, *F. occidentalis* and *T. tabaci*, were observed over a specific period. Figure 1 displays the population counts based on sampling dates for 20 plants. As depicted in Figure 1, *T. tabaci* showed varying abundance in different cultivated plants, excluding field crops. In winter vegetables, the population density of *T. tabaci* remained relatively steady throughout the sampling dates, ranging between 6 and 8 individuals per 20 plants. Conversely, *F. occidentalis* was rarely found in the group of winter vegetables, with the highest count being 1 individual per 20 plants. Within the summer vegetable group, adult *T. tabaci* individuals were more prevalent from March to June, reaching the peak on May 7th with 31 individuals per 20 plants. After June, harmful *T. tabaci* individuals were not found on the plants. On the other hand, the highest population density of *F. occidentalis* in summer vegetables was observed on May 27th, with 4.5 individuals per 20 plants, coinciding with a significant decrease in the *T. tabaci* population. Regarding fruit trees (both soft and hard-tissue fruits), the highest number of *T. tabaci* individuals was recorded on February 5th, at the beginning of the flowering period. For *F. occidentalis*, they were rarely found on fruit trees in several sampling dates, with the highest count being 0.44 individuals per 20 plants on April 29th. In contrast to other cultivated plants, *F. occidentalis* individuals were more prominent in field crops such as cotton, soybean, and sesame. They were mainly collected from cotton flowers, with its highest population density being 6.5 individuals per 20 plants on August 24th. This information highlights the population fluctuations of the two thrips species over the specified period, providing valuable insights for further study and management strategies.

4. Discussion

Thrips tabaci and *F. occidentalis* species were the most commonly recorded in this study (Tables 1 and 2). It is evident that these two species were also common in previous studies conducted in Adana Province (Atakan 2007a, b). In other words, it was concluded that the introductions of 2 new invasive thrips species (*T. hawaiiensis* and *S. dorsalis*) into Adana Province did not cause any change in thrips composition in the sampling area, known as the polyculture area. Individuals of *T. tabaci* in winter vegetables and *F. occidentalis* individuals in summer vegetables appeared at relatively higher densities (Figure 1). This may be related to the bio-ecological demands of the species, their preferences for specific plant species (such as plant morphology and anatomy, and chemical content). Thrips were mainly recorded in the flowers of the sampled plants, and population

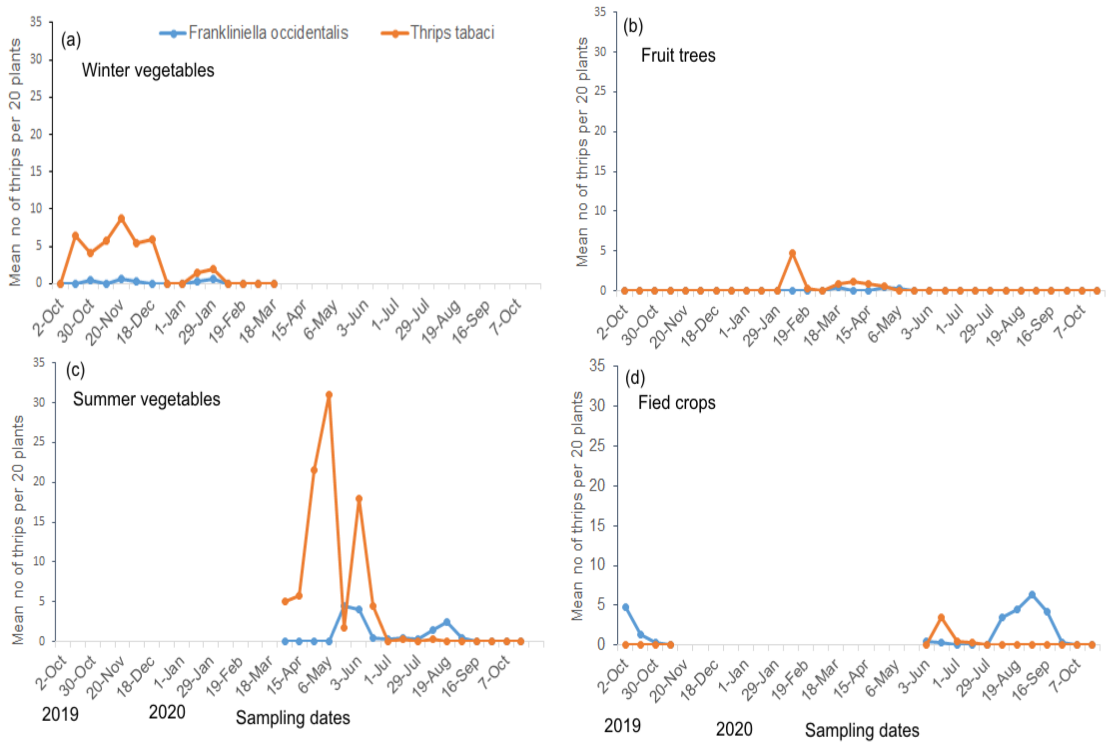

Figure 1. Population density patterns of two common Thysanoptera species on diverse crop plants during 2019-2020; winter vegetables (a), fruit trees (b), summer vegetables (c), field crops (d).

patterns in different plant groups changed according to the flowering status of the plants. In other words, their population densities depended on flower formations and flower densities in plants. Nectars, protein and carbohydrate nutrition in the flowers of plant species may have attracted thrips more in terms of their development and reproduction (Yudin 1986; Lewis 1997). The fact that *T. tabaci* was not found in many sampling dates after June may be related to its lower tolerance to summer temperatures compared to *F. occidentalis* (Lewis 1997).

When evaluating the numbers of thrips individuals from different plants, it is evident that the number of individuals, including common species, is low (Table 2). This situation, along with the diversity of plant and insect species, may indicate that maintaining a natural balance in agricultural ecosystems is possible with good habitat planning, allowing beneficial insects to easily suppress harmful species (Barbosa and Wratten 1998; Landis et al. 2000; Gurr et al. 2017). In this study, particularly, the predator *Orius* species (Hemiptera: Anthocoridae) were frequently collected alongside thrips, which may account for the absence of thrips larvae (Tatlıcıoğlu et al. 2022). The low mobility and soft body of thrips larvae make them vulnerable to such predatory insects (Funderburk et al. 2000; Osekre et al. 2008). Predatory thrips species were observed in the sampled crop plants after March, but only a low number of them were recorded, indicating their inactivity during the winter vegetation. The low individual numbers might be influenced by their habitat preferences, plant choices, and predation by larger general predators of the order Hemiptera (e.g., *Geocoris*, *Orius*, *Piocoris* and *Nabis* species) (Asghar et al. 2008). However, predatory Aeolothripidae species were recorded in large numbers on the flowers of the weeds in the same sampling area in previous works (Atakan and Uygur 2004, 2005). This may be related to the habitat and plant preferences of the predatory thrips of the genus *Aeolothrips*.

In conclusion, thrips were found to be more abundant on annual herbaceous plants compared to perennial arboreal plants. It was observed that thrips densities are closely linked to the flowering phenology of plants sampled. The richness of plant species diversity in a specific agro-ecosystem can promote healthier and more dynamic relationships between plants, harmful insects, and beneficial organisms i.e. predators and parasitoids (trophic relations). Moreover, investigating harmful or beneficial insect species in different targeted ecosystems, as demonstrated in this study, allows for early detection of invasive harmful species entering the ecosystem. This early detection of pest thrips on crop plants may contribute to planning of control tactics in preventing their spread over large agricultural areas. *Scirtothrips dorsalis*, which is often considered an important pest of pepper plants in the literature, was mostly collected from pepper flowers in the present study. Although *F. occidentalis* is known as a common pest insect in pepper production areas in Türkiye, it is thought to be beneficial to investigate this recently introduced thrips species in crop plants particularly in green peppers across a large region.

References

Asghar S, Askari FA, Sedghi M (2008) Interaction of *Aeolothrips intermedius* and *Orius niger* in controlling *Thrips tabaci* on potato. International Journal of Agriculture and Biology 10(5): 521-525.

Atakan E, Uygur S (2004) Seasonal abundance of the some thrips species and their predators on weeds. Türkiye Entomoloji Dergisi 28(2): 123-132.

Atakan E, Uygur S (2005) Winter and spring abundance of *Frankliniella* spp and *Thrips tabaci* Lindeman (Thysan, Thripidae) on weed host plants in Türkiye. Journal of Applied Entomology 129: 17-26.

Atakan E (2007a) Thrips (Thysanoptera) species occurring on fruit orchards in Çukurova Region of Türkiye. Book of Abstracts of Second Symposium on Palaearctic Thysanoptera, Strunjan, Slovenia, pp. 7.

Atakan E (2007b) Thrips (Thysanoptera) species occurring on winter vegetables crops in Çukurova Region of Türkiye. Book of Abstracts of Second Symposium on Palaeartic Thysanoptera, Strunjan, Slovenia, pp. 24.

Atakan E (2008) Adana ilinde bazı ılıman iklim meyvelerinde iki thrips (Thysanoptera) türünün populasyon değişimleri ve zararı üzerinde araştırmalar. Türkiye Entomoloji Dergisi 32(4): 255-271.

Atakan E (2011) Adana İlinde merkez parklarında bazı süs bitkilerinde bulunan Thysanoptera (Thrips) türleri. Türkiye IV Bitki Koruma Kongresi Bildirileri, Kahramanmaraş, s.183.

Atakan E, Ölçülü M, Pehlivan S, Satar S (2015) Türkiye'de yeni zararlı bir thrips türü: *Thrips hawaiiensis* (Morgan, 1913) (Thysanoptera: Thripidae). Türkiye Entomoloji Bülteni 5(2): 77-84.

Atakan E, Pehlivan S (2021) A new harmful thrips species in orange in Antalya Province: *Scirtothrips dorsalis* Hood (Thysanoptera: Thripidae). Mediterranean Agricultural Sciences 34(3): 273-277.

Atakan E, Pehlivan S, Achiri TD (2021) Pest status of the Hawaiian flower thrips, *Thrips hawaiiensis* (Morgan) (Thysanoptera: Thripidae) in lemons in the Mediterranean region of Türkiye. Phytoparasitica 49(3): 1-13.

Barbosa P, Wratten SD (1998) Influence of plants on invertebrate predators: implications to conservation biological control. In Barbosa P (ed.), Conservation biological control. Academic Press, San Diego, CA, pp. 83-100.

Funderburk JE, Stavisky J, Olson S (2000) Predation of *Frankliniella occidentalis* (Thysanoptera: Thripidae) in field peppers by *Orius insidiosus* (Hemiptera: Anthocoridae). Environmental Entomology 29: 376-382.

Funderburk J, Reitz S, Stansly P, Freeman J, Miller C, Mcavoy G, Whidden A, Demirozer O, Nuessly G, Leppla N (2018) Managing Thrips in Pepper and Eggplant. IFAS Extension, University of Florida, pp. 1-10.

Goldaranzena A (2011) First record of *Thrips hawaiiensis* (Morgan, 1913) (Thysanoptera: Thripidae), an Asian pest thrips in Spain. Bulletin OEPP/EPPO 41(2): 170-173.

Gurr GM, Wratten SD, Landis DA, You M (2017) Habitat management to suppress pest populations: progress and prospects. Annual Review of Entomology 62: 91-109.

Landis DA, Wratten SD, Gurr GM (2000) Habitat management to conserve natural enemies of arthropod pests in agriculture. Annual Review of Entomology 45(1): 175-201.

Lewis T (1997) Thrips: Their Biology, Ecology and Economic Importance. London, UK: Academic press.

Morse JG, Hoddle MS (2006) Invasion Biology of thrips. Annual Review of Entomology 51: 67-89.

Osekre EA, Wright DL, Marios JJ, Mailhot DJ (2008) Predator-prey interactions between *Orius insidiosus* (Hetereoptera: Anthocoridae) and *Frankliniella tritici* (Thysanoptera: Thripidae) in cotton blooms. Journal of Cotton Science 12: 195-201.

Priesner H (1951) Zwei neue Thysanopteren aus der Türkei. Zeitschrift fur Pflanzenkrankheiten und Pflanzenschutz 58: 255-258 (in Germany).

Tatlıcıoğlu Ş, Atakan E, Pehlivan S (2022) Investigation of predatory *Orius* species (Hemiptera: Anthocoridae) in Balcalı District of Adana Province, Türkiye. Türkiye Biyolojik Mücadele Dergisi 13(1): 25-36. doi: 10.31019/tbmd.1056178.

Tunç İ, Hastenpflug-Vesmanıs A (2016) Records and checklist of Thysanoptera in Türkiye. Turkish Journal of Zoology 40: 769-778 doi: 10.3906/zoo-1512-37.

Yakhontov VV (1964) Order Thysanoptera (Physopoda) In: Bei-Bienko GY (Ed), Keys to the Insects of the European USSR, 1. pp. 1119-1160.

Yudin LS, Tabashnik BE, Cho JJ, Mitchell WC (1986) Host range of western flower thrips *Frankliniella occidentalis* (Thysanoptera: Thripidae), with special reference to Leucaena glauca. Environmental Entomology 15: 1292-1295.

zur Strassen R (2003) Die Terebranten Thysanopteren Europas und des Mittel Gebietes. Die tierwelt deutschlauds, Beggründet 1925 von Friedrich Dahl, 74. Teil. Goecke und Evers, Keltern, Deutschland, p. 277. (in Germany).

Research Article

www.dergipark.org.tr/en/pub/mediterranean

MEDITERRANEAN AGRICULTURAL SCIENCES
(2023) 36(3): 123-127
DOI: 10.29136/mediterranean.1344488

Exploration of ITS region as DNA barcode for *Kakothrips priesneri* Pelikan phylogeny

Inci SAHIN NEGIS

Selcuk University, Agricultural Faculty, Department of Plant Protection, 42030, Konya, Türkiye

Corresponding author: I. Sahin Negis, e-mail: incisahin@selcuk.edu.tr

ARTICLE INFO

Received: August 16, 2023
Received in revised form: November 7, 2023
Accepted: November 7, 2023

Keywords:

Kakothrips
DNA barcoding
BLAST
Phylogenetic tree
ITS

ABSTRACT

The Thripidae family is a diverse group of insects with global distribution and significant economic importance as pests of agricultural crops. Accurate identification and classification of Thripidae species are critical for their effective management and control. To aid in this effort, DNA barcoding using the ITS gene region has proven to be an efficient and reliable tool for identifying and differentiating Thripidae species. The high variability rate of the ITS region makes it particularly effective for identifying and classifying closely related species, providing valuable insight for understanding and preserving the taxonomic diversity of the Thripidae family. The present study aims to explore the effectiveness of the ITS gene region for DNA barcoding of *Kakothrips priesneri* Pelikan species. For the first time, the ITS data obtained from this study showed the placement of *Kakothrips priesneri* in both common methods, using DNA barcoding (BLAST and MEGA), produced positive results for the ITS analysis, indicating that the region may be suitable for the *Kakothrips* species.

1. Introduction

Thrips (Thysanoptera) are very small insects, with an average length of 1-2 mm, found all over the world (Mehle and Trdan 2012). The order is considered complex due to its high level of homoplasy, which means that different species may have similar characteristics that have evolved independently (Gauld and Mound 1982; Mound and Palmer 1983; Retana-Salazar 1998; 2000). Thrips are divided into two suborders: Terebrantia, which has nine extant families, and Tubulifera (Moritz 1994; Moritz et al. 2000; ThripsWiki 2023). While the Aeolothripidae family is not known to include any pest species (Mound 1997), the Thripidae family, which includes three subfamilies: Panchaetothripinae, Dendrothripinae, and Sericothripinae, has some species that can cause serious crop losses (Mound et al. 2022). Out of approximately 6000 Thysanoptera species identified so far (ThripsWiki 2023), some species are known to be pollinators or biological control agents (Mound and Kibby 1998; Trdan et al. 2005).

The genus *Kakothrips* Williams comprises of eight known species (ThripsWiki 2023). Records of *K. dentatus* Knechtel from Greece (zur Strassen 1996) and Türkiye (Şahin et al. 2019) confirm the Mediterranean affiliation of the genus, despite *Kakothrips dentatus* being primarily distributed in northern Europe (zur Strassen 2003). *Kakothrips acanthus* Berzosa was described by Berzosa (1994) from various locations in Spain and Sicily, and until recently, it was only known from the western and central Mediterranean regions. However, the occurrence of *Kakothrips acanthus* has been documented by Şahin Negiş (2023) in Konya, expanding its distribution range towards the eastern Mediterranean. Among all *Kakothrips* species, females of *Kakothrips priesneri* Pelikan are particularly unique as they possess a pair of pore plates on the third abdominal segment. This species is noted at couplet 6(4) in zur Strassen (2003) as having females with a single pore plate on sternite III, and an image of such a structure in *K. priesneri* is provided by Şahin Negiş (2023). Additionally, *K. priesneri* has longitudinal rows of thickened setae on the tibia (zur Strassen 2003).

Several studies have demonstrated the efficacy of using the ITS gene region for identifying and classifying different species within the Thripidae family. The ITS2 region is a commonly employed DNA barcoding region for identifying Thysanoptera species, as it exhibits sufficient variability among different species. Ashfaq and Hebert (2016) utilized DNA barcoding to investigate Thrips diversity in Pakistan and revealed the existence of cryptic species complexes. Almási et al. (2016) employed the ITS gene region for identifying *Thrips tabaci* Lindeman and determining cryptic species complexes. Tyagi et al. (2017) used DNA barcoding to discover a new cryptic species in *Thrips palmi* Karny in India. These studies highlight the significance of the ITS gene region as a valuable tool in identifying species within the Thripidae family and determining cryptic species complexes.

In this study, two commonly used methods were employed to analyze the DNA barcoding results. The first approach involved using BLAST (Basic Local Alignment Search Tool) to match the query sequences against a reference database and identify the closest match. The second method utilized taxonomic assignment algorithms, such as MEGA, to assign species identities and construct a phylogenetic tree based on sequence alignments. Both methods were applied to analyze both query and *K. priesneri* sequences. The results from both methods were found to be favorable for the ITS analysis, indicating that this region may be suitable for identifying *Kakothrips* species. Furthermore, the barcode sequence of *K. priesneri* species was uploaded to the GenBank for the first time as part of the present study (accession number OQ779479), contributing to the growing database of genetic information for this species.

2. Materials and Methods

The sample was diagnosed following the method described in zur Strassen (2003). DNA isolation was carried out using the CTAB protocol, with each sample processed individually according to the method described by Doyle and Doyle (1987). The ITS F/R primers, which amplify a region of approximately 650 bp, were used for PCR amplification of the ITS gene region, and the PCR reaction and cycling conditions were the same as those described in Şahin Negiş et al. (2022) (Fig. 1). Sanger sequencing was performed at BM Labosis. After obtaining the sequencing results, each sample was individually analyzed using the Neighbor-Joining (NJ) and UPGMA (unweighted pair group method with arithmetic mean) methods in the Mega11 program. The Kimura 2-parameter model, as proposed by Kimura (1980), was used for calculating genetic distances and time of divergence between sequences. An outgroup sequence, from GenBank accession number MW1865502.1 for *Limenitis archippus* (Cramer) (Lepidoptera: Nymphalidae), was used in the phylogenetic tree as a reference for comparison.

3. Results and Discussion

Thysanoptera, a group of insects commonly known as thrips, presents challenges in taxonomic and phylogenetic studies. Some researchers have argued against relying solely on molecular data for taxonomic decisions in Thysanoptera, as highlighted by Mound and Morris (2007), and have considered it an insufficient solution for addressing taxonomic issues, as noted by Lee (2004). However, recent studies have shown promising results in using molecular techniques for defining species in Thysanoptera. Further research, that compares morphological and molecular data, may help establish more confidence in the use of molecular techniques for taxonomic purposes in this group. Recognition of invasive species is crucial for effective management, and it is important that the process is rapid, cost-effective, technically accessible, and accurate, as emphasized by Darling and Blum (2007). Molecular techniques, such as DNA barcoding, can provide a valuable tool for identifying invasive species, as they can be applied to a wide range of samples and can yield accurate results with relatively small sample sizes. Combining molecular data with other taxonomic approaches, such as morphological and ecological data, can enhance our understanding of invasive species and aid in their management and control.

The utilization of BLAST and taxonomic assignment algorithms in this study allowed for a thorough analysis of the DNA barcoding results for *Kakothrips priesneri* Pelikan. The BLAST method, which compares query sequences to a reference database, and the taxonomic assignment algorithms (MEGA), which assign species identities and generate a phylogenetic tree, were both applied to analyze the query and *K. priesneri* sequences. The positive results obtained from both methods (BLAST and MEGA) in the ITS analysis indicate that the ITS gene region may be appropriate for identifying *Kakothrips* species. Additionally, the barcode sequence of *K. priesneri* was uploaded to the GenBank for the first time, with the accession number OQ779479, contributing to the growing genetic database for this species. These findings highlight the importance of utilizing multiple methods for DNA barcoding analysis, as it allows for a comprehensive and reliable identification of species. The combination of molecular techniques, such as DNA barcoding, with traditional taxonomic approaches can greatly enhance our understanding of species identification, phylogenetics, and biodiversity studies.

The BLAST program was used in this study to search the reference database using the ITS sequence of *Kakothrips priesneri* as the query sequence. The top BLAST hits for the query sequence were mainly from *Frankliniella* species, with a similarity of 97.5%. Other hits with similarity ranging from 92-97% were from various other thrips species. This indicates that the query sequence of *K. priesneri* showed high similarity to *Frankliniella* species, which was expected based on previous knowledge. However, it is also important to note that there were hits with slightly lower similarity to other thrips species, which could potentially indicate close genetic relationships or shared genetic regions among thrips species. The use of BLAST in this study allowed for the comparison of the query sequence against a reference database, providing valuable information on potential matches and similarities with other known thrips species. However, further analysis and verification using additional molecular and morphological techniques would be necessary to confirm the taxonomic identification of *K. priesneri* and related species.

