PLANT PROTECTION BULLETIN

Bitki Koruma Bülteni

Volume 63 Number 3 July-September 2023 ISSN 0406-3597 E-ISSN 1308-8122

Published by Plant Protection Central Research Institute Ankara, Turkey

TAGEM JOURNALS

PLANT PROTECTION BULLETIN / BİTKİ KORUMA BÜLTENİ

Volume 63	No 3	July-September 2023

Aims and Scope

Plant Protection Bulletin has been published by Plant Protection Central Research Institute since 1952. The journal is published four times a year with original research articles in English or Turkish languages on plant protection and health. It includes research on biological, ecological, physiological, epidemiological, taxonomic studies and control methods of disease, pest, weed, and natural enemies that cause damage in plants and plant products. In addition, studies on residue, toxicology and formulations of plant protection products and plant protection machinery are also included. Article evaluation process is based on double blind referee system and published as open access. Annual biological studies, short communication, first report and compilations do not publish in the journal.

Abstracted/indexed by EBSCOhost, CAB Abstracts, Clarivate Analytics-Zoological Record, TR-Dizin.

Plant Protection Bulletin is quarterly publication of the Directorate of Plant Protection Central Research Institute on behalf of General Directorate of Agricultural Research and Policies.

Correspondence Address : Zirai Mücadele Merkez Araştırma Enstitüsü Müdürlüğü

Gayret Mahallesi Fatih Sultan Mehmet Bulvarı No.66 PK 49 06172 Yenimahalle, Ankara / TÜRKİYE

+90 (0312) 344 59 93 (4 lines)

+90 (0312) 315 15 31

bitkikorumabulteni@zmmae.gov.tr

http://dergipark.gov.tr/bitkkorb

Grafik Tasarım : Filiz Eryılmaz

Printing: Tarım ve Orman Bakanlığı - Eğitim ve Yayım Dairesi Başkanlığı İvedik Caddesi Bankacılar Sokak No: 10 Yenimahalle, Ankara Türkiye

Tel: (0312) 315 65 55 - Fax: 0312 344 81 40

E-Posta : yayin@tarim.gov.tr

PLANT PROTECTION BULLETIN / BİTKİ KORUMA BÜLTENİ

Volume 63 | No 3 | July-September 2023

Contents / İçindekiler

Bitki Koruma Bülteni / Plant Protection Bulletin

http://dergipark.gov.tr/bitkorb

Original article

New data on plant hosts of Longidoridae and Trichodoridae nematodes in Türkiye

Türkiye'de Longidoridae ve Trichodoridae familyalarına bağlı nematod türlerinin konukçularına ilişkin yeni veriler

Lerzan ÖZTÜRK[a]*, Tohid BEHMAND[b], Atilla ÖCAL[c], Gürkan Güvenç AVCI[d], İbrahim Halil ELEKCİOĞLU[e]

[a]https://orcid.org/0000-0003-2199-6807, [b]https://orcid.org/0000-0001-7227-2484, [c]https://orcid.org/0000-0003-1638-0485, [d]https://orcid.org/0000-0002-2760-0773, [e]https://orcid.org/0000-0002-1707-7392

[a]*Viticulture Research Institute, 59100, Süleymanpaşa, Tekirdag, Türkiye*
[b]*Oragro, 07800, Korkuteli, Antalya, Türkiye*
[c]*Atatürk Horticultural Central Research Institute, 77102 Yalova, Türkiye*
[d]*Atatürk Soil Water and Agricultural Meteorology Research İnstitute, 39100, Kırklareli, Türkiye*
[e]*Çukurova University, Faculty of Agriculture, Department of Plant Protection, 01330, Sarıçam, Adana, Türkiye*

ARTICLE INFO

Article history:
DOI: 10.16955/bitkorb.1245271
Received : 31-01-2023
Accepted : 14-08-2023

Keywords:
Xiphinema, *Longidorus*, *Trichodorus*, geographic distribution, agricultural areas, Türkiye

* Corresponding author: Lerzan ÖZTÜRK
lerzanozturk@gmail.com

ABSTRACT

In this study, it was aimed to determine nematode species belonging to Longidoridae and Trichodoridae families in agricultural areas of the Thrace Region. The study was carried out between 2015-2022. For this purpose, soil samples were collected from fruit, vegetable, vineyard, and forest areas. Eleven nematode species belonging to *Xiphinema*, *Longidorus*, and *Trichodorus* were obtained from soils around the rhizosphere of 28 plants. Identified species include *Xiphinema pachtaicum* (26 plants), *X. turcicum* (grapevine), *X. pyrenaicum* (grapevine and fig), *X.ingens* (grapevine), *X. italiae* (grapevine and olive), *X. index* (nine plants), *X. diversicaudatum* (grapevine and fig), *X. opisthohysterum* (grapevine), *Longidorus elongatus* (four plants), *L. attenuatus* (olive and grapevine) and *Trichodorus similis* (grapevine and walnut). All 22 plants are a new record for nematodes of the Longidoridae and Trichodoridae families in Turkey. This article also includes information generated on a national scale for *Xiphinema* spp., *Longidorus* spp., and *Trichodorus* spp. being identified in Türkiye.

INTRODUCTION

Unlike other pathogens that can be controlled with pesticides, virus diseases are considered the most significant threat in agriculture due to the ineffective control measurements on the infected plants. The virus infections affect plant growth parameters and reduce the quality of plant products. The initial impact of viruses may be severe depending on the virulence of the virus isolate and the cultivar susceptibility (De Klerk and Loubser 1988).

Viruses are highly distributed among cultivated plants. To date, more than 80 viruses belonging to 21 families and 35 viruses from 15 families have been reported associated with grapevines and fruit trees, respectively (Umer et al. 2019). According to recent records at least 44 viruses were identified in *Prunus* species (Rubio et al. 2017).

The transmission of virus particles from plant to plant occurs with the help of several vectors. Approximately 55% of this transmission occurs with aphids, 11% with grasshoppers, 11% with coleopters, 9% with whiteflies, 7% with nematodes, 5% with fungi, and 2% with thrips (Astier et al. 2001). Among pest vectors, nematodes play a leading role in disease dispersal. Longidoridae and Trichodoridae are two families of harmful nematodes that include species capable of transmitting virus diseases. Thirty-seven nematode species from these families can vector 23 nepoviruses and two tobraviruses. For instance, grapevine fanleaf virus is semi-persistently vectored by both juvenile stages and adults of the ectoparasitic nematodes *Xiphinema index* and *X. italiae*, and arabis mosaic virus is transmitted by *X. diversicaudatum* (Demangeat et al. 2010, Taylor 1962). *Longidorus elongatus* is the other nematode vectoring raspberry ringspot virus (Comoviridae: Nepovirus) and tomato black ring virus (Comoviridae: Nepovirus) (Harrison et al. 1961).

Main factor in the transfer of plant viruses from plants and areas is the use of infected production material. Even a small number of nematodes that will be found in the soil residues in the root zone of plants such as seedlings taken from infected areas can cause diseases to be transmitted to new planting areas. As a matter of fact, the introduction of GFLV and *Xiphinema index* from Europe to other countries and the ToRSV virus and vector from America to other countries was through transfer of infected material. In addition, a single vector nematode individual can feed on the root and infect the healthy plant in a short time and maintain its vitality in the soil for a long time even if the host plant is not present (Das and Raski 1969, Taylor and Raski 1964). In this respect, determining the existing nematode species in the soil in the production areas and knowing whether they carry viruses will help prevent the transmission of infections to new production areas.

Thrace Region is located in the north-western European part of Türkiye and consists of Edirne, Kırklareli, and Tekirdağ, as well as European parts of İstanbul and Çanakkale provinces. The region has 160.000 ha of irrigable and 1.225.000 ha of non-irrigable agricultural lands (TUIK 2017). Although the vast majority of areas are not irrigated, drip irrigation is preferred in large-scale production. Agriculture is conducted mainly by smallholder farmers, and sunflower (*Helianthus annuus* L.), wheat (*Triticum aestivum* L.), corn (*Zea mays* L.), cherry (*Prunus avium* L.), walnut (*Juglans regia* L.) and grapevine (*Vitis vinifera* L.) production is intensive. Viruses are highly distributed disease agents causing substantial crop losses in Thrace Region. In our earlier studies in Thrace Region, three nematode-vectored viruses, including grapevine fanleaf virus (GFLV), tobacco black ringspot virus (TBRV), and arabis mosaic virus (ArMV), were detected in vineyards and orchards. GFLV was the most predominant found in 277 of 434 sampled vineyards (Öztürk et al, 2017). Because of the higher incidence of viruses in the Thrace Region, we decided to conduct studies to identify nematodes belonging to the families Longidoridae and Trichodoridae, especially virus vector species, and record their geographical distribution in agricultural areas. Another objective was to record the presence, the occurrence rate, and the plant species growing in co-infested regions. Distribution maps of all species in Türkiye have also been prepared by reviewing the results of studies conducted by other researchers throughout the country.

MATERIALS AND METHODS

Nematological field surveys were carried out twice a year in autumn and spring from 2015 to 2022 in vineyards, vegetable fields, pastures, and forests in Thrace Region, Türkiye. The study area covered Tekirdağ, Edirne, and Kırklareli provinces (Figure 1). A total of 1514 soil samples (274 for vineyards; 803 for orchards (olive, cherry, walnut, plum, quince, apple, pear, pomegranate, kiwi, mulberry, peach, and almond); 105 for sunflower fields; 85 for wheat fields; 28 for maize fields; 120 for vegetable fields; 50 for pastures; 49 for forest trees) were collected from Edirne (6 districts, 20 localities), Kırklareli (4 districts, 13 localities) and Tekirdağ (5 districts, 38 localities) provinces. The soil sampling depth was 0-90 cm. Sampling was done by moving in a zigzag pattern between plants in randomly selected areas. Six sub-samples were taken from different points of the plant rhizosphere or tree canopy, and a total of 1 kg of soil was collected.

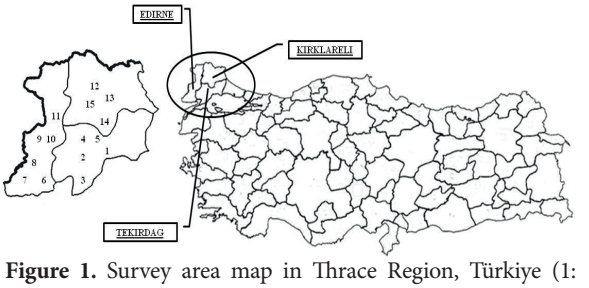

Figure 1. Survey area map in Thrace Region, Türkiye (1: Süleymanpaşa; 2: Şarköy; 3: Malkara; 4: Hayrabolu; 5: Muratlı; 6: Keşan; 7: Enez; 8: İpsala; 9: Meriç; 10: Uzunköprü; 11: Havsa; 12: Merkez; 13: Pınarhisar; 14: Lüleburgaz; 15: Babaeski)

Nematodes were extracted from 200 g sub-samples by using the decanting-sieving and centrifugal flotation methods (Brown and Boag 1988, Jenkins 1964). At first, isolated nematodes were heat-killed and then fixed in a double-strengthened triethanolamine-formalin solution [constituting 8 ml formalin (of 40% formaldehyde) + 2 ml triethanolamine + 90 ml water] for two days. Fixed nematodes were suspended in Seinhorst I (20 parts 95% ethanol, 1 part glycerin, and 79 parts distilled water) and Seinhorst II (5 parts glycerine and 95 parts 96% ethanol) solutions. Nematodes were identified by genus level and mounted on slides (Seinhorst 1959). The identifications of *Xiphinema* and *Longidorus* species were carried out based on the morphology and morphometrics of female individuals (Loof and Luc 1990). Conversely, Trichodorus was identified from females and males (Decraemer and Baujard 1998). After examining slides with a Leica DM1000 microscope, images of females were taken with a Leica ICC50 W camera, and morphometric measurements were conducted with Leica Application Suite. Texture and pH analyzes were also made in soil samples, to assess the interaction with nematode prevalence. Texture analysis was made from 200 g soil using the Bouycous hydrometer method using sodium hexametaphosphate ($NaPO_3$)6. Soil pH was measured with a pH meter (Gülçur 1974).

RESULTS

Eight *Xiphinema* species [*Xiphinema index* Thorne and Allen, 1950; X. *italiae* Meyl 1953; *X. pachtaicum* (Tulaganov 1938) Kirjanova 1951; *X. turcicum* Luc & Dalmasso 1964; *X. ingens* Luc & Dalmasso, 1964; *X. pyrenaicum* Dalmasso 1964; *X. opisthohysterum* Siddiqi 1961; *X. diversicaudatum* (Micoletzky 1927) Thorne 1939], two *Longidorus* species (*Longidorus elongatus* (de Man 1876) Micoletzky 1922; *L. attenuatus* Hoper 1961) and one *Trichodorus* (*Trichodorus similis* Seinhorst 1963) species were identified in three provinces (Table 1). Within *Xiphinema*, two species (*X. opistohysterum* and *X. pachtaicum*) were regarded as the *X. americanum* subgroup (Kumari and Decraemer 2007). *Xiphinema* individuals were obtained from soil samples collected from the rhizosphere soil of 26 different plants, while *Longidorus* was recorded from the rhizosphere soil of four plants and *Trichodorus* in the rhizosphere soil of two plants.

All 11 species were found in the province of Tekirdağ, while 10 were found in Edirne and seven in Kırklareli. In terms of prevalence, while *X. pachtaicum* was detected in all provinces and districts, *X. italiae*, X. *ingens*, *X.pyrenaicum*, *X. turcicum*, X. *diversicaudatum*, *L. attenuatus*, and *T. similis* were not found in Edirne. In contrast, *L. elongatus*, *X. index*, *X. diversicaudatum*, and *X. pyrenaicum* were not found in Kırklareli.

Table 1. Occurrence of Longidoridae and Trichodoridae species in Thrace, Türkiye

PROVINCES	LOCATIONS	*Longidorus elongatus*	*Longidorus attenuatus*	*Xiphinema pachtaicum*	*Xiphinema index*	*Xiphinema italiae*	*Xiphinema pyrenaicum*	*Xiphinema turcicum*	*Xiphinema ingens*	*Xiphinema opistohysterum*	*Xiphinema diversicaudatum*	*Trichodorus similis*
EDİRNE	[7]Enez			+	+							
	[8]İpsala			+						+		
	[11]Havsa			+								
	[6]Keşan			+	+							
	[9]Meriç			+								
	[10]Uzunköprü	+		+						+		+
KIRKLARELİ	[15]Babaeski			+								
	[13]Pınarhisar			+								
	[14]Lüleburgaz			+								
	[12]Merkez		+	+		+		+	+	+		+
TEKİRDAĞ	[4]Hayrabolu			+								
	[2]Malkara	+		+	+		+	+				
	[5]Muratlı			+								
	[1]Süleymanpaşa	+	+	+	+	+	+		+	+	+	
	[3]Şarköy	+	+	+	+	+	+			+		+

Table 2. Plants associated with Longidoridae and Trichodoridae in Thrace Region, Türkiye

Nematode species	Associated plants	Location
Xiphinema pachtaicum	*Acacia (*Acacia* spp.)	Edirne, Kırklareli, Tekirdağ
	*Almond (*Amygdalus* communis L.)	Edirne, Kırklareli, Tekirdağ
	*Apple (*Malus domestica* L.)	Edirne, Kırklareli, Tekirdağ
	*Apricot (*Prunus armeniaca* L.)	Edirne, Kırklareli, Tekirdağ
	**Cherry (*Prunus avium* L.)*	Edirne, Kırklareli, Tekirdağ
	*Corn (*Zea mays* L.)	Tekirdağ, Kırklareli
	*Cypress (*Cypressus* sp.)	Edirne, Kırklareli, Tekirdağ
	*Fig (*Ficus carica* L.)	Edirne, Kırklareli, Tekirdağ
	Grapevine (*Vitis* spp.)	Edirne, Kırklareli, Tekirdağ
	Loquat (*Eriobotrya* sp.)	Tekirdağ
	*Mulberry (*Morus* spp.)	Tekirdağ
	Melon (*Cucumis melo* L.)	Tekirdağ
	Olive (*Olea europaea* L.)	Tekirdağ
	Onion (*Allium cepa* L.)	Tekirdağ
	*Peach (*Prunus persica* (L.) Batsch)	Tekirdağ, Edirne, Kırklareli
	*Pear (*Pyrus communis* L.)	Tekirdağ, Edirne, Kırklareli
	*Pine (*Pinus* spp.)	Tekirdağ, Edirne, Kırklareli
	*Plum (*Prunus domestica* L.)	Tekirdağ, Edirne, Kırklareli
	*Pomegranate (*Punica granatum* L.)	Tekirdağ
	*Poplar (*Populus* sp.)	Tekirdağ, Kırklareli
	*Quince (*Cydonia oblonga* L.)	Tekirdağ, Edirne
	Rose (*Rosa* sp.)	Tekirdağ
	*Sunflower (*Helianthus annuus* L.)	Tekirdağ, Edirne, Kırklareli
	*Spruce (*Picea* sp.)	Tekirdağ, Edirne, Kırklareli
	*Wheat (*Triticum aestivum* L.)	Tekirdağ, Edirne, Kırklareli
	*Walnut (*Juglans regia* L.)	Tekirdağ, Edirne, Kırklareli
Xiphinema opisthohysterum	Grapevine (*Vitis* spp.)	Edirne, Kırklareli, Tekirdağ
X. italiae	Grapevine (*Vitis* spp.)	Kırklareli, Tekirdağ
	*Olive (*Olea europaea* L.)	Tekirdağ
X.diversicaudatum	Fig (*Ficus carica* L.)	Tekirdağ
	Grapevine (*Vitis* spp.)	Tekirdağ
X. ingens	Grapevine (*Vitis* spp.)	Kırklareli, Tekirdağ
X. pyrenaicum	*Fig (*Ficus carica* L.)	Tekirdağ
	Grapevine (*Vitis* spp.)	Tekirdağ
X. turcicum	Grapevine (*Vitis* spp.)	Kırklareli
X. index	*Almond (*Amygdalus communis* L.)	Tekirdağ
	*Cherry (*Prunus avium* L.)	Tekirdağ
	*Cypress (*Cypressus* sp.)	Tekirdağ
	Fig (*Ficus carica* L.)	Edirne, Tekirdağ
	Grapevine (*Vitis* spp.)	Edirne, Tekirdağ
	*Olive (*Olea europaea* L.)	Tekirdağ
	*Peach (*Prunus persica* (L.) Batsch)	Tekirdağ
	*Pear (*Pyrus communis* L.)	Tekirdağ
	*Walnut (*Juglans regia* L.)	Tekirdağ
Longidorus elongatus	Grapevine (*Vitis* spp.)	Tekirdağ
	*Kiwi (*Actinidia deliciosa* L.)	Tekirdağ
	*Chickpea *Cicer arietinum* L.)	Tekirdağ
	*Cherry (*Prunus avium* L.)	Edirne, Tekirdağ
L. attenuatus	*Olive (*Olea europaea* L.)	Tekirdağ
	Grapevine (*Vitis* spp.)	Tekirdağ
Trichodorus similis	Grapevine (*Vitis* spp.)	Kırklareli, Tekirdağ
	*Walnut (*Juglans regia* L.)	Edirne

*New record for the recorded host plant in Türkiye

In this research, *X. pachtaicum* was found in the soils around the rhizosphere of 26 plants. An average of seven individuals were obtained from 200 cm^3 soil taken from 0-30 cm soil depth, and the highest population was determined as 30±2.2 individuals on average in heavily contaminated areas. The higher number of *X. index* was recovered from soils around nine plants, and *X. italiae* from two plants. An average of 3.48±1.3 and 2.41 individuals were caught in 200 cm^3 of soil taken from 0-30 cm, respectively, in vineyards and orchards, and the number increased to 17 in the rhizosphere of fig trees. In Thrace, higher populations of these species (15.5 ± 1.5 for *X. index* and 16.2 ± 2.1 for *X. italiae*) were recorded at 30-60 cm soil depth in heavily infested vineyards and reached up to 26 individuals in fig plantations. *X. index* was most widespread in fig cultivation areas (42%) and vineyards (20%) in Tekirdağ.

The impact of soil texture on nematode distribution was observed during the study. *Xiphinema* species were mostly found in areas with sandy clay loam soil structures. Soil pH and altitude of the sampling area did not impact the nematode's prevalence. The species were present even in soils with a pH of 5.40 in Ipsala and a pH of 7.61 in Keşan. The nematode was found at an altitude of 10 m in Edirne, 175 m in Tekirdağ, and 234 m in Kırklareli.

Of the *Longidorus* species, *L. elongatus* was detected in soils around the root zone of four plants and *L. attenuatus* of two plants. Kiwifruit (*Actinidia deliciosa* L.), chickpea (*Cicer arietinum* L.), and cherry (*Prunus avium* L.) constitute new hosts for *L. elongatus*. The frequency of occurrence of *L. elongatus* in vineyards was found to be 4.49% in Tekirdağ and 1.5% in Edirne. The population of *L. elongatus* increased at 60-90 cm 26 individuals were counted in an average of 200 cm^3 of soil in areas with intense co-infestation at 60 cm, and a peaked number of individuals (77 ± 1.4 / 100 g soil) was recovered in two vineyards from 90 cm soil depth. The altitude, soil structure, and pH were not observed as limiting factors in nematode distribution. Only the number of individuals at 0-60 cm depth in irrigated vineyards was slightly higher than in non-irrigated vineyards. *L. elongatus* was found in samples taken from 103 and 143 m altitudes, in soil with a pH of 6.28 and 7.45 in Edirne Keşan district, at 65 m altitude and 7.60 pH in Tekirdağ Süleymanpaşa district.

In the study, *T. similis* was found in vineyards and walnut (*Juglans regia* L.) growing areas in Şarköy and Süleymanpaşa districts of Tekirdağ province and Uzunköprü district of Edirne province. Occurrence rates in vineyards and walnut areas were 1.43% and 0.8%, respectively. Male, female, and young individuals of *T. similis were* caught from a soil sample collected from 0-30 cm depth in the walnut orchard, and the prevalence rate was 0.79%. The number of individuals in 100 cm^3 of vineyard soil collected from a depth of 0-30 cm was counted as four in two areas, and males were not recovered. By contrast, seven individuals per 100 cm^3 of soil were recovered from the walnut orchard.

Grapevine hosts the most species, namely eight *Xiphinema*, two *Longidorus*, and one *Trichodorus* species. The rate of the species in vineyards was found as follows; *X. index* 12%, *X. pachtaicum* 77%, *X. italiae* 3.2%, *X. opistohysterum* 1.2%, *X. pyrenaicum* 10%, *X. turcicum* 0.4%, *X. ingens* 0.8%, *L. elongatus* 2%, *L. attenuatus* 0.35%, *T. similis* 0.35%. In terms of the number of species identified, walnut and olive orchards followed vineyards.

The associated plants of nematodes from Longidoridae and Trichodoridae in Thrace were included in Table 2. The comparable morphometrics of species are represented in Table 3-8.

