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Orijinal araştırma (Original article)

Evaluation of Turkish wild Emmers (*Triticum dicoccoides* Koern.) and wheat varieties for resistance to the root lesion nematodes (*Pratylenchus thornei* and *Pratylenchus neglectus*)[1]

Türkiye'de bulunan yabani Emmer buğdayları (*Triticum dicoccoides* Koern.) ve buğday çeşitlerinin Kök lezyon nematodları (*Pratylenchus thornei* and *P. neglectus*)'na karşı dayanıklılıklarının belirlenmesi

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Summary

Root-lesion nematodes (RLN; *Pratylenchus thornei* and *Pratylenchus neglectus*) are serious plant parasitic nematodes that attack wheat roots and cause significant losses in grain quality and quantity worldwide. They are widely distributed in Turkish wheat growing areas and can be only controlled by growing resistant cultivars and non-host crops. So far, there is no commercial wheat cultivar identified as completely resistant to the RLN is available. The objective of this research was to evaluate some Turkish wild Emmers (*Triticum dicoccoides*) and national spring wheat varieties for resistant to *P. thornei* and *P. neglectus*. In the experiments, 32 wild Emmers and 42 wheat cultivars were inoculated with *P. thornei* or *P. neglectus* individuals and grown under controlled conditions in a growth room for 9 weeks. The results showed that 25 and 35 wheat varieties were moderately resistant to *P. thornei* and *P. neglectus*, respectively. In total, 17 lines exhibiting multiple resistance reactions to both nematodes were in the same group with the check lines ($P<0.05$). The identified resistant wheat accessions showed improved levels of RLN resistance over current parents and could serve as an excellent genetic pool to be used in an efficient Turkish wheat-breeding programme.

Keywords: Wheat, wild emmer, root lesion nematodes, *Pratylenchus* spp., resistance

Özet

Kök lezyon nematodları (RLN; *Pratylenchus thornei* ve *P. neglectus*) buğday köklerine saldırarak, tahıl kalitesi ve miktarında küresel olarak önemli kayıplara neden olan önemli bir bitki paraziti nematod gurubudur. Türkiye'de buğday ekiliş alanlarında yaygın olarak bulunan bu nematodlarla ancak dayanıklı çeşitler ve rotasyonla mücadele etmek mümkündür. Maalesef bugüne kadar bu nematod gurubuna karşı tamamen dayanıklı olarak tanımlanan hiçbir ticari buğday çeşidi bulunamamıştır. Bu nedenle, buğday ıslah programlarında yüksek verimli ve kaliteli buğday çeşitlerine orta derecede (kısmi) dayanıklı ebeveynler kullanılarak dayanıklılık entegre edilmeye çalışılmıştır. Bu araştırmanın amacı bazı Türk yabani Emmer'lerinin (*Triticum dicoccoides*) ve bazı ulusal yazlık buğday çeşitlerinin *P. thornei* ve *P. neglectus*'a karşı dayanıklılık durumlarını ortaya koymaktır. Bu amaçla her iki nematoda karşı yapılan iki farklı denemede, 32 yabani Emmer buğdayı ve 42 buğday çeşidinin, *P. thornei* veya *P. neglectus* bireylerine karşı dayanıklıkları 9 hafta boyunca kontrollü koşullar altında testlenmiştir. Sonuç olarak, *P. thornei* ve *P. neglectus*'a karşı sırasıyla 25 ve 35 buğday çeşidinin orta derecede dayanıklılık reaksiyonu sağladığı belirlenmiştir. Toplamda 17 çeşit her iki nematoda karşı dayanıklılık göstermiştir ($p<0.05$). Elde edilen verilere göre, tespit edilen dayanıklı buğday çeşitlerinin mevcut hatlardan daha dayanıklı olduğu belirlenmiş ve bu hatların Türkiye'deki ıslah programlarında mükemmel bir şekilde kullanılabileceği ortaya konmuştur.

Anahtar sözcükler: Buğday, yabani emmer, kök yara nematodları, *Pratylenchus* spp., dayanıklılık

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Introduction

Turkey is considered 10th wheat (*Triticum aestivum*) producing country in the world with 20 million tonnes in 9 million hectares per year (TUIK, 2013). Plant parasitic nematodes are important biotic agents that cause substantial yield loss in semiarid wheat growing regions. Root-lesion nematodes (RLN; *Pratylenchus* spp.) damage plants mechanically and chemically, reduce plant vigour and can cause root lesions that predispose plants to infection by root-infecting fungi, which may lead to the formation of a disease complex. There are eight species of RLN that have been recorded for small grains (Rivoal & Cook, 1993). Of these four species (*Pratylenchus thornei, Pratylenchus crenatus, Pratylenchus neglectus* and *Pratylenchus penetrans*) have worldwide distribution, especially in the temperate zones (Rivoal & Cook, 1993). Nicol et al. (2002) reported that both *P. thornei* and *P. neglectus* can occur individually or mixed in soil of Turkish wheat growing regions.

RLN have a wide host range and can be difficult to identify and control. Symptoms on plants caused by RLN are non-specific and easily confused with stress from nutrient deficiency, drought and/or disease. The use of host-plant resistance is one of the most effective methods of controlling RLN. Resistance is defined as the ability of the host to inhibit nematode multiplication in plant (Rivoal & Cook, 1993). It can range from low to moderate (partial or intermediate) resistance, to high resistance. Preferably, resistant germplasm should be combined with tolerance, which is the ability of the host plant to maintain high yield potential in the presence of the nematode (Rivoal & Cook, 1993). The use of resistant varieties is an effective option to control RLN and it also environmentally sound, has no cost and requires no additional equipment. So far, there are no commercial wheat varieties with complete resistance to RLN (*P. thornei* and *P. neglectus*). But, there are some alternative sources of partial resistance to RLN which have been identified in wild forms of wheat (Thompson & Haak, 1997; Sheedy & Thompson, 2009; Toktay et al., 2012a).

The best strategy for wheat improvement against diseases and pest is to the use of the valuable genetic resources of its wild progenitors (Peng et al., 2011). Emmer wheat (*Triticum dicoccoides*) is one of the earliest domesticated plants and has been a staple crop over millennia (Zahireva et al., 2010; Ozkan et al., 2002). *T. dicoccoides*, occurs naturally across the Fertile Crescent (Peng et al., 2011) and is a rich genetic resource to improve resistance to diseases and pest for breeding programs.

The objectives of this study were to screen wild emmer wheat and some national spring wheat varieties to find new sources of resistance to both *P. thornei* and *P. neglectus* and to identify varieties with superior resistance to current wheat varieties.

Material and Methods

In this study, 42 Turkish spring wheat varieties and 32 wild Emmer accessions as well as reference cultivars (check lines) were screened for resistance to *P. thornei* and *P. neglectus* under controlled growth room conditions.

The wild Emmers were collected from South-eastern Anatolia during 2004 and 2006 and were maintained in a wheat nursery collection by the Department of Crop Science in Cukurova University.

The wheat cultivars Gatcher and Seri were used as susceptible control and (CROC_1/AE.SQUARROSA (224)//OPATA) was used as a moderately resistant control against both *P. thornei* and *P. neglectus* (Sheedy & Thompson, 2009). The Australian wheat cultivar GS50a was used as a moderately resistant control for *P. thornei* and a susceptible control for *P. neglectus* (Thompson, 2008; Sheedy et al., 2012).

Experimental procedures

Seeds were surface sterilized with 1% sodium hypochlorite solution for 1 min and then rinsed 3 times with sterilized distilled water. To enhance seed germination, about 20 seeds of each accession were placed in 9 cm diameter petri dishes. These petri dishes were provided with a filter paper, which was moistened with sterile water and kept incubated at 20^{o}C for 48 hours. After then, semi-rooted plants with root length about 3 cm were transplanted into the tubes (3 cm diam. x13 cm in long) filled with mixture of sand and soil (9:1 v/v). Plants were left to grow in a growth room at 23^{o}C and 16 hours of artificial supplementary light (Toktay et al., 2012b).

Plants were harvested after 9 weeks of nematodes inoculation. Final numbers of nematodes were extracted from both soil and roots using a Baermann funnel technique and kept in a misting chamber for 6 days to facilitate extraction. The nematodes were counted to determine resistant reaction of the different germplasm and compared them to known check lines. Each plant was replicated 7 times placed in a randomized block design in this experiment.

Nematode inoculum culture and inoculation procedure

Nematodes were grown *in-vitro* on carrot cultures according to Moody et al. (1973). The nematodes were extracted from the carrot culture by chopping the carrot disks and placing them into a misting chamber for 2-4 days. Nematodes were collected and amended to the required concentration per one ml of water. *P. thornei* and *P. neglectus* individuals collected from Southeastern part of Turkey, were counted under a binocular microscope and suspended tap water in 50 ml flasks. Then one week of sowing, each plant was injected with 1 ml of water consisting of 400 individuals in 1 ml water. Plants were planted in growth room at 23-25^{o}C temperature and during 16 hours of illumination for 9 weeks (Toktay et al., 2012b).

Nematode extraction from plant roots

After 9 weeks, plant shoots were removed and the nematodes were extracted from the soil and the roots using a Baermann funnel kept in a misting chamber for 6 days to extract the nematodes. One millilitre of nematode suspension was counted with three replications in a counting slide under a stereomicroscope at 32-fold magnification. The number of extracted nematodes per plant was calculated.

Statistical design and analysis

The number of nematodes per plant is an appropriate estimate of plant resistance (Keil et al., 2009; Sheedy et al., 2009; Farsi et al., 1995; Toktay et al., 2012b). The Reproduction factor (Rf) generally used in resistance experiments in nematology were used to give quantitative value of resistance (Keil et al., 2009). In the screening experiments under the laboratory conditions, nematode initial population (Pi) set as inoculum level and nematode final population (Pf) and Pf / Pi which defined as the reproduction factor of nematodes should be carefully emphasise to identify resistant reaction of the germplasm (Toktay et al., 2012b).

Wheat varieties were classified as completely resistant (R), if no nematode multiplication in plant roots and soil was observed. Moderately resistant if nematode reproduction factor was under 1 (RF < 1), and finally if the RF is higher than 1 this line considers as susceptible. Number of nematodes were compared also according to susceptible and resistance check lines.

The total number of RLN's both root and soil in the pot for each plant were counted under microscope. Resistance was evaluated from the reproduction factor calculated by dividing the final by the initial P. thornei numbers in soil plus roots (Sheedy et al., 2009; Keil et al., 2009). Data were also analyzed by ANOVA and means were separated using Duncan's Multiple Range Test at $P < 0.05$ significance level. All statistical analyses were performed by SPSS 16.0 (SPSS Inc. Illinois, USA).

Results and Discussion

None of the screened wheat germplasm (15 bread wheat, 27 durum wheat and 32 emmer wheat genotypes collected from southeast part of Turkey) has completely resistance reaction against *P. thornei* and *P. neglectus* (Table 1, 2). Four check lines were used in the experiment (2 susceptible and 2 resistant) for *P. thornei*. Eleven durum wheat cultivars and six emmer lines were moderately resistant to the two root lesion nematodes. Four bread, twelve durum and nine emmers were moderately resistant against *P. thornei* (Table 1).