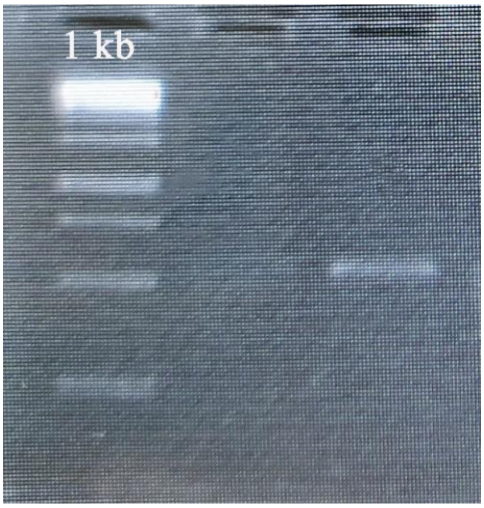

Figure 1. The amplification of *Kakothrips priesneri* in the ITS gene region is approximately 650 bp (Marker 1 Kb).

In this study, the UPGMA (unweighted pair group method with arithmetic mean) and NJ (Neighbor-Joining) methods (Schlee 1975) were used to infer the evolutionary history of 27 nucleotide sequences, with the ITS gene region being the focus of analysis were used . The Maximum Composite Likelihood method (Tamura et al. 2004) was used to calculate evolutionary distances, and all ambiguous positions were removed for each sequence pair using pairwise deletion option, resulting in a final dataset of 603 positions. MEGA11 (Kumar et al. 2016) was used for all evolutionary analyses. The overall mean distance (OMD), which represents the average evolutionary distance between all taxa, was calculated to understand the structure of the phylogenetic tree. OMD is a metric that ranges between 0 and 1, with values close to 0 indicating low genetic differences between taxa, suggesting that the phylogenetic tree accurately reflects the relationships between taxa. In the case of the ITS gene region, a value of 0.03 between taxa is considered to reflect a good relationship in the phylogenetic trees, indicating that the analysis results are reliable in terms of the ITS gene region. This information provides insights into the robustness and accuracy of the phylogenetic analysis conducted in the study, indicating that the relationships between taxa in the phylogenetic tree are well-supported by the ITS gene region data, as reflected by the OMD value of 0.03. The phylogenetic trees constructed in this study were based on the available sequences of *Frankliniella* species and other thrips species from the GenBank database, as there were no sequences available for *K. priesneri* or other *Kakothrips* species. This was done because *K. priesneri* is morphologically similar to *Frankliniella* species (zur Strassen 2003), and thus *Frankliniella* sequences were used as a proxy for inferring the evolutionary relationships of *K. priesneri*.

In both the UPGMA (Fig. 2) and NJ trees (Fig. 3), the outgroup was placed outside the main tree. However, there were some differences observed between the two trees. For example, in the NJ tree, *Parabaliothrips* sp., which is characterized by its abdominal ctenidia terminating at the median lateral seta on tergites VI-VII and pronotum posteroangular setae positioned upper side the posteromarginal setae (OzThrips 2023), was positioned closer to the outgroup compared to the UPGMA tree. This suggests that the evolutionary relationship of *Parabaliothrips* sp. is slightly different when analyzed using the NJ method compared to the UPGMA method. Similarly, in the NJ tree (Fig.2), two species belonging to the genus *Aptinothrips* were observed to be close to each other, while they were positioned far apart in the UPGMA tree (Fig. 3). This indicates that the evolutionary relationship between these two species may vary depending on the method used for phylogenetic analysis. These differences in the positioning of species in the phylogenetic trees may be attributed to the different algorithms used in the UPGMA and NJ methods, as well as the evolutionary distance calculation method (Maximum Composite Likelihood) used in this study. It highlights the importance of using multiple methods and considering different factors when interpreting phylogenetic trees, and the need for further studies with more sequences and additional molecular markers for a comprehensive understanding of the evolutionary relationships among thrips species, including *K. priesneri*.

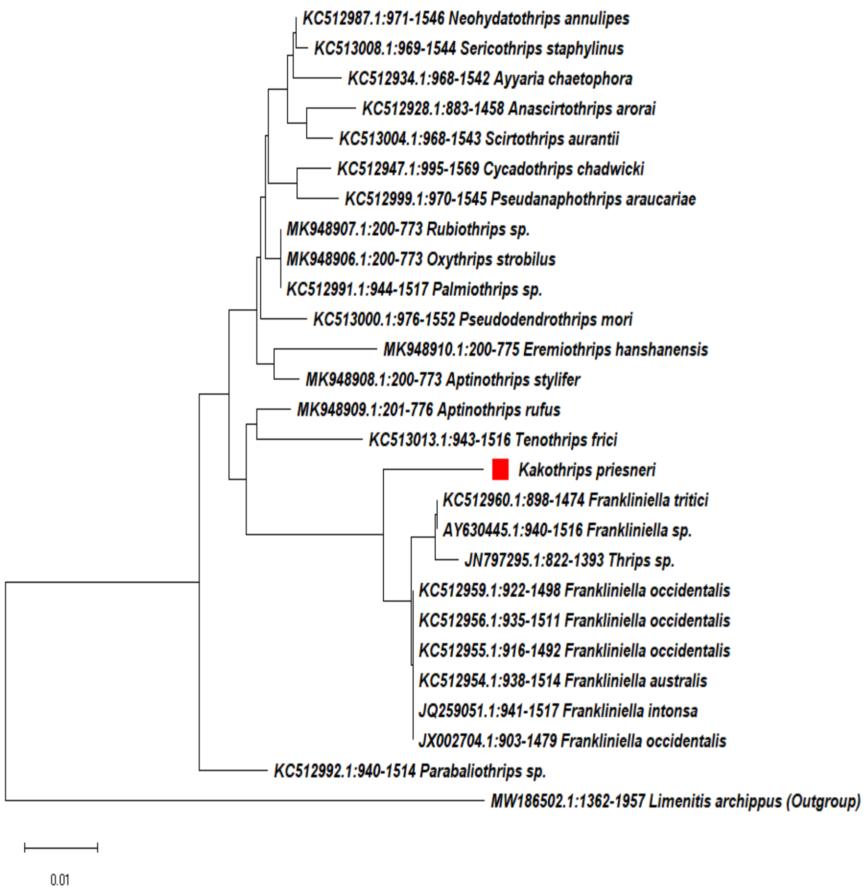

Figure 2. The *Kakothrips priesneri* NJ phylogenetic tree according to the ITS gene region of 27 nucleotide sequences studied with MEGA11. *Limenitis archippus* (Cramer) was used as an outgroup. (Overall mean distance: 0.03).

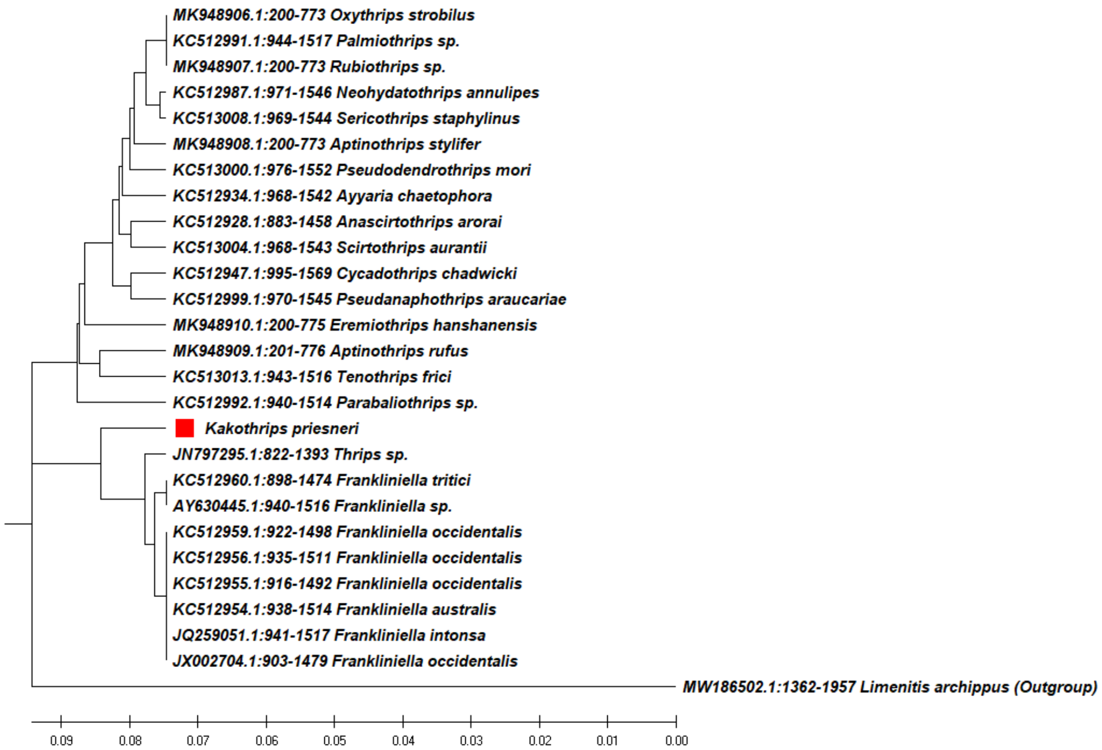

Figure 3. The *Kakothrips priesneri* UPGMA phylogenetic tree according to the ITS gene region of 27 nucleotide sequences studied with MEGA11. *Limenitis archippus* (Cramer) was used as an outgroup. (Overall mean distance: 0.03).

The study suggests further evaluation of the ITS gene region for distinguishing thrips species, as it is important to test it on a larger number of thrips species, particularly those that are phylogenetically distant and dissimilar, in both phylogenetic trees where thrips species are separated. Additionally, the study by Şahin Negiş et al. (2022) reported that the ITS gene region was effective in distinguishing thrips species at the genus level, indicating its potential for higher taxonomic classifications. Therefore, a greater number of thrips species and expanding the taxonomic coverage in the phylogenetic analysis using the ITS gene region may provide more comprehensive information about this gene region and its utility in thrips systematics, classification, and identification.

4. Conclusions

The previous studies have documented the presence of certain thrips species, specifically those belonging to the Thysanoptera family Thripidae, including the newly identified *Kakothrips priesneri* Pelikan species, ITS gene region sequencing in this study. Morphological diagnosis was also implemented to validate the findings. Furthermore, the sequence data was compared with the GenBank references detailed in the figures. The result of this study supports the use of ITS DNA barcode primers for diagnosis. Notably, this study also marks the inclusion of *K. priesneri* species sequence data in GenBank (OQ779479) for the first time. Future studies should focus on expanding thrips sequences in a well-designed network for accurate and robust diagnosis.

Acknowledgments

This study was orally presented at the IMPPC (2nd International Molecular Plant Protection Congress) conference held on May 15-18, 2023. Furthermore, the abstract has been published in the congress book.

References

Almási A, Tóbiás I, Bujdos L, Jenser G (2016) Molecular characterisation of Thrips tabaci Lindeman, 1889 (Thysanoptera: Thripidae) populations in Hungary based on the ITS2 sequences. Acta Zoologica Academiae Scientiarum Hungaricae 62(2): 157-164. doi: 10.17109/AZH.62.2.157.2016.

Ashfaq M, Hebert PD (2016) DNA barcodes for bio-surveillance: regulated and economically important arthropod plant pests. Genome 59(11): 933-945. doi: 10.1139/gen-2016-0024.

Berzosa J (1994). Two new species of Kakothrips Williams 1914 (Insecta: Thysanoptera: Thripidae). Senckenbergiana biologica 73(1-2): 77-82.

Darling JA, Blum MJ (2007) DNA-based methods for monitoring invasive species: a review and prospectus. Biological Invasions 9(7): 751-765. doi: 10.1007/s10530-006-9079-4.

Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. 19(1): 11-15.

Gauld ID, Mound LA (1982) Homoplasy and the delineation of holophyletic genera in some insect groups. Systematic Entomology 7(1): 73-86. doi: 10.1111/j.1365-3113.1982.tb00127.x.

Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of molecular evolution 16(2): 111-120. doi: 10.1007/BF01731581.

Kumar S, Stecher G, Tamura K (2016) MEGA 11: Molecular Evolutionary Genetics Analysis Version 11. Molecular Biology and Evolution 33(7): 1870-1874. doi: 10.1093/molbev/msw054.

Lee MS (2004). The molecularisation of taxonomy. Invertebrate Systematics 18(1): 1-6. doi: 10.1071/IS03021.

Mehle N, Trdan S (2012) Traditional and modern methods for the identification of thrips (Thysanoptera) species. Journal of Pest Science 85(2): 179-190. doi: 10.1007/s10340-012-0423-4.

Moritz G (1994) Pictorial key to the economically important species of Thysanoptera in Central Europe. EPPO Bulletin 24(1): 181-208. doi: 10.1111/j.1365-2338.1994.tb01060.x.

Moritz G, Delker C, Paulsen M, Mound L, Burgermeister W (2000) Modern methods for identification of Thysanoptera. EPPO Bulletin 30(3-4): 591-593. doi: 10.1111/j.1365-2338.2000.tb00953.x.

Mound L, Palmer J (1983) The generic and tribal classification of spore-feeding Thysanoptera (Phlaeothripidae: Idolothripinae), Bulletin of the British Museum (Natural History). Entomology 46(1): 1-174.

Mound LA (1997) Biological diversity. In: Lewis, T. (ed.) Thrips as crop pests. , In, Eds, CAB International. Wallingford, UK. pp. 197-215.

Mound LA, Kibby G (1998) Thysanoptera: an identification guide. Cab International, Wallingford pp. 70.

Mound LA, Morris DC (2007) The insect order Thysanoptera: classification versus systematics. Zootaxa 1668(1): 395-411. doi: 10.11646/zootaxa.1668.1.21.

Mound LA, Wang Z, Lima ÉF, Marullo R (2022) Problems with the concept of “pest” among the diversity of pestiferous thrips. Insects 13(1): 61. doi: 10.3390/insects13010061.

OzThrips (2023) Tysanoptera in Australia. http://www.ozthrips.org/. Accessed 8 March, 2023.

Retana-Salazar AP (1998) Reestablecimiento de los géneros *Frankliniella*, Exophtalmothrips y *Bolbothrips* (Thysanoptera: Thripidae). Revista de Biología Tropical 46(2): 385-396. doi: 10.15517/rbt.v46i2.19629.

Retana-Salazar AP (2000) Revision y filogenia del grupo generico Pseudothrips (Thysanoptera: Thripidae). Brenesia 54: 51-62.

Şahin İ, Ünlü L, İkten C, Tunç İ, Minaei K (2019) The first report of *Kakothrips dentatus* (Thysanoptera: Thripidae) from Turkey. Journal of Entomological Society of Iran 34(4): 435-438.

Şahin Negiş İ, İkten C, Ünlü L, Tunç İ (2022) Molecular Characterization of Thripidae (Thysanoptera) Species in Karaman, Konya and Mersin (Turkey). Selcuk Journal of Agriculture and Food Sciences 36(2): 203-211. doi: 10.15316/SJAFS.2022.026.

Şahin Negiş İ (2023) *Kakothrips acanthus* Berzosa (Thysanoptera: Thripidae) in Türkiye, with an illustrated key to the species of *Kakothrips*. Zootaxa 5258(1): 141-145. doi: 10.11646/zootaxa.5258.1.7.

Schlee D (1975) Numerical taxonomy. The principles and practice of numerical classification. JSTOR (for the Society of Systematic Biologists) 24(2): 263-268. doi: 10.2307/2412767.

Tamura K, Nei M, Kumar S (2004) Prospects for inferring very large phylogenies by using the neighbor-joining method. Proceedings of the National Academy of Sciences 101(30): 11030-11035. doi: 10.1073/pnas.0404206101.

ThripsWiki (2023) Thrips Wiki-providing information on the World's thrips. http://thrips.info/wiki/Main_Page. Accessed 8 March, 2023.

Trdan S, Andjus L, Raspudić E, Kač M (2005) Distribution of Aeolothrips intermedius Bagnall (Thysanoptera: Aeolothripidae) and its potential prey Thysanoptera species on different cultivated host plants. Journal of Pest Science 78(4): 217-226. doi: 10.1007/s10340-005-0096-3.

Tyagi K, Kumar V, Singha D, Chandra K, Laskar BA, Kundu S, Chakraborty R, Chatterjee S (2017) DNA Barcoding studies on Thrips in India: Cryptic species and Species complexes. Scientific Reports 7(1): 1-14. doi: 10.1038/s41598-017-05112-7.

zur Strassen R (1996) Neue daten zur Systematik und Verbreitung einiger west-paläarktischer Terebrantia-Arten (Thysanoptera), Entomologische Nachrichten und Berichte 40(2): 111-118.

zur Strassen R (2003) Die terebranten Thysanopteren Europas und des Mittelmeer-Gebietes. Die Tierwelt Deutschlands 74: 1-271.

Research Article

www.dergipark.org.tr/en/pub/mediterranean

MEDITERRANEAN AGRICULTURAL SCIENCES
(2023) 36(3): 129-134
DOI: 10.29136/mediterranean.1318007

Analysing crisis situations of seedlings and crisis conditions in palmyra palm stands in Burkina Faso

Babou SOGUÉ[1], Burhan OZKAN[2]

[1]University of Nazi BONI, Legal, Political, Economic, and Management Training and Research Unit Sciences, Bobo-Dioulasso, Burkina Faso
[2]Akdeniz University, Faculty of Agriculture, Department of Agricultural Economics, 07070, Antalya, Türkiye

Corresponding author: B. Sogué, e-mail: babousogue@gmail.com
Author(s) e-mail: bozkan@akdeniz.edu.tr

ARTICLE INFO

Received: June 21, 2023
Received in revised form: August 20, 2023
Accepted: August 21, 2023

***Keywords*:**

Bioeconomic model
Borassus aethiopum
Degeneration rate
Equilibrium price
Exploitation rate

ABSTRACT

The regeneration system of Borassus aethiopum in Burkina Faso is the subject of several hypotheses. However, it is challenging to implement appropriate conservation strategies due to the lack of usage of bioeconomic approaches to support this or that perspective. The objective of this study is to settle this debate by assessing the status of palm tree stands using a bioeconomic model based on a dynamic equilibrium price. The data used in the study was gathered from thirty farming households in the study area in 2010, 2015, and 2020, as well as from monitoring thirty farm sites. The results show that, on average, there is an equilibrium between the rate of harvesting and the rate of regeneration of the palmyra palm. Nonetheless, market prices do not reflect the species' value, as they are between 28% and 166% below their implicit level. To ensure a proportionality coefficient between the equilibrium price and the resource constraint, a dynamic Pigouvian tax should be applied, taking into account the gap, the rate of exploitation, and regeneration.

1. Introduction

Borassus aethiopum is a species of dioecious palm native to Africa, where it grows in savannahs and forests (Bayton 2007). It is particularly abundant in eastern Burkina Faso in the Kompienga watershed. The province of Kompienga spans 7280 km^2 and is located in the far eastern region of Burkina Faso. However, since the 1990s, it has been experiencing accelerated degradation due to the exploitation of young shoots for their roots (hypocotyls) (Guinko and Ouédraogo 2005), leading to the ageing of the palm stand. The species' regeneration system is therefore affected by these practices, while there is virtually no appropriate mechanism for internalising the external effects of the actions of local populations.

The social cost of overexploitation of in situ seedlings in certain localities is characterised by the perceptible ageing of palm stands, the fragmentation of their plant formations and the decline in their productivity, on the one hand, but also by the fall in agricultural yields and the income of farmers dependent on forestry, the silting up of watercourses and the destruction of crops located downstream of the banks, on the other. This is a case of the presence of negative externalities specific to common goods. It is also accepted that the market fails in the presence of externalities, common goods, and collective goods, as in the present case (IRP 2020). For the time being, decisions on the preservation of the species combine Ronald Coase and Pigouvian approaches, i.e. the organisation of neighbouring populations and the application of a flat-rate tax on the exploitation of hypocotyls. Coase (1960) was highly critical of standard neo-classical theory, which emphasised public solutions to the harmful effects of activities. Instead, he put forward private solutions to deal with so-called externalities. The "Coase theorem" states that "the final result is independent of the legal system if the pricing system is assumed to work". So, in the absence of transaction costs, property rights are sufficient to achieve the social optimum. Unfortunately, in the hypocotyl market, prices do not work in the way Coase wants (Chu 2022) and there are no property rights over the resource. However, local people have put in place strategies to conserve the resource, such as the development of seeding to reduce the extraction of seedlings in situ, and the protection of banks overgrown with palm trees. Although this decision takes into account the ecological footprint, i.e. the equalisation between exploitation and regeneration rates, its effectiveness is ignored. Several methods of economic evaluation of the degradation of renewable resources have been proposed for this purpose. Among others, the Chevassus-au-Louis (2009) report proposes valuing biodiversity on the basis of the ecosystem services from which society benefits. Gastineau et al. (2023) used a multi-criteria approach to discuss the modalities of ecological compensation measures in response to environmental damage when ecological, economic, and societal criteria are taken into account Gastineau et al. (2021). They showed that it is necessary to find a compromise between ecological criteria and criteria that take into account the preservation of the quality of life of inhabitants, which they call the "social well-being" criterion (IRP 2020). The principle of monetarisation was used to evaluate, in monetary terms, the impact of a variation in the quantity of an environmental good on the well-being of an individual consisted in determining how much an individual would be prepared to pay to avoid the degradation of an element of his environment. However, this method itself contains intrinsic biases linked to the moral hazard of actors. L'Écuyer-Sauvageau (2022) analysed the supply and demand of water-related ecosystem services in the agricultural sector in Quebec using the benefit transfer method and methods derived from market prices. This approach has certainly provided a picture of the value of ecosystem services associated with agricultural environments, but it does not take into account the imperfect nature of markets, which makes the price incapable of internalising the externalities associated with the exploitation of environmental heritage. Similarly, several studies have addressed the degradation of palm trees in eastern Burkina Faso (Sogué 2010; Yaméogo 2016). However, all these studies have merely presented the results obtained without explicitly developing the techniques, methods and hypotheses. Njomgang (2002) developed an innovative dynamic method based on the equilibrium price between the resource constraint and human activity. This approach seems better suited to our context and is used in this study to analyse the crisis situation and the crisis state of plant exploitation in the Kompienga watershed, with variances. We integrated the price of a perfect substitute for hypocotyl as the social opportunity cost of the depletion of the palm tree stock, to determine the crisis situation and the state of crisis of the economic exploitation of this natural good. In what follows, the methodology used is first presented, then the results of the data analysed are presented and discussed, and finally, we drew the inherent conclusion and recommendations.