Table 3. Morphometrics of *Xiphinema pachtaicum* and *Xiphinema opistohysterum* females from the Thrace, Türkiye

	Thrace	*Xiphinema pachtaicum*		*Xiphinema opistohysterum*	
		Lazarova et al. 2016	Bonta et al. 2013	Thrace	Siddiqi 1961
n	10	6	10	2	20
L (mm)	1.7 (1.5 - 1.9)	1.7 (1.5 - 2.0)	2 (1.8 - 2.1)	1.7 - 1.8	1.8 - 1.9
a	58.6 (53 - 69.7)	58.7 (53.3 - 65.7)	66.4 (62.3 - 73)	61 - 62.4	56 - 63
b	5.8 (5.2 - 6.4)	5.9 (5.3 - 6.4)	6.3 (5.6 - 7.3)	5.8 - 6	7.4 - 7.5
c	58.4 (51.6 - 58.4)	58.2 50.9 - 66.3)	64 (56.5 - 74.1)	58.6 - 61.5	50 - 52
c'	1.6 (1.4 - 1.8)	1.7 (1.6 - 1.8)	1.8 (1.7 - 2.1)	1.7	1.9 - 2
Odontophore	46.7 (38.8 - 54.6)	48.9 (46 - 51)	50 (48 - 52)	36.7 - 37.6	34 - 38
Odontostyle	90.2 (83.3 - 107.2)	84.2 (77 - 88.5)	90 (88 - 92)	70 - 70.7	64 - 68
Style	138.1 (128 - 146)	-	-	106.7 - 107.4	-
%Vulva	57.9 (56 - 60)	58.6 (57 - 60.4)	57.5 (56.5 - 58.6)	56 - 58	50 - 56
Guide ring-oral aperture	77.8 (71.4 - 83.1)	76.8 (73 - 80)	80.5 (71 - 83)	58.4 - 59.7	-
Body diameter at guide ring	21.3 (71.4 - 83.1)	21.5 (20.5 - 23)	22.5 (22 - 23)	18.3 - 18.5	17
Tail	30.2 (27.1 - 32.3)	29.8 (28 - 30)	31.4 (29 - 35)	27.9 - 29	30 - 36

Table 4. Morphometrics of *Xiphinema index* and *Xiphinema italiae* females from the Thrace, Türkiye

	Xiphinema italiae			*Xiphinema index*		
	Thrace	Martelli et al. 1966	Mistanoğlu et al. 2015	Thrace	Mistanoğlu et al. 2015	Meza et al. 2011
n	4	12	10	30	7	25
L (mm)	2.8 (2.8 - 2.9)	3.0 (2.6 - 3.5)	3.1 (2.8 - 3.5)	2.9 (2.8 - 3.2)	3.0 (2.1 - 3.3)	3.0 (2.6 - 3.4)
a	88.6 (84.6 - 91.6)	97 (84 - 109)	102.1 (90.8 - 112.0)	65.2 (57.7 - 66)	66.7 (61.9 - 64.2)	57.6 (50.3 - 65.2)
b	7 (6.9 - 7.3)	8.1 (7.5 - 8.8)	7.4 (6.3 - 8.6)	7.0 (5.7 - 8.0)	7.3 (5.3 - 10.6)	6.9 (4.9 - 7.8)
c	37.6 (37.7 - 41.1)	42 (38 - 47)	33.29 (30.1 - 38.8)	79.0 (80.8 - 76)	71.5 (48.2 - 86.93)	91.0 (78.2 - 113.5)
c'	3.8 (3.4 - 3.9)	3.5 (3.2 - 3.9)	4.62 (3.8 - 5)	0.8 (0.8 - 0.9)	1.2 (1.0 - 1.5)	0.9 (0.7 - 1)
Odontophore	57.5 (57.0 - 60.2)	57 (55 - 58)	63.4 (58.5 - 71.2)	74 (70 - 80)	69.0 (53.5 - 86.7)	68.8 (56.3 - 74.5)
Odontostyle	99.2 (89 - 106)	94 (87 - 99)	97.9 (87.7 - 112.3)	133 (121 - 139)	120.7 (86.1 - 145.0)	123.1 (116.8 - 129.5)
Style	-		-	205.9 (191 - 212)	-	-
%Vulva	46	45 (43 - 48)	45.92 (42.3 - 47.9)	42 (41 - 43)	41.3 (33.9 - 48.8)	39.4 (36.2 - 42.9)
Guide ring-oral aperture	87.5 (85.9 - 93.5)	-	-	126.4 (120.9 - 131)	-	111.9 (100.7 - 121)
Body diameter at guide ring	25.6 (24.9 -28.0)	-	-	30.6.7 (29.9 - 31)	-	35.2 (32.1 - 38)
Tail	72.7 (70.4 - 76.2)	-	92.6 (84.1 - 100.3)	32.6 (28 - 34.6)	40.26 (25 - 50.7)	32.9 (27.1 - 39.4)

Table 5. Morphometrics of *Xiphinema pyrenaicum*, *Xiphinema turcicum*, and *Xiphinema ingens* females from the Thrace, Türkiye

	Xiphinema pyrenaicum			*Xiphinema turcicum*		*Xiphinema ingens*	
	Thrace	Baujard et al. 1996	Arias et al. 2005	Thrace	Lamberti et al. 1983	Thrace	Mirzaei et al. 2015
n	4	12	11	2	7	1	20
L (mm)	3.5 (3.3-3.8)	4.0 (3.5 - 4.5)	3.6 (3 - 3.9)	3.95 - 4.05	3.5 - 4.1	5.8	5 (5.0 - 5.9)
a	58.3 (54.1 - 60.1)	69 (56 - 79)	58.5 (53.3 - 64)	64.9 - 63.4	61 - 71	83.3	58.5 (53.3 - 64)
b	7.7 (7.5 - 7.9)	8.6 (7.1 - 11.1)	7.2 (5.9 - 8.1)	7.81 - 10.5	8.3 - 9.6	9	7.2 (5.9 - 8.1)
c	88.5 (93.6 - 101)	98.4 (90 - 126)	91.1 (76.1 - 105.8)	105.6 - 108.8	106 - 109	150	91.1 (76.1 - 105.8)
c'	0.8 (0.86 - 0.74)	0.8 (0.73 - 0.93)	0.8 (0.7 - 1)	0.86 - 0.86	0.9	0.7	0.8 (0.7 - 1)
Odontophore	78.2 (76.6 - 79)	82 (76 - 90)	82.1 (80-88)	79.7 - 87.6	77 - 78	83	51 (48 - 53)
Odontostyle	151.4 (148 -151)	137 (127 - 149)	135.9 (134 - 142)	156.4 - 155.3	135 - 149	190	135.9 (134 - 142)
Style	244.6 (240 - 250)	219 (211 - 230)	-	-	-	273	82.1 (80 - 88)
% Vulva	47.1 (46 - 48)	48 (45 - 52)	51 (48 - 53)	46.1 - 50.4	50 - 51	48	218 (207 - 232)
Guide ring-oral aperture	135.8 (132.3 - 137)	123 (108 - 136)	122 (112 - 135)	147.6 - 152.5	119 - 123	135	122 (112 - 135)
Body diameter at guide ring	43.1 (42.7 - 43.9)	-	-	44 - 52	34 - 41	-	-
Tail	41.5 (36.1 - 37.1)	38 (34 - 41)	39.5 (35.2 - 49.6)	37.2 - 37.8	39 - 41	47	39.5 (35.2 - 49.6)

Table 6. Morphometrics of *Xiphinema diversicaudatum* females from the Thrace, Türkiye

	Xiphinema diversicaudatum		
	Thrace	Goodey et al. 1960	Chizhov et al. 2014
n	1	43	25
L (mm)	4.1	4.9 (4 - 5.5)	4.29 (3.7 - 4.7)
a	83.6	74 (57 - 92)	75.6 63.9 - 86.8)
b	-	9.1 (6.6 - 11.4)	9.5 (7.3 - 10.7)
c	99.2	78 (61 - 134)	85.4 (69.4 - 108.3)
c'	1.43	-	1.1 (0.9 - 1.3)
Odontophore	70	60 (50 - 70)	76 (67 - 82)
Odontostyle	128	85 (70 - 97)	126 (114 - 132)
Style	198	143 (130 - 157)	203 (190 - 211)
%Vulva	45	43 (39 - 46)	42 (39 -50)
Guide ring-oral aperture	-	-	110 (91 - 135)
Body diameter at guide ring	-	-	-
Tail	41.3	-	51 (41 - 65)

Table 7. Morphometrics of *Longidorus elongatus* and *Longidorus attenuatus* females from the Thrace, Türkiye

	Longidorus elongatus			*Longidorus attenuatus*		
	Thrace	Kumari and Decraemer 2007	Kepenekci 2014	Thrace	Kepenekci 2014	Susulovska et al. 2016
n	10	12	199	2	5	25
L (mm)	5.9 (5.8 - 6.2)	5.0 (4.1 - 5.8)	5.8 (4.6 - 7.1)	6.7 - 6.8	6.3 (5.8 - 6.8)	6.3 (5.2 - 6.9)
a	96.7 (94.8 - 99.6)	86.7 (68.6 - 104.2)	91.8 (69.2 - 119.1)	115.6 - 117	189 (175 - 195)	139.7 (123.8 - 151.1)
b	19.7 (18 - 21.3)	12.8 (10 - 15.8)	13.8 (10 - 20.3)	15.6 - 17.3	19.7 (17 - 22)	16.2 (13.9 - 18.5)
c	119.6 (118 - 124)	118.9 (100.4 - 132.7)	114.7 (89.6 - 156.3)	128 - 131	111.8 (102 - 122)	127.9 (111.9 - 144.3)
c'	1.06 (1.03 - 1.09)	1.2 (1.06 - 1.45)	1.3 (1 - 1.6)	1.1 - 1.2	1.66 (1.4 - 1.8)	1.53 (1.4 - 1.6)
Style	138.2 (136 - 142)	121 (117 - 128)	-	137 - 141	-	142.1 ± 3.6 (137 - 147)
%Vulva	49.30 (49 - 50)	55 (48.9 - 60.2)	-	50 - 51	48.8 (48 - 50)	49.1 (47.5 - 52.2)
Guide ring-oral aperture	37.0 (36 - 39.4)	29 (26 - 33)	-	27 - 30	-	29.9 (28 - 31)
Body diameter at guide ring	25.2 (24 - 27.3)	18 (17 - 19)	-	18 - 19	-	20.2 (19 - 22)
Tail	32.6 (28 - 34.6)	42 (36 - 48)	47.6 (41.8 - 55.7)	51.9 - 52.3	53.7 (47 - 58)	49.7 (44 - 56)

Table 8. Morphometrics of *Trichodorus similis* females and males from the Thrace, Türkiye

	Trichodorus similis			
	Thrace (Female)	Thrace (Male)	Kepenekci 2014	Seinhorst 1963
n	3	3	5	9
L (mm)	0.82 (0.77 - 0.87)	0.81 (0.80 - 0.82)	0.89 (0.88 - 0.89)	0.75 - 0.83
a	30 (28.8 - 30.0)	29.5 (28.5 - 30)	28.6 (28.2 - 29.4)	21 - 27
b	-	5.6 (5.6 - 5.7)	5.6 (5.5 - 5.8)	5 - 6
c	-	51.8 (50.4 - 52.9)	-	-
c'	-	0.6	-	-
Onchiostyle	44.8 (45 - 45.6)	42.6 (41 - 44)	40.6 (40 - 41)	38 - 42
% Vulva	61.3 (61 - 63)	-	55 (52 - 56)	50.2 - 62.3
Tail	-	15.3 (14.8 - 15.7)	3.4 (2 - 4)	-

Other studies on nematodes from Longidoridae and Trichodoridae in Türkiye

Studies on *Xiphinema* and *Longidorus* species in agricultural areas in Türkiye date back to the 1960s, and 15 species (11 *Xiphinema* species, 4 *Longidorus* species, 1 *Trichodorus* species) have been identified in 12 different crop plantations located in 17 provinces. The studies were mainly conducted in vineyards in the provinces located in the western and southern parts of Türkiye. The distribution map of Longidoridae and Trichodoridae species in Türkiye is given in Figure 2. Table 9 represents associated host plants and provinces where they were detected.

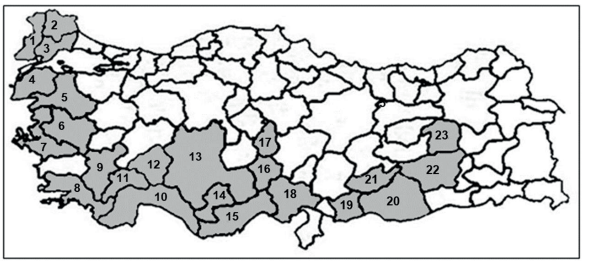

Figure 2. Distribution map of *Xiphinema*, *Longidorus*, and *Trichodorus* species in Türkiye

Edirne (1), Kırklareli (2), Tekirdağ (3), Çanakkale (4), Balıkesir (5), Manisa (6), İzmir (7), Muğla (8), Denizli (9), Antalya (10), Burdur (11), Isparta (12), Konya (13), Karaman (14), Mersin (15), Niğde (16), Nevşehir (17), Adana (18), Gaziantep (19), Şanlıurfa (20), Adıyaman (21), Diyarbakır (22), Bingöl (23)

DISCUSSION

This study provides the results of our preliminary nematode survey focused on determining the occurrence of Longidoridae and Trichodoridae species in the Thrace part ofTürkiye. At the end of the study, eight *Xiphinema*, two *Longidorus,* and one *Trichodorus* species were identified in the region. The species were mainly found in vineyards and orchards. No significant morphological and morphometric differences were observed when the identified species were compared with published species from other countries.

Xiphinema pachtaicum was common in our survey locations in Thrace Region. The specimen was more abundant in mulberry (*Morus* spp.) plantations and vineyards, and the prevalence was 8.3% and %77, respectively. Several researchers indicate the distribution and association *X. pachtaicum* with vineyards in Türkiye. For instance, nematode prevalence was reported as 88% and 87.3% in vineyards in the Manisa and İzmir provinces of the Aegean Region, respectively. In addition, the prevalence of the *X. index* was 72% in the study of Karakaş (2013) in the same region, and Mistanoğlu et al. (2015) determined the prevalence of *X. index* and *X. italiae* as 19.0% and 9.52%, respectively.

L. elongatus and *L. attenuatus* were two species that in this

Table 9. *Xiphinema*, *Longidorus*, and T*richodorus* species and associated hosts plants in Türkiye

Nematode Species	Associated Plants	Location	Reference
Xiphinema pachtaicum	Forage crops	23	Yıldız et al. 2012
X. pachtaicum	Grapevine	15, 18	Elekcioğlu 1992
X. pachtaicum	Grapevine	14, 17	Kepenekci et al. 2014
X.pachtaicum	Barley, wheat, lentil	20	Yıldız and Elekcioğlu 2011
X. pachtaicum	Grapevine	6, 7	Mistanoğlu et al. 2015
X. pachtaicum	Grapevine	19	Kasapoğlu et al. 2018
X. pachtaicum	Grapevine	1, 2, 3	Özturk et al. 2017
X. pachtaicum	Chickpea	3, 9, 10, 18	Behmand et al. 2019
Xiphinema index	Pistachio	19	Kasapoğlu et al. 2018
X. index	Forage crops	23	Yıldız et al. 2012
X. index	Pistachio, wheat, grapevine	20	Yıldız and Elekcioğlu, 2011
X. index	Grapevine	1, 3	Ozturk et al. 2017
X. index	Grapevine	6, 7	Mistanoğlu et al. 2015
X.index	Chickpea	3, 4, 8, 10	Behmand et al. 2019
Xiphinema italiae	Grapevine	2, 3	Ozturk et al. 2017
X. italiae	Grapevine	6, 7	Mistanoğlu et al. 2015
Xiphinema diversicaudatum	Grapevine	22	İmren and Elekcioğlu 2008
X.diversicaudatum	Grapevine	11, 12, 13	Kepenekci et. al. 2014
Xiphinema brevicolle	Grapevine, olive, cypress	6, 5	Arınç 1982
Xiphinema pyrenaicum	Grapevine, walnut	9	Arınç 1982
Xiphinema turcicum	Grapevine	Thrace	Arseven 1969
Xiphinema opisthohysterum	Grapevine	Thrace	Arseven 1969
Xiphinema ingens	Grapevine	Thrace	Arseven 1969
Longidorus attenuatus	Alfa alfa	Central Anatolia	Öztürk and Enneli 1994
Longidorus elongatus	Alfa alfa	Central Anatolia	Öztürk and Enneli 1994
Longidorus euonymus	Alfa alfa	Central Anatolia	Öztürk and Enneli 1994
Longidorus leptocephalus	Alfa alfa	Central Anatolia	Öztürk and Enneli 1994
Trichodorus similis	Grapevine	12, 17	Kepenekci et al. 2014

study observed at the root zone of four plants including olive and grapevine. Among these, *L. attenuatus*, previously reported in plants such as artichokes in many countries, has not been found in olive orchards in our country, and has not been detected in vineyards in the Thrace region before.

Of all identified Londigoridae and Trichodoridae species, eight have been reported as vectors of plant virus diseases. *Xiphinema* index and *X. italiae* transmit the grapevine fanleaf virus, while *X. diversicaudatum* is a vector of the arabis mosaic virus. *L. elongatus* can transmit peach rosette mosaic virus (PRMV), raspberry ringspot virus (RRV), tomato black ring virus (TBRV), and artichoke italian latent virus (AILV). L. attenuatus vectors tomato black ring virus (TBRV) and artichoke italian latent virus (AILV) nepoviruses to a wide range of susceptible plants (Brown et al. 1994). In addition, T. similis has been found to transmit the tobacco rattle virus (TRV) tobravirus, which infects more than 400 plant species from 50 families.

The number of areas infected with Longidoridae and Trichodoridae members is regularly increasing in Türkiye and the world. There is an increase, mainly due to the uncontrolled transport of production materials from region to region, unconscious practices, and inadequate quarantine procedures. The prevalence of Xiphinema species in 60% of soil samples taken from Spain, 23% of samples from Lebanon, 71% of samples from Chile, 49% of samples from Germany, 71% of samples from Samos island of Greece, and the occurrence of Longidorus species in countries such as Bulgaria, Australia, Germany, Slovakia, Greece, and Italy show how Longidoridae and Trichodoridae species are common in the world (Aballay et al. 2009, Arias and Fresno 1994, Avgelis and Tzortzakakis 1997, Bleyer et al. 1993, Coiro et al. 1991 Gangl et al. 2009, Hanna et al. 2008, Peneva et al. 2012, Sırca and Urek 2009, Tzortzakakis 2008). Such an identification is possible only in adult nematodes. Diagnosis using juvenile nematodes can only be made by molecular methods by using the internal transcribed spacer (ITS) region of ribosomal DNA, cytochrome c oxidase subunit I (COI), and some other genetic markers and should also be done by experts. In many countries, specialist researchers are limited in number, and diagnostic facilities are insufficient. For this reason, the existence of these species has not yet been determined in many countries and locations. As well in Türkiye, studies are generally carried out in provinces where nematologists working in institutes or faculties. For this reason, species were mostly found in the Aegean, Mediterranean, Marmara, and Southeastern Anatolia regions.

ACKNOWLEDGEMENTS

This research paper (10 of 30 plants) includes some results of the PhD thesis of Lerzan Öztürk conducted at Çukurova University (Adana, Türkiye), and some results were represented as a poster abstract at the European Society of Nematologists Congress in 2018 in Belgium.

ÖZET

Bu çalışmada, Trakya Bölgesi tarım alanlarında Longidoridae ve Trichodoridae familyalarına ait nematod türlerinin belirlenmesi amaçlanmıştır. Çalışma 2015-2022 yılları arasında gerçekleştirilmiştir. Bu amaçla, meyve, sebze, bağ ve orman alanlarından toprak örnekleri alınmıştır. *Xiphinema*, *Longidorus* ve *Trichodorus*'a ait 11 nematod türü, 28 bitkinin rizosferi etrafındaki topraklardan elde edilmiştir. Tanımlanan türler arasında *Xiphinema pachtaicum* (26 bitki), *X. turcicum* (asma bitkisi), *X. pyrenaicum* (asma bitkisinin ve incir rizosferi), *X.ingens* (asma bitkisi), *X. italiae* (asma ve zeytin bitkisi), *X. index* (9 bitki), *X. diversicaudatum* (asma bitkisi), *X. opisthohysterum* (asma bitkisi), *Longidorus elongatus* (4 bitki), *L. attenuatus* (zeytin ve asma bitkisi) ve *Tirichodorus similis* (asma ve ceviz) yer almaktadır. Saptanan 22 bitkinin tamamı, Türkiye'deki Longidoridae ve Trikodoridae familyalarına bağlı nematodlar için yeni kayıt durumundadır. Bu makale ayrıca ülkemizde teşhis edilmiş *Xiphinema* spp., *Longidorus* spp. ve *Trichodorus* spp. için bilgiler de içermektedir.

Anahtar kelimeler: *Xiphinema*, *Longidorus*, *Trichodorus*, coğrafik dağılım, tarım alanları, Türkiye

REFERENCES

Aballay E., Persson P., Martensson A., 2009. Plant-parasitic nematodes in Chilean vineyards. Nematropica, 39, 85-97.

Arınç Y., 1982. Research on occurrence, distribution and host range of *Xiphinema* spp. associated with grapevines in Aegean Region. İzmir Directorate of Agriculture Quarantine Research Series, 41, 83 pp.

Arias M., Fresno J., 1994. Agroecological characterization of *Xiphinema index* in Spain. Bulletin OEPP/EPPO Bulletin, 24, 403-411. https://doi.org/10.1111/j.1365-2338.1994.tb01397.x.

Arias M.,Escuer M., Cobacho A.S., Bello A., 2005. Distribution of *Xiphinema pyrenaicum* Dalmasso, 1969 with notes on X. aceri Chizhov, Tiev & Turkina, 1986 (Nematoda: Longidoridae). Nematology, 7, 45-51. 10.1163/1568541054192144.

Astier S., Albouy J., Maury Y., Lecoq H., 2001. Principes de virologie végétale. INRA, Paris, France.

Avgelis D., Tzortzkakis E.A., 1997. Occurrence and distribution of *Xiphinema* species and grape Fanleaf nepovirus in vineyards of the Greek Island of Samos. Journal

of Nematologia Mediterranea, 25 (2),177-182.

Baujard P., Luc M., Loof P.A.A., 1996. *Xiphinema pyrenaicum* Dalmasso, 1964 and its synonyms (Nematoda: Longidoridae). Fundamental and Applied Nematology, 19, 293-296.

Behmand T., Elekcioğlu N.Z., Berger J., Can C., Elekcioğlu İ.H., 2019. Determination of plant parasitic nematodes associated with chickpea in Turkey. Turkish Journal of Entomology, 43 (4), 357-366. doi: 10.16970/entoted.578081

Bleyer G., Kassemeyer H.H., 1993. Investigations on the occurrence of the nematode genera *Xiphinema, Longidorus,* and Paralongidorus in vineyards of Baden-Württemberg (Germany), p. 118. In: P. Gugerli (ed.), Extended Abstracts 11th Meeting ICVG, Montreux, Switzerland. Federal Agricultural Research Station of Changins, CH-1260 Nyon, Switzerland.

Bontă (Groza) M., Lazarova S., Roşca I., Peneva V., 2013. New data on the morphology and distribution of two species of the *Xiphinema americanum* group (Nematoda: Dorylaimida) from Romania. Scientific Papers Series A Agronomy, 56, 513-519 .

Brown D.J.F., Boag B., 1988. An examination of methods used to extract virus-vector nematodes (Nematoda: Longidoridae and Trichodoridae) from soil samples. Nematologia Mediterranea, 16, 93-99.

Brown D.J.F., Halbrendt J.M., Jones A.T., Vrain T.C., Robbins R.T., 1994. Transmission of three North American nepoviruses by populations of four distinct species of the *Xiphinema americanum* group. Phytopathology, 84, 646–649. doi: 10.1094/Phyto-84-646

Chizhov V.N.,. Pridannikov M.V., Peneva V., Subbotin S., 2014. Morphological and molecular characterization of the Saratov population of the European dagger nematode, *Xiphinema diversicaudatum* (Nematoda: Dorylaimida), with notes on the phylogeography of the species. Nematology, 16, 847-862. 10.1163/15685411-00002813

Coiro M., Agostinelli A., 1991. The development of juvenile stages of *Xiphinema index* (Nematoda: Dorylamida) on *Vitis vinifera*. Revue de Nematologie, 14 (1),181-182.

Das S., Raski D.J., 1969. Effect of grapevine fanleaf virus on the reproduction and survival of its nematode vector, *Xiphinema index* Thorne & Allen. Journal of Nematology, 1, 107-110.

Decraemer W., Baujard P., 1998. A polytomous key for the identification of species of the family Trichodoridae Thorne, 1935 (Nematoda: Triplonchida). Fundamental and Applied Nematology, 21, 37-62.

De Klerk C.A., Loubser J.T., 1988. Relationship between grapevine roots and soil-borne pests. In: The grapevine root and its environment. J.L. van Zyl, (ed.). South African Department of Agriculture and Water Supply, pp 88-105.

Demangeat G., Komar V., Van-Ghelder C., Voisin R., Lemaire O., Esmenjaud D., Fuchs M., 2010. Transmission competency of single-female *Xiphinema index* lines for grapevine fanleaf virus. Phytopathology, 100, 384-389. doi: 10.1094/PHYTO-100-4-0384

Elekcioğlu İ.H., 1992. Untersuchungen zum Auftreten and zur Verbreitung Phytoparasitaerer Nematoden in den landwirtschaftlichen Hauptkulturen des ostmediterranen Gebietes der Türkei. Plits, 10 (5), 120 p.

Gangl H., Leitner G., Hack C., Tiefenbrunner W., 2009. Rebschädigende viren, bakterien und bodenbürtige vektoren im Nordburgenland. Mitteilungen Klosterneuburg, , 59 (3), 134-143.