Table 1. The resistance reaction of Turkish wheat varieties and wild emmers with resistant and susceptible check lines against Turkish population of *Pratylenchus thornei*

Accession no	Genotypes	Wheat type	Pi (Initial *P. thornei* population)	Pf (Final nematod Population) ± SE	Rf (Reproduction factor)	*Reaction**
1	*Gatcher	Bread	400	399,29±3,62	1,00	S
2	*Croc.	Bread	400	205,14±3,14	0,51	MR
3	*Serı	Bread	400	564,86±2,77	1,41	S
4	*GS50a	Bread	400	117,57±4,88	0,29	MR
12	Adana 99	Bread	400	213,86±2,89	0,53	MR
9	Doğankent-1	Bread	400	378,00±4,63	0,95	MR
17	Ceyhan-99	Bread	400	380,29±3,43	0,95	MR
10	Karatopak	Bread	400	383,57±3,4	0,96	MR
14	Pandas	Bread	400	390,29±3,16	0,98	S
18	Yüregir-89	Bread	400	412,43±3,87	1,03	S
6	Karakılçık	Bread	400	475,29±5,61	1,19	S
8	Cemre	Bread	400	478,00±4,77	1,20	S
15	Çukurova-86	Bread	400	483,57±3,73	1,21	S
13	Seri-82	Bread	400	499,71±4,11	1,25	S
5	Karacadağ 98	Bread	400	501,57±5,68	1,25	S
11	Seyhan 95	Bread	400	505,29±4,23	1,26	S
16	Osmaniyem	Bread	400	518,71±2,28	1,30	S
7	Nurkent	Bread	400	532,43±8,73	1,33	S
40	Sogol Acırlı	Durum	400	207,14±3,21	0,52	MR
19	Fuatbey 208	Durum	400	209,43±3,72	0,52	MR
39	Sarı bursa	Durum	400	218,00±3,32	0,55	MR
44	Minoret	Durum	400	218,57±2,52	0,55	MR
36	Siverek	Durum	400	221,29±2,35	0,55	MR
27	Hacıhalil	Durum	400	268,57±5,52	0,67	MR
21	Amanos-97	Durum	400	300,71±4,33	0,75	MR
31	Zenit	Durum	400	313,86±3,17	0,78	MR
41	İskenderi	Durum	400	349,71±13,9	0,87	MR
37	Şırnak	Durum	400	373,00±4,06	0,93	MR
26	Bagacak96m	Durum	400	376,43±3,18	0,94	MR
33	Giberunda	Durum	400	385,57±3,43	0,96	MR
30	Dicle 74-M	Durum	400	396,71±2,67	0,99	S
23	Menceki-2	Durum	400	398,57±3,54	1,00	S
38	Menceki-M	Durum	400	405,86±4,36	1,01	S
20	Gediz.75	Durum	400	412,43±4,24	1,03	S

* RF < 1= R, 0,5<RF<1=MR, RF>1=S

Table 1. (continued)

Accession no	Genotypes	Wheat type	Pi (Initial *P. thornei* population)	Pf (Final nematod Population) ± SE	Rf (Reproduction factor)	*Reaction**
42	Kurtalan	Durum	400	415,86±2,2	1,04	S
32	Şiraslan	Durum	400	417,29±2,9	1,04	S
46	Şırnak Akkaya	Durum	400	419,14±2,96	1,05	S
28	Akbugday	Durum	400	419,29±3,69	1,05	S
22	Sham-1	Durum	400	442,57±3,28	1,11	S
43	Sorgül	Durum	400	442,57±2,25	1,11	S
35	Beyaziyem	Durum	400	474,29±2,93	1,19	S
45	Sorgül-2	Durum	400	474,57±3,32	1,19	S
25	Selçuklu	Durum	400	481,86±4,02	1,20	S
29	Havrani	Durum	400	504,86±3,28	1,26	S
34	A-97	Durum	400	517,29±3,39	1,29	S
24	Hav-27	Durum	400	517,43±2,62	1,29	S
73	*Triticum.dicoccoides*	Emmer	400	198,29±9,4	0,50	MR
57	*T. dicoccoides*	Emmer	400	204,29±8,52	0,51	MR
64	*T. dicoccoides*	Emmer	400	205,14±8,61	0,51	MR
70	*T. dicoccoides*	Emmer	400	211,86±10,39	0,53	MR
51	*T. dicoccoides*	Emmer	400	237,29±13,13	0,59	MR
58	*T. dicoccoides*	Emmer	400	280,86±22,89	0,70	MR
63	*T. dicoccoides*	Emmer	400	332,71±14,14	0,83	MR
67	*T. dicoccoides*	Emmer	400	333,29±7,68	0,83	MR
48	*T. dicoccoides*	Emmer	400	333,43±13,49	0,83	MR
74	*T. dicoccoides*	Emmer	400	389,57±10,13	0,97	S
61	*T. dicoccoides*	Emmer	400	430,14±13,72	1,08	S
52	*T. dicoccoides*	Emmer	400	440,43±12,19	1,10	S
66	*T. dicoccoides*	Emmer	400	444,29±12,05	1,11	S
71	*T. dicoccoides*	Emmer	400	449,43±12,36	1,12	S
78	*T. dicoccoides*	Emmer	400	450,57±11,29	1,13	S
75	*T. dicoccoides*	Emmer	400	451,29±18,14	1,13	S
55	*T. dicoccoides*	Emmer	400	453,00±11,69	1,13	S
47	*T. dicoccoides*	Emmer	400	466,00±12,51	1,17	S
72	*T. dicoccoides*	Emmer	400	494,00±11,74	1,24	S
69	*T. dicoccoides*	Emmer	400	508,71±15,44	1,27	S
60	*T. dicoccoides*	Emmer	400	525,86±12,47	1,31	S
50	*T. dicoccoides*	Emmer	400	527,29±10,15	1,32	S
53	*T. dicoccoides*	Emmer	400	527,29±14,5	1,32	S
56	*T. dicoccoides*	Emmer	400	527,43±14,13	1,32	S
62	*T. dicoccoides*	Emmer	400	529,14±11,72	1,32	S
77	*T. dicoccoides*	Emmer	400	531,14±14,46	1,33	S
59	*T. dicoccoides*	Emmer	400	544,86±13,93	1,36	S
65	*T. dicoccoides*	Emmer	400	546,00±15,75	1,37	S
54	*T. dicoccoides*	Emmer	400	550,43±13,9	1,38	S
49	*T. dicoccoides*	Emmer	400	558,29±20,4	1,40	S
76	*T. dicoccoides*	Emmer	400	571,29±13,91	1,43	S
68	*T. dicoccoides*	Emmer	400	584,57±14,17	1,46	S

* RF < 1= R, 0,5<RF<1=MR, RF>1=S

There is one cultivar was as resistant control in the experiment against *P. neglectus*. GS50A was resistant to *P. thornei,* whereas it was susceptible against *P. neglectus.*

In this experiment, only two bread wheat, twenty durum wheat and thirteen emmers of wheat were found moderately resistant against *P. neglectus* (Table 2).

Table 2. The resistance reaction of Turkish wheat varieties and wild emmers with resistant and susceptible check lines against Turkish population of *Pratylenchus neglectus*

Accession no	Genotypes	Wheat type	Pi (İnitial *P. neglectus* population)	Pf (Final nematod Population) ± SE	Rf (Reproduction factor	*Reaction**
1	*Gatcher	Bread	400	402,71±5,62	1,01	S
2	*Croc.	Bread	400	196,71±3,73	0,49	MR
3	*Serı	Bread	400	483,29±4,63	1,21	S
4	*GS50a	Bread	400	477,43±3,64	0,69	S
15	Çukurova-86	Bread	400	376,29±3,26	0,94	MR
18	Yüregir-89	Bread	400	377,43±4,85	0,94	MR
6	Karakılçık	Bread	400	394,14±3,32	0,99	S
11	Seyhan 95	Bread	400	399,86±4,01	1,00	S
5	Karacadağ 98	Bread	400	405,29±4,37	1,01	S
17	Ceyhan-99	Bread	400	414,43±3,61	1,04	S
12	Adana 99	Bread	400	416,57±2,60	1,04	S
14	Pandas	Bread	400	440,57±3,94	1,10	S
10	Karatopak	Bread	400	454,57±3,37	1,14	S
9	Doğankent-1	Bread	400	471,57±3,38	1,18	S
16	Osmaniyem	Bread	400	471,57±3,80	1,18	S
8	Cemre	Bread	400	502,71±5,02	1,26	S
7	Nurkent	Bread	400	505,29±3,70	1,26	S
13	Seri-82	Bread	400	506,86±3,72	1,27	S
41	İskenderi	Durum	400	197,71±3,23	0,49	MR
43	Sorgül	Durum	400	206,71±3,56	0,52	MR
24	Hav-27	Durum	400	208,14±3,28	0,52	MR
31	Zenit	Durum	400	213,71±3,95	0,53	MR
37	Şırnak	Durum	400	217,71±2,23	0,54	MR
26	Bagacak96m	Durum	400	249,57±3,08	0,62	MR
30	Dicle 74-M	Durum	400	250,57±3,26	0,63	MR
27	Hacıhalil	Durum	400	273,43±3,00	0,68	MR
33	Giberunda	Durum	400	286,71±3,28	0,72	MR
29	Havrani	Durum	400	305,86±3,79	0,76	MR
19	Fuatbey 208	Durum	400	307,00±4,04	0,77	MR
39	Sarı bursa	Durum	400	307,14±6,15	0,77	MR
20	Gediz.75	Durum	400	313,57±4,38	0,78	MR
32	Şiraslan	Durum	400	315,71±2,75	0,79	MR
40	Sogol Acırlı	Durum	400	338,14±3,61	0,85	MR
21	Amanos-97	Durum	400	339,29±3,22	0,85	MR
42	Kurtalan	Durum	400	341,86±3,32	0,85	MR
23	Menceki-2	Durum	400	346,57±2,69	0,87	MR
38	Menceki-M	Durum	400	378,71±3,34	0,95	MR
44	Minoret	Durum	400	380,71±2,83	0,95	MR
35	Beyaziyem	Durum	400	398,29±2,88	1,00	S
46	Şırnak Akkaya	Durum	400	394,43±3,52	0,99	S
28	Akbugday	Durum	400	401,00±15,49	1,00	S
36	Siverek	Durum	400	415,86±2,34	1,04	S
34	A-97	Durum	400	441,71±3,34	1,10	S
45	Sorgül-2	Durum	400	469,14±3,97	1,17	S

* RF < 1= R, 0,5<RF<1=MR, RF>1=S

Table 2. (continued)

Accession no	Genotypes	Wheat type	Pi (İnitial *P. neglectus* population)	Pf (Final nematod Population) ± SE	Rf (Reproduction factor	*Reaction**
22	Sham-1	Durum	400	473,71±2,69	1,18	S
25	Selçuklu	Durum	400	492,00±3,70	1,23	S
57	*Triticum.dicoccoi*	Emmer	400	193,71±15,98	0,48	MR
67	*T. dicoccoides*	Emmer	400	194,29±11,67	0,49	MR
64	*T. dicoccoides*	Emmer	400	201,14±7,56	0,50	MR
73	*T. dicoccoides*	Emmer	400	206,86±12,54	0,52	MR
77	*T. dicoccoides*	Emmer	400	294,71±16,66	0,74	MR
62	*T. dicoccoides*	Emmer	400	314,43±17,22	0,79	MR
61	*T. dicoccoides*	Emmer	400	328,14±12,97	0,82	MR
58	*T. dicoccoides*	Emmer	400	330,29±14,70	0,83	MR
54	*T. dicoccoides*	Emmer	400	332,71±17,80	0,83	MR
74	*T. dicoccoides*	Emmer	400	356,71±13,97	0,89	MR
66	*T. dicoccoides*	Emmer	400	358,57±16,04	0,90	MR
47	*T. dicoccoides*	Emmer	400	373,71±13,38	0,93	MR
70	*T. dicoccoides*	Emmer	400	391,00±15,90	0,98	MR
53	*T. dicoccoides*	Emmer	400	427,57±12,18	1,07	S
78	*T. dicoccoides*	Emmer	400	433,14±12,87	1,08	S
75	*T. dicoccoides*	Emmer	400	436,14±12,28	1,09	S
65	*T. dicoccoides*	Emmer	400	441,86±15,06	1,10	S
48	*T. dicoccoides*	Emmer	400	447,29±15,05	1,12	S
72	*T. dicoccoides*	Emmer	400	449,43±14,83	1,12	S
59	*T. dicoccoides*	Emmer	400	452,43±12,22	1,13	S
51	*T. dicoccoides*	Emmer	400	453,00±16,53	1,13	S
50	*T. dicoccoides*	Emmer	400	480,00±22,81	1,20	S
55	*T. dicoccoides*	Emmer	400	481,29±11,03	1,20	S
68	*T. dicoccoides*	Emmer	400	526,29±11,17	1,32	S
69	*T. dicoccoides*	Emmer	400	528,71±19,45	1,32	S
49	*T. dicoccoides*	Emmer	400	529,14±10,05	1,32	S
60	*T. dicoccoides*	Emmer	400	531,71±13,87	1,33	S
63	*T. dicoccoides*	Emmer	400	533,86±12,72	1,33	S
76	*T. dicoccoides*	Emmer	400	536,71±15,14	1,34	S
56	*T. dicoccoides*	Emmer	400	538,43±11,37	1,35	S
71	*T. dicoccoides*	Emmer	400	538,71±15,55	1,35	S
52	*T. dicoccoides*	Emmer	400	539,14±15,82	1,35	S

* RF < 1= R, 0,5<RF<1=MR, RF>1=S

Using resistant varieties to control the root lesion nematodes is the main target of many nematologists and breeders around all over the world due to its adaptability and easy to be applied once identified. There are very limited researches to determine resistance of local cultivars to root lesion nematodes in Turkey. Toktay et al. (2008) found resistant in some local varieties in East Mediterranean region of Turkey. İmren et al. (2013), reported that wheat cv. Adana 99 is moderately resistant to both cereal cyst nematode (*Heterodera avenae*) pathotype Ha 21 and root lesion nematode (*P. thornei*).

