ISSN 1010-6960
E-ISSN 2536-491X

Türkiye Entomoloji Dergisi

Turkish Journal of Entomology

Cilt (Vol.): 46 Sayı (No.): 2 2022

Türkiye Entomoloji Dergisi
(Turkish Journal of Entomology)

Cilt (Vol.) **46** **Sayı** (No.) **2** **Haziran** (June) **2022**

Sahibi (Owner)
Ferit TURANLI

Sorumlu Müdür (Editor in Chief)
Galip KAŞKAVALCI

Dil Editörü (Linguistic Editor)
Ian Timothy RILEY

Turkish Journal of Entomology

The Turkish Journal of Entomology is a quarterly journal which has been published by the Entomological Society of Turkey. İt accepts original research articles in the fields of entomology and agricultural zoology in Turkish or English.

Abstracted/Indexed in Biological Abstracts, BIOSIS Previews, CABAbstracts, FAO AGRIS, Elsevier Scopus, Global Health, Information Reference Library, Review of Agricultural Entomology, SCI-E, TÜBİTAK/ULAKBİM, VINITI, Zoological Record.

Annual subscription price:€75 Price of asingle issue: €20

Corresponding address:
Turkish Journal of Entomology
Ege Üniversitesi Kampüsü PTT Şubesi, P.O. Box: 10, 35100 Bornova, İzmir, Turkey
e-mail: dergi@entomoloji.org.tr
web : http://www.entomoloji.org.tr

Türkiye Entomoloji Dergisi
(Turkish Journal of Entomology)

Cilt (Vol.) **46** **Sayı** (No.) **2** **Haziran** (June) **2022**

İçindekiler (Contents)

Orijinal araştırmalar (Original articles)

Türk. entomol. derg., 2022, 46 (2): 131-138
DOI: http: //dx.doi.org/10.16970/entoted.1061222

ISSN 1010-6960
E-ISSN 2536-491X

Original article (Orijinal araştırma)

The effect of three plant extracts on *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 (Tylenchida: Meloidogynidae)

Üç bitki ekstraktının *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 (Tylenchida: Meloidogynidae) üzerindeki etkinliği

Fatma Dolunay ERDOĞUŞ[1*]

Abstract

The use of plant extracts for biotechnical control root-knot nematodes is increasingly important. In this study, the efficacy of three plant extracts, *Thymbra spicata* L. (Lamiales: Lamiaceae) (Mediterranean thyme), *Plantago lanceolata* L. (Lamiales: Plantaginaceae) (ribwort plantain) and *Rosmarinus officinalis* L. (Lamiales: Lamiaceae) (Rosemary), for controlling of *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 (Tylenchida: Meloidogynidae) was examined. The experiments were conducted twice with four replicates under controlled conditions at the Laboratories of Plant Protection Central Research Institute of the Ministry of Agriculture of Turkish Republic in Ankara, Türkiye in 2020. The extracts were prepared at 1.2, 2.5 and 5%. The egg mass number, plant height, fresh and dry plant weight, and fresh and dry root weight of each plant were evaluated in the pot trials. The lowest number of in egg masses was with *T. spicata* (100 ± 2.3) followed by *R. officinalis* then *P. lanceolata* with (104 ± 2.6) and (113 ± 2.1). The least effect was observed at 1.2% concentration of *P. lanceolata* (217 ± 5.5).

Keywords: *Meloidogyne incognita,* plant extract, *Plantago lanceolata*, *Rosmarinus officinalis, Thymbra spicata*

Öz

Kök-ur nematodlarının biyoteknik mücadelesinde bitki ekstraktlarının kullanımı giderek daha fazla önem kazanmaktadır. Bu çalışmada 3 bitki ekstraktı *Thymbra spicata* L. (Lamiales: Lamiaceae) (Zahter, Kekik), *Plantago lanceolata* L. (Lamiales: Plantaginaceae) (Dar yapraklı sınırlı ot) ve *Rosmarinus officinalis* L. (Lamiales: Lamiaceae) (biberiye)'nin *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 (Tylenchida: Meloidogynidae)'nın kontrolündeki etkinliği araştırılmıştır. Denemeler 2020 yılında Zirai Mücadele Merkez Araştırma Enstitüsü Müdürlüğü Laboratuvarlarında (Türkiye Cumhuriyeti Tarım ve Orman Bakanlığı, Ankara, Türkiye) kontrollü koşullarda 4 tekerrürlü ve 2 tekrarlı olarak yürütülmüştür. Ekstraktlar (%1.2, 2.5 ve 5 dozlarda hazırlanmıştır. Sera-saksı denemelerinde yumurta paketi sayısı, bitki boyu, bitki taze ve kuru ağırlığı, köklerin taze ve kuru ağırlığı değerlendirilmiştir. En düşük yumurta paketi oluşumu *T. spicata 'da* meydana gelirken (100 ± 2.3) bunu *R. officinalis* (104 ± 2.6) ve 113 ± 2.1 ile *P. lanceolata* izlemiştir. En düşük etki ise *P. lanceolata*'nın %1.2'lik konsantrasyonunda (217 ± 5.5) görülmüştür.

Anahtar sözcükler: *Meloidogyne incognita*, bitkisel ekstrakt, *Plantago lanceolata*, *Rosmarinus officinalis, Thymbra spicata*

[1] General Directorate of Agricultural Research and Policies, Department of Plant Health, 06800, Ankara, Türkiye
* Corresponding author (Sorumlu yazar) e-mail: dolerkoll@gmail.com
Received (Alınış): 21.01.2022 Accepted (Kabul ediliş): 01.05.2022 Published Online (Çevrimiçi Yayın Tarihi): 14.05.2022

Introduction

Plant parasitic nematodes are of great importance among the various agricultural production problems. The root-knot nematodes (*Meloidogyne* spp.) are the most important of all plant parasitic nematodes due to the damage they cause to their hosts (Javed et al., 2006). The root-knot nematodes are found commonly in the greenhouse vegetable cultivation areas especially on the coastal regions of Türkiye. This group has a large host range and causes large yield loses in vegetables all over the world (e.g., 42-54% in tomato and 30-60% in eggplant) (Netscher & Sikora, 1990). Highly toxic nematicides can be used for the control of the root-knot nematodes. The difficulties of controlling with these pests are increasing and some nematicides have been banned. Although nematicides are the main method of control; the increasing concerns for human health and the environment have led to the banning of nematicides and fumigants like methyl bromide, 1,3-dicloropropen, carbofuran and aldicarb. Therefore, alternative control methods need to be developed and applied. Studies on the use of volatile oils, plant extracts and plant-based compounds obtained from plants for controlling pests, have gained momentum and useful results have been obtained (Lee et al., 2001a, b; Varma & Dubey, 2001; Lee & Annis, 2004; Polatoğlu et al., 2015a, b). Plants have several defense mechanisms for pests. Among these mechanisms, various secondary metabolites synthesized within the living organisms have an important place. Secondary metabolites are important chemical compounds that are not primarily related to the vital activities of the plant, and some can provide defense against herbivores (Taiz & Zeigeri, 2002). The compounds with insecticidal and behavioral effects against pests can be classified as alkaloids, glycosides, phenols, terpenoids, tannins and saponins (Shanker & Solanki, 2000; Güncan & Durmuşoğlu, 2004). The use of herbal extracts against root-knot nematodes is widely practiced and there are many studies on the subject (Malik et al., 1987; Taba et al., 2008) In these studies, many plants in 57 families have been found to have nematicidal effects and these families include Lamiaceae, Asteraceae, Rutaceae and Lauraceae (Sukul, 1992; Andres et al., 2012; Kepenekci & Sağlam, 2015).

In this study, the effects of three plant extracts obtained from three plant species that grow naturally in Türkiye on *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 (Tylenchida: Meloidogynidae) were determined under controlled conditions. The plants were *Thymbra spicata* L. (Lamiales: Lamiaceae) (Mediterranean thyme), *Plantago lanceolata* L. (Lamiales: Plantaginaceae) (ribwort plantain) and *Rosmarinus officinalis* L. (Lamiales: Lamiaceae) (rosemary).

Materials and Methods

Plant material

Thymbra spicata, *P. lanceolata* (from Erzurum) and *R. officinalis* (from Muğla) were collected in Anatolia, Türkiye, during flowering in June and September in the year. Aerial parts of the plants dried in the shade and ground in a grinder.

Extraction of plant material extract

Plant leaves were collected and spread on polythene sheets in the laboratory for 10 days for air drying. The plants were then dried at 80°C for 3-4 days. The dried materials were ground into a fine powder in a blender. Ethanol was added to the ground plant material and shaken in a rotary shaker at 120 rpm for 48 h. The ethanol was filtered and vacuumed in a rotary evaporator at 50-60°C to obtain organic crude extracts free of ethanol (Brauer & Devkota, 1990). Each plant extract was used immediately in all tests. Concentrations of 1.2, 2.5 and 5.0% was prepared in distilled water (Orisajo et al., 2007).

Nematode culture

Pure cultures of *M. incognita* were grown on tomato cv. Tueza F1. The infected roots were washed carefully washed under tap water and egg masses collected from the roots under the microscope and left

to hatch at room temperature. The juveniles that hatched in the first 24 h were discarded and subsequent hatching was monitored and juveniles were collected daily. The suspension with nematodes obtained was adjusted to 1,000 juveniles ml^{-1}.

Greenhouse pot trials

Tomato seeds were placed in 3% NaOCI for 1 min for surface sterilization, then washed with sterile water and dried on blotting paper before planting in tubes containing peat. The tubes were placed in a climate room (25 ± 2ºC, 14:10 h L:D photoperiod). Tomato plants with two to four leaves in the climate room were transplanted into pots (760 ml, 10 x 10 x 11 cm) containing sterile soil-sand mixture (2:1) with one seedling per pot. After 15 days, when the seedlings reached ~10 cm, *M. incognita* larvae were inoculated by pipette into 2 cm deep holes around each seedling at 1,250 juveniles ml^{-1}. The plant extracts were applied in different 2-cm deep holes around each seedling. One ml of extract was applied per plant or pot. Water was used in the controls. The experiments included four replicates and were conducted twice under controlled room conditions. A single control group was used because all trials were set up on the same day and under the same conditions. Eight weeks after the applications, the plants were harvested by cutting 1 cm above the soil surface. The number of egg masses per plant, shoot height, shoot fresh and dry weight, root fresh and dry weight were determined. Before counting the egg mass, the roots were soaked in a aqueous solution of red food-coloring (0.15 g L^{-1}) for 15-20 min, washed and counted under the microscope (Fenner, 1962; Dickson & Struble, 1965; Holbrook et al., 1983). For dry weight determination the samples dried in an oven at 70ºC for 48 h (Mohammad et al., 2007).

Statistical analysis

Statistical analyzes were made using the MINITAB 18 package program. Analyzed by Tukey's multiple comparison test.

Results and Discussion

The effects of three concentrations of *T. spicata*, *P. lanceolata* and *R. officinalis* on the number of egg mass, shoot and root fresh and dry weights are given in Table 1.

There was a decrease in egg mass formation with all three plant extracts compared to the control (Table 1) The different extract concentrations caused a different rate of decrease in egg mass formation compared to the control, and this was statistically significant ($P < 0.05$) (Table 1). Among the doses used, the greatest effect was seen at the highest dose of 5% in all three extracts. *Thymbra spicata* extract that caused the greatest decrease in egg mass formation, followed by *R. officinalis* and *P. lanceolata*, respectively. The lowest effect was observed at 1.2% concentration of *P. lanceolata*

There was an increase in shoot fresh weight for all extracts, but no dose effect, compare to the control (Table 1). However, this effect was not evident for shoot dry weight. The effect on root fresh weight was less consistent. Only *T. spicata* differed from the control. Across extract concentrations, there were some slight suppression in root fresh weight with the extracts at 2.5%. These differences were not evident when root dry was determined. There was not statistically significant effect on shoot height.

Table 1. Effective of three concentrations of *Thymbra spicata, Plantago lanceolata* and *Rosmarinus officinalis* extract on *Meloidogyne incognita* egg mass production and plant growth at three concentrations

Parameters	Extract	1.2%	2.5%	5%
Egg masses	*T. spicata*	195 ± 6.2 b[1]A[2]	128 ± 6.1 bc	100 ± 2.3 bC
	P. lanceolata	217 ± 5.5 bA	149 ± 4.8 bB	113 ± 2.1 bC
	R. officinalis	199 ± 5.5 bA	121 ± 3.7 cB	104 ± 2.6 bC
	Control	309 ± 18.6 aA	309 ± 18.6 aA	309 ± 18.6 aA
		F[3] = 32.4; P < 0.05	F = 104; P < 0.05	F = 207; P < 0.05
Shoot fresh weight (g)	*T. spicata*	8.0 ± 0.4 aA	7.9 ± 0.4 aA	8.1 ± 0.5 aA
	P. lanceolata	6.9 ± 0.2 aA	6.8 ± 0.3 aA	7.2 ± 0.4 aA
	R. officinalis	7.1 ± 0.4 aA	7.3 ± 0.3 aA	7.6 ± 0.4 aA
	Control	4.8 ± 0.4 bA	4.8 ± 0.4 bA	4.8 ± 0.4 bA
		F = 7.63; P < 0.05	F = 10.4; P < 0.05	F = 6.79; P < 0.05
Shoot dry weight (g)	*T. spicata*	1.9 ± 0.1 aA	1.8 ± 0.1 aA	2.8 ± 0.1 aA
	P. lanceolata	1.7 ± 0.2 aA	1.6 ± 0.2 aA	1.8 ± 0.2 aA
	R. officinalis	1.6 ± 0.1 aA	1.6 ± 0.1 aA	1.1 ± 0.1 aA
	Control	1.5 ± 0.1 aA	1.5 ± 0.1 aA	1.5 ± 0.1 aA
		F = 0.90; P > 0.05	F = 0.42; P > 0.05	F = 2.65; P > 0.05
Root fresh weight (g)	*T. spicata*	9.1 ± 0.6 aA	6.8 ± 0.4 aB	9.8 ± 0.3 aA
	P. lanceolata	7.4 ± 0.7 aAB	5.1 ± 0.5 aB	8.6 ± 0.3 abA
	R. officinalis	7.5 ± 0.3 aB	6.1 ± 0.4 aC	8.9 ± 0.2 abA
	Control	7.5 ± 0.8 aA	7.5 ± 0.8 aA	7.5 ± 0.8 bA
		F = 1.87; P > 0.05	F = 1.57; P > 0.05	F = 6.62; P < 0.05
Root dry weight (g)	*T. spicata*	2.4 ± 0.2 aA	1.5 ± 0.1 aB	2.3 ± 0.1 aA
	P. lanceolata	1.9 ± 0.1 abA	1.3 ± 0.1 aB	2.0 ± 0.1 aA
	R. officinalis	1.9 ± 0.1 abA	1.4 ± 0.1 aB	2.1 ± 0.1 aC
	Control	1.6 ± 0.2 bA	1.6 ± 0.2 aA	1.6 ± 0.2 bA
		F = 3.90; P < 0.05	F = 0.51; P > 0.05	F = 7.26; P < 0.05
Shoot height (cm)	*T. spicata*	34.0 ± 1.7 aA	31.0 ± 3.0 aA	31.9 ± 1.9 aA
	P. lanceolata	31.2 ± 1.5 aA	28.1 ± 1.9 aA	27.5 ± 1.1 aA
	R. officinalis	30.7 ± 1.4 aA	26.3 ± 1.7 aA	29.0 ± 1.3 aA
	Control	28.6 ± 1.1 aA	28.6 ± 1.1 aA	28.6 ± 1.1 aA
		F = 1.79; P > 0.05	F = 0.83; P > 0.05	F = 1.75; P > 0.05

[1] Means followed but the same lowercase letter within columns are not significantly different (P < 0.05, Tukey test).
[2] Means followed but the same uppercase letter within rows are not significantly different (P < 0.05, Tukey test).
[3] F value degrees of freedom were all 3,31.

In the study of Oka et al. (2000), in which the nematicidal activity of volatile oils obtained from 27 spices was investigated in laboratory and controlled room conditions, *Carum carvi* L., *Foeniculum vulgare* Mill. (Apiales: Apiaceae), *Mentha longifolia* (L.) Huds. and *M. spicata* L. (Lamiales: Lamiaceae) had the greatest effect under the laboratory conditions at a dose of 1,000 µl L^{-1}. A mixture of *Origanum vulgare* L., *Thymus syriacus* Boiss., and Thymbra capitata (L.) Cav. (Lamiales: Lamiaceae) reduced gall numbers in the roots of cucumber at doses of 100 and 200 mg/kg.

Aydınlı & Mennan (2014) investigated the effectiveness of 12 plant extracts, including *Viscum album* L. (Santalales: Viscaceae), *R. officinalis, Cirsium arvense* (L.) Scop. (Asterales: Asteraceae), prepared in cold and hot form, on *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949 (Tylenchida: Meloidogynidae) under laboratory and controlled room conditions. Cold extracts, except *R. officinalis*, caused higher larval mortality and lower egg hatching than hot extracts, and that the cold extract of *C. arvense* completely inhibited egg hatching. A study was conducted to evaluate the activity of the extract of *R. officinalis* in forming resistance to *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949 *(*Tylenchida: Meloidogynidae) and *Pratylenchus brachyurus* (Godfrey, 1929) Filipjev *&* Schuurmans Stekhoven, 1941 (Tylenchida: Pratylenchidae) in soybean. Herbal volatile oil prepared at 0, 1, 2 and 3% were applied to the upper parts of the plants once every 2 weeks and once per month. Rosemary oil was phytotoxic to soybean and caused significant decrease in plant height, shoot fresh and dry weight, and root fresh weight of plants that treated every 2 weeks (Mattei et al., 2013). In another study with *R. officinalis*, activity on *M. incognita* was investigated in soybean cvs CD206 and CD215 under controlled conditions. Rosemary extract prepared at three concentrations (1, 5 and 10%) was applied to the plants weekly. The number of eggs in the root and soil, the number of infective juveniles, the number of knots on the roots and the reproduction factor were evaluated. Rosemary extract reduced egg hatching. Likewise, while the number of galls on cv. CD206 decreased by 46.5%, there was a decrease in both galling and the number of juveniles in the soil with cv CD215 (Müller et al., 2016). Bajestani et al. (2017) tested the activity of marigold (*Tagetes* spp.) and rosemary (*R. officinalis*) and black cumin (*Nigella sativa* L. (Ranunculales: Ranunculaceae) on *M. javanica* on tomato plant. There was a significant decrease in root infection rate and root weight in all applications. Rosemary extract gave the greatest effect with a 40% reduction in the nematode population compared to the control.

Kepenekci & Sağlam (2015) investigated the nematicidal effects of the extracts of five plants [pepper, *C. frutescens*); henbane, *Hyoscyamus niger* L. (Solanales: Solanaceae) ; bead tree, *Melia azedarach* L. (Sapindales: Meliaceae); common cocklebur, *Xanthium strumarium* L. (Asterales: Asteraceae); and yarrow *Achillea santolinoides* subsp. *wilhelmsii* (K.Koch) Greuter (Asterales: Asteraceae)]) on *M. javanica*. Extract concentrations of 3.6% and 12% for *H. niger*, *X. strumarium* and *M. azedarach* completely inhibited hatching, but the effect was less with by *C. frutescens* and *A. santolinoides* subsp. *wilhelmsii*. Considering the mortality rate of infective juveniles, the most effective extracts were from *X. strumarium* and *M. azedarach*.

In another study, the efficacy of eucalyptus [*Eucalyptus camaldulensis* Dehnh. (Myrtales: Myrtaceae)], garlic [*Allium sativum* L. (Asparagales: Amaryllidaceae)], marigold [*Tagetes erecta* L. (Asterales: Asteraceae)] and neem tree [(*Azadirachta indica* A. Juss. (Sapindales: Meliaceae)] extracts and volatile oils on *M. incognita* was tested under laboratory, field and greenhouse conditions. Neem extract provided the highest mortality rate (65%) in laboratory conditions. This was followed by neem volatile oil (64%) and marigold extract (61%), respectively. Likewise, the most effective results were obtained in the applications of neem extract 44% and essential oil 33% under greenhouse and field conditions (Kamal et al., 2009).

In another study, the effects of aqueous extracts of *Euphorbia myrsinites* L. (Malpighiales: Euphorbiaceae) and *Drimia maritima* (L.) Stearn (Asparagales: Asparagaceae) on the damage of *M.*

incognita were evaluated. All dilutions of the extracts (1, 2 and 4%) applied to the soil, reduced root galling caused by *M. incognita* and increased tomato yield compared to the control (Kaşkavalcı & Civelek, 2009). Leaves of the castor oil plant [*Ricinus communis* L. (Malpighiales: Euphorbiaceae)] and oleander [*Nerium oleander* L. (Gentianales: Apocynaceae)], fruit of squirting cucumber [*Ecbalium elaterium* (L.) A.Rich. (Cucurbitales: Cucurbitaceae)] and whole plants of marigold (*T. erecta*) was tested on root-knot nematode (*M. incognita*). All treatments resulted in a less galling compared to a positive control (Hatipoğlu & Kaşkavalcı, 2007).

The nematicidal effect of methanol extracts of *Sylibum marianum* (L.) Gaertn. (Asterales: Asteraceae), *P. lanceolata* and *Cassia fistula* L. (Fabales: Fabaceae) on *M. incognita* was tested under laboratory conditions. *Plantoago lanceolata* was the most effective extract and reduced egg hatching by 75% and killed infective juveniles within 5 days (Adekunle et al., 2006). In another study conducted by Kepenekci et al. (2017), three concentrations (3, 6 and 12%) of the extracts obtained from *Capsicum frutescens* L. (Solanales: Solanaceae), *H. niger*, *X. strumarium*, *A. antolinoides subsp. wilhelmsii* and *M. azedarach* were tested against root-knot nematodes (*M. incognita* race 2 and *M. arenaria* race 2). As a result of the study, 12% concentrations of *H. niger* and 12% of *X. strumarium* concentrations were found to be effective on egg hatching of *M. arenaria* the concentration of 12% of *X. strumarium* was effective on egg hatching. A concentration of 12% of *M. azedarach* was effective against juveniles of *M. arenaria* and *M. incognita*.

Considering all studies, it is clear that some plant extracts give effective results against root-knot nematodes. In the present study, it was found that *T. spicata*, *P. lanceolata* and *R. officinalis* can be effective against root-knot nematodes under controlled conditions. It is therefore possible that these plant extracts could be of valued in the control programs against root-knot nematodes, but further studies are needed to confirm their value under field conditions.

Acknowledgments

Prof. Dr. Şaban Kordalı (Muğla Sıtkı Koçman University, Muğla, Türkiye) is thanked for provision of the plant extracts, Prof. Dr. İlker Kepenekci (Gaziosmanpaşa University, Tokat, Türkiye) for his help during the establishment of the experiments and Assoc. Prof. Dr. Mustafa Alkan (Directorate of Plant Protection Central Research Institute, Ankara, Türkiye) for help with statistical analysis.

References

Adekunle, O. K., N. Singh, N. Kumar & B. Singh, 2006. Nematicidal action of some plant extracts against *Meloidogyne incognita* and isolation of nematicidal fraction from *Plantago lanceolata*. Pakistan Journal of Nematology, 25 (1): 189-197.

Andres, M. F., A. Coloma, G. J. Sanz, J. Burillo & P. Sainz, 2012. Nematicidal activity of essential oils: a review. Phytochemistry Reviews, 11 (4): 371-390.

Aydınlı, G. & S. Mennan, 2014. Effect of some plant extracts on *Meloidogyne arenaria* Neal, 1889 (Tylenchida: Meloidogynidae) and tomato. Bazı bitki ekstraktlarının *Meloidogyne arenaria* Neal, 1889 (Tylenchida: Meloidogynidae) ve domatese etkisi. Turkish Journal of Entomology, 38 (3): 323-332 (in Turkish with abstract in English).

Bajestani, M. S., K. Dolatabadi & E. Mahdikhani-Moghadam, 2017. Effect of medicinal plant extracts on inoculated *Meloidogyne javanica* in tomato. Pakistan Journal of Nematology, 35 (1): 73-78.

Brauer, M. & B. Devkota, 1990. Control of *Thaumatopoea pityocampa* (Den. & Schiff) by extracts of *Melia azedarach* L. (Meliaceae). Journal of Applied Entomology, 110 (1-5): 128-135.

Dickson, D. W. & F. B. Struble, 1965. A sieving-staining technique for extraction of egg masses of *Meloidogyne incognita* from soil. Phytopathology, 55: 497.

Fenner, L. M., 1962. Determination of nematode mortality. Plant Disease Reports, 46: 383.

Güncan, A. & E. Durmuşoğlu, 2004. Bitkisel kökenli doğal insektisitler üzerine bir değerlendirme. Hasad Dergisi, 233: 26-32 (in Turkish).

Hatipoğlu, A. & G. Kaşkavalcı, 2007. Kök-ur Nematodları [*Meloidogyne incognita* (Kofoid&White) Chitwood]'na karşı savaşta bazı bitki kısımlarının etkileri üzerine araştırmalar. Türkiye Entomoloji Dergisi, 31 (2): 139-151 (in Turkish with abstract in English).

Holbrook, C. C., D. A. Knauft & D. W. Dickson, 1983. A technique for screening peanut for resistance to *Meloidogyne arenaria*. Plant Disease, 67 (9): 957-958.

Javed, N., S. A. Anwar, M. Inam-ul-haq, R. Ahmad & H. U. Khan, 2006. "Effect of neem formulations applied as soil drenching on invasion and development of root-knot nematode, *Meloidogyne javanica*, 244-247". Proceeding of International Symposium on Sustainable Crop Improvement and Integrated Management (14-16 September 2006, University of Agriculture, Faisalabad, Pakistan).

Kamal, A. M., A. Elyousr, M. M. Avad & M. A. A. Gaid, 2009. Management of tomato root-knot Nematode *Meloidogyne incognita* by plant extracts and essential oils. The Plant Pathology Journal, 25 (2): 189-192.

Kaşkavalcı, G. & H. S. Civelek, 2009. Effects of two plant extracts on the damage of *Meloidogyne incognita* in tomato plants. Ekoloji, 18 (72): 16-22.

Kepenekci, İ., T. Katı Çekengil, F. D. Erdoğuş, P. Erdoğan & H. D. Sağlam, 2017. Beş farklı bitki ekstraktının domateste zararlı kök-ur nematod (*Meloidogyne incognita* Irk 2 ve *M. arenaria* Irk 2) (Tylenchida: Meloidogynidae)'larına karşı sera koşullarındaki etkisinin belirlenmesi. Turkish Journal of Weed Science, 20 (1): 36-47 (in Turkish with abstract in English).

Kepenekci, İ. & D. Sağlam, 2015. Extracts of some indigenous plants affecting hatching and mortality in the root-knot nematode [*Meloidogyne javanica (*Treub) Chitwood]. Egyptian Journal of Biological Pest Control, 25 (1): 39-44.

Lee, B. H., P. C. Annis & W. S. Choi, 2004. Fumigant toxicity of essential oils from the Myrtaceae family and 1, 8-cineole against 3 major stored-grain insects. Journal of Stored Products Research, 40 (5): 553-564.

Lee, B. H., W. S. Choi, S. E. Lee & B. S. Park, 2001a. Fumigant toxicity of essential oils and their constituent compounds towards the rice weevil, *Sitophilus oryzae* (L.). Crop Protection, 20 (4): 317-320.

Lee, S. E., B. H. Lee, W. S. Choi, B. S. Park, J. G. Kim & B. C. Campell, 2001b. Fumigant toxicity of volatile natural products from Korean spices and medicinal plants towards the rice weevil, *Sitophilus oryzae* (L). Pest Management Science, 57 (6): 548-553.

Malik, M. S., N. K. Sangwan, K. S. Dhindsa & D. S. Bahatti, 1987. Nematicidal activity of extracts of *Xanthium strumarium* L. Weeb Abstract, 21 (10): 19-20.

Mattei, D., C. R. Dias-Arieira, H. H. Puerari, T. S. Dadazio, M. Roldi & T. R. Benetoli da Silva, 2013. Evaluation of R*osmarinus officinalis* essential oil in inducing resistance to *Meloidogyne javanica* and *Pratylenchus brachyurus* in soybean. Journal of Food, Agriculture & Environment, 11 (3-4): 1171-1175.

Mohammad, A. B., Z. Eskandar, S. Saeid, J. Mohammad & F. Mariba, 2007. Evaluation of sulfosulfuran for broadleaved and grass weed control in wheat (L.) in Iran. Crop Protection, 26 (9): 1385-1389.

Müller, M. A., T. M. Mioranza, J. R. Stangarlin, O. K. Kuhn & A. Gustav, 2016. In vitro toxicity and control of *Meloidogyne incognita* in soybean by rosemary extract Toxicidade (In vitro e controle de *Meloidogyne incognita* em soja por extrato aquoso de alecrim). Semina: Ciências Agrárias, Londrina, 37 (1): 103-110 (in Portuguese with abstract in English).

Netscher, C. & R. A. Sikora, 1990. "Nematode Parasites of Vegetables, 237-284". In: International, Wallingford, U.K., 877 pp.

Oka, Y., Ş. Nacar, E. Putievsky, U. Ravid, Z. Yaniv & Y. Spiegel, 2000. Nematicidal activity of essential oils and their components against the root-knot nematode. Nematology, 90 (7): 710-715.

Orisajo, S. B., M. O. Okeniyi, O. A. Fademi & L. N. Dongo, 2007. Nematicidal effects of water extracts of A*calypha ciliate*, *Jatropha gosssypifolia*, *Azadiractha indica* and *Allium ascalonicum* on *Meloidogyne incognita* infection on cacao seedlings. Journal of Research in Biosciences, 3 (3): 49-53.

Polatoğlu, K., Ö. C. Karakoç, B. Demirci, N. Gören & K. H. C. Başer, 2015a. *Sitophilus granarius* L. (Coleoptera) Toxicity and biological activities of the essential oils of *Tanacetum macrophyllum* (Waldst. & Kit.) Schultz Bip. Journal of Oleo Science, 64 (8): 881-893.

Polatoğlu, K., Ö. C. Karakoç, Y. Y. Yücel, B. Demirci, N. Gören & K. H. C. Başer, 2015b. Composition, insecticidal activity and other biological activities of *Tanacetum abrotanifolium* Druce. essential oil. Industrial Crops & Products, 71: 7-14.

Shanker, C. & K. R. Solanki, 2000. Botanical insecticides: A historical perspective. India, Asian Agrihistory, 4 (2): 21-30.

Sukul, N. C., 1992. Plant antagonist to plant-parasitic nematodes. Indian Review of Life Science, 12: 23-52.

Taba, S., J. Sawada & Z. Moromizato, 2008. Nematicidal activity of Okinawa Island plants on the root-knot nematode *Meloidogyne incognita* (Kofoid & White) Chitwood. Plant Soil, 303 (1-2): 207-216.

Taiz, L. & E. Zeigeri, 2002. Plant Physiology. Sinauer Associates, Sunderland, Massachusetts, USA, 603 pp.

Varma, J. & N. K. Dubey, 2001. Efficacy of essential oils of *Caesulia axillaris* and *Mentha arvensis* against some storage pests causing biodeterioration of food commodities. International Journal of Food Microbiology, 68 (5): 207-210.

Türk. entomol. derg., 2022, 46 (2): 139-148
DOİ: http://dx.doi.org/10.16970/entoted.1074947

ISSN 1010-6960
E-ISSN 2536-491X

Original article (Orijinal araştırma)

Biodiversity and distribution of soil nematodes at surroundings of the ancient city of Prusias ad Hypium, Konuralp, Düzce (Türkiye)[1]

Prusias ad Hypium (Konuralp) Antik Kenti, Düzce (Türkiye)'deki toprak nematodların biyolojik çeşitliliği ve dağılımı

Taylan ÇAKMAK[2*]

Abstract

The diversity and distribution of soil nematodes were studied in Prusias ad Hypium (Konuralp) Ancient City, Düzce (Türkiye) in 2021. A total of 1.388 individuals were discovered within 17 families, 25 genera and 30 species. Species richness and the nematode abundance show closely related distribution at each ecological characteristic and the number of species reaches a maximum where low disturbance of soil is observed. Nematodes feeding with bacteria are dominating the total community (53%) in all sampling sites, followed by omnivorous (31.6%), plant parasitic (6%), fungivorous (4.8%) and predatory nematodes (4.6%).

Keywords: Community analysis, distribution, ecology, habitat

Öz

Bu çalışmada, 2021 yılında Düzce İli (Türkiye) Prusias (Konuralp) Antik Kenti ve çevresinde yaşayan toprak nematodlarının çeşitliliği ve yayılışları araştırılmıştır. 17 familya, 25 cins ve 30 türe ait toplam 1.388 birey tespit edilmiştir. Örnek alınan alanlara göre nematod çeşitliliği (tür zenginliği) ve nematod sayısı, düşük insan etkisinin gözlemlendiği yerlerde maksimum sayıya ulaştığı, habitatlar arası yakın ilişkili bir dağılım göstermiştir. Tüm örnekleme alanlarında bakterivor nematodlar (%53) nematod topluluğuna sayıca hakimdir. Bakterivor nematodları sayıca omnivor nematodlar (%31,6), bitki paraziti nematodlar (%6), fungivor nematodlar (%4,8) ve predatör nematodlar (%4,6) takip etmektedir.

Anahtar sözcükler: Topluluk analizi, dağılım, ekoloji, habitat

[1] This study was supported by Düzce University, Faculty of Agriculture, Department of Agricultural Biotechnology, Düzce, Türkiye
[2] Düzce University, Faculty of Agriculture, Department of Agricultural Biotechnology, 81620, Düzce, Türkiye
* Corresponding author (Sorumlu yazar) e-mail: cakmaktaylan@gmail.com
Received (Alınış): 24.02.2022 Accepted (Kabul ediliş): 09.05.2022 Published Online (Çevrimiçi Yayın Tarihi): 18.05.2022

Introduction

Many biogeographic reasons explain the biodiversity of Türkiye. Türkiye is at the intersection of Asia and Europe and has a diversity of geographic features and climatic conditions, consequently the country has rich biodiversity. As a result of the ice ages, which lasted between 1.8 million and 10 thousand years ago, a wide range of species richness evolved and is distributed throughout the country. The map of biodiversity hotspots defined on a global scale indicates the rich species diversity found in Türkiye (Demirsoy, 1998).

Nematodes are animals that comprise an important part of ecdysozoan with a wide range of life strategies and feeding habits. They have adapted to many environmental conditions by being able to feed on many other species. These features involve a crucial role in soil nematodes in the food webs in soil. According to biodiversity and conservation studies, nematodes (Zhang, 2012), currently identified with some 25 to 30 thousand named species, constitute the highest diversity group with 1 million species (Hugot et al., 2001) in terms of estimated species number after Arthropoda. Free-living animals comprise the most abundant group of them, which inhabit soils and (both freshwater and marine) sediments (Yeates et al*.,* 1993). Other species have become plant and animal (including human) parasites, causing important diseases and pests (Lee, 2002). Their adaptability, diversity and abundance make nematodes useful bioindicators of soil health (Wilson & Khakouli-Duarte, 2009). Various ecological indices (Bongers, 1990; Bongers et al., 1997; Ferris & Bongers, 2009) have been proposed to use them to monitor natural (successional) or artificial (disturbances caused by human activities) changes that occurred in these systems (Bongers & Ferris, 1999).

The ancient city Prusias ad Hypium (Konuralp) is located in Düzce Province (40°54’20" N, 31°08’53" E), of Türkiye between Sakarya and Bolu Provinces in the Western Black Sea Region. It is unique place having the only antique theater in Black Sea Region with an archeological park. Today, the ancient city is located in a town called Konuralp. The natural habitat is mainly dominated by forest and cultivated trees such as *Fagus sylvatica* L. and *Quercus robur* f. *fastigiata* (Lam.) O. Schwarz (Fagales: Fagaceae). The main agriculture in the region is *Corylus avellana* L. (Fagales: Betulaceae) trees. Also, wild plant meadow and mountain grassland are common plants of the landscape. In Düzce, which reflects the characteristics of the climate of the Western Black Sea Region surrounded by dense forest areas, studies on the diversity of soil nematodes are quite limited. Therefore, as might be expected, nematode species distribution, ecology and diversity in the surrounding areas of Prusias Ancient City are largely unknown. This study aimed to examine the soil nematode fauna surrounding areas of Prusias ad Hypium. More specifically, characterization of the soil nematode fauna, description of distribution patterns of nematode species, and analysis of soil maturity indices associated with nematode assemblages in the studied area.

Materials and Methods

Sampling

This study was conducted at Düzce University, Faculty of Agriculture, Department of Agricultural Biotechnology. Soil samples were collected in Konuralp, Düzce, Türkiye, between July and September 2021 (Figure 1). The sampling was conducted along three different eco-habitats, namely forest (Sessiz Bahçe, 1-10), farms (surroundings of antique theater, 11-20) and fruit orchards (21-30). These zones were determined in order to cover different habitats in the region. Thirty soil samples were collected from three sampling locations (Table 1). Samples were collected from a depth of 30 cm from a 15 x 15 cm plot. Soil samples were then placed in zip-lock sample bags, kept in a mobile refrigerator during transport, and taken to the laboratory for nematode extraction.

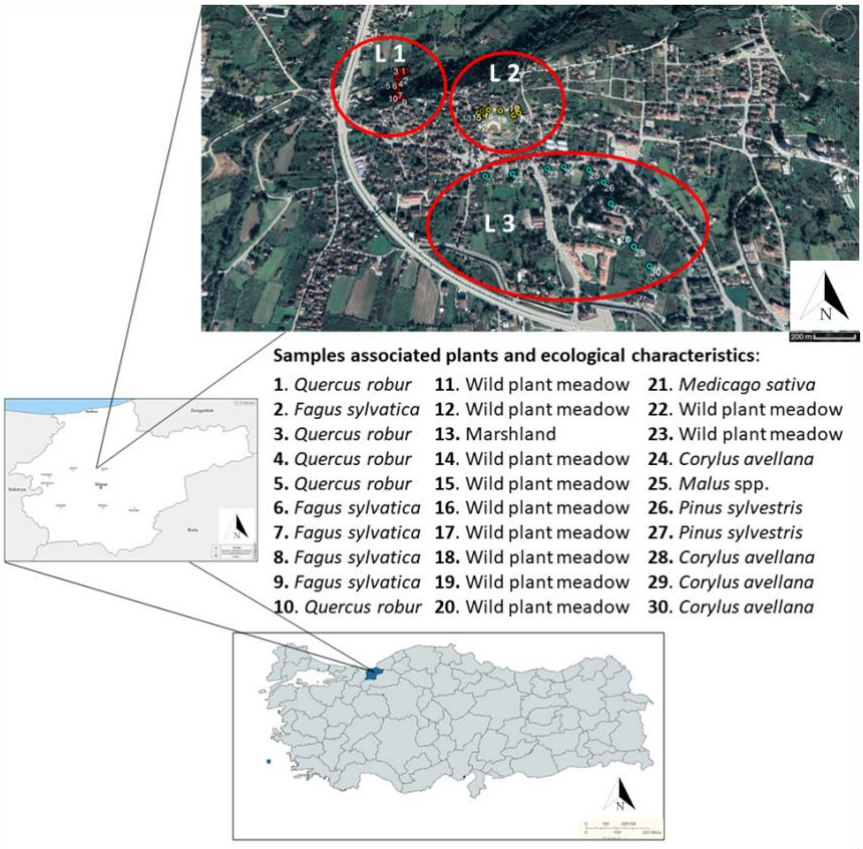

Figure 1. Location of the sampling sites; L1: Sessiz Bahçe natural park, L2: Surrounding area of Prusias Antique Theatre, L3: Orchards around the town.

Table 1. Associated plants and geographical coordinates of the sampling sites

Sample	Associated plant	Coordinates	Sample	Associated plant	Coordinates
1 (L1)	*Quercus robur*	40°54'27" N, 31°8'41" E	16 (L2)	Wild plant meadow	40°54'23" N, 31°8'54" E
2 (L1)	*Fagus sylvatica*	40°54'27" N, 31°8'40" E	17 (L2)	Wild plant meadow	40°54'23" N, 31°8'55" E
3 (L1)	*Quercus robur*	40°54'27" N, 31°8'40" E	18 (L2)	Wild plant meadow	40°54'22" N, 31°8'56" E
4 (L1)	*Quercus robur*	40°54'27" N, 31°8'40" E	19 (L2)	Wild plant meadow	40°54'23" N, 31°8'56" E
5 (L1)	*Quercus robur*	40°54'26" N, 31°8'40" E	20 (L2)	Wild plant meadow	40°54'23" N, 31°8'56" E
6 (L1)	*Fagus sylvatica*	40°54'26" N, 31°8'40" E	21 (L3)	*Medicago sativa*	40°54'16" N, 31°8'52" E
7 (L1)	*Fagus sylvatica*	40°54'25" N, 31°8'40" E	22 (L3)	Wild plant meadow	40°54'16" N, 31°8'56" E
8 (L1)	*Fagus sylvatica*	40°54'24" N, 31°8'40" E	23 (L3)	Wild plant meadow	40°54'17" N, 31°09'00" E
9 (L1)	*Fagus sylvatica*	40°54'24" N, 31°8'41" E	24 (L3)	*Corylus avellana*	40°54'17" N, 31°09'20" E
10 (L1)	*Quercus robur*	40°54'24" N, 31°8'40" E	25 (L3)	*Malus* spp.	40°54'17" N, 31°09'05" E
11 (L2)	Wild plant meadow	40°54'22" N, 31°8'51" E	26 (L3)	*Pinus sylvestris*	40°54'15" N, 31°9'07" E
12 (L2)	Wild plant meadow	40°54'22" N, 31°8'52" E	27 (L3)	*Pinus sylvestris*	40°54'13" N, 31°09'08" E
13 (L2)	Wild plant meadow	40°54'23" N, 31°8'51" E	28 (L3)	*Corylus avellana*	40°54'10" N, 31°09'10" E
14 (L2)	Wild plant meadow	40°54'23" N, 31°8'51" E	29 (L3)	*Corylus avellana*	40°54'09" N, 31°09'10" E
15 (L2)	Wild plant meadow	40°54'23" N, 31°8'52" E	30 (L3)	*Corylus avellana*	40°54'07" N, 31°09'12" E

Nematode extraction

The modified Baermann (1917) funnel technique was used to extract nematodes. Rock and large organic particles were separated from the samples. A 100-g subsample of soil was used for extraction. Samples were incubated on plastic mesh sieves covered with paper towel. Live nematodes were collected after 48 h and placed in 4% formalin solution. Then, each extract was labeled with the relevant sample number and transferred to Düzce University Faculty of Agriculture, Department of Agricultural Biotechnology Nematology Laboratory in plastic tubes.

Slide preparation of nematodes

After obtaining the nematodes, all nematodes were rinsed with distilled water to remove possible residues and transferred to 1.25 cm deep staining blocks containing 96% ethanol and incubated at 40°C. Then, a few drops of glycerol in 4% formalin (1:99) were added and left at room temperature overnight. The next morning, a few drops of glycerol in 95% ethanol were added and two-thirds of its cavity was covered with a glass lid. A few drops of the glycerol-ethanol (5:95) solution were added every 2 h for a gradual transition to glycerol. At the end of the day, two drops of glycerol-ethanol (50:50) were added to the staining block (Seinhorst, 1959). The next day, individual nematodes were covered with glycerol and permanent glass slides prepared (Yoder et al., 2006).

Identification and data analyses

Identification and observations of nematodes were made under an Olympus CH microscope (Olympus Optical, Tokyo, Japan). Using the keys of the classification provided by De Ley and Blaxter (2004) with additional information from Hodda et al. (2006) and Andrássy (2002, 2005, 2009), nematodes were identified to genus level. Nematode life cycle properties according to colonizer-persister classification (1-5) were obtained based on Bongers (1990) and Bongers and Ferris (1999). The classification of feeding types was established in line with to Yeates et al. (1993). To indicate the maturity degree referring to the nematode community composition in the ecosystem, several maturity indices were calculated according to Ferris & Bongers (2009). The nematode indicator joint analysis calculation system (Sieriebriennikov et al., 2014) was used for diagnostics on feeding types, maturity indices and food web structure. Diversity indices were calculated by performing the Shannon-Wiener index and Simpson Index.

Results and Discussion

A total of 1,388 nematodes have been collected and identified. These belonged to 30 species, 25 genera, 17 families and 6 orders (Table 2). About 26 to 76 individuals per 100 g of soil were collected from each sampling site (Table 3).

Table 2. Number of nematodes taxa collected from Prusias ad Hypium, Türkiye

Class	Order/Suborder	Family	Genera	Species	Specimen
Enoplea	Enoplida	1	1	1	2
	Mononchida	1	2	2	65
	Dorylaimida	6	8	10	522
Chromadorea	Monhysterida	1	3	4	269
	Plectida	2	3	3	359
Rhabditida	Tylenchina	6	8	10	171
Total		17	25	30	1388

Table 3. Species abundance and occurrence

Genus Name	Phylum: Family	Total Abundance	Relative Abundance	% Occurrence	C-p Class
Anaplectus Coninck & Schuurmans Stekhoven, 1933	Plectidae	256	18.5	100	2
Mesodorylaimus Andrássy, 1959	Dorylaimidae	212	15.3	93	4
Geomonhystera Andrássy, 1981	Monhysteridae	206	14.9	86	2
Eudorylaimus Andrássy, 1959	Dorylaimidae	150	10.8	83	4
Plectus Bastian, 1865	Plectidae	99	7.14	83	2
Panagrolaimus Fuchs, 1930	Rhabditidae	63	4.55	53	1
Monhystera de Man, 1881	Monhysteridae	59	4.26	30	2
Aporcelaimus Thorne et Swanger, 1936	Aporcelaimidae	44	3.17	30	5
Tylencholaimus De Man, 1876	Tylencholaimidae	41	2.96	40	4
Eucephalobus Steiner, 1936	Cephalobidae	38	2.74	30	2
Belondira Thorne, 1939	Belondiridae	34	2.45	30	0
Mylonchulus Cobb, 1916	Mylonchulidae	33	2.38	46	4
Clarkus Jairajpuri, 1970	Mononchidae	32	2.31	37	4
Hoplolaimus Daday, 1905	Hoplolaimidae	23	1.66	17	0
Aporcelaimellus Heyns, 1965	Aporcelaimidae	21	1.52	20	5
Pungentus Thorne & Swanger, 1936	Nordiidae	20	1.44	23	4
Tylenchorhynchus Cobb, 1913	Tylenchida	18	1.30	7	0
Aphelenchoides Fischer, 1894	Aphelenchida	16	1.15	17	2
Tylenchus Bastian, 1865	Tylenchida	9	0.65	10	0
Eumonhystera Andrássy, 1981	Monhysteridae	4	0.29	13	2
Alaimus de Man, 1880	Alaimidae	4	0.29	3	4
Acrobeloides Cobb, 1924	Cephalobidae	3	0.22	10	2
Tylocephalus Crossman, 1933	Plectidae	2	0.14	7	2
Cervidellus Thorne, 1937	Cephalobidae	1	0.07	7	2

Community analysis

The mean of Shannon-Wiener index from 30 samples was 1.74 ± 0.15 with this index indicating higher diversity in the sampling zone around the town (1.86 ± 0.24), then around Prusias ad Hypium (1.73 ± 0.42) and the forested area (1.62 ± 0.18). The average value of the maturity index was 2.86 ± 0.21. However, any significant differences based on ANOVA ($p > 0.05$) were detected in terms of diversity and maturity indices between the sampling sites. Different maturity indices (sigma maturity and maturity 2-5) showed a similar trend of variability on the different sampling sites (average value of sigma maturity was 2.90 ± 0.22, and maturity 2-5 was 2.94 ± 0.19). Enrichment Index (20 ± 11) showed a high variability between the sampling sites. Structure index (74 ± 7.0) revealed that most of the samples (83%) shows highly mature soil profile.

There were five samples (7, 11, 12, 19 and 26) with semi-endoparasites predominant among the plant parasites, two samples (15 and 23) with epidermal/root hair-feeders predominant and in the remaining samples the ectoparasites were predominant a (Table 4).

Table 4. Maturity, maturity 2-5, Shannon-Wiener, sigma maturity, enrichment and structure indices of all sampling sites

Sample	Index					
	Maturity	Maturity 2-5	Sigma Maturity	Shannon-Wiener	Enrichment	Structure
1	3.4	3.5	3.4	1.9	26.7	91.1
2	2.6	2.6	2.6	1.9	0	62.6
3	2.6	2.8	2.6	1.7	35.3	71.2
4	2.5	2.6	2.6	1.2	21.1	61.5
5	3.6	3.7	3.6	1.9	34.8	91.7
6	3.0	3.0	3.0	1.2	0	80.4
7	3.2	3.2	3.2	1.8	0	84.7
8	2.7	3.0	2.7	2.1	55.0	78.3
9	3.9	3.9	4.3	1.8	0	97.8
10	2.2	2.2	2.2	0.8	0	32.8
11	2.8	2.8	2.9	1.6	0	74.1
12	3.5	3.5	3.5	1.7	0	92.3
13	2.9	2.9	2.9	1.6	0	75.3
14	2.4	2.5	2.4	1.7	25.8	53.8
15	2.6	2.6	2.5	1.9	13.3	64.9
16	2.9	2.9	2.9	1.4	13.8	77.9
17	2.4	2.5	2.6	1.8	25.8	54.9
18	2.1	2.5	2.1	1.6	71.6	59.6
19	2.7	3.1	2.8	2.1	64.9	82.2
20	3.0	3.1	3.1	1.9	25.0	81.7
21	3.1	3.2	3.1	2.1	13.3	81.8
22	2.6	2.8	2.6	2.2	47.8	71.5
23	2.6	2.8	2.6	1.9	40.0	71.0
24	2.9	3.3	2.9	1.8	71.4	88.5
25	2.9	2.9	3.2	2.0	0	76.3
26	2.6	2.6	2.6	1.8	23.3	61.0
27	3.1	3.1	3.1	1.5	0	82.5
28	3.1	3.1	3.1	1.5	0	81.9
29	3.3	3.3	3.3	2.0	0	85.6
30	2.5	2.5	2.6	1.7	3.9	56.1
Mean ± SD	2.86 ± 0.21	2.94 ± 0.19	2.9 ± 0.22	1.74 ± 0.15	20 ± 11	74 ± 7

Feeding type composition

For each sample, the relative abundance of feeding types is presented (Figure 2). Bacterivorous nematodes dominated the community (53%) in all sampling sites. This was followed by omnivores (32%), plant parasites (6%), fungivores (5%) and predatory nematodes (5%). There was no clear correlation between nematode communities and ecological characteristics. Omnivore and predatory nematodes were detected more frequently in sampling sites of similar ecological characteristics. Fungivore nematodes were of low abundance in all sampling zones.

Of the plant parasitic nematodes, the ectoparasites were the most abundant (56%) followed by semi-endoparasites (29%). Epidermal/root hair feeders were the third most common group at 15% and algal/lichen/moss feeders are following with 9.5%. Migratory endoparasites and sedentary endoparasites were not found in any samples.

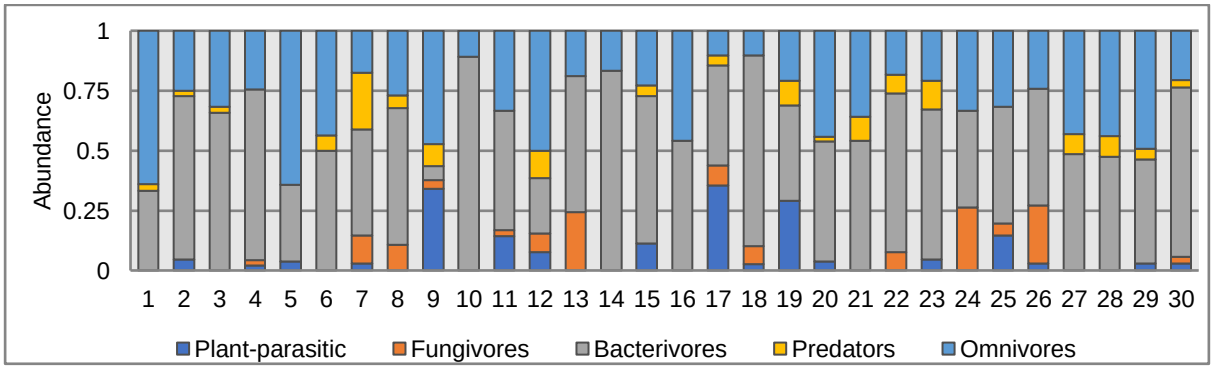

Figure 2. Relative abundance of feeding types in the sampling sites.

Community structure of free-living nematode assemblages

Based on the life cycle properties, free-living nematodes were classified from c-p 1 (colonizers, enrichment opportunists) to c-p 5 (persisters with long life cycle) groups. The free-living nematode community in Prusias ad Hypium did has a recognizable pattern. Opportunistic taxa (c-p = 1) are re-evaluated because they are considered enrichment opportunists and their population densities increase rapidly in response to addition of nutrients to soil and may not necessarily reflect long-term changes in soil ecological condition. Those with c–p values between 2 and 5 are more stable temporally and may provide relatively long-term information about environmental conditions (Hodda et al., 2006). Sampling sites were dominated by colonizer nematodes with c-p value 1-2 (57%). Enrichment opportunist nematodes with c-p value 4-5 were present 43% of the sampling sites (Figure 3).

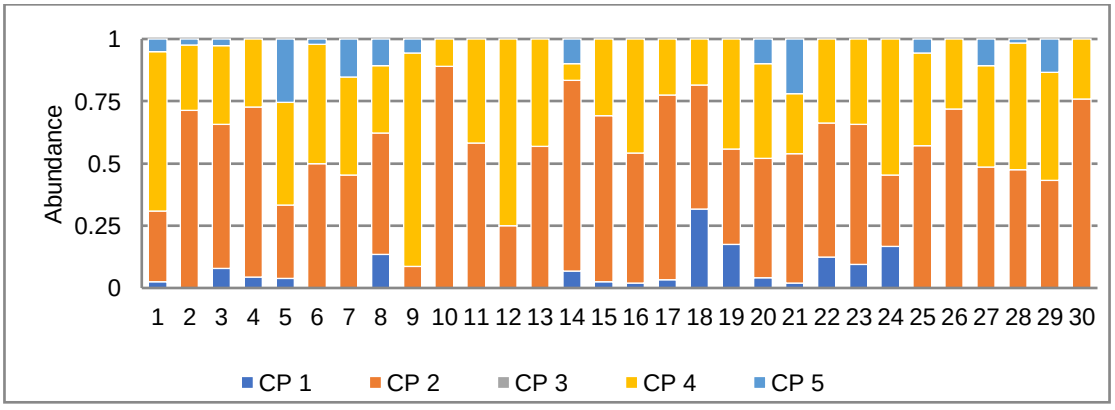

Figure 3. Coloniser-persister structure of free-living nematode assemblages. Maturity Indices (MI) are computed as a weighted mean frequency, $\sum\left(\frac{vi\ X\ fi}{n}\right)$ where *vi* is the c–p value assigned to family, *fi* is the frequency of family *i* in sample, and *n* is the total number of individuals in a sample (Bongers, 1990).

Food web analysis

The analysis of food web structure using the colonizer-persister maturity concept allows evaluation soil community structure and enrichment state of the samples (Ferris et al., 2001). Only one sample (10) showed a poor profile in terms of the structure of the soil. Four of 30 samples resulted in position within the maturing, N-enriched, low C:N cycle, high bacterial suppressive and regulated soil classification, while 25 soil samples resulted within a matured, fertile, moderate cycle of C/N, bacterial/fungal and suppressive soil type (Figure 6).

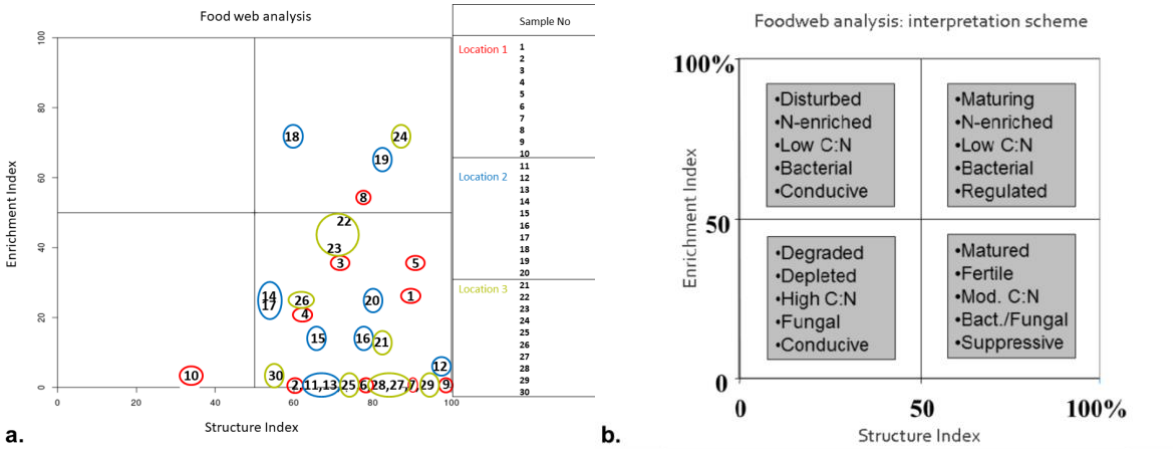

Figure 4. a) Food web analysis of 30 sample sites and b) the interpretation scheme (Ferris et al., 2001).

Discussion

Faunistic

In recent years, there have been a global trend to examine how nematode communities behave in the soil food web by monitoring their role in the ecosystem. In Türkiye, soil nematode diversity, especially free-living forms, are so far poorly investigated. This study aimed to determine soil nematode fauna in one location of the West Black Sea Region using a holistic approach and to identify all forms of soil nematodes. *Anaplectus, Cervidellus, Pungentus* and *Tylocephalus* genera were some of a few records that have been reported in the entire country. This original study, therefore not only significantly expands on what is known about nematode genera in Türkiye, but also improves knowledge of the geographic records in the Western Black Sea Region of Türkiye.

Previously, Yıldız et al. (2021) and Imren et al. (2015) investigated nematode diversity in three habitat types around Bolu Province, Türkiye. Most of the genera has similar patterns in both Düzce and Bolu but they represented by different species in general. Although most of these taxa are globally distributed, it is clear that most of the free-living nematode species are not well known in Türkiye. Regarding the total diversity, this study provides a contribution on the importance of faunistic studies of soil nematodes.

