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Original article (Orijinal araştırma)

Determination of plant parasitic nematodes associated with chickpea in Turkey[1]

Türkiye'de nohut alanlarındaki bitki paraziti nematodların belirlenmesi

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Abstract

A survey of plant parasitic nematodes associated with chickpea was conducted in the chickpea growing areas of Turkey including 37 districts in 17 provinces during spring and summer of 2014-2016. A total of 211 soil and root samples were collected. Nematodes were extracted from soil by different extraction methods to ensure all kinds of nematode groups. Nematodes were identified using morphological and morphometric features. In addition, *Pratylenchus* spp. Filipjev, 1936 were determined using species-specific primers. *Ditylenchus dipsaci* (Kühn, 1857), *Pratylenchus neglectus* (Rensch, 1924) and *Pratylenchus thornei* Sher & Allen, 1953 were the most common of the plant parasitic nematodes associated with chickpea in the areas surveyed. *Pratylenchus neglectus*, *P. penetrans* (Cobb, 1917) and *P. thornei* were present in almost all samples. In descending order, *P. thornei*, *P. neglectus* and *D. dipsaci* were detected in 179, 138 and 95 in samples (84, 65 and 45% of samples, respectively). Other nematodes found at lower frequency were species of *Aphelenchus* Bastian, 1965, *Criconemoides* Taylor, 1936*,* Dorylaimida species, *Helicotylenchus* Steiner, 1945, *Merlinius* Siddiqi, 1970, *Paratrophurus* Arias, 1970*, Paratylenchus* Micoletzky, 1922*, Trophurus* Loof, 1957, *Tylenchorhynchus* Cobb, 1930, *Tylenchus* Bastian, 1865 and *Xiphinema* Cobb, 1913.

Keywords: Chickpea, plant parasitic nematodes, molecular identification

Öz

Türkiye nohut üretim alanlarında nematod türlerini belirlemek amacıyla 17 ile bağlı 37 ilçede 2014-2016 yılları arasında yürütülen sürvey çalışmasında toplam 211 toprak ve kök örnekler toplanmıştır. Elde edilen örneklerde tüm nematod gruplarını elde etmek amacıyla, topraktan farklı ekstraksiyon yöntemleriyle elde edilmiştir. Nematod türlerinin teşhisi, morfolojik ve morfometrik özellikler kullanılarak klasik teşhis yöntemlerine göre yapılmıştır. Ayrıca, *Pratylenchus* Filipjev, 1936 türlerinin teşhisi için türe özgü primer yardımıyla moleküler yöntemleri kullanılmıştır. *Ditylenchus dipsaci* (Kühn, 1857), *Pratylenchus neglectus* (Rensch, 1924) ve *Pratylenchus thornei* Sher & Allen, 1953, sürvey yapılan nohut alanlarda en yaygın bitki paraziti nematodları tespit edilmiştir. *Pratylenchus neglectus, Pratylenchus penetrans* (Cobb, 1917) ve *P. thornei* tüm örneklerde tespit edilmiştir. *Pratylenchus thornei*, *P. neglectus* ve *D. dipsaci* incelenen toprak ve köklerde sırasıyla 179, 138 ve 95 örnekte (toplam örneklerin sırasıyla %84, 65 ve 45'inde) tespit edilmiştir. Toprak örneklerinde daha düşük *Aphelenchus* Bastian, 1965, *Criconemoides* Taylor, 1936, Dorylaimida species, *Helicotylenchus* Steiner, 1945, *Merlinius* Siddiqi, 1970, *Paratrophurus* Arias, 1970, *Paratylenchus* Micoletzky, 1922, *Trophurus* Loof, 1957, *Tylenchorhynchus* Cobb, 1930, *Tylenchus* Bastian, 1865 ve *Xiphinema* Cobb, 1913 cinslerine bağlı türler belirlenmiştir.

Anahtar sözcükler: Nohut, bitki paraziti nematodlar, moleküler teşhis

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Introduction

Chickpea (*Cicer arietinum* L.) has a prominent place in total legume production in the world. Turkey is ranked fifth in the world for chickpea production (FAO, 2017). The most important chickpea producing countries in the world are India, Australia, Myanmar, Ethiopia, Turkey, Pakistan, Russia, Iran, Mexico, the USA and Canada (FAO, 2017). Chickpea originated in the Fertile Crescent, which borders the southeastern regions of Turkey, and spread west and south via the historically called Silk Route. The average global chickpea yield is changing due to the effect of many biotic and abiotic limitations that can cause an important reduction in grain quantity and quality of chickpea (Singh & Sharma, 1994; Sudupak et al., 2002). Plant parasitic nematodes have been reported an economically important pest affecting chickpea as the biotic factors (Castillo & Vovlas, 2007). Plant parasitic nematodes generally feed on different parts of the plant, especially on roots and other subterranean plant structure such as rhizomes of some legumes. Many researchers have shown that plant parasitic nematodes cause damage to food legumes (Greco, 1985; Greco & Vitro, 1988; Greco & Sharma, 1990; Sikora & Greco, 1990).

The root lesion nematodes (RLNs), *Pratylenchus* spp. Filipjev, 1936 (Tylenchida: Pratylenchidae), are the most widespread nematodes in legume crops, such as alfalfa (*Medicago sativa* L.), chickpea (*Cicer arietinum* L.), faba bean (*Vicia faba* L.) and lentil (*Lens culinaris* Medikus) in Mediterranean regions (Greco et al., 1984). Similarly, Hollaway et al. (2000) reported that chickpea is generally considered as more susceptible to RLNs than faba bean, field pea and lupin but less so than wheat. Vanstone et al. (1998) also reported that *Pratylenchus crenatus* Loof, 1960, *Pratylenchus neglectus* (Rensch, 1924), *Pratylenchus penetrans* (Cobb, 1917) and *Pratylenchus thornei* Sher & Allen, 1953 are the most important *Pratylenchus* species worldwide. In addition, chickpea crops infested with RLNs show symptoms of stunted growth and may have some yellowing of foliage, but often have no obvious foliar symptoms of the disease. When many nematodes attack chickpea roots, the affected tissues can turn dark brown-black, have a reduction in root hairs or nodules, and discolored root tissue. Discoloration often appears as brown or black stripes along the roots. However, diagnosis of root symptoms is usually difficult in the chickpea and are normally not observed until plants are older than 8 weeks (Pulse Australia, 2013). In a survey of chickpea in Turkey (Di Vito et al., 1994), the other plant parasitic nematodes species found were *Helicotylenchus* Steiner, 1945 (Tylenchida: Hoplolaimidae), *Longidorus* Micoletzky, 1922 (Dorylaimida: Longidoridae), *Paratylenchus* Micoletzky, 1922 (Tylenchida: Paratylenchinae), *Trichodorus* Cobb, 1913 (Tylenchida: Trichodoridae), *Trophurus* Loof, 1956 (Tylenchida: Dolichodoridae), *Tylenchus* Bastian, 1865 (Tylenchida: Tylenchidae), *Xiphinema index* Thorne & Allen, 1950 and *Xiphinema pachtaicum* (Tulaganov, 1938) (Dorylaimida: Longidoridae).

The detection of new or potentially harmful species of nematode in the chickpea is important for in success of agriculture, and aids in the improvement and evaluation of quarantine or regulatory operation to minimize their spread. Correct identification of nematode species is basic to effective nematode control and successful plant quarantine procedure. Also, surveys in southern Spain chickpea fields showed that the legume and cereal root lesion nematodes such as *P. neglectus and P. thornei* were the most important and widespread plant parasitic nematodes (Castillo et al., 1996). RLNs are microscopic organisms and cannot be detected with the naked eye in the soil or in plants. Coolen (2013) reported that DNA analysis or direct counting (under a microscope) are the best ways to determine the presence of RLNs in the soil. Additionally, identification of *Pratylenchus* species is difficult because of the high degree of morphological similarity within the genus. Recently, Subbotin et al. (2008) stated that the different molecular techniques are needed to identify nematode species that have a close morphological similarity together.

Species of *Pratylenchus* Filipjev, 1936 infest a wide range of crops and causes important economic damage in global grain production. These nematodes have been found widely distributed in wheat field in Turkey. Toktay et al. (2006) reported that *P. thornei* is responsible for up to 19% of total losses in wheat fields in Turkey. Information on the species of plant parasitic nematodes infesting chickpea crops in Turkey is limited. A comprehensive study was done by Behmand (2018) on resistance of chickpea genotypes from Turkey against *P. neglectus*, *P. thornei* and *Ditylenchus dipsaci* (Kühn, 1857). The present study was undertaken to identify the most important plant parasitic nematode species potentially causing damage and yield loss in chickpea growing areas of Turkey.

Materials and Methods

Survey

A survey was conducted in 37 districts in 17 provinces in the Aegean, Central Anatolia, Central East Anatolia, East Marmara, Eastern Anatolia, Mediterranean, Southeastern Anatolia, Trace and West Marmara Regions of Turkey, during spring and summer 2014-2016 (Figure 1). A total of 211 soil and root samples (74 in 2014, 69 in 2015 and 68 in 2016) were collected using the sampling method of Bora & Karaca, (1970). Five to ten composite subsamples were taken from one location.

A soil auger was used to sample soil to 20 cm and combined to give 500-ml composite samples. Then, samples were individually packed in sealed plastic bags and brought to the laboratory as quickly as possible.

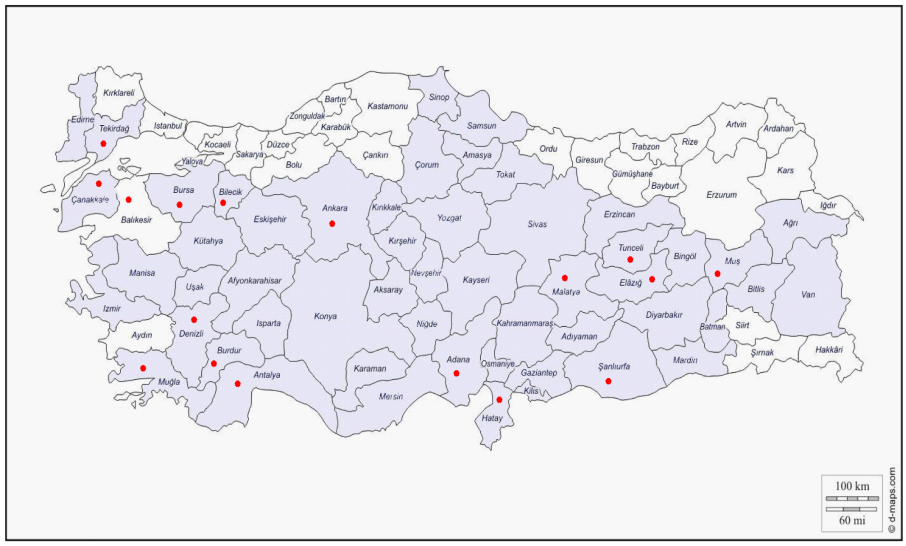

Figure 1. Location of sampling sites in 17 provinces of Turkey. Provinces in with over 10 ha of chickpea production are shown in gray.

Laboratory assessments

In the laboratory, plant shoots were removed and nematodes were extracted from the 500-ml soil samples by Cobb's sieving, centrifugal flotation (Jenkins, 1964) and modified Baermann funnels (Hooper, 1986), and extracted from roots by using an incubation technique (Young, 1954; Coolen, 1979). Then nematodes were killed at 60ºC for 1 min, fixed in a TAF solution and mounted on slides by wax-ring method (Seinhorst, 1959). The permanent slides were examined under a light microscope to identify specimens to species when possible. Also, for molecular confirmation, *P. neglectus* and *P. thornei* were identified by morphology (Handoo & Golden, 1989) and individually transferred in a small tube using a bamboo sliver under a light microscope, then placed onto surfaced sterilized carrot disk and incubated at 23±1°C for several generations to make a pure culture.

DNA was extracted from each nematode culture according to Waeyenberge et al. (2000), with some modification. From each *Pratylenchus* culture, five to ten second-stage juveniles were transferred with 25 µl sterile distilled water into an Eppendorf tube. Then, 10 µl of a suspension containing nematodes was pipetted into a 0.2-ml sterile Eppendorf tube with 8 µl of lysis buffer (500 mM KCl; 100 mM Tris-Cl, pH 8.3; 15 mM MgCl2; 10 mM dithiothreitol; 4.5% Tween 20; and 0.1% gelatin). The tube contents were frozen at -20°C for at least 20 min, then thawed, and 2 µl of proteinase K at 600 µg/ml added. The tubes were incubated for 60 min at 65°C and finally transferred to the thermocycler for 10 min at 95°C to inactivate proteinase. The tubes were then centrifuged at 16,000 rpm for 5 min and stored at -20°C until use as the DNA template.

A species-specific polymerase chain reaction (PCR) was used to identify the RLNs. The common reverse primer D3B5 and the primers PTHO D3B PNEG-F1 were used to identify *P. neglectus* and *P. thornei*, respectively (Table 1).

Table 1. Primer sequences and expected band sizes for *Pratylenchus neglectus* and *P. thornei*

Species Primer	Primer name*	Sequence (5′-3′)	Band size (bp)	Reference
P. neglectus	F: PNEG-F1	CGCAATGAAAGTGAACAATGTC	144	Yan et al. (2008)
	R: D3B5	AGTTCACCATCTTTCGGGTC		
P. thornei	F: PTHO	GAAAGTGAAGGTATCCCTCG	288	Al-Banna et al. (2004)
	R: D3B	TCGGAAGGAACCAGCTACTA		

* F, forward primer; R, reverse primer.

Results

From the 211 soil and root samples were collected from chickpea production areas surveyed, RLNs were determined in the Aegean, Central Anatolia, Central East Anatolia, East Marmara, Eastern Anatolia, Mediterranean, Southeastern Anatolia, Trace and West Marmara Regions of Turkey. *Pratylenchus* were observed in all samples in locations that were collected on chickpea growing areas. Of the *Pratylenchus* species, *P. thornei* and *P. neglectus* were identified by molecular methods in 179 (84%) and 138 (65%) samples, respectively. Chickpea plants infested with root lesion nematode had stunted growth, fewer leaves and branching. Symptoms of nematode infestation in roots were included loss of root hairs or nodules and poor root structure. Where the high population densities of nematodes attack chickpea roots, often show symptoms such as dark brown-black and discolored root tissue. Higher population densities of the RLNs was found in the Mediterranean and Aegean Provinces when compared with other regions of Turkey. A lower population density was determined in the West Marmara and Central Anatolia Regions (Figure 2). PCR with PNEG-F1/D3B5 primers and PTHO/D3B produced products of 144 and 288 bp for all the *P. neglectus and P. thornei* populations, respectively. (Figures 3 & 4). In addition, *D. dipsaci* was found in 95 soil samples (45% of the total samples). Chickpea fields infested with *D. dipsaci* showed symptoms of leaf and stem necrosis and pod deformity. Other plant parasitic nematodes found in the samples included species of *Aphelenchus* Bastian, 1965 (Aphelenchida: Aphelenchidae) (59%), *Helicotylenchus* (38%), *Merlinius* Siddiqi, 1970 (Tylenchida: Telotylenchidae) (37%), Dorylaimida (35%), *Tylenchus* (42%), *Tylenchorhynchus* Cobb, 1930 (Tylenchida: Dolichodoridae) (20%), *Paratylenchus* (10%), *Trophurus* (7%), *Paratrophurus* Arias, 1970 (Tylenchida: Dolichodoridae) (6%), *Paratylenchoides* Raski, 1973 (Tylenchida: Paratylenchidae) (8%), *X. pachtaicum* (3%), *X. index* (2%) and *Criconemoides* Taylor, 1936 (Tylenchida: Criconematidea) (2%). Generally, chickpea crops infested with these nematodes showed no symptoms and plant damage (Table 2).

Pratylenchus neglectus, P. thornei and *D. dipsaci* were observed in most samples and found to be causing damage to chickpea plants in the field. Geographical distribution of the most important plant parasitic nematodes in chickpea growing fields is shown in Figure 5. The four most common species were *P. thornei* (85% of samples), *P. neglectus* (65%), *D. dipsaci* (45%) and *P. penetrans* (18%) (Table 2).

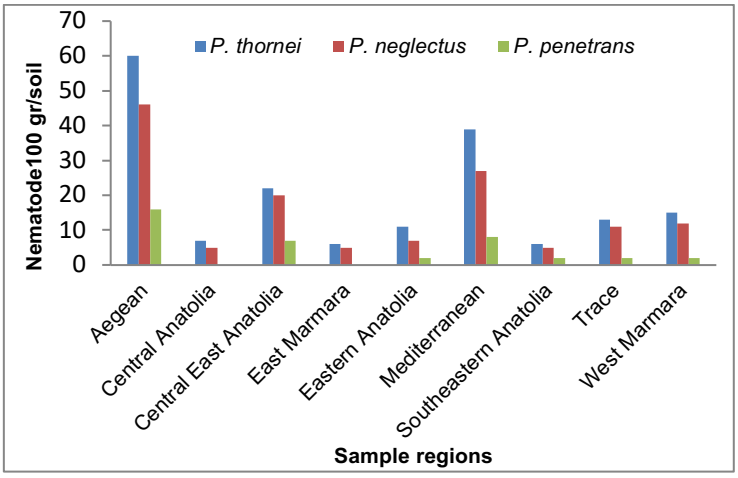

Figure 2. Frequency of RLNs (*Pratylenchus neglectus, P. penetrans* and *P. thornei*) in different chickpea production regions in Turkey.

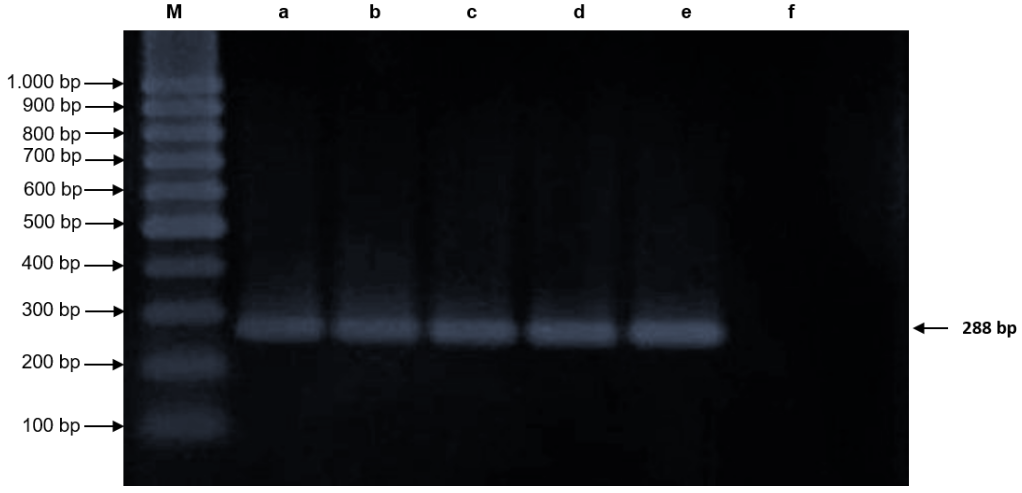

Figure 3. PCR patterns of *Pratylenchus thornei* amplified (288 bp) with specific primer set PTHO/D3B M: DNA molecular weight ladder (100 bp), a-e: samples, f: negative control.

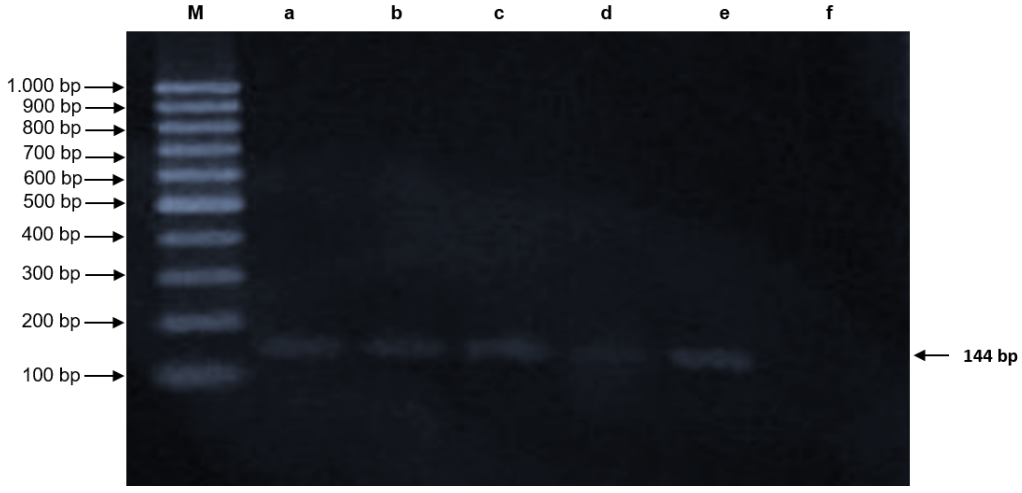

Figure 4. PCR patterns of *Pratylenchus neglectus* amplified (144 bp) with specific primer set PTHO/D3B M: DNA molecular weight ladder (100-bp), a-e: samples, f: negative control.

Table 2. Details of sampling locations and occurrence (number of positive samples per province) of identified nematodes

No	Region	Province	The number of samples collected	Latitude (N)	Longitude (E)	*D. dipsaci*	*P. thornei*	*P. neglectus*	*P. penetrans*	*Aphelenchus* spp	*Helicotylenchus* spp	Dorylaimida species	*Merlinius* spp	Other plant-parasitic nematodes*
1	Aegean	Balıkesir	7	40°15'21"	27°50'14"	2	4	3	2	1	-	2	-	*Tylenchorhynchus* spp (5), *Trophurus* spp (3)
2		Balıkesir	10	40°12'56"	27°45'33"	3	5	3	1	10	5	3	2	*Paratylenchus* spp (4), *Pratylenchoides* spp
3		Balıkesir	4	40°12'56"	27°46'2"	2	6	4	1	1	-	-	1	*Criconemoides* spp, *Tylenchus* spp (6)
4		Bursa	3	40°12'47"	28°41'13"	0	6	0	0	5	2	1	4	*Tylenchus* spp (9), *Trophurus* spp (2)
5		Denizli	5	37°34'13"	29°19'36"	2	5	5	2	1	-	-	1	*Tylenchus* spp (5), *Xiphinema pachtaicum* (2)
6		Denizli	4	37°37'5"	29°14'53"	3	6	4	1	4	5	2	-	*Paratylenchus* spp (5), *Criconemoides* spp
7		Denizli	10	37°50'0"	29°6'39"	0	6	5	1	5	4	1	3	*Tylenchorhynchus* spp (3), *Tylenchus* spp (5)
8		Denizli	8	37°34'54"	29°17'46"	2	5	6	2	6	10	5	1	*Paratylenchus* spp (3), *Xiphinema index*
9		Denizli	4	37°37'38"	29°12'37"	4	4	5	2	2	4	-	7	*Tylenchorhynchus* spp (4), *Paratylenchoides* spp (2)
10		Denizli	7	37°34'34"	28°59'24"	3	4	4	1	1	1	1	1	*Paratylenchoides* spp, *Tylenchus* spp (3)
11		Mugla	8	36°35'53"	29°35'53"	4	5	4	1	2	1	3	-	*Xiphinema index*, *Tylenchus* spp (2)
12		Mugla	5	36°51'19"	29°43'26"	3	4	3	2	1	-	-	2	*Paratrophurus* spp (2), *Trophurus* spp (3)
13	Central Anatolia	Ankara	4	39°55'32"	32°51'256"	5	7	5	0	2	1	3	2	*Paratylenchus* spp (3), *Tylenchus* spp (5)
14	Central East Anatolia	Malatya	4	38°41'36"	37°33'12.8	0	4	3	1	1	1	5	12	*Tylenchus* spp (8), *Trophurus* spp (2)
15		Malatya	8	38°20'59.7	37°40'56.5	2	4	2	2	1	1	1	2	*Tylenchus* spp (8), *Criconemoides* spp (2)
16		Malatya	3	38°16'29"	38°4'13"	2	5	5	1	1	2	-	-	*Paratylenchus* spp (3), *Tylenchus* spp (3)
17		Mus	5	38°52'52"	41°14'12"	3	4	3	2	1	2	-	-	*Tylenchus* spp (4), *Tylenchorhynchus* spp (3)
18		Mus	4	38°53'31"	41°26'5"	4	5	5	1	7	5	2		*Paratrophurus* spp (5), *Tylenchorhynchus* spp (5)
19		Tunceli	3	39°21'26"	39°30'55"	0	0	2	0	2	12	-	-	*Tylenchus* spp (6)
20	East Marmara	Bilecik	7	39°52'0"	30,°6'9"	0	6	5	0	10	2	3	4	*Paratylenchus* spp (3), *Pratylenchoides* spp (2)
21	Eastern Anatolia	Elazıg	4	38°34'22"	38°44'4"	3	5	3	1	1	1	-	-	*Paratrophurus* spp (2), *Tylenchorhynchus* spp (2)
22		Elazıg	5	38°38'50"	39°10'56"	4	6	4	1	5	2	5	7	*Tylenchorhynchus* spp (3)
23	Mediterranean	Adana	8	37°0'6"	35°19'44"	4	6	6	0	4	3	1	2	*X. pachtaicum, Tylenchus* spp (3)
24		Antalya	3	37°13'3"	30°30'23"	3	5	4	0	3	-	3	-	*X. pachtaicum, X. index*
25		Antalya	5	36°53'34"	30°21'94"	0	5	3	1	5	-	2	-	*Paratrophurus* spp (2)
26		Antalya	8	37°17'7"	30°19'39"	2	5	4	1	1	1	-	2	-
27		Burdur	3	37°26'11"	30°33'19"	3	4	3	1	10	4	2	3	*Tylenchorhynchus* spp (6)
28		Burdur	6	37°21'55"	30°30'41"	4	5	4	2	8	1	1	-	*Tylenchus* spp (4), *Tylenchorhynchus* spp (5)
29		Burdur	6	37°18'20"	30°28'6"	3	4	3	1	10	2	5	-	*Paratylenchus* spp (2)
30		Hatay	11	36°28'36"	36°17'3"	4	5	0	2	2	-	5	2	*Tylenchus* spp (3), *Tylenchorhynchus* spp (4)
31	Southeastern Anatolia	Sanliurfa	13	37°08'29"	38°46'30"	3	6	5	2	4	1	2	-	*Tylenchus* spp (4), *Heterodera ciceri*
32	Trace	Tekirdag	8	40°38'41"	26°59'8"	4	4	3	1	1	1	3	-	*Xiphinema index*, *Tylenchus* spp (2)
33		Tekirdag	2	40°49'48"	27°2'52"	3	4	4	0	-		-	-	*Paratylenchoides* spp (2), *Tylenchus* spp (3)
34		Tekirdag	5	40°38'37"	26°59'53"	2	5	4	1	2	4	8	4	*Paratrophurus* spp (2), *Xiphinema pachtaicum* (2)
35	West Marmara	Canakkale	2	39°42'27"	26°29'56"	4	4	5	2	2	-	4	7	*Trophurus* spp (2), *Tylenchus* spp (4)
36		Canakkale	7	40°16'30"	27°25'47"	3	6	4	0	1	-	2	-	*Xiphinema index*, *Tylenchorhynchus* spp (3)
37		Canakkale	2	39°41'32"	26°25'26"	2	5	3	0	2	2	-	9	*Trophurus* spp (5), *Tylenchus* spp (3)
Total			211			95	179	138	39	125	80	75	78	-
Percentage (%)						45	84	65	18	59	38	35	37	-

* Number nematodes found for each genus is given in parentheses.

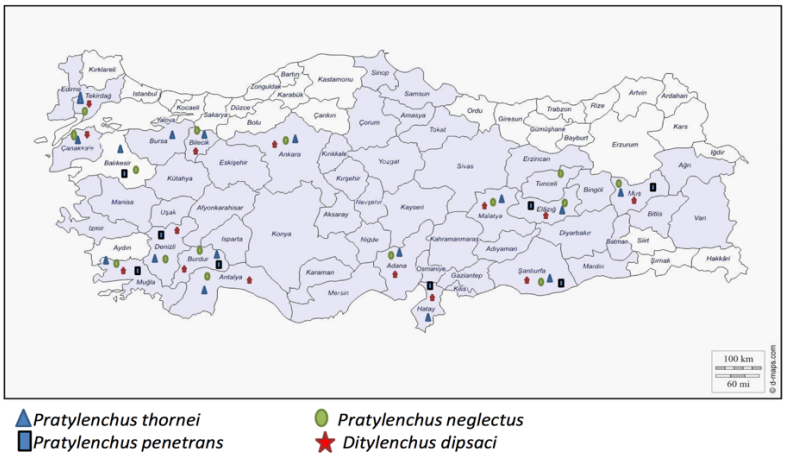

Figure 5. Geographical distribution of the four most important plant parasitic nematodes in chickpea growing areas of Turkey.

Discussion

Chickpea is a component of many Mediterranean and semiarid subtropical crop rotation systems (Whish et al., 2007; Chattopadhyay & Mohapatra, 2015). It is susceptibility to diseases and environmental conditions remains a challenge for optimizing productivity (Ghosh et al., 2013; Rubiales et al., 2015). Plant parasitic nematodes cause important damage to legumes (including chickpea) in different Mediterranean countries (Greco, 1985; Greco & Di Vito, 1988; Sikora & Greco, 1990; Greco et al., 1992; Di Vito et al., 1994). Sharma et al. (1992) reported that plant parasitic nematodes caused 14% yield loss in chickpea worldwide, but there is no information on crop losses in chickpea caused by nematodes in Turkey.

Pratylenchus spp. are found worldwide and infest a wide range of plant species. This study determined the distribution of RLNs in 17 chickpea growing provinces of Turkey. *Pratylenchus neglectus*, *P. penetrans* and *D. dipsaci* were the most important plant parasitic nematodes after *P. thornei* in all sampling sites in Turkey. Similarly, Di Vito et al. (1994) indicated that although different species of RLNs were found in different part of Turkey, *P. thornei* was dominant in Central Anatolia. Survey of plant parasitic nematodes in chickpea and lentil production areas in Syria and North Africa indicated that *P. neglectus*, *P. penetrans* and *P. thornei* were the most common nematodes and *P. thornei* the most common (Greco et al., 1992 & Di Vito et al., 1994). Consistent with those findings, *P. penetrans* was detected in 39 soil and root samples (18% of samples) in the present study. GRDC research on chickpea also reported that chickpea was susceptible to *P. neglectus*, *P. thornei* and *P. penetrans* (Grain Research Chickpea, 2015). Similarly, Greco & Di Vito (1988) reported that all these nematodes caused damage to chickpea around the world. Castillo et al. (1998) indicated that infestation of chickpea by *P. thornei* caused increases in the severity of root necrosis and enhances the root colonization by *Fusarium*. Similarly, Castillo & Vovlas (2007) indicated that these nematodes caused lesions on the roots that affect the growth and development of the crop and lead to significant yield loss. Di Vito et al. (1992) showed that among RLNs, *P. thornei* could cause yield loss of 50% in chickpea in Syria. *Pratylenchus* species ranked second after root-knot nematodes among the nematodes which cause damage to crops and chickpea (Barker & Noe, 1987; Jatala & Bridge, 1990; Castillo & Vovlas, 2007). Also, about 70 species of *Pratylenchus* have been described globally (Castillo & Vovlas, 2007). These species nematode reduce of the resistance of plants and damage by feeding roots (Orion et al., 1982). Similarly, Riley & Wouts (2001), Riley & Kelly (2002), Hollaway et al. (2008) and Thompson et al. (2010) showed that *P. thornei* and *P. neglectus* were a significant problem in chickpea production regions of Australia.

Di Vito et al. (1994) reported *Heterodera ciceri* Vovlas et al., 1986 (Tylenchoidea: Heteroderidae) as the first cyst nematode recorded in Siverek Province in Southeastern of Turkey. Similarly, *H. ciceri* was the first cyst nematode found in two samples collected at Şanliurfa Province in Southeastern Anatolia Region. Imren et al. (2012) reported *H. ciceri* was found as the first record in Adıyaman Province of the Southeastern Anatolia Region.

In the present survey, *D. dipsaci* was found in nearly half of root and soil samples. Similarly, it was reported *D. dipsaci* is one of the most detrimental pests of chickpea after root lesion, root-knot and cyst nematodes*i* (Barker & Noe, 1987; Jatala & Bridge, 1990). Chitwood & Krusberg (1977) indicated that the population densities of *D. dipsaci* can cause a gall formation in seedlings of a resistant cultivars of legumes.

Identification of *P. neglectus* and *P. thornei* based on morphological characteristics requires detailed microscopic measurements by an experienced nematologist. The genetic similarity between *P. neglectus* and *P. thornei* is reflected in their morphological similarities. Also, *P. neglectus* and *P. thornei* share some important morphological characters. Waeyenberge et al. (2000) reported that a PCR technique is rapid, efficient and can be used as a rapid identification tool for *Pratylenchus* species. Subbotin et al. (2008) reported that PCR methods can be used for identifying species of *Pratylenchus*. Whereas, Loof (1991) reported that the identification of *Pratylenchus* genus based on morphology and morphometric methods takes considerable time, requires skill and training in the observer and it is frequently ineffective because individual specimens often vary considerably within a population (Loof,1991). In the current study, *P. neglectus* and *P. thornei* were identified using molecular markers. Correct identification of important species of nematodes is critical to the success of chickpea production and integrated pest management strategies. Results of the present study will be helpful for setting priorities for further studies on of plant parasitic nematodes in chickpea production in Turkey.

References

Al-Banna, L., A. T. Ploeg, V. M. Williamson & I. Kaloshian, 2004. Discrimination of six *Pratylenchus* species using PCR and species- specific primers. Journal of Nematology, 36: 142-146.

Barker, K. R. & J. P. Noe, 1987. Establishing and using threshold population levels. Vistas on Nematology, 75-81.

Behmand, T., 2018. Screening of chickpea genotypes collected from Turkey against to the root lesion nematodes, *Pratylenchus neglectus*, *P. thornei* and *Ditylenchus dipsaci* for resistance. Çukurova University, Faculty of Agriculture, Department of Plant Protection, (Unpublished) PhD Thesis, Adana, Turkey, 163 pp.

Bora, T. & İ. Karaca, 1970. Kültür Bitkilerinde Hastalığın ve Zararın Ölçülmesi. Ege Üniversitesi Yardımcı Ders Kitabı, Yayın No: 167, E.Ü. Matbaası, Bornova-İzmir, 8s.

Castillo, P., A. Gomez-Barcina & R. M. Jimenez-Diaz, 1996. Plant parasitic nematodes associated with chickpea in southern Spain and effect of soil temperature on reproduction of *Pratylenchus thornei*. Nematologica, 42 (2): 211-219.

Castillo, P. & N. Vovlas, 2007. "*Pratylenchus* (Nematoda: *Pratylenchidae*): Diagnosis, Biology, Pathogenicity and Management, 305-324". In: Biology and Ecology of *Pratylenchus* (Eds. D. J. Hunt & R. N. Perry). Nematology Monographs Perspectives 6, Brill, Leden-Boston, MA, 529 pp.

Castillo, P., N. Vovlas & R. M. Jimenez-Diaz, 1998. Pathogenicity and histopathology of *Pratylenchus thornei* populations on selected chickpea genotypes. Plant Pathology, 47: 370-376.

Chattopadhyay, C. & S. D. Mohapatra, 2015. Perception of constraints in chickpea production in India. Indian Journal of Agricultural Sciences, 85: 287-289.

Chitwood, D. J. & L. R. Krusberg, 1977. Pectolytic enzymes in three populations of *Ditylenchus dipsaci*. Journal of Nematology, 9: 187-192.

Coolen, W. A., 1979. "Methods for the Extraction of *Meloidogyne* spp. and Other Nematodes from Roots and Soil, 317-329". In: Root-Knot Nematodes (*Meloidogyne* Species) Systematics, Biology and Control (Eds. F. Lamberti & C. E. Taylor). Academic Press, London, 802 pp.

Di Vito, M., N. Greco, H. M. Haula Mabsout, M. Labdi, S. P. S. Beniwal, M. C. Saxena, K. B. Singh & M. B. Solh, 1994. Nematodes of winter season legumes in North Africa. Nematologia Mediterranea, 22: 3-10.

Di Vito, M., N. Greco, G. Ores, M. C. Saxena, K. B. Singh & I. Kusmenoglu, 1994. Plant parasitic nematodes of legumes in Turkey. Nematologia Mediterranea, 22: 245-251.

Di Vito, M., N. Greco & M. C. Saxena, 1992. Patogenicity of *Pratylenchus thornei* on chickpea in Syria. Nematologia Mediterranea, 20: 71-73.

FAO, 2017. Food and Agriculture Organization of the United Nations Statistical Data. (Web page: www.fao.org/faostat/en) (Date accessed: 02.11.2017).

Ghosh, R., M. Sharma, R. Telangre & S. Pande, 2013. Occurrence and distribution of chickpea diseases in central and southern parts of India. American Journal of Plant Sciences, 4: 940-944.

GRDC, 2015. Tips and tactics: Root lesion nematodes Western region. (Web page: grdc.com.au/resources-and-publications/all-publications/factsheets/2015/03/tt-rootlesionnematodes) (Date accessed: 03.03.2015).

Greco, N., 1985. "Nematodes of faba beans, chickpeas, and lentils in the Mediterranean region and their control, 179-187". In: Proceedings of International Workshop on Faba Beans, Kabuli Chickpeas, and Lentils in the 1980s (Eds. M. C. Saxena & S. Varma), (16-20 May 1983, ICARDA, Aleppo, Syria), 457 pp.

Greco, N. & M. Di Vito, 1988. "The importance of plant parasitic nematodes in food legume production in the Mediterranean region, 28-45". In: Proceedings of Workshop on nematodes Parasitic to Cereals and Legumes in Temperate Semi-arid Regions (1-5 March 1987, Larnaca, Cyprus), 217 pp.

Greco, N., M. Di Vito, M. V. Reddy & M. C. Saxena, 1984. A preliminary report of the survey of plant parasitic nematodes of leguminous crops in Syria. Nematologia Mediterranea, 12: 87-93.

Greco, N., M. Di Vito & C. Saxena, 1992. Plant parasitic nematodes of cool season food legumes in Syria. Nematologia Mediterranea, 20: 37-46.

Greco, N. & S. B. Sharma, 1990. "Progress and problems in the management of nematode diseases, 135-137". In: Chickpea in the Ninetie, Proceedings of the Second International Workshop on Chickpea Improvement (4-8 December 1989, ICRISAT Center, India. Patancheru, Andhra Pradesh, India) (Eds. H. A. van Rheenen, M. C. Saxena, B. J. Walby & S.D. Hall), 403 pp.

Handoo, Z. A. & M. A. Golden, 1989. A key and diagnostic compendium to the species of the genus *Pratylenchus* Filipjev, 1936 (lesion nematodes). Journal of Nematology, 21: 202-218.

Hollaway, G. J., P. T Sharyn, F. E Russeli & H. H Colleen, 2000. Effect of field crops on density of *Pratylenchus* in South Eastern Australia; Part 2: *P. thornei*. Journal of Nematology, 32 (4S): 600-608.

Hollaway, G. J., V. A. Vanstone, J. Nobbs, J. G. Smith & J. S. Brown, 2008. Pathogenic nematodes of cereal crops in south-west Victoria, Australia. Australia Plant Pathology, 37: 505-510.

Hooper, D. J., 1986. "Extraction of Free-Living Stages from Soil, 5-30". In: Laboratory Methods for Work with Plant and Soil Nematodes (Ed. J. F. Southey), CAB International, London, UK, 629 pp.

Imren, M., L. Waeyenberge, N. Viaene, H. Toktay, A. Dababat & I. H. Elekcioğlu, 2012.Molecular characterization of cereal cyst nematodes from South Anatolian Region in Turkey using ITS-rDNA sequences. Turkish Journal of Entomology 36 (4): 491-499.

Jatala, P. & J. Bridge, 1990. "Nematode Parasites of Root and Tuber Crops,137-180". In: Plant Parasitic Nematodes in Subtropical and Tropical Agriculture (Eds. M. Luc, A. Sikora & J. Bridge). CAB International, Wallingford, UK, 221 pp.

Jenkins, W. R., 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. Plant Disease Reports, 48: 692.

Loof, P. A. A., 1991. "The Family *Pratylenchidae* Thorne, 1949, 363-421". In: Manual of Agricultural Nematology (Ed. W. R. Nickle). Marcel Dekker, New York NY, USA, 503 pp.

Orion, D., J. Krikun & J. Amir, 1982. "Population dynamics of *Pratylenchus thornei* and its effect on wheat in a semi-arid region, 48". Abstracts of the 16th International Symposium of the European Society of Nematologists (30 August- 3 September 1982, St. Andrews, Scotland, UK), 160 pp.

Pulse Australia, 2013. Northern chickpea best management practices training course manual 2013. Pulse.

Riley, I. T. & S. J. Kelly, 2002. Endoparasitic nematodes in cropping soils of Western Australia. Australian Journal of Experimental Agriculture, 42: 49-56.

Riley, I. T. & W. M. Wouts, 2001. *Pratylenchus* and *Radopholus* species in agricultural soils and native vegetation in southern Australia. Transactions and Proceedings of the Royal Society of South Australia, 125: 147-153.

Rubiales, D., S. Fondevilla, W. Chen, L. Gentzbittel, T. J. Higgins, M. A. Castillejo & N. Rispail, 2015. Achievements and challenges in legume breeding for pest and disease resistance. Critical Reviews in Plant Sciences, 34: 195-236.

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

Sharma, S. B., D. H. Smith & D. I. McDonald, 1992. Nematode constraints of chickpea and pigeon pea production in the semi-arid tropics. Plant Disease, 76 (9): 868-874.

Sikora, A. & N. Greco, 1990. “Nematode Parasites of Food Legumes, 181-235”. In: Plant Parasitic Nematodes in Subtropical and Tropical Agriculture (Eds. M. Luc, R. A. Sikora & J. Bridge). CAB International, Wallingford, UK, 261 pp.

Singh, M. & S. B. Sharma, 1994. Temperature effects on development and reproduction of *Heterodera cajani* on pigeon pea. Journal of Nematology, 26: 241-248.

Subbotin, S. A., E. J. Ragsdale, T. Mullens, P. A. Roberts, M. Mundo-Ocampo & J. G. Baldwin, 2008. A phylogenetic framework for root lesion nematodes of the genus *Pratylenchus* (Nematoda): evidence from 18s and D2-D3 expansion segments of 28s ribosomal RNA genes and morphological characters. Molecular Phylogenetics and Evolution, 48: 491-505.

Sudupak, A., M. S. Akkaya & A. Kence, 2002. Analysis of genetic relationships among perennial and annual *Cicer* species growing in Turkey using RAPD markers. Theoretical and Applied Genetics, 105: 1220-1228.

Thompson, J. P., T. G. Clewett, J. G. Sheedy, 2010. Occurrence of root-lesion nematodes (*Pratylenchus thornei* and *P. neglectus*) and stunt nematode (*Merlinius brevidens*) in the northern grain region of Australia. Australasian Plant Pathology, 39: 254-264.

Toktay, H., L. McIntyre, J. M. Nicol, H. Ozkan & H. I. Elekcioglu, 2006. Identification of common root lesion nematode (*Pratylenchus thornei* Sher et Allen) loci in bread wheat. Genome, 49: 1319-1323.

Vanstone, V. A., A. J. Rathjen, A. H. Ware & R. D. Wheeler, 1998. Relationship between root lesion nematodes (*Pratylenchus negleclus* and *P. thornei*) and performance of wheat varieties. Australian Journal of Experimental Agriculture, 38: 18-188.

Waeyenberge, L., A. Ryss, M. Moens, J. Pinochet & T. C. Vrain, 2000. Molecular characterization of 18 *Pratylenchus* species using rDNA restriction fragment length polymorphism. Nematology, 2: 135-142.

Whish, J. P. M., P. Castor & P. S. Carberry, 2007. Managing production constraints to the reliability of chickpea (*Cicer arietinum* L.) within marginal areas of the northern grains region of Australia. Crop and Pasture Science, 58: 396-405.

Yan, G., S. Richard, A. Patricia, P. A. Okubara, A. Skantar, A. E Sandra, G. S. Jason & T. Alison, 2008. Detection and Discrimination of *Pratylenchus neglectus* and *P. thornei* in DNA Extracts from Soil, Plant Disease, 92: 1480-1487.

Young, T. W., 1954. An incubation method for collecting migratory endoparasitic nematodes. Plant Disease Reporter, 38: 794-795.

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Original article (Orijinal araştırma)

Purification and characterization of an esterase from larval *Diplolepis fructuum* (Rübsaamen, 1895) (Hymenoptera: Cynipidae)[1]

Larva dönemindeki *Diplolepis fructuum* (Rübsaamen, 1895) (Hymenoptera: Cynipidae)'dan bir esterazın saflaştırılması ve karakterizasyonu

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Abstract

Diplolepis fructuum (Rübsaamen, 1895) (Hymenoptera: Cynipidae) is one of the important insect species that causes damages on Rosaceae species. With this study commenced in 2018 at the laboratory of Department of Biochemistry, Faculty of Science, Cumhuriyet University to get a biochemical data, an esterase (EC 3.1.1.X) from the larvae of *D. fructuum* was purified using Q Sepharose anion exchange, phenyl Sepharose CL-4B and Sephacryl S100 HR gel filtration chromatography, respectively. The enzyme had 6.94 U/mg protein specific activity, about 29-fold purity, and 8.8% yield. Only one activity band was observed in native-PAGE studies. The molecular weight of the esterase was estimated as 60 kDa using native-PAGE and SDS-PAGE techniques. By the kinetic data, optimum temperature and pH for the enzyme was determined as 40ºC and 9.0, respectively. The enzyme was stable for 4 h at 40ºC and pH 8.0. K_m and V_{max} values were found to be 0.035 mM and 1.41 µmol/mL.min., using 4-nitrophenyl butyrate (p-NPB) as substrate. The enzyme exhibited its highest activities on p-NPB (100%) and 4-nitrophenyl acetate (52%). All of these data indicate that the enzyme might be a typical esterase with different kinetic properties and molecular weight than esterolytic enzymes reported from other insect species.

Keywords: Column chromatography, *Diplolepis fructuum*, esterase, larvae, purification

Öz

Diplolepis fructuum (Rübsaamen, 1895) (Hymenoptera: Cynipidae) Rosaceae türlerinde zararlara yol açan en önemli böcek türlerinden birisidir. Biyokimyasal veri elde etmek için Cumhuriyet Üniversitesi, Fen Fakültesi, Biyokimya Anabilim Dalı laboratuvarında 2018 yılında başlatılan bu çalışma ile *D. fructuum*'un larvasından bir esteraz (EC 3.1.1.X) Q Sefaroz anyon değişim, fenil Sefaroz CL-4B ve Sefakril S 100 HR jel filtrasyon kromatografisini kullanarak saflaştırılmıştır. Enzim 6.94 U/mg protein spesifik aktivite, yaklaşık 29 kat saflık ve %8.80 verime sahipti. Nativ-PAGE çalışmalarında sadece bir aktivite bandı gözlenmiştir. Nativ-PAGE ve SDS-PAGE tekniklerini kullanarak, esterazın molekül kütlesi yaklaşık olarak 60 kDa olarak tahmin edilmiştir. Kinetik datadan, enzimin optimum sıcaklık ve pH'ı sırasıyla 40ºC ve 9.0 olarak belirlenmiştir. Enzim, 40ºC ve pH 8.0'da 4 saat kararlıydı. 4 nitrofenil butirat (p-NPB) substrat olarak kullanılarak, K_m ve V_{max} değerlerinin 0.035 mM and 1.41 µmol/mL.dk olduğu bulunmuştur. Enzim en yüksek aktivitesini p-NPB (%100) ve 4-nitrofenil asetat (%52) üzerinde sergilemiştir. Tüm bu veriler, enzimin diğer böcek türlerinden bildirilen esterolitik enzimlerden farklı kinetik özellik ve molekül kütlesi ile klasik bir esteraz olabileceğini göstermektedir.