Durable resistance has generally been transferred from wild relatives to cultivated crops (Boerma & Hussey, 1992). The southeastern Anatolia region Karacadağ-Diyarbakir is rich in genetic resources of wild emmer, *T. dicoccoides, T. urartu,* and *T. boeticum* as important wheat wild relatives of wheat (Nesbit & Samuel, 1998; Salamina et al., 2002; Ozkan et al., 2011). Therefore wild emmer of wheat has been obtained from the Southeastern Anatolia region to assess resistance reaction of RLN.

There have been many studies to identify new sources of resistance to RLN on Middle Eastern wheat varieties and landraces (Nicol et al., 2005; Schimdt et al., 2005; Zwart et al., 2005; Thompson,

2008; Thompson & Seymour 2011; Toktay et al., 2012a). One of the particular relevance to Australian wheat improvement programs were the *P. thornei* resistant accessions identified among Iranian landraces (Sheedy & Thompson, 2009) and wild relatives of wheat including *T. dicoccoides* (Sheedy et al., 2012). In this study, most varieties and wild relatives from this region in Turkey were resistant; these results were in agreement with those of Toktay et al. (2012a). Farsi et al. (1995) showed that GS50a was susceptible to *P. neglectus*, indicating that resistance to *P. thornei* does not convey resistance to *P. neglectus*. Imren et al. (2013) found 17 emmer wheat and 4 local wheat varieties resistant against Ha 21 pathotype of *H. avenae.*

In Turkey, the soil borne diseases, especially plant parasitic nematodes, have not been given attention and are under exploited by breeders. Also, the Turkish farmers have been planting their local seeds without having any idea of nematodes and their potential to cause significant losses. Such ignorant and continued plantation of susceptible wheat cultivar is expected to accelerate the nematode population that will ultimately lead to huge amount of their grain yield losses. Both emmer and cultivated wheat collections germplasms are useful to understanding the genetic basis for resistance, determining the gene(s) responsible, and identifying which may be of great benefit to breeding programs by pyramiding different resistance genes into single lines.

In this this study, a total of 74 lines were evaluated for RLN resistance. Unfortunately, there were no cultivars with complete resistance to either RLN species. But, 17 durum wheat and four wild emmers were moderately resistant to both nematodes. These cultivars can be used to improve RLN resistance in commercial cultivars and to identify molecular markers for resistance to RLN through association mapping. More detailed experiments with local varieties and wild relatives are required to find new sources of resistance that can be used in both national and international wheat breeding programs.

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Orijinal araştırma (Original article)

Efficacy of entomopathogenic nematodes against the Tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in tomato field[1]

Entomopatojen nematodların Domates güvesi *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae)'ya karşı domates tarlasındaki etkinliği

Çiğdem GÖZEL[2*] İsmail KASAP[2]

Summary

The tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is a very challenging pest that causes economical losses in tomato production. This devastating pest originated from South America was the first time detected in İzmir province of Turkey in August 2009. The efficacy of the infective juveniles (IJs) of four native entomopathogenic nematode (EPN) species, *Steinernema affine* (Bovien) (isolate 46), *S. carpocapsae* (Weiser) (isolate 1133), *S. feltiae* (Filipjev) (isolate 879) and *Heterorhabditis bacteriophora* (Poinar) (isolate 1144) was investigated against the larvae of *T. absoluta* in the field during the tomato production seasons of 2012-2013 in Çanakkale. Individuals of *T. absoluta* were collected from infested tomato fields in Çanakkale and mass produced on tomato plants in a climate controlled room. EPNs were isolated from different parts of Turkey and mass produced by using *Galleria mellonella* larvae in the laboratory. The tomato leaf miners were exposed to each nematode species at the rate of 50 IJs/cm^2 on tomato plants in cages. *T. absoluta* were susceptible to all EPNs tested but the degree of susceptibility of the larvae to EPN infection varied according to the species. The most effective nematode species on *T. absoluta* larvae was *S. feltiae* (isolate 879) with 90.7% and 94.3% mortality in 2012 and 2013, respectively, whereas the least effective species was *S. affine* (isolate 46) with 39.3% and 43.7% mortality in 2012 and 2013, respectively. EPNs can be potential canditates to control tomato leafminer, so the integration possibility of these biological agents into the *T. absoluta* management programme is discussed.

Keywords: Biological control, entomopathogenic nematodes, *Heterorhabditis, Steinernema, Tuta absoluta*

Özet

Domates güvesi, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) domates üretiminde ekonomik kayıplara neden olan, mücadelesi zor bir zararlıdır. Tahrip gücü yüksek bu zararlı Güney Amerika orijinli olup, ülkemizde ilk olarak 2009 Ağustosu'nda İzmir'de tespit edilmiştir. Dört yerel entomopatojen nematod türü; *Steinernema affine* (Bovien) (izolat 46), *S. carpocapsae* (Weiser) (izolat 1133), *S. feltiae* (Filipjev) (izolat 879) ve *Heterorhabditis bacteriophora* (Poinar) (izolat 1144)'nın *T. absoluta*'ya karşı etkinliği tarlada 2012-2013 Çanakkale domates üretim sezonu süresince araştırılmıştır. *T. absoluta* bireyleri Çanakkale'deki bulaşık domates tarlalarından toplanmış ve iklim odasında domates bitkileri üzerinde kitle üretimi yapılmıştır. EPN'ler ise ülkemizin farklı bölgelerinden elde edilmiş ve laboratuarda *Galleria mellonella* larvalarında kitle üretimi yapılmıştır. Her bir nematod türü domates güvesine kafeslerdeki domates bitkileri üzerinde 50 IJs/cm^2 olacak şekilde uygulanmıştır. *T. absoluta*'nın, denemede kullanılan tüm EPN'lere karşı duyarlı olduğu tespit edilmiş, ancak larvaların infeksiyona karşı gösterdiği duyarlılık nematod türüne bağlı olarak değişiklik göstermiştir. *S. feltiae* (izolat 879) 2012 ve 2013 yıllarında sırası ile meydana getirdiği %90.7 ve %94.3 ölüm oranları ile en etkili tür olarak tespit edilmişken, *S. affine* (izolat 46) 2012 ve 2013 yıllarında sırası ile meydana getirdiği %39.3 ve %43.7 ölüm oranları ile en az etkili tür olarak tespit edilmiştir. EPN'ler domates güvesini kontrol etmek için potansiyel adaylar olabilir, bu nedenle bu biyolojik ajanların *T. absoluta*'nın mücadele programına dahil edilme olasılığı üzerinde durulmalıdır.

Anahtar sözcükler: Biyolojik mücadele, entomopatojen nematodlar, *Heterorhabditis, Steinernema, Tuta absoluta*

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Introduction

The tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), is a Neotropical, oligophagous pest of solanaceous crops that originates from South America (Lietti et al., 2005; Urbaneja et al., 2007). This devastating pest has spread throughout the Mediterranean Basin, dispersing to other European and Middle East Asian countries, and within a 15-year period, it is expected to reach the Pacific Asian Coast (Potting, 2009; Desneux et al., 2011; Germain et al., 2009). It has been listed with the code GNORAB in the A1 quarantine list of the European and Mediterranean Plant Protection Organization (EPPO, 2009). In Turkey it was first recorded in 2009 in the Urla District of Izmir Province (Kılıç, 2010) and has been a serious problem to tomato production in Çanakkale since the first detection in Turkey (Kasap et al., 2011).

Tuta absoluta is a holometabolous insect with a high rate of reproductive capacity. It can overwinter in the egg, pupal, or adult stage, is multivoltine and can complete 12 generations per year depending on environmental conditions. Adults are silvery gray with black spots on the forewings and a wingspan reaching 10 mm. Their activity is concentrated in the early morning and dusk; during the rest of the day, they remain hidden among the leaves. Adult lifespan ranges between 10 and 15 days for females and 6-7 days for males. The number of eggs per female is usually between 40 and 50 and may reach 260. Eggs are small, 0.35 mm long, cylindrical and creamy white to yellow. Egg hatching takes 4-6 days. Larval development goes through four stages and pupation may take place in the soil, on the leaves and even within the galleries or other parts of the plant. The pupa is cylindrical and greenish when recently formed, later turning brown. It may be protected by a silky white cocoon (Anonymous, 2010).

Females generally prefer to lay eggs on leaves (73%), leaf veins and stem margins (21%), sepals (5%) or green fruits (1%) (Estay, 2000). After hatching, larvae enter the plant tissue and begin feeding. These feeding mines affect the photosynthetic capacity of the plant and enable attacks by plant pathogens. The galleries produced by young larvae may be confused with those produced by leafminers (*Liriomyza* spp.), but the gallery produced by *T. absoluta* subsequently widens and the damaged tissue dries. During development the larvae may change gallery several times. Young larvae can mine leaves, stems, shoots, flowers, and developing fruit; later instars can attack mature tomato fruit and infested fruit usually falls to the ground (Vargas, 1970). This devastating pest can attack all parts and stages of the tomato plant, overwinter in the egg, pupal, or adult stage and can cause up to 100% losses in tomato crops (EPPO, 2005).

Tomato leaf miner primarily attacks cultivated and non-cultivated tomato plants and other members of the family Solanaceae but it can also feed, develop and reproduce on other naturally available host-plants such as *Datura ferox* L., *D. stramonium* L. and *Nicotiana glauca* Graham (Garcia & Espul, 1982; Larrain, 1986). Different plant species have been reported as alternative hosts of this insect as Cape gooseberry (*Physalis peruviana* L.), bean (*Phaseolus vulgaris* L.), *Lycium* sp. L. and *Malva* sp. L. (Caponero, 2009; EPPO, 2009; Tropea Garzia, 2009). This range indicates that *T. absoluta* shows a high propensity to use various plants as secondary hosts.

Since its dispersal in the 1970s, chemical control has been the main method of controlling this pest. Farmers have tried to reduce its damages by applying insecticides two times a week during a single cultivation period, sometimes every 4-5 days/season with minimum and maximum numbers of 8 to 25 sprays (Temerak, 2011). Even with the numerous applications of chemicals, effective control is difficult to achieve due to the mine-feeding behaviour of the larvae. Furthermore, the use of pesticides in crop production has many disadvantages such as pesticide residues on human health and on the environment. Thus, biological control can be considered as an alternative method to chemical control. In this respect, entomopathogenic nematodes (EPNs), which have great potential as biological control agent of insects, can be an alternative to chemicals.