Goodey J.B., Peacock F.C., Pitcher R.S., 1960. A redescription of *Xiphinema diversicaudatum* (Micoletzky, 1923 & 1927) Thorne, 1939 and observations on its larval stages. Nematologica, 5, 127-135.

Gülçur F., 1974. Toprağın fiziksel ve kimyasal analiz metodları. İstanbul Üniversitesi Orman Fakültesi Yayınları, Yayın no: 201,, Kutulmuş, İstanbul, 225 p..

Hanna E., Digiaro M., Elbeaino T., Choueiri E., Jawhar J., Martelli G.P., 2008. Incidence of viruses and nematode vectors in Lebanese vineyards. Journal of Phytopathology, 156 (5), 304-310. https://doi.org/10.1111/j.1439-0434.2007.01336.x.

Harrison B.D., Winslow R.D., 1961. Laboratory and field studies on the relation of arabis mosaic virus to its nematode vector *Xiphinema diversicaudatum* (Micoletzky). Annals Applied of Biology, 49 (4), 621-633.

İmren M., Elekcioğlu İ.H., 2008. Determination of plant parasitic nematodes in vegetable, wheat and grapevine fields in Diyarbakır province. Çukurova University, Institute of Natural and Applied Sciences Journal, 17, 2, 116-121.

Jenkins W.R., 1964. Rapid centrifugal-flotation technique for separating nematodes from soil. Plant Disease Reporter, 48, 692.

Karakaş M., 2013. Population density of dagger nematode, *Xiphinema index* (Dorylaimida: Longidoridae) in vineyards in Manisa, Turkey. Mehmet Akif Ersoy University Institute of Natural and Applied Sciences 4 (2), 8-12. https://dergipark.org.tr/tr/pub/makufebed/issue/19420/206535.

Kasapoğlu Uludamar E.B., Yıldız Ş., İmren M., Öcal

A., Elekçioğlu İ.H., 2018. Occurrence of plant parasitic nematode species in important crops in the Southeast Anatolia Region of Turkey. Turkish Journal of Entomology, 42 (1), 63-74. doi: 10.16970/entoted.359616

Kepenekci İ., 2014. A new genus *Trichodorus* Cobb (stubby root nematode) (Triplonchida: Trichodoridae) and a preliminary list of virus vector nematodes associated in Turkey. Munis Entomology & Zoology, 9 (1), 227-244.

Kepenekci İ, Toktay H, Evlice E (2014). Plant parasitic and virus vector nematodes associated with vineyards in the Central Anatolia Region of Turkey. Pakistan J. Zool. 46 (3):866-870.

Kumari S., Decraemer W., 2007. The genus *Longidorus* (Nematoda: Longidoridae) from Bohemia and South Moravia in the rhizosphere of fruit orchards and vineyards. Helminthologia, 44 (4), 193-203. https://doi.org/10.2478/s11687-007-0031-7

Lamberti F., Choleva B., Agostinelli A., 1983. Longidoridae from Bulgaria (Nematoda, Dorylaimida) with descriptions of three new species of *Longidorus* and two new species of *Xiphinema*. Nematologia Mediterranea, 11, 49-72.

Lazarova S., Peneva V., Kumari S., 2016. Morphological and molecular characterization and phylogenetic position of *X. browni* sp. n., *X. penevi* sp. n. and two known species of *Xiphinema americanum*-group (Nematoda, Longidoridae). ZooKeys, 574, 1-42. 10.3897/zookeys.574.8037

Loof P.A.A., Luc M.A., 1990. A revised polytomous key for the identification of species of the genus *Xiphinema* Coob, 1913 (Nematoda: Longidoridae) with exclusion of the *X. americanum* group. Systematic Parasitology, 16, 35-66.

Martelli G.P., Cohn E., Dalmasso A., 1966. A redescription of *Xiphinema italiae* Meyl, 1953 and its relationship to *Xiphinema arenarium* Luc et Dalmasso, 1963 and *Xiphinema conurum* Siddiqi, 1964. Nematologica, 12, 183-194.

Meza P., Aballay E., Hinrichsen P., 2011. Molecular and morphological characterization of species within the *Xiphinema americanum*-group (Dorylaimida: Longidoridae) from the central valley of Chile. Nematology, 13 (3), 295-306. https://doi.org/10.1163/138855410X518498

Mirzaei S., Pourjam E., Pedram M., 2015. Molecular characterization of and new data on, two populations of *Xiphinema ingens* Luc & Dalmasso, 1964 (Nematoda: Longidoridae) from Iran. Nematology, 17 (2),125-138. https://doi.org/10.1163/15685411-00002858

Mistanoğlu İ., Kaşkavalcı G., Devran Z., 2015. Identification of the economically important plant parasitic nematodes in vineyards areas of İzmir and Manisa provinces by morphological and molecular techniques. Turkish Journal of Entomology, 39 (3), 297-309. https://doi.org/10.16970/ted.65336

Öztürk L., Avci G.G., Behmand T., Elekcioğlu H.I., 2017. "Incidence of viruses and vector nematodes in Thrace vineyards, Turkey." International Journal of Agriculture and Environmental Research, 3 (6), 4078-4089.

Öztürk G., Enneli S., 1994. Distribution of plant parasitic nematodes in alfalfa-growing areas in Central Anatolia Region of Turkey. Proceedings of 9th Congress of the Mediterranean Phytopathological Union, Kuşadası, Aydın, Türkiye, 537-538.

Peneva V.K., Urek G., Lazarova S., Šırca S., Knapıč M., Elshishka M., Brown D.J.F., 2012. Longidoridae and nepoviruses in Bulgaria and Slovenia. Helminthologia, 49 (1), 49-56. https://doi.org/10.2478/s11687-012-0008-z

Rubio M., Martínez-Gómez P., Marais A., Sánchez-Navarro J.A., Pallás V., Candresse T., 2017. Recent advances and prospects in Prunus virology. Annals of Applied Biology, 171, 125-138. doi: 10.1111/aab.12371

Seinhorst J.W., 1959. A rapid method for the transfer of nematodes from fixative to anhydrous glycerin. Nematologica, 4 (1), 67-69.

Seinhorst J.W., 1963. A redescription of the male of *Trichodorus primitivus* (de Man), and the description of a new species T. similis. Nematologica, 9, 125-130. https://doi.org/10.1163/187529263X00232

Šırca S., Urek G., 2009. Morphological and molecular characterization of six *Longidorus* species (Nematoda: Longidoridae) from Slovenia. Russian Journal of Nematology, 17 (2), 95–105.

Siddiqi M.R., 1961. On *Xiphinema opisthohysterum* n. sp., and *X. pratense* Loos, 1949, two dorylaimid nematodes attacking fruit trees in India. Zeitschrift für Parasitenkunde, 20, 457-465.

Susulovska S., Susulovsky A., Kornobis F.W., 2016. Morphometrical and molecular data on plant parasitic nematodes *Longidorus attenuatus* Hooper, 1961 and *L. danuvii* Barsi et al., 2007 (Nematoda: Longidoridae) reported from Ukraine for the first time. Helminthologia, 53 (4), 396-400. https://doi.org/10.1515/helmin-2016-0040

Taylor C.E., 1962. Transmission of raspberry ringspot virus by *Longidorus elongatus* (de Man) (Nematoda: Dorylaimidae). Virology, 17, 493-494.

Taylor C.E., Raski D.J., 1964. On the transmission of grape fanleaf by *Xiphinema index*. Nematologica, 10, 489-495.

Tuik 2017. Turkish Statistical Institute. Available from: https://www.tuik.gov.tr.

Tzortzakakis E.A., Peneva V., Brown D.J.F., Avgelis A.D., 2008. A literature review on the occurrence of nematodes of the family Longidoridae in Greece. Nematologia Mediterranea, 36 (2), 153-156.

Umer M., Liu J., You H., Xu C., Dong K., Luo N,., Kong L., Li X., Hong N., Wang G., Fan X., Kotta-Loizou I., Xu W., 2019. Genomic, morphological and biological traits of the viruses infecting major fruit trees. Viruses, 11, 1-12. doi: 10.3390/v11060515

Yıldız S., Handoo Z.A., Carta L.K., Skantar A.M., Chitwood D.J., 2012. A survey of plant-parasitic nematodes associated with forage crops in Bingol, Turkey. Nematologia Mediterranea, 40 (1), 73-77.

Yıldız Ş., Elekcioğlu İ.H., 2011. Şanlıurfa ilinde tarımsal ve doğal alanlarda nematod biyoçeşitliliği. Turkish Journal of Entomology, 35 (2), 381-394. https://dergipark.org.tr/en/pub/entoted/issue/64049/969263.

Cite this article: Öztürk, L., Behmand, T., Öcal, A., Avcı, G. G. & Elekcioğlu, İ. H. (2023). New data on plant hosts of Longidoridae and Trichodoridae nematodes in Türkiye. Plant Protection Bulletin, 63-3. DOI: 10.16955/bitkorb.1245271

Atıf için: Öztürk, L., Behmand, T., Öcal, A., Avcı, G. G. & Elekcioğlu, İ. H. (2023). Türkiye'de Longidoridae ve Trichodoridae familyalarına bağlı nematod türlerinin konukçularına ilişkin yeni veriler. Bitki Koruma Bülteni, 63-3. DOI: 10.16955/bitkorb.1245271

Bitki Koruma Bülteni / Plant Protection Bulletin

http://dergipark.gov.tr/bitkorb

Original article

Isolation and identification of entomopathogenic fungi from coastal districts of Ordu province, Turkey

Ordu ili kıyı ilçelerinden entomopatojen fungusların izolasyonu ve tanımlanması

Funda ŞAHİN[a]*, Yusuf YANAR[b]

[a]https://orcid.org/0000-0001-9150-7066, [b]https://orcid.org/0000-0002-5795-6340

[a]*Tokat Gaziosmanpaşa University, Faculty of Agriculture, Department of Plant Protection, Tokat, Türkiye*

ARTICLE INFO

Article history:

DOI: 10.16955/bitkorb.1296436

Received : 12-05-2023

Accepted : 23-06-2023

Keywords:

entomopathogenic fungi, isolation, biological control, forest, hazelnut, Black Sea

* Corresponding author: Funda ŞAHİN

funda.sahin@gop.edu.tr

ABSTRACT

A total of 250 soil samples were taken from the forest, hazelnut, kiwi, vegetable, and meadow-rangeland areas in the coastal regions of Ordu province, Turkey. Entomopathogenic fungi were isolated from these soil samples using the *Galleria*-bait method. Eighty-five fungal isolates were isolated from these soil samples, after which they were morphologically and molecularly identified. After morphological characterization, 64 out of 85 isolates were identified molecularly. Based on the molecular characterization results, twenty-three out of the 64 isolates were *Beauveria bassiana* (35.94%), 11 isolates were *Metarhizium brunneum* (17.19%), 8 isolates were *Metarhizium anisopliae* (12.5%), 6 isolates were *Metarhizium robertsii* (9.38%), 4 isolates were *Purpureocillium lilacinum* (6.25%), 4 isolates were *Clonostachys rogersoniana* (6.25%), 3 isolates were *Fusarium solani* (4.69%), 1 isolate was *Clonostachys rossmaniae* (1.56%), 1 isolate was *Aspergillus flavus* (1.56%), 1 isolate was *Cordyceps cicadae* (1.56%), 1 isolate was *Cordyceps fumosorosea* (1.56%), and 1 isolate was *Fusarium oxysporum* (1.56%). In the coastal area of Ordu province, the most common entomopathogen fungal genus is *Metarhizium* followed by *Beauveria bassiana*.

INTRODUCTION

Ordu is located in the Eastern Black Sea region of Turkey. It has a mild climate with an average temperature of 7 ℃ in January and 23.2 ℃ in July. The average annual temperature is 14.4 ℃, the highest temperature is 37.3 ℃, and the lowest is -7.2 ℃. It has a humid climate with cool winters and warm summers. The average annual precipitation is 1045.2 mm, and seasonal precipitation is observed in all months. Ordu has a coastline length of 100 km in the Black Sea. The annual drought index is very humid. The vegetation is classified as a very humid forest. Humid areas increase towards the East. The annual average relative humidity is 74.7%, the average seawater temperature is 15.4 ℃, and the average number of sunny days is 58 days. The northern part, which receives abundant rainfall, is quite rich in terms of vegetation compared to the southern (inner) part, where the continental climate is dominant. The forests, which cover the largest area with 202.893 hectares in the land distribution, constitute 34% of the land of Ordu. The hazelnut orchards, which constitute the most important product of the provincial economy, dominate the areas up to 800 m high from the coast. Beside the hazelnut orchards, there are

kiwi orchards, and field lands where corn, potatoes, beans, cabbage, and other field products are grown. There are also forests consisting of chestnut, alder, hornbeam, oak, beech, elm, and maple species. Areas over 1.000 meters form pastures and plateaus. In general, 43.9% of Ordu's land is agricultural, 33.8% is forestland, 8.4% is meadow-rangelands, and 13.9% is residential and non-agricultural land (Anonymous 2023).

It can be thought that the climatic characteristics of Ordu province may provide an advantage in biological control, which is one of the important branches of pest control, as well as agricultural production. Biological control is the use of living organisms/agents (fungi, bacteria, viruses, or insects) to suppress the population density or effect of the pest organism or to reduce its damage (Eilenberg et al. 2001). Entomopathogenic fungi (EPF), which constitute an important class of biological control agents, are used in the control of many pest groups. EPF infect their target host by cuticle penetrating and using their nutrient resources. This fungal attack causes mechanical damage to the tissues of the target host. EPF secrete enzymes, toxins, and secondary metabolites, which are host-directed during this process (Shin et al. 2013). In this way, they can directly kill the host or weaken the vital activities of the host (Kulkarni 2015).

The soil is an essential environment for EPF, and the majority of EPF species sustain in the soil (Abdullah et al. 2015, Majchrowska-Safaryan and Tkaczuk 2021). Although soil provides an excellent habitat for EPF, factors such as temperature, humidity, pH, and soil microbiota affect their survival and sustainability (Niu et al. 2019).

Abiotic factors, such as temperature and humidity, play an important role in the germination and development of fungal spores and can limit the pathogenicity of even a strong EPF under unsatisfied conditions. Mishra et al. (2015) reported that when *Beauveria bassiana* isolate was sprayed on the house flies under different temperature and humidity conditions, the highest mortality rate was observed to be 25-30 ℃ at 75-100% relative humidity, fungal growth slowed down at lower and higher temperatures, and the mortality decreased.

The presence and distribution of EPF in the soil can be affected ways differently by geographical location, habitat, soil type, and cultural practices (Vänninen 1996). Knowledge of the presence, distribution, and diversity of indigenous EPF species have importance in the biological control of insect pest populations (Meyling and Eilenberg 2006). The possibility of getting a successful mycoinsecticide that controls pest insects can enhance by choosing and testing various indigenous EPF isolates having different characteristics (Şahin and Yanar 2021). Based upon this idea, it was aimed to investigate the presence of EPF and their molecular identification in the soil samples taken from the coastal districts of the Ordu province in this study.

MATERIALS AND METHODS

Collection of soil samples

A total of 250 soil samples were collected from different sampling sites in fields, forests, and meadow-rangeland areas in Ünye, Fatsa, Perşembe, Center, and Gülyalı districts located in the coastal part of Ordu province during 2019 and 2020 (Figure 1). The areas to be sampled were determined using the random sampling method. Thus, 50 soil samples were obtained from each of the districts. Soil samples were taken from 5 different points at a depth of 0-20 cm with a shovel in ways that represent the entire field and mixed. Approximately 1 kg of soil taken from the mixture was placed in 25 x 42 cm polyethylene bags and labeled. GPS data, including altitude information, were recorded using a Magellan Explorist 310 (Magellan, Santa Clara, CA, USA) handheld GPS receiver.

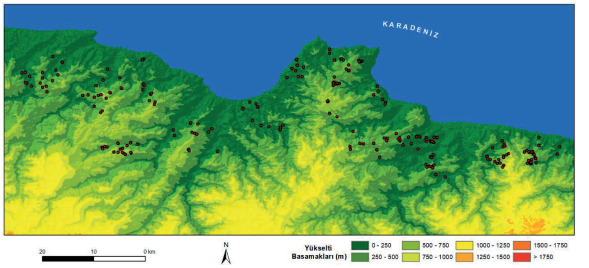

Figure 1. Distribution of soil sampling points in coastal part of Ordu province

Isolation of entomopathogenic fungi using the Galleria bait method

EPF was isolated using the "*Galleria bait method*" proposed by Zimmermann (1986). *Galleria mellonella* larvae were reared in closed double-layer glass jars with filter paper on the artificial diet by Han and Ehlers (2000) in the laboratory under 16 h light and 8 h dark conditions at 26 ℃. The larvae were soaked in 55 ℃ water for 5-10 s to reduce their silk webbing formation. Ten-fourth or fifth instar larvae of *G. mellonella* were baited in each soil and placed in a 90 mm glass Petri dish moistened with distilled water. The Petri dishes were kept at room temperature for 10-15 days. Soil moisture was kept to approximate field capacity by moistening it daily. Petri dishes were regularly turned upside down to ensure the larvae had contact with the soil. The infected insect cadavers were subjected to surface sterilization in 1% NaClO solution for 2-3 min, washed three times in sterile distilled water then the cross-sections from cadavers were placed on water agar (1.5% w/v). After mycelia had grown on the cadaver, they were transferred to PDA to get pure fungal culture. The isolates obtained were incubated at 25 ℃ for 15-30 days.

Colony morphology and spore structures of the isolates were evaluated according to the key described by Humber (1997), and isolates showing similar morphology were grouped at the genus level. Agar block smear preparation was used to examine the conidial structures of the fungi (Woo et al. 2010).

Fungal DNA extraction, polymerase chain reaction (PCR) and sequencing

Genomic DNA isolation of fungi was performed with a Turkuaz DNA purification kit (patent pending), adapted by Saygılı (2019) and Keskin et al. (2014). One hundred/one-hundred-fifty milligrams of mycelia were scraped from pure culture and taken into 1.5 ml microcentrifuge tubes. Liquid nitrogen was added to the tube and the tissues were crushed using a pipette tip. Two hundred-fifty microliters of 1X TE buffer was added and vortexed. Then, 500 µl TLB and 40 µl Proteinase K solution were added and mixed gently. The tubes were incubated at 65 °C water bath for 1 h. Seven hundred-fifty microliter of chloroform and isoamyl alcohol in a ratio 24:1 was added to the tubes and mixed thoroughly to form an emulsion. The mixed tubes were centrifuged at 12 000 rpm for 5 min. The supernatant was taken into a new tube and 500 µl of cold 2-propanol was added. After inverting the tubes for a few min, they were centrifuged at 12 000 rpm for 2 min, and the supernatant was discarded. Two microliters of RNase (10 mg/ml) and 24 µl of 5 M NaCl solution were added onto the pellets and incubated at 65 °C water bath for 30 min. The tubes were turned upside down after adding 750 µl of 96% cold ethanol to the melted pellet. Then, the DNA was precipitated by keeping the tubes at -20 °C for 10 min. The tubes were centrifuged at 12 000 rpm for 10 min after reaching room temperature then the liquids were discarded. The tubes were centrifuged at 12 000 rpm for 10 min, and the supernatant was discarded. The pellets were washed slowly with 70% cold ethanol, and they were dried at room temperature for 2 h. Genomic DNA isolation was performed by dissolving the dried pellets in 50 µl of 1X TE buffer. Resultant DNAs were electrophoresed in 1X TBE buffer on 1% agarose gel added 0.5 µg/ml ethidium bromide and checked with a UV imaging system (Vilber Lourmat CN-08). The displayed bands were recorded using the BioCapt v.11.02 program. Purified DNA was stored at -20 °C until PCR experiments. ITS (Internal Transcribed Spacer) and EF1-α (elongation factor 1-alpha) gene regions were preferred for the amplification of purified DNA. ITS amplification was achieved using the ITS5 (5'–GGAAGTAAAAGTCGTAACAAGG–3') / ITS4 (5'-TCCTCCGCTTATTGATATTGC–3') primers (White et al. 1990); EF1-α amplification was achieved using the 1567R (5'–ACHGTRCCRATACCACCSATCTT–3') and 1577F (5'–CARGAYGTBTACAAGATYGGTGG–3').primers (D'Alessandro et al. 2014). PCR reactions and conditions used were as described by Gül (2016).

The PCR products were subjected to single-direction sequence analysis by a commercial sequencing service (Atlas Biotechnologies Inc, Ankara, Turkey). Sequence chromatograms were arranged with MEGA X program and compared with NCBI BLAST (Basic Local Alignment Search Tool). Phylogenetic trees were created using the Maximum Likelihood method using BioEdit 7.2 and MEGA X programs to determine the genetic relationships between DNA sequences (Şahin and Yanar 2021).

RESULTS

The number of EPF obtained from soil samples taken from Ünye, Center, Merkez, Perşembe, and Gülyali districts of Ordu province are listed in Table 1. In total, 85 entomopathogenic fungi were isolated from 250 soil samples. The 85 isolates were identified based on morphological characteristics.

Morphological identification

The isolates were grouped to the genus level according to their colony morphology. Then, the isolate groups were classified by examining their spore structures using the agar block method under the microscope.

Beauveria spp.