Anahtar sözcükler: Kolon kromatografisi, *Diplolepis fructuum*, esteraz, larva, saflaştırma

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Introduction

Arthropods have the highest number of individuals on the earth (Ødegaard, 2000; Canavaso et al., 2001). In addition to this phenomenon, insect-derived diseases are also showing important increases worldwide. For this reason, there are great efforts to control the size of the insect populations showing vector or pest features. Control programs, such as the use of insect growth regulators, depend on the use of the chemical insecticides (Montella et al., 2012). However, repeated applications of the insecticides have led to resistant-insect populations (Shin & Smartt, 2016).

Glutathione-S-transferases, cytochrome P450 monooxygenases, and esterases, especially carboxylesterases, are the important enzymes which have important roles in the metabolic resistance against insecticides (Li et al., 2007). It appears that a common mechanism of the resistance is increased or reduced levels of these enzymes, depending on single or multiple mutations within their genes (Li et al., 2007; Gong et al., 2017).

Carboxylesterases (CarEs) are involved in both in the detoxification processes of the harmful exogenous compounds and in the metabolism of compounds having physiological importance in the metabolism in insects (Ma et al., 2018) and other organisms (Satoh et al., 2002; Satoh, 2005). For this reason, esterases (EC 3.1.1.X) have been given considerable attention (Montella et al., 2012), which was reviewed by Nauen (2007) and Li et al. (2007) due to their roles in insecticide resistance that are develops during pest or vector-control programs. Also, using the inhibition criteria of the insecticide applied is a reliable experimental method to classify esterases. For example, three kinds of inhibitors organophosphates, eserine sulfate and sulfydryl reagent, are used to inhibit carboxylesterase, cholinesterase and arylesterase activities, respectively. The acetylesterases, the fourth class of esterases, are not affected by these chemicals (Dahan-Moss & Koekemoer, 2016). There are available studies on the contribution of esterases from different insect species, such as *Oryzaephilus surinamensis* (Linnaeus, 1758) (Coleoptera: Silvanidae) (Rossiter et al., 2001), *Aedes aegypti* (Linnaeus, 1762) (Diptera: Culicidae) (Yaicharoen et al., 2005), *Aphis gossypii* (Glover, 1877) (Hemiptera: Aphididae) (Tabasian et al., 2010), *Dendrolimus superans* (Buttler, 1877) (Lepidoptera: Lasiocampidae) (Zou et al., 2014), *Anopheles funestus* (Giles, 1900) (Diptera: Culicidae) (Dahan-Moss & Koekemoer, 2016) and *Apis cerana cerana* (Fabricius, 1793) (Hymenoptera: Apidae) (Ma et al., 2018). However, many of these studies have been focused on the esterolytic activity assays without performing an esterase purification study.

Diplolepis fructuum is a member of Cynipidae family containing about 1400 insect species (Ronquist, 1999; Katılmış & Kıyak, 2009). This insect species is capable of making abnormal growths (galls) in the tissues of the plants such as *Rosa canina* (Linnaeus, 1753) from the family Rosaceae, resulting in damage to produce (Lotfalizadeh et al., 2009; Raman, 2011; Akpınar et al., 2017). The level of enzyme expression might be related to the age and life stage of the insects, and this is an important factor to consider during efforts to control harmful insect populations (Dahan-Moss & Koekemoer, 2016). In this context, no studies have been found on the purification of the esterase of *D. fructuum*'s. For this reason, for the first time in this study, an esterase fraction from the larvae of *D. fructuum,* a holometabol insect species, was purified and kinetically characterized to understand its biochemical function.

Materials and Methods

Larvae samples

Diplolepis fructuum (Rübsaamen, 1895) (Hymenoptera: Cynipidae) were collected from the galls on the plant *Rosa canina* L. from different localities in Sivas Province, Turkey between November 2012 and November 2013. Gall samples were also taken to the laboratory and kept in glass bottles. Embryological periods were observed and the larvae samples were obtained by observing the successive embryological periods of the insect. The larvae obtained were preserved at -80ºC until used (Akpınar et al., 2017). From these larvae samples, purification of esterase was attempted using chromatographic techniques in the laboratory as explained below.

Preparation of enzyme extract and purification

The preparation of enzyme extract was performed according to Görgün & Akpınar (2012) with slight modification. The larvae samples (3 g) were homogenized in buffer (buffer A; 50 mM Tris-HCl, pH= 7.4, 1 mM DTT, 1 mM Na4EDTA, 5 mM D-Mannit) with a Wise Tis homogenizer on ice for 5 min at 22,000 rpm. The resulting homogenate was clarified by centrifuging at 10,000 g for 15 min at 4ºC with Sanyo MSE MS 60 ultracentrifuge. The supernatant was obtained and the pellet was re-homogenized in homogenate buffer and then re-centrifuged. The supernatants from both centrifuge steps were combined for purification.

Chromatographic procedures were performed according to Görgün & Akpınar (2012) with modifications. Q Sepharose fast flow column chromatography was the first chromatographic step in the purification studies. The column material was suspended in a column (1 x 20 cm) and ethanol was removed by washing with distilled water. After this procedure, the column was equilibrated with 20 mM Tris-HCl at pH 7.80 (buffer B). The sample was applied into the column and washed with two column volumes of buffer B to elute unbounded fractions. Then, the bound protein fractions were eluted from the column by washing with 0.1, 0.2, 0.4 and 1 M NaCl series of buffer B with a peristaltic pump. The tubes showing esterase activity were combined and concentrated using a Millipore ultra-centrifugal filter unit (MWCO 10 kDa). The concentrated protein fraction was applied into phenyl Sepharose CL-4B hydrophobic interaction column (1 x 20 cm) that was equilibrated with 20 mM Tris-HCl buffer at pH 7.80 containing 0.1 M ammonium sulfate (buffer C). To obtain unbound protein fractions, the column was washed with two column volumes of buffer C then two column volumes of buffer B. The retained proteins in the hydrophobic interaction column were eluted with 40 mL of 10, 20 and 50% isopropanol series in buffer B. The last chromatographic step was Sephacryl S 100 HR gel filtration chromatography (1 x 30 cm) that is equilibrated with buffer B. Activity tubes concentrated from the previous chromatographic step were introduced into the column and elution tubes were collected until protein absorbance reached zero at 280 nm in the spectrophotometer.

Esterase and protein assay

Esterase activity measurements were performed according to Bülow & Mosbach (1987) using 4-nitro phenyl butyrate (p-NPB) as substrate at 405 nm against blank tube in a double beam spectrophotometer. The sample tube consisted of 10 µL of sample, 20 µL of 50 mM p-NPB dissolved in acetonitrile and 970 µL of activity buffer (pH 8.0 Tris-HCl with 4% ethanol). The blank tube contained 980 µL of activity buffer and 20 µL of p-NPB. During chromatographic steps, protein amounts from the elution tubes were recorded at 280 nm absorbance in 1 mL cuvettes. The method of Bollag et al. (1996) was used in the determination of protein amounts of the purification steps, using BSA (bovine serum albumin) as a standard. Every measurement consisted of three repeats.

Kinetic characterization

The method of Görgün & Akpınar (2012) with some modifications was used to obtain the kinetic data. The effects of temperature (between 4 to 60ºC) and pH (from 5.7 to 10) were assessed under standard activity assay conditions by incubating the enzyme solution for 15 min in related parameters. The effect of substrate concentration was evaluated at nine different concentrations between 0.025 and 1.25 mM of p-NPB, using constant amount of enzyme. Substrate chain length were evaluated using 4-nitrophenyl acetate (p-NPA), 4-nitrophenyl butyrate (p-NPB), 4-nitrophenyl dodecanoate (p-NPD) and 4-nitrophenyl palmitate (p-NPP). The stability of the enzyme was also assayed at 40ºC and pH 8.0 between 1 to 4 h. All studies were repeated three times under the standard activity measurements by changing the regarding parameters. All the data obtained were tested statistically using SPSS 11.0 for windows (Görgün & Zengin, 2015). One-way analysis of variance was used to analyze the repeated experiments (mean±SE). Differences between means were evaluated with Tukey's test at 0.05 significance level.

Electrophoretic studies

Native-PAGE studies without using SDS were applied according to Görgün & Zengin (2015). The equal amounts of samples from different purification fractions were loaded onto 10% native gels consisting of only stacking gel. Electrophoresis was performed at a constant 100 mA in Tris-glycine buffer (pH 8.3, 0.025 M Tris and 0.192 M glycine) for 80 min under a cooling system. To detect the esterase bands in the samples, the gels were stained with 1 naphthyl acetate. Later the same gels were stained with Coomassie Brilliant Blue and then silver staining method (Bollag et al., 1996) to follow the progress of different purification stages. Denature SDS-PAGE studies were also conducted on the samples under the conditions mentioned for the native-PAGE studies.

Results and Discussion

The summary of the purification of esterase from the larval stage of *Diplolepis fructuum* can be seen from Table 1. Sequences of Q Sepharose anion exchange, phenyl Sepharose CL-4B and Sephacryl S100 HR gel filtration chromatography were conducted to purify an esterase fraction from the larval stage of *D. fructuum*. These data are presented in Figure 1. A specific activity of 0.246 U/mg protein was found in the homogenate. Using Q Sepharose column, four major protein peaks corresponding to two esterase activity peaks were detected. There was no esterase activity in the unbound protein fraction by the washing with buffer B. Also, elution tubes with 0.1 M NaCl did not show any esterase activities. However, the tubes that have the highest esterase activities were found to be between tubes 22 and 27, obtained by washing with 0.2 M NaCl (in buffer B). The tubes 32 and 33 had minor esterase activities and these fractions have been ignored due to their very little specific activities. This chromatographic step provided about a 6-fold purification and 60% yield with a specific activity of 1.45 U/mg protein. Using DEAE-cellulose anion exchange chromatography, Fahmy et al. (2004) found six esterase forms (from E1 to E6) corresponding to six protein peaks by eluting with sequential NaCl concentrations between 0 and 1 M during the embryogenesis of *Hyalomma dromedarii* (Koch, 1844) (Acari: Ixodidae). Among these bands, E3 that has the highest esterase activity was eluted with 0.2 M NaCl. The chromatographic result of this step was a 5-fold purification with a yield of 5.23%, showing similarities with our results obtained in the ion exchange chromatography step. After this step, they obtained a 19-fold purification parameter of esterase from *H. dromedarii* by gel filtration (Sepharose 6B) chromatography.

Table 1. Purification steps of esterase from the larval period of *Diplolepis fructuum*

Purification step	Volume (mL)	Total protein (mg) (mean±SE)		Total activity (µmol/mL.min)** (mean±SE)		Specific activity (µmol/mL.min./mg) (mean±SE)		Purification factor	Yield (%)
Homogenate	31	166.61±0.28	a*	40.93±1.03	a	0.246±0.06	a	1.00	100.00
Q Sepharose	20	14.74±2.42	b	24.89±0.63	b	1.450±0.04	b	5.90	60.81
Phenyl Sepharose CL-4B	12	3.72±0.75	b	6.31±0.13	c	1.697±0.04	c	6.91	15.42
Sephacryl S100 HR	2.5	0.52±0.03	b	3.60±0.04	c	6.940±0.08	d	28.30	8.80

* Means are for three repeat experiments. Means followed by the same letter are not significantly different at $P \leq 0.05$.

** One unit of esterase activity is defined as the amount of enzyme that catalyze the release of p-nitrophenol (p-NP) per min under assay conditions explained in material and method section.

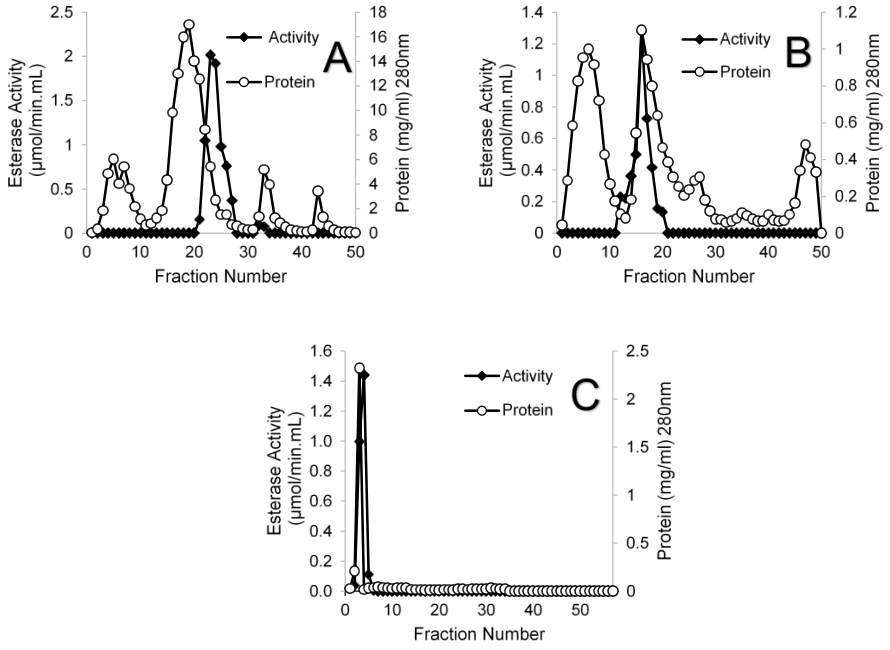

Figure 1. Purification of esterase from *Diplolepis fructuum* larvae by chromatographic series: A) Elution profile of Q-Sepharose of anion exchange chromatography with tubes collected as 4 mL at 3 mL/min flow rate; B) elution profile of phenyl Sepharose CL-4B hydrophobic interaction chromatography with tubes eluted as 4 mL at 2 mL/min flow rate; and C) elution profile of Sephacryl S100-HR gel filtration chromatography with tubes collected as 2.5 mL at 1 mL/min flow rate.

Hydrophobic interaction chromatography has been reported to be useful for the purification of lipolytic enzymes such as lipases and esterases (Bompensieri et al., 1996; Qerioz et al., 2001; Bhardwaj et al., 2001; Görgün & Akpınar, 2012). In the present study, phenyl Sepharose CL-4B column resulted in purification parameters of 1.69 U/mg protein specific activity and about a 7-fold purification of *D. fructuum* larval esterase (Table 1). We obtained three protein peaks corresponding to only one activity peak. These results suggested that this chromatographic step resolved the protein peaks and discharged contaminant proteins, but we had activity losses resulting in a similar specific activity to that of previous chromatographic step (Table 1). In their key study, Arrese & Wells (1994) purified an insect triacylglycerol lipase from the insect fat body of *Manduca sexta* (Linnaeus, 1763) using five different chromatographic steps in which step three was phenyl Sepharose column chromatography, which resulted in a 939-fold purification of the enzyme. Later, the same group reported that this enzyme is an active phospholipase (Arrese et al., 2006).

Sephacryl S100 HR gel filtration chromatography was the last purification step of the present study. Purification parameters of this step were determined as 6.94 U/mg protein specific activity, 8.80% yield, and 28-fold purification (Table 1). In this analysis, the enzyme activity appeared in the first five tubes corresponding to a single protein peak. Despite this finding, electrophoretic data showed that there was a contaminant protein band that lack of esterase activity. The protein content of the purified fraction was 0.52 mg and this contaminant band was also purified with our target enzyme. For this reason, we were not able to get higher purification factor, using Sephacryl S100 chromatography. In the present study, Q Sepharose anion exchange chromatography was able to capture the esterase from the crude homogenate. Hydrophobic interaction chromatography did not show substantial purification factor with the application of the sample obtained from Q Sepharose to phenyl Sepharose CL-4B hydrophobic interaction chromatography. However, this step was important to eliminate contaminant protein bands. The last step, gel filtration chromatography, contained only one protein peak that correspond to one activity peak. However, this protein peak contained two protein bands in denaturing electrophoresis, with one of the protein bands was

contaminant as revealed by native-PAGE studies. There were important statistical differences ($P \leq 0.05$) between the specific activities through the purification process with the chromatographic techniques used in the present study. Purification studies were performed on some insect species, applying the combination of different chromatographic steps, and these studies had both similar and different results to our study. The same sequences of the chromatographic techniques also reported enzyme preparations in different purity. For example, two different researchers dealt with the purification of larval mid gut lipase, using ammonium sulfate precipitation, Sephacryl G-100 gel filtration and DEAE-cellulose anion exchange chromatography. From these studies, a digestive lipase from *Pieris brassicae* (Linnaeus, 1758) (Lepidoptera: Pieridae) larvae was purified with 39.9 U/mg protein specific activity, 18.1% yield and 42-fold purification (Zibaee, 2012), while the second study achieved a 12-fold purification, 8.21% recovery and 5.60 U/mg protein specific activity in *Naranga aenescens* (Moore, 1881) (Lepidoptera: Noctuidae) (Zibaee & Fazeli-Dinan, 2012).

In this study, kinetic data including optimum temperature, optimum pH, K_m and V_{max} values, enzyme stability, effect of the substrate chain length to the enzyme activity were assessed (Figure 2). Optimum temperature and pH were 40ºC and 9.0, respectively. Purified enzyme retained 88% of activity for 4 h at 40ºC and pH 8.0. The relative activities were 100% for p-NPB, 52% for p-PNA, 5% p-NPD, and 1% for p-NPP. This finding suggests that the purified enzyme is more active with short-chain substrates and might be an esterase (Fojan et al., 2000). Using p-NPB as a substrate, K_m and V_{max} values were found to be 0.035 mM and 1.41 µmol/mL.min respectively. The data from the present study has similarities and differences from published reports. The differences that are determined might be a result of the tissue investigated or the purified enzymes are a lipase or esterase. For example, lipolytic enzymes from *Ectomyelois ceratoniae* (Zeller, 1839) (Lepidoptera: Pyralidae) (Ranjbar et al., 2015), *Rhynchophorus palmarum* (Linnaeus, 1758) (Santana et al., 2017) and *Chilo suppressalis* (Walker, 1863) (Lepidoptera, Pyralidae) (Zibaee et al., 2008) exhibited optimum temperatures of 30, 37, and 37-40ºC, and optimum pH of 7, 6.5 and 10, respectively. At the same time, Ranjbar et al. (2015) indicated that the purified enzyme was active for 3 h at 30ºC. Regarding with the substrate specificities, Santana et al. (2017) was assessed the substrates ranging from 10 (p-NPD) to 16 (p-NPP) carbon chain length and they found the highest activity in p-NPP, indicating that the enzyme was a lipase. Esterase (E3) from *H. dromedarii* was showing a great affinity for the short-chain substrate (p-NPA) with a K_m value of 1.43 mM (Fahmy et al., 2004). In *P. brassicae*, V_{max} and K_m values were reported as 30.3 U/mg protein and 2.72 mM p-NPB, respectively (Zibaee, 2012).

In the present study, at the end of the purification experiments, native-PAGE studies were conducted to determine esterase activities both in the homogenate and the purification series, using a native substrate (1-naphthyl acetate). Only one band with esterase activity band and a molecular weight between 60-62 kDa was present in all of the samples (Figure 3A). After defining the location of this band using molecular weight markers, the same gels were stained by silver staining to determine the number of the bands. This showed that purified fraction consisted of only two bands in which one of them was a contaminant protein band with a molecular weight of about 80 kDa that lacked esterase activity (Figure 3B). These experiments were repeated using denaturing SDS-PAGE under the same conditions as used for native-PAGE, and the same findings were obtained (Figure 3C). When compared to the literature, reported molecular weights for the lipolytic enzymes from different purification studies in the insects were 76 kDa in *M. sexta* fat body (Arrese & Wells, 1994; Arrese et al., 2006), 45 kDa in *H. dromedarii* larvae (Fahmy et al., 2004), 72.3 kDa in the midgut of *P. brassicae* (Zibaee, 2012), 84.8 kDa in *D. superans* larvae (Zou et al., 2014), and 25 kDa in the middle gut of *E. ceratoniae* (Ranjbar et al., 2015). Esterases are important enzymes in the living systems to digest both endogenous substrates and exogenous xenobiotics. The present study is the first report on the purification and biochemical characterization of an esterase from *D. fructuum* larvae, which is economically important because it induces galls on *R. canina*. The estimated 60 kDa lipolytic enzyme from *D. fructuum* might be an esterase with slight differences in kinetic properties from lipolytic enzymes reported

for other insect species. The literature data given in above together with our findings suggest that purification parameters obtained might depend on factors such as the insect species under investigation, the tissue of insect, growth stage, expression level of esterases, chromatographic techniques and their application sequences. The kinetic assays that were performed on crude homogenates might show differences with the purified fractions. From the present study, we suggest a method to purify an esterase from *D. fructuum* larvae, a damaging pest of *R. canina*. Likewise, further studies should be undertaken on the purification and characterization of esterases from insect species to inform efforts to manage pest or vectors.

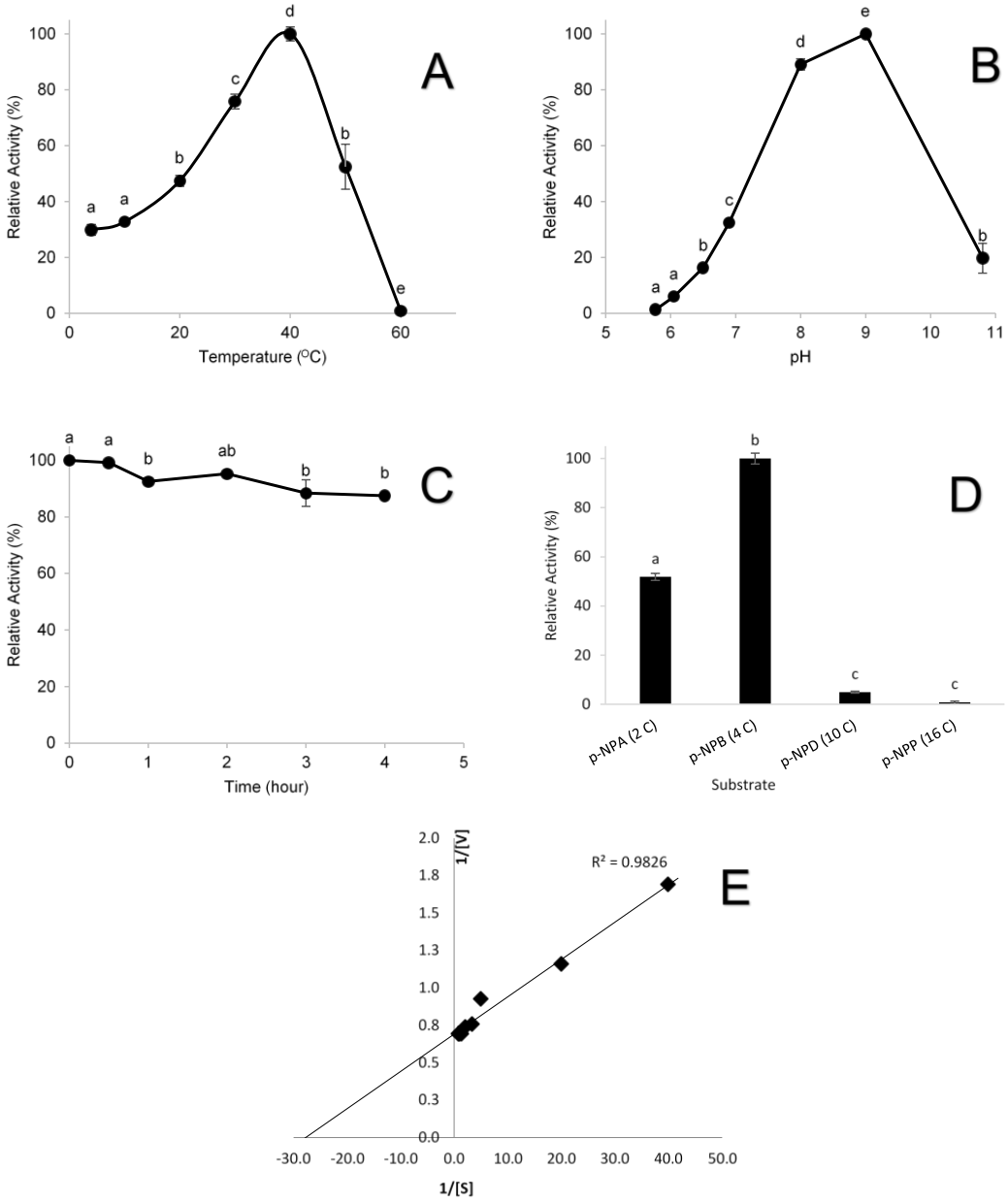

Figure 2. Kinetic characterization of the esterase from *Diplolepis fructuum* larvae: A) Effect of temperature; B) effect of pH; C) stability at 40ºC and pH 8.0; D) effect of substrate chain length; and E) effect of substrate concentration. Values with the same letter are not significantly different at P ≤ 0.05.

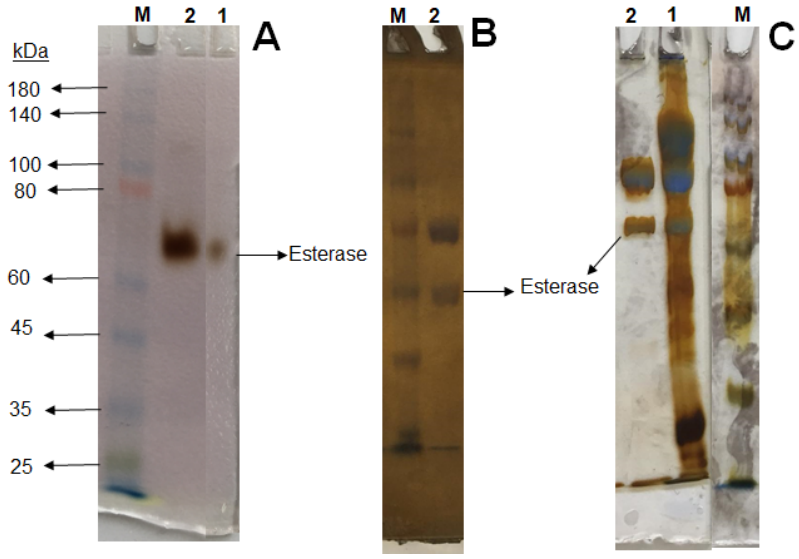

Figure 3. Electrophoretic analyses of the purification steps of esterase from *Diplolepis fructuum* larvae: A) A native-PAGE analysis of homogenate (lane 1) (20 µg protein) and the purified fraction (lane 2) (20 µg protein); B) silver staining of a native-PAGE analysis of the purified fraction (lane 2); and C) SDS-PAGE electrophoretic pattern of homogenate (lane 1) (20 µg protein) and purified fraction (lane 2) (20 µg protein), using coomassie and silver staining. M, molecular weight marker.

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References

Akpınar, M. A., S. Görgün, L. Gençer & A. Aktümsek, 2017. Fatty acid composition of *Diplolepis fructuum* (Rübsaamen, 1895) (Hymenoptera: Cynipidae) during its developmental stages. Journal of the Entomological Research Society, 19 (3): 109-118.

Arrese, E. L. & M. A. Wells, 1994. Purification and properties of a phosphorylatable triacylglycerol lipase from the fat body of an insect, *Manduca sexta*. Journal of Lipid Research, 35: 1652-1660.

Arrese, E. L., R. T. Patel & J. L. Soulages, 2006. The main triglyceride-lipase from the insect fat body is an active phospholipase A1: identification and characterization. Journal of Lipid Research, 47: 2656-2667.

Bhardwaj, K., A. Raju & R. Rajasekharan, 2001. Identification, purification, and characterization of a thermally stable lipase from rice bran. A new member of the (phospho) lipase family. Plant Physiology, 127 (4): 1728-1738.

Bollag, D. M., M. D. Rozycki & S. J. Edelstein, 1996. Protein Methods. A John Wiley and Sons Inc. Publication, 432pp.

Bompensieri, S., R. Gonzalez, R. Kok, M. V. Miranda, I. Nutgeren-Roodzant, K. J. Hellingwerf, O. Cascone & B. C. Nudel, 1996. Purification of lipase from *Acinetobacter calcoaceticus* AAC323-1 by hydrophobic interaction methods. Biotechnology and Applied Biochemistry, 23 (1): 77-81.

Bülow, L. & K. Mosbach, 1987. The expression in *E. coli* of a polymeric gene coding for an esterase mimic catalyzing the hydrolysis of p-nitrophenyl esters. Federation of European Biochemical Societies, 210 (2): 147-152.

Canavaso, L.E., Z. E. Jouni, K. J. Karnas, J. E. Pennington & M. A. Wells, 2001. Fat metabolism in insects. Annual Review of Nutrition, 21: 23-46.

Dahan-Moss, Y. L. & L. L. Koekemoer, 2016. Analysis of esterase enzyme activity in adults of the major malaria vector *Anopheles funestus*. Parasites & Vectors, 9: 110.

Fahmy, A. S., S. S. Abdel-Gany, T. M. Mohamed & S. A. Mohamed, 2004. Esterase and lipase in camel tick *Hyalomma dromedarii* (Acari: Ixodidae) during embryogenesis. Comparative Biochemistry and Physiology Part B, 137: 159-168.

Fojan, P., P. H. Jonson, M. T. N. Petersen & S. B. Petersen, 2000. What distinguishes an esterase from a lipase: A novel structural approach. Biochimie, 82 (11): 1033-1041.

Gong, Y. H., G. M. Ai, M. Li, X. Y. Shi, Q. Y. Diao & X. W. Gao, 2017. Functional characterization of carboxylesterase gene mutations involved in *Aphis gossypii* resistance to organophosphate insecticides. Insect Molecular Biology, 26 (6): 702-714.

Görgün, S. & M. A. Akpınar, 2012. Purification and characterization of lipase from the liver of carp, *Cyprinus carpio* L. (1758), living in Lake Tödürge (Sivas, Türkiye). Turkish Journal of Fisheries and Aquatic Sciences, 12 (2): 207-215.

Görgün, S. & G. Zengin, 2015. Determination of fatty acid profiles and esterase activities in the gills and gonads of *Vimba vimba* (L., 1758). Journal of the American Oil Chemist's Society, 92 (3): 353-360.

Katılmış, Y. & S. Kıyak, 2009. The oak gall wasp *Aphelonyx persica*: a new record from Turkey, with some new host records. Phytoparasitica, 37: 95-97.

Li, X., M. A. Schuler & M. R. Berenbaum, 2007. Molecular mechanisms of metabolic resistance to synthetic and natural xenobiotics. Annual Review of Entomology, 52: 231-253.

Lotfalizadeh, H., R. Ezzati-Tabrizi & A. Masnadi-Yazdinejad, 2009. *Diplolepis fructuum* (Rübsaamen) (Hym.: Cynipidae) a new host for *Exeristes roborator* (Fabricius) (Hym.: Ichneumonidae) in Iran. Biharean Biologist, 3: 171-173.

Ma, M., H. Jia, X. Cui, N. Zhai, H. Wang & X. Guo, 2018. Isolation of carboxylesterase (esterase FE4) from *Apis cerana cerana* and its role in oxidative resistance during adverse environmental stress. Biochimie, 144: 85-97.

Montella, I. R., R. Schama & D. Valle, 2012. The classification of esterases: an important gene family involved in insecticide resistance-A review. Memorias do Instituto Oswaldo Cruz, Rio *de* Janeiro, 107 (4): 437-449.

Nauen, R., 2007. Insecticide resistance in disease vectors of public health importance. Pest Management Science, 63: 628-633.

Ødegaard, F., 2000. How many species of arthropods? Erwin's estimate revised. Biological Journal of the Linnean Society, 71 (4): 583-597.

Qerioz, J. A., C. T. Tomaz & J. M. S. Cabral, 2001. Hydrophobic interaction chromatography of proteins. Journal of Biotechnology, 87: 143-159.

Raman, A., 2011. Morphogenesis of insect-induced plant galls: facts and questions. Flora, 206: 517-533.

Ranjbar, M., A. Zibaee & J. J. Sendi, 2015. Purification and characterization of a digestive lipase in the midgut of *Ectomyelois ceratoniae* Zeller (Lepidoptera: Pyralidae). Frontiers in Life Science, 8 (1): 64-70.

Ronquist, F., 1999. Phylogeny, classification and evolution of the Cynipoidea. Zoologica Scripta, 28: 139-164.

Rossiter, L. C., C. M. Conyers, A. D. MacNicoll & H. A. Rose, 2001. Two qualitatively different B-esterases from two organophosphate-resistant strains of *Oryzaephilus surinamensis* (Coleoptera: Silvanidae) and their roles in fenitrothion and chlorpyrifos-methyl resistance. Pesticide Biochemistry and Physiology, 69: 118-130.

Santana, C. C., L. A. Barbosa, I. D. B. Junior, T. G. do Nascimento, C. B. Dornelas & L. A. M. Grillo, 2017. Lipase activity in the larval midgut of *Rhynchophorus palmarum*: Biochemical characterization and the effects of reducing agents. Insects, 8 (100): 1-7.

Satoh, T., 2005. Toxicological implications of esterases-From molecular structures to functions. Toxicology and Applied Pharmacology, 207: 11-18.

Satoh, T., P. Taylor, W. F. Bosron, S. P. Sanghanı, M. Hosokawa & B. N. La Du, 2002. Current progress on esterases: From molecular structure to function. Drug Metabolism and Disposition, 30 (5): 488-493.

Shin, D. & C. T. Smartt, 2016. Assessment of esterase gene expression as a risk marker for insecticide resistance in Florida *Culex nigripalpus* (Diptera: Culicidae). Journal of Vector Ecology, 41 (1): 63-71.

Tabasian, H., S. Ravan, A. R. Bandani & B. A. Siahsar, 2010. The effect of esterase activity in resistance of *Aphis gossypii* to selective insecticides. Journal of Food, Agriculture & Environment, 8 (3&4): 1108-1112.

Yaicharoen, R., R. Kiatfuengfoo, T. Chareonviriyaphap & P. Rongnoparut, 2005. Characterization of deltamethrin resistance in field populations of *Aedes aegypti* in Thailand. Journal of Vector Ecology, 30 (1): 144-150.

Zibaee, A., 2012. A digestive lipase of *Pieris brassicae* L. (Lepidoptera: Pieridae): purification, characterization, and host plants effects. Archieves of Insect Biochemistry and Physiology, 81 (1): 1-19.

Zibaee, A., A. R. Bandani & S. Ramzi, 2008. Lipase and invertase activities in midgut and salivary glands of *Chilo suppressalis* (Walker) (Lepidoptera, Pyralidae), rice striped stem borer. Invertebrate Survival Journal, 5: 180-189.

Zibaee, A. & M. Fazeli-Dinan, 2012. Purification and characterization of a digestive lipase in rice green semi-looper, *Naranga aenescens* Moore (Lepidoptera: Noctuidae). Signpost Open Access Journal of Entomological Studies, 1: 38-54.

Zou, C.-S., C.-W. Cao, G.-C. Zhang & Z.-Y. Wang, 2014. Purification, characterization, and sensitivity to pesticides of carboxylesterase from *Dendrolimus superans* (Lepidoptera: Lasiocampidae). Journal of Insect Science, 14 (260): 1-6.

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Original article (Orijinal araştırma)

Resilience of the date stone beetle, *Coccotrypes dactyliperda* Fabricius, 1801 (Coleoptera: Curculionidae), following periods of exposure to subzero temperature[1]

Hurma böceği, *Coccotrypes dactyliperda* Fabricius, 1801 (Coleoptera: Curculionidae)'nın sıfır altındaki sıcaklıklara esnekliği

Dirk H. R. SPENNEMANN[2*]

Abstract

The date stone beetle, *Coccotrypes dactyliperda* Fabricius, 1801 (Coleoptera: Curculionidae), is a cold-sensitive agricultural pest that goes into hibernation inside infested seeds, ready to breed as temperature increases. To assess their resilience following exposure to cold, specimens reared from infested *Phoenix canariensis* Chabaud seeds were exposed prolonged subzero temperature in a laboratory setting at Charles Sturt University (Albury, Australia) in 2018. Specimens which had been allowed to tunnel into *P. canariensis* seeds as well as those held in vials were exposed to -8ºC for durations between 5 min and 7 h. Unprotected beetles survived a 75-min exposure to -8ºC, but the beetles in their brood galleries inside the seeds survived for over 7 h.

Keywords: Biogeography, climatic extremes, insect pests, physiology

Öz

Hurma böceği, *Coccotrypes dactyliperda* Fabricius, 1801 (Coleoptera: Curculionidae), sıcaklık arttıkça üremeye hazır hale gelen ve bulaşık tohumların içinde kışı geçiren soğuğa duyarlı tarımsal zararlıdır. Soğuğa karşı dayanıklılıklarını değerlendirmek için, bulaşık *Phoenix canariensis* Chabaud tohumlarından elde edilen örnekler, 2018 yılında Charles Sturt Üniversitesi (Albury, Avustralya)'ndeki bir laboratuvar ortamında uzun süreli sıfırın altındaki sıcaklıklara maruz bırakılmıştır. *Phoenix canariensis* tohumlarının içinde galeri açmalarına izin verilen örnekler ve şişelerde tutulan örnekler 5 ila 420 dakika arasında -8ºC'ye maruz bırakıldı. Korunmasız böcekler -8ºC'ye 75 dakika hayatta kalabilirken, tohumların içindeki gizlendikleri galerilerindeki böcekler 7 saatten fazla yaşamışlardır.

Anahtar sözcükler: Biyocoğrafya, iklim aşırı uçlar, zararlı böcekler, fizyoloji

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Introduction

The spermatophagus date stone beetle, *Coccotrypes dactyliperda* Fabricius, 1801 (Coleoptera: Curculionidae), is a pest in commercial or domestic date (*Phoenix dactylifera* L., Arecaceae) plantations throughout Northern Africa and the Middle East where it can cause extensive fruit losses (known as the July drop) (Mostafa, 1997; Anber et al., 1998; Helal, 1998; Bar-Shalom & Mendel, 2001; Ali et al., 2002; 2003). They have since infested ornamental palms, primarily Canary Island date palm (*Phoenix canariensis* Chabaud) throughout the Mediterranean and beyond.

Closely associated with the date palm complex and originally endemic to the Middle East and North Africa, *C. dactyliperda* has become a true cosmopolitan species that can be found in most subtropical and temperate zones (Spennemann, 2018a). As with other crypto-parasites, the entire life cycle of *C. dactyliperda* occurs inside the seed. Mated females start to deposit eggs 3 to 5 d after inhabiting a new seed. Unmated *C. dactyliperda* females are able to deposit eggs that produce male offspring and then proceed to mate with these to produce offspring of mixed sex. Depending on the size of the seed, multiple broods and even generations of beetles can hatch and reproduce inside a seed before emerging. A single date seed can concurrently contain large numbers of eggs, larvae, pupae and imagines in excess of 80 individuals (females, males, pupae, larvae and eggs) (see review by Spennemann, 2019a and references cited therein).

Coccotrypes dactyliperda is a cold-sensitive species. During the winter imagines of female *C. dactyliperda* enter a hibernation or dormancy period inside the seeds in which they hatched. Hibernation in the northern hemisphere is usually from November or December to February (El-Bahria Oasis, Egypt) (Ali et al., 2003), or even to May (Abd-Allah & Tadros, 1994 cited after Boraei, 1994; Helal, 2014). The commencement and termination of hibernation are linked to both temperature and photoperiod.

Although the extant literature is silent on resilience of *C. dactyliperda* following exposure to subzero temperature, some resilience can be inferred from the spatial distribution of the species (Spennemann, 2018a). In North Africa and the Middle East, where *C. dactyliperda* is considered an endemic, the temperature can drop below zero, for example, -1.7ºC (Tripoli, Libya), -4.0ºC (Jerusalem, Israel), -6.1ºC (Tunis, Tunisia) and -8.0ºC (Amman, Jordon). In the south of France, where *C. dactyliperda* has become naturalized, the temperature can drop to -13.0ºC (Toulon, France) (NCDC, 2018). Annual minimum temperatures vary and are thus of indicative value only (Figure 1). Although temperature extremes in North Africa and the Middle East can drop to these minima, the average minima are higher by 5-8ºC (Ageena et al., 2013; World Bank Group, 2019). There is no evidence that any of the areas where date palm and the date stone beetle occur have multiday events with temperature below -5ºC.

Over recent decades, the Middle East and North Africa have experienced warming trends. Significantly for *C. dactyliperda*, these are accompanied by fewer cold days and nights, and shorter cold periods (Zhang et al., 2005; Almazroui et al., 2012; Ageena et al., 2013; Donat et al., 2014; El Kadi, 2016). Also, on a broad spatial scale, this trend is predicted to continue if not accelerate (Samuels et al., 2011), although it is not uniform on a finer scale. Indeed, the opposite trend is likely to occur in some smaller areas (Matouq et al., 2013).

Although it is understood that adult beetles in their galleries will be somewhat protected from low temperature, at least for short periods, compared to beetles that are fully exposed, there are no reliable experimental data of the ability of *C. dactyliperda* to withstand exposure to subzero temperature. Cooperband et al. (2016) found for another scolytinid species (*Euwallacea fornicatus* Eichhoff, 1868) (Coleoptera: Curculionidae) that 4-h exposure to a temperature between -5 and -1ºC led to mortality of 100% of larvae, 95.7% of pupae and 69.2% of adults. Longer exposure killed all adults.

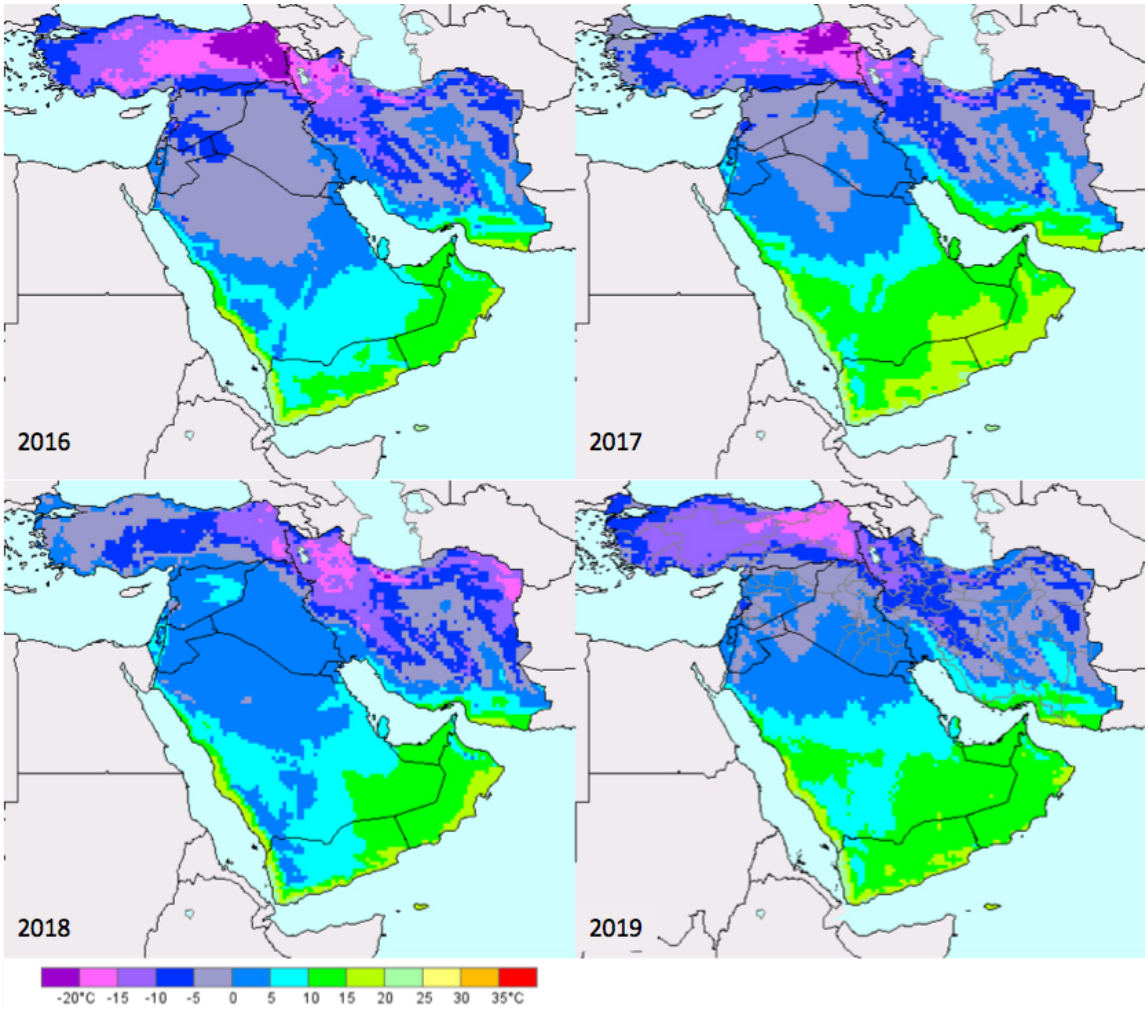


Figure 1. Minima for the Middle East for the winters of 2016-2019. Data: USAF 557th Weather Wing, via International Production Assessment Division, Foreign Agricultural Service, United States Department of Agriculture.

The resilience of *C. dactyliperda* following exposure to subzero temperature has implications of the future spread of the beetle into currently uncolonized areas. Knowledge of death threshold temperature informs on past and present biogeography of the species. This paper reports the results of an experiment exposing date stone beetles to subzero temperature, both on their own and inside seeds.

Materials and Methods

The beetles used were came from a *C. dactyliperda* population bred for multifactorial experiment designed at assessing food choices and emergence times (Spennemann, 2019b). The original beetle population stemmed from Canary Island date palm seeds collected at Alma Park, NSW, Australia (Spennemann et al., 2018a). The experiment was conducted in October 2018 in the PC2 laboratory of the Peter Till Laboratories, Faculty of Science, Charles Sturt University (Albury, Australia).

To assess the ability of *C. dactyliperda* to withstand subzero temperature, two sets of samples were prepared: 1) 16 vials with 25 ml air volume, containing 25 specimens each without any substrate; and 2) 16 same-sized vials populated with 10 specimens each that had been allowed to tunnel into 10 fresh *P. canariensis* seeds for 49 h. One set of beetles and one set in other Canary Island date palm seeds in a food preference experiment were used as controls.

The sample vials were placed on an expanded polystyrene tray in a laboratory freezer at -8ºC. After 5, 10, 15, 20, 30, 45, 60, 75, 90, 120, 150, 180, 240, 300, 360 and 420 min, one sample of each set was removed from the freezer, and left to thaw on a laboratory bench at room temperature (about 20ºC) for 60 min before assessing the viability of the beetles.

The samples with beetles in the seeds were placed between moistened sheets of tissue in a germination chamber at 28ºC for 1 week. Experience has shown that live beetles will cause frass to emerge from entrance hole in infested seeds, which is readily apparent in a germination chamber (Spennemann et al., 2018b). After 1 week, the seeds were dissected to expose and assess the viability of the beetles (found to crawl from opened seed or on filter paper when shaken from the seed). The sample vials with specimens without any substrate were emptied on the center of 100-mm circular filter paperr. Survival was defined as the ability of the beetle to crawl from the center of the filter paper to its edge, i.e., a distance of at least 50 mm (i.e., 25x a beetle's body length). To meet the phytosanitary requirements of the PC2 laboratory, any specimens found alive were killed in 90% alcohol.

Statistical analysis of the small sample was limited to exploratory analysis (Tukey, 1980). Survivorship curves were calculated based on straight percentages.