EPNs are a group of soil-dwelling organisms that attack soilborne insect pests that live in, on, or near the soil surface and can be used effectively to control important pests. EPNs of the families Steinernematidae and Heterorhabditidae are symbiotically associated with bacteria in the genera *Xenorhabdus* (Thomas and Poinar) and *Photorhabdus* (Boemare, Akhurst and Mourant), respectively (Boemare et al., 1997; Burnell & Stock, 2000). The bacteria kill the host by producing toxins, provide nematodes with nutrition, and prevent secondary invaders from contaminating the host cadaver (Forst & Clarke, 2002). Infective juveniles (IJs) enter the host body mainly through natural openings such as the mouth, spiracles, anus or thin parts of the host cuticle and release their bacteria inside the hemocoel. Most biological agents require days or weeks to kill the host, yet nematodes can kill insects usually in 24-48 hours.

EPNs have many advantages; they are easy and relatively inexpensive to culture, live from several weeks up to months in the infective stage, are capable of infecting a broad range of insect species, occur in soil and have been isolated from most regions of the world except Antarctica (Griffin et al., 1990; Kaya & Gaugler, 1993). Foliar applications of nematodes have been successfully used to control the quarantine leaf eating caterpillars on various crops and have the potential for controlling various other insect pests. Application of EPNs does not require masks or other safety equipment as chemicals. EPNs and their associated bacteria have no detrimental effect to mammals or plants (Poinar et al., 1982; Boemare et al., 1996; Akhurst & Smith, 2002).

Discovery and development of new nematode species and strains and further improvement in formulation to enhance the biological control potential of entomopathogenic will further expand the options for implementation of nematodes against a wider range of targeted pests and also improvements in production technology, distribution, and application will be key to increasing nematode use.

The aims of the work were to determine the efficacy of EPNs against *T. absoluta* and to reduce the use of pesticides. This paper covers the efficacy of native EPNs against *T. absoluta* larvae in a tomato field in Çanakkale.

Materials and Methods

Entomopathogenic nematodes culture

Four native species of nematodes; *Steinernema affine* (Bovien) (isolate 46) *S. carpocapsae* (Weiser) (isolate 1133), *S. feltiae* (Filipjev) (isolate 879) and *Heterorhabditis bacteriophora* (Poinar) (isolate 1144) were evaluated against the tomato leaf miner larvae. Each isolates was reared in the last instar of wax moth larvae *Galleria mellonella* L., which is the most commonly used insect host for in vivo production of EPNs (Bedding & Akhurst, 1975; Lindegren et al., 1993; Kaya & Stock, 1997). *G. mellonella* was preferred because of its high susceptibility to the most nematodes, wide availability, ease in rearing, and high yields (Shapiro-Ilan & Gaugler, 2002; Woodring & Kaya, 1988).

Nematode-killed *G. mellonella* larvae were placed on White traps (White, 1927) at 25 ^{o}C and IJs that emerged from cadavers were harvested. These IJs were rinsed in distilled water and used within a week for the experiments. Before using the nematodes, their viability was checked under the stereomicroscope.

Tuta absoluta culture

Larvae, pupae and adults of *T. absoluta* used in the trials were obtained from infested tomato fields in Çanakkale. They reared in wooden rearing cages (50x50x50 cm) covered with organza on tomato plants at 25±1 ^{o}C, 65±5% RH, with a 16:8 L:D photoperiod in climate room. Male and female adults of *T. absoluta* were used to establish larval infestation on the tomato plants for the trials. A continuous mass-rearing of all development stages of *T. absoluta* was maintained on tomato plants in a climate room in cages.

Field trials

Field trials were carried out in the training and research area of Agriculture Faculty in Dardanos Campus in Çanakkale in 2012-2013. In both seasons, approximately 1000 m^2 area was cultivated with tomato. The tomato cultivar, Troy F1, was used in the trials because it is the most suitable for the Çanakkale climate. Seedlings were watered and fertilized periodically and closed by a cage when they reached 20 cm height. Each tomato plant was grown in a single cage (50x50x50 cm) covered with organza to prevent the entry of natural enemies and other unwanted organisms. An iron frame structure was used for the cages to prevent them from falling over. When the plants were 30 days old, 2 males and 2 females were released into each cage.

EPNs were applied at dusk to utilise the higher air humidity for the nematodes using a conventional airblast-sprayer at a rate of 50 IJs/cm^2. This application rate was calculated based on the ground surface area of the cage and recommended dose of a commercial company called e-nema. No adjuvants were added while spraying and no IJs were sprayed on control plants, only water was sprayed with the same volume as in nematode suspension on the tomato plants. Tomato plants remained wet in cages after application for a couple hours and that provides EPNs enough time with perfect condition to find and infect the target pest.

A zip was sewn up on the organza to control the leaves, fruits inside of the cages easily. Periodic observation of the cages allowed to control the damages of *T. absoluta* on tomato plants. The experiment was carried out with 2 replicates per nematode species and exposure day and repeated twice. Three plants were cut to determine the leaf miner mortality on each control days.

After releasing the adults of *T. absoluta*, EPNs were sprayed on tomato plants at the 7^{th}, 14^{th}, and 21^{st} days. Tomato plants were cut from the soil line at the 3^{rd}, 5^{th} 7^{th}, 9^{th}, 11^{th}, 13^{th} and 15^{th} days after EPN applications and analysed to determine the mortality of *T. absoluta.* Dead *T. absoluta* larvae were immediately dissected and checked for nematode infection.

Statistical analysis

To evaluate the efficacy of EPNs against *T. absoluta,* larval percentage mortalities were Arcsine transformed before analysis (Anscombe transformation) (Zar, 1999). Factorial Design ANOVA was used to test significant differences among treatments. Afterwards a Tukey's multiple range test was performed to separate means. A level of significance of $P < 0.05$ was used. All statistical analyses were performed using Minitab 16 software version (Minitab Inc., State College, PA, USA).

Results

Efficacy of entomopathogenic nematodes in the first year

The efficacy of EPNs in field in 2012 varied between 0 and 90.7±1.5%. The least efficient day post treatment was found on the 3^{rd} and the most efficient day was found on the 15^{th}. After the emergence of *T. absoluta* adults, the lowest mortality occurred on the 7^{th} day and the highest mortality was observed on the 21^{st} day. The least efficient species was *S. affine* (isolate 46) and the most efficient species was *S. feltiae* (isolate 879) with the mortality of 39.3±1.5% and 90.7±1.5%, respectively (Table 1). The temperature and humidity was recorded from June to October and found between 21 and 26 ^{o}C and 57 and 65%, respectively in 2012.

Steinernema affine caused 0-39.3±1.5% mortality and found as the least efficient species. *S. carpocapsae* caused 0-43.7±1.5% mortality while *S. feltiae* caused 0-90.7±1.5% mortality. Among the *Steinernema* species, *S. feltiae* was found to be the most efficient species. *H. bacteriophora* caused 0-81±3.5% mortality and was the second efficient species after *S. feltiae* against *T. absoluta* in tomato field

in 2012. The differences between control days and EPNs application days were significant (*F*= 28.40; *df*= 12; *P*<0.000). The differences between control days and EPN isolates (*F*= 11.88; *df*= 18; *P*<0.000), EPN application days and EPN isolates (*F*= 63.65; *df*= 6; *P*<0.000), control days, EPN application days and EPN isolates were also found significant (*F*= 2.50; *df*= 36; *P*<0.000).

Table 1. Mortality of *Tuta absoluta* larvae caused by EPNs in field in 2012[a, b, c] Mean (%)±SE

Day	*Heterorhabditis bacteriophora* (isolate 1144)*			*Steinernema affine* (isolate 46)			*Steinernema carpocapsae* (isolate 1133)			*Steinernema feltiae* (isolate 879)		
	7**	14	21	7	14	21	7	14	21	7	14	21
3***	0±0 C f I	15.7±1.5 B e II	33.0±1.7 A d II	0±0 B c I	9.7 ±0.9 A c II	14.7±1.8 A c III	0±0 B e I	16.0±1.2 A d II	17.3±2.3 A d III	0±0 C e I	42.0±1.7 B e I	58.3±2.3 A e I
5	23.3±1.2 B e I	21.7±2.6 B e II	38.3±1.5 A d II	10.3±1.5 B b II	11.0±1.7 AB c III	17.0±2.3 A c III	9.3±0.9 B d II	19.7±2.3 A cd II	21.3±2.0 A cd III	14.3±2.0 C d II	54.3±2.3 B d I	65.7±1.8 A de I
7	28.7±2.3 B de I	33.7±2.6 B d II	44.7±2.3 A cd II	13.3±1.5 B ab II-III	20.7±1.8 A b III	19.7±1.2 AB bc III	11.0±1.7 B d III	21.3±1.8 A cd III	22.3±1.5 A cd III	20.3±2.0 B d II-III	66.7±2.3 A c I	70.3±3.5 A cd I
9	35.0±2.7 B cd I	41.0±2.3 B cd II	52.3±2.6 A c II	14.3±2.3 B ab II	23.0±2.3 A ab III	21.3±2.3 AB bc III	16.3±2.3 B cd II	29.3±1.8 A bc III	29.7±2.3 A bc III	36.3±2.6 B c I	77.7±2.0 A b I	77.3±3.5 A bc I
11	44.3±2.6 B bc I	48.3±2.6 B bc II	64.3±2.6 A b II	17.3±2.6 B ab II	24.0±2.3 AB ab III	28.3±2.6 A ab III	20.7±2.3 B bc II	32.0±1.7 A ab III	36.3±3.2 A ab III	49.7±2.6 B b I	81.3±4.1 A ab I	81.0±2.9 A b I
13	51.0±3.5 B b I	53.7±3.5 B b II	71.3±3.2 A ab II	20.0±2.1 B a II	31.7±3.2 A a III	34.3±2.3 A a III	28.0±2.7 B ab II	39.0±2.1 A ab III	41.0±2.1 A ab III	59.7±2.6 B b I	84.7±3.8 A ab I	85.7±3.2 A ab I
15	64.0±2.3 B a I	73.7±2.6 A a II	81.0±3.5 A a II	22.0±1.7 B a III	32.7±2.6 A a III	39.3±1.5 A a III	37.7±2.6 A a II	41.3±2.0 A a III	43.7±1.5 A a III	72.3±2.6 B a I	86.3±5.4 A a I	90.7±1.5 A a I

[a] The EPN isolate (*) means within column followed by the same capital letter for the control day are not statistically different by Tukey's multiple range test $P < 0.05$

[b] The EPNs application day (**) means within column followed by the same small letter for each EPN isolate are not statistically different by Tukey's multiple range test $P < 0.05$

[c] The control day (***) means in a row followed by the same roman numeral for the EPN application day and EPN isolate are not statistically different by Tukey's multiple range test $P < 0.05$.

Efficacy of entomopathogenic nematodes in the second year

The efficacy of entomopathogenic nematodes in field in 2013 varied between 0-94.3±2.0%. Similar to the results obtained in 2012, the least efficient day was found as the 3rd and the most efficient day was found as the 15th. After the emergence of *T. absoluta* adults, the lowest mortality occurred on the 7th day and the highest mortality occurred on the 21st day. The least efficient species was *S. affine* (isolate 46) and the most efficient species was *S. feltiae* (isolate 879) with the mortality of 43.7±2.3% and 94.3±2.0%, respectively (Table 2). The temperature and humidity was recorded from June to October and found between 19.9-25.5 °C and 50.4-60.3%, respectively in 2013.