Species distribution

An integrative approach to the species relative abundance and occurrence was used for the determination of species distribution patterns. The outcomes of this study revealed a widespread group of nematodes (*Anaplectus, Mesodorylaimus, Geomonhystera, Eudorylaimus* and *Plectus*) that were most abundant and frequent, and this contrast with the least abundant/frequetn group (*Eumonhystera, Alaimus, Acrobeloides, Tylocephalus* and *Cervidellus*). However, some genera had different distributions. For example, *Hoplolaimus* and *Tylenchorhynchus* may occur abundantly but less frequently within the samples since these taxa occur with high numbers in a limited number of soil samples. Host plant availability may explain this situation as this is the main limiting factor for the distribution of plant parasitic nematodes.

Nematode abundance

The environmental variables are optimal for a nematode survey during the late summer season according to Yeates & Bird (1994) and Stamou et al. (2005). In this survey, the total nematode abundance ranged from 26 to 76 individuals per 100 g of soil, so the sampling time was suitable. The faunistic results, however, show similarities with the characteristics of a temperate humid climate and environmental features at the genus level (Andrássy, 2005; 2009). However, future identification to species level might demonstrate variation and richness within these genera.

Nematode community

The trophic groups in the nematode assemblages varied between the sampled zones. Mean abundance and fraction of bacterivorous nematodes did not differ significantly between sites (Figure 2). A high number of bacterivorous nematodes were found in all sampling sites. The order Dorylaimida appeared as the most diverse group in terms of genera richness. Predatory nematodes were found at all sampling sites and are classified as a persister group, which have a long life and tend to occur at mature and fertile soils. Also, little variation was seen in the abundance of omnivorous, fungivorous and plant parasitic nematodes. Also, maturity indices had similar values at all sites and did not differ significantly between sites. Schnürer et al. (1986) and Yeates (2007) considered that the effect of local, regional, and seasonal differences (soil chemical differences, texture and structure, moisture, and organic matter) and environmental disturbances caused by humans were the most important factors affecting the nematode community. Although Prusias ad Hypium is a protected area, such disturbances may affect the nematode community structure around the town, since it is a permanent settlement for humans. Overall, nematode assemblages showed similar trends by means of maturity indices between 30 sampling sites which is divided into 3 groups namely forest (1–10), farms (11–20) and fruit orchards (21–30). The impact of environmental changes did not show any consistent pattern at the surroundings of Prusias ad Hypium Ancient City. The soil properties may be studied further for explaining this matter.

Soil food web analysis

Nematodes have many advantages as a bioindicators in assessing soils. Nematodes high abundance make statistically valid sampling possible and their broad range of biodiversity facilitates observing expanded physiologies and feeding types that are spread to any environmental conditions worldwide. This definitely positions them at a key positions in the food webs of soil. Their high range of tolerance occurs under all climatic conditions in pristine to highly polluted habitats; include tolerant species and sensitive species. Life-cycle times ranging from 6 days to over 2 years, and rapid responses to disturbance and enrichment provides for wide perspectives when monitoring soil and biological indicators for changes in the environment and soil health.

Soil food web analysis revealed the dominance of highly structured and enriched soil profiles around the Prusias ad Hypium according to nematofauna data (Ferris et al., 2001). Most of the samples clustered within the moderate C:N cycle and suppressive profile of soil structure, which indicates high similarities on the soil characteristics of the area around the antique city. This contribution provides data on the fauna of nematodes in Prusias and its surroundings, the structure of trophic groups, colonizer-persister groups in the nematode community, maturity and soil food web analysis which reveals that the knowledge related to the diversity of nematodes might give beneficial for conservation of biodiversity and soil health monitoring.

Acknowledgments

The essential help with the identification of the order Dorylaimida kindly provided Prof. Reyes Peña Santiago from the University of Jaen, Spain, is most appreciated.

References

Andrássy, I., 2002. “Free-Living Nematodes from the Fertő-Hanság National Park, Hungary, 21-97”. In: The Fauna of The Fertő-Hanság National Park, Budapest, Hungary (Ed. S. Mahunka), 829 pp.

Andrássy, I., 2005. Free-Living Nematodes of Hungary, I (Nematoda Errantia). Hungarian Natural History Museum and Systematic Zoology Research Group of the Hungarian Academy of Science, Budapest, Hungary, 518 pp.

Andrássy, I., 2009. Free-Living Nematodes of Hungary (Nematoda Errantia), Vol. III. Hungary, Hungarian Natural History Museum, 608 pp.

Baermann, G., 1917. Eine einfache methode zur auffindung von Ancylostomum (Nematoden) larven in Erdproben. Geneeskd Tijdschr Ned Indie, 57: 131-137 (in German).

Bongers, T. & H. Ferris, 1999. Nematode community structure as a bioindicator in environmental monitoring. Trends in Ecology & Evolution, 14 (6): 224-228.

Bongers, T., 1990. The maturity index: an ecological measure of environmental disturbance based on nematode species composition. Oecologia, 83 (1):14-19.

Bongers, T. & R. G. M de Goede, 2001. A framework for soil food web diagnostics: extension of the nematode faunal analysis concept. Applied Soil Ecology, 18 (1): 13-29.

Bongers, T., H. van der Meulen & G. Korthals, 1997. Inverse relationship between the nematode maturity index and plant parasite index under enriched nutrient conditions. Applied Soil Ecology, 6 (2): 195-199.

De Ley, P. & M. Blaxter, 2005. A new system for Nematoda: combining morphological characters with molecular trees, and translating clades into ranks and taxa. Nematology Monographs & Perspectives, 2 (1): 633-653.

Demirsoy, A., 1998. Yaşamın Temel Kuralları: Omurgalılar. Meteksan, Ankara, Volume I, Part II, 830 pp (in Turkish).

Ferris, H. & T. Bongers, 2009. "Indices for Analysis of Nematode Assemblages, 124-145". In: Nematodes as Environmental Bioindicators (Eds. M. Wilson & T. Kakouli-Duarte). CABI, Wallingford, USA, 352 pp.

Ferris, H., T. Bongers & R. G. de Goede, 2001. A framework for soil food web diagnostics: extension of the nematode faunal analysis concept. Applied Soil Ecology, 18 (1): 13-29.

Hodda, M., A. Ocana & W. Traunspurger, 2006. "Nematodes from Fresh Water Habitats, 179-211". In: Freshwater Nematodes: Ecology and Taxonomy (Eds. E. Abebe, I. Andrássy & W. Traunspurger). CABI, Wallingford, USA, 752 pp.

Hugot, J. P., P. Baujard & S. Morand, 2001. Biodiversity in helminths and nematodes as a field of study: an overview. Nematology, 3 (3): 199-208.

Imren, M., Ş. Yıldız, E. Kasapoğlu, H. Toktay, H. Kütük & A. A. Dababat, 2015. "The plant-parasitic nematodes associated with cereal crops In Bolu, Turkey, 131-140". Proceedings of the Nematodes of Small Grain Cereals: Current Status and Research, Fifth International Cereal Nematode Initiative Workshop (12-15 September 2015, Ankara, Turkey), (Eds. A. A. Dababat, H. Muminjanov & R. W. Smiley), Food and Agriculture Organization of United Nations, Ankara, Turkey, 384 pp.

Lee, D. L., 2002. The Biology of Nematodes. CRC Press, 338 pp.

Schnürer, J., M. Clarholm, S. Boström & T. Rosswall, 1986. Effects of moisture on soil microorganisms and nematodes: a field experiment. Microbial Ecology, 12 (2): 217-230.

Sieriebriennikov, B., H. Ferris & R. G. M. de Goede, 2014. NINJA: An automated calculation system for nematode-based biological monitoring. European Journal of Soil Biology, 61 (1): 90-93.

Stamou, G. P., E. M. Papatheodorou, A. Hovardas & M. D. Argyropoulou, 2005. Some structural and functional characteristics of a soil nematode community from a Mediterranean grassland. Belgian Journal of Zoology, 135 (2): 253-259.

Wilson, M. J. & T. Khakouli-Duarte, 2009. Nematodes as Environmental Indicators. CABI, USA, 326 pp.

Yeates, G. W. & A. F. Bird, 1994. Some observations on the influence of agricultural practices on the nematode faunae of some South Australian soils. Fundamental & Applied Nematology, 17 (2): 133-145.

Yeates, G. W., T. Bongers, R. G. M. De Goede, D. W. Freckman & S. S. Georgieva, 1993. Feeding habits in soil nematode families and genera-an outline for soil ecologists. Journal of Nematology, 25 (3): 315-331.

Yeates, G., 2007. Abundance, diversity, and resilience of nematode assemblages in forest soils. Canadian Journal of Forest Research, 37 (2): 216-225.

Yıldız, Ş., G. Emine, Ö. Göksel & M. İmren, 2021. Investigations on soil nematode diversity in three contrasting habitat types in Bolu, Turkey. Turkish Journal of Entomology, 45 (4): 451-461.

Yoder, M., I. T. De Ley, I. Wm King, M. Mundo-Ocampo, J. Mann, M. Blaxter, L. Poiras & P. De Ley, 2006. DESS: a versatile solution for preserving morphology and extractable DNA of nematodes. Nematology, 8 (3): 367-376.

Zhang, M., W. J. Liang & X. K. Zhang, 2012. Soil nematode abundance and diversity in different forest types at Changbai Mountain, China. Zoological Studies, 51 (5): 619-626.

Türk. entomol. derg., 2022, 46 (2): 149-158
DOI: http://dx.doi.org/10.16970/entoted.1039327

ISSN 1010-6960
E-ISSN 2536-491X

Original article (Orijinal araştırma)

Influence of dietary indole-3-acetic acid on phenoloxidase and hemolytic activities in *Pimpla turionellae* L., 1758 (Hymenoptera: Ichneumonidae) and *Galleria mellonella* L., 1758 (Lepidoptera: Pyralidae) in a host-parasitoid system[1]

Gülşah BECERİKLİ AKSAN[2] **Fevzi UÇKAN[2]** **Aylin ER[3*]**

Indole-3 asetik asit'in *Pimpla turionellae* L., 1758 (Hymenoptera: Ichneumonidae) ve *Galleria mellonella* L., 1758 (Lepidoptera: Pyralidae)'nın konukçu-parazitoit sisteminde fenoloksidaz ve hemolitik aktivitelerine beslenme yoluyla etkileri

Abstract

The continuity of food demand has caused modern agriculture to be heavily dependent on chemical inputs. Plant growth regulators (PGRs) are naturally occurring or synthetic compounds that have the potential to control pest insects through their chemosterilant activity. Along with pests, non-target organisms such as parasitoids in agroecological systems are likely to be influenced by indirect contact via their hosts or direct contact with hosts and plants at the tritrophic level. This study demonstrated the dietary effects of PGR indole-3-acetic acid (IAA) on hemolytic activity and phenoloxidase activity in stored product pest *Galleria mellonella* L., 1758 (Lepidoptera: Pyralidae) and parasitoid *Pimpla turionellae* L., 1758 (Hymenoptera: Ichneumonidae). The significant increase in hemolytic activity in *G. mellonella* and *P. turionellae* hemolymph were observed in 500, 5,000 and 10,000 ppm IAA-treated groups. Phenoloxidase activity in *G. mellonella* hemolymph significantly decreased with all IAA doses compared to control, however, the reductions in phenoloxidase activity in *P. turionellae* hemolymph were significant between 500 and 10,000 ppm. Since IAA interacts with both host and parasitoid immunity, it should be used with caution in agricultural areas with high host-parasitoid population.

Keywords: *Galleria mellonella*, hemolytic activity, Indole-3-acetic acid, phenoloxidase activity, *Pimpla turionellae*

Öz

Gıda ihtiyacının sürekliliği, modern tarımın büyük ölçüde kimyasal girdilere bağımlı hale gelmesine neden olmuştur. Bitki büyüme düzenleyicileri, kemosterilant aktiviteye sahip doğal olarak oluşan veya sentetik bitki kaynaklı kimyasallardır ve zararlı böcekleri baskılama potansiyeline sahiptir. Zararlılarla birlikte, agroekolojik sistemlerdeki parazitoitler gibi hedef olmayan organizmaların, konukçuları aracılığıyla dolaylı temastan veya konukçu ve bitkilerle tritrofik etkileşim yoluyla doğrudan temastan etkilenmesi muhtemeldir. Bu çalışma bitki büyüme düzenleyicisi indol-3-asetik asidin (IAA) depolanmış ürün zararlısı *Galleria mellonella* L., 1758 (Lepidoptera: Pyralidae) ve parazitoit *Pimpla turionellae* L., 1758 (Hymenoptera: Ichneumonidae)'nın hemolitik aktivitesi ve fenoloksidaz aktivitesi üzerindeki besinsel etkilerini göstermektedir. *Galleria mellonella* ve *P. turionellae* hemolenfinin hemolitik aktivitelerinde istatistiksel olarak önemli artışlar 500, 5000 ve 10000 ppm IAA dozları tatbik edilen gruplarda gözlenmiştir. *Galleria mellonella* hemolenfinin fenoloksidaz aktivitesi, kontrole kıyasla tüm IAA uygulanan dozlarda önemli ölçüde azalırken, *P. turionellae* hemolenf fenoloksidaz aktivitesindeki azalmalar 500 ve 10000 ppm arasında anlamlı bulundu. IAA, hem konukçu hem de parazitoit bağışıklığı ile etkileşime girdiğinden, konukçu-parazitoit popülasyonu yüksek olan tarım alanlarında dikkatle kullanılmalıdır.

Anahtar sözcükler: *Galleria mellonella*, hemolitik aktivite, Indole-3-asetik asit, fenoloksidaz aktivite, *Pimpla turionellae*

[1] This study was supported by Kocaeli University, Scientific Research Unit, Kocaeli, Türkiye, Grant Project No: 2014/068.
[2] Kocaeli University, Faculty of Science and Literature, Department of Biology, 41380, Umuttepe, Kocaeli, Türkiye
[3] Balıkesir University, Faculty of Science and Literature, Department of Biology, 10100, Çağış, Balıkesir, Türkiye
* Corresponding author (Sorumlu yazar) e-mail: asahin@balikesir.edu.tr
Received (Alınış): 29.12.2021 Accepted (Kabul ediliş): 24.05.2022 Published Online (Çevrimiçi Yayın Tarihi): 01.06.2022

Introduction

The term chemical control in agricultural systems covers a wide range of substances including herbicides, insecticides, rodenticides, fungicides, plant growth regulators and others. The indiscriminate uses of chemical compounds against insect pests possess hazards to non-target organisms due to the accumulation of chemicals in nature and their toxic effects on the environment (Brzozowski & Mazourek, 2018). The repeated applications of insecticides lead to the transmission of genes encoding rapid biochemical detoxification processes on the succeeding generations. Eventually, the agricultural pest populations can become totally immune and resistant, and not controlled effectively. The problems caused by chemical control increase the importance of biological control. Among the agents used in biological control, parasitoids are perhaps the most suitable, least risky and most effective (Wang et al., 2019). However, during chemical control, these non-target beneficial insect species with parasitoid character are also directly or indirectly exposed to various chemicals. Concerns about chemicals in the natural environment and their possible ecotoxic risks have focused more on pesticides. In addition to pesticides, other chemicals to which parasitoids are indirectly and / or directly exposed are plant growth regulators (PGRs) (Uçkan et al., 2008, 2011a, b). Many authors have reported that PGRs have the potential to diminish pest insect populations by influencing reproduction, development, fecundity and egg viability (Er & Keskin, 2016; Zhao et al., 2017), to effect biochemical and histological parameters (Abdellaoui et al., 2013), and induce oxidative stress (Altuntaş, 2015). Indole-3-acetic acid (IAA) is such a commercial PGR and also a common endogenous auxin class synthesized from an amino acid tryptophan (Ahmad et al., 2005). IAA is also known as a universal signal molecule existing in a large range of organisms such as bacteria, fungus, plants and animals, including mammals (Lins et al., 2006; Kaya et al., 2021b). The various commercial and exogenous uses of IAA in various crops and its physiological impacts on plants can negatively affect plant-insect interactions (Prado & Frank, 2013). These compounds are used not only to altered agronomic characteristics in plant growth processes, but also as chemosterilants for harmful insects (Abdellaoui et al., 2013; Özyılmaz et al., 2019). Despite their widespread use in agriculture, limited numbers of studies are included in literature and still more work needs to be done concerning their side-effects on non-target parasitoids, pest insects and their interactions in tritrophic contexts (Prado & Frank, 2013). Detrimental effects of IAA on insect survival, metabolism and reproduction, life table and some biochemical parameters have been particularly demonstrated in different agricultural pests (Uçkan et al., 2011a, 2014, 2015). IAA has shown to reduce longevity and increase larval development time of the biocontrol agent endoparasitoid *Apanteles galleriae* Wilkinson, 1932 (Hymenoptera: Braconidae) reared on its host the lesser wax moth *Achroia grisella* (Fabricius, 1794) (Lepidoptera: Pyralidae) (Uçkan et al., 2011a). Dose-responsive influences of IAA on total protein, lipid, and glycogen levels on *A. galleria* were also demonstrated (Uçkan et al., 2014). Adverse effects of plant growth regulators on life table parameters and biochemical markers in a host-parasitoid interaction suggest that PGRs have the potential to influence the functioning of immune system in pest insects and their parasitoids. In addition, recent studies showed elevated total hemocyte counts in *A. grisella* (Çelik et al., 2017) and *Galleria mellonella* L., 1758 (Lepidoptera: Pyralidae) (Kaya et al., 2021b) due to IAA application interrelated with the increased granulocyte incidences, but no changes in the parasitoid *Pimpla turionellae* L., 1758 (Hymenoptera: Ichneumonidae) hemocyte counts that are the main components of the insect cellular immunity (Kaya et al., 2021b).

Insect innate immune system relies on humoral and cellular components that function interactively in order to provide a response for pathogen infections and foreign materials such as parasitoid eggs (Strand, 2008). Cellular components consist of hemocytes that are analogous of macrophages in vertebrates having multiple functions such as nodulation, encapsulation, and phagocytosis (Wojda, 2017). While humoral immunity entails enzymatic cascades, which orchestrate coagulation and melanization reactions (Gillespie et al., 1997), synthesis of antimicrobial peptides, intermediates of reactive oxygen and nitrogen, and lipid peroxidation (Altuntaş et al., 2021). Among them one important enzyme in insect

immunity is phenoloxidase (PO) that is responsible for the conversion of phenols to quinones which subsequently turn into melanin form against pathogens and parasitoid eggs (Eleftherianos & Revenis, 2011). In the process melanogenesis both intermediary products such as superoxide, quinones, diphenols, hydrogen peroxide and PO have unique roles in humoral immunity (Santoyo & Aguilar, 2011). Several studies have demonstrated that cellular immunity of insects and the survival ability under stress conditions are susceptible to different environmental factors such as botanical sourced xenobiotics (Zibaee & Bandani, 2010; James & Xu, 2012; Altuntaş et al., 2021). However, our knowledge on the effects of plant-derived compounds concerning the PO enzyme, which is an important component of both cellular and humoral immunity, is mostly dependent on plant extracts or essential oils. Zibaee & Bandani (2010) demonstrated the negative effect of *Artemisia annua* L. (Asterales: Asteraceae) extract on nodule formation and PO activity in *Eurygaster integriceps* Puton, 1881 (Hemiptera: Scutelleridae) (Zibaee & Bandani, 2010) whereas Kaya et al. (2021b) showed an increase in PO activity in *G. mellonella* due to injection with *Helichrysum arenarium* L. (Asterales: Asteraceae) extract. A dose-dependent increase was also observed in PO enzyme activity in non-mulberry silkworm *Antheraea assamensis* Helfer, 1837 (Lepidoptera: Saturniidae) with topical application of essential oils obtained from *Ocimum sanctum* L. (Lamiales: Lamiaceae), *Ocimum gratissimum* L. (Lamiales: Lamiaceae) and *Ageratum conyzoides* L. (Asterales: Asteraceae). However, we could not find any report demonstrating the effects of a plant growth regulator IAA on PO activity in *G. mellonella* and its parasitoid *P. turionellae*. Another parameter thought to be related to humoral immunity is hemolytic activity which also represents the common initial toxicity assessment (Greco et al., 2020). Several studies demonstrated the hemolytic activity in hemolymph in many invertebrate groups including *G. mellonella* (Phipps et al., 1989). Also, fluctuations of hemolytic activity paralleled the pattern of antimicrobial activity (Phipps et al., 1989) that is one of the main characteristics of insect immunity. Nevertheless, to our knowledge, there are no reports on the effects of plant growth regulators on hemolytic activity in insect hemolymph.

Galleria mellonella is a ubiquitous pest of honeycombs and honey bee colonies and also used as a powerful model organism to test the ecotoxicological, immune and physiological effects of environmental pollutants (Altuntaş et al., 2021). Endoparasitoid *P. turionellae* uses many agricultural pests that feed on plants for oviposition and it is probable that its larvae developing inside the host may be exposed to agricultural chemicals that accumulates in hosts and adult parasitoids by feeding on honey and fruit nectar. This kind of tritrophic interaction between plants, pest insects and their natural enemies are an integral part of agricultural ecosystems. Understanding the tritrophic interactions is essential for monitoring the roles of parasitoid insects in biological control and IPM programs. IAA is a universal signal molecule and also a widely used plant growth regulator in agricultural systems. Therefore, it is most likely that parasitoid *P. turionellae* may be exposed to IAA via host insects or plants. To further clarify the immune modulator effects of IAA, we studied PO and hemolytic activities in insect hemolymph as indicators of immune function in the model host-parasitoid system of *G. mellonella-P. turionellae*.

Materials and Methods

Insects

Model organism *G. mellonella* were reared at 25 ± 5ºC, 60 ± 5% RH and 12:12 h L:D photoperiod conditions at Kocaeli University Animal Physiology Laboratory. *Galleria mellonella* larva were fed with artificial Bronskill (1961) diet. Parasitoid *P. turionellae* cultures were reared under the same laboratory conditions with their host *G. mellonella*. Individuals of the parasitoids were cultured by parasitizing *G. mellonella* pupae. Obtained *P. turionellae* adults were fed with a 30% honey solution diluted with water daily and kept in wire cages of 20 × 20 × 20 cm. Pupal hemolymph of *G. mellonella* was also provided to parasitoid adults twice a week for obtaining protein by hemolymph feeding.

Experimental groups

IAA (Merck 10 g, Darmstadt, Germany) doses (0, 50, 500, 1,000, 5,000 and 10,000 ppm) were selected according to Kaya et al. (2021b) and used in all experimental analyses. Newly hatched *G. mellonella* larvae were fed with 10 g artificial diet including different doses of IAA. Control experiments included *G. mellonella* larvae fed with diet containing distilled water instead of IAA. Larval feeding process was repeated until the host insects reached last instars. For each control and experimental assay 10 last instar larvae were used in three replicates (n = 30). To determine the influence of IAA on parasitoid hemolytic and phenoloxidase activity last instar IAA-fed host larvae were pupated and provided to parasitoid *P. turionellae* as host for oviposition in three replicates. Parasitization was conducted on day 2 of the IAA-fed *G. mellonella* pupae by exposing one individual pupa to an 15-20-d-old *P. turionellae* female previously determined to lay the highest number of eggs in this period (Şeker & Yanıkoğlu, 1999).

Sampling hemolymph

Ten µl of hemolymph from IAA-fed *G. mellonella* last instar larvae was sampled from the first abdominal proleg. Each larva was pierced on the abdominal proleg with a sterile microneedle and the drawn hemolymph was collected with the aid of a scaled microcapillary tube (Sigma-Aldrich, St Louis, MO, USA). In terms of *P. turionellae*, larvae collected from parasitized host pupae 8 days post-parasitization that is large enough to obtain clear hemolymph (Kaya et al., 2021b) and hemolymph collected with a scaled microcapillary tube. Hemolymph collected from 10 individuals were pooled in an ice-cold 1.5 ml eppendorf tube containing 1 µg N-phenlythiourea (Sigma) and stored at -80 ºC for hemolytic activity.

In parallel set of experiments, 100 µl hemolymph obtained by the same method from 10 individual IAA-fed last instars and *P. turionellae* larval hemolymph dissected from parasitized host pupae were transferred to ice-cold 1.5 ml eppendorf tubes containing 1 µg N-phenlythiourea (Sigma) and used immediately for phenoloxidase enzyme activities without freezing in three replicates.

Assaying hemolytic activity

The rat has been sedated with xylene from the femur head and its cardiac environment was sterilized with alcohol and 3 ml of blood was taken from the heart by intracardiac puncture method using a 21 gauge cannula. The blood was then mixed with sterile Alsever solution at a ratio of 1:5 in a 30-ml Falcon tube and stored at +4ºC (Guzman et al., 1993) (Kocaeli University ethics committee decision no: KOÜ HADYEK 8/2-2013).

Cells were subsequently centrifuged in a salt solution (NaCl 0.15 M) and TBS-Ca (Tris buffered saline: 50 mM Tris-HCl, 100 mM NaCl, pH 7.5 with 10 mM $CaCl_2$) twice at 800 g and 10°C for 10 min and then washed. Finally, erythrocytes were resuspended in 8% (v/v) TBS-Ca. Hemolytic activity was measured by taking 850 µl of TBS-Ca, 100 µl of 8% rat erythrocytes and 50 µl of hemolymph sample. This mixture was incubated at 25°C for 30 min and microfuge for 30 s. The resulting hemoglobin was measured as an end point in the ELISA plate reader at a wavelength of 540 nm. TBS-Ca buffer was used as control. Full hemolysis was achieved by mixing 900 µl of ammonium chloride (0.15 M) with 100 µl of rat erythrocytes (8%). The resulting result gave the percentage of hemolysis (Guzman et al., 1993).

Assaying phenoloxidase activity

Hemolymph from 10 final instars *G. mellonella* larvae and 5 parasitoid larvae was collected for every independent experiment. Eight µl of pooled hemolymph was transferred into 800 of PBS. 100 µl hemolymph PBS mixture from both control and experimental groups was added into a 96-well microplate. 100 µl of 20 mM dihydroxy-L-phenylalanine (L-DOPA, Sigma-Aldrich) dissolved in phosphate buffer solution was added on the hemolymph samples and incubated for 30 min at 25°C. After the incubation, the microplate was read in ELISA plate reader at 490 nm absorbance at intervals of 1 min from 0 to 30 min. PBS was used instead of hemolymph in controls. The data obtained for each subject was determined as U/mg protein. The protein concentration was assayed using the method of Bradford (1976).

Statistical analyses

The obtained experimental results were compared with one-way ANOVA in the SPSS statistical program (SPSS, 2018). Differences between means were evaluated with Tukey HSD. Percentage data was normalized by arcsine transformation before analyses. The results were statistically significant at the $P < 0.05$.

Results

Effects of IAA on hemolytic activities in *G. mellonella* and *P. turionellae* hemolymph

Results obtained from the hemolytic activity assays of *G. mellonella* hemolymph demonstrate that the mean percentage of hemolytic activity was 22 ± 1.6 in control groups. An increase in hemolytic activity was observed in all IAA-treated groups. However, the elevations were only significant in 500, 5,000 and 10,000 ppm compared to control ($F = 92.0$; $df = 5,24$; $p = 0.00$) (Figure 1). In addition, comparisons between doses revealed that 5,000 ppm was more effective than any other dose.

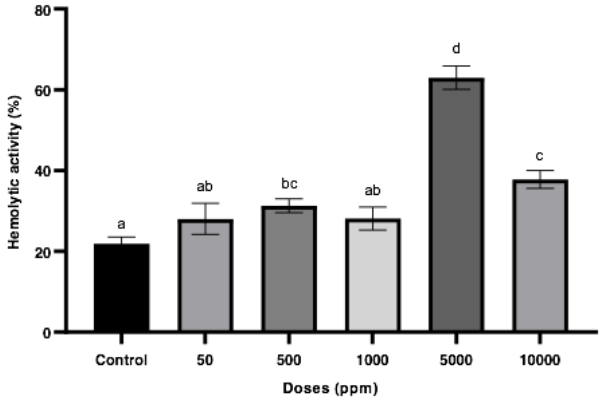

Figure 1. IAA-induced effects on hemolytic activity in *G. mellonella* larval hemolymph. Means with the same letters are not significantly different (one-way ANOVA followed Tukey's HSD, $P < 0.05$).

The hemolytic activity obtained from untreated hemolymph samples of *P. turionellae* displayed 13 ± 3.3. Treatment of different concentrations of IAA resulted in a remarkable increase in hemolytic activity in *P. turionellae* hemolymph (Figure 2). The increase in 500, 5,000 and 10,000 ppm was statistically significant ($F = 86.5$; $df = 5,9$; $p = 0.00$). Maximum hemolytic activity was observed at 5,000 ppm.

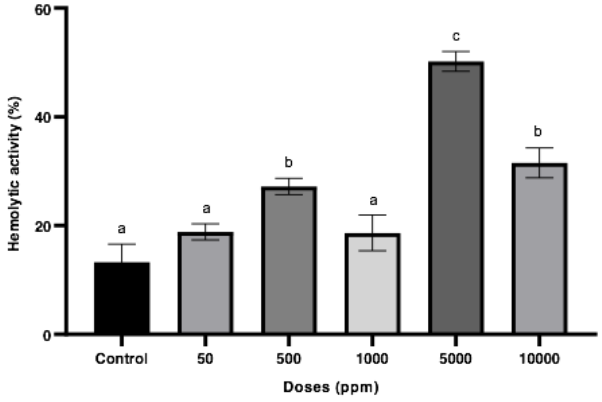

Figure 2. IAA-induced changes on hemolytic activity in *P. turionellae* larval hemolymph. Columns with the same letters do not differ significantly (one-way ANOVA followed Tukey's HSD, $P < 0.05$).

Effects of IAA on phenoloxidase activity in *G. mellonella* and *P. turionellae* hemolymph

The effects of IAA treatment on phenoloxidase enzyme activity in *G. mellonella* hemolymph is shown in Figure 3. Phenoloxidase activity in hemolymph significantly decreased in all IAA-treated doses compared to control ($F = 224$; $df = 5,24$; $p = 0.00$). Among the experimental groups, the lowest phenoloxidase activity was 0.223 u/mg protein at 1,000 ppm (Figure 3).

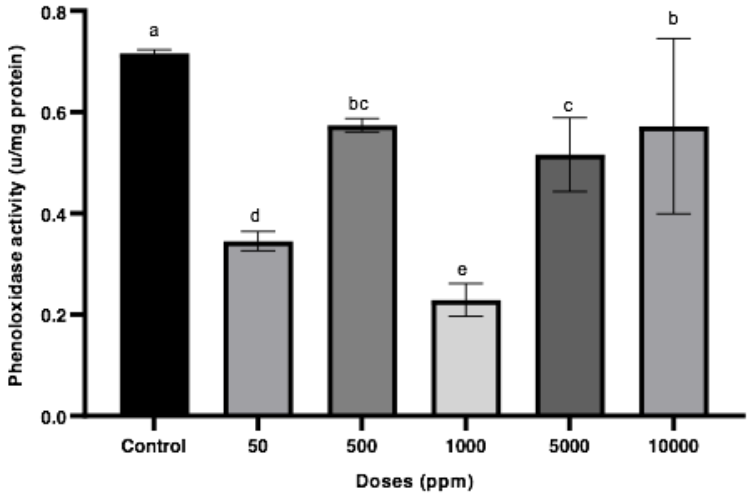

Figure 3. IAA-induced changes on phenoloxidase activity in *G. mellonella* larval hemolymph. Columns with the same letters do not differ significantly (one-way ANOVA followed Tukey's HSD, $P < 0.05$).

Phenoloxidase activity in *P. turionellae* hemolymph treated with different doses of IAA is shown in Figure 4. Phenoloxidase activity in the hemolymph of parasitoid *P. turionellae* decreased with IAA doses more than 50 ppm. However, the reductions were only statistically significant in 500 and 10,000 ppm compared to control ($F = 25.0$; $df = 5,9$; $p = 0.00$). The lowest phenoloxidase activity was determined as 0.04 u/mg at 500 ppm IAA treatment.

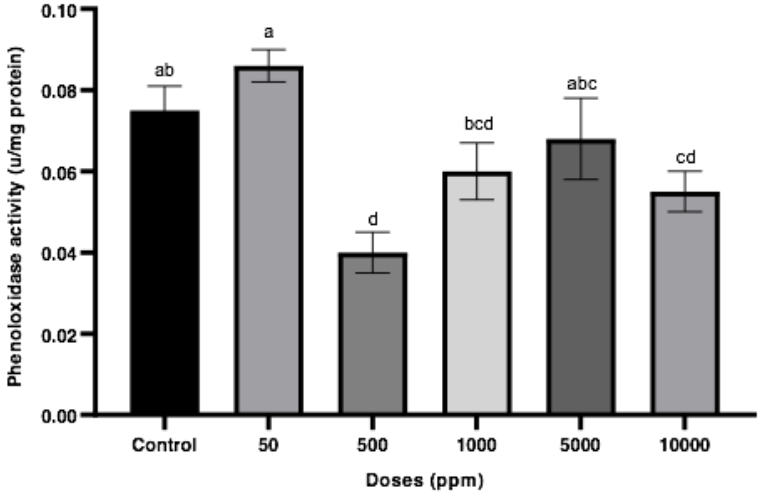

Figure 4. IAA-induced changes on phenoloxidase activity in *P. turionellae* larval hemolymph. Columns with the same letters do not differ significantly (one-way ANOVA followed Tukey's HSD, $P < 0.05$).

Discussion

Recent studies have focused on providing new strategies that can be an alternative to intensive insecticide use in the effective suppression of agricultural pests in agroecological systems. Literature in the last four decades (Ahmad et al., 2003; Ghoneim, 2018) demonstrated that plant growth regulators can be used as an alternative of chemical insecticides that have detrimental effects on human health, environment and non-target organisms. Plant growth regulators are now considered as candidate plant-derived bioinsecticides that have potential for control of pest insects by affecting reproductive potential, hormonal balance, food metabolism and other physiological processes (Mu et al., 2003; Abdellaoui et al., 2013; Özyılmaz, 2019; Kaya et al., 2021a). Along with pest insects, other non-target organisms, such as parasitoids, are likely to be influenced by indirect contact via their hosts or direct contact with plants (Zhao et al., 2017; Kaya et al., 2021a). The present study focused on indirect effects of IAA on hemolytic and phenoloxidase activity in *G. mellonella* and its parasitoid *P. turionellae* developed inside IAA ingested host model.

Data from our experiments showed that nutrient-mediated IAA application caused a dose-dependent increase in hemolytic activity in *G. mellonella* hemolymph and the elevation was significant at 500, 5,000 and 10,000 ppm compared to the control group. Although an increase was observed at 5,000 ppm, there was a sharp decrease in the hemolytic activity in the larval *G. mellonella* at 10,000 ppm. It was discussed in a previous study that high doses of a common plant growth regulator GA_3 might exceed the ability of cellular defense system to response and the toxicological influences of PGRs are variable depending on concentrations and physiological repair mechanisms (Altuntaş, 2015). In combination with previous studies, we can conclude that reduction of this immune response at 10,000 ppm may be associated with a physiological adaptation to compensate for IAA-induced stress. Similar results were obtained in the hemolytic activity in *P. turionellae* final instar larval hemolymph exposed indirectly to IAA via its host on rat erythrocytes. In a previous study it was demonstrated that hemolytic activity in arthropod hemolymph was increased by microbial infection or parasitization associated with insect immunity by lysing cells of invading organisms (Wang et al., 2015). IAA-induced elevated hemolytic activity in *G. mellonella* and *P. turionellae* in our study demonstrates that both the host and the parasitoid contain hemolysin to perform immune function and the expression of hemolytic genes might be affected by IAA. However, it is unlikely that the increased hemolytic activity in both the host and the parasitoid was part of the PO system because, despite the elevated hemolytic activity, we observed reduced PO activity in *G. mellonella* and *P. turionellae* hemolymph. These results indicate that the hemolytic activity is not associated with hemocytes of both insects and further studies are necessary to elucidate the specific hemolysis mechanisms. The cell-free hemolytic activity in *G. mellonella* hemolymph was detected in a previous study and the hemolysin protein of *G. mellonella* was shown to be larger than hemolysins of other invertebrates (Phipps et al., 1989). In the same study conducted on erythrocytes taken from three experimental animals (rabbit, guinea pig and sheep), it was determined that hemolytic activity increased if the host hemolymph was infected with a bacterial species, *Pseudomonas aeruginosa* S. (Pseudomonadales: Pseudomonadaceae) (Phipps et al., 1989). Sasaki et al. (2010) found that the hemolymph of the mosquito *Armigeres subalbatus* (Coquillett, 1898) (Diptera: Culicidae) induced hemolytic activity against human red blood cells and lectin was responsible for the hemolysis of the cells. Even though hemolysis represents one of the most commonly performed toxicity tests (Greco et al., 2020), we could not find any report on hemolytic activity of a plant growth regulator on an agricultural pest and their parasitoids. However, a few studies have demonstrated the adverse effects of IAA on life history traits, immune functions and antioxidant systems of the endoparasitoid *P. turionellae* (Uçkan et al., 2008, 2011a; Kaya et al., 2021b). In addition to the existing studies the present study demonstrates the elevated levels of hemolytic activity in the host depending on IAA application that will damage the parasitoid develop inside the host. These adverse effects will reduce the effectiveness of parasitoids in sustainable agriculture by disrupting the host-parasitoid relationship.

In insects, the phenoloxidase cascade is important for the melanization process (Pech & Strand, 2000) and also an important indicator of immune stimulation. Numerous studies reported the effects of plant growth regulators on cellular immunity of various insect pests (Çelik et al., 2017; Kaya et al., 2021b). However, to our knowledge none of these studies discussed the effects of IAA on phenoloxidase activity in an insect pest and its parasitoid. The obtained data in this study demonstrates that IAA decreased the phenoloxidase activity in both the parasitoid *P. turionellae* and host *G. mellonella*. At the lowest dose of 50 ppm IAA caused nearly 50% reduction of PO activity in *G. mellonella* hemolymph compared to control, however, at higher doses the reductions were more moderate except for 1,000 ppm. Our results are consistent with a previous study demonstrating the sharp reduction in melanization response of *G. mellonella* due to 50 ppm IAA application (Kaya et al., 2021b). In the same study IAA-related elevations of total hemocyte counts was found to be quite remarkable at 50 ppm compared to other doses. Since hemocytes are the predominant source of PO activity in insects, the increase in hemocyte count at 50 ppm in the previous study (Kaya et al., 2021b) seems to be the reason for the increase in PO activity in the current study at the same dose of IAA. Although, IAA was strongly immunosuppressive at 50 ppm on the host insect it had an opposite effect on the parasitoid PO activity at 50 ppm. We suggest a hormetic-like effect triggered by low dose stimulation of toxic materials on the parasitoid *P. turionellae* (Kefford et al., 2008). Low dose stimulation effect of IAA seems advantageous for successful parasitization, however, this is not the case at higher doses. In a previous work it was determined that insect growth regulators buprofezin and pyriproxyfen reduced the phenoloxidase activity in *Spodoptera littoralis* (Boisduval, 1833) (Lepidoptera: Noctuidae) (Nasr et al., 2010). Another report demonstrated the oxidative stress induced by IAA on *G. mellonella* and the parasitoid *P. turionellae* larvae (Özyılmaz et al., 2019). It is obvious that along with pest insects, non-target biological control agents are likely to be affected indirectly from IAA by trophic alteration.

Phenoloxidases are expressed in insects as inactive proPOs and are converted to active PO when required (Santoyo & Aguilar, 2011). PO activation is triggered by a serine protease cascade that is highly dependent on Ca^{2+} concentration or pH, which is somewhat analogous to the coagulation pathway and complement system in human plasma (An et al., 2013). In a previous study, it was demonstrated that IAA can strongly inhibit human blood coagulation with antithrombotic and antiplatelet activities with reduced intracellular Ca^{2+} (Lee et al., 2016). This study showing the IAA-inhibiting effects of coagulation pathway is in line with our study. It is possible that IAA suppresses PO activity in insects by inhibiting the serine proteases and coagulation pathway that is analogous to human plasma. Non-target effects of IAA on developmental biology such as parasitism abilities and rates, emergence rates, immune parameters, and antioxidant defense system were discussed before (Uçkan et al., 2011a, 2014, 2015; Çelik et al., 2017; Zhao et al., 2017; Özyılmaz et al., 2019). These adverse effects on parasitoid insects may be the result of suppressed phenoloxidase activity that is one of the main characters of humoral immunity. Combined with the previous studies demonstrating the adverse effects we can conclude that IAA induced changes on hemolytic and phenoloxidase activities in host and parasitoid insects could be a potential risk on host-parasitoid interactions in biologic control programs.

References

Abdellaoui, K., M. B. Halima-Kamel, N. Soltani, N. Aribi & M. H. B. Hamouda, 2013. Biochemical and histological effects of gibberellic acid on *Locusta migratoria* fifth instar larvae. Pesticide Biochemistry & Physiology, 107 (1): 32-37.

Ahmad, F., I. Ahmad & M. S. Khan, 2005. Indole acetic acid production by the indigenous isolates of azotobacter and fluorescent *Pseudomonas* in the presence and absence of tryptophan. Turkish Journal of Biology, 29 (1): 29-34.

Altuntaş, H., 2015. Determination of gibberellic acid (GA_3)-induced oxidative stress in a model organism Galleria mellonella L. (Lepidoptera: Pyralidae). Environmental Entomology, 44 (1): 100-105.

Altuntaş, H., R. Gwokyalya & N. Bayram, 2021. Immunotoxic effects of force-fed ethephon on model organism *Galleria mellonella* (Lepidoptera: Pyralidae). Drug and Chemical Toxicology, 18: 1-8.

An, C., M. Zhang, Y. Chu & Z. Zhao, 2013. Serine protease MP2 activates prophenoloxidase in the melanization immune response of *Drosophila melanogaster*. PloS One, 8 (11): e79533.

Bradford, M. M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, 72 (1): 248-254.

Bronskill, J. F., 1961. A cage to simplify the rearing of the greater wax moth, *Galleria mellonella* (Pyralidae). Journal of the Lepidopterists' Society, 15 (1): 102-104.

Brzozowski, L. & M. Mazourek, 2018. A sustainable agricultural future relies on the transition to organic agroecological pest management. Sustainability, 10 (6): 2023.

Çelik, D., R. Özbek & F. Uçkan, 2017. Effects of indole-3-acetic acid on hemocytes of *Achoria grisella* Fabr. (Lepidoptera: Pyralidae). Journal of the Entomological Research Society, 19 (2): 83-93.

Eleftherianos, I. & C. Revenis, 2011. Role and importance of phenoloxidase in insect hemostasis. Journal of Innate Immunity, 3 (1): 28-33.

Er, A. & M. Keskin, 2016. Influence of abscisic acid on the biology and hemocytes of the model insect *Galleria mellonella* (Lepidoptera: Pyralidae). Annals of the Entomological Society of America, 109 (2): 244-251.

Ghoneim, K., 2018. Role of plant growth regulators in the insect pest control: a quick outlook. Environmental Analyses & Ecological Studies, 4 (1): 1-2.

Gillespie, J. P., M. R. Kanost & T. Trenczek, 1997. Biological mediators of insect immunity. Annual Review of Entomology, 42 (1): 611-643.

Greco, I., N. Molchanova & E. Holmedal, 2020. Correlation between hemolytic activity, cytotoxicity and systemic in vivo toxicity of synthetic antimicrobial peptides. Scientific Reports, 10 (1): 13206.

Guzman, M. A., J. L. Ochoa & F. Vargas-Albores, 1993. Hemolytic activity in the brown shrimp (*Penaeus californiensis* Holmes) haemolymph. Comparative Biochemistry & Physiology, 106A (2): 271-275.

James, R. R. & J. Xu, 2012. Mechanisms by which pesticides affect insect immunity. Journal of Invertebrate Pathology, 109 (2):175-182.

Kaya, S., G. Akkuş, S. Türkdoğan & B. Gündüz, 2021a. Influence of *Helichrysum arenarium* on hemocyte-mediated immune responses and phenoloxidase enzyme activity of model organism *Galleria mellonella* (L.). International Journal of Tropical Insect Science, 41 (4): 2521-2528.

Kaya, S., F. Uçkan & A. Er, 2021b. Influence of Indole-3-Acetic Acid on Cellular Immune Responses of *Galleria mellonella* L. (Lepidoptera: Pyralidae) and *Pimpla turionellae* L. (Hymenoptera: Ichneumonidae) in a host-parasitoid system. International Journal of Tropical Insect Science, 41 (1): 169-179.

Kefford, B. J., L. Zalizniak, M. S. J. Warne & D. Nugegoda, 2008. Is the integration of hormesis and essentiality into ecotoxicology now opening Pandora's box? Environmental Pollution, 151 (3): 516-523.

Lee, W., J. Lee, R. Kulkarni, M.-A. Kim, J. S. Hwang M. K. Na & J.-S. Bae, 2016. Antithrombotic and antiplatelet activities of small-molecule alkaloids from *Scolopendra subspinipes mutilans*. Scientific Reports, 6: 21956 (12pp).

Lins, P. G., C. R. Valle, S. M. P. Pugine, D. L. Oliveira, M. S. L. Ferreira, E. J. X. Costa & M. P. De Melo, 2006. Effect of indole acetic acid administration on the neutrophil functions and oxidative stress from neutrophil, mesenteric lymph node and liver. Life Sciences, 78 (6): 564-570.

Mu, J., T. Uehara & T. Furuno, 2003. Effect of bamboo vinegar on regulation of germination and radicle growth of seed plants. Journal of Wood Science, 49 (3): 262-270.

Nasr, H. M., M. E. I. Badawy & E. Rabea, 2010. Toxicity and biochemical study of two insect growth regulators, buprofezin and pyriproxyfen, on cotton leafworm *Spodoptera littoralis*. Pesticide Biochemistry & Physiology, 98 (2): 198-205.

Özyılmaz, D., R. Özbek, H. Altuntaş & F. Uçkan, 2019. Indole-3-Acetic Acid induced oxidative stress in model host *Galleria mellonella* L. (Lepidoptera: Pyralidae) and its endoparasitoid *Pimpla turionellae* (L.) (Hymenoptera: Ichneumonidae). Invertebrate Survival Journal, 16 (1): 184-188.

Pech, L. L. & M. R. Strand, 2000. Plasmatocytes from the moth *Pseudoplusia includens* induce apoptosis of granular cells. Journal of Insect Physiology, 46 (12): 1565-1573.

Phipps, D. J., J. S. Chadwick, R. G. Leeder & W. P. Aston, 1989. The hemolytic activity of *Galleria mellonella* hemolymph. Developmental and Comparative Immunology, 13 (2): 103-111.

Prado, S. G. & S. D. Frank, 2013. Tritrophic effects of plant growth regulators in an aphid-parasitoid system. Biological Control, 66 (1): 72-76.

Santoyo, I. G. & A. C. Aguilar, 2011. Phenoloxidase: a key component of the insect immune system. Entomologia Experimentalis et Applicata, 142 (1): 1-16.

Sasaki, T., T. Hiraoka & M. Kobayashi, 2010. Hemolytic activity is mediated by the endogenous lectin in the mosquito hemolymph serum. Journal of Insect Physiology, 56 (9): 1032-1039.

SPSS, 2018. IBM SPSS Statistics for Windows, Version 20. Armonk, NY.

Şeker, D. A., A. Yanıkoğlu, 1999. *Pimpla turionellae* L. (Hymenoptera: Ichneumonidae)'nın açlık, beslenme, parazitleme ve yaşlılık durumlarında glikojen seviyesindeki değişmeler. Turkish Journal of Zoology, 23 (1): 289-296 (in Turkish with abstract in English).

Strand, M. D., 2008. The insect cellular immune response. Insect Science, 15 (1): 1-14.

Uçkan, F., İ. Haftacı & E. Ergin, 2011a. Effects of indol-3-acetic acid on biological parameters of the larval endoparasitoid *Apanteles galleriae* (Hymenoptera: Braconidae). Annals of the Entomological Society of America, 104 (1): 77-82.

Uçkan, F., R. Özbek & E. Ergin, 2015. Effects of Indol-3-Acetic Acid on the biology of *Galleria mellonella* and its endoparasitoid *Pimpla turionellae*. Belgian Journal of Zoology, 145 (1): 49-58.

Uçkan, F., Z. Öztürk, H. Altuntaş & E. Ergin, 2011b. Effects of gibberellic acid (GA3) on biological parameters and hemolymph metabolites of the pupal endoparasitoid *Pimpla turionellae* (Hymenoptera: Ichneumonidae) and its host *Galleria mellonella* (Lepidoptera: Pyralidae). Journal of the Entomological Research Society, 13 (3): 1-14.

Uçkan, F., H. K. Soydabaş & R. Özbek, 2014. Effect of indol-3-acetic acid on the biochemical parameters of *Achoria grisella* hemolymph and *Apanteles galleriae* larva. Pakistan Journal of Biotechnology, 11 (2): 163-171.

Uçkan, F., A. Tüven, A. Er & E. Ergin, 2008. Effects of gibberellic acid on biological parameters of the larval endoparasitoid *Apanteles galleriae* (Hymenoptera: Braconidae). Annals of the Entomological Society of America, 101 (3): 593-597.

Wang, X. Y., S. F. Bai, X. Li, X. M. Yin & X. C. Li, 2015. The endoparasitoid *Campoletis chlorideae* induces a hemolytic factor in the herbivorous insect *Helicoverpa armigera*. Archives of Insect Biochemistry and Physiology, 90 (1): 14-27.

Wang, Z. Z., Y. Q. Liu, M. Shi, J. H. Huang & X. X. Chen, (2019). Parasitoid wasps as effective biological control agents. Journal of Integrative Agriculture, 18 (4): 705-715.

Wojda, I., 2017. Immunity of the greater wax moth *Galleria mellonella*. Insect Science, 24 (3): 342-357.

Zhao, H., H. H. Cao & M. Z. Pan, 2017. The role of plant growth regulators in a plant-aphid-parasitoid tritrophic system. Journal of Plant Growth Regulation, 36 (4): 868-876.

Zibaee, A. & A. R. Bandani, 2010. Effects of *Artemisia annua* L. (Asteracea) on the digestive enzymatic profiles and the cellular immune reactions of the sunn pest, *Eurygaster integriceps* (Heteroptera: Scutellaridae), against *Beauveria bassiana*. Bulletin of Entomological Research,100 (2): 185-196.

Türk. entomol. derg., 2022, 46 (2): 159-173
DOI: http://dx.doi.org/10.16970/entoted.1071347

ISSN 1010-6960
E-ISSN 2536-491X

Original article (Orijinal araştırma)

Efficacy of local entomopathogenic fungi isolated from forestlands in Tokat Province (Türkiye) against the Colorado potato beetle, *Leptinotarsa decemlineata* (Say, 1824) (Coleoptera: Chrysomelidae)[1]

Tokat İli (Türkiye) orman alanlarından izole edilen yerel entomopatojen fungusların patates böceği *Leptinotarsa decemlineata* (Say, 1824) (Coleoptera: Chrysomelidae)'ya etkisi

İlker POLAT[2*] **Yusuf YANAR[3]** **Dürdane YANAR[3]**

Abstract

Effects of the entomopathogenic fungi, isolated from forest soil samples gathered from Tokat Province (Türkiye) and its 11 districts between 2014-2017, were evaluated on the third instars and adults of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say, 1824) (Coleoptera: Chrysomelidae) under laboratory conditions. Initially, single-dose efficacy experiments were conducted to determine the effect of the 33 isolates on *L. decemlineata* larvae and adults at 1×10^8 conidia/ml. The four isolates giving the highest mortality in single-dose efficacy experiments, GOPT-498-4, GOPT-529-2, GOPT-552, GOPT-562 that included *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin (Hypocreales: Cordycipitaceae), were used in dose-mortality experiments at 1×10^3, 1×10^5, 1×10^7, 1×10^8, 1×10^9 conidia/ml. The lowest LC_{50} and LT_{50} values were recorded for GOPT-552 with 1.4×10^6 conidia/ml and 10.6 days, respectively, followed by GOPT-562 and GOPT-529-2. *Beauveria bassiana* (GOPT-552 and GOPT-562) isolates were more effective against *L. decemlineata* larvae and adults. Accordingly, GOPT-552 and GOPT-562 isolates are considered to have potential for biological control of Colorado potato beetle.

Keywords: *Beauveria bassiana*, microbial control, mycopesticide, pests, virulence

Öz

Tokat İli (Türkiye) ve 11 ilçesinden 2014-2017 yılları arasında toplanan orman toprak örneklerinden izole edilen entomopatojenik fungusların, Kolorado patates böceği, *Leptinotarsa decemlineata* (Say, 1824) (Coleoptera: Chrysomelidae)'nın üçüncü dönem larva ve erginleri üzerindeki etkileri laboratuvar koşullarında değerlendirilmiştir. İlk olarak 33 izolatın *L. decemlineata* larvaları ve erginleri üzerindeki etkinliğini belirlemek için 1×10^8 konidi/ml'de tek doz etkinlik denemeleri yapılmıştır. Tek doz etkinlik denemelerinde en yüksek ölüm oranlarını veren dört izolat olan *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin (Hypocreales: Cordycipitaceae)'yı içeren GOPT-498-4, GOPT-529-2, GOPT-552, GOPT-562 izolatları 1×10^3, 1×10^5, 1×10^7, 1×10^8, 1×10^9 konidi/ml'de doz-ölüm denemelerinde kullanılmıştır. En düşük LC_{50} ve LT_{50} değerleri sırasıyla 1.4×10^6 konidi/ml ve 10.6 gün ile GOPT-552 izolatı için kaydedilmiş olup, bunu GOPT-562 ve GOPT-529-2 izolatları takip etmiştir. *Beauveria bassiana* (GOPT-552, GOPT-562) izolatları *L. decemlineata* larva ve erginlerine karşı daha etkili olmuştur. Dolayısıyla GOPT-552 ve GOPT-562 izolatlarının Kolorado patates böceğinin biyolojik kontrolü için potansiyele sahip olduğu görülmüştür.

Anahtar sözcükler: *Beauveria bassiana*, mikrobiyal mücadele, mikopestisit, zararlılar, virulans

[1] This study was supported by Tokat Gaziosmanpasa University, Scientific Research Projects Unit, Türkiye, Grant Project No: 2015/84.
[2] Middle Black Sea Transitional Zone Agricultural Research Institute, Department of Plant Health, 60250, Tokat, Türkiye
[3] Gaziosmanpasa University, Faculty of Agriculture, Department of Plant Protection, 60250, Tasliciftlik Campus, Tokat, Türkiye
* Corresponding author (Sorumlu yazar) e-mail: ikrpolat@gmail.com
Received (Alınış): 10.02.2022 Accepted (Kabul ediliş): 09.06.2022 Published Online (Çevrimiçi Yayın Tarihi): 17.06.2022

Introduction

Colorado potato beetle, *Leptinotarsa decemlineata* (Say, 1824) (Coleoptera: Chrysomelidae), is one of the most devastating pests of potato that cause significant damage by eating the leaves. Both larvae and adults can cause complete defoliation of potato plants, with considerable yield losses of up to 60% (Jacques & Fasulo, 2015). This pest is a native of North America and has become a devastating pest worldwide. The beetle survives by feeding on cultivated and wild solanaceous plants, like potato, eggplant and tomato (Kıvan & Aysal, 2014).

The most widely used biological agents in biological control after predators and parasitoid insects are entomopathogens (Deacon, 1983). Generally, four groups of microbial pathogens are found in insects. These are bacteria, fungi, viruses and protozoa. Among these biological control agents, entomopathogenic fungi (EPF) especially infect the pest through integument, their ease of production and high adaptability make them potentially more useful than the others. EPF have three mechanisms of action including causing mechanical damage to tissues and producing toxic metabolites. In this way, they can cause the direct death of insects or weaken them, and limit their vital activities (Kulkarni, 2015).

Beauveria bassiana (Balsamo-Crivelli) Vuillemin (Hypocreales: Cordycipitaceae), *Beauveria brongniartii* (Sacc.) Petch (Hypocreales: Cordycipitaceae), *Metarhizium anisopliae* (Metschn.) Sorokin (Hypocreales: Clavicipitaceae), *Cordyceps fumosorosea* (Wize) Kepler, B. Shrestha & Spatafora (Hypocreales: Cordycipitaceae) and *Cordyceps farinosa* (Holmsk.) Kepler, B. Shrestha & Spatafora (Hypocreales: Cordycipitaceae) are the most commonly used EPF for biological control of soilborne pests (Dragonova et al., 2008). The commercially available *B. bassiana* product has been applied to pests such as *Curculio elephas* J.C.Fabricius, 1781 (Coleoptera: Curculionidae) (chestnut borer) and *Curculio nucum* (L., 1758) (Coleoptera: Curculionidae) (hazelnut borer) that have pupae and adults in the soil, with instar mortality of 35% (Paparatti & Speranza 1999; 2005). This showed that the soil phase has an important stage in the life cycle of pests such as potato beetle, which pupae in the soil and overwinter in the soil as an adult. In addition, EPF are used to control other pests such as rose aphid *Macrosiphum rosae* (L., 1758) (Hemiptera: Aphididae) (Soy, 2017); alfalfa weevil *Hypera postica* (Gyllenhal, 1813) (Coleoptera: Curculionidae) and lucerne beetle *Gonioctena fornicata* (Brüggemann, 1873) (Coleoptera: Chrysomelidae) (Baysal, 2017); *Tetranychus urticae* (Koch, 1836) (Acari: Tetranychidae) (Ateş, 2020); apple blossom beetle *Tropinota* (*Epicometis*) *hirta* (Poda, 1761) (Coleoptera: Scarabaeidae) (Uçar, 2021); common grasshopper species *Poecilimon glandifer* (Karabag, 1950) (Orthoptera: Tettigoniidae) (Doğan, 2022); rice weevil *Sitophilus oryzae* (L., 1763) (Coleoptera: Curculionidae) (Solmaz, 2022). Boverin, a mycoinsecticide derived from *B. bassiana*, was produced in 1965 for the control of Colorado potato beetle and codling moth in the former USSR (Kendrick, 2000).