Results and Discussion

No mortality was observed in any sample during the first 75 min. Thereafter, the survivorship curves diverged (Figure 2). Unprotected beetles showed a gradual increase in mortality, with 72% alive after 180 min and all dead after 240 min. Beetles, partially protected from the cold in their galleries, began to die after 150 min. By 180 min, survivorship had dropped to 70%, dropping to 60% at 300 min and reaching 20% at the termination of the experiment after 420 min (7 h). None of the beetles in the seeds kept at room temperature died.

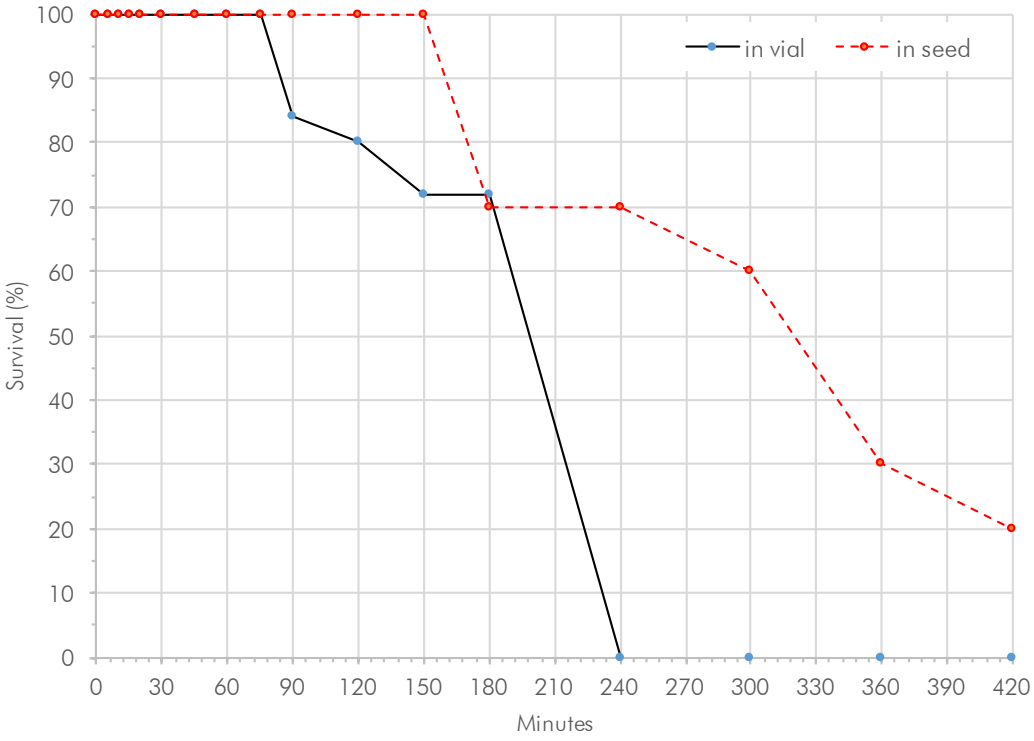


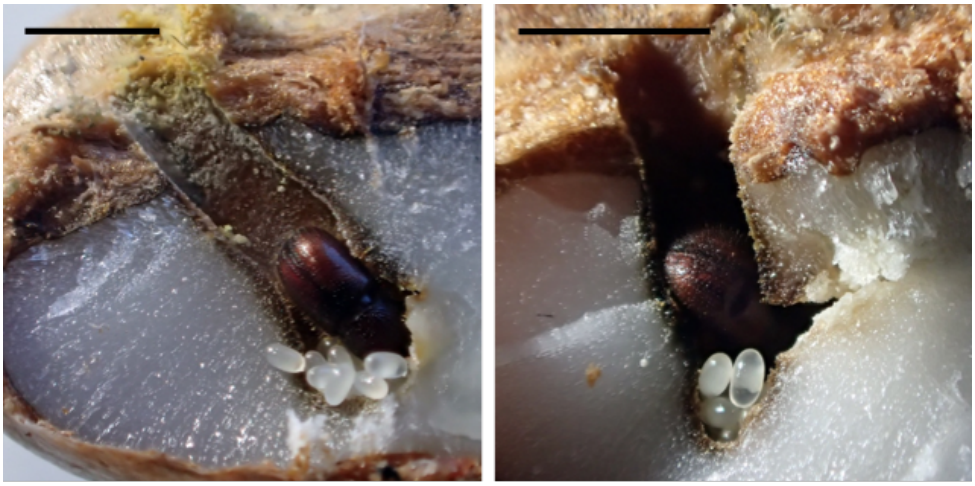
Figure 2. Survivorship curves (in %) for date stone beetles exposed to the air and tunneled into seed.

The total time between exposure of the seeds to the beetles and termination of the experiment was 8 d (2 d penetration and 6 d post cold exposure). Given tunneling speeds observed among siblings of the beetles used for the experiment (unpublished data), it can be assumed that after 24 h a rudimentary tunnel would have been excavated, with the tunnel being at least twice the length of the beetle after 48 h. As fertilized females start to deposit eggs between 1 and 3 d after entering a new seed, with an incubation period of 4-6 d (see review by Spennemann, 2019a and references cited therein), it can also be assumed that few, if any, of the females would have deposited eggs at the time of exposure to subzero temperature. This was confirmed by dissecting some seeds (Figure 3).

a b

Figure 3. Brood galleries in Canary Island date palm seed with dead date stone beetles (*Coccotrypes dactyliperda*) after 4 h exposure to -8ºC following 7 d incubation in 28ºC. Scale bar = 2 mm.

In seed exposed to shorter periods of subzero temperature, however, there was evidence of live beetles tending their eggs (Figure 4). One of these had at least 21 eggs (Figure 4b). Given the size of the seed and the nature of brood chambers, it is unlikely that a longer period of tunneling would have altered the outcome of the experiment. Although speculative, it can be assumed that adult beetles would have a lower mortality rate than eggs and larvae due to the latter's higher, unstructured moisture content and thus effects of ice crystal formation.



a b

Figure 4. Brood galleries in Canary Island date palm seed with live date stone beetles (*Coccotrypes dactyliperda*) and their eggs after 3 h exposure to -8ºC following 7 d incubation in 28ºC. Scale bar = 2 mm.

Conclusions

The design of this experiment represents an extreme scenario. In the real world, it is unlikely that beetles would be crawling about at a time when the temperature dropped below 10ºC, let alone into the subzero range. Moreover, it would be expected that some of the infested seed may be covered by leaf litter and thus further protected from the ambient air temperature. The exposure of unprotected *C. dactyliperda* beetles to such temperatures, however, underlines the resilience of the species at least after exposure to short periods of cold. Notably, 20% of the specimens survived a 7 h exposure to -8ºC. This demonstrates that although cold winters will severely impact a hibernating population, sufficient numbers will survive to ensure the survival of that population.

The findings reported here have implications for the viability of *C. dactyliperda* populations in marginal environments. Although this beetle species was originally confined to the Middle East and North Africa, where it was closely associated with the date palm complex, it has been able adapt to a range of host plants, in particular the Canary Islands date palm. Largely facilitated by the rapid and widespread horticultural dispersal of that ornamental palm (Spennemann 2018b, 2019c; Zona 2008), *C. dactyliperda* has been able to establish itself as a true cosmopolitan species in warm temperate zones (Spennemann, 2018a). The data presented here indicates that the cold tolerance of *C. dactyliperda* mirrors that of the Canary Islands date palm (Larcher & Winter, 1981). Therefore, the distribution of the Canary Islands date palm can act as a proxy for the maximum biogeographic dispersal of *C. dactyliperda*.

References

Abd-Allah, F. F. & A. W. Tadros, 1994. Biological studies on the date stone beetle, *Coccotrypes dactyliperda* Fabricius (Coleoptera: Scolytidae). Alexandria Science Exchange, 15 (1): 1-9.

Ageena, I., N. Macdonald & A. Morse, 2013. Variability of minimum temperature across Libya (1945-2009). International Journal of Climatology, 33 (3): 641-653.

Ali, M. A., M. M. Metwally & A. R. E. Hussain, 2002. Pest suppression of date palm insect populations and effects on yield as a component of sustainable development of El-Bahria Oases, Egypt. Bulletin of the Entomological Society of Egypt, 79 (2001/2002): 89-102.

Ali, M. A., M. M. Metwally & A. R. E. Hussain, 2003. Infestation levels and population density of insect pests attacking stored dates under the conditions of Elbahria Oases, Egypt. Bulletin of the Entomological Society of Egypt, 80: 133-146.

Almazroui, M., M. Nazrul Islam, H. Athar, P. Jones & M. A. Rahman, 2012. Recent climate change in the Arabian Peninsula: annual rainfall and temperature analysis of Saudi Arabia for 1978-2009. International Journal of Climatology, 32 (6): 953-966.

Anber, H. A. I., R. M. Y. Helal & R. El-Sufty, 1998. Efficiency of some insecticides in controlling the date stone beetle, *Coccotrypes dactyliperda* Fabricius (Coleoptera, Scolytidae) in palm plantations. Mansoura Journal of Agricultural Science, 23 (10): 4597-4602 [not seen].

Bar-Shalom, O. & Z. Mendel, 2001. Seasonal changes in the seed bank in date palm (*Phoenix dactylifera*) orchards and the involvement of the date-stone beetle (*Coccotrypes dactyliperda*). Phytoparasitica, 29 (1): 84-85.

Boraei, H. A., 1994. Effect of temperature and photoperiod on the dormancy of the date stone beetle, *Coccotrypes dactyliperda*. Journal of Agricultural Research, Tanta University, 20 (1): 72-79.

Cooperband, M. F., R. Stouthamer, D. Carrillo, A. Eskalen, T. Thibault, A. A. Cossé, L. A. Castrillo, J. D. Vandenberg & P. Rugman-Jones, 2016. Biology of two members of the *Euwallacea fornicatus* species complex (Coleoptera: Curculionidae: Scolytinae), recently invasive in the USA, reared on an ambrosia beetle artificial diet. Agricultural and Forest Entomology, 18: 223-237.

Donat, M. G., T. C. Peterson, M. Brunet, A. D. King, M. Almazroui, R. K. Kolli, D. Boucherf, A. Y. Al-Mulla, A. Y. Nour, A. A. Aly, T. A. A. Nada, M. M. Semawi, H. A. Al Dashti, T. G. Salhab, K. I. El Fadli, M. K. Muftah, S. Dah Eida, W. Badi, F. Driouech, K. El Rhaz, M. J. Y. Abubaker, A. S. Ghulam, A. S. Erayah, M. B. Mansour, W. O. Alabdouli, J. S. Al Dhanhani & M. N. Al Shekaili, 2014. Changes in extreme temperature and precipitation in the Arab region: long-term trends and variability related to ENSO and NAO. International Journal of Climatology, 34 (3): 581-592.

El Kadi, A., 2016. Global warming: a study of the Gaza temperature variations in the period 1976-1995. الجامعة مجلة الإنسانية للبحوث الإسلامية [IUG Journal for Humanities Research], 13 (2): 1-19.

Helal, R. M. Y., 1998. Plant extracts and microbial insecticides as protectants against the date stone beetle *Coccotrypes dactyliperda* F. (Col. Scolytidae). Menofiya Journal of Agricultural Research, 23 (6): 1669-1682 [not seen].

Helal, R. M., 2014. Studies on the date stone beetle *Coccotrypes dactyliperda* (F.) (Coleoptera: Scolytidae). The Blessed Tree, 6 (1): 13-21.

Larcher, W. & A. Winter, (1981). Frost susceptibility of palms: experimental data and their interpretation. Principes, 25: 143-152.

Matouq, M., T. El-Hasan, H. Al-Bilbisi, M. Abdelhadi, M. Hindiyeh, S. Eslamian & S. Duheisat, 2013. The climate change implication on Jordan: A case study using GIS and artificial neural networks for weather forecasting. Journal of Taibah University for Science, 7 (2): 44-55.

Mostafa, A. M., 1997. Survey of Date Palm Insect Pests in Northern Delta, with the Study of seasonal abundance of certain Species. Alexandria University.

NCDC, 2018. NNDC Climate Data Online. In: National Climatic Data Center, National Oceanic and Atmospheric Administration (Web page: https://www7.ncdc.noaa.gov/CDO/cdo) (Date accessed 12 August 2018).

Samuels, R., G. Smiatek, S. Krichak, H. Kunstmann & P. Alpert, 2011. Extreme value indicators in highly resolved climate change simulations for the Jordan River area. Journal of Geophysical Research: Atmospheres, 116: D24123, 9 pp.

Spennemann, D. H. R., 2018a. Global distribution of the date stone beetle, *Coccotrypes dactyliperda* (Coleoptera: Curculionidae, Scolytinae). Journal of Insect Biodiversity and Systematics, 4 (3): 203-226.

Spennemann, D. H. R., 2018b. Canary date palms (*Phoenix canariensis*) in Australia: introduction and early dispersal. Palms, 62 (4): 185-201.

Spennemann, D. H. R., 2019a. Biology, ecology and distribution of the date stone beetle, *Coccotrypes dactyliperda* (Scolytinae, Coleoptera). Zoology in the Middle East, 65 (2): 163-182.

Spennemann, D. H. R., 2019b. An Experimental Evaluation of Food Preferences and Associated Hatching Times of the Date Stone Beetle, *Coccotrypes dactyliperda* (Scolytinae, Coleoptera). Institute for Land, Water and Society Report No 120. Institute for Land, Water and Society, Charles Sturt University, Albury, NSW, Australia, 78 pp.

Spennemann, D. H. R., 2019c. Canary date palms (*Phoenix canariensis*) as ornamental plants. The first thirty years of the horticultural trade. Huntia, 17 (2): [in press].

Spennemann, D. H. R., K. Kent & R. Cook, 2018. Uninvited guests: Mass Emergence of Scolytinid Beetles in a Seed Germination Experiment and its Management. Institute for Land, Water and Society Report No: 118. Institute for Land, Water and Society, Charles Sturt University, Albury, NSW, Australia, 33 pp.

Tukey, J. W., 1980. We need both exploratory and confirmatory. The American Statistician, 34 (1): 23-25.

World Bank Group, 2019. Climate Change Knowledge Portal. (Web page: https://climateknowledgeportal.worldbank.org) (Date accessed: 20 January 2019).

Zhang, X., E. Aguilar, S. Sensoy, H. Melkonyan, U. Tagiyeva, N. Ahmed, N. Kutaladze, F. Rahimzadeh, A. Taghipour, T. H. Hantosh, P. Albert, M. Semawi, M. Karam Ali, M. H. Said Al-Shabibi, Z. Al-Oulan, T. Zatari, I. Al Dean Khelet, S. Hamoud, R. Sagir, M. Demircan, M. Eken, M. Adiguzel, L. Alexander, T. C. Peterson & T. Wallis, 2005. Trends in Middle East climate extreme indices from 1950 to 2003. Journal of Geophysical Research: Atmospheres, 110: D22104, 12 pp.

Zona, S., 2008. The horticultural history of the Canary Island date palm (*Phoenix canariensis*). Garden History, 36: 301-308.

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Original article (Orijinal araştırma)

Residual efficacy of methoxyfenozide applied on different grain commodities for the control of three stored-product insect pests

Depolanan ürün zararlısı üç böcek türünün mücadelesinde farklı tahıl ürünlerine uygulanan methoxyfenozidin kalıntı etkinliği

Muhammad YASIR[1*] **Mansoor ul HASAN[1]**

Muhammad SAGHEER[1] **Nazir JAVED[2]**

Abstract

The residual efficacy of methoxyfenozide was assessed by exposing last instar larvae of *Oryzaephilus surinamensis* (Linnaeus, 1758) (Coleoptera: Silvanidae)*, Tribolium castaneum* Herbst, 1797 (Coleoptera: Tenebrionidae) and *Trogoderma granarium* Everts, 1898 (Coleoptera: Dermestidae) to the treated grain commodities (maize, oat, rice and wheat) at concentrations of 1, 2 and 4 mg kg^{-1} of active ingredient under laboratory conditions. This trial was performed at the Department of Entomology, University of Agriculture, Faisalabad, Pakistan during the year 2018. Six bioassays were conducted by releasing the insects on treated commodities after different post treatment periods (0, 2, 4, 8, 12 and 16 weeks). At 4 mg kg^{-1}, the adult emergence from larvae exposed to treated commodities did not exceed 15% at week 0 and it was less than 36% at week 12 in all the tested insect species on all the tested commodities. The methoxyfenozide was generally more effective on oats followed by wheat, maize and rice in the *O. surinamensis* and *T. castaneum* while against *T. granarium* it was more effective in wheat followed by oats, maize and rice. Results show that methoxyfenozide possess great potential for residual control of *O. surinamensis, T. castaneum* and *T. granarium*, and can be used for replacement of conventional neurotoxic insecticides.

Keywords: Ecdysone agonist, grain commodities, insect growth regulator, residual efficacy, stored grain insects

Öz

Laboratuvar koşullarında 1, 2 ve 4 mg kg^{-1} aktif madde konsantrasyonlarında uygulama yapılmış (buğday, mısır, çeltik ve yulaf) ürünlerde, *Oryzaephilus surinamensis* (Linnaeus, 1758) (Coleoptera: Silvanidae), *Tribolium castaneum* Herbst, 1797 (Coleoptera: Tenebrionidae) ve *Trogoderma granarium* Everts, 1898 (Coleoptera: Dermestidae)'un son dönem larvaları üzerinde metoksifenozidin kalıntı etkinliği değerlendirilmiştir. Bu deneme, Ziraat Üniversitesi, Entomoloji Bölümü (Faisalabad, Pakistan)'nde 2018 yılında gerçekleştirilmiştir. Uygulama yapılmış ürünlere zararlı böcekler bırakıldıktan farklı süreler (0, 2, 4, 8, 12 ve 16 hafta) sonrasında, altı biyolojik analiz yapılmıştır. Uygulama yapılmış ürünlerdeki larvaların ergin olma oranı, 4 mg kg^{-1}'de 0. haftada %15'i geçmezken, test edilen tüm ürünlerde ve tüm böcek türlerinde 12. haftada %36'dan daha az olarak saptanmıştır. Metoksifenozid, genellikle yulaf üzerinde sırasıyla buğday, mısır ve pirince göre *T. granarium*'a göre *O. surinamensis* ve *T. castaneum* açısından daha fazla etkiliyken; *T. granarium*'a karşı ise yulaf, mısır ve pirince göre buğday üzerinde daha etkili olmuştur. Sonuçlar, *O. surinamensis*, *T. castaneum* ve *T. granarium* açısından metoksifenozidin kalıntı kontrolü için büyük potansiyele sahip olduğunu ve geleneksel sinir sistemi insektisitlerinin yerini alabileceğini göstermektedir.

Anahtar sözcükler: Ecdysone agonisti, tahıl ürünleri, böcek büyüme düzenleyicisi, kalıntı etkinliği, depolanmış tahıl böcekleri

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Introduction

Insect pests of stored commodities are not only the pests of bulk grains but also of many value-added food products in mills, processing plants and facilities where these products are stored (Gorham, 1991). These insects cause considerable postharvest losses; annually about 9% in developed countries whereas 20% or more in developing countries (Phillips & Throne, 2010). These insects are also responsible for contamination of food products with arthropod fragments and other related contaminants which may be allergic and even carcinogenic (Hubert et al., 2018).

Widely used control methods for stored product insects comprise the application of pyrethroid insecticides (Ghimire et al., 2016; Arthur et al., 2018), use of diatomaceous earth (Korunic, 1998; Kavallieratos et al., 2015, 2018) and phosphine fumigation (Carpaneto et al., 2016). However, the synthetic neurotoxic insecticides possess certain limitations for their usage, like the possible presence of insecticide residues in food (Phillips & Throne, 2010), development of insecticide resistance by the target pest species (Zettler & Cuperus, 1990; Boyer et al., 2012), health hazards (Arthur, 1996), increase in the management expenditures (Hagstrum & Subramanyam, 2006) and unfriendly for non-target organisms (Fields, 1992; Hagstrum & Subramanyam, 2006). To overcome these problems, safe alternate approaches are required for the management of insect pests of stored products. Insect growth regulators (IGRs) are the chemicals that can provide various benefits as alternatives to conventional neurotoxin products (Oberlander et al*.,* 1997; Mondal & Parween, 2000). Generally, these compounds lack harmful effects on the humans or environment and are compatible with other pest management approaches (Staal, 1975). These products possess great potential for acceptance in the food industry (Phillips & Throne, 2010). The IGRs control the pest species through inhibiting adult emergence, and are comparatively friendly for the non-target organisms than most of the conventional neurotoxic grain protectants (Oberlander et al*.,* 1997; Oberlander & Silhacek, 2000).

Mainly, the IGRs include the compounds that interfere by three different modes of action: cuticle formation; growth and development of immature insects or stimulation of metamorphosis (Oberlander et al., 1997; Oberlander & Silhacek, 2000) and are termed as chitin synthesis inhibitors, juvenile hormone agonists and ecdysteroid agonists, respectively (Oberlander et al., 1997; Mondal & Parween, 2000). Among these, the ecdysteroid agonists causes the premature synthesis of the insect's cuticle and feeding inhibition (Schneiderman, 1972; Fox, 1990; Wing & Aller, 1990). These compounds have the ability to penetrate the insect's cuticle (Schneiderman, 1972) and have chemosterilant activity in females (Heller et al*.,* 1992) through stomach and contact (Fox, 1990). Several studies have been conducted for evaluating the potential of other insect growth regulators (juvenile hormone analogues and chitin synthesis inhibitors) against different stored-grain insect pests (Elek, 1998a, b; Parween et al., 2001; Abo-Elghar et al., 2004; Arthur et al., 2009, 2018; Sagheer et al., 2011, 2012; Yasir et al., 2012, 2019; Trostanetsky et al., 2015; Ali et al., 2016, 2017, 2018; Malik et al., 2017; Arthur & Hartzer, 2018).

However, very few studies have evaluated the potential of ecdysteroid agonists against stored-grain insect pests (Oberlander et al*.,* 1998; Kostyukovsky et al., 2000; Kavallieratos et al., 2012; Ali et al., 2016, 2017). Methoxyfenozide, an ecdysteroid agonist, is the member of the diacylhydrazine class (Carlson et al., 2001), novel and potent molt-accelerating compound that targets Lepidoptera (Enríquez et al., 2010), but is harmless to beneficial insects. This compound mimics the natural insect molting hormone by binding competitively to ecdysteroid receptors in insect cells, thus to prematurely inducing larval molt (Chen et al., 2019). It is registered for use on several agricultural and horticultural plants including cotton in the United States (Alavo et al., 2011). It is important to assess the long-term residual efficacy of methoxyfenozide against stored-grain insect pests which have not been tested in these studies. Therefore, the current study was planned to evaluate the residual efficacy of methoxyfenozide (ecdysteroid agonist) against three stored-grain insects *Oryzaephilus surinamensis* (Linnaeus, 1758) (Coleoptera: Silvanidae), *Tribolium castaneum* Herbst, 1797 (Coleoptera: Tenebrionidae) and *Trogoderma granarium* Everts, 1898 (Coleoptera: Dermestidae).

Materials and Methods

The current studies were conducted at the Grain Research, Training and Storage Management Cell, Department of Entomology, University of Agriculture, Faisalabad, Pakistan during 2018.

Test insects and insecticide

The heterogeneous culture of *O. surinamensis, T. castaneum* and *T. granarium* was collected from household granaries, grain markets and stores of Punjab Food Department located at Faisalabad, Pakistan. The collected insect species were reared in sterilized glass jars placed in incubator (SANYO, MIR-254) at 30±2ºC and 65±5% RH to achieve uniform-aged first generation. The culture medium was cracked wheat grains, sterilized wheat flour and whole wheat grains for rearing of *O. surinamensis, T. castaneum* and *T. granarium*, respectively. The methoxyfenozide 240 SC (Runner®) was obtained from Arysta Life Sciences, Pakistan.

Grain commodity and treatment

Untreated, clean and infestation free four different grain commodities maize, oats, rice and wheat were obtained from the local market and used in these tests. The moisture content of these grain commodities was determined by a Dickey-John moisture meter (Dickey-John multigrain CAC-II, Dickey-John Co., Auburn, IL, USA) and it was recorded 12.4, 10.4, 12.1 and 11.2% for maize, oats, rice and wheat, respectively. For evaluating methoxyfenozide at concentration of 1, 2 and 4 mg kg^{-1} of active ingredient, the serial solution of 4 mg kg^{-1} was prepared in acetone and further concentrations were prepared from this stock solution. Commodities treatment with different concentrations of methoxyfenozide was performed by following the method of Vayias et al. (2010) with little modifications. Briefly, a lot of 4 kg grains from each commodity were prepared and treated with the desired concentrations. Separate 4 kg grain lot from each commodity was treated with acetone only to act as a control treatment. Treatment with different tested concentrations was carried out in a tray containing thin layer spread into it. All the treated grain lots were kept at 25±2ºC, 65±5% RH and continuous darkness to dry for 24 h. Then, the grain lots were placed in sealed 5 L plastic containers and stored at the above conditions for the total period of 16 weeks. For bioassays, the grains samples were obtained at the day of storage (24 h after treatment) and after the period of 2, 4, 8, 12 and 16 weeks.

Bioassays

Three samples of 50 g each were acquired from each lot and placed in glass jars. Thirty last instar larvae (3 weeks old) of each of the tested insect species were placed into each glass jar and the jars were kept in incubator (SANYO, MIR-254) at 28±2ºC, 65±5% RH and continuous darkness. The emergence of adults from larvae exposed to treated commodities were recorded after period of 30 days. Separate trials were conducted at the 2, 4, 8, 12 and 16 weeks post treatment, as described above. The entire experiment was repeated four times by using new treated lots every time.

Statistical analysis

All the treatments were performed under completely randomized design and replicated four times. The collected data was statistically analyzed by using the R-software (version 3.5.2) (R Core Team, 2013) and the means of the treatments were compared by using Tukey-Kramer HSD test at 0.05 (Sokal & Rohlf, 1995).

Results and Discussion

Efficacy on *Oryzaephilus surinamensis*

The ANOVA for *O. surinamensis* shows that all the main effects and their interactions were significant (Table 1). From week 0-16, the effect of concentration on adult emergence from exposed larvae was significant on specific commodity (Table 2). However, the effect of commodity on adult emergence was only significant from week 8-16 at 4 mg kg^{-1} concentration (Table 2). At week 0, the adult emergence from larvae exposed to 4 mg kg^{-1} did not exceeded 14.1% on all the tested commodities compared to control where the emergence was above 97.5% (Table 2). Similarly, at week 12, among the tested commodities, the adult emergence was minimum (29.1%) in oats and not exceeded 37.5% in other commodities at 4 mg kg^{-1} (Table 2). However, at week 16, the emergence of adults reached to 78.3-91.7% at concentration of 4 mg kg^{-1} (Table 2).

Table 1. ANOVA for main effects and interactions for adult emergence of *Oryzaephilus surinamensis*, *Tribolium castaneum* and *Trogoderma granarium* (error df = 288)

Source	df	*O. surinamensis*		*T. castaneum*		*T. granarium*	
		F	P	F	P	F	P
Week	5	4016	< 0.01	2073	< 0.01	3246	< 0.01
Concentration	3	18661	< 0.01	9514	< 0.01	14743	< 0.01
Commodity	3	44.0	< 0.01	9.81	< 0.01	20.8	< 0.01
Week × Concentration	15	544	< 0.01	267	< 0.01	425	< 0.01
Week × Commodity	15	4.15	< 0.01	2.28	< 0.01	2.13	< 0.01
Concentration × Commodity	9	7.21	< 0.01	5.02	< 0.01	6.75	< 0.01
Week × Concentration × Commodity	45	1.51	0.025	0.88	0.689	1.27	0.127

Efficacy on *Tribolium castaneum*

The ANOVA for *T. castaneum* shows that all the main effects and their interactions were significant except the interaction week by concentration by commodity (Table 1). From week 0-16, the effect of concentration on adult emergence from exposed larvae was significant on specific commodity (Table 3). However, the effect of commodity on adult emergence was only significant from week 8-16 at 4 mg kg^{-1} concentration (Table 3). At week 0, the adult emergence from larvae exposed to 4 mg kg^{-1} did not exceeded 13.3% on all the tested commodities compared to control where the emergence was above 98.3% (Table 3). Similarly, at week 12, among the tested commodities, the adult emergence was minimum (28.3%) in oats and not exceeded 35.8% in other commodities at 4 mg kg^{-1} (Table 3). However, at week 16, the emergence of adults increased to 77.5-88.3% at concentration of 4 mg kg^{-1} (Table 3).

Efficacy on *Trogoderma granarium*

The ANOVA for *T. granarium* shows that all the main effects and their interactions were significant except the interaction week by concentration by commodity (Table 1). From week 0-16, the effect of concentration on adult emergence from exposed larvae was significant on specific commodity (Table 4). However, the effect of commodity on adult emergence was only significant from week 8-16 at 4 mg kg^{-1} concentration (Table 4). At week 0, the adult emergence from larvae exposed to 4 mg kg^{-1} did not exceeded 15% on all the tested commodities compared to control where the emergence was above 98.3% (Table 4). Similarly, at week 12, among the tested commodities, the adult emergence was minimum (32.5%) in wheat and not exceeded 38.3% in other commodities at 4 mg kg^{-1} (Table 4). However, at week 16, the emergence of adults increased to 80.0-90.8% at concentration of 4 mg kg^{-1} (Table 4).

Table 2. Percentage emergence of *Oryzaephilus surinamensis* normal adult (mean±SE) from last instar larvae exposed at different post-treatment periods on treated grain commodities with different concentrations of methoxyfenozide at 28±2ºC

Week	Concentration (mg kg^{-1})	Commodity			
		Maize	Oats	Rice	Wheat
0	0	98.3±0.10 Aa*	99.2±0.08 Aa	97.5±0.08 Aa	97.5±0.08 Aa
	1	51.7±0.10 Ab	51.7±0.10Ab	52.5±0.08 Ab	53.3±0.14 Ab
	2	28.3±0.10 Ac	28.3±0.10 Ac	30.8±0.08 Ac	27.5±0.08 Ac
	4	12.5±0.08 Ad	13.3±0.14 Ad	14.2±0.08 Ad	11.7±0.10 Ad
2	0	98.3±0.10 Aa	98.3±0.10 Aa	99.2±0.08 Aa	99.2±0.08 Aa
	1	53.3±0.14 Ab	52.5±0.08 Ab	53.3±0.14 Ab	53.3±0.14 Ab
	2	30.8±0.08 Ac	30.8±0.08 Ac	31.7±0.10 Ac	30.0±0.14 Ac
	4	13.3±0.00 Ad	14.2±0.25 Ad	15.8±0.08 Ad	14.2±0.08 Ad
4	0	98.3±0.10 Aa	98.3±0.17 Aa	100.0±0.00 Aa	97.5±0.08 Aa
	1	56.7±0.14 Ab	53.3±0.14 Ab	57.5±0.08 Ab	54.2±0.08 Ab
	2	34.2±0.08 Ac	31.7±0.10 Ac	34.2±0.08 Ac	32.5±0.08 Ac
	4	17.5±0.16 Ad	15.0±0.10 Ad	17.5±0.08 Ad	15.8±0.08 Ad
8	0	98.3±0.10 Aa	99.2±0.08 Aa	98.3±0.10 Aa	98.3±0.10 Aa
	1	62.5±0.08 Ab	57.5±0.08 Bb	60.8±0.08 ABb	60.0±0.14 ABb
	2	40.8±0.08 ABc	37.5±0.08 Bc	41.7±0.10 Ac	38.3±0.14 ABc
	4	25.0±0.10 ABd	20.0±0.14 Cd	27.5±0.08 Ad	22.5±0.08 BCd
12	0	99.2±0.08 Aa	99.2±0.08 Aa	98.3±0.10 Aa	100.0±0.00 Aa
	1	66.7±0.14 Ab	61.7±0.10 Bb	67.5±0.08 Ab	64.2±0.08 ABb
	2	48.3±0.10 Ac	40.8±0.08 Bc	50.8±0.08 Ac	44.2±0.08 Bc
	4	36.7±0.14 Ad	29.1±0.10 Bd	37.5±0.16 Ad	32.5±0.08 ABd
16	0	99.2±0.08 Aa	98.3±0.10 Aa	97.5±0.16 Aa	99.2±0.08 Aa
	1	93.3±0.14 Ab	90.8±0.08 Ab	94.2±0.08 Aab	92.5±0.16 Ab
	2	91.7±0.10 Ab	87.5±0.21 Ab	93.3±0.14 Aab	89.2±0.08 Ab
	4	89.2±0.08 Ab	78.3±0.10 Cc	91.7±0.10 Ab	84.2±0.08 Bc

* Significant difference between treatments (columns) denoted by different lower-case letters, significant difference between commodities (rows) denoted by different upper-case letters.

Among the tested stored grain insect species, *T. granarium* was the least susceptible to methoxyfenozide irrespective of the type of grain commodity, concentration and the post-treatment period. While, *T. castaneum* was definitely the most susceptible among the tested insect species. A similar trend between stored-grain insect species has already been reported by Arthur et al. *(*2009) by exposing the larvae of *O. surinamensis* and *T. castaneum* to pyriproxyfen and hydroprene treated surfaces, and found that *O. surinamensis* was least susceptible compared to *T. castaneum.* Similarly, in another study Arthur & Hartzer (2018) found *Trogoderma variabile* Ballion, 1878 more tolerant than *T. castaneum* due to the combination treatment of novaluron and pyriproxyfen. Similar results were also found in other studies

(Scheff et al., 2016, 2017), in which *T. castaneum* and *T. variabile* larvae were exposed to methoprene-treated packaging materials; *T. variabile* was found to be the more tolerant species. This wavering susceptibility can be related to specific IGRs, or most probably it may be due to the morphological characters of the larvae of *Trogoderma* spp. that possess bristles and hairs on the body that might be responsible for the less exposure of the insecticide from the treated grain commodities.

Table 3. Percentage emergence of *Tribolium castaneum* normal adult (mean ± se) from last instar larvae exposed at different post-treatment periods on treated grain commodities with different concentrations of methoxyfenozide at 28±2ºC

Week	Concentration (mg kg^{-1})	Commodity			
		Maize	Oats	Rice	Wheat
0	0	99.2±0.08 Aa*	98.3±0.10 Aa	98.3±0.10 Aa	98.3±0.10 Aa
	1	47.5±0.16 Ab	48.3±0.22 Ab	45.8±0.21 Ab	50.8±0.25 Ab
	2	26.7±0.14 Ac	27.5±0.16 Ac	28.3±0.17 Ac	25.0±0.10 Ac
	4	9.2±0.16 Ad	12.5±0.16 Ad	13.3±0.14 Ad	10.0±0.14 Ad
2	0	99.2±0.08 Aa	98.3±0.10 Aa	99.2±0.08 Aa	100.0±0.00 Aa
	1	48.3±0.10 Ab	49.2±0.21 Ab	45.8±0.21 Ab	51.7±0.22 Ab
	2	31.7±0.10 Ac	32.5±0.08 Ac	31.7±0.10 Ac	28.3±0.22 Ac
	4	15.8±0.08 Ad	13.3±0.24 Ad	15.3±0.16 Ad	13.3±0.14 Ad
4	0	99.2±0.08 Aa	97.5±0.16 Aa	98.3±0.17 Aa	98.3±0.10 Aa
	1	50.0±0.14 ABb	50.0±0.14A Bb	47.5±0.08 Bb	53.3±0.14 Ab
	2	32.5±0.08 Ac	32.5±0.08 Ac	33.3±0.14 Ac	30.0±0.14 Ac
	4	16.7±0.14 Ad	14.2±0.16 Ad	16.7±0.14 Ad	14.2±0.16 Ad
8	0	99.2±0.08 Aa	97.5±0.08 Aa	98.3±0.10 Aa	99.2±0.08 Aa
	1	55.8±0.16 Ab	52.5±0.16 Ab	55.8±0.16 Ab	59.2±0.16 Ab
	2	35.8±0.16 Ac	34.2±0.08 Ac	38.3±0.10 Ac	35.0±0.10 Ac
	4	20.0±0.14 Bd	18.3±0.10 Bd	25.0±0.10 Ad	19.2±0.08 Bd
12	0	98.3±0.17 Aa	98.3±0.10 Aa	97.5±0.16 Aa	98.3±0.10 Aa
	1	64.2±0.16 Ab	60.8±0.38 Ab	65.8±0.16 Ab	63.3±0.14 Ab
	2	44.2±0.25 Ac	40.0±0.24 Ac	48.3±0.22 Ac	41.7±0.22 Ac
	4	32.5±0.16 ABd	28.3±0.10 Bd	35.8±0.16 Ad	30.8±0.25 ABd
16	0	99.2±0.08 Aa	98.3±0.10 Aa	98.3±0.10 Aa	98.3±0.17 Aa
	1	94.2±0.16 Aab	90.0±0.14 Ab	95.8±0.08 Aab	91.7±0.17 Ab
	2	90.0±0.14 Abc	88.3±0.22 Ab	91.7±0.10 Abc	89.2±0.10 Abc
	4	85.8±0.16 Ac	77.5±0.16 Bc	88.3±0.22 Ac	85.0±0.10 Ac

* Significant difference between treatments (columns) denoted by different lower-case letters, significant difference between commodities (rows) denoted by different upper-case letters.

Table 4. Percentage emergence of *Trogoderma granarium* normal adult (mean ± se) from last instar larvae exposed at different post-treatment periods on treated grain commodities with different concentrations of methoxyfenozide at 28±2ºC

Week	Concentration (mg kg^{-1})	Commodity			
		Maize	Oats	Rice	Wheat
0	0	98.3±0.10 Aa*	98.3±0.10 Aa	98.3±0.10 Aa	99.2±0.10 Aa
	1	53.3±0.14 Ab	49.2±0.16 Ab	50.0±0.14 Ab	54.2±0.21 Ab
	2	29.2±0.16 Ac	28.3±0.10 Ac	33.3±0.14 Ac	28.3±0.10 Ac
	4	13.3±0.14 Ad	13.3±0.14 Ad	15.0±0.10 Ad	14.2±0.16 Ad
2	0	98.3±0.10 Aa	99.2±0.08 Aa	99.2±0.08 Aa	100.0±0.00 Aa
	1	55.0±0.22 Ab	50.8±0.08 Ab	51.2±0.22 Ab	56.7±0.14 Ab
	2	32.5±0.08 Ac	33.3±0.14 Ac	34.2±0.08 Ac	30.8±0.16 Ac
	4	15.8±0.08 Ad	15.0±0.22 Ad	16.7±0.14 Ad	15.8±0.16 Ad
4	0	99.2±0.08 Aa	97.5±0.16 Aa	97.5±0.16 Aa	99.2±0.08 Aa
	1	57.5±0.08 Ab	55.8±0.08 Ab	59.2±0.08 Ab	58.3±0.10 Ab
	2	35.8±0.16 Ac	34.2±0.08 Ac	35.8±0.08 Ac	35.0±0.10 Ac
	4	18.3±0.17 Ad	15.8±0.08 Ad	18.3±0.10 Ad	17.5±0.08 Ad
8	0	99.2±0.08 Aa	98.3±0.10 Aa	99.2±0.08 Aa	100.0±0.00 Aa
	1	62.5±0.08 Ab	57.5±0.16 Ab	61.7±0.10 Ab	60.0±0.14 Ab
	2	41.7±0.10 ABc	40.8±0.08 ABc	44.2±0.08 Ac	39.2±0.08 Bc
	4	29.2±0.08 ABd	26.7±0.14 Abd	30.0±0.14 Ad	25.0±0.10 Bd
12	0	98.3±0.17 Aa	98.3±0.10 Aa	99.2±0.08 Aa	99.2±0.08 Aa
	1	68.3±0.10 Ab	67.5±0.08 Ab	68.3±0.10 Ab	65.8±0.08 Ab
	2	49.2±0.08 ABc	46.7±0.14 Bc	51.7±0.10 Ac	45.0±0.10 Bc
	4	37.5±0.08 ABd	33.3±0.14 ABd	38.3±0.17 Ad	32.5±0.08 Bd
16	0	99.2±0.08 Aa	98.3±0.10 Aa	99.2±0.08 Aa	100.0±0.00 Aa
	1	95.0±0.10 Ab	92.5±0.08 Ab	94.2±0.08 Ab	93.3±0.14 Ab
	2	91.7±0.10 Abc	90.0±0.14 Ab	93.3±0.00 Ab	90.8±0.08 Ab
	4	89.2±0.08 Ac	81.7±0.10 Bc	90.8±0.16 Ab	80.0±0.14 Bc

* Significant difference between treatments (columns) denoted by different lower-case letters, significant difference between commodities (rows) denoted by different upper-case letters.

The efficacy of methoxyfenozide was not affected due to the type of commodity for initial bioassays (week 0-4) in all the tested insect species. However, this effect become prominent in later bioassays (week 8-16), particularly at 4 ppm in all species tested. The methoxyfenozide was generally more effective on oats followed by wheat, maize and rice in the *O. surinamensis* and *T. castaneum* while against *T. granarium* it was more effective in wheat followed by oats, maize and rice at bioassays performed after 16 weeks post treatment at the higher concentration. The present results are opposite to those of Athanassiou et al., (2011) who found methoprene more effective on maize as compared to rice and wheat. These results are in contrast to those of Kavallieratos et al. (2009), who reported abamectin more effective in maize than that

in wheat against stored-grain insect species tested. Similarly, Vayias et al. (2010) reported that spinosad was more persistent in barley and wheat than that in maize against stored-grain insects tested during storage period of six month. These results are also in accordance with those of Ali et al. (2017) in which *T. castaneum* and *T. granarium* were exposed to methoxyfenozide treated gain commodities and it was found that its efficacy was better on wheat than maize and rice. Therefore, it is proposed that there may be interactions between methoxyfenozie and specific features (morphological or physiological) of the grain, which may be responsible for the process of methoxyfenozide degradation over time.

With respect to residual activity, the residue of methoxyfenozide degraded over time (week 1-16) as the exposed larvae were able to complete their development to the adult stage near the end of the test period (week 16). These results are similar to those of Arthur & Hartzer (2018) by using combination treatment of novaluron and pyriproxyfen against *T. variabile.* These results are consistent with other studies in which larvae of *O. surinamensis* and *T. castaneum* were exposed to the hydroprene and pyriproxyfen treated surfaces at 1, 28 and 56 d post treatment. It was concluded that pyriproxyfen was more persistent in the entire testing period, while hydroprene lost its effectiveness after 28 d (Arthur et al., 2009). The results of methoxfenozide persistence are in contrast to those obtained by Liu et al. (2016) who found that methoprene (a juvenoid IGR) was effective up to 40 weeks at 1 ppm due to its residual activity in treated wheat. Similarly, Arthur (2019) reported that efficacy of the IGR increased with different combinations of deltamethrin and methoprene were applied to grain, all treatments were effective against *T. castaneum* for 15 months on maize.

In conclusion, the methoxyfenozide evaluated in this study provide adequate control of the insect species tested. However, it is advisable to further evaluate this product in combination with other insecticides that are suitable for the complete control of these three pest species.

References

Abo-Elghar, G. E., A. E. El-Sheikh, F. M. El-Sayed, H. M. El-Maghraby & H. M. El-Zun, 2004. Persistence and residual activity of an organophosphate, pirimiphos-methyl, and three IGRs, hexaflumuron, teflubenzuron and pyriproxyfen, against the cowpea weevil, *Callosobruchus maculates* (Coleoptera: Bruchidae). Pest Management Science, 60: 95-102.

Alavo, T. B. C., K. J. C. Tégbéssou & B. B. Yarou, 2011. Potentialities of methoxyfenozide for the integrated management of *Helicoverpa armigera* (Lepidoptera: Noctuidae) on cotton in Benin, West Africa. Archives of Phytopathology and Plant Protection, 44: 813-819.

Ali, Q., M. Hasan, L. J. Mason, M. Sagheer & N. Javed, 2016. Biological activity of insect growth regulators, pyriproxyfen, lufenuron and methoxyfenozide against *Tribolium castaneum* (Herbst). Pakistan Journal of Zoology, 48 (5): 1337-1342.

Ali, Q., M. Hasan, M. Sagheer, S. Saleem, M. Faisal, A. Naeem & J. Iqbal, 2017. Screening of seven insect growth regulators for their anti-insect activity against the larvae of *Trogoderma granarium* (Everts) and *Tribolium castaneum* (Herbst). Pakistan Journal of Agricultural Sciences, 54 (3): 589-595.

Ali, Q., M. Hasan, H. U. Shakir, N. A. Anjum, S. Saleem, M. Y. Iqbal, M. U. Qasim & M. Faisal, 2018. Transgenerational effect of insect growth regulators on the *Trogoderma granarium* (Everts) (Coleoptera: Dermestidae) under different abiotic factors. Pakistan Journal of Agricultural Sciences, 55 (4): 897-903.

Arthur, F. H., 1996. Grain protectants: current status and prospects for the future. Journal of Stored Products Research, 32: 293-302.

Arthur, F. H., 2019. Efficacy of combinations of methoprene and deltamethrin as long-term commodity protectants. Insects, 10 (2): 50.

Arthur, F. H., M. N. Ghimire, S. W. Myers & T. W. Phillips, 2018. Evaluation of pyrethroid insecticides and insect growth regulators applied to different surfaces for control of *Trogoderma granarium* (Coleoptera: Dermestidae) the khapra beetle. Journal of Economic Entomology, 111 (2): 612-619.

Arthur, F. H. & K. L. Hartzer, 2018. Susceptibility of selected stored product insects to a combination treatment of pyriproxyfen and novaluron. Journal of Pest Science, 91 (2): 699-705.

Arthur, F. H., S. Liu, B. Zhao & T. W. Phillips, 2009. Residual efficacy of pyriproxifen and hydroprene applied to wood, metal and concrete for control of stored-product insects. Pest Management Science, 65: 791-797.

Athanassiou, C. G., F. H. Arthur & J. E. Throne, 2011. Efficacy of layer treatment with methoprene for control of *Rhyzopertha dominica* (Coleoptera: Bostrychidae) on wheat, rice and maize. Pest Management Science, 67: 380-384.

Boyer, S., H. Zhang & G. Lempérière, 2012. A review of control methods and resistance mechanisms in stored-product insects. Bulletin of Entomological Research, 102 (2): 213-229.

Carlson, G. R., T. S. Dhadialla, R. Hunter, R. K. Jansson, C. S. Jany, Z. Lidert & R. A. Slawecki, 2001. The chemical and biological properties of methoxyfenozide, a new insecticidal ecdysteroid agonist. Pest Management Science, 57: 115-119.

Carpaneto, B., R. Bartosik, L. Cardoso & P. Manetti, 2016. Pest control treatments with phosphine and controlled atmospheres in silo bags with different airtightness conditions. Journal of Stored Products Research, 69: 143-151.

Chen J., W. Jiang, H. Hu, X. Ma, Q. Li, X. Song, X. Ren & Y. Ma, 2019. Joint toxicity of methoxyfenozide and lufenuron on larvae of *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae). Journal of Asia-Pacific Entomology, 22: 795-801.

Elek, J. A., 1998a. Treatment of adult Coleoptera with a chitin synthesis inhibitor affects mortality and development time of their progeny. Entomolgia Experimentalis et Applicata, 88: 31-39.

Elek, J. A., 1998b. Interaction of treatment of both adult and immature Coleoptera with a chitin synthesis inhibitor affects mortality and development time of their progeny. Entomolgia Experimentalis et Applicata, 89: 125-136.

Enríquez, C. L. R., S. Pineda, J. I. Figueroa, M. I. Schneider & A. M. Martínez, 2010. Toxicity and sublethal effects of methoxyfenozide on *Spodoptera exigua* (Lepidoptera: Noctuidae). Journal of Economic Entomology, 103: 662-667.

Fields, P. G., 1992. The control of stored-product insects and mites with extreme temperatures. Journal of Stored Products Research, 28: 89-118.