This fungus group initially formed a white mycelium. Within 14-21 days, it generated white powdery spores on PDA media. The colony's color turned yellowish-white over time. Conidiophores were zigzag-shaped and transparent,

Table 1. The number of EPF obtained from soil samples taken from Ünye, Center, Merkez, Perşembe, and Gülyalı districts of Ordu province

Locality	The number of			
	Soil samples	Entomopathogenic fungi isolated	Presence of entomopathogenic fungi (%)	Isolates identified
Ünye	50	17	34	8
Fatsa	50	20	40	12
Merkez	50	14	28	10
Perşembe	50	25	50	25
Gülyalı	50	9	18	9
Total	250	85	34	64

Table 2. Species names, strains, gene regions, sampling data, GenBank accession numbers, and percent identity of the isolates identified

No	Strain no	Gene Region	Habitat	Altitude (m)	Sampling date	Species	Accession no	Percent Identity
1	ORU-11	ITS	Hazelnut Orchard	609	29.06.2019	*Beauveria bassiana*	MW410165	100%
2	ORU-21	ITS	Hazelnut Orchard	236	29.06.2019	*Beauveria bassiana*	MW410166	100%
3	ORU-23	ITS	Hazelnut Orchard	101	29.06.2019	*Beauveria bassiana*	MW410167	99.79%
4	ORU-25	ITS	Hazelnut Orchard	170	29.06.2019	*Metarhizium robertsii*	MW410168	100%
5	ORU-40	ITS	Hazelnut Orchard	580	30.06.2019	*Metarhizium robertsii*	MW410169	100%
6	ORU-50	ITS	Hazelnut Orchard	427	10.03.2020	*Beauveria bassiana*	MW410170	100%
7	ORF-3	ITS	Hazelnut Orchard	626	28.04.2019	*Beauveria bassiana*	MW410171	100%
8	ORF-8	ITS	Hazelnut Orchard	560	28.04.2019	*Beauveria bassiana*	MW410172	99.79%
9	ORF-9	ITS	Hazelnut Orchard	396	28.04.2019	*Beauveria bassiana*	MW410173	100%
10	ORF-11	ITS	Hazelnut Orchard	353	28.04.2019	*Beauveria bassiana*	MW410174	100%
11	ORF-17	ITS	Hazelnut Orchard	106	29.04.2019	*Beauveria bassiana*	MW410175	100%
12	ORF-22-a	ITS	Hazelnut Orchard	243	29.04.2019	*Beauveria bassiana*	MW410176	100%
13	ORF-23	ITS	Hazelnut Orchard	146	29.04.2019	*Beauveria bassiana*	MW410177	100%
14	ORF-25	ITS	Kiwi Orchard	6.6	29.04.2019	*Beauveria bassiana*	MW410178	100%
15	ORF-30	ITS	Hazelnut Orchard	426	7.03.2020	*Metarhizium brunneum*	MW410179	100%
16	ORF-42	ITS	Vegetable Garden	194	8.03.2020	*Beauveria bassiana*	MW410180	100%
17	ORF-43	ITS	Hazelnut Orchard	90	8.03.2020	*Beauveria bassiana*	MW410181	100%
18	ORM-8	ITS	Vegetable Garden	313	13.07.2019	*Metarhizium anisopliae*	MW410182	100%
19	ORM-14	ITS	Vegetable Garden	444	13.07.2019	*Aspergillus flavus*	MW410183	99.80%
20	ORM-21	ITS	Hazelnut Orchard	276	13.07.2019	*Purpureocillium lilacinum*	MW410184	100%
21	ORM-39	ITS	Hazelnut Orchard	175	10.03.2020	*Beauveria bassiana*	MW410185	100%
22	ORM-40	ITS	Vegetable Garden	83	10.03.2020	*Metarhizium brunneum*	MW410186	100%
23	ORM-45	ITS	Hazelnut Orchard	221	10.03.2020	*Beauveria bassiana*	MW410187	100%
24	ORM-47	ITS	Hazelnut Orchard	55	10.03.2020	*Metarhizium anisopliae*	MW410188	100%
25	ORM-48	ITS	Hazelnut Orchard	45	10.03.2020	*Clonostachys rogersoniana*	MW410189	99.57%
26	ORM-50	ITS	Hazelnut Orchard	88	10.03.2020	*Beauveria bassiana*	MW410190.1	100%
27	ORP-1	ITS	Hazelnut Orchard	243	23.11.2019	*Metarhizium brunneum*	MW410191.1	100%
28	ORP-2	ITS	Vegetable Garden	275	23.11.2019	*Metarhizium anisopliae*	MW410192.1	99.34%
29	ORP-4	ITS	Vegetable Garden	344	23.11.2019	*Metarhizium robertsii*	MW410193.1	99.78%
30	ORP-9	ITS	Vegetable Garden	289	23.11.2019	*Cordyceps cicadae*	MW410194.1	99.39%
31	ORP-13	ITS	Hazelnut Orchard	467	23.11.2019	*Metarhizium brunneum*	MW410195.1	99.78%
32	ORP-14	ITS	Hazelnut Orchard	392	23.11.2019	*Metarhizium brunneum*	MW410196.1	99.78%
33	ORP-15	ITS	Hazelnut Orchard	371	23.11.2019	*Beauveria bassiana*	MW410197.1	100%
34	ORP-16	ITS	Forestland	402	23.11.2019	*Metarhizium robertsii*	MW410198.1	98.01%
35	ORP-17	ITS	Forestland	351	23.11.2019	*Metarhizium brunneum*	MW410199.1	99.78%
36	ORP-18	ITS	Hazelnut Orchard	344	23.11.2019	*Metarhizium brunneum*	MW410200.1	97.57%
37	ORP-22	ITS	Hazelnut Orchard	472	24.11.2019	*Metarhizium brunneum*	MW410201.1	98.67%
38	ORP-24	ITS	Forestland	462	24.11.2019	*Beauveria bassiana*	MW410202.1	100%
39	ORP-26	EF1-α	Hazelnut Orchard	675	24.11.2019	*Purpureocillium lilacinum*	MW464658.1	100%
40	ORP-27	ITS	Vegetable Garden	683	24.11.2019	*Metarhizium brunneum*	MW410203.1	99.56%
41	ORP-29	ITS	Hazelnut Orchard	523	24.11.2019	*Clonostachys rogersoniana*	MW410204.1	100%
42	ORP-30	ITS	Hazelnut Orchard	456	24.11.2019	*Metarhizium anisopliae*	MW410205.1	94.03%
43	ORP-34-a	ITS	Hazelnut Orchard	221	24.11.2019	*Cordyceps fumosorosea*	MW410206.1	99.39%
44	ORP-34-b	ITS	Hazelnut Orchard	221	24.11.2019	*Metarhizium brunneum*	MW410207.1	94.69%
45	ORP-35	ITS	Forestland	30	24.11.2019	*Beauveria bassiana*	MW410208.1	100%
46	ORP-36	EF1-α	Hazelnut Orchard	53	24.11.2019	*Purpureocillium lilacinum*	MW464659.1	100%
47	ORP-37	ITS	Hazelnut Orchard	101	24.11.2019	*Metarhizium brunneum*	MW410209.1	96.46%
48	ORP-39	ITS	Forestland	33	24.11.2019	*Metarhizium anisopliae*	MW410210.1	98.67%
49	ORP-40	ITS	Forestland	23	24.11.2019	*Metarhizium anisopliae*	MW410211.1	94.91%
50	ORP-46	ITS	Hazelnut Orchard	206	9.03.2020	*Beauveria bassiana*	MW410212.1	100%
51	ORP-48	ITS	Meadow-Rangelands	151	9.03.2020	*Metarhizium robertsii*	MW410213.1	94.47%
52	ORG-1	ITS	Hazelnut Orchard	147	11.03.2020	*Beauveria bassiana*	MW410214.1	100%
53	ORG-2	ITS	Kiwi Orchard	303	11.03.2020	*Beauveria bassiana*	MW410215.1	100%
54	ORG-5	ITS	Hazelnut Orchard	296	11.03.2020	*Clonostachys rogersoniana*	MW410216.1	99.36%
55	ORG-6	ITS	Hazelnut Orchard	299	11.03.2020	*Metarhizium anisopliae*	MW410217.1	99.34%
56	ORG-21	ITS	Hazelnut Orchard	314	11.03.2020	*Metarhizium anisopliae*	MW410218.1	99.56%
57	ORG-24	ITS	Hazelnut Orchard	199	11.03.2020	*Clonostachys rossmaniae*	MW410219.1	99.36%
58	ORG-35	ITS	Hazelnut Orchard	401	12.03.2020	*Clonostachys rogersoniana*	MW410220.1	99.57%
59	ORG-42	ITS	Vegetable Garden	472	12.03.2020	*Metarhizium robertsii*	MW410221.1	96.46%
60	ORG-48	EF1-α	Kiwi Orchard	151	12.03.2020	*Purpureocillium lilacinum*	MW464660.1	100%
61	ORM-7	ITS	Hazelnut Orchard	299	13.07.2019	*Fusarium solani*	MW410222.1	100%
62	ORU-10	ITS	Hazelnut Orchard	476	29.06.2019	*Fusarium solani*	MW410223.1	100%
63	ORU-39	ITS	Hazelnut Orchard	477	30.06.2019	*Fusarium solani*	MW410224.1	100%
64	ORF-22-b	ITS	Hazelnut Orchard	243	29.04.2019	*Fusarium oxysporum*	MW410225.1	99.78%

and their base parts were swollen. Conidia were 1.5-3 µm in size, transparent, spherical, and densely clustered.

Metarhizium spp.

Mycelia, which initially started to form in white color, turned yellowish-green in a few days. The colony morphologies, spore development and colors differed according to the isolates. Spores were dark green and clustered on white mycelium. There were vertically branched conidiophores and Penicillium-like phialides. The conidia were cylindrical, average 5-7 µm long, 2-3 µm wide, and were densely clustered or chained at the ends of the phialides.

Purpureocillium spp.

Mycelia, which initially started to form in white color on PDA medium, turned into a purplish-gray color in a few days. Purple-violet powdery sporulation was seen on the mycelium over time. Conidiophores had slender phialides, and the conidia extended in the form of chains at the ends of these phialides. Conidia were transparent, spherical, and approximately 2.5 x 2 µm in size.

Clonostachys spp.

Mycelia grew as white concentric circles and formed reddish-orange sporulation on PDA medium. Reddish-orange pigment formation was also observed on the colony reverse. Conidia were clustered at the tip of the phialides of the verticillate conidiophore. Their conidial structures were elliptical and narrower at one end than the other. They were approximately 3-7 x 1-2 µm in size. The fungus generated chlamydospores which are 4-7 x 3-6 µm in size, larger than the conidia.

Fusarium spp.

Mycelia, which were initially white-colored overhead, turned reddish-orange, yellowish, or purplish depending on their species on PDA media in the following days. Chlamydospores were occasionally seen on the mycelium. The macroconidia formed on the sporodochia were pointed sickle-shaped and had 3-6 septa. Microconidia varied in shape from elliptical to cylindrical, septate to aseptate.

Aspergillus spp.

Brownish sporulation generated on white mycelium on PDA medium. The vesicles at the ends of the conidiophores had phialides, and the conidia extended in short chains at the ends of these phialides. The spherical conidia were approximately 2-3 x 2-2.5 µm in size.

Molecular identification

Twenty-five of these fungi were morphologically similar to *Fusarium* spp. The use of entomopathogenic *Fusarium* isolates in biological control is still debated because they are weak pathogens, saprophytic strains are high on cadavers, they have the potential to be plant pathogens, and they can produce mycotoxins (Teetor-Barsch and Roberts 1983). Due to these disadvantages, only 4 representatives of *Fusarium isolates*, which are thought to have different morphological characters, were selected for molecular diagnosis.

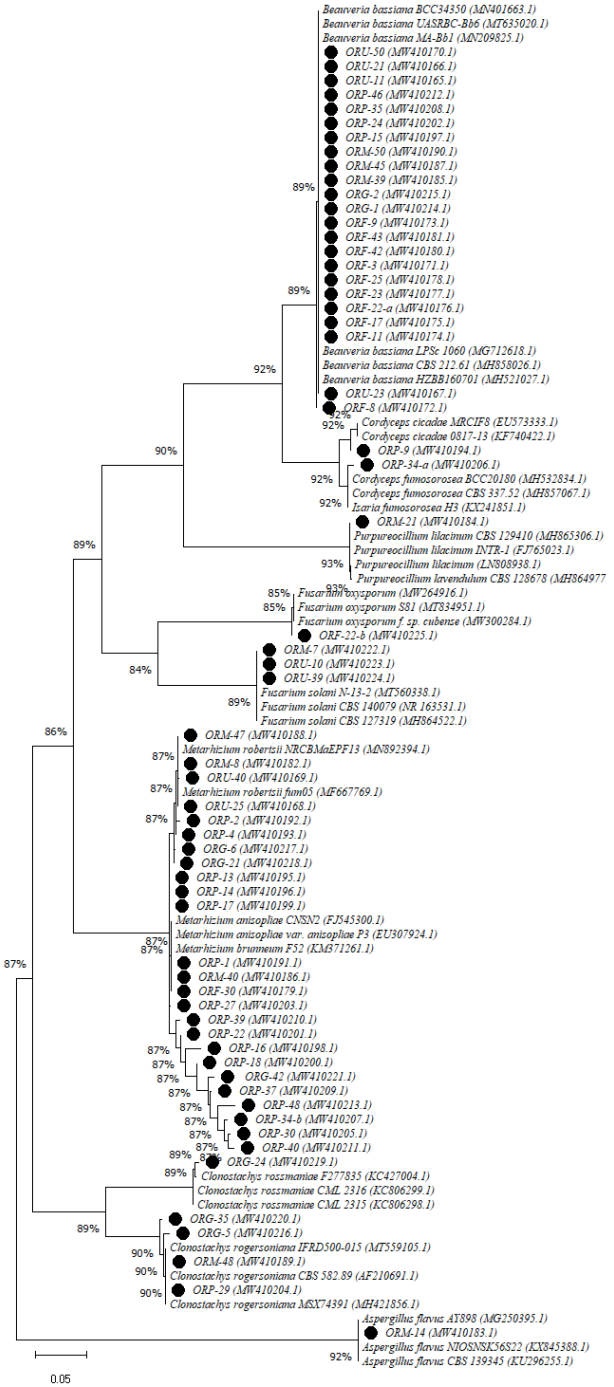

Figure 2. Phylogenetic tree of isolates identified by amplifying the ITS4-5.8S-ITS5 gene region

Bands with a size of approximately 600 bp were obtained in the PCR products obtained from the ITS gene region, and approximately 700 bp in the PCR products were obtained from the EF1-α gene region. One-way sequence analysis results were arranged with the MEGA X program and compared with other sequences registered in the GenBank database using the BLAST tool. The names of the species identified according to the percent identity as a result of the comparison and the recorded accession numbers are given in Table 2. Accordingly, 64 out of 23 isolates were *Beauveria bassiana* (35.94%), 11 isolates were *Metarhizium brunneum* (17.19%), 8 isolates were *Metarhizium anisopliae* (12.5%), 6 isolates were *Metarhizium robertsii* (9.38%), 4 isolates were *Purpureocillium lilacinum* (6.25%), 4 isolates were *Clonostachys rogersoniana* (6.25%), 3 isolates were *Fusarium solani* (4.69%), 1 isolate was *Clonostachys rossmaniae* (1.56%), 1 isolate was *Aspergillus flavus* (1.56%), 1 isolate was *Cordyceps cicadae* (1.56%), 1 isolate was *Cordyceps fumosorosea* (1.56%) and 1 isolate was *Fusarium oxysporum* (1.56%).

Phylogenetic similarities between the species identified by amplifying the ITS4-5.8S-ITS5 region were determined by reference to other species in the GenBank database, and the dendrogram is shown in Figure 2. The similarity rate between taxa is above the branches. Accordingly, it was observed that ORU-23 and ORF-8 isolates among B. bassiana species were more similar to each other than other Beauveria spp., and other B. bassiana species were also highly similar. It was determined that *Metarhizium* spp. showed more diversity among each other compared to other species. The affinities in the remaining species were among themselves at varying rates.

DISCUSSION AND CONCLUSION

EPF isolated from the soil of a region is more effective in the management of local pests in that region (Liu et al. 2021). EPF isolation is relatively affected by geographic regions. This may be related to changes in the climatic conditions of geographic areas, agricultural practices, or sampling timing (Ali-Shtayeh et al. 2002). The soil environment is usually the classical isolation zone for EPF species in the Hypocreales, and various EPF species can be found in both arable soils and more natural environments (Meyling and Eilenberg 2007). Keller et al. (2003) reported that *M. anisopliae* is common in arable soils and meadows with higher density. While Vänninen (1996) reported that *M. anisopliae* was isolated more frequently from the southern parts of Finland, and tillage did not adversely affect the isolation of this fungus. Steenberg (1995) suggested that in Danish soil, *M. anisopliae* is more common in open areas than in shady habitats (Meyling and Eilenberg 2007). Similarly, Bidochka et al. (1998) reported that *M. anisopliae* is more common in agricultural areas compared to forests in Canada, and *B. bassiana* is mostly found in shady and natural habitats such as forests. Contrary to these studies, Meyling and Eilenberg (2006) found that *B. bassiana* was also frequently seen in agricultural soil in a part of Denmark. Also Mietkiewski et al. (1997) found that *B. bassiana* was the dominant species in arable land. Gebremariam (2021) emphasized that the difference in fungal species and the number isolated between cultivated and uncultivated soils may be caused by the insect host, soil structure, shading area that protects the region from UV radiations, and any pesticide application. In the current study, based on the results of molecular identification, no clear relationship was found between the entomopathogen fungi species and the sampling habitat. While different entomopathogenic fungal species are encountered in hazelnut orchards, only *B. bassiana* and *Metarhizium* spp. was obtained from forest soil; *B. bassiana* and *P. lilacinum* were obtained from kiwi orchards, *B. bassiana*, *Metarhizium* spp, and *C. cicadae* species were obtained from vegetable fields isolate. Sevim et al. (2010) conducted an EPF survey on hazelnut-growing areas of the Black Sea region, including Ordu, and determined 3 different species of EPF, which *M. anisopliae*, *B. bassiana*, *I. fumosorosea*, and *Evlachovaea* spp. from 301 soil samples. The sampling location involved hazelnut, meadow, tea, vegetable, apple, poplar, and oak vegetation. *M. anisopliae* was the most commonly detected fungus in all vegetations, which were mainly tea and hazelnut, followed by *B. bassiana.* The fact remains that *I. fumosorose*a was only isolated in agricultural fields. Similarly, *Cordyceps* (=*Isaria*) species detected in the present study were isolated from hazelnut and vegetable vegetation. On the contrary, Vänninen (1996) asserted that *I. fumosorosea* occurred only in natural habitats and was never isolated from intensively cultivated soil. Ali-Shtayeh et al. (2002) defended that EPF isolation and diversity were not significantly affected by soil pH and chemical characteristics. In conclusion, entomopathogenic fungi species diversity is not clarified yet, so further study will be conducted to determine the interaction between soil chemical structure and entomopathogen fungi species diversity.

This study contributes to the understanding relationship between the natural distribution and vegetation of EPF and increases the number of EPF species previously described in the Ordu province of the Black Sea Region of Turkey. The European Council Farm to Fork Strategy, which was published recently, targeted removing especially more toxic synthetic pesticides up to 50% of pesticides markets by 2030. So biological control agents come forward, and their use in pest management programs will be increased for

the establishment of agricultural sustainability (European Commission 2021).

ACKNOWLEDGEMENTS

We acknowledge Tokat Gaziosmanpaşa University Scientific Research Projects Coordination for financial support.

This article is prepared from a part of the Master of Science thesis of Funda ŞAHİN submitted to the Graduate School of Natural and Applied Sciences, Tokat Gaziosmanpaşa University, Türkiye.

This research was presented as an "Oral Presentation" at 2nd International Molecular Plant Protection Congress in 2023.

ÖZET

Ordu ili kıyı bölgelerindeki orman, fındık, kivi, sebze ve çayır-mera alanlarından toplam 250 adet toprak örneği alınmıştır. Bu toprak örneklerinden *Galleria*-tuzak yöntemi kullanılarak entomopatojen funguslar izole edilmiştir. İzolasyonlar sonucunda 85 fungal izolat elde edilmiştir. Morfolojik karakterizasyondan sonra 85 izolattan 64'ü moleküler olarak tanımlanmıştır. Moleküler karakterizasyon sonuçlarına göre 64 izolattan 23'ü *Beauveria bassiana* (%35.94), 11 izolat *Metarhizium brunneum* (%17.19), 8 izolat *Metarhizium anisopliae* (%12.5), 6 izolat *Metarhizium robertsii* (%9.38), 4 izolat *Purpureocillium lilacinum* (%6.25), 4 izolat *Clonostachys rogersoniana* (%6.25), 3 izolat *Fusarium solani* (%4.69), 1 izolat *Clonostachys rossmaniae* (%1.56), 1 izolat *Aspergillus flavus* (%1.56), 1 izolat *Cordyceps cicadae* (%1.56), 1 izolat *Cordyceps fumosorosea* (%1.56) ve 1 izolat *Fusarium oxysporum* (%1.56) idi. Ordu ilinin kıyı kesimlerinde en yaygın entomopatojen mantar türü *Metarhizium*'dur ve bunu *Beauveria bassiana* izlemektedir.

Anahtar kelimeler: entomopatojen fungus, izolasyon, biyolojik mücadele, orman, fındık, Karadeniz

REFERENCES

Abdullah S.K., Mustafa R.A., Assaf L.H., 2015. Isolation of entomopathogenic and opportunistic fungi from soil in Duhok province, Kurdistan region of Iraq by different selective isolation media. Journal of Biology, Agriculture, and Healthcare, 5, 73-79.

Ali-Shtayeh M.S., Mara'i A.B.B., Jamous R.M., 2003. Distribution, occurrence ,and characterization of entomopathogenic fungi in agricultural soil in the Palestinian area. Mycopathologia, 156 (3), 235-244. https://doi.org/10.1023/a:1023339103522

Anonymous, 2023. General statistical data of the cities https://www.mgm.gov.tr/veridegerlendirme/il-ve-ilceler-istatistik.aspx?k=A&m=ORDU (accessed date: 29.08.2023).

Bidochka M.J., Kasperski J.E., Wild G.A., 1998. Occurrence of the entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana* in soils from temperate and near-northern habitats. Canadian Journal of Botany, 76 (7), 1198-1204. https://doi.org/10.1139/b98-115

D'Alessandro C.P., Jones L.R., Humber R.A., López Lastra C.C., Sosa-Gomez D.R., 2014. Characterization and phylogeny of *Isaria* spp. strains (Ascomycota: Hypocreales) using ITS1-5.8S-ITS2 and elongation factor 1-alpha sequences. Journal of Basic Microbiology, 54, 21-31. https://doi.org/10.1002/jobm.201300499

Eilenberg J., Hajek A., Lomer C., 2001. Suggestions for unifying the terminology in biological control. BioControl, 46 (4), 387-400. https://doi.org/10.1023/A:1014193329979

European Commision 2021. Farm to fork targets – progress. https://food.ec.europa.eu/plants/pesticides/sustainable-use-pesticides/farm-fork-targets-progress_en

Gebremariam A., Chekol Y., Assefa F., 2021. Phenotypic, molecular, and virulence characterization of entomopathogenic fungi, *Beauveria bassiana* (Balsam) Vuillemin, and *Metarhizium anisopliae* (Metschn.) Sorokin from soil samples of Ethiopia for the development of mycoinsecticide. Heliyon, 7 (5), e07091. https://doi.org/10.1016/j.heliyon.2021.e07091

Gül E., 2016. Morphological and molecular identification of entomopathogenic fungi isolated from sunn pests (*Eurygaster* spp.) and determination of their pathogenicity. Ankara University, Doctoral dissertation, Ankara, 70 p.

https://dergiler.ankara.edu.tr/xmlui;/bitstream/handle/20.500.12575/84201/434744.pdf?sequence=1&isAllowed=y

Han R., Ehlers R.U., 2000. Pathogenicity, development, and reproduction of *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* under axenic in vivo conditions. Journal of Invertebrate Pathology, 75 (1), 55-58. https://doi.org/10.1006/jipa.1999.4900

Humber R.A., 1997. Fungi: identification. Manual of Techniques in Insect Pathology, 153-185. https://doi.org/10.1016/B978-012432555-5/50011-7

Keller S., Kessler P., Schweizer C., 2003. Distribution of insect pathogenic soil fungi in Switzerland with special reference to *Beauveria brongniartii* and *Metarhizium anisopliae*. BioControl, 48, 307-319. https://doi.org/10.1023/A:1023646207455

Keskin A., Koprulu T.K., Bursali A., Ozsemir A.C., Yavuz K.E. Tekin S., 2014. First record of *Ixodes arboricola* (Ixodida: Ixodidae) from Turkey with presence of *Candidatus Rickettsia* vini (Rickettsiales: Rickettsiaceae). Journal of Medical Entomology, 51, 864-7. https://doi.org/10.1603/ME13169

Kulkarni S.A., 2015. Biochemical and molecular studies of chitin deacetylase from Metarhizium species. Savitribai Phule Pune University, Doctoral dissertation, Pune, India. http://ndl.iitkgp.ac.in/document/d2lzYlFSMWhDSTFQNHRvOGtpVmhEZDJDZVBuRDVOWlBTTTFDdmV1Q3 lrYz

Liu Y.C., Ni N.T., Chang J.C., Li Y.H., Lee M.R., Kim J.S., Nai Y.S., 2021. Isolation and selection of entomopathogenic fungi from soil samples and evaluation of fungal virulence against insect pests. Journal of Visualized Experiments, 175, e62882. https://doi.org/ 10.3791/62882

Majchrowska-Safaryan A., Tkaczuk C., 2021. Abundance of entomopathogenic fungi in leaf litter and soil layers in forested habitats in Poland. Insects, 12 (2), 134. https://doi.org/10.3390/insects12020134

Meyling N.V., Eilenberg J., 2006. Isolation and characterization of Beauveria bassiana isolates from phylloplanes of hedgerow vegetation. Mycological Research, 110 (2), 188-195. https://doi.org/10.1016/j.mycres.2005.09.008

Meyling N.V., Eilenberg J., 2007. Ecology of the entomopathogenic fungi *Beauveria bassiana* and Metarhizium anisopliae in temperate agroecosystems: potential for conservation biological control. Biological control, 43 (2), 145-155. https://doi.org/10.1016/j.biocontrol.2007.07.007

Mietkiewski R.T., Pell J.K., Clark S.J., 1997. Influence of pesticide use on the natural occurrence of entomopathogenic fungi in arable soils in the UK: field and laboratory comparisons. Biocontrol Science and Technology, 7 (4), 565-576. https://doi.org/10.1080/09583159730622

Mishra S., Kumar P., Malik A., 2015. Effect of temperature and humidity on pathogenicity of native Beauveria bassiana isolate against Musca domestica L. Journal of Parasitic Diseases, 39 (4), 1-8. https://doi.org/10.1007/s12639-013-0408-0

Niu X., Xie W., Zhang J., Hu Q., 2019. Biodiversity of entomopathogenic fungi in the soils of South China. Microorganisms, 7 (9), 311. https://doi.org/10.3390/microorganisms7090311

Saygılı İ., 2019. Intogression of barley drought tolerance QTL using marker-assisted selection. Tokat Gaziosmanpaşa University, Doctoral Dissertation, 120 p., Tokat. https://acikbilim.yok.gov.tr/bitstream/handle/20.500.12812/684229/yokAcikBilim_10237873.pdf?sequence=-1&isAllowed=y

Sevim A., Demir I., Höfte M., Humber R.A., Demirbag Z., 2010. Isolation and characterization of entomopathogenic fungi from hazelnut-growing region of Turkey. Biocontrol, 55, 279-297. https://doi.org/10.1007/s10526-009-9235-8

Shin T.Y., Ko S.H., Lee W.W., Bae S.M., Choi J.B., Woo S.D., 2013. Screening and evaluation of antibacterial metabolites from entomopathogenic fungi. International Journal of Industrial Entomology, 26 (2), 89-94. https://doi.org/10.7852/ijie.2013.26.2.89

Steenberg T., 1995. Natural occurrence of Beauveria bassiana (Bals.) Vuill. with focus on infectivity to Sitona species and other insects in lucerne. Royal Veterinary and Agricultural University, Unpublished doctoral dissertation, Copenhagen, Denmark, 126 p.