Steinernema affine caused from 0 to 43.7±2.3% mortality and was the least efficient species. *S. carpocapsae* caused from 0 to 49.3±2.4% mortality, whereas *S. feltiae* caused from 0 to 94.3±2.0% mortality. Among the *Steinernema* species, *S. feltiae* was the most efficient species. *H. bacteriophora* caused from 0 to 83.0±2.1% mortality and was the second efficient species after *S. feltiae* against *T. absoluta* in field in 2013. The differences between control days and EPNs application days were significant (*F*= 37.79; *df*= 12; *P*<0.000). The differences between control days and EPN isolates (*F*= 15.47; *df*= 18; *P*<0.000), EPN application days and EPN isolates (*F*= 78.35; *df*= 6; *P*<0.000), control days, EPN application days and EPN isolates were also found significant (*F*= 2.94; *df*= 36; *P*<0.000).

Table 2. Mortality of *Tuta absoluta* larvae caused by EPNs in field in 2013 [a, b, c] Mean (%)±SE

Day	*Heterorhabditis bacteriophora* (isolate 1144)*			*Steinernema affine* (isolate 46)			*Steinernema carpocapsae* (isolate 1133)			*Steinernema feltiae* (isolate 879)		
	7**	14	21	7	14	21	7	14	21	7	14	21
3***	0±0 C f I	17.3±1.5 B f II	34.3±2.3 A e II	0±0 B d I	12.3±0.9 A d II	16.3±1.5 A d III	0±0 B f I	18.0±1.7 A d II	20.3±2.0 A b III	0±0 C f I	48.0±4.0 B d I	61.0±2.3 A e I
5	21.0±2.3 B e I	25.3±2.0 B ef II	39.7±2.0 A de II	11.3±1.5 B c II	13.7±0.9 AB cd III	18.0±1.7 A d III	10.3±0.9 B e II	21.3±1.8 A d II	22.0±1.7 A b III	14.3±2.0 B e I-II	58.3±3.2 A d I	66.7±2.0 A de I
7	31.0±2.1 B d I	34.7±2.0 B de II	46.0±2.1 A cd II	15.3±2.0 A bc II-III	20.7±1.9 A bc III	21.7±1.5 A cd III	12.3±1.5 B de III	23.7±0.9 A cd III	23.3±2.0 A b III	21.7±1.5 B e II	70.3±3.8 A c I	74.3±3.2 A cd I
9	36.7±2.0 B cd I	43.7±2.0 B cd II	53.7±2.0 A c II	15.7±2.0 B bc II	23.0±1.2 A b III	24.7±2.0 A cd III	18.3±2.0 B cd II	31.3±2.0 A bc III	29.3±2.3 A b III	38.7±2.0 B d I	80.0±2.7 A b I	79.7±3.2 A bc I
11	46.0±2.3 B bc I	51.0±2.7 B bc II	65.7±2.6 A b II	19.0±2.1 B abc II	25.3±2.0 AB ab IV	30.3±1.5 A bc IV	22.0±1.2 B bc II	35.3±2.0 A ab III	41.3±2.0 A a III	51.7±2.6 B c I	84.3±2.6 A b I	82.7±3.5 A bc I
13	53.7±2.3 B b II	57.7±3.5 B b II	72.7±4.1 A b II	20.7±1.8 B ab IV	29.7±1.5 A ab IV	36.7±2.6 A ab III	31.7±2.0 B ab III	40.3±1.5 A ab III	44.7±2.3 A a III	63.3±1.5 B b I	86.0±3.8 A ab I	85.7±2.6 A b I
15	67.7±2.6 B a I	78.0±3.5 A a II	83.0±2.1 A a II	24.7±2.0 C a III	34.0±1.7 B a III	43.7±2.3 A a III	41.3±2.0 A a II	42.3±2.0 A a III	49.3±2.4 A a III	75.0±2.3 B a I	92.0±2.3 A a I	94.3±2.0 A a I

[a] The EPN isolate (*) means within column followed by the same capital letter for the control day are not statistically different by Tukey's multiple range test $P < 0.05$

[b] The EPNs application day (**) means within column followed by the same small letter for each EPN isolate are not statistically different by Tukey's multiple range test $P < 0.05$

[c] The control day (***) means in a row followed by the same roman numeral for the EPN application day and EPN isolate are not statistically different by Tukey's multiple range test $P < 0.05$.

Discussion

Tuta absoluta is considered as one of the most important lepidopterous pests associated with tomato crops and because of its biology and behavior, it is a very challenging pest to control. At high densities and without adequate controls, infestations of *T. absoluta* can result in 90 to 100% loss of field-produced tomatoes by losing their commercial value (Estay, 2000; Vargas, 1970). Effective chemical control is difficult because *T. absoluta* feeds internally within the plant tissues. Also resistance to insecticides is another significant problem in chemical control of *T. absoluta* because of its high reproduction capacity, short generation cycle and intensive use of insecticides (Salazar & Araya, 1997, 2001; Siqueira et al., 2000, 2001). Additionally, the widespread use of pesticides disturbs populations of natural enemies and consequently reduces natural control of this pest. Due to these negative aspects of chemical insecticides other approaches need to be found for this pest.

Some insects may be controlled by a combination of practices that are not fully effective when used alone. *T. absoluta* is one of these insects that require more than one practice to be controlled successfully. Therefore, integrated pest management (IPM) programs are being developed in several countries to manage infestations of *T. absoluta*. EPN species belonging to the families Steinernematidae and Heterorhabditidae have been considered as potential control agents for leafminers in recent years (Olthof & Broadbent, 1990). EPNs can be applied, in combination with other biological and chemical pesticides, fertilizers and soil amendments and in the form of adjuvants or antidesiccants (Glazer & Navon, 1990; Baur et al., 1997). Progress in nematode commercialization during the 1990s was substantial. Development of large-scale production technology and easy-to-use formulations led to the expanded use of nematodes. These developments led to the use of nematodes against various insect species (Georgis et al., 2006).

Many studies on EPNs have been conducted throughout the world, but little research has been conducted on the efficacy of EPNs against tomato leaf miner. This is the first study conducted both in Çanakkale and in Turkey that focused on the efficacy of native EPNs against *T. absoluta* in a tomato field. EPNs most likely entered feeding canals in the leaves of tomatoes. Many larvae of *T. absoluta* died inside these galleries, which indicate that IJs were able to find and infect them.

In a similar study by Batalla-Carrera et al. (2010), the efficacy of the three nematode species after foliar application to potted tomato plants was evaluated under greenhouse conditions. They reported high larval mortality (78.6-100%) and low pupal mortality (<10%) in laboratory experiments. In the leaf bioassay a high level of larval parasitisation (77.1-91.7%) was recorded. In the pot experiments, they determined that nematode treatment reduced insect infestation of tomato plants by 87-95%. Their findings demonstrate the suitability of EPNs for controlling *T. absoluta*.

In another study, the efficacy of soil treatments of three native EPNs (*Steinernema carpocapsae*, *S. feltiae* and *Heterorhabditis bacteriophora*) against *T. absoluta* larvae, pupae and adults was determined under laboratory conditions (Garcia-del Pino et al., 2013). They also evaluated the effect of three insecticides commonly used against *T. absoluta*, in the survival, infectivity and reproduction of these nematode species. When the larvae dropped into the soil to pupate, soil application of nematodes resulted in a high mortality of larvae: 100%, 52.3% and 96.7% efficacy for *S. carpocapsae*, *S. feltiae* and *H. bacteriophora,* respectively. No mortality of pupae was observed and mortality of adults emerging from soil was 79.1% for *S. carpocapsae* and 0.5% for *S. feltiae*. They reported that the insecticides tested, had a negligible effect on nematode survival, infectivity and reproduction. They didn't observe any sublethal effects. Their results suggest that larvae of *T. absoluta*, falling from leaves following insecticide application, could be suitable hosts for nematodes, thereby increasing their concentration and persistence in the soil.

Kaya & Gaugler (1993) emphasized that there is a need for more in-depth basic information on EPNs biology, including ecology, behavior, and genetics, to help understand the underlying reasons for their successes and failures as biological control agents. Selecting the most appropriate nematode species and/or strain is important for efficacy and abiotic factors such as soil type, soil temperature and moisture. Proper match of the nematode to the host entails virulence, host finding, and ecological factors are essential before application to the field. There is little hope of success if a nematode does not possess a high level of virulence toward the target pest. In rare cases, persistence may compensate for moderate virulence (Shields et al., 1999). Matching the appropriate nematode host-seeking strategy with the pest is also essential. Poor host suitability has been the most common cause of failure in EPN applications (Gaugler, 1999). Furthermore, high virulence under laboratory conditions has often been inappropriately extrapolated to field efficacy (Georgis & Gaugler, 1991). Application strategies, including field dosage, volume, irrigation and appropriate application methods, are very important. Besides, crop morphology and phenology must be considered in predicting whether nematodes are viable control candidates (Georgis et al., 2006).

Our results clearly demonstrate that larvae of *T. absoluta* were highly susceptible to the EPNs tested and these EPNs can be used as efficient biological control agents against *T. absoluta*. All four EPNs tested showed efficacy at different rates against *T. absoluta*. EPNs were able to find and infect *T. absoluta* larvae both inside and outside of the tomato leaf. In conclusion, it could be suggested that EPNs have a great potential to use as biocontrol agents for the management of *T. absoluta*. Typical feeding galleries made by *T. absoluta* larvae provide EPNs an excellent environment to penetrate the pest easily and also avoid negative factors (desiccation, ultraviolet light, etc.). However, to control *T. absoluta* effectively, it is critical to combine all available control measures including cultural methods, other biological control agents, and the proper and judicious use of registered pesticides.

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Orijinal araştırma (Original article)

Effects of entomopathogenic nematodes, *Heterorhabditis bacteriophora* (Poinar) and *Steinernema carpocapsae* (Weiser), in biological control of *Agrotis segetum* (Denis & Schiffermüller) (Lepidoptera: Noctuidae)

Agrotis segetum (Denis & Schiffermüller) (Lepidoptera: Noctuidae)' un biyolojik mücadelesinde entomopatojen nematodlar, *Heterorhabditis bacteriophora* (Poinar) ve *Steinernema carpocapsae* (Weiser)' ın etkinlikleri

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Summary

Agrotis segetum (Denis & Schiffermüller) (Lepidoptera: Noctuidae) is one of the most serious pests in Iran that attacks nearly all vegetables. Using synthetic insecticides is the main controlling method of this pest. Human and environmental health hazards on insecticides usage encourage scientists to search for alternative safer methods. This research is devised to evaluate the potential of two indigenous entomopathogenic nematodes against this pest. Ability of different concentrations of infective juveniles to infect penultimate and last instars larvae, pre-pupa and pupa was assessed under laboratory condition after 12, 24 and 48 h. Susceptibility of larval stages, pre-pupa and pupa to different concentrations of both entomopathogenic nematodes was evaluated in a pot experiment. Final instar larvae was the most susceptible stage in both laboratory and greenhouse condition. Pre-pupa was more vulnerable to entomopathogenic nematodes as compared with pupa. The mortality increased with increasing in the time of exposure. After 12 hours, the LD_{50} of *Heterorhabditis bacteriophora* (Poinar) and *Steinernema carpocapsae* (weiser) on final instar larvae were 34 and 56 infective juveniles per 10 cm Petri dish respectively. About 98 and 90% of final instar larvae were parasitized five days after exposing to *H. bacteriophora* and *S. carpocapsae* in the greenhouse. According to the results, these two indigenous entomopathogenic nematodes have good potentials in managing *A. segetum*.