Fox, P., 1990. Insect Growth Regulators. Richmond, PJB Publ. Ltd. UK, 102 pp.

Ghimire, M. N., F. H. Arthur, S. M. Myers & T. W. Phillips, 2016. Residual efficacy of deltamethrin and b-cyfluthrin against *Trogoderma variabile* and *Trogoderma inclusum* (Coleoptera: Dermestidae). Journal of Stored Products Research, 66: 6-11.

Gorham, J. R, ed. 1991. Ecology and Management of Food Industry Pests. Association of Official Analytical Chemists, Arlington, Virginia, USA, 595 pp.

Hagstrum, D.W. & B. Subramanyam, 2006. Fundamentals of Stored-product Entomology. AACC International, St. Paul, Minnesota, USA, 215 pp.

Heller, J. J., H. Mattioda, E. Klein & A. Sagenmuller, 1992. “Field evaluation of RH5992 on lepidopterous pests in Europe, 59-65”. Proceedings of the Bright on Crop Protection Conference, Pests and Diseases, (23 to 26 November 1992, Brighton, UK), British Crop Protection Council, Farnham, UK, 1500 pp.

Hubert, J., V. Stejskal, C. Athanassiou & J. E. Throne, 2018. Health hazards associated with arthropod infestation of stored products. Annual Review of Entomology, 63: 553-573.

Kavallieratos, N. G., C. G. Athanassiou, G. G. Peteinatos, M. C. Boukouvala & G. Benelli, 2018. Insecticidal effect and impact of fitness of three diatomaceous earths on different maize hybrids for the eco-friendly control of the invasive stored-product pest *Prostephanus truncatus* (Horn). Environmental Science and Pollution Research, 25: 10407-10417.

Kavallieratos, N. G., C. G. Athanassiou, B. J. Vayias, S. B. Mihail & Z. Tomanovic, 2009. Insecticidal efficacy of abamectin against three stored-product insect pests: influence of dose rate, temperature, commodity, and exposure interval. Journal of Economic Entomology, 102 (3): 1352-1359.

Kavallieratos, N. G., C. G. Athanassiou, B. J. Vayias & Z. Tomanovic, 2012. Efficacy of insect growth regulators as grain protectants against two stored-product pests in wheat and maize. Journal of Food Protection, 75 (5): 942-950.

Kavallieratos, N. G., C. G. Athanassiou, Z. Korunic & N. H. Mikeli, 2015. Evaluation of three novel diatomaceous earths against three stored-grain beetle species on wheat and maize. Crop Protection, 75: 132-138.

Korunic, Z., 1998. Diatomaceous earths, a group of natural insecticides. Journal of Stored Products Research, 34: 87-97.

Kostyukovsky, M., B. Chen, S. Atsmi & E. Shaaya, 2000. Biological activity of two juvenoids and two ecdysteroids against three stored product insects. Insect Biochemistry and Molecular Biology, 30 (8-9): 891-897.

Liu, S. S., F. H. Arthur, D. Van Gundy & T. W. Phillips, 2016. Combination of methoprene and controlled aeration to manage insects in stored wheat. Insects, 7 (2): 25.

Malik, G., A. Qadir, H. A. A. Khan & A. Aslam, 2017. Toxicity of lufenuron and thiamethoxam against five Pakistani strains of *Sitophilus oryzae* on wheat, rice and maize. Pakistan Entomologist, 39 (1): 41-47.

Mondal, K. A. M. S. H. & S. Parween, 2000. Insect growth regulators and their potential in the management of stored-product insect pests. Integrated Pest Management Reviews, 5: 255-295.

Oberlander, H. & D. Silhacek, 2000. "Insect Growth Regulators, 147-163". In: Alternatives to Pesticides in Stored-Product IPM (Eds. B. Subramanyam & D. W. Hagstrum), Kluwer Academic Publishers, New York, USA, 437 pp.

Oberlander, H., D. Silhacek & C. E. Leach, 1998. Interactions of ecdysteroid and juvenoid agonists in *Plodia interpunctella* (Hubner). Archives of Insect Biochemistry and Physiology, 38: 91-99.

Oberlander, H., D. L. Silhacek, E. Shaaya & I. Ishaaya, 1997. Current status and future perspectives of the use of insect growth regulators for the control of stored product pests. Journal of Stored Products Research, 33: 1-6.

Parween, S., S. I. Faruki & M. Begum, 2001. Impairment of reproduction in the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) due to larval feeding on triflumuron-treated diet. Journal of Applied Entomology, 125: 413-416.

Phillips, T. W. & J. E. Throne, 2010. Biorational approaches to managing stored-product insects. Annual Review of Entomology, 55: 375-397.

R Core Team, 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Sagheer, M., M. Yasir, M. Hasan & M. Ashfaq, 2012. Impact of triflumuron on reproduction and development of red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). Pakistan Journal of Agricultural Sciences, 49 (2): 173-178.

Sagheer, M., M. Yasir, B. S. Khan & M. Hasan, 2011. Ovicidal and reproduction inhibition activity of flufenoxuron, an acylurea insect growth regulator, against *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). Pakistan Entomologist, 33 (2): 131-136.

Scheff, D. S., B. Subramanyam & F. H. Arthur, 2016. Effect of methoprene treated polymer packaging on fecundity, egg hatchability, and egg-to-adult emergence of *Tribolium castaneum* and *Trogoderma variabile*. Journal of Stored Products Research, 69: 227-234.

Scheff, D. S., B. Subramanyam & F. H. Arthur, 2017. Susceptibility of *Tribolium castaneum* and *Trogoderma variabile* larvae and adults exposed to methoprene-treated woven packaging material. Journal of Stored Products Research, 73: 142-150.

Schneiderman, H. A., 1972. "Insect Hormones and Insect Control, 3-29". In: Insect Juvenile Hormones: Chemistry & Action (Eds. J. J. Menn & M. Beroza). Academic Press, London, 358 pp.

Sokal, R. R. & F. J. Rohlf, 1995. Biometry: The Principles and Practice of Statistics in Biological Research. 3rd edition, W.H. Freeman and Co., New York, USA, 880 pp.

Staal, G. B., 1975. Insect growth regulators with juvenile hormone activity. Annual Review of Entomology, 20: 417-460.

Trostanetsky, A., M. Kostyukovsky & E. Quinn, 2015. Transovarial effect of novaluron on *Tribolium castaneum* (Coleoptera: Tenebrionidae) after termination of direct contact. Journal of Insect Science, 15 (1): 125.

Vayias, B. J., C. G. Athanassiou, D. N. Milonas & C. Mavrotas, 2010. Persistence and efficacy of spinosad on wheat, maize and barley grains against four major stored product pests. Crop Protection, 29 (5): 496-505.

Wing, H. D. & H. E. Aller, 1990. "Ecdysteroid Agonists as Novel Insect Regulators, 251-257". In: Pesticides and Alternatives: Innovative Chemical and Biological Approaches to Pest Control (Ed. J. E. Casida). Elsevier, Amsterdam, the Netherlands, 586 pp.

Yasir, M., M. Sagheer, S. K. Abbas, M. Hasan, S. Ahmad & M. Ijaz, 2019. Bioactivity of lufenuron against *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). Sains Malaysiana, 48 (1): 75-80.

Yasir, M., M. Sagheer, M. Hasan, S. K. Abbas & S. Ahmad, 2012. Bioactivity of a chitin synthesis inhibitor, triflumuron, against red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). Sarhad Journal of Agriculture, 28 (4): 603-609.

Zettler, J. L. & G. W. Cuperus, 1990. Pesticide resistance in *Tribolium castaneum* (Coleoptera: Tenebrionidae) and *Rhyzopertha dominica* (Coleoptera: Bostrichidae) in wheat. Journal of Economic Entomology, 83: 1677-1681.

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Original article (Orijinal araştırma)

Optimizing container size and rearing density for rapid and economic mass rearing of *Oenopia conglobata* (Linnaeus, 1758) (Coleoptera: Coccinellidae)[1]

Oenopia conglobata (Linnaeus, 1758) (Coleoptera: Coccinellidae)'nın hızlı ve ekonomik kitle üretiminde optimum kap büyüklüğü ve yetiştirme yoğunluğunun belirlenmesi

Mehmet MAMAY[2*] **Çetin MUTLU[2]**

Abstract

A study was conducted to determine the optimum container size and rearing density to economize and optimize the mass rearing of *Oenopia conglobata* (Linnaeus, 1758) (Coleoptera: Coccinellidae) during 2017 in Şanlıurfa, Turkey. The experiment consisted of three types of containers of varying size (106, 310 and 785 ml, regarded as small, medium and large, respectively) and four different rearing densities of *O. conglobata* (1, 5, 10 and 20 larvae/container, regarded as low, moderate, medium and high rearing density, respectively). *Oenopia conglobata* was fed with the eggs of *Ephestia kuehniella* Zeller, 1879 (Lepidoptera: Pyralidae) throughout the study. The highest (100%) larval survival rate was recorded for all containers with low rearing density, whereas the lowest (31.5-35.0%) larval survival was observed in medium containers with medium and high rearing density. Medium containers at low rearing density had the shortest larval development period (8.6 d), while the shortest pupal development period (4.3-4.4 d) was in medium and large containers at medium and moderate density, respectively. The highest rearing cost was computed for small and medium containers at low rearing density, whereas the lowest rearing cost was incurred with large containers at high rearing density. Considering the survival rates, development periods and economic cost incurred; large containers with high rearing density is recommended for the economic and rapid mass rearing of *O. conglobata*.

Keywords: Container size, *Ephestia kuehniella,* mass rearing, *Oenopia conglobata*, rearing density

Öz

Bu çalışma, 2017 yılında Şanlıurfa ilinde (Türkiye) *Oenopia conglobata* (Linnaeus, 1758) (Coleoptera: Coccinellidae)'nın laboratuvar koşullarında ekonomik ve hızlı kitle üretimi için optimum kap büyüklüğü ve yetiştirme yoğunluğunu belirlemek amacıyla yapılmıştır. Farklı şekil ve büyüklükte üç farklı kap (106, 120 ve 785 ml; sırasıyla küçük, orta ve büyük olarak isimlendirilen) ve dört farklı *O. conglobata* yetiştirme yoğunluğu (1, 5, 10 ve 20 larva/kap; sırasıyla düşük, orta, orta-yüksek ve yüksek yoğunluk olarak isimlendirilen) çalışmaya dahil edilmiştir. *Oenopia conglobata* çalışma boyunca *Ephestia kuehniella* Zeller, 1879 (Lepidoptera: Pyralidae) yumurtaları ile beslenmiştir. En yüksek (%100) larva canlılık oranı düşük yetiştirme yoğunluğunun denenen tüm kapların interaksiyonunda belirlenirken, en düşük (%31,5-35,0) larva canlılık oranı ise, orta ve yüksek yetiştirme yoğunluğunun orta büyüklükteki kap ile interaksiyonunda kaydedilmiştir. Düşük yoğunluk ve orta boy kap en kısa larva gelişim periyoduna (8,6 gün) sahipken, en kısa pupa gelişim periyodu (4,3-4,4 gün) sırasıyla orta ve orta-yüksek yoğunluğun orta ve büyük kap interaksiyonunda kaydedilmiştir. En yüksek yetiştirme maliyeti, düşük yoğunluğun küçük ve orta boy kap interaksiyonu için belirlenirken, en düşük yetiştirme maliyeti, yüksek yoğunluğa sahip büyük kaplarda olmuştur. Hayatta kalma oranları, gelişme dönemleri ve meydana gelen ekonomik maliyet dikkate alındığında; *O. conglobata*'nın ekonomik ve hızlı kitle üretimi için büyük kaplarda yüksek yetiştirme yoğunluklu üretim önerilmektedir.

Anahtar sözcükler: Kap büyüklüğü, *Ephestia kuehniella,* kitle üretimi, *Oenopia conglobata*, yetiştirme yoğunluğu

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Introduction

Predatory insects occupy an important place among the natural enemies that are effective in biological control of agricultural pests. Coccinellids or ladybirds (Coleoptera: Coccinellidae) are among the most familiar beetles. Most of the species belonging to Coccinellidae are predatory; hence, are efficient biological control agents of economically important pests in agricultural habitats (Santos et al., 2009; Kundoo & Khan, 2017). The coccinellids are reported to have been feeding on many important agricultural pests such as whiteflies (Gerling, 1990), aphids (Hodek, 1973; Frazer, 1988), mealybugs (Hodek, 1973; Herren, 1991), Psyllidae (Michaud, 2002), scale insects (Drea & Gordon, 1990) and spider mites (Lucas et al., 1997; Obrycki & Kring, 1998; Villanueva et al., 2004).

Coccinellids can predate both larval and adult stages of the same pest species. Moreover, they are found in diverse habitats, have a wide variety of prey, their adults and larvae are predatory, can move rapidly and are voracious (Kundoo & Khan, 2017). *Oenopia conglobata* (Linnaeus, 1758) (Coleoptera: Coccinellidae) is well known as an efficient predator of many harmful species of sap-sucking insects such as psyllids, diaspids, coccids, and especially aphids (Erol & Yaşar, 1996; Mehrnejad, 2002; Mojib-Haghghadam et al., 2002, 2009; Bolu, 2004; Erler, 2004; Aslan & Uygun, 2005; Bolu & Uygun, 2005; Özgen & Karsavuran, 2005; Almatni & Khalil, 2008; Güncan et al., 2008).

Modern biological control has started since the end of 19th century; however, it is being used for at least 2000 years (DeBach, 1964; Van Lenteren & Godfray, 2005). Biological control can be divided into four main types, i.e., natural, conservation, classical, and augmentative biological control (Eilenberg et al., 2001; Cock et al., 2010). Natural populations of predators and parasitoids in agro-ecosystem may be insufficient for keeping the density of harmful insect pests below economic threshold level. The increased need and awareness about integrated pest management among farmers have emphasized the utilization of biological control agents for the management of agricultural pests. Though their demand is increasing, yet their availability is far from sufficient. Therefore, mass rearing of biological control agents could possibly fulfill the increased demands of different biological control agents. For this reason, the natural enemies are mass-reared in controlled laboratory conditions and released in large numbers for pest control in a specific crop (Cock et al., 2010; Lorito et al., 2010; Van Lenteren, 2012; Parnell et al., 2016). Biological control agents have shorter life spans; therefore, cannot be stored for longer periods (Kumar et al., 2017), which further necessitate the need of mass rearing. Therefore, mass rearing and release of the effective natural enemies is a pre-requisite to suppress the populations of insect pests (Van Lenteren, 2000; Van Lenteren et al., 2018).

The optimum conditions (food, space and environment) for mass rearing of natural enemies under laboratory conditions may vary according to species. The container size, space allocated to each individual and the amount of food provided become more important in species with cannibalistic behavior, such as *O. conglobata* (Rodriguez & Rabinovich, 1980; Abdel-Salam & Abdel-Baky, 2001; Silva et al., 2008; Riddick & Wu, 2015). Moreover, rearing density is another important factor that may affect mass rearing. The higher rearing density of *O. conglobata* decreases the space allocated to each individual thereby leading to increased mortality of the predator during pre-adult stages (unpublished data).

The successful augmentative biological control program must be economic and time saving. It is difficult to estimate the overall cost of a biological control program; however, cost incurred on mass rearing, research and release of biological control agents could be easily computed (van den Bosch et al., 1982). Reducing the rearing cost of biological control agents ultimately results in lower cost of a biological control program. Unfortunately, no study to best of our knowledge has reported the effects of container size and rearing density on mass rearing of *O. conglobata*. The knowledge of optimum population density and container size would help in rapid, economic and productive mass rearing of *O. conglobata*. Moreover, limited studies report the cost incurred on rearing of biological control agents. Nonetheless, no study has reported the economics of mass rearing of *O. conglobata.*

This study was therefore conducted to determine the optimum container size and rearing density for rapid and economic mass rearing of *O. conglobata*. It was hypothesized that varying rearing densities and container sizes will affect the survival rate of *O. conglobata*. It was further hypothesized that time required to complete different life stages (i.e., larvae, pupa and adult) will also be influenced by container

size and rearing density. Further, high rearing density in large container would be more economical than low rearing density in all containers. The results of the study will help to identify the optimal container size and rearing density for rapid and economic mass rearing of *O. conglobata*.

Materials and Methods

Experimental site

The mass rearing studies were conducted in controlled insectarium of Department of Plant Protection, Faculty of Agriculture, Harran University, Sanliurfa, Turkey (37.170951ºN, 39.003401ºE) during 2017. Sanliurfa is located in southeastern Anatolia region, Turkey. Summers are hot and dry with temperatures >30ºC in the region. Spring and autumn seasons are generally mild; however, sudden heat and cold episodes are frequently observed in the region.

Rearing containers

Three types of rearing container were used (Figure 1). The containers were selected based on their easy availability, low price and feasibility for the mass rearing of *O. conglobata*. Moreover, all these containers are being used in mass rearing studies in Turkey; therefore, these containers were used to find the optimum container size for mass rearing of *O. conglobata.* All these containers were made of plastic. The containers were different in size and regarded as small, medium and large. The small container was a Petri dish 1.5 cm high, 9.5 cm in diameter and 106 ml volume (Figure 1a). The medium container was a plastic jar 7 cm high, 7.5 cm diameter and 310 ml volume (Figure 1b). The large container was a plastic jar 10 cm high, 10 cm diameter and 785 ml volume (Figure 1c).

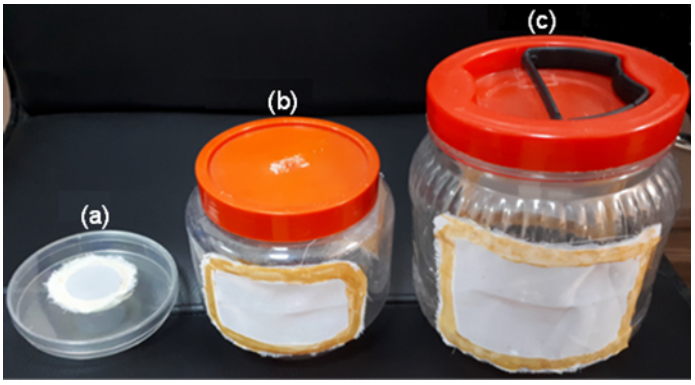

Figure 1. Containers: a) small, b) medium and c) large, used in the mass rearing studies of *Oenopia conglobata*.

Rearing densities

Four rearing densities were tested during the experiment. These rearing densities were regarded as low, medium, moderate and high; 1, 5, 10 and 20 individuals per container, respectively.

Rearing of *Ephestia kuehniella*

Eggs of *E. kuehniella* were used as food (being practical and economical) for rearing *O. conglobata* larvae and adults. Two generations of *O. conglobata* were reared on *E. kuehniella* before this study. *E. kuehniella* was reared under controlled conditions, i.e., 25±1ºC, 65±5% RH and 16:8 h L:D photoperiod. Flour and bran mixture in a proportion of 2:1 was used as a medium and food during rearing procedure (Bulut & Kılınçer, 1987). Flour-bran mixture was sterilized in a drying oven at 60ºC for 3-3.5 h and then kept in a refrigerator. About 2 kg sterilized mixture was placed in plastic containers (27 × 37 × 7 cm). Fifty mg of *E. kuehniella* eggs were sprinkled into each container, and the containers were covered with muslin cloth. The adults of *E. kuehniella* appeared 35-40 d after the initiation of rearing process. The adults were collected with an aspirator and transferred to plastic egg-laying containers with wired edges to deposit eggs. These containers were placed in plastic tubs, which had white paper at the bottom. Eggs were taken from these containers once in 2 d. Some of the fresh eggs collected were reused for the *E. kuehniella* culture, while the remainders were stored in a deep freezer for feeding *O. conglobata*.

Rearing of *Oenopia conglobata*

Oenopia conglobata was reared in transparent plastic jars, with a capacity of 1.5 L, covered with thin muslin cloth in controlled insectarium at 25±1ºC, 65±5% R.H and 16:8 h L:D photoperiod. Eggs of *E. kuehniella* were used as food for rearing *O. conglobata* larvae and adults. The eggs *E. kuehniella* were sprinkled over black cardboard moistened with distilled water. The eggs get attached to the cardboard as it dries. The cardboard strips were prepared and provided to *O. conglobata* for feeding. Tissue papers crumpled by hand were placed in the containers for egg deposition of *O. conglobata*. The containers were observed at 2-d interval, and deposited eggs were collected and transferred to another jar having cardboard strips with *E. kuehniella* eggs. The emerging larvae were raised inside these jars until adult stage. To prevent larvae from starvation, *E. kuehniella* eggs in excess of the daily consumption were provided. Yanık (2011) reported that *O. conglobata* consumed 565 eggs over the develop of one generation, an average of 35 eggs/d. Therefore, 60 eggs/larvae were provided to prevent starvation and cannibalism.

Determination of optimum rearing density and container size for mass rearing of *Oenopia conglobata*

Newly emerged (0-24 h old) *O. conglobata* larvae obtained from stock cultures were used in the experiments. All experiments were performed at 25±1ºC, 65±5% R.H and 16:8 h L:D photoperiod. The larvae were given enough food (*E. kuehniella* eggs) to avoid starvation (see above for details). The food was checked once in 3 d and if necessary new food was provided.

To determine the optimum container size and rearing density, experiments were conducted in a factorial design. The container size was considered as main factor, whereas rearing density was regarded as sub-factor. The experiment had 20 replicates. Tissue paper was fixed at the bottom of all containers to facilitate the movement of the larvae. The containers were monitored daily at 0900 h for recording survival rate, larval and pupal development periods, and maturation rate. Container size and rearing density with high survival rate, shorter development period and low rearing cost were regarded as optimum for mass rearing of *O. conglobata.*

Statistical analysis

The collected data on survival rate and development time were analyzed using Fisher's analysis of variance (ANOVA) technique (Steel et al., 1997). The normality in the dataset was tested by Shapiro-Wilk normality test, which indicated a normal distribution. Therefore, the analyses were performed on original data. The data variance was visually inspected by plotting the residuals to confirm homogeneity of variance before statistical analysis. Two-way ANOVA was used to infer the differences among container size, rearing density and their interaction. Least significant difference test at 99% probability was used as post-hoc test to separate the means where ANOVA indicated significance. All statistical analyses were performed on SPSS statistical software (IBM, 2013).

Economic analysis

An economic analysis was conducted to evaluate the economic feasibility of different container sizes and rearing densities to find the lowest cost incurring combination. The cost of rearing 1000 individuals was computed. The fixed cost included labor cost incurred to monitor the containers during rearing period, whereas variable cost was the price of different containers required to 1000 insect. The food cost was not considered while computing the rearing cost as same amount per individual was provided in all combinations. The number of larvae reaching to adult stage in container size by rearing density interaction was used to compute the number of containers required for rearing 1000 individuals and then other costs were calculated. The labor charges were computed according to existing minimum wages rate (12 USD/8 h) in Turkey. The current container price was taken from different laboratory equipment suppliers and averaged to get the price. Total number of days required to monitor the containers were computed based on total developmental period. The duration required to monitor one container was accepted as one minute. The number of days were computed based on 8 h working day. The fixed and variable costs were added to get the total rearing cost for 1000 individuals. The treatment having the lowest cost of rearing 1000 individuals was accepted as the optimum container size and rearing density for rearing *O. conglobata.*

Results

Determination of optimum rearing density and container size for mass rearing of *Oenopia conglobata*

The results revealed that different container sizes, rearing densities and the effect of their interaction on survival and development period of *O. conglobate* significantly influenced the larval survival rate (Table 1).

Table 1. Analysis of variance of different container sizes, rearing densities and their interaction on survival and developmental period of *Oenopia conglobata*

Source	DF	Sum of squares	Mean squares	F value	P value
Larvae survival rate					
Container size (C)	2	228223	11411	247	0.0001*
Rearing density (D)	3	32627	10875	235	0.0001*
C × D	6	8999	1500	32.4	0.0001*
Pupa survival rate					
Container size (C)	2	109	54	5.50	0.005*
Rearing density (D)	3	302	101	10.20	0.0001*
C × D	6	326	54	5.50	0.0001*
Larvae reaching adult stage					
Container size (C)	2	15121	7561	118	0.0001*
Rearing density (D)	3	40556	13519	211	0.0001*
C × D	6	7524	1254	19.6	0.0001*
Larval development period					
Container size (C)	2	78	39	142	0.0001*
Rearing density (D)	3	88	29	106	0.0001*
C × D	6	42	7	25.4	0.0001*
Pupal development period					
Container size (C)	2	1	0.4	3.43	0.036^{NS}
Rearing density (D)	3	5	1.6	13.30	0.0001*
C × D	6	6	1.0	8.56	0.0001*
Total (larval + pupal) development period					
Container size (C)	2	78	39	106	0.0001*
Rearing density (D)	3	65	22	58.2	0.0001*
C × D	6	61	10	27.2	0.0001*

* Significant ($p \leq 0.01$), NS = non-significant ($p > 0.01$).

The highest larval survival rate was in large sized containers, whereas the lowest was in medium sized containers (Figure 2).

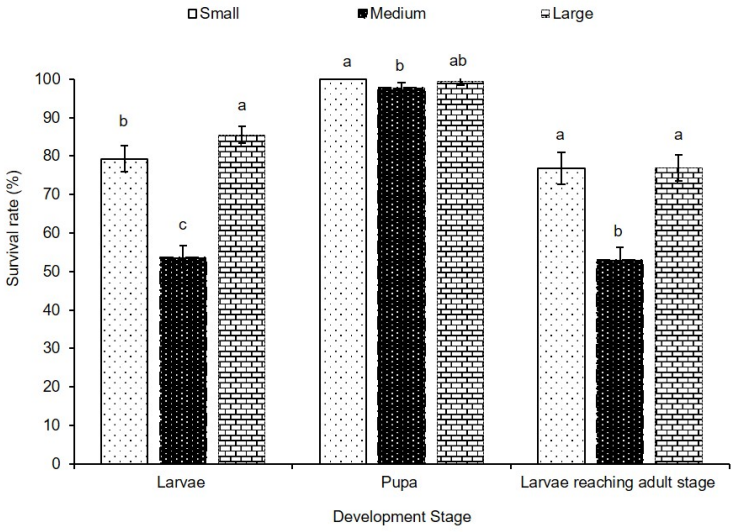

Figure 2. The influence of different container sizes (small, medium and large) on the survival rate of *Oenopia conglobata* at different developmental stages. The vertical bars represent the standard errors of means. Any two bars sharing different letters within a development stage are statistically significant ($p \leq 0.01$).

Similarly, the highest and the lowest larval survival rate was recorded for low and high rearing density, respectively (Figure 3).

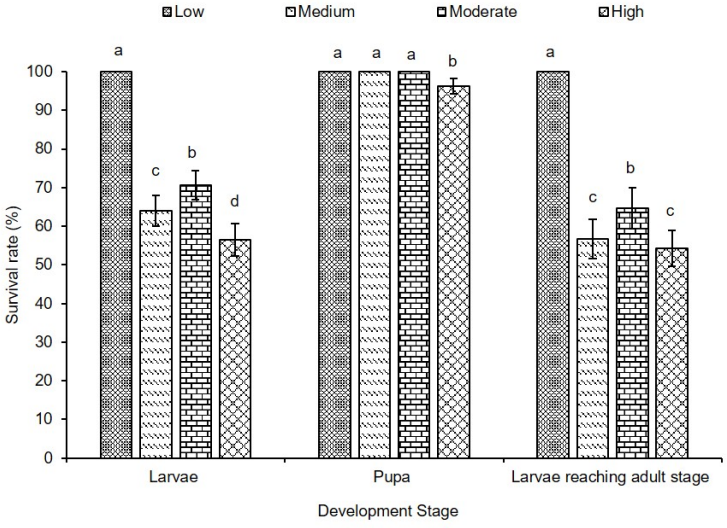

Figure 3. The influence of different rearing densities (low, medium, moderate and high) on the survival rate of *Oenopia conglobata* at different developmental stages. The vertical bars represent the standard errors of means. Any two bars sharing different letters within a development stage are statistically significant ($p \leq 0.01$).

The highest larval survival rate was at low rearing density in all sized containers, whereas the lowest larval survival rate was observed in medium sized containers at moderate and high rearing densities (Table 2).

Table 2. The influence of container sizes by rearing densities interaction on survival rate of different development stages and development period of *Oenopia conglobata*

Treatments	Larvae survival rate (%)	Pupa survival rate (%)	Larvae reaching adult stage (%)	Larval development period (d)	Pupal development period (d)	Total (larval + pupal) development period (d)
S × Lw	100 a*	100 a	100 a	9.6 c	5.3 ab	14.8 d
S × Md	76 cd	100 a	70 b	12.5 a	4.8 def	17.2 ab
S × Mo	72 d	100 a	70 b	9.8 c	4.7 ef	14.5 de
S × H	69 d	100 a	67 b	12.3 a	5.1 bcd	17.4 a
M × Lw	100 a	100 a	100 a	8.6 e	4.8 cdef	13.4 g
M × Md	35 f	100 a	30 d	9.4 c	4.4 g	13.8 fg
M × Mo	48 e	100 a	54 c	9.5 c	5.4 a	14.8 d
M × H	32 f	91 b	28 d	8.9 de	5.1 bc	14.0 ef
L × Lw	100 a	100 a	100 a	9.4 cd	5.1 abc	14.5 de
L × Md	81 c	100 a	70 b	12.3 a	4.5 fg	16.8 b
L × Mo	92 b	100 a	70 b	9.7 c	4.4 g	14.0 ef
L × H	69 d	98 a	68 b	10.5 b	5.0 bcde	15.5 c
LSD 0.01	7.97	3.68	9.38	0.46	0.30	0.53

* Means sharing the same letter within a column are statistically non-significant (p >0.01), NS = non-significant;
S = small container, M = medium container, L = large container;
Lw = low density, Md = medium density, Mo = moderate density, H = high density.

Pupal survival rate was significantly altered by container size, rearing density and their interaction (Table 1). The highest and the lowest pupal survival was in small and medium sized containers, respectively (Figure 2). Likewise, low, medium and moderate rearing density had similar and the highest pupal survival rate, whereas the lowest pupal survival was in high rearing density (Figure 3). In the interactive effect of container size by rearing density, all combinations had similar survival rate except medium container and high-density combination where survival was lower than the other combinations (Table 2).

Different container size, rearing density and their interaction significantly influenced the number of larvae reaching adult stage (Table 1). Small and large sized containers yielded the highest number of larvae reaching adult stage, whereas the lowest number of larvae reaching adult stage was in medium sized containers (Figure 2). Similarly, the highest number of larvae reached adult stage in low rearing density, whereas the lowest number of larvae reached adult stage in medium and high rearing density (Figure 3). Regarding interactions, the combination of all container sizes with low rearing density observed the highest number of larvae reaching adult stage (Table 2). The lowest number of larvae reaching adult stage was recorded in medium sized containers with medium and high rearing density (Table 2).

Larval developmental period was significantly affected by container size, rearing density and their interaction (Table 1). The shortest time to complete larval stage was observed for the individuals reared in medium sized containers, whereas the individuals reared in small sized containers took the longest time to complete the larval stage (Figure 4).

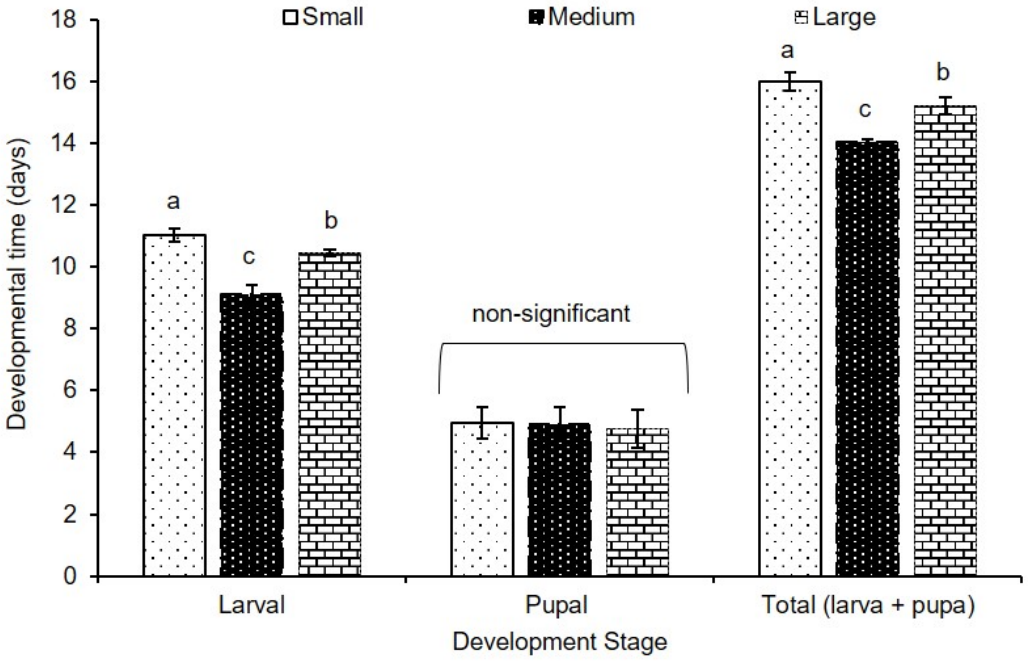

Figure 4. The influence of different container sizes (small, medium and large) on time required to complete different developmental stages of *Oenopia conglobata*. The vertical bars represent the standard errors of means. Any two bars sharing different letters within a development stage are statistically significant ($p \leq 0.01$).

Similarly, the longest and the shortest time to complete the larval stage was at moderate and low rearing density, respectively (Figure 5).

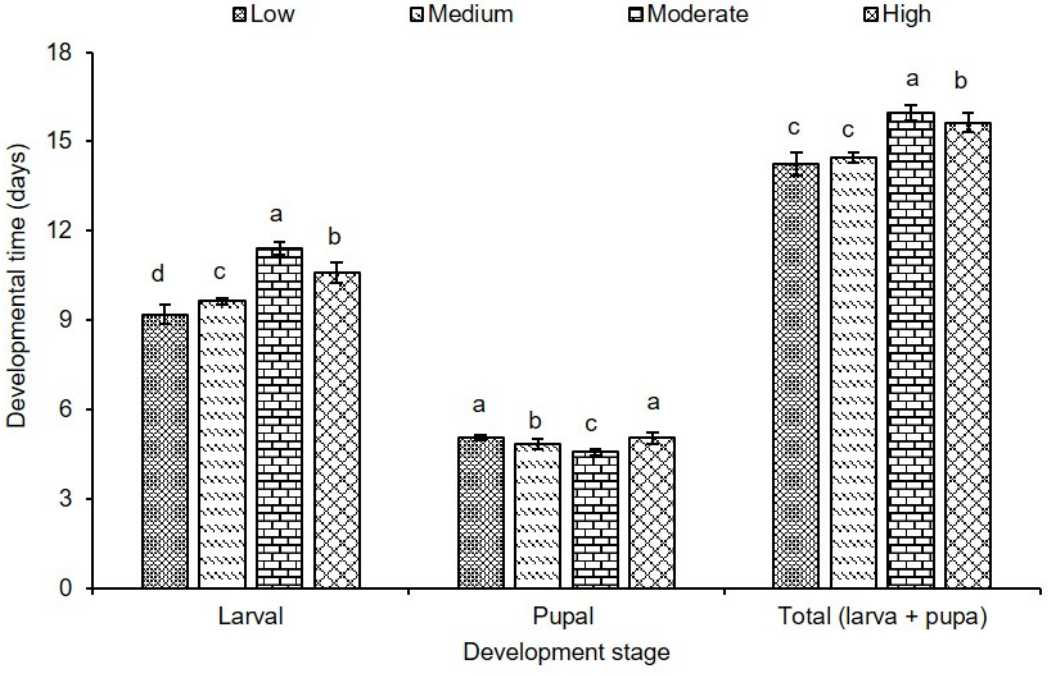

Figure 5. The influence of different rearing densities (low, medium, moderate and high) on time required to complete different developmental stages of *Oenopia conglobata*. The vertical bars represent the standard errors of means. Any two bars sharing different letters within a development stage are statistically significant ($p \leq 0.01$).

Considering the effect interaction of container size and rearing density, the shortest larval developmental period was in medium sized containers with low rearing density. However, the longest larval developmental period was observed in small sized containers at high rearing density, small sized containers at medium rearing density and large sized containers at medium rearing density (Table 2).

Pupal developmental period was not affected by container size; however, rearing density and interaction among container size and rearing density had significant effect on pupal developmental period (Table 1). Slight differences were observed between rearing densities for pupal developmental period. The moderate population density resulted in the shortest pupal developmental period, whereas the longest pupal development period was at low and high rearing density (Figure 5). The longest pupal development period was in medium sized containers at moderate rearing density and small sized containers at low rearing density (Table 2). The shortest pupal development period was in medium sized containers at medium rearing density, and large containers at moderate rearing density (Table 2).

The shortest larval developmental period was in medium sized containers at low rearing density. However, the longest larval developmental period was in small sized containers at high rearing density, small sized containers at medium rearing density and large sized containers at medium rearing density (Table 2).

The total developmental period from larvae to adult stage was significantly altered by container size, rearing density and their interaction (Table 1). The longest and the shortest total developmental period was for the individuals reared in small and medium sized containers, respectively (Figure 4). Similarly, low and medium rearing density had the shortest total developmental period, whereas the longest total developmental period was observed at moderate rearing density (Figure 5). The small sized containers with medium and high rearing density had the longest total developmental period. The shortest total developmental period was in medium sized containers at low population density (Table 2).

Different combinations of container size and rearing density differed for the costs incurred to rear 1000 individuals of *O. conglobata* (Table 3).

Table 3. Economic analysis of rearing 1000 *Oenopia conglobata* individuals with different container sizes and rearing densities

Treatments	Number of containers required	Container price (US$)	Total container cost (US$)	Total number of days required for monitoring	Total labor cost (US$)	Total cost (US$)
S × Lw	1000	0.24	240.00	30.9	371.00	611.00
S × Md	286	0.24	68.57	10.3	123.07	191.64
S × Mo	143	0.24	34.28	4.3	51.85	86.14
S × H	75	0.24	17.91	2.7	32.39	50.29
M × Lw	1000	0.28	280.00	27.9	335.00	615.00
M × Md	667	0.28	186.66	19.2	230.66	417.33
M × Mo	185	0.28	51.85	5.7	68.70	120.55
M × H	179	0.28	50.00	5.2	62.59	112.59
L × Lw	1000	0.22	220.00	30.3	363.00	583.00
L × Md	286	0.22	62.86	10.0	120.07	182.93
L × Mo	143	0.22	31.43	4.2	50.14	81.57
L × H	74	0.22	16.30	2.4	28.76	45.05

S = small container, M = medium container, L = large container;
Lw = low density, Md = medium density, Mo = moderate density, H = high density.

The highest rearing cost for 1000 individuals was for low rearing density in small and medium sized containers. The lowest cost to rear 1000 individuals was for high rearing density in small and large sized containers (Table 3).

Discussion

Different container sizes and rearing densities, as hypothesized, significantly affected the mass rearing of *O. conglobata*. The differences were noted in terms of survival rate and developmental periods of different growth stages. Overall, increasing the container size showed an increase in the survival rate of different developmental stages, whereas increasing the rearing density lowered the survival rate of different developmental stages of *O. conglobata* (Figure 3). The increasing survival rate with increasing container size is thought to be the result of increased space available per individual. Similarly, the decreasing survival rate with increasing rearing density could be linked to the lower space and food available per individual. At higher predator density, nutrition may become scare due high population pressure and hence, to avoid starvation, cannibalism apparently occurs (Michaud, 2003). The higher density could have increased the competition of food and space, which affected the survival rate of different developmental stages in the current study. As there was no food limitation in the current study, the difference in survival rate are thought to be the direct result of competition for space.

Various researchers have reported that rearing density significantly alters population parameters during mass rearing of different biological control agents (Rodriguez & Rabinovich, 1980; Harada & Spence, 2000; Silva et al., 2008). The space limitation in high rearing density treatments led to higher mortality in these studies. Overcrowding during mass rearing could lead to suffocation, competition for diet and cannibalism. Moreover, these studies have reported that rearing density-induced survival rates are species dependent. There was no cannibalism in the current study as more than enough food was given to the larvae; therefore, the differences in population parameters are thought to be the result of space limitation.

Time required to complete different developmental stages was also affected by container size and rearing density (Figures 3 & 4). The time required to complete larval and pupal stages ranged between 8.60-12.28 and 4.37-5.39 d, respectively. Mehrnejad & Jalali (2004) determined that under the same humidity and temperature conditions of the current study, larval and pupal development stages of *O. □onglobate □onglobate□* (Ménétriés, 1849) (Coleoptera: Coccinellidae) fed with *Agonoscena pistaciae* Burckhardt & Lauterer, 1989 (Hemiptera: Psyllidae) were completed in 8.3 and 5.3 d, respectively. Like survival rate of different developmental stages, increasing container size decreased the time required to complete different developmental phases. Whereas, increasing rearing density increased the time required to complete different developmental stages. These results can also be linked with the availability of space and nutrition. Several studies have reported that rearing density alters the population parameters of different species during mass rearing (Hodjat, 1969; Kiritani & Kimura, 1966; Abdel-Salam & Abdel-Baky, 2001; Sahayaraj, 2002). Since increasing container size decreased the time required to complete different developmental phases, increasing rearing density increased it. Therefore, it becomes obvious that space is the main limitation for parameters studied.

The increasing density, in some cases, also had positive impacts on survival rate and developmental period of different insect species. For example, Yanık (2011) reported that larval and pupal development periods of *O. □onglobate* were considerably shortened as larval density increased. Sasaki et al. (2002) also pointed out that insect population density influences rearing environment. Nonetheless, non-significant effects of rearing density on survival and time required to complete different developmental stages have also been reported (Bista et al., 2012). Riddick & Wu (2015) also reported that development period of *Coleomegilla □ongloba* (De Geer, 1775) (Coleoptera: Coccinellidae) was not affected by population density.

Shorter developmental period and high survival rate are considered as optimal for mass rearing of predatory insects. The highest number of larvae reaching adult stage was at low rearing density in all container sizes. Medium sized container had the lowest number of larvae reaching adult stage. These results could again be linked with the availability of space and nutrition during rearing process. Several studies support our results where the highest number of larvae reached adult stage in low rearing density and the survival rate was decreased with increasing density (Ito, 2007; Omkar & Pathak, 2009; Riddick & Wu, 2015).

As hypothesized, container size and rearing density significantly varied for economic costs incurred during rearing process. The economic analysis indicated that large containers with a high population density had the lowest rearing cost. Moreover, the same combination allowed completion of the different developmental stages in relatively less time. The adaptability of any technique is dependent on its economic feasibility (Shah et al., 2013). Thus, the lower cost of large container with better survival rate and shorter developmental time makes it the most economical option for the mass rearing of *O. onglobate.* However, higher densities need to be tested in the same container sizes for their economic feasibility.

Conclusion

It seems that both small and large sized containers with high rearing density could be effectively used for economic and rapid mass production of *O. onglobate.* However, small containers with high rearing density had the longest total development period, which increases the length of time required to rear 1000 insects. Therefore, large container with high rearing density proved the most rapid and economic combination for mass rearing of 1000 *O. onglobate* individuals. Therefore, it is recommended that large container with high rearing density could effectively be used for rapid and economical mass rearing of *O. onglobate*.

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References

Abdel-Salam, A. H. & N. F. Abdel-Baky, 2001. Life table and biological studies of *Harmonia axyridis* Pallas (Col., Coccinellidae) reared on the grain moth eggs of *Sitotroga cerealella* Olivier (Lep., Gelechiidae). Journal of Applied Entomology, 125 (8): 455-462.

Almatni, W. & N. Khalil, 2008. "A primary survey of aphid species on almond and peach, and natural enemies of *Brachycaudus amygdalinus* in As Sweida, southern Syria, 109-115". Proceedings Ecofruit, 13th International Conference on Cultivation Technique and Phytopathological Problems in Organic Fruit- Growing (18-20 February, 2008, Weinsberg, Germany), 347 pp.

Aslan, M. M. & N. Uygun, 2005. The Aphidophagus Coccinellid (Col.: Coccinellidae) Species in Kahramanmaraş, Turkey. Turkish Journal of Zoology, 29: 1-8.

Bista, M., G. Mishra & O. Omkar, 2012. Influence of crowding and diet on the development and survival of the ladybird *Brumoides suturalis* (Coleoptera: Coccinellidae) reared on two aphid species. International Journal of Tropical Insect Science, 32 (1): 64-68.

Bolu, H., 2004. Coccinellidae species, their distribution areas and their impact on population fluctuations of *Agonoscena pistaciae* at Pistachio orchards in Southeastern Anatolia Region in Turkey. Turkish Bulletin of Entomology, 44 (1-4): 69-77.

Bolu, H. & N. Uygun, 2005. Population development of *Suturaspis pistaciae* Lindinger (Hem.: Diaspididae) and its natural enemies. Plant Protection Bulletin, 45 (1-4): 61-78.

Bulut, H. & N. Kılınçer, 1987. "Rearing of egg parasitoid *Trichogramma* spp. (Hymenoptera: Trichogrammatidae) on eggs of *Ephestia kuehniella* Zell. (Lepidoptera: Pyralidae) and interaction of prey-parazitoid, 563-572''. Proceeding of the First Turkish National Congress of Entomology (13-16 October, İzmir, Turkey), 753 pp.

Cock, M. J. W., J. C. Van Lenteren, J. Brodeur, B. I. P. Barratt, F. Bigler, K. Bolckmans, F. L. Cônsoli, F. Haas, P. G. Mason & J. R. P. Parra, 2010. Do new access and benefit sharing procedures under the convention on biological diversity threaten the future of biological control? BioControl, 55: 199-218.

DeBach, P., 1964. Biological Control of Insect Pests and Weeds. Chapman and Hall, London, UK, 844 pp.

Drea, J. J. & R. D. Gordon, 1990. "Coccinellidae in Armored Scale Insects: Their Biology, Natural Enemies, and Control, 19-40''. In: Integrated Control of Citrus Pests in the Mediterranean Region (Ed. D. Rosen), Elsevier, New York, USA, 281 pp.

Eilenberg, J., A. Hajek & C. Lomer, 2001. Suggestions for unifying the terminology in biological control. BioControl, 46: 387-400.

Erler, F., 2004. Natural enemies of the pear psylla *Cacopsylla pyri* in treated *vs.* untreated pear orchards in Antalya Turkey. Phytoparasitica, 32 (3): 295-304.

Erol, T. & B. Yaşar, 1996. Studies on determination of harmful and beneficial fauna in the apple orchards in Van province. Turkish Journal of Entomology, 20 (4): 281-293.

Frazer, B. D., 1988. "Coccinellidae, 231-247". In: Aphids, Their Biology, Natural Enemies and Control (Eds. A. K. Minks & P. Harrewijn), Elsevier, New York, USA, 364 pp.

Gerling, D., 1990. "Natural Enemies of Whiteflies: Predators and Parasitoids, 147-185". In: Whiteflies, Their Bionomics, Pest Status and Management (Ed. D. Gerling), Intercept Ltd., Andover, UK. 348 pp.

Güncan, A., Z. Yoldaş & T. Koçlu, 2008. Studies on pest and beneficial insects of citrus in İzmir province (Turkey). Control in Citrus Fruit Crops. IOBC/wprs Bulletin, 38: 268-274.

Harada, T. & J. R. Spence, 2000. Nymphal density and life histories of two water striders (Hemiptera: Gerridae). The Canadian Entomologist*,* 132 (3): 353-363.

Herren, H. R. & P. Neuenschwander, 1991. Biological control of cassava pests in Africa. Annual Review of Entomology, 36: 257-83.

Hodek, I., 1973. Biology of Coccinellidae. Springer Netherlands, 260 pp.