Colorado potato beetle populations in cultivated potato fields have developed resistance to many insecticides used for control, and this species is becoming the most destructive pest of potatoes in the northeast of the USA (Zhao et al., 2000). Since then, a range of control methods have been implemented. Unfortunately, despite all efforts to control this pest, potato beetle has developed resistance to DDT, other chlorinated hydrocarbons, organophosphorus, carbamates and some pyrethroids (Grafius, 1997). Therefore, it is necessary to search for alternative methods to chemical control for potato beetle and to put them into practice, and many studies have been conducted for this purpose worldwide (Kepenekci et al., 2015; Zemek et al., 2021). For example, in a study conducted by Güven et al. (2015), four *B. bassiana* isolates from different sources and sites were tested for their pathogenicity against larvae and adult of *L. decemlineata* with spraying, dipping and residue methods under laboratory conditions at 10^8 conidia/ml, and *B. bassiana* was found to be the more effective on larvae than adults. It was reported that mortality of the adult insect was low but larval mortalities obtained with the spraying, dipping, and residual methods for BMAUM-001 were 73, 65 and 68%; for BMAUM-002 were 84, 93 and 91%; and for BMAUM-003 were 84,

60 and 79%, respectively. In another study Öztürk et al. (2015) evaluated the efficacy of commercial mycopesticides containing entomopathogenic fungi; Priority [*Paecilomyces fumosoroseus* (Wize) A.H.S. Br. & G. Sm.], Nibortem [*Verticillium lecanii* (Zimm.) Viégas], Nostalgist (*B. bassiana*), Bio-Magic (*M. anisopliae*), Bio-Nematon (*Paecilomyces* sp., Bainier) and plant extracts; Nimbedicine EC (azadirachtin) on adult and larva of *L. decemlineata* under laboratory conditions. The biological control agents were applied to second-third instars, fourth instars and adults with spray and leaf dipping methods. Single concentration (10^8 conidia/ml) of entomopathogenic fungi and recommended dose of bioinsecticides were prepared for application. Entomopathogenic fungi and bioinsecticides were found to be more effective against early instars than fourth instars and adults. In spray methods, Bio-Magic, Nibortem, and Nostalgist caused 96, 93 and 82% mortality on second instars and 20, 37 and 33% mortality on adults, respectively, while all local *B. bassiana* isolates caused 100% mortality on second and fourth instars of the insect. Adults showed 59-86% mortality. Similarly, in another study four *B. bassiana* isolates (BbDm-1, BbDs-2, BbMg-2 and BbMp-1) at 1×10^7 conidia/ml were more pathogenic than the others against *L. decemlineata*, causing mortality between 97% and 100% on first and second instars, respectively, between 92 and 97% on the third and fourth instars, respectively, and between 93 and 97% on 0-48h adults, respectively, 9 days post treatment. The highest mortality was seen in larvae; but first and second instars were more susceptible to fungal isolates than third and fourth instars (Baki et al., 2021).

In forest areas, unlike cultivated areas, no pesticides are used and no tillage is done. Therefore, the presence of entomopathogenic fungi in these regions is much higher than in cultivated soils (Vänninen et al., 1989; Miętkiewski et al., 1991; Vänninen, 1995; Chandler et al., 1997; Bałazy, 2004). The isolation of entomopathogenic fungi from forest areas makes the present study different from other studies and adds to its originality. The aim of the present study was to evaluate the efficacy of local *B. bassiana* and *Talaromyces* spp. isolates from forest soils in Tokat Province, Türkiye against third instars and adult of Colorado potato beetle under laboratory conditions.

Materials and Methods

The main material of the experiment was third instars (which consume the most potato leaves) and adults of the Colorado potato beetle (*L. decemlineata*) and the entomopathogenic fungi isolates of forest soil (Table 2) applied against the pest.

Soil sampling

Soil samples were collected in forest areas of different elevation in districts of Tokat Province, Türkiye (Table 1). Soil samples were collected after removing the surface soil at depths of ~5-20 cm. Soil sampling was made from five points at each sampling site. The samples were mixed in a plastic bag, ~750 g of soil from the mixture was placed in polyethylene bags and brought to the laboratory and examined for entomopathogenic fungi as soon as possible (Ali-Shtayeh et al., 2002). Soils brought to the laboratory from forest areas were dried for 4 days in the climate chamber (25 ± 2ºC) and then passed through 2 mm sieves in order to purify them from foreign materials (e.g., leaves, stones and wood chips). Fifty g of each soil sample was then placed in 90 mm sterile glass Petri dishes and moistened to field capacity by spraying 10 ml of sterile distilled water. Fungi were isolated from soil samples using a *Galleria* bait method (Zimmermann, 1986). In order to prevent the *Galleria mellonella* (L., 1758) (Lepidoptera: Pyralidae) larvae from forming pupa, the larvae were transferred to Petri dishes after they were kept at 50°C for 120 s. Five fourth or fifth instar *Galleria* of the same age and size were added to each soil sample in glass Petri dishes, each of which was prepared in three replicates. The Petri dishes were wrapped with Parafilm and incubated at 25 ± 2ºC in the dark for 7-14 days. The Petri dishes were turned and agitated daily to ensure that the larvae came into maximum contact with the soil (Mietkiewski et al., 1991). The larvae were examined once every 2 days for a period of 2 weeks. Dead larvae were removed and surface-sterilized in 1% sodium hypochlorite for 2-3 min, then washed two times in sterile distilled water (Quesada-Moraga et al., 2007) and they were taken

into sterile Petri dishes with moist filter paper and incubated at 25 ± 2 ºC (Ali-Shtayeh et al., 2002). The fungi obtained from the samples showing external fungal growth during the incubation were purified using a needle in a sterile cabinet (Sevim, 2010). For this, potato dextrose agar with 1% yeast extract was used. To prevent bacterial contamination, 50 µg/ml ampicillin, 20 µg/ml broad spectrum antibiotic tetracycline and 200 µg/ml streptomycin were added to the medium (Ihara et al., 2001).

Table 1. Number of soil samples collected in Tokat Province and its districts and number of EPF isolates obtained

Districts of Tokat Province	Forest area* (ha)	Samples collected	EPF isolates obtained
Tokat (Central)	94033	47	11
Almus	71355	36	11
Artova	14520	7	0
Başciftlik	5259	3	0
Erbaa	42000	21	0
Niksar	46244	23	2
Pazar	3355	4	0
Reşadiye	47129	23	1
Sulusaray	9350	4	0
Turhal	42332	21	4
Yeşilyurt	7598	4	1
Zile	26925	13	3
Total	410100	206	33

*Area values are from 2013 (Anonymous, 2014).

Rearing of third-instars and adults of *L. decemlineata*

Potato plants (cv. Marabel) grown during the production season in a 72 m^2 screen house at the Middle Black Sea Transitional Zone Agricultural Research Institute were used for potato beetle rearing. Third-instars were separated based on their pronotum coloration. Insects used in the experiments were first generation larvae and adults that emerged from the underground overwintered adults that came to the soil surface, mated and deposited eggs in the spring with the potato planting. No pesticide application was applied to the potato plants grown in the screen house during the vegetation period.

Fungal isolates

In total, 33 EPF isolates (Table 1) were isolated from soil samples collected from forestlands in Tokat Province in Türkiye in 2014-17 by using the *G. mellonella* bait method (Zimmermann, 1986). These isolates were identified morphologically and molecularly at the genus and species level by Prof. Dr. İsmail Demir (Karadeniz Technical University Faculty of Arts and Sciences Department of Biology in Trabzon Province, Türkiye) (Samson et al., 1988; White et al., 1990; Humber, 2005; 2012). Based on the sequence analysis, 25 isolates were identified as *B. bassiana*, and eight of isolates were identified as *Talaromyces* spp. (C.R. Benjamin) (Eurotiales: Trichocomaceae). All isolates were cultured on potato dextrose agar (PDA) medium at 25 ± 1°C for 15-30 days.

Table 2. Coordinates and altitude of local fungus isolates obtained from forest vegetation soils by trap insect method

Isolates	Sampling site	GPS Coordinates		Altitude (m)	EPF Scientific Name
		N	E		
GOPT-479	Tokat	40°22'20"	36°32'48"	872	*Talaromyces* spp.
GOPT-483	Tokat	40°19'33"	36°33'53"	661	*B. bassiana*
GOPT-498-1	Tokat	40°21'32"	36°42'35"	754	*Talaromyces* spp.
GOPT-498-2	Tokat	40°21'32"	36°42'35"	754	*Talaromyces* spp.
GOPT-498-4	Tokat	40°21'32"	36°42'35"	754	*B. bassiana*
GOPT-501-1	Tokat	40°19'56"	36°30'07"	628	*Talaromyces* spp.
GOPT-510	Tokat	40°19'46"	36°30'30"	606	*Talaromyces* spp.
GOPT-517	Tokat	40°23'42"	36°40'13"	769	*B. bassiana*
GOPT-528	Tokat	40°11'16"	36°29'00"	1161	*B. bassiana*
GOPT-529-1	Tokat	40°12'38"	36°30'36"	1054	*B. bassiana*
GOPT-529-2	Tokat	40°12'38"	36°30'36"	1054	*B. bassiana*
GOPT-537	Almus	40°22'22"	36°52'00"	1004	*B. bassiana*
GOPT-541-1	Almus	40°24'02"	36°54'18"	870	*B. bassiana*
GOPT-541-2	Almus	40°24'02"	36°54'18"	870	*B. bassiana*
GOPT-547-1	Almus	40°25'09"	36°54'29"	808	*B. bassiana*
GOPT-547-2	Almus	40°25'09"	36°54'29"	808	*Talaromyces* spp.
GOPT-547-4	Almus	40°25'09"	36°54'29"	808	*B. bassiana*
GOPT-547-5	Almus	40°25'09"	36°54'29"	808	*B. bassiana*
GOPT-549	Almus	40°24'54"	36°54'42"	872	*B. bassiana*
GOPT-551	Almus	40°24'27"	36°55'17"	810	*B. bassiana*
GOPT-552	Almus	40°24'21"	36°55'20"	837	*B. bassiana*
GOPT-557	Almus	40°21'31"	37°01'22"	866	*B. bassiana*
GOPT-562	Niksar	40°31'29"	36°53'11"	576	*B. bassiana*
GOPT-563	Niksar	40°34'16"	36°55'06"	270	*Talaromyces* spp.
GOPT-580-2	Turhal	40°14'21"	36°06'52"	708	*B. bassiana*
GOPT-580-3	Turhal	40°14'21"	36°06'52"	708	*Talaromyces* spp.
GOPT-583	Turhal	40°14'03"	36°04'03"	711	*B. bassiana*
GOPT-584	Turhal	40°13'42"	36°04'09"	783	*B. bassiana*
GOPT-597	Zile	40°13'29"	36°00'37"	952	*B. bassiana*
GOPT-615	Zile	40°14'12"	35°57'29"	820	*B. bassiana*
GOPT-617	Zile	40°13'08"	35°58'10"	970	*B. bassiana*
GOPT-624	Reşadiye	40°22'45"	37°21'19"	516	*B. bassiana*
GOPT-665	Yeşilyurt	40°00'32"	36°16'32"	1129	*B. bassiana*

Inoculum production

The fungi isolates were subcultured on PDA medium and incubated for 4 weeks. At the end of the incubation period, 10 ml of sterile distilled water with 0.02% Tween 80 was poured into the Petri dishes and spores were harvested with glass spreader. The spore suspension was filtered through three layers of sterile cheesecloth and the mycelia and agar pieces were removed. Spore density in stock solutions was determined using a hemocytometer. Spore concentration was adjusted to 1×10^8 conidia/ml for each isolate.

Single-dose efficacy experiments

Third instars and adults of *L. decemlineata* were immersed in the spore suspensions (1×10^8 conidia/ml) of each fungal isolate for 8-10 s. The excess water on the larvae and adults was removed with filter paper. Treated larvae and adults were put in Petri dishes (9 cm) containing fresh potato leaves their stem wrapped with wet cotton. Larvae and adults in control treatment were immersed in distilled water containing 0.02% Tween 80. Each treatment had a group of five larvae and adults. Mortality of the larvae and adults were registered daily from days 1 to 7 and days 1 to 11 of incubation under the laboratory conditions, respectively. The experiments had three replicates and were conducted twice.

Dose-mortality experiments

Only adults were evaluated in dose-mortality studies because the mortality was high in the control larvae 7 day after treatments. As mentioned above adults of *L. decemlineata* were immersed with different spore concentrations (1×10^3, 1×10^5, 1×10^7, 1×10^8 and 1×10^9 conidia/ml) and in distilled water containing 0.02% Tween 80 (control). Mortality of the adults were registered daily from day 1 to 14 of incubation. Experiments included three replicates and were conducted twice.

Statistical analysis

The obtained mortality data were then analyzed by one-way ANOVA using the SPSS 17 package program (IBM, Armonk, NY, USA). Prior to ANOVA, the mortality data were tested for normality (Kolmogorov Smirnov test) and then arcsine transformed ($n' = \arcsin \sqrt{n}$) to obtain a normally distributed data set. Post-hoc Tukey's HSD test was performed to separate and compare means ($P = 0.05$) were detected. LC_{50} and LT_{50} values of isolates were calculated by probit analysis of dose-mortality experiments with Polo-PC program (LeOra, 1994).

Results

Single-dose efficacy experiments

The results in Tables 3 and 4 show the mortality of *L. decemlineata* larvae and adults treated with 1×10^8 conidia/ml of each isolate, respectively.

There were no significant differences in the mortality caused by the isolates after 1 day ($F = 1.3$; $df = 33$; $p > 0.05$) and 3 days ($F = 3.8$; $df = 33$; $p > 0.05$). After 3 days, mortality observed in GOPT-597 and GOPT-549 (75%) isolates belonging to *Beauveria* genera were higher than the control and other isolates. Significant differences in mortality were evident on day 5 compared to the control ($F = 6.5$; $df = 33$; $p < 0.05$). Isolates GOPT-624 (85%), GOPT-547-1, GOPT-617, GOPT-549 and GOPT-665 (90%), GOPT-584 and GOPT-552 (95%), GOPT-498-4 (100%) caused a higher mortality than others after 5 days. The differences in mortality were also significant on day 7 compared to the control ($F = 11.6$; $df = 33$; $p < 0.05$). GOPT-597, GOPT-617, GOPT-549, GOPT-624, GOPT-665, GOPT-557, GOPT-583, GOPT-584, GOPT-541-2, GOPT-541-1, GOPT-580-2, GOPT-547-5, GOPT-498-4, GOPT-551, GOPT-552, GOPT-562 (100%) isolates belonging to *Beauveria* genera caused high mortality. GOPT-498-4 caused highest mortality (100%) in third instars on day 5. Among the *Talaromyces* isolates, GOPT-547-2 caused the highest mortality (85%) after 7 days. Although both GOPT-510 and GOPT-498-2 *Talaromyces* isolates caused mortality of 70%, there was no a statistically significant difference ($F = 11.6$; $df = 33$; $p > 0.05$) between them when compared to the control (Table 3).

Table 3. Mortality (%) of *Leptinotarsa decemlineata* larvae treated with suspension of EPF isolates at 1×10^8 conidia/ml

Fungal isolate	Species	Mortality ± SDM*(%)			
		1 DAT	3 DAT	5 DAT	7 DAT
Control		0 ± 0 a	0 ± 0 a	5 ± 10 a	15 ± 10 a***
GOPT-498-1	*Talaromyces* spp.	0 ± 0 a	20 ± 23 ab	30 ± 25 abcd	65 ± 30 abc
GOPT-479	*Talaromyces* spp.	0 ± 0 a	30 ± 11 abc	35 ± 19 abcde	60 ± 16 abcde
GOPT-547-2	*Talaromyces* spp.	0 ± 0 a	40 ± 28 abc	55 ± 19 abcdef	85 ± 19 bcdef
GOPT-580-3	*Talaromyces* spp.	0 ± 0 a	20 ± 23 ab	45 ± 30 abcde	55 ± 19 abc
GOPT-501-1	*Talaromyces* spp.	0 ± 0 a	20 ± 16 ab	30 ± 11 abc	40 ± 0 ab
GOPT-510	*Talaromyces* spp.	5 ± 10 a	45 ± 10 abc	65 ± 10 abcdefgh	70 ± 11 abc
GOPT-615	*B. bassiana*	0 ± 0 a	50 ± 11 abc	75 ± 25 efghi	90 ± 11 def
GOPT-483	*B. bassiana*	0 ± 0 a	45 ± 10 abc	65 ± 25 abcdefghi	80 ± 23 cdef
GOPT-547-1	*B. bassiana*	0 ± 0 a	65 ± 19 bc	90 ± 11 efghi	95 ± 10 def
GOPT-563	*Talaromyces* spp.	0 ± 0 a	0 ± 0 a	10 ± 11 ab	25 ± 10 abc
GOPT-498-2	*Talaromyces* spp.	5 ± 10 a	25 ± 25 abc	45 ± 10 abcdefg	70 ± 11 abcd
GOPT-597	*B. bassiana*	10 ± 11 a	75 ± 37 c	80 ± 28 bcdefghi	100 ± 0 def
GOPT-537	*B. bassiana*	5 ± 10 a	35 ± 19 abc	60 ± 16 bcdefghi	85 ± 19 def
GOPT-617	*B. bassiana*	5 ± 10 a	70 ± 11 bc	90 ± 11 ghi	100 ± 0 f
GOPT-549	*B. bassiana*	10 ± 20 a	75 ± 10 c	90 ± 11 fghi	100 ± 0 f
GOPT-517	*B. bassiana*	0 ± 0 a	30 ± 25 abc	70 ± 20 bcdefghi	95 ± 10 def
GOPT-624	*B. bassiana*	10 ± 11 a	50 ± 11 abc	85 ± 19 efghi	100 ± 0 f
GOPT-665	*B. bassiana*	5 ± 10 a	45 ± 10 abc	90 ± 11 defghi	100 ± 0 ef
GOPT-557	*B. bassiana*	5 ± 10 a	50 ± 11 abc	80 ± 16 efghi	100 ± 0 ef
GOPT-583	*B. bassiana*	10 ± 20 a	50 ± 11 abc	85 ± 19 fghi	100 ± 0 f
GOPT-529-1	*B. bassiana*	15 ± 10 a	35 ± 19 abc	65 ± 19 cdefghi	95 ± 10 ef
GOPT-584	*B. bassiana*	0 ± 0 a	65 ± 19 bc	95 ± 10 i	100 ± 0 f
GOPT-528	*B. bassiana*	15 ± 19 a	50 ± 28 abc	80 ± 16 cdefghi	95 ± 10 def
GOPT-529-2	*B. bassiana*	0 ± 0 a	35 ± 30 abc	70 ± 34 cdefghi	95 ± 10 ef
GOPT-541-2	*B. bassiana*	5 ± 10 a	45 ± 19 abc	80 ± 16 efghi	100 ± 0 f
GOPT-541-1	*B. bassiana*	20 ± 16 a	35 ± 19 abc	60 ± 16 cdefghi	100 ± 0 f
GOPT-547-4	*B. bassiana*	5 ± 10 a	40 ± 0 abc	70 ± 25 efghi	95 ± 10 ef
GOPT-580-2	*B. bassiana*	0 ± 0 a	50 ± 25 abc	75 ± 19 efghi	100 ± 0 f
GOPT-547-5	*B. bassiana*	0 ± 0 a	40 ± 16 abc	70 ± 11 abcdefghi	100 ± 0 f
GOPT-498-4	*B. bassiana*	5 ± 10 a	55 ± 10 bc	100 ± 0 hi	100 ± 0 ef
GOPT-551	*B. bassiana*	10 ± 11 a	50 ± 25 abc	75 ± 10 defghi	100 ± 0 f
GOPT-552	*B. bassiana*	0 ± 0 a	50 ± 11 abc	95 ± 10 fghi	100 ± 0 f
GOPT-562	*B. bassiana*	0 ± 0 a	20 ± 0 ab	70 ± 11 cdefghi	100 ± 0 f

* SDM Standard deviation of the mean. **DAT, days after treatment. ***Means followed by the same letter within columns are not significantly different (Tukey's test, $P < 0.05$).

There were no significant differences in mortality between isolate or compared to the control after 1 day ($F = 0.7$; $df = 33$; $p > 0.05$), 3 days ($F = 1.0$; $df = 33$; $p > 0.05$) and 5 days ($F = 0.9$; $df = 33$; $p > 0.05$). Significant differences in mortality compared to the control were evident after 7 days ($F = 11.5$; $df = 33$; $p < 0.05$). *Beauveria* isolates GOPT-541-1 (53%), GOPT-547-4 (56%), GOPT-529-2 (63%), GOPT-498-4 (66%), GOPT-547-5 and GOPT-551 (70%), GOPT-562 (93%), GOPT-552 (100%) caused high mortality (>50%) after 7 days. Differences in mortality on day 11 were also significant compared to control ($F = 18.3$; $df = 33$; $p < 0.05$). GOPT-584 (80%), GOPT-528 (83%), GOPT-529-2 and GOPT-541-2 (86%), GOPT-541-1, GOPT-547-4 and GOPT-580-2 (90%), GOPT-547-5 (93%), GOPT-498-4 and GOPT-551 (96%), GOPT-552 and GOPT-562 (100%) caused high mortality after 11 days. Mortality caused by *Talaromyces* isolates after 11 days did not differ from the control or from each other ($F = 18.3$; $df = 33$; $p > 0.05$) (Table 4).

Table 4. Mortality (%) of *Leptinotarsa decemlineata* adults treated with a suspension of EPF isolates at 1×10^8 conidia/ml

Fungal isolate	Species	Mortality ± SDM*(%)				
		1DAT	3DAT	5DAT	7DAT	11DAT
Control		0 ± 0 a	0 ± 0 a	0 ± 0 a	6 ± 10 ab	10 ± 10 ab***
GOPT-498-1	*Talaromyces* spp.	3 ± 8 a	6 ± 10 a	6 ± 10 a	6 ± 10 ab	6 ± 10 a
GOPT-479	*Talaromyces* spp.	0 ± 0 a	6 ± 16 a	10 ± 16 a	10 ± 16 ab	10 ± 16 ab
GOPT-547-2	*Talaromyces* spp.	6 ± 16 a	10 ± 16 a	10 ± 16 a	10 ± 16 ab	10 ± 16 ab
GOPT-580-3	*Talaromyces* spp.	3 ± 8 a	6 ± 10 a	6 ± 10 a	6 ± 10 ab	10 ± 16 ab
GOPT-501-1	*Talaromyces* spp.	0 ± 0 a	10 ± 10 a	13 ± 10 a	13 ± 10 abc	16 ± 8 abc
GOPT-510	*Talaromyces* spp.	3 ± 8 a	6 ± 10 a	10 ± 10 a	13 ± 10 abc	16 ± 15 ab
GOPT-615	*B. bassiana*	0 ± 0 a	3 ± 8 a	10 ± 10 a	16 ± 8 abcd	16 ± 8 abc
GOPT-483	*B. bassiana*	0 ± 0 a	0 ± 0 a	3 ± 8 a	6 ± 10 ab	20 ± 12 abc
GOPT-547-1	*B. bassiana*	0 ± 0 a	10 ± 10 a	13 ± 16 a	16 ± 19 abc	20 ± 21 ab
GOPT-563	*Talaromyces* spp.	0 ± 0 a	6 ± 10 a	6 ± 10 a	16 ± 15 abcd	20 ± 17 abc
GOPT-498-2	*Talaromyces* spp.	6 ± 16 a	16 ± 19 a	23 ± 23 a	23 ± 23 abcde	23 ± 23 abc
GOPT-597	*B. bassiana*	3 ± 8 a	13 ± 16 a	13 ± 16 a	16 ± 19 abc	23 ± 23 abc
GOPT-537	*B. bassiana*	0 ± 0 a	0 ± 0 a	3 ± 8 a	3 ± 8 a	26 ± 10 abc
GOPT-617	*B. bassiana*	3 ± 8 a	13 ± 20 a	20 ± 25 a	23 ± 29 abcd	26 ± 35 abc
GOPT-549	*B. bassiana*	0 ± 0 a	6 ± 16 a	10 ± 16 a	16 ± 15 abcd	33 ± 24 abcd
GOPT-517	*B. bassiana*	3 ± 8 a	16 ± 15 a	16 ± 15 a	23 ± 15 abcdefg	36 ± 19 abcde
GOPT-624	*B. bassiana*	0 ± 0 a	6 ± 10 a	16 ± 15 a	26 ± 16 abcdefg	40 ± 21 abcde
GOPT-665	*B. bassiana*	0 ± 0 a	0 ± 0 a	10 ± 16 a	30 ± 20 abcdefg	40 ± 25 abcde
GOPT-557	*B. bassiana*	3 ± 8 a	3 ± 8 a	10 ± 10 a	23 ± 19 abcdef	50 ± 20 bcdef
GOPT-583	*B. bassiana*	0 ± 0 a	0 ± 0 a	10 ± 16 a	16 ± 15 abcd	50 ± 16 bcdef
GOPT-529-1	*B. bassiana*	0 ± 0 a	6 ± 10 a	10 ± 10 a	36 ± 15 abcdefg	66 ± 16 cdefg
GOPT-584	*B. bassiana*	6 ± 10 a	6 ± 10 a	6 ± 10 a	30 ± 20 abcdefg	80 ± 21 efg
GOPT-528	*B. bassiana*	0 ± 0 a	6 ± 10 a	10 ± 10 a	43 ± 15 bcdefg	83 ± 8 defg
GOPT-529-2	*B. bassiana*	3 ± 8 a	10 ± 16 a	10 ± 16 a	63 ± 26 defgh	86 ± 16 fg
GOPT-541-2	*B. bassiana*	0 ± 0 a	6 ± 16 a	6 ± 16 a	37 ± 8 abcdefg	86 ± 24 fg
GOPT-541-1	*B. bassiana*	0 ± 0 a	0 ± 0 a	3 ± 8 a	53 ± 10 cdefg	90 ± 10 fg
GOPT-547-4	*B. bassiana*	0 ± 0 a	0 ± 0 a	0 ± 0 a	56 ± 15 cdefgh	90 ± 10 fg
GOPT-580-2	*B. bassiana*	3 ± 8 a	6 ± 10 a	10 ± 10 a	36 ± 15 abcdefg	90 ± 10 fg
GOPT-547-5	*B. bassiana*	0 ± 0 a	6 ± 10 a	6 ± 10 a	70 ± 20 fghi	93 ± 10 g
GOPT-498-4	*B. bassiana*	6 ± 16 a	16 ± 15 a	16 ± 15 a	66 ± 27 ghi	96 ± 8 g
GOPT-551	*B. bassiana*	0 ± 0 a	6 ± 10 a	16 ± 15 a	70 ± 16 efghi	96 ± 8 g
GOPT-552	*B. bassiana*	0 ± 0 a	10 ± 16 a	13 ± 16 a	100 ± 0 i	100 ± 0 g
GOPT-562	*B. bassiana*	0 ± 0 a	13 ± 16 a	20 ± 17 a	93 ± 10 hi	100 ± 0 g

* SDM Standard deviation of the mean. **DAT, days after treatment. ***Means followed by the same letter within columns are not significantly different (Tukey's test, $P < 0.05$).

Dose-mortality experiments

The results of a single-dose screening of both larvae and adult experiments in Tables 3 & 4 were used to select isolates causing high mortality for further testing. GOPT-498-4 (*B. bassiana*) (100-96%), GOPT-529-2 (*B. bassiana*) (95-86%), GOPT-552 (*B. bassiana*) (100-100%), GOPT-562 (*B. bassiana*) (100-100%) were selected for dose-mortality experiments (1×10^3-1×10^9 conidia/ml) (Table 5).

The highest mortality was observed with GOPT-552 at 1×10^3 conidia/ml compared to other isolates. Mortality increased with increasing dose and 90% mortality was observed at the 1×10^9 conidia/ml, the highest mortality with this isolate. GOPT-498-4 caused the second highest mortality at 1×10^3 conidia/ml. Mortality increased with increasing dose, and a mortality of 81% was observed at the 1×10^9 conidia/ml.

With GOPT-562, mortality was the same as the control at 1×10^3 conidia/ml ($F = 41$; $df = 5$; $p > 0.05$), and a significant increase from 16 to 70% at the 1×10^7 conidia/ml was observed on day 14 ($F = 41$; $df = 5$; $p < 0.05$). Mortality of 86% was observed at 1×10^9 conidia/ml. With GOPT-529-2 at 1×10^9 conidia/ml mortality of 86% was observed, similar to GOPT-562. After 14 days, the mortality observed at 10^7, 10^8 and 10^9 conidia/ml with all isolates were significantly different from the control, but there was no significant differences between isolates (Table 5). The data acquired from the dose-death experiments were examined by probit analysis.

Table 5. Mortality percentage of *Leptinotarsa decemlineata* adults treated with a range of doses of the four selected *Beauveria bassiana* isolates

Fungal isolate	Doses (conidia/ml)	Mortality ± SDM*(%) 1DAT**	3DAT	5DAT	7DAT	9DAT	11DAT	14DAT
Control		0 ± 0 a*	0 ± 0 a	0 ± 0 a	3 ± 8 a	3 ± 8 a	3 ± 8 a	3 ± 8 a
GOPT-498-4 *B. bassiana*	1×10^3	0 ± 0 a	0 ± 0 a	0 ± 0 a	3 ± 8 a	3 ± 8 a	6 ± 10 a	6 ± 10 a***
	1×10^5	0 ± 0 a	3 ± 8 a	3 ± 8 a	3 ± 8 a	6 ± 10 a	10 ± 10 a	20 ± 25 a
	1×10^7	0 ± 0 a	0 ± 0 a	0 ± 0 a	21 ± 4 b	25 ± 5 b	40 ± 0 b	60 ± 0 b
	1×10^8	3 ± 8 a	3 ± 8 a	6 ± 16 a	38 ± 4 bc	48 ± 9 c	66 ± 8 bc	73 ± 8 b
	1×10^9	0 ± 0 a	0 ± 0 a	3 ± 8 a	50 ± 8 c	60 ± 8 c	73 ± 8 c	81 ± 9 b
Control		0 ± 0 a	0 ± 0 a	0 ± 0 a	3 ± 8 a	3 ± 8 a	3 ± 8 a	3 ± 8 a
GOPT-529-2 *B. bassiana*	1×10^3	0 ± 0 a	0 ± 0 a	0 ± 0 a	3 ± 8 a	3 ± 8 a	3 ± 8 a	3 ± 8 a
	1×10^5	0 ± 0 a	0 ± 0 a	0 ± 0 a	3 ± 8 a	3 ± 8 a	16 ± 15 a	26 ± 10 b
	1×10^7	3 ± 8 a	3 ± 8 a	3 ± 8 a	26 ± 10 b	41 ± 9 b	53 ± 8 b	63 ± 5 c
	1×10^8	0 ± 0 a	0 ± 0 a	0 ± 0 a	33 ± 10 b	56 ± 8 bc	70 ± 8 bc	76 ± 5 cd
	1×10^9	0 ± 0 a	3 ± 8 a	3 ± 8 a	40 ± 0 b	66 ± 10 c	81 ± 9 c	86 ± 10 d
Control		0 ± 0 a	0 ± 0 a	0 ± 0 a	3 ± 8 a	3 ± 8 a	3 ± 8 a	3 ± 8 a
GOPT-552 *B. bassiana*	1×10^3	3 ± 8 a	3 ± 8 a	6 ± 10 a	6 ± 10 a	6 ± 10 a	6 ± 10 a	13 ± 16 a
	1×10^5	0 ± 0 a	0 ± 0 a	0 ± 0 a	3 ± 8 a	6 ± 10 a	20 ± 25 a	23 ± 32 a
	1×10^7	0 ± 0 a	6 ± 10 a	6 ± 10 a	26 ± 10 b	43 ± 8 b	60 ± 0 b	63 ± 5 b
	1×10^8	0 ± 0 a	6 ± 10 a	6 ± 10 a	40 ± 0 b	60 ± 0 b	68 ± 7 b	78 ± 4 b
	1×10^9	0 ± 0 a	0 ± 0 a	3 ± 8 a	46 ± 10 b	66 ± 10 b	85 ± 12 b	90 ± 10 b
Control		0 ± 0 a	0 ± 0 a	0 ± 0 a	3 ± 8 a	3 ± 8 a	3 ± 8 ab	3 ± 8 a
GOPT-562 *B. bassiana*	1×10^3	0 ± 0 a	0 ± 0 a	0 ± 0 a	0 ± 0 a	0 ± 0 a	0 ± 0 a	3 ± 8 a
	1×10^5	0 ± 0 a	0 ± 0 a	0 ± 0 a	0 ± 0 a	0 ± 0 a	16 ± 15 b	16 ± 15 a
	1×10^7	0 ± 0 a	0 ± 0 a	0 ± 0 a	26 ± 10 b	50 ± 10 b	60 ± 6 c	70 ± 6 b
	1×10^8	0 ± 0 a	0 ± 0 a	0 ± 0 a	41 ± 4 c	53 ± 10 b	56 ± 8 c	78 ± 4 b
	1×10^9	0 ± 0 a	3 ± 8 a	6 ± 16 a	50 ± 6 c	60 ± 6 b	70 ± 8 c	86 ± 10 b

* SDM Standard deviation of the mean. **DAT, days after treatment. ***Means followed by the same letter within columns are not significantly different (Tukey's test, $P < 0.05$).

The doses that killed 50% (LC_{50}) of the population on day 14 and the assay parameters are presented in Table 6. The minimum LC_{50} values were with GOPT-552 with 1.4×10^6 conidia/ml. Therefore, the most virulent isolate was GOPT-552, followed by GOPT-562.

Table 6. LC_{50} values (conidia/ml) of the isolates against adults of *Leptinotarsa decemlineata* at 14 days after treatment

Fungal isolate	Slope ± SE	X^2	LC_{50} (conidia/ml)	95% confidence interval
GOPT-498-4	0.319 ± 0.060	31.1	3.8×10^7	$8x10^6$-$2.4x10^8$
GOPT-529-2	0.405 ± 0.064	18.7	1.1×10^7	$3x10^6$-$3.8x10^7$
GOPT-552	0.390 ± 0.060	36.4	1.4×10^6	$2.9x10^5$-$5.4x10^6$
GOPT-562	0.438 ± 0.067	21.7	9.5×10^6	$3.3x10^6$-$2.7x10^7$

Also, in order to determine the effect of time on death, LT_{50} values calculated at 1×10^8 conidia/ml is given in Table 7. GOPT-529-2 caused the fastest death (LT_{50} = 10.2) of the isolates tested, followed by GOPT-552 (LT_{50} = 10.6) and GOPT-562 (LT_{50} = 10.7).

Table 7. LT_{50} values (days) of *Leptinotarsa decemlineata* adults treated with suspension of selected isolates at a dose of $1x10^8$ conidia/ml

Fungal isolate	Slope ± SE	X^2	LT_{50} (days)	95% confidence interval
GOPT-498-4	2.32 ± 0.477	97.4	15.1	10.9-36.4
GOPT-529-2	5.74 ± 0.927	15.7	10.2	9.47-11.2
GOPT-552	3.58 ± 0.608	28.2	10.6	9.43-12.5
GOPT-562	4.34 ± 0.753	13.2	10.7	9.64-12.1

Discussion

Mortality of adult insects caused by 33 different fungal isolates tested in this study on days 7 and 11 (Table 4) were lower than mortality of the third instars on days 5 and 7 (Table 3). The probable reason for this lower efficacy against adult beetles is the hardening of the insect cuticle as it develops from a larva to an adult. The insect cuticle is an important barrier against the invasion of fungal pathogens and the initial condition for the initiation of infection is the adhesion of the spore to the integument of the pest. Cuticular waxes accumulated during insect molting and subsequent intermolt period of insect life contain chemical components that inhibit the growth and penetration of microorganisms (David, 1967). In the instar cuticle, by comparison, the chemical components differentiate with maturation and cause the hardening of the cuticle, as well as an increase in the internal defense mechanisms against microbial infections. (Boman, 1981). According to the study of Wraight & Ramos (2002), *B. bassiana* was found to be more virulent against the early instars of the potato beetle. In another study, the mortality of insects due to *B. bassiana* infection in the late stage instars was low due to the hardening of the insect cuticle (Charnley, 2003). In our study of third instars, mortality ranging from 20 to 100% were observed among all isolates. The diversity in the virulence of isolates, and the amount of enzyme produced by them should be considered as the reason for this difference. The results obtained in this study show similarities and differences with previous studies. Çam et al. (2002), in their preliminary study to determine the effect of *B. bassiana* isolate at 1×10^8 conidia/ml on the third instars of potato beetle, reported that 89% mortality from fungal infection occurred after 6 days, and this mortality was different from the control but not imidacloprid application. McCoy et al. (1988) reported that the application of *B. bassiana* at 2×10^9 spore/g resulted in high mortality in potato beetle larvae. Akbarian et al. (2012) immersed the second and fourth instars of potato beetle in suspension of *B. bassiana* at 1×10^8 conidia/ml. Mortality after 15 days were 39.3% for the second instars and 25.6% for the fourth instars, which was lower mortality than found in our study. Shafighi et al. (2012) applied five concentrations of two local isolates of *B. bassiana*, DEBI007 and IR1217C, on second instars using the immersion method. Mortality after 15 days were 60 and 58% for the high concentration (1×10^9 conidia/ml) DEBI007 and IR1217C isolates, respectively. These results generally had low mortality compared to the mortality after 3, 5 and 7 days in our study.

Çam et al. (2002) in their preliminary study to determine the effect of a *B. bassiana* isolate at 1×10^8 conidia/ml on potato beetle adults, while the mortality observed in adult potato beetles was 11% after 3 days, it increased slightly with the extension of the incubation period and was 20% to 6 days. In our study, mortality in adults was generally the same as the mortality on day 3 of Çam et al. (2002), but were generally higher than the mortality on day 7. Karaman (2019) determined that *Simplicillium lamellicola* ((F.E.V.Sm.) Zare & W.Gams (Hypocreales: Cordycipitaceae), *Lecanicillium muscarium* (Petch) Zare & Gams (Hypocreales: Cordycipitaceae), *B. bassiana*, *M. anisopliae*, *C. fumosorosea* entomopathogenic fungi caused 80, 90, 90, 100 and 95% mortality in potato beetle adults after 7 days, respectively, under laboratory conditions.

Metarhizium anisopliae showed the highest effect with 100%. When these values are compared to our study, GOPT-552 gave the same mortality (100%) as *M. anisopliae*. Kılınç (2020), in his study, found the highest mortality (72%) after 7 days with isolate GOPT-258, followed by GOPT-321 with a mortality of 66%. In our study, the mortality after 7 days was the highest. The highest mortality (100%) was obtained with GOPT-552, followed by GOPT-562 with a mortality of 93%. However, there was no significantly difference between these isolates ($F = 11.5$; $df = 5$; $p > 0.05$) (Table 4). Watt & LeBrun (1984) reported similar results to our study, and found that soil treatment with *B. bassiana* was effective in controlling the first and second progeny pupae of *L. decemlineata* with a reduction of 74 and 77%, respectively. The treatment caused a decrease in the adults emerging from the pupa and an increase in the formation of mycosis. Todorova et al. (2000) determined that different *B. bassiana* isolates were highly effective at 100, 93, 90 and 87% against potato beetle adults 8 days after the application, and showed similarity with the high mortality of 86, 93 and 100% obtained after 7 and 11 days in our study (Table 4). However, it was low at 1×10^9 conidia/ml in the dose-mortality experiment (Table 5). The reason for this is thought to be the continuous subculture of EPF from PDA to PDA after their initial isolation from the insect. Güven et al. (2015) determined the effects of four *B. bassiana* isolates at 1×10^8 conidia/ml on third instars and adults of potato beetle using three application methods (spraying, dipping and residue method). *Beauveria bassiana* isolates were found to be more effective on larvae than adults in the three application methods, similar to our study. Mortality of third instars in the immersion method were 93% for BMAUM-002, 65% for BMAUM-001 and 60% for BMAUM-003, and mortality on day 5 for GOPT-498-4, GOPT-552 and GOPT-562 isolates in our study were 100, 95 and 70%, respectively (Table 3). Öztürk (2016), *B. bassiana* BMAUM-LDE-001, BMAUM-LDL-002 and BMAUM-LDE-002 isolates were sprayed on the second and third instars of potato beetle. Similar to our study, after 7 days, the same high (100%) mortality was found with GOPT-597, GOPT-617, GOPT-549, GOPT-624, GOPT-665, GOPT-557, GOPT-583, GOPT-584, GOPT-541-2, GOPT-541-1, GOPT-580-2, GOPT-547-5, GOPT-498-4, GOPT-551, GOPT-552, GOPT-562. The same isolates caused mortality of 59, 62, 86% in adults after 7 days with the three spray methods, respectively, and they were generally higher than after 7 days in our study. The reason for this difference is thought to be due to the different application methods (dipping-spraying) of the fungi solutions to adults.

In dose-mortality experiments, there was no significant differences ($p > 0.05$) between doses in terms of mortality caused by fungal isolates after 1, 3 and 5 days. From day 7 onwards, there was an increase in the mortality due to the increasing dose. Noronha & Goettel (2009) reported that inoculation concentration in adult potato beetles significantly affects the mortality and that most adult insects are infected when exposed to a surface concentration of 10^7 conidia/cm^2 compared to a surface concentration of 10^6 conidia/cm^2. Kılınç (2020) investigated the effects of two different isolates (GOPT-228 and GOPT-375) of *B. bassiana* against the adults of potato beetle at five concentrations (1×10^3, 1×10^5, 1×10^7, 1×10^8 and 1×10^9 conidia/ml) and observed 64-48, 72-52, 80-52, 80-68, and 88-72% mortality, respectively, after 11 days. This was higher mortality than at our day 11. Taking into account the mortality, mycosis and sporulation density in the culture media in single-dose efficacy experiments, Kılınç (2020) conducted a dose-mortality experiment on potato beetle adults using isolates GOPT-228 and GOPT-375. In that study, isolates GOPT-228 and GOPT-375 of *B. bassiana* gave low mortality of 48 and 32%, respectively, after 7 days. Likewise, in our dose-mortality experiment with four isolates, mortality at 1×10^8 conidia/ml was lower than in the single-dose efficacy experiments. In addition, Çerçi (2010) indicated that the results of the similar dose acquired from the single-dose efficacy experiments and dose-death experiments varied. These variations may be due to the difference in application date or the continuous subculture of fungus to purify and reproduce them. Butt & Goettel (2000) argued that when an isolate is used in biological activity experiments, its virulence should be enhanced by subculture through an insect host before being cultured in an artificial medium. Yıldırım (2021) determined the adult LC_{50} value of *B. bassiana* isolate LdA 1 as 0.17×10^8 conidia/ml. The LC_{50} of 0.17×10^8 conidia/ml obtained was less virulent than the LC_{50} of 1.4×10^6 conidia/ml for GOPT-552, which was the most virulent isolate in our study (Table 6). This high virulence in our isolate is attributable to its

isolation from forest soil rather than potato beetle cadavers. Despite the fact that both *B. bassiana* and *M. anisopliae* are common, *B. bassiana* is known to be very sensitive to the harmful effects of tillage and thus its natural habitat is limited. The persistence of *M. anisopliae* in cultivated soil has been determined as more common in the forest soil than in arable land (Rath et al., 1992; Vänninen, 1995; Quesada-Moraga et al., 2007; Sánchez-Peña et al., 2011). Most reports indicate that the incidence of entomopathogenic fungi in heavily cultivated soils is lower than in forest soils (Vänninen et al., 1989; Miętkiewski et al., 1991; Vänninen, 1995; Chandler et al., 1997; Bałazy, 2004).

In conclusion, *B. bassiana* isolates, GOPT-552, GOPT-562 and GOPT-529-2, were been found to be promising for biocontrol of *L. decemlineata* larvae and adults. However, further studies under field conditions is needed to confirm their usefulness.

References

Akbarian, J., Y. Ghosta, N. Shayesteh & S. A. Safavi, 2012. Pathogenicity of some isolates of *Beauveria bassiana* (Bals.) Vuill. and *Metarhizium anisopliae* (Metsch.) Sorokin on 2nd and 4th larval instars of Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Col.: Chrysomelidae), under laboratory conditions. African Journal of Microbiology Research, 6 (34): 6407-6413.

Ali-Shtayeh, M. S., A. B. Marai & R. M. Jamous, 2002. Distribution, occurrence and characterization of entomopathogenic fungi in agricultural soil in the Palestinian Area. Mycopathologia, 156 (3): 235-244.

Anonymous, 2014. Tokat Gıda, Tarım ve Hayvancılık İl Müdürlüğü ÇKS Kayıtları, 77 s (in Turkish).

Ateş, K., 2020. Entomopatojen Fungus İzolatlarının *Tetranychus urticae* (Koch) (Acari: Tetranychidae)'nin Bazı Biyolojik Parametreleri Üzerine Etkisi. Gaziosmanpaşa Üniversitesi Fen Bilimleri Enstitüsü, Bitki Koruma Anabilimdalı, (Unpublished) Master Sciences Thesis, Tokat, Türkiye, 49 s (in Turkish with abstract in English).

Baki, D., H. S. Tosun & F. Erler, 2021. Efficacy of indigenous isolates of *Beauveria bassiana* (Balsamo) Vuillemin (Deuteromycota: Hyphomycetes) against the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae). Egyptian Journal of Biological Pest Control, 31 (1): 1-11.

Bałazy, S., 2004. Significance of protected areas for the preservation of entomopathogenic fungi. Kosmos, 53 (1): 5-16 (in Polish with abstract in English).

Baysal, E., 2017. Bazı Entomopatojen Fungus İzolatlarının, Yonca Yaprak Böceği, [*Gonioctena fornicata* (Brüggeman) (Coleoptera: Chrysomelidae)] ve Yonca Hortumlu Böceği, [*Hypera postica* (Gyllenhal) (Coleoptera: Curculionidae)]'ne Karşı Etkinliklerinin Araştırılması. Gaziosmanpaşa Üniversitesi Fen Bilimleri Enstitüsü, Bitki Koruma Anabilimdalı, (Unpublished) Master Sciences Thesis, Tokat, Türkiye, 61 s (in Turkish with abstract in English).

Boman, H. G., 1981. "Insect Responses to Microbial infections, 769-784". In: Microbial Control of Pests and Plant Diseases (Ed. H. D. Burges). Academic Press, London, UK, 949 pp.

Butt, T. M. & M. S. Goettel, 2000. "Bioassays of Entomogenous Fungi, 144-197". In: Bioassays of Entomopathogenic Microbes and Nematodes (Eds. A. Navon & K. R. S. Ascher). CABI Publishing, Wallingford, Oxon, UK, 324 pp.

Çam, H., A. Gökçe, Y. Yanar & İ. Kadıoğlu, 2002. "Entomopatojen fungus *Beauveria bassiana* (Bals.) Vuill.'nın patates böceği, *Leptinotarsa decemlineata* (Say.) üzerindeki etkisi. 359-364." Türkiye 5. Biyolojik Mücadele Kongresi, (4-7 Eylül 2002, Erzurum, Türkiye), 483 s (in Turkish).

Çerçi, F. S., 2010. Bazı Fungus İzolatlarının Pamuk Yaprakkurdu, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae), Larvalarına Ölüm Etkilerinin Belirlenmesi. Kahramanmaraş Sütçü İmam Üniversitesi Fen Bilimleri Enstitüsü Bitki Koruma Ana Bilim Dalı, (Unpublished) Master Sciences Thesis, Kahramanmaraş, Türkiye, 38 s (in Turkish with abstract in English).

Chandler, D., D. Hay & A. P. Reid, 1997. Sampling and occurrence of entomopathogenic fungi and nematodes in UK soils. Applied Soil Ecology, 5 (2): 133-141.

Charnley, A. K., 2003. Fungal pathogens of insects: Cuticle Degrading Enzymes and Toxins. Advances in Botanical Research, 40: 241-321.

David, W. A. L., 1967. "The Physiology of the Insect Integument in Relation to the Invasion of Pathogens, 17-35". In: Insects and Physiology. (Eds. J. W. L. Bearnent & J. E. Trehere), Oliver-Boyd, Edinburg, London, 378 pp.

Deacon, J. W., 1983. Microbial Control of Plant Pests and Diseases. Van Nostrand Reinhold Company, New York, US, 88 pp.

Doğan, M. K., 2022. Tokat İlinde Yaygın Olarak Bulunan Çekirge Türü *Poecilimon glandifer* (Karabag) (Orthoptera: Tettiigonidae) Mücadelesinde Bazı Entomopatojen Fungusların Kullanım Potansiyeli Üzerine Araştırmalar. Gaziosmanpaşa Üniversitesi Fen Bilimleri Enstitüsü, Bitki Koruma Anabilimdalı, (Unpublished) Master Sciences Thesis, Tokat, Türkiye, 58 s (in Turkish with abstract in English).

Dragonova, S., R. Donkova & D. Geogieva, 2008. Impact of strains of entomopathogenic fungi on some main groups of soil microorganisms. Journal of Plant Protection Research, 48 (2): 169-179.

Grafius, E., 1997. Economic impact of insecticide resistance in the Colorado potato beetle (Coleoptera: Chrysomelidae) on the Michigan potato industry. Journal of Economic Entomology, 90 (5): 1144-1151.

Güven, Ö., D. Çayır, R. Baydar & İ. Karaca, 2015. Entomopatojen fungus *Beauveria bassiana* (Bals.) Vull. izolatlarının patates böceği [*Leptinotarsa decemlineata* Say. (Coleoptera: Chrysomelidae)] üzerindeki etkisi. Türkiye Biyolojik Mücadele Dergisi, 6 (2): 105-114 (in Turkish with abstract in English).

Humber, R. A., 2005. Entomopathogenic Fungal Identification. USDA-Agricultural Research Service, New York, USA, 32 pp.

Humber, R. A., 2012. "Identification of Entomopathogenic Fungi, 151-187" In: Manual of Techniques in Invertebrate Pathology (Second Edition) (Ed. L. A. Lacey). Academic Press, New York, 504 pp.

Ihara, F., K. Yaginuma, N. Kobayashi, K. Mishiro & T. Sato, 2001. Screening of entomopathogenic fungi against the brown-winged green bug, *Plautia stali* Scott (Hemiptera: Pentatomidae). Applied Entomology and Zoology, 36 (4): 495-500.

Jacques R. L. & T. R. Fasulo, 2015. Colorado potato beetle *Leptinotarsa decemlineata*. DPI Entomology Circular 271. Florida Department of Agriculture and Consumer Services, Division of Plant Industry and University of Florida, 6 pp.

Karaman, S. Y., 2019. Bazı Entomopatojen Fungusların Patates Böceği [*Leptinotarsa decemlineata* Say. (Coleoptera: Chrysomelidae)] Üzerine Etkilerinin Belirlenmesi. Ondokuz Mayıs Üniversitesi Fen Bilimleri Enstitüsü Bitki Koruma Anabilimdalı, (Unpublished) Master Sciences Thesis, Samsun, Türkiye, 61 s (in Turkish with abstract in English).

Kendrick, B., 2000. The Fifth Kingdom, Third Edition. Mycologue Publications, Sidney, Australia, 386 pp.

Kepenekci, I., E. Oksal, H. D. Saglam, T. Atay, A. Tulek & E. Evlice, 2015. Identification of Turkish isolate of the entomopathogenic fungi, *Purpureocillium lilacinum* (syn: *Paecilomyces lilacinus*) and its effect on potato pests, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae) and *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae). Egyptian Journal of Biological Pest Control, 25 (1): 121-127.

Kılınç, M., 2020. Bazı Entomopatojen Fungus İzolatlarının *İn-Vivo* ve *İn-Vitro* Şartlarda Patates Böceği'ne [*Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae)] İnsektisidal Etkisinin Belirlenmesi Üzerine Araştırmalar. Gaziosmanpaşa Üniversitesi Fen Bilimleri Enstitüsü, Bitki Koruma Anabilimdalı, (Unpublished) Master Sciences Thesis, Tokat, Türkiye, 73 s (in Turkish with abstract in English).

Kıvan, M. & T. Aysal, 2014. Laboratuvar koşullarında *Perillus bioculatus* (F.) (Hemiptera: Pentatomidae)'un gelişme süresi ve ölüm oranı üzerinde ön çalışmalar. Türkiye Biyolojik Mücadele Dergisi, 5 (1): 23-29 (in Turkish with abstract in English).

Kulkarni, S. A., 2015. Biochemical and Molecular Studies of Chitin Deacetylase from *Metarhizium* species. Savitribai Phule Pune University, Biochemical Sciences Division CSIR-National Chemical Laboratory, (Unpublished) Ph.D Thesis, India, 196 pp.

LeOra, S., 1994. LeOra software, Polo-Pc: probit and logit Analysis. Berkeley CA, the US, 28 pp.

McCoy, C. W., R. A. Samson & D. G. Boucias, 1988. "Entomogenous Fungi, 151-236". In: CRC Handbook of Natural Pesticides, Microbial Insecticides, Part A, Entomogenous Protozoa and Fungi (Eds. C. M. Ignoffo & N. B. Mandava). CRC Press, Inc., Boca Raton, Florida, USA, 262 pp.

Miętkiewski, R., M. Żurek, C. Tkaczuk & S. Bałazy, 1991. Occurrence of entomopathogenic fungi in arable soil, forest soil and litter. Roczniki Nauk Rolniczych, Seria E, 21 (1/2): 61-68 (in Polish with abstract in English).

Noronha, C. & M. S. Goettel, 2009. The effect of digging by the Colorado Potato Beetle (*Leptinotarsa decemlineata*) (Coleoptera: Chrysomelidae) on the acquisition and retention of *Beauveria bassiana* conidia. Fruit, Vegetable and Cereal Science and Biotechnology, 3 (1): 59-64.

Öztürk, H. E., 2016. Bazı Biyoinsektisit ve Entomopatojen Fungusların *Leptinotarsa decemlineata* (Say, 1824) (Coleoptera: Chrysomelidae)'ya Etkileri. Süleyman Demirel Üniversitesi Fen Bilimleri Enstitüsü Bitki Koruma Anabilimdalı, (Unpublished) Master Sciences Thesis, Isparta, Türkiye, 25 s (in Turkish with abstract in English).

Öztürk, H. E.., Ö. Güven & İ. Karaca, 2015. Effects of some bioinsecticides and entomopathogenic fungi on Colorado potato beetle (*Leptinotarsa decemlineata* L.). Communications in Agricultural and Applied Biological Sciences, 80 (2): 205-211.

Paparatti, B. & S. Speranza, 1999. "Biological control of chestnut weevil (*Curculio elephans* Gyll.; Coleoptera, Curculionidae) with the entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuill. (Deuteromycotina, Hyphomycetes), 459-464". Acta Horticulture 494 2th International Symposium on Chestnut (1 July 1999, Bordeaux, France), 528 pp.

Paparatti, B. & S. Speranza, 2005. "Biological control of hazelnut weevil (*Curculio nucum* L., Coleoptera, Curculionidae) using the entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuill. (Deuteromycotina, Hyphomycetes), 407-412". Acta Horticulture 686 7th International Congress on Hazelnut (30 July 2005, Tarragona-Reus, Spain), 676 pp.

Quesada-Moraga, E., J. A. Navas-Cortés, E. A. A. Maranhao, A. Ortiz-Urquiza & C. Santiago-Álvarez, 2007. Factors affecting the occurrence and distribution of entomopathogenic fungi in natural and cultivated soils. Mycological Research, 111 (8): 947-966.

Rath, A. C., T. B. Koen & H. Y. Yip, 1992. The influence of abiotic factors on the distribution and abundance of *Metarhizium anisopliae* in Tasmanian pasture soils. Mycological Research, 96 (5): 378-384.

Samson R. A., H. C. Evans & J. P. Latge, 1988. Atlas of Entomopathogenic Fungi. Springer Verlag, Berlin, 187 pp.

Sánchez-Peña, S. R., J. S. Juan Lara & R. F. Medina, 2011. Occurrence of entomopathogenic fungi from agricultural and natural ecosystems in Santillo, Mexico, and their virulence towards thrips and whiteflies. Journal of Insect Science, 11 (1): 1-10.

Sevim, A., 2010. Doğu Karadeniz Bölgesi'nden Entomopatojenik Fungusların İzolasyonu, Karakterizasyonu ve Virülanslarının Belirlenmesi. Karadeniz Teknik Üniversitesi, Fen Bilimleri Enstitüsü Biyoloji Anabilimdalı, (Unpublished) Ph.D Thesis, Trabzon, 168 s (in Turkish with abstract in English).

Shafighi, Y., M. H. Kazemi, Y. Ghosta & J. Akbarian, 2012. Insecticidal efficacy of two isolates of *Beauveria bassiana* (Bals.) (Vuill.), on the second larval stage of *Leptinotarsa decemlineata* (Say) (Col: Chrysomelidae). Archives of Phytopathology and Plant Protection, 45 (15): 1852-1860.

Solmaz, B., 2022. Bazı Entomopatojen Fungus İzolatlarının ve Diatom Toprağının Pirinç Biti, *Sitophilus oryzae* (L.) (Coleoptera:Curculionidae) Üzerine Etkileri. Gaziosmanpaşa Üniversitesi Fen Bilimleri Enstitüsü, Bitki Koruma Anabilimdalı, (Unpublished) Master Sciences Thesis, Tokat, Türkiye, 46 s (in Turkish with abstract in English).

Soy, B., 2017. Tokat İli Sebze Alanlarından İzole Edilen Bazı Entomopatojen Fungusların Gül Yaprak Biti [*Macrosiphum rosae* (L.) (Hemiptera:Aphididae)]'ne Etkilerinin Belirlenmesi. Gaziosmanpaşa Üniversitesi Fen Bilimleri Enstitüsü, Bitki Koruma Anabilimdalı, (Unpublished) Master Sciences Thesis, Tokat, Türkiye, 43 s (in Turkish with abstract in English).

Todorova, S. I., D. Coderre & J. C. Côté, 2000. Pathogenicity of *Beauveria bassiana* isolates toward *Leptinotarsa decemlineata* [Coleoptera: Chrysomelidae], *Myzus persicae* [Homoptera: Aphididae] and their predator *Coleomegilla maculata lengi* [Coleoptera: Coccinellidae]. Phytoprotection, 81 (1): 15-22.