Şahin F., Yanar Y. 2021. Pathogenicity of some local entomopathogenic fungus isolates on the cotton leafworm larvae, Spodoptera littoralis (Boisd.) (Lepidoptera: Noctuidae). Egyptian Journal of Biological Pest Control, 31, 1-6. https://doi.org/10.1186/s41938-021-00494-3

Teetor-Barsch G.H., Roberts D.W., 1983. Entomogenous Fusarium species. Mycopathologia, 84 (1), 3-16. https://doi.org/10.1007/BF00436991

White T.J., Bruns T., Lee S.J.W.T., Taylor J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR Protocols: A guide to methods and applications, 18 (1), 315-322. http://dx.doi.org/10.1016/B978-0-12-372180-8.50042-1

Woo P.C., Ngan A.H., Chui H.K., Lau S.K., Yuen K.Y., 2010. Agar block smear preparation: a novel method of slide preparation for preservation of native fungal structures for microscopic examination and long-term storage. Journal of Clinical Microbiology, 48 (9), 3053-3061. https://doi.org/10.1128/JCM.00917-10

Vänninen I., 1996. Distribution and occurrence of four entomopathogenic fungi in Finland: effect of geographical location, habitat type and soil type. Mycological Research, 100 (1), 93-101. https://doi.org/10.1016/S0953-7562(96)80106-7

Zimmermann G., 1986. The 'Galleria bait method' for detection of entomopathogenic fungi in soil. Journal of applied Entomology, 102 (1-5), 213-215. https://doi.org/10.1111/j.1439-0418.1986.tb00912.x

Cite this article: Şahin, F. & Yanar, Y. (2023). Isolation and identification of entomopathogenic fungi from coastal districts of Ordu province, Türkiye. Plant Protection Bulletin, 63-3. DOI: 10.16955/bitkorb.1296436

Atıf için: Göze Özdemir, F. G. (2023). Ordu ili kıyı ilçelerinden entomopatojen fungusların izolasyonu ve tanımlanması. Bitki Koruma Bülteni, 63-3. DOI: 10.16955/bitkorb.1296436

Bitki Koruma Bülteni / Plant Protection Bulletin

http://dergipark.gov.tr/bitkorb

Original article

Important insect pests in winter vegetables grown in Beydere Seed Certification Test Directorate

Beydere Tohum Sertifikasyon Test Müdürlüğünde yetiştirilen kışlık sebzelerde görülen önemli zararlı böcek türleri

Fatih YILDIZ[a]*, Erol YILDIRIM[b]

[a]https://orcid.org/0009-0002-5371-8561, [b]https://orcid.org/0000-0002-3509-425X

[a]*Directorate of Horticultural Research Institute, 24060, Erzincan, Türkiye*
[b]*Atatürk University, Agriculture Faculty, Department of Plant Protection, 25240, Erzurum, Türkiye*

ARTICLE INFO

Article history:
DOI: 10.16955/bitkorb.1274312
Received : 31-03-2023
Accepted : 11-08-2023

Keywords:
winter vegetable pests, Brassicaceae, winter vegetables, Manisa, Türkiye

* Corresponding author: Fatih YILDIZ
fatihyildiz2425@gmail.com

ABSTRACT

This study was carried out in Beydere Seed Certification Test Directorate (Manisa) between 2016-2018 with the aim of detecting important insect pests species in winter-grown vegetables such as artichoke, cauliflower, broccoli, spinach, cress, lettuce, parsley, arugula, dill, beet, carrot, white cabbage, red cabbage and Brussels sprouts. In the result of study, as insect species *Gryllotalpa gryllotalpa* (Linnaeus) (Orthoptera: Gryllotalpidae), *Brevicoryne brassicae* (Linnaeus) (Hemiptera: Aphididae), *Eurydema ornata* (Linnaeus) (Hemiptera: Pentatomidae), *Sphaeroderma rubidum* (Graells), *Phyllotreta* sp. and *Cassida rubiginosa*, (Müller) (Coleoptera: Chrysomelidae), *Mamestra brassicae* (Linnaeus) (Lepidoptera: Noctuidae), *Hellula undalis* (Fabricius) (Lepidoptera: Pyralidae), *Phragmacossia albida* (Erschoff) (Lepidoptera: Cossidae), *Pieris brassicae* (Linnaeus), *Pieris napi* (Linnaeus) and *Pieris rapae* (Linnaeus) (Lepidoptera: Pieridae), *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae) and *Delia radicum* (Linnaeus) (Diptera: Anthomyiidae) were determined. Among them, the most important pests species were determined as *B. brassicae*, *E. ornata*, *P. brassicae* and *D. radicum*. It was determined that these pests species caused intense damage in head (white-red) cabbage, but the density was low in broccoli.

INTRODUCTION

Compared to many countries, Turkey has a very rich fauna and flora in its climate zone and is among the important countries of the world in terms of natural richness. In terms of vegetable growing, it is among the most important vegetable producer countries in the world and Europe in terms of both the number of species and varieties and the amount of production (Yanmaz et al. 2015).

Red, white and brussels sprouts, cauliflower, broccoli, arugula, cress, from leafy vegetables lettuce, cabbage, spinach, chard, purslane, edible vegetables, tomato, pepper, eggplant, cucumber, pumpkin, melon, watermelon, flower and flower table edible vegetables, cauliflower, broccoli, artichoke, okra, fragrant herbs, parsley, dill, mint, cress, arugula, sorrel and many other wild and other vegetable

species are grown in Turkey (Faydaoğlu and Sürücüoğlu 2011). It is stated that there are many pests and beneficial species in vegetable growing areas, and the pests do not only harm the vegetables, but also negatively affect the economy by reducing the market value of the products. As in cultivated plants, it is of great importance to detect and management diseases and pests with an appropriate method, together with fertilization, irrigation and other agricultural techniques, in increasing the yield of Cruciferae vegetables (Tozlu et al. 2002).

Vegetable production can be increased in various ways. One of them, to obtain more and quality products from the existing area, it is the correct and timely application of management methods against diseases and pests that cause quality and quantity losses in vegetables. The way to achieve this is possible with full knowledge of diseases and pests. No study has been carried out on pest species found in winter vegetable species in the area where the study was conducted. The aim of this study is to determine pest species and their density in winter vegetable species, and to obtain some information about pests species by making some biological observations.

MATERIALS AND METHODS

The material of the study, it was consisted of samples of pest species in winter vegetables grown in Beydere Seed Certification Test Directorate (Figure 1) located in Selimşahlar neighborhood of Şehzadeler district of Manisa province.

Figure 1. Study area in Beydere Seed Certification Test Directorate (Latitude 27.511536º E Longitude 38.732966º N)

Collection of material

In order to determine the harmful insect species in the study area, weekly surveys were carried out and pest species were collected in artichoke (*Cynara scolymus*), cauliflower (*Brassica oleracea* var. *botrytis*), broccoli *(Brassica oleracea* var. *italica*), spinach (*Spinacia oleracea*), cress (*Lepidium sativum*), lettuce (*Lactuca sativa*), parsley (*Petroselinum crispum*), arugula (*Eruca vesicaria*), dill (*Anethum graveolens*), beet (*Beta vulgaris*), carrot (*Daucus carota* subsp. *sativus*), white cabbage (*Brassica oleracea* var. *capitate*), red cabbage (*Brassica oleracea* var. *capitata f. rubra*) and Brussels sprouts (*Brassica oleracea* var. *gemmifera*) during the winter vegetable production vegetation in 2016-2018 (January-December). For this purpose, the root collar, stem, branch, shoot, leaves, flowers and fruits of the plants were visually examined from each vegetable field cultivated, and samples belonging to the adult and pre-adult periods were taken.

Coordinates and heights of the study areas were determined by using the GPS (Global Positioning Systems) device. In addition, the host plant information from which the samples collected was recorded. To the collection of samples sweep net, visual control, leaf counting and culturing methods were used in the study.

Sweeping net method

This method was used to capture motile or low-motility species in vegetable production areas and to determine their population densities. In this context, starting from the inside of each trial area, a total of 50 sweep nets, 10 of them, were shaken by contacting randomly and sweeping the plants in every 15-20 steps in the direction of the diagonal by contacting the plants in a way that sweeps the plants (Anonymous 2023a, Kaya and Kornoşor 2008). Pests caught in the sweep net were put in kill bottles or in transparent polyethylene bags, killed with a few drops of ethyl acetate dripped on blotting paper, and brought to the laboratory in an ice container with the label containing the collection information (Ölmez et al. 2021). The samples were classified at the order and family, and after they were labeled appropriately, they were identified.

Some insects that did not come to the sweep net were taken from the plants using a sable brush and aspirator, and the necessary information was recorded. In the observations made in the field, yellowing, wilting, drying or the underground and above-ground parts of the plants that were eaten by the pest and the soil or weeds around it were also examined, and it was investigated whether there were harmful insects.

Visual control method

This method was used to determine the population densities of some pest species in the experimental areas from the beginning of flowering during the vegetation period. For this purpose, the surveyed fields were visited once a week, each field was entered in the direction of the diagonals, the plants examined according to the size of the field was chosen randomly, all parts of the selected plants (stem, branch, shoot, leaf, fruit, etc.) were checked. In large-leaf lettuce and spinach, 10-20 plants in 100-1000 m^2, and in small-leaved parsley, arugula, cress, dill, mint, basil and purslane, 50 plants in 100-1000 m2 were examined (Anonymous 2023b). While sampling, the plants were checked one by one and the

pests obtained were taken into the killing bottle. Then, the insects were taken from the killing bottles with the help of a mouth aspirator and put into plastic-lidded boxes with label information.

Cultivation method

Pre-adult specimens such as larvae and pupae in the survey areas were cut together with the plant organ they were in or taken with the help of a forceps, and put together with their label information in plastic storage boxes that are suitable for air intake and adult emergence was achieved by culturing in the laboratory (at 25 ± 1 °C, 60% ± 10% proportional humidity, 16 hours of light and 8 hours of dark). The cultured samples were checked daily, and the nutrients in the culture boxes were replaced with new ones as long as the feeding process of the larvae continued (Kaya and Kornoşor 2008). Adult individuals of the pest species obtained from the culture were prepared in accordance with the diagnosis together with the label containing the collection information and separated according to their families.

Preparation of samples

Adult specimens collected in the survey areas were brought to the laboratory and separated from plant particles, and small-sized adults were glued on triangular papers, and large-sized individuals were pinned directly. From the pre-adult periods, firstly, adults were obtained, and then sticking and pinning processes were started. Water-soluble glue (glotofix) was used as the adhesive material. Care was taken not to damage the parts of the insect body used as a diagnostic character while pinning and sticking on triangular cardboard, and samples made ready for diagnosis. The species were identified by Erol Yıldırım by looking at the diagnosed specimens in Atatürk University, Faculty of Agriculture, Department of Plant Protection Entomology Museum.

RESULTS AND DISCUSSION

This study was carried out between 2016-2018 to determine the pest species seen in the vegetables artichoke, cauliflower, broccoli, spinach, cress, lettuce, parsley, arugula, dill weed, beet, white cabbage, red cabbage, and Brussels sprouts produced in Beydere Seed Certification Test Directorate in

Table 1. Orders, families and host plant species of the species determined in the study

Order	Family	Species	Host plant species
Orthoptera	Gryllotalpidae	*Gryllotalpa gryllotalpa* (Linnaeus)	Brassica oleracea var. capitata f. alba, B. oleracea var. capitata f. rubra, B. oleracea gemmifera, B. oleracea var. botrytis L., B. oleracea var. italica Plenck
Hemiptera	Aphididae	*Brevicoryne brassicae* (Linnaeus)	B. oleracea var. capitata f. alba, B. oleracea var. capitata f.rubra, B. oleracea gemmifera, B. oleracea var. botrytis L., B. oleracea var. italica Plenck, Sinapis arvensis L.
	Pentatomidae	*Eurydema ornata* (Linnaeus)	B. oleracea var. capitata f. alba, B. oleracea var. capitata f.rubra, B. oleracea var. botrytis L., B. oleracea var. italica Plenck
		Sphaeroderma rubidum (Graells)	Cynara scolymus L.
Coleoptera	Chrysomelidae	*Phyllotreta* sp.	B. oleracea var. capitata f. alba, B. oleracea var. botrytis L., B. oleracea var. italica Plenck, Wild crucifers
		Cassida rubiginosa O.F. Muller	Cynara scolymus L., Beta vulgaris L., Spinacia oleracea L.
Lepidoptera	Noctuidae	*Mamestra brassicae* (Linnaeus)	B. oleracea var. capitata f. alba, B. oleracea var. botrytis L., B. oleracea var. italica Plenck, B. oleracea gemmifera, Spinacia oleracea L., Lactuca sativa var. capitata L.
	Pyralidae	*Hellula undalis* (Fabricius)	B. oleracea var. capitata f. alba, B. oleracea var. capitata f. rubra, B. oleracea var. botrytis L.
	Cossidae	*Phragmacossia albida* (Erschoff)	Cynara scolymus L.
	Pieridae	*Pieris brassicae* (Linnaeus)	B. oleracea var. capitata f. alba, B. oleracea var. capitata f. rubra, B. oleracea var. botrytis L., B. oleracea var. italica Plenck, B. oleracea gemmifera
		Pieris napi (Linnaeus)	B. oleracea var. capitata f. alba, B. oleracea var. capitata f. rubra, B. oleracea var. botrytis L.
		Pieris rapae (Linnaeus)	B. oleracea var. capitata f. alba, B. oleracea var. capitata f. rubra, B. oleracea var. botrytis L., B. oleracea var. italica Plenck, Wild crucifers
	Plutellidae	*Plutella xylostella* (Linnaeus)	B. oleracea var. capitata f. alba, B. oleracea var. capitata f. rubra, B. oleracea gemmifera, B. oleracea var. italica Plenck, B. oleracea var. botrytis L.
Diptera	Anthomyiidae	*Delia radicum* (Linnaeus)	B. oleracea var. capitata f. alba, B. oleracea var. botrytis L., B. oleracea gemmifera, B. oleracea var. italica Plenck

Selimşahlar neighborhood of Şehzadeler district of Manisa province. As a result of the study, a total of 14 pests species [(*Gryllotalpa gryllotalpa* (Linnaeus), *Brevicoryne brassicae* (Linnaeus), *Eurydema ornata* (Linnaeus), *Sphaeroderma rubidum* (Graells), *Phyllotreta* sp., *Cassida rubiginosa* Müller, *Mamestra brassicae* (Linnaeus), *Hellula undalis* (Fabricius), *Phragmacossia albida* (Erschoff), *Pieris brassicae* (Linnaeus), *P. napi* (Linnaeus), *P. rapae* (Linnaeus), *Plutella xylostella* (Linnaeus) and *Delia radicum* (Linnaeus)] belonging to 5 orders and 10 families were determined (Table 1). As a result of a study carried out in Bitlis, Muş and Van provinces, 21 pests species from 12 families belonging to 4 orders were determined in the cabbage cultivation areas, and among them *Pieris rapae* L., *P. brassicae* (Cabbage butterfly), *Hellula undalis* (Fabricius) (Pyralidae) (Cabbage bellyworm), *Plutella xylostella* L., (Yponomeutidae) (Cabbage leaf moth), *Spodoptera littoralis* (Bois.) belonging to the Lepidoptera order and *Aleyrodes proletella* L. (Aleyrodidae) (Cabbage whitefly) belonging to the Hemiptera order were determined as the main important damage species (Ölmez et al. 2021).

As a result of field studies, it was observed that *Pieris brassicae* was the most economically important species among the pests' species detected. When monitoring the population of this species, it was determined that adults were showed in nature in the second week of February or first week of March, and it was observed that it reached the highest population in May and June. In the study, pheromone trap counting was not performed. These are purely observational findings. It was also determined that the adult and pre-adult stages of *P. brassicae* remained active in nature for about eight months, from the second week of February to the second week of October. It was observed that *P. brassicae* prefer cabbage (white and red), cauliflower, broccoli, Brussels sprouts, and many wild crucifers. It was indicated that they heavily damaged cabbage (white and red) and cauliflower, and they preferred broccoli, Brussels sprouts and many wild crucifers less in the study area. In a study conducted in İzmir, it was stated that *P. brassicae* is the most common pest of cabbage and cauliflower (Uzun 1987). In another study conducted in the Eastern Mediterranean Region, it was indicated that this species harms cabbage (*B. oleracae*) and cauliflower (*B. oleracae* var. *botrytis*) (Bayhan et al. 2002). It was determined that the larvae initially gnawed superficially between the veins of the leaves, and over time they left only the thick veins by eating the leaves of the plant they were feeding on. Extremely damaged plants show a bushy appearance (Figure 2), rain and dew droplets and excrement accumulated between the leaves of the plant cause the cabbage to become inedible. It was determined that the biology of *Pieris napi* (Linnaeus), another species belonging to the same genus, is similar to *P. brassicae*, but laid its eggs one by one. It was determined that *Pieris rapae* (Linnaeus) fed on white and red cabbage, cauliflower, broccoli and many wild crucifers, and laid its eggs on the cabbage plant one by one, like *P. napi*. It was determined that *Phragmacossia albida* (Erschoff) preferred artichoke. It was found that the larvae of the pest caused the roots in which they were found to rot by hollowing out, and the root of the plant, which was hollowed out, rots after a while. It has been stated that the main host of this species is artichoke and it damages this plant, and it spends the winter in the plant body and in a strong cocoon, usually in the seventh and later larval stages. In addition, it has been stated that the larvae emerging from the eggs laid near the root collar and root of the plants enter the plant through fresh shoots, cracks and nipples and feed (Özbek and Hayat 2003). Another lepidopteran species, *P. xylostella*, was detected in white and red cabbage, Brussels sprouts, broccoli and cauliflower plants. In a conducted study, it was stated that *P. xylostella* harmed broccoli (*B. oleracea* var. *italica* Plenck), brussels sprouts (*B. oleracea* var. *gemmifera*), cabbage (*B. oleracea* var. *capitata*), Chinese cabbage (*B. rapa* subsp. *pekinensis* Lour), cauliflower (*B. oleracea* var. *botrytis* L.), collard (*B. oleracea* var. *viridis* D.C.L.), kale (*B. oleracea* var. *sabellica* L.), kohlrabi (*B. oleracea gongylodes* D.C.L.), mustard (*B. juncea*), radish (*Raphanus sativus* var. *longipinnatus*), turnip (*B. rapa* subsp. *rapa*), and watercress (*Nasturtium officinale* W.T.Aiton) and a few wild crucifers (Saran and Genç 2021). It was found that the larvae of the pest went out of the leaf and fed by gnawing the leaves from the bottom to the upper epidermis, and only a thin membrane remained on the upper side of the gnawed parts. After a while, it was determined that the leaves turned into a very perforated appearance, both large and small. In addition, it was determined that this species was densely populated on wild crucifer plants around cabbage fields. Cabbage moth, *M. brassicae* was determined to cause damage to cabbage, cauliflower, broccoli, brussels sprouts, spinach and lettuce. It was determined that the pest fed on especially on the navel section of the cabbage plant, and the flowers and leaves of the cauliflower. It was reported that M. brassicae is the most serious pest of vegetables in the Brassica genus in Asia and Europe (Finch and Thompson 1992). In another study, it was stated that *M. brassicae* caused up to 80% damage in *Brassica* (broccoli, arugula, bok Choy, Brussels sprouts, cabbage, cauliflower, radish, turnip and watercress) vegetables (Cartea et al. 2009). Cabbage belly worm, *H. undalis*, was observed to prefer white, red cabbage and cauliflower. In the areas where the study was carried out, it was determined that it caused damage to cabbage and cauliflower plants in July from the seedling production date to the end of vegetation; however, the population density was low. In a study in the Eastern Mediterranean Region,

it was determined that the damage caused by *H. undalis* on white cabbage (*B. oleracea* var. *capitata f. alba*), red cabbage (*B. oleracea* var. *capitata f. rubra*) and cauliflower (*B. oleracea* var. *botrytis*) is very important. It was stated that the damage especially in cauliflower (*B. oleracea* var. *botrytis*) increased up to 100% and no product could be obtained (Yabaş and Zeren 1990).

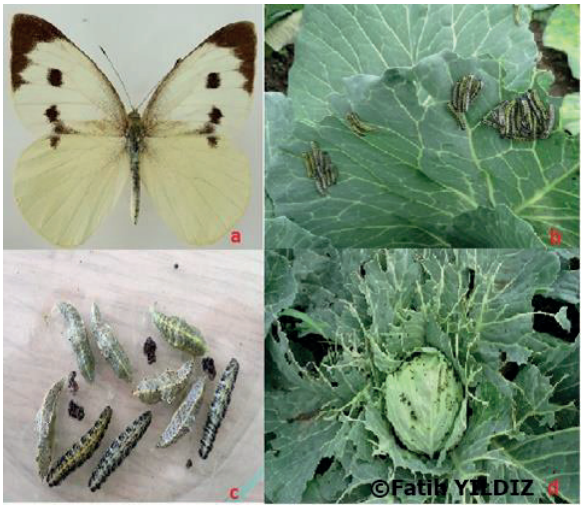

Figure 2. *Pieris brassicae* (Linnaeus) adult (a), larvae (b), mature larvae and pupae preparing to pupate (c), damage to cabbage plant (d)

When monitoring the population of *Eurydema ornata*, it was observed as the second pest species that is important in terms of density and economy in vegetable cultivation areas. It was determined that the pest was seen intensely with the seedling period in September and in the flowering periods in June and July. It was found that reached to the highest population in April and June, causing color change in the leaves and drying in advanced cases. It was determined that the population density of *E. ornata* was high in white cabbage, red cabbage and Brussels sprouts belonging to the Cruciferae family, while the population density was low in cauliflower and broccoli in the study area. These are purely observational findings. In a study conducted in the Aegean region, it was stated that seven species of *Eurydema* were detected and these pest species damaged cabbage and cauliflower seedlings belonging to the Cruciferae family. In addition, the researchers stated that the dominant species was *Eurydema ventral* Klt. while the second most common species was *E. ornata* (Atalay and Çağlayan 1990). It was determined that the pest caused damage by sucking the sap of the host plant and was caused curling in the leaves, whitish-yellow spots on sucking sites, and that in time, the spots combine and was caused them to drying and spilling (Figure 3). It was stated that except for the first instar nymphs of the pests, the nymphs and adults of the other instar is fed on the leaves and was caused sucking spots with a diameter of 1.06-3.72 mm (Atalay and Çağlayan 1990). As a result of feeding, it was determined that the vascular tissues of the host plant were damaged, especially in the seedling period, as they caused a lot of damage, preventing the development of the seedling and causing them to dry out. In addition, it was determined that this species released a foul odor in the places where it was fed, unlike other species.

Figure 3. *Eurydema ornata* (Linnaeus) adult (a) and egg (b)

According to observational findings, *B. brassicae* was observed to be dense in white cabbage (*B. oleracea* var. *capitata f. alba*), red cabbage (*B. oleracea* var. *capitata f.rubra*), Brussels sprouts (*B. oleracea gemmifera*) and wild mustard plants (*Sinapis arvensis*) belonging to the Cruciferae family, and a lower population was observed in cauliflower (*B. oleracea* var. *botrytis*) and broccoli (*B. oleracea* var. *italica*) in the study area. It was determined that this species caused curling and deformities in the leaves and fresh shoots they feed on (Figure 4). It was detected for the first time in our country on cabbage (Avcı and Özbek 1991, Bodenheimer and Swirski 1957, Düzgünes and Tuatay 1956, Düzgünes et al. 1982, Giray 1974). In another study, it was stated that it fed on white head cabbage (*B. oleracea* var. *capitata f. alba*), leaf cabbage (*B. oleraceae* var. *acephala*), red head cabbage (*B. olareacae* var. *capitata* subsp. *rubra*), *Brassica* sp., *Ochtodium aegyptiorum*, radish (*Rhaphanus sativus* L.), wild mustard (*Sinapis arvensis* L.), *Sinapis* sp., *Brassica* sp. and canola (*B. napus* var. *oleifera*) (Toros et al. 2002).