Hodjat, S. H., 1969. The effects of crowding on the survival, rate of development, size, color and fecundity of *Dysdercus fasciatus* Sign. (Hem., Pyrrhocoridae) in the laboratory. Bulletin of Entomological Research, 58 (3): 487-504.

IBM, C., 2012. SPSS Statistics for Windows. IBM Corp. Released 2012, Version 20, 440 pp.

Ito, K., 2007. A simple mass-rearing method for predaceous *Orius* bugs in the laboratory. Applied Entomology and Zoology*,* 42 (4): 573-577.

Kiritani, K. & K. Kimura, 1966. A study on the nymphal aggregation of the cabbage stink bug, *Eurydema rugosum* Motschulsky (Heteroptera: Pentatomidae). Applied Entomology and Zoology*,* 1 (1): 21-28.

Kumar, P., J. Kaur, J. C. Sekhar, S. P. Lakshmi & S. B. Suby, 2017. "Mass Production of Biocontrol Agents of Insect Pests, 451-465". In: Industrial Entomology (Ed. O, Omkar), Springer, Singapore, 465 pp.

Kundoo, A. A. & A. A. Khan, 2017. Coccinellids as biological control agents of soft bodied insects: a review. Journal of Entomological and Zoological Studies, 5 (5): 1362-1373.

Lorito, M., S. L. Woo, G. E. Harman & E. Monte, 2010. Translational research on Trichoderma: from 'omics to the field. Annual Review of Phytopathology, 48: 395-417.

Lucas, E., D. Coderre & C. Vincent, 1997. Voracity and feeding preferences of two aphidophagous coccinellids on *Aphis citricola* and *Tetranychus urticae*. Entomologia experimentalis et applicata, 85 (2): 151-159.

Mehrnejad, M. R., 2002. "Bionomics of the common pistachio psylla *Agonoscena pistaciae* in Iran, 535-539". Proceedings of the, III. International Symposium on Pistachios and Almonds (30 November 2002, Zaragoza, Spain), 591 pp.

Mehrnejad, M. R. & M. A. Jalali, 2004. Life history parameters of the Coccinellid beetle, *Oenopia conglobata contaminata,* an important predator of the common pistachio psylla, *Agonoscena pistaciae* (Hemiptera: Psylloidea). Biocontrol Science and Technology, 14 (7): 701-711.

Michaud, J. P., 2002. Biological control of Asian citrus psyllid, *Diaphorina citri* (Hemiptera:Psyllidae) in Florida: A preliminary report. Entomological News, 113: 216-222.

Michaud, J. P., 2003. A comparative study of larval cannibalism in three species of ladybird. Ecological Entomology*,* 28 (1): 92-101.

Mojib-Haghghadam, Z., J. Jalali Sendi, S. E. Sadeghi & J. Hajizadeh, 2002. Effects of temperature on developmental time and oviposition rate of *Oenopia conglobata* L. (Col.: Coccinellidae) fed on *Chaitophorus populeti*. Journal of Entomological Society of Iran*,* 22 (1): 1-11.

Mojib-Haghghadam, Z., J. Jalali Sendi, S. E. Sadeghi & M. Uosefpour, 2009. Introduction of lady beetle *Oenopia conglobata* (L.) as predator of ulmus aphid *Tinocallis saltans* Nevsky in Guilan Province and biology of lady beetle in laboratory conditions. Iranian Journal of Biology*,* 22 (2): 363-370.

Obrycki, J. J. & T. J. Kring, 1998. Predaceous Coccinellidae in biological control. Annual Review of Entomology, 43: 295-321.

Omkar, O. & S. Pathak, 2009. Crowding affects the life attributes of an aphidophagous ladybird beetle, *Propylea dissecta.* Bulletin of Insectology, 62 (1): 35-40.

Özgen. İ. & Y. Karsavuran, 2005. "The investigations on the Coccinellidae (Coleoptera) species, their density and hosts in pistachio (*Pistacia vera*) agroecosystem in Siirt province (Turkey), 1393-1396". GAP IV. Agricultural Congress, (21-23 September 2005, Şanlıurfa, Turkey), 1753 pp.

Parnell, J. J., R. Berka, H. A. Young, J. M. Sturino, Y. Kang, D. M. Barnhart & M. V. DiLeo, 2016. From the lab to the farm: an industrial perspective of plant beneficial microorganisms. Frontiers in Plant Science, 7: 1110.

Riddick, E. W. & Z. Wu, 2015. Effects of rearing density on survival, growth, and development of the Ladybird *Coleomegilla maculata* in culture. Insects, 6: 858-868.

Rodriguez, D. & J. Rabinovich, 1980. The effect of density on some population parameters of *Rhodnius prolixus* (Hemiptera: Reduviidae) under laboratory conditions. Journal of Medical Entomology*,* 17 (2): 165-171.

Sahayaraj, K., 2002. Small scale laboratory rearing of a reduviid predator, *Rhynocoris marginatus* FAB. (Hemiptera: Reduviidae) on *Corcyra cephalonica* Stainton larvae by larval card method. Journal of Central European Agriculture, 3 (2): 137-148.

Santos, S. A., J. A. Pereira, L. Torres & A. J. A. Nogueira, 2009. Voracity of coccinellid species on different phenological stages of the olive pest *Saissetia oleae* (Homoptera, Coccidae). Applied Ecology and Environmental Research, 7 (4): 359-365.

Sasaki, R., F. Nakasuji, & K. Fujisaki, 2002. Environmental factors determining wing form in the Lygaeid bug, *Dimorphopterus japonicus* (Heteroptera: Lygaeidae). Applied Entomology and Zoology*,* 37 (2): 329-333.

Shah, M. A., A. Manaf, M. Hussain, S. Farooq & M. Zafar-ul-Hye, 2013. Sulphur fertilization improves the sesame productivity and economic returns under rainfed conditions. International Journal of Agriculture and Biology. 15: 1301-1306.

Silva, C. C., R. A. Laumann, M. C. Blassioli, M. Pareja, & M. Borges, 2008. *Euschistus heros* mass rearing technique for the multiplication of *Telenomus podisi*. Pesquisa Agropecuaria Brasileira*,* 43 (5): 575-580.

Steel, R. G. D., J. H. Torrie & D. A. Dickey, 1997. Principles and Procedures of Statistics: A Biological Approach. McGraw-Hill, 666 pp.

Van den Bosch, R., P. S. Messenger & A. P. Gutierrez, 1982. An Introduction to Biological Control. Springer, Boston, USA, 247 pp.

Van Lenteren, J. C., 2000. A green house without pesticides: Fact of fantasy? Crop Protection, 19: 375-384.

Van Lenteren, J. C., 2012. The state of commercial augmentative biological control: plenty of natural enemies, but a frustrating lack of uptake. BioControl, 57: 1-20.

Van Lenteren, J. C. & H. C. J. Godfray, 2005. European science in the enlightenment and the discovery of the insect parasitoid life cycle in The Netherlands and Great Britain. Biological Control, 32: 12-24.

Van Lenteren, J. C., K. Bolckmans, J. Köhl, W. J. Ravensberg & A. Urbaneja, 2018. Biological control using invertebrates and microorganisms: plenty of new opportunities. BioControl, 63 (1): 39-59.

Villanueva, R. T., J. P. Michaud & C. C. Childers, 2004. Ladybeetles as predators of pest and predacious mites in citrus. Journal of Entomological Science, 39 (1): 23-29.

Yanık, E., 2011. Effect of different temperatures on biological characteristics of *Oenopia conglobata* (L.) (Coleoptera: Coccinellidae) reared on *Ephestia kuehniella* Zell. (Lepidoptera: Pyralidae) eggs. Harran University Journal of the Faculty of Agriculture, 15 (1): 21-28.

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Original article (Orijinal araştırma)

Orientation of some *Heterorhabditis bacteriophora* (Poinar, 1976) (Rhabditida: Heterorhabditidae) strains to *Lolium perenne* L. (Poales: Poaceae) and *Galleria mellonella* (L., 1758) (Lepidoptera: Pyralidae)

Bazı *Heterorhabditis bacteriophora* (Poinar, 1976) (Rhabditida: Heterorhabditidae) ırklarının *Lolium perenne* L. (Poales: Poaceae) ve *Galleria mellonella* (L., 1758) (Lepidoptera: Pyralidae)'ya yönelimi

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Abstract

Entomopathogenic nematodes (EPNs) have different host-finding (foraging) behavior that varies from species to species. Besides their foraging behavior, the orientation of some EPNs can vary depending on plant roots. In the present study, the orientation of some *Heterorhabditis bacteriophora* (Poinar, 1976) (Rhabditida: Heterorhabditidae) strains to perennial ryegrass *Lolium perenne* L. (Poales: Poaceae) roots and greater wax moth *Galleria mellonella* (L., 1758) (Lepidoptera: Pyralidae) larvae were investigated. For this purpose, three different strains of *H. bacteriophora* were used. One of them was a hybrid strain (HBH) and the two were parents (HB1138 and HB4) of the hybrid strain. Three experimental combinations were conducted in the study conducted in 2019 under laboratory conditions in Bursa, Turkey: (A) plant roots, larvae and control; (B) plant roots, control I and control II; and (C) mechanically damaged plant roots, control-I and control-II in Y-tube olfactometers filled with moist sand. The results indicated that for combination A, most orientation to plants and larvae was seen in HB1138; for combination B, most orientation to plant roots was was seen in HBH; and for combination C, strain HB4 showed the most orientation to mechanically damaged plant roots. According to the results, each strain of the same EPN species may have a different response to plant roots and host insects.

Keywords: Entomopathogenic nematodes, perennial ryegrass, orientation

Öz

Entomopatojen nematodlar (EPN'ler) türlere bağlı olarak, birbirinden farklı konukçu bulma davranışlarına sahiptirler. Konukçu arama davranışlarının yanı sıra, bazı EPN'lerin topraktaki yönelimleri bitki köklerine bağlı olarak değişebilir. Bursa'da laboratuvar koşullarında 2019 yılında yapılan bu çalışmada bazı *Heterorhabditis bacteriophora* (Poinar, 1976) (Rhabditida: Heterorhabditidae) ırklarının İngiliz çimi *Lolium perenne* L. (Poales: Poaceae) köklerine ve Petek Güvesi *Galleria mellonella* (L., 1758) (Lepidoptera: Pyralidae) larvalarına yönelimleri araştırılmıştır. Bu amaçla, üç farklı *H. bacteriophora* ırkı kullanılmıştır. Bunlardan birisi hibrit tür (HBH) diğer ikisi ise hibrid türün ebeveynidir (HB1138, HB4). Çalışma, üç farklı deneysel kombinasyon ile laboratuvar koşullarında yapılmıştır. Y-Olfaktometre düzeneğinde A kombinasyonu bitki kökleri, larvalar ve kontrol; B kombinasyonu bitki kökleri, kontrol-I ve kontrol-II; C kombinasyonu ise mekanik olarak yaralanmış bitki kökleri, kontrol-I ve kontrol-II bölümlerini içermektedir. Elde edilen sonuçlar; Kombinasyon A'da bitki köklerine ve böcek larvalarına karşı en yüksek yönelimi HB1138 göstermiştir. Kombinasyon B'de bitki köklerine en yüksek yönelimi HBH ve kombinasyon C'de mekanik olarak yaralı bitki köklerine en yüksek yönelimi HB4 sergilemiştir. Bu çalışmada, aynı türe ait farklı EPN ırklarının bitki köklerine ve konukçu böceklere yönelimi değişebileceği tespit edilmiştir.

Anahtar sözcükler: Entomopatojen nematodlar, İngiliz çimi, yönelim

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Introduction

Entomopathogenic nematodes (EPNs) belonging to the families Heterorhabditidae and Steinernematidae are used commercially in place of chemical pesticides in biological control against insect pests, especially of soil inhabitants (Ehlers, 2001; Susurluk, 2011; Lacey & Georgis, 2012). Heterorhabditidae and Steinernematidae are associated symbiotically with *Photorhabdus* and *Xenorhabdus* bacteria, respectively (Boemare et al., 1993). In the soil, only the third-stage larvae (infective juveniles, IJs) of EPNs actively move to find their hosts. The IJs release the bacteria when they reach to the host insect hemocoel, so the bacteria cause the insect to die from septicemia within 36-48 h (Kaya & Gaugler, 1993). EPNs have different host-finding strategies (foraging behavior), known as ambusher, cruiser and intermediate (Lewis et al., 1993; Grewal et al., 1994; Susurluk et al., 2004). Cruiser IJs move continuously to find their hosts and they are mostly more effective against sedentary insect pests (Lewis et al., 1992). *Steinernema glaseri* (Steiner, 1929) (Rhabditida: Steinernematidae) and *Heterorhabditis bacteriophora* (Poinar, 1976) (Rhabditida: Heterorhabditidae) have cruiser behavior (Susurluk et al., 2004; Bal & Grewal, 2015). However, the IJs that have ambusher behavior are more effective at infecting mobile insect hosts and tend to stay in the same place waiting for their hosts (Campbell & Gaugler, 1993; Kaya & Gaugler, 1993). *Steinernema carpocapsae* (Weiser, 1955) (Rhabditida: Steinernematidae) shows ambusher host finding strategy (Bal & Grewal, 2015). The intermediate IJs have behavior between the two foraging strategies, which are cruiser and ambusher. This foraging behavior is shown by *Steinernema feltiae* (Filipjev, 1934) (Rhabditida: Steinernematidae) (Susurluk, 2009). The difference in host-finding strategies depends on the species of EPNs (Grewal et al., 1994).

EPNs tend to respond to the chemical cues of their hosts, such as carbon dioxide, feces and plant roots (Boff et al., 2002; Webster & Cutler, 2003; Bal & Grewal, 2015). Plant roots have chemical and physical defense mechanisms against harmful insects and can secrete volatile chemicals as a sign to allure natural enemies of herbivores (Turlings & Wäckers, 2004; Rasmann et al., 2005; Tonelli et al., 2016). For example, carbon dioxide is an important signal for EPNs secreted by both plant roots and host insects (Susurluk, 2009; Turlings et al., 2012). However, the plant roots also can produce a repellent effect against EPNs (Boff et al., 2002; Bal & Grewal, 2015).

The aim of the study was to determine the orientation of three *H. bacteriophora* strains (one a hybrid strain *H. bacteriophora* HBH and two parents of the hybrid strain *H. bacteriophora* HB1138 and *H. bacteriophora* HB4) to the perennial ryegrass roots, *Lolium perenne* L. (Poales: Poaceae) and insect host larvae, *Galleria mellonella* (L., 1758) (Lepidoptera: Pyralidae). The experiment was performed in three combinations: A) plant roots, larvae and control; B) plant roots, control and control II; and C) mechanically damaged plant roots, control I and control II in moist sand filled Y-tube olfactometers.

Materials and Methods

Culture of the EPN strains

In this study, the hybrid strain *H. bacteriophora* HBH and their parents that isolated from Antalya (*H. bacteriophora* HB1138) and Şanlıurfa (*H. bacteriophora* HB4) in Turkey were used. The last-instar larvae of the *G. mellonella* were selected as host for the production of infective juveniles (IJs). IJs were extracted using the White trap method (White, 1927) and stored in standard Ringer's solution (distilled water + KCl + $CaCl_2 \cdot 2H_2O$ + NaCl + $NaHCO_3$) at 4°C. The experiments were conducted with 1-week-old IJs.

Culture of *Galleria mellonella*

The greater wax moth, *G. mellonella,* larvae have been used as a host since it can be grown easily and have a suitable size for the production of EPNs. Sufficient number of EPNs can be obtained by in vivo production using the last-instar larvae of the *G. mellonella* (Kaya & Stock, 1997). Honey (200 g), glycerin

(200 g), yeast (50 g), milk powder (100 g), soybean flour (100 g), cornstarch (150 g) and bran (200 g) were used in the mass production of the larvae (Wiesner, 1993). The insect larvae with the food were kept in glass jars at 30ºC.

Culture of *Lolium perenne*

Lolium perenne plants with about 10 cm long roots were taken from the Department of Field Crops (Bursa Uludağ University, Faculty of Agriculture). Two kinds of roots were used as healthy roots and damaged roots of the grass, which were cut 1 cm from end by scissors.

Experimental design and methodology

The Y-tube olfactometer (Boff et al., 2001; Susurluk, 2011) was used to test the orientation of the strains to perennial ryegrass root, insect hosts *G. mellonella* and control arms. Olfactometer has three separate arms (diameter 2.5 cm by 12.5 cm long), which were filled with sterilized quartz sand (particle size: 300-400 µm) 10% moistened by sterilized tap water. Three separate experiments were established simultaneously with the different combinations (Figure 1). This study was performed in nematology laboratory in Department of Plant Protection in Faculty of Agriculture in University of Bursa Uludağ in 2019.

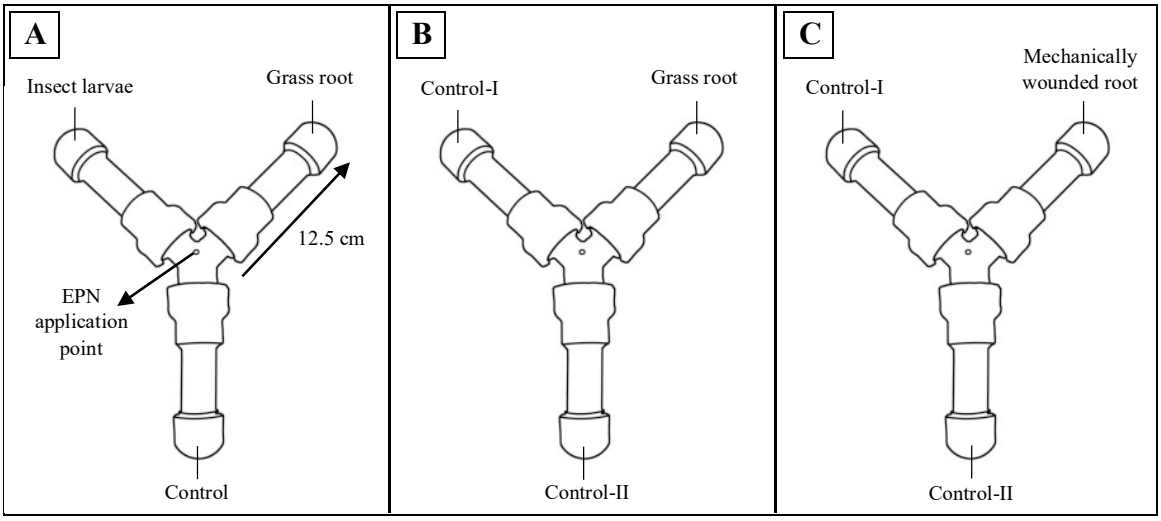

Figure 1. Schematic drawing of used Y-tube olfactometer in the study. A, B and C show the experimental combinations.

In the experimental combination A, the insect host larvae and the plant roots were placed at the end of the first two olfactometer arms. The other arm was kept as a control without any treatment. In the experimental combination B, only the plant roots were transferred at the end of one arm. The other arms, control-I and control-II were without any treatment. The experimental combination C was constructed in a similar manner to the B. However, the plant roots used in this experiment were mechanically damaged (Figure 1).

Before adding the strains in the center of the olfactometers, the different combinations were incubated horizontally at 25ºC for 24 h to for chemical gradient into the olfactometer. Then, about 1000 IJs were applied to the center of each olfactometer. After inoculation of the IJs, incubation was continued for a further 96 h (4 d). Four d after the inoculation, the IJs were recovered from the quartz sand using the Cobb's (1918) decanting and sieving method (Klein et al., 1994). Afterwards, the number of the IJs showing orientation towards different arms was determined and each combination was repeated three times.

Statistical analysis

Statistical differences were detected in the orientations of the strains by using one-way ANOVA in JMP®7.0 Software. LSD test ($P < 0.05$) was used to settle the difference between means.

Results and Discussion

The orientations of the strains towards *Lolium perenne* roots and *Galleria mellonella* larvae (combination A)

Among the strains, HB1138 showed statistically higher orientation to *L. perenne* roots and *G. mellonella* larvae than HB4 and HBH. The statistically highest orientation towards control was shown by HB4, while the lowest by HB1138, HBH and HB4 strains showed more tendency towards the control arm. Also, the orientations of the HBH, HB4 and HB1138, to *G. mellonella* larvae and *L. perenne* roots were not statistically different ($F = 32.6$, $df = 8,18$, $P < 0.0001$) (Figure 2).

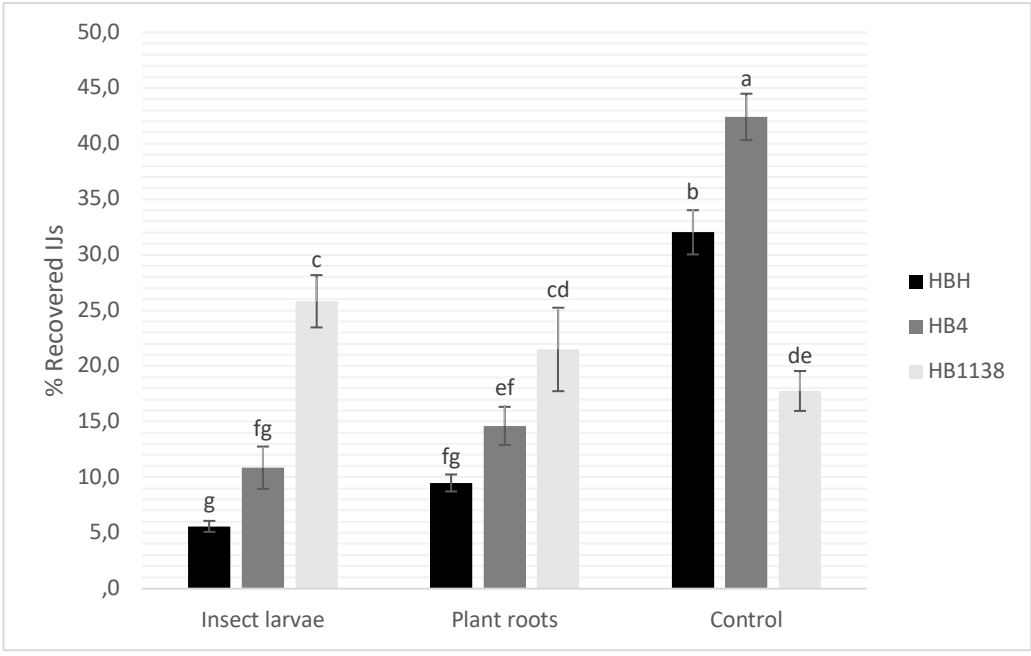

Figure 2. *Heterorhabditis bacteriophora* strain (HBH, HB4 and HB1138) orientations towards perennial ryegrass roots and wax moth.

The orientations of the strains towards the healthy roots of *Lolium perenne* (combination B)

Among the strains, HBH showed significantly highest orientation to the grass roots. The orientations of HB1138 and HB4 to plant roots were not statistically different. HBH and HB1138 strains showed more tendency towards the control-II, but the orientations of the three strains were not statistically different towards control-I. ($F = 22.0$, $df = 8,18$, $P < 0.0001$) (Figure 3).

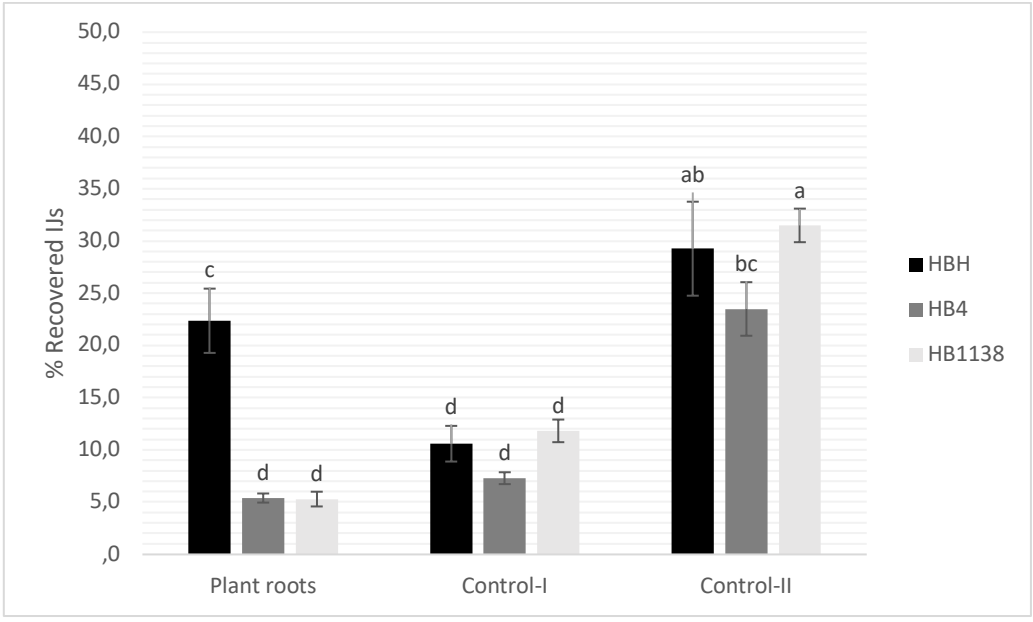

Figure 3. *Heterorhabditis bacteriophora* strains (HBH, HB4, HB1138) orientations towards perennial ryegrass roots.

The orientations of the strains towards the damaged roots of *Lolium perenne* (combination C)

Among the strains, HB4 had the significantly highest orientation to the damaged plant roots. The orientations of HB1138 and HBH to the damaged plant roots were not statistically different. Also, HB4 showed the statistically highest orientation towards control-II ($F = 91.8$, $df = 8,18$, $P < 0.0001$) (Figure 4).

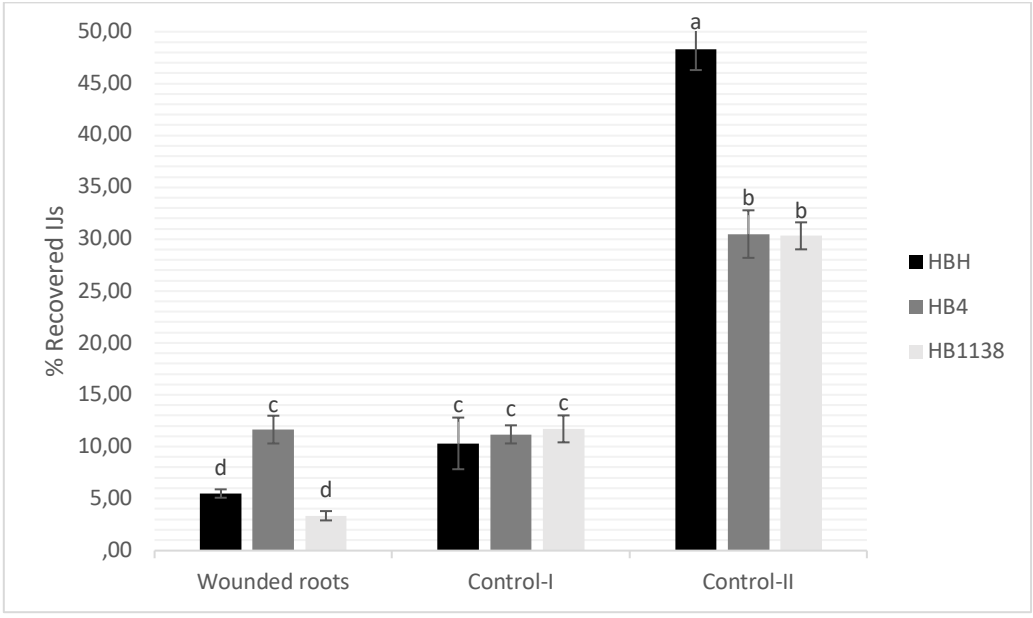

Figure 4. *Heterorhabditis bacteriophora* strains (HBH, HB4, HB1138) orientations towards perennial ryegrass roots that were damaged.

In biological control, the effectiveness of entomopathogenic nematodes (EPNs) is mainly determined by host-finding (foraging) strategy, insect species and soil conditions (Shapiro-Ilan et al., 2006). It is known that the foraging strategies (ambusher, cruiser and intermediate) depend on the EPN species (Grewal et al., 1994). Campbell & Gaugler (1993) described *H. bacteriophora* as a cruiser. All the strains (HBH, HB4

and HB1138) used in this trial belong to *H. bacteriophora* species. The plant roots of *L. perenne* and *G. mellonella* larvae were selected to determine the orientations between the *H. bacteriophora* strains. The effectiveness and dispersal of *H. bacteriophora* are better for sedentary insect hosts as evidenced in the research of Bal & Grewal (2015). Investigations have proved that EPNs use chemical signals secreted by plants or insects to find their hosts (Boff et al., 2001; Rasmann et al., 2005; Rasmann & Turlings, 2008; Turlings et al., 2012; Lortkipanidze et al., 2016; Tonelli et al., 2016). EPNs use volatile chemicals released from the plant roots and insect, such as carbon dioxide, to find their hosts (Susurluk, 2009; Turlings et al., 2012; Bal & Grewal, 2015; Tonelli et al., 2016). However, there should be some EPN strains that do not show any inclination to elevated amounts of the plant volatiles (Anbesse & Ehlers, 2013; Laznik & Trdan, 2013).

In the present study, both mechanically damaged and healthy plant roots of *L. perenne* were tested separately. In the experimental combination A, which contained both the insect larvae and plant roots in their separate arms, had the statically highest orientation to *G. mellonella* and *L. perenne* roots for HB1138. The orientation of HBH, HB4 and HB1138 individually to the insect larvae and the plant roots was not statistically different. Similarly, Rasmann & Turlings (2008) investigated the tritrophic interactions containing maize plant roots, herbivore insects and EPNs. They found that the orientation of *H. bacteriophora* towards healthy plant roots and insect larvae was not statically different, consistent with the results of the present study. However, the nematode *Heterorhabditis megidis* Poinar, Jackson & Klein, 1987 (Rhabditida: Heterorhabditidae) showed more orientation to plant roots than to insect larvae (Rasmann & Turlings, 1987). Susurluk (2011) studied the larvae of *Delia radicum* L. (1758) (Diptera: Anthomyiidae) (Diptera: Anthomyiidae), oilseed rape roots and in a combination of both using Y-tube olfactometer at 8 and 15ºC. In contrast, in that study the maximum orientation of the *S. feltiae* was to the insect larvae of *D. radicum* at both temperature values. Different result might be obtained when using other *S. feltiae* strains. Although the response of HB1138 to the insect was statically more significant than other strains (HBH and HB4) and the control. The orientation of HBH and HB4 towards control was higher compared to the plant roots and the insect larvae. However, Rasmann & Turlings (2008) indicated that using maize plant roots, *H. bacteriophora* did not have a statistically significant response towards healthy plant roots and insect larvae only *Diabrotica virgifera virgifera* LeConte, 1868 (Coleoptera: Chrysomelidae) or the control. However, *H. megidis* migrated significantly towards undamaged plant roots than control (Rasmann et al., 2005). The nematode, *H. megidis*, had different behavior from strains of *H. bacteriophora* in the present study. Tonelli et al. (2016) found, when the roots of sugarcane were used, that bothof *Heterorhabditis indica* (Poinar, 1992) (Rhabditida: Heterorhabditidae) and *S. carpocapsae* responded equally to the plant roots and the control of moistened sand only.

In combination B; among the strains, HBH had the highest orientation to the undamaged *L. perenne* roots. Rasmann & Turlings (2008) found that the orientation of *H. megidis* to maize and cotton plant roots was significantly higher than to damaged cowpea roots, which is consistent with the findings of the present study. In combination C; in all the strains, HB4 had the highest orientation to the mechanically damaged *L. perenne* roots. However, Rasmann et al. (2005), using a six-arm olfactometer, found the orientation of *H. megidis* towards mechanically damaged and undamaged maize plants roots were not statically different. Boff et al. (2001) found that *H. megidis* had more orientation towards mechanically damaged strawberry roots than to roots damaged by *Otiorhynchus sulcatus* (Fabricius, 1775) (Coleoptera: Curculionidae) larvae, which is again consistent with the results of the current study. Whereas, *Thuja occidentalis* L. (Pinales: Cupressaceae) roots damaged by *O. sulcatus* larvae were more attractive to *H. megidis* than to mechanically damaged roots (Boff et al., 2002), which differs from the findings of the present study, because there might be a difference between roots damaged with scissors and those damaged by insect. Additionally, Ali et al. (2010) reported that when citrus roots were damaged by insect larvae, *Diaprepes abbreviates* (L., 1758) (Coleoptera: Curculionidae), more EPNs were attracted than to mechanically damaged roots. According to those results, roots with an insect bite can be more attractive to EPNs. To

date, all studies on the orientation of EPNs have been conducted with different EPN species. However, in the present study different strains of the same *H. bacteriophora* were used.

The results of the present study indicate that the orientations between the strains of *H. bacteriophora* towards *G. mellonella* larvae and undamaged or mechanically damaged *L. perenne* roots may be significantly different. The response of the EPN species and strains on plant roots or insect hosts can significantly affect the biological control potential of the EPNs. However, further studies should be conducted in the field to confirm these findings. This would allow the proper application of EPNs and enhance biological control.

References

Ali, J. G., H. T. Alborn & L. L. Stelinski, 2010. Subterranean herbivore-induced volatiles released by citrus roots upon feeding by *Diaprepes abbreviatus* recruit entomopathogenic nematodes. Journal of Chemical Ecology, 36 (4): 361-368.

Anbesse, S. & R. U. Ehlers, 2013. *Heterorhabditis* sp. not attracted to synthetic (E)-β-caryophyllene, a volatile emitted by roots upon feeding by corn rootworm. Journal of Applied Entomology, 137 (1-2): 88-96.

Bal, H. K. & P. S. Grewal, 2015. Lateral dispersal and foraging behavior of entomopathogenic nematodes in the absence and presence of mobile and non-mobile hosts. PloS ONE, 10 (6): e0129887.

Boemare, N. E., R. J. Akhurst & R. G. Mourant, 1993. DNA relatedness between *Xenorhabdus* spp. (Enterobacteriaceae), symbiotic bacteria of entomopathogenic nematodes, and a proposal to transfer *Xenorhabdus luminescens* to a new genus, *Photorhabdus* gen. nov. International Journal of Systematic and Evolutionary Microbiology, 43 (2): 249-255.

Boff, M. I. C., R. H. W. M. Van Tol & P. H. Smits, 2002. Behavioural response of *Heterorhabditis megidis* towards plant roots and insect larvae. Biocontrol, 47 (1): 67-83.

Boff, M. I., F. C. Zoon & P. H. Smits, 2001. Orientation of *Heterorhabditis megidis* to insect hosts and plant roots in a Y-tube sand olfactometer. Entomologia Experimentalis et Applicata, 98 (3): 329-337.

Campbell, J. F. & R. Gaugler, 1993. Nictation behaviour and its ecological implications in the host search strategies of entomopathogenic nematodes (Heterorhabditidae and Steinernematidae). Behaviour, 155-169.

Cobb, N. A., 1918. Estimating the Nematode Population of the Soil. Agricultural Technology Circular 1, Bureau of Plant Industry, United States Department of Agriculture, 48 pp.

Ehlers, R. U., 2001. Mass production of entomopathogenic nematodes for plant protection. Applied Microbiology and Biotechnology, 56 (5-6): 623-633.

Grewal, P. S., E. E. Lewis, R. Gaugler & J. F. Campbell, 1994. Host finding behaviour as a predictor of foraging strategy in entomopathogenic nematodes. Parasitology, 108 (2): 207-215.

Kaya, H. K. & R. Gaugler, 1993. Entomopathogenic nematodes. Annual Review of Entomology, 38 (1): 181-206.

Kaya, H. K. & S. P. Stock, 1997. "Techniques in Insect Nematology, 281-324". In: Manual of Techniques in Insect Pathology (Ed. L. A. Lacey). Academic Press, San Diego, CA, USA, 409 pp.

Klein, B. M. Z., G. L. Wiegers & P. H. Smits, 1994. Biological control of cockchafer larvae (*Melolontha melolontha*) with the entomopathogenic nematode *Steinernema glaseri*. Mededelingen van de Faculteit der Landbouwwetenschappen, Rijksuniversiteit Gent, 59: 411-419.

Lacey, L. A. & R. Georgis, 2012. Entomopathogenic nematodes for control of insect pests above and below ground with comments on commercial production. Journal of Nematology, 44 (2): 218-225.

Laznik, Ž. & S. Trdan, 2013. An investigation on the chemotactic responses of different entomopathogenic nematode strains to mechanically damaged maize root volatile compounds. Experimental Parasitology, 134 (3): 349-355.

Lewis, E. E., R. Gaugler & R. Harrison, 1992. Entomopathogenic nematode host finding: response to host contact cues by cruise and ambush foragers. Parasitology, 105 (2): 309-315.

Lewis, E. E., R. Gaugler & R. Harrison, 1993. Response of cruiser and ambusher entomopathogenic nematodes (Steinernematidae) to host volatile cues. Canadian Journal of Zoology, 71 (4): 765-769.

Lortkipanidze, M. A., O. A. Gorgadze, G. S. Kajaia, N. G. Gratiashvili & M. A. Kuchava, 2016. Foraging behavior and virulence of some entomopathogenic nematodes. Annals of Agrarian Science, 14 (2): 99-103.

Rasmann, S., T. G. Köllner, J. Degenhardt, I. Hiltpold, S. Toepfer, U. Kuhlmann & T. C. Turlings, 2005. Recruitment of entomopathogenic nematodes by insect-damaged maize roots. Nature, 434 (7034): 732-737.

Rasmann, S. & T. C. Turlings, 2008. First insights into specificity of belowground tritrophic interactions. Oikos, 117 (3): 362-369.

Shapiro-Ilan, D. I., D. H. Gouge, S. J. Piggott & J. P. Fife, 2006. Application technology and environmental considerations for use of entomopathogenic nematodes in biological control. Biological Control, 38 (1): 124-133.

Susurluk, A., 2009. Seasonal and vertical distribution of the entomopathogenic nematodes *Heterorhabditis bacteriophora* (TUR-H2) and *Steinernema feltiae* (TUR-S3) in turf and fallow areas. Nematology, 11 (3): 321-327.

Susurluk, I. A., 2011. Potential of *Steinernema feltiae* (Rhabditida: Steinernematidae) as a biological control agent against the cabbage maggot *Delia radicum* (Diptera: Anthomyiidae) in oilseed rape. Turkish Journal of Agriculture and Forestry, 35 (4): 413-419.

Susurluk, I. A., I. O. Ünlü & I. Kepenekci, 2004. Host finding behavior of two different Turkish isolates of entomopathogenic nematode species, *Heterorhabditis bacteriophora*, Poinar 1976 (Rhabditida: Heterorhabditidae). Turkish Journal of Biology, 27 (4): 203-207.

Tonelli, M., M. F. G. V. Peñaflor, L. G. Leite, W. D. Silva, F. Martins & J. M. S. Bento, 2016. Attraction of entomopathogenic nematodes to sugarcane root volatiles under herbivory by a sap-sucking insect. Chemoecology, 26 (2): 59-66.

Turlings, T. C., I. Hiltpold & S. Rasmann, 2012. The importance of root-produced volatiles as foraging cues for entomopathogenic nematodes. Plant and Soil, 358 (1-2): 51-60.

Turlings, T. C. & F. Wäckers, 2004. Recruitment of predators and parasitoids by herbivore-injured plants. Advances in Insect Chemical Ecology, 2: 21-75.

Webster, J. & G. C. Cutler, 2003. Host-finding ability of three entomopathogenic nematode isolates in the presence of plant roots. Nematology, 5 (4): 601-608.

White, G. F., 1927. A method for obtaining infective nematode larvae from cultures. Science, 66: 302- 303.

Wiesner, A., 1993. Die Induktion Der Immunabwehr Eines Insekts (*Galleria mellonella*, Lepidoptera) Durch Synthetische Materialien Und Arteigene Haemolymphfaktoren. Verlag nicht ermittelbar, (Unpublished) Doctoral dissertation, Berlin,107 pp.

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Original article (Orijinal araştırma)

Reproduction of root-knot nematode isolates from the middle Black Sea Region of Turkey on tomato with *Mi-1.2* resistance gene[1]

Türkiye'nin Orta Karadeniz Bölgesi'nden elde edilen kök-ur nematodu izolatlarının *Mi-1.2* dayanıklılık geni taşıyan domateste üremesi

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

Abstract

Research was conducted to evaluate the reproduction of 90 *Meloidogyne* isolates including *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949 (38 isolates), *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 (4 isolates), *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949 (11 isolates) and *Meloidogyne luci* Carneiro et al., 2014 (37 isolates) (Tylenchida: Meloidogynidae) from the middle Black Sea Region of Turkey on susceptible and resistant tomato cultivars in a greenhouse with temperature controlled between 2013 and 2014. Galling index and reproduction factor of nematode isolates were assessed in a pot assay and resistance-breaking isolates were determined according to their reproduction index. Among the isolates, only two isolates of *M. luci* (Or-2 and Pr-1) had a similar galling index on susceptible and resistant tomato ($P \leq 0.05$). Four isolates of *M. luci* (Çr-19, Or-2, Pr-1 and Pr-2), two isolates of *M. arenaria* (A-7 and Sn-11) and an isolate of *M. incognita* (A-11) produced more eggs than the initial inoculum on resistant tomato. These root-knot nematode isolates, except two isolates of *M. arenaria*, were classified as resistance-breaking isolates having a reproduction index higher than 10%. These findings showed that the *Mi-1.2* gene confers resistance to *M. luci* but four isolates of *M. luci* could overcome this resistance. This is the first report for resistance-breaking isolates of *M. luci* in tomato.

Keywords: *Meloidogyne, Mi-1.2* gene, resistance, tomato, virulent

Öz

Araştırma, Türkiye'nin Orta Karadeniz Bölgesi'nden elde edilen *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949 (38 izolat), *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 (4 izolat), *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949 (11 izolat) ve *Meloidogyne luci* Carneiro et al., 2014 (37 izolat) (Tylenchida: Meloidogynidae)'den oluşan 90 *Meloidogyne* izolatının hassas ve dayanıklı domates çeşitlerindeki üremesini değerlendirmek için 2013-2014 yılları arasında sıcaklık kontrollü bir serada yürütülmüştür. Nematod izolatlarının üreme faktörü ve urlanma indeksi saksı denemesi ile değerlendirilmiş ve üreme indeksi değerlerine göre dayanıklılığı kıran izolatlar belirlenmiştir. İzolatlar arasından sadece iki *M. luci* izolatı (Or-2 ve Pr-1) hassas ve dayanıklı domateste benzer bir urlanma indeksine sahiptir ($P \leq 0.05$). *M. luci*'nin dört izolatı (Çr-19, Or-2, Pr-1 ve Pr-2), *M. arenaria*'nın iki izolatı (A-7 ve Sn-11) ve *M. incognita*'nın bir izolatı (A-11), dayanıklı domateste başlangıç inokulumundan daha fazla yumurta meydana getirmiştir. Bu kök-ur nematodu izolatları, *M. arenaria*'nın iki izolatı hariç, %10'dan fazla üreme indeksine sahip olarak dayanıklılığı kıran izolatlar olarak sınıflandırıldı. Bu bulgular, *Mi-1.2* geninin *M. luci*'ye dayanıklılık sağladığını ancak dört izolatın bu dayanıklılığı kırabileceğini göstermiştir. İlk defa, domateste *M. luci*'nin dayanıklılığı kıran izolatları kayıt edilmiştir.

Anahtar sözcükler: *Meloidogyne*, *Mi-1.2* geni, dayanıklılık, domates, virülent

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Introduction

Root-knot nematodes (RKN), *Meloidogyne* spp., are polyphagous plant parasites and they are among the most destructive pests in protected vegetable cropping areas in different geographic regions of Turkey (Söğüt & Elekçioğlu, 2000; Devran & Söğüt, 2009; Akyazı & Ecevit, 2011; Aydınlı & Mennan, 2016; Uysal et al., 2017). While *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949, *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949 and *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949 (Tylenchida: Meloidogynidae) are widely distributed throughout the country, *Meloidogyne ethiopica* Whitehead, 1968 was encountered only in the middle Black Sea coastal areas of Turkey (Aydınlı & Mennan, 2016). *Meloidogyne ethiopica* was detected in Turkey for the first time in tomato (*Solanum lycopersicum* L.) and cucumber (*Cucumis sativus* L.) greenhouses located in Samsun Province in 2009 (Aydınlı et al., 2013). In 2014, *Meloidogyne luci* Carneiro et al., 2014 was described as a new RKN species detected in vegetables, flowers and fruits in Brazil, Iran and Chile (Carneiro et al., 2014). This new species, very close to *M. ethiopica*, raised doubts about the accuracy of the earlier reported *M. ethiopica* isolates from Europe (Carneiro et al., 2014; Gerič Stare et al., 2017). Soon after, the Slovenian isolate known as the first record of *M. ethiopica* in Europe was identified as *M. luci* (Janssen et al., 2016). Recently, Gerič Stare et al. (2017) re-examined several isolates previously identified as *M. ethiopica* from different countries in Europe, including Turkey, and re-identified them as *M. luci*. Therefore, all the RKN isolates previously recorded as *M. ethiopica* from Turkey are now considered to be *M. luci*. To date, this nematode has been identified from Europe (Slovenia, Italy, Greece, Turkey and Portugal), America (Brazil, Chile and Guatemala) and Asia (Iran) (Conceição et al., 2012; Carneiro et al., 2014; Janssen et al., 2016; Gerič Stare et al., 2017; Maleita et al., 2018).

The management of RKN is generally practiced through four main methods, chemical treatment, cultural practices, biological control and host plant resistance (Nyczepir & Thomas, 2009). Among these methods, the use of resistance is the most convenient since it does not require special equipment and does not have any adverse effects on human health and the environment (Boerma & Hussey, 1992; Jacquet et al., 2005; Sorribas et al., 2005; Cortada et al., 2008; Verdejo-Lucas et al., 2009). Additionally, resistant plants can also be easily integrated with other control methods (Roberts, 1982). Resistant cultivars can efficiently suppress nematode reproduction; thus, they should be included in crop rotations to reduce nematode density in soils and to protect subsequent susceptible cultivars without extra cost (Mukhtar et al., 2013).

The *Mi-1.2* gene in tomato is one of the well-known resistance genes effective against three major RKN species (*M. arenaria, M. incognita* and *M. javanica*) (Verdejo-Lucas et al., 2009). Therefore, commercial tomato cultivars and rootstock cultivars with the *Mi-1.2* gene have been widely grown in protected vegetable production areas (Cortada et al., 2009). However, resistance-breaking or virulent populations can reproduce on resistant *Mi-1.2* plants and compromise the durability of the resistance against these nematodes (Verdejo-Lucas et al., 2009; Devran & Söğüt, 2010). Virulent nematodes can exist naturally in the field without previous exposure to resistant cultivars or through repeated culture of resistant tomatoes with the *Mi-1.2* gene (Roberts, 1995; Tzortzakakis & Gowen, 1996; Eddaoudi et al., 1997; Castagnone-Sereno, 2002). In many countries, wide geographical distribution of *Mi-1.2* virulent populations of *M. arenaria*, *M. incognita* and *M. javanica* have been reported (Riggs & Winstead, 1959; Prot, 1984; Kaloshian et al., 1996; Eddaoudi et al., 1997; Ornat et al., 2001; Tzortzakakis et al., 2005; Devran & Söğüt, 2010).

In a recent extensive survey, Aydınlı & Mennan (2016) observed RKN on tomato rootstocks in protected vegetable areas in the middle Black Sea Region of Turkey, where tomato is grown as a monoculture. Furthermore, *M. luci* (formerly known as *M. ethiopica*) was detected only on the weeds in greenhouses where resistant rootstocks or resistant tomato cultivars were grown. Based on these findings, researchers hypothesized that (i) nematode populations detected on rootstocks in tomato monocultures are *Mi-1.2* virulent and (ii) the *Mi-1.2* gene confers resistance to *M. luci*. Therefore, this study was conducted to assess the reproduction of 90 RKN isolates, obtained from the same region in Turkey, on resistant and susceptible tomato cultivars.