2. Materials and Methods

2.1. Method

We started from the work of Njomgang (2002) to define the crisis in relation to the equilibrium, symbolized by: $\mu = \omega t \omega o^{-1} = 1$. Where: μ is the rate of depletion of the palmyra palm wood resource, ωt is the effective rate of exploitation of the forest capital Wt in t, ωo is the optimal exploitation rate, i.e. the rate compatible with forest regeneration.

To determine ωo in practice, we conducted an inventory of a dozen hectares of natural palmyra palm tree stands, which we compared to the stratum of young palmyra palm trees ranging in age from six months to six years at 30 logging sites selected based on the density of the stands and their accessibility. This stratum is considered by Guinko and Ouédraogo (2005) and Yaméogo et al. (2016) as the regenerated palmyra palm franche. If, for example, the inventory yields 60 six-month-old to six-year-old seedlings per hectare out of a total of 300 palmyra palm trees, then the regeneration rate will be 60/300. And if the net natural regeneration (dead seedlings and surviving saplings) from harvesting sites yields 2 out of 300 trees per hectare per year, then the regeneration cycle (equal, in equilibrium, to the rotation period) is:

$T = 60(300)^{-1}(2(300))^{-1} = 30$ years. We will then have $\omega o = 1{*}T^{-1} = 1{*}30^{-1}$ (1)

That is to say, the forest formation considered can be divided into thirty equal parcels, of which one can be exploited each year without danger to the forest capital. The effective rate of extraction is defined as the ratio between the quantity of seedlings harvested Qt and the forest capital Wt, i.e.

$$\omega_t = Q_t W_t^{-1} \quad (2)$$

The equilibrium situation (the crisis condition) is thus symbolized by:

$$\mu = \omega t \omega o^{-1};\ \mu = 1, \text{ i.e. } \omega t = \omega o \text{ or } Q_t W_t^{-1} = 1{*}T^{-1} \quad (3)$$

The indicator μ makes it possible to distinguish between the two types of planting crisis that occur in the Kompienga watershed: The over-exploitation crisis, characterized by $\mu>1$, is prevalent in the Kompienga watershed. The under-exploitation crisis, characterized by $\mu<1$, is rampant in the forestry sector.

The equilibrium price is an implicit price. That is to say, it results from the process of maximizing, under the constraint of the scarcity of young shoots, the net social benefit provided by the regeneration of palmyra palm trees, instead of being a parameter (market price or public price) in this process (Njomgang 2002). It indicates the price of a unit of resource in terms of net social benefit when the constraint is varied by one unit. It is therefore a decision variable in our problem of rationalizing reforestation or natural regeneration policies, allowing us to assess the impact of human activities on the palmyra palm seedlings and not an exogenous variable. In the context of the study, the research on the equilibrium price aims at providing a criterion for quantitative analysis of the interface exploitation of the seedlings (hypocotyl), and the regeneration of the species. These seedlings are viewed in terms of their dual function as a source of food and income on the one hand, and as a factor in future ecosystem regulation on the other. This is an implicit macro-price (Njomgang 1993), in the sense that it results from a joint maximisation in the ecosystem and in the economic exploitation of the young shoots, under the hypothesis of proportionality between the economic value of the palmyra palm tree and its regeneration cycle. As assumptions**,** we place ourselves in the eyes of the state, whose objective it is to maximise the satisfaction of the needs for non-wood forest products under the constraint of ecological balance, i.e., with natural regeneration. The only way to increase the stock of palmyra palm tree stands is through natural regeneration. We consider that the rate of assisted regeneration of the palmyra palm tree asset is very weak because of the absence of action for reforestation of the palmyra palm trees on the site. The policy of natural regeneration is taken into account through the rate of replacement of the old or dead Borassus aethiopum each year by the survivors of the young palmyra palmer shoots. The first relationship is a defining relationship of cut replacement:

$$R_t = r\, Q_t \quad (4)$$

Where R_t is the number of trees to be retained in the harvested area to ensure stand regeneration, and Q_t is the number of seedlings removed for their roots. The rate r is constant (the mortality rate is considered to be identical in the whole area). The second relationship is a relation of definition of the forest capital.

$$W_t = W_0 - \left(\int_0^t Q\tau\, d\tau - \int_0^t R\tau\, d\tau\right) \quad (5)$$

Expression in which: W_t and W_0 denote, respectively, the stand capital of the palmyra palm tree at t and the initial stand capital of the palmyra palm tree. The sums in parentheses denote the quantities harvested and retained to t, respectively. This is again written as:

$$W_t = W_0 - \left(\omega \int_0^t W\tau\, d\tau - r\omega \int_0^t W\tau\, d\tau\right) \quad (6)$$

$$W_t = W_0 - \omega(1-r)\int_0^t W\tau\, d\tau \quad (7)$$

In this expression, (1-r) denotes the effective exploitation rate in the presence of natural regeneration. One has in effect:

$$\omega(1-r) = \frac{Q_t}{W_t}\left(1 - \frac{R_t}{Q_t}\right) = \frac{Q_t - R_t}{W_t} \quad (8)$$

The definition of the net social benefit provided by the operation of palmyra palm seedlings is:

$$B_s = U(Q) - C_s \quad (9)$$

Where: U(Q) is the social utility of a seedling. This utility is defined, in the context of the study, as the sum of the income of the hypocotyl producers and the consumption and production services rendered by the palmyra palm in the various uses. These include food, energy, handicrafts, fertilisation of agricultural soils, etc. However, in this study, the utility will be summarized as the income provided to hypocotyl producers because of the difficulties in evaluating the other services provided by the palmyra palm to society. Thus, the utility is underestimated (Yaméogo 2008). C_s is the social cost of the palmyra palm tree. This cost includes environmental costs and opportunity costs measured in terms of the comparative yield of alternative uses of non-wood palmyra products (timber, in particular, food, medicine, etc.) and substitutes for hypocotyls in food uses (tubers and agricultural roots, in particular). The social costs were assessed in this study through the value of substitutes for hypocotyls in food uses, including yam, sweet potato, and cassava. According to Sogué (2010), cassava is the closest substitute to hypocotyl with a cross-price elasticity equal to +1.16. That is, a 1% increase in the price of cassava leads to a 1.16% increase in the consumption of hypocotyl. Thus, the social cost of hypocotyl is equal to its opportunity cost, i.e., the price of cassava times the quantity. U(Q) measures the sum of the utility flows Pt (q), where q denotes the flow of wood production. Thus we have:

$$U(Q) = \int_0^q Pt(q)\, dt \quad (10)$$

The problem thus amounts to maximizing: $B_s = U(Q) - C_s$.

Under the ecological equilibrium constraint ω= ωo, where R_t= rQ_t in terms of replacement, r= 1. The Lagrangian is written:

$$V(Q,\lambda,t) = (U(Q) - C_s) - P'_t Q_t - \lambda(t)(Q_t - R_t) \quad (11)$$

Where again:

$$V(Q,\lambda,t) = (U(Q) - C_s(Q)) - P'_t Q_t - \lambda(t)(1-r)Q_t \quad (12)$$

By canceling the partial derivatives, we obtain:

$$\frac{\partial V}{\partial Q} = U'(Q) - P'_t - \lambda(t)(1-r) = 0 \quad (13)$$

and

$$\frac{\partial V}{\partial \lambda} = Q_t(1-r) = 0 \quad (14)$$

The relation (13) gives:

$$U'(Q) = P'_t + \lambda(t)(1-r) \quad (15)$$

With P'_t the price of cassava. Again, knowing that:

$$U(Q) = \int_0^q Pt(q)\, dq. \text{ And } P_t = P'_t + \lambda(t)(1-r) \quad (16)$$

Where (t) is a time-dependent Lagrange multiplier, playing the role of a proportionality coefficient between the equilibrium price and the resource constraint. According to equation (8) $\omega(1-r) = \frac{Q_t}{W_t}\left(1 - \frac{R_t}{Q_t}\right) = \frac{Q_t - R_t}{W_t}$ hence $\lambda(t) = \omega(t) = \frac{Q_t}{W_t}$ i.e. the effective rate of hypocotyl extraction. Here, P_t is the inverse demand expression for hypocotyl with λ(t) (1-r) as the quantity demanded. (1-r) being constant, the increase in quantity demanded depends on (t) which in turn is a function of the rate of resource depletion which is analytically positive λ(t)= $\mu^*\omega_0$. This is consistent with the economic theory of natural resource depletion where P_t is an increasing function of time and depletion of the palmyra palm resource in the situation of overexploitation of the resource (μ>1), and decreasing in the situation of under exploitation of the resource (μ<1), and roughly equal to $P'_t + (1-r)$ in the equilibrium situation. To summarise, regardless of the resource's exploitation crisis, its price must be higher than its closest substitute in order to reduce demand.

2.2. Data

Secondary and primary data were used in this research. For this purpose, the study reports of the environmental institutions established in the study area were consulted. Part of the data was extracted from the master's thesis of Sogué (2010) and supplemented by a survey of households growing palmyra palm from 2010 to 2020; because of the remoteness of the households' compounds, insecurity in the area and the recent presence of armed groups in the area, the sample size was reduced to thirty households. In addition, in 2010, twelve control sites were identified and the Borassus aethiopum was inventoried by age group. The exploitable seedlings are those between 1 and 3 months old. At this age, they have one to two leaves. Seedlings over one meter in height are considered regenerated. The same approach was adopted for thirty harvesting sites in 2010, 2015, and 2020. Of the thirty sites monitored, 17 were close to the concessions (2 to 3 km) and were considered "high accessibility" while those located between 3 and 5 km were considered "medium accessibility", of which there were seven. Finally, the remaining six were either on the riverbank or on the other side of the riverbank. They were accessible only in the dry season after January or March. They were qualified as sites with low accessibility.

3. Results and Discussions

The average density of the park is 436 mature palmyra palm trees per hectare in the inventory areas. If the youngest individuals are taken into account, the average density increases to 3876 trees ha^{-1}, of which 430 seedlings are extracted per year on each site, i.e., an exploitation rate of 0.11. This density is well above that of agroforestry parks in southwestern Burkina Faso (Cassou and Depommier 1997).

There is little variation in density at the plot level as indicated by coefficients of variation (CV) ranging from 22% to 33%. However, there are more intermediate strata in the sparse sites generally closer to the concessions than in the dense, inaccessible

Table 1. Descriptive statistics of the data

Variables	Unit	Average	Median	SD[a]	CV[b]
Seedlings	feet	3440.23	3146.50	1123.19	0.33
Palmyra palm	feet	435.74	417.50	96.77	0.22
W_t	feet	3875.98	3542.50	1204.56	0.31
Q_t	feet	429.90	420.00	109.20	0.25
ω_t	-	0.11	0.11	0.02	0.17
ω_0	-	0.11	0.11	0.04	0.33
$\mu = \omega_t \omega_o^{-1}$	-	1.00	0.97	0.17	0.17
Price of hypocotyl	USD	0.25	0.21	0.11	0.00
Cassava price	USD	0.54	0.36	0.69	0.00

a; standard deviation, b; coefficient of variation.

areas. The results in Table 1 also show a natural regeneration rate in the control areas of ωo= 0.11 giving a seedling depletion rate of μ= 1. This is an equilibrium situation that ensures compatibility between the regeneration cycle of the resources and the rotation rate. Overall, there is no seedling crisis condition in the study area. Table 2 shows that the situation is not static. In 2010, the natural asset was facing an overexploitation crisis with a depletion rate of μ= 1.07, i.e. an overexploitation of 7%. Of the 30 plots monitored, 53.3% were overexploited compared to 43.3% and 26.7%, respectively, in 2015 and 2020. In addition, the table 2 shows that the plots wereincreasingly under-exploited over time. The security crisis has caused the displacement of populations from the areas and reduced the accessibility of production sites. However, this generates a crisis of under-exploitation where the potential resources are enormous, but the poor accessibility of the deep forest limits the exploitable resources and has accentuated the over-resourcing around the concessions and refusal areas and the generalisation of the production of hypocotyl in germinators. The result is a scarcity of hypocotyls that is as constraining in its effects on prices and the environment as the over-exploitation crisis. Njomgang (1993, 2005) found similar results in the context of fuelwood exploitation in Cameroon.

In addition to the safety measures that slowed down the overexploitation of palm trees, the awareness-raising actions of rural development actors has promoted the awareness of the existence of damage on an even larger scale, such as global warming, the disruption of ecosystems, the occurrence of extreme weather events, etc. In this perspective, the analysis of

Table 2. Interannual seedling exploitation crisis situation

Years	Under-exploited	Over-exploited	μ
2010	46.7%	53.3%	1.07
2015	56.7%	43.3%	0.98
2020	73.3%	26.7%	0.96

the interactions between human activities and nature has become crucial. Indeed, Figure 1 shows a gradual shift of human pressure on stands towards highly accessible areas and the substitution of in-situ sapling extraction for controlled agricultural production of hypocotyls. The determination of equilibrium prices of hypocotyls recorded in Table 3 shows the extent of hypocotyl undervaluation over time. These prices observed in 2010, 2015 and 2020 are respectively 0.13 USD, and USD 0.24, USD 0.37 per hypocotyl. They should be USD 0.35, USD 0.40 and USD 0.48 per hypocotyl to take into account the social cost of their depletion. The social cost generated represents an undervaluation of the producer price of hypocotyls of 166%, 70%, and 28% of their current price.

Baral et al. (2008) used a contingent valuation method to determine the price of entry into Nepal's largest protected area, Annapurna. Taking into account biodiversity conservation, ecosystem protection and sustainable development, they found that the proposed fees ranged from USD 30 to USD 120, with a survey price of USD 27. The study indicates that the entry fee may well be increased incrementally from USD 27 to USD 50. Thus, the implicit prices of environmental assets are generally reservation prices for the consumers and therefore reduce their surplus to zero.

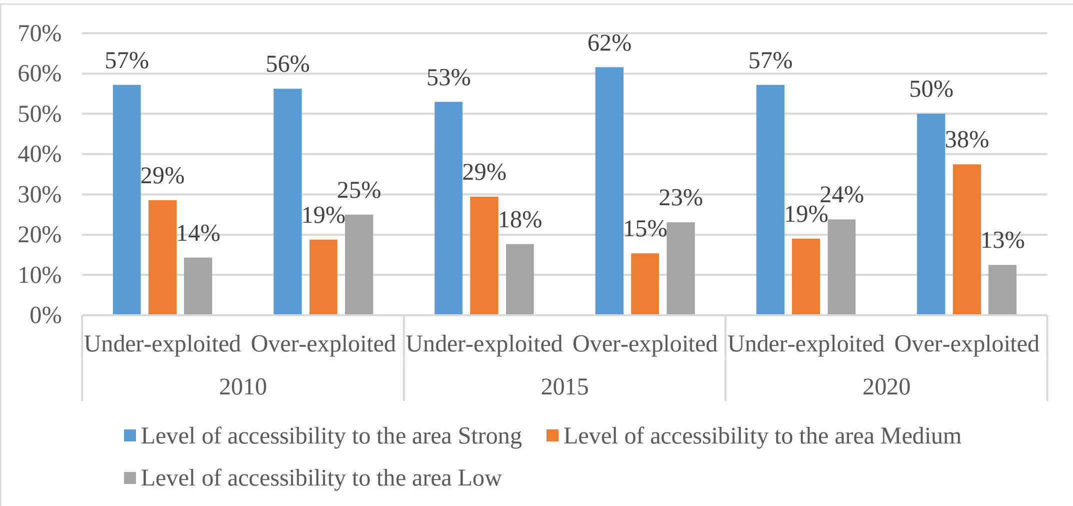

Figure 1. Dynamics of the nature of the crisis.

Figure 2 provides a comparative view of price structures over this decade. A visualisation of the implicit prices of hypocotyls shows little difference with those of their direct substitute over time. This implies a transition to green and sustainable growth of palmyra palm tree services. However, this requires taking the analysis further and looking beyond production as described above by requiring the asset base to remain intact. Indeed, the current depletion of the asset base poses a risk to growth, and this type of risk must be avoided. Until now, the price of hypocotyls on the market essentially integrated private costs, i.e., the costs borne by those involved in the young growth sector of the palmyra palm tree for the production and marketing of hypocotyls. The social costs include forestry taxes and fees, taxes and other regulatory costs whose objective is to reduce harvesting and safeguard forest resources are derisory (Sogué 2010) to dissuade consumers. As shown in Table 3, these taxes should represent 166%, 70% and 28% of the market price of hypocotyl in 2010, 2015 and 2020, respectively, in order to reflect the real use value of the seedlings and to preserve the regeneration of the species, whereas Sogué (2010) reported that the tax represented only 6% of the price of hypocotyl in 2010.

As shown in Figure 2, implicit prices are variable both intra- and inter-annually. Similar results were found by Revéret et al. (2019). Indeed, he conducted a case study in the Tioga classified forest in Burkina Faso on a sample of 300 surveyed households. The author shows two types of average Willingness To Pay (WTP) for riparian households: an average monthly WTP of USD 1.03 to obtain a field in the forest (alternative use), and an average WTP of USD 0.80 for forest maintenance. In the same vein, Ouédraogo (2015), based on the work of Yaméogo et al. (2016), showed that between 1985 and 1995, the real price of firewood sold to consumers increased by 28.54%. However, this increase in the price of fuelwood is explained by simultaneous increases of 124.96% in real transport costs, 39.87% in real retail costs, and 42.16% in real wholesale profit margins; real retail profit margins have decreased by 16.27% between 1985 and 1995, and not by the price of the social costs of producing fuelwood. Finally, Figure 2 shows the evolution of observed prices at an increasing rate from 2015 and the possibility of convergence of the three prices if this 2015 growth rate is maintained. Observed prices rose on average from USD 0.13 in 2010 to USD 0.24 in 2015, and to USD 0.37 in 2020. This represents a 2010-based price index of 100; 181.25; and 287.68 respectively, a tripling of prices in a decade. In addition, in some sites where the depletion rate is very low, the observed price is higher than that determined.

4. Conclusion

Our approach to developing this methodology has been based on the most recent developments in the economic evaluation of environmental degradation. We started with the work of Claude Njomgang (1993, 2002) to determine an equilibrium price for young palmyra palm tree shoots in the Kompienga watershed. The exploitation of seedlings is in equilibrium. In fact, at equilibrium, the rate of depletion (μ) of wood resources is equal to 1 and ensures compatibility between the regeneration cycle of the resources and the rotation rate. Moreover, with the generalisation of germination and the deterioration of the security situation in eastern Burkina Faso since 2015, the demand for hypocotyl has decreased, also leading to a reduction in the rate of exploitation of seedlings in natural formations. While these two phenomena have helped to rebalance the flow of energy in the ecosystem, they also create situations of under-exploitation crises which, like over-exploitation, constitute an inefficiency from an economic point of view. The prices observed during the three periods do not allow for optimal exploitation of the resource. Producer prices are between 28% and 166% lower than their implicit level. The Pigouvian tax currently applied by the public

Table 3. Determination of equilibrium prices of the hypocotyl

Years	Average prices determined in USD	Average prices observed in USD	Differences in USD	Extent of price underpricing
2010	0.35	0.13	0.22	166%
2015	0.40	0.24	0.16	70%
2020	0.48	0.37	0.10	28%

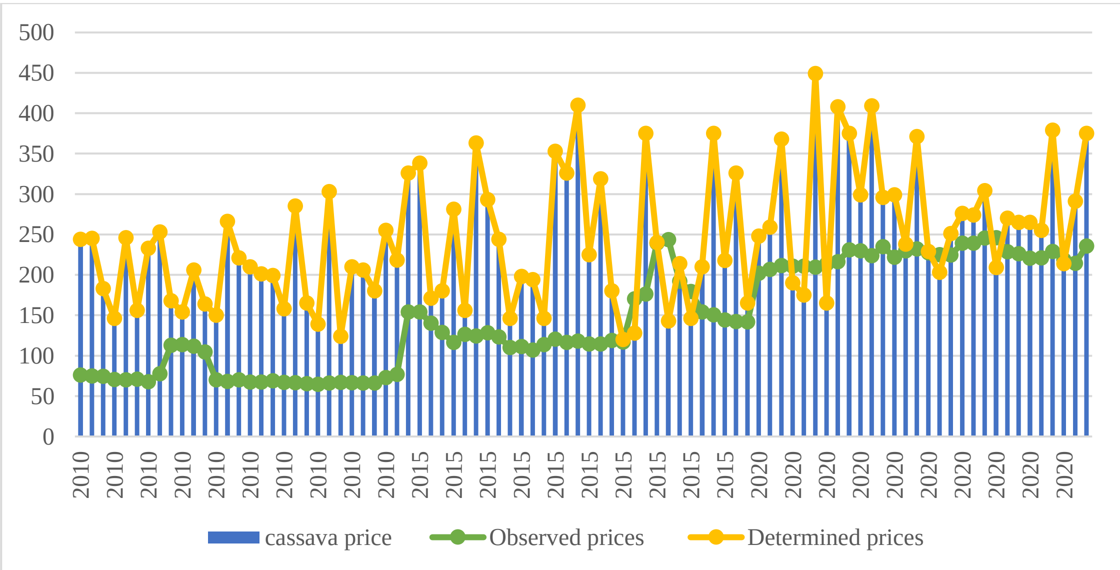

Figure 2. Evolution of observed and determined prices of hypocotyl and cassava in space.

administration to ensure price convergence is modest enough to be effective. It is barely 6% of the observed price, whereas it should have been 166% in 2010, 70% in 2015, and 28% in 2020 of the price of the producer of the hypocotyl in order to reflect the unit use value of the seedlings and to preserve the regeneration of the species. The convergence of the observed prices and the determined prices is accidental because it is essentially created by the security situation, which reduced the accessibility of the operators of the zones of production and generated a supply of hypocotyls lower than their demand. It is recommended that the public administration should apply a tax that varies over time according to the rate of exploitation and natural regeneration of palmyra palm tree seedlings. This dynamic tax should act as a proportionality coefficient between the equilibrium price and the resource constraint.