Uçar, S., 2021. Bazı Entomopatojen Fungus İzolatlarının Bakla Zınnı [*Tropinota* (*Epicometis*) *hirta* (Poda) (Coleoptera: Scarabaeidae)]'na Karşı Etkinliklerinin Araştırılması. Gaziosmanpaşa Üniversitesi Fen Bilimleri Enstitüsü, Bitki Koruma Anabilimdalı, (Unpublished) Master Sciences Thesis, Tokat, Türkiye, 54 s (in Turkish with abstract in English).

Vänninen, I., 1995. Distribution and occurrence of four entomopathogenic fungi in Finland: effect of geographical location, habitat type and soil type. Mycological Research, 100 (1): 93-101.

Vänninen, I., G. B. Husberg & H. M. T. Hokkanen, 1989. Occurrence of entomopathogenic fungi and entomopathogenic nematodes in cultivated soils in Finland. Acta Entomologica Fennica, 53: 65-71.

Watt, B. A. & R. A. LeBrun, 1984. Soil effects of *Beauveria bassiana* on pupal populations of the Colorado potato beetle (Coleoptera: Chrysomelidae). Environmental Entomology, 13 (1): 15-18.

White, T. J., T. D. Bruns, S. B. Lee & J. W. Taylor, 1990. "Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics, 315-322". In: PCR Protocols: A Guide to Methods and Applications (Eds. M. A. Innis, D. H. Gelfand, J. J. Sninsky & T. J. White). Academic Press, San Diego, California, USA, 482 pp.

Wraight, S. P. & M. E. Ramos, 2002. Application parameters affecting field efficacy of *Beauveria bassiana* foliar treatments against Colorado potato beetle *Leptinotarsa decemlineata*. Biological Control, 23 (2): 164-178.

Yıldırım, K., 2021. Patates Böceği (*Leptinotarsa decemlineata*, Coleoptera Chrysomelidae)'nin Fungal Patojenlerinin Araştırılması ve Prototip Fungal Mücadele Ürününün Geliştirilmesi. Karadeniz Teknik Üniversitesi Fen Bilimleri Enstitüsü Biyoloji Anabilimdalı, (Unpublished) Master Sciences Thesis, Samsun, Türkiye, 117 s (in Turkish with abstract in English).

Zemek, R., J. Konopická, E. Jozová & O. Skoková Habuštová, 2021. Virulence of *Beauveria bassiana* strains isolated from cadavers of Colorado potato beetle, *Leptinotarsa decemlineata*. Insects, 12 (12): 1-13.

Zhao, J. Z., B. A. Bishop & E. J. Grafius, 2000. Inheritance and synergism of resistance to imidacloprid in the Colorado potato beetle (Coleoptera: Chrysomelidae). Journal of Economic Entomology, 93 (5): 1508-1514.

Zimmermann, G., 1986. The '*Galleria* bait method' for detection of entomopathogenic fungi in soil. Journal of Applied Entomology, 102 (1-5): 213-215.

Türk. entomol. derg., 2022, 46 (2): 175-186
DOI: http://dx.doi.org/10.16970/entoted.1016214

ISSN 1010-6960
E-ISSN 2536-491X

Original article (Orijinal araştırma)

Life table parameters of *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) on four wild tomato species

Tuta absoluta (Meyrick, 1917) (Lepidoptera: Gelechiidae)'nın dört yabani domates türü üzerinde yaşam çizelgesi parametreleri

Baran ASLAN[1*] Ali Kemal BİRGÜCÜ[2] Selman ULUIŞIK[1] İsmail KARACA[2]

Abstract

Tuta absoluta (Meyrick, 1917) (Lepidoptera: Gelechiidae) is an important tomato pest that also feeds on other plants in the Solanaceae. The effects of four wild tomato species (*Solanum arcanum* Peralta, *Solanum habrochaites* S.Knapp & D.M.Spooner, *Solanum peruvianum* L., *Solanum pimpinellifolium* L.) and two accession of *Solanum lycopersicum* L. (LA0292 and cv. 112-432) on the life table parameters of *T. absoluta* were determined. Larval development time, lifespan, pupal period, fecundity, and longevity were also estimated. The study was conducted in Isparta University of Applied Sciences, Agriculture Faculty, Plant Protection Department, Isparta, Türkiye in 2020-2021. *Solanum lycopersicum* was the most suitable species for the development of *T. absoluta*. Among the wild tomato species, *S. pimpinellifolium* for intrinsic rate of increase, *S. arcanum,* and *S. pimpinellifolium* for net reproductive rate, *S. habrochaites* and *S. pimpinellifolium* for mean generation time and doubling time*, S. pimpinellifolium* and *S. arcanum* for finite rate of increase were higher than the others. Although the results showed significant differences between the tested wild tomato species, *S. pimpinellifolium* and *S. arcanum* were the most effective wild hosts.

Keywords: Life table, *Solanum*, *Tuta absoluta*, wild tomato species

Öz

Tuta absoluta (Meyrick, 1917) (Lepidoptera: Gelechiidae) önemli bir domates zararlısıdır ve Solanaceae familyasındaki diğer konukçu bitkiler ile de beslenebilmektedir. Bu çalışmada dört yabani domates türü (*Solanum arcanum* Peralta, *Solanum habrochaites* S.Knapp & D.M.Spooner, *Solanum peruvianum* L., *Solanum pimpinellifolium* L.) ve iki *Solanum lycopersicum* L. (LA0292 ve cv. 112-432) aksesyonunun *T. absoluta*'nın yaşam tablosu parametreleri üzerindeki etkileri belirlenmiştir. Ayrıca larva gelişim süresi, yaşam süresi, pupa dönemi, doğurganlık ve yaşam ömrü de hesaplanmıştır. Çalışma Isparta Uygulamalı Bilimler Üniversitesi, Ziraat Fakültesi, Bitki Koruma Bölümü (Isparta, Türkiye)'nde 2020-2021 yılları arasında yürütülmüştür. *Solanum lycopersicum*, *T. absoluta*'nın gelişmesi bakımından en uygun tür olarak belirlenmiştir. Yabani domates türlerinden, kalıtsal üreme yeteneği için *S. pimpinellifolium*, net üreme gücü için *S. arcanum* ve *S. pimpinellifolium*, ortalama döl süresi ve popülasyonun ikiye katlanma süresi için *S. habrochaites ve S. pimpinellifolium*, artış oranı sınırı için *S. pimpinellifolium* ve *S. arcanum* türleri en etkili konukçular olarak belirlenmiştir. Sonuçlar, çalışılan yabani domates türlerinde önemli farklılıklar göstermiş olmasına rağmen *S. pimpinellifolium* ve *S. arcanum* en etkili yabani konukçu türler olarak belirlenmiştir.

Anahtar sözcükler: Yaşam çizelgesi, *Solanum*, *Tuta absoluta*, yabani domates türleri

[1] Burdur Mehmet Akif Ersoy University, Burdur Food Agriculture and Livestock Vocational School, 15030, Burdur, Türkiye
[2] Isparta University of Applied Sciences, Faculty of Agriculture, Department of Plant Protection, 32260, Isparta, Türkiye
* Corresponding author (Sorumlu yazar) e-mail: aslanb@mehmetakif.edu.tr
Received (Alınış): 29.10.2021 Accepted (Kabul ediliş): 09.06.2022 Published Online (Çevrimiçi Yayın Tarihi): 18.06.2022

Introduction

The tomato leafminer, *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae), is the main pest in field and greenhouse cultivation of tomato because of the economic damage it causes in growing areas (Biondi & Desneux, 2019). *Tuta absoluta* is on the A1 quarantine list of EPPO (as South American tomato pinworm because the pest was first described in Peru in 1917), and has spread to North African countries and caused serious damage to the tomato growing areas in the region (Desneux et al., 2011). The first record of the pest in Europe was in Spain in 2006 and it spread to all Mediterranean countries including Türkiye within a short time (Kılıç, 2010; Campos et al., 2017). The distribution of pest continued to expand quickly and it is now also found in the majority of European countries, North African countries (Son et al., 2017; Sylla et al., 2017; Mukwa et al., 2021), Middle East countries and Arabian Peninsula (Biondi et al., 2018), Asian countries, especially India and Afghanistan (Han et al., 2019). In infested growing areas, if effective controls are not implemented, it causes 80-100% product losses in tomato plants (Cocco et al., 2013). Due to its fast spread, damage to almost all plant parts and year-round suitable climatic conditions, a highly intense control is made against the pest. The pesticides applied intensively have led to development resistance in the pest populations and caused residue problems in the crops (Gontijo et al., 2013; Guedes et al., 2019). Since chemical control alone is not sufficient, researchers are now focusing on other control techniques, including biological control (Al-Jboory et al., 2012; Zappalà et al., 2013; Gervassio et al., 2019), biotechnological control (Caparros Medigo et al., 2013; Aksoy & Kovancı, 2016) and IPM (Giorgini et al., 2019) techniques, but generally it is concluded that effective control can only be achieved on low pest populations.

Currently, cultivated tomatoes contain only <5% of the genetic diversity of wild species (Miller & Tanksley, 1990). Wild seeds have a high level of genetic diversity compared to modern cultivars (Zhang et al., 2016). Genes cloned from wild relatives have been often used in crossbreeding and molecular studies to improve various crop species (Maxted et al., 2013). Although resistant cultivars have been developed, they could not be a long-term strategy due to the continued use of limited resources, the disease agent rapid adaptation and behavior development of insects. Nowadays, the development of tomato cultivars resistant to biotic factors has been achieved to a great extent by transferring various characteristics from the wild relatives of tomatoes; *Solanum chilense* (Dunal) Reiche (Zamir et al., 1994), *Solanum habrochaites* S.Knapp & D.M.Spooner (Prasanna et al., 2015), *Solanum peruvianum* L. (Seah et al., 2004; Lanfermeijer et al., 2005), *Solanum pennellii* Correll (Parniske et al., 1999) and *Solanum pimpinellifolium* Mill. ex Dunal (Chunwongse et al., 2002).

The life table parameters are an important tool for pest control because they provide a detailed description of the growth, survival and fecundity. This study aimed to determine the life table parameters of *T. absoluta* on wild tomato species and to identify which species showed low growth and reproduction rates. The data obtained in this study include development time, fecundity and survival of *T. absolut*a on four wild tomato species, and two accessions of *Solanum lycopersicum* L. (LA0292 and cv. 112-432).

Materials and Methods

Plant and pest culture

Seeds of *Solanum arcanum* Peralta (LA2152), *S. habrochaites* (LA0094), *S. peruvianum* (LA0445), *S. pimpinellifolium* (LA0100) and *S. lycopersicum* (LA0292) used in this study were obtained from Tomato Genetics Resource Center (tgrc.ucdavis.edu; UC Davis, CA, USA). Tomato plants and *T. absoluta* population were grown in two growth cabinets in Isparta University of Applied Sciences, Agriculture Faculty, Plant Protection Department, Isparta, TÜRKİYE during 2020-2021.

Four wild tomato species and two accessions of *S. lycopersicum* (LA0292 and cv. 112-432; the latter being a commercial cultivar and included as a control in this study) were grown together with the same time and same conditions. All tomato seeds were sized using standard seedling growing practices, and then they were transplanted into 15 × 9 cm pots (4 L) containing a mixture of peat and perlite (1:1). While irrigation and maintenance procedures were applied to all tomato seedlings grown, no chemical fertilizers or pesticides were used. In case of any disease or pest contamination, the damaged part of the plants was removed; if the whole plant was affected, it was immediately removed from the growth cabinet.

Rearing of *Tuta absoluta*

Larvae, pupae and adult samples of *T. absoluta* were collected from infested tomato growing areas and were placed in a climate room. Healthy individuals were selected and applied to commercial tomato plants. To get a stock culture, healthy individuals of *T. absoluta* were reared on cv. 112-432 for at least three generations and then used in experiments.

Survival experiments

All cultures and experiments were kept and conducted in a controlled climate chamber (25±1 ºC; 60±5% RH and 16:18 h L:D photoperiod).

At first, 20 individuals of *T. absoluta* adults taken from the stock culture were placed in net covered plastic boxes (10 × 8 × 8 cm), a complete compound leaf of the tomato species that is suitable for deposition of eggs. To prevent water loss of the tomato leaves, the stem was wrapped with wet cotton and placed in eppendorf tubes containing water. With the daily controls, the eggs contained tomato leaves were transferred into new Petri dishes, and again a clean tomato leaf was left in the plastic boxes containing the adults. The eggs were left on the studied tomato species on the fourth day with leaf parts. After hatching, the larvae were taken into Petri dishes covered with a net together with the leaflet. Each larva considered as a separate replicate. The development and survival of pest species were checked daily. The sexes of individuals were distinguished as male or female at the pupal stage. After determining the sexes, adults were left in plastic boxes with clean tomato leaves with one female and at least two males. A 5% sugar-water solution soaked in blotting paper was placed in the boxes for adult feeding. The tomato leaves were removed from the boxes, and clean tomato leaves of the same species were replaced in the boxes daily. The experiment continued until the last adult died. All treatments had 30 replicates for each plant to give a total 180 replicates.

Life tables analyses

The obtained daily data were analyzed based on the theory of age-stage, two-sex life table (Chi & Liu 1985; Chi, 1988; Chi et al., 2020). According to this theory, calculated parameters:

Age-specific survival rate (l_x), age-specific fecundity rate, age-stage-specific survival rate (the possibility that newly laid eggs will live or exist to age *x* and *j*),

Mean fecundity (F) (eggs/female), $F = \frac{\sum_{x=1}^{N_f} E_x}{N_f}$

Net reproductive rate (R_0) (females/female), $R_0 = \sum_{x=0}^{\infty} l_x . m_x$

Intrinsic rate of increase (*r*) (female/female/day), $1 = \sum_{x=0}^{\infty} \left(e^{-r(x+1)} \sum_{j=1}^{m} f_{xj} s_{xj} \right)$

Mean generation time (T) (day), $T = \frac{lnR_0}{r}$

Gross reproduction rate (GRR) (larvae/female), $GRR = \sum m_x$ (Birch, 1948),

Finite rate of increase (λ) (larvae/female/day), $1 = \sum_{x=0}\left(\lambda^{-(x+1)} \sum_{j=1}^{m} f_{xj}\, s_{xj}\right)$

Population doubling time (DT) (day) $DT = \frac{ln\,2}{r}$ (Kairo & Murphy, 1995).

To be used in a comparison test, the mean and standard errors of the *r* values gained from the populations were calculated by the Bootstrap resampling method with the estimates 100,000 times (Meyer et al., 1986; Lawo & Lawo, 2011; Huang & Chi, 2012; Yu et al., 2013a,b). Before the Tukey multiple comparisons test (Tukey, 1949), One-Way ANOVA was applied on the bootstrap values of the intrinsic rates. IBM SPSS Statistics (Version 20.0, August 2011, SPSS Inc., Chicago, IL, USA) and MS Excel 2010 (Version 14.0, June 2010, Microsoft Corporation, Redmond, WA, USA) were used for statistical analyses.

In order to define l_x of tomato leaf miner on different tomato species, a two-parameter Weibull distribution model was used (Deevey, 1947; Pinder et al., 1978; Tingle & Copland, 1989; Wang et al., 2000). The parameters of this distribution model formula:

$$S_p(x) = e^{\left[-\left(\frac{x}{b}\right)^c\right]} x, b, c > 0$$

The probability of survival at the age $S_p(x)$ of females in days, b is a scale parameter, and c is a shape parameter. The parameters and curves of Weibull distributions were performed by using SigmaPlot (Version 11.0, Systat Software, Inc., San Jose, CA, USA).

Results

No significant difference was detected between the egg period on control, *S. habrochaites,* and *S. lycopersicum* species; however, there were significant differences between *S. arcanum, S. peruvianum,* and *S. pimpinellifolium* (Table 1). Table 1 shows the developmental times and Figure 1 gives life table graphics of *T. absoluta* on tomato species. The developmental factors of the tomato leafminer were affected by the wild tomato species, especially on larval periods, pupal period and lifespan. The larval period was determined the longest on *S. habrochaites* (43.7 days) and the shortest on control (*S. lycopersicum*) (29.6). Pupal development time of *T. absoluta* was determined the highest on *S. habrochaites* (11.2 days). The shortest pupal period was determined on *S. peruvianum* and control (*S. lycopersicum*) (8.13 and 8.16 days, respectively). Among the experimented host species, the longest lifespan determined on *S. habrochaites,* and the shortest on control (*S. lycopersicum* cv. 112-432) (Table 1).

Table 1. Preadult development periods (mean±SE days)* of *Tuta absoluta* on control (*Solanum lycopersicum* cv. 112-432), *S. lycopersicum* (LA0292) and four wild tomato species

Species	n	Egg		L_1		L_2		L_3		L_4		Pupal period		Lifespan	
Control	30	5.26±0.10	b	4.13±0.15	c	3.93±0.10	d	3.90±0.10	c	4.31±0.10	d	8.16±0.39	c	29.6±0.59	d
S. arcanum	29	5.75±0.09	a	4.69±0.11	ab	5.34±0.18	ab	4.75±0.16	b	9.06±0.33	b	9.62±0.29	b	39.2±0.39	b
S. *habrochaites*	31	5.38±0.08	b	4.35±0.10	bc	5.38±0.17	ab	4.96±0.19	b	12.12±0.50	a	11.3±0.23	a	43.7±0.47	a
S. lycopersicum	31	5.16±0.06	b	4.48±0.12	bc	5.00±0.13	bc	6.16±0.18	a	6.87±0.35	c	10.9±0.33	ab	38.7±0.48	b
S. peruvianum	30	5.43±0.09	ab	4.36±0.08	bc	4.50±0.11	cd	5.86±0.14	a	6.10±0.27	c	8.13±0.33	c	34.4±0.48	c
S. pimpinellifolium	33	5.48±0.08	ab	5.03±0.11	a	5.87±0.17	a	5.18±0.11	b	6.48±0.23	c	10.6±0.35	ab	39.5±0.45	b

* Different letters in each column show significant differences among biological periods at 5% level. (Tukey's HSD test, P > 0.05); (F_{Egg} = 5.04; df = 5,178; P = 0.000; F_{L1} = 7.22; df = 5,178; P = 0.000; F_{L2} = 21.4; df = 5,178; P = 0.000; F_{L3} = 27.769; df = 5,18; P = 0.000; F_{L4} = 70.048; df = 5,177; P = 0.000; F_{Pupa} = 16.842; df = 5,173; P = 0.000; $F_{Lifespan}$ = 101.802; df = 5,1167; P = 0.000).

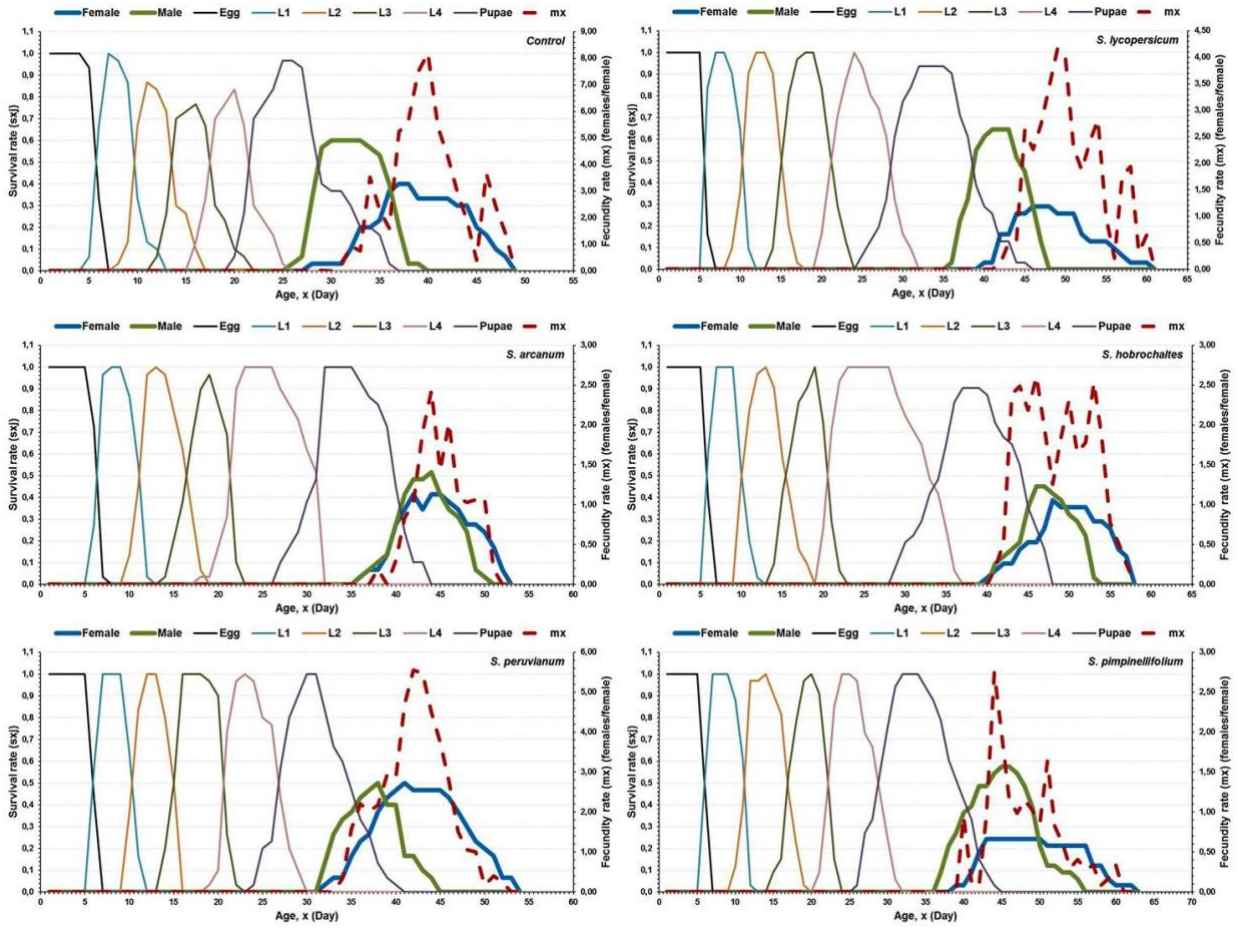

Figure 1. Age-stage-specific survival rate$_j$ and fecundity curves of *Tuta absoluta* on control (*Solanum lycopersicum* cv. 112-432), *S. lycopersicum* (LA0292) and four wild tomato species; *Solanum arcanum*, *S. habrochaites*, *S. peruvianum* and *S. pimpinellifolium*.

There was no significant difference between the oviposition periods of adult tomato leafminer affected by host plants except for *S. arcanum* and *S. habrochaites*. Although the values of adult pre-oviposition periods were similar to each other, statistical differences were observed. However, female adult longevity was not different from each other except for *S. peruvianum.* The shortest total longevity was observed on control (*S. lycopersicum* cv. 112-432) (34.6 days), and the longest was on *S. habrochaites* (46.5 days). The results were not different from the overall longevity on *S. arcanum* (42.3 days) and *S. pimpinellifolium* (43.0 days) (Table 2).

Table 2. Total longevity (days), adult pre-oviposition period (APOP), oviposition and post-oviposition periods, adult (female, male) longevity of *Tuta absoluta* on control (*Solanum lycopersicum* cv. 112-432), *S. lycopersicum* (LA0292) and four wild tomato species

Species	Total longevity*	APOP*	Oviposition period*	Post-oviposition period*	Adult longevity*	
					Female	Male
Control	34.8±0.77 d	0.83±0.11 a	9.75±0.65 a	0.67±0.19 bc	11.3±0.55 a	8.61±0.22 b
S. arcanum	42.3±0.59 b	1.92±0.16 b	5.00±0.54 b	1.92±0.24 a	8.9±0.68 a	7.60±0.19 c
S. habrochaites	46.5±0.81 a	1.00±0.15 b	5.87±0.88 b	0.93±0.23 abc	9.6±0.60 a	7.26±0.18 c
S. lycopersicum	44.1±0.51 ab	1.10±0.18 b	8.90±1.64 a	0.40±0.22 c	11.6±1.42 a	7.85±0.13 bc
S. peruvianum	38.7±0.71 c	1.93±0.20 a	8.86±1.34 a	1.80±0.20 ab	12.6±1.22 ab	7.60±0.16 c
S. pimpinellifolium	43.0±0.46 b	1.00±0.23 b	9.18±2.06 a	1.63±0.52 ab	16.3±1.25 a	10.63±0.26 a

* Different letters in each column show significant differences among biological periods at 5% level. (Tukey's HSD test, P > 0.05; ($F_{Total\ longevity}$ = 36.481; df = 5,65; P = 0.000; F_{APOP} = 8.00; df = 5,72; P = 0.00; $F_{Oviposition\ period}$ = 2.75; df = 5,72; P = 0.025; $F_{Post\text{-}oviposition\ period}$ = 5.10; df = 5,72; P = 0.000; $F_{Adulu\ female\ longevity}$ = 6.09; df = 5,65; P = 0.000; $F_{Adult\ male\ longevity}$ = 41.0; df = 5,96; P = 0.000).

The main fecundity was highest on control (149 eggs/female) and the lowest on *S. arcanum* (43.7 eggs/female) (Table 3). The *r* and finite rates of increase (λ) were the highest on the control (0.086 and 1.09 day^{-1}, respectively). Among the studied tomato species, *S. lycopersicum* had the lowest *r* and λ rates (0.049 and 1.05, respectively). The highest net reproductive rate (R_0) was seen on the *S. peruvianum* with the value of 29.9 (females/female). The lowest R_0 was recorded on *S. pimpinellifolium* (10.5 females/female) and *S. arcanum* (11.0 females/female). Mean generation time (T) and population doubling time (DT) were the shortest on control with the 37.5 and 8.02 days, respectively, and the highest T (47.6 days) and DT (14.0 days) were on *S. lycopersicum* (Table 3).

Table 3. Fecundity and the population parameters (mean±SE)* of *Tuta absoluta* on control (*Solanum lycopersicum* cv. 112-432), *Solanum lycopersicum* (LA0292) and four wild tomato species

	Control	*S. arcanum*	*S. habrochaites*	*S. lycopersicum*	*S. peruvianum*	*S. pimpinellifolium*
F	149±11.9 a	43.7±6.00 c	63.0±10.25 bc	110±21.6 ab	108±18.6 abc	123±23.9 ab
r	0.086±0.001 a	0.054±0.001 d	0.063±0.001 c	0.049±0.004 f	0.084±0.003 b	0.051±0.002 e
R_0	25.5±0.30 b	11.0±0.13 d	18.8±0.22 c	10.5±0.13 d	29.9±0.28 a	10.5±0.14 d
T	37.5	44.2	46.9	47.6	40.3	45.8
GRR	58.4	14.6	27.1	35.7	45.2	17.0
DT	8.02	12.8	11.1	14.0	8.22	13.5
λ	1.09	1.06	1.07	1.05	1.09	1.05

* Different letters in each row show significant differences among biological periods at 5% level. (Tukey's HSD test, P > 0.05); (F_F = 6.937; df = 5, 65; P = 0.000; F_r = 2760; df = 5, 326; P = 0.000; F_{R0} = 1630; df = 5,326; P = 0.000).

Weibull distribution models were applied to the age-specific survival rate of the tomato leafminer individuals reared on control, *S. lycopersicum* (LA0292), and wild tomato species (Figure 2). When the model and parameters are examined together, control (*S. lycopersicum* cv. 112-432) has the lowest age (day), b and c values.

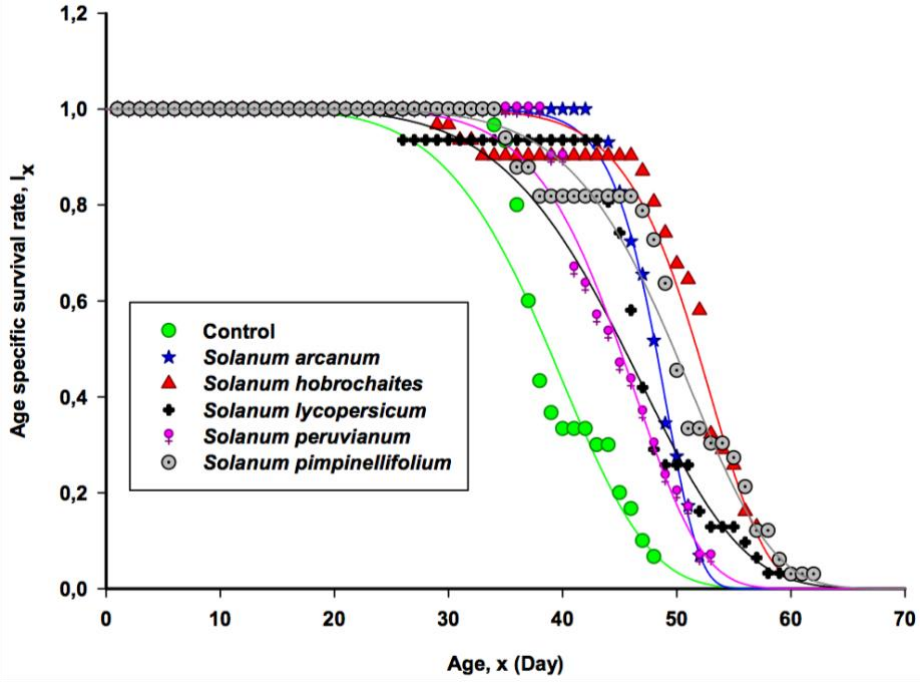

Figure 2. Weibull distribution models applied on the age-specific survival rate (l_x) of *Tuta absoluta* on control and four wild tomato species.

Among the wild species, the highest to the lowest b values were in *S. habrochaites, S. pimpinellifolium, S. arcanum*, and *S. peruvianum*, respectively. When c values are examined, the order was different; the highest to lowest were *S. arcanum, S. habrochaites, S. peruvianum* and *S. pimpinellifolium.*

Considering the values taken by the parameter c determines that the shape of the slope in the modeling, c > 1 indicates a developing population, c = 1 a stable populations and c < 1 a regressed population. When these c values are examined, it is observed that *T. absoluta* population represented an increasing population on four wild tomatoes, *S. lycopersicum* (LA0292) and the control cultivar. R^2 coefficient ranges between 0 and 1 as a measure of the predictive power of a Weibull model. A value closer to 1 indicates that the predictive power of the model has increased. All parameters for the pests on all studied tomato species were effectively predictive (Table 4).

Table 4. Weibull distribution model parameters applied on the age-specific survival rate (l_x) of *Tuta absoluta* on control (*Solanum lycopersicum* cv. 112-432), *S. lycopersicum* (LA0292) and four wild tomato species

Species	b*	c*	R^2	RSS*
Control	41.2±0.25	6.24±0.316	0.975	0.746
Solanum arcanum	49.2±0.04	17.6±0.30	0.999	0.049
Solanum habrochaites	53.4±0.11	11.8±0.48	0.990	0.246
Solanum lycopersicum	48.2±0.31	6.22±0.249	0.973	1.275
Solanum peruvianum	46.9±0.13	8.77±0.273	0.991	0.309
Solanum pimpinellifolium	52.1±0.23	8.06±0.270	0.983	0.856

* Values of parameters b and c are given with their standard errors (P < 0.0001). RSS: residual sum of squares.

Discussion

This is the first study of the life tables of the tomato leafminer on different wild tomato species including *S. arcanum* (LA2152)*, S. habrochaites* (LA0094)*, S. peruvianum* (LA0445)*, S. pimpinellifolium* (LA0100). In order to compare the effectiveness of the results, *S. lycopersicum* (LA0292) and control (cv. 112-432) were also used in experiments. There were significant differences on the life table parameters of the pest even between these two *S. lycopersicum* accessions.

Pereyra and Sánchez (2006) reported that *T. absoluta* females laid an average of 133 eggs. The data in this study are similar with our results. However, in another study by Uchoa-Fernandes et al., (1995), it was reported that females can lay up to 260 eggs during their life. This result was much higher than our data but, this may not be due to the difference in tomato species used, but due to the fact that the females mate with only one or two males.

Life tables are very useful tools to determine the susceptible phases of pests and can be used to gain applied and practical information about the population parameters of pests like survival, developmental time and fecundity (Özgökçe & Atlıhan, 2005). In the last decade, tomato leaf miner population parameters have been studied in many times on different tomato cultivars. Erdoğan & Babaroğlu (2014) studied on unknown cultivar of tomatoes in laboratory conditions. They estimated the intrinsic rate of increase (r_m) (0.132 day^{-1}), λ (1.141 day^{-1}), R_0 (42.01) and T (28.3 days) of *T. absoluta.* Gharekhani & Salek-Ebrahimi (2014) studied on three greenhouse cultivars of tomatoes. As a result of their study, they estimated the mean T (23.8, 23.8 and 24.3 days), *r* (0.13, 0.12 and 0.13 day^{-1}), gross reproductive rate (GRR) (35.8, 30.6 and 68.54), R_0 (24.5, 19.2 and 30.5) and mean generation DT (5.18, 5.68 and 4.99) on Atabay, Cluse and Perenses, respectively. Cluse was showed negative influences among the studied species. Çekin & Yaşar (2015) determined r_m as 0.173, 0.169, 0.159 and 0.150 day^{-1} on four tomato cultivars, Torry, Newton, Caracas and Şimşek c.v.s., respectively. Duarte et al. (2015) determined the tomato leaf miner's population parameters on the Cuban tomato (*S. lycopersicum* L.) cultivar Vyta. As a result of their study, the population growth parameters of *T. absoluta*, R_0, T, r_m, DT and λ were 9.36 and 8.90, 5.52 and 5.75, 0.02 and 0.01, 34.6 and 117, and 1.02 and 1.01 at 25 and 20/30ºC, respectively. Rostami et al. (2017) studied population parameter of *T. absoluta* on three cultivated tomatoes, Falkato, Grandella and Isabella. They estimated *r* (0.091, 0.074 and 0.095 day^{-1}), R_0 (15.7, 10.03 and 17.7 offspring), T (30.1, 31.1 and 30.2 days), fecundity (56.2, 45.9, and 59.1 eggs/female), and longevity (26.9, 18.0 and 27.2 days) on Falkato, Grandella and Isabella, respectively. Actually, they recommended that Grandella cultivation would be best in infested areas.

Studies on the population parameters of the studied wild tomato species are limited. Maluf et al. (2010) reported that the total preadult development time of *T. absoluta* showed the slowest development on *S. habrochaites* with 43.7 days. *Solanum habrochaites* was reported to be resistant to *T. absoluta* and other herbivorous pests. Silva et al. (2021) calculated the life table data of *T. absoluta* on six cultivars and four wild species from the Solanaceae family (with only one wild tomato species the same as in our study; *S. habrochaites*). As a result of their study, they determined that the finite rate of increase of *T. absoluta* was 1.15 on the cultured *S. lycopersicum* species and 1.12 on the wild species *S. habrochaites*.

Results of the present study revealed significant differences between population parameters across the examined tomato species. However, it is possible that the control (cv. 112-432) is the most susceptible host for tomato leafminer in terms of preadult development periods, fecundity and population parameters. Among the wild species the longest development periods were determined on *S. pimpinellifolium* in the first and second larval stages and S. *peruvianum* species in the third larval stage. However, the longest lifespan was observed on S. *habrochaites* as fourth larval stage, pupal stage, development time and longevity. The lowest fecundity was observed in *S. arcanum*. In life table parameters, *S. pimpinellifolium* for *r*, *S. arcanum*, *S. pimpinellifolium* for R_0, *S. pimpinellifolium* for T and DT, *S. arcanum* and *S. pimpinellifolium* for GRR was the most susceptible wild tomato species.

These results clearly demonstrate that tomato leaf miner deposits eggs on all host plants, but the effects of the pest were diverse on wild tomato species. There were differences observed on the population parameters between hosts. These differences could be related to insect feeding deterrents present in these wild tomato species. Development of resistant tomato cultivars, by the transfer of resistance factors to commercial cultivars, may be useful in pest management programs for *T. absoluta* (Sohrabi et al., 2016). The most promising genetic sources of resistance are from wild tomato species (Biondi et al., 2018). Firdaus et al. (2012) reported that *S. habrochaites* have a high density of trichome type IV and could be resistant to *Bemisia tabaci* (Gennadius, 1889) (Hemiptera: Aleyrodidae). This trichome structure may have adversely affected the egg-deposition by *T. absoluta*. Trichomes are a common morphological defense against pests; in particular, type IV glandular trichomes have been associated with resistance against different invertebrates. Mata-Nicolás et al. (2021) reported that *S. pimpinellifolium* has a high density of this type of trichomes. Due to close relationship with *S. lycopersicum*, *S. pimpinellifolium* has been recognized the wild progenitor of cultivated tomatoes (Zuriaga et al., 2009; Wang et al., 2020).

In conclusion, commercial tomato cultivars with resistance quantitative trait loci or genes to *T. absoluta* from *S. arcanum* and *S. pimpinellifolium* would be useful in future breeding programs. However, more research is needed on what caused these differences in order to achieve practical applications. Also, these contributing genetic factors in these wild species could be considered as candidates for use in integrated management programs of tomato leaf miner.

References

Aksoy, E. & O. B. Kovancı, 2016. Mass trapping low-density populations of *Tuta absoluta* with various types of traps in field-grown tomatoes. Journal of Plant Diseases and Protection, 123 (2): 51-57.

Al-Jboory, I. J., A. Katbeh-Bader & S. Al-Zaidi, 2012. First observation and identification of some natural enemies collected from heavily infested tomato by *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in Jordan. Middle-East Journal of Scientific Research, 11 (6): 787-790.

Biondi, A. & N. Desneux, 2019. Special issue on *Tuta absoluta*: recent advances in management methods against the background of an ongoing worldwide invasion. Journal of Pest Science, 92 (4): 1313-1315.

Biondi, A., R. N. C. Guedes, F. H. Wan & N. Desneux, 2018. Ecology, worldwide spread, and management of the invasive South American tomato pinworm, *Tuta absoluta*: past, present, and future. Annual Review of Entomology**,** 63 (1): 239-258.

Birch, L. C., 1948. The intrinsic rate of natural increase of an insect population. Journal of Animal Ecology, 17 (1): 15-26.

Campos, M. R., A. Biondi, A. Adiga, R. N. C. Guedes & N. Desneux, 2017. From the western Palaearctic region to beyond: *Tuta absoluta* ten years after invading Europe. Journal of Pest Science, 90 (3): 787-796.

Caparros Megido, R., E. Haubruge & F. J. Verheggen, 2013. Pheromone-based management strategies to control the tomato leafminer, *Tuta absoluta* (Lepidoptera: Gelechiidae). Biotechnology, Agronomy and Society and Environment, 17 (3): 475-82.

Çekin, D. & B. Yaşar, 2015. The life table of *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) on different tomato varieties. Journal of Agricultural Sciences, 21 (2): 199-206 (in Turkish with abstract in English).

Chi, H., 1988. Life table analysis incorporating both sexes and variable development rates among individuals. Environmental Entomology, 17 (1): 26-34.

Chi, H. & H. Liu, 1985. Two new methods for the study of insect population ecology. Bulletin of the Institute of Zoology, Academia Sinica. 24 (2): 225-240.

Chi, H., M. You, R. Atlıhan, C. L. Smith, A. Kavousi, M. S. Özgökçe, A. Güncan, S. J. Tuan, J. W. Fu, Y. Y. Xu, F. Q. Zheng, B. H. Ye, D. Chu, Y. Yu, G. Gharekhani, P. Saska, T. Gotoh, M. I. Schneider, P. Bussaman, A. Gökçe & T. X. Liu, 2020. Age-stage, two-sex life table: An introduction to theory, data analysis, and application. Entomologia Generalis, 40 (2): 103-124.

Chunwongse, J., C. Chunwongse, L. Black & P. Hanson, 2002. Molecular mapping of the Ph-3 gene for late blight resistance in tomato. The Journal of Horticultural Science and Biotechnology, 77 (3): 281-286.

Cocco, A., S. Deliperi & G. Delrio, 2013. Control of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in greenhouse tomato crops using the mating disruption technique. Journal of Applied Entomology, 137 (1): 16-28.

Deevey, E. S., 1947. Life Tables for natural populations of animals. The Quarterly Review of Biology, 22 (4): 283-314.

Desneux, N., M. G. Luna, T. Guillemaud & A. Urbaneja, 2011. The invasive South American tomato pinworm, *Tuta absoluta*, continues to spread in Afro-Eurasia and beyond: the new threat to tomato world production. Journal of Pest Science, 84 (4): 403-408.

Duarte, L., M. D. A. Martinez & V. H. P. Bueno, 2015. Biology and population parameters of *Tuta absoluta* (Meyrick) under laboratory conditions. Revista Protección Vegetal, 30 (1): 19-29.

Erdoğan, P. & N. Babaroğlu, 2014. Life table of the tomato leaf miner, *Tuta abosluta* (Meyrick) (Lepidoptera: Gelechiidae). Journal of Agricultural Faculty of Gaziosmanpaşa University, 2014 (2): 80-89.

Firdaus, S., A. W. van Heusden, N. Hidayati, E. D. J. Supena, R. G. F. Visser & B. Vosman, 2012. Resistance to *Bemisia tabaci* in tomato wild relatives. Euphytica, 187 (1): 31-45.

Gervassio, N. G. S., D. Aquion, C. Vallina, A. Biondi & M. G. Luna, 2019. A re-examination of *Tuta absoluta* parasitoids in South America for optimized biological control. Journal of Pest Science, 92 (4): 1343-1357.

Gharekhani, G. H. & H. Salek-Ebrahimi, 2014. Life table parameters of *Tuta absoluta* (Lepidoptera: Gelechiidae) on different varieties of tomato. Journal of Economical Entomology, 107 (5): 1765-1770.

Giorgini, M., E. Guerrieri, P. Cascone & L. Guntuo, 2019. Current strategies and future outlook for managing the neotropical tomato pest *Tuta absoluta* (Meyrick) in the Mediterranean Basin. Neotropical Entomology, 48 (1): 1-17.

Gontijo, P. C., M. C. Picanço, E. J. G. Pereira, J. C. Martins, M. Chediak & R. N. C. Guedes, 2013. Spatial and temporal variation in the control failure likelihood of the tomato leaf miner, *Tuta absoluta.* Annals of Applied Biology, 162 (1): 50-59.

Guedes, R. N. C., E. Roditakis, M. R. Campos, K. Haddi, P. Bielza, H. A. A. Siqueira, A. Tsagkarakou, J. Vontas & R. Nauen, 2019. Insecticide resistance in the tomato pinworm *Tuta absoluta*: patterns, spread, mechanisms, management and outlook. Journal of Pest Science, 92 (4): 1329-1342.

Han, P., Y. Bayram, L. Shaltiel-Harpaz, F. Sohrabi, A. Saji, U. P. T. Esenali, A. Jalilov, A. Ali, P. R. Shashank, K. Ismoilov, Z. Lu, S. Wang, G. Zhang, F. Wan, A. Biondi & N. Desneux, 2019. *Tuta absoluta* continues to disperse in Asia: damage, ongoing management and future challenges. Journal of Pest Science, 92 (4): 1317-1327.

Huang, Y. B. & H. Chi, 2012. Assessing the application of the jackknife and bootstrap techniques to the estimation of the variability of the net reproductive rate and gross reproductive rate: a case study in *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae). Journal of Agriculture and Forestry, 61 (1): 37-45.

Kairo, M. T. K. & S. T. Murphy, 1995. The life history of *Rodolia iceryae* Janson (Coleoptera:Coccinellidae) and the potential for use in innoculative releases against *Icerya pattersoni* Newstead (Homoptera: Margarodidae) on coffee. Journal of Applied Entomology, 119 (4): 487-491.

Kılıç, T., 2010. First record of *Tuta absoluta* in Turkey. Phytoparasitica, 38 (3): 243-244.

Lanfermeijer, F. C., J. Warmink & J. Hille, 2005. The products of the broken Tm-2 and the durable Tm-22 resistance genes from tomato differ in four amino acids. Journal of Experimental Botany, 56 (421): 2925-2933.

Lawo, J. P. & N. C. Lawo, 2011. Misconceptions about the comparison of intrinsic rates of natural increase. Journal of Applied Entomology, 135 (10): 715-725.

Maluf, W., V. Fátima Silva, M. Graças Cardoso, L. Gomes, Á. Neto, G. Maciel & D. Nízio, 2010. Resistance to the South American tomato pinworm *Tuta absoluta* in high acylsugar and/or high zingiberene tomato genotypes. Euphytica, 176 (1): 113-123.

Mata-Nicolás, E., J. Montero-Pau, E. Gimeno-Paez, A. García-Pérez, P. Ziarsolo, J. Blanca, E. van der Knaap, M. J. Díez & J. Cañizares, 2021. Discovery of a major QTL controlling trichome IV density in tomato using K-seq genotyping. Genes, 12 (243):1-18.

Maxted, N., B. J. M. Brehm & S. Kell, 2013. Resource Book for Preparation of National Conservation Plans for Crop Wild Relatives and Landraces. Food and Agriculture Organization of the United Nations Commission on Genetic Resources for Food and Agriculture, Rome, Italy, 456 pp.

Meyer, J. S., C. G. Ingersoll, L. L. McDonald & M. S. Boyce, 1986. Estimating uncertainty in population growth rates: jackknife vs. bootstrap techniques. Ecology, 67 (5): 1156-1166.

Miller, J. C. & S. D. Tanksley, 1990. RFLP analysis of phylogenetic relationships and genetic variation in the genus *Lycopersicon*. Theoretical and Applied Genetics, 80 (4): 437-448.

Mukwa, L. F. T., J. Mukendi, F. G. Adakate, D. M. Bugeme, A. Kalonji-Mbuyi & S. Ghimire, 2021. First report of the South American tomato pinworm *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) and its damage in the Democratic Republic of Congo. BioInvasions Records, 10 (1): 33-44.

Özgökçe, M. S. & R. Atlıhan, 2005. Biological features and life table parameters of the mealy plum aphid *Hyalopterus pruni* on different apricot cultivars. Phytoparasitica, 33 (1): 7-14.

Parniske, M., B. B. H. Wulff, G. Bonnema, C. M. Thomas, D. A. Jones & J. D. G. Jones, 1999. Homologues of the Cf-9 disease resistance gene (Hcr9s) are present at multiple loci on the short arm of tomato chromosome 1. Molecular Plant- Microbe Interactions, 12 (2): 93-102.

Pereyra, P. C. & N. E. Sánchez, 2006. Effect of two solanaceous plants on developmental and population parameters of the tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera:Gelechiidae). Neotropical Entomology, 35 (5): 671-676.

Pinder, J. E., J. G. Wiener & M. H. Smith, 1978. The Weibull distribution: a new method of summarizing survivorship data. Ecology, 59 (1): 175-179.

Prasanna, H. C., D. P. Sinha, G. K. Rai, R. Krishna, S. P. Kashyap, N. K. Singh & V. G. Malathi, 2015. Pyramiding Ty-2 and Ty-3 genes for resistance to monopartite and bipartite tomato leaf curl viruses of India. Plant Pathology, 64 (2): 256-264.

Rostami, E., H. Madadi, H. Abbasipour, H. Allahyari & A. G. S. Cutbertson, 2017. Life table parameters of the tomato leaf miner *Tuta absoluta* (Lepidoptera: Gelechiidae) on different tomato cultivars. Journal of Applied Entomology, 141 (1): 88-96.

Seah, S., J. Yaghoobi, M. Rossi, C. A. Gleason & V. M. Williamson, 2004. The nematode-resistance gene, Mi-1, is associated with an inverted chromosomal segment in susceptible compared to resistant tomato. Theoretical and Applied Genetics, 108 (8): 1635-1642.

Silva, G. A., E. A. Queiroz, L. P. Arcanjo, M. C. Lopes, T. A. Araújo, T. S. V. Galdino, R. I. Samuels, N. Rodrigues-Silva & M. C. Picanço, 2021. Biological performance and oviposition preference of tomato pinworm *Tuta absoluta* when offered a range of Solanaceous host plants. Scientific Reports, 11 (1153): 1-10.

Sohrabi, F., H. Nooryazdan, B. Gharati & Z. Saeidi, 2016. Evaluation of ten tomato cultivars for resistance against tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) under field infestation conditions. Entomologia Generalis, 36 (2): 163-175.

Son, D., S. Bonzi, I. Somda, T. Bawin, S. Boukraa, F. Verheggen, F. Francis, A. Legrève & B. Schiffers, 2017. First record of *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) in Burkina Faso. African Entomology, 25 (1): 259-263.

Sylla, S., T. Brévault, A. B. Bal, A. Chailleux, M. Diatte, N. Desneux & K. Diarra, 2017. Rapid spread of the tomato leafminer, *Tuta absoluta* (Lepidoptera, Gelechiidae), an invasive pest in sub-Saharan Africa. Entomologia Generalis, 36 (3): 269-283.

Tingle, C. C. D. & M. J. W. Copland, 1989. Progeny production and adult longevity of the mealybug parasitoids *Anagyrus pseudococci*, *Leptomastix dactylopii* and *Leptomastidea abnormis* (Hymenoptera: Encyrtidae) in relation to temperature. Entomophaga, 34 (1): 111-120.

Tukey, J. W., 1949. Comparing individual means in the analyses of variance. Biometrics, 5 (2): 99-114.

Uchoa-Fernandes, M., L. T. Della & E. Vilela, 1995. Mating, oviposition and pupation of *Scrobipalpuloides absoluta* (Meyr.) (Lepidoptera: Gelechiidae). Anais da Sociedade Entomológica do Brasil, 24(1): 159-164.

Wang, X., L. Gao, J. C. Jiao, S. Stravoravdis, P. S. Hosmani, S. Saha, J. Zhang, S. Mainiero, S. R. Strickler, C. Catala, G. B. Martin, L. A. Mueller, J. Vrebalov, J. J. Giovannoni, S. Wu & Z. Fei, 2020. Genome of *Solanum pimpinellifolium* provides insights into structural variants during tomato breeding. Nature Communications, 11 (5817): 1-11.

Wang, J. J., J. H. Tsai, Z. M. Zhao & L. S. Li, 2000. Development and reproduction of the psocid *Liposcelis bostrychophila* (Psocoptera: Liposcelididae) as a function of temperature. Annals of the Entomological Society of America, 93 (2): 261-270.

Yu, L. Y., Z. Z. Chen, F. Q. Zheng, A. J. Shi, T. T. Guo, B. H. Yeh, H. Chi & Y. Y. Xu, 2013a. Demographic analysis, a comparison of the jackknife and bootstrap methods, and predation projection: A Case Study of *Chrysopa pallens* (Neuroptera: Chrysopidae). Journal of Economical Entomology, 106 (1): 1-9.

Yu, J. Z., H. Chi & B. H. Chen, 2013b. Comparison of the life tables and predation rates of *Harmonia dimidiata* (F.) (Coleoptera: Coccinellidae) fed on *Aphis gossypii* Glover (Hemiptera: Aphididae) at different temperatures. Biological Control, 64 (1): 1-9.

Zamir D., I. Eksteinmichelson, Y. Zakay, N. Navot, M. Zeidan, M. Sarfatti & H. Czosnek, 1994. Mapping and introgression of a Tomato yellow leaf curl virus tolerance gene, Ty-1. Theoretical and Applied Genetics, 88 (2): 141-146.

Zappalà, L., A. Biondi, A. Alma, I. J. Al-Jboory, J. Arno, A. Bayram, A. Chailleux, D. El-Arnaouty, Y. Gerling & Y. Guenaoui, 2013. Natural enemies of the South American moth, *Tuta absoluta*, in Europe, North Africa and Middle East, and their potential use in pest control strategies. Journal of Pest Science, 86 (4): 635-647.

Zhang, H., C. Li, E. L. Davis, J. Wang, J. D. Griffin, J. Kofsky & B. H. Song, 2016. Genome-wide association study of resistance to soybean cyst nematode (*Heterodera glycines*) HG Type 2.5.7 in wild soybean (*Glycine soja*). Frontiers in Plant Science, 7 (1214): 1-11.

Zuriaga, E., J. M. Blanca, L. Cordera, A. Sifres, W. G. Blas-Cerdán, R. Morales & F. Nuez, 2009. Genetic and bioclimatic variation in *Solanum pimpinellifolium*. Genetic Resources and Crop Evolution, 56 (1): 39-51.

Türk. entomol. derg., 2022, 46 (2): 187-197
DOI: http://dx.doi.org/10.16970/entoted.1092654

ISSN 1010-6960
E-ISSN 2536-491X

Original article (Orijinal araştırma)

Prevalence of root-knot nematodes and their effects on fruit yield in kiwifruit orchards in Samsun Province (Türkiye)[1]

Samsun İli (Türkiye) kivi bahçelerindeki kök-ur nematodlarının yaygınlığı ve meyve verimine etkileri

Gökhan AYDINLI[2*] **Sevilhan MENNAN[3]**

Abstract

The aims of this study were to determine the distribution of root-knot nematodes (*Meloidogyne* spp.) in kiwifruit orchards in Samsun Province (Türkiye) and assess the effect of *Meloidogyne* spp. on fruit yields in naturally infested orchards. The survey was conducted in 25 kiwifruit orchards in September-November 2017. In addition, the data on fruit yields was obtained in two orchards at the harvest time in 2018. Fifty-six soil and root samples were collected from infested orchards. Species identification was performed by esterase phenotype and PCR with species-specific primers. *Meloidogyne* spp. were found in 92% of the orchards surveyed. *Meloidogyne luci* (Carneiro et al., 2014) (Tylenchida: Meloidogynidae) was detected in 59% of the samples, followed by *Meloidogyne hapla* (Chitwood, 1949) in 41%, *Meloidogyne arenaria* (Neal, 1889) in 27% and *Meloidogyne incognita* (Kofoid & White, 1919) in 2%. Regarding the distribution of *Meloidogyne* spp. in kiwifruit orchards, *M. luci* was found in 74%, *M. hapla* in 57%, *M. arenaria* in 39% and *M. incognita* in 4% of orchards infested. *Meloidogyne luci* was found for the first time in the kiwifruit orchards of Türkiye in this study. It was also determined that *Meloidogyne* spp. caused significant yield losses in kiwifruit orchards, and yield losses of 36 and 49% were detected in two orchards infested with *Meloidogyne* spp., respectively.

Keywords: *Actinidia deliciosa*, distribution, identification, *Meloidogyne*, yield

Öz

Bu çalışmanın amacı, Samsun İli (Türkiye) kivi bahçelerinde kök-ur nematodlarının (*Meloidogyne* spp.) dağılımının belirlenmesi ve doğal olarak bulaşık bahçelerde meyve verimi üzerine *Meloidogyne* spp.'nin etkisinin değerlendirilmesidir. Sürvey 2017 yılı Eylül-Kasım aylarında 25 kivi bahçesinde yürütülmüştür. Ayrıca, 2018 yılı hasat zamanında iki bahçede meyve verimlerine ilişkin veriler elde edilmiştir. Bulaşık bahçelerden 56 toprak ve kök örneği alınmıştır. Tür teşhisleri esteraz fenotipi ve türe özgü primerler ile yapılan PCR ile gerçekleştirilmiştir. *Meloidogyne* spp., sürvey yapılan bahçelerin %92'sinde bulunmuştur. Örneklerin %59'unda *Meloidogyne luci* (Carneiro et al., 2014) (Tylenchida: Meloidogynidae) tespit edilmiş, bunu %41 ile *Meloidogyne hapla* (Chitwood, 1949), %27 ile *Meloidogyne arenaria* (Neal, 1889) ve %2 ile *Meloidogyne incognita* (Kofoid & White, 1919) izlemiştir. *Meloidogyne* spp.'nin kivi bahçelerindeki dağılımı ile ilgili olarak, bulaşık bahçelerin %74'ünde *M. luci*, %57'sinde *M. hapla*, %39'unda *M. arenaria* ve %4'ünde *M. incognita* bulunmuştur. *Meloidogyne luci*, Türkiye'deki kivi bahçelerinde ilk kez bu çalışmada bulunmuştur. Ayrıca, *Meloidogyne* spp.'nin kivi bahçelerinde önemli verim kayıplarına neden olduğu ve *Meloidogyne* spp. ile bulaşık iki bahçede sırasıyla %36 ve %49 verim kaybı olduğu belirlenmiştir.

Anahtar sözcükler: *Actinidia deliciosa*, dağılım, teşhis, *Meloidogyne*, verim

[1] This study was partly presented as a poster at the 33th Symposium of the European Society of Nematologists (9-13 September 2018, Ghent, Belgium) and published as an abstract in the abstract book.

[2] Ondokuz Mayıs University, Vocational High School of Bafra, 55400, Bafra, Samsun, Türkiye
[3] Ondokuz Mayıs University, Faculty of Agriculture, Department of Plant Protection, 55139, Atakum, Samsun, Türkiye
* Corresponding author (Sorumlu yazar) e-mail: gokhanay@omu.edu.tr
Received (Alınış): 24.03.2022 Accepted (Kabul ediliş): 14.06.2022 Published Online (Çevrimiçi Yayın Tarihi): 18.06.2022

Introduction

Actinidia chinensis Planch. and *Actinidia deliciosa* (A. Chev.) C.F.Liang et A.R.Ferguson, which are closely related species, are cultivated for commercial kiwifruit production globally, with most of the production from the latter as in Türkiye (Ferguson & Seal, 2008; Atak, 2015). Kiwifruit has a significant nutritional content that is beneficial to human health. Particularly, a high concentration of vitamin C and a variety of other nutrients, including vitamins, fiber, and antioxidants in its nutrient content have been linked to digestive, immune, and metabolic health advantages (Richardson et al., 2018).

World kiwifruit production was about 4.4 Mt in 2020 (FAO, 2022). China was largest producer with 50% of global production (2.2 Mt), followed by New Zealand (625 kt), Italy (522 kt), Greece (307 kt), Iran (290 kt) and Chile (159 kt). Türkiye ranked seventh with an annual production of 74 kt (FAO, 2022). The kiwifruit cultivation in Türkiye began at the Atatürk Central Horticultural Research Institute in Yalova in 1988 (Atak, 2015). The plantation area has rapidly expanded, which almost doubled in the last decade, from 1,720 ha in 2010 to 3,260 ha in 2020 (TUIK, 2022). In 2021, the average yield was 45 kg per fruiting vine (TUIK, 2022). The Black Sea Region is the second-largest regional production area with 40% of kiwifruit plantations of Türkiye, and Samsun, Ordu and Rize Provinces are the primary producers in this region. In recent years, interest in kiwifruit production has increased as an alternative to hazelnut in Samsun Province.