Keywords: Biocontrol, common cutworm, lethal dose, pest management, turnip moth

Özet

Agrotis segetum (Denis & Schiffermüller) (Lepidoptera: Noctuidae), İran'da hemen hemen tüm sebze bitkilerine saldıran en önemli zararlılardan birisidir. Zararlının mücadelesinde sentetik kimyasallar kullanılmaktadır. İnsektisit kullanımının çevreye insan hayatına vermiş olduğu olumsuz etkileri ortadan kaldırmak için bilim insanları zararlılar ile mücadele için alternatif ve daha güvenli metotlar araştırmaktadırlar. Bu çalışma bu zararlıya karşı iki entomopatojen nematod türünün potansiyelini belirlemek için planlanmıştır. Farklı konsantrasyondaki nematod juvenillerinin zararlının sondan bir önceki, son, pre-pupa ve pupa dönemleri üzerindeki etkisi laboratuvar koşullarında uygulamadan 12, 24 ve 48 saat sonra değerlendirilmiştir. Larva dönemlerinin, pre-pupa ve pupa dönemlerinin her iki nematodun farklı konsantrasyonlarına uygunluğu saksı denemeleri ile belirlenmiştir. Zararlının son larva dönemi hem laboratuvar hem de sera koşullarında nematodlar için en uygun dönem olarak belirlenmiştir. Pupa dönemi ile karşılaştırıldığında pre-pupa dönemi nematodlara karşı daha hassas bulunmuştur. Ölüm oranı zararlının nematodla muamele süresi arttıkça artmıştır. Uygulamadan 12 saat sonra *Heterorhabditis bacteriophora* (Poinar) ve *Steinernema carpocapsae* (Weiser)' nın son larva dönemine karşı 10 cm çapındaki Petri kabında LD50 değeri sırasıyla 34 ve 56 juvenil bulunmuştur. Serada *H. bacteriophora* ve *S. carpocapsae* uygulamalarından beş gün sonra son larva dönemlerinin yaklaşık % 98 ve 90'nın nematodlar tarafından parazitlendiği tespit edilmiştir. Araştırma sonuçlarına göre her iki entomopatojen nematod türünün *A. segetum* mücadelesinde önemli bir potansiyele sahip olduğu belirlenmiştir.

Anahtar sözcükler: Biyolojik mücadele, bozkurt, letal doz, zararlılarla mücadele, şalgam güvesi

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Introduction

Larvae of *Agrotis segetum* (Denis & Schiffermüller) (Lepidoptera: Noctuidae) have a widespread host range and can attack the roots and lower stems of their host plants. Their presence is often revealed only when the plants are already damaged severely (Bourner et al., 1992; Bowden et al., 1983). This moth is considered as one of the most serious pests on vegetables and cereals throughout Asia, Europe and parts of Africa that could impose a considerable economic loss (Jakubowska et al., 2005). Management of this pest is difficult due to their soil-dwelling habit. The current control method is mainly based on different kinds of insecticides that have met varying success (Sevim et al., 2010). Hazards on environment and human health have encouraged researchers to find some alternative safe measures for chemicals (Moosavi & Zare, 2012).

Biological control by entomopathogenic nematodes (EPNs) has attracted much attention in the last few decades (Hunt, 2007). Among all EPNs, members of the families Steinernematidae and Heterorhabditidae are considered effective (Hominick, 2002; Adams et al., 2006) with a great potential for biological control, especially against soil-inhabiting insects (Ehler, 1990; Koppenhöfer, 2000; Sharma et al., 2011). Rapid death of the host insects, searching ability of hosts, easy to use, long-term effect, surviving ability in environment, being safer to non-target organisms and compatibility with many chemical insecticides are some of EPNs' favorable features that make them suitable as biocontrol agents (Koppenhöfer & Kaya, 2002; Vashisth et al., 2013). There is significant variation among different species/isolates of EPNs in their host range, environmental requirement for survival and pathogenicity (Bedding, 1990; Lacey & Georgis, 2012). Indigenous isolates of EPNs may have greater potential in biocontrol as a result of their compatibility to native habitats (Griffin et al., 2005); therefore, it is rational to evaluate the ability of locally adapted species or isolates in controlling significant pests of that region (Moosavi & Zare, 2015).

Lepidopterans are considered as a susceptible host for Steinernematids and Heterorhabditids (Vashisth et al., 2013). Additionally, existence of the common cutworm's larval stages below ground, make them an appropriate target for biocontrol by EPNs (Georgis et al., 2006). Thus the present study was designed to evaluate the effect of two Iranian species of EPNs (Damani Zamani et al., 2015) on biological control of the common cutworm, *Agrotis segetum*, in laboratory and greenhouse.

Materials and Methods

Preparation of entomopathogenic nematodes inoculum

Two indigenous nematode species maintained alive in the Marvdasht branch, Islamic Azad University EPN Collection were used in this research. Enough population of nematodes were reared on *Galleria mellonella* L. (Lepidoptera: Pyralidae) larvae. The greater wax moth larvae was collected from infected hives and reared on an artificial medium (Metwally et al., 2012).

Petri dishes (100 × 15 mm) were lined with two pieces of filter papers and were moistened with 2 or 3 ml of water containing 200-300 infective juveniles (IJs) of *Steinernema carpocapsae* (Sc) or *Heterorhabditis bacteriophora* (Hb). Ten late instar larvae of the greater wax moth were exposed to IJs of nematodes by putting on filter papers (Nguyen, 2007). The IJs migrated away from the host cadaver upon emergence and were harvested on the White trap (White, 1927; Ehlers & Shapiro-Ilan, 2005). IJs were stored at 12°C (Stock & Goodrich-Blair, 2012) for one week and were permitted to acclimatize at room temperature for 1 h before using as inoculum. The viability of IJs in nematode suspension was assessed by observation of movement under a stereomicroscope.

Collecting and rearing of *Agrotis segetum*

The common cutworm larvae were collected from different tomato fields near Marvdasht, south west of Iran. In order to prepare enough larvae for the experiment, sufficient population of *Agrotis segetum* was established in growth chamber at a temperature of 23°C, relative humidity 60% and a photoperiod of 17:7 (L:D) (Rosen, 2002). Different healthy larval stages and pupae were selected to be used in pathogenicity test.

Pathogenicity test

Laboratory experiment

The virulence of two indigenous nematode species against pre-pupa, pupa and two last larval stages (4^{th} and 5^{th} instar larvae) of common cutworm was evaluated in a laboratory experiment. Ten larvae in fourth or fifth instar stage were put on two moist filter papers in a 100 × 15 mm Petri dish inoculated with 1 ml of water containing different concentrations of IJs. The experiment was established in a completely randomized design with a factorial treatment arrangement consisting of two nematode species (Hb and Sc) and 4 application rates (25, 50, 75, and 100 IJs/dish). The number of dead larvae was recorded at 3 different exposure times (24, 48 and 72 hour). Control plates were treated with distilled water only. Five replicates were considered for each treatment and Petri dishes were kept at 27 ± 1 °C. Dead larvae were recognized according to change in their body color. Cadavers were transferred to White trap to confirm nematode infection.

The susceptibility of pre-pupal and pupal stages of the common cutworm was also assessed in soil. Ten insect's pre-pupa or pupa was placed at the bottom of a dish with 3 cm depth and the dish was filled with 23 g of moistened sterile sandy loam soil (sand 67.3%, clay 12.1%, silt 20.6%, organic matter 3.5% and pH 7.5). The experiment was conducted at 27 ± 1 °C in a factorial arrangement consisting of two nematode species (Hb and Sc) and 3 application rates (50, 100 and 200 IJs/ cm^2 of soil) with five replicates. IJs of two nematode species were applied on the soil surface. The amount of water in nematode suspension was adjusted that the final soil moisture level reached to 10% (w/w). Control plates were treated with distilled water only. Mortality of the pre-pupae and pupae was recorded 48 and 72 h after inoculation by transferring on individual White trap to verify the mortality was due to nematode infection.

Greenhouse experiment

500 g plastic pots (15 cm diameter, 15 cm depth) were filled with sterile sandy loam soil (sand 67.3%, clay 12.1%, silt 20.6%, organic matter 3.5% with pH 7.5) and two disinfected tomato seeds (*cv*. Early Urbana) were sown in each pot. After two weeks, one seedling was selected and the other was eliminated. Each nematode species was evenly applied on to the soil surface of each pot at the rate of 8, 10 and 20 IJs/g soil (respectively equal to 25.5, 28.3 and 56.6 IJs cm^{-2} soil). After 48 h, the pots were separately inoculated with ten larvae (2^{nd} to 5^{th} larval stages), pre-pupae and 3-days-old pupae of the common cutworm. Different developmental stages of insect were put at depth of 2 cm and were covered with soil. After five days, the number of dead insects was counted. Cadavers were transferred to White trap to confirm nematode infection. The experiment was carried out in a completely randomized design with five replicates.

Statistical analysis

The mortality percentage of each treatment was corrected according to the control treatment values by Abbott's formula (Abbott, 1925). Statistical analyses were carried out by SAS software (version 9.1.3; SAS Institute, Cary, NC) (1990). Mean values were separated using Duncan's Multiple Range Test ($P < 0.05$). Polynomial regression was performed on larval mortality data in the laboratory experiment to determine the lethal dosage which kills 50% (LD_{50}) and 90% (LD_{90}) of insect's population for each nematode species (Sigmaplot 11, Systat Software Inc., San Jose, CA). The effect of same concentrations of *H. bacteriophora* and *S. carpocapsae* on mortality of each developmental stage of *A. segetum* in greenhouse was compared by the Independent-Samples T Test.

Results

Laboratory experiment

The mortality of insect's larvae was significantly influenced by nematode species (N), nematode concentration (C), and their interaction (N × C) (Table1). At all exposure times, *H. bacteriophora* caused a significantly greater mortality in 4th and 5th larval stages of *A. segetum* than *S. carpocapsae* did. Similarly, the highest larval mortality was achieved when EPNs were applied at a dose of 100 IJs / dish.

Table 1. Analysis of variance for mortality of 4th and 5th instar larvae of the common cutworm (*Agrotis segetum*) on filter paper when they exposed to four different concentrations (25, 50, 100 and 200 IJs / Petri dish) of *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* in a factorial design

Source[a]	Mean squares					
	L4 mortality percent [b]			L5 mortality percent [b]		
	12 h	24 h	48 h	12 h	24 h	48 h
N	390.6**	1322.5**	1232.5**	1050.6**	1500.6**	1322.5**
C	3377.3**	3407.5**	4026.7**	3029**	3914**	4689.2**
N × C	167.3**	174.2**	142.5**	139**	174**	149.2**
Error	31.87	24.37	22.2	35.3	20.6	20
C.V. (%)	18.3	32.7	24.5	15.2	27.4	29.2

**: significant at 1% probability levels
[a] N = nematode species, C = nematode concentration
[b] The corrected mortality percents were considered for analysis.

The mortality percent of 4th and 5th instar larvae was increased with increase in exposure time. When L_4 and L_5 were exposed to EPNs for similar time span, L_5 was more vulnerable than L_4 was. After 48 h, *H. bacteriophora* and *S. carpocapsae* caused 98% and 87% mortality respectively in last instar larvae when they were applied at a dose of 100 IJs / dish (Table 2).