References

Baral N, Stern MJ, Ranju B (2008) Contingent valuation of eco-tourism in Annapurna Conservation Area, Nepal: Implication for sustainable park finance and local development. Ecological Economics 66: 218-227.

Bayton RP (2007) A revision of Borassus L. Arecaceae. Kew Bulletin, 62: 561-585.

Cassou J, Depommier D (1997) Le parc à rônier (Borassus aethiopum Mart.) de Wolokonto dans le Sud-Ouest du Burkina faso: Structure, dynamique et usages de la rôneraie.

Chevassus-au-Louis B (2009) Approche économique de la biodiversité et des services liés aux écosystèmes: contribution à la décision publique. Ministère de l'Alimentation, de l'Agriculture et de la Pêche. 18: 320-400.

Chu LK (2022) Determinants of ecological footprint in OCED countries: do environmental-related technologies reduce environmental degradation?. Environmental Science and Pollution Research 29(16): 23779-23793.

Coase RH (1960) Law economics. Journal of Law and Economics 3: 1-44.

Gastineau P, Mossay P, Taugourdeau E (2021) Ecological compensation: how much and where? Ecological Economics 190: 107-191.

Gastineau P, Mossay P, Taugourdeau E (2023) La compensation écologique: un enjeu environnemental, économique et sociétal, Regards économiques: 177.

Guinko S, Ouédraogo A (2005) Usages et enjeux de conservation du palmyra palm (Borassus L.) à l'Est et à l'Ouest du Burkina Faso. In: Boussim I.J., Lykke A.M., Nombré I., Nielsen I. and Guinko S. (Eds). Homme, plantes et environnement au Sahel occidental. Serein Occasional Paper N°; 19, pp: 1-6.

IRP (2020) Resource Efficiency and Climate Change: Material Efficiency Strategies for a Low-Carbon Future. A report of the International Resource Panel. United Nations Environment Programme, Nairobi, Kenya. doi: 10.5281/zenodo.3542680.

L'Écuyer-Sauvageau C (2022) Analyse de l'offre et de la demande de services écosystémiques liés à l'eau en milieu agricole au Québec. Thèse de doctorat. Université du Québec en Outaouais.

Njomgang C (1993) La substitution au bois de feu dans un système dualiste: le cas de quelques villes de la zone soudano-sahélienne du Cameroun. Liaison-Énergie-Francophonie 18: 22-56.

Njomgang C (2002) Esquisse d'un cadre d'analyse du marché du bois de feu au Cameroun. Cahiers Agricultures 11: 207-212.

Njomgang C (2005) Théorie économique et dimension environnementale du développement durable. Liaison énergie francophonie 66-67: 24-26.

Ouédraogo B (2015) Tarification du bois-énergie au Burkina Faso : la problématique de l'internalisation des coûts sociaux liés à l'exploitation de l'énergie.12.

Revéret JP, Yelkouni, M, Babin D, Froger G, Harou PA, Henry F, Yonkeu S (2019) Économie et gestion de l'environnement et des ressources naturelles. Institut de la Francophonie pour le développement durable, pp: 26-266.

Sogué B (2010) Exploitation économique et stratégies de conservation de Borassus aethiopum Mart. Dans le bassin versant du Kompienga. Mémoire de DESS, CEPAPE à l'Université de Ouagadougou.

Yaméogo J (2008) Contribution des parcs à akeassii Bayton, Ouedraogo et Guinko au fonctionnement des systèmes de production dans le sud-ouest du Burkina Faso. Thèse de Doctorat. Université de Ouagadougou.

Yaméogo J, Samandoulgou Y, Belem M (2016) Le palm tree Borassus akeassii B.O.G. dans les parcs agroforestiers à Kokologho, Sakoinsé et Ramongo dans la province du Boulkiemdé, Centre-ouest du Burkina Faso. Journal of Applied Biosciences 100: 9557-9566.

Research Article

www.dergipark.org.tr/en/pub/mediterranean

MEDITERRANEAN AGRICULTURAL SCIENCES
(2023) 36(3): 135-141
DOI: 10.29136/mediterranean.1292860

Achieving high buckwheat sorting accuracy in a deep learning based model by applying fine scaling method

Hakan AKTAS[1], Ovunc POLAT[2]

[1]Niğde Ömer Halisdemir University, Engineering Faculty, Computer Engineering Department, 51240, Niğde, Türkiye
[2]Akdeniz University, Engineering Faculty, Electrical and Electronics Engineering Department, 07070, Antalya, Türkiye

Corresponding author: H. Aktas, e-mail: hakanaktas5151@gmail.com
Author(s) e-mail: ovuncpolat@akdeniz.edu.tr

ARTICLE INFO

Received: May 5, 2023
Received in revised form: September 25, 2023
Accepted: October 2, 2023

***Keywords*:**

Image processing
Dataset generation
Seed sorting
Convolutional neural networks

ABSTRACT

Automated seed sorting is widely used in the agricultural industry. Deep learning is a new field of study in agricultural seed sorting applications. In this study, a classification of buckwheat seeds and foreign materials, such as sticks, chaff, stones was performed using deep learning. The main purpose of the study was to show the effect of scaling the images on the classification results, while creating a dataset. An industrial experimental setup was used to generate the datasets of buckwheat seeds and foreign materials to be sorted by deep learning. The images in the created dataset were rescaled with two different techniques, precision scaling and direct scaling, which were labelled as Type1 dataset and Type2 dataset, respectively. To classify buckwheat seeds and foreign materials, AlexNet architecture was used. The classification accuracy was calculated as 98.57% for Type1 Dataset and 97.34% for Type2 Dataset. As a result, it was concluded that the Type1 dataset had a higher accuracy and the use of precision scaling can be used to improve the classification results in industrial applications.

1. Introduction

Deep learning architectures are widely used in many applications such as object classification (Sharma et al. 2018), sorting food and beverage (Nasiri et al. 2020), face detection (Sun et al. 2018), speech recognition (Zoughi et al. 2020), plate recognition (Omar et al. 2020) etc. Object classification is one of the main applications, which is used in many industries as well as in food and agriculture (Unal 2020). In 2012 and with the achievement of AlexNet (Krizhevsky et al. 2012) in ImageNet Large Scale Visual Recognition Challenge, convolutional neural network (CNN) architectures became much more popular in classification applications. Following AlexNet, many other CNN structures were proposed in literature such as VGGNet (Simonyan and Zisserman 2015), GoogleNet (Szegedy et al. 2015), ResNet (He et al. 2016), MobileNet (Howard et al. 2017).

Classification algorithms are also widely used in agriculture. The use of deep learning for classification problems in agriculture makes these problems more applicable and achieves a high accuracy of results. One of the problems, which is widely covered in the literature, is the problem of seed classification. Kurtulmus et al. 2016 proposed a neural network to classify eight different varieties of pepper seeds. The proposed network had three layers, including an input layer, a hidden layer with 30 neurons and an output layer. Although the proposed network was not a deep convolutional neural network, it had a classification accuracy of 84.94%. In a similar study, to classify Chinese cabbage seeds, the features were extracted from seeds' images and for the classification of features back-propagation neural network was proposed (Huang and Cheng 2017). According to this study, classification accuracy was calculated as 91.53% and 88.95% for good and bad seeds respectively. In another study, a custom CNN architecture was proposed to classify diploid and haploid maize seeds (Veeramani et al. 2018). The proposed custom CNN architecture classification accuracy was calculated as 96.70% and SVM accuracy was calculated as 87.60%. To compare machine learning algorithms and deep learning algorithms, another study was done by Huang et al. 2019. In this study, they classified defect and good maize seeds with deep learning (GoogleNet) and machine learning techniques, such as speeded up robust features, (SURF) and support vector machines (SVM). Experimental results showed that GoogleNet had an accuracy of 95% and SURF+SVM had an accuracy of 79.2%. In a recent study, to identify sunflower seeds, three deep learning architectures (AlexNet, GoogleNet and Resnet) were investigated. The highest classification accuracy (95%) was calculated from GoogleNet algorithm (Kurtulmus 2021).

Sorting the seeds from all kinds of foreign materials such as sticks, chaff, stones etc. before the packaging stage, and even classifying them according to their size, color and shape, provides a higher quality product for the market. The process of classifying seeds with a deep learning algorithm consists of three main sub-processes such as image acquisition, image pre-processing and image classification (Khirade and Patil 2015). Although feature extraction is not required in deep learning algorithms, acquisition of images that reflects important information clearly is very important (Devaraj et al. 2019). In industrial applications, it is not possible to take such images of seeds as they move on the conveyor belt or fall from the reservoir. For this reason, seeds must be extracted from current frames, which is called blob analysis (Yusuf et al. 2018). The other important case is that the images need to be resized to adapt to the requirements of the Deep Learning model (Kamilaris and Prenafeta-Boldú 2018). It was found in the literature review, that there are many studies on seed classification with CNN architectures. However, there are a lack of studies in the literature that explain in detail how to create a seed dataset for a CNN architecture, how to scale the seed data precisely to required size, and which emphasizes how these processes will affect the classification.

In this study, buckwheat seeds were sorted from foreign materials, such as sticks, chaff and stones with deep learning. the generation of datasets were performed by using an industrial experimental setup which is described in the materials and methods section. In order to show the scaling effect to the classification results, the images in the initial dataset were processed to form two datasets which were labeled as Type1 and Type2 (Aktaş 2020). Type1 Dataset was obtained using precision scaling by locating the blob in the middle of NxN Template Image. Type2 Dataset was obtained using direct scaling by generating NxN data from raw blob. To classify buckwheat seeds and foreign materials, AlexNet architecture was used. The classification accuracy was calculated as 98.57% for Type1 Dataset and 97.34% for Type2 Dataset.

2. Materials and Methods

2.1. Experimental setup and image collection

The experimental setup shown in Figure 1a consists of four mechanical parts including hopper, groove, vibration motor and a mechanical body. Inside the body there is a Basler 107649 acA1440-73gc 1440 x 1080, 73 fps, color 1/3” CMOS camera, MBJ imaging DTL-ic-1010 Diffuse Flat Dome Light and other automation cards. The parts and components of the system are shown in Figure 1b. To collect the buckwheat and foreign materials images the experimental setup works as follows: First of all the hopper is filled with buckwheat seeds. The vibration motor is run by 220v AC. A potentiometer is used to adjust the speed of the vibration motor. The vibration motor starts to work by adjusting the potentiometer. In this way, the seeds in the hopper start to move on the groove. The seeds that reach the end of the groove start to fall down. As the seeds fall down, clear images of the seeds are obtained by an industrial camera and lighting system. The industrial camera and lighting system work synchronously with each other; when the camera is ready to take an image, the lighting system is activated by the camera through I/O connection. In this way, the images are taken by the camera and saved to the computer using the pylon viewer interface program. In the next stage, all of these operations were performed for foreign objects. This experimental setup was used to collect images of buckwheat seeds and foreign materials. The classification algorithm was tested on Intel i7, 3.6 GHz, 32 GB DDR3 1600 MHz RAM, Nvidia RTX 2080 GPU, Windows 10 Pro Desktop computer. All of the image processing and deep learning applications were performed with Python version 3.8, OpenCV 4.5 and Keras Library version 2.5.

2.2. Dataset generation

Dataset generation is one of the most important steps before applying deep learning architectures. The blob detection algorithm is used widely during dataset generation to detect and extract the objects, (Dewi et al. 2021). The aim of blob detection is to find regions in an image that differ from the surroundings. Using properties such as intensity and shape, the contours of the objects are defined (Ter Haak 2018). After applying the blob detection algorithm the images need to be resized to adapt to the requirements of the Deep Learning model (for example 227x227 or 224x224) (Kour and Arora 2019). In other words, the size of the extracted blob from current frame must fit to the model input image size. Basically, this can be done by overlapping the center of the blobs with the center of the desired sized frame, and the resulting segment can be subtracted from the entire image, thus producing an image of the desired dimensions. This technique has some disadvantages such as other objects which appear on the edge of the extracted image. Another problem of this technique arises when the natural shape and size of the objects to be classified are quite different from the model input dimensions.

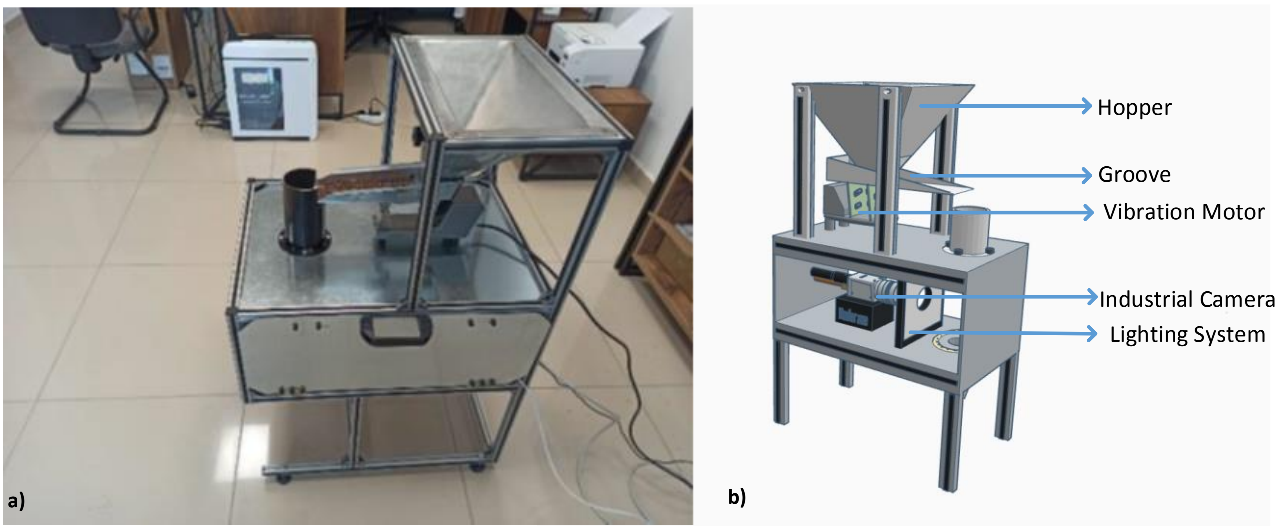

Figure 1. (a) Experimental setup (b) Working principle of experimental setup.

Then the object needs to be stretched or shrunk to fit in the desired frame. To demonstrate the effect of these problems on the classification results, Type2 Dataset was generated using the technique explained above. As a solution to the aforementioned problems it is better to extract the blob from it's borders (x_min,x_max,y_min,y_max) and then locate the blob in the middle of NxN Template Black Image. To demonstrate the effect of this solution, Type1 Dataset was generated using NxN Template Black Image. The proposed method (Aktaş 2020) using the blob detection algorithm is given in Figure 2, where it is clearly seen that in Type2 Dataset the natural shape of the classified object is distorted.

The scaling processes are explained in depth in Figure 3, in order to better understand how all of these image resize operations are done for Type1 datasets . Because the images were grabbed while the objects (buckwheat seeds and garbage objects) were in free fall, the size of buckwheat and foreign materials change too much. The maximum size of the x or y coordinate of one buckwheat seed can be 150 pixels and the minimum size can be 70 pixels. So, in order to locate buckwheat seeds in the middle of these templates, no image resize operation is needed. Because of the nature of free fall, seeds turn and move on x and/or z coordinates. This turn and move effect can be seen a lot more in foreign materials. The size of foreign materials can change over a wide range, which can be seen in Figure 4.

2.3. AlexNet architecture

Alexnet became popular with its success in the ImageNet Large Scale Visual Recognition Challenge (ILSVRC) competition and revolutionized the use of CNN structures in classification problems (Khan et al. 2020). AlexNet Architecture consists of 5 convolution layers, 3 max pooling layers, 2 normalization layers, 2 fully connected layers and 1 softmax layer. A total of 1376 filters, 96, 256, 384, 384 and 256, were used in 5 convolution layers, respectively (Krizhevsky et al. 2012). In this study, the AlexNet structure was produced using Keras library with Python programming language in accordance with the literature. Since there are two classes in the application, the fully connected layers wereupdated as 256, 128, and the last layer, the softmax layer, as 2, in order to classify the two classes. AlexNet architecture with modified output layers is given in Figure 5. The AlexNet architecture can be used as a pre-trained version for the classification problems. Seed classification is an industrial application, in this study instead of using a pre-trained AlexNet structure the network structure created with the keras library was trained from scratch with the created dataset.

After generating 227x227 input images as AlexNet requires for each class, each dataset must be split into training, validation and test datasets to evaluate the performance of the network. In the literature different ratios are used while creating training, validation and test datasets, and the most popular rates such as 70%, 15% and 15% were used in this study (Islam and Raj 2017, Aktaş 2022). After applying these ratios the training, validation and test dataset of each class obtained were 1141, 245 and 245, respectively. The AlexNet architecture, which was modified as in Figure 5, was used for training and testing processes. Accuracy is expected to be high when the network is trained in high epochs. However, after a while the learning process slows down and as the training process continues the network does not learn any more or starts to memorize the dataset, which causes over fitting (Li et al. 2019). One of the solutions to avoid over fitting is to use the early stopping parameter in the keras library, which is used to limit the training of the network to a certain epoch. If val_loss is low 20 times in a row, an automatic training stop has been set as the stopping criterion. The parameters used in the training are as follows: optimizer= SGD, learning rate= 1e-04, batch size= 64, loss_function= categorical_crossentropy.

3. Results and Discussion

In this study, two types of dataset were used for training and tested with the AlexNet structure. Firstly, the AlexNet structure was trained with Type1 dataset. In order to evaluate the success of this trained network, it was tested with 245 images in the test dataset and the test accuracy was calculated as 98.57%. The training of the network ended in the 43rd epoch. Then the AlexNet structure was trained with Type2 dataset from scratch. The training of the network ended at the 46th epoch. Test accuracy was calculated as 97.34% when 245 test images from Type2 dataset were applied. Considering the classification results, the network structure trained with the Type1 dataset produced a higher accuracy.

In the next step, the number of train, validate and test data was halved in order to understand the effect of the number of images in the dataset to its accuracy. Using this new number of datasets and also using the same training options, the training and test process wasrepeated. Using halved Type1 dataset (570 train, 122 validation and 122 test data for each class) the training stopped at 60th epochs by using the early stop class and the test accuracy was calculated as 95.49%. In the next step using halved Type2 dataset (570 train, 122 validation and 122 test data for each class) the training stopped at 56th epochs by using the early stop class and the test accuracy was calculated as 94.67%. The results of using the full number of datasets and half the number of datasets and the comparison of the results are shown in Table 1.

For better representation of the effect of different scaling techniques on the accuracy, the confusion matrices from which the test results were calculated are given in Figure 6a and 6b. The confusion matrix obtained from the network structure trained with Type1 dataset is given in Figure 6a and classification accuracy calculated from that matrix was calculated as 98.57%. It was easier for the network to detect buckwheat seeds because the shapes and sizes of the buckwheat seeds were similar to each other and the foreign materials had various shapes and colors. As seen in Figure 6a, only one out of 245 buckwheat seed images were misclassified, and the number of misclassified images was 6 out of the same amount of foreign matter images.

The confusion matrix obtained from the network structure trained with Type2 dataset is given in Figure 6b and classification accuracy calculated from that matrix was calculated as 97.34%. As seen in Figure 6a, 6 out of 245 buckwheat seed images were misclassified, and the number of misclassified images was 8 out of the same amount of foreign matter images. It can be clearly seen that the number of misclassified classes in the Type2 Dataset is higher than in the Type1 Dataset, which underlines the importance of precision scaling during image processing.