Root-knot nematodes (RKN), *Meloidogyne* spp., are among the potential threats to limiting kiwifruit production worldwide. Their obligate sedentary parasitism in plant roots alters the physiological processes of plants that can result in the reduction quantity and quality of yield. Di Vito et al. (1988) reported that *Meloidogyne incognita* (Kofoid & White, 1919) (Tylenchida: Meloidogynidae) caused a substantial reduction in the relative aerial growth of plants in pot experiments in a greenhouse. El-Borai & Duncan (2005) reported that RKN infestations in kiwifruit orchards of Italy and France were associated with poor plants. It has been estimated that RKN caused up to 40% yield losses in some kiwifruit orchards of China (as cited in Tao et al., 2017). *Meloidogyne incognita* and *Meloidogyne hapla* (Chitwood, 1949) have been the most commonly encountered species among eight species of RKN (*Meloidogyne aberrans* Tao et al., 2017, *Meloidogyne actinidiae* Li & Yu, 1991, *Meloidogyne arenaria* Neal, 1889, *Meloidogyne ethiopica* Whitehead, 1968, *M. hapla, M. incognita*, *Meloidogyne javanica* Treub, 1885, *Meloidogyne luci* Carneiro et al., 2014) found associated with kiwifruit in different countries of the world (Scotto La Massese, 1973; Vovlas & Roca, 1976; Haygood et al., 1990; Watson et al., 1992; Castillo et al., 1993; Philippi et al., 1996; Maafi & Mahdavian, 1997; Waliullah, 2005; Carneiro et al., 2007; Ma et al., 2007; Somavilla et al., 2011; Tzortzakakis et al., 2011; Conceição et al., 2012; Akyazi et al., 2017; Tao et al., 2017; Shokoohi & Mashela, 2020). Similarly, these two species were found to commonly occur in Türkiye when a survey of RKN in kiwifruit orchards was conducted in a few provinces (Akyazı & Felek, 2013; Akyazi et al., 2017; Evlice & Özdemir, 2021). In addition to these two species, *M. arenaria* was found only in a kiwifruit orchard in Ordu Province (Akyazi et al., 2017).

Previous surveys revealed the widespread distribution of RKN in vegetable-growing areas in Samsun Province of Türkiye (Katı & Mennan, 2006; Aydınlı & Mennan, 2016; Aydınlı, 2018). Particularly, *M. luci* was commonly found (Aydınlı & Mennan, 2016; Aydınlı, 2018). The esterase phenotype (M3) of one population from Türkiye reported as an unidentified RKN population by Esbenshade & Triantaphyllou (1985) is very similar to that of *M. luci* (L3). Thus, this nematode species may have been present in Türkiye for many years. We hypothesized that this species might occur in kiwifruit orchards in Samsun Province. Therefore, the first objective of this study was to identify the RKN species in kiwifruit roots. Despite a few studies indicating the effect of *Meloidogyne* on the plant growth in kiwifruit (Di Vito et al., 1988; Philippi & Budge, 1992), to date, there are no studies on the effect of RKN on fruit yield. Therefore, the second objective of this study was to determine the relative fruit yield losses in kiwifruit vines naturally infested with RKN compared to non-infested ones.

Materials and Methods

Survey

The survey was conducted in September-November 2017 in 25 kiwifruit orchards in six districts (Atakum, Bafra, Çarşamba, Ondokuzmayıs, Salıpazarı and Terme) of Samsun Province, Türkiye. The roots of 10-20 plants from each kiwifruit orchard were examined for galls caused by root-knot nematode. When kiwifruit roots with galls were detected, roots and rhizosphere soils were collected. According to the size of the kiwifruit orchard, the sampling area in each orchard was divided into two to four subsections. In each subsection, roots and soils from three to five kiwifruit plants were placed together into a labeled polyethylene bag to obtain a composite sample and transferred to the Nematology Laboratory at Ondokuz Mayıs University, Samsun. A total of 56 samples were processed within 3 days.

Nematode extraction and identification

Egg masses and females were collected randomly from infested kiwifruit roots using forceps, which was sterilized between samples to prevent cross-contamination. Five to 10 egg masses per sample were packed in an Eppendorf tube and placed at -20°C until DNA extraction. Young egg-laying females were macerated with a pestle in a tube with 5 µL of extraction buffer (20% sucrose with 1% Triton X-100), then the specimens were immediately placed in a freezer at -20°C. Females and egg masses were used for biochemical (esterase phenotype, EST) and molecular (PCR with species-specific primers) identification of samples, respectively. In some samples, there were unsuitable females for the esterase study. Thus, soil samples were placed into 600 mL pots, and individual tomato (*Solanum lycopersicum* L. cv. Falcon) seedlings were planted singly. The tomato plants were maintained in a greenhouse at 22-30°C. After 6-8 weeks, young egg-laying females were handpicked and prepared as described above. For each sample, at least 20 females collected from roots of kiwifruit and tomato were analyzed for their esterase phenotypes. Electrophoresis was performed according to Aydınlı & Mennan (2016). Following electrophoresis, the gels were removed from the glass plates and stained for esterase activity. Females from pure isolate of *M. javanica* were included in each gel as a reference.

Total DNA was extracted from the egg masses for molecular identification using the DNeasy Tissue & Blood Kit (QIAGEN, Hilden, Germany). PCRs were performed with specific primers, namely Far/Rar (Zijlstra et al., 2000) for *M. arenaria*, inc-K14F/R (Randig et al., 2002) for *M. incognita*, JMV1/JMV2/JMVhapla (Wishart et al., 2002) for *M. hapla*, and Me309F/Me549R (Gerič-Stare et al., 2019) for *M. luci*. The PCR mixture (25 µL) contained 12.5 µl of BioMix Red (Bioline), 1 µl of each primer (0.4 µM of each primer), and 5 µl of DNA template. A positive control with reference DNAs and a negative control (water) were included. PCR amplification was done using a T-100 Thermal Cycler (Bio-Rad Laboratories, Hercules, CA, USA). Samples were amplified using cycling conditions of 94ºC for 3 min, followed by 35 cycles of 94ºC for 30 s, annealing temperature for 30 s and 72ºC for 1 min, and a final cycle of 72ºC for 7 min. Annealing temperatures were 50ºC for primers Me309F/Me549R, 55ºC for primers JMV1/JMV2/JMVhapla, 56ºC for primers Far/Rar, and 60ºC for primers inc-K14F/R. All primer pairs were used for all samples in the study. The products from PCRs were separated by electrophoresis in 2% agarose gel stained with ethidium bromide and visualized under UV light.

Fruit yield

Two commercial kiwifruit (cv. Hayward) orchards in Çarşamba District were selected to evaluate the effect of RKN on fruit yield. The data on fruit yield was obtained at harvest time on 26 October 2018. Orchard A located in Dikbıyık (41°13'13" N, 36°37'17" E) was in a 9-year-old, while orchard B located in Eğrikum (41°08'07" N, 36°43'07" E) was in a 6-year-old kiwifruit plantation. According to survey in this study, both orchards had been previously known naturally infested with mixed *Meloidogyne* populations. Each orchard was initially checked for root-knot galls at harvest time in 2018, and four nematode-infested vines were

determined. The soil samples were collected, and second-stage juveniles (J2) were obtained from one 100 mL soil subsample per vine using the tray extraction method (Whitehead & Hemming, 1965). Four vines having no gall symptoms caused by root-knot nematode were marked as non-infested vines. Fruits were harvested from nematode-infested and non-infested vines, and yields were expressed in kg/vine. Yields were compared within each kiwifruit orchard by t-test at $P \leq 0.05$ significance level using SPSS statistical software to determine the significance of differences between nematode-infested vines and non-infested vines.

Results

RKNs were present in all surveyed districts in Samsun Province (Figure 1). Of the 25 kiwifruit orchards surveyed, 23 (92%) were infested with RKN (Table 1). In 56 samples collected from infested orchards, species identification was done using biochemical (EST) and molecular (PCR with species-specific primers) analysis. In 18 root samples of kiwifruit, females were not suitable for biochemical identification. Thus, EST phenotypes of these samples were obtained on females collected roots of tomatoes cultured in their soil samples. Four EST phenotypes from 56 samples analyzed were identified associated with the four RKN species: *M. arenaria* (A2), *M. hapla* (H1), *M. incognita* (I2) and *M. luci* (L3) (Figure 2). As expected, species-specific primers, Far/Rar, inc-K14F/R, JMV1/JMV2/JMVhapla and Me309F/Me549R, used in this study that produced a single band of 420 bp for *M. arenaria*, 400 bp for *M. incognita*, 440 bp for *M. hapla*, and 240 bp for *M. luci*, respectively (Figure 3). A species-specific EST phenotype detected in all samples, except four samples (namely 11-3, 11-4, 13-2 and 14-2), was consistently associated with each of the *Meloidogyne* spp. identified by PCR with species-specific primers. In three samples (11-3, 11-4 and 13-2), *M. hapla* species-specific EST phenotype H1 was not observed whereas PCR amplifications were positive for JMV1/JMV2/JMVhapla primers that produced a single band of ~440 bp. Conversely, in sample 14-2, *M. arenaria* species-specific phenotype was detected, but the primer Far/Rar did not give an amplification product.

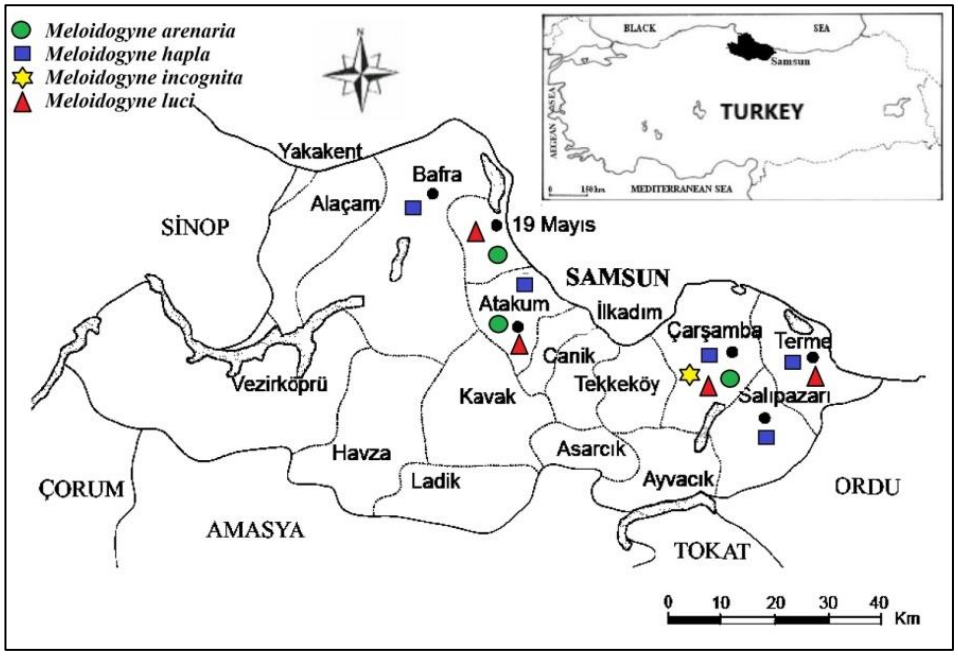

Figure 1. Distribution of *Meloidogyne* spp. in kiwifruit orchards surveyed in Samsun, Türkiye.

Table 1. *Meloidogyne* spp. identified in samples (n = 56) from 23 kiwifruit orchards in Samsun Province, Türkiye

District	Location	Geographic coordinates	Sample no	Esterase phenotype		PCR*	*Meloidogyne* spp.
				Kiwifruit	Tomato		
Atakum	Taflan	41°26'10" N	1-1	L3	L3	Ml	*M. luci*
		36°09'01" E	1-2	H1, L3	L3	Mh, Ml	*M. hapla, M. luci*
		41°26'11" N	2-1	L3	L3	Ml	*M. luci*
		36°09'05" E	2-2	L3	L3	Ml	*M. luci*
		41°26'07" N	3-1	-	A2, L3	Ma, Ml	*M. arenaria, M. luci*
		36°08'43" E	3-2	-	L3	Ml	*M. luci*
		41°24'57" N	4-1	-	L3	Ml	*M. luci*
		36°05'20" E	4-2	-	L3	Ml	*M. luci*
		41°25'07" N	5-1	H1	H1	Mh	*M. hapla*
		36°06'06" E	5-2	H1	H1	Mh	*M. hapla*
		41°26'06" N	6-1	L3	L3	Ml	*M. luci*
		36°09'03" E	6-2	-	L3	Ml	*M. luci*
Bafra	Sürmeli	41°28'52" N	7-1	H1	-	Mh	*M. hapla*
		35°56'32" E	7-2	H1	-	Mh	*M. hapla*
	Darboğaz	41°25'59" N	8-1	H1	-	Mh	*M. hapla*
		35°55'03" E	8-2	H1	H1	Mh	*M. hapla*
Çarşamba	Eğrikum	41°07'41" N	9-1	H1, L3	L3	Mh, Ml	*M. hapla, M. luci*
		36°42'40" E	9-2	L3	L3	Ml	*M. luci*
		41°07'17" N 36°42'57" E	10-1	H1, L3	-	Mh, Ml	*M. hapla, M. luci*
			10-2	H1	-	Mh	*M. hapla*
			10-3	I2	-	Mi	*M. incognita*
		41°08'07" N 36°43'07" E	11-1	A2, L3	A2, L3	Ma, Ml	*M. arenaria, M. luci*
			11-2	H1, L3	L3	Mh, Ml	*M. hapla, M. luci*
			11-3	L3	-	Mh, Ml	*M. hapla, M. luci*
			11-4	L3	L3	Mh, Ml	*M. hapla, M. luci*
	Karamustafalı	41°19'03" N 36°40'46" E	12-1	A2	-	Ma	*M. arenaria*
			12-2	L3	L3	Ml	*M. luci*
			12-3	L3	L3	Ml	*M. luci*
	Dikbıyık	41°13'13" N	13-1	H1	-	Mh	*M. hapla*
		36°37'17" E	13-2	L3	L3	Mh, Ml	*M. hapla, M. luci*
	Yeşilova	41°15'28" N 36°34'05" E	14-1	-	A2	Ma	*M. arenaria*
			14-2	A2, L3	A2, L3	Ml	*M. arenaria, M. luci*
			14-3	A2	A2	Ma	*M. arenaria*
	Demiraslan	41°09'36" N 36°59'02" E	15-1	-	A2, H1	Ma, Mh	*M. arenaria, M. hapla*
			15-2	A2	A2	Ma	*M. arenaria*
			15-3	-	A2, L3	Ma, Ml	*M. arenaria, M. luci*
			15-4	-	A2	Ma	*M. arenaria*
	Beylerce	41°12'25" N 36°40'39" E	16-1	L3	-	Ml	*M. luci*
			16-2	-	L3	Ml	*M. luci*
			16-3	L3	-	Ml	*M. luci*
	Hacılıçay	41°14'20" N	17-1	-	A2	Ma	*M. arenaria*
		36°41'37" E	17-2	-	L3	Ml	*M. luci*
		41°14'48" N 36°41'27" E	18-1	H1	-	Mh	*M. hapla*
			18-2	H1	-	Mh	*M. hapla*
			18-3	-	A2	Ma	*M. arenaria*
		41°14'02" N 36°41'46" E	19-1	-	L3	Ml	*M. luci*
			19-2	L3	L3	Ml	*M. luci*
			19-3	-	A2, L3	Ma, Ml	*M. arenaria, M. luci*

* Ma: *M. arenaria*, Mh: *M. hapla*, Mi: *M. incognita*, Ml: *M. luci*

Table 1. Continued

District	Location	Geographic coordinates	Sample no	Esterase phenotype		PCR*	*Meloidogyne* spp.
				Kiwifruit	Tomato		
Ondokuzmayıs	Engiz	41°29'34" N	20-1	-	A2, L3	Ma, Ml	*M. arenaria, M. luci*
		36°05'44" E	20-2	A2	A2, L3	Ma, Ml	*M. arenaria, M. luci*
Salıpazarı	Yavaşbey	41°06'20" N	21-1	-	H1	Mh	*M. hapla*
		36°50'18" E	21-2	H1	H1	Mh	*M. hapla*
	Kızılay	41°00'23" N	22-1	H1	-	Mh	*M. hapla*
		36°52'11" E	22-2	H1	H1	Mh	*M. hapla*
Terme	Hüseyinmescit	41°09'36" N	23-1	L3, H1	L3	Mh, Ml	*M. hapla, M. luci*
		36°59'02" E	23-2	-	L3	Ml	*M. luci*

* Ma: *M. arenaria*, Mh: *M. hapla*, Mi: *M. incognita*, Ml: *M. luci*

When EST phenotypes and DNA analyses were assessed together, *M. luci* detected in 33 samples (59%) was the most prevalent RKN species. This nematode species was found associated with 18 samples alone and in 15 samples as mixed populations with *M. hapla* (8 samples) or *M. arenaria* (7 samples). *Meloidogyne hapla* was detected in 23 samples (41%), of which one was a mixed population with *M. arenaria*. In 15 samples (27%), *M. arenaria* was found alone (7 samples) or mixed populations with *M. luci* or *M. hapla*. The presence of *M. incognita* was detected only in one sample (2%), having as a single nematode species.

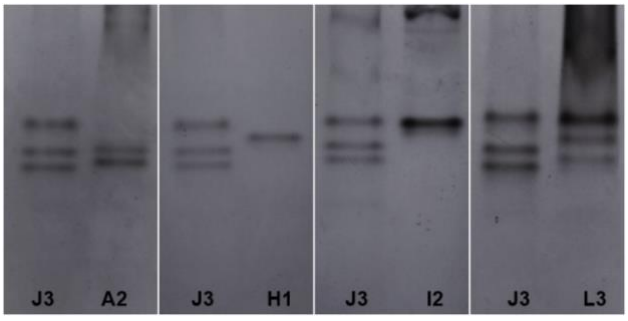

Figure 2. Esterase phenotypes of *Meloidogyne* spp. detected in kiwifruit orchards in Samsun Province, Türkiye (J3, *M. javanica* as reference isolate; A2, *M. arenaria*, H1, *M. hapla*; I2, *M. incognita*; L3, *M. luci*).

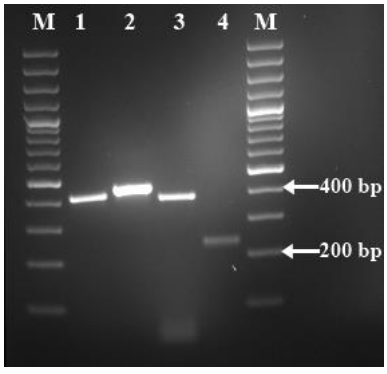

Figure 3. DNA amplification products with *Meloidogyne arenaria*-specific Far/Rar primers (Line 1), *Meloidogyne hapla*-specific JMV primers (Line 2), *Meloidogyne incognita*-specific inc-K14F/R primers (Line 3), *Meloidogyne luci*-specific Me309F/Me549R primers (Line 4) on *Meloidogyne* populations detected in kiwifruit orchards in Samsun, Türkiye (M: molecular marker with 100 bp).

Considering the distribution of *Meloidogyne* spp. in kiwifruit orchards, *M. luci* predominated, being occurred in 74% (17 orchards) of orchards infested, followed by *M. hapla* in 57% (13 orchards), *M. arenaria* in 39% (9 orchards) and *M. incognita* in 4% (1 orchard) (Figure 4). Multiple RKN species, having two or

three species, occurred in 61% (14 orchards) of the orchards infested. Remaining orchards were infested with *M. luci* or *M. hapla*.

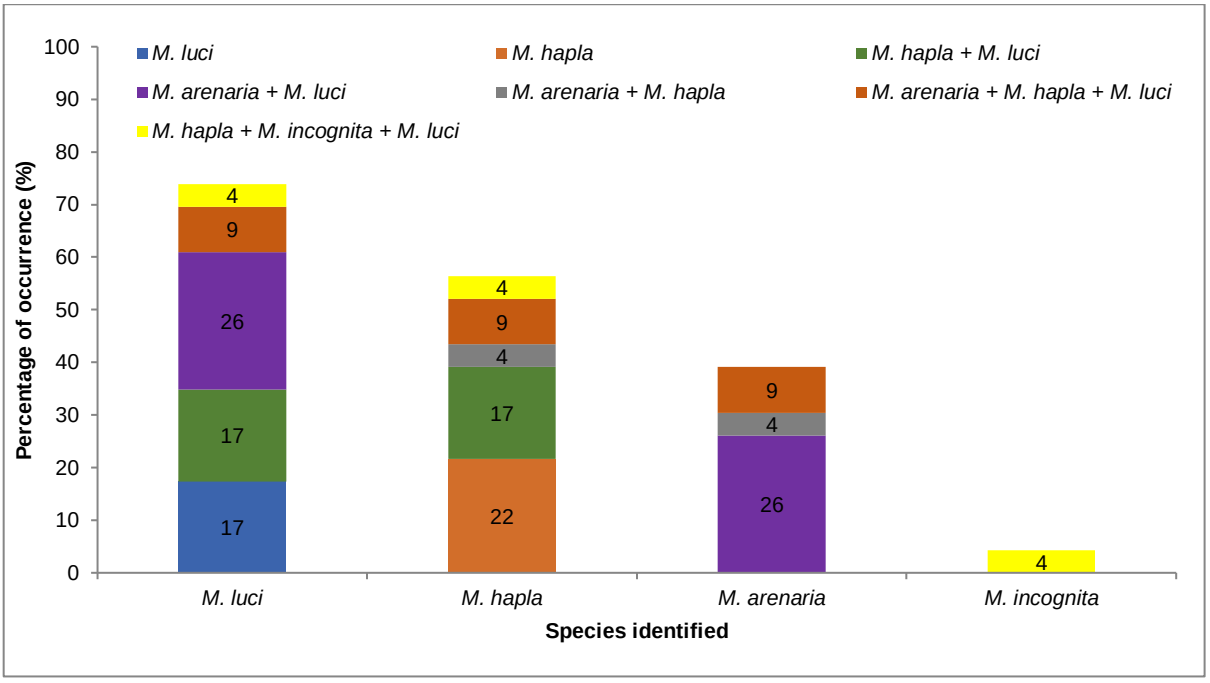

Figure 4. Frequency of *Meloidogyne* spp. identified in kiwifruit orchards in Samsun, Türkiye.

All *Meloidogyne* spp. identified in this study were detected in Çarşamba District (Figure 1). *Meloidogyne hapla* was present in all surveyed districts except Ondokuzmayıs, where *M. arenaria* and *M. luci* occurred. *Meloidogyne luci* was also found in Atakum and Terme Districts. *Meloidogyne arenaria* was detected in Atakum, Çarşamba and Ondokuzmayıs Districts whereas *M. incognita* was only found in Çarşamba District.

Fruit yields were obtained from two orchards (Orchard A and B) at the harvest period to estimate yield losses caused by RKN in kiwifruit. The mean population densities (mean ± SD) of *Meloidogyne* in vines infested in orchard A and B were determined as 526 ± 345 and 333 ± 233 J2/100 mL soil, respectively. According to the survey study, *M. luci* and *M. hapla* were found in orchard A, and *M. arenaria, M. hapla* and *M. luci* in orchard B (Table 1). In both orchards, the presence of *Meloidogyne* spp. was significantly reduced kiwifruit yield ($P < 0.05$) (Table 2). Relative yield losses compared to non-infested plants were determined as 36% in orchard A and 49% in orchard B. Fruits harvested from nematode-infested vines were smaller than that of non-infested vines in the orchards having the same cultural treatments (Figure 5).

Table 2. Kiwifruit yields (kg/vine, mean ± SD, n = 4) of *Meloidogyne*-infested and non-infested vines in two orchards in Samsun Province, Türkiye

Kiwifruit	Orchard A	Orchard B
Nematode-infested tree	100 ± 23	32 ± 9
Non-infested tree	158 ± 18	62 ± 10

Figure 5. Fruits harvested from *Meloidogyne*-infested (upper row) and non-infested (bottom row) vines in a kiwifruit orchard in Samsun, Türkiye.

Discussion

The present study revealed the wide distribution of RKN in kiwifruit plantations of Samsun Province. In addition to *M. arenaria, M. hapla* and *M. incognita* that have been which have previously been identified in kiwifruit orchards of Türkiye (Akyazı & Felek, 2013; Akyazi et al., 2017; Evlice & Özdemir, 2021), *M. luci* was reported for the first time with this study. Also, it was determined as the most common *Meloidogyne* spp. in kiwifruit orchards. Similar results on the prevalent of *M. luci* in Samsun were obtained in previous survey studies conducted on vegetable plants in greenhouses and open fields (Aydınlı & Mennan, 2016; Aydınlı, 2018). The esterase phenotype L3 is species-specific and the most useful character for differentiating *M. luci* from other species (Carneiro et al., 2014; Gerič Stare et al., 2017). An atypical (unidentified) population with L3 esterase phenotype on kiwifruit was detected first time by Somavilla et al. (2011) in Brazil. Subsequently, this population was reported as *M. luci* by Carneiro et al. (2014) in the original description of this nematode species. Before this description, a population of *Meloidogyne* obtained from soil sample of kiwifruit in Greece was reported as *M. ethiopica* by Conceição et al. (2012). However, this population was reclassified as *M. luci* along with all *M. ethiopica* populations detected in Europe (Gerič Stare et al., 2017). To our knowledge, the present study represents the third detection of *M. luci* on kiwifruit.

Taking into account the geographical distribution of RKN species reported on kiwifruit in the world, *M. hapla* is the most prevalent species and reported from Brazil (Somavilla et al., 2011), Chile (Philippi et al., 1996; Carneiro et al., 2007), France (Scotto La Massese, 1973), Greece (Tzortzakakis et al., 2011), India (Waliullah, 2005), Iran (Maafi & Mahdavian, 1997), Italy (Vovlas & Roca, 1976), New Zealand (Watson et al., 1992), South Africa (Shokoohi & Mashela, 2020), South Korea (Ma et al., 2007), Spain (Pinochet et al., 1990; Verdejo-Lucas, 1992) and Türkiye (Akyazi et al., 2017; Evlice & Özdemir, 2021). This nematode species had the widest distribution, occurring in five of the six surveyed districts, in this study, although it was the second highest frequency. With the third highest frequency, *M. arenaria* was detected in three districts of Samsun. Globally, *M. arenaria* has been recorded from kiwifruit in Australia (Castillo et al., 1993), Brazil (Somavilla et al., 2011), Iran (Maafi & Mahdavian, 1997), Italy (Castillo et al., 1993), Spain (Verdejo-Lucas, 1992), the USA (Haygood et al., 1990) and Türkiye (Akyazi et al., 2017; Evlice & Özdemir, 2021). In a survey conducted by Somavilla et al. (2011) in the Brazil, this nematode was detected as the most frequent *Meloidogyne* spp. in the samples. Akyazi et al. (2017) reported *M. arenaria* and *M. hapla* in kiwifruit roots for the first time in Türkiye, where both species were obtained from a commercial orchard in the neighboring province of Ordu. Evlice & Özdemir (2021) reported *Meloidogyne* infestation in four of eight sampled orchards in Bartın, Düzce, and Zonguldak from the Black Sea Region of Türkiye, and detected *M. hapla* in two orchards from Düzce and Zonguldak, where one of them also had *M. incognita*. The remaining infested orchards had *M. incognita*. In the first study conducted in kiwifruit orchards of Türkiye for RKN,

only *M. incognita* was found in all surveyed 17 orchards from Ordu (Akyazı & Felek, 2013). However, in the present study, *M. incognita* occurred only in one kiwifruit orchard from Çarşamba, where multiple RKN species infestation was found. The low frequency of this nematode in Samsun was also reported in recent surveys conducted in vegetable areas (Aydınlı & Mennan, 2016; Aydınlı, 2018). *Meloidogyne incognita* has also been found in Australia (Castillo et al., 1993), Brazil (Somavilla et al., 2011), Chile (Philippi et al., 1996), China (Tao et al., 2017), India (Khan, 2000), Iran (Maafi & Mahdavian, 1997), Italy (Castillo et al., 1993), Spain (Verdejo-Lucas, 1992) and the United States (Haygood et al., 1990).

Two or three nematode species were detected together in many orchards in this study. The occurrence of multiple *Meloidogyne* spp. in a kiwifruit orchard was also recorded in previous survey studies in kiwifruit orchards of Türkiye (Akyazi et al., 2017; Evlice & Özdemir, 2021). The multiple species that occurred in a kiwifruit orchard have also been reported in other countries (Verdejo-Lucas, 1992; Carneiro et al., 2007; Somavilla et al., 2011). The situation reveals that species identification must be performed on several specimens obtained from the same orchard since the accurate diagnosis of the species composition is required for developing appropriate nematode management practices (Kolombia et al., 2017).

Assessment of crop damage is a critical factor in deciding the phytosanitary importance of a pest species (Singh et al., 2013). In kiwifruit, there is little data on the damage potential of *Meloidogyne* (Di Vito et al., 1988; Philippi & Budge, 1992). In a pot experiment on the relationship between the initial population densities of *M. incognita* and plant growth in kiwifruit seedlings, Di Vito et al. (1988) found a growth suppression of 40 and 55% at eight and 32 eggs and juveniles per mL soil, respectively. However, Philippi & Budge (1992) indicated tolerance of kiwifruit seedlings to *M. hapla* in pot trials. Similarly, Pinochet et al. (1990) observed that the nematode population did not affect vegetative growth, yield, or fruit size, indicating the tolerance of plants. However, this consideration was not based on data. In our study, fruit yield decreased 36% in orchard A and 49% in orchard B in the *Meloidogyne*-infested vines compared to non-infested vines. To our knowledge, this is the first report on fruit yield losses caused by *Meloidogyne* in kiwifruit globally. However, yield loss was not associated with any RKN species since species identification was not done in sampled vines in harvest time.

The distribution of RKN in kiwifruit orchards in the Black Sea Region reveals a problem that should be addressed to ensure kiwifruit productivity in the near future. Currently, no registered nematicide is available for use on kiwifruit in Türkiye. Therefore, it is essential to be aware of the preventive measures. Especially, producers should obtain the seedlings free of nematode in planting in new areas. Only two kiwifruit orchards in this study, belonging to the same producer, were not infested with RKN. In this case, the producer had produced the seedlings himself whereas other producers obtained their seedlings from nurseries. The dissemination and wide distribution of RKN in a kiwifruit orchard and kiwifruit-producing areas of Samsun is most likely due to the planting of infested seedlings.

Acknowledgments

The authors thank Mr. İrfan Öztürk (Çarşamba Directorate of County Agriculture and Forestry, Samsun, Türkiye), Ms. Fadime Şen, Mr. Hoshang Kanabi Hamad, Mr. Hissein Mahamad Haroun and Ms. Merve Gümüş (Ondokuz Mayıs University, Samsun, Türkiye) for their assistance throughout sample collection.

References

Akyazı, F. & A. F. Felek, 2013. Molecular identification of root-knot nematode *Meloidogyne incognita* from kiwifruit orchards in Ordu province, Turkey. Turkish Journal of Entomology, 37 (4): 449-456.

Akyazi, F., S. Joseph, A. F. Felek & T. Mekete, 2017. Mitochondrial haplotype-based identification of root-knot nematodes, *Meloidogyne arenaria* and *Meloidogyne hapla*, infecting kiwifruit in Turkey. Nematropica, 47 (1): 34-48.

Atak, A., 2015. Kiwifruit research and production in Turkey. Acta Horticulturae, 1096 (1): 63-67.

Aydınlı, G., 2018. Detection of the root-knot nematode *Meloidogyne luci* Carneiro et al., 2014 (Tylenchida: Meloidogynidae) in vegetable fields of Samsun Province. Turkish Journal of Entomology, 42 (3): 229-237.

Aydınlı, G. & S. Mennan, 2016. Identification of root-knot nematodes (*Meloidogyne* spp.) from greenhouses in the Middle Black Sea Region of Turkey. Turkish Journal of Zoology, 40 (5): 675-685.

Carneiro, R. M. D. G., M. R. A. Almeida, E. T. Cofcewicz, J. C. Magunacelaya & E. Aballay, 2007. *Meloidogyne ethiopica*, a major root-knot nematode parasitizing *Vitis vinifera* and other crops in Chile. Nematology, 9 (5): 635-641.

Carneiro, R. M. D. G., V. R. Correa & M. R. A. Almeida, 2014. *Meloidogyne luci* n. sp. (Nematoda: Meloidogynidae), a root-knot nematode parasitising different crops in Brazil. Nematology, 16 (3): 289-301.

Castillo, P., N. Vovlas & A. Gomes Barcina, 1993. Nematodos fitoparásitos del kiwi (*Actinidia deliciosa*). Phytoma-España, 46: 3-8.

Conceição, I. L., E. A. Tzortzakakis, P. Gomes, I. Abrantes & M. J. Cunha, 2012. Detection of the root-knot nematode *Meloidogyne ethiopica* in Greece. European Journal of Plant Pathology, 134 (3): 451-457.

Di Vito, M., N. Vovlas & A. M. Simeone, 1988. Effect of root-knot nematode *Meloidogyne incognita* on the growth of kiwi (*Actinidia deliciosa*) in pots. Advances in Horticultural Science, 2 (3): 109-112.

El-Borai, F. E. & L. W. Duncan, 2005. "Nematode Parasites of Subtropical and Tropical Fruit Tree Crops, 467-492". In: Plant Parasitic Nematodes in Subtropical and Tropical Agriculture (Eds. M. Luc, R. A. Sikora & J. Bridge). CAB International, Oxfordshire, UK, 871 pp.

Esbenshade, P. R. & A. C. Triantaphyllou, 1985. Use of enzyme phenotypes for identification of *Meloidogyne* species (Nematoda: Tylenchida). Journal of Nematology, 17 (1): 6-20.

Evlice, E. & E. Özdemir, 2021. Distribution and occurrence of root-knot nematodes (*Meloidogyne* spp.) in kiwi orchards in Western Black Sea Region. KSU Journal of Agriculture and Nature, 24 (5): 1093-1099.

FAO, 2022. Food and Agriculture Organization of the United Nations Statistical Data. (Web page: www.fao.org/faostat) (Date accessed: February 2022).

Ferguson, A. R. & A. G. Seal, 2008. "Kiwifruit, 235-264". In: Temperate Fruit Crop Breeding: Germplasm to Genomics (Ed. J. F. Hancock). Springer, Dordrecht, Netherlands, 455 pp.

Gerič Stare, B., G. Aydınlı, Z. Devran, S. Mennan, P. Strajnar, G. Urek & S. Širca, 2019. Recognition of species belonging to *Meloidogyne ethiopica* group and development of a diagnostic method for its detection. European Journal of Plant Pathology, 154 (3): 621-633.

Gerič Stare, B., P. Strajnar, N. Susic, G. Urek & S. Širca, 2017. Reported populations of *Meloidogyne ethiopica* in Europe identified as *Meloidogyne luci*. Plant Disease, 101 (9): 1627-1632.

Haygood, R. A., J. A. Saunders & J. A. Miller, 1990. Widespread occurrence of *Meloidogyne incognita* on kiwifruit in the Coastal areas of South Carolina. Plant Disease, 74 (1): 81.

Katı, T. & S. Mennan, 2006. "Researches on species and race determination of root-knot nematodes (*Meloidogyne* spp.) found in greenhouse of Samsun, Turkey, 130". Abstracts of the 28th International Symposium of the European Society of Nematologists (5-9 June 2006, Blagoevgrad, Bulgaria), 168 pp.

Khan, M. L., 2000. Occurrence of root-knot nematode (*Meloidogyne incognita*) and other plant parasitic species on kiwi fruit (*Actinidia delicious* Chev.) in Himachal Pradesh. Indian Journal of Nematology, 30 (2): 245.

Kolombia, Y. A., G. Karssen, N. Viaene, P. L. Kumar, N. de Sutter, L. Joos, D. L. Coyne & W. Bert, 2017. Diversity of root-knot nematodes associated with tubers of yam (*Dioscorea* spp.) established using isozyme analysis and mitochondrial DNA-based identification. Journal of Nematology, 49 (2): 177-188.

Ma, K. C., Y. S. Jo, B. H. Kim & D. G. Lim, 2007. Seasonal occurrence and aspects of root-knot nematodes in major kiwifruit cultivation areas of Korea. Acta Horticulturae, 753 (2): 719-724.

Maafi, Z. T. & S. Mahdavian, 1997. Species and physiological races of root knot nematodes (*Meloidogyne* spp.) on kiwifruit and the effect of *M. incognita* on kiwifruit seedlings. Applied Entomology and Phytopathology, 65 (1): 1-3.

Philippi, I. & A. Budge, 1992. Effects of *Meloidogyne hapla* on young kiwi plants. Nematropica, 22 (1): 47-54.

Philippi, I., B. A. Latorre, G. F. Pérez & L. Castillo, 1996. Identification of the root-knot nematodes (*Meloidogyne* spp.) on kiwifruit by isoenzyme analysis in Chile. Fitopatologia, 31 (2): 96-101.

Pinochet, J., S. Verdejo & A. Soler, 1990. Observations on the seasonal fluctuation of *Meloidogyne hapla* on kiwi (*Actinidia deliciosa*) in Spain. Nematropica, 20 (1): 31-37.

Randig, O., M. Bongiovanni, R. M. D. G. Carneiro & P. Castagnone-Sereno, 2002. Genetic diversity of root-knot nematodes from Brazil and development of SCAR markers specific for the coffee-damaging species. Genome, 45 (5): 862-870.

Richardson, D. P., J. Ansell & L. N. Drummond, 2018. The nutritional and health attributes of kiwifruit: a review. European Journal of Nutrition, 57 (8): 2659-2676.

Scotto La Massese, C., 1973. Nouvel hóte Européen de *Meloidogyne hapla* et *Rotylenchus robustus*: *Actinidia chinensis*. Nematologia Mediterranea, 1 (1): 55-59.

Shokoohi, E. & P. W. Mashela, 2020. First report of *Meloidogyne hapla* on kiwifruit in South Africa. Journal of Nematology, 52 (1): 1-5.

Singh, S. K., M. Hodda & G. J. Ash, 2013. Plant-parasitic nematodes of potential phytosanitary importance, their main hosts and reported yield losses. EPPO Bulletin, 43 (2): 334-374.

Somavilla, L., C. B. Gomes, J. J. Carbonari & R. M. D. G. Carneiro, 2011. Survey and characterization of root-knot nematode species in kiwi in Rio Grande do Sul State, Brazil. Tropical Plant Pathology, 36 (2): 89-94.

Tao, Y., C. Xu, C. Yuan, H. Wang, B. Lin, K. Zhuo & J. Liao, 2017. *Meloidogyne aberrans* sp. nov. (Nematoda: Meloidogynidae), a new root-knot nematode parasitizing kiwifruit in China. PLoS ONE, 12 (8): e0182627.

TUIK (2022). Turkish Statistical Institute. (Web page: www.tuik.gov.tr) (Date accessed: February 2022).

Tzortzakakis, E. A., I. L. P. M. da Conceição, M. C. V. dos Santos & I. M. de O. Abrantes, 2011. Root-knot nematodes (*Meloidogyne* spp.) in Greece. Hellenic Plant Protection Journal, 4 (1): 25-30.

Verdejo-Lucas, S., 1992. Seasonal population fluctuations of *Meloidogyne* spp. and the *Pasteuria penetrans* group in kiwi orchards. Plant Disease, 76 (12): 1275-1279.

Vovlas, N. & F. Roca, 1976. *Meloidogyne hapla* su *Actinidia chinensis* en Italia. Nematologia Mediterranea, 4 (1): 115-116.

Waliullah, M. I. S., 2005. Nematodes associated with kiwi (*Actinidia deliceous* Chev.) in Kashmir valley, India. Indian Journal of Nematology, 35 (2): 227.

Watson, R. N., E. A. Wilson & R. S. Marsden, 1992. Distribution of plant parasitic nematodes in the rhizosphere of kiwifruit. Acta Horticulturae, 297 (2): 537-543.

Whitehead, A. G. & J. R. Hemming, 1965. A comparison of some quantitative methods extracting small vermiform nematodes from the soil. Annals of Applied Biology, 55 (1): 25-38.

Wishart, J., M. S. Phillips & V. C. Blok, 2002. Ribosomal intergenic spacer: A polymerase chain reaction diagnostic for *Meloidogyne chitwoodi*, *M. fallax*, and *M. hapla*. Phytopathology, 92 (8): 884-892.

Zijlstra, C., D. T. H. M. Donkers-Venne & M. Fargette, 2000. Identification of *Meloidogyne incognita*, *M. javanica* and *M. arenaria* using sequence characterised amplified region (SCAR) based PCR assays. Nematology, 2 (8): 847-853.

Türk. entomol. derg., 2022, 46 (2): 199-210
DOI: http://dx.doi.org/10.16970/entoted.1049424

ISSN 1010-6960
E-ISSN 2536-491X

Original article (Orijinal araştırma)

Investigation of thrips (Thysanoptera) species, their population changes and damage rates in vineyards of Mersin Province (Türkiye)[1]

Mersin İli (Türkiye) bağ üretim alanlarında thrips (Thysanoptera) türleri, popülasyon değişimleri ve zarar oranlarının araştırılması

İbrahim TEKE[2] **Ekrem ATAKAN[3*]**

Abstract

This study was conducted to determine thrips (Thysanoptera) species, their population changes as well as damage rates in vineyard areas in Mersin Province (Türkiye) in 2019 and 2020. Fourteen Thysanoptera species were identified. *Rubiothrips vitis* (Priesner, 1933) (Thysanoptera: Thripidae) was found to be more common (56%). This pest thrips was followed by *Mycterothrips tschirkunae* (Yakhontov, 1961) (Thysanoptera: Thripidae) with the ratio of 23% in total adults. The adult thrips species were collected mostly from fruit (88%). Population changes of the two common thrips species was assessed in the two vineyards in Hacıahmetli and Bağcağız. Population densities of *R. vitis* and *M. tschirkunae* coincided with the mature fruiting period of the vines. Damage ratios on grape fruits due to thrips feeding varied between 10 and 15% in Mersin Province.

Keywords: Damage, population density, thrips, Türkiye, vineyard

Öz

Bu çalışma Mersin İli (Türkiye) bağ alanlarında bulunan thrips (Thysanoptera) türlerinin tespiti, popülasyon değişimleri ve zarar oranlarını belirlemek amacıyla 2019 ve 2020 yıllarında yürütülmüştür. Çalışma sonucunda 14 Thysanoptera türü belirlenmiştir. En fazla tespit edilen tür *Rubiothrips vitis* (Priesner, 1933) (Thysanoptera: Thripidae) (%56) olmuş ve onu *Mycterothrips tschirkunae* (Yakhontov, 1961) (Thysanoptera: Thripidae) (%23) izlemiştir. Thrips erginleri en fazla oranda (%88) meyvelerde örneklenmiştir. Popülasyon takibi Hacıahmetli ve Bağcağız yörelerinde belirlenen iki bağ alanında yürütülmüştür. *Rubiothrips vitis* ve *M. tschirkunae*'nin popülasyon yoğunlukları olgun meyve döneminde yüksek bulunmuştur. Mersin İli genelinde bağlarda thrips beslenmesi nedeniyle üzüm meyvelerinde zarar oranı %10-15 arasında değişmiştir.

Anahtar sözcükler: Zarar, popülasyon yoğunluğu, thrips, Türkiye, bağ

[1] This study was produced by a part of the master thesis of the first author accepted by Çukurova University Graduate School of Natural and Applied Sciences, Department of Plant Protection on 28 December, 2020.

[2] Ministry of Agriculture and Forestry, Oil Seeds Research Institute, 80000, Cevdetiye, Osmaniye, Türkiye
[3] Çukurova University, Faculty of Agriculture, Department of Plant Protection, 01380, Sarıçam, Adana, Türkiye
* Corresponding author (Sorumlu yazar) e-mail: eatakan@mail.cu.edu.tr
Received (Alınış): 28.12.2021 Accepted (Kabul ediliş): 23.06.2022 Published Online (Çevrimiçi Yayın Tarihi): 29.06.2022

Introduction

Grapes are an important export crop for Türkiye. According to the data of FAO in 2019, Türkiye is an important country with the sixth place in the ranking made according to the vineyard areas of the countries around the world. Grapes are grown on an area of about 400 kha in Türkiye producing 4 Mt of fruit (FAO, 2019). Mersin Province, located in the eastern Mediterranean Region of Türkiye, has an important place in terms of grape cultivation, and 383 kt of grapes are produced on an area of about 19 kha. Of this, 360 kt was for table grapes, 2 kt dried grapes and 21 kt wine grapes (TUIK, 2020).

There are many harmful factors that reduce the quality and yield in grape production. There are 267 pest species known to occur in vineyards in Türkiye, although these pest species differ with region, 25 to 30 species are common and 8-10 of them are economically important (Öztürk et al., 2005). Among these pests, thrips belonging to the order Thysanoptera constitute a group that causes significant damage (Kaplan & Bayhan, 2018). Thrips cause direct damage to grapevines through their feeding activities and indirectly by carrying pathogen (Bournier, 1970). Thrips, which are seen with the opening of the buds in the spring, continue to cause damage during the vegetative growth. With their stinging and sucking mouth structures, they first scratch the plant tissue (needle of mandible) and then suck the sap (needles of maxilla), causing silvery spots, scar tissues and necrosis. They suck fluid from the flowers, stems and buds, and cause them to fall, thus keeping the clusters sparse and reducing yield. In the unripe grape period, the tissues around the wound open while laying eggs on the grape florets turn white and adversely affect the quality of grapes. Both larvae and adult thrips cause scars on grapes, decreasing market value of the grapes (Roditakis & Roditakis, 2007).

The thrips species and their economic importance have been reported by the previous studies conducted in the vineyard production areas in Türkiye (Kaplan et al., 2016; Kaplan & Bayhan, 2017, 2018). The Ministry of Agriculture technical staff report indicates that thrips are problem in vineyards of Mersin Province particularly in locations Mut and Tarsus. In addition, some grape producers in the region have reported that thrips are harmful and they use pesticides. There is no study yet on the current status and damage patterns of pest thrips species in the region. In this context, it is necessary to determine the thrips species, their population changes and degree of damage, that is, their economic importance in vineyards cultivated in these areas. Composition of thrips species and degree of thrips damage in vineyards may differ from region to region. For example, Özsemerci (2007) reported that *Rubiothrips vitis* (Priesner, 1933) (Thysanoptera: Thripidae) was the most common species among 31 thrips species detected in vineyards of Manisa Province (Türkiye), while *Haplothrips globiceps* Bagnall, 1934 (Thysanoptera: Phlaeothripidae) was reported as the most common species among 19 thrips species identified in vineyards of Mardin Province (Türkiye). Doğanlar & Yiğit (2002) found *Retithrips syriacus* (Mayet, 1890) (Thysanoptera: Thripidae) as a common thrips species seriously damaging grapevines grown in the home gardens in Hatay Province (Türkiye). Sezginalp (2006) determined that *Thrips tabaci* Lindeman, 1889 (Thysanoptera: Thripidae) and *Frankliniella occidentalis* (Pergande, 1895) (Thysanoptera: Thripidae) are common in the vineyards of Bursa Province (Türkiye). This study aimed to determine the Thysanoptera species and to obtain basic information about the damage status of the thrips in the vineyards in Tarsus and Mut Districts of Mersin Province.

Materials and Methods

Detection of Thysanoptera species in vineyards

In the vineyard areas of Mut and Tarsus districts of Mersin Province (Figure 1), in 2019, non-periodic surveys for thrips were performed according to the phenological (shoot, flowering, fruit formation, harvest period and leaf fall) periods of the vineyards between the awakening of the buds in the vines in April-

October. Fifty-five surveys were conducted. Field surveys were performed in the morning as much as possible and as many vineyards were sampled as possible.

Figure 1. The locations where thrips species are sampled in vineyard areas of Mersin Province (Türkiye) in 2019.

In order to represent the vineyard area in thrips sampling, 30 grapevines were randomly selected for sampling and each grapevine were shaken into the white container with 34 × 23 × 7 cm for 5-10 s. The fallen thrips were collected with the help of a fine brush or the suction tube, and they were put in the plastic Eppendorf tubes (2 ml) containing 60% ethanol. The label information of the samples taken (the place of collection, date, geographic coordinates and phenology of the vineyards sampled) were recorded. The locations where Thysanoptera species were sampled are given in Figure 1 and coordinates of the vineyards surveyed in Table 1.

Thrips samplings

Two vineyards were selected in Hacıahmetli and Bağcağız, Mut District of Mersin Province in 2019. Insecticide and acaricide were not used in the selected vineyards during the sampling periods. Only bluestone (copper preparation) was applied in vineyards in Hacıahmetli, and no other chemical application was made in the other vineyard. Thirty shoots and 30 ripe grapes were sampled from both vineyards between 11 June and 8 October at weekly intervals, especially in the morning in 2019. Shoots and grapes were tapped onto a white plastic container for 5-10 s. Thrips were collected with the help of fine brush or suction tube. Sample individuals were placed in the plastic tubes containing 60% ethanol. Collected samples were prepared and recorded.

The vineyard (1667 m^2) in Hacıahmetli was 24 km away from the district center of Mut, at 36°40’03" N and 33°36’02" E, and at an altitude of about 1100 m and a slope of 25-30% from north to south. There are pine groves to its east and south, and vineyards to its west and north. The soil structure is low in yield under arid conditions, moderately stony and permeable. The vineyard was about 25 years old, and was planted to cv. Göğüzüm. The vineyard in Bağcağız, where the population study was conducted, was 18 km away from the center of Mut at 36°41’04" N and 33°32’27" E, with an area of 1024 m^2, at an altitude of about 900 m and slope of 15-20% from east to west. There was a forest in the east, apricot orchard in the west and bare land in the north. The soil was infertile under arid conditions, moderately stony and permeable. The vineyard was about 25 years old, and also planted to cv. Göğüzüm.

Table 1. Geographic coordinates of the vineyards where thrips surveys were performed in Mersin Province (Türkiye) in 2019

No	Location	Coordinates	No	Location	Coordinates
1	Mut-Bağcağız	36°41'04" N, 33°32'27" E	17	Mut-Kıravga	36°47'11" N, 33°10'33" E
2	Mut-Cumhuriyet	36°38'56" N, 33°26'43" E	18	Mut-Kültür	36°38'21" N, 33°26'33" E
3	Mut-Cumhuriyet	36°38'60" N, 33°26'46" E	19	Mut-Meydan	36°38'57" N, 33°26'03" E
4	Mut-Cumhuriyet	36°39'00" N, 33°26'46" E	20	Mut-Meydan	36°38'54" N, 33°26'03" E
5	Mut- Cumhuriyet	36°38'56" N, 33°26'44" E	21	Mut-Sakız	36°37'43" N, 33°17'55" E
6	Mut- Deveci	36°38'11" N, 33°25'37" E	22	Mut-Yatırtaş	36°38'08" N, 33°25'40" E
7	Mut- Deveci	36°38'11" N, 33°25'40" E	23	Mut-Yatırtaş	36°38'08" N, 33°25'39" E
8	Mut-Doğancı	36°38'25" N, 33°26'28" E	24	Tarsus-Gaziler	36°55'54" N, 34°51'39" E
9	Mut-Güllük	36°38'30" N, 33°26'35" E	25	Tarsus-Sucular	36°57'24" N, 34°48'41" E
10	Mut-Güllük	36°38'30" N, 33°26'33" E	26	Tarsus-Sucular	36°57'49" N, 34°49'31" E
11	Mut-Güllük	36°38'32" N, 33°26'34" E	27	Tarsus-Sucular	36°57'14" N, 34°48'44" E
12	Mut-Güllük	36°38'35" N, 33°26'34" E	28	Tarsus-Sucular	36°58'01" N, 34°49'43" E
13	Mut-Güllük	36°38'34" N, 33°26'33" E	29	Tarsus-Takbaş	36°56'52" N, 34°49'20" E
14	Mut-Hacıahmetli	36°40'03" N, 33°36'02" E	30	Tarsus-Ulaş	36°59'13" N, 34°47'27" E
15	Mut-İbrahimli	36°41'37" N, 33°37'34" E	31	Tarsus-Ulaş	36°59'05" N, 34°47'31" E
16	Mut-Kelceköy	36°38'52" N, 33°27'57" E	32	Tarsus-Ulaş	37°00'21" N, 34°46'12" E

Thrips identification

Thrips samples were placed in the plastic tubes (2 ml) with AGA (10 parts 60% ethanol, 1 part glycerin and 1 part glacial acetic acid) liquid (Lewis, 1973). Thrips samples, which were kept in this liquid for 2 days, were taken again into tubes containing 60% ethanol. The preparation of microscope slides for the specimens were made according to Mound & Kibby (1998). In temporary preparations, thrips were kept in 10% sodium hydroxide until slight color change appear on their bodies on a hot plate at 48°C for nearly 1 h, then specimens were washed in 96% ethanol, they were prepared in the Hoyer medium. Adult and larval thrips species were identified to species by the second author using published keys (Priesner, 1951; Nakahara, 1994; zur Strassen, 2003; Masumoto & Okajima, 2006; Minaei & Mound, 2008; Vierbergen et al., 2010; Mirab-Balou et al., 2012).

Determination of thrips damage

In the vineyards at Hacıahmetli and Bağcağız rates of thrips infestation were assessed and also the symptoms of thrips damage recorded. After the buds bust and the shoots began to grow (from the appearance of the leaves to the appearance of the clusters), 30 shoots and 10 bunches of grapes were examined for possible thrips damage in each vineyard in 2019. For this, shoots were examined along a row of the vineyard. Shoots with thrips and shoots with thrips damage were recorded separately. The number of infected shoots recorded and the number of damaged shoots were added separately and proportioned to the total number of shoots (Bora & Karaca, 1970). In 2019, 10 grape clusters were taken randomly from each vineyard in the fixed trial areas (Hacıahmetli and Bağcağız). In 2020, 10 vineyards, 5 in each region (Mut and Tarsus), were sampled (Table 2), and 10 unripe/ripe grape clusters samples were taken from each vineyard. The samples of grape clusters were brought to the laboratory of Mut District Directorate of Agriculture of the Ministry of Agriculture and Forestry, and grapes were examined for the thrips damage.

Table 2. Geographic coordinates of the vineyards where thrips damage detection were performed in Mersin Province (Türkiye) in 2019 and 2020

No	Location	Coordinates	No	Location	Coordinates
1	Mut-Bağcağız	36°41'04" N, 33°32'27" E	6	Tarsus-Sucular	36°58'01" N, 34°49"43" E
2	Mut-Hacıahmetli	36°40'03" N, 33°36'02" E	7	Tarsus-Sucular	36°57'49" N, 34°49'31" E
3	Mut-İlice	36°41'47" N, 33°11'55" E	8	Tarsus-Ulaş	36°59'13" N, 34°47'27" E
4	Mut-İlice	36°41'39" N, 33°11'54" E	9	Tarsus-Ulaş	36°58'55" N, 34°47'46" E
5	Mut-İlice	36°41'32" N, 33°11'49" E	10	Tarsus-Ulaş	36°59'14" N, 34°47'23" E

Evaluation of data

In total, 783 thrips specimens (adult and larvae) were examined. The frequency of identified thrips species, their total numbers and percentages in total adult were calculated (Table 3). The numbers of individuals of thrips species in different phenological periods of the grapevine in the sampling locations and also their percentages found in different plant organs were also evaluated (Table 4). Percentage of damaged fruits was found by dividing the total number of damaged fruits recorded individually with the total number of fruit (Bora & Karaca, 1970). In 2020, data collected on thrips damage rate from five vineyards in each district were combined; thus, the damage rates were evaluated on a district basis.

Results and Discussion

Thrips species

The frequency, total numbers and percentages of thrips species collected and identified from the sampling locations are shown in Table 3. In Mut and Tarsus, between April and October 2019, 55 surveys were conducted to determine the thrips species in the vineyards, and as a result, 783 thrips individuals (adults) were collected. *Rubiothrips vitis* (Priesner, 1933) was the most common thrips species with 444 specimens and it constituted 57.0% of the total adult thrips. The second most collected species was *Mycterothrips tschirkunae* (Yakhontov, 1961) with its 183 specimens and it constituted 23.3% of the total adult individuals. As a result of the study conducted in the surveyed vineyard areas in 2019, 14 thrips species, one from the family Aeolothripidae, 12 from the family Thripidae and one other from the family Phlaeothripidae, are new records for the vineyards of Mersin Province.

Thysanoptera species detected as a result of this study showed parallelism with the Thysanoptera species detected in previous studies. However, in addition to the thrips species detected in previous studies, vineyards in different locations of Mersin province, *Thrips euphorbiae* Knechtel, 1923, *Thrips hawaiiensis* (Morgan, 1913) and *Thrips pillichi* Priesner, 1924 species from the family Thripidae were recorded the first time in very low numbers in 2019. *Haplothrips globiceps* and *Anaphothrips vitis* Priesner, 1933 were determined in the vineyard areas of the southeastern and eastern Anatolia regions of Türkiye (Günaydın, 1972; Maçan, 1984; Kaplan & Çınar, 1998). Although *A, vitis* is not a registered thrips species in the Thysanoptera Checklist for Türkiye (Tunç & Hastenpflug-Vesmanis, 2016), Cengiz (1974) detected 25 thrips species in the vineyards of İzmir and Manisa Provinces, Türkiye, they reported that *A. vitis,* together with *Drepanothrips reuteri* Uzel, 1895 (Thysanoptera: Thripidae) and *H. globiceps*, was more intense. Karagöz (1987), found that *Haplothrips reuteri* (Karny, 1907) and *R. vitis* were common in vineyards of Aydın Province (Türkiye). Özsemerci (2007) determined 31 thrips species in the seedless vineyards in Manisa Province and reported that *R. vitis, H. globiceps*, *H. reuteri* and *Aeolothrips collaris* were denser. Kaplan et al. (2016) detected 19 thrips species in the vineyard areas of Mardin Province and he also found that *H. globiceps* species was more common.