Figure 4. *Brevicoryne brassicae* (Linnaeus) on cabbage plant preparing to seed

Gryllotalpa gryllotalpa (Figure 5), which is one of the harmful species, was observed to damage white cabbage, red cabbage, Brussels sprouts, cauliflower and broccoli in the study area. It was stated that adults and nymphs of *G. gryllotalpa* move forward by opening a gallery in the soil, gnawing and damaging all kinds of plant materials such as seeds, roots and tubers; they caused drying by cutting the roots of newly planted or newly germinated vegetable seedlings. On the other hand, it was also stated that the pest gnawed the tubers of tuberous vegetables and damaged the roots by cutting almost all in vegetable seedlings (Erdoğan 2006). In another study, it was indicated that the pest caused significant damage in the seedling period of cabbage, tomato, eggplant, pepper, potato and onion plants in Elazığ, Mardin, Malatya, Tunceli, Erzincan and Siirt provinces, which are important in terms of vegetable growing in the Eastern and Southeastern Anatolia Regions (Asena 1972).

Figure 5. *Gryllotalpa gryllotalpa* (Linnaeus) adult

It was observed that the artichoke leaf beetle, *S. rubidum* created high populations on the artichoke plant and fed on the leaves. It was indicated that the early instar larvae of *S. rubidum* fed on the primary and secondary leaf veins, and the next instar larvae fed on all leaf texture between the upper and lower epidermis (Anonymous 2023c). On the other hand, it was observed that *C. rubiginosa* caused damage on artichoke leaves and also fed on beet (*Beta vulgaris* L.), spinach (*Spinacia oleracea* L.) and some wild cruciferous herbs. In a study conducted in the artichoke fields in Center and Ezine districts of Çanakkale province, it was stated that *C. rubiginosa* caused intense damage to the leaves of the artichoke plant, and they also damaged the outside parts of the flowers that had not yet opened, but this damage remained at a small level (Efil 2018). It was observed that *Phyllotreta* sp., which is one of the soil fleas, created dense population in cabbage, cauliflower, broccoli and wild crucifers. In addition, during the visual inspections, it was observed that the adults of the pest damaged on the leaves of carrot, spinach, lettuce, eggplant, beet, and they ate the leaves in the young stages of the plants and adversely affected the leaf quality. Although *Phyllotreta* spp. was the predominant pest of the canola plant, it was stated that it harms vegetables belonging to the Cruciferae family (*Brassicae* spp.) (Burgess 1977, Wylie 1979).

It was observed that the larvae of *D. radicum* L. from the order Diptera, which is one of the harmful species, caused damage to cabbage, cauliflower, Brussels sprouts, broccoli and turnip plants, respectively. It was stated that *D. radicum* damaged cultivated plants belonging to the Cruciferae family, such as cabbage (*B. oleracea* var. *capitata* L.), cauliflower (*B. oleracea* var. *botrytis* L.), turnip (*B. napus* subsp. *rapifera*) and radish (*Raphanus sativus* L.) (Maack 1977, McKinlay and Birch 1991). It was determined that pest larvae caused damage and stress to the host plants as a result of fed on the root and root collar of the host. It was observed that as the number of larvae per plant increased, the damage rate also increased. In addition, when the plants took a certain size, it was seen that the head binding (plate) was not at the desired level, although the damage was tolerated.

In vegetable production, which is an important source of income for the regional economy, experiences product and quality losses due to various factors. Among the factors affecting this yield and quality, pests hold an important place. As a result of this study, pests species found in winter vegetables produced in Manisa province were determined, among these species, the leading species were determined in terms of density and damage observationally. In addition, some data about the damage shape of the species and the time of their have in nature are given. These results are extremely important both in terms of shedding light on future studies and in terms of producers making use of these data.

ACKNOWLEDGEMENTS

We would like to thank the Beydere Seed Certification Test Directorate for giving the opportunity to benefit from the opportunities, especially T.S.T.M. manager and to my Variety Registration Coordinator and my colleagues who conduct vegetable trials for which they are responsible in my field studies.

ÖZET

Bu çalışma Beydere Tohum Sertifikasyon Test Müdürlüğünde (Manisa) kışlık yetiştirilen enginar, karnabahar, brokoli, ıspanak, tere, marul, maydanoz, roka, dereotu, pancar, havuç, beyaz lahana, kırmızı lahana ve brüksel lahanası gibi sebzelerde görülen önemli zararlı böcek türlerini tespit etmek amacı ile 2016-2018 yıllarında yürütülmüştür. Çalışma sonucunda zararlı türlerden *Gryllotalpa gryllotalpa* (Linnaeus) (Orthoptera: Gryllotalpidae), *Brevicoryne brassicae* (Linnaeus)

(Hemiptera: Aphididae), *Eurydema ornata* (Linnaeus) (Hemiptera: Pentatomidae), *Sphaeroderma rubidum* (Graells), *Phyllotreta* sp. ve *Cassida rubiginosa* (Müller) (Coleoptera: Chrysomelidae), *Mamestra brassicae* (Linnaeus) (Lepidoptera: Noctuidae), *Hellula undalis* (Fabricius) (Lepidoptera: Pyralidae), *Phragmacossia albida* (Erschoff) (Lepidoptera: Cossidae), *Pieris brassicae* (Linnaeus), *Pieris napi* (Linnaeus), *Pieris rapae* (Linnaeus) (Lepidoptera: Pieridae), *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae) ve *Delia radicum* (Linnaeus) (Diptera: Anthomyiidae) türleri tespit edilmiştir. Bunlar içerisinde en önemli zararlı türlerin; *B. brassicae*, *E. ornata*, *P. brassicae* ve *D. radicum* olduğu belirlenmiştir. Bu zararlı türlerin baş (beyaz-kırmızı) lahanada yoğun olarak zarar verdiği, ancak brokolide yoğunluğun düşük olduğu belirlenmiştir.

Anahtar kelimeler: kışlık sebze zararlıları, Brassicaceae, kışlık sebzeler, Manisa, Türkiye

REFERENCES

Anonymous 2023a. New and revised integrated control technical instructions (2022). Cotton integrated control technical instruction.pdf https://www.tarimorman.gov.tr/TAGEM/Belgeler/Entegre/Pamuk%20Entegre%20M%C3%BCcadele%20Teknik%20Talimat%C4%B1.pdf (accessed date: 12.02.2023).

Anonymous 2023b. New and revised integrated control technical instructions (2022). Vegetables with edible leaf integrated control technical instruction.pdf. https://www.tarimorman.gov.tr/TAGEM/Belgeler/Entegre/Yapra%C4%9F%C4%B1%20Yenen%20Sebzeler%20Entegre%20M%C3%BCcadele%20Teknik%20Talimat%C4%B1.pdf (accessed date: 12.02.2023).

Anonymous 2023c. *Sphaeroderma* spp. - UK Beetles. https://www.ukbeetles.co.uk/sphaeroderma-spp. (accessed date: 09.03.2023)

Asena N., 1972. Studies on vegetable pests in Eastern and Southeastern Anatolia Regions. Plant Protection Research Annual, The Ministry of Agriculture, The General Directorate of Plant Protection and Plant Quarantine, Research Department, no. 6, 9 p.

Atalay R., Çağlayan L., 1990. Pest *Eurydema ornatum* L. in cabbage and cauliflower seedlings of (Heteroptera: Pentatomidae) studies on the economic damage threshold. Turkish Journal of Entomology, 14 (4), 215-226.

Avci U., Özbek H., 1991. In Erzurum, cabbage aphid, *Brevicoryne brassicae* (L.) a study on natural enemies of (Homoptera, Aphididae). Turkish Journal of Entomology, 15 (1), 37-41.

Bayhan E., Ölmez S., Ulusoy M.R., 2002. Species harmful to cabbage (*Brassicae oleracae* L.) and cauliflower (*Brassicae oleracae* L. var. botrytis L.) and their predators and parasitoids in the Eastern Mediterranean Region. Çukurova University Journal of the Faculty of Agriculture, 17 (3), 85-92.

Bodenheimer F.S., Swirski E., 1957. The Aphidodea of the Middle East. The Weizmann Science Press of Israel, Jerusalem, 378 pp.

Burgess L., 1977. Flea beetles (Coleoptera:Chrysomelidae) attacking rape crop in the Canadian Prairie Provinces. The Canadian Entomologist, 109, 21–32.

Cartea M.E., Padilla G., Vilar M., Velasco P., 2009. Incidence of the major Brassica pests in northwestern Spain. Journal of Economic Entomology, 102, 767-773.

Düzgüneş Z., Tuatay N., 1956. Türkiye Aphids. Ministry of Agriculture, Ankara Plant Protection Institute Publications, Ankara, 64 pp.

Düzgüneş Z., Toros S., Kılınçer N., Kovancı B., 1982. Detection of parasites and predators of aphidoidea species in Ankara province. The Ministry of Agriculture and Forestry, Publications of General Directorate of Agricultural Protection and Quarantine, Ankara, 251 pp.

Efil L., 2018. New pests in Çanakkale province in artichoke plant: *Acanthiophilus helianthi* (Rossi, 1794) *Terellia fuscicornis* (Loew, 1844) (Diptera: Tephritidae) and *Cassida rubiginosa* (Müller, 1776) (Coleoptera: Chrysomelidae). Turkish Journal of Agriculture and Natural Sciences, 5 (3), 291–297.

Erdoğan P., 2006. Pests in Vegetables and Forage Crops and Control Methods. Journal of Field Crops Central Research Institute, 15 (1-2), 1-10.

Faydaoğlu E., Sürücüoğlu M.S., 2011.The use and economic importance of medicinal and aromatic plants from past to present. Kastamonu University Journal of Forestry Faculty, 11, 52-67.

Finch S., Thompson A.R., 1992. Pests of cruciferous crops. In: Vegetable crop pests. R.G., McKinlay (Ed.). Palgrave Macmillan, London, pp. 87-138. https://doi.org/10.1007/978-1-349-09924-5_4

Giray H., 1974. With the first list of species of the family Aphididae (Homoptera) around İzmir province notes on their hosts and shape of damage. Review of the Faculty of Agriculture Ege University, 11 (1), 39-69.

Kaya K., Kornoşor S., 2008. Harmful Lepidoptera species and parasitoids found in important winter vegetable areas in Hatay province and population fluctuations of important pest species. Turkish Journal of Entomology, 32 (3), 195-209.

Maack, G. 1977. Schadwirkung der kleinen kohlfliege (*Phorbia brassicae* Bouche´) und mo¨glichkeiten zur reduzierung des insektizidaufwandes bei der Bekampfung. Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirschaft, Berlin-Dahlem, 177, 135 pp

Mckinlay R.G., Birch A.N.E., 1991. Integrated control of rootflies in Swedes. Proceedings Working Group Meeting, 28-30 October, 62-67, Vienna.

Ölmez M., Sertkaya E., Büyük M., Alaserhat İ., 2021. Determination of harmful and beneficial ınsect species and population development of ımportant species in cabbage (*Brassica oleraceae* L.) cultivation areas of Bitlis, Muş and Van provinces. European Journal of Science and Technology, 31 (Supp. 1), 256-267.

Özbek H., Hayat R., 2003. Grain, vegetable, feed and industrial plant pests. Atatürk University Publication No: 930, Faculty of Agriculture Publication no: 340, Erzurum, 320 p.

Saran C., Genç H., 2021. Determination of biological properties of *Plutella xylostella* (Linnaeus, 1758) (Lepidoptera: Plutellidae). COMU Journal of Agricultural Faculty, 9 (1), 21–30.

Toros S., Uygun N., Ulusoy R., Satar S., Özdemir I., 2002. Aphidoidea species in the eastern mediterranean region. T.C. Ministry of Agriculture and Rural Affairs, General Directorate of Agricultural Research, 108 p.

Tozlu G., Gültekin L., Hayat R., Güçlü Ş., 2002. Studies on the natural enemies of cabbage pests in Erzurum. Proceedings of the Fifth Turkish National Congress of Biological Control. Özbek, H., Güçlü, Ş., Hayat, R., (Eds.), Faculty of Agriculture, Atatürk University, Erzurum, Turkey, pp. 227-235.

Uzun S., 1987. Parasites of the cabbage butterfly (*Pieris brassicae* L.) (Lepidoptera: Pieridae) causing damage to cabbage and cauliflower in İzmir province. Turkish Journal of Entomology, 11 (4), 237-245.

Wylie H.G., 1979. Observations on distribution, seasonal life history, and abundance of flea beetles (Coleoptera: Chrysomelidae) that infest rape crops in Manitoba. The Canadian Entomologist, 111, 1345–1353.

Yabaş C., Zeren O., 1990. Studies on the bioecology and struggle of cabbage bellywolf (*Hellula undalis* Fab.) (Lep.: Pyralidae) in the Eastern Mediterranean Region. T.C. Ministry of Agriculture and Rural Affairs. General Directorate of Agricultural Research, Plant Protection Research Department, Plant Protection Research Yearbook, 24-25:30-31.

Yanmaz R., Duman İ., Yaralı F., Demir K., Sarıkamış G., Sarı N., Balkaya A., Kaymak H.Ç., Akan S., Özalp R., 2015. Changes and new searches in vegetable production. Türkiye Agricultural Engineering VIII. Technical Congress, 12-16 January 2015, volume 1, 579-605 pp, Ankara.

Cite this article: Yıldız, F. & Yıldırım, E. (2023). Important insect pests in winter vegetables grown in Beydere Seed Certification Test Directorate. Plant Protection Bulletin, 63-3. DOI: 10.16955/bitkorb.1274312

Atıf için: Yıldız, F. & Yıldırım, E. (2023). Beydere Tohum Sertifikasyon Test Müdürlüğünde yetiştirilen kışlık sebzelerde görülen önemli zararlı böcek türleri. Bitki Koruma Bülteni, 63-3. DOI: 10.16955/bitkorb.1274312

Bitki Koruma Bülteni / Plant Protection Bulletin

http://dergipark.gov.tr/bitkorb

Original article

Faunistic contributions on the subfamily Agathidinae (Hymenoptera: Braconidae) of Central Black Sea Region of Türkiye

Orta Karadeniz Bölgesi (Türkiye) Agathidinae (Hymenoptera: Braconidae) altfamilyası üzerine faunistik katkılar

Özlem ÇETİN ERDOĞAN[a]*

[a]https://orcid.org/0000-0001-6465-4060

[a] *Trakya University, Science Faculty, Department of Biology, 22030 Edirne, Türkiye*

ARTICLE INFO

Article history:
DOI: 10.16955/bitkorb.1261587
Received : 07-03-2023
Accepted : 14-06-2023

Keywords:
distribution, endoparasitoid, koinobiont, Türkiye, Agathidinae

* Corresponding author: Özlem ÇETİN ERDOĞAN
ozlemerdogan@trakya.edu.tr

ABSTRACT

This study was conducted between 2003-2004 to investigate the Agathidinae (Hymenoptera: Braconidae) fauna of the Central Black Sea Region in Türkiye. For this purpose, Agathidinae samples were collected from different altitudes and habitats of Amasya, Çorum, Ordu, Samsun and Tokat provinces in the region. Seventeen species were determined and identified. These were twelve species in the genus *Agathis*, four species in the genera *Bassus* and one species in the genera *Disophrys* were recorded. Although all identified species were recorded before Türkiye, they are new records for the research area. The distributions and detailed locality records of the identified species are given.

INTRODUCTION

The Agathidinae is a large subfamily, represented all over the world, comprising 52 genera and about 1213 known species (Yu et al. 2016). More than 76 of these have been reported in Europe and 90 in the West Palearctic region (Yu et al. 2016). Eleven genera are recorded from the West Palearctic region. So far 5 genera and 40 species of Agathidinae have been recorded from Türkiye: Çetin and Beyarslan (2001), Çetin Erdoğan (2005, 2010, 2013, 2014), Çetin Erdoğan and Beyarslan (2004, 2006, 2009, 2016), Çetin Erdoğan et al. (2009), Güçlü and Özbek (2002), Zettel and Beyarslan (1992).

Agathidines are koinobiont endoparasitoids of larvae of Lepidoptera. The species with a short ovipositor select exposed larvae and those with a long ovipositor use larvae with a concealed way of life (van Achterberg and Long 2010).

Central Black Sea Region which is located in the north part of Türkiye conforms 7% of the total area of the country. The area includes Çorum, Amasya, Samsun, Tokat and Ordu provinces; Akdağ, Kocadağ and Canik Mountains and Kızılırmak, Yeşilırmak and Kelkit rivers. This region contains 25% of Türkiye's forests.

The aim of the present study is to document the Agathidinae fauna in Central Black Sea region and to contribute to the knowledge to the braconid fauna of Türkiye.

MATERIALS AND METHODS

Samples were collected from different habitats in Amasya, Çorum, Ordu, Samsun and Tokat provinces with the help of a net from June to September 2003 and from June to July 2004 (Figure 1). Agathidins were treated with 70% ethanol and then placed on a filter paper for drying. The dried specimens were card-mounted and labeled. All the agathidin specimens were deposited in the Entomological Museum, Trakya University (EMTU), Department of Biology, Türkiye. The works of Simbolotti and van Achterberg (1992, 1999) were used for taxonomical examination and identification of the materials. Information on parasitoids, hosts, general distributions are given according to Yu et al. (2016).

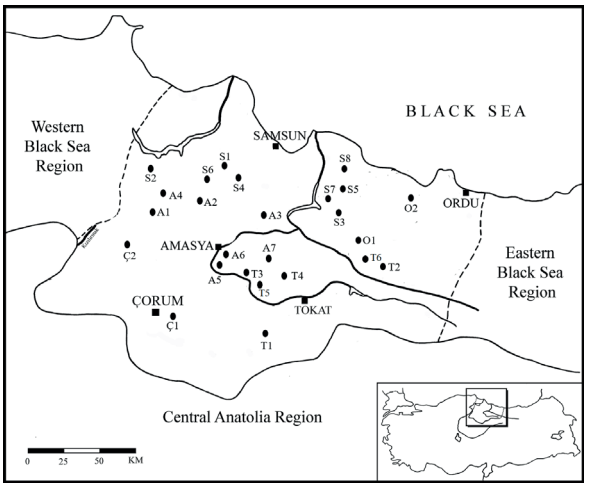

Figure 1. The Central Black Sea Region of Türkiye and sampling sites

A list of localities is presented in Table 1 from provinces of Central Black Sea region. The localities are numbered with the full locality data given. A locality number and date are indicated for each record. The total number of species identified for each genus and at each province is given in

Table 2. Number of species identified according to genus and provinces

Genus	Number of species	Number of species identified				
		Amasya	Çorum	Ordu	Samsun	Tokat
Agathis	12	10	3	5	3	6
Bassus	4	-	-	1	4	-
Disophrys	1	1	1	-	-	1
Total	17	11	4	6	7	7

RESULTS AND DISCUSSION

Agathidinae

Agathis Latreille, 1804

Agathis anglica Marshall, 1885

Material examined: Amasya: Merzifon-Tavşan Dağları-Esenköy Yaylası, 41°00'37"N 35°17'23"E,09.07.2003, 23 ♀♀; Suluova, 40°49' 53"N 35°38'52"E, 03.09.2003, 2 ♀♀; Çorum: Kuşsaray, 40°35'44"N 35°08'36"E, 29.06.2004, 5♀♀; Ordu: Akkuş-Yukarıdüğencili, 40°47'35"N 37°00'59"E, 05.07.2003, 11♀♀, 2♂♂; Ünye-Çatalpınar, 41°07'00"N 37°15'00"E, 05.07.2003, 1♀, 1♂; Samsun: Kavak-Boğaziçi, 41°12'49"N 36°14'24"E, 02.07.2003, 1♀; Tokat: Pazar-Ballıca, 40°15'44"N 36°18'48"E, 07.07.2003, 1♀; Turhal-Üçyol, 40°46'69"N

Table 1. List of sampling sites in provinces of Central Black Sea region

Province	Sampling site	Coordinates	Altitude (m)	Locality number
AMASYA	Merzifon-Tavşan Dağları-Esenköy Yaylası	41°00'37"N 35°17'23"E	1700	A1
	Suluova	40°49' 53"N 35°38'52"E	470	A2
	Taşova-Borabay	40°80'98"N 36°18'32"E	740	A3
	Merzifon-Tavşandağı-Uzunağaç	41°00'30"N 35°21'38"E	1600	A4
	Çakallar	40°64'54"N 35°82'69"E	510	A5
	Yolyanı	40°34'22"N 36°06'38"E	700	A6
	Karaibrahim-Çengel	40°31'20"N 36°10'29"E	670	A7
ÇORUM	Kuşsaray	40°35'44"N 35°08'36"E	1015	Ç1
	Dodurga-Yeniköy	40°49'27"N 34°42'34"E	1035	Ç2
ORDU	Akkuş-Yukarıdüğencili	40°47'35"N 37°00'59"E	1340	O1
	Ünye-Çatalpınar	41°07'00"N 37°15'00"E	80	O2
SAMSUN	Kavak-Boğaziçi	41°12'49"N 36°14'24"E	350	S1
	Vezirköprü-Kızılcaören	41°06'00"N 35°30'00"E	500	S2
	Salıpazarı-Aspete mevkii	41°05'02"N 36°49'49"E	600	S3
	Kocadağ-TRT İstasyonu	41°32'81"N-36°10'43"E	1100	S4
	Salıpazarı-Kayaköprü	41°10'18"N 36°28'16"E	100	S5
	Havza-Mismilliağaç,	40°99'63"N 35°70'33"E	610	S6
	Salıpazarı-Derbentaltı,	41°10'10"N 36°10'20"E	970	S7
	Salıpazarı-Soyuk	41°28'16"N 36°25'10"E	650	S8
TOKAT	Pazar-Ballıca	40°15'44"N 36°18'48"E	1020	T1
	Turhal-Üçyol	40°46'69"N 36°29'45"E	1040	T2
	Turhal-Doğanlı Çiftliği	40°18'14"N 36°19'29"E	554	T3
	Reşadiye-Çayırpınar	40°24'16"N 37°16'16"E	672	T4
	Turhal-Çamlıca	40°41'09"N 36°22'99"E	900	T5
	Niksar-Çamiçi	40°59'02"N 36°95'12"E	1120	T6

36°29'45"E, 07.07.2003, 6♀♀; Turhal-Doğanlı Çiftliği, 40°18'14"N 36°19'29"E, 03.09.2003, 1♂.

General distribution: Palearctic and Oriental regions (Albania, Armenia, Bulgaria, China, Croatia, Cyprus, Finland, former Yugoslavia, France, Germany, Greece, Hungary, Iran, Italy, Kazakhstan, Macedonia, Mongolia, Morocco, Netherlands, Poland, Romania, Russia, Slovenia, Spain, Sweden, Switzerland, Syria, Tajikistan, Türkiye, Ukraine, United Kingdom).

Hosts: *Agonopterix nervosa, Agonopterix pallorella, Aproaerema anthyllidella, Coleophora adjunctella, Coleophora albitarsella, Coleophora argentula, Coleophora discordella, Coleophora laricella, Coleophora lusciniaepennella, Epinotia mercuriana, Loxostege sticticalis, Nothris verbascella, Pexicopia malvella, Pyrausta aurata, Syncopacma taeniolella, Teleiodes saltuum.*

Agathis assimilis Kokujev, 1895

Material examined: Amasya: Merzifon-Tavşan Dağları-Esenköy Yaylası, 41°00'37"N 35°17'23"E, 09.07.2003, 1♀; Taşova-Borabay, 40°80'98"N 36°18'32"E, 08.07.2003, 1♀.

General distribution: Palaearctic Region (Austria, Azerbaijan, Bulgaria, Croatia, former Yugoslavia, France, Germany, Hungary, Iran, Italy, Kazakhstan, Korea, Lithuania, Macedonia, Moldova, Mongolia, Netherlands, Norway, Poland, Russia, Tajikistan, Türkiye, Ukraine, United Kingdom, Uzbekistan).

Hosts: *Coleophora astragalella*

Agathis fulmeki Fischer, 1957

Material examined: Amasya: Merzifon-Tavşan Dağları-Esenköy Yaylası, 41°00'37"N 35°17'23"E, 09.07.2003, 1♀; Ordu: Ünye-Çatalpınar, 41°07'00"N 37°15'00"E, 05.07.2003, 6♀♀, 1♂

General distribution: Palaearctic Region (Austria, Bulgaria, France, Greece, Hungary, Morocco, Spain, Türkiye).