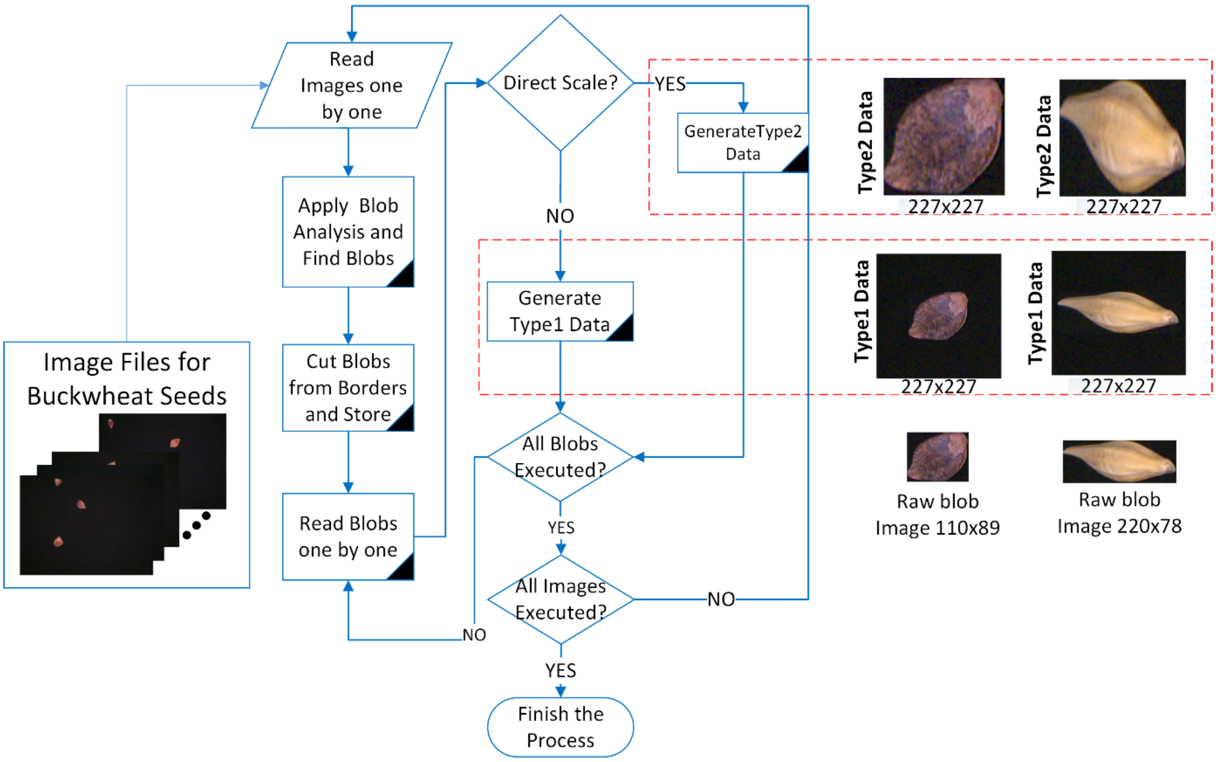

Figure 2. Flowchart for two different dataset type generation. Type1 dataset (locate the blob in the middle of NxN template) and Type2 dataset (NxN data generated from raw blob).

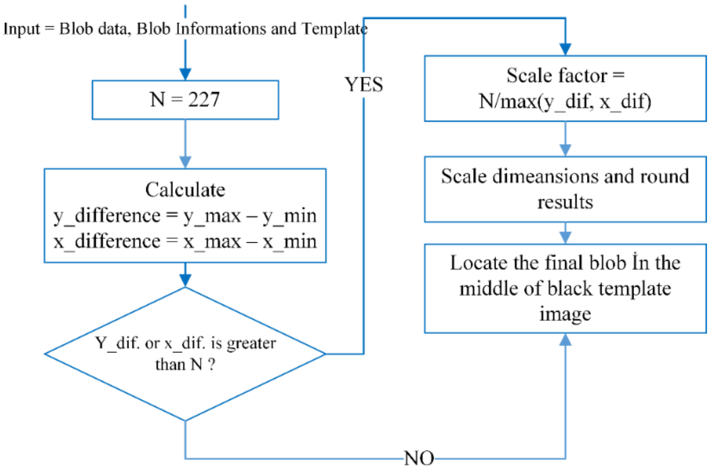

Figure 3. Image resize operations for Type1 dataset (locate the blob in the middle of NxN template black image).

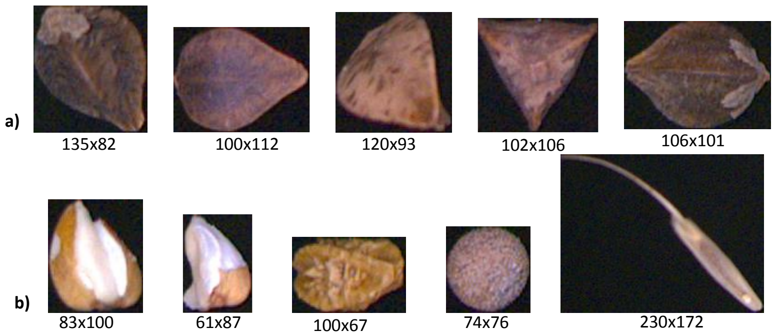

Figure 4. (a) Example of raw buckwheat seed images generated from buckwheat seed image files (b) Example of raw foreign materials images generated from foreign materials image files.

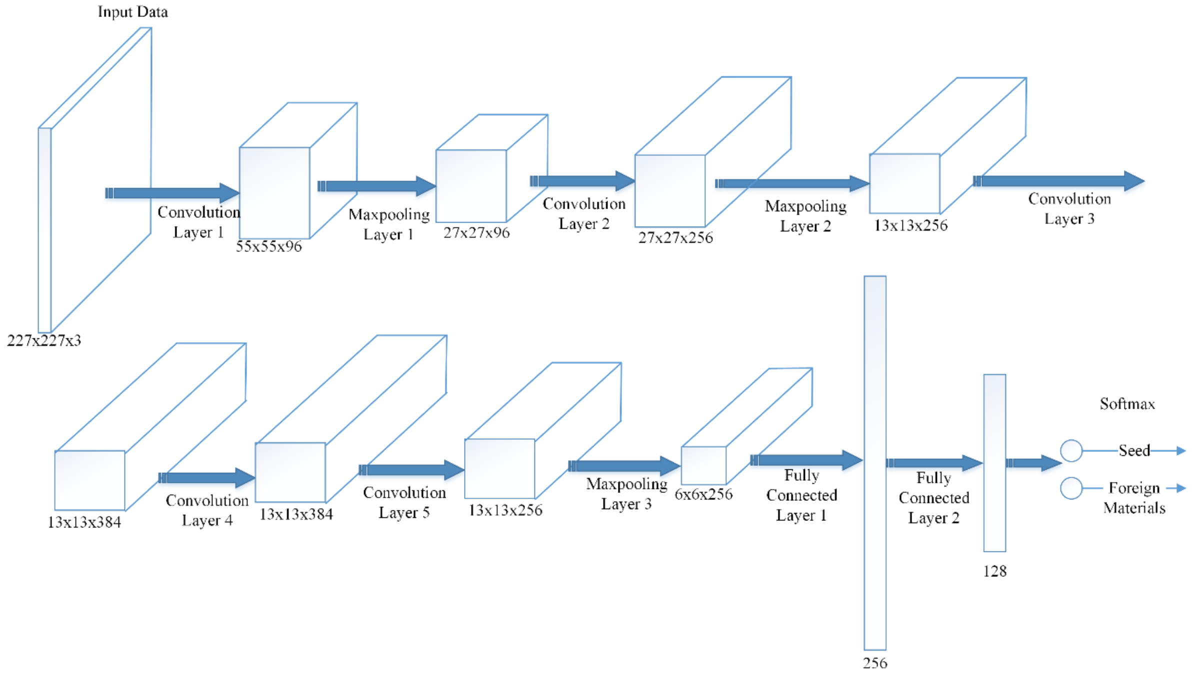

Figure 5. AlexNet architecture.

Table 1. Comparison of AlexNet accuracy results for different types and amount of datasets

Type1_Dataset				Type2_Dataset			
Train= 1141x2, Validation= 245x2, Test= 245x2		Train= 570x2, Validation= 122x2, Test= 122x2		Train= 1141x2, Validation= 245x2, Test= 245x2		Train= 570x2, Validation= 122x2, Test= 122x2	
Epochs	Test Accuracy	Epochs	Test Accuracy	Epochs	Test Accuracy	Epochs	Test Accuracy
43	98.57%	60	95.49%	46	97.34%	56	94.67%

a)

OUTPUT		TARGET: Seed	TARGET: Foreign Materials
	Seed	244	6
	Foreign Materials	1	239

b)

OUTPUT		TARGET: Seed	TARGET: Foreign Materials
	Seed	239	8
	Foreign Materials	6	237

Figure 6. (a) Confusion matrix for Type1 dataset (b) Confusion matrix for Type2 dataset.

Although the numerical difference between these two datasets is not very high, it may mean a lot for farmers and commercial enterprises. The crops collected after harvest must be packaged in order to be offered for sale. Making this packaging process as error-free as possible is appreciated by the buyer, which naturally reflects on the prices. For this reason, it is very important to carefully sort the product before packaging.

The comparison of the classification made with the Type1 and Type2 datasets and the obtained confusion matrix results from Figure 6a and 6b in terms of economic value for farmers and commercial enterprises can be seen in Table 2. There is not a specific ratio in seed sorting, but it is expected that the percentage of foreign materials should be less than that of seeds. For example, as a result of sorting a 100 kg product, the amount of foreign materials removed can reach up to 1 kg. If in sorting application Type1 dataset is being used to train and test (in real time), according to the Figure 6a the false seed percentage is calculated as: 1 / 245 * 100= 0.40. That means 0.40% of the 99 kg sorted seeds were misclassified. That means 0.39 kg seeds misclassified as foreign materials. Again using Type1 dataset, from Figure 6a the false foreign materials percentage calculated as: 6 / 245 * 100= 2.44. That means 2.44% of the 1 kg sorted foreign materials were misclassified. That means 0.02 kg foreign materials misclassified as seeds. In this way, for Type1 dataset sorting 100 kg products the total misclassification is calculated as: 0.39 kg + 0.02 kg= 0.41 kg. Using the same calculations for Type2 dataset the misclassification is calculated as 2.44 kg. Although the effect of Type1 and Type2 datasets on classification is 98.57% - 97.34%= 1.23%, it can be said that the effect in practice is 5.9 times (2.44 / 0.41= 5.9) according to Table 2. Considering this information, the wrong classification of buckwheat products as foreign materials constitutes a serious loss for farmers and commercial enterprises.

Table 2. Economic effect of wrong classification in practice

Dataset Type	Seed in kg	Foreign Materials in kg	False Seed Rate	False Foreign Materials Rate	Wrong Classification of Seeds in kg	Wrong Classification of Foreign Materials in kg	Total Wrong Classification in kg
Type 1	99	1	0.40%	2.44%	0.39	0.02	0.41
Type 2	99	1	2.44%	3.26%	2.41	0.03	2.44

4. Discussion

In this study, the effect of the datasets generation methods used in the sorting processes on the test accuracy was investigated and datasets with different scaling methods have been proposed to develop high-accuracy datasets. The classification of buckwheat seeds and foreign materials using deep learning techniques was chosen as the sorting problem. AlexNet architecture was used for the classification process. Two different data types, Type1 and Type2, which were generated with different scaling methods, were used to train the AlexNet architecture. As a result of training the AlexNet structure with these datasets, the test accuracies for Type1 and Type2 datasets were calculated as 98.57% and 97.34%, respectively. It is concluded that Type1 dataset has a better test accuracy than Type2 dataset. More importantly, when Type1 dataset is used, the network structure classifies buckwheat seeds data with a higher accuracy. The effect of scaling techniques were also investigated for the economic effects of industrial sorting applications. If these scaling methods are used in industrial applications, Type1 dataset will make it possible to carry out more efficient sorting processes.

Seed sorting is an application that requires high speed and accuracy. With the use of deep learning in many classification problems, it is expected that deep learning-based sorting machines will be used more widely in the future. Since the results obtained in this study will be applicable in industrial sorting machines in the future, the aim is to realize higher efficiency sorting processes. In future studies, the aim is to develop sorting systems that can operate in real time at high speeds and accuracy by using these datasets. In order to make the system applicable in real-time, the aim is to develop optimized network structures with low processing load and high accuracy. In future studies, the aim is also to use these scaling techniques for different datasets and compare the classification results.

Acknowledgements

This research was supported by The Scientific and Technological Research Council of Turkey (TUBITAK) (grant number BIDEB/2211-C/1649B031900774) and also supported by Akdeniz University BAP Coordinate (grant number FDK-2019-4879). This study was produced from a doctoral thesis.

References

Aktaş H (2020) Development of Optimized Network Architectures for High Speed Industrial Applications Using Deep Learning, PhD Thesis, Akdeniz University, Antalya.

Aktaş H (2022) Antep fıstığının derin öğrenme ile dış kabuk rengine göre sınıflandırılması. Niğde Ömer Halisdemir Üniversitesi Mühendislik Bilimleri Dergisi 11(3): 461-466. doi: 10.28948/ngumuh.1064522.

Devaraj A, Rathan K, Jaahnavi S, Indira K (2019) Identification of plant disease using image processing technique. In: Proceedings of the IEEE International Conference on Communication and Signal Processing. ICCSP, 749-753. doi: 10.1109/ICCSP.2019.8698056.

Dewi T, Mulya Z, Risma P, Oktarina Y (2021) BLOB analysis of an automatic vision guided system for a fruit picking and placing robot. International Journal of Computational Vision and Robotics 11(3), 315-327. doi: 10.1504/IJCVR.2021.115161.

He K, Zhang X, Ren S, Sun J (2016) Deep residual learning for image recognition. In: Proceedings of the IEEE Computer Society Conference on Computer Vision and Pattern Recognition. CVPR, Las Vegas, NV, USA, pp. 770-778. doi: 10.1109/CVPR.2016.90.

Howard AG, Zhu M, Chen B, Kalenichenko D, Wang W, Weyand T, Andreetto M, Adam H (2017) Mobilenets: Efficient convolutional neural networks for mobile vision applications. arXiv preprint arXiv:1704.04861. doi: 10.48550/arXiv.1704.04861.

Huang KY, Cheng JF (2017) A novel auto-sorting system for Chinese cabbage seeds. Sensors 17(4): 886. doi: 10.3390/s17040886.

Huang S, Fan X, Sun L, Shen Y, Suo X (2019) Research on Classification Method of Maize Seed Defect Based on Machine Vision. Journal of Sensors 2019: 1-9. doi: 10.1155/2019/2716975.

Islam KT, Raj RG (2017) Real-time (Vision-based) road sign recognition using an artificial neural network. Sensors 17.4: 853. doi: 10.3390/s17040853.

Kamilaris A, Prenafeta-Boldú FX (2018) Deep learning in agriculture: A survey. Computers and Electronics in Agriculture 147: 70-90. doi: 10.1016/j.compag.2018.02.016.

Khan A, Sohail A, Zahoora U, Qureshi AS (2020) A survey of the recent architectures of deep convolutional neural networks. Artificial Intelligence Review 53(8): 5455-5516. doi: 10.1007/s10462-020-09825-6.

Khirade SD, Patil AB (2015) Plant disease detection using image processing. In: 1st International Conference on Computing, Communication, Control and Automation. ICCUBEA, Pune, India, pp. 768-771. doi: 10.1109/ICCUBEA.2015.153.

Kour VP, Arora S (2019) Fruit Disease Detection Using Rule-Based Classification. Advances in Intelligent Systems and Computing 851: 295-312. doi: 10.1007/978-981-13-2414-7_28.

Krizhevsky A, Sutskever I, Hinton GE (2012) ImageNet classification with deep convolutional neural networks. Advances in Neural Information Processing Systems 25: 1097-1105.

Kurtulmus F, Aliba İ, Kavdir I (2016) Classification of pepper seeds using machine vision based on neural network. International Journal of Agricultural and Biological Engineering 9.1: 51-62. doi: 10.3965/ijabe.v9i1.1790.

Kurtulmus F (2021) Identification of sunflower seeds with deep convolutional neural networks. Journal of Food Measurement and Characterization 15.2: 1024-033. doi: 10.1007/s11694-020-00707-7.

Li H, Li J, Guan X, Liang B, Lai Y, Luo X (2019) Research on Overfitting of Deep Learning. In: 15th International Conference on Computational Intelligence and Security (CIS) Macao, SAR, China, pp. 78-81. doi: 10.1109/CIS.2019.00025.

Nasiri A, Omid M, Taheri-Garavand A (2020) An automatic sorting system for unwashed eggs using deep learning. Journal of Food Engineering 283: 110036. doi: 10.1016/j.jfoodeng.2020.110036.

Omar N, Sengur A, Al-Ali SGS (2020) Cascaded deep learning-based efficient approach for license plate detection and recognition. Expert Systems with Applications 149: 113280 doi: 10.1016/j.eswa.2020.113280.

Sharma N, Jain V, Mishra A (2018) An Analysis of Convolutional Neural Networks for Image Classification. Procedia Computer Science 132: 377-384. doi: 10.1016/j.procs.2018.05.198.

Simonyan K, Zisserman A (2015) Very deep convolutional networks for large-scale image recognition. 3rd International Conference on Learning Representations. ICLR2015, San Diego, CA, USA.

Sun X, Wu P, Hoi SCH (2018) Face detection using deep learning: An improved faster RCNN approach. Neurocomputing 299: 42-50. doi: 10.1016/j.neucom.2018.03.030.

Szegedy C, Liu W, Jia Y, Sermanet P, Reed S, Anguelov D, Erhan D, Vanhoucke V, Rabinovich A (2015) Going deeper with convolutions. In: Proceedings of the IEEE Computer Society Conference on Computer Vision and Pattern Recognition. CVPR, Boston, MA, USA, pp. 1-9. doi: 10.1109/CVPR.2015.7298594.

Ter Haak M (2018) Machine learning for blob detection in high-resolution 3D microscopy images. Master thesis, KTH Royal Institute of Technology, Stockholm. doi: 10.13140/RG.2.2.10635.75045.

Unal Z (2020) Smart Farming Becomes even Smarter with Deep Learning - A Bibliographical Analysis. IEEE Access 8.105587–105609. doi: 10.1109/ACCESS.2020.3000175.

Veeramani B, Raymond JW, Chanda P (2018) DeepSort: Deep convolutional networks for sorting haploid maize seeds. BMC Bioinformatics 19: 1-9. doi: 10.1186/s12859-018-2267-2.

Yusuf MD, Kusumanto R, Oktarina Y, Dewi T, Risma P (2018) BLOB Analysis for Fruit Recognition and Detection. Computer Engineering and Applications Journal, 7.1: 23-32. doi: 10.18495/comengapp.v7i1.237.

Zoughi T, Homayounpour MM, Deypir M (2020) Adaptive windows multiple deep residual networks for speech recognition. Expert Systems with Applications 139: 112840. doi: 10.1016/j.eswa.2019.112840.

Research Article

www.dergipark.org.tr/en/pub/mediterranean

MEDITERRANEAN AGRICULTURAL SCIENCES
(2023) 36(3): 143-146
DOI: 10.29136/mediterranean.1338041

Races of stripe rust (*Puccinia striiformis* f. sp. *tritici*) identified in Central Anatolia

Emine Burcu TURGAY, Merve Nur ERTAS OZ, Sibel BULBUL

Field Crops Central Research Institute, Ankara, Türkiye

Corresponding author: E. B. Turgay, e-mail: cercospora79@gmail.com
Author(s) e-mail: m.nur.ertas@gmail.com, sibel.bulbul.391@gmail.com

ARTICLE INFO

Received: August 4, 2023
Received in revised form: November 3, 2023
Accepted: November 6, 2023

***Keywords*:**

Stripe rust
Puccinia striiformis
Race analysis
Phenotyping

ABSTRACT

Wheat is one of the most important cereal groups, with large cultivation areas in Türkiye and around the world. Stripe rust **(***Puccinia striiformis* f. sp. *tritici***)** is one of the biotic factors limiting the yield and quality of wheat. It is significant to determine its race/races in a region in terms of breeding studies and suggestions on varieties of wheat for farmers. This study aims to detect the stripe rust races in Central Anatolia, Türkiye. Wheat leaves with stripe rust were collected in Ankara, Eskişehir, Kırkkale, Kırşehir, and Yozgat provinces in 2020. Urediniospores for each isolate were collected from leaves, and a set of differentials containing different resistance genes was used to determine the virulence of the isolates. Inoculation was done by spraying 7-days-old seedlings, and reactions were assessed. Results showed that isolates were effective on *Yr2, Yr6, Yr7, Yr8, Yr9, Yr10, Yr17, Yr24, Yr25, and Yr32* resistance genes in the entire region. Along with this phenotyping study, further studies on these isolates will be done genotypically.

1. Introduction

Wheat belongs to the cereal crops with the largest cultivation area worldwide due to its significance in human nutrition. Globally, it is predicted that 785.1 million tonnes of wheat will be produced by the end of 2023 (FAO 2023). The forecast for the wheat production in 2023 has increased considerably by 6.6 million tonnes, reaching 783.3 million tonnes in the world (FAO 2023). In addition to this, it is estimated that there are 7.2 million ha of wheat cultivation areas with a projected 19.5 million metric tonnes of production for 2023/24 in Türkiye (USDA 2023). However, there are several factors threatening wheat cultivation areas in terms of quality and yield, such as biotic and abiotic stresses. Abiotic stresses result from environmental factors whereas biotic factors, consist of diseases, pests, weeds, etc. Among the biotic stresses, stripe rust is a disease caused by *Puccinia striiformis*, limiting both the quality and the yield of wheat. The annual wheat yield loss is estimated at 5.5 million tonnes worldwide due to stripe rust (Beddow et al. 2015).

There are various approaches to controlling wheat rust diseases, and one of the most widely recommended is the cultivation of rust-resistant wheat varieties. To date, several resistance genes have been identified (Chen and Kang 2017). Seedling resistance is expressed through a single major gene; on the other hand, more than one minor gene controls adult plant resistance. Since seedling resistance is controlled by a single major gene, resistance can be overcome easier by pathogens evolving rapidly (Roelfs et al. 1992). Though adult plant resistance is accepted as more durable, it is more effective in high temperatures rather than lower temperatures (Chen 2013; Chen et al. 2021). Due to these disadvantages of seedling resistance and adult plant resistance, gene pyramiding, which combines several resistance genes, is the finest option for breeders (Chen et al. 2021). However, it is crucial to picture pathogen populations in related regions through annual surveys (Park et al. 2011). Information obtained from these surveys will shed light on breeding programmes for disease-resistant varieties.