Table 2. The effect of different concentrations of *Heterorhabditis bacteriophora* (Hb) and *Steinernema carpocapsae* (Sc) on mortality of 4th and 5th instar larvae of the common cutworm (*Agrotis segetum*) on filter paper

EPN species	No. of IJs[a]	% dead L4 (mean ± SE) [b]			% dead L5 (mean ± SE) [b]		
		12 h	24 h	48 h	12 h	24 h	48 h
Hb	25	33 ± 2.5 e E	39 ± 1.8 d CD	41 ± 1.9 d BC	39 ± 1.9 c CD	46 ± 1.8 e AB	49 ± 1.9 e A
	50	55 ± 3.5 c C	70 ± 2.2 b AB	75 ± 2.3 b A	65 ± 4.5 b B	73 ± 2.5 bc AB	79 ± 1.9 c A
	100	72 ± 2.5a D	83 ± 1.2 a BC	89 ± 2.9 a B	77 ± 2.5 a CD	88 ± 1.2 a B	98 ± 1.2 a A
	200	61 ± 1.9 bc D	70 ± 2.2 b BC	76 ± 1.7 b AB	67 ± 2.5 b C	73 ± 1.2 bc BC	80 ± 2.2 c A
Sc	25	23 ± 2.5 f B	29 ± 1.9 e AB	32 ± 2.6 e A	27 ± 2.5d AB	28 ± 2.5 f AB	34 ± 1.9 f A
	50	41 ± 1.8 d D	49 ± 1.8 c BC	54 ± 1.9 c AB	46 ± 1.9 c CD	53 ± 2.5 d B	60 ± 2.2 d A
	100	66 ± 2.9 ab D	69 ± 2.7 b CD	76 ± 1.8 b BC	68 ± 2.5 b D	79 ± 1.9 b B	87 ± 2.5 b A
	200	66 ± 1.7 ab C	69 ± 2.9 b BC	73 ± 1.2 b B	66 ± 1.8 b C	71 ± 1.9 c BC	79 ± 1.9 c A

[a] the number of infective juveniles in each 100 × 15 mm Petri dish.
[b] Mean values followed by different lowercase letters in the same column, or followed by different uppercase letters on the same row are significantly different according to Duncan's test ($P < 0.05$). Each treatment had five replications.

After 48 h, the mortality of pre-pupa and pupa of *A. segetum* significantly differed according to nematode species (N), nematode concentration (C), and their interaction (N × C) (Table 3). However, no significant effect of nematode species and nematode concentration was observed after 72 h of exposure (Table 3). After 48 or 72 h, *H. bacteriophora* caused greater mortality to pre-pupa and pupa than did *S. carpocapsae*. At the similar time of exposure, the highest level of parasitizing occurred at the highest dose of EPNs (200 IJs cm^{-2} soil).

Pre-pupae were more vulnerable to both EPN species than pupae. Mortality percent increased as the time of exposure increased (Table 4). When IJs of *H. bacteriophora* were applied at a dose of 200 IJs cm^{-2} of soil, 80 and 66 % of pre-pupa and pupa were respectively killed after 72 h. Infection rate of pre-pupa and pupa by *S. carpocapsae* was respectively 76 and 50 percent 72 h after inoculation with a 200 IJs cm^{-2} soil (Table 4).

Table 3. Analysis of variance for mortality of pre-pupae and pupae of *Agrotis segetum* in soil when they exposed to three different concentrations (50, 100 and 200 IJs/cm^2 soil) of *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* in a factorial design

Source[a]	Mean squares			
	Pre-pupae mortality percent [b]		pupae mortality percent [b]	
	48 h	72 h	48 h	72 h
N	907.5**	163.3*	1333.3**	213.3*
C	6070**	5923.3**	6107.5**	2822.5**
N ×C	270**	3.3^{ns}	425.8**	55.8^{ns}
Error	20	29.2	22.9	27.1
C.V. (%)	35.6	19.1	21.8	33.4

^{ns}, *, **: Non significant, significant at 5% and 1% probability levels, respectively.
[a] N = nematode species, C = nematode concentration
[b] The corrected mortality percents were considered for analysis.

Table 4. The effect of different concentrations of *Heterorhabditis bacteriophora* (Hb) and *Steinernema carpocapsae* (Sc) on mortality of pre-pupa and pupa of the common cutworm (*Agrotis segetum*) in soil in laboratory experiment

EPN species	No. of IJs[a]	% dead pre-pupa (mean ± SE) [b]		% dead pupa (mean ± SE) [b]	
		48 h	72 h	48 h	72 h
Hb	50	15 ± 1.6 d B	32 ± 2.5 c A	5 ± 1.6 e C	20 ± 3.2 d B
	100	35 ± 2.2 c B	50 ± 2.2 b A	24 ± 2.9 c C	40 ± 1.6 b B
	200	73 ± 2.5 a AB	80 ± 2.2 a A	50 ± 1.6 a C	66 ± 2.9 a B
Sc	50	10 ± 1.6 d B	28 ± 2.5 c A	2 ± 1.2 e C	13 ± 1.2 e B
	100	30 ± 2.2 c B	44 ± 1.9 b A	15 ± 2.3 d C	31 ± 1.9 c B
	200	50 ± 1.6 b B	76 ± 2.9 a A	38 ± 1.2 b C	50 ± 3.5 a B

[a] the number of infective juveniles per cm^2 of soil. IJs were applied onto the soil surface in Petri dish.
[b] Mean values followed by different uppercase letters on the same row, or followed by different lowercase letters in the same column are significantly different according to Duncan's test ($P < 0.05$). Each treatment had five replications.

The estimated LD_{50} of each nematode species on two developmental stages (L_4 and L_5) of the common cutworm at certain time after inoculation is presented in table 5. The LD_{90} was calculated when it was applicable. The required number of both EPNs for killing 50% of each developmental stage of the pest decreased with increase in exposure time.

Table 5. The calculated LD_{50} or LD_{90} of *H. bacteriophora* and *S. carpocapsae* on the penultimate and last instar larvae of the common cutworm at different exposure times

Growth stage	Time (h)	*Heterorhabditis bacteriophora*			*Steinernema carpocapsae*		
		LD_{50}	LD_{90}	R^{2a}	LD_{50}	LD_{90}	R^{2a}
L_4	12	43	–	0.87	65	–	0.94
	24	32	–	0.95	51	–	0.92
	48	30	–	0.94	45	–	0.95
L_5	12	34	–	0.84	56	–	0.93
	24	28	–	0.95	47	–	0.95
	48	25	65	0.96	39	114	0.96

[a] R^2 shows the coefficient of determination for fitting data in each regression model.

Greenhouse experiment

The virulence of the both EPNs against the common cutworm was similar in greenhouse. No significant difference was found between *H. bacteriophora* and *S. carpocapsae* when the same concentration of them was applied on the same developmental stage of *A. segetum*.

The last instar larvae of *A. segetum* were more vulnerable to both nematode species than other developmental stages were (Figures 1 and 2). The least susceptible developmental stages to *H. bacteriophora* were second and third instar larvae while the least susceptible developmental stage to *S. carpocapsae* was pupa. The best nematode dose for control of nearly all developmental stages of the common cutworm was 10 IJs per g of soil (equal to 28.3 IJs cm^{-2} soil). Higher or lesser concentrations of EPNs resulted in lesser efficacy against *A. segetum*; however, 8 IJs g^{-1} soil (= 25.5 IJs cm^{-2} soil) was more effective compared with 20 IJs g^{-1} soil (= 56.6 IJs cm^{-2} soil).

Application of *H. bacteriophora* on soil surface of tomato pots resulted in a maximum 98% kill of fifth instar larvae five days after inoculation with a dose of 10 IJs g^{-1} soil. Only 54% of 2nd instar larvae were infected by *H. bacteriophora* when it was applied at a dose of 20 IJs g^{-1} soil (Figure 1).

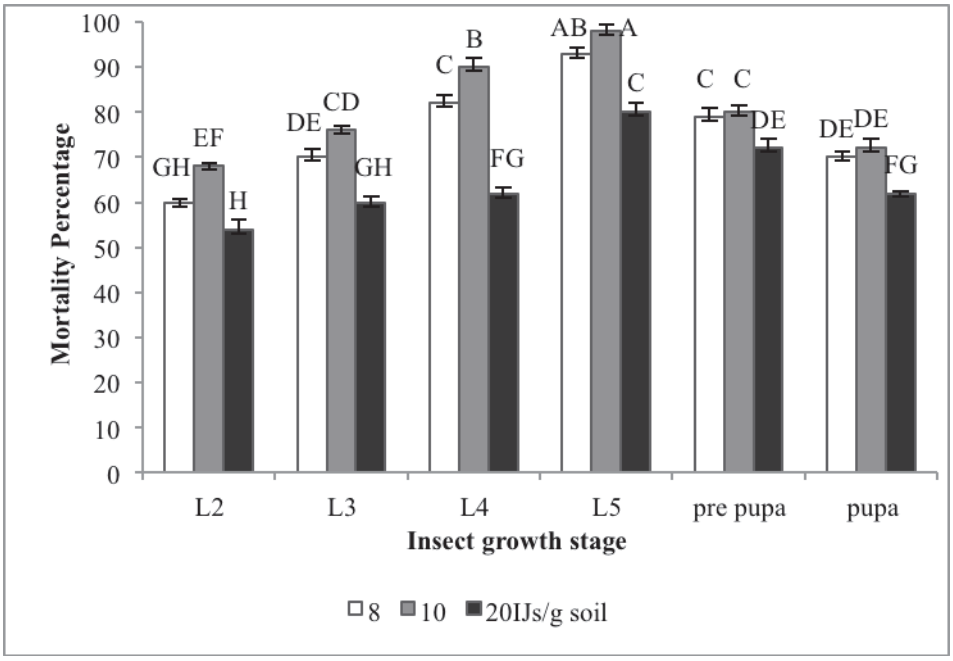

Figure 1. Corrected mortality percent of different developmental stages of the common cutworm five days after inoculation with three concentrations of *Heterorhabditis bacteriophora* in tomato pots. Bars on the columns correspond to standard error. Each treatment had five replications. Columns with the similar letter(s) are not significantly different ($P < 0.05$).

The *S. carpocapsae* respectively caused 90% and 85% mortality in 5th and 4th instar larvae when it was applied at a dose of 10 IJs g^{-1} soil. Eight IJs g^{-1} soil was more virulent to all insect's developmental stages than 20 IJs g^{-1} soil was, except for pupal stage. Contrary to other treatments, pupal's infection by *S. carpocapsae* increased when the application dose of IJs per gram of soil was increased (Figure 2).

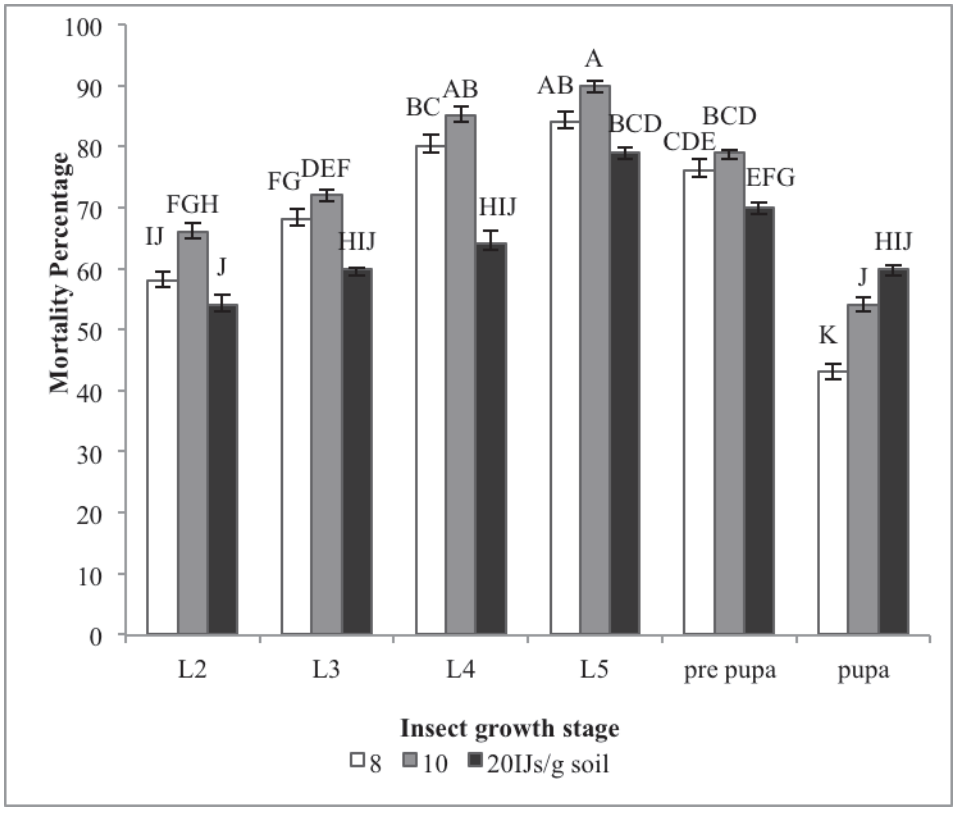

Figure 2. Corrected mortality percent of different developmental stages of the common cutworm five days after inoculation with three concentrations of *Steinernema carpocapsae* in tomato pots. Bars on the columns correspond to standard error. Each treatment had five replications. Columns with the similar letter(s) are not significantly different ($P < 0.05$).