Hosts: Unknown.

Agathis fuscipennis (Zetterstedt, 1838)

Material examined: Amasya: Merzifon-Tavşan Dağı-Uzunağaç, 41°00'30"N 35°21'38"E, 09.07.2003, 1♀, Merzifon-Tavşan Dağları-Esenköy Yaylası, 41°00'37"N 35°17'23"E, 09.07.2003, 1♀; Ordu: Ünye-Çatalpınar, 41°07'00"N 37°15'00"E, 05.07.2003, 1♀.

General distribution: Palearctic Region (Armenia, Austria, Bosnia and Hercegovina, Bulgaria, Croatia, Finland, former Yugoslavia, France, Germany, Greece, Hungary, Iran, Ireland, Italy, Kazakhstan, Korea, Latvia, Lithuania, Macedonia, Mongolia, the Netherlands, Poland, Russia, Spain, Sweden, Switzerland, Tajikistan, Tunisia, Türkiye, United Kingdom).

Hosts: *Aproaerema anthyllidella, Caryocolum saginella, Chrysoesthia hermannella, Chrysoesthia sexguttella, Coleophora albicostella, Coleophora albitarsella, Coleophora artemisiae, Coleophora artemisicolella, Coleophora chamaedriella, Coleophora conspicuella, Coleophora conyzae, Coleophora cracella, Coleophora dianthi, Coleophora follicularis, Coleophora granulatella, Coleophora inulae, Coleophora laripennella, Coleophora linosyridella, Coleophora meridionella, Coleophora salicorniae, Coleophora salinella, Heliodines roesella, Ochromolopis ictella, Olethreutes arbutella, Scrobipalpa atriplicella, Scrobipalpa gallicella, Scrobipalpa ocellatella, Scrobipalpula absoluta, Spilonota ocellana, Thiotricha subocellea.*

Agathis glaucoptera Nees, 1834

Material examined: Amasya: Çakallar, 40°64'54"N 35°82'69"E, 28.05.2002, 1♀.

General distribution: Palearctic Region (Azerbaijan, former Yugoslavia, France, Germany, Hungary, Iran, Italy, Kazakhstan, Macedonia, Russia, Spain, Türkiye, Ukraine).

Hosts: Unknown.

Agathis lugubris (Foerster, 1862)

Material examined: Ordu: Akkuş-Yukarıdüğencili, 40°47'35"N 37°00'59"E, 05.07.2003, 1♀, 2♂♂

General distribution: Palearctic Region (Finland, former Czechoslovakia, Germany, Greece, Hungary, Iran, Ireland, Mongolia, the Netherlands, Norway, Poland, Switzerland, Türkiye, Ukraine, United Kingdom).

Hosts: *Coleophora alticolella, Coleophora glaucicolella.*

Agathis malvacearum Latreille, 1805

Material examined: Amasya: Merzifon-Tavşan Dağları-Esenköy Yaylası, 41°00'37"N 35°17'23"E, 09.07.2003, 7♀♀, 5♂♂; Merzifon-Tavşan Dağı-Uzunağaç, 41°00'30"N 35°21'38"E, 09.07.2003, 1♀; Çorum: Kuşsaray, 40°35'44"N 35°08'36"E, 29.60.2004, 1♀, Dodurga-Yeniköy, 40°49'27"N 34°42'34"E, 28.06.2004, 2♀♀; Ordu: Akkuş-Yukarıdüğencili, 40°47'35"N 37°00'59"E, 05.07.2003, 6♀♀, 2♂♂; Samsun: Kavak-Boğaziçi, 41°12'49"N 36°14'24"E, 02.07.2003, 1♀; Tokat: Pazar-Ballıca, 40°15'44"N 36°18'48"E, 07.07.2003, 1♀.

General distribution: Palearctic and Nearctic regions (Albania, Armenia, Azerbaijan, Bulgaria, Canada, Croatia, Finland, former Czechoslovakia, former Yugoslavia, France, Georgia, Germany, Greece, Hungary, Iran, Italy, Kazakhstan, Latvia, Lithuania, Macedonia, Moldova, Mongolia, the Netherlands, Poland, Romania, Russia, Slovakia, Slovenia,

Spain, Switzerland, Tajikistan, Türkiye, Ukraine, United Kingdom, United States, Uzbekistan).

Hosts: *Coleophora galbulipennella, Coleophora graminicolella, Hellinsia didactylites, Metzneria aestivella, Metzneria lappella, Pexicopia malvella, Rhyacionia resinella.*

Agathis montana Shestakov, 1932

Material examined: Amasya: Merzifon-Tavşan Dağları-Esenköy Yaylası, 41°00'37''N 35°17'23''E, 09.07.2003, 1♀, 5♂♂.

General distribution: Palaearctic and Oriental Regions (Andorra, Armenia, Azerbaijan, Bulgaria, China, Czech Republic, former Yugoslavia, France, Greece, Hungary, Iran, Israel, Kazakhstan, Korea, Kyrgyzstan, Macedonia, Moldova, Mongolia, Poland, Russia, Switzerland, Türkiye, Ukraine, United Kingdom, Uzbekistan).

Hosts: *Pandemis cerasana, Pyrausta aurata.*

Agathis nigra, Nees, 1814

Material examined: Tokat: Pazar-Ballıca, 40°15'44''N 36°18'48''E, 07.07.2003, 1♀.

General distribution: Palearctic Region (Austria, Belgium, Bulgaria, Croatia, Finland, former Czechoslovakia, former Yugoslavia, France, Germany, Greece, Hungary, Iran, Israel, Italy, Kazakhstan, Korea, Latvia, Lithuania, Macedonia, Moldova, Mongolia, Morocco, the Netherlands, Poland, Russia, Slovenia, Spain, Sweden, Switzerland, Türkiye, Ukraine, United Kingdom).

Hosts: *Acleris quercinana, Apodia bifractella, Coleophora argentula, Coleophora laripennella, Coleophora meridionella, Coleophora vestianella, Eupoecilia roseana, Isophrictis striatella, Metzneria lappella, Metzneria metzneriella, Monochroa striatella, Ortholepis betulae, Phlyctaenia coronata, Ptycholoma lecheana, Pyrausta aurata, Scrobipalpa atriplicella.*

Agathis rufipalpis Nees, 1814

Material examined: Amasya: Merzifon-Tavşan Dağları-Esenköy Yaylası, 41°00'37''N 35°17'23''E, 09.07.2003, 1♀; Yolyanı, 40°34'22''N 36°06'38''E, 30.06.2004, 1♀; Çorum: Kuşsaray, 40°35'44''N 35°08'36''E, 29.06.2004, 1♀; Tokat: Pazar-Ballıca, 40°15'44''N 36°18'48''E, 07.07.2003, 1♀.

General distribution: Palaearctic Region (Belgium, Bulgaria, former Czechoslovakia, former Yugoslavia, Finland, France, Germany, Hungary, Ireland, Israel, Italy, Netherlands, Norway, Poland, Portugal, Romania, Slovenia, Spain, Sweden, Switzerland, Türkiye, United Kingdom).

Hosts: *Agonopterix kaekeritziana, Chrysoesthia hermannella, Coleophora alcyonipennella, Pyrausta aurata.*

Agathis umbellatarum Nees, 1814

Material examined: Amasya: Merzifon-Tavşan Dağları-Esenköy Yaylası, 41°00'37''N 35°17'23''E, 09.07.2003, 1♀; Tokat: Turhal-Çamlıca, 40°41'09''N 36°22'99''E, 07.07.2003, 1♂; Niksar-Çamiçi,40°59'02''N 36°95'12''E, 05.07.2003, 1♀.

General distribution: Palearctic Region (Algeria, Azerbaijan, Bulgaria, Croatia, Cyprus, former Yugoslavia, France, Germany, Greece, Hungary, Iran, Israel, Italy, Kazakhstan, Kyrgyzstan, Macedonia, Moldova, Mongolia, Portugal, Russia, Spain, Tajikistan, Tunisia, Türkiye, Turkmenistan, Ukraine, Uzbekistan).

Hosts: *Depressaria sp., Metzneria aestivella, Metzneria lappella.*

Agathis varipes Thomson, 1895

Material examined: Samsun: Kocadağ-TRT İstasyonu, 41°32'81''N-36°10'43''E, 29.8.2003, 1♀; Salıpazarı-Kayaköprü, 41°10'18''N 36°28'16''E, 03.07.2003, 1♀; Tokat: Pazar-Ballıca, 40°15'44''N 36°18'48''E, 07.07.2003, 1♀.

General distribution: Palearctic Region (Finland, former Yugoslavia, Germany, Greece, Hungary, Italy, Kazakhstan, Macedonia, Mongolia, the Netherlands, Norway, Russia, Slovakia, Sweden, Switzerland, Tajikistan, Türkiye, Ukraine, United Kingdom, Uzbekistan).

Hosts: *Apodia bifractella, Metzneria lappella, Myelois cirrigerella.*

Bassus Fabricius, 1804

Bassus conspicuus (Wesmael, 1837)

Material examined: Ordu: Ünye-Çatalpınar, 41°07'00''N 37°15'00''E, 05.07.2003, 1♀; Samsun: Vezirköprü-Kızılcaören 41°06'00''N 35°30'00''E, 02.07.2003, 1♀.

General distribution: Holarctic Region (Belgium, Canary Islands, China, Croatia, Finland, former Yugoslavia, France, Germany, Greece, Hungary, Ireland, Italy, Japan, Korea, Netherlands, Poland, Russia, Slovenia, Spain, Sweden, Switzerland, Türkiye).

Hosts: *Cydia pomonella, Grapholita molesta, Gypsonoma nitidulana, Pammene regiana, Phalonidia manniana, Rhopobota ustomaculana, Scoparia crataegella.*

Bassus dimidiator (Nees, 1834)

Material examined: Samsun: Salıpazarı-Aspete mevkii, 41°05'02''N 36°49'49''E, 03.07.2003, 2♀♀.

General distribution: Palaearctic, Nearctic and Oriental regions (Armenia, Azerbaijan, Belarus, Bulgaria, Canada, China, Finland, former Czechoslovakia, former Yugoslavia, France, Germany, Greece, Hungary, Iran, Italy, Kazakhstan,

Latvia, Lithuania, Malta, Moldova, Netherlands, Poland, Portugal, Russia, Slovakia, Switzerland, Türkiye, U.S.A., Ukraine, United Kingdom).

Hosts: *Acleris forsskaleana, Acleris variana, Aleimma loeflingiana, Anthonomus pomorum, Archips cerasivorana, Archips crataegana, Archips rosana, Archips xylosteana, Argyrotaenia velutinana, Blastodacna atra, Choristoneura rosaceana, Coleophora spinella, Croesia bergmanniana, Cydia latiferreana, Dichelia histrionana, Epiblema scutulana, Epinotia tetraquetrana, Grapholita interstinctana, Grapholita molesta, Hedya nubiferana, Pandemis cerasana, Pandemis heparana, Recurvaria leucatella, Recurvaria nanella, Spilonota ocellana, Tortrix viridana, Yponomeuta malinella.*

Bassus graecus Achterberg, 1992

Material examined: Samsun: Havza-Mismilliağaç, 40°99'63''N 35°70'33''E, 02.07.2003, 1♀.

General distribution: West Palaearctic Region (Greece, Türkiye).

Hosts: Unknown.

Bassus tumidulus (Nees, 1814)

Material examined: Samsun: Vezirköprü-Kızılcaören 41°06'00''N 35°30'00''E,02.07.2003, 4♀♀; Salıpazarı-Aspete mevkii, 41°05'02''N 36°49'49''E, 03.07.2003, 4♀♀, 2♂♂; Salıpazarı-Derbentaltı, 41°10'10''N 36°10'20''E, 03.07.2003, 6♀♀, 2♂♂; Salıpazarı-Soyuk, 41°28'16''N 36°25'10''E, 03.07.2003, 1♀.

General distribution: Palearctic and Oriental regions (Austria, Azerbaijan, Belarus, Belgium, Bulgaria, China, Croatia, Finland, former Czechoslovakia, former Yugoslavia, France, Georgia, Germany, Greece, Hungary, Iran, Ireland, Italy, Japan, Kazakhstan, Korea, Latvia, Lithuania, Moldova, Mongolia, Morocco, Netherlands, Norway, Poland, Portugal, Russia, Spain, Sweden, Switzerland, Türkiye, Ukraine, United Kingdom).

Hosts: *Agonopterix atomella, Cydia compositella, Cydia delineana, Cydia pallifrontana, Cydia splendana, Cydia tenebrosana, Dichrorampha acuminatana, Epiblema cirsiana, Epiblema scutulana, Gypsonoma aceriana, Gypsonoma minutana, Lathronympha strigana, Lobesia botrana, Lobesia euphorbianus, Mompha epilobiella, Ptocheuusa inopella, Rhopobota ustomaculana, Sparganothis pilleriana, Utetheisa jacobaeae.*

Disophrys caesa (Klug, 1835)

Material examined: Amasya: Karaibrahim-Çengel, 40°31'20''N 36°10'29''E, 03.09.2003, 1♀; Çorum: Kuşsaray, 40°35'44''N 35°08'36''E, 29.06.2004, 1♂; Tokat: Reşadiye-Çayırpınar, 40°24'16''N 37°16'16''E, 01.07.2004, 2♂♂.

General distribution: Palearctic Region (Algeria, Armenia, Azerbaijan, Bulgaria, Croatia, former Yugoslavia, France, Germany, Hungary, Iran, Italy, Morocco, Portugal, Romania, Russia, Spain, Switzerland, Türkiye).

Hosts: Unknown

As a result of this study caried out in the Central Black Sea Region, 17 species of the genera Agathis, Bassus and Disophrys belong to Agathidinae were reported. All of the species are new to the fauna of Central Black Sea Region of Türkiye.

Agathis anglica and *A. malvacearum* are the most common species in the studied areas in all provinces where the study was conducted (Amasya, Çorum, Ordu, Samsun and Tokat) in the Central Black Sea Region of Türkiye. The highest number of specimens (50 females and 4 males) belongs to *A. anglica*.

The highest species diversity was found in Amasya. Compared with other provinces in the Central Black Sea Region, Çorum and Ordu have the lowest numbers of Agathidinae species (Table 2). Compared to the other studied locations, these locations are poorly studied, however, new records have been found for the provinces.

Agathidins are koinobiont endoparasitoids of lepidopteran larvaes, which are plant pests, and thus these insects are important in natural regulation of pests in agriculture and forest areas. Therefore, it is essential to determine species diversity, habitat and host complexes of these beneficial insects so as to understand and protect their populations in nature.

Some identified parasitoid species in this study were reported as parasitoids in important agricultural and forest pests in previous studies. Among the reported species, *Agathis fuscipennis* is the parasitoid of the harmful tomato moth *Tuta absoluta*; *Agathis montana* is the *parasitoid* of *Pandemis cerasana*, which causes direct economic damage to flowers, fruits or grains of some pome fruits; *Bassus conspicuus* and *Bassus dimidiator* are *parasitoids* of apple pests *Cydia pomonella* and *Choristoneura rosaceana* and *Bassus tumidulus* is parasitoid of poplar borer *Gypsonoma aceriana* (Güçlü and Özbek 2007, Loni et al. 2011, Mills 2005, Wilkinson et al. 2004, Žikić et al. 2013).

Consequently, identifying these parasitic wasps which are quite considerable in biological control studies from the study area will guide future studies and also the identified species will contribute to the fauna of Türkiye.

ACKNOWLEDGEMENTS

Thanks are due to the Scientific Research Fund of Trakya University (TÜBAP-553) for financial support.

ÖZET

Bu çalışma Türkiye'nin Orta Karadeniz Bölgesine ait Agathidinae (Hymenoptera: Braconidae) faunasını ortaya koymak için 2003-2004 yılları arasında gerçekleştirilmiştir. Bu amaçla bölgedeki, Amasya, Çorum, Ordu, Samsun ve Tokat illerinin farklı yükseklik ve habitatlarından Agathidinae örnekleri toplanmıştır. Toplam 17 tür belirlenmiş ve tanımlanmıştır. Bunlar, *Agathis* cinsine ait on iki tür, *Bassus* cinsine ait dört tür ve *Disophrys* cinsine ait bir türdür. Tespit edilen tüm türler Türkiye'den daha önce kaydedilmiş olmasına rağmen araştırma bölgesi için yeni kayıt niteliğindedir. Tespit edilen türlerin dağılışları ve detaylı lokalite kayıtları hakkında bilgiler verilmiştir.

Anahtar kelimeler: dağılım, endoparazitoid, koinobiont, Türkiye, Agathidinae

REFERENCES

Çetin Ö., Beyarslan A., 2001. The Agathidinae (Hymenoptera: Braconidae) fauna of the Marmara Region. Turkish Journal of Zoology, 25 (3), 257-268.

Çetin Erdoğan Ö., 2005. *Bassus beyarslani* sp. n. (Hymenoptera, Braconidae, Agathidinae) from Turkey. Biologia, 60 (2), 129- 132.

Çetin Erdoğan Ö., 2010. A new species, *Agathis berkei* sp. n. from Eastern Anatolia, Turkey (Hymenoptera, Braconidae, Agathidinae). Turkish Journal of Zoology, 34 (2), 177-180.

Çetin Erdoğan Ö., 2013. Contributions to the knowledge of Agathidinae fauna of the Eastern Anatolia Region of Türkiye. Turkish Journal of Zoology, 37 (2), 195-199.

Çetin Erdoğan Ö., 2014. A contribution on the subfamily Agathidinae (Hymenoptera: Braconidae) in Diyarbakır, Mardin, and Şanlıurfa provinces of Turkey. Turkish Journal of Zoology, 38 (1), 102-103.

Çetin Erdoğan Ö., Beyarslan A., 2004. First record of *Agathis rubens* Tobias from Türkiye (Hymenoptera: Braconidae: Agathidinae). Acta Entomologica Slovenica, 12 (2), 253-254.

Çetin Erdoğan Ö., Beyarslan A., 2006. New records of endoparasitoid *Bassus Fabricius*, 1804 (Hymenoptera: Braconidae: Agathidinae) species from Turkey. Phytoparasitica, 34 (4), 353-356.

Çetin Erdoğan Ö., Beyarslan A., 2009. Doğu Karadeniz Bölgesi Agathidinae Haliday, 1833 (Braconidae: Hymenoptera) türleri üzerine bir araştırma. Türk Entomoloji Dergisi, 33 (1), 73-80 (in Turkish).

Çetin Erdoğan Ö., Beyarslan A., 2016. Faunistic survey on the Agathidinae (Hymenoptera: Braconidae) in the Central Anatolia Region with a new record for the Turkish fauna: *Bassus tegularis* (Thomson, 1895). Turkish Journal of Zoology, 40 (3), 448-453.

Çetin Erdoğan Ö., van Achterberg C., Beyarslan A., 2009. On the zoogeographical distribution of the genus *Agathis Latreille*, 1804 (Hymenoptera: Braconidae: Agathidinae) in Türkiye. Journal of the Entomological Research Society, 11 (1), 17-25.

Güçlü C., Özbek H., 2002. The Subfamily Agathidinae (Hymenoptera, Braconidae) of Erzurum Province. Journal of the Entomological Research Society, 4 (2), 7-19.

Güçlü C., Özbek H., 2007. *Agathis montana* Shestakov (Hymenoptera: Braconidae), a new parasitoid of *Pandemis cerasana* Huebner (Lepidoptera: Tortricidae) in Turkey. Entomological News. 118 (5), 534.

Loni A., Rossi E., van Achterberg K., 2011. First report of *Agathis fuscipennis* in Europe as parasitoid of the tomato leafminer *Tuta absoluta*. Bulletin of Insectology, 64 (1), 115-117.

Mills N., 2005. Selecting effective parasitoids for biological control introductions: Codling moth as a case study. Biological Control, 34 (3), 274-282.

Simbolotti G., van Achterberg C., 1992. Revision of the West Palearctic species of the genus *Bassus Fabricius* (Hymenoptera: Braconidae). Zoologische Verhandelingen, 281 (1), 1-80.

Simbolotti G., van Achterberg C., 1999. Revision of the West Palearctic species of the genus *Agathis Latreille* (Hymenoptera: Braconidae). Zoologische Verhandelingen, 325 (1), 1-167.

van Achterberg C., Long K.D., 2010. Revision of the Agathidinae (Hymenoptera, Braconidae) of Vietnam, with the description of forty-two new species and three new genera. ZooKeys, 54, 1-184.

Wilkinson T.K., Landis D.A, Gut L.J., 2004. Parasitism of oblique banded leafroller (Lepidoptera: Tortricidae) in commercially managed Michigan apple orchards. Journal of Economic Entomology, 97 (5), 1524-1530.

Yu D.S., van Achterberg C., Horstmann K., 2016. Home of Ichneumonoidea. Ottawa, Canada. Available online at www.taxapad.com (accessed date: 26.02.2023).

Zettel H., Beyarslan A., 1992. Über Agathidinae aus der Türkei (Hymenoptera: Braconidae). Entomofauna, 13, 121-132.

Žikić V., Stanković S.S., Ilić M., Kavallieratos N.G., 2013. Braconid parasitoids (Hymenoptera: Braconidae) on poplars and aspen (*Populus* spp.) in Serbia and Montenegro. North-Western Journal of Zoology, 9 (2), 264-275.

Cite this article: Erdoğan, Ö. (2023). Faunistic contributions on the subfamily Agathidinae (Hymenoptera: Braconidae) of Central Black Sea Region of Türkiye, Türkiye. Plant Protection Bulletin, 63-3. DOI: 10.16955/bitkorb.1261587

Atıf için: Sırrı, M. & Özaslan, C. (2023). Orta Karadeniz Bölgesi (Türkiye) Agathidinae (Hymenoptera: Braconidae) altfamilyası üzerine faunistik katkılar. Bitki Koruma Bülteni, 63-3. DOI: 10.16955/bitkorb.1261587

Bitki Koruma Bülteni / Plant Protection Bulletin

http://dergipark.gov.tr/bitkorb

Original article

Diagnosis of Peach latent mosaic viroid (PLMVd) and Hop stunt viroid (HSVd) by RT-PCR using different extraction protocols

Peach latent mosaic viroid (PLMVd) ve Hop stunt viroid (HSVd)'in farklı ekstraksiyon metotları kullanılarak RT-PCR ile teşhisi

Kamil DUMAN[a]*, Mustafa GÜMÜŞ[b]

[a]https://orcid.org/0000-0003-4240-9253, [b]https://orcid.org/0000-0002-1603-8666

[a]*Directorate of Plant Protection Central Research Institute, Gayret Mah. Fatih Sultan Mehmet Bulv. 06172 Yenimahalle, Ankara, Türkiye*
[b]*Ege University, Faculty of Agriculture, Plant Protection Department, İzmir, Türkiye*

ARTICLE INFO

Article history:
DOI: 10.16955/bitkorb.1239183
Received : 19-01-2023
Accepted : 14-08-2023

Keywords:
TNA extraction, RT-PCR, viroid, stone fruit, nucleic acid

* Corresponding author: Kamil DUMAN
ziraatcitr@outlook.com

ABSTRACT

RT-PCR method was performed using four different nucleic acid extraction methods to identify viroids; peach latent mosaic viroid (PLMVd) and hop stunt viroid (HSVd) which causes serious damage to stone fruits. Leaf samples were collected from fruit orchards showing viroid-like symptoms in İzmir province. Silica capture, citric buffer, lithium chloride, and ames buffer methods were used to extract total nucleic acids. The four extraction methods were compared using samples collected during the vegetation period from naturally infected trees (plum, apricot, and peach). They were evaluated with RT-PCR tests. In 64 stone fruit tree samples, only the silica capture method gave reliable and accurate results in RT-PCR molecular tests for the detection of PLMVd and HSVd. The other wielded nucleic acid extraction methods were found to be ineffective for the isolation of the viroid RNAs.

INTRODUCTION

Stone fruits are significant in the agricultural economy and contain 30% of fruit production; in total 3 million tons of stone fruits are produced in Türkiye (TÜİK 2019). Plant pathogenic viroids are small, single-stranded RNA molecules that infect plants and cause disease (Góra-Sochacka 2004). Viroids do not code or change proteins. They show their effects in host plants with plant enzymes. They cause alteration of the genes that regulate plants' growth and development. In previous studies, it was determined that viroids cause disease not only in highly structured plants but also induce disorders in hosts from a lot of various families and genera (Góra-Sochacka 2004). Potato, tomato, hop, coconut, grapevine, citrus, avocado, peach, apple, pear, and chrysanthemum are among the hosts that viroids cause disease. Therefore, the wide host range of viroids facilitates their spread and transmission. Viroids are transmitted by grafting, Cuscuta spp., vector insects, seed, pollen, and mechanical ways (Hataya et al. 2017).