In this study, a survey was conducted in Central Anatolia (Ankara, Eskişehir, Kırıkkale, Kırşehir, Yozgat provinces) in 2020. Stripe rust isolates wereisolated from leaf samples collected from diverse locations. Hence, this study investigates phenotypically the isolates via race analysis.

2. Materials and Methods

2.1. Plant materials

The differential sets consisted of the European and World stripe rust differential sets, Avocet S near-isogenic lines (NILs) and additional supplementary European wheat varieties with different stripe rust resistance gene(s) (de Vallavieille-Pope et al. 2012; Johnson 1992; Wellings 2007) (Table 1).

2.2. Stripe rust samples

A survey of stripe rust was conducted during the May 2020 cropping season in five important wheat-producing provinces (Ankara, Eskişehir, Kırkkale, Kırşehir, and Yozgat) and surrounding areas of the Central Anatolia Region of Türkiye. A total of 34 durum (*Triticum durum*) and bread wheat (*Triticum aestivum*) fields were randomly selected and assessed in the Central Anatolia Region of Türkiye. Stripe rust disease was detected in 15 of these fields.

Tablo 1. Wheat differential lines for the assessment of the virulence phenotype of isolates of *Puccinia striiformis f. sp. tritici*

Virulence inferred	Differential lines	Resistance genes
S	Morocco	*Susceptible check*
vAvR	Avocet 'R'	*A*
vAvs	Avocet 'S'	*As*
v1	Chinese 166	*Yr 1*
v2	Kalyansona	*Yr 2*
v3	Vilmorin 23	*Yr 3*
v4	Hybrid 46	*Yr 4*
v5	*Yr5*/6* Avocet 'S'	*Yr5, Yr18, AvS*
v6	*Yr6/6* Avocet 'S'*	*Yr6, AvS*
v7	*Yr7/6* Avocet 'S'*	*Yr7, AvS*
v8	*Yr8*/6* Avocet 'S'	*Yr 8*
v9	*Yr9/6* Avocet 'S'*	*Yr9*
v10	Moro	*Yr10*
v15	Yr15/6*Avocet 'S'	*Yr15, Yr18, AvS*
v17	*Yr17*/6*Avocet 'S'	*Yr17, Avs*
v24	*Yr24*/6*Avocet 'S'	*Yr24, AvS*
v25	TP 981	*Yr25, +*
v27	OPATA 85	*Yr27, Yr18, +*
v32	Carstens V	*Yr32, Yr25, +*
vSp	*YrSP*/6*Avocet 'S'	*YrSp, YrAvS*
vSu	Suwon 92/ Omar	*YrSu*
vND	Nord Desprez	*YrND*
vSD	Strubes Dickkopf	*YrSD*
vTre	Tres/6* Avocet 'S'	*YrTre*
vCham	Cham 1	*YrCh1*

2.3. Recovery of the stripe rust urediniospores

Collected leaf samples were washed under tap water and then placed on a double layer of wet filter paper in a petri dish, followed by incubation for 2-3 hours at room temperature. After that, the 10-day seedlings of the susceptible cultivar "Morocco" were then rub inoculated using those samples (Roelfs et al. 1992). After 14-15 days, the stripe rust urediniospores that developed in the susceptible cultivar "Morocco" were collected, and these isolates were stored at – 80°C until used.

2.4. Race Analysis

Six seeds of each genotypes of the stripe rust differential set (Tablo1) were sown individually into a plastic pot (7X7X9 cm) containing a ready-to-use sterilised peat moss and were grown under greenhouse conditions at 18-20°C for 16 hours light and 8 hours of darkness. Stored urediniospores of the stripe rust isolates were suspended in mineral oil (Soltrol 170®) and then inoculated into seedling-stage genotypes (Zadoks 11-12). Following the inoculation process, all testing materials were placed in a dew chamber at 9±1°C for 24 hours for incubation and then transferred into a greenhouse with a temperature of 15±2°C. After 15-17 days of inoculation, seedlings were evaluated using a 0-9 scale described by McNeal et al. 1971. Genotypes with infection types (IT) between 0-6 were considered as low infection types and indicated an absence of virulence, while those with 7-9 were considered high infection types and indicated the presence of virulence (McNeal et al. 1971). Symbols designate virulence and avirulence (-), corresponding to stripe rust resistance genes.

3. Results and Discussion

A total of 34 wheat fields were examined, and 15 samples of stripe rust were obtained during the survey. Eight out of 15 stripe rust samples were recovered, and the urediniospores of the samples were multiplied for race identification. Collected stripe rust samples from Kırşehir and Kırıkkale did not recover and did not multiply. For this reason, isolates from these provinces were not obtained. The identified races of eight samples are shown in Table 2.

The core set of the stripe rust differential set, which consisted of 25 differential genotypes carrying *YrAs*, *YrA*, *Yr1*, *Yr2*, *Yr3*, *Yr4*, *Yr5*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr10*, *Yr15*, *Yr17*, *Yr24*, *Yr25*, *Yr27*, *Yr32*, *YrSP*, *YrSu*, *YrND*, *YrSD*, and *YrTre* were used to determine the race identification.

Three obtained stripe rust isolates from Yozgat (ID: 1, 3, and 4) were found to be resistant on *Yr2, Yr 6, Yr 7, Yr 8, Yr 17, Yr 25, YrSu*, and *YrND* resistance genes. Only one isolate from Yozgat (ID: 2) was also effective on the *Yr24* resistance gene, in addition to other genes (Table 2). Both races from Eskişehir had virulence on *Yr2, Yr6, Yr7* and *Yr8* resistance genes. In addition to these genes, isolates 5 and 6 were effective on *Yr9* and *Yr17*, respectively. Only the isolate from Beylikova had a wider virulence phenotype profile, with *Yr10*, *Yr17*, *Yr24*, and *Yr32* resistance genes susceptible to this isolate. One isolate was obtained from Ankara; Haymana had more virulence than all other isolates, and *Yr1, Yr 2, Yr 3, Yr 4, Yr 6, Yr 7, Yr 9, Yr 17, Yr 25* and *Yr 32* resistance genes were found to be susceptible to this isolate.

Table 2. Virulence phenotypes of stripe rust races identified in Central Anatolia region

ID	Province/district	Virulence phenotype
1	Yozgat /Sekili	AvS,A,-,2,-,-,-,6,7,8,-,-,-,17,-,25,-,-,-,YrSu,YrND,-,-,-
2	Yozgat/Sekili	AvS, A,-,2,-,-,-,6,7,8,-,-,-,17,24,25,-,-,-, YrSu,YrND,-,-,
3	Yozgat/Yerköy	AvS, A,-,2,-,-,-,6,7,8,-,-,-,17,-,25,-,-,-,-, YrSu,YrND,-,-,-
4	Yozgat/ Yerköy	AvS, A,-,2,-,-,-,6,7,8,-,-,-,17,-,25,-,-,-,YrSP,YrSu,YrND,-,-,-
5	Eskişehir/Sivrihisar	AvS, A,-,2,-,-,-,6,7,8,9,-,-,-,-,25,-,-,-,YrSu,YrND,-,-,
6	Eskişehir/Sivrihisar	AvS, A,-,2,-,-,-,6,7,8,-,-,-,17,-,25,-,-,-,YrSu,YrND,-,-,-
7	Eskişehir/Beylikova	AvS ,A,-,2,-,-,-,6,7,8,-,10,-,17,24,-,-,32,-,YrSP,YrSu,YrND,YSD,-,-
8	Ankara/Haymana	AvS, A,1,2,3,4,-,6,7,-,9,-,-,17,-,25,-,32, YrSP,YrSu,YrND, YrTre,-

4. Conclusion

The first record ofstripe rust was made in 1886, afterwards, some epidemics were recorded in Central Anatolia and West Anatolia between 1936 and 1963 (Braun and Saari 1992; İren 1964). Especially, the race coming from East Africa to Türkiye overcomes the *Yr9* resistance gene in the variety called Seri-82, which was cultivated widely in the Çukurova region of Türkiye. The anticipated expense of the damage caused by the stripe rust epidemic in Cukurova is $7 million (Braun and Saari 1992; Mamluk et al. 1997).

In 1998, it was stated that the wheat-growing area in Central Anatolia, which has a cold and humid environment, incurred losses ranging from 26.5% to 50% as a result of the yellow rust epidemic.

The stripe rust study has been conducted for over 20 years. According to the outcomes of this project between 1995 and 2009, stripe rust has been detected as virulent on *Yr2*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, and *Yr25* resistance genes (Çat et al. 2017). In 2010, a new race acquired virulence to the *Yr27* resistance gene (Hovmøller 2012), and it has been projected that the loss in wheat production due to stripe rust was 10 million dollars in the same year (Beddow et al. 2015).A new race known as the *Warrior* was identified and was thought to have originated from the northwest region of the country (virulent on *Yr1*, *Yr2*, *Yr3*, *Yr4*, *Yr6*, *Yr7*, *Yr9*, *Yr17*, *Yr25*, *Yr32*, and *YrSp*) (Mert et al. 2016). For the last four years, PstS11, PstS13, and PstS14 race groups have been detected (Hovmøller et al. 2020), as well as races such as *Yr10* and *Yr24* virulent pathogens (Hovmøller et al. 2020).

Türkiye has one of the largest wheat cultivation areas in Central Anatolia. The varieties widely grown in this area have been found to be mostly susceptible to stripe rust, especially *Warrior* race, according to the seedling and adult plant resistance tests done under artificial inoculations (Mert et al. 2016). Utilisation of the resistance genes in new wheat varieties developed by breeding programmes plays a very important role in decreasing the spread of rusts in wheat-growing areas (Ellis et al. 2014). Therefore, cultivation of susceptible varieties in the region might enable the dispersion of rust /races.

In addition to host susceptibility, climatic changes may promote the emergence of new races of *Puccinia striiformis*. Some wheat rusts have genetic mechanisms that adapt to the changing climate (Ali et al. 2014), and geographic distributions related to global warming may play a significant role in these mechanisms. For example, two races of *P. striiformis* that are thought to have originated from the Middle East or East Africa and spread to North America, Europe, and Australia are highly adapted to temperature variations (Ali et al. 2014). It is projected that this disease will increase by 5-20% in Southern Spain and Italy by 2030 (Bregaglio et al. 2013). These adaptations of wheat pathogens to climate change are related to genetic differentiations (Zhan and McDonal 2011) rather than phenotypic plasticity (i.e., the ability of genotypes to produce phenotypes under various environmental conditions) (Fusco and Minelli 2010). Climatic changes are favoured by airborne and polycyclic pathogens such as rust or Septoria (i.e., pathogens that have multiple infection cycles in a season (West et al. 2012) because they help them spread and complete their life cycles in a shorter time, resulting in large populations. Due to these large populations, the interaction between individuals is likely to increase, which may enable them to evolve rapidly. Consequently, new races or race groups will appear (Chakraborty 2013; Fones et al. 2017).

Türkiye has one of the largest wheat cultivation areas in Central Anatolia. It is significant to identify the rust races in that region since stripe rust is the most common and devastating disease in the area. It is thought that these outcomes will help to enlighten the breeding programmes and also provide guidance for the wheat producers in Central Anatolia.

Acknowledgement

This study was conducted under the project TAGEM/TBAD/20/A12/P01/002.

References

Ali S, Gladieux P, Leconte M, Gautier A, Justesen AF, Hovmøller MS, Enjalbert J, de Vallavieille-Pope C (2014) Origin, Migration Routes and Worldwide Population Genetic Structure of the Wheat Yellow Rust Pathogen *Puccinia striiformis* f.sp. *tritic*i. PLoS Pathogens 10(1).

Beddow JM, Pardey PG, Chai Y, Hurley TM, Kriticos DJ, Braun HJ, Park RF, Cuddy WS, Yonow T (2015) Research investment implications of shifts in the global geography of wheat stripe rust. Nature Plants 1(10): 15132.

Braun H, Saari E (1992) An assesment of the potential of *Puccinia striiformis* f.sp. *tritici* to cause yield losses in wheat on the Anatolian Plateau of Turkey. Vortrage Pflanzenzucht (24): 121-123.

Bregaglio S, Donatelli M, Confalonieri R (2013) Fungal infections of rice, wheat, and grape in Europe in 2030–2050. Agronomy for Sustainable Development 33(4): 767-776.

Chakraborty S (2013) Migrate or evolve: options for plant pathogens under climate change. Global Change Biology 19(7): 1985-2000.

Chen X (2013) Review Article: High-Temperature Adult-Plant Resistance, Key for Sustainable Control of Stripe Rust. American Journal of Plant Sciences 04(03): 608-627.

Chen X, Kang Z (2017) History of research, symptoms, taxonomy of the pathogen, host range, distiribution, and impact of stipe rust. Chen X, Kang Z (Ed.) Stripe Rust, Dordrecht: Springer Netherlands : Imprint: Springer. doi: doi.org/10.1007/978-94-024-1111-9_5.

Chen X, Wang M, Wan A, Bai Q, Li M, López PF, Maccaferri M, Mastrangelo AM, Barnes CW, Cruz DFC, Tenuta AU, Esmail SM, Abdelrhim AS (2021) Virulence characterization of *Puccinia striiformis* f. sp. *tritici* collections from six countries in 2013 to 2020. Canadian Journal of Plant Pathology 43(2): 308-322.

Çat A,Tekin M,Çatal M,Akan K, Akar T (2017) Buğdayda sarı pas hastalığı ve dayanıklılık ıslahı çalışmaları. Mediterranean Agrıcultural Sciences 30(2): 97-105.

de Vallavieille-Pope C, Ali S, Leconte M, Enjalbert J, Delos M, Rouzet J (2012) Virulence dynamics and regional structuring of Puccinia striiformis f. sp. tritici in France between 1984 and 2009. Plant Disease 96(1): 131-140.

Ellis JG, Lagudah ES, Spielmeyer W, Dodds PN (2014) The past, present and future of breeding rust resistant wheat. Frontiers in Plant Science 5: 1-13.

FAO (Food and Agriculture Organization) (2023) Cereal Supply and Demand Brief | World Food Situation | Food and Agriculture Organization of the United Nations. [online] Available at: https://www.fao.org/worldfoodsituation/csdb/en/. Accessed 28 Jul. 2023.

Fones HN, Fisher MC, Gurr SJ (2017) Emerging Fungal Threats to Plants and Animals Challenge Agriculture and Ecosystem Resilience. Microbiology Spectrum 5(2): 1-23.

Fusco G, Minelli A (2010) Phenotypic plasticity in development and evolution: facts and concepts. Philosophical Transactions of the Royal Society B Biological Sciences 365(1540): 547-556.

Hovmøller MS (2012) Report on Puccinia striiformis race analyses 2011, Slagelse, Denmark, Global Rust Reference Center (GRRC), Aarhus University.

Hovmøller MS, Patpour M, Rodriguez-Algaba J, Thach T, Fejer Justesen A, Hansen J G (2020) GRRC annual report 2019: Stem- and yellow rust genotyping and race analyses, GRRC, Aarhus University.

İren S (1964) Türkiye’de 1963 yılı hububat pas türleri ve zarar ve yayılışları üzerinde araştırmalar. Bitki Koruma Bülteni 4: 141-159.

Johnson R (1992) Reflections of a plant pathologist on breeding for disease resistance, with emphasis on yellow rust and eyespot of wheat. Plant Pathology 41(3): 239-254.

Mamluk O, Çetin L, Braun H, Bolat N, Bertschinger L, Makkouk K, Yıldırım A, Saari E, Zencirci, N, Albustan S, Cali S, Beniwal S, Düşünceli F (1997) Current status of wheat and barley diseases in the Central Anatolian Plateau of Turkey. Phytopathology (36): 167-181.

McNeal FH, Konzak CF, Smith EP, Tate WS, Russell TS (1971) A Uniform System for Recording and Processing Cereal Research Data. Washington, DC: USDA-ARS Bull. pp. 34121.

Mert Z, Nazari K, Karagöz E, Akan K, Öztürk İ, Tülek A (2016) First Incursion of the Warrior Race of Wheat Stripe Rust (*Puccinia striiformis* f. sp. *tritici*) to Turkey in 2014. Plant Disease 100(2): 528-528.

Park R, Fetch T, Hodson D, Jin Y, Nazari K, Prashar M, Pretorius Z (2011) International surveillance of wheat rust pathogens: progress and challenges. Euphytica 179(1): 109-117.

Roelfs A, Singh R, Saari E (1992) Rust diseases of wheat: concepts and methods of disease management. Mexico, CIMMYT.

USDA (United States Department of Agriculture) (2023) Turkey Wheat Area, Yield and Production. [online] Available at: https://ipad.fas.usda.gov/countrysummary/Default.aspx?id=TU&crop=Wheat. Accessed 28 Jul. 2023.

Wellings CR (2007) Puccinia striiformis in Australia: a review of the incursion, evolution, and adaptation of stripe rust in the period 1979 - 2006. Australian Journal of Agricultural Research 58(6): 567-575.

West JS, Townsend JA, Stevens M, Fitt BDL (2012) Comparative biology of different plant pathogens to estimate effects of climate change on crop diseases in Europe. European Journal of Plant Pathology 133(1): 315-331.

Zhan J, McDonal BA (2011) Thermal adaptation in the fungal pathogen Mycosphaerella graminicola. Molecular Ecology 20(8): 1689-1701.

Research Article

www.dergipark.org.tr/en/pub/mediterranean

MEDITERRANEAN AGRICULTURAL SCIENCES
(2023) 36(3): 147-152
DOI: 10.29136/mediterranean.1325684

Evaluation of sewage sludge effects on soil fertility and silage maize growth

Aylin ZAMBAK OZGUR[1], Sule ORMAN[1], Huseyin OK[2], Ismail Emrah TAVALI[1]

[1]Department of Soil Science and Plant Nutrition, Faculty of Agriculture, Akdeniz University, Antalya, Türkiye
[2]Department of Plant Production and Technologies, Faculty of Agricultural Sciences and Technologies, Cyprus International University, 99258 Nicosia, Türkiye

Corresponding author: S. Orman, e-mail: suleorman@akdeniz.edu.tr
Author(s) e-mail: aylin__z@hotmail.com, hok@ciu.edu.tr, etavali@akdeniz.edu.tr

ARTICLE INFO

Received: July 11, 2023
Received in revised form: September 7, 2023
Accepted: September 8, 2023

Keywords:

Heavy metals
Organic waste
Soil quality
Sustainability

ABSTRACT

Different methods are being tried all over the world for the safe disposal of sewage sludge. One of these methods is to evaluate the waste in plant production by applying it to the soil. In this study, the potential of using stabilized and dried sewage sludge in silage maize cultivation was investigated. Silage maize was grown by applying the sewage sludge to the soil at different rates [0 t ha^{-1} (SS0-Control), 20 t ha^{-1} (SS2), 40 t ha^{-1} (SS4), 60 t ha^{-1} (SS6), and 80 t ha^{-1} (SS8)]. Thus, the material's effects on soil fertility and plant growth were examined. According to the results, the sewage sludge enriched the soil, which contains restrictive factors in terms of fertility and on which maize growth is carried out, with humus and nutrients. However, it was observed that the material does not cause salinity problems in the soil. It was determined that sewage sludge positively affected the growth of silage maize but could not provide sufficient nutrition for the plant in terms of macronutrients. Considering the economic benefits and environmental risks, applying the sewage sludge with a maximum of 60 t ha^{-1} in silage maize growth is appropriate. However, it would be incredibly beneficial to support the material with chemical fertilizers to increase the silage yield and quality of the plant.

1. Introduction

Sewage sludge is an excellent source of nitrogen and phosphorus, and when applied to the soil, it increases the content of mineral nutrients and positively affects soil fertility (Gasco and Lobo 2007). One of the main factors limiting the use of sewage sludge in plant production is heavy metal content (Sims and Kline 1991). Although heavy metals are generally toxic to some extent, they tend to accumulate cumulatively in the food chain (Zhang and Ke 2004). In addition, the reaction of the soil (pH) where the sewage sludge is applied is another limiting factor. As a matter of fact, it was determined that sewage sludge applied to low pH soils increases heavy metal adsorption in the soil (Sauvé et al. 1997).

Silage maize (*Zea mays* L.) is an indispensable input for animal production, as it provides quality and low cultivation costs in addition to rations. The maize planting area for silage produced in Türkiye is approximately 420 thousand hectares. Total maize cultivation for silage is 20.1 million tons (Tezel 2018). However, silage maize removes many plant nutrients, especially nitrogen, from the soil where it is produced. Plant nutrients removed from the soil by plant cultivation and lost or turned into an unavailable form because of other factors, must be returned to the soil (Sağlam et al. 1993). Maintaining productivity and quality in plant cultivation is only possible by improving the physical, chemical and biological properties of the soil, in other words, by preserving soil fertility. For this reason, the use of organic wastes in agricultural production has become more and more common in recent years. Among organic wastes, especially stabilized and dried sewage sludge is an organic material/soil conditioner with strong potential due to its properties. In this study, the effects of sewage sludge amendments on soil fertility and silage maize growth were investigated.

2. Material and Methods

The experiment was carried out at Akdeniz University Faculty of Agriculture Research and Application Farm. The stabilized and dried sewage sludge was obtained from the Hurma Wastewater Treatment Plant in Antalya, Türkiye. In this context, the sewage sludge and test soil properties are given in Table 1. In the experiment area, soil tillage was carried out first, and the physical conditions were suitable for vegetative production. 6 m^2 (1.5x4) plots were created following the experiment plan. The sewage sludge was weighed by the application rates [0 t ha^{-1} (SSO)-(Control), 20 t ha^{-1} (SS2), 40 t ha^{-1} (SS4), 60 t ha^{-1} (SS6), and 80 t ha^{-1} (SS8)] and mixed homogeneously with the soil in the plots. A drip irrigation system was installed on the plots, ensuring that each plot was irrigated equally during the experiment.