Materials and Methods

Nematode isolates

Ninety RKN isolates including *M. arenaria* (38), *M. incognita* (4), *M. javanica* (11) and *M. luci* (formerly reported as *M. ethiopica*, 37) from greenhouses located in the middle Black Sea Region of Turkey were collected and identified in a previous study (Aydınlı & Mennan, 2016) (Table 1). After reclassification of all *M. ethiopica* isolates reported from Europe (Gerič Stare et al., 2017), the identification of *M. ethiopica* isolates, reported in a previous study (Aydınlı & Mennan, 2016), was re-examined for esterase isozyme marker, the most distinguishing character between *M. ethiopica* and *M. luci*, and all the isolates were identified as *M. luci*. Additionally, the identification of several *M. luci* isolates, included in this study, was also confirmed by molecular analysis (Gerič Stare et al., 2017; Maleita et al., 2018).

Pure RKN isolates were obtained from single egg masses and maintained on susceptible tomato cv. Falcon plants (May Seed, Turkey) in pots in greenhouses with controlled temperature (24±2°C). These pot cultures were used to obtain nematode inoculum.

Pot assays

The study was conducted between 2013 and 2014 at University of Ondokuz Mayıs, Turkey. Reproduction of *Meloidogyne* isolates on a resistant tomato cv. Alsancak RN (Yuksel Seeds, Turkey) bearing the *Mi-1.2* gene was compared with a susceptible tomato cv. Beril (Rito Seeds, Turkey). Whether tomato cultivars carried the *Mi-1.2* resistance gene was assess using the molecular marker Mi23 and the presence of this gene in the resistant tomato cultivar was confirmed (Seah et al., 2007).

Tomato seedlings with 3-4 true leaves were transplanted singly into plastic pots containing 500 g sterilized sandy-loam soil and sand (2:1). Nematode eggs were obtained by rinsing the infected tomato roots from the pure nematode cultures using 10% commercial bleach solution (0.52% sodium hypochlorite) (Hussey & Barker, 1973). Five or 7 d after transplanting, for each of the 90 RKN isolates, five pots of each of the resistant and susceptible tomato cultivars were inoculated with 1000 RKN eggs (initial population density, Pi). Pots were arranged in a randomized block design and were grown in greenhouses at 24±2°C. Eight weeks after the inoculation, root systems were removed from the pots, washed with tap water, and rated for gall index (GI) using a 0-10 scale (Bridge & Page, 1980). Then, the roots were macerated in a blender with 20% commercial bleach solution for 30-40 s, the suspension poured onto 200 and 500 mesh sieves, and eggs were collected from the 500 mesh sieve for counting. Final population densities (Pf) of each nematode isolate on resistant and susceptible tomato cultivars were determined. The reproduction factor (Rf = Pf/Pi) and reproduction index (RI = Pf on the resistant cultivar/Pf on susceptible cultivar X 100) were calculated (Ornat et al., 2001; Cortada et al., 2008).

The resistance level of tomato cultivar containing *Mi-1.2* gene to each RKN isolate was categorized according to the RI as highly resistant (RI < 10%), moderately resistant (10% ≤ RI < 50%) or susceptible (RI ≥ 50%) (Cortada et al., 2009). Isolates having a RI value higher than 10% were considered as resistance-breaking and those isolates that caused moderately resistant or susceptible reactions on this cultivar were classified as partially virulent or virulent, respectively.

Data analysis

All data were analyzed with SAS statistical software. Each *Meloidogyne* species was evaluated separately. GI and Rf on resistant plants were log-transformed [log10(x+1)] before analysis. Data from both resistant and susceptible plants were subjected to analysis of variance. Differences between isolates from the same nematode species were evaluated using the Duncan's multiple range test at P ≤ 0.05 significance level. GI and Rf of each RKN isolate on susceptible and resistant cultivars were compared with t-test at P ≤ 0.05 significance level (Devran & Söğüt, 2010).

Results

Molecular marker Mi23 indicated that cv. Alsancak RN is a hybrid and heterozygous for the *Mi* locus (*Mi/mi*) with 380 and 430 bp DNA fragments, whereas susceptible tomato cultivar displayed a single DNA fragment with 430 bp, confirming the absence of the *Mi-1.2* gene (*mi/mi*).

The reproduction of the RKN isolates on the susceptible and resistant cultivars was evaluated within each nematode species (Table 1). As expected, all isolates reproduced on the susceptible tomato cultivar (Pf of 6 to 47.8 times greater than Pi) and had greater Rf values on susceptible than on the resistant tomato cultivar ($P < 0.05$).

Meloidogyne arenaria isolates had lower GI on the resistant than on the susceptible tomato cultivar ($P < 0.05$). However, *M. arenaria* A-7 and Sn-11 had a Rf higher than 1.0 on resistant tomato, but the RI were 4.77 and 4.13%, respectively. Although the highest RI of *M. arenaria* was 5.67% for A-12, this isolate on the resistant cultivar produced lower number of eggs than the Pi. Overall, the tomato cultivar with *Mi-1.2* gene displayed high level of resistance (RI < 10%) against all *M. arenaria* isolates.

On the susceptible cultivar, of the four *M. incognita* isolates A-11 had significantly lower Rf than the other isolates ($P < 0.05$). However, this isolate showed the highest GI (4.8) and Rf (4.8) on the resistant tomato cultivar in contrast to the other *M. incognita* isolates that were unable to reproduce on the resistant tomato. Similarly, the resistant tomato cultivar was highly resistant (RI < 10%) to all *M. incognita*, except for A-11 (RI = 15.1%) that caused a moderately resistant reaction on this cultivar.

GI values of the *M. javanica* isolates on the resistant cultivar ranged from 0 to 2.8 and the nematodes did not reproduce (Rf < 1) on this cultivar indicating high level of resistance (RI < 10%).

For four isolates of *M. luci* (Çr-19, Or-2, Pr-1 and Pr-2) on the resistant cultivar, the Rf values were >1, ranging from 3.6 to 12.6 times greater than the Pi. Similarly, on the resistant tomato, the GI values of these four *M. luci* isolates were the highest ranging from 5.6 to 7.0 which did not significantly differ ($P < 0.05$). Moreover, the GI values of two isolates (Or-2 and Pr-1) were not significantly different on the resistant and susceptible tomato cultivars. Remarkable differences were detected for the RI values for *M. luci*, ranging from 0 to 51.2%. Isolate Or-2 had the highest RI (51.2%) on the cv. Alsancak, with *Mi-1.2* gene, responding as susceptible. However, Alsancak was moderately resistant (10% ≤ RI < 50%) to three isolates (RI: Pr-2 = 46.4%, Pr-1 = 21.1% and Çr-19 = 16.0%) and highly resistant (RI < 10%) to the remaining (33) *M. luci* isolates.

Table 1. Gall index (GI), reproduction factor (Rf) and reproduction index (RI) of *Meloidogyne* spp. isolates on susceptible (S) and resistant (R) tomato cultivars, 8 weeks after inoculation of 1000 eggs/plant in a pot assay conducted in a temperature-controlled greenhouse (24±2°C)*

Code[a]	*Meloidogyne* species	Host Plant	GI (0-10)[b]		Rf[c]		RI%[d]
			S	**R**	**S**	**R**	
Er-1	*M. arenaria*	Cucumber	7.2±0.20 d-f	2.2±0.20 b-f	18.8±1.91 m-o	0.31±0.12 de	1.65
Er-2			7.0±0.00 e-g	2.4±0.24 b-f	15.8±1.24 o-q	0.22±0.08 d-f	1.39
Er-4			7.6±0.24 b-e	2.4±0.24 b-f	33.4±1.54 e-g	0.21±0.07 d-f	0.63
Er-5			7.6±0.24 b-e	1.6±0.24 e-h	24.0±1.73 j-l	0.12±0.04 e-k	0.50
Er-6			7.8±0.20 a-d	0.6±0.24 i-k	16.8±1.36 o-q	0.03±0.02 h-k	0.18
A-5			8.0±0.00 a-c	1.8±0.20 d-g	25.6±2.20 i-k	0.11±0.03 f-k	0.43
A-7			8.2±0.20 ab	4.0±0.00 a	30.8±2.22 f-h	1.47±0.11 a	4.77

Table 1. Continued

Code[a]	*Meloidogyne* species	Host Plant	GI (0-10)[b]		Rf[c]		RI%[d]
			S	**R**	**S**	**R**	
A-8			7.8±0.20 a-d	3.0±0.00 a-d	28.2±1.66 h-j	0.64±0.07 c	2.27
A-12			7.8±0.20 a-d	3.2±0.20 a-c	16.4±1.03 o-q	0.93±0.12 b	5.67
A-13			6.0±0.32 i	0.6±0.24 i-k	12.8±0.97 p-s	0.08±0.03 f-k	0.63
A-17			8.2±0.20 ab	3.6±0.24 ab	37.4±1.29 de	0.68±0.13 c	1.82
A-18			6.4±0.24 g-i	0.8±0.20 h-j	9.6±0.75 rs	0.06±0.03 f-k	0.63
Sn-7			8.4±0.24 a	2.2±0.20 b-f	35.2±1.59 d-f	0.16±0.02 d-j	0.45
Sn-9			8.4±0.24 a	2.0±0.00 c-f	47.6±1.03 a	0.12±0.01 e-k	0.25
Sn-11			8.2±0.20 ab	3.2±0.20 a-c	29.8±1.53 g-i	1.23±0.05 a	4.13
Om-2			7.2±0.20 d-f	0.2±0.20 k	18.8±1.43 m-o	0.02±0.02 i-k	0.11
B-3			7.8±0.20 a-d	1.4±0.24 f-h	12.4±1.03 q-s	0.20±0.07 d-g	1.61
B-17			7.4±0.24 c-f	0.2±0.20 k	9.8±0.66 rs	0.00±0.00 k	0.00
B-19			7.6±0.24 b-e	0.6±0.24 i-k	21.8±2.44 k-n	0.11±0.05 f-k	0.50
B-20			8.2±0.20 ab	0.6±0.24 i-k	30.6±1.44 f-h	0.11±0.04 f-k	0.36
B-27			7.0±0.00 e-g	0.4±0.24 jk	8.2±0.58 s	0.01±0.01 jk	0.12
B-30			6.8±0.20 f-h	0.4±0.24 jk	17.3±0.97 n-p	0.03±0.02 h-k	0.17
Çr-1			8.0±0.00 a-c	2.2±0.20 b-f	10.6±1.08 rs	0.21±0.07 d-f	1.98
Çr-23			7.0±0.00 e-g	1.6±0.24 e-h	8.6±0.68 rs	0.10±0.03 f-k	1.16
Çr-30			7.8±0.20 a-d	0.2±0.20 k	42.0±1.45 bc	0.02±0.02 i-k	0.05
Çr-33			7.8±0.20 a-d	0.4±0.24 jk	17.4±1.57 n-p	0.05±0.03 f-k	0.29
Çr-35			7.6±0.24 b-e	0.4±0.24 jk	26.8±1.36 h-j	0.00±0.00 k	0.00
Ço-2			8.2±0.20 ab	1.0±0.00 g-i	38.8±1.59 cd	0.33±0.05 d	0.85
Ço-3			8.0±0.00 a-c	0.8±0.20 h-j	23.4±1.86 j-m	0.18±0.05 d-i	0.77
Ço-6			7.6±0.24 b-e	1.0±0.00 g-i	43.8±1.46 ab	0.17±0.08 d-j	0.39
Çr-26		Melon	7.8±0.20 a-d	0.6±0.24 i-k	25.2±1.69 i-k	0.06±0.02 f-k	0.24
Er-8		Nightshade	7.6±0.24 b-e	2.6±0.24 a-e	17.2±2.06 n-q	0.19±0.05 d-h	1.10
A-4			7.8±0.20 a-d	1.6±0.24 e-h	23.4±1.94 j-m	0.15±0.05 e-k	0.64
Tk-1			6.8±0.20 f-h	0.8±0.20 h-j	36.3±1.46 de	0.08±0.02 f-k	0.22
Çr-28			8.0±0.00 a-c	1.6±0.24 e-h	44.2±1.43 ab	0.15±0.05 e-k	0.34
A-9		Tomato[Rt]	7.4±0.24 c-f	2.8±0.20 a-e	19.4±1.50 l-o	0.56±0.08 c	2.89
A-15		Tomato	6.2±0.20 hi	0.6±0.24 i-k	13.2±1.32 p-r	0.07±0.03 f-k	0.53
Pr-7			6.4±0.24 g-i	0.4±0.24 jk	33.7±1.50 e-g	0.04±0.03 g-k	0.12

Table 1. Continued

Code[a]	*Meloidogyne* species	Host Plant	GI (0-10)[b]		Rf[c]		RI%[d]
			S	**R**	**S**	**R**	
Çr-20	*M. incognita*	Cucumber	8.2±0.20 a	1.2±0.20 b	38.2±0.86 a	0.00±0.00 b	0.00
Pr-4			6.8±0.20 b	0.6±0.24 c	35.9±1.03 a	0.06±0.03 b	0.18
Tr-14		Pepper	7.6±0.24 a	0.0±0.00 d	36.0±1.14 a	0.00±0.00 b	0.00
A-11		Tomato[Rt]	8.2±0.20 a	4.8±0.20 a	31.8±0.97 b	4.80±0.42 a	15.09
Sn-4	*M. javanica*	Cucumber	8.0±0.00 ab	2.8±0.20 a	44.8±0.86 ab	0.48±0.05 b	1.07
Sn-5			7.0±0.00 e	2.2±0.20 ab	35.4±1.21 d	0.21±0.03 cd	0.60
Tr-8			7.4±0.24 c-e	0.8±0.20 cd	42.3±0.90 b	0.09±0.02 d-f	0.20
B-1			7.2±0.20 de	1.4±0.24 bc	24.2±1.16 f	0.24±0.05 cd	0.98
B-7			8.2±0.20 a	2.6±0.24 a	45.8±1.39 a	0.81±0.14 a	1.76
B-18			7.6±0.24 b-d	0.4±0.24 de	38.6±1.03 c	0.16±0.10 c-e	0.41
Çr-41			7.6±0.24 b-d	0.2±0.20 e	31.5±0.94 e	0.02±0.02 e-f	0.07
Ço-1			8.0±0.00 ab	0.0±0.00 e	47.8±1.02 a	0.00±0.00 f	0.00
B-6		Eggplant	7.8±0.20 a-c	0.0±0.00 e	10.2±0.86 g	0.00±0.00 f	0.00
B-26		Tomato	8.0±0.00 ab	1.8±0.20 ab	47.6±1.21 a	0.25±0.05 c	0.52
Çr-27			8.2±0.20 a	0.8±0.20 cd	38.4±0.93 cd	0.10±0.03 c-f	0.26
Sn-3	*M. luci*	Cucumber	7.6±0.24 a-d	1.2±0.20 c-e	21.4±1.50 f-i	0.07±0.03 g-i	0.32
Sn-12			7.8±0.20 a-c	0.4±0.24 fg	21.4±1.36 f-i	0.03±0.02 hi	0.15
Tk-2			7.4±0.24 b-e	0.2±0.20 g	7.4±0.53 rs	0.03±0.03 hi	0.43
Al-2			7.6±0.24 a-d	0.4±0.24 fg	14.6±1.21 k-o	0.05±0.03 g-i	0.36
Al-3			8.0±0.00 ab	0.2±0.20 g	23.4±1.63 e-g	0.02±0.02 hi	0.08
Al-4			7.4±0.24 b-e	0.4±0.24 fg	9.8±1.07 p-s	0.04±0.03 hi	0.43
Om-1			7.4±0.24 b-e	0.0±0.00 g	25.6±1.50 de	0.00±0.00 i	0.00
Tr-1			8.0±0.00 ab	0.4±0.24 fg	29.8±1.36 c	0.03±0.02 hi	0.11
Tr-2			7.6±0.24 a-d	0.4±0.24 fg	29.8±1.69 c	0.08±0.05 g-i	0.27
Tr-3			6.6±0.24 fg	0.4±0.24 fg	9.2±0.86 q-s	0.00±0.00 i	0.00
Tr-5			8.0±0.00 ab	0.4±0.24 fg	23.4±0.81 e-g	0.06±0.04 g-i	0.26
Tr-13			8.0±0.00 ab	0.4±0.24 fg	36.2±1.20 b	0.14±0.09 g-i	0.39
Çr-3			7.6±0.24 a-d	1.2±0.20 c-e	6.9±0.48 s	0.05±0.03 g-i	0.67
Çr-5			7.8±0.20 a-c	0.2±0.20 g	27.4±1.89 cd	0.02±0.02 hi	0.08
Çr-7			7.6±0.24 a-d	2.2±0.20 bc	20.2±1.77 g-j	0.37±0.06 ef	1.81
Çr-9			8.0±0.00 ab	2.4±0.24 b	30.0±1.22 c	0.48±0.14 e	1.59

Table 1. Continued

Code[a]	*Meloidogyne* species	Host Plant	GI (0-10)[b]		Rf[c]		RI%[d]
			S	**R**	**S**	**R**	
Çr-10			7.8±0.20 a-c	0.6±0.24 e-g	9.8±0.72 p-s	0.11±0.05 g-i	1.12
Çr-11			8.0±0.00 ab	0.6±0.24 e-g	21.8±1.07 e-h	0.07±0.03 g-i	0.32
Çr-19			7.4±0.24 b-e	5.6±0.24 a	42.5±0.91 a	6.8±0.83 c	16.00
Çr-25			8.00±0.00 ab	0.2±0.20 g	15.2±0.86 k-n	0.03±0.03 hi	0.17
Çr-34			7.6±0.24 a-d	1.8±0.20 b-d	6.0±0.35 s	0.19±0.04 f-h	3.23
Çr-36			7.0±0.00 d-f	0.2±0.20 g	12.0±1.22 n-q	0.01±0.01 i	0.08
Çr-39			7.0±0.00 d-f	1.0±0.00 d-f	13.4±1.21 m-p	0.00±0.00 i	0.00
Çr-40			8.2±0.20 a	0.6±0.24 e-g	17.8±1.85 i-l	0.07±0.04 g-i	0.42
Pr-1[e]			6.4±0.24 gh	5.8±0.20 a	17.1±1.04 j-m	3.60±0.58 d	21.05
Pr-2			7.2±0.20 c-e	5.6±0.24 a	22.0±1.21 e-h	10.20±0.75 b	46.36
Om-3		Eggplant	7.8±0.20 a-c	0.2±0.20 g	11.2±0.86 o-r	0.00±0.00 i	0.00
Tk-4		Nightshade	6.0±0.00 h	1.4±0.24 b-d	9.7±0.98 p-s	0.15±0.05 g-i	1.53
Tr-19			8.0±0.00 ab	0.4±0.24 fg	14.6±1.44 k-o	0.03±0.03 hi	0.19
Çr-2			6.8±0.20 e-g	0.0±0.00 g	19.4±1.21 h-j	0.00±0.00 i	0.00
Çr-12			7.6±0.24 a-d	1.4±0.24 b-d	13.0±1.00 n-q	0.09±0.04 g-i	0.68
Çr-15			8.0±0.00 ab	0.6±0.24 e-g	22.2±1.16 e-h	0.09±0.04 g-i	0.42
Or-2[e]			7.4±0.24 b-e	7.0±0.32 a	24.6±0.53 d-f	12.60±1.02 a	51.22
Tr-18		Tomato	7.8±0.20 a-c	0.4±0.24 fg	18.4±0.93 h-k	0.07±0.04 g-i	0.38
B-32			7.8±0.20 a-c	1.8±0.20 b-d	14.8±1.55 k-o	0.22±0.03 fg	1.50
Çr-24			7.8±0.20 a-c	0.4±0.24 fg	14.8±1.36 k-o	0.04±0.02 hi	0.26
Or-1			6.8±0.20 e-g	0.2±0.20 g	13.9±0.43 l-o	0.02±0.02 hi	0.13

* Data are means of five replicates±standard errors. Statistical analyses of the data were based on log10(x+1) transformed data. Root-knot nematode species were analyzed separately and grouped to form clusters within each species. For each root-knot nematode species, values in the same column followed by the same letter are not significantly different according to Duncan's multiple range test at $P < 0.05$;

S: Beril F1 (Rito Seeds, Turkey), R: Alsancak RN F1 (Yuksel Seeds, Turkey);

[a] Letters in the isolate codes indicate the location of the isolates collected from Black Sea Region of Turkey. A: Amasya, Al: Alaçam, B: Bafra, Ço: Çorum, Çr: Çarşamba, Er: Erbaa, Om: Ondokuzmayıs, Or: Ordu, Pr: Perşembe, Sn: Sinop, Tk: Tekkeköy, and Tr: Terme

[b] Based on a scale from 0 (none) to 10 (dead plants);

[c] final population density (Pf) / initial population density (Pi);

[d] (Pf on resistant cultivar / Pf on susceptible cultivar) x 100;

[e] GI of nematode isolate on susceptible and resistant cultivars are not significantly different according to t-test;

[Rt] Rootstock.

Discussion

The potential use of a resistant cultivar for nematode control depends on nematode virulence. This study reports the response of the resistant tomato cv. Alsancak, heterozygous for the *Mi* locus (*Mi/mi*), against 90 RKN isolates compared to that on the susceptible tomato cv. Beril. The resistant tomato cultivar exhibited wide variations in its response to RKN isolates, which was highly resistant to 85 isolates but moderately resistant to four isolates and susceptible to one isolate.

One *M. incognita* (A-11) and four *M. luci* (Çr-19, Or-2, Pr-1 and Pr-2) with RI > 10% were considered as resistance-breaking isolates and characterized as partially virulent or virulent according to host reaction of resistant cv. Alsancak based on RI. Among these isolates, only *M. luci* Or-2 isolate showed RI higher than 50% and was classified as virulent, whereas other isolates were partially virulent because of the moderately resistant response of the cv. Alsancak to these isolates (10% ≤ RI < 50%). However, all isolates of the *M. arenaria* and *M. javanica* were considered as avirulent, despite *M. arenaria* A-7 and Sn-11 isolates on resistant cultivar reproduced without substantial increases (Rf close to 1.0). *Meloidogyne arenaria* A-9 and *M. incognita* A-11 were expected to be resistance-breaking isolates as they were isolated from tomato rootstocks cv. King Kong RZ F1 (Rijk Zwaan) and cv. Kemerit RZ F1 (Rijk Zwaan), respectively, both presumed to have the *Mi-1.2* gene. Nevertheless, in the pot assays, the *M. incognita* A-11 reproduced in the resistant cultivar, but the *M. arenaria* A-9 did not (Rf < 1). Similarly, Sorribas & Verdejo-Lucas (1994) reported that three isolates of *Meloidogyne* species reproduced in resistant tomato in the field, and were considered as resistance-breaking isolates, but two of these isolates did not reproduce in resistant tomato in greenhouse assays. Eddaoudi et al. (1997) also reported that an isolate obtained from resistant tomato plant in the field did not reproduce in resistant cultivars in pot assays. Probably, there is more than one reason why an isolate that reproduced in resistant tomato in the field or greenhouse did not reproduce on resistant tomato under temperature-controlled conditions (<28°C). The *Mi-1.2* gene efficiency is lost at temperatures above 28°C (Dropkin, 1969; Ammati et al., 1986). Recently, Özalp & Devran (2018) reported that the resistance controlled by *Mi-1.2* gene in tomato plants held at 32ºC soil temperature for ≥48 h lost its effect. Therefore, nematodes will be to reproduce on resistant tomato plants in a greenhouse during the summer period when the temperature in the pots exceeds 28°C. An isolate of *M. javanica* did not reproduce in resistant cultivars in a controlled environment (soil temperature 22-24°C) and registered as avirulent but it reproduced under greenhouse conditions with soil temperatures ranging between 22°C to 42°C (Tzortzakakis & Gowen, 1996). Genetic background of tomato cultivars and rootstocks bearing the *Mi-1.2* resistance gene may cause differences on the virulence level of a nematode population (Roberts & Thomason, 1986; Jacquet et al., 2005; Cortada et al., 2009). Another reason for the difference in virulence level of a nematode isolate in a greenhouse compared to controlled conditions might be related to the nematode inoculum density. Within an avirulent population, low numbers of virulent nematodes can be present and consequently at a high density of nematodes the number of virulent individuals will increase resulting in high levels of infection on resistant roots (Tzortzakakis & Gowen, 1996; Castagnone-Sereno et al., 2007; Verdejo-Lucas et al., 2013). Moreover, Tzortzakakis et al. (2008) noted that the assessment of the virulence of RKN isolates for resistant cultivars in pot assays may not always be reliable because limited quantity of inoculum used in these assays may reduce the possibility to detect the virulent individuals with low densities within a population. It is therefore important to emphasize that repeated culture of resistant tomato cultivars in the same field may increase the proportion of virulent individuals (Sorribas et al., 2005). Virulent populations may be selected from an initial avirulent population because of selection pressure (Jarquin-Barberena et al., 1991; Castagnone-Sereno et al., 2007). Rotation of susceptible and resistant tomato cultivars instead of continuously cultivation of resistant cultivars in the same field would delay the occurrence of virulent populations and provide stability of the plant resistance (Castagnone-Sereno et al., 2007; Djian-Caporalino et al., 2011).

A large number of *M. luci* isolates were tested against the resistant tomato cv. Alsancak and the results showed that *Mi-1.2* gene confers resistance to *M. luci*. Previously, Strajnar & Širca (2011) reported that tomato cv. Venezia with *Mi-1.2* resistance gene was resistant to a Slovene *M. luci* isolate. Similarly, attempts to establish two Greek *M. luci* isolates on resistant tomato cv. Silvana by Conceição et al. (2012) failed. However, in this study, we identified a *M. luci* isolate (Or-2) that reproduces (51.2% RI) in the resistant cv. Alsancak. Or-2 was obtained from the roots of a weed (*Solanum nigrum* L.) growing in a greenhouse with grafted tomato. Notably, the roots of the grafted tomato plants near this weed had low numbers of galls. Although this isolate had an avirulent reaction in tomato rootstocks grown in a greenhouse, this isolate was classified as virulent based on the pot assay described in this study. Though no information exists on the tomato rootstock used in the greenhouse, it is likely that the genetic background of the rootstock was different from that of the resistant cultivar used in our pot assay. The other resistance-breaking isolates of *M. luci* (Çr-19, Pr-1 and Pr-2) were collected from cucumbers planted during two summer cropping cycles in greenhouses and the cropping history before those two years is unknown. Therefore, it is likely that these three *M. luci* isolates are naturally virulent to the *Mi-1.2* gene.

Mi-1.2 virulent isolates of *M. incognita* and *M. javanica* were reported for the first time in the Mediterranean Region of Turkey by Devran & Söğüt (2010). However, resistance-breaking isolates of *M. arenaria* were not recorded in Turkey (Devran & Söğüt, 2010; Özarslandan & Elekçioğlu, 2010). This study presents the first report of *Mi-1.2* resistance-breaking isolates of *M. luci*. Together with previous findings, it is concluded that *M. luci* is a potential threat for economically important crops grown in vegetable cropping areas (Aydınlı et al., 2013; Aydınlı & Mennan, 2016; Aydınlı, 2018). Thus, further research should be conducted to elucidate the mechanisms related to virulence of this nematode species in resistant cultivars.

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References

Akyazı, F. & O. Ecevit, 2011. Identification and distribution of root knot nematode species (*Meloidogyne* spp.) in vegetable fields in Tokat Province. Anadolu Journal of Agricultural Sciences, 26: 1-9.

Ammati, M., I. J. Thomason & H. E. McKinney, 1986. Retention of resistance to *Meloidogyne incognita* in *Lycopersicon* genotypes at high soil temperature. Journal of Nematology, 18 (4): 491-495.

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

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

Aydınlı, G., S. Mennan, Z. Devran, S. Širca & G. Urek, 2013. First report of the root-knot nematode *Meloidogyne ethiopica* on tomato and cucumber in Turkey. Plant Disease, 97: 1262.

Boerma, H. R. & R. S. Hussey, 1992. Breeding plants for resistance to nematodes. Journal of Nematology, 24: 242-252.

Bridge, J. & S. L. J. Page, 1980. Estimation of root-knot nematodes infestation levels on roots using a rating chart. Tropical Pest Management, 26: 296-298.

Carneiro, R. M. D. G., V. R. Correa, M. R. A. Almeida, A. C. M. M. Gomes, A. M. Deimi, P. Castagnone-Sereno & G. Karssen, 2014. *Meloidogyne luci* n. sp. (Nematoda: Meloidogynidae), a root-knot nematode parasitising different crops in Brazil, Chile and Iran. Nematology, 16 (3): 289-301.

Castagnone-Sereno, P., 2002. Genetic variability of nematodes: a threat to the durability of plant resistance genes. Euphytica, 124: 193-199.

Castagnone-Sereno, P., M. Bongiovanni & E. Wajnberg, 2007. Selection and parasite evolution: a reproductive fitness cost associated with virulence in the parthenogenetic nematode *Meloidogyne incognita*. Evolutionary Ecology, 21: 259-270.

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

Cortada, L., F. J. Sorribas, C. Ornat, M. F. Andres & S. Verdejo-Lucas, 2009. Response of tomato rootstocks carrying the *Mi*-resistance gene to populations of *Meloidogyne arenaria, M. incognita* and *M. javanica*. European Journal of Plant Pathology, 124: 337-343.

Cortada, L., F. J. Sorribas, C. Ornat, I. Kaloshian & S. Verdejo-Lucas, 2008. Variability in infection and reproduction of *Meloidogyne javanica* on tomato rootstocks with the *Mi* resistance gene. Plant Pathology, 57: 1125-1135.

Devran, Z. & M. A. Söğüt, 2009. Distribution and identification of root-knot nematodes from Turkey. Journal of Nematology, 41: 128-133.

Devran, Z. & M. A. Söğüt, 2010. Occurrence of virulent root-knot nematode populations on tomatoes bearing the *Mi* gene in protected vegetable-growing areas of Turkey. Phytoparasitica, 38 (3): 245-251.

Djian-Caporalino, C., S. Molinari, A. Palloix, A. Ciancio, A. Fazari, N. Marteu, N. Ris & P. Castagnone-Sereno, 2011. The reproductive potential of the root-knot nematode *Meloidogyne incognita* is affected by selection for virulence against major resistance genes from tomato and pepper. European Journal of Plant Pathology, 131: 431-440.

Dropkin, V. H., 1969. The necrotic reaction of tomatoes and other hosts resistant to *Meloidogyne*: Reversal by temperature. Phytopathology, 59: 1632-1637.

Eddaoudi, M., M. Ammati & A. Rammah, 1997. Identification of resistance breaking populations of *Meloidogyne* on tomatoes in Morocco and their effect on new sources of resistance. Fundamental and Applied Nematology, 20: 285-289.

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

Hussey, R. S. & K. R. Barker, 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. Plant Disease Reporter, 57: 1025-1028.

Jacquet, M., M. Bongiovanni, M. Martinez, P. Verschave, E. Wajnberg & P. Castagnone-Sereno, 2005. Variation in resistance to the root-knot nematode *Meloidogyne incognita* in tomato genotypes bearing the *Mi* gene. Plant Pathology, 54: 93-99.

Janssen, T., G. Karssen, M. Verhaeven, D. Coyne & W. Bert, 2016. Mitochondrial coding genome analysis of tropical root-knot nematodes (*Meloidogyne*) supports haplotype-based diagnostics and reveals evidence of recent reticulate evolution. Scientific Reports, 6: 22591.

Jarquin-Barberena, H., A. Dalmasso, G. de Guiran & M. C. Cardin, 1991. Acquired virulence in the plant parasitic nematode *Meloidogyne incognita*. I. Biological analysis of the phenomenon. Revue de Nématologie, 14: 299-303.

Kaloshian, I., V. M. Williamson, G. Miyao, D. Lawn & B. B. Westerdahl, 1996. Resistance-breaking nematodes identified in California tomatoes. California Agriculture, 50: 18-19.

Maleita, C., I. Esteves, J. M. S Cardoso, M. J. Cunha, R. M. D. G. Carneiro & I. Abrantes, 2018. *Meloidogyne luci*, a new root-knot nematode parasitizing potato in Portugal. Plant Pathology, 67 (2): 366-376.

Mukhtar, T., M. Z. Kayani & M. A. Hussain, 2013. Response of selected cucumber cultivars to *Meloidogyne incognita*. Crop Protection, 44: 13-17.

Nyczepir, A. P. & S. H. Thomas, 2009. "Current and Future Management Strategies in Intensive Crop Production Systems, 412-443". In: Root-knot Nematodes (Eds. R. N. Perry, M. Moens, J. L. Starr), CAB International Wallingford, UK, 488 pp.

Ornat, C., S. Verdejo-Lucas & F. J. Sorribas, 2001. A population of *Meloidogyne javanica* from Spain virulent to the *Mi* resistance gene in tomato. Plant Disease, 85: 271-276.

Özalp, T. & Z. Devran, 2018. Response of tomato plants carrying *Mi-1* gene to *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 under high soil temperatures. Turkish Journal of Entomology, 42 (4): 313-322.

Özarslandan, A. & İ. H. Elekçioğlu, 2010. Investigation on virulence of *Meloidogyne incognita* (Kofoid & White, 1919), *M. arenaria* (Neal, 1889) and *M. javanica* (Treub, 1885) (Tylenchida: Meloidogynidae) populations on resistant and susceptible tomato cultivars. Turkish Journal of Entomology, 34 (4): 495-502.

Prot, J. C., 1984. A naturally occurring resistance breaking biotype of *Meloidogyne arenaria* on tomato. Reproduction and pathogenicity on tomato cultivars Roma and Rossol. Revue de Nématologie, 7: 23-28.

Riggs, R. D. & N. N. Winstead, 1959. Studies on resistance in tomato to root-knot nematodes and occurrence of the pathogenic biotypes. Phytopathology, 49: 716-724.

Roberts, P. A., 1982. Plant resistance in nematode pest management. Journal of Nematology, 14: 24-33.

Roberts, P. A., 1995. Conceptual and practical aspects of variability in root-knot nematodes related to host plant resistance. Annual Review Phytopathology, 33: 199-221.

Roberts, P. A. & I. J. Thomason, 1986. Variability in reproduction of isolates of *Meloidogyne incognita* and *M. javanica* on resistant tomato genotypes. Plant Disease, 70: 547-551.

Seah, S., V. M. Williamson, B. E. Garcia, L. Mejia, M. S. Salus, C. T. Martin & D. P. Maxwell, 2007. Evaluation of a codominant SCAR marker for detection of the *Mi-1* locus for resistance to root-knot nematode in tomato germplasm. Report of the Tomato Genetics Cooperative, 57: 37-40.

Söğüt, M. A. & İ. H. Elekçioğlu, 2000. Determination of *Meloidogyne* spp. Goeldi, 1892 (Nemata: Heteroderidae) species races found in vegetable growing areas of the Mediterranean Region of Turkey. Turkish Journal of Entomology, 24: 33-40.

Sorribas, F. J., C. Ornat, S. Verdejo-Lucas, M. Galeano & J. Valero, 2005. Effectiveness and profitability of the Mi-resistant tomatoes to control root-knot nematodes. European Journal of Plant Pathology, 111: 29-38.

Sorribas, F. J. & S. Verdejo-Lucas, 1994. Survey of *Meloidogyne* spp. in tomato production fields of Baix Llobregat county, Spain. Journal of Nematology, 26 (4S): 731-736.

Strajnar, P. & S. Širca, 2011. The effect of some insecticides, natural compounds and tomato cv. Venezia with *Mi* gene on the nematode *Meloidogyne ethiopica* (Nematoda) reproduction. Acta Agriculturae Slovenica, 97 (1): 5-10.

Tzortzakakis, E. A., M. A. M. Adam, V. C. Blok, C. Paraskevopoulos & K. Bourtzis, 2005. Occurrence of resistance-breaking populations of root-knot nematodes on tomato in Greece. European Journal of Plant Pathology, 113: 101-105.

Tzortzakakis, E. A., I. L. P. M. da Conceição, M. C. V. dos Santos & I. M. de O. Abrantes, 2008. Selection of virulent *Meloidogyne* individuals within mixed isolates by continuous cultivation on a *Mi* gene resistant tomato genotype. Journal of Plant Diseases and Protection, 115 (5): 234-237.

Tzortzakakis, E. A. & S. R. Gowen, 1996. Occurrence of a resistance breaking pathotype of *Meloidogyne javanica* on tomatoes in Crete, Greece. Fundamental and Applied Nematology, 19 (3): 283-288.

Uysal, G., M. A. Söğüt & İ. H. Elekçioğlu, 2017. Identification and distribution of root-knot nematode species (*Meloidogyne* spp.) in vegetable growing areas of Lakes Region in Turkey. Turkish Journal of Entomology, 41 (1): 105-122.

Verdejo-Lucas, S., M. Blanco, L. Cortada & F. J. Sorribas, 2013. Resistance of tomato rootstocks to *Meloidogyne arenaria* and *Meloidogyne javanica* under intermittent elevated soil temperatures above 28°C. Crop Protection, 46: 57-62.

Verdejo-Lucas, S., L. Cortada, F. J. Sorribas & C. Ornat, 2009. Selection of virulent populations of *Meloidogyne javanica* by repeated cultivation of *Mi* resistance gene tomato rootstocks under field conditions. Plant Pathology, 58: 990-998.

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Original article (Orijinal araştırma)

Characterization of a novel baculovirus isolate from *Malacosoma neustria* (Linnaeus, 1758) (Lepidoptera: Lasiocampidae) in Samsun and its pathogenicity in different hosts

Samsun'da *Malacosoma neustria* (Linnaeus, 1758) (Lepidoptera: Lasiocampidae)'dan yeni bir bakülovirüs izolatının ve farklı konukçularda patojenitesinin belirlenmesi

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Abstract

Malacosoma neustria (Linnaeus, 1758) (Lepidoptera: Lasiocampidae) causes economic losses in apple, pear, plum, willow, oak and other economically important trees. In this study, an *Alphabaculovirus* was isolated from the larval population of *M. neustria* from Samsun in the central Black Sea Region of Turkey between 2015 and 2016. Electron microscope analysis of occlusion bodies (OBs) obtained from dead larvae showed that the nucleocapsids of a new isolate (ManeNPV-T4) are multiply enveloped. The Kimura two-parameter analysis and the phylogenetic tree were performed based on concatenated nucleotide and amino acid sequences of the partial *lef-8, lef-9* and *polh* genes from ManeNPV-T4 isolate compared to those of other 51 baculoviruses. Insecticidal activity tests against third instar *M. neustria* larvae produced 48 to 100% mortalities. The LC_{50} of ManeNPV-T4 was 0.78 x 10^3 OBs/ml in *M. neustria*. Additionally, the isolate caused mortalities lower than 50% in *Spodoptera exigua* (Hübner, 1808) (Lepidoptera: Noctuidae), *Lymantria dispar* (Linnaeus, 1758) (Lepidoptera: Erebidae), *Hyphantria cunea* (Drury, 1773) (Lepidoptera: Arctiidae) and *Helicoverpa armigera* (Hübner, 1805) (Lepidoptera: Noctuidae) larvae. Consequently, the new nucleopolyhedrovirus is infectious on *M. neustria* larvae and other lepidopterans.

Keywords: Baculovirus, identification, *Malacosoma neustria*, mortality

Öz

Malacosoma neustria (Linnaeus, 1758) (Lepidoptera: Lasiocampidae), Avrupa, Asya ve Kuzey Afrika'ya dağılmış dünya çapında bir zararlıdır. Elma, armut, erik, söğüt ve meşede ekonomik kayıplara neden olur. Bu çalışmada, Türkiye'nin Orta Karadeniz Bölgesi'nde bulunan Samsun'dan 2015-2016 yıllarında toplanan *M. neustria* larvalarından bir *Alphabakülovirüs* izole edilmiştir. Ölü larvalardan elde edilen oklüzyon badilerin (OB) elektron mikroskobu analizi, çoklu nükleokapsidlere sahip yeni bir izolat (ManeNPV-T4) olduğunu gösterdi. Kimura iki-parametre analizi ve filogenetik ağaç, ManeNPV-T4 izolatından elde edilen kısmi *lef-8, lef-9* ve *polh* genlerinin birleştirilmiş nükleotit ve amino asit dizilerine dayanarak, diğer 51 bakülovirüsünki ile karşılaştırıldı. Üçüncü evre *M. neustria* larvalarına uygulanan insektisidal aktivite testleri sonucunda %48 ile %100 arasında ölüm gözlendi. ManeNPV-T4'ün *M. neustria* için LC_{50} değeri 0,78 x 10^3 OB/ml olarak belirlendi. Ek olarak, viral izolat *Spodoptera exigua* (Hübner, 1808) (Lepidoptera: Noctuidae), *Lymantria dispar* (Linnaeus, 1758) (Lepidoptera: Erebidae), *Hyphantria cunea* (Drury, 1773) (Lepidoptera: Arctiidae) ve *Helicoverpa armigera* (Hübner, 1805) (Lepidoptera: Noctuidae) larvaları üzerinde %50'den düşük ölüm oranı oluşturdu. Sonuç olarak, bu yeni nükleopolihedrovirüs *M. neustria* ve diğer lepidopter larvalarına karşı da enfektiviteye sahiptir.

Anahtar sözcükler: Bakülovirüs, tespit, *Malacosoma neustria*, ölüm oranı

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Introduction

The baculoviruses are a family (Baculoviridae) of large, enveloped viruses, with circular covalently closed double stranded DNA genomes ranging from 80 to 180 kbp in size (Rohrmann, 2013). Baculoviruses infect mainly insects from the orders Diptera, Hymenoptera and Lepidoptera (Slack & Arif, 2007). A major distinctive feature is that these viruses have occlusion bodies (OBs), major proteins that are produced by the virus genes (*polyhedrin* or *granulin*), assembled into a protective paracrystalline matrix around the enveloped virions. The family Baculoviridae consists of four genera: *Alpha*-, *Beta*-, *Gamma*- and *Deltabaculovirus*. While the lepidopterans are infected by *Alphabaculovirus* and *Betabaculovirus*, hymenopterans and dipterans are infected by *Gammabaculovirus* and *Deltabaculovirus*, respectively (Jehle et al., 2006).

Malacosoma neustria (Linnaeus, 1758) (Lepidoptera: Lasiocampidae), also known as the European tent caterpillar, often cause serious damage to economically important fruit trees including apple, plum, hazelnut and pear; wild shrubs and ornamental trees including oleaster and oak, rose species, sea buckthorn, poplar, barberry, elm trees, willow and aspen, particularly in central and eastern Turkey (Ozbek & Calmasur, 2005; Ozbek & Coruh, 2010). The hatchlings migrate directly towards the new branches of the plant after emerging from egg clusters. The caterpillars feed on buds then on the upper epidermis and finally the parenchyma of the leaf tissue. In some years, the host plants are completely defoliated due to high numbers of this pest. Caterpillars are susceptible to parasitoids, predators and entomopathogens, which vary in their contributions to the larval mortality across regions.

As the European tent caterpillar larvae live together in a community, an infectious virus like a baculovirus may be effective for their control. Larvae emerging from tents may contact foliage contaminated with virus during feeding, and then infect other larvae upon returning to the tent. The use of baculovirus as a biological control agent has already been proven effective with agricultural pests (Granados, 1980).

The susceptibility of *Malacosoma* spp. to baculoviruses had been noted on numerous occasions (Jankevica et al., 2002; Progar et al., 2010). Moreover, the presence of baculoviruses in various *Malacosoma* spp. populations had been previously reported in Turkey (Yaman, 2003; Demir et al., 2013, 2014).

Host range is critical for determining the persistence of baculovirus isolates in an ecosystem, depending on the availability of primary and alternative hosts. Wider host ranges facilitate coevolution of baculoviruses and their hosts (Herniou et al., 2004). Likewise, broad host range is an important feature for developing effective commercial biocontrol agents (Brodeur, 2012).

In 2015, during field research in Samsun in the central Black Sea Region of Turkey, we observed baculovirus epizootics among *Malacosoma neustria* larvae populations. In this study, we aimed to characterize the biological properties of this baculovirus. We determined the morphological properties, molecular structure and phylogenetic position of new Malacosoma neustria nucleopolyhedrovirus isolate (ManeNPV-T4) from Turkey. Also, the pathogenicity of the virus was tested on various hosts including *Malacosoma neustria*, *Helicoverpa armigera* (Hübner, 1805) (Lepidoptera: Noctuidae)*, Hyphantria cunea* (Drury, 1773) (Lepidoptera: Arctiidae), *Lymantria dispar* (Linnaeus, 1758) (Lepidoptera: Erebidae) and *Spodoptera exigua* (Hübner, 1808) (Lepidoptera: Noctuidae) larvae.

Materials and Methods

Insect

Spodoptera exigua and *H. armigera* eggs used were obtained from Keyun Biocontrol Company, China. The eggs were allowed to hatch under laboratory conditions. After hatching, the neonate larvae were reared on a semisynthetic diet (Poitout & Bues 1974). *Malacosoma neustria*, *H. cunea* and *L. dispar* larvae were collected near Samsun and Trabzon in the Black Sea Region of Turkey. Collected larvae were fed with fresh leaves obtained from hazelnut (for *M. neustria*), mulberry (*H. cunea*) and oak (for *L. dispar*) trees till pupal stage in the laboratory. The pupae were then placed inside the oviposition chamber. After adult emergence, cotton soaked with 10% (w/v) sugar solution was provided for as a food source to increase the fecundity. Filter paper was placed inside adult emergence cage for egg laying. The eggs were then hatched and reared under laboratory conditions. Third instar larvae were used in the experiments described below. The eggs and the larvae were reared in an incubator at 65% RH, 25±1°C and 16:8 h L:D photoperiod.

Virus detection, isolation and propagation

Dead larvae of *M. neustria* were collected from various fruit trees in Samsun and brought to the microbiology laboratory of the Biology Department, Karadeniz Technical University, Trabzon during 2015 and 2016. These larvae were checked under phase-contrast microscope (Nikon Eclipse E600) for baculovirus infection. After detecting baculovirus infection, OBs were isolated using the procedure of Muñoz et al. (1997). These OBs were used for propagating the virus in healthy field collected *M. neustria* larvae. Subsequently, the larvae, fed with the leaf pieces (~30 mm^2) contaminated with OBs (10^6), were placed in Petri dishes. Insects were supplied with fresh leaves following consumption of the inoculum and maintained at 28°C until virus symptoms were observed. The OBs, observed under phase-contrast microscope, were reisolated from dead larvae and stored at -20°C. The isolate was designated as ManeNPV-T4.

Electron microscopy

The suspension of the purified OBs was transferred onto the coverslips and then air-dried. The sample was sputtered with gold for 3 min and examined with a Zeiss EVO LS10 scanning electron microscope. SmartSEM program was used for measuring the diameters of OBs (30 OBs/isolate). Purified OBs, fixed in 2% glutaraldehyde, 0.1 M phosphate buffer (pH 7.2) and post fixed in 1% OsO_4, analyzed using transmission electron microscope. For transmission electron microscopy (Zeis EM900), OB's were embedded in resin and ultra-thin sections stained with uranyl acetate were examined at 80 kV.

Restriction analysis

Viral DNA isolation was performed according to the method of Reed et al. (2003). The quantity and quality of the isolated DNA were determined spectrophotometrically (260/280 nm). For restriction enzyme analyses, 5 µg of DNA, was digested with *Hind*III (New England Biolabs, Ipswich, MA, USA) at 37°C for 5 h. Digestion reaction were electrophoresed in a 0.8% agarose gel containing ethidium bromide in TAE buffer (1 mM EDTA and 40 mM Tris-acetate at pH 8.0) at 25 V for 15 h. Fragments were displayed under UV light.