Table 3. Frequency of the sampled Thysanoptera species, numbers of adults and their ratios in total adult individuals (%) in Mersin Province (Türkiye) in 2019

Species	Frequency	Total no of species	Ratio (%)
Aeolothripidae			
Aeolothrips collaris Priesner, 1919*	4	4	0.5
Thripidae			
Drepanothrips reuteri Uzel, 1895	11	29	3.7
Frankliniella occidentalis (Pergande, 1895)	12	24	3.0
Mycterothrips tschirkunae (Yakhontov, 1961)	44	183	23.3
Neohydatothrips sp.	2	2	0.3
Rubiothrips vitis (Priesner, 1933)	75	444	56.0
Thrips major Uzel, 1895	1	2	0.3
Thrips meridionalis (Priesner, 1926)	1	2	0.3
Thrips euphorbiae Knechtel, 1923	1	2	0.3
Thrips hawaiiensis (Morgan, 1913)	1	2	0.3
Thrips tabaci Lindeman, 1889	21	29	3.7
Thrips pillichi Priesner, 1924	1	1	0.1
Thrips physapus Linnaeus, 1758	1	2	0.3
Phlaeothripidae			
Haplothrips globiceps (Bagnall, 1934)*	20	57	7.2
Total	**195**	**783**	**100**

* Predatory thrips

Distribution of thrips species according to plant phenology of grapevine

The distributions of thrips species according to plant phenology (shoot/leaf, fruit and harvest-postharvest period) in the vineyard areas of Mut and Tarsus are shown in Table 4. Fourteen thrips species and 783 adult thrips individuals were identified in both districts. In Mut, 679 thrips individuals were detected, and the most common species was *R. vitis* (369 specimens). The second most collected species in Mut was *M. tschirkunae* with its 181 specimens collected. In Tarsus, *R. vitis* was the most common Thripidae species with 75 specimens, and *F. occidentalis* (17 specimens) was the second most collected species.

Distributions of thrips species according to plant phenology were also examined; two thrips species were identified in the shoot/leaf period of the vineyards in Mut. In this district, the most common Phlaeothripidae species was *H. globiceps* with its two specimens, while *M. tschirkunae* (1 individual) was found in the second rank. Thirteen Thysanoptera species (527 adults) were collected in the fruiting period of the vineyards. The most common thrips species was *R. vitis* with its 260 specimens. In the harvest-postharvest period of the vineyards, six thrips species (149 adults) were identified, and the most common species was *R. vitis* with its 109 specimens. Two Thysanoptera species (9 individuals) were detected in the shoot/leaf period of the vineyards, and the most common Thripidae species was *F. occidentalis* with eight specimens in Tarsus. Seven Thysanoptera species (88 adults) were detected during fruiting of the grapevines, and the most common species was *R. vitis* with its 71 specimens. In the harvest-postharvest period in the vineyards, a four Thysanoptera species were detected, and *R. vitis* was relatively more common.

The highest frequency (%) based on the total number of thrips species sampled was on fruits (87.7%), followed by harvest-postharvest (8.8%) and shoot/leaf rates (3.5%) (Table 4). Özsemerci (2007) reported that the *R. vitis* was found had high frequency in the vineyards of Manisa Province during bud burst period, and it caused damage to the buds. Kaplan & Bayhan (2018) determined that the *R. vitis* species was first seen in the vineyards of Mardin Province, at low density, from April when the buds began to awaken. In Mersin Province, *M. tschirkunae* was first seen from the shoot elongation period, and it was

recorded intensively during the fruiting period (160 individuals and 87% frequency). Özsemerci (2007) reported that *M. tschirkunae* was first seen in the vineyards of Manisa Province from the shoot elongation, and it was most common in October (harvet-postharvest). *Haplothrips globiceps* was first seen from the shoot elongation period, and it was recorded in high number (50 individuals and 87.7% frequency) during the fruiting period. This species has been recorded in the vineyards in Fars Province, Iran (Minaei & Mound, 2008). In the current study, although there is no data on the pest status *H. globiceps*, Cengiz (1974) reported that *H. globiceps*, like *A. vitis* and *D. reuteri*, feed on both vegetative and fruiting organs of the vines, and therefore this thrips was recognized as harmful. Kaplan (2014) also determined that *H. globiceps* first appeared in the shoot elongation period of grapevines in Mardin Province, and caused serious damage to grapevines in the flowering period. However, Minaei & Mound (2008) reported that 20 *Haplothrips* spp. in Iran, of which *Haplothrips kermanensis* zur Strassen, 1975 and *Haplothrips flavicinctus* (Karny, 1910) are harmful, and *H. globiceps* is a predatory species. According to that study, the feeding behavior of other 10 species was unknown. *Haplothrips globiceps* has been recognized as a predatory thrips in the oak plantations of Iran (Mirab-Balou & Miri, 2021). *Drepanothrips reuteri* was found intensively during the fruiting period (25 individuals and 86% frequency) in the surveyed vineyard areas, and it continued to exist until the end of the harvest (Table 4). Özsemerci (2007) reported that *D. reuteri* is first seen in the vineyards of Manisa Province in May (shoot/leaf period), and it was detected at high density in July-September (the fruiting period).

Table 4. Distribution (%) of sampled Thysanoptera species according to regions and plant phenology in Mersin Province (Türkiye) in 2019

Species	Mut			Tarsus			
	Shoots/leaves	Fruits	Harvest	Shoots/leaves	Fruits	Harvest	Total
Aeolothripidae							
Aeolothrips collaris	0	3	0	0	1	0	4
Thripidae							
Drepanothrips reuteri	0	24	3	0	1	1	29
Frankliniella occidentalis	0	4	3	8	9	0	24
Mycterothrips tschirkunae	1	158	22	0	2	0	183
Neohydatothrips sp.	0	2	0	0	0	0	2
Rubiothrips vitis	0	260	109	0	71	4	444
Thrips major	0	2	0	0	0	0	2
Thrips meridionalis	0	2	0	0	0	0	2
Thrips euphorbiae	0	0	0	0	2	0	2
Thrips hawaiiensis	0	2	0	0	0	0	2
Thrips tabaci	0	17	8	1	2	1	29
Thrips pillichi	0	1	0	0	0	0	1
Thrips physapus	0	2	0	0	0	0	2
Phlaeothripidae							
Haplothrips globiceps	2	50	4	0	0	1	57
Total	3	527	149	9	88	7	783

Population changes of thrips species

This study was conducted in two vineyards in Hacıahmetli and Bağcağız locations in 2019 (Figures 2 and 3). Population fluctuation of *R. vitis* and *M. tschirkunae*, which were more common species, were examined in the weekly survey. Population fluctuation of thrips species according to plant phenology in the vineyard in the Hacıahmetli are shown in Figure 2. *Rubiothrips vitis* was first seen on 25 June (0.53 individuals/unripe fruit) and it was detected in all samples during the growing season. This harmful thrips species reached the highest density (0.60 individuals/fruit) on 23 July. The average population density of the pest species varied between 0.20-0.46 thrips individuals between 6 August and 10 September, and the

population density was found to be quite low in the samples taken after 17 September (Figure 2). The first adults (males and females) of *M. tschirkunae* were recorded on 11 June (0.03 individuals/shoot). The population density of this harmful thrips species varied between 0-0.03 individuals/unripe fruit. *Mycterothrips tschirkunae* reached the highest density (0.5 individuals/ripe fruit) on 2 July (during fruiting). After that date, the population was variable with an average density from 9 July to 3 September of 0.06-0.43 individuals (Figure 2). The combined average population densities of other thrips species during the unripe fruiting period of the vineyard were low, varying from 0 to 0.10 individuals/unripe fruit (Figure 2).

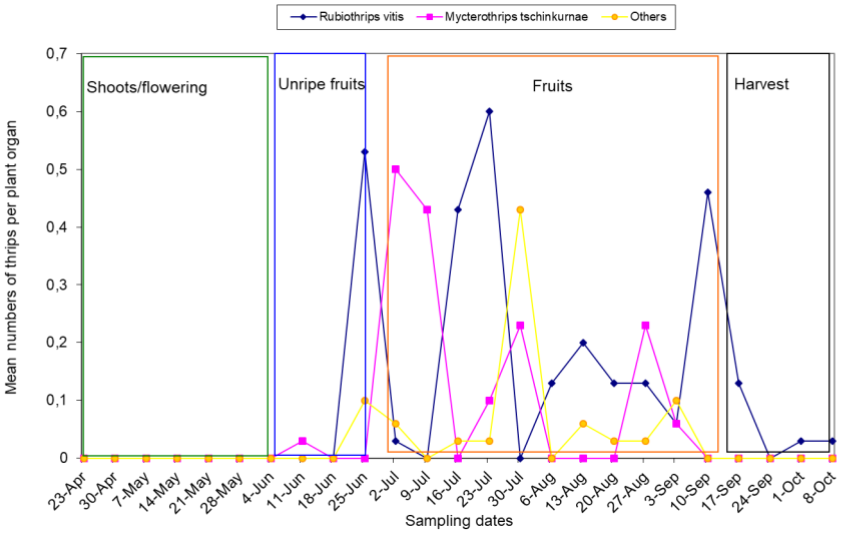

Figure 2. Mean number (adults) of two Thysanoptera species according to plant phenology in the vineyard in Hacıahmetli in Mersin Province (Türkiye) in 2019.

Population fluctuation of thrips species according to plant phenology in the vineyard in Bağcağız location in 2019 are given in Figure 3. *Rubiothrips vitis* started to be seen on 18 June (0.33 individuals/unripe fruit). This harmful thrips species reached its highest density (0.36 individuals/ripe fruit) on 10 September. This species had varying population densities throughout the season, with an average density of 0.26-0.36 individuals from 18 June to 10 September.

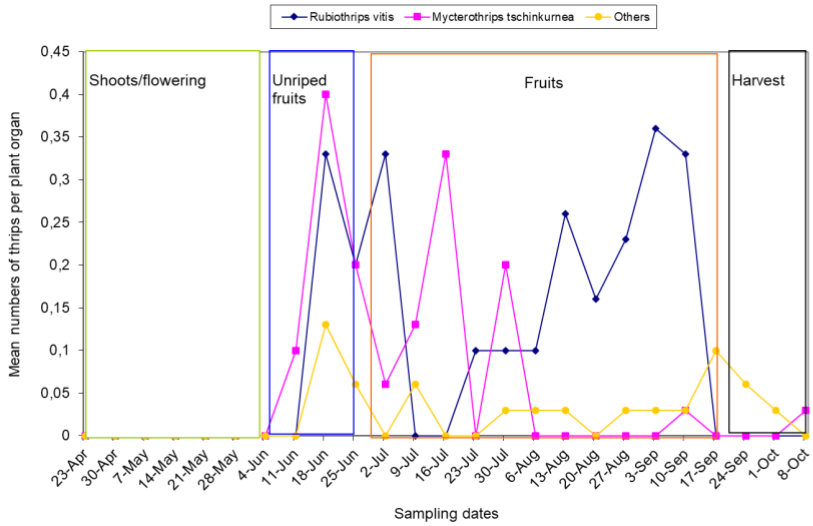

Figure 3. Mean number (adults) of two Thysanoptera species according to plant phenology in the vineyard in Bağcağız in Mersin Province (Türkiye) in 2019.

Mycterothrips tschirkunae was first seen during the unripe fruiting period. The pest thrips reached its highest density (0.40 individuals/unripe fruit) on 18 June. After that date, its population varied with an average population density between 0.20-0.33 individuals from 25 June to 30 July (Figure 3). In both locations (Hacıahmetli and Bağcağız), no thrips were found between 23 April and 11 June. This was thought to be due to unsuitable plant phenology or it being on other host plants, and thus it may have not yet moved into the vineyards.

Thrips damage symptoms in vineyards

Damage views due to thrips on grapevine leaves and fruits are shown in Figure 4. Thrips, which are first seen with the opening of the buds of vines in the spring, continue their damage during vegetative growth. At the beginning of the vegetative period in the vineyards, the pest thrips feeds by sucking on the lower surface of the leaves, and as a result, it forms a light yellow halo spots which eventually turn brown. As a result of intensive thrips feeding, the leaves may dry out. Thrips caused damage of black and brown spots on the fruits, and then these spots expand forming wounds. The brown spots get bigger and covered the florets in the cluster of fruits during the period of fall. Following this period, these spots caused cracking in the florets, finally the fruits to rot and thus the fruit-quality loss in the cluster (Figure 4). In the study conducted by Yokoyama (1977) in the USA, it was found that thrips lay eggs on the newly forming florets and cause injuries in the tissues where the eggs are deposited. Lewis (1997) reported that in table grapes, *T. tabaci* caused silvery or bronze colored spots on the florets in the fruit ripening period. In vineyards in Türkiye, similar damage symptoms on berries have been reported (Özsemerci, 2007; Kaplan, 2014).

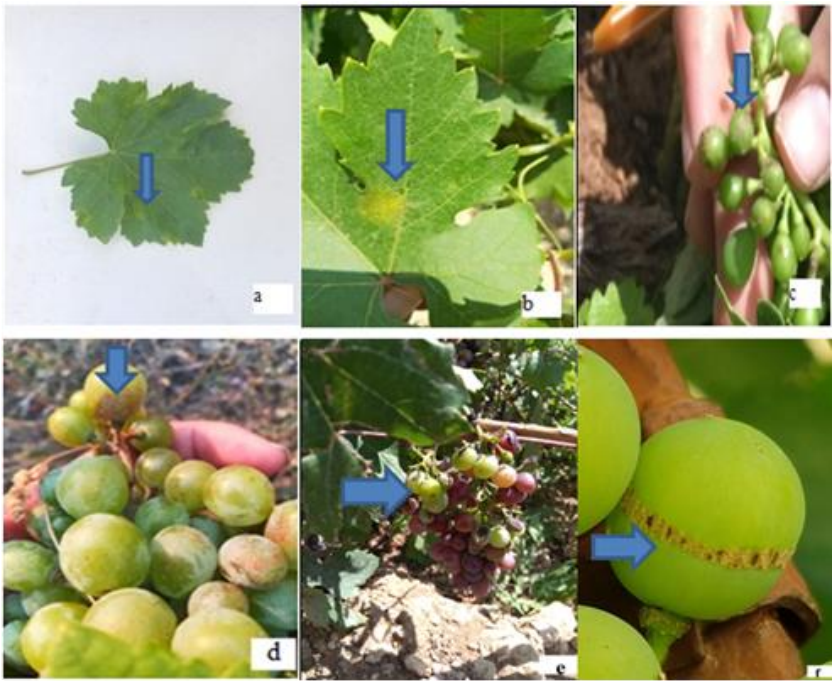

Figure 4. Damage symptoms due to the thrips attacks on grape leaves (a & b), unripe fruits (c & d) and matured fruits (e & f) in Mersin Province (Türkiye) in 2019.

Determination of thrips damage

The rate of damage (%) caused by the thrips in the vineyards in Hacıahmetli and Bağcağız are given in Table 5. The percentage of damage in Hacıahmetli and Bağcağız (when all clusters are included) was determined as 14.8 and 13.8%, respectively (Table 5). The damage in the vineyards in Mut and Tarsus (when all clusters were included) was 14.2 and 9.9%, respectively (Table 6).

In order to determine the percentage of damage, due to thrips on the grape leaves in Tarsus and Mut in 2020, thrips damage (%) was determined for 100 leaves randomly selected on 30 June and 8 August before the harvesting time. Percentage of thrips damage on grape leaves in Tarsus and Mut were found as 34 and 42%, respectively. Yokoyama (1977), reported that thrips cause 50% damage to the berries in the clusters, and this damage was due to the feeding of thrips larvae as well as adults in California, USA. Özsemerci (2007) reported that thrips species cause damage between 0.04 and 0.60% in seedless grapes in Manisa Province. Roditakis & Roditakis (2007) determined that the most damaging thrips species on grapes were *F. occidentalis*, *T. tabaci* and *D. reuteri*, and they determined the damage rates due to the thrips attacks under the laboratory conditions. In their experiments, they found that *F. occidentalis* caused significant injury (90%) in the first 6 days and 100% in the next 16 days, while *T. tabaci* and *D. reuteri* had caused 77 and 45% damage in the fruits after 16 days, respectively. Kaplan (2014), reported that the damage rate of thrips on grapes ranged from 15.34% to 21% in Mardin Province. Based on the previous studies, degree of damage caused by the thrips in the vineyards varied. This issue may be related to harmful thrips species, grape cultivars, pesticide applications in vineyards and other ecological factors.

Table 5. Percentage of damage, due to thrips feedings in the vineyards in the locations Hacıahmetli and Bağcağız s in Mut district in Mersin Province (Türkiye) in 2019

	Bağcağız			Hacıahmetli		
No of clusters	No of berries in clusters	No of damaged berries	Damage ratio (%)	No of Berries in clusters	No of damaged berries	Damage ratio (%)
1	60	8	13.3	36	7	19.4
2	50	7	14.0	60	10	16.7
3	45	4	8.9	42	8	19.0
4	47	7	14.9	75	9	12.0
5	69	5	7.2	47	1	2.1
6	51	8	15.7	75	5	6.7
7	27	6	22.2	22	3	13.6
8	28	6	21.4	23	7	30.0
9	25	4	16.0	38	11	28.9
10	34	5	14.7	27	5	18.5
Mean	43.6	6	13.8	44.5	6.6	14.8

Table 6. Damage rates (%) due to thrips feeding in the vineyards in Mut and Tarsus districts in Mersin Province (Türkiye) in 2020

	Mut			Tarsus		
No of clusters	No of berries in clusters	No of damaged berries	Damage ratio (%)	No of Berries in clusters	No of damaged berries	Damage ratio (%)
1	57	7	12.3	87	10	11.5
2	69	8	11.6	76	10	13.2
3	69	9	13.0	83	9	10.8
4	77	14	18.2	76	7	9.2
5	74	11	14.9	84	8	9.5
6	66	17	25.8	99	11	11.1
7	74	10	13.5	89	7	7.9
8	80	10	12.5	91	6	6.6
9	83	8	9.6	81	8	9.9
10	75	8	10.7	78	7	9.0
Mean	72.4	10.2	14.2	84.4	8.3	9.9

In the current study, mainly adults (both female and male) of thrips were collected. Few larvae were recorded. Larvae were mostly collected together with adults of *R. vitis*. This species was the main thrips in the vineyards sampled (Table 4). The majority of larvae belonged to this species. In other words, only *R. vitis* could multiply in the vineyards in the Eastern Mediterranean Region of Türkiye. We observed that the thrips damage in the vineyards sampled was mainly due to *R. vitis*. Similar to findings of the current study, Shoukat & Shayesteh (2006) reported that *R. vitis* was a common pest thrips species in both vegetative and fruiting organs of grapevines in vineyards in Western Azarbaijan in Iran.

Conclusions

In this study, harmful grapevine thrips species such as *R. vitis* was identified in important grape production areas of Mersin Province. The damage due to thrips feedings (mainly *R. vitis*) in the vineyards sampled varied between 10-15%. Thrips damage to grapes is, therefore, of potential importance in the grape cultivation in the region. However, further study is needed to clarify pest status of the thrips in the vineyards including an economic analysis of the yield loss due to thrips. Grape growers in the region do not know enough about thrips species and their damage. Probably, pesticides they use against other harmful insects such as the European grapevine moth in the vineyards also suppress sucking pest insects such as thrips. It would be useful to solve the thrips problem in the vineyards in the region within the scope of integrated pest management principles including education of growers about grapevine thrips in the region.

References

Bora, T. & İ. Karaca, 1970. Kültür Bitkilerinde Hastalığın ve Zararın Ölçülmesi. Ege Üniversitesi Ziraat Fakültesi Yardımcı Ders Kitabı Yayın No: 167, Ege Üniversitesi Matbaası, İzmir, 43 s (in Turkish).

Bournier, A., 1970. Principaux types de degats de thysanopterés sur les plantes cultivées. Annales du Zoologie et Ecologie Animale, 2 (2): 237-259.

Cengiz, F., 1974. İzmir ve Manisa Dolaylarında Bağlara Arız Olan Thysanoptera Türleri, Tanınmaları, Konukçuları, Zararları ve Tabii Düşmanları Üzerinde Araştırmalar. Türkiye Cumhuriyeti Tarım Bakanlığı Zirai Mücadele ve Zirai Karantina Genel Müdürlüğü Araştırma Eserleri Serisi Teknik Bülten No:22, İstiklal Matbaası, İzmir, 86 s (in Turkish with abstract in English).

Doğanlar, M. & A. Yiğit, 2002. Hatay'da yeni bir potansiyel meyve ve bağ zararlısı siyah bağ thripsi, *Retithrips syriacus* (Mayet) (Thysanoptera: Thripidae). Türkiye Entomoloji Dergisi, 26 (4): 283-294 (in Turkish with abstract in English).

FAO, 2019. Food and Agricultura Organization of the United Nations. (Web page: http//www.fao.org/faostat) (Date accessed: 05.01.2020).

Günaydın, T., 1972. A Survey of Vine Pests in South- East and East Anatolia. Plant Protection Research Annual, Zirai Mücadele Araştırma Yıllığı, 42:170 s (in Turkish).

Kaplan, M., 2014. Mardin İli Bağ Alanlarındaki Thysanoptera Türleri, Yayılışları, Zarar Oranları, Doğal Düşmanları ve Popülasyon Değişimleri ile Mücadele Olanaklarının Belirlenmesi. Dicle Üniversitesi, Fen Bilimleri Enstitüsü Bitki Koruma Anabilim Dalı (Unpublished) Doktora Tezi, Diyarbakır, 169 s (in Turkish with abstract in English).

Kaplan M. & E. Bayhan, 2017. Mardin ili bağ alanlarında zararlı thrips türleri ile mücadele olanaklarının belirlenmesi. Bitki Koruma Bülteni, 57 (4): 433-446 (in Turkish with abstract in English).

Kaplan, M. & E. Bayhan, 2018. Mardin ili bağ alanlarındaki Thysanoptera türlerinin popülasyon değişimlerinin belirlenmesi. Bitki Koruma Bülteni, 58 (1): 24-31 (in Turkish with abstract in English).

Kaplan, M., E. Bayhan & E. Atakan, 2016. Mardin İli bağ alanlarındaki Thysanoptera türleri, mevsimsel yoğunlukları ve yayılış alanlarının belirlenmesi. Türkiye Entomoloji Bülteni, 6 (2): 161-168 (in Turkish with abstract in English).

Kaplan, C. & M. Çınar, 1998. Güneydoğu Anadolu Bölgesi Bağlarında Ekonomik Öneme Sahip Zararlılar ile Yararlıların Yıllık Popülasyon Değişimleri ve Zararlıların Mücadeleye Esas Kritik Biyolojik Dönemlerinin Saptanması. Diyarbakır Zirai Mücadele Araştırma Enstitüsü, Meyve ve Bağ Zararlıları Alanında Yayınlar (unpublished Final Report), (Web page: http://web.ttnet.com.tr//users/dbzmae/entomeyve.html) (Date accessed: 20 July, 2020) (in Turkish).

Karagöz, M., 1987. Trakya Bölgesinde Bağlarda Zarar Yapan Böcek Türleri, Önemlilerinin Tanınmaları, Yayılışları, Zarar Şekilleri ve Ekonomik Önemleri Üzerinde İncelemeler. Trakya Üniversitesi, Fen Bilimleri Enstitüsü, Bitki Koruma Anabilim Dalı (Unpublished) Yüksek Lisans Tezi, Edirne, 40 s (in Turkish with abstract in English).

Lewis, T., 1973. Thrips, Their Biology, Ecology and Economic Importance. Academic Press, London and New York, USA, 267 pp.

Lewis, T., 1997. Thrips as Crop Pests. Institute of Arable Crops Research-Rothamsted, Harpenden, Herts, England, 740 pp.

Maçan, S., 1984. Güneydoğu Anadolu Bölgesi'nde Bağlarda Zarar Yapan Böcek Türleri, Önemlilerinin Tanınmaları, Yayılışları ve Ekonomik Önemleri Üzerinde İncelemeler. T.C. Tarım Orman ve Köy İşleri Bakanlığı, Zirai Mücadele Zirai Koruma Genel Müdürlüğü, Diyarbakır Bölge Zirai Mücadele Araştırma Enstitüsü, Ankara, 47 s (In Turkish with abstract in English).

Masumoto, M. & S. Okajima, 2006. A revision of and key to world species of *Mycterothrips* Trybom (Thysanoptera, Thripidae). Zootaxa, 1261: 1-90.

Minaei, K. & L. A. Mound, 2008. The Thysanoptera Haplothripini (Insecta: Phlaeothripidae) of Iran. Journal of Natural History, 42 (41-42): 2617-2658.

Mirab-Balou, M. & B. Miri, 2021. A survey of diversity and frequency of oak thrips during different seasons in Eyvan county (Ilam Province). Taxonomy & Biosystematics, 12 (42): 83-92.

Mirab-Balou, M., C. Xue-Xin & T. Xiao-Li, 2012. A review of the *Anaphothrips* genus-group (Thysanoptera: Thripidae) in China, with descriptions of two new species. Acta Entomologica Sinica, 55 (6): 719-726.

Mound, L. A. & G. Kibby, 1998. Thysanoptera an Identification Guide (2nd Edition). CABI Publishing, CAB International, Wallingford, Oxon OX10 8DE, UK, 70 pp.

Nakahara, S., 1994. The genus *Thrips* Linnaeus (Thysanoptera: Thripidae) of the new world. United States Department of Agriculture Technical Bulletin, 1822: 1-183.

Özsemerci, F., 2007. Manisa İlinde Çekirdeksiz Üzüm Bağlarında Bulunan Thysanoptera Türlerinin Yayılışı, Popülasyon Değişimi ve Önemli Zararlı Türün Biyolojisi Üzerinde Araştırmalar. Ege Üniversitesi, Fen Bilimleri Enstitüsü, Bitki Koruma Anabilim Dalı, (Unpublished) PhD Thesis, İzmir, 121 s (in Turkish with abstract in English).

Öztürk, N., A. Hazır & M. R. Ulusoy, 2005. "Türkiye bağlarında saptanan zararlı türler ile doğal düşmanları, 575-588". Türkiye 6. Bağcılık Sempozyumu Bildirileri (19-23 September 2005, Tekirdağ), 646 s (in Turkish with abstract in English).

Priesner, H., 1951. Zwei neue Thysanopteren aus der Türkei. Zeitschrift fur Pflanzenkrankheiten und Pflanzenschutz, 58 (7/8): 255-258.

Roditakis, E. & N. E. Roditakis, 2007. Assessment of the damage potential of three thrips species on white variety table grapes in vitro experiments. Crop Protection, 26 (4): 476-483.

Sezginalp, K., 2006. İznik (Bursa) Yöresinde Bağlarda Bulunan Arthropod Türlerinin Saptanması ve Önemli Olanların Popülasyon Dalgalanmaları Üzerinde Çalışmalar. Uludağ Üniversitesi Fen Bilimleri Enstitüsü, Bitki Koruma Anabilim Dalı, (Unpublished) Yüksek Lisans Tezi, Bursa, 89 s (in Turkish with abstract in English).

Shoukat, G. A. & N. Shayesteh, 2006. Thrips species found in West Azarbaijan (Orumieh vineyards, and seasonal abundance of the predominant species (*Rubiothrips vitis*). Journal of Agricultural Science & Technology, 8 (2): 133-139.

TUIK, 2020. Bitkisel Üretim İstatistikleri. (Web page: biruni.tuik.gov.tr/medas/?kn=92&locale=tr) (Date accessed: 05.01.2020) (in Turkish).

Tunç, I. & A. Hastenpflug-Vesmanis, 2016. Records and checklist of Thysanoptera in Turkey. Turkish Journal of Zoology, 40 (5): 769-778.

Vierbergen, G., H. Kucharczyk & W. D. J. Kirk, 2010. A key to second instar larvae of the Thripidae of the Western Palaeartic region (Thysanoptera). Tijdschrift voor Entomologie, 153 (1): 99-160.

Yokoyama, Y. V., 1977. *Drepanothrips reuteri* on Thompson seedless grapes. Environmental Entomology, 1 (6): 21-24.

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, 277 pp (in German).

Türk. entomol. derg., 2022, 46 (2): 211-226
DOİ: http://dx.doi.org/10.16970/entoted.1079195

ISSN 1010-6960
E-ISSN 2536-491X

Original article (Orijinal araştırma)

Efficacy of *Nicotiana tabacum* L. (Solanaceae), *Allium sativum* L. (Amaryllidaceae) and soft soap for controlling *Polyphagotarsonemus latus* (Banks, 1904) (Acari: Tarsonemidae)[1]

Polyphagotarsonemus latus (Banks, 1904) (Acari: Tarsonemidae)'un kontrolünde *Nicotiana tabacum* L. (Solanaceae), *Allium sativum* L. (Amaryllidaceae) ve arap sabununun etkinliği

Rana AKYAZI[2*] Mete SOYSAL[2] Yunus Emre ALTUNÇ[2] Duygu AKYOL[2]

Abstract

The toxicity of bulb extract of garlic, *Allium sativum* L. (Amaryllidaceae), the leaf extract of tobacco, *Nicotiana tabacum* L. (Solanaceae), soft soap and the mixtures of soft soap with the garlic and tobacco extracts on *Polyphagotarsonemus latus* (Banks, 1904) (Acari: Tarsonemidae) were investigated in the Plant Protection Department of Ordu University between 2014 and 2018 under controlled conditions. The ovicidal and adulticidal effects were tested against 0-24-h-old eggs and newly emerged adult females (1-2 days old) of *P. latus*, respectively at five concentrations of each extract. The efficacies of the same solutions in controlling the *P. latus* population were investigated on potted Barbunia bean, *Phaseolus vulgaris* L., 1753 cv. Barbunia (Fabaceae) plants in a climatic room as well. The results demonstrated that the soft soap and the garlic bulb extract+soft soap mixture are promising for controlling *P. latus*. The ovicidal effects of soap at 10% and garlic+soap mixture at 20% were 100% and 97.8%, respectively. Additionally, the adulticidal effects of soap at 7.5 and 10% and garlic+soap mixture at 10% were not significantly different and ranged between 90-100%. Also, the soap (10%) and garlic+soap (20%) treatments were able to keep the mite population on bean plants below the economic threshold for 1 week.

Keywords: Adulticidal, garlic, indirect ovicidal activity, tobacco, yellow tea mite

Öz

Sarımsak, *Allium sativum* L. (Amaryllidaceae) yumru ekstraktı, tütün, *Nicotiana tabacum* L. (Solanaceae) yaprak ekstraktı, arap sabunu ile sarımsak ve tütün ekstraklarının sabun ile karışımlarının *Polyphagotarsonemus latus* (Banks, 1904) (Acari: Tarsonemidae)'a karşı toksik etkileri, Ordu Üniversitesi, Bitki Koruma Bölümü'nde, 2014-2018 yılları arasında araştırılmıştır. Ovisidal ve adultisidal etkiler, sırası ile *P. latus*'un 0-24 saatlik yumurtaları ve genç ergin dişilerine (1-2 günlük) karşı beş farklı konsantrasyonda test edilmiştir. Aynı solüsyonların *P. latus* popülasyonunu kontroldeki etkinlikleri ise saksılı fasulye, *Phaseolus vulgaris* L., 1753 cv. Barbunia (Fabaceae) bitkileri üzerinde, iklim odası koşullarında araştırılmıştır. Sonuçlar, arap sabunu ve sarımsak+sabun karışımının *P. latus* kontrolü açısından umut verici olduğu göstermiştir. Arap sabununun %10, sarımsak+sabun karışımının %20'lik konsantrasyonlarındaki ovisidal etkileri sırası ile %100 ve %97.8 olarak tespit edilmiştir. Sabunun %7.5 ve %10'luk konsantrasyonları ile sarımsak+sabun karışımının %10'luk konsantrasyonunun istatistiksel olarak aynı ve %90-100 arasında değişen adultisidal etkiye sahip oldukları da belirlenmiştir. Ayrıca, sabun (10%) ve sarımsak+sabun (20%) uygulamaları, fasulye bitkilerindeki akar popülasyonunu 1 hafta boyunca Ekonomik Zarar Eşiği altında tutabilmiştir.

Anahtar sözcükler: Adultisidal, sarımsak, ovisidal, tütün, sarı çay akarı

[1] This study was partly presented as a poster at the 15th International Conference of Acarology (ICA) (2-8 September 2018, Antalya, Türkiye) and the International Conference on Biopesticide 7 (ICOB7) (19-25 October 2014, Antalya, Türkiye) and published as an abstract in the abstract book.

[2] Ordu University, Faculty of Agriculture, Department of Plant Protection, 52200, Altınordu, Ordu, Türkiye
* Corresponding author (Sorumlu yazar) e-mail: ranaakyazi@odu.edu.tr
Received (Alınış): 25.02.2022 Accepted (Kabul ediliş): 16.06.2022 Published Online (Çevrimiçi Yayın Tarihi): 29.062022

Introduction

Polyphagotarsonemus latus (Banks, 1904) (Acari: Trombidiformes: Prostigmata: Tarsonemidae) is an economically important pest mite on more than 60 plant families including Solanaceae, Cucurbitaceae, and Malvaceae (Grinberg et al., 2005). This species attacks growing young parts of the plants and is generally found on the undersides of young leaves. It can also inject toxic saliva into plant tissues (Rodriguez et al., 2017). Its feeding causes twisted, hardened and distorted growth in the terminal of a plant (Rogers et al., 2010; Rodriguez et al., 2017). Damaged leaves turn to coppery colors and bend. Also, the leaves of the heavily infested plants fall, they often fail to flower and produce bronze, cracked or blemished fruits (Gerson, 1992). Additionally, because individuals of this mite are microscopic (0.1-0.3 mm long), they can be noticed just when the symptoms occur (Venzon et al., 2008).

The control of *P. latus* is mostly based on the use of pesticides (Kousik et al., 2007). However, some common pesticides have unwanted various negative impacts on the non-target organisms and the environment (Aktar et al., 2009). So, there is an urgent need for the discovery of new non-toxic natural products as an alternative to conventional pesticides in the control of *P. latus.* Tobacco, *Nicotiana tabacum* L. (Solanaceae) used as an insecticide since 1960, garlic, *Allium sativum* L. (Amaryllidaceae) used for more than 7,000 years as a medicinal plant by humans (Pavela, 2016) and soft soap, which has been used as a pesticide since the 1700s (Olkowski et al., 1993), are among alternative natural pesticidal materials.

Several studies have focused on the control of *P. latus* using garlic, tobacco and soft soap on different host plants except bean plants. Among these, Hossain et al. (2013) investigated the efficacy of garlic against this mite on jute, *Corchorus capsularis* L. and *Corchorus olitorius* L. (Malvaceae). Uraisakul (2003) and Mari et al. (2013) used the tobacco extract to control *P. latus* on chili, *Capsicum* spp. (Solanaceae). A study on the management of the *P. latus* population using soft soap on chili under both screen house and field conditions was conducted by David et al. (2009). Akyazi et al. (2019) investigated the effect of garlic and tobacco extracts, soft soap against *P. latus* on the tea plant, *Camellia sinensis* (L.) Kuntze (Theaceae). However, no study has yielded the efficiency of the soft soap, garlic, and tobacco extract against *P. latus* on bean plants. It is known that the different plant species have different leaf morphology. Leaf morphology may affect spray deposition (de Ruiter et al., 1990; Himel et al., 1990; Smith et al., 2000). Also, in particular, no study, to our knowledge, has considered the effect of the mixtures of soft soap with garlic and tobacco extracts on *P. latus* on any plant.

Additionally, although Almansour & Akbar (2013) and Akyazi et al. (2018) have illuminated the ovicidal effects of garlic and tobacco extracts, soft soap on *Tetranychus urticae* Koch., 1836 (Trombidiformes: Tetranychidae) eggs, a still-unanswered question is whether these treatments and their mixtures also have a toxic effect on *P. latus* eggs.

Also, few studies have focused on the adulticidal efficiency of the tested solutions in the current study on plant-parasitic mites. Previous studies such as Attia et al. (2002), Hincapie et al. (2008), Erdogan et al. (2012), Almansour & Akbar (2013), Nour El-Deen & Abdallah (2013), Akyazi et al. (2018) have almost exclusively focused on *T. urticae*. Also, it should be aware that the potential effect in the mixture may be less or greater than that of individual substances.

To fill these literature gaps, this paper identifies the effects of the aqueous garlic bulb and tobacco leaf extracts, soft soap, aqueous garlic bulb extract+soft soap, and aqueous tobacco leaf extract+soft soap mixtures against *P. latus* on bean plants, and their ovicidal and adulticidal effects on *P. latus* under controlled conditions.

Materials and Methods

Plant rearing as the host for *Polyphagotarsonemus latus* and the main material for test substrate

Barbunia bean, *Phaseolus vulgaris* L. cv. Barbunia (Fabaceae) plants were grown from seed in plastic pots in a plant growth room (25 ± 2°C, 70-80% RH, 16:8 h L:D photoperiod, 12.000 lux, daylight). The plants were used as a host for *P. latus*. Bean leaf discs (3 cm) served as a substrate for releasing the mites on potted bean plants and for adult oviposition to assess the ovicidal and adulticidal activities.

Mite origin and rearing

The original population of the *P. latus* was obtained from an organic tea plantation at the Rize Atatürk Tea and Horticultural Research Institute in 2014. The stock cultures of *P. latus* were maintained on potted bean plants in a climate room (25 ± 2°C, 70-80% RH, 16:8 h L:D photoperiod, 12.000 lux, daylight). Heavily infested bean plants were replaced with fresh ones for maintaining the mite population.

Zero-24-h-old eggs of *P. latus* were used to test the ovicidal efficacy of different solutions. To obtain eggs of the same age, five adult females of *P. latus* were transferred from the stock colony to each bean leaf disc and kept overnight for oviposition. After 24 h, adult females were removed from each disc. Discs including 10 eggs were used for ovicidal activity assessments.

The adulticidal assay was performed using 24-48-h-old adult females. For this purpose, 20 *P. latus* females taken from the stock culture were placed on each leaf disc. Mites were allowed to lay eggs for 24 h, then they were removed the disc. The leaf discs with the *P. latus* eggs were kept in a growth chamber (25 ± 2°C, 70-80% RH, 16:8 h L:D photoperiod, 12.000 lux, daylight). After egg hatching, the adult females from these experimental units were used for the adulticidal bioassays.

Larva and quiescent nymph stages were not included in the experiment because these stages generally lasted about 24 h or less under the study conditions.

Preparing stock solutions

Samsun Canik tobacco, Taşköprü garlic, and soft soap (5-10% KOH, 15-30% sunflower oil, distilled water) were used for preparing the stock solutions.

Tobacco was harvested using the priming method (the leaves are harvested as and when they are fully developed and matured) on 10 August 2013 in Vezirköprü, Samsun, Türkiye. The leaves were dried using the sun-curing method (leaves were spread out on racks and placed outdoor when the sun rises, at night or in rainy weather, the racks were brought indoor until the leaves were sun-cured).

The garlic was harvested when the stem turned yellow and fell over and the bottom few leaves turned brown on 15 July 2013 and 2018 in Çetmi village in Taşköprü, Kastamonu, Türkiye. The harvested garlic was cured in a warm, dry, shaded area with good ventilation by tying the stem together and hanging to dry for 3-4 weeks.

The impact of each solution on the different stages of *P. latus* was different. So, the content of each stock solution and their used concentrations were adjusted by preliminary trials so that the mortality rate was almost equally distributed (Table 1).

For preparing the aqueous garlic bulb extract stock solution, peel garlic cloves (50 g for ovicidal and acaricidal efficiency and 125 g for adulticidal efficiency) were crushed and mixed up with distilled water (500 ml) and then incubated into a tightly sealed glass jar at 25 ± 1°C for 24 h. After incubation, the product was separated using a fine muslin cloth and then filtered through Whatman No. 1 filter paper.

To obtain the aqueous tobacco leaf extract stock, the dried tobacco leaves (50 g) were mixed with 500 ml distilled water and incubated in a tightly sealed glass jar at 70°C for 24 h. After incubations, the obtained product was passed through firstly a fine muslin and then a filter paper (Whatman No. 1).

To the stock solution of soft soap, soft soap (50 g) was mixed with distilled water (500 ml), and then shaken into a tightly sealed glass jar at 25 °C for 24 h at 200 rpm until the soap was fully dissolved.

For the aqueous garlic bulb extract+soft soap mixture, 25 g soft soap was mixed up with the garlic stock solution (125 ml) and shacked until the soap was fully dissolved.

For the aqueous tobacco leaf extract+soft soap mixture, 25 g soft soap was mixed up with the tobacco stock solution (125 ml) and shacked until the soap was fully dissolved.

Table 1. Stock solutions, stock contents and tested concentrations

Stock	Stock content	Ovicidal effect dose (%) (v/v) *	Adulticidal effect dose (%) (v/v) *	Acaricidal effect on bean plants dose (%) (w/v)
Aqueous garlic bulb extract 1	50 g garlic + 500 ml water (ovicidal and acaricidal)	1, 2.5, 5, 7.5, 10	-	10
Aqueous garlic bulb extract 2	125 g garlic + 500 ml water (adulticidal effect)	-	2.5, 6.25, 12.5, 18.75, 25	-
Aqueous tobacco leaf extract	50 g tobacco + 500 ml water	1, 2.5, 5, 7.5, 10	1, 2.5, 5, 7.5, 10	10
Soft soap	50 g soap + 500 ml water	1, 2.5, 5, 7.5, 10	1, 2.5, 5, 7.5, 10	10
Aqueous garlic bulb extract + soft soap	25 g soap + 125 ml garlic stock	2, 5, 10, 15, 20	2, 5, 10, 15, 20	20
Aqueous tobacco leaf extract + soft soap	25 g soap + 125 ml tobacco stock	2, 5, 10, 15, 20	2, 5, 10, 15, 20	20

* w/v for 10% of single solutions, 25% of garlic extract and 20% of mixture solutions.

Preparing required concentrations

Each treatment was evaluated at five concentrations for assessing ovicidal and adulticidal activities. The concentrations used for the ovicidal and adulticidal effects were chosen after preliminary bioassays to obtain mortality rates almost equally distributed between 0 and 100% (Table 1) (Tuncer, 2001). So, different concentrations of garlic extract were evaluated for ovicidal and adulticidal efficiencies. However, since some treatments had phytotoxic effects at high concentrations in the preliminary bioassay, their lower concentrations were used in the study.

To test the ovicidal and adulticidal effects, 1, 2.5, 5, 7.5 and 10 ml of each stock solution were diluted separately with distilled water to a total volume of 10 ml (final volume) to get five concentrations of each tested solution (Table 1).

However, the stock solutions were directly used to control the *P. latus* population on potted bean plants (Table 1). Due to the phytotoxic effect of the stock solution of garlic extract at 25% on bean leaf disc, the stock solution of garlic extract at 10% was used for the acaricidal effect experiment on potted bean plants. The other stock solutions and all tested concentrations had not any phytotoxic effect on bean leaf disc. So, they were used for the acaricidal effect experiment on potted bean plants.

Ovicidal effect on *Polyphagotarsonemus latus* eggs (0-24 h old)

The ovicidal effects of seven treatments including soft soap, garlic extract, tobacco extract and the mixtures of garlic and tobacco extracts with soap were investigated against *P. latus* eggs (0-24 h old). Each treatment was evaluated at five concentrations (Table 1). Five replicate bioassays with five concentrations and two controls (negative and positive) were performed. Distilled water and abamectin 18 g/L (25 ml/100 L water) were used as a negative and positive control, respectively. Abamectin is a natural fermentation product of the *Streptomyces avermitilis* (ex Burg et al., 1979) (Actinomycetales: Streptomycetaceae) (Siddique et al., 2013).

It has also translaminar or local systemic activity (Cloyd, 2016). It is used commonly and effectively against *T. urticae* on some vegetables. So, it was chosen as a positive control for the current study to test if it has also an effect on *P. latus*. A modified leaf disc dip method (de Silva et al., 2008) was used to evaluate the ovicidal activities of treatments. The discs with eggs were dipped in the solutions, distilled water or abamectin for 5 s. Treated leaf discs were dried at room temperature and placed underside up on moistened cotton in a plastic tray (15 x 11 cm). The number of hatched eggs in each treatment was recorded at 1- and 2-day intervals for 10 days following the application to be completely sure of the egg hatching rates in the treatments. Those eggs that did not hatch after this period were regarded as non-viable.

Adulticidal effect on *Polyphagotarsonemus latus* adult females (1-2 days old)

Adulticidal bioassay was performed by the leaf disc spraying method since the leaf disc dip method used to assess ovicidal efficacy was not appropriate for the adulticidal activity bioassays. Solutions at five different concentrations were evaluated for the adulticidal effect (Table 1). A single bioassay with five concentrations and two controls (negative and positive) was replicated five times. The negative control discs were treated with distilled water. Abamectin 18 g/L (25 ml/100 L water) was used for the positive control discs. A modified leaf disc spraying method described by Zalom et al. (2010) was used to assess the adulticidal activity of the tested solutions. For this purpose, ten adult female mites (1-2 days old) were gently transferred on each leaf disc by using a 5/0 size brush. The solutions were applied using a small hand sprayer held 15 cm away from the leaf discs resulting in a 3.08 ± 0.39 µl/cm^2 deposit. Dead mites were counted 1, 24, 48 and 72 h after solution applications (Akyazi et al., 2018).

Acaricidal effect on *Polyphagotarsonemus latus* population on potted bean plants

The method to detect the acaricidal effect on the *P. latus* population was spraying the potted bean plants infested by *P. latus*. In the experiment, there were seven treatments: soft soap, tobacco leaf extract, garlic bulb extract, the mixtures of garlic and tobacco extracts with soap, Abamectin 18 g/L (25 ml/100 L water) and untreated control. Five replicates were used per treatment. Each replicate contained six plants giving a total of 30 plants for each treatment. Seven days after planting bean plants, *P. latus* was released to the plants at about 15 cm in length and two-leaf-stage. The plants were infested with adult females of *P. latus* at a density of 4-5 mites/plant. Seven days after the release, the leaf sampling was started. The population of *P. latus* was evaluated weekly for 4 weeks. At each sampling date, 15 young leaves from the top of the plants were randomly examined for each treatment. All mites present on both sides of the leaves were counted using a stereomicroscope (Leica DM 2500). The spraying was done when the density of *P. latus* motile stages exceeds an average of 4 mites/leaf (Anonymous, 2008) using a hand-operated sprayer for each treatment. Untreated control plants were included. The experiment was conducted in a climate room (25 ± 2°C, 70-80% RH, 16:8 h L:D photoperiod, 12.000 lux, daylight).

Data analysis

The data on ovicidal efficacy was corrected using Abbott's formula (Abbott, 1925). The Kolmogorov-Smirnov and Levene's tests were applied to test normality and homogeneity of variance, respectively. Two-way ANOVA was used for analyzing data sets. Means were compared by letters with Duncan's multiple range test (DMRT). The significance level was set at 0.05 (5%). Minitab 17 statistical package program was used for the statistical analysis.

The data on adulticidal efficacy were also corrected using Abbott's formula (Abbott, 1925). Data normality was tested by the Kolmogorov-Smirnov test. Levene's and Bartlett's tests were applied to test the homogeneity of variances. If the assumption fitted, the variables were analyzed by one-way ANOVA. Minitab 17 software program was used for all calculations and the comparison of the groups in letters was displayed by Tukey's test. The alpha level was also preferred as 0.05 (5%).

Probit analysis was not applied for the ovicidal and adulticidal data because the dead effects of some treatments did not show a distribution between 20% and 80% (Simon, 2014).

The non-continuous data sets of effect against *P. latus* population on bean plants were analyzed with Kruskal Wallis and Ranks were compared by letters with Dunn multiple comparison test. The significance level was set at 5%. The SPSS 24 statistical package program was used for the statistical analysis.

Results

Assessment of ovicidal efficacy

The results of the ovicidal bioassay of the tested solutions were given in Table 2. In some cases, mortality was lower than the control. According to the single solution data, at a concentration of 1%, the ovicidal effect of tobacco leaf extract (T) (28.3%) was greater than the efficacy of garlic bulb extract (G) (-6.9%) and soft soap (S) (-5.0%). However, at a concentration of 2.5%, the toxic effects on *P. latus* eggs of T (39.9%) and G (28.8%) were the not significantly different and the effect of T was higher than the effect of S (15.4%). There were also no significant differences between the effects of G (53.3%) and T (66.2%) at a concentration of 5%. Also, the ovicidal effect of S (31.3%) was lower than the other two extracts at that concentration. S (62.1%), G (75.7%), and T (75.9%) had no significantly different ovicidal effects at 7.5%. Notable, the ovicidal efficacy of S increased to 100% at a concentration of 10%. Additionally, there were no significant differences between its effect and the effect of T (86.1%). Also, the effect of G (84.9%) was not significantly different from T (86.1%) but lower than S (100%).

Table 2. Ovicidal effects of tested solutions on the *Polyphagotarsonemus latus* eggs (0-24 h old)

Dose single (%)	Ovicidal effect of a single solution (%, mean ± SE)			Dose mixture (%)	Ovicidal effect of mixture solution (%, mean ± SE)		Ovicidal effect of abamectin (%, mean ± SE)
	T	G	S		T+S	G+S	
1	28.3 ± 4.9 Ca	*-6.9 ± 2.9 Db	*-5 ± 6.1 Eb	2	*-7.2 ± 4.9 Db	14.8 ± 6.9 Da	*-9.2 ± 2.3 b
2.5	39.9 ± 2.1 Cab	28.8 ± 2.2 Cbc	15.4 ± 2.4 Dc	5	24.6 ± 2.3 Cc	51.1 ± 2.1 Ca	*-9.2 ± 2.3 d
5	66.2 ± 4.5 Bb	53.3 ± 1.4 Bbc	31.2 ± 2.6 Cd	10	41.9 ± 6.1 Bcd	84.1 ± 3.2 Ba	*-9.2 ± 2.3 e
7.5	75.9 ± 3.6 ABab	75.7 ± 1.5 Aab	62.1 ± 2.9 Bbc	15	55.6 ± 3.1 Ac	89.1 ± 3.2 ABa	*-9.2 ± 2.3 d
10	86.1 ± 4.5 Aab	84.9 ± 4.3 Ab	100 ± 0.0 Aa	20	69.0 ± 1.6 Ac	97.8 ± 2.2 Aab	*-9.2 ± 2.3 d

Means followed by the same uppercase or lowercase letters for doses and extracts, respectively, are not significantly different ($P < 0.05$) (SE: Standard Error). T, tobacco; G, garlic; S, soft soap; T+S, tobacco+ soft soap; and G+S: garlic+ soft soap.

* Negative mortality values indicate lower mortality than in the control.

According to the data of binary mixtures of tested extracts with soap (Table 2), the ovicidal effect of the garlic extract+soft soap mixture (G+S) (14.8%) was higher than the effect of tobacco extract+soft soap mixture (T+S) (-7.2%) at 2%. At 5%, the effect of T+S and G+S were significantly different and again the toxic efficacy of G+S (51.1%) was higher than that of T+S (24.6%). Also, the G+S (84.11%) showed significantly greater efficacy than the T+S (41.9%) at 10% as well. At 15%, there were significant differences between the ovicidal effect of T+S (55.6%) and G+S (89.1%). It was clear that the significantly high level of egg mortality was caused by the G+S (97.8%) at 20%. Also, the effect of T+S (69.0%) was lower than the effect of G+S.

In conclusion, it would appear that the ovicidal effects of all tested solutions generally increased with increasing concentration. However, there were no significant differences between the effect of 7.5 and 10% of T. The G extract also had no significantly different ovicidal effects at 7.5 and 10%. However, the ovicidal

effect of S at 10% was the highest in its other concentrations tested. The effects of T+S at 15 and 20% were also not significantly different. Also, the mean effects of 15 and 20% of G+S were not significantly different (Table 2).

A comparison of the effect of the mixture and single solutions revealed that there were no significant differences between the effects of T at 1% and G+S at 2%. T at 2.5% and G+S at 5% did not have significantly different ovicidal effects. The effect of G+S at 10% was the highest in the tested solutions at 5% (single) and 10% (mixture). The ovicidal effects of the T and G at 7.5% and G+S at 15% were not significantly different. There were also no significant differences between the ovicidal effects of S at 10%, T at 10% and G+S at 20%. The effects of these three solutions ranged from 86 to 100% (Table 2).

Additionally, it was observed that unhatched eggs treated with T (10%), G (10%) or G+S (10%, 15%, 20%) lost their original shape, then seemed as if they melted 10 days after treatment (Figure 1).

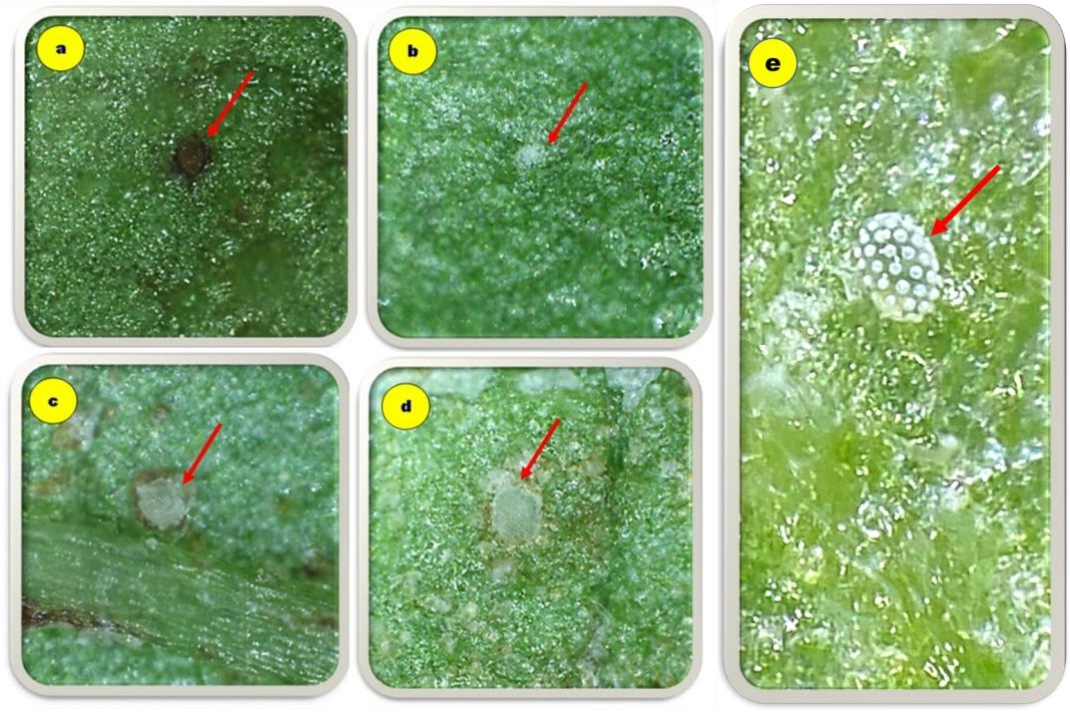

Figure 1. Treated with a) T and b) G at 10%, G+S at c) 10%, d) 20% concentrations ten days after treatment and, e) healthy *Polphagotarsonemus latus* eggs.

Another finding was that the ovicidal efficacy of abamectin was even lower compared with control. Its toxic effect on *P. latus* eggs was the lowest in all treatments (Table 2). However, we observed that young larvae died shortly (0-24 h) after hatching (Figure 2).

Figure 2. Hatched eggs treated with Abamectin and dead neonate larvae of *Polyphagotarsonemus latus* from abamectin sprayed eggs.

Assessment of adulticidal efficacy

The adulticidal bioassay results of tested solutions were given in Table 3. According to the single solution data, 72-h after exposure, there were no significant differences between the adulticidal effects of G at 2.5 and 6.25%, T and S at 1% and 2.5%. However, the effects of G at 12.5% and T at 5%, G at 18.75%, and T at 7.5% were also not significantly different but were lower than the effect of S at 5 and 7.5%. No adult mites exposed to the S at a concentration of 10% were found to survive 72 h after treatment. Also, there were no significant differences between the effects of S at 7.5% and 10% (97.8 and 100%, respectively) and abamectin (100%). However, it was observed that G at 25% had a phytotoxic effect on bean leaf disc.

According to the results for binary mixtures of tested extracts with soap 72-h after exposure, the adulticidal effects of G+S and T+S mixtures at 2%, and also 5, 10 and 15% were not significantly different. The G+S showed significantly greater efficacy than the T+S (66.2%) at 20%. The significantly high level of adult mortality (89.3%) was caused by the G+S at 20% (Table 3).

A comparison of the effect of the mixture and single solutions revealed that there were not any significant differences between the effect of all single and mixture solutions at the lowest two dose levels of each solution. The effect of S (80.2-97.8%) was the highest in the tested single solutions at 5, 7.5, 12.25 and 18.75% and mixture solutions at 10 and 15%. However, the effects of the S (100%) at 10% and G+S (89.3%) at 20% were not significantly different but were higher than the effects of tested other single and mixture solutions at the same concentrations including the 25% of single solutions (Table 3).

It can be also concluded that the adulticidal effects of all tested solutions generally increased with increasing extract concentration. However, there were no significant differences between the effect of 18.7 and 25% of G and also between the effect of T or S at 7.5 and 10% (Table 3).

We also found that all the adult mites died 1 h after application with abamectin. This is an important finding in the understanding of the adulticidal efficacy of abamectin against *P. latus*. Additionally, there were no significant differences between the effect of S at 7.5 and 10% 72-h after exposure and abamectin. The G+S mixture at 20% after 72 h of treatment also had no significantly different adulticidal effect to abamectin (Table 3).