The sewage sludge applied to the soil was incubated for 3 weeks to begin mineralization with the effect of water and other factors (temperature, pH, microorganisms etc.). At the end of this period, the first soil sampling was performed from a 0-30 cm depth of each plot. Later, the seeds of the silage maize (Burak cv) were planted in plots (two for each drip). On the 10^{th} day following the planting date, the observation was made of two maize plants per drop in each plot, and the dilution process was carried out by leaving the homogeneously growing ones in the plots. The second soil sampling was done when the plant cob tassels had completed their growth. Silage maize, which completed its vegetation period, was harvested. At the same time, third soil samples were taken, and the experiment was concluded. In addition, chlorophyll measurements of leaves were made in field conditions ten weeks after sowing. Then, samples were taken from these leaves and measured, and other plant analyses were carried out under laboratory conditions. Silage maize plants that completed their vegetation period were harvested by cutting them from the root neck. Measurements and analysis were made for the whole shoots (excluding the cob) and the cob.

The analysis made to determine the characteristics of the sewage sludge are as follows: organic matter (Kacar 2009), pH and EC (Jackson 1967), total nitrogen (N) (Kacar and İnal 2008), total phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sodium (Na), iron (Fe), zinc (Zn), manganese (Mn), copper (Cu), lead (Pb), cadmium (Cd), chromium (Cr), nickel (Ni) (U.S. EPA 2007). The experiment soils were made ready for analysis by sifting through a 2 mm sieve after they were dried. The analysis performed are as follows: Soil texture (Bouyoucos 1955), lime (Çağlar 1949), pH and EC (Jackson 1967), soil organic matter (Black 1965), total N (Kacar 2009), available P (Olsen and Sommers 1982), exchangeable K, Ca, and Mg (Kacar 2009).

For the height of the first cob in the plant, the vertical distance between the node to which the cobs are attached and the soil surface was measured. For the number of cobs, the number of cobs in the plant selected from each plot was determined in units. For the cob/plant ratio, the cobs of the plant selected from each plot were separated from the stem and leaf and weighed, and the calculation was made in proportion to the weight of the whole shoot (excluding the cob). The whole shoot (excluding the cob) and the cob were calculated by weighing them separately for wet weight. Plant samples were dried at 65°C and weighed for dry matter yield. The values obtained were calculated as kg ha^{-1}. The amount of acid detergent fiber (ADF), neutral detergent fiber (NDF) and crude cellulose (CC) in the plants were determined according to Van Soest et al. (1991). The value of chlorophyll in the plant was measured with a chlorophyll meter (Minolta SPAD-502 plus) under field conditions. Chlorophyll-a and chlorophyll-b were determined using acetone extraction (Williams 1984). Total N, P, K, Ca, and Mg concentrations in leaves of plants were determined according to the wet digestion method (Kacar and İnal 2008). After determining the N content of the plant samples, the value obtained was calculated by multiplying the protein conversion coefficient of 6.25 for maize.

The significance levels of the obtained data were determined by analysis of variance using the MSTAT-C package program. In addition, the differences between the MINITAB package program and the means were determined in the LSD test, and the 5% significance level was taken as a basis for the letters of the different groups (Minitab 2010).

3. Results and Discussion

3.1. pH, EC, and organic matter

The change in pH values according to the sampling period was statistically significant (P<0.001). It was determined that the effect of the sewage sludge on the EC of the soil is significant at the level of 5% (Table 2). The highest EC value was measured in soils where SS6 (0.53 dS m^{-1}) and SS8 (0.57 dS m^{-1}) were applied. It is reported that the sewage sludge application causes an increase in the EC of the soil but does not reach salinity levels (Aldatmaz 2006). The data also supports that the sewage sludge application does not create any salinity risk. On the other hand, the change in EC values of the soil was found to be statistically significant according to the sampling period (P<0.001). Soil EC values decreased from the first sampling to the third. The sewage sludge takes longer to break down and mineralize in the soil than other organic substances due to the high heat it is exposed to during the drying phase. This process slows the passage of plant nutrients from the sewage sludge to the soil. Silage maize is a plant that absorbs high levels of nutrients. The results of the second and third sampling is thought to be affected by the

Table 1. Selected physico-chemical properties of the sewage sludge, experiment soil, and certificated materials

Parameter		Sewage sludge	Soil	Certificated Clay loam soil material (RTC CRM052)		Certificated Sewage sludge material (CRM029-50G)		Certificated Plant material (Peach Leaves) (CRM-1547)	
				Referance range	Value	Referance range	Value	Referance range	Value
pH		6.42	7.06	-	-	-	-	-	-
EC dS m^{-1}		5.44	0.44	-	-	-	-	-	-
$CaCO_3$ %		-	43	-	-	-	-	-	-
Texture		-	Clay loam	-	-	-	-	-	-
Organic Matter %		74	1.39	-	-	-	-	-	-
Tot.[1]	N %	4.9	0.13	3.97±0.562	3.42	3.26±0.56	4.58	2.94	3.09
	P mg kg^{-1}	16144	118	168±30.7	102.67	2.11±0.26	1.51	1371	1184
	K mg kg^{-1}	4484	8226	2390 ±76.2	1858.40	3540±290	3604	24330	24120
	Ca mg kg^{-1}	61400	53512	2860±118	1680.97	51600±1650	55620	15590	16744
	Mg mg kg^{-1}	6506	4267	1690±74.8	1593.10	10400±395	10428	4320	4326
Avai.[2]	P mg kg^{-1}	-	3.14	-	-	-	-	-	-
Exc.[3]	K mg kg^{-1}	-	250	-	-	-	-	-	-
	Ca mg kg^{-1}	-	410	-	-	-	-	-	-
	Mg mg kg^{-1}	-	210	-	-	-	-	-	-

[1]Total concentration, [2]Available concentration, [3]Exchangeable concentration.

absorption of ions that can accumulate in the soil and cause salinity by the plant. This result may explain the relatively higher EC value of the first sampling period. Göçmez (2006) observed an increase in soil EC with increasing rates of sewage sludge and found the highest EC value in the highest sewage sludge application. However, he reported that the increase in soil EC is far from changing the salinity level of the soil.

The effect of increasing rates of sewage sludge on the organic matter was found to be statistically significant at the level of 5% (Table 2). The sewage sludge increased the organic matter compared to the control. The highest organic matter value was determined in the soil treated with SS2 (2.77%). The sewage sludge used in the experiment has higher organic matter content (74%) than many organic fertilizers or soil conditioners. In addition, the fact that the experiment soil is poor in humus (1.39%) explains the increase in the sewage sludge compared to the control. The change in organic matter values according to the sampling period was also statistically significant (P<0.001). Although organic matter values determined in the second and third sampling periods were similar, it was determined that they increased compared to the first. One of the most important benefits of applying sewage sludge in soils where the organic matter content is low and decomposition is intense, due to the climate, is the improvement provided in the organic matter. A multi-year study by Angın (2008) shows that sewage sludge application supports organic matter for the following two years.

3.2. Total N, available P, and exchangeable K, Ca, Mg

The effect of the sewage sludge on the total N of the soil was found to be statistically significant at the level of 1% (Table 3). When the total N content of the soil was evaluated according to Loue (1968), it was at a reasonable level at the beginning, while 20 t ha^{-1} and more sewage sludge made it to an outstanding level (0.130%<). In addition, in the soil analysis before the experiment was established, the total N was determined as 0.130%, and the highest soil N value was determined in SS6 (0.21%) and SS8 (0.20%) of the sewage sludge. In this way, an increase of 61.5% was achieved in the N content of the soil compared to the beginning. It was reported that increasing sewage sludge rates regularly increased the soil's total N content (Navas et al. 1998). The change in the total N values according to the sampling period was statistically significant (P<0.001). While the N value of the soil was in the same statistical group in all application rates at the first and second sampling periods, it was determined as a different group and lower in the third sampling. This situation may have been caused by the increase in nutrient absorption during the growth of the maize. In addition, the N released by mineralization may have partially evaporated (due to high pH) and leached losses.

The effect of the sewage sludge on the available P content of the soil was found to be statistically significant at the 0.1% level, and the available P content of the soil increased in all other application rates compared to the control (Table 3). The available P of the soil sample taken before the experiment was determined as 3.14 mg kg^{-1}. The highest value was determined in SS6 (12.30 mg kg^{-1}), and an increase of 292% was achieved compared to the control. In addition, when evaluated according to Olsen and Sommers (1982), the soil available P content, which was in the low level (5 mg kg^{-1}>) with 3.14 mg kg^{-1}, had increased to the high level (10<) with 12.30 mg kg^{-1}. The change in the available P values of the soil according to the sampling period was significant (P<0.001). From the first sampling to the third, the available P value of the soil increased. It is thought that during the mineralization process of the sewage sludge, the release of P increases with time and that the P uptake by the maize takes place. The effect of sewage sludge application on the P content of the soil was evaluated among the most critical agricultural benefits of the material (Schowanek et al. 2004).

Table 2. Effects of sewage sludge on soil pH, EC, and organic matter

Application	pH	EC (dS m^{-1})	Organic matter (%)
SS0	7.36	0.33c[2]	2.24d
SS2	7.35	0.41b	2.77a
SS4	7.33	0.46b	2.45c
SS6	7.27	0.53a	2.65b
SS8	7.23	0.57a	2.66b
ANOVA			
Sewage Sludge (SS)	NS[5]	*[4]	*
Sampling period (SP)	***	***	***[3]

[1]Values are the means of three different soil sampling period, [2]Means in the same column followed by the same letter are not significantly different at the 5% level according to LSD test, [3]*** P<0.001, [4]* P<0.05, [5]NS: Not significant.

Table 3. Effects of sewage sludge on macronutrients of soil

Application	Total					Exchangeable			Available
	N %	P mg kg^{-1}	K mg kg^{-1}	Ca mg kg^{-1}	Mg mg kg^{-1}	K mg kg^{-1}	Ca mg kg^{-1}	Mg mg kg^{-1}	P mg kg^{-1}
SS0	0.13c[2]	115d	7724b	84102a	4540a	230ab	4500	230c	2.52d
SS2	0.17b	138c	8226a	87489a	4380b	270a	4800	260b	8.89c
SS4	0.18b	157b	7821b	63952c	4360b	200b	4500	280a	10.38b
SS6	0.21a	161a	8577a	60571c	4359b	230ab	4600	290a	12.30a
SS8	0.20a	156b	6847c	80141b	4213c	190c	4600	260b	10.04b
ANOVA									
Sewage Sludge (SS)	**[4]	***	***	***	***	***	NS[6]	***	***[3]
Sampling period (SP)	***	***	***	***	***	***	*[5]	***	***

[1]Values are the means of three different soil sampling period, [2] Means in the same column followed by the same letter are not significantly different at the 5% level according to LSD test, [3] *** P<0.001, [4]** P<0.01, [5] * P<0.05, [6] NS: Not significant.

It was determined that the effect of the sewage sludge on the exchangeable K content of the soil is statistically significant at the level of 0.1% (Table 3). Considering the differences between application rates, the highest exchangeable K value SS2 (0.027% - 270 mg kg^{-1}) was determined in the applied soil. When evaluated according to Pizer (1967), it was determined that the exchangeable K content of the control soil was 0.024% (230 mg kg^{-1}), and it was at a reasonable level (199-249 mg kg^{-1}). The change of K values of the soil according to the sampling period was found to be significant (P<0.001). Since the maize seed was planted at the end of the incubation period of the material in the soil, the second and third samplings are within the plant's growing period. Considering that the plant meets the K requirement in this process, the soil's K content is expected to decrease gradually. There was a 26.08% decrease in exchangeable K values from 270 mg kg^{-1} to 190 mg kg^{-1}. It was reported that the sewage sludges are poor in K compared to the significant amount of N and P they contain (Angın and Yağanoğlu 2009).

The sewage sludge on the exchangeable Ca content of the soil were statistically insignificant (Table 3). On the other hand, the change in Ca values in terms of sampling periods was significant (P<0.05). The effect of the sewage sludge on the exchangeable Mg content of the soil was found to be statistically significant at the 0.1% level (Table 3). Considering the Mg values between the application rates, the highest value was determined in the soils where SS4 (0.028%) and SS6 (0.029%) were applied. When evaluated according to Loue (1968), the exchangeable Mg content of the control soil is 0.023% (230 mg kg^{-1}), and it is at a reasonable level (114 mg kg^{-1}<). Similarly, it was determined that the sewage sludge brought the exchangeable Mg content of the soil to a reasonable level and provided increases ranging from 13.04% to 26.08%. The change in the Mg values of the soil was also found significant in terms of sampling period (P<0.001). The values decreased by 0.027% (272 mg kg^{-1}) in the first sampling, 0.026% (261 mg kg^{-1}) in the second, and 0.025% (252 mg kg^{-1}) in the third. Considering that the plant meets the Mg requirement in this process, it is usual to decrease the exchangeable Mg content of the soil. The content of exchangeable Mg has decreased by 7.56% from 272 mg kg^{-1} to 252 mg kg^{-1}.

3.3. Plant Growth and Yield

The analysis results of the sewage sludge regarding the first cob height, number of cobs, cob/plant ratio, wet weight, dry weight, and dry matter yield of silage maize are given in Table 4. The effect of the sewage sludge on the first cob height was found to be statistically significant at the level of 0.1%. As the applied sewage sludge rates increased, the height of the first cob increased. The highest value was obtained in SS8 with 153.2 cm. The effect of sewage sludge on the number of cobs was found to be statistically significant at 5%. In the SS6 application, the highest number of cobs occurred, with an average of 8. It was determined that the effect of sewage sludge on the cob/plant ratio was statistically 0.1%. While the highest cob/plant ratio is under control, these values decrease as the application rates increase. The effects of the sewage sludge on the plant's wet and dry weight values were found to be statistically significant at a 0.1% level. According to this, the wet weight in the whole shoots was determined as 3808 g in SS8.

When the wet and dry weights of the cob samples were examined, the values were higher in the control and SS2, while the wet and dry weights of the cob samples decreased as the application rates increased. The effect of the sewage sludge on the dry matter yield of whole shoots and cob samples was found to be statistically significant at the level of 0.1%. It was determined that the sewage sludge applied at increasing rates increased the dry matter yield in the whole shoots and decreased the dry matter yield in the cob samples. The highest dry matter yield was detected in SS8 with 19000 kg ha^{-1} in the whole shoot samples. In the cob samples, the highest yield was observed in SS2 with 3900 kg ha^{-1}. Hallauer and Miranda (1987) reported that the morphological characteristics of maize, plant height and first cob height mainly depend on genetic factors. In addition, factors such as the amount of light, intensity, and plant nutrients are influential on the first cob height and plant height. In rotations sensitive to maize, if the net assimilation rate of the plant slows down due to light and nutrients, the cob stalk is negatively affected by this situation (Uyanık 1984).

3.4. ADF, NDF, CC and Chlorophyll (a, b, SPAD value)

The effect of sewage sludge on ADF, NDF and CC values in whole shoots samples was found to be statistically insignificant (Table 5). The ADF values of the cob samples were found to be statistically significant at the 0.1% level. It was determined that the highest ADF value was 32.0% in SS6. The effect of the sewage sludge on the NDF values of the cob samples was found to be statistically significant at a 5% level. It is seen that the highest NDF value is 60.6% in SS6. The effect of the sewage sludge on the amount of CC of the cob samples was found to be statistically insignificant. Khan et al. (2015) reported that NDF and ADF contents of maize silages decreased as they progressed from early maturity to 2/3 milk maturity period but did not change from 2/3 milk to full maturity. It is reported that the water-soluble dry matter, NDF, ADF, and crude protein contents of the corn plant decreased while the dry matter and starch contents of the corn plant increased as the vegetation period progressed (Dwyer et al. 1998).

The effect of sewage sludge on chlorophyll-a, chlorophyll-b, and SPAD value of silage maize was statistically significant at the level of 0.1% (Table 5). Accordingly, in SS6 and SS8, the chlorophyll content of the plant doubled compared to the control and other application rates. It is known that there is an important relationship between the N, which is a critical element in the chlorophyll molecule, and the chlorophyll content. The chlorophyll content of plants with good N nutrition is also expected to be relatively high (Türkan 2008). The obtained chlorophyll results show that the N nutritional status of the plants in the SS6 and SS8 applied plots is better than the others. It is thought that even at these high rates, it will not cause stress in the plant because it is known that the chlorophyll content of plants decreases due to increasing stress conditions (Manios and Stentiford 2003).

3.5. Mineral nutrition status of plant

The effect of sewage sludge on both the total N concentration and protein amount of leaf samples was found to be statistically significant at a 0.1% level (Table 6). The protein and N content of the plant similarly increased due to sewage sludge. SS8 (2.22%) and SS6 (2.19%) had the highest total N values. The effect of the sewage sludge on P concentrations was found to be statistically significant at 0.1% level. It was determined that as the application rates increased, the P concentration also

Table 4. Effects of sewage sludge on growth and yield of silage maize

Application	First cob height (cm)	Number of cob	Cob/Plant ratio	Wet weight (g)		Dry weight (g)		Dry matter yield (kg ha^{-1})	
				Whole shoots	Cob	Whole shoots	Cob	Whole shoots	Cob
SS0	110.50c[1]	5b	0.30a	2270e	682b	1078e	353b	10780c	3500b
SS2	132.05b	6b	0.22b	3373d	718a	1625d	392a	16000b	3900a
SS4	138.75ab	6b	0.13c	3474c	486d	1628c	277c	16000b	2770c
SS6	146.80ab	8a	0.14c	3651b	500c	1666b	244d	16000b	2430d
SS8	153.20a	7ab	0.13c	3808a	482e	1906a	241e	19000a	2410e
ANOVA (LSD 5%)									
Sewage sludge (SS)	***[2]	*[3]	***	***	***	***	***	***	***

[1] Means in the same column followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test, [2] *** P<0.001, [3] * P<0.05.

Table 5. Effects of sewage sludge on acid detergent fiber (ADF), neutral detergent fiber (NDF), crude cellulose (CC), and chlorophyll of silage maize

Application	ADF (%)	NDF (%)	CC (%)	Chlorophyll		
				a (mg kg^{-1})	b (mg kg^{-1})	SPAD value
SS0	21.8c[1]	50.7b	18.6	6.630b	2.888b	30.025c
SS2	22.7bc	47.2b	19.8	5.801b	2.701b	38.095b
SS4	25.9b	53.7ab	22.2	6.990b	3.128b	42.495b
SS6	32.0a	60.6a	22.8	12.322a	4.550a	47.795a
SS8	25.7bc	47.5b	19.4	10.496a	4.414a	47.880a
ANOVA (LSD 5%)						
Sewage sludge (SS)	***[2]	*[3]	NS[4]	***	***	***

[1] Means in the same column followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test, [2] *** P<0.001, [3] * P<0.05, [4] NS: Not significant.

Table 6. Effects of sewage sludge on concentration of nutrient elements of silage maize

Application	Protein (%)	Total (%)				
		N	P	K	Ca	Mg
SS0	9.40d[1]	1.51c	0.17c	1.67b	0.71c	0.13c
SS2	11.85c	1.90b	0.19bc	1.92a	0.97ab	0.16bc
SS4	12.57bc	2.02b	0.20b	2.00a	0.98ab	0.20ab
SS6	13.06ab	2.19a	0.24a	1.92a	0.92b	0.21ab
SS8	13.89a	2.22a	0.26a	1.97a	1.02a	0.24a
ANOVA (LSD 5%)						
Sewage sludge (SS)	***	***	***[2]	**[3]	***	**

[1] Means in the same column followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test, [2] *** P<0.001, [3] ** P<0.01.

increased. The highest P values (0.26%) were determined in SS8 and (0.24%) SS6. The effects of the sewage sludge on K concentrations were found to be statistically significant at the level of 1%. The sewage sludge increased the K concentration of the leaves compared to the control, but all application doses were in the same group.

The effect of sewage sludge on Ca concentrations in leaf samples was found to be statistically significant at 0.1%. The highest Ca was determined with the value of 1.02% in SS8. The effects of the sewage sludge on Mg concentrations in leaf samples were found to be statistically significant at 1%. The Mg value, which was 0.13% in the control, increased as the application rates increased, and the highest concentration of 0.24% was determined in SS8. According to Yılmaz (2004), the normal limits for macronutrients in silage maize plants according to leaf analysis are as follows: N 3.50-5.0%, P 0.35-0.60%, K 3.0-4.50%, Ca 0.30-1.0%, Mg 0.25-0.50%. Accordingly, sewage sludge could not nourish the plant sufficiently regarding the nutrients (excluding Ca) examined. It is known that a plant with high vegetative and generative yield potential, such as silage maize, exploits high amounts of nutrients from the soil in a short time. Therefore, it is thought that the sewage sludge should be supplemented with chemical fertilizers.

4. Conclusion

As well as maintaining the fertility of the soil, it is imperative that the yield and quality of the plant grown on it is high. For this reason, the fertilizing materials (organic or chemical) must be in a suitable composition for both the soil and the plant. For this purpose, the effects of sewage sludge on soil fertility and plant growth in silage maize, a forage crop, were investigated. According to the results, all application rates of sewage sludge increased the soil fertility where maize was grown. While this material enriches the soil with humus and plant nutrients, it was determined that it does not cause salinity risk. However, it was determined that sewage sludge positively affected the growth of silage maize but could not provide sufficient nutrition for the plant in terms of macronutrients. As a result, considering the economic benefits and environmental risks, it is appropriate to apply sewage sludge as a soil conditioner at a maximum of 60 t ha^{-1} in silage maize cultivation. However, applying the sewage sludge and chemical fertilizers would significantly benefit the plant's silage yield and quality. In this way, soil fertility would be preserved, and there will be no decrease in plant yield and quality using sewage sludge. It is thought that it would be beneficial to continue extensive studies using this material to increase the reliability of the results obtained from existing scientific studies and eliminate the uncertainties regarding the use of sewage sludge in plant cultivation.