PCR amplification and phylogenetic analysis

Genes, *lef-8*, *lef-9* and *polh,* of ManeNPV-T4 were amplified using the degenerate primers of De Moraes and Maruniak (1997), Herniou et al. (2003) and Jehle et al. (2006). PCR reactions were performed as previously described (Demir et al., 2014). Amplified fragments were cloned into a pJET1.2 (Thermo Fisher Scientific, Waltham, MA, USA) vector and subsequently sequenced by Macrogen Inc. (Amsterdam, The Netherlands). These sequences were searched for similarity with those of the other baculovirus genes and submitted to National Center for Biotechnology Information under accession numbers, MH809425, MN218195 and MN218194 for*, lef-8*, *lef-9* and *polh* genes, respectively.

Nucleotide distance matrices between ManeNPV-T4 and other baculoviruses were determined for concatenated partial *lef-8*, *lef-9* and *polh,* gene sequences using the Kimura 2-parameter (K2P) analysis (Wenmann et al., 2018). Additionally, a phylogenetic tree was built based on the concatenated amino acid sequences of these three genes using MEGA7. Bootstrap analysis was used for testing the robustness of the phylogenetic tree. Phthorimaea operculella granulovirus, Spodoptera frugiperda granulovirus, Mythimna unipuncta granulovirus, Mocis latipes granulovirus and Pseudaletia unipuncta granulovirus were used as out-groups in the phylogenetic analysis.

Pathogenicity experiments

The pathogenicity of ManeNPV-T4 was tested in five potential hosts (*S. exigua, H. armigera, H. cunea, L. dispar* and *M. neustria*) at five OB concentrations (10^3, 10^4, 10^5, 10^6 and 10^7 OBs/ml). Thirty third-instar larvae were used per concentration and all treatments were repeated three times. The larvae were fasted for 12 h and then fed with leaves inoculated with 100 µl of the different OB concentrations. After consumption of the inoculated leaves, fresh diets were added and incubated at 28°C under a 16:8 h L:D photoperiod. Water treated leaves were used for feeding the control group larvae. Mortality was checked daily for 14 d. At the end of the experiment, dead larvae were collected and checked for the presence of OBs under phase-contrast microscope for NPV infection. The mortality levels varied for all host over the 14 d period. Mortality data were evaluated by using the Schneider-Orelli formula (Püntener, 1981) and the LC_{50} values necessary for *M. neustria* host were calculated by probit analysis using IBM SPSS statistics 23 software.

Results

Electron microscopy

The electron micrograph study revealed typical baculovirus OBs. Scanning electron microscopy results showed irregularly shaped OBs that were measured as 1.94 ± 0.23 µm in diameter (Figure 1A). Transmission electron micrograph of the ManeNPV-T4 OB isolated from infected larvae is occupied by the virion envelope with many virions occluded in the occlusion body (Figure 1B). Nucleocapsid sizes were 224.07 nm long by 46.29 nm wide.

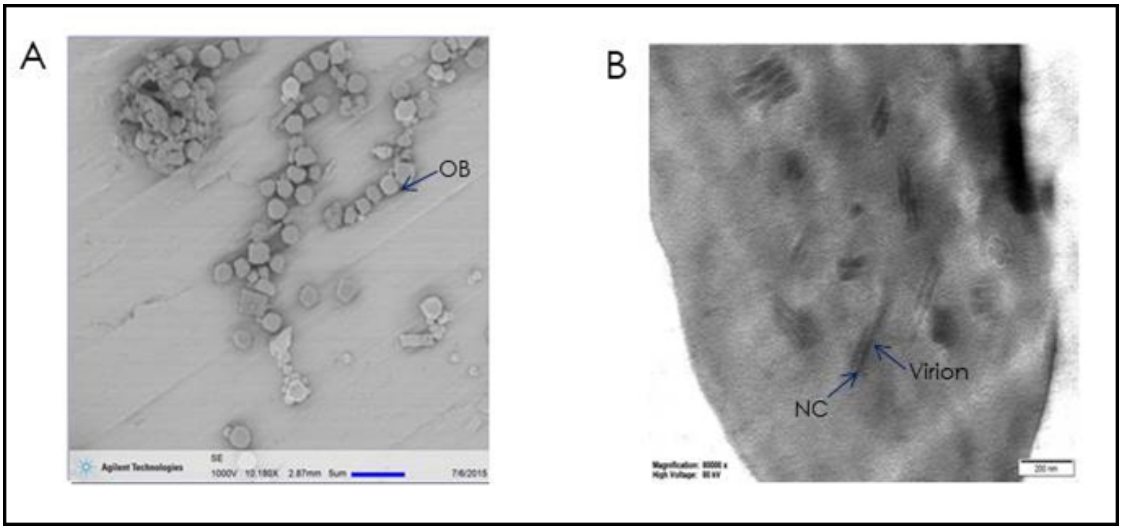

Figure 1. The electron micrographs of ManeNPV-T4: A) Scanning electron microscopy; B) Transmission electron micrographs. Virions are seen as dark rods and points in OBs; OB: occlusion bodies, NC: nucleocapsid.

Restriction endonuclease analysis of viral DNA

Restriction endonuclease (RE) analysis of the ManeNPV-T4 DNA, purified from viral OBs, yielded 15 *Hind*III fragments. These fragments were named alphabetically. *Hind*III digestion of the completely sequenced ManeNPV-T2 genome (Gencer et al., 2018) was done with Benchling Life Sciences program and yielded 20 different bands (Figure 2). The sizes of the fragments were estimated according to *Hind*III digested λ DNA and 1 kb markers (Table 1). The ManeNPV-T4 complete genome was estimated to be 119.7 kbp consistent with the *Hind*III restriction profile.

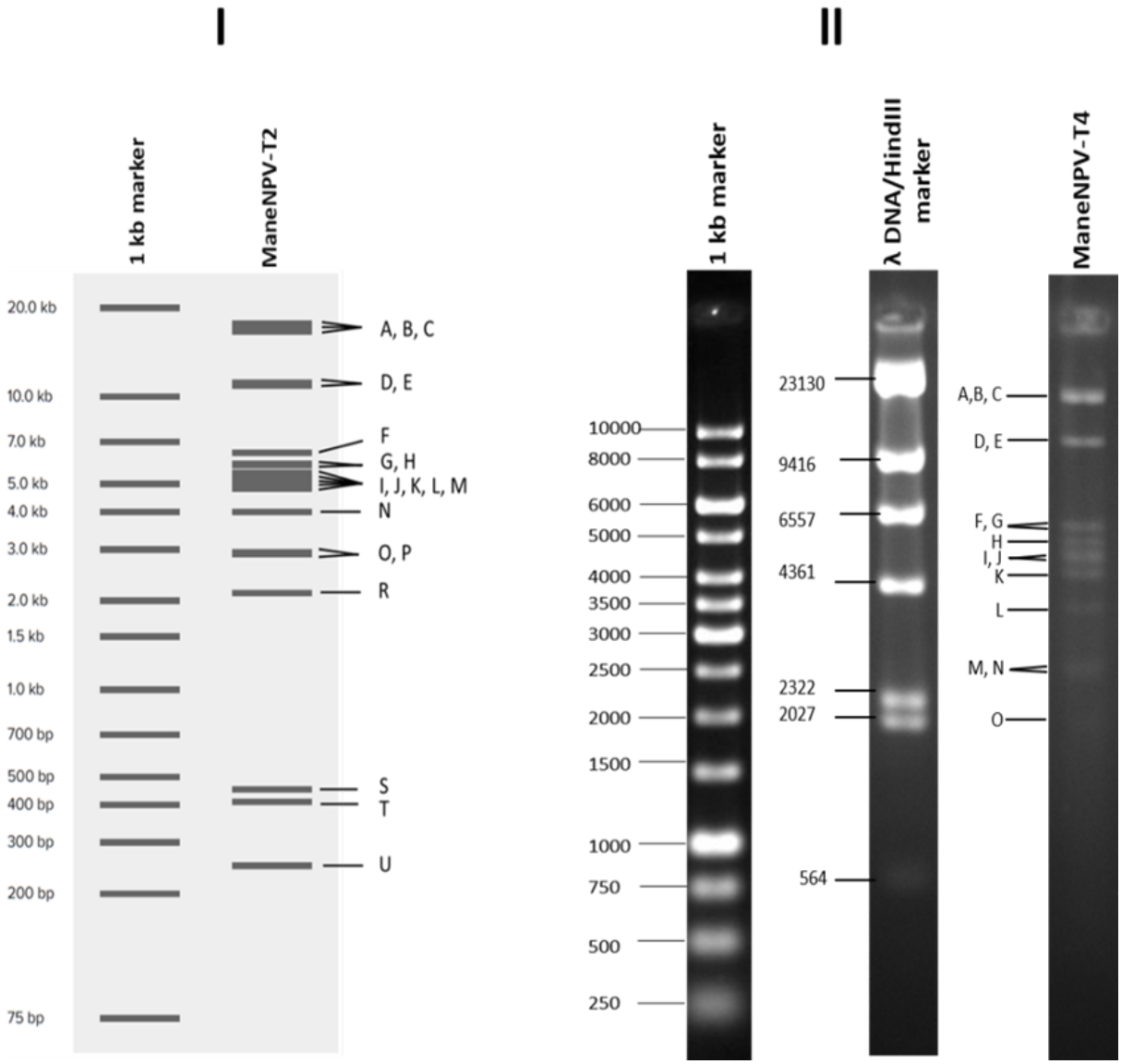

Figure 2. Restriction endonuclease mapping of ManeNPV-T4 DNA with *Hind*III. Fragments generated by *Hind*III enzyme are indicated by the letters near columns. I) ManeNPV-T2 digested with *Hind*III in silico. 1 kb marker: GeneRuler 1 kb Plus DNA Ladder, Thermo Scientific. II) ManeNPV-T4 DNA digested with *Hind*III in vitro. 1 kb marker: GeneRuler 1 kb DNA Ladder, Thermo Scientific. λ DNA/*Hind*III marker, Bio Basic.

Table 1. Fragments and total genome size of ManeNPV-T4 isolate digested with *Hind*III enzyme

Fragment	ManeNPV-T4
A	17.7
B	17.0
C	16.7
D	11.1
E	11.0
F	6.4
G	5.9
H	5.8
I	5.5
J	5.4
K	5.2
L	4.0
M	3.0
N	2.9
O	2.1
Total (kbp)	119.7

Phylogenetic analysis

K2P analysis was performed to define baculovirus species based on nucleotide sequence distances (Jehle et al., 2006). In this study, we used K2P analysis to address the position of ManeNPV-T4 among the species from *Alphabaculovirus* Groups I and II, and *Betabaculoviruses*.

According to K2P analysis, two viruses are considered to be the same species if the nucleotide locus distances value is less than 0.015 and are considered as different virus species if the distance is greater than 0.050 (Jehle et al., 2006). Based on concatenated nucleotide sequences of the partial*, lef-8*, *lef-9* and *polh,* genes, the nucleotide locus distance between the previous Turkish isolate (ManeNPV-T2) and ManeNPV-T4 is found as 0.053 which indicates that these two viruses are different species according to Jehle et al. (2006) (Figure 3). However, the amino acid sequence identities of each three marker genes of both ManeNPV-T2 and ManeNPV-T4 was 100%. The nucleotide locus distance between two viruses may be high because of the different nucleotides, coding the same amino acids. Additionally, in a study performed with concatenated 38 baculovirus core gene sequences, it was reported that baculoviruses that have nucleotide distances between 0.021 and 0.072 can be classified as same species (Wenmann et al., 2018). Thus, ManeNPV-T4 can be thought as same species of *M. neustria* NPVs in Turkey.

The phylogenetic tree has been constructed based on the concatenated POLH, LEF-8 and LEF-9 amino acid sequences of baculoviruses from *Alphabaculovirus* groups I and II, and *Betabaculoviruses*. The resulted tree demonstrated that ManeNPV-T4 is closely related with ManeNPV-T2 (Figure 4).

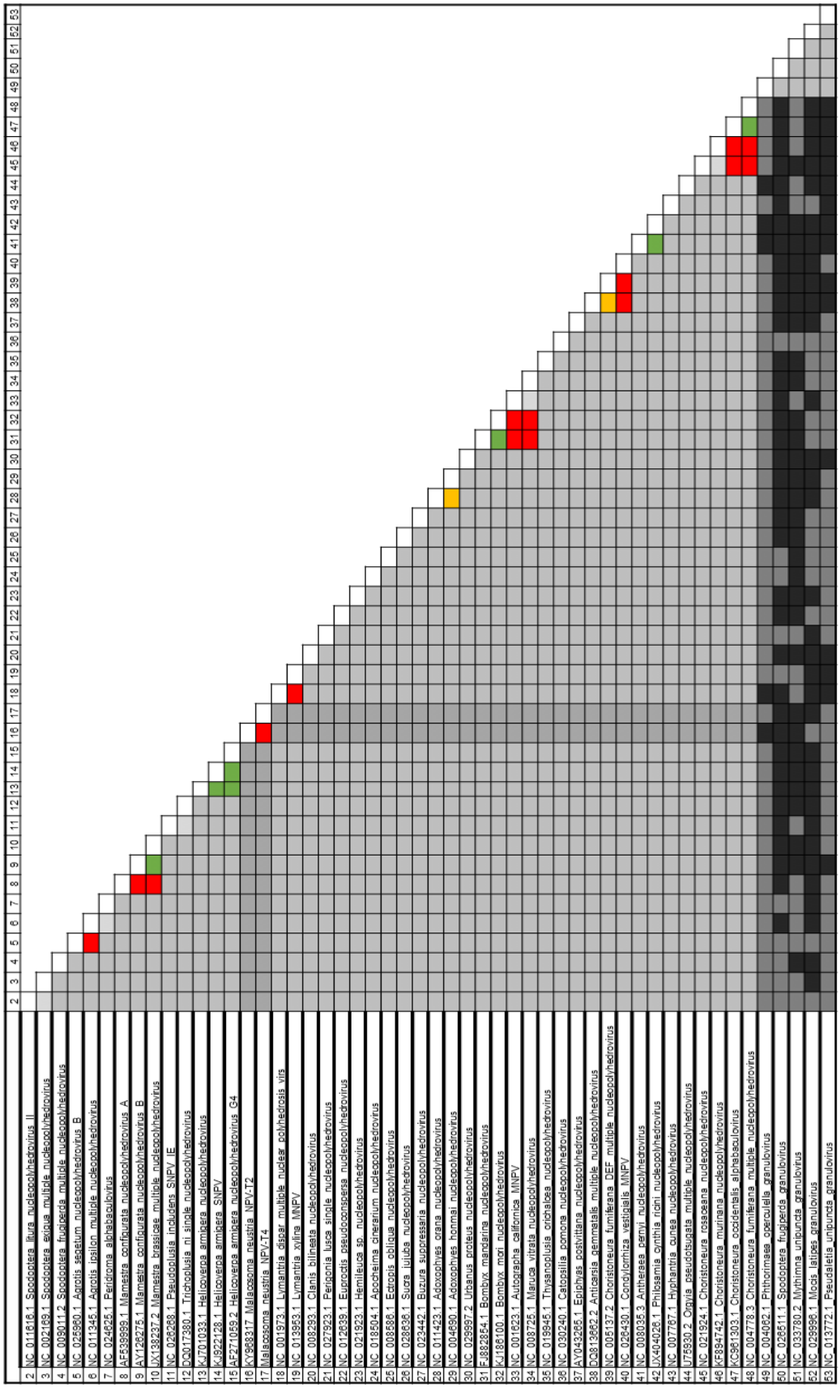

Figure 3. Pairwise distances of the nucleotide sequences of concatenated lef-8, lef-9 and polh fragments of baculoviruses. Kimura two-parameter thresholds were applied to indicate different colors as 0.000-0.015 green, 0.015-0.021 yellow, 0.021-0.05 orange, 0.05-0.072 red, 0.072 (light gray) >1 (black).

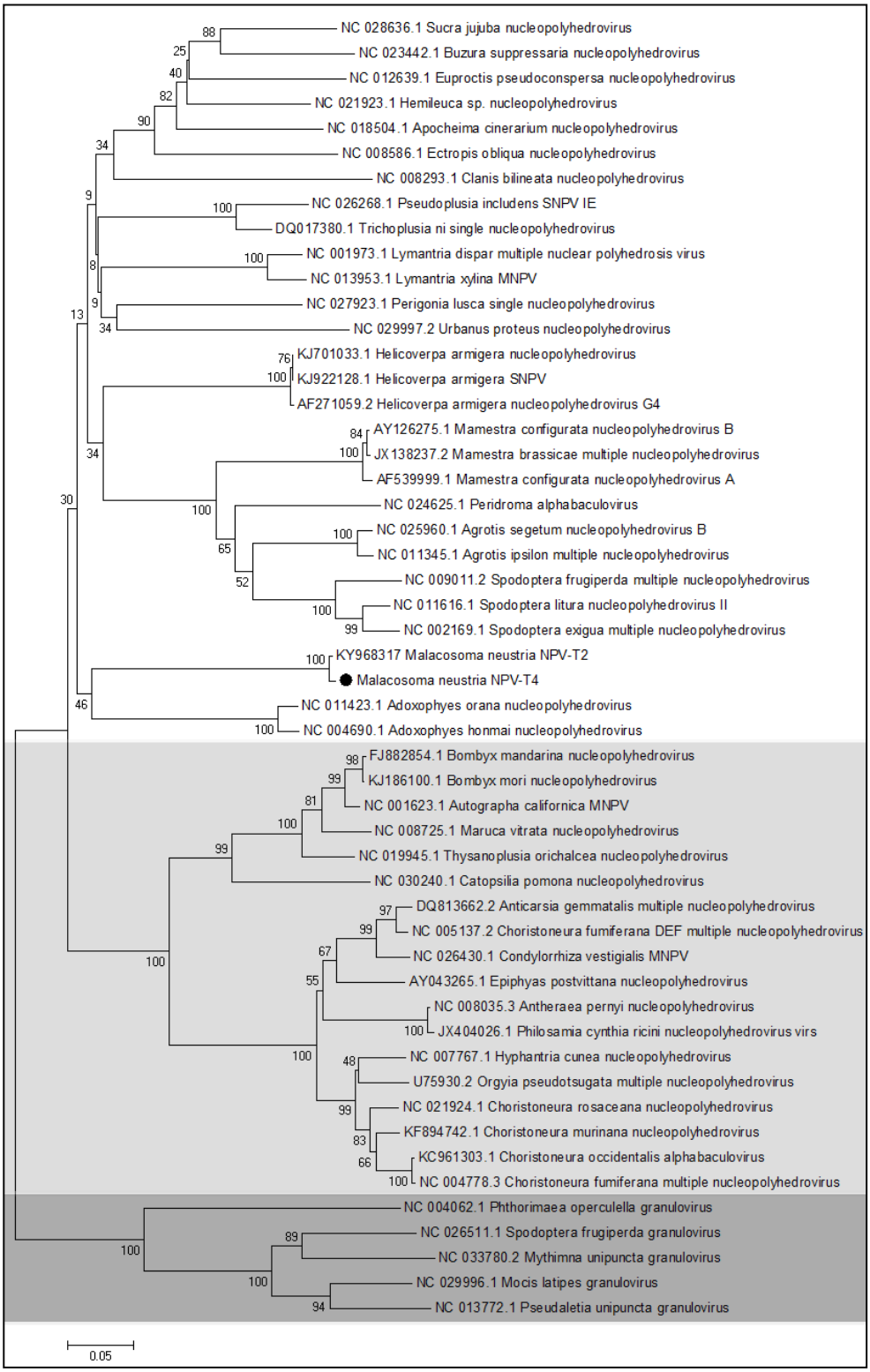

Figure 4. A phylogenetic tree (neighbor joining) according to the amino acid sequences of concatenated partial *lef-8*, *lef-9* and *polh*, genes. Bootstrap scores were showed with numbers on branches. Black dot indicates the location of the ManeNPV-T4. White, gray and dark gray areas show *Alphabaculovirus* group II, *Alphabaculovirus* group I and *Betabaculoviruses*, respectively.

Pathogenicity

The infectivity of the ManeNPV-T4 isolate was determined in *M. neustria* and four other lepidopteran hosts. Thirty third-instar larvae were used from each host at five virus concentrations, and tests were performed three times. Mortalities were assessed daily for 14 d. Mortality of *M. neustria*, ranged from 28 to 100%. The mortality of *M. neustria* larvae reached 100% with 10^7 OBs/ml within 14 d, whereas, this concentration caused 28, 30, 36 and 42% mortality of *H. armigera, H. cunea, S. exigua* and *L. dispar* larvae, respectively (Figure 5). The LC_{50} of ManeNPV-T4 in *M. neustria* was 0.78 x 10^3 (slope±se = 0.448±0.481; df = 3; X2 = 0.903). Since mortality of the hosts, other than *M. neustria*, did not exceed 50%, the LC_{50} for these hosts could not be calculated.

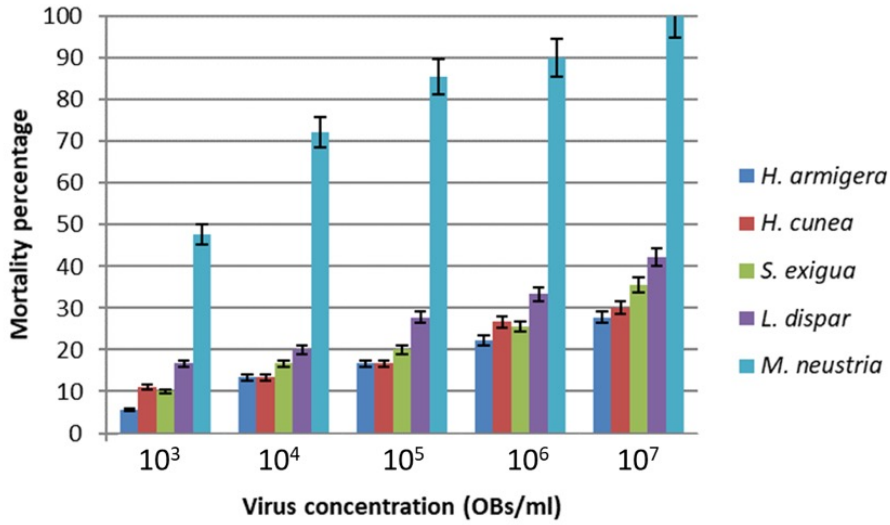

Figure 5. Pathogenicity of the ManeNPV-T4 isolate between 10^3-10^7 OBs/ml concentrations on third instar *Helicoverpa armigera*, *Hyphantria cunea*, *Spodoptera exigua*, *Lymantria dispar*, *Malacosoma neustria* larva 14 d after infection. Mortality data were corrected with Schneider-Orelli's formula (Püntener, 1981). Different lowercase letters represent statistically significant differences between mortalities according to LSD multiple comparison test (P < 0.05). Concentrations were evaluated each other. Bars show standard error.

Discussion

In recent years, baculoviruses have been used as biopesticide due to the negative effects of chemicals on environment and other non-target organisms. However, geographical baculovirus variants have different effects on local populations in laboratory and field conditions (Haase et al., 2015). In current study, we obtained a new isolate from *M. neustria* near Samsun, Turkey, and characterized its biological properties. Additionally, the pathogenicity of this isolate was determined in five species of lepidopteran insect larvae. This new isolate was designated ManeNPV-T4 to indicate its difference from previous Turkish isolates (Yaman, 2003; Demir et al., 2013, 2014). Scanning electron microscopy observations showed that the OBs of ManeNPV-T4 are irregularly shaped and about 1.5**-**2.17 µm in diameter. The OB dimension of ManeNPV-T4 was compared to those of other ManeNPVs, and ManeNPV-T4 OBs was found to be larger than the Latvian isolates (Jankevica et al., 1998), which have OBs of 0.85**-**1.4 µm, and also larger than previous Turkish isolates, which have OBs of 0.87-1.75 µm (Demir et al., 2013) and 1.0-2.1 µm (Demir et al., 2014). However, ManeNPV-T4 OBs are smaller than the other Turkish ManeNPV isolates (Yaman, 2003), Polish isolate (Lipa et al., 1968) and the isolate used by Ponsen et al. (1964) which have OB of 0.76-3.85, 0.9**-**2.0 and 1**-**3.5 µm, respectively.

The transmission electron microscopy results demonstrated that ManeNPV-T4 has multinucleocapsids in its OBs. The nucleocapsid size of ManeNPV-T4, 224 × 46 nm, was compared with

the nucleocapsid sizes of other ManeNPV isolates. The nucleocapsid size of ManeNPV-T4 was similar to the Turkish isolates, ManeNPV (Yaman, 2003), ManeNPV-T2 and ManeNPV-T3, which have nucleocapsid sizes of 240 × 35, 250 × 50 and 194.5 × 40 nm, respectively. However, the Polish isolate, the Latvian isolate and the isolate used by Bergold (1953) being 310 × 50, 360 × 80 and 315-324 × 40-46 nm, respectively, had larger nucleocapsids than ManeNPV-T4.

The RE profile of the genome has been used to examine geographical variation within a single virus (Murillo et al., 2001). For further characterization, ManeNPV-T4 genome was digested with *Hind*III and the profile was compared with other Turkish isolates (Demir et al., 2013). The RE analysis of the new isolate showed that it is different to the other Turkish isolates. While the genome of another Turkish isolate (ManeNPV-T2) yielded 20 different fragments with *Hind*III enzyme in silico, ManeNPV-T4 genome yielded 15 fragments for the same enzyme. The pattern of digested viral genomes showed similarities among major fragments of both isolates. Restriction endonuclease digestion profiles are used in genome size calculation. The size of the ManeNPV-T4 genome was estimated at 119 kbp (Table 1). This size is compatible with the completely sequenced ManeNPV-T2 genome (130 kbp) (Gencer et al., 2018) and the range for the baculovirus genomes (80-180 kb) (Rohrmann, 2013). The profile obtained with RE analysis supported the microscopic observations and we can conclude that ManeNPV-T4 is a new M. neustria nucleopolyhedrovirus isolate from Turkey.

The genes*, lef-8, lef-9* and *polh/gran***,** and 38 core genes nucleotide sequences are used to identify similarities between baculovirus species using K2P (Jehle et al., 2006; Wennmann et al., 2018). This method does not necessarily prove whether viruses are different, but rather indicates similarity of virus species. According to the K2P results, ManeNPV-T4 and ManeNPV-T2 are similar species, but different isolates. However, more information is required to prove this outcome according to the distance parameters. The morphological and molecular characterization of these isolates showed that they are quite similar rand but different from other baculoviruses **(**Figure 3).

Concatenated partial POLH, LEF-8 and LEF-9 amino acid sequences of ManeNPV-T4 were used to construct a phylogenetic tree. This tree showed that Malacosoma NPVs cluster together as other NPVs, such as Lymantria or Spodoptera NPVs. ManeNPV-T4 isolate clustered with ManeNPV-T2 and these two isolates close to Adoxophyes NPV isolates. Although this phylogenetic tree, demonstrated that ManeNPV-T4 and ManeNPV-T2 are almost the same species with high similarity, these two isolates have different nucleotide sequences. This nucleotide diversification might be the result of geographical differences.

Bioassays were used to determine the pathogenicity of ManeNPV-T4 in different lepidopteran insect pests. The mortality with 10^7 OBs/ml reached 100% in *M. neustria* larvae after 14 d. Whereas 10^6 OBs/ml of ManeNPV-T2 was enough to cause 100% mortality on *M. neustria* larvae after 10 d (Demir et al., 2013). Furthermore, in another study, 10^6 OBs/ml concentration of ManeNPV-T3 caused 100% mortality in *M. neustria* larvae within 10 d (Demir et al., 2014). Additionally, ManeNPV-T4, at 10^7 OBs/mln, caused mortality between 28 and 42% in *H. armigera, H. cunea, S. exigua* and *L. dispar* larvae within 14 d. *Hyphantria cunea* is the most resistant to these baculovirus isolates and *M. neustria* is most susceptible of the species tested. It is important to note that the larvae used in bioassay were collected from different locations of Turkey, not from the same place as the isolate. These data indicate that ManeNPV-T4 caused different mortality tin *M. neustria* and other lepidopteran pests used in this study and has the potential to be used for improving the biological control programs.

As expected, ManeNPV-T4 caused high mortality in its source host. However, its pathogenicity was lower than that of other Turkish isolates, ManeNPV-T2 and ManeNPV-T3. Therefore, it is concluded that ManeNPV-T4 has relatively large OBs with less nucleocapsids compared to other isolates indicating that, different geographic isolates can have different microscopic properties, nucleotide sequences and pathogenicity.

References

Bergold, G. H., 1953. Insect viruses. Advances in Virus Research, 1: 91-139.

Brodeur, J., 2012. Host specificity in biological control: insights from opportunistic pathogens. Evolutionary Applications, 5: 470-480.

De Moraes, R. R. & J. E. Maruniak, 1997. Detection and identification of multiple baculoviruses using the polymerase chain reaction (PCR) and restriction endonuclease analysis. Journal of Virological Methods, 63: 209-217.

Demir, I., R. Nalçacioğlu, L. Mohammad Gholizad & Z. Demirbağ, 2013. Characterization of a new isolate of *Malacosoma neustria* nucleopolyhedrovirus (ManeNPV) from Turkey. Turkish Journal of Biology, 37: 385-391.

Demir, I., R. Nalçacioğlu, L. Mohammad Gholizad & Z. Demirbağ, 2014. A highly effective nucleopolyhedrovirus against *Malacosoma* spp. (Lepidoptera: Lasiocampidae) from Turkey: isolation, characterization, phylogeny, and virulence. Turkish Journal of Agriculture and Forestry, 38: 462-470.

Gencer, D., R. Nalcacioglu, Z. Demirbag & I. Demir, 2018. Complete genome sequence analysis of the *Malacosoma neustria* nucleopolyhedrovirus from Turkey. Virus Genes, 54: 706-718.

Granados, R. R., 1980. Infectivity and mode of action of Baculoviruses. Biotechnology and Bioengineering, 22: 1377-1405.

Haase, S., A. Sciocco-Cap & V. Romanowski, 2015. Baculovirus Insecticides in Latin America: Historical Overview, Current Status and Future Perspectives. Viruses, 7: 2230-2267.

Herniou, E. A., J. A. Olszewski, J. S. Cory & D. R. O'Reilly, 2003. The genome sequence and evolution of baculoviruses. Annual Review of Entomology, 48: 211-234.

Herniou, E. A., J. A. Olszewski, D. R. O'Reilly & J. S. Cory, 2004. Ancient coevolution of Baculoviruses and their insect hosts. Journal of Virology, 78: 3244-3251.

Jankevica, L., Z. Cudare & V. Ose, 1998. New isolate of *Malacosoma neustria* nuclear polyhedrosis virus in Latvia. Journal of Invertebrate Pathology, 71: 283-285.

Jankevica, L., M. Kropa, N. Savenkovs & R. Jankevics, 2002. Presence of nucleopolyhedroviruses in natural populations of *Malacosoma neustria* L. (Lepidoptera, Lasiocampidae). Latvijas Entomologs, 39: 30-37.

Jehle, J. A., M. Lange, H. Wang, Z. Hu, Y. Wang & R. Hauschild, 2006. Molecular identification and phylogenetic analysis of baculoviruses from Lepidoptera. Virology, 346: 180-193.

Lipa, J. J., S. M. Gershenson & A. P. Gudz-Gorban, 1968. Electron microscopy, histopathology and pathogenicity of nuclear polyhedrosis virus of *Malacosoma neustria* L. Acta Microbiologica Polonica, 17: 191-201.

Muñoz, D., J. M. Vlak & P. Caballero, 1997. In vivo recombination between two strains of the genus Nucleopolyhedrovirus in its natural host, *Spodoptera exigua*. Applied and Environmental Microbiology, 63: 3025-3031.

Murillo, R., M. D. Muñoz, J. J. Lipa & P. Caballero, 2001. Biochemical characterization of three nucleopolyhedrovirus isolates of *Spodoptera exigua* and *Mamestra brassicae*. Journal of Applied Entomology, 125: 267-270.

Ozbek, H. & O. Calmasur, 2005. A review of insects and mites associated with roses in Turkey. Acta Horticulturae, 690: 167-174.

Ozbek, H. & S. Coruh, 2010. Egg parasitoids of *Malacosoma neustria* (Linnaeus, 1758) (Lepidoptera: Lasiocampidae) in Erzurum province of Turkey. Turkish Journal of Entomology, 34: 551-560.

Poitout, S. & R. Bues, 1974. Elevage de chenilles de vingt huit especes de lepidoptere Noctuidae et de deux especes d' Arctiidae sur milieu artificiel simple. Particularites de l' elevage selon les especes. Annales de Zoologie Ecologie Animale, 6: 431-441.

Ponsen, M. B., S. Henstra & C. Scheer, 1964. Electron microscope observations of nuclear polyhedra from *Malacosoma neustria* (Lepidoptera: Lasiocampidae). Netherlands Journal of Plant Pathology, 70: 101-104.

Progar, R. A., M. J. Rinella, D. Fekedulegn & L. Butler, 2010. Nuclear polyhedrosis virus as a biological control agent for *Malacosoma americanum* (Lepidoptera: Lasiocampidae). Journal of Applied Entomology, 134: 641-646.

Püntener, W., 1981. Manual for Field Trials in Plant Protection (2nd Edition). Agricultural Division, Ciba-Geigy Limited, Basel, Switzerland, 205 pp.

Reed, C., I. S. Otvos, R. Reardon, I. Ragenovich & H. L. Williams, 2003. Effects of long-term storage on the stability of OpMNPV DNA contained in TM Biocontrol-1. Journal of Invertebrate Pathology, 84: 104-113.

Rohrmann, G., 2013. Baculovirus Molecular Biology (3rd Edition). National Center for Biotechnology Information, Bethesda, Maryland, USA, 154 pp.

Wennmann, T. J., J. Keilwagen & J. A. Jehle, 2018. Baculovirus Kimura two-parameter species demarcation criterion is confirmed by the distances of 38 core gene nucleotide sequences. Journal of General Virology, 99: 1307-1320.

Yaman, M., 2003. Isolation and Characterization of Virus from *Malacosoma neustria* (Lepidoptera: Lasiocampidae) and Its Use in Microbial Control. Graduate School of Natural and Applied Science, Karadeniz Technical University, (Unpublished) PhD Thesis, Trabzon, Turkey, 83 pp.

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Original article (Orijinal araştırma)

Effect of temperature on insecticidal efficiency of local diatomaceous earth against stored-grain insects[1]

Yerel diatomit topraklarının depolanmış tahıl zararlılarına karşı insektisidal etkinliği üzerine sıcaklığın etkisi

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Abstract

A study was conducted in 2017 in Entomology Laboratory of Kahramanmaraş Sütçü İmam University to determine effect of temperature on insecticidal efficacy of local diatomaceous earth (DE), collected from Turkey, against the rice weevil, *Sitophilus oryzae* (L., 1763) (Coleoptera: Curculionidae), the confused flour beetle, *Tribolium confusum* du Val., 1863 (Coleoptera: Tenebrionidae), and the lesser grain borer, *Rhyzopertha dominica* (F., 1792) (Coleoptera: Bostrichidae). Bioassays were performed at three temperatures (20, 25 and 30ºC) and 55% RH on wheat treated with 0, 100, 300, 500, 900 and 1500 ppm (mg DE/kg grain) concentrations of local DE. Temperature had significant effect on insecticidal efficacy of local DE against the tested stored-grain insects. The effect of temperature on the insecticidal efficacy of local DE varied with insect species and concentration. Mortality of *S. oryzae* and *T. confusum* adults generally increased with increasing temperature and mortality at 30ºC was significantly higher than at 20 and 25ºC. However, for *R. dominica* adults treated with local DE, mortality at 20ºC was significantly higher than at 25 and 30ºC. The results indicated that complete mortality of *T. confusum* and *S. oryzae* can be achieved at lower concentrations ranging from 500 to 900 ppm. In conclusion, local DE formulation (ACN-1) has potential to be used for control of stored-grain insects.

Keywords: Local diatomaceous earth, *Rhyzopertha dominica*, *Sitophilus oryzae*, temperature, *Tribolium confusum*

Öz

Bu çalışma, 2017 yılında Kahramanmaraş Sütçü İmam Üniversitesi'nin Entomoloji Laboratuvarı'nda Türkiye'nin farklı bölgelerinden elde edilen yerel diatom toprağının depolanmış tahıl zararlısı, Pirinç biti, *Sitophilus oryzae* (L., 1763) (Coleoptera: Curculionidae), Kırma un biti, *Tribolium confusum* Du Val., 1863 (Coleoptera: Tenebrionidae) ve Ekin kambur biti *Rhyzopertha dominica* (F., 1792) (Coleoptera: Bostrichidae)'ne karşı insektisidal etkinliği üzerine sıcaklığın etkisini belirlemek için yürütülmüştür. Bu amaçla buğday üzerinde üç farklı sıcaklıklarda (20, 25 ve 30ºC) ve %55 nispi nem ortamında buğday üzerinde yerel diatom toprağının 0, 100, 300, 500, 900 ile 1500 ppm (mg/kg) konsantrasyonlarında biyolojik testler yürütülmüştür. Mevcut çalışma sıcaklığın test edilen yerel diatom toprağın depolanmış tahıl zararlılarının karşı etkinliği üzerine önemli etkiye sahip olduğunu göstermiştir. Çalışmada sıcaklığın yerel diatom toprağının etkinliğine etkisi test edilen böcek türüne ve diatom konsantrasyonuna göre değişiklik gösterdiği görülmüştür. Yerel diatom toprağı uygulamalarında sıcaklık artışıyla *S. oryzae* ve *T. confusum* erginlerinin ölüm oranlarında artış saptanırken 30ºC'deki ölüm oranlarının 20ºC ve 25ºC'deki ölüm oranlarından önemli derecede daha yüksek olduğu görülmüştür. Ancak, yerel diatom toprağı uygulanan *R. dominica* erginlerinde ise 20ºC sıcaklıkta elde edilen *R. dominica* erginlerinin ölüm oranlarının 25ºC ve 30ºC sıcaklıktan elde edilen ölüm oranlarından daha yüksek olduğu bulunmuştur. Bu çalışmanın sonuçları, 500 ppm ila 900 ppm arasında değişen düşük konsantrasyonlarda *T. confusum* ve *S. oryzae* erginlerinde %100 ölüm oranının elde edildiğini göstermiştir. Sonuç olarak, bu çalışmada yürütülen biyolojik test sonuçlarına göre, yerel diatom toprak formülasyonun (ACN-1), buğday üzerinde depolanmış tahıl böceklerinin kontrolünde kullanılma potansiyeline sahip olabileceği sonucuna varılmıştır.

Anahtar sözcükler: Yerel diatom toprağı, *Rhyzopertha dominica*, *Sitophilus oryzae*, sıcaklık, *Tribolium confusum*

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Introduction

Stored grains and their byproducts are the most important durable food category for human nutrition. During grain storage, damage can be caused by a numerous pest, particularly insect species, known as stored product pests, which cause very serious quantitative loss and qualitative degradation (Hill, 2002; Rees, 2004). In grain commodities, residual contact insecticides, primarily pyrethroid and organophosphorus compounds, applied directly to grain when it is loaded into storage facilities have been the main components of grain management programs to maintain grain quality. The residues from single application of these insecticides protects the grains from the insects in stored grain. However, intensive research has focused on alternative control methods that can replace insecticides in stored grain due to the development of resistance to commonly used grain protectant insecticides (Subramanyam & Hagstrum, 1995), an increasing attention on testing and evaluating nontoxic and environmentally-friendly control methods (Arthur, 1996), and a possible loss of organophosphorus grain protectants in Europe and the USA due to regulatory action.

The use of diatomaceous earth (DE) is one of the most promising alternatives to insecticides in stored grains. The insecticidal activity of DE currently mined varies depending upon diatom species composition, geological and geographical origin as well as certain physical and chemical characteristics, such as particle size, silicon dioxide content, pH and density (Korunic, 1997). DEs are natural resource-based dry substances that can be used as insecticides (Korunic, 1998). They act on the insect cuticle by absorbing the lipids or cuticular abrasion, resulting in insect death through rapid desiccation. DEs are non-toxic to mammals (acute oral toxicity for the rats; LD_{50} > 5 g/kg of body weight), leave no toxic residues on the commodity and according to the US Environmental Protection Agency. Since DEs are classified in the category of GRAS (generally recognized as safe) they are used as food or feed additives (FDA, 1995). Regarding their insecticidal use, there is no specialized equipment required for DE treatment since they can be applied with the same application technology as other grain protectants (Athanassiou et al., 2005). They persist in the treated commodity, providing a long-term protection against insect pests, which is currently a major problem for the use of synthetic pesticides. Eventually, DEs are also completely compatible with organic food production (Subramanyam & Roesli, 2000).

Several DE formulations, based on natural deposits, are now commercially available, and have proven to be very effective against stored-grain pests (Subramanyam & Roesli, 2000; Athanassiou et al., 2011). However, the investigation to discover new naturally-occurring DEs that are more effective in insect control is still in progress, especially in areas rich in siliceous rocks. Based on the initial evidence and preliminary results, Turkey is considered to have rich natural DE deposits, and there is clear evidence for the existence of large DE deposits in different regions of Turkey (Özbey & Atamer, 1987; Mete, 1988; Sıvacı & Dere, 2006; Çetin & Taş, 2012). Diatomite reserves of Turkey are about 125 Gt. Hırka (Kayseri) in Turkey has the largest diatomite reserve (106 Gt) (Çetin & Taş, 2012). However, there are only a few published reports on the potential use of local DEs against stored-product insect pests. Doğanay (2013) reported that 750 and 1000 ppm of a Turkish DE formulation (Turco 1) applied to wheat and paddy rice resulted in high mortalities of S*itophilus granarius* (L., 1758) (Coleoptera: Curculionidae) and *Rhyzopertha dominica* (F., 1792) (Coleoptera: Bostrichidae) (lesser grain borer) adults and significantly reduced progeny production, while 500 ppm and lower concentrations of Turco 1 had low efficacy against both tested insects and did not prevent reproduction. Akçalı et al. (2018) investigated efficacy of nine local DEs collected from different regions of Turkey against stored-grain insects, *Sitophilus oryzae* (L., 1763) (Coleoptera: Curculionidae) (rice weevil), *Tribolium confusum* du Val., 1863 (Coleoptera: Tenebrionidae) (confused flour beetle) and *R. dominica* and reported that CB2N-1 and BGN-1 local DE had high efficacy against *S. oryzae*, *T. confusum* and *R. dominica* adults and thus have potential to be successfully used for controlling stored-grain insect pests as a grain protectant. Sağlam et al. (2017) also evaluated some physical properties of local DEs collected from different locations of Turkey.

Different factors such as insect species, commodity, grain moisture and temperature are important for the efficiency of DEs in controlling stored-grain insects (Korunic, 1999; Fields & Korunic, 2000), and can be somewhat limiting for effective use of DEs in grain protection. Temperature is a crucial factor for the efficiency of DEs against stored-grain insects. Generally, increasing temperature increases the insecticidal efficiency of DE formulations against stored-grain insects such as *R. dominica* and *S. oryzae* (Fields & Korunic, 2000; Athanassiou et al., 2005). At higher temperatures, water loss of the insects is faster since insects are more mobile and more DE particles are adsorbed onto the cuticle (Fields & Korunic, 2000; Subramanyam & Roesli, 2000). Recent research has shown that DEs from different geographical locations can have different insecticidal activity, diatom species, pH, density, particle size distribution, internal surface area, lipid adsorption capability and effects on grain bulk density (Korunic, 1997). A few reports have indicated that DE deposits from Turkey are potentially effective for the control of stored-product insect pests (Doğanay, 2013; Sağlam et al., 2017; Akçalı et al., 2018). However, there is still inadequate information about the effectiveness of local DE mined from different locations of Turkey against stored-grain insects and the effect of abiotic factors (temperature and relative humidity) on the efficacy of these local DEs against stored-grain insects. The lack of information on toxic effects of these local DEs on stored-grain insects under different abiotic conditions justifies the study of these DE deposits for control of these insect pests. The objective of this study was to evaluate the efficiency of local DE formulation against *S. oryzae*, *R. dominica* and *T. confusum* adults in wheat at various temperatures.

Materials and Methods

Test insects

Sitophilus oryzae, T. confusum and *R. dominica* adults used in the bioassays were from stock cultures maintained in Entomology Laboratory of Plant Protection Department, Kahramanmaraş Sütçü İmam University. *Sitophilus oryzae* and *R. dominica* were reared on whole soft wheat with11% moisture content at 26±1ºC, 65±5% RH and 30±1ºC, 65±5% RH, respectively, and *T. confusum* were reared at 26±1ºC and 65±5% RH on a diet of wheat flour mixed with yeast (17:1, w/w) using standard culture techniques. Seven to 10 d-old adults of *S. oryzae*, *T. confusum* and *R. dominica* were exposed to DE treatments in bioassays.

Wheat cultivar

Untreated, clean, low admixture (0.8%) and infestation-free soft wheat (*Triticum aestivum* L., cv. Elbistan Yazlığı) was used in bioassays. One g of wheat corresponded to 21.3 individual grains. The moisture content of wheat used in bioassays, as determined by a Dickey-John moisture meter (Dickey-John Multigrain CACII, DICKEY-John Co., Lawrence, KS), ranged between 11.0 and 11.4%.

Local diatomaceous earth formulation

A local DE formulation (ACN-1) was used in bioassays. The DE was collected from diatomite reserves in Ankara Province located in Central Anatolia, Turkey. With the DE samples were crashed coarsely with a laboratory type knife hammer mill (LB 160, Mertest, Eskişehir, Turkey), they were oven dried oven (UF260 Memmert, Germany) at 100ºC for 48 h to give 3-4% moisture content. These samples were ground to powder using the same laboratory mill. With the DE powder was sieved by using metal sieve with 140 µm diameter (Retsch, Germany), natural grade local DE was obtained and used in bioassays. Some physical and chemical properties of the DE formulation (ACN-1) used in bioassays are given in Table 1 & 2.

Table 1. Some physical properties of local diatomaceous earth formulation (ACN-1)

DE formulation	Diatom type	Median particle Diameter (d (0.5)) (µm)[1]	Adherence of DE on wheat kernels (%)[2]	Percentage of crystalline silica (%)[3]	Colour
ACN-1	Freshwater	14.2	91.9	<3	Yellowish-white

[1] Median particle diameter value that corresponds to 50% of the total particle volume in the volumetric cumulative particle size distribution. Particle size analysis was conducted using laser light diffraction technique by Accredited Mineralogy and Petrography Laboratory of General Directorate of Mineral, Research and Exploration of Turkey.
[2] Tests for adherence rate of DE on wheat kernels were performed using method of Korunic (1997) in Stored Product Insects Laboratory of Kahramanmaraş Sütçü Imam University.
[3] Mineral/phase analysis of diatomaceous earth sample was performed using analytical Emperian X-Ray Diffraction device by the chemical-analytical laboratory of Advance Technologies Center of Kütahya Dumlupınar University.

Table 2. Total quantitative chemical analysis of local diatomaceous earth formulation (ACN-1)[1]

DE formulation	Loss on ignition (%)	Al_2O_3 (%)	CaO (%)	Fe_2O_3 (%)	K_2O (%)	MgO (%)	Na_2O (%)	SO_3 (%)	SiO_2 (%)	TiO_2 (%)
ACN-1	6.25	11.75	1.80	2.40	0.90	0.80	1.30	0.07	73.80	0.55

[1] Quantitative chemical analysis was conducted by Accredited Mineralogy and Petrography Laboratory of General Directorate of Mineral, Research and Exploration of Turkey. Atomic absorption spectroscopy device was also used in the analysis of the elements following melting and acid removal processes.