They systematically consist of two families. These families are; Pospiviroidae and Asunviroidae. In viroid diseases, it is crucial to identify the viroid with all aspects, determine its strain and hosts, and finally detect the viroid with the rapid diagnostic methods for the control (Flores et al. 2004). They cannot be detected by the serological methods because of the absence of their protein coats but can be detected by

molecular methods such as hybridization and RT-PCR in the diagnosis of plant viroid diseases (Hataya et al. 2017, Ragozzino et al. 2004). It is often necessary to extract the nucleic acids from plant tissues to detect and study these viroids. There are many different nucleic acid extraction protocols available, and the choice of method depends on several factors such as the nature of the sample, the downstream application, and the sensitivity of the detection method.

Diagnosis and detection are as considerable as cultural and sanitation practices in preventing the spread of viroid diseases. Indicator plants, molecular hybridization, and Reverse Transcription Polymerase Chain Reaction (RT-PCR) methods are reliable and the most used among the detection methods.

RT-PCR has been widely used for a long time in molecular biology studies. Extraction and purification methods are also very important in obtaining the desired amount of virus and viroid RNAs from plants (Akbas and Degirmenci 2010, Schepetiuk et al. 1997, Wiedbrauk et al. 1995). There are many different nucleic acid extraction protocols available, and the choice of method depends on several factors such as the nature of the sample, the downstream application, and the sensitivity of the detection method. Many materials existing in the structure of the plant as cytosol, gum, and phenolic compounds play a preventative role in the reaction of RT-PCR (Martelli et al. 1994). The main challenge in RT-PCR application occurs in the process of the preparation of nucleic acid extraction in good quality. This problem is encountered generally in studies conducted on woody plants, especially with the origin of Malus and Prunus (Korschineck et al. 1991). Mostly used nucleic acid extraction procedures cannot terminate the connection with polysaccharides and phenolic compounds preventing RT-PCR (Demeke and Adams 1992).

Herein, it was aimed to determine the best RNA extraction method for agents representing both viroid families causing diseases in orchards in Türkiye and the world. For this, peach latent mosaic viroid (PLMVd) belonging to the family Pospiviroidae and hop stunt viroid (HSVd) belonging to the family Asunviroidae, which are the most significant viroids that cause diseases wherever stone fruits are grown, were selected.

MATERIALS AND METHODS

PLMVd and HSVd isolates from the previous studies and the 64 leave samples collected from apricot, peach, and plum trees, positive and negative controls consist of the main materials of the study. These isolates were used in RT-PCR tests..

Field surveys and sampling

Plant leaves showing viroid-like symptoms were collected from various fruit orchards in İzmir province. Sampling was conducted during the spring and summer months. Collected leaf samples were put into the polyethylene bags, marked with a code, and kept in an icebox during transportation to the laboratory. All samples were kept at -20 °C in deep freeze until nucleic acid extraction.

Total nucleic acid (TNA) extraction

Total nucleic acid extraction was carried out using four different methods. These methods are; silica capture (Foissac et al. 2000), citric buffer (Wetzel et al. 1992), lithium chloride (Hughes and Galau 1988), and ames/chloroform buffer methods (Laulhere and Rozier 1976, Podleckis et al. 1993).

RT-PCR (Reverse transcriptase polymerase chain reaction) method

In the PCR reaction, complementary DNA (cDNA) was synthesized for the total nucleic acid of viroids extracted from plants. For this purpose, protocols of the cDNA (Super Script RNAase H) kit supplied by Invitrogen (Invitrogen, TECH-LINESM U.S.A.) company were followed. The PCR process was handled at 50 µl volume according to the procedure advised by Fermantas Company. Primers used in the RT-PCR test were given in Table 1.

25 µl 2x PCR master mix. 1 µl primer 1, 1 µl primer 2, 2 µl cDNA, and 21 µl nuclease-free water were added to the sterile PCR tubes. Amplifications were carried out in an analytikjena thermal cycler in the cycling conditions, given in Table 2 for each viroid (Candresse et al. 1995). PCR products were separated by electrophoresis in 1.5% agarose gel in TAE buffer at 105 V for 60 minutes and stained with ethidium bromide and visualized under UV light.

Table 1. Viroid primers used in RT-PCR analysis

Viroid	Primer	Base Pair	Sequence	Position	Reproduced DNA (bp)	References
PLMVd	cPLMVd	25	5'-AACTGCAGTGCTCCGAATAGGGCAC-3'	91-115	337	Loreti et al. (1999)
	hPLMVd	25	5'-CCCGATAGAAAGGCTAAGCACCTCG-3'	116-140		
HSVd	VP19	26	5'-GCCCCGGGGCTCCTTTCTCAGGTAAG-3'	60-85	300	Astruc et al. (1996)
	VP20	25	5'-CCCGGGGCAACTCTTCTCAGAATCC-3'	80-102		

PLMVd, peach latent mosaic viroid; HSVd, hop stunt viroid.

Table 2. Followed programs in RT-PCR analysis

PLMVd	30 cycles 95 °C 3 min	30 cycles 95 °C – 30 s 60 °C – 45 s 72 °C - 45 s	30 cycles 72 °C 7 min
HSVd	1 cycle 95 °C 3 min	30 cycles 95 °C – 30 s 60 °C – 45 s 72 °C - 45 s	1 cycle 72 °C 7 min

RESULTS AND DISCUSSION

A total of 64 samples, 2 positive control, and 2 negative control plants were employed in the study. For the four nucleic acid extraction methods; two different primer pairs were put into account. Both primer pairs were worked with the silica capture method in previous assays and their existence was validated. PLMVd was found in 12 out of 64 tested samples, and hop stunt viroid was found in 8 samples by using the silica capture method. Citric buffer, lithium chloride, and ames buffer methods did not work properly in extracting nucleic acids and they did not show any bands formations on agarose gel after running the RT-PCR products in the electrophoresis. The best option for extracting nucleic acids from plant pathogenic viroids may depend on several factors such as the nature of the sample, the downstream application, and the sensitivity of the detection method.

The TNA extraction method has a highly significant impact on the constitution of RT-PCR final productions. The results indicated that the silica capture method provided suitable for extracting nucleic acids from plant tissues. Sipahioglu et al. (2007) also obtained very good results with the silica capture method based on Foissac et al. (2000), who reported that nucleic acid extraction was realized at the highest rate and quality with the silica capture method. That result was also confirmed by our study and the best result was obtained with this method. As compared to the other three extraction methods, this method was simple and fast and had a high yield of purified nucleic acids. Positive results could not be taken with the other three extraction methods tried. In addition, it was seen that this method was fast and easy to perform when compared to the others.

Although positive results were obtained from the lithium chloride method in some studies (Cieslinska 2004, Hataya et al. 1999, Loreti et al. 1999, Ragozzino et al. 2003, Shamloul et al. 2002), we could not get positive results with this extraction method. This may be derived from its requiring special handling of the RNA pellet, particularly during the washing steps. In the same way, certain types of RNA, including viral RNA, may not be as effective using this method. Navarro et al. (2000) confirmed our results in their study. They reported that lithium chloride was less effective than other methods for isolating viroid RNA from infected plants (Navarro et al. 2000). On the contrary, Cieslinska (2004) reported that even diluted ratios of the lithium chloride method were more successful and efficient than the silica capture method in obtaining strawberry mottle virus RNA in strawberry tissues. This may depend on several factors, such as the nature of the sample, the downstream application, and the sensitivity of the detection method. However, the silica capture method as mentioned above was found to be more suitable for extracting nucleic acids from plant tissues in many plant virology studies (Rott and Jelkmann 2001, Sipahioglu et al. 2007, Zacharzewska et al. 2014)

The ames buffer method has the advantage of being the fastest method for the total nucleic acid extraction process compared to the other nucleic acid extraction methods (Laulhere and Rozier 1976, Podleckis et al. 1993). However, the purity of the RNA produced by the process is just as important as how long it took. However, in our study, we could not get good results with that extraction method.

It seems perfectly reasonable that the citric acid method we used in our study did not yield positive results. Because it was noted that the citric buffer method is a more practical way to isolate DNA from small amounts of fresh plant tissue (Doyle and Doyle 1987). Although it was reported to successfully isolate viroid RNA from infected plant tissues (Diener and Lawson 1984), it is particularly useful for extracting DNA from plant tissues. Therefore, it can be said that the citric buffer method may not be as effective at isolating RNA, and it may not be appropriate for all kinds of materials. Additionally, it was thought that these two methods deactivate the phenolic and polysaccharide compounds in the plant during nucleic acid extraction and reverse transcriptase. Furthermore, these compounds inhibit detection in RT-PCR tests. The nucleic acid used for the detection of viroids by PCR should be as pure as possible. Wilde et al. (1990) proved that reverse transcriptase with its over-sensibility has a great significance on the appearance of the inhibitive and regulative characteristics of the substances in the amplification reactions. This also demonstrates that the extraction methods used in the study played a significant role in removing the inhibitory substance. The usage of silica capture minimizes the deterioration of PCR products. RNA isolated and PCR products formed by the silica capture method can stay without spoiling for a couple of months and can be kept to work on it again. This shows the existence of a very useful method for long-term analysis of both plants and products and as well as for PCR products and RNA-isolated from them (Sipahioglu et al. 2006a).

This shows how the reverse transcription stage is important while working with viroids on RT-PCR and how the phenolic compounds existing in plant tissues can affect the result of extraction high in a contrary way.

The lithium chloride and the silica capture methods which contain mercaptoethanol can minimize the effect of polyphenols and polysaccharides in plant tissues thus both methods can work on various plant species in the extraction of nucleic acids very well (Cieslinska 2004, Sipahioglu et al. 2006b). After getting the results in that variation, it is obvious that how silica capture method is the correct method in use for nucleic acid extraction. Our study showed that the silica capture method is the most reliable and it can be used successfully in the detection of PLMVD and HSVd on stone fruits. The study also showed that using different methods for distinct viroids on various plant species can show dissimilar results like the success of the silica capture method and failure of the lithium chloride, citric acid, and ames buffer method. Obtaining results by using these methods can be very fruitful from the perspective of today's fruit-growing sector, in the production of rootstock, sapling, and grafting materials for obtaining disease-free and certified production materials.

In conclusion, the silica capture method appears to be the best option for isolating total nucleic acids of PLMVd and HSVd from stone viroids. However, when choosing an extraction method, it is crucial to keep in mind that the particular type of viroid and the characteristics of the sample being analyzed may have an impact on the choice of extraction method. Finally, this study revealed once again that the silica capture extraction method is the most suitable extraction method, which ensures that the nucleic acid of plant pathogenic viruses and viroids is obtained in the highest yield, purest, and without causing any loss or degradation.

ACKNOWLEDGEMENTS

I would like to offer my special thanks to Assoc. Prof. Dr. İsmail Can PAYLAN for his guidance during my studies and Assoc. Prof. Dr. Birol AKBAŞ for his support and help in the preparation of my manuscript.

ÖZET

Sert çekirdekli meyve ağaçlarında ekonomik zarara neden olan peach latent mosaic viroid (PLMVd) ve hop stunt viroid (HSVd)'leri RT-PCR ile tanılamak için 4 farklı nükleik asit ekstraksiyon yöntemi kullanılmıştır. Yaprak örnekleri İzmir ilinde farklı meyve bahçelerinden viroid benzeri belirti gösteren ağaçlardan alınmıştır. Silica capture, lithium chloride, citric buffer ve ames buffer yöntemleri kullanılarak total nükleik asit ekstraksiyonu gerçekleştirilmiştir. Dört farklı ekstraksiyon yöntemi erik, kayısı ve şeftali ağaçlarından farklı dönemlerde toplanan örnekler kullanılarak birbiri ile karşılaştırılmıştır. Altmış dört farklı sert çekirdekli bitki örneği RT-PCR test yöntemi ile değerlendirilmiş ve silica capture ekstraksiyon yöntemi, kullanılan yöntemler arasında en güvenilir sonucu vermiştir. Diğer 3 ekstraksiyon yöntemi ile yapılan ekstraksiyonlarda istenilen sonuç alınamamıştır.

Anahtar kelimeler: TNA ekstraksiyonu, RT-PCR, viroid, sert çekirdekli meyve, nükleik asit

REFERENCES

Akbas B., Degirmenci K., 2010. Simultaneous detection of Apple mosaic virus in cultivated hazelnuts by one-tube RT-PCR. African Journal of Biotechnology, 9 (12), 10.5897/AJB10.1749

Astruc N., Marcos J.F., Macquaire G., Candresse T., Pallás V., 1996. Studies on the diagnosis of hop stunt viroid in fruit trees: Identification of new hosts and application of a nucleic acid extraction procedure based on non-organic solvents. European Journal of Plant Pathology, 102, 837-846.

Candresse T., Lanneau T., Revers F., Grasseau N., Macquaire G., German S., Malinowsky T., Dunez J., 1995. An immunocapture PCR assay adapted to the detection and the analysis of the molecular variability of apple chlorotic leaf spot virus. Acta Horticulturae, 386, 136-147.

Cieslinska M., 2004. Detection of strawberry mottle virus (SMoV) using RT-PCR: comparison of two RNA extraction methods. Journal of Fruit and Ornamental Plant Research, 12, 17-22.

Demeke T., Adams R.P., 1992. The effects of plant polysaccharides and buffer additives on PCR. Biotechniques, 12 (3), 332-334.

Diener T.O., Lawson R.H., 1973. Chrysanthemum stunt: a viroid disease. Virology, 51 (1), 94-101.

Doyle J.J., Doyle J.L., 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin, 19, 11-15.

Flores R., Delgado S., Gas M.E., Carbonell A., Molina D., Gago S., De La Pena M., 2004. Viroids: The minimal non-coding RNAs with autonomous replication. FEBS Letter, 567 (1), 42-48.

Foissac X., Savalle-Dumas L., Gentit P., Dulucq M.J., Candresse T., 2000. Polyvalent detection of fruit tree Tricho, Capillo and Favea viruses by nested RT-PCR using degenerated and inosine-containing primers (PDORT-PCR). Acta Horticulturae, 357, 52–59.

Góra-Sochacka A., 2004. Viroids; unusual small pathogenic RNAs. Acta Biochimica Polonica, 51 (3), 587-607.

Hataya T., Nakahara K., Furuta K., Shikata E., 1999. Comparisons of gene diagnostic methods for the practical diagnosis of chrysanthemum stunt viroid in chrysanthemum plants. Archives of Phytopathology and Plant Protection, 32 (3), 179–192.

Hataya T., Tsushima T., Sano T., 2017. Hop stunt viroid. In: Viroids and satellites. Hadidi, A., Flores, R., Randles J.W., Paukaitis, P. (Eds.). Oxford, UK: Academic Press, 199-210 pp.

Hughes D.W., Galau G., 1988. Preparation of RNA from cotton leaves and pollen. Plant Molecular Biology Reporter, 6, 253-257.

Korschineck I., Himmler G., Sagl R., Steinkellner H., Katinger H.W., 1991. A PCR membrane spot assay for the detection of plum pox virus RNA in bark of infected trees. Journal of Virological Methods, 31 (2-3), 139-145.

Laulhere J-P., Rozier C., 1976. One-step extraction of plant nucleic acids, Plant Science Letters, 6 (4), 237-242.

Loreti S., Faggioli F., Cardoni M., Mordenti G., Babini A.R., Poggi Pollini C., Barba M., 1999. Comparison of different diagnostic methods for detection of peach latent mosaic viroid. EPPO Bulletin, 29 (4), 433 – 438.

Martelli G.P., Candresse T., Namba S., 1994. Trichovirus, a new genus of plant viruses. Archives of Virology, 134, 451-455.

Navarro J.A, Vera A., Flores R., 2000. A chloroplastic RNA polymerase resistant to tagetitoxin is involved in replication of avocado sunblotch viroid. Virology, 268 (1), 218-225. doi 10.1006/viro.1999.0161.

Podleckis E.V., Hammond R.W., Hurtt S.S., Hadidi A., 1993. Chemiluminescent detection of potato and pome fruit viroids by digoxigenin-labeled dot-blot and tissue blot hybridization. Journal of Virological Methods, 43, 147-158.

Ragozzino E., Faggioli F., Alioto D., Barba M., 2003. Detection and differentiation of peach latent mosaic viroid and hop stunt viroid in stone fruit trees in Italy using multiplex RT-PCR. Phytopathologia Mediterranea, 42 (1), 79-84.

Ragozzino E., Faggioli F., Barba M., 2004. Development of a one tube-one step RT-PCR protocol for the detection of seven viroids in four genera: Apscaviroid, Hostuviroid, Pelamoviroid, and Pospiviroid. Journal of Virological Methods, 121 (1), 25-29.

Rott M.E., Jelkmann W., 2001. Characterization and detection of several filamentous viruses of cherry: adaptation of an alternative cloning method (DOP-PCR), and modification of an RNA extraction protocol. European Journal of Plant Pathology, 107, 411-420.

Schepetiuk S., Kok T., Martin L., Waddell R., Higgins G., 1997. Detection of Chlamydia trachomatis in urine samples by nucleic acid tests: comparison with culture and enzyme immunoassay of genital swab specimens. Journal of Clinical Microbiology, 35 (12), 3355-3357.

Shamloul A.M., Faggioli F., Keith J.M., Hadidi A., 2002. A novel multiplex RT-PCR probe capture hybridization (RT-PCR-ELISA) for simultaneous detection of six viroids in four genera: Apscaviroid, Hostuviroid, Pelamoviroid, and Pospiviroid. Journal of Virological Methods, 105 (1), 115-121.

Sipahioglu H.M., Usta M., Ocak M., 2006a. Use of dried high-phenolic laden host leaves for virus and viroid preservation and detection by PCR methods. Journal of Virological Methods, 137 (1), 120-124.

Sipahioğlu H.M., Demir S., Myrta A., Al Rwahnih M., Polat B., Schena L., Usta M., Akkopru A., Selcuk M., Ippolito A., Minafra A., 2006b. Viroid, phytoplasma, and fungal diseases of stone fruit in eastern Anatolia, Turkey. New Zealand Journal of Crop and Horticultural Science, 34, 1, 1-6, doi: 10.1080/01140671.2006.9514380

Sipahioglu H.M., Ocak M., Usta M., 2007. Comparison of three conventional extraction methods for the detection of plant virus/viroid RNAs from heat dried high-phenolic host leaves. Asian Journal of Plant Sciences, 6 (1), 102-107.

TÜİK, 2019. Bitkisel Üretim İstatistikleri. Turkiye İstatistik Kurumu (TUİK), (http://www.tuik.gov.tr/). (accessed date: 09.09.2023).

Wetzel T., Candresse T., Macquaire G., Ravelonandro M., Dunez J., 1992. A highly sensitive immunocapture polymerase chain reaction method for plum pox potyvirus detection. Journal of Virological Methods, 39 (1-2), 27-37.

Wilde J., Eiden J., Yolken R., 1990. Removal of inhibitory substances from human fecal specimen for detection of group A rotaviruses by reverse transcriptase and polymerase chain reactions. Journal of Clinical Microbiology, 28, 1300-1307.

Zacharzewska B., Przewodowska A., Treder K., 2014. The adaptation of silica capture RT-PCR for the detection of Potato Virus Y. American Journal of Potato Research, 91, 525–531. https://doi.org/10.1007/s12230-014-9383-y

Cite this article: Duman, K. & Gümüş, M. (2023). Diagnosis of Peach latent mosaic viroid (PLMVd) and Hop stunt viroid (HSVd) by RT-PCR using different extraction protocols. Plant Protection Bulletin, 63-3. DOI: 10.16955/bitkorb.1239183

Atıf için: Duman, K. & Gümüş, M. (2023). Peach latent mosaic viroid (PLMVd) ve Hop stunt viroid (HSVd)'in farklı ekstraksiyon metotları kullanılarak RT-PCR ile teşhisi. Bitki Koruma Bülteni, 63-3. DOI: 10.16955/bitkorb.1239183

PLANT PROTECTION BULLETIN PRINCIPLES OF PUBLISHING

1. All responsibility for the published article belongs to authors.

2. Plant Protection Bulletin publishes the researches on taxonomic, biological, ecological, physiological and epidemiological studies and methods of protection against diseases, pest, and weed which cause damages on plant products as well as researches on residue, toxicology, and formulations of pesticides and plant protection machinery.

3. The publishing language of the journal is English and Turkish. Turkish abstract would be prepared by the editorial office, if necessary.

4. It is not accepted in Plant Protection Bulletin that biological observations carried out in a single year and in one orchard or field, and short biological notes reported one species of first records for Turkey.

5. The articles submitted to the journal should not have been published in any publication or at the same time in the evaluation phase of another publication.

6. The articles containing the results of postgraduate theses or the projects supported by various institutions such as TÜBİTAK, SPO, TAGEM, BAP should be prepared for publication after the necessary permissions are obtained from the related persons. This must be stated in the "acknowledgments".

7. Submission of article requested to be published in the journal should be made via Dergipark system (http://dergipark.gov. tr/bitkorb).

8. The article uploaded to the system should be prepared according to the "Manuscript template" in the "For authors" tab. It should be uploaded together with " Manuscript cover page" and the "Copyright release form" and "Conflict of Interest and Reviewer Proposal Form" completed and signed by all authors.

9. In the journal, a blind review process for designated reviewers is being followed.

10. The articles included in the evaluation process are reviewed by subject editors and the designated reviewers and published after the corrections have been completed by their authors in accordance with recommendations.

11. There is no printing fee for articles published in the journal.

BİTKİ KORUMA BÜLTENİ YAYIN İLKELERİ

1. Yayınlanan eserlere ait tüm sorumluluk yazarlarına aittir.

2. Bitki Koruma Bülteni bitkisel ürünlerde zarar oluşturan hastalık, zararlı ve yabancı ot konularında yapılan taksonomik, biyolojik, ekolojik, fizyolojik ve epidemiyolojik çalışmaların ve mücadele yöntemleri ile ilgili araştırmaların yanı sıra, zirai mücadele ilaçlarının kalıntı, toksikoloji ve formülasyonları ile zirai mücadele alet ve makinaları ilgili araştırmaları yayınlamaktadır.

3. Bitki Koruma Bülteni'nin yayın dili İngilizce ve Türkçe'dir. Gerekli hallerde Türkçe özet editör ofisi tarafından hazırlanır.

4. Bitki Koruma Bülteni'nde tek yıllık ve tek bir bahçe veya tarlada gerçekleştirilmiş biyolojik gözlemler, Türkiye için tek bir türe ait ilk kayıtları bildirilen kısa biyolojik notlar gibi eserler kabul edilmemektedir.

5. Bitki Koruma Bülteni'ne gönderilen makaleler, daha önce herhangi bir yayın organında yayınlanmamış veya aynı zamanda başka bir yayın organında değerlendirme aşamasında olmamalıdır.

6. Lisansüstü tezler veya TÜBİTAK, DPT, TAGEM, BAP gibi çeşitli kurumlarca desteklenen projelerin sonuçlarından kısımlar içeren eserler ilgililerinden gerekli izinler alındıktan sonra yayına hazırlanmalı, bu durum teşekkür kısmında mutlaka belirtilmelidir.

7. Bitki Koruma Bülteni'nde yayınlanması istenilen eserler için makale başvurusu DERGİPARK sistemi (http://dergipark.gov.tr/bitkorb) üzerinden yapılmalıdır.

8. Sisteme yüklenen makale "Yazarlar için" sekmesinde yer alan "Makale taslağı"na göre hazırlanmalı, sisteme "Makale giriş sayfası" ve tüm yazarlar tarafından doldurulup imzalanan "Bitki Koruma Bülteni Telif Hakkı Devir Formu" ve "Çıkar Çakışması ve Hakem Önerileri Formu" ile birlikte yüklenmelidir.

9. Bitki Koruma Bülteni'nde kör hakemlik değerlendirme süreci izlenmektedir.

10. Değerlendirme sürecine dahil edilen makaleler konu editörü ve belirlenen hakemler tarafından incelenip, onların önerileri doğrultusunda yazarları tarafından düzeltildikten sonra yayınlanır.

11. Bitki Koruma Bülteni'nde yayınlanan makaleler için baskı ücreti alınmamaktadır.