Acknowledgements

This study was financially supported by the Scientific Research Projects Coordination Unit of Akdeniz University (Project no. FYL-2017-2688).

References

Aldatmaz G (2006) Toprağa uygulanabilir nitelikteki arıtma çamurlarının topraktaki bazı enzim aktiviteleri üzerine etkileri. Yüksek lisans tezi, Uludağ Üniversitesi, Bursa, s. 108.

Angın İ (2008) Arıtma çamurlarının fiziksel ve kimyasal toprak düzenleyicisi olarak kullanımı. Doktora tezi, Atatürk Üniversitesi, Erzurum.

Angın İ, Yağanoğlu AV (2009) Arıtma çamurlarının fiziksel ve kimyasal toprak düzenleyicisi olarak kullanımı. Ekoloji 19(73): 39-47.

Black CA (1965) Methods of Soil Analysis. Part 2. AmericanSociety of Agronomy Inc., Publisher Madison, Wisconsin, USA.

Bouyoucos GJ (1955) A Recalibration of the Hydrometer Method for making mechanical analysis of the soils. Agronomy Journal 4(9): 434.

Çağlar KÖ (1949) Toprak Bilgisi. Ankara Üniversitesi Ziraat Fakültesi Yayınları, Sayı: 10.

Dwyer LM, Stewart DW, Glenn F (1998) Silage yields of leafy and normal hybrids. Com and Sorghum Conf. Am. Seed Trade. Assoc.Washington D.C.

Gasco G, Lobo MC (2007) Composition of a Spanish sewage sludge and effects on treated soil and olive trees. Waste Management 27(11): 1494-1500.

Göçmez S (2006) Menemen Ovası Topraklarında İzsu Kentsel Arıtma Çamuru Uygulamalarının Mikrobiyal Aktivite ve Biyomas ile Bazı Fiziksel ve Kimyasal Toprak Özellikleri Üzerine Etkisi. Doktora Tezi, Ege Üniversitesi Fen Bilimleri Enstitüsü, Toprak Anabilim Dalı, İzmir.

Hallauer AB, Miranda JB (1987) Quantitative Genetics in Maize Breeding. Lowa State Unıv. Press, Ames, Lowa, pp. 118-119.

Jackson ML (1967) Soil Chemical Analysis. Prentice Hall of India Private Limited, New Delhi, India.

Kacar B, İnal A (2008) Bitki Analizleri. Nobel Yayınları, Ankara, No: 1241.

Kacar B (2009) Toprak Analizleri. Nobel Yayın No: 1387, Fen Bilimleri: 90, Nobel Bilim ve Araştırma Merkezi Yayın No. 44 ISBN 978-605-395-184-1. s. 467.

Khan NA, Yu P, Ali M, Cone JW, Hendriks WH (2015) Nutritive value of maize silage in relation to dairy cow performance and milk quality. Journal of the Science of Food and Agriculture 95(2): 238-252.

Loue A (1968) DiagonisticPetiolaire de Prospection. Etudes Sur la Nutrition et al Fertilisation Potassiques de la Vigne. Societe Commerciale des Potasses d'Alsace Sevices Agronomiques pp. 31-41.

Manios T, Stentiford E (2003) The effect of heavy metals accumulation on the chlorophyll concentration of typha latifolia plants growing in a substrate containing sewage sludge compost and watered with metalliferous water. Ecological Engineering 20: 65-74.

Minitab (2010) Minitab Inc. MINITAB: Minitab for Windows Version Release 17. State College, PA, USA: Minitab Inc.

Navas A, Bermudez F, Machin J (1998) Influence of sewage sludge application on physical and chemical properties of Gypsisols. Geoderma 87: 123-135.

Olsen SR, Sommers LE (1982) Phosphorus. In: Page, A.L., Miller, R.H. and Keeney, D.R., Eds., Methods of Soil Analysis. Part 2: Chemical and Microbiological Properties, American Society of Agronomy, Inc., Madison, pp. 403-427.

Pizer NH (1967) Some advisory aspects of potassium and magnesium. Technology Bulletin 14: 184.

Sağlam MT, Bahtiyar M, Cangir C, Tok HH (1993) Toprak Bilimi. Trakya Üniversitesi Tekirdağ Ziraat Fakültesi Toprak Bölümü Ders Kitabı, Anadolu Matbaa Tic. Koll. Şti., Tekirdağ.

Sauvé S, McBride MB, Hendershot WH (1997) Copper solubility and speciation of in-situ contaminated soils: effect of copper level, pH and organic matter. Water, Air and Soil Pollution 100: 133-149.

Schowanek D, Carr R, David H, Douben P, Hall J, Kirchmann H, Patria H, Sequi P, Smith S, Webb S (2004) A risk-based methodology for deriving quality standards for organic contaminants in sewage sludge for use in agriculture conceptual framework. Regulatory Toxicology and Pharmacology 40: 227-251.

Sims JT, Kline JS (1991) Chemical fractionation and plant uptake of heavy metals in soil amended with co-composted sewage sludge. Journal of Environmental Quality 20: 387-395.

Tezel M (2018) Türkiye'de silajlık mısır üretimi ve hayvan beslemede yeri. TÜRKTOB Dergisi 25: 17-19.

Türkan İ (2008) Bitki Fizyolojisi, Palme Yayınları: 455, Palme Yayıncılık.

U.S. EPA (2007) Method 3051A (SW-846): Microwave Assisted Acid Digestion of Sediments, Sludges, and Oils. Revision 1. Washington, DC.

Uyanık M (1984) Mısır Bitkisinin Botanik Özellikleri. Karadeniz Bölge Zirai Araştırma Enstitüsü Yayınları No: 1984-1, Samsun.

Van Soest PJ, Robertson JB, Lewis BA (1991) Method for Dietary Fiber, Neutral Detergent Fiber, and Nostarch Polysaccharides in Relation to Animal Nutrition. Journal of Dairy Science 74: 3583-3597.

Williams S (1984) Official Methods of Analysis of the Association of Official Analytic Chemists. Published by the Association of Official Analytical Chemists. Inc. Wircini, 22.209, USA, pp. 59-60.

Yılmaz C (2004) Bitkisel Üretimde Besin Elementleri. Hasad Yayıncılık, ISBN 975-8377-36-1, İstanbul.

Zhang MK, Ke ZX (2004) Heavy metals, phosphorus and some other elements in urban soils of Hangzhou City, China. Pedosphere 14(2): 177-185.

Acknowledgement of reviewers

MEDITERRANEAN AGRICULTURAL SCIENCES thanks to reviewers listed below for their enormous contribution to the articles published in Volume 36.

Akan, Kadir
Akpinar, Aysegul
Aktaş, Ali Rıza
Aktaş, Hakan
Aktaş, Türkan
Aslan, Baran

Başer, İsmet
Bayaner, Ahmet
Bayindir Erol, Alime
Binici, Turan
Bozbuğa, Refik
Bölük, Gülden

Ceylan, Figen

Dağli, Fatih
Dellal, İlkay
Demirel, Onur
Dinçer, Cüneyt
Direk, Mithat
Dube, Ahmed Kasim

Ergin, Firuze
Erper, İsmail
Erşahin, Sabit

Gözlekçi, Şadiye
Gülmezoğlu, Nurdilek
Gültekin, Rohat
Güneş, Erdoğan
Gürbüz, Ramazan

Hatipoğlu, Rüştü

Iftikhar, Romana
Imran, Muhammed
İkten, Cengiz
İrget, Eşref

Kabaş, Önder
Karabıyık, Şenay
Kaşkavalci, Galip
Kılıç, Tülin
Kırıcı, Saliha
Kıyak, Ali
Korkmaz, Kürşat
Kök Taş, Tugba
Külcü, Recep

Mohanraj Kanagaraj,

Oluk, Celile Aylin

Özgönen Özkaya, Hülya
Özpinar, Sakine

Rajaram, Sangeetha

Sarıçoban, Kazım
Sogue, Babou
Solmaz, İlknur

Telci, İsa
Topakci, Mehmet
Turgut, Kenan

Ulukan, Hakan
Üstün, Hayri

Vursavuş, Kubilay Kazım

Yağdı, Köksal
Yıldırım, Ertan
Yılmaz, İbrahim
Yilmaz, Erdem
Yol, Engin

Volume content, Volume 36

***Number*: 1 (April 2023)**

***Number*: 2 (August 2023)**

Number: 3 (December 2023)

Author index

INSTRUCTIONS FOR AUTHORS

Scope

MEDITERRANEAN AGRICULTURAL SCIENCES is a multidisciplinary platform for the related scientific areas of agriculture and life sciences. Therefore, the journal primarily publishes original research articles and accepts a limited number of invited reviews in agricultural biotechnology, agricultural economics, agricultural machinery, animal husbandry, bioenergy, biostatistics and genetics, farm structure and irrigation, field crops, food science and technology, horticulture, landscape and nature conservation, natural resources, plant protection, soil science and plant nutrition.

General rules

Manuscripts within the scope of MEDITERRANEAN AGRICULTURAL SCIENCES can be submitted. The submitted manuscript must be unpublished, must not be simultaneously submitted for publication elsewhere, nor can the copyright be transferred somewhere else. Responsibility for the work published in this journal remains with the author(s). Moreover, the author(s) must comply with the ethical rules of science and scientific publications (International Committee of Medical Journal Editors and Committee on Publication Ethics). The journal is not responsible for these issues.

Manuscript submission

The manuscripts should be submitted to the journal by using online system: www.dergipark.org.tr/en/pub/mediterranean. A copy of the "Copyright Transfer Agreement" signed by all authors who contributed to the manuscript should be submitted by the corresponding author. Those manuscripts requiring an Ethics Committee Report should be supplied a copy of the report by the Ethics Committee. Ethics Committee Report which is the name of the board, date and number of approval, etc. information should be specified in the manuscript (on the Manuscript Cover Page and Material-Method). A plagiarism document is requested from the authors during the first submission.

Review process, proof and publishing

The manuscript submitted to the journal is subject to preliminary assessment by the Editorial Board. The Board has the right to decline the manuscript without initiating the peer review process in the event the manuscript does not meet the journal's criteria.

Manuscripts that meet the basic requirements of the journal are sent to three referees for review by experts in the particular field of science.

If all or a majority of the reviewers do not find the manuscript suitable for publication, the author is informed and documents are not returned.

Should the manuscript as is be found suitable for publication by reviewers; the author is informed of the final decision.

Should the manuscript is found publishable but requires revision as suggested by the review team; the areas where revisions are required are sent to the author with the referee's suggestions. The author is expected to return the corrected manuscript, or a letter of rebuttal within thirty days, including the last revised version of the manuscript and correction list sent to Editor. Should the author(s) do not accept the reasons for the revision, they are required to present scientific evidence and record the sources giving reason for this rejection in the letter of rebuttal. The Editorial Board takes the final decision by taking the referee reports into account and the compliance with the requirements for correction and the authors are notified of the final decision for publication.

Before publishing, the proof of the accepted manuscript is sent to the corresponding author for a final check. The corresponding author is expected to return the corrected final proof within 10 days. All authors can access their article on the web page of the journal (www.dergipark.org.tr/en/pub/mediterranean).

***MEDITERRANEAN AGRICULTURAL SCIENCES*is free of charge.**

Manuscript preparation guidelines

Manuscript submitted to the journal should consist of main two parts: the coverpage and the manuscript.

1.The coverpage: Should contain the title, names of the author(s) and addresses including the corresponding author's name and full contact details in addition the author(s) must contain the ORCID number(s), as well as declarations.

2. Manuscript: Manuscripts should be prepared on A4-size paper in 12 point, Times New Roman font, double line spaced, leaving 3 cm blank spaces on all four margins of each page. Each page of the manuscript and each line on page should be numbered.

Copyright Transfer Agreement: Should also be sent after being signed by all the authors during the first submission.

The manuscript should not be longer than **16** pages, double line spaced, including the "References" section (excluding any figures and tables). The information (name and title) of the author(s) should not be given in the article. A total of Tables or Figures should not be more than 8 in the manuscript, and must have the following sections:

Title: Must be short and inclusive, not to exceed **15** words, and the first letter of the first word to be written in uppercase and rest in lowercase letters, in bold.

Abstract: The abstract should not exceed **250** words, and it should summarize the objective of the study, the methods employed and the results.

Keywords: A maximum of **5** keywords, directly related to the subject matter and not employed in the title, should be recorded directly below the abstract.

Introduction: In this section, the subject of the study should be summarized, previous studies directly related to the study should be evaluated with the current knowledge of the subject, and the issues associated with production of the information needed are highlighted. Finally, the objective of the study should be clearly and explicitly stated. *Section titles within the manuscript:* except for the "References" all the main and sub-titles should be numbered. The first letters of the first words in the titles should be written in capital letters. Main titles should be written in bold and the sub-titles in italics.

Material and methods: In this section, all the materials employed in the study, the methods used, criteria evaluated, sampling methods applied, experimental design with statistical analysis and the confidence limits should be clearly explained. In addition, information regarding the permit (name of the board, date and number no of approval) of the studies requiring Ethics Committee permit should be provided in this section (Method).

Results: In this section the findings of the study should be presented clearly and explicitly with the help of figures, tables, and statistical analysis. Duplication of data presented in the Figures and Tables should be avoided, and the most appropriate tool should be employed.

Discussion and Conclusion: The findings of the study should be discussed with the results of previous studies, in terms of their similarity and contrast, and information gap filled by the study should be emphasized. Finally, conclusions and recommendations should be given. The manuscript layout of this section can be entitled "Results and Discussion" and / or "Conclusions" depending on author(s) preference.

For the reviews, the author(s) can make appropriate title arrangements.

Acknowledgement: People who contribute to the manuscript and/or the study and the funding agency (project numbers, if any) must be specified.

References: In the text, "the author's surname and the year" method should be used for identification of references. A reference identified by means of an author’s surname should be followed by the date of the reference in parentheses. For identification of references provided by two authors, “and” should be used between the surnames of authors. When there are more than two authors, only the first author’s surname should be mentioned, followed by ‘et al.’. In the event that an author cited has had two or more works published in the same year, the reference, both in the text and in the reference list, should be identified by a lower case letter like ‘a’ and ‘b’ after the date to distinguish between the works. When more than one reference is given at the end of a sentence, the references should be chronologically ordered, those of same date in alphabetical order.

Examples:

Burton (1947), Sayan and Karaguzel (2010), Keeve et al. (2000), (van Harten 2002), (Karaguzel and Altan 1995), (Burton 1947; Keeve et al. 2000; Yilmaz 2004a, b; Karaguzel 2005, 2006; Gulsen et al. 2010; Sayan ve Karaguzel 2010).

References should be listed at the end of the manuscript in alphabetical order in the References section. The original language of reference should be employed and journal’s name should not be abbreviated. Authors are fully responsible for the accuracy of the references they provide.

Examples:

Journal:

Karagüzel O (2003) Farklı tuz kaynak ve konsantrasyonlarının Güney Anadolu doğal *Lupinusvarius*’larının çimlenme özelliklerine etkisi. Akdeniz Üniversitesi Ziraat Fakültesi Dergisi 16: 211-220.

Keeve R, Loupser HL, Kruger GHJ (2000) Effect of temperature and photoperiod on days to flowering, yield and yield components of *Lupinusalbus* (L.) under field conditions. Journal of Agronomy and Crop Science 184: 187-196.

Book:

Taiz L, Zeiger E (2002) Plant Physiology. 3rd Edition, Sinauer Associates, Massachusetts.

Book chapter:

Van HartenAM (2002) Mutation breeding of vegetatively propagated ornamentals. In: Vainstein A (Ed), Breeding for ornamentals: Classical and Molecular Approaches. Kluwer Academic Publishers, Dordrecht, pp. 105-127.

Institution publications with unknown author name(s):

TSI (2005) Agricultural Structure.T.C. Prime Ministry State Institute of Statistics, Publication No. 1579, Ankara.

DOI and received information from the internet:

Gulsen O, Kaymak S, Ozongun S, Uzun A (2010) Genetic analysis of Turkish apple germplasm using peroxidase gene-based markers. doi:10.1016/j.scienta.2010.04.023.

FAO (2010) Statistical database.http://faostat.fao.org/site/339/default.aspx. Accessed 27 July, 2010.

Theses:

Sever Mutlu S (2009) Warm-season turfgrass species: Adaptation, drought resistance and response to trinexapac-ethyl application. PhD Thesis, The University of Nebraska, Nebraska.

Girmen B (2004) Gazipaşa yöresinde doğal yayılış gösteren hayıtların (*Vitexagnus-castus* L.) seleksiyonu ve çoğaltılabilme olanakları. Yüksek Lisans Tezi, Akdeniz Üniversitesi Fen Bilimleri Enstitüsü, Antalya.

Full-text congress/symposium book:

Hawkes JG (1998) Current status of genetic diversity in the world. In: Zencirci N, Kaya Z, Anikster Y, Adams WT (Eds), The Proceedings of International Symposium on *In Stu* Conservation of Plant Genetic Diversity. CRIFC, Ankara, Turkey, pp. 1-4.

Kesik T (2000) Weed infestation and yield of onion and carrot under no-tillage cultivation using four crops. In: 11th International Conference on Weed Biology. Dijon, France, pp. 437–444.

Figures and tables: In submitted manuscripts all photographs, graphics, figures, diagrams and the like must be named as "Figure", and lists of numerical values as "Table". All figures and tables should be numbered and placed at the end of the manuscript. The font of the letters within Figures and Tables used should be no larger than 8 points. Figure and table widths should be 8 cm or 17 cm and, if necessary, dimensions of up to 17x23 cm. The images should be in JPG format with 600 dpi resolution and should be informative in explaining the results. The authors must check the printing quality of the figures and should use high quality figures suitable for printing. Use of vertical lines in the tables is unacceptable, statistical significance should be stated using *P* values as much as possible, or using the "*" symbols for which description should be given. Small case lettering should be used for statistical groupings, and the statistical comparison method and significance level specified. Table and figure captions and descriptions should be short, concise, and descriptive. Abbreviations should be explained immediately if used within the Figures and tables. Those images composed of pieces should be grouped and converted into high-quality TIF format.

Units: For manuscripts SI (System International d'Units) unit system is used. In units, "/" should not be used and there should be a space between the units (for example: 5.6 kg ha^{-1}, instead of 5.6 kg/ha; 18.9 g cm^{-3}, instead of 18.9 g/cm^3; 1.8 µmol s^{-1} m^{-2}, instead of 1.8 µmol/s/m^2).

Abbreviations and symbols: Abbreviations should not be used in the manuscript title or in the subtitles. The necessary abbreviations at their first mention should be given in parentheses. Universal rules must be followed in the use of abbreviations and symbols.

Latin names and chemicals: The authority should not be used in the manuscript title when Latin names are used. The authority should be given when the Latin names are first used in the abstract and the text. For example: "*Lupinusvarius* (L.) is ....", "*L. varius* ... grown in the.. " Latin names should be written in italics. The trade mark of chemicals used in the studies should not be given unless it is absolutely necessary to do so.

Formulas: In manuscripts, formulas should be called "Equation", numbered as necessary, the numbers next to the formulas leaning right shown in brackets and the equations should be fitted in a single line (double-column, 8 cm), if possible.

The author (s) is encouraged to visit the web site (www.dergipark.org.tr/en/pub/mediterranean) to see the latest issue of the journal.

MEDITERRANEAN AGRICULTURAL SCIENCES

e-ISSN 2528-9675

Journal web page: www.dergipark.org.tr/en/pub/mediterranean

Address:

Faculty of Agriculture
Akdeniz University
07058 Antalya, Türkiye

Phone: +90 242 310 2412
Fax: +90 242 310 2479
E-mail: masjournal@akdeniz.edu.tr

COPYRIGHT TRANSFER AGREEMENT

Please note that publication of this article **can not** proceed until this signed form is submitted.

Author(s)	
Article title	

Corresponding Author's Contact Information

Name		Address	
E-mail			
Phone		Fax	

As the author (s) of the article submitted, we hereby accept and agree to the following terms and conditions.

a) I/We acknowledge that the Faculty of Agriculture at Akdeniz University does not carry any responsibility until the article arrives at the Bureau of Editor in Chief of the MEDITERRANEAN AGRICULTURAL SCIENCES.

b) I/We confirm that this article is in compliance with ethical rules, carries the ethical permission documents for the conditions required and will not cause any damage or injury when the materials and methods described herein are used.

c) The author(s) here take the full responsibility for the contents of the article.

d) The article has not been previously published and has not been submitted for publication elsewhere.

e) All the authors have seen, read and approved the article.

f) We accept that by disclaiming the copyright of the article, we transfer this right to the Faculty of Agriculture at Akdeniz University and authorize the Faculty of Agriculture at Akdeniz University in respect to publication of the article.

Except for the above issues, the author (s) reserve (s) the following rights

a) The author(s) retain (s) all proprietary rights, other than copyright, such as patent rights.

b) The author(s) can use the whole article in their books, teachings, oral presentations and conferences.

c) The author (s) has/have the right to reprint/reproduce the article for noncommercial personal use and other activities.

Whether accepted for publication or not, articles submitted to the journal are not returned and all the materials (photographs, original figures and tables, and others) is withheld for two years and is destroyed at the end of this period of time.

This document must be signed by all of the authors. If the authors are from different institutions, the signatures can be submitted on separate forms. Nevertheless, all the signatures must be wet signatures.

***Author(s) Name(s)**	**Address**	**Date**	**Signature**

*: The number of colon must be equal to the number of authors. If insufficient, it must be increased.

If the submitted article is not accepted for publication, this document is null and void.

PLEASE SIGN THE FORM AND UPLOAD ONLINE SYSTEM.