Bioassays

Bioassays were performed according to randomized parcel design with 3 x 5 factorial layout and with three replicates at three temperatures (20, 25 and 30±1ºC), 55±3% RH and five concentrations of local DE formulation (ACN-1): 100, 300, 500, 900 and 1500 ppm (mg DE/kg wheat). The desired relative humidity was maintained using saturated salt solution of magnesium nitrate as recommend by Greenspan (1977). For each experiment (temperature-DE concentration combination), five samples of 50 g wheat were taken. Each grain sample was placed in a small cylindrical self-standing centrifuge tube that was closed, apart from a hole 1.5 cm in diameter (at the top of the tube), and that was covered with muslin cloth to allow sufficient ventilation. The grain samples were treated individually with the respective DE concentration and then shaken manually for 5 min to achieve equal distribution of DE dust in the entire grain quantity. Three additional tubes containing untreated wheat served as control in each case. Subsequently, 30 1-wk-old adults of *S. oryzae* were introduced into each tube. The same procedure was followed with *T. confusum* and *R. dominica* adults. All tubes containing DE-treated wheat were placed in the lockable 80 l (26 × 36.5 × 15 cm) plastic container which contained saturated salt solution of magnesium nitrate under the plastic. The plastic containers were then placed in the incubator (IPP55 Plus, Memmert, Germany) set at the desired temperature. Three replicates were used for each trial (temperature-DE concentration combination). Dead adults of three species were counted 7 and 14 d after DE exposure. Temperature and relative humidity during bioassays were monitored by using HOBO digital recorders (HOBO H8, Onset Computers, Bourne, MA, USA).

Data analysis

Generally, for three species, the control mortality ranging from 0 to 7.7% was very low and therefore, no corrections were used. All mortality data for each species were normalized using arcsine transformation and then subjected to two-way ANOVA with main factors, temperature and DE concentration by using the GLM Procedure of SAS/STAT® 12.1 (SAS Institute, 2012). Mean mortality percentages for each species were separated by using the Duncan's multiple range test at 5% significance level.

Results

The ANOVA analysis for mortality of *S. oryzae* indicated significant differences for the main effects, DE concentration (7-d exposure $F_{4,30} = 126$, $P<0.0001$; 14-d exposure $F_{4,30} = 109$, $P < 0.0001$), temperature (for 7-d exposure $F_{2,30} = 37.8$, $P < 0.0001$; 14-d exposure $F_{2,30} = 26.5$, $P < 0.0001$) and DE concentration x temperature interaction (7-d exposure $F_{8,30} = 11.4$, $P < 0.0001$; 14-d exposure $F_{8,30} = 13.7$, $P < 0.0001$). With *S. oryzae* at each temperature, increasing DE concentration from 100 to 300 ppm and from 300 to 500 ppm at 20 and 25°C resulted in significant increases in mortality with 7-d and 14-d exposure, and increasing DE from 500 to 900 ppm and 1000 ppm at the three temperatures did not produce any significant increase in mortality with 7-d and 14-d exposure (Table 3). With 7-d exposure, complete mortality was obtained at 1500, 900 and 500 ppm DE at 20, 25 and 30°C, respectively. However, with 14-d exposure, compete mortality was obtained at 900, 500 and 500 ppm DE at 20, 25 and 30°C, respectively. With *S. oryzae,* temperature had no significant effect on mortality at 500, 900 and 1500 ppm DE with 7-d and 14-d exposure (Table 3). At 100 ppm DE, there was no significant difference in mortality of *S. oryzae* at 20 and 25°C, while mortality at 30°C was significantly higher than at 20 and 25°C. At 300 ppm DE, mortality at 30°C was significantly higher than at 25°C, while there was no significant difference between mortality at 20 and 30°C.

Table 3. Mean mortality (%) of *Sitophilus oryzae* adults exposed to wheat-treated with local diatomaceous earth at five concentrations and three temperatures with 7-d and 14-d exposure

Concentration (ppm)	Mean mortality rate (%)±S.E.			F and P value	Mean mortality rate (%)±S.E.			F and P value
	7.day				14. day			
	20ºC	25ºC	30ºC		20ºC	25ºC	30ºC	
100 ppm	16.6±1.9 Bc*	33.3±3.0 Bc	88.3±4.6 Ab	$F_{2,6}$=83.15 P<0.0001	22.1±3.0 Bc	46.5±4.1 Bc	90.3±6.3 Aa	$F_{2,6}$=23.70 P=0.0019
300 ppm	76.6±6.6 BAb	67.8±5.0 Bb	95.3±3.0 Aba	$F_{2,6}$=8.40 P=0.0182	93.1±0.0 BAb	81.4±7.0 Bb	98.7±1.2 Aa	$F_{2,6}$=7.81 P=0.0214
500 ppm	95.5±2.2 Aa	98.8±1.1 Aa	100.0±0.0 Aa	$F_{2,6}$=2.04 P=0.2111	98.8±1.1 Aa	100.0±0.0 Aa	100.0±0.0 Aa	$F_{2,6}$=1.00 P=0.4219
900 ppm	97.7±2.2 Aa	100.0±0.0 Aa	100.0±0.0 Aa	$F_{2,6}$=1.00 P=0.4219	100.0±0.0 Aa	100.0±0.0 Aa	100.0±0.0 Aa	-
1500 ppm	100.0±0.0 Aa	100.0±0.0 Aa	100.0±0.0 Aa	-	100.0±0.0 Aa	100.0±0.0 Aa	100.0±0.0 Aa	-
Control	0.0±0.0	3.3±1.9	4.4±1.1		4.4±1.1	4.4±1.1	7.7±1.1	
F and P value	$F_{4,10}$=50.77 P<0.0001	$F_{4,10}$=120 P<0.0001	$F_{4,10}$=8.20 P=0.0034		$F_{4,10}$=198.38 P<0.0001	$F_{4,10}$=63.71 P<0.0001	$F_{4,10}$=2.51 P=10.810	

* Two-way ANOVA was applied to the data. Means within a row with the same upper-case letter and a column with the same lowercase letter are not significantly different (Duncan test at 5% level).

With *T. confusum*, the analysis for mortality indicated significant differences for the main effects, DE concentration (7-d exposure $F_{4,30} = 527$, $P < 0.0001$; 14-d exposure $F_{4,30} = 712$, $P < 0.0001$), temperature (7-d exposure $F_{2,30} = 64.5$, $P < 0.0001$; 14-d exposure $F_{2,30} = 20.8$, $P < 0.0001$) and DE concentration x temperature interaction (7-d exposure $F_{8,30} = 12.4$, $P < 0.0001$; for 14-d exposure $F_{8,30} = 5.37$, $P = 0.0003$). At 20°C, mortality significantly increased with increasing DE concentration with 7-d exposure. At 25°C, there was no significant difference between mortality at 100 and 300 ppm DE, while increasing DE from 300 to 500, 900 and 1500 ppm resulted in significant increases in mortality. At 30°C, DE from 100 to 300 ppm and from 500 to 900 and 1500 ppm resulted in significant increase in mortality with 7-d exposure, while increasing DE from 900 to 1500 ppm did not produce significant increase in mortality. With 14-d

exposure, mortality of *T. confusum* at 100 ppm DE was significantly lower than at 300 ppm DE at all temperatures, while there were not significant differences between mortality at 500, 900 and 1500 ppm DE. With 14-d exposure, complete mortality of *T. confusum* was obtained with 1500, 900 and 500 ppm DE at 20, 25 and 30°C, respectively (Table 4). For *T. confusum* at 100 and 300 ppm DE, there was no significant temperature effect on mortality at 20 and 25°C, while mortality at 30°C was significantly higher than at 20 and 25°C with 7-d and 14-d exposure. At 900 and 1500 ppm DE, there was no significant difference in mortality at 25 and 30°C, while mortality at 25 and 30°C was significantly higher than at 20°C with 7-d exposure. With 14-d exposure, at 900 and 1500 ppm DE, there was no significant difference between mortality between the temperatures.

Table 4. Mean mortality (%) of *Tribolium confusum* adults exposed to wheat-treated with local diatomaceous earth at five concentrations and three temperatures with 7-d and 14-d exposure

Concentration (ppm)	Mean mortality rate (%)±S.E.			F and P value	Mean mortality rate (%)±S.E.			F and P value
	7.day				14. day			
	20ºC	25ºC	30ºC		20ºC	25ºC	30ºC	
100 ppm	0.0±0.0 Bc*	1.1±1.1 Bd	8.8±2.2 Ad	$F_{2,6}$=14.49 P=0.0050	13.3±3.3 Bc	14.4±4.1 Bc	55.5±4.8 Ab	$F_{2,6}$=43.96 P=0.0003
300 ppm	3.3±1.9 Bc	1.1±1.1 Bd	46.6±3.3 Ac	$F_{2,6}$=39.10 P=0.0004	58.8±2.9 Bb	62.2±4.8 Bb	94.4±2.9 Aa	$F_{2,6}$=17.89 P=0.0030
500 ppm	23.3±3.3 Cb	48.8±2.9 Bc	85.5±6.1 Ab	$F_{2,6}$=37.00 P=0.0004	90±3.8 Ba	97.7±1.1 ABa	100.0±0.0 Aa	$F_{2,6}$=8.56 P=0.0175
900 ppm	28.8±2.2 Bb	90±1.9 Ab	97.7±2.2 Aa	$F_{2,6}$=73.89 P<0.0001	93.3±5.0 Aa	100.0±0.0 Aa	100.0±0.0 Aa	$F_{2,6}$=2.74 P=0.1430
1500 ppm	87.7±5.5 Ba	100±0.0 Aa	100±0.0 Aa	$F_{2,6}$=17.85 P=0.0030	100±0.0 Aa	100.0±0.0 Aa	100.0±0.0 Aa	-
Control	0.0±0.0	0.0±0.0	0.0±0.0		0.0±0.0	0.0±0.0	0.0±0.0	
F and P value	$F_{4,10}$=77.58 P<0.0001	$F_{4,10}$=248.83 P<0.0001	$F_{4,10}$=81.32 P<0.0001		$F_{4,10}$=49.90 P<0.0001	$F_{4,10}$=207.08 P<0.0001	F_{410}=41.10 P<0.0001	

* Two-way ANOVA was applied to the data. Means within a row with the same upper-case letter and a column with the same lower-caseletter are not significantly different (Duncan test at 5% level).

With *R. dominica*, the analysis for mortality indicated significant differences for the main effects, DE concentration (7-d exposure $F_{4,30}$ = 339, P<0.0001; 14-d exposure $F_{4,30}$ = 191, P<0.0001), temperature (7-d exposure $F_{2,30}$ = 87.3, P<0.0001; 14-d exposure $F_{2,30}$ = 33.9, P<0.0001) and DE concentration x temperature interaction (7-d exposure $F_{8,30}$ = 10.7, P<0.0001; 14-d exposure $F_{8,30}$ = 3.80, P = 0.0035). In most cases, the increasing DE concentration increased mortality of *R. dominica* (Table 5). With 7-d exposure at 20ºC, and with 14-d exposure at 20 and 30ºC, increasing DE concentration from 100 to 300 ppm resulted in a significant increase in mortality. With 7-d and 14-d exposure at 30ºC, mortalities at 900 and 1500 ppm were significantly higher than at all other DE concentrations, and at 25ºC, mortality at 1500 ppm was significantly higher than at all other concentrations. Complete mortality of *R. dominica* was only recorded at 20ºC at 1500 ppm DE with 14-d exposure (Table 5). For *R. dominica* at 100 ppm, there was no significant effect of temperature with 7-d and 14-d exposure. At the concentrations above 100 ppm, mortality at 20ºC was significantly higher than at 25ºC with 7-d and 14-d exposure. At all concentrations, 20 and 30ºC gave similar mortality with 7-d and 14-d exposure, other than at 300 and 500 ppm with 7-d exposure.

Table 5. Mean mortality (%) of *Rhyzopertha dominica* adults exposed to wheat-treated with local diatomaceous earth at five concentrations and three temperatures with 7-d and 14-d exposure

Concentration (ppm)	Mean mortality rate (%)±S.E			F and P value	Mean mortality rate (%)±S.E			F and P value
	7. day				14. day			
	20ºC	25ºC	30ºC		20ºC	25ºC	30ºC	
100 ppm	3.3±1.9 Ad*	0.0±0.0 Ad	0.0±0.0 Ac	$F_{2,6}$=3.67 P=0.0912	5.9±3.1 Ad	1.8±1.8 Ad	0.0±0.0 Ad	$F_{2,6}$=3.40 P=0.1032
300 ppm	20.0±5.0 Ac	3.3±1.9 Bdc	0.0±0.0 Bc	$F_{2,6}$=16.5 P=0.0036	30.6±4.2 Ac	8.9±3.8 Bdc	18.4±2.2 BAc	$F_{2,6}$=7.57 P=0.0228
500 ppm	77.7±2.9 Ab	6.6±1.9 Cc	43.2±6.2 Bb	$F_{2,6}$=74.84 P<0.0001	87±4.7 Ab	20.2±2.9 Bc	66.6±6.9 Ab	$F_{2,6}$=32.06 P=0.0006
900 ppm	87.7±2.2 Aba	54.4±2.2 Bb	83.1±3.3 Aa	$F_{2,6}$=36.69 P=0.0004	95.2±3.1 Aba	71.9±2.2 Bb	90.8±3.0 BAa	$F_{2,6}$=8.75 P=0.0166
1500 ppm	95.5±2.2 Aa	75.5±2.9 Ba	87.6±1.1 BAa	$F_{2,6}$=9.71 P=0.0132	100.0±0.0 Aa	89.8±3.3 Aa	94.2±4.1 Aa	$F_{2,6}$=1.67 P=0.2654
Control	0.0±0.0	0.0±0.0	1.1±1.1		5.5±1.1	1.1±1.1	3.3±0.0	
F and P value	$F_{4,10}$=71.34 P<0.0001	$F_{4,10}$=113.76 P<0.0001	$F_{4,10}$=284.7 P<0.0001		$F_{4,10}$=71.17 P<0.0001	$F_{4,10}$=53.29 P<0.0001	$F_{4,10}$=75.96 P<0.0001	

* Two-way ANOVA was applied to the data. Means within a row with the same upper-case letter and a column with the same lowercase letter are not significantly different (Duncan test at 5% level).

Discussion

Insecticidal efficacy of DE is highly influenced by several factors including temperature and DE formulation and concentration (Kavallieratos et al., 2007). The present study indicated that temperature had a significant effect on the insecticidal efficacy of local DE when tested on stored-grain insects. The temperature effects on insecticidal efficacy varied with insect species and DE concentration. Mortality of *S. oryzae* and *T. confusum* adults generally increased with increasing temperature and mortality at 30ºC was significantly higher than at 20ºC and 25ºC. Studies of the influence of temperature on efficiency of some commercial DEs against *S. oryzae* and *T. confusum* adults indicated that increasing temperature generally resulted in increasing insecticidal efficiency against *S. oryzae* adults (Fields & Korunic, 2000; Arthur, 2002; Athanassiou et al., 2005; Vassilakos et al., 2006; Rojht et al., 2010). Arthur (2000) reported that *T. castaneum* and *T. confusum* exposed directly to the DE formulation, Protec-It, at controlled temperatures showed a progressive increase in mortality as temperature increased from 22 to 27 and 32°C. Similarly, Vassilakos et al. (2006) reported that insecticidal efficacy of the commercial DE formulation, Silisosec®, against *S. oryzae* adults increased with increasing temperature. These results parallel those obtained in the present study of local DE. This could be attributed to the fact that at higher temperatures insects are usually more mobile and the possibility of picking up dust particles is increased (Arthur, 2000, 2001; Fields & Korunic, 2000; Rigaux et al., 2001). In addition, water loss is likely to be increased at higher temperatures (Arthur, 2000; Fields & Korunic, 2000). Also, the rate of cuticular transpiration rises only slightly with temperature until the transition temperature, which for most insects is above 30°C (Wigglesworth, 1972). However, increased temperature would also increase feeding and therefore moisture replacement through the food and production of metabolic water. The synthesis of cuticular waxes may be faster at higher temperatures because of temperature effects on the biochemical pathways. This positive increase in toxicity with temperature is also similar to data reported for exposure studies with organophosphate insecticides (Turnbull & Harris, 1986).

In the present study, inconsistent results for *R. dominica* were obtained regarding the effect of temperature on the insecticidal efficacy of the local DE. The mortality at 20ºC was significantly higher than at 25ºC and 30ºC. It is clear that there is not a positive correlation between temperature and mortality of *R. dominica* adults exposed to the local DE. Inconsistent results have been reports for the effect of temperature on the insecticidal efficacy of DEs against *R. dominica* adults. Several studies regarding efficiency of some commercial DEs against *R. dominica* adults indicated that increasing temperature generally resulted in increasing mortality (Fields & Korunic, 2000; Athanassiou et al., 2005; Vassilakos, 2006). These results do not parallel those obtained in present study of local DE. However, while using the DE formulation, Protec-It, Vardeman et al. (2006) noted that temperature did not significantly affect mortality of *R. dominica* adults. These results parallel those obtained in present study.

Studies of several commercially available DEs demonstrated that a satisfactory level of grain protection is achieved with application rates that are much lower than 1000 ppm (Arthur, 2000; Fields & Korunic, 2000; Arthur & Throne, 2003; Athanassiou et al., 2003, 2005; Ceruti et al., 2008; Kljajic et al., 2010; Athanassiou et al., 2014; Baldassari & Martini, 2014; Nesvorna & Hubert, 2014). Athanassiou et al. (2011) noted that DEs mined from several parts of Europe were effective at 900 ppm. In the present study, complete mortality was obtained against adults of *S. oryzae* and *T. confusum* with concentrations ranging from 500 to 900 ppm with 14-d DE exposure. However, complete mortality of *R. dominica* was obtained only at 1500 ppm DE at 20°C. Generally, lower efficacy of local DE against *R. dominica* adults was observed at all tested temperatures compared with those reported for commercial DE formulations (Silicosec, Insecto and others). This difference can be attributed to physical, morphological and chemical characters of the local DE formulation, and internal characteristics or physical properties of the wheat cultivar used. Other studies have shown that *S. oryzae* is the most DE-susceptible, followed by *R. dominica* and *T. confusum* (Korunic, 1998; Arthur, 2000; Subramanyam & Roesli, 2000; Fields & Korunic, 2000; Kavallieratos et al., 2005; Athanassiou et al., 2014). In the present study, *S. oryzae* was the most DE-susceptible, followed by *T. confusum* and *R. dominica*. It seems that *R. dominica* was the most tolerant species to the local DE. *Rhyzopertha dominica* adults are not very agile (Flinn & Hagstrum, 2011), so the possibility of picking up DE particles is decreased and this might be why this species is among the most DE-tolerant species (Korunic, 1998; Fields & Korunic, 2000). Kavallieratos et al. (2005) also reported that 750 ppm DEs (SilicoSec and Insecto) were needed to obtain high mortality of *R. dominica* adults in wheat and maize.

The results of the present study indicated that complete mortality of *T. confusum* achieved at lower concentrations, ranging from 500 to 900 ppm. This is particularly important, since other studies with other DEs suggest that the same mortality can be achieved with 1000 ppm or higher (Aldryhim, 1990; Athanassiou et al., 2004, 2005; Vayias & Athanassiou, 2004). In conclusion, the present study indicated that temperature had significant effect on the insecticidal efficacy of a local DE against stored-grain insects. Generally, higher temperature increased the efficacy of the local DE with an exception of species of *R. dominica*, which showed greater tolerance at 30°C than that at 20°C. Therefore, temperature effects on insecticidal efficacy of the DE tested varied with insect species and concentration of DE. Moreover, based on the results of the bioassays in this study, the local DE (ACN-1) has potential to be used for control of stored-grain insects in wheat. Additional studies are required to determine the effects of biotic and abiotic factors on efficacy of the local DE deposit against other stored-grain insects and then to evaluate its insecticidal performance under field conditions.

References

Akçalı, S., A. A. Işıkber, Ö. Sağlam, H. Tunaz & M. K. Er, 2018. “Laboratory evaluation of Turkish diatomaceous earths as potential stored grain protectants, 739-743”. Proceedings of Twelfth International Working Conference on Stored Product Protection (IWCSPP) (7-11 October 2018, Berlin, Germany), 1130 pp.

Aldryhim, Y. N., 1990. Efficacy of the amorphous silica dust, Dryacide, against *Tribolium confusum* Jacquelin du Val and *Sitophilus oryzae* (L.) (Coleoptera: Tenebrionidae and Curculionidae). Journal of Stored Products Research, 26: 207-210.

Arthur, F. H., 1996. Grain protectants: current status and prospects for the future. Journal of Stored Products Research, 32: 293-302.

Arthur, F. H., 2000. Toxicity of diatomaceous earth to red flour beetles and confused flour beetles (Coleoptera: Tenebrionidae): effects of temperature and relative humidity. Journal of Economic Entomology, 93 (2): 526-532.

Arthur, F. H., 2001. Immediate and delayed mortality of *Oryzaephilus surinamensis* (L.) exposed on wheat treated with diatomaceous earth: effects of temperature, relative humidity, and exposure interval. Journal of Stored Products Research 37: 13-21.

Arthur, F. H., 2002. Survival of *Sitophilus oryzae* (L.) on wheat treated with diatomaceous earth: impact of biological and environmental parameters on product efficacy. Journal of Stored Products Research, 38 (3): 305-313.

Arthur, F. H. & J. E. Throne, 2003. Efficacy of diatomaceous earth to control internal infestation of rice weevil and maize weevil (Coloeoptera: Curculionidae). Journal of Economic Entomology, 96: 510-518.

Athanassiou, C. G., N. G. Kavallieratos & N. S. Andris, 2004. Insecticidal effect of three diatomaceous earth formulations against adults of *Sitophilus oryzae* (Coleoptera: Curculionidae) and *Tribolium confusum* (Coleoptera: Tenebrionidae) on oat, rye and triticale. Journal of Economic Entomology, 97: 2160-2167.

Athanassiou, C. G., N. G. Kavallieratos & F. A. Lazzari, 2014. Insecticidal effect of Keepdry® for the control of *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) and *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) on wheat under laboratory conditions. Journal of Stored Products Research, 59: 133-139.

Athanassiou, C. G., N. G. Kavallieratos, F. C. Tsaganou, B. J. Vayias, C. B. Dimizas & C. Th. Buchelos, 2003. Effect of grain type on the insecticidal efficacy of SilicoSec® against *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae). Crop Protection, 22: 1141-1147.

Athanassiou, C. G., N. G. Kavallieratos, B. J. Vayias, Z. Tomanovi´c, A. Petrovi´c, V. Rozman, C. Adler, Z. Korunic & D. Milovanovi´c, 2011. Laboratory evaluation of diatomaceous earth deposits mined from several locations in central and southeastern Europe as potential protectants against coleopteran grain pests. Crop Protection, 30: 329-339.

Athanassiou, C. G., B. J. Vayias, C. B. Dimizas, N. G. Kavallieratos, A. S. Papagregoriou & C. Th. Buchelos, 2005. Insecticidal efficacy of diatomaceous earth against *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) and *Tribolium confusum* du Val (Coleoptera: Tenebrionidae) on stored wheat: influence of dose rate, temperature and exposure interval. Journal of Stored Products Research, 41: 47-55.

Baldassari, N. & A. Martini, 2014. The efficacy of two diatomaceous earths on the mortality of *Rhyzopertha dominica* and *Sitophilus oryzae*. Bulletin of Insectology, 67: 51-55.

Ceruti, F. C., S. M. N. Lazzari, F. A. Lazzari & A. R. Pinto Junior, 2008. Efficacy of diatomaceous earth and temperature to control the maize weevil in stored maize. Scientia Agraria, 9: 73-78.

Çetin, M. & B. Taş, 2012. A natural mineral with biological origin: Diatomite. Turkish Science-Research Foundation (Türk Bilim Araştırma Vakfı (TÜBAV) Science Journal, 5 (2): 28-46.

Doğanay, Ş. İ., 2013. Determination of Efficiency of Some Diatomaceous Earths against to Stored-Grain Insects, *Sitophilus granarius* (L.) and *Rhyzopertha dominica* (F.). Kahramanmaraş Sütçü İmam University, Institute of Natural and Applied Science, Plant Protection Department (Unpublished) Master Thesis, Kahramanmaraş, Turkey, 55 pp.

FDA (Food and Drug Administration, USA), 1995. Specifications for diatomaceous earths as a maximum 2% animal feed additive. 21 CFR Section 573.340.

Fields, P. & Z. Korunic, 2000. The effect of grain moisture content and temperature on the efficacy of diatomaceous earths from different geographical locations against stored-product beetles. Journal of Stored Products Research, 36: 1-13.

Flinn, P. W. & D. W. Hagstrum, 2011. Movement of *Rhyzopertha dominica* in response to temperature gradients in stored wheat. Journal of Stored Products Research, 47: 407-410.

Greenspan, L., 1977. Humidity fixed points of binary saturated aqueous solutions. Journal of Research of the National Bureau of Standards- A. Physics and Chemistry, 81A (1): 89-96.

Hill, D. D., 2002. Pests of Stored Foodstuffs and Their Control. Kluwer Academic Publisher, Netherlands, 478 pp.

Kavallieratos, N. G., C. G. Athanassiou, B. J. Vayias & S. N. Maistrou, 2007. Influence of temperature onsusceptibility of *Tribolium confusum* (Coleoptera: Tenebrionidae) populations to three modifieddiatomaceous earth formulations. Florida Entomologist, 90: 616-625.

Kavallieratos, N. G., C. G. Athanassiou, F. G. Pashalidou, N. S. Andris & Z. Tomanovic, 2005. Influence of grain type on the insecticidal efficacy of two diatomaceous earth formulations against *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae). Pest Management Science, 61: 660-666.

Kljajic, P., G. Andric, M. Adamovic, M. Bodroza-Solarov, M. Markovic & I. Peric, 2010. Laboratory assessment of insecticidal effectiveness of natural zeolite and diatomaceous earth formulations against three stored-product beetle pests. Journal of Stored Products Research, 46: 1-6.

Korunic, Z., 1997. Rapid assessment of the insecticidal value of diatomaceous earths without conducting bioassays. Journal of Stored Products Research, 33: 219-229.

Korunic, Z., 1998. Diatomaceous earths, a group of natural insecticides. Journal of Stored Products Research, 34: 87-97.

Korunic, Z., 1999. Enhanced diatomaceous earth: an alternative to methyl bromide. Australian Journal of Technology, 2: 95-104.

Mete, Z., 1988. Enrichment of diatomite reserve in Kutahya-Alayunt region. Journal of The Mediterranean University Isparta Engineering Faculty, 1: 184-201.

Nesvorna, M. & J. Hubert, 2014. Effect of diatomaceous earth-treated wheat on population growth of stored product mites under laboratory test. International Journal of Acarology, 40: 269-273.

Özbey, G. & N. Atamer, 1987. "Some knowledge on Kizelgur (Diatomite), 493-502". 10th Turkish Scientific and Technical Congress of Mining (11-15 May 1988, Ankara, Turkey) 550 pp.

Rees, D., 2004. Insects of Stored Products. CSIRO Publishing, Collingwood VIC, Australia. 181 pp.

Rigaux, M., E. Haubruge & P. G. Fields, 2001. Mechanisms for tolerance to diatomaceous earth between strains of *Tribolium castaneum*. Entomologia Experimentalis et Applicata, 101: 33-39.

Rojht, H., A. Horvat, C. G. Athanassiou, B. J. Vayias, Ž. Tomanović & S. Trdan, 2010. Impact of geochemical composition of diatomaceous earth on its insecticidal activity against adults of *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae). Journal of Pest Science, 83 (4): 429-436.

Sağlam, Ö., A. A. Işıkber, H. Tunaz, M. K. Er, F. Bahadır & R. Şen, 2017. Preliminary checking of some Turkish diatomaceous earth similarities with commercial diatomaceous earths under scanning electron microscope (SEM). Journal of Tekirdağ Agriculture Faculty (JOTAF), The Special Issue of the Second International Balkan Agriculture Congress (16-18 May 2017, Tekirdağ, Turkey): 13-19.

SAS Institute Inc., 2012. SAS / STAT® User's Guide, Version 12.1, 2nd Ed. SAS Institute Inc., Cary, NC. 21 pp.

Sıvacı, R. & Ş. Dere, 2006. Seasonal change of Diatomic flora of Melendiz Stream. Ç.U. Science and Art Faculty Journal of Science, 27 (1): 1-12.

Subramanyam, B. & D. W. Hagstrum, 1995. "Resistance Measurement and Management, 331-398". In: Integrated Management of Insects in Stored Products (Eds. Bh. Subramanyam & D. W. Hagstrum). Marcel Decker Inc., New York, USA, 432 pp.

Subramanyam, B. & R. Roesli, 2000. "Inert Dust, 321-379". In: Alternatives to Pesticides in Stored-Product IPM (Eds. Bh. Subramanyam & D. W. Hagstrum). Kluwer Academic Publishers, Springer, Boston, USA, 437 pp.

Turnbull, S. A. & C. R. Harris, 1986. Influence of post treatment temperature on the contact toxicity of ten organophosphorus and pyrethroid insecticides to onion maggot adults (Diptera: Anthomyiidae). Proceedings of the Entomological Society of Ontario, 117: 41-44.

Vardeman, E. A., F. H. Arthur, J. R. Nechols & J. F. Campbell, 2006. Effect of temperature, exposure interval and depth of diatomaceous earth on distribution, mortality, and reproduction of the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) in stored wheat. Journal of Economic Entomology, 99: 1017-1024.

Vassilakos, T. N., C. G. Athanassiou, N. G. Kavallieratos & B. J. Vayias, 2006. Influence of temperature on the insecticidal effect of *Beauveria bassiana* in combination with diatomaceous earth against *Rhyzopertha dominica* and *Sitophilus oryzae* on stored wheat. Biological Control, 38 (2): 270-281.

Vayias, B. J. & C. G. Athanassiou, 2004. Factors affecting the insecticidal efficacy of the diatomaceous earth formulation SilicoSec® against adults and larvae of the confused flour beetle, *Tribolium confusum* du Val. (Coleoptera: Tenebrionidae). Crop Protection, 23 (7): 565-573.

Wigglesworth, V. B., 1972. The Principles of Insect Physiology. London: Chapman & Hall, London, 827 pp.

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Original article (Orijinal araştırma)

A new Diptera family (Pallopteridae Loew, 1862) for the fauna of Turkey with four new records

Türkiye faunası için dört yeni kayıtla yeni bir Diptera familyası (Pallopteridae Loew, 1862)

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Abstract

Family Pallopteridae Loew, 1862 is a small Diptera family which is well known in the Palearctic Region. Species of this family have been reported especially in the many countries of Europe. However, there has been no study of the distribution of the family Pallopteridae in Turkey. This study was based on fly specimens collected from Amasya, Çorum and Sinop Provinces of Turkey between 2017 and 2018. Four species [*Palloptera ustulata* Fallen, 1820, *Palloptera umbellatarum* (Fabricius, 1775), *Toxoneura basimaculata* (Czerny, 1934) and *Toxoneura trimacula* (Meigen, 1826)] of Pallopteridae (Diptera) recorded for the first time in Turkey. A key for the Turkish Pallopteridae species, zoogeographic distribution, and figures of wing and adult of each species are included.

Keywords: Fauna, new record, *Palloptera*, Pallopteridae, *Toxoneura*, Turkey

Öz

Pallopteridae Loew, 1862 familyası Palearktik bölgede iyi bilinen küçük bir sinek ailesidir. Özellikle Avrupa'nın birçok ülkesinde bu familyaya ait türlerin varlığı bildirilmiştir. Bununla birlikte Pallopteridae familyasının Türkiye'deki yayılışı ile ilgili bir çalışma bulunmamaktadır. Bu çalışma, Türkiye'den Amasya, Çorum ve Sinop illerinden, 2017 ve 2018 yılları arasında toplanan sinek örneklerine dayanmaktadır. Pallopteridae (Diptera) familyasının dört türü [*Palloptera ustulata* Fallen, 1820, *Palloptera umbellatarum* (Fabricius, 1775), *Toxoneura basimaculata* (Czerny, 1934) ve *Toxoneura trimacula* (Meigen, 1826)] Türkiye'de ilk kez kaydedilmiştir. Türkiye Pallopteridae türleri için teşhis anahtarı, her bir türün zoocoğrafik yayılışları ve ergin ve kanat fotoğrafları sunulmuştur.

Anahtar sözcükler: Fauna, yeni kayıt, *Palloptera*, Pallopteridae, *Toxoneura*, Türkiye

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Introduction

The order Diptera one of the largest insect orders, including 159 294 species worldwide (Pape et al., 2011). According to Koçak & Kemal (2013), 2 992 species of fly have been recorded from Turkey.

Tephritoidea is one of the large superfamilies consisting of 10 acalyptrate fly families (Ctenostylidae, Eurygnathomyiidae, Lonchaeidae, Pallopteridae, Piophilidae, Platystomatidae, Pyrgotidae, Richardiidae, Tephritidae and Ulidiidae) including over 7750 valid species worldwide (Korneyev, 1999; Han & Ro, 2005; Pape et al., 2011). The family Pallopteridae is a sister group of Richardiidae and Piophilidae in the superfamily Tephritoidea (Korneyev, 1999).

In the superfamily Tephritoidea, Tephritidae is one of the better-known families in Turkey with more than 160 species recorded and described (Kütük, 2009; Kütük et al., 2011, 2012; Yaran & Kütük, 2014, 2015, 2016; Korneyev & Kolcsar, 2015; Namin & Korneyev, 2015; Yaran et al., 2018a, b; Keçe et al., 2019). Also, the families Platystomatidae and Ulidiidae families have nearly 20 species in Turkey (Mesci & Hasbenli, 2015a, b). The family Pallopteridae includes 71 valid species in worldwide. This family also includes five fossil species (Gentilini et al., 2006; Pape et al., 2011). Thirty-nine species of Pallopteridae are currently recognized in the Palearctic Region (Han, 2013). Members of Pallopteridae known for their small to medium sized (about 2.5-7 mm), are slender flies varying in color from pale yellow to reddish, gray or black (Oosterbroek, 2006). According to Korneyev (1999), they usually infest shoots and stems of herbaceous plants, or live under bark, often in association with wood-boring Coleoptera.

The main purpose of this study was to contribute to the Diptera fauna of Turkey. Currently, there is no published report on the presence of the family Pallopteridae in Turkey.

This study is the first to record four members [*Palloptera ustulata* Fallen, 1820, *Palloptera umbellatarum* (Fabricius, 1775), *Toxoneura basimaculata* (Czerny, 1934) and *Toxoneura trimacula* (Meigen, 1826)] of the Pallopteridae. In the paper, a key for Turkish Pallopteridae species, zoogeographic distribution, and wing and adult photos of each species are presented.

Materials and Methods

Thirteen specimens were collected from various habitats in Amasya, Çorum and Sinop Provinces of Turkey in the summers of 2017 and 2018 (Figure 1). Fly samples were collected randomly from possible host plants using an insect net. After the collection, the flies were killed in jars containing ethyl acetate. The fruit flies collected were brought to the laboratory and prepared by standard museum methods. Thus, all the specimens were made ready for the identification to the species level. The specimens were identified according to McAlpine (1981), Merz (1998), Ozerov (2009) and Han (2013). Identification key for Turkish Pallopteridae species modified from Ozerov (2009). Specimens were deposited in Entomology Laboratory of Biology Department, Faculty of Science and Arts, Gaziantep University, Gaziantep, Turkey.

Figure 1. Distribution of the sampling sites in Turkey.

Results

Four species of the Pallopteridae were collected and identified from Amasya, Çorum and Sinop Provinces of Turkey. Species are listed below alphabetical order. Also, an identification key for the species in Turkey is included.

Key for Turkish Pallopteridae species

1. Anepisternum bare.....2 (*Palloptera,* Fallen)

- Anepisternum with bristles or setulae, or both .....3 (*Toxoneura,* Macquart)

2. Wings hyaline, at apex with dark smoky spot (Figure 2c, d)..... *Palloptera ustulata* Fallen

- The wing is darkened along the costal margin, including the Sc cell, at the apex of the veins R_{4+5} and M, and also with spots at the base of the veins R_{2+3} and R_{4+5} (Figure 2a, b).... *Palloptera umbellatarum* (Fabricius)

3. Veins M and Cu are darkened at base (Figure 3a, b)..... *Toxoneura basimaculata* (Czerny)

- Veins M and Cu are light at base (Figure 3c, d) ..... *Toxoneura trimacula* (Meigen)

Genus *Palloptera* Fallen, 1820

Palloptera umbellatarum (Fabricius, 1775) (Figure 2a, b)

Specimens examined. Çorum: Bayat, Kunduzlu, 26.VII.2017, 40°45' N, 34°14' E, 1360 m, 1 ♀, 2 ♂♂, leg. M. Yaran.

Remarks. Biology of the species is unknown. According to Rotheray (2014), larvae are found under bark of fallen *Tilia*. Adult members of *P. umbellatarum* were collected from a humid and wet habitat with abundant *Petasites hybridus* (L.) Gaertner on the floor of a *Cedrus libani* A. Richard forest (Figure 4).

Global distribution. Andorra, Austria, Belgium, Britain, Bulgaria, Corsica, Czech Republic, Danish mainland, Finland, French mainland, Germany, Hungary, Ireland, Italian mainland, Liechtenstein, Northern Ireland, Northwest European Russia, Norwegian mainland, Poland, Romania, Sicily, Slovakia, Spanish mainland, Sweden, Switzerland, The Netherlands and Ukraine (Merz, 2011).

Figure 2. Adult and wing figures of *Palloptera* species: a) *Palloptera umbellatarum* (♂), b) wing; c) *Palloptera ustulata* (♀), d) wing.

Palloptera ustulata Fallen, 1820 (Figure 2c, d)

Specimens examined. Amasya: Gümüşhacıköy, Sekü, 27.VII.2017, 40°57' N, 36°06' E, 809 m, 2 ♀♀, 1 ♂, leg. M. Yaran.

Remarks. Larvae develop beneath the bark of the tress where they probably feed on mycelium (Merz, 1998). Adult members of *P. ustulata* were collected from a humid and wet habitat with abundant *P. hybridus* and some gramineous species.

Global distribution. Andorra, Austria, Belgium, Britain, Bulgaria, Corsica, Czech Republic, Danish mainland, Finland, French mainland, Germany, Hungary, Ireland, Italian mainland, Liechtenstein, Northern Ireland, Northwest European Russia, Norwegian mainland, Poland, Romania, Sicily, Slovakia, Spanish mainland, Sweden, Switzerland, The Netherlands and Ukraine (Merz, 2011).

Genus *Toxoneura* Macquart, 1835

Toxoneura basimaculata (Czerny, 1934) (Figure 3a, b)

Specimens examined. Çorum: Bayat, Kunduzlu, 26.VII.2017, 40°45' N, 34°14' E, 1360 m, 3 ♂♂, leg. M. Yaran.

Remarks. Adult members of *T. basimaculata* were collected from a humid and wet habitat with abundant *P. hybridus* on the floor of a *C. libani* forest (Figure 4).

Global distribution. Austria, Croatia, Czech Republic, East Palearctic, Germany, Hungary, Italian mainland, Near East and Poland (Merz, 2011).

Figure 3. Adult and wing figures of *Toxoneura* species: a) *Toxoneura basimaculata* (♂), b) wing; c) *Toxoneura trimacula* (♂), d) wing.

Toxoneura trimacula (Meigen, 1826) (Figure 3c, d)

Specimens examined. Çorum: İskilip, Ahlatçık, 31.VII.2017, 40°46' N, 34°18' E, 1374 m, 1 ♂, leg. M. Yaran; Sinop: Ayancık, Gökçukur, 17.VII.2017, 41°39' N, 34°40' E, 866 m, 1 ♂, leg. M. Yaran; Ayancık, Gökdere, 21.VI.2018, 41°39' N, 34°40' E, 801 m, 2 ♂♂, leg. M. Yaran.

Remarks. Larvae of species develop in the stems of *Heracleum* sp. (Chandler 1991) and *Angelica sylvestris* (Rotheray, 2014). Adult members of *T. trimacula* were collected from a humid and wet habitat with abundant *P. hybridus* on the floor of a *C. libani* forest (Figure 4).

Global distribution. Austria, Belgium, Bosnia and Herzegovina, Britain, Czech Republic, Danish mainland, Faroe Is., Finland, French mainland, Germany, Hungary, Iceland, Ireland, Italian mainland, Lithuania, Northern Ireland, Northwest European Russia, Norwegian mainland, Poland, Romania, Slovakia, Slovenia, Spanish mainland, Sweden, Switzerland, The Netherlands and Ukraine (Merz, 2011).

Figure 4. Habitats of the Pallopteridae species collected: a, b) Çorum, Bayat, Kunduzlu, c) Çorum, İskilip, Ahlatçık, d) Sinop, Ayancık, Gökçukur.

Discussion

The family Pallopteridae includes 71 valid species worldwide and 39 species distributed in the Palearctic Region (Pape et al., 2011; Han, 2013). There is no information about distribution of this family in Turkey. This is the first record of the family Pallopteridae in Turkey. In the study, four species of Pallopteridae (*P. ustulata*, *P. umbellatarum*, *T. basimaculata* and *T. trimacula*) were identified for the first time.

Turkey is the only country covered almost entirely by three of the 34 global biodiversity hotspots: the Caucasus, Irano-Anatolian and the Mediterranean. At the nexus of Europe, the Middle East, Central Asia and Africa, Turkey's location, mountains, and its encirclement by three seas have resulted in spectacular biodiversity, making Turkey "the biodiversity superpower of Europe" (Şekercioğlu, 2011). It is clear that Turkey has many different biotopes and ecological conditions. Many species, genera and families will be waiting to be discovered in Turkey, and some species might become extinct before they can be collected and described.

References

Gentilini, G., V. A. Korneyev & E. P. Kameneva, 2006. Fossil tephritoid flies (Diptera: Pallopteridae, Ulidiidae, Tephritidae) from the Upper Miocene of Monte Castellaro, Italy, and a review of fossil European tephritoids. Instrumenta Biodiversitatis, 7: 85-104.

Han, H. Y., 2013. A Checklist of the families Lonchaeidae, Pallopteridae, Platystomatidae, and Ulidiidae (Insecta: Diptera: Tephritoidea) in Korea with notes on 12 species new to Korea. Animal Systematics, Evolution and Diversity, 29 (1): 56-69.

Han, H. Y. & K. E. Ro, 2005. Molecular phylogeny of the superfamily Tephritoidea (Insecta: Diptera): new evidence from the mitochondrial 12S, 16S, and COII genes. Molecular Phylogenetics and Evolution, 34: 416-430.

Keçe, A. F. Ç., B. Ö. Çatal & M. R. Ulusoy, 2019. A new invasive species in Turkey: *Dacus ciliatus* Loew, 1862 (Diptera: Tephritidae). Turkish Journal of Entomology, 43 (1): 25-30.

Koçak, A. Ö. & M. Kemal, 2013. Diptera of Turkey. Priamus (Suppl.), 28: ii+ 1-411.

Korneyev, V. A. & L. P. Kolcsar, 2015. First records of Trypetini (Diptera: Tephritidae: Trypetinae) from Europe. Ukrainska Entomofaunistyka, 6 (3): 34.

Korneyev, V. A., 1999. "Phylogenetic Relationships among Higher Groups of the Superfamily Tephritoidea, 3-22". In: Fruit Flies (Tephritidae): Phylogeny and Evolution of Behavior (Eds. M. Aluja & A. L. Norrbom). CRC Press, Boca Raton, XVI + 944 pp.

Kütük, M., 2009. A new species of Urophora Rob-Des, 1830 (Diptera: Tephritidae) from Turkey. Entomological News 120 (1): 101-105.

Kütük, M., N. Bayrak & R. Hayat, 2011. A new species of Terellia (Diptera: Tephritidae) from Turkey with a key to Turkish species of the subgenus Cerajocera. Turkish Journal of Entomology, 35 (2): 207-213.

Kütük, M., N. Bayrak & R. Hayat, 2012. A new species of Tephritis Latreille (Diptera: Tephritidae) from Turkey. Turkish Journal of Zoology, 36: 475-480.

McAlpine, J. F., 1981. *Morgea freidbergi* new species, a living sister-species of the fossil species *M. mcalpinei*, and a key to world genera of Pallopteridae (Diptera). The Canadian Entomologist, 113 (2): 81-91.

Merz, B., 1998. "Family Pallopteridae, 201-210". In: Contributions to a Manual of Palaearctic Diptera (with Special Reference to Flies of Economic Importance, Vol. 3. Higher Brachycera) (Eds. L. Papp & B. Darvas). Science Herald, Budapest, 880 pp.

Merz, B., 2011. "Fauna Europaea: Pallopteridae". In: Fauna Europaea: Diptera, Brachycera (Eds. T. Pape & P. Beuk). Fauna Europaea version 2.4. (Web page: https://fauna-eu.org) (Date accessed: February 2012).

Mesci, S. & A. Hasbenli, 2015a. Contributions to the genus Platystoma (Diptera: Platystomatidae) fauna of Turkey with seven new records. Turkish Journal of Zoology, 39: 804-810.

Mesci, S. & A. Hasbenli, 2015b. Contributions to subfamily Otitinae (Diptera: Ulidiidae) fauna of Turkey with four new records. Turkish Journal of Zoology, 39: 1090-1094.

Namin, S. M. & S. V. Korneyev, 2015. Revision of *Heringina* Aczél, 1940 (Diptera: Tephritidae), with description of a new species from Iran and Turkey. Zootaxa, 3949 (1): 111-122.

Oosterbroek, P., 2006. The European Families of the Diptera: Identification, Diagnosis, Biology. KNNV Publishing, Utrecht, 205 pp.

Ozerov, A. L., 2009. Review of the family Pallopteridae (Diptera) of the fauna of Russia. Russian Entomological Journal, 18: 129-146.

Pape, T., V. Blagoderov & M. B. Mostovski, 2011. "Order Diptera Linnaeus, 1758". In: Animal biodiversity: An outline of higher-level classification and survey of taxonomic richness (Ed. Z-Q Zhang). Zootaxa, 3148: 222-229.

Rotheray, G. E., 2014. Development sites, feeding modes and early stages of seven European *Palloptera* species (Diptera, Pallopteridae). Zootaxa, 3900 (1): 50-76.

Şekercioğlu, Ç., 2011. "Turkey's Conservation Crisis: Global Biodiversity Hotspots under Threat". National Geographic. (Web page: https://blog.nationalgeographic.org/2011/12/31/turkeys-conservation-crisis-global-biodiversity-hotspots-under-threat/) (Date accessed: December 2011).

Yaran, M. & M. Kütük, 2014. A new species of *Urophora* Robineau-Desvoidy, 1830 (Diptera: Tephritidae) from Turkey with a key to the *dzieduszyckii* group. Turkish Journal of Entomology, 38: 149-155.

Yaran, M. & M. Kütük, 2015. Two new records of fruit flies (Diptera: Tephritidae) with fauna of Aksaray and Mersin provinces. Turkish Journal of Zoology, 39: 1056-1070.

Yaran, M. & M. Kütük, 2016. Fruit flies (Diptera: Tephritidae) fauna in Nevşehir and Niğde provinces with a new record from Turkey. Turkish Journal of Zoology, 40 (5): 785-800.

Yaran, M., M. Kütük, V. Görmez & M. Ö. Koyuncu, 2018a. A new species and additional record of *Terellia* Robineau-Desvoidy (Diptera: Tephritidae) from Turkey with a key for the *Cerajocera* group. Turkish Journal of Zoology, 42: 661-665.

Yaran, M., M. Kütük, V. Görmez & M. Ö. Koyuncu, 2018b. Some additional notes on fruit fly (Diptera: Tephritidae) fauna and a new genera and species record from Turkey. Biological Diversity and Conservation, 11 (3): 141-144.