Table 3. Adulticidal effects of the tested solutions against *Polyphagotarsonemus latus* adult females (1-2 days old)

Time (h)	Effects of single solutions (%) (Mean ± SE)					Effects of mixture solutions (%) (Mean ± SE)		
	Dose (%)	G	Dose (%)	S	T	Dose (%)	T+S	G+S
1	2.5	0 ± 0.0 Ca i	1	0 ± 0.0 Da iii	0 ± 0.0 Da ii	2	0 ± 0.0 Da i	2 ± 2.0 Da i
	6.25	2 ± 2.0 Ca ii	2.5	2 ± 2.0 Da ii	4 ± 2.5 Da i	5	2 ± 2.0 Da i	8 ± 3.5 Da ii
	12.25	4 ± 2.5 Cb ii	5	38 ± 4.9 Ca ii	4 ± 2.5 Db ii	10	14 ± 7.5 CDb ii	12 ± 3.8 Db ii
	18.75	28 ± 3.8 Bb ii	7.5	62 ± 8.6 Ba ii	20 ± 5.5 Cb ii	15	26 ± 2.5 Cb ii	32 ± 2.0 Cb ii
	25	24 ± 2.5 Bc iii	10	76 ± 4.0 Ba ii	38 ± 3.8 Bc ii	20	58 ± 4.9 Bb ii	68 ± 3.8 Bab iii
	Abamectin	100 ± 0.0 Aa i	Abamectin	100 ± 0.0 Aa i	100 ± 0.0 Aa i	Abamectin	100 ± 0.0 Aa i	100 ± 0.0 Aa i
24	2.5	2 ± 2.0 Da i	1	6.0 ± 4.0 Ca ii,iii	6 ± 4.0 Ca i,ii	2	4 ± 2.5 Da i	10 ± 3.2 Ca i
	6.25	2 ± 2.0 Da ii	2.5	13.8 ± 6.9 Ca i,ii	8 ± 3.7 Ca i	5	4 ± 4.0 Da i	20 ± 8.4 Ca i,ii
	12.25	16 ± 4.0 Cab i	5	36.4 ± 4.8 Ba ii	8 ± 2.0 Cb i,ii	10	22 ± 7.4 CDab i,ii	22 ± 4.9 Cab iii
	18.75	34 ± 4.0 Bb i,ii	7.5	86.0 ± 5.1 Aa i	40 ± 8.9 Bb i	15	36 ± 5.1 Cb i,ii	46 ± 5.1 Bb i
	25	36 ± 2.5 Bc ii	10	91.8 ± 3.8 Aa i	58 ± 7.4 Bbc i	20	66 ± 8.1 Bb i	80 ± 5.5 Aab ii
	Abamectin	100 ± 0.0 Aa i	Abamectin	100 ± 0.0 Aa i	100 ± 0.0 Aa i	Abamectin	100 ± 0.0 Aa i	100 ± 0.0 Aa i
48	2.5	1.8 ± 3.9 Da i	1	12.4 ± 4.0 Ca i,ii	10 ± 4.5 Ca i,ii	2	8.0 ± 5.8 Da i	14 ± 5.1 Ca i
	6.25	4.0 ± 2.5 Da i,ii	2.5	26.7 ± 7.6 Ca i	10 ± 3.6 Ca i	5	9.8 ± 5.7 Da i	22 ± 10.2 Ca i,ii
	12.25	18.2 ± 1.8 Cb i	5	65.3 ± 3.9 Ba i	10 ± 0.0 Cb i,ii	10	24.2 ± 7.8 CDbi	26 ± 5.1 BCb i,ii
	18.75	36.4 ± 4.7 Bb i,ii	7.5	94.0 ± 2.5 Aa i	44 ± 10.3 Bb i	15	40.9 ± 5.6 Cbi	48 ± 4.9 Bb i
	25	40.7 ± 2.7 Bd i,ii	10	100 ± 0.0 Aa i	62 ± 5.8 Bc i	20	67.1 ± 7.6 Bbci	86 ± 5.1 Aab i,ii
	Abamectin	100 ± 0.0 Aa i	Abamectin	100 ± 0.0 Aa i	100 ± 0.0 Aa i	Abamectin	100 ± 0.0 Aai	100 ± 0.0 Aa i
72	2.5	5.8 ± 5.3 Ca i	1	17.3 ± 5.6 Ca i	12 ± 5.8 Ca i	2	10 ± 5.5 Da i	8.7 ± 7.4 Ca i
	6.25	10.2 ± 3.2 Ca i	2.5	34.5 ± 8.3 Ca i	12 ± 2.0 Ca i	5	12 ± 6.8 Da i	28.7 ± 12.6 BCa i
	12.25	20.2 ± 2.9 Cb i	5	80.2 ± 1.9 Ba i	14 ± 2.5 Cb i	10	29.3 ± 5.3 CDb i	32.2 ± 7.1 BCb i
	18.75	40.4 ± 5.1 Bb i	7.5	97.8 ± 2.2 ABa i	48 ± 9.7 Bb i	15	39.1 ± 7.3 Cb iii	47.6 ± 4.7 Bb i
	25	46.9 ± 2.0 Bc i	10	100 ± 0.0 Aa i	66 ± 5.1 Bbc i	20	66.2 ± 7.9 Bb i	89.3 ± 3.2 Aa i
	Abamectin	100 ± 0.0 Aa i	Abamectin	100 ± 0.0 Aa i	100 ± 0.0 Aa i	Abamectin	100 ± 0.0 Aa i	100 ± 0.0 Aa i

Means followed by the same uppercase and lowercase letters or Roman numerals for doses, extracts and times, respectively, are not significantly different ($P < 0.05$). T, tobacco; G, garlic; S, soft soap; T+S, tobacco+ soft soap; and G+S: garlic+ soft soap.

Assessment of acaricidal efficacy to control of *Polyphagotarsonemus latus* on barbunia bean plants

For four weeks, the acaricidal effects of G, T, S, T+S and G+S were also investigated against the *P. latus* population on barbunia bean plants (Table 4). In the S treatment plants, mite density exceeded the ET in the first (10 mites/leaf) and third week (9.5 mites/leaf) of the experiment. After spraying in those weeks, the population was maintained below damaging levels for 1 week. During the application period, the highest weekly mean number of *P. latus* in the S treatment group was 9.5 mites/leaf.

Table 4. Densities of *Polyphagotarsonemus latus* active stages (mean ± SE) on bean plants during the 4-week experimental period

Treatment	Mean mite (all motile stages) / leaf ± SE											
	Week 1*			Week 2			Week 3			Week 4		
	Mean ± SE	Median	Rank	Mean ± SE	Median	Rank	Mean ± SE	Median	Rank	Mean ± SE	Median	Rank
Untreated Control	6.9 ± 1.3	5	39.9	56.4 ± 6.7 a	57	91.3	128 ± 27 a	45	93.3	47.7 ± 5.6 a	9	94.0
Abamectin	12.1 ± 1.9	11	62.3	0.0 ± 0.0 b	0	12.5	0.0 ± 0.0 b	0	8.0	0.5 ± 0.2 b	0	25.0
S	10.0 ± 1.6	9	55.0	0.9 ± 0.3 bc	1	24.0	9.5 ± 2.1 bc	1	31.5	2.6 ± 0.7 b	0	46.5
G	9.0 ± 1.7	12	48.9	10.8 ± 2.0 cd	8	55.0	19.5 ± 2.7 cd	10	47.5	26.7 ± 5.5 ac	3	83.2
T	10.0 ± 1.7	9	54.9	26.4 ± 3.4 ad	28	74.7	19.3 ± 2.9 cd	16	47.6	1.3 ± 0.6 b	0	31.4
T+S	12.5 ± 2.2	9	63.5	39.7 ± 10.7 ad	25	76.9	47.0 ± 12.6 ac	30	64.1	4.3 ± 1.1 bc	4	49.9
G+S	8.4 ± 1.6	7	46.5	3.6 ± 1.4 bc	2	36.7	65.6 ± 10.3 ad	52	79.0	2.9 ± 1.1 b	1	40.9
P-value	0.314			0.000			0.000			0.000		

Means followed by the same letters within columns are not significantly different (P<0.05).

***** Mite densities before the spraying. T, tobacco; G, garlic; S, soft soap; T+S, tobacco+ soft soap; and G+S: garlic+ soft soap.

The G applications were not able to maintain the mite density below the ET. The mite density was always above the ET despite the three times of spraying during the experiment. In this treatment group, the highest mite level was 26.7 mites/leaf.

The T and T+S applications were also not able to maintain the mite density below the ET except the last week of the experiment. However, we observed that both of them had a phytotoxic effect on bean plants. We think that the decrease in mite density is due to the deterioration of plant quality as a result of the phytotoxic effect. The highest mite levels were 47.0 mites/leaf in the T treatment group and 26.4 mites/leaf in the T+S treatment group during the experiment.

In the G+S treatment plants, the mite density exceeded the ET two times (week 1 and 3). G+S treatments were able to keep the population below the ET for 1 week after application. The highest population density (65.0 mites/leaf) was detected on the third week of the experiment.

In the unsprayed control plants, the mite population was above the ET during the entire experiment. In this treatment group, the highest mite level was 128 mites/leaf.

However, in the sprayed control plants, the mite population was above the ET just in the first week of the experiment (pre-infestation period). After spraying with abamectin at that time, the population was maintained below the ET during the remaining experimental period. The highest mite level was 0.5 mites/leaf in this treatment group during the experiment after the pre-infestation period.

It was also clear in the results that the *P. latus* egg population followed almost the same pattern of variation as the motile stage population during the experimental period (Table 5).

Table 5. The densities of *Polyphagotarsonemus latus* eggs (mean ± SE) on bean plants during the 4- week experimental period

Treatment	Mean mite (egg stage)/leaf ± SE											
	Week 1*	Week 2			Week 3			Week 4				
	Mean ± SE	Median	Rank	Mean ± SE	Median	Rank	Mean ± SE	Median	Rank	Mean ± SE	Median	Rank
Untreated Control	13.6 ± 2.9 a	11	35.3	150 ± 23 a	183	94.0	214 ± 31 a	226	92.4	42.6 ± 10.2 a	21	90.4
Abamectin	19.8 ± 3.3 ab	19	51.4	0.0 ± 0.0 b	0	17.5	0.0 ± 0.0 b	0	12.5	1.0 ± 0.5 b	0	38.1
S	25.5 ± 4.5 ab	23	59.6	1.1 ± 0.5 b	0	25.9	11.0 ± 4.3 bc	4	38.2	2.8 ± 0.7 bc	2	56.9
G	14.7 ± 3.4 a	11	37.6	16.8 ± 3.2 cd	14	59.8	27.0 ± 5.8 acd	19	59.9	24.4 ± 6.6 ac	12	85.2
T	19.8 ± 2.4 ab	19	53.6	20.1 ± 3.4 ac	18	63.5	8.2 ± 1.9 bd	5	40.9	0.2 ± 0.2 b	0	26.7
T+S	36.4 ± 5.6 b	33	75.8	68.8 ± 15.8 ac	46	82.0	41.6 ± 18.7 bce	8	47.5	0.2 ± 0.2 b	0	28.2
G+S	23.4 ± 3.8 ab	20	57.8	2.0 ± 1.2 bd	0	28.3	102 ± 19 ae	71	79.6	4.2 ± 1.9 b	0	45.5
P-value	0.314			0.000			0.000			0.000		

Means followed by the same letters within columns are not significantly different ($P < 0.05$),

*****Mite densities before the spraying. T, tobacco; G, garlic; S, soft soap; T+S, tobacco+ soft soap; and G+S: garlic+ soft soap.

Discussion

About a hundred species of plants have been determined to have the potential to suppress phytophagous mites (Peng-ying et al., 2011). Among these, *N. tabacum* (Almansour & Akbar, 2013) and *A. sativum (*Attia et al., 2012) are well known for their acaricidal properties. Soft-bodied mites including the two-spotted spider mite are susceptible to soap applications as well (Cloyd, 2018).

However, to our knowledge, no study has tested the G, T, S, and their mixtures on *P. latus* eggs. Also, only a few studies have demonstrated their ovicidal effect on mites. Among them, the effect of aqueous extracts of *N. tabacum* on *T. urticae* eggs was researched by Almansour & Akbar (2013). The mortality rate of eggs ranged between 16.2-90.0% at 0-100%. They also showed that the LC_{50} value of tobacco extract against the egg stages of *T. urticae* was 0.25 g/100 ml. A recent study by Akyazi et al. (2018) declared that the LC_{50} values of S+T mixture, S, S+G mixture, G and T extracts were 12.5, 7.4, 5.2%, 3.8%, 3.4%, respectively for *T. urticae* eggs. The same solutions had the LC_{90} values ranging from 6.9 to 38.3%.

Also, to our knowledge, no prior study has also examined the effect of T+S and G+S mixtures on *P. latus* eggs. However, it should be aware that the potential effect in the mixture may be less (antagonistic) or greater (synergistic) than that of individual substances (Larsen et al., 2003). This finding is consistent with Akyazi et al. (2018) showing that the ovicidal efficiency of soft soap on *T. urticae* can be increased by the addition of the garlic extract. Until the current study, a still-unanswered question is whether these mixtures have the same effect on *P. latus.* In agreement with Akyazi et al. (2018), our result also showed that the toxicity of garlic extract on *P. latus* egg can be generally increased by the addition of soft soap. However, the tobacco showed a lower toxic effect when mixed with the soft soap.

To our knowledge, no study has yielded the ovicidal efficacy of abamectin on *P. latus* eggs. In the current study, abamectin has been reported as having extremely low toxicity against the egg stage of *P. latus*. However, we observed that young larvae died shortly after hatching (Figure 2). This effect has been evaluated as an indirect ovicidal effect by Ismail et al. (2007). Based on this, it can be concluded that abamectin has indirect ovicidal properties against *P. latus* under laboratory conditions. Previous studies have almost exclusively focused on *T. urticae*. For example, Kumar & Singh (2004) reported it did not have any ovicidal

effect on *T. urticae* eggs. Consistent with the results of our study, Ismail et al. (2007) found the abamectin effectively lacked of ovicidal action and had a clear toxic effect against newly hatched larva of *T. urticae.*

Only a few studies have shown the adulticidal effects of tested solutions on plant pest mites. Also, previous studies have almost exclusively focused on *T. urticae.* Among examples of previous research, the adulticidal effect of aqueous extract of tobacco was investigated by Almansour & Akbar (2013). They found that the adult mortality rate was about 82% at the highest dose tested. LC_{50} values of the same treatment against the adult stage of *T. urticae* was 0.12 g/100 ml. Hincapie et al. (2008) declared that the mortality rate of water extract of garlic bulb on *T. urticae* adult females was less than 40%. They used the leaf disc-dipping method. The toxic effect of the garlic steam distillate on the adult females of *T. urticae* was investigated by Attia et al. (2012). They found that the LC_{50} and LC_{90} values of garlic were 7.49 and 13.5 mg/L, respectively. It was also reported that the garlic application caused significant mortality at low concentrations. Erdogan et al. (2012) also found that the efficiency of the ethanol extract of garlic against *T. urticae* adults on bean leaf disc at 12% was 39.5%. Nour El-Deen & Abdallah (2013) tested the adulticidal effect of garlic seed extract against *T. urticae* using the leaf dip method under laboratory conditions. They found that the LC_{50} value of garlic was 8.4 ml/L while the LC_{90} value was 1678.3 ml/L. Akyazi et al. (2018) investigated the effectiveness of the soft soap, garlic bulb, tobacco leaf extracts and their mixtures against the adult females of *T. urticae.* It was found that the garlic extract had the lowest toxic effect in the tested solutions. However, its mixtures with soft soap and tobacco extract gave higher toxic effects in combination. They also found that the LC_{50} and LC_{90} values of the same treatments ranged between 0.07-9.35 and 0.28-40.4%, respectively against *T. urticae* adults. Our results showed that the toxicity of garlic extract on *P. latus* adults can be increased by the addition of the soft soap only at its concentration of 10%. The adulticidal effect of soft soap did not change when mixed with the garlic extract in combinations at that concentration. The adulticidal effect of tobacco extract generally did not change when mixed with the soap in combinations. However, the soft soap showed a lower toxic effect when mixed with the tobacco extract at 5, 7.5 and 10%. A literature review revealed that no study to date has examined the efficacy of tested solutions on *P. latus* adults.

To our knowledge, no study has also yielded the adulticidal efficacy of abamectin against *P. latus* on bean leaf discs. In the current study, abamectin has been reported as having high toxicity against the adult stage of *P. latus*. After a 1 h exposure to abamectin, no mites were alive. Also, the effect of S at 7.5 and 10% and the G+S at 10% after 72 h of treatment had no significantly different adulticidal effect than abamectin. On this basis, we conclude that especially the S and the G+S mixture have the potential in the control *P. latus* adults.

According to the data on acaricidal efficacy on bean plants, both S and G+S treatments were able to keep the population below the ET for 1 week after each application. Although the mite density increased to 65.6 mites/leaf 1 week after the G+S application, a single G+S application at that time was able to reduce the mite density by 2.9 mites/leaf. However, the G applications were not able to maintain the mite density on bean plants below the ET. T and T+S had phytotoxic effects on bean plants. So, the main conclusion that can also be drawn is that especially S but also G+S are promising for controlling *P. latus* on the bean plants. Many studies have shown that some plant extracts have a toxic effect on *P. latus* (Ponte, 1996; Palaniswamy & Ragini, 2000; Al-Ani et al., 2008; Venzon et al., 2008; Mari et al., 2013; Hossain et al., 2013; Hasyim et al., 2017). There are also reports on the effectiveness of tobacco on different mite species (McIndoo, 1943; Madanlar et al., 2000; Almansour & Akbar, 2013; Akyazi et al., 2018). However, a literature review revealed that garlic has also acaricidal properties on some mite species (Hincapie et al., 2008; Attia et al., 2012; Nour El-Deen & Abdallah, 2013; Akyazi et al., 2018, 2019). Also, a series of studies such as Madanlar et al. (2000, 2002), Koçar et al. (2003), Güncan et al. (2006), Çobanoğlu & Alzoubi (2013), Skorupska (2013), Akyazi et al. (2018, 2019) have indicated soft soap toxicity to several mite species. However, to date, the influences of garlic, tobacco, and soap against *P. latus* on bean plants have been

insufficiently examined. Most early studies, as well as current work, have almost exclusively focused on the influences of garlic, tobacco and soap against *P. latus* on different plants especially pepper. For example, the effects of tobacco extract on chili (Uraisakul, 2003; Mari et al., 2013), garlic extract on jute (Hossain et al., 2013) and soft soap on chili (David et al., 2009) against *P. latus* were researched. In a recent study by Akyazı et al. (2019), the effectiveness of soft soap, garlic and tobacco extracts in the control of *P. latus* was investigated on tea plants under field conditions. It is known that the different plant species have different leaf morphology. Also, leaf morphology may affect spray deposition (de Ruiter et al., 1990; Himel et al., 1990; Smith et al., 2000). So, before this study, a still-unanswered question was the potential of G, S and T against *P. latus* on a bean plant.

Also, as far as we know, no study to date has also examined the effect of their mixtures on the *P. latus* population on bean plants. As mentioned before, the interactions may result in either a weaker or stronger combined effect than the toxicity and mode of action of each compound (Larsen et al., 2003). To fill this literature gap, this paper also identified the effect of T+S and G+S mixtures on the *P. latus* population on bean plants. The result showed that the toxicity of garlic extract against *P. latus* on bean plants can be increased by the addition of soft soap. Given that the G applications were not able to maintain the mite density below the ET. The mite density was always above the ET despite the three times spraying during the experiment. In the S and G+S treated plants, the mite density exceeded the ET two times. Both of these treatments were able to keep the population below the ET for 1 week after each application on these dates. However, the effect of tobacco extract against *P. latus* on bean plants did not generally change when mixed with the soap in combinations. However, the effectiveness of soap alone was higher than its effectiveness in the mixture with tobacco. Also, the T+S at 20% had a phytotoxic effect on bean plants.

Our results cast a new light on the high efficacy of abamectin against *P. latus* on bean plants. A single abamectin application after the pre-infestation period maintained the mite population below the economic threshold during the experiment. Also, to our knowledge, no study has yielded the effect of abamectin on the *P. latus* population on bean plants. However, Goldsmith & James (2002), Montasser et al. (2011), and Singh & Singh (2013) reported that the abamectin gave satisfactory control against this mite on pepper plants.

We need to consider which ingredients are responsible for the toxic effects of the solutions tested in the current study. Consistent with Singh et al. (2001), the toxic effect that has been attributed to garlic are thought to be related to the high organosulfur substances content. Also, the water extracts of tobacco have been used for garden insect control as early as 1690. Consistent with previous studies (George et al., 2000; Ntalli & Menkissoglu-Spiroudi, 2011), the nicotine in tobacco can have a toxic effect. Soaps are prepared from animal fats or vegetable oils and sodium or potassium salts of fatty acids. So, it is assumed that they may kill mites in different ways (Cloyd, 2018). Abamectin has both acaricidal and insecticidal properties (Lewis et al., 2016). The toxic effect of abamectin may be attributed to its binding effect to the glutamate-gated chloride channels causing hyperpolarization of the nerve and muscles cell membrane (Montasser et al., 2011). The affected pest becomes paralyzed, stops feeding, and dies after a few days (El-Ashry & Ramadan, 2021).

In summary, this study has shown that especially soft soap (10%) but also garlic+soft soap mixture (20%) may be non-toxic alternative control options to current pesticides used to control *P. latus.* However, further work is required to determine the efficiency of these natural products under greenhouse and field conditions. Another area for future research would be to investigate the effect of the same solutions on natural enemies for pest mites.

Acknowledgments

The authors would like to thank the anonymous reviewers for their deep, thorough review and constructive comments.

References

Abbott, W. S., 1925. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology, 18 (2): 265-267.

Aktar, W., D. Sengupta & A. Chowdhury, 2009. Impact of pesticides use in agriculture: their benefits and hazards. Interdisciplinary Toxicology, 2 (1): 1-12.

Akyazi, R., R. Sekban, M. Soysal, D. Akyol, J. Colee & S. Z. Bostan, 2019. Ecofriendly control approaches for *Polyphagotarsonemus latus* (Acari: Tarsonemidae) on tea (*Camellia sinensis* L.). International Journal of Acarology, 45 (1-2): 79-89.

Akyazi, R., M. Soysal, E. Y. Altunç, A. Lisle, E. Hassan & D. Akyol, 2018. Acaricidal and sublethal effects of tobacco leaf and garlic bulb extract and soft soap on *Tetranychus urticae* Koch (Acari: Trombidiformes: Tetranychidae). Systematic & Applied Acarology, 23 (10): 2054-2070.

Al-Ani, N., L. K. Al-Ani & A. Gatta, 2008. Effect of China berry crude extracts on broad mites in vivo and in vitro. Journal of Biotechnology Research Center, 2 (1): 12-18.

Almansour, N. A. & M. M. Akbar, 2013. The effect of some plant extracts in the biology of *Tetranychus urticae* (Acarina: Tetranychidae). Journal of Purity, Utility Reaction and Environment, 2 (6): 153-159.

Anonymous, 2008. Agricultural Control Technical Instructions. No:3, 332 pp.

Attia, S., K. L. Grissa, A. C. Mailleux, G. Lognay, S. Heuskin, S. Mayoufi & T. Hance, 2012. Effective concentrations of garlic distillate (*Allium sativum*) for the control of *Tetranychus urticae* (Tetranychidae). Journal of Applied Entomology, 136 (4): 302-312.

Cloyd, R. A., 2016. What impacts the effectiveness of translaminar pesticides? GrowerTalks - Pest Management, p.4 [online]. (Web page: https://www.growertalks.com/Article/?articleid=22487) (Date accessed: November 2021).

Cloyd, R. A., 2018. “Soaps” and detergents: should they be used on roses? Nashville Rose Society Newsletter Article, p.4 (Web page: https://www.rose.org/single-post/2018/03/20/-soaps-and-detergents-should-they-be-used-on-roses) (Date accessed: November 2021).

Çobanoğlu, S. & S. Alzoubi, 2013. Effects of soft soap and abamectin on the two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) and predatory mite *Phytoseiulus persimilis* A-H (Acari: Phytoseiidae) under laboratory conditions. Turkish Journal of Entomology, 37 (1): 31-38.

David, P. M. M., K. Rajkumar, K. Elanchezhyan, T. A. Razak, S. J. Nelson, P. Nainar & R. K. Muralibaskaran, 2009. Effect of soft soaps on broad mite, *Polyphagotarsonemus latus* (Banks) on chilli. Karnataka Journal of Agricultural Sciences, 22 (3): 554-556.

de Ruiter, H. D., A. J. M. Uffing, E. Meinen & A. Prins, 1990. Influence of surfactants and plant species on leaf deposition of spray solutions. Weed Science, 38 (6): 567-572.

de Silva, W., G. Manuweera & S. Karunaratne, 2008. Insecticidal activity of *Euphorbia antiquorum* L. latex and its preliminary chemical analysis. Journal of the National Science Foundation of Sri Lanka, 36 (1): 15-23.

El-Ashry, R. & M. Ramadan, 2021. In Vitro Compatibility and combined efficacy of entomopathogenic nematodes with abamectin and imidacloprid against the white grub, *Pentodon bispinosus* Kust. Toxicology & Pest Control (EAJBSF), 13 (1): 95-114.

Erdogan, P., A. Yildirim & B. Sever, 2012. Investigations on the effects of five different plant extracts on the two-spotted mite *Tetranychus urticae* Koch (Arachnida: Tetranychidae). Psyche: A Journal of Entomology 2012 (Article ID 125284): 5.

George, J., H. P. Bais & G. A. Ravishankar, 2000. Biotechnological production of plant-based insecticides. Critical Reviews in Biotechnology, 20 (1): 49-77.

Gerson, U., 1992. Biology and control of the broad mite, *Polyphagotarsonemus latus* (Banks) (Acari: Tarsonemidae). Experimental & Applied Acarology, 13 (3): 163-178.

Goldsmith, J. & O. James, 2002. Chemical control of the broad mite (*Polyphagotarsonemus latus* Banks) on hot pepper. The Bulletin of the Jamaican Society for Agricultural Sciences 14 (1): 105-109.

Grinberg, M., R. Perl-Treves, E. Palevsky, I. Shome & V. Soroker, 2005. Interaction between cucumber plants and the broad mite, *Polyphagotarsonemus latus* from damage to defense gene expression. Entomologia Experimentalis et Applicata, 115 (1): 135-144.

Güncan, A., N. Madanlar, Z. Yoldaş, F. Ersin & Y. Tüzel, 2006. Pest status of organic cucumber production under greenhouse conditions in İzmir (Turkey). Turkish Journal of Entomology, 30 (3): 183-193.

Hasyim, A., W. Setiawati, L. Marhaeni, L. Lukman & A. Hudayya, 2017. Bioaktivitas Enam Ekstrak Tumbuhan untuk Pengendalian Hama Tungau Kuning Cabai *Polyphagotarsonemus latus* Banks (Acari: Tarsonemidae) di Laboratorium. Jurnal Hortikultura, 27 (2): 217-230 (in Indonesian).

Himel, C. M., H. Loats & G. W. Bailey, 1990. "Pesticide Sources to the Soil and Principles of Spray Physics, 7-50" In: Pesticides in the Soil Environment: Processes, Impacts, and Modeling (Ed. H. H. Cheng). Madison, Wisconsin, USA, 530 pp.

Hincapie, C. A., G. E. Lopez & Y. R. Torres, 2008. Comparison and characterization of Garlic (*Allium sativum* L.) bulbs extracts and their effects on mortality and repellency of *Tetranychus urticae* Koch (Acari: Tetranychidae). Chilean Journal of Agricultural Research, 68 (4): 317-327.

Hossain, M. D., S. Yasmin, M. A. Latif & N. Akhter, 2013. Effect of neem (*Azadirachta indica*) and other plant extracts on yellow mite of jute. International Journal of Stress Management, 4 (3): 412-417.

Ismail, M. S. M., M. F. M. Soliman, M. H. El Naggar & M. M. Ghallab, 2007. Acaricidal activity of spinosad and abamectin against two-spotted spider mites. Experimental & Applied Acarology, 43 (2): 129-135.

Koçar, G., A. Gül, N. Madanlar, Z. Yoldaş & E. Durmuşoğlu, 2003. Effects of using natural pesticides against pests on yield and fruit quality of cucumbers grown under greenhouse conditions. Ege Üniversitesi Ziraat Fakültesi Dergisi, 40 (1): 33-40.

Kousik, C. S., B. M. Shepard, R. Hassell, A. Levi & A. M. Simmons, 2007. Potential Sources of Resistance to Broad Mites (*Polyphagotarsonemus latus*) in Watermelon Germplasm. Hortscience, 42 (7): 1539-1544.

Kumar S. & R. N. Singh, 2004. Ovicidal action of certain pesticides against eggs of two-spotted mite, *Tetranychus urticae* Koch under laboratory condition. Resistant Pest Management Newsletter, 14 (1): 8-10.

Larsen, J. C., M. L. Binderup, M. Dalgaard, L. O. Dragsted, A. Hossaini, O. Ladefoged, H. R. Lam, C. Madsen, O. Meyer, E. S. Rasmussen, T. K. Reffstrup, I. Soborg, A. M. Vinggaard & G. Ostergard, 2003. Combined Actions and Interactions of Chemicals in Mixtures, the Toxicological Effects of Exposure to Mixtures of Industrial and Environmental Chemicals. Danish Veterinary and Food Administration, Fødevare Rapport, 158 pp.

Lewis, K. A., J. Tzilivakis, D. Warner & A. Green, 2016. An international database for pesticide risk assessments and management. Ecological Risk Assessment: An International Journal, 22 (4): 1050-1064.

Madanlar, N., Z. Yoldaş & E. Durmuşoğlu, 2000. Laboratory investigations on some natural pesticides for use against pests in vegetable greenhouses. Integrated Control in Protected Crops, Mediterranean Climate. IOBC/WPRS Bulletin, 23 (1): 281-288.

Madanlar, N., Z. Yoldaş & E. Durmuşoğlu, A. Gül, 2002. Investigations on the natural pesticides against pests in vegetable greenhouses in İzmir (Turkey). Turkish Journal of Entomology, 26 (3): 181-195 (in Turkish with an abstract in English).

Mari, J. M., R. B. Laghri, A. S. Mari & A. K. Shahzadi, 2013. Eco-friendly pest management of chili crop. International Journal of Agricultural Technology, 9 (7): 1981-1992.

McIndoo, N. E., 1943. Insecticidal Uses of Nicotine and Tobacco; a Condensed Summary of the Literature, 1690-1934. Physical Description, 16 pp.

Montasser, A. A., A. M. Taha, A. R. I. Hanafy & G. M. Hassan, 2011. Biology and control of the broad mite *Polyphagotarsonemus latus* (Banks, 1904) (Acari: Tarsonemidae). International Journal of Environmental Science and Engineering (IJESE), 1: 26-34.

Nour El-Deen, M. E. M. & A. A. M. Abdallah, 2013. Effect of different compounds against *Tetranychus urticae* Koch and its predatory mite *Phytoseiulus persimilis* A.H. under laboratory conditions. Journal of Applied Sciences Research, 9 (6): 3965-3973.

Ntalli, N. G. & U. Menkissoglu-Spiroudi, 2011. "Pesticides of Botanical Origin: A Promising Tool in Plant Protection, 3-24". In: Pesticides - Formulations, Effects, Fate *(*Ed. M. Stoytcheva). InTech, Croatia, 824 pp.

Olkowski, W., S. Daar & H. Olkowski, 1993. Common-Sense Pest Control: Least Toxic Solutions for Your Home, Garden, Pets and Community. Taunton Press, Newtown, Connecticut, USA, 736 pp.

Palaniswamy, S. & J. C. Ragini, 2000. Influence of certain plant extracts on yellow mite *Polyphagotarsonemus latus* (Banks) on chillies. Insect Environment, 6 (1): 25-26.

Pavela, R., 2016. History, presence and perspective of using plant extracts as commercial botanical insecticides and farm products for protection against insects - a Review. Plant Protection Science, 52 (4): 229-241.

Peng-ying, M. E. I, P. Chong, Z. Yong-qiang & D. Wei, 2011. Research and application of botanical acaricides. African Journal of Agricultural Research, 6 (12): 2634-2637.

Ponte, J. J. da, 1996. Efficiency of manipueira for the control of papaya white mite (preliminary note). Revista de Agricultura (Piracicaba), 71 (2): 259-261.

Rodriguez, I. V., M. C. N. Cristina, M. O. Valencia & O. Julian, 2017. Population parameters and damage of *Polyphagotarsonemus latus* (Acari: Tarsonemidae) in Valencia orange (*Citrus sinensis* L. Osbeck) crop. Acta Agronómica, 66 (4): 633-640.

Rogers, M. E., P. A. Stansly, C. C. Childers, C. W. Mccoy & H. N. Nigg, 2010. Florida Citrus Pest Management Guide: Rust Mites, Spider Mites, and Other Phytophagous Mites. New York, USA, University of Florida. IFAS Extension, 603 pp.

Siddique, S., Q. Syed, A. Adnan, M. Nadeem, M. Irfan & F. A. Qureshi, 2013. Production of avermectin B1b from *Streptomyces avermitilis* 41445 by batch submerged fermentation. Jundishapur. Journal of Microbiology, 6 (8): e7198.

Simon, Y. J., 2014. The Toxicology and Biochemistry of Insecticides. CRC Press, London, UK, 276 pp.

Singh, U. P., B. Prithiviraj, B. K. Sarma, M. Singh & A. B. Ray, 2001. Role of garlic (*Allium sativum* L.) in human and plant diseases. Indian Journal of Experimental Biology, 39 (4): 310-322.

Singh, A. P. & R. N. Singh, 2013. Management of yellow mite, *Polyphagotarsonemus latus* (Acari: Tarsonemidae) in chili. The Indian Journal of Agricultural Sciences, 83 (11): 1250-1252.

Skorupska, A., 2013. The possibilities of reduction of the two-spotted spider mite (*Tetranychus urticae* Koch) population with aqueous extracts of selected plant species. Progress in Plant Protection, 49 (1): 383-386.

Smith, D. B., S. D. Askew, W. H. Morris & M. Boyette, 2000. Droplet size and leaf morphology effects on pesticide spray deposition. Trans ASAE (American Society of Agricultural Engineers), 43 (2): 255-259.

Tuncer, C., 2001. Üç farklı bilgisayar programı (POLO, SPSS ve PROBIT ANALYSIS) ile probit analizinin uygulanması ve yorumu. Ondokuz Mayıs Üniversitesi Ziraat Fakültesi Dergisi,16 (3): 63-77(in Turkish with an abstract in English).

Uraisakul, K., 2003. "Annona seed extract and some herb extracts on chili yield and control broad mite (*Polyphagotarsonemus latus* (Bank)) and some key pests in chilli, 354-361". Proceedings of 41st Kasetsart University Annual Conference (3-7 February 2003, Kasetsart University, Thailand), 760 pp.

Venzon, M., M. C. Rosado, A. J. Molina-Rugama, V. S. Duarte, R. Dias & A. Pallini, 2008. Acaricidal efficacy of neem against *Polyphagotarsonemus latus (*Banks) (Acari: Tarsonemidae). Crop Protection, 27 (3/5): 869-872.

Zalom, F. G. & F. J. S. C. Irigaray, 2010. "Integrating Pesticides and Biocontrol of Mites in Agricultural Systems, 471-476". In: Trends in Acarology (Eds. M. Sabelis & J. Bruin). Springer, Dordrecht, the Netherlands, 566 pp.

Türk. entomol. derg., 2022, 46 (2): 227-237
DOI: http://dx.doi.org/10.16970/entoted.1017243

ISSN 1010-6960
E-ISSN 2536-491X

Original article (Orijinal araştırma)

Local isolate of *Bacillus thuringiensis* (Berliner, 1915) (Bacteria: Bacillaceae) from *Cydalima perspectalis* (Walker, 1859) (Lepidoptera: Crambidae: Spilomelinae) includes cry1, cry3 and cry4 genes and their insecticidal activities

Cydalima perspectalis (Walker, 1859) (Lepidoptera: Crambidae: Spilomelinae)'ten izole edilen cry1, cry3 ve cry4 genlerini içeren yerel *Bacillus thuringiensis* (Berliner, 1915) (Bacteria: Bacillaceae) bakterisine ait insektisidal aktivite

Mehtap USTA[1*]

Abstract

Cydalima perspectalis (Walker, 1859) (Lepidoptera: Crambidae: Spilomelinae) is a primary pest on boxwood. The larvae of this pest cause damage by eating the leaves. They prevent the plant from performing photosynthesis, gnaw the bark of the shoots, damage the cambium layer and thus dry the boxwood shoots and branches. The boxwood moth, which is an exotic species in Türkiye, was first found in gardens and parks in Istanbul in 2011 and subsequently in Artvin and Düzce in 2015, and Bartın in 2016. The aim of this study is to develop a control method for this pest by considering the damage of the species. In this context, bacteria were isolated from the larvae collected from Artvin in May 2021 and bacteria screening was conducted to obtain an effective isolate. At the end of this isolation, *Bacillus thuringiensis* (Berliner, 1915) (Bacteria: Bacillaceae) was found. When cry gene analysis of this bacterium was performed, it was determined that it contained cry1, cry3 and cry4 genes. The insecticidal activity of this bacterium was tested on *C. perspectalis* and a mortality rate of 85% was obtained.

Keywords: *Bacillus thuringiensis,* biological control, cry genes, *Cydalima perspectalis,* microbiology

Öz

Cydalima perspectalis (Walker, 1859) (Lepidoptera: Crambidae: Spilomelinae) şimşir üzerinde birincil zararlıdır. Bu zararlının larvaları yaprakları yiyerek zarar verir. Bitkinin fotosentez yapmasını engeller, sürgünlerin kabuğunu kemirir, kambiyum tabakasına zarar verir ve böylece şimşir sürgünlerini ve dallarını kuruturlar. Türkiye'de egzotik bir tür olan şimşir güvesi ilk olarak 2011 yılında İstanbul'da bahçe ve parklarda bulunmuş ve daha sonra; 2015 yılında Artvin ve Düzce'de, 2016 yılında Bartın'da yapılmıştır. Bu çalışmanın amacı, türün zararını göz önünde bulundurarak bu zararlı için bir kontrol yöntemi geliştirmektir. Bu kapsamda Mayıs 2021'de Artvin ilinden toplanan larvalardan bakteri izolasyonu yapılmış ve etkili bir izolat elde etmek için bakteri taraması yapılmıştır. Bu izolasyon sonunda *Bacillus thuringiensis* (Berliner, 1915) (Bacteria: Bacillaceae) bulunmuştur. Bu bakterinin cry gen analizi yapıldığında cry1, cry3 ve cry4 genlerini içerdiği belirlendi. Bu bakterinin böcek öldürücü aktivitesi *C. perspectalis* üzerinde test edilmiş ve %85'lik bir ölüm oranı elde edilmiştir.

Anahtar sözcükler: *Bacillus thuringiensis*, biyolojik mücadele, cry genleri, *Cydalima perspectalis,* mikrobiyoloji

[1] Trabzon University, Tonya Vocational School, Health and Care Program, 61500, Tonya, Trabzon, Türkiye
* Corresponding author (Sorumlu yazar) e-mail: mehtapyakupoglu@trabzon.edu.tr
Received (Alınış): 04.11.2021 Accepted (Kabul ediliş): 29.06.2022 Published Online (Çevrimiçi Yayın Tarihi): 30.06.2022

Introduction

Cydalima perspectalis (Walker, 1859) (Lepidoptera: Crambidae: Spilomelinae) originates from East Asia (China, Japan and Korea) and is an exotic species in Türkiye (Öztürk et al., 2016). It was first found in Europe in 2007 in Germany. Its presence and damage have been determined in southern Russia (Mally & Nuss, 2010; Wan et al., 2014; Santi et al., 2015) and it was reported that can cause damage to Buxus sempervirens L. (Buxales: Buxaceae) in Georgia in 2015 (Matsiakh et al., 2016). In Türkiye, it was observed to cause damage in gardens and parks in Sarıyer, Istanbul in 2011 (Hizal, 2012). Than It was observed in Düzce (Öztürk et al., 2016) and Artvin (Anonymous, 2011) in 2015, and in Bartın in 2016 (Toper Kaygın & Taşdeler, 2018; Yıldız et al., 2018). It was also reported to have caused intense damage in Artvin in 2016-2017 (Göktürk, 2017). *Cydalima perspectalis* feeds on *B. sempervirens* and *Buxus microphylla* (Sieb. & Zucc.,1965) (Buxales: Buxaceae) in Europe (Leuthardt et al., 2010). The pest has also been reported to feed on *Buxus sinica* (Rehder & E.H.Wilson, 1959) (Buxales: Buxaceae) (Wan et al., 2014). Also, *Euonymus japonicus* Thunb. (Linne, 1880) (Celastrales: Celastraceae) and *Ilex purpurea* Hassk. (Sims, 1819) (Aquifoliales: Aquifoliaceae) are host plants in Asia (Muus et al., 2009). *Buxus sempervirens* occurs in Artvin, Rize, Trabzon, Osmaniye, Kastamonu, Bolu, Karabük, Denizli, Kocaeli, Hatay and Kahramanmaraş Provinces of Türkiye; *Buxus balearica* Lam. (Buxales: Buxaceae) occurs naturally in Adana, Antalya and Hatay (Anonymous, 2021a, b), and is also grown as an ornamental in parks and gardens in many provinces.

Damage caused by *C. perspectalis* in *B. sempervirens* has been reported in Türkiye (Toper Kaygın & Taşdeler, 2018). Boxwood blight disease caused by *Cylindrocladium buxicola* Henricot (Hypocreales: Nectriaceae) (Henricot & Culham, 2002) has previously been reported on boxwood in areas where damage appears. This pathogen was detected in Artvin and Trabzon Provinces for the first time in 2011. Destructive effects have been reported in the infested regions. About 90% defoliation of boxwood can be caused by this fungal disease (Lehtijärvi et al., 2014, 2017).

In Türkiye, damage caused by the boxwood moth, the reduction of the natural boxwood population, and the damage to the forest ecosystem in large devastated forest areas is of great concern. Boxwood contributes to the livelihood of the local people and therefore to the economy of the country with its valuable wood and shoots in the regions where it grows. In addition, since it is a preferred species in landscaping, it is seen as an undesirable and alarming situation by the public to be damaged or even killed by boxwood moth. It is hoped to be able to prevent the spread of *C. perspectalis*, especially in the Black Sea Region, using biological methods to combat this pest.

Considering the damage caused by the pest, this study aimed to determine a local isolate for control. In this context, the effect of the bacteria obtained from the pest and the crystal proteins of this bacteria on the pest was determined and the most effective material was determined and the difference between them investigated.

Materials and Methods

Sample collection

Samples of *C. perspectalis* larvae were collected from central Artvin, Türkiye. About 75 larvae were collected from the boxwood trees at the sampled locations. Collected *C. perspectalis* larvae were placed in sterile tubes and brought to the laboratory condition. Collected larvae samples were obtained in May 2021.

Bacterial isolation

Before starting the bacterial isolation, surface sterilization was performed with 70% alcohol. After surface sterilization, the larvae washed with sterile distilled water, homogenized and in sterilized nutrient broth (NB) using a glass tissue grinder and filtered through cheesecloth. The filtered homogenates were

diluted to 1 × 10^{-5} and 0.1 ml spread on agar medium (nutrient agar, NA) (Thiery & Frachon, 1997). The homogenate was heated to 80°C for 20 min before spreading onto plates to isolate bacteria in the genus *Bacillus*. Petri dishes were incubated at 30°C for 2 days. Colonies obtained after 2-3 days were expected to be *Bacillus*. The isolates were determined according to the color and morphology of the colonies. Identification of bacterial colonies was made according to morphological, biochemical and molecular methods (16S rRNA).

Analysis of scanning electron microscope and physiological and morphological properties of isolate

Selection of bacterial isolates was made according to Bergey's Manual of Systematic Bacteriology via morphological, physiological and molecular methods according to Sneath (1986). The phenotypic characterization was determined by looking at the colony morphology on NA. The optimum pH value of the bacteria was obtained after 16 h at 30°C (Ben-Dov et al., 1995).

For the SEM image, the bacterial sample was first incubated for 24 h at 30°C on NA. The collected samples were dropped on filter membrane and air dried. Bacteria samples were coated with gold before SEM. Then fixed in phosphate buffered saline (PBS). Five µl of the bacterial solution was taken and dried on a SEM sample set (Carl Zeiss, Jena, Germany). The voltage of the microscope was adjusted to be 5 to 10 kV.

Molecular identification of bacterium

Genomic DNA isolation from bacterial sample was done according to Sambrook et al. (1989). Genomic DNA isolation from bacterial sample was done according to Sambrook et al. (1989). PCR amplification of 16S rRNA gene of bacterial isolate was performed with the following universal primers (William et al., 1991); UNI 16S-L: 5-ATTCTAGAGTTTGATCATGGCTCA-3 as forward and UNI 16S-R: 5-ATGGTACCGTGTGTGACGGGCGGTGTGTA-3 as reverse. PCR conditions were adjusted according to William et al. (1991). Reactions were totally in 50 µl; 1 µl of template DNA was mixed with 5 µl reaction buffer, 0.2 mM of each deoxynucleoside triphosphate, 0.5 µM (each) with primer and 0.5 U with Taq DNA polymerase. Amplification was performed with 30 cycle programs (each cycle consisting of denaturation at 94°C for 3 min, annealing at 55°C for 60 s and extension at 72°C for 3 min), followed by a final extension step at 72°C for 5 min, by using thermal cycler (BioRad, Hercules, CA, USA). The reaction was made in total of 50 ml. Each PCR reaction was performed using the negative control (without DNA template). PCR product was analyzed on a 1.2% agarose gel. The visible band on gel was sent for sequencing. Sequencing of the amplified 16S rRNA samples was done by SenteBiolab (Ankara, Türkiye) using universal primers. The resulting sequences were analyzed for BLAST using the NCBI GenBank database.

Determination of cry genes

Screening of cry genes by PCR was done according to the method of Nishiwaki et al. (2007). The primer list of cry genes is given in Table 1. The reaction was made in total of 50 ml. Each PCR reaction was performed using the negative control (without DNA template). Amplified PCR products were visualized with a 1.2 % agarose gel. PCR products were transferred into to pJET1.2 Blunt Vector (Thermo Fisher Scientific, Waltham, MA, USA) and transferred to *Escherichia coli* (Migula,1895), (Enterobacterales: Enterobacteriaceae) DH10β cells. Vectors containing cry genes of *Bacillus thuringiensis* (Berliner, 1915) (Bacteria: Bacillaceae) were selected via white colonies. All white colonies growing on the Petri dish indicate clones that are include cry genes. Sequence analyzes were performed by checking each one for confirmation. Purification of recombinant plasmids were done by the Wizard Plus SV Minipreps DNA Purification System (Promega, Madison, WI, USA) and sequence analyzes were performed by SenteBiolab.

Table1. Primers used for cry gene sequencing (after Ben-Dov et al., 1995)

Primer	Primer	Amplicon length (bp)	Tm (°C)
cry1Fw	CATGATTCATGCGGCAGATAAC	277	55
cry1Rv	TTGTGACACTTCTGCTTCCCATT		
cry2Fw	GTTATTCTTAATGCAGATGAATGGG	701	52
cry2Rv	CGGATAAAATAATCTGGGAAATAGT		
cry3Fw	CGTTATCGCAGAGAGATGACATTAAC	604	54
cry3Rv	CATCTGTTGTTTCTGGAGGCAAT		
cry4Fw	GCATATGATGTAGCGAAACAAGCC	439	59
cry4Rv	GCGTGACATACCCATTTCCAGGTCC		

Expression of *cry* genes in *Escherichia coli*

Recombinant pJET 1.2 Blunt vectors containing cry1, cry 3 and cry 4 genes were cut with appropriate enzymes (BamHI and NcoI) and cloned into pET-28a+ expression vector. These generated recombinant vectors were transferred into *E. coli* BL21 (DE3) component cells for construct plasmids (pET-cry1, pET-cry3 and pET-cry4). These were incubated for 16 h at 37°C by spreading on LB agar medium containing antibiotic (kanamycin). One each was selected from the colonies growing in Petri dishes and incubated at 37°C in liquid medium (LB broth) supplemented with kanamycin. When the optical density (OD; 600 nm) reached 0.6, expression was induced with 1 mM isopropyl β-D-1-thiogalactoside. After incubation of the cells for 120 min at 37°C, they were centrifuged at 5,000 x g for 5 min at 4°C. Since the expressed proteins contain His-tag, their purification was performed according to the MagneHis Protein Purification System (Promega) manual. Protein concentrations were obtained according to procedures outlined by Bradford (1976). Purified cry proteins were screened by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (10% PAGE).

Insecticidal activities

For insecticidal activity, firstly, the bacterial isolate was incubated in NB at 30ºC for 72 h (for sporulation). After incubation, the bacterial sample was centrifuged at 3,000 rpm for 10 min (Ben-Dov et al., 1995). The resulting pellet was suspended in sterile PBS (or sterile H_2O). The density of the cells was adjusted to OD 600 nm and five concentrations were applied. (0.945, 1.80, 2.84, 3.78 and 5.67 x 10^9 cfu/ml) (Moar et al., 1995). The boxwood leaves that used during the application were obtained from the boxwood trees, which are the natural environment where insects are also collected. When these leaves were brought to the laboratory, surface disinfection was performed with 70% ethanol before application. Boxwood leaves prepared in small pieces were wetted with bacterial suspensions and dried at room temperature. (Hernández et al., 2005). Petri dishes (16 cm) were used in the bioanalysis. Samples of *C. perspectalis* larvae were collected in central Artvin. The insects used were collected from the boxwood trees in the central Artvin. Since the larvae were obtained from the natural environment, so no pesticides had been applied and the larvae were used directly in the assay. Third instar *C. perspectalis* larvae were used to determine the insecticidal activities of bacterial isolates. During the collection of the larvae to be used in the application, only the third instar larvae were collected with the guidance of the entomologist. For the bacterial isolate, 10 larvae were tested. Experiments were performed in triplicate. Sterile PBS was used as control. The Petri dishes were incubated at room temperature and death recorded daily for 10 days. The insecticidal activities of cry proteins were determined according to Sun et al. (2004).

Crystal enzyme solutions with five concentrations (500, 750, 1000, 1500 and 2000 U/ml) enzyme activity were prepared from His-tag purified cry enzymes (Binod et al., 2007). Small boxwood leaves with a diameter of 5 mm were prepared, soaked with cry proteins for 1 min, dried at room temperature and given to previously starved larvae. The larvae were starved for 8 h and then fed on leaves coated with enzyme

solutions. The control groups were fed with the same nutrient impregnated with the elution solution.10 larvae were tested for each protein isolate. Experiments were performed in triplicate and deaths recorded daily for 10 days (Ding et al., 2008).

Statistical Analysis

Mortality data were corrected by Abbott's formula (Abbott, 1925). Lethal concentrations (LC50) for the bacterial isolate (Finney, 1952) and cry proteins against third-stage larvae of hosts were calculated by probit analysis using MS Excel.

Results

In this study, *B. thuringiensis* was isolated from *C. perspectalis*. The colony color was creamy and optimum pH was pH 5 for bacterial isolate. The 16S rRNA partial gene sequence generated in this study has accession number OK513177. Also, for identification scanning electron microscopy photography is shown in Figure 1.

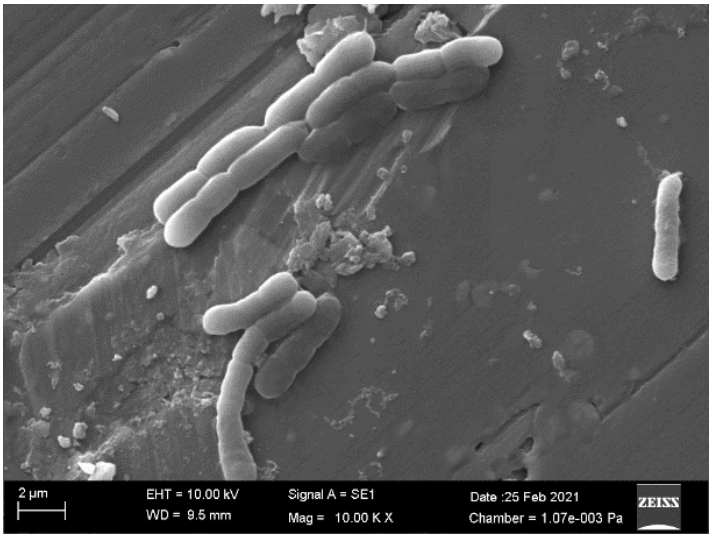

Figure 1. Scanning electron microscope image of the bacterium.

The PCR amplification of cry genes (cry1, cry2, cry3 and cry4) of the bacterium was scanned. According to amplification results only cry1, cry3 and cry4 genes were found. According to these results, expected sizes were obtained as indicated in Table 1 (Figure 2). The bands obtained were for cry1 (277 bp), cry3 (604 bp) and cry4 (439 bp) (Figure 2).

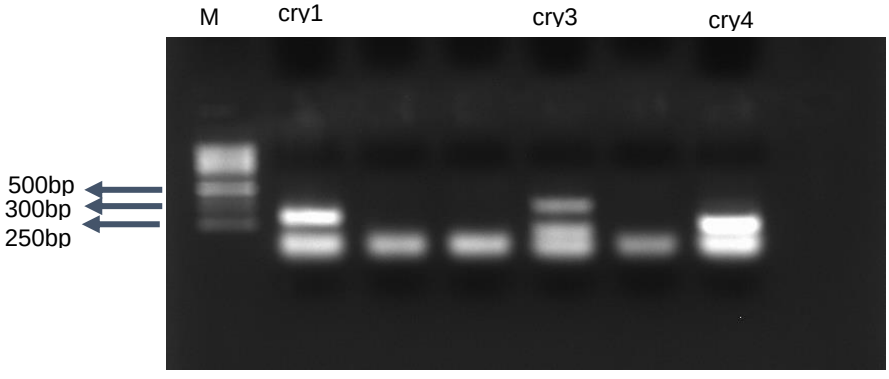

Figure 2. PCR amplicon of cry genes of *Bacillus thuringiensis.*

The proteins of cry1, cry3 and cry4 were coding approximately 130, 75 and 70 kDa, respectively (Figure 3).

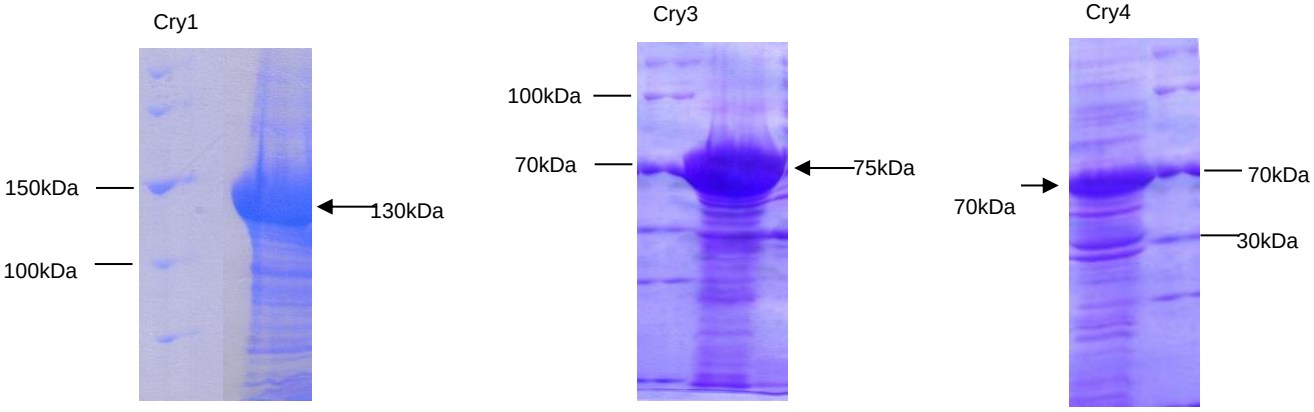

Figure 3. SDS-PAGE analysis of cry1, cry3 and cry4 proteins.

The insecticidal activity study found that the bacterial isolate has 85% insecticidal activity on *C. perspectalis* larvae.

The insecticidal activity of cry proteins was evaluated against the larvae of *C. perspectalis*. Test results showed that, cry1, 3 and 4 proteins have 75, 50 and 45% insecticidal activities against *C. perspectalis* larvae, respectively (Figure 4). The mortalities of all doses of insects infected with cry proteins are shown in Figure 5. The highest mortalities with cry1, cry3 and cry4 treatment were 85, 70 and 60% for *C. perspectalis*, respectively, at the 2000 U/ml. Also, mortality of all doses of insects infected with *B. thuringiensis* as shown in Figure 6. The highest mortality with bacterium treatment was 90% for *C. perspectalis* at 3.78 x 10^9 cfu/ml.

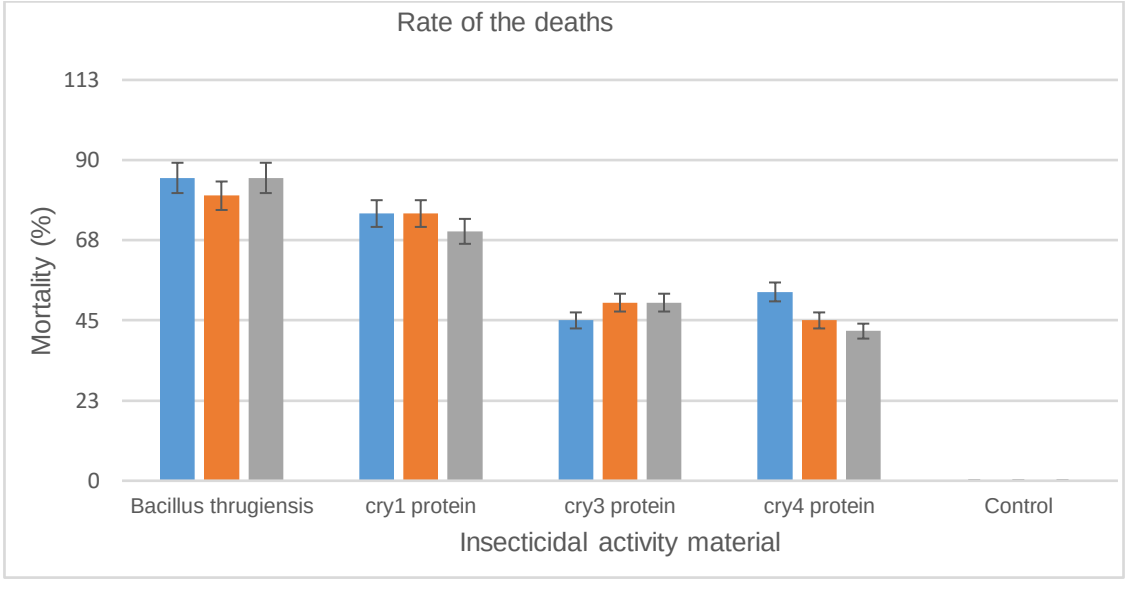

Figure 4. Insecticidal activity of *Bacillus thuringiensis* and cry proteins. Blue bars are the ratios in the first iteration, orange bars the ratios in the second iteration, and gray bars the ratios in the third iteration).

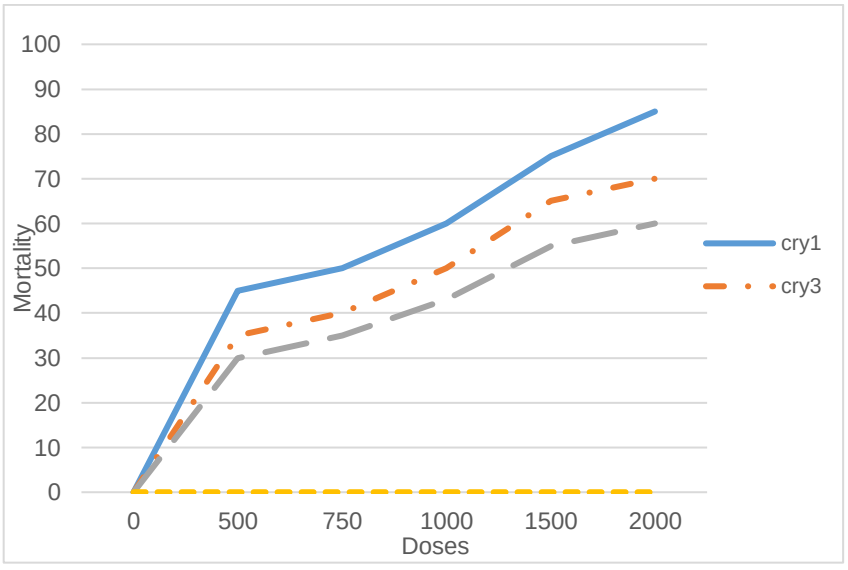

Figure 5. Mortality of insect larvae resulting from cry proteins.

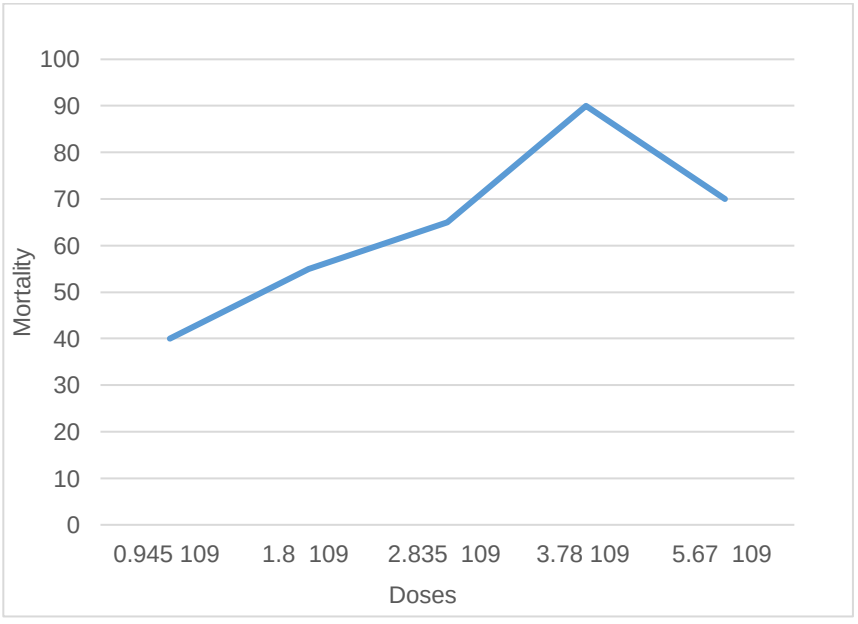

Figure 6. Mortality of insect larvae resulting from *Bacillus thuringiensis*.

The LC_{50} values calculated by probit analysis are given in Table 2. For the proteins, the LC_{50} was lowest cry3 and cry4 proteins and highest cry1 protein for *C. perspectalis.* The LC_{50} for bacterial isolate was higher than each of the individual proteins.

Table 2. Median lethal concentration (LC_{50}) of bacterial isolate and cry proteins assayed against *Cydalima perspectalis*

Isolate	LC 50 U/ml	df1	*df2*	X^2	SS	F	Slope ± SE
Bacillus thuringiensis	4070	1	3	0.92	0.554	3.01	0.325 ± 0.525
Cry1	3580	1	3	0.87	0.492	2.79	0.304 ± 0.507
Cry3	1020	1	3	0.54	0.213	1.58	0.236 ± 0.452
Cry4	1000	1	3	0.42	0.188	1.00	0.226 ± 0.423

Discussion

The first detection of *C. perspectalis* in Türkiye was in 2011 in parks and gardens located in the Sarıyer District of Istanbul (Bahçeköy, Emirgan, Hacıosman, Zekeriyaköy) (Hizal, 2012). Türkiye has favorable conditions for the spread of *C. perspectalis*, especially in the western and northern regions (especially Artvin), as the average temperature is above 30°C, and boxwood species, which are also the host of the pest, are widely used in parks and gardens.

There are various methods for control of *C. perspectalis*, including biological control with *B. thuringiensis*. This bacterium is the most important entomopathogenic bacterium. The crystal proteins/genes of this bacterium is ingested by the caterpillars during feeding, passes into the stomach, and then the active toxin crystal dissolved in the midgut passes into the blood, preventing skin formation and causing their death.

In this study, *B. thuringiensis* was isolated from *C. perspectalis.* PCR amplification of cry genes (cry1, cry2, cry3 and cry4) of the bacterium was scanned. According to amplification results only cry1, cry3 and cry4 genes were found. The cloning of the crystal protein gene (cry) of *B. thuringiensis* was first reported by Schnepf & Whiteley (1981). Since then, more than 120 cry genes have been cloned, characterized, and proteins classified based on amino acid sequence similarity (Crickmore et al., 2018). Cry1 proteins are effective against Lepidoptera species, cry3 proteins against Coleoptera species and cry4 proteins against on Diptera species. Cry genes are expressed and their products (proteins) accumulate to form a crystal inclusion, which can usually constitute 20 to 30% of the dry weight of cells (Schnepf et al., 1998). Few studies have reported a detailed characterization of Bt strain collections in terms of cry gene content. In this study, cry1 protein (90%) gave the highest mortality. In a study on Alper et al. (2014) the cry1 protein gave the highest mortality (36%), cry3 protein (1%) and cry4 protein (4%) gave lower mortality. In another study at Uribe et al. (2003) cry1 gave highest mortality again. And also, they scanned the samples for cry1 because of prevalence. In a study from Bravo et al. (1998) the cry1 genes were the most frequently found in the Mexican strain collection. A high frequency of cry1 genes seems to be common to all *B. thuringiensis* strains. As seen in the literature cry1 is commonly found to give high mortality. The second abundant genes and proteins were cry3 and cry4. In this study, *B. thuringiensis* isolated had cry1, cry3 and cry4 genes and also proteins. In a study of Brazilian *B. thuringiensis* they observed frequencies of the cry genes in *B. thuringiensis* isolates obtained from stored grain (48), compared to other isolates (180) in their collection. Using specific primers for *cry1*, 77% of the isolates from grain were positive but only 41% of the others.

Bacillus thuringiensis (Bt) is a gram-positive, spore-forming bacterium that produces insecticidal crystal proteins. Bt exerts its insecticidal activity in the orders Lepidoptera, Coleoptera, Hymenoptera, Diptera, Hemiptera, Orthoptera, and Mallophaga and among nematodes, mites, and protozoa, and by producing parasporal crystals that are toxic to a wide variety of insect species (Schnepf et al., 1998). The fact that insecticidal products obtained from *B. thuringiensis* bacteria do not cause infection in humans, non-target organisms and beneficial insects has increased the effective use of these products in the control of harmful insects (Lacey et al., 2001). *Bacillus thuringiensis* derivative products constitute 95% of the world biopesticide market. Many commercial companies have introduced and are promoting products of *B. thuringiensis* to the market. By 1998, more than 200 products of *B. thuringiensis* origin are used against pests only in the USA (Schnepf et al., 1998, Sanahuja Solsona et al., 2011). In addition, many *B. thuringiensis*-derived products are susceptible to synthetic chemical pesticides, obtained at a lower cost. Some other species belonging to the genus *Bacillus* are also used in the control of harmful insects. *Paenibacillus popilliae* (Dutky, 1940) Pettersson et al.,1999 (Paenibacillales: Paenibacillaceae) is used in the control of some species belonging to the family Scarabaeidae, while *Lysinibacillus sphaericus* Ahmed et al.,2007 (Bacillales: Planococcaceae) is used in the control of mosquito larvae (Jackson & Klein, 2006). *Paenibacillus popilliae* needs to be produced *in vivo*, and lower than expected levels of infection in many

field applications reduces the potential of this bacterium to be used in large areas (Klein & Kaya, 1995). Although it is more resistant to various factors, its biggest disadvantage is that the host spectrum is narrow (Lacey & Undeen, 1986, Charles et al., 1996, Nicolas et al., 1994). Also, some fly species are resistant to this bacterium (Rao et al., 1995, Nielsen-Leroux et al., 1997).

In conclusion, the results presented here show cry proteins (especially cry1) and the bacterium can use for control the *C. perspectalis*. Considering the effects of cry proteins in the literature and considering the harmful effect of the insect, these proteins have the potential to be developed as biopesticides and used against pests. Apart from laboratory application, it can be used against both *C. perspectalis* and other Lepidoptera by developing both bacteria and proteins as biopesticides, supported by field application.

References

Abbott, W. S., 1925. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology, 18 (2): 265-267.

Alper, M., H. Güneş, A. Tatlipinar, B. Çöl, H. S. Civelek, C. Özkan & B. Poyraz, 2014. Distribution, occurrence of cry genes, and lepidopteran toxicity of native *Bacillus thuringiensis* isolated from fig tree environments in Aydın Province. Turkish Journal of Agriculture & Forestry, 38 (6): 898-907.

Anonymous, 2011. Box tree caterpillar *Cydalima perspectalis*. Plant pest factsheet, the Food and Environment Research Agency (Fera). (Web page: www.defra.gov.uk/fera) (Date accessed: 5 August 2021).

Anonymous, 2021a. Bitki Veritabanı, Ağaçlar.net. (Web page: www.agaclar.net/forum/bitki-veritabani-www-agaclar-org/7734.htm) (Date accessed: 4 May 2021) (in Turkish).

Anonymous, 2021b. Türkiye Bitkileri Veri Servisi. (Web page: 194.27.225.161/yasin/tubives/index.php?sayfa=karsilastir) (Date accessed: 5 May 2021) (in Turkish).

Ben-Dov, E., S. Boussiba & A. Zaritsky, 1995. Mosquito larvicidal activity of *Escherichia coli* with combinations of genes from *Bacillus thuringiensis* subsp. *israelensis*. Journal of Bacteriology, 177 (10): 2581-2587.

Binod, P., R. K. Sukumaran, S. V. Shirke, J. C. Rajput & A. Pandey, 2007. Evaluation of fungal culture filtrate containing chitinase as a biocontrol agent against *Helicoverpa armigera*. Journal of Applied Microbiology, 103 (5): 1845-1852.

Bradford, M. M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, 72 (1-2): 248-254.

Bravo, A., S. Sarabia, L. Lopez, H. Ontiveros, C. Abarca, A. Ortiz, M. Ortiz, L. Lina, F. J. Villalobos, G. Peña, M.-E. Nuñez-Valdez, M. Soberón & R. Quintero, 1998. Characterization of cry genes in a Mexican *Bacillus thuringiensis* strain collection. Applied and environmental microbiology, 64 (12): 4965-4972.

Charles, J. F., C. Nielson-LeRoux & A. Delecluse, 1996. *Bacillus sphaericus* toxins: molecular biology and mode of action. Annual Review of Entomology, 41 (1): 451-472.

Crickmore, N., J. Baum, A. Bravo, D. Lereclus, K. Narva, K. Sampson, E. Schnepf, M. Sun & D. R. Zeigler, 2018. *Bacillus thuringiensis* toxin nomenclature. (Web page: http://www.vifabio.de/iqfBio/detail/1274) (Date accessed: September 2021).

Ding, X., Z. Luo, L. Xia, B. Gao, Y. Sun & Y. Zhang, 2008. Improving the insecticidal activity by expression of a recombinant cry1Ac gene with chitinase-encoding gene in acrystalliferous *Bacillus thuringiensis*. Current Microbiology, 56: 442-446.

Finney, D. J., 1952. Probit Analysis: A Statistical Treatment of the Sigmoid Response Curve. Cambridge University Press, Cambridge, England, 256 pp.

Göktürk, T., 2017. "The effect of dipel and spruzit against *Cydalima perspectalis* (Walker, 1859) (Lepidoptera: Crambidae), 125". In: The Abstract Book of International Forestry and Environment Symposium "Climate Change and Tree Migration" (7-10 November 2017, Trabzon, Türkiye), 146 pp.

Henricot, B. & A. Culham, 2002. *Cylindrocladium buxicola*, a new species affecting *Buxus* spp. and its phylogenetic status. Mycologia, 94 (6): 980-997.

Hernández, C. S., R. Andrewa, Y. Bel & J. Ferré, 2005. Isolation and toxicity of *Bacillus thuringiensis* from potato-growing areas in Bolivia. Journal of Invertebrate Pathology, 88 (1): 8-16.

Hizal, E., 2012. Two invasive alien insect species, *Leptoglossus occidentals* (Heteroptera: Coreidae) and *Cydalima perspectalis* (Lepidoptera: Crambidae), and their distribution and host plants in Istanbul Province, Turkey. Florida Entomologist, 95 (2): 344-350.

Jackson, T. A. & M. G. Klein, 2006. Scarabs as pests: a continuing problem. The Coleopterists Bulletin, 60 (5): 102-119.

Klein, M. G. & H. Kaya, 1995. *Bacillus* and *Serratia* species for scarab control. Memórias do Instituto Oswaldo Cruz, 90 (1): 87-95.

Lacey, L. A., R. Frutos, H. K. Kaya & P. Vail, 2001. Insect pathogens as biological control agents: do they have a future? Biological Control, 21 (3): 230-248.

Lacey, L. A. & A. H. Undeen, 1986. Microbial control of black flies and mosquitoes. Annual Review of Entomology, 31 (1): 265-296.

Lehtijärvi, A., H. T. Doğmuş-Lehtijärvi & F. Oskay, 2014. *Cylindrocladium buxicola* is threatening the native *Buxus sempervirens* populations in Turkey. Plant Protection Science, 50 (4): 227-229.

Lehtijärvi, A., H. T. Doğmuş-Lehtijärvi & F. Oskay, 2017. Boxwood blight in Turkey: Impact on natural boxwood populations and management challenges. Baltic Forestry, 23 (1): 274-278.

Leuthardt, F. L. G., W. Billen & B. Baur, 2010. Spread of the box-tree pyralid *Diaphania perspectalis* (Lepidoptera: Pyralidae) in the region of Basel-a pest species new for Switzerland. Entomology Helvetica, 3 (6): 51-57.

Mally, R. & M. Nuss, 2010. Phylogeny and nomenclature of the box tree moth, *Cydalima perspectalis* (Walker, 1859) comb. n., which was recently introduced into Europe (Lepidoptera: Pyraloidea: Crambidae: Spilomelinae). European Journal of Entomology, 107 (3): 393-400.

Matsiakh, I., V. Kramarets, M. Kavtarishvili & G. Mamadashvili, 2016. Distribution of invasive species and their threat to natural populations of boxwood (*Buxus colchica* Pojark) in Georgia. PPT Presentation. National Forestry Agency of Georgia, ENPI East Fleg II Program (Web page: www.observatree.org.uk/wp-content/uploads/2016/03/Matsiakh%20I._Threats%20to%20boxwood%20in%20Georgia.pdf) (Date accessed: 20 May 2021).

Moar, W. J., M. Pusztzai-Carey & T. P. Mack, 1995. Toxity of purified proteins and the HD-1 strain from *Bacillus thuringiensis* against lesser cornstalk borer (Lepidoptera: Pyralidae). Journal of Economic Entomology, 88 (3): 606-609.

Muus, T. S. T., E. J. Van Haaften & L. J. Van Deventer, 2009. De buxusmot *Palpita perspectalis* (Walker) in Nederland (Lepidoptera: Crambidae). Entomologische Berichten, 69 (2): 66-67.

Nicolas, L., L. N. Regis & E. M. Rios, 1994. Role of the exosporium in the stability of the *Bacillus sphaericus* binary toxin. FEMS Microbiology Letters, 124 (3): 271-275.

Nielsen-Leroux, C., F. Pasquier, J. F. Charles, G. SinÈGre, B. Gaven & N. Pasteur, 1997. Resistance to *Bacillus sphaericus* involves different mechanisms in *Culex pipiens* (Diptera: Culicidae) larvae. Journal of Medical Entomology, 34 (3): 321-327.

Nishiwaki, H., K. Nakashima, C. Ishida, T. Kawamura & K. Matsuda, 2007. Cloning, functional characterization, and mode of action of a novel insecticidal pore-forming toxin, sphaericolysin, produced by *Bacillus sphaericus*. Applied and Environmental Microbiology, 73 (10): 3404-3411.

Öztürk, N., S. Akbulut & B. Yüksel, 2016. Düzce için yeni bir zararlı *Cydalima perspectalis* (Walker, 1859) (Lepidoptera: Crambidae). Düzce Üniversitesi Ormancılık Dergisi, 12 (1): 112-121 (in Turkish).

Rao, D. R., T. R. Mani, R. Rajendran, A. S. Joseph, A. Gajanana & R. Reuben, 1995. Development of a high level of resistance to *Bacillus sphaericus* in a field population of *Culex quinquefasciatus* from Kochi, India. Journal of the American Mosquito Control Association-Mosquito News, 11 (1): 1-5.

Sambrook, J., E. F. Fritsch & T. Maniatis, 1989. Molecular Cloning: a Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1659 pp.

Sanahuja Solsona, G., R. Banakar, R. M. Twyman, T. Capell & P. Christou, 2011. *Bacillus thuringiensis*: a century of research, development and commercial applications. Plant Biotechnology Journal, 9 (3): 283-300.

Santi, F., P. Radeghieri, G. I. Sigurtà & S. Maini, 2015. Sex pheromone traps for detection of the invasive box tree moth in Italy. Bulletin of Insectology, 68 (1): 158-160.

Schnepf, E., N. V. Crickmore, J. Van Rie, D. Lereclus, J. Baum, J. Feitelson & D. Dean, 1998. *Bacillus thuringiensis* and its pesticidal crystal proteins. Microbiology and Molecular Biology Reviews, 62 (3): 775-806.

Schnepf, H. E. & H. R. Whiteley, 1981. Cloning and expression of the *Bacillus thuringiensis* crystal protein gene in *Escherichia coli*. Proceedings of the National Academy of Sciences, 78 (5): 2893-2897.

Sneath, P. H. A., 1986. "Regular, Nonsporing Gram Positive Rods, 1208-1260". In: Bergey's Manual of Systematic Bacteriology, Vol. 2. (Eds. P. H. A. Sneath, N. S. Mair, M. E. Sharpe & J. G. Holt). Williams and Wilkins, Baltimore, 1203 pp.

Sun, X., H. Wang, X. Sun, X. Chen, C. Peng, D. Pan, J. A. Jehle, W. Werf, J. M. Vlak & Z. Hu, 2004. Biological activity and field efficacy of a genetically modified *Helicoverpa armigera* single-nucleocapsid nucleopolyhedrovirus expressing an insect-selective toxin from a chimeric promoter. Biological Control, 29 (1):124-137.

Thiery, I. & E. Frachon, 1997. "Identification, Isolation, Culture and Preservation of Entomopathogenic Bacteria, 55-73". In: Manual of Techniques in Insect Pathology (Ed. A. L. Lacey). Academic Press, London, 409 pp.

Toper Kaygın, A. & C. Taşdeler, 2018. "*Cydalima perspectalis* (Walker,1859) (Lepidoptera: Crambidae) şimşirlerimiz için bir tehdit mi*?* 21". Proceedings of III. Türkiye Orman Entomolojisi ve Patolojisi Sempozyumu (10-12 May 2018, Artvin, Türkiye), 113 pp (in Turkish).

Uribe, D., W. Martinez & J. Ceron, 2003. Distribution and diversity of cry genes in native strains of *Bacillus thuringiensis* obtained from different ecosystems from Colombia. Journal of Invertebrate Pathology, 82 (2): 119-127.

Wan, H., T. Haye, M. Kenis, S. Nacambo, H. Xu, F. Zhang & H. Li, 2014. Biology and natural enemies of *Cydalima perspectalis* in Asia: Is there biological control potential in Europe? Journal of Applied Entomology, 138 (10): 715-722.

William, G. W., M. B. Susan, A. P. Dale & J. L. David, 1991. 16S ribosomal DNA amplification for phylogenetic study. Journal of Bacteriology, 173 (2): 697-703.

Yıldız, Y., İ. Yıldırım & C. Bostancı, 2018. "Bartın İlinin İstilacı Böcek Türleri, 25". Proceedings of III. Türkiye Orman Entomolojisi ve Patolojisi Sempozyumu (10-12 May 2018, Artvin, Türkiye), 113 pp (in Turkish).

Türk. entomol. derg., 2022, 46 (2): 239-247
DOI: http://dx.doi.org/10.16970/entoted.1096288

ISSN 1010-6960
E-ISSN 2536-491X

Original article (Orijinal araştırma)

Size and shape variability of the wing in burnet moth, *Zygaena ephialtes* (L., 1767) (Lepidoptera: Zygaenidae)[1]

Burnet güvesi, *Zygaena ephialtes* (L., 1767) (Lepidoptera: Zygaenidae)'nde kanadın boy ve şekil değişkenliği

Tina KLENOVŠEK[2*] **Predrag JAKŠIĆ[3]** **Franc JANŽEKOVIČ[4]**

Abstract

The burnet moth, *Zygaena ephialtes* (L., 1767) (Lepidoptera: Zygaenidae) is a distinctly polymorphic species of moth appearing in several color morphotypes. This study examined whether the variability of the *Z. ephialtes* forewing could be explained by geometric morphometric methods. The analysis included 70 male specimens from five localities in Montenegro (Plužine, Tepca, Dobrilovina, Gusinje and Rugovska Gorge), and one locality in North Macedonia (Kožuf Mountain) belonging to the subspecies *Zygaena ephialtes pannonica* Holik, 1937 and *Zygaena ephialtes istoki* Silbernagel, 1944, respectively, collected between 1981 to 2018. The forewing outline and the aposematic color pattern formed by five spots were analyzed separately. Neither forewing size nor forewing spots pattern size exhibited interpopulation heterogeneity. Size variation of the basal spot was independent from the rest. Considering the forewing outline shape, the apical portion was the most variable and different among populations. Two morphological groups were recognized: a group with a narrower pointed forewing (samples: Kožuf Mountain, Rugovska Gorge and Tepca), and a group with a wider forewing with a blunt apex (Plužine, Gusinje). Neither the outline or the spots pattern of the forewing supported the distinction between the subspecies *Z. e. pannonica* and *Z. e. istoki*.

Keywords: Aposematic coloration, Balkan Peninsula, geographical variability, geometric morphometrics, wing pattern

Öz

Burnet güvesi, *Zygaena ephialtes* (L., 1767) (Lepidoptera: Zygaenidae) farklı renk morfotiplerinde görülen belirgin şekilde polimorfik bir güve türüdür. Bu çalışmada, *Z. ephialtes*'in ön kanatlarındaki değişkenliğin geometrik morfometri yöntemleri ile açıklanıp açıklanamayacağını incelenmiştir. Analizde, 1981 ile 2018 yılları arasında toplanan sırasıyla *Zygaena ephialtes pannonica* Holik, 1937 ve *Zygaena ephialtes istoki* Silbernagel, 1944 alt türlerine ait Karadağ'daki beş bölgeden (Plužine, Tepca, Dobrilovina, Gusinje, Rugovska Gorge) ve Kuzey Makedonya'daki bir bölgeden (Kožuf Dağı) 70 erkek örnek kullanılmıştır. Beş noktanın oluşturduğu ön kanat taslağı ve aposematik renk modeli ayrı ayrı analiz edilmiştir. Hem ön kanat boyu hem de nokta desen boyu, popülasyonlar arası heterojenlik göstermemiştir. Bazal noktanın boyut değişimi diğerlerinden bağımsızdır. Ön kanadın anahat şekli göz önüne alındığında apikal kısım, popülasyonlar arasında en değişken ve farklı olanıdır. Daha dar sivri uçlu bir grup (Örneğin: Kožuf Dağı, Rugovska Gorge ve Tepca) ve küt bir tepeye sahip daha geniş bir ön kanatlı bir grup (Plužine, Gusinje) olmak üzere iki morfolojik grup tanımlanmıştır. Ön kanadın ne anahattı ne de benek deseni, *Z. e. pannonica* ve *Z. e. istoki* alt türleri arasındaki ayrımı desteklememiştir.

Anahtar sözcükler: Aposematik renklenme, Balkan Yarımadası, coğrafi değişkenlik, geometrik morfometrik, kanat deseni

[1] This study was partly presented as a poster at the Fifth Slovenian Entomological Symposium with International Attendance (Maribor, 2018) and published as an abstract in the abstract book. This study was supported by the Slovenian Research Agency and the Research Programme Computationally Intensive Complex Systems (P1-0403).
[2] University of Maribor, Faculty of Natural Sciences and Mathematics, Department of Biology, 2000 Maribor, Slovenia
[3] University of Priština, Faculty of Natural Sciences and Mathematics, 38220 Kosovska Mitrovica. Serbia
[4] University of Maribor, Faculty of Natural Sciences and Mathematics, Department of Biology, 2000 Maribor, Slovenia
* Corresponding author (Sorumlu yazar) e-mail: tina.klenovsek@um.si
Received (Alınış): 31.03.2022 Accepted (Kabul ediliş): 29.06.2022 Published Online (Çevrimiçi Yayın Tarihi): 30.06.2022

Introduction

The burnet moth, *Zygaena ephialtes* (L., 1767) (Lepidoptera: Zygaenidae) is a larger representative of the burnet family with a forewing 15-19 mm long. Adult animals show a pronounced aposematic pattern of coloration (Figure 1). The forewing is black with five or six spots (stigma), in white and yellow or red. The abdomen is black, with yellow or red abdominal belt (cingulum) on only one segment. The species is polymorphic, with two basic forms: peucedanoid and ephialtoid. Both forms come in red and yellow. *Z. ephialtes* is also characterized by mimetic forms, the coloration of the ephialtoid form is similar to the *Amata* genus Fabricius, 1807 (Lepidoptera: Erebidae) (Batesian-Müller mimicry). Only one generation is present in the life cycle, adult specimens having a short life span, and flying from late may to august (Hofmann & Tremewan, 2020). The species inhabits thermophilic dry grasslands on limestone. Its areal entails central and southern Europe, extending to Asia Minor (Türkiye) (Naumann et al., 1999).

Understanding the pronounced polymorphism of the *Z. ephialtes* is a challenge for researchers (Hofmann, 2003a, b, c). The entire Zygaenoidea group is the subject of in-depth phylogenetic analyses (Yen et al., 2005 and the literature collected there). Attempts have been made to explain interpopulation variability by morphometric and genetic approaches (Hofmann, 2003a, b, c). Researchers have been looking for links to evolutionary changes during the last glacial period, where populations survived in various refuges in which they had previously evolved. Zoogeographic interpretation of morphological variability seeks answers in recolonizations from multiple refuges with partially specialized populations, which has led to contact between different haplotypes, thereby also greatly increasing morphological variability (Hofmann, 2003a, b, c). Genomic analysis does not yield unambiguous results on subspecific taxonomy. Taxonomic affiliation at the subspecies level is based primarily on geographical distribution. According to Hofmann (2003c), populations of *Z. ephialtes* used in this study belong to the subspecies *Zygaena ephialtes pannonica* Holik, 1937 and *Zygaena ephialtes istoki* Silbernagel, 1944. The discriminating characteristic in taxonomy for the subspecies *Z. e. pannonica* and *Z. e. istoki* is that specimens of subspecies *Z. e. pannonica* are always five-spotted with spots 1 and 2 and cingulum on abdomen yellow, whereas specimens of *Z. e. istoki* are five-spotted with spots 1 and 2 and abdominal ring (cingulum) either red or yellow. The percentage of red and yellow forms differs according to the region (more yellow in the north, more red towards the south of the range of this subspecies). However, research on morphological characteristics and anatomical features conducted so far does not allow a reliable distinction between subspecies (Hofmann, 2003a, b, c). Another source of phenotypic variability may be due to adaptations to local ecological conditions, leading to ecotypic diversity (Bai et al., 2015; Martin et al., 2016).

The primary purpose of this study was to contribute to the understanding of the phenotypic variability of the burnet moth, *Z. ephialtes* in the western Balkan Peninsula. We analyze the forewing outline size and shape variability as well as the spots pattern using methods of geometric morphometrics (Adams et al., 2013). More specifically, we intend to find answers about (1) whether the morphometric variability of the forewing coincides with the geographical distribution of the populations, and (2) whether the interpopulation variability corresponds with the subspecies affiliation.

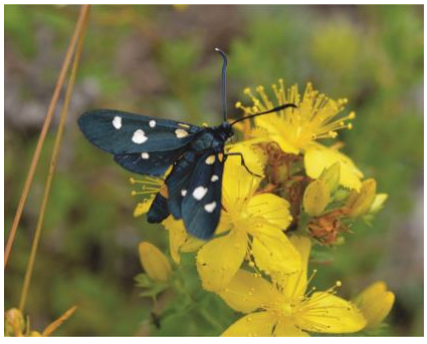

Figure 1. The burnet moth, *Zygaena ephialtes* is a characteristic species of dry grasslands. Suva Planina Mountain, photo by P. Jakšić.

Materials and Methods

The sample comprised 70 specimens of *Z. ephialtes* collected at six localities in Montenegro and North Macedonia (Table 1 and Figure 2) in grassland habitats at altitudes between approx. 690 and 925 m. To exclude variability associated with sexual dimorphism, only males were used in the analysis. The specimens were collected and identified by coauthor Predrag Jakšić (PJ) in the period from 1981 to 2018 as part of various biodiversity research projects, and are deposited in a dry collection of PJ in Belgrade.

Table 1. Localities of populations (MN, Montenegro and MK, North Macedonia), subspecies, altitude, geographic coordinates and number of specimens of *Zygaena ephialtes*

Locality	Subspecies	Altitude (m)	Coordinates	Specimens
Plužine (MN)	*Z. e. pannonica*	690	43°09'41" N, 18°47'41" E	15
Tepca, Tara River Canyon (MN)	*Z. e. pannonica*	900	43°12'08" N, 19°04'26" E	7
Dobrilovina, Tara River Canyon (MN)	*Z. e. pannonica*	800	43°01'37" N, 19°23'50" E	5
Gusinje, Prokletije Mountain (MN)	*Z. e. pannonica*	925	42°32'50" N, 19°49'31" E	12
Rugovska Gorge, Prokletije Mountain (MN)	*Z. e. pannonica*	820	42°41'09" N, 20°10 40" E	14
Kožuf Mountain (MK)	*Z. e. istoki*	810	41°11'29" N, 22°18'37" E	17
Total				70

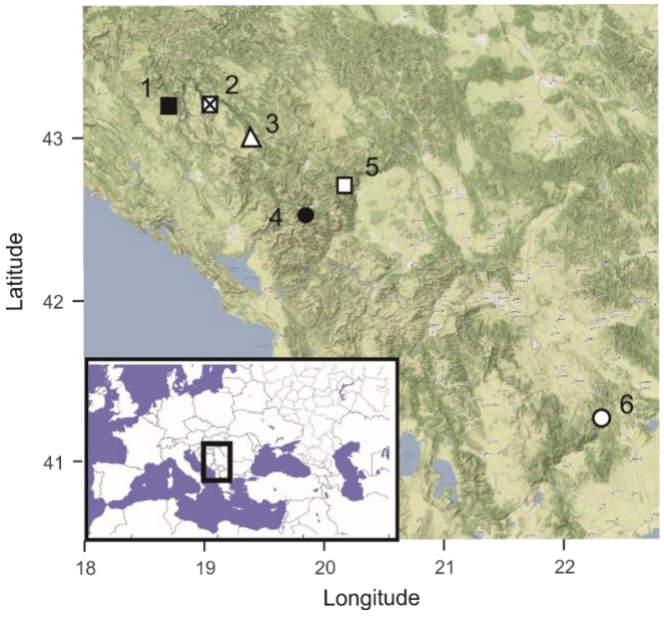

Figure 2. Balkan Peninsula depicting localities of *Zygaena ephialtes* (1, Plužine; 2, Tepca; 3, Dobrilovina; 4, Gusinje; 5, Rugovska Gorge; and 6, Kožuf Mountain).

Images of the dorsal side of left forewings were taken under constant conditions with a Nikon D 3200 camera with AF-S Micro Nikon lens. Landmarks were used to describe the forewing morphology from two aspects, the forewing outline and the pattern of color spots (Figure 3). In total, 14 landmarks were used to describe the outline of the wing, and 22 landmarks to describe the shape and arrangement of the five color spots. Landmarks were digitized using the TpsDig2 software (Rohlf, 2015). Generalized Procrustes analysis (GPA) was performed to standardize size and remove the differences in landmark configurations due to position and orientation. Size information was preserved as centroid size (Bookstein, 1991) and shape information as Procrustes coordinates (Zelditch et al., 2012). The GPA and all subsequent analyses were performed for the forewing outline and the spots separately. Therefore, we analyzed the centroid size and shape of the forewing outline, as well as the centroid size and shape of the forewing spots pattern. Additionally, to get centroid sizes of individual spots, we performed a GPA for each spot.

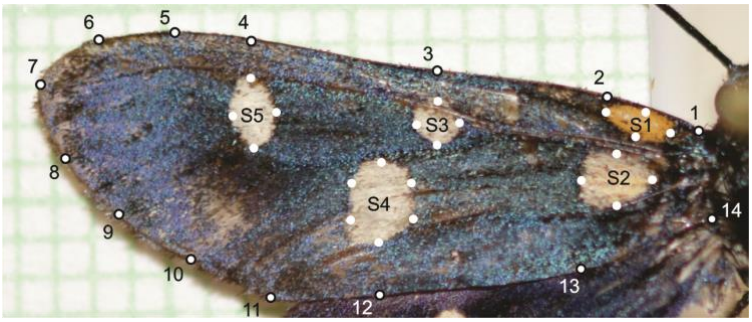

Figure 3. Left forewing of the *Zygaena ephialtes* with 36 landmarks (LM). Landmarks on the outline of the wing (LM 1-14), and spots S1-5 (LM 15-36). Forewing outline: 1, base of the wing; 2, at distal edge of S1; 3, at midst of S3; 4, at midst of S5; 5 and 6, at 1/3 and 2/3 outline distance between LM 4 and 7; 7, apex; 8, 9 and 10, at 1/4, 2/4 and 3/4 outline distance between LM 7 and LM 11; 11, juncture of the outer and the rear wing edge; 12, at midst of S4; 13, at distal edge of S2; and 14, base of the wing. Spots: S1-5: landmarks at proximal, distal, upper and lower edges of spots (in S4 proximal and distal edges described with two equally distanced landmarks).

Analysis of variance was used to examine wing and spots pattern size variability between geographic localities (populations). The strength of connection in size among individual spots was analyzed by the Pearson correlation coefficient. Principal component analysis (PCA) was used to explore the pattern of variation among specimens in the forewing and spots shape as well as to reduce the number of shape variables. Scatter plots of the first two principal components (PCs) were used for visualization. Shape changes along the first PC were presented by wire-frame graphs based on the thin plate spline algorithm (Bookstein, 1991). PCA was used also to search for outliers. One outlier from Kožuf Mountain and one from Rugovska Gorge were excluded from forewing shape and spots shape analyses, respectively. Differences among localities with larger sample sizes ($n > 10$) were tested using multivariate analysis of variance (MANOVA) and nonparametric permutation test of Procrustes distances (Bookstein, 1991) among populations mean shapes. To control results for increased likelihood of type I error from multiple comparisons, we used a Bonferroni correction. Given the relatively small sample sizes, MANOVA was performed with a reduced set of shape variables (first 10 PCs explaining 94.2% and 82.3% of total variance in the forewing outline and spots pattern, respectively). We estimated the effect of wing and spots size on overall variation in wing and spots shape by multivariate regression of shape onto size and estimated the statistical significance of the regression by a permutation test with 10,000 iterations against the null hypothesis of complete independence between shape and size. All analyses were performed using the MorphoJ software (Klingenberg, 2011) and SPSS Version 28.0.

Results

Forewing size and shape

There was no statistically significant variation in forewing size ($F_{5,64} = 0.63$, $p = 0.674$) among the six geographic samples of *Z. ephialtes*. However, highly significant differences were observed in the outline shape (Wilks' $\lambda < 0.001$, $F_{30,129} = 109$, $p < 0.001$). A PC 1 vs 2 scatter plot, explaining 52.58% of the total shape variation, revealed grouping of specimens from the Kožuf Mountain, Rugovska Gorge and Tepca. Specimens from these locations had mostly positive PC 1 scores (Figure 4) and were characterized by a narrower distal (costal and apical) part and wider basal part of the forewing (Figure 4). Specimens from Plužine and Gusinje had the widest distribution interval and mostly negative PC 1 scores, which was characterized by a broader apical part of the wing. Specimens from Dobrilovina had average values on both PCs and therefore the mean forewing shape. No distinction among the six samples was visible along PC 2. Wing shape differences between the two groups of samples according to the PC 1 vs 2 graph (Kožuf Mountain, Rugovska Gorge, Tepca vs Plužine and Gusinje) were statistically significant (Wilks' $\lambda = 0.266$, $F_{10,53} = 14.6$, $p < 0.001$).

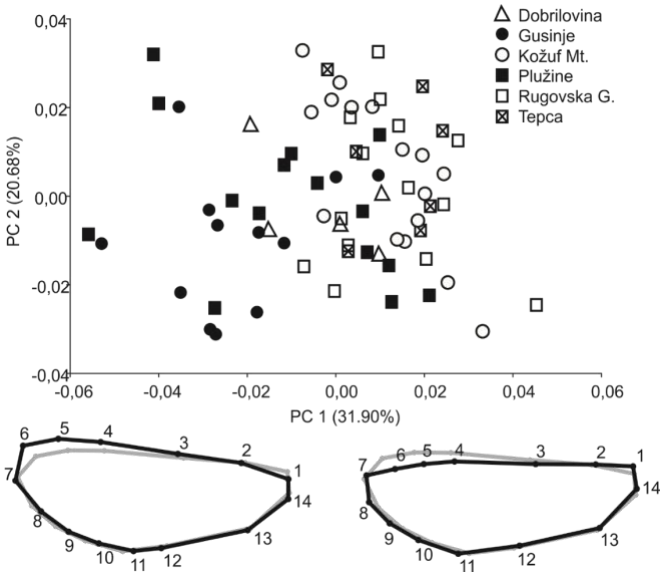

Figure 4. Scatter plot of the first two PCs for the forewing outline of the six populations of *Zygaena ephialtes* with wire-frame graphs showing wing shape changes along the first PC axis for the unit of 0.1 (black) in the negative (left) or positive direction (right) compared to the mean of PC 1 (gray).

Pair wise tests for differences in the mean wing outline shape (permutation test for Procrustes distances between mean shapes) showed specimens from Gusinje had in average the most unique wing shape, especially compared to specimens from Kožuf Mountain and Rugovska (Procrustes distances >0.035 and p<0.001; Table 2). Specimens from Rugovska and Kožuf Mountain were the most similar. The influence of allometry on the wing outline shape was statistically significant (p = 0.001) but low 6.05%.

Table 2. Procrustes distances and p-values from permutation tests for Procrustes distances among populations (n > 10) of *Zygaena ephialtes*. Significance after Bonferroni correction for multiple comparisons is p<0.0125

	Gusinje	Kožuf Mountain	Plužine
Kožuf Mountain	0.041; <.001		
Plužine	0.025; 0.007	0.025; 0.003	
Rugovska G.	0.041; <.001	0.010; 0.654	0.024; 0.007

Forewing spots pattern

There was no statistically significant variation in the total spots size ($F_{5,64} = 1.72$, p = 0.143) among the six populations. We also investigated whether the variability in the sizes of individual wing spots is related. The correlation analysis showed significant but weak (r < 0.5) positive correlations between spots S2-5 (Table 3). The variability in the size of spot S1 was completely independent and did not correlate with any other spot.

Table 3. Correlation coefficients and p-values among centroid size values for individual spots (CS_{S1-5})

	CS_{S1}	CS_{S2}	CS_{S3}	CS_{S4}
CS_{S2}	0.17; 0.159			
CS_{S3}	0.14; 0.261	0.44; <0.001		
CS_{S4}	-0.12; 0.311	0.32; 0.007	0.27; 0.023	
CS_{S5}	-0.17; 0.168	0.19; 0.107	0.38; 0.001	0.34; 0.004

No differences were observed between geographic locations in the wing spots shape pattern (Wilks' $\lambda = 0.456$, $F_{30, 132} = 1.36$, p = 0.124). Also, PC 1 vs 2 scatter plot, explaining only 33.0% of the total shape variation, showed no grouping of the specimens (Figure 5). The scatter of specimens along PC 1 showed the main variation in relative position and shape of spots S3 and S4 compared to the whole pattern (Figure 5).

In specimens with positive values on PC 1, S3 had a more proximal position, closer to S1 and S2, and S4 was, compared to specimens with negative values on PC 1, narrower. PCA was repeated separately for S3 and S4 landmark subsets (results not shown). Similarly, to the whole set of spots, no grouping of specimens according to populations was visible either for S3 or S4 along PC 1 or 2.

Pairwise tests for differences in the mean shape of spots pattern showed significant differences ($p < 0.0125$ after Bonferroni correction for multiple comparisons) only between specimens from Gusinje and Kožuf Mountain ($p = 0.006$). The influence of allometry on the wing spots pattern was not significant ($p = 0.145$).

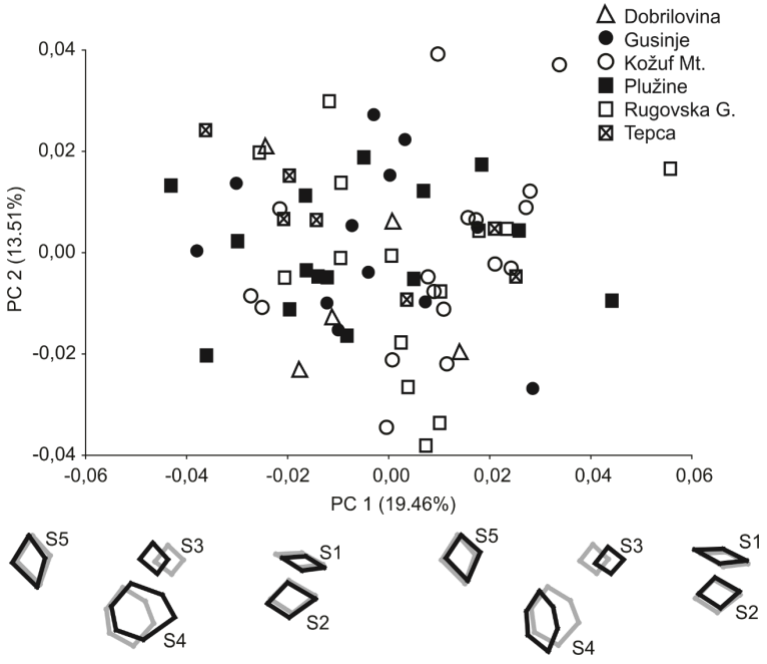

Figure 5. Scatter plot of the first two PCs for the pattern of forewing spots of the six geographical localities of *Zygaena ephialtes* with wire frame graphs showing spots pattern shape changes along the first PC axis for the unit of 0.1 (black) in the negative (left) or positive direction (right) compared to the mean of PC1 (gray).

Discussion

The morphometric study of the forewing of the burnet moth, *Z. ephialtes* in the western Balkan Peninsula showed no difference in the size of either the wing or the spots pattern among the populations. However, the wing outline shape distinguished two groups, one with narrow pointed forewings and the other with broad forewings with a blunt apex. Morphometric variation did not coincide with the geographical distribution of the populations. In the spots pattern, the relative position of the middle two spots was the most variable, without relation to population structuring. The assumption of a morphological distinction between subspecies *Z. e. pannonica* and *Z. e. istoki* was not apparent in any aspect of forewing morphology.

Forewing size and shape

Phenotypic variability in body size and shape is well expressed in insects (Blanckenhorn, 2009; Whitman & Agrawal, 2009). Larger specimens exhibit greater fitness, and usually come from environment with rich and quality food supply. Body size of insects is directly correlated with the quality and quantity of nutrients (Blanckenhorn, 2009; Whitman & Agrawal, 2009). Inadequate nutrition has a strong inhibitory effect on physical development of all organ systems in insects, as well as a strong effect on reduced fertility, especially evident from smaller number and size of eggs. Environmental variability (e.g., climatic conditions)

indirectly affects insect body size, but according to Whitman & Agrawal (2009), short-lived insects with an r-strategy do not have a large need for plasticity. They build their evolutionary success on a large number of offspring, the life cycle of short-lived adults being too short for selection pressures for phenotypic adaptations to particular climate environment to be asserted. In our study, we did not confirm significant differences in forewing size of the *Z. ephialtes* from different locations (populations), which could also be explained by a pronounced r-strategy of *Z. ephialtes*, as adult *Z. ephialtes* are short-lived and live only a few days, maximum two to three weeks (Naumann et al., 1999). In accordance with Whitman & Agrawal (2009), the absence of interpopulation variability in body size can also be explained by similar environmental and dietary conditions, such as food quantity and quality. The climatic conditions, in which the studied populations live, are probably different, especially in the southern Kožuf Mountain, but analysis of climate has not been carried out due to the small number of populations.

Interpopulation variability of the forewing of *Z. ephialtes* was well expressed in the wing shape. The apical portion of the forewing was the most variable feature and also discriminated between two groups of populations. An example of the phenotypic response of butterfly populations living in different environments is given by Bai et al. (2015) for the *Pieris rapae* (L., 1758) (Lepidoptera: Pieridae) species. It mentions that the shape of the fore and hindwings differ between populations, and that interpopulation variability is consistent with zoogeographical distribution, while boundaries between morphotypes run along zoogeographical boundaries. In our study, variability in the shape of the forewing of the *Z. ephialtes* was most evident in the distal area of the wing, the costal-apical region widening and narrowing. The pattern of forewing shape variability did not coincide with the geographical distribution of the populations. The geographically distant population from the Kožuf Mountain belonged to the group with a narrower wing alongside populations from the Tara River Canyon (Tepca) and the Rugovska Gorge. Except from geographic variation, Shkurikhin & Oslina (2016) discovered seasonal variation of the forewing in the polyvoltine *Pieris napi* (L., 1758) and *P*). *rapae.* Spring generations had smaller, elongated and pointed wings, and summer (and autumn) generations had larger, broad and rounded wings, which was an adaptation to dispersal. *Z. ephialtes* is not polyvoltine, therefore seasonal variation is less probable. Also, the effect of forewing size on the overall shape variation was minimal, which means the pattern of shape variability could not be explained by allometry. Wing shape affects flight type, which is anteromotoric in lepidopterans, i.e., the forewings have the leading locomotor role (Dudley, 2002). Longer and more slender forewings produce greater acceleration (Berwaerts et al., 2002) and large broader wings enable longer-distance flights. The flight of *Z. ephialtes* has been described as slow and undulating, nevertheless the species has also been found during quick flying events over longer distances (Horák, 2013). Relationships between forewing morphometry and possible sources of variability (e.g., geographical distance, bioclimatic conditions, diet and dispersion) were not tested in our study. This challenge remains for future research, especially with a larger number of specimens and more populations.

Forewing spots pattern

The aposematic coloration of the whole body, and especially the color pattern formed by the size and shape of the wing spots, has an important defensive function against predators of the *Z. ephialtes*, especially against insectivorous birds and lizards. Moths larvae feed on plants that contain cyanogenic glucosides from which they synthesize the toxic linamarin and lotaustralin (Zagrobelny & Møller, 2011; Nishida, 2017). Cyanogenic toxins remain in the body even after the transformation of larvae into adult animals, the highest concentration of toxins in adult butterflies remaining in the wings (Muhtasib & Evans, 1987). The defense mechanism in the wings is reasonable, as the wings first come into contact with the predator. The color pattern might be under strong selection pressure exerted by butterfly predators, yet evidence also show no association between spots size and toxicity across species of aposematic burnet moths (Briolat et al., 2019). We studied the five spots pattern (S1-5) on the forewing of *Z. ephialtes*. The first two, S1 and S2 were yellow in subspecies *Z. e. pannonica*, and red or yellow in 60 or 40% ratio in

subspecies *Z. e. istoki*, while the remaining three, S3-5, were white in both subspecies. We expected a close correlation between the sizes of individual spots, but the relationships among them were more complex. In other words, the size of each spot was more or less independent of the size of the others. Of particular note was spot S1, which varied completely independently of the rest. Among the remaining spots, the association was weak to moderately strong. The size and the shape of the forewing color pattern showed no population or subspecies structuring. Due to the lack of comparable data with other populations or species in the genus *Zygaena*s, these findings remain a reference for further comparisons.

None of the four datasets, of the forewing size and shape or the size and shape of the spots pattern, showed a recognizable morphometric distinction between subspecies *Z. e. istoki* and *Z. e. pannonica*. Nevertheless, research on a higher number of populations, especially of subspecies *Z. e. istoki*, is needed for meaningful conclusions.

References

Adams, D. C., F. J. Rohlf & D. E. Slice, 2013. A field comes of age: geometric morphometrics in the 21st century. Hystrix, 24 (1): 1-8.

Bai, Y., L. Bin Ma, S.-Q. Xu & G.-H. Wang, 2015. A geometric morphometric study of the wing shapes of *Pieris rapae* (Lepidoptera: Pieridae) from the Qinling Mountains and adjacent regions: An environmental and distance-based consideration. Florida Entomologist, 98 (1): 162-169.

Berwaerts, K., Van H. Dyck & P. Aerts, 2002. Does flight morphology relate to flight performance? An experimental test with the butterfly *Pararge aegeria*. Functional Ecology, 16 (4): 484-491.

Blanckenhorn, W. U., 2009. "Causes and Consequences of Phenotypic Plasticity in Body Size: The Case of the Yellow Dung Fly *Scathophaga stercoraria* (Diptera: Scathophagidae), 369-422". In: Phenotypic Plasticity of Insects: Mechanism and Consequences. (Eds. D. W. Whitman & T. N. Ananthakrishnan). Science Publishers, Enfield, NH, USA, 904 pp.

Bookstein, F. L., 1991. Morphometric Tools for Landmark Data. Cambridge University Press, Cambridge, UK, 456 pp.

Briolat, E. S., M. Zagrobelny, C. E. Olsen, J. D. Blount & M. Stevens, 2019. No evidence of quantitative signal honesty across species of aposematic burnet moths (Lepidoptera: Zygaenidae). Journal of Evolutionary Biology, 32 (1): 31-48.

Dudley, R., 2002. The Biomechanics of Insect Flight: Form, Function, Evolution, Princeton, NJ: Princeton University Press, USA, 496 pp.

Hofmann, A., 2003a. *Zygaena* (*Zygaena*) *ephialtes* (L., 1767) in südlichen Balkan Peninsula nebst Anmerkungen zur Entstehung von Polymorphismus sowie melanistischer Zygaena-Formen im Mittelmeerraum (Lepidoptera: Zygaenidae). Entomologische Zeitschrift mit Insekten-Börse, 113 (2): 50-54 (in German).

Hofmann, A., 2003b. *Zygaena* (*Zygaena*) *ephialtes* (L., 1767) in südlichen Balkan Peninsula nebst Anmerkungen zur Entstehung von Polymorphismus sowie melanistischer Zygaena-Formen im Mittelmeerraum (Lepidoptera: Zygaenidae). Entomologische Zeitschrift mit Insekten-Börse, 113 (3): 75-86 (in German).

Hofmann, A., 2003c. *Zygaena* (*Zygaena*) *ephialtes* (L., 1767) in südlichen Balkan Peninsula nebst Anmerkungen zur Entstehung von Polymorphismus sowie melanistischer Zygaena-Formen im Mittelmeerraum (Lepidoptera: Zygaenidae). Entomologische Zeitschrift mit Insekten-Börse, 113 (4): 108-120 (in German).

Hofmann, A. F. & W. G. Tremewan, 2020. The Natural history of Burnet moths (*Zygaena* Fabricius, 1775) (Lepidoptera: Zygaenidae), Part 3. Proceedings of the Museum Witt Munich, 6 (3.1): 1-508; 6 (3.2): 509-1097.

Horák, J., 2013. Habitat requirements of conspicuous burnet moth *Zygaena ephialtes* (L., 1767) (Lepidoptera: Zygaenidae). North-Western Journal of Zoology, 9 (1): 1-5.

Klingenberg, C. P., 2011. MorphoJ: an integrated software package for geometric morphometrics. Molecular Ecology Resources, 11 (2): 353-357.

Martin, M. A., C. Craioveanu, C. Sitar & L. Rákosy, 2016. Why morphometrics: a short review and a case study on *Zygaena carniolica* (Scopoli, 1763). Entomologica Romanica, 20 (1): 57-62.

Muhtasib, H. & D. L. Evans, 1987. Linamarin and histamine in the defense of adult *Zygaena filipendulae*. Journal of Chemical Ecology, 13: 133-142.

Naumann, C. M., G. M. Tarmann & W. G. Tremewan, 1999. The Western Palaearctic Zygaenidae. Apollo Books, Stenstrup, Denmark, 304 pp.

Nishida, R., 2017. “Chemical Ecology of Poisonous Butterflies: Model or Mimic? A Paradox of Sexual Dimorphisms in Mullerian Mimicry, 205-220”. In: Diversity and Evolution of Butterfly Wing Patterns (Eds. T. Sekimura & H. F. Nijhout). Springer, Singapore, 333 pp.

Rohlf, F. J., 2015. The tps series of software. Hystrix, 26 (1): 9-12.

Shkurikhin, A. O. & T. S. Oslina, 2016. Seasonal variation of the forewing in polyvoltine whites *Pieris rapae* L. and *P. napi* L. (Lepidoptera: Pieridae) in the forest-steppe zone of the Southern Urals. Russian Journal of Ecology, 47 (3): 296-301.

Whitman, D. W. & A. A. Agrawal, 2009. “What is Phenotypic Plasticity and Why is it Important?, 1-63”. In: Phenotypic Plasticity of Insects: Mechanism and Consequences (Eds. D. W. Whitman & T. N. Ananthakrishnan). Science Publishers, Enfield, NH, USA, 904 pp.

Yen, S.-H., G. S. Robinson & D. L. J. Quicke, 2005. The phylogenetic relationships of Chalcosiinae (Lepidoptera, Zygaenoidea, Zygaenidae). Zoological Journal of the Linnean Society, 143 (2): 161-341.

Zagrobelny, M. & B. L. Møller, 2011. Cyanogenic glucosides in the biological warfare between plants and insects: The Burnet moth-Birdsfoot trefoil model system. Phytochemistry, 72 (13): 1585-1592.

Zelditch, M. L., D. L. Swiderski & H. D. Sheets, 2012. Geometric Morphometrics for Biologists: A Primer. Elsevier, Academic Press, London, UK, 488 pp.

Türkiye Entomoloji Dergisi Yayın İlkeleri

Derginin yayın ilkeleri aşağıda özet olarak sunulmuştur. Ayrıntılar için web adresine (www.entomoloji.org.tr) bakınız.

1. Dergi, entomoloji ve tarımsal zooloji bilim dallarıyla ilişkili konulara açıktır.
2. Dergide Türkçe veya İngilizce yazılmış orijinal araştırmalar yayımlanır.
3. Yayımlanması istenilen eserlerin kısmen veya tamamen herhangi bir yerde yayınlanmamış veya yayımlanmayacak olması zorunludur.
4. Daha önce Kongre/Sempozyum vs. de sözlü/poster bildiri olarak sunulmuş ancak sadece kısa özet olarak basılmış eserler, dipnotta belirtilmesi koşuşuyla kabul edilir.
5. Lisansüstü tezleri veya TÜBİTAK, DPT, BAP gibi çeşitli kurumlarca desteklenen proje bulgularından kısımlar içeren eserler ilgililerinden gerekli izinler alındıktan sonra hazırlanmalı, ilgi durum dipnotta mutlaka belirtilmelidir.
6. Türkiye veya herhangi bir bölge için, başta karantina listesinde bulunan türler olmak üzere, yeni tür kayıtlarını içeren eserler gönderilmeden önce mutlaka ilgili kurumlara bilgi verilmiş olmalıdır.
7. Dergide yayımlanması istenilen eserler, web sayfasında sunulan “eser başvurusu” bölümünde açıklandığı gibi hazırlanarak, üst yazı, imzalı telif hakları formu ve başvuru ücreti dekontu ile dergi e-posta adresine gönderilmelidir.
8. Yayımlanması istenilen eserler web sayfasında sunulan “örnek makale taslağı” kullanılarak, gereksiz tekrar, şekil ve cetvellerden kaçınılarak, özden uzaklaşmayacak şekilde hazırlanmalı ve 16 sayfadan fazla olmamalıdır.
9. Yayın ilkelerine uygun olmayan eserler istenilen şekle göre yeniden düzenlenmek üzere yazara geri gönderilir. Detaylar için web sayfasında sunulan “eser değerlendirme süreci” ne bakınız.
10. Bir eser yayıma kabul edildiğinde, telif hakları formu tüm yazarlar tarafından imzalanıp dergimize gönderilmeden yayımlanmaz. Sorumlu yazara eserin pdf formatında hazırlanmış hali e-posta ile gönderilir, ayrıca telif ücreti ödenmez. Yayımlanan eserlere ait şekil dışı sorumluluklar yazarlarına aittir.

Türkiye Entomoloji Dergisi

Türkiye Entomoloji Dergisi, Türkiye Entomoloji Derneği tarafından yılda dört kez yayınlanır. Dergide, entomoloji ve tarımsal zooloji bilim dallarıyla ilişkili konularda, Türkçe veya İngilizce yazılmış orijinal araştırmalaryayımlanır.

Makale Özetleri, Biological Abstracts, BIOSIS Previews, CABAbstracts, FAOAGRIS, Elsevier Scopus, Global Health, Information Reference Library, Review of Agricultural Entomology, SCI-E, TÜBİTAK/ULAKBİM, VINİTİ, Zoological Record tarafından taranmaktadır.

Yıllık abone bedeli: 150 TL Tek sayı bedeli: 50 TL

Yazışma adresi:

Türkiye Entomoloji Dergisi
Ege Üniversitesi Kampüsü PTT Şubesi, PK. 10, 35100 Bornova, İzmir
e-posta: dergi@entomoloji.org.tr
web : http://www.entomoloji.org.tr