Discussion

EPNs have been successfully employed as efficient biocontrol agents against the larvae of some noctuids, including *Agrotis ipsilon* (Hufnagel) and *A. segetum* in some Asian or European countries (Georgis et al., 2006; Kaya et al., 2006; Lacey & Georgis, 2012). Both *Heterorhabditis* spp. and *Steinernema* spp. are considered as promising biological control agents of important insect pests due to their ability to seek target hosts, kill hosts rapidly, being safe to non-target organisms and having no threat to the environment-as compared to chemical insecticides (Vashisth et al., 2013).

On filter paper, *H. bacteriophora* provided a more efficient control of the common cutworm's larvae. Increasing in time of exposure caused more mortality in 4th and 5th instar larvae for both nematode species (Table 2). Similar results have been repeatedly reported in which increasing exposure time raised the mortality rate of most developmental stages (Capinera et al., 1988; Jackson & Brooks, 1995; Ansari et al., 2006; Ebssa & Koppenhöfer, 2012; Gökçe et al., 2013; Kamali et al., 2013) due to increasing the possibility of encountering with and penetrating into the insect by IJs.

Insect's larval (L_4 and L_5) mortality was IJs-does-dependant. It is suggested that exposing insect hosts to higher dose of EPNs may obfuscate host recognition and decrease host mortality (Lewis, 2002). Though the filter paper technique cannot simulate field conditions accurately, it is usually used as a quick method of efficacy assessment (Capinera et al., 1988).

The common cutworm pre-pupa was more susceptible to both EPNs rather than pupa. *H. bacteriophora* appeared to act better since it inflicted higher mortality in pre-pupal and pupal stages of insect than *S. carpocapsae* did. The soil technique surely is more similar to natural conditions (Stock & Goodrich-Blair, 2012). Pre-pupa further susceptibility may be due to the fact that the natural opening of this developmental stage is not completely sealed yet and its cuticle is thinner.

The LD_{50} of *H. bacteriophora* on fourth and fifth larval stages of *A. segetum* was lower than those of *S. carpocapsae* (at similar time span). Because of decline in larval mortality at doses more than 100 IJs, LD_{90} was only calculable after 48 h and on L_5 where the mortality percent surpassed 90%. The pathogenicity of EPNs may greatly differ according to different species / isolates (Bedding, 1990; Lacey & Georgis, 2012). The LD_{50} of *Steinernema kraussei* (Steiner) against *A. segetum* third instar larvae was 99 IJs g^{-1} dry sand seven days after treatment (Gökçe et al., 2013). Application of a Japanese isolate of *S. carpocapsae* against *A. segetum* larvae at a rate of 5×10^5 and 10^6 IJs/m^2 soil respectively caused 67% and 80% mortality (Yokomizo & Kashio, 1996). Among three Turkish *Steinernema* species, *S. feltiae* (Filipjev), *S. weiseri* (Mrácek, Sturhan & Reid) and *S. carpocapsae*, the latter was more effective when applied at 10, 25, 50 and 100 IJs per larva (Unlu et al., 2007). The LD_{50} of two isolates of *S. carpocapsae* (in the original article the species of the nematode was identified as *S. feltiae* according to confusion on the nomenclature at that time) against *A. ipsilon* was determined as 16 and 486 IJs per 100 g soil (Capinera et al., 1988). Significant variation was also reported for different strains of *S. carpocapsae* against *Curculio caryae* (Horn) (Shapiro-Ilan et al., 2003).

Mortality of successive larval stages of common cutworm progressively increased in pot experiment by both EPN's species with the maximum value seen in 5^{th} instar larvae. Thereafter the susceptibility reduced for pre-pupae and pupae. This pattern of susceptibility is consistent with earlier studies (Shannag et al., 1994; Jackson & Brooks, 1995; Kim et al., 2004; Ebssa & Koppenhöfer, 2012). Variation in vulnerability of different developmental stages of insect hosts has been chiefly ascribed to differences in the size or accessibility of body natural openings (mouth, spiracles and anus) which serve as a gate for entrance of nematode IJs (Dowds & Peters, 2002). On the other hand, older larvae with larger body size may attract more IJs (Smits et al., 1994). For noctuids, stage susceptibility may also be affected by variation in feeding activity. Susceptibility of unfed insect larvae was more than fed ones at lower nematode inoculum doses. Emergence of IJs from unfed insect larval cadaver was also faster and more frequent than from cadavers of fed ones (Kondo, 1987).

Pre-pupa and pupa were less susceptible to tested EPNs than most larval stages were. Less available natural opening for entering IJs to pupae can reduce pupal vulnerability. Only spiracles remain open at pupal stage and the mouth and anus are sealed. The cuticle of pupae is also thicker than in the larval stages and resists more against IJs penetration (Dowds & Peters, 2002). Pupae are considered as a relative inactive stage with minimum metabolism. At this stage, volatile cues emission (especially CO_2) by pupae is significantly decreased than by larval stages making pupae less attractive to IJs (Lewis et al., 1993, 1995).

There was no significant difference between virulence of *H. bacteriophora* and *S. carpocapsae* against the common cutworm in pot experiment. Search tactics and dispersal pattern of IJs in soil govern infectivity level (Griffin et al., 2005). Foraging strategy of *S. carpocapsae* IJs is ambushing (Campbell & Gaugler, 1993) while *H. bacteriophora* IJs are cruiser (Lewis, 2002). *S. carpocapsae* IJs usually inhabit the upper 1-2 cm of the soil while *H. bacteriophora* IJs are distributed evenly in the top 8 cm of soil (Ferguson et al., 1995; Campbell et al., 1996). As well, IJs of *S. carpocapsae* tend to move toward the surface of the soil (Georgis & Poinar, 1983) but IJs of *H. bacteriophora* move both upwards and throughout the soil column (Schroeder & Beavers, 1987). Though *S. carpocapsae* is a sit-and-wait strategist (ambusher), it can efficiently parasitize mobile host species (like cutworms) near the soil surface (Hazir et al., 2003). *H. bacteriophora* is highly mobile and orients to volatile host cues (Hazir et al., 2003). In this study the different developmental stages of the pest were put at the depth of 2 cm. Movement of the common cutworm's larval stages in the soil enhances the chance of confronting with the infective juveniles of the both species of EPNs. These factors along with dispersal pattern of both EPNs may be the reason of similar virulence against *A. segetum*.

The efficacy of *S. carpocapsae* and *H. bacteriophora* against cutworms were inconsistent in previous studies. *S. carpocapsae* was highly virulent (95%) to *A. ipsilon*, while *H. bacteriophora* did not provided sufficient control (62%) (Georgis & Poinar, 1994). When four commercial products containing *H. bacteriophora*, *S. carpocapsae*, *S. feltiae* and *S. riobrave* (Cabanillas, Poinar & Raulston) were applied against the fourth-instar of *A. ipsilon* in turfgrass, *S. carpocapsae* was the best performing species. Though the efficacy of *H. bacteriophora* and *S. feltiae* was often like to *S. carpocapsae*, their overall consistency were lesser (Ebssa & Koppenhöfer, 2011). Six isolates of EPNs (*S. carpocapsae* All, *S. carpocapsae* Mex, *S. feltiae* T319, *S. longicaudum* X-7 (Shen & Wang), *H. bacteriophora* H06 and *H. indica* LN2 (Poinar, Karunakar & David)) were tested against *A. ipsilon* in a laboratory and field experiments in china. *S. carpocapsae* Mex and *Heterorhabditis indica* LN2 were the most effective species and could respectively kill 80 and 83.3 % of 3rd instar larvae after 72 h (Yan et al., 2014). The pathogenicity of ten Canadian (from three species: *S. carpocapsae*, *S. feltiae,* and *S. kraussei* ((Steiner) Travassos)) and two commercial (from two species: *S. carpocapsae* and *S. feltiae*) isolates of EPNs were tested against *A. ipsilon* in a laboratory experiment. Both commercial (98% at the 1000 IJs concentration after 72 h) and a Canadian (94% at 250 IJs / larva / Petri dish after 72 h) isolates of *S. carpocapsae* could effectively control the pest (Bélair et al., 2013). When *S. carpocapsae* and *H. bacteriophora* were sprayed on above ground of corn at a rate of 5×10^5 IJs / 150 plants, they could respectively kill 96.7 and 68.7% of *A. ipsilon* larvae (Saleh et al., 2015). *Steinernema websteri* was recently isolated from *A. segetum* in Turkey and could efficiently control this pest. The nematode killed 100% of third instar larvae in 5 days when it was applied with the concentration of 500 IJs / g dry sand (Gökçe et al., 2015).

The results of current research suggest that both *H. bacteriophora* and *S. carpocapsae* can be used to control the common cutworm. Future work in an open field situation should be done to emphasize the potential of these two candidates in biological control of *A. segetum*.

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Orijinal araştırma (Original article)

Distribution of nematodes on onion and their relationship with soil physicochemical characteristics in Karaman province, Turkey[1,2]

Türkiye Karaman ilinde soğan ekiliş alanlarında bulunan nematodların dağılımı ve toprak fizikokimyasal özellikleri ile ilişkileri

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Summary

The distribution of plant-parasitic and free-living nematodes on onion rhizospheres and plant material and their relationship with soil physicochemical properties was investigated in Karaman province. Plant and soil samples totalling 100 were collected from onion fields during July, 2012. Nematode population density per three plants and 100 g of dry soil were determined for each sample. *Ditylenchus dipsaci* (Kühn 1857) (Tylenchida: Anguinidae) was found in 15% of plants and 61% of soil samples. Nematode numbers ranged between 0 and 140 (mean: 5) nematodes/three plants and 0–165 (mean: 33) nematodes/100 g dry soil. Other abundant plant-parasitic nematode genera were *Paratylenchus* (Micoletzky 1922) (Tylenchida: Paratylenchidae) (56%) and *Tylenchus* (Bastian 1865) (Tylenchida: Tylenchidae) (49%). The most abundant free-living nematodes were bacterivorous nematodes, which were found in 98% of samples, and were dominated by the *Cephalobus* (Bastian 1863) (Rhabditida: Cephalobidae) and *Eucephalobus* (Steiner 1936) (Rhabditida: Cephalobidae) genera. The majority of soil samples (68%) contained fungivorous nematodes belonging to the *Aphelenchus* (Bastian 1865) (Aphelenchida: Aphelenchidae) and *Aphelenchoides* (Fischer 1894) (Aphelenchida: Aphelenchoididae) genera. Omnivorous nematodes in the Dorilaimida order were found in 23% of soil samples. The distribution of plant- and bacterial-feeding nematodes were negatively related to the soil silt content and positively related to the soil sand content. Hyphal-feeding nematodes were negatively related to soil organic matter.

Keywords: Nematode, onion, soil physicochemical characteristics, Karaman

Özet

Soğan ekiliş alanlarındaki bitki paraziti ve serbest yaşayan nematodlar tespit edilmiş ve toprak fizikokimyasal özellikleriyle ilişkileri araştırılmıştır. Yüz soğan ekili alandan 2012 Temmuz ayında 100 adet bitki ve toprak örneği alınmıştır. Nematod sayıları 3 bitki başına ve 100 g kuru topraktaki nematod sayısı olarak verilmiştir. *Ditylenchus dipsaci* (Kühn 1857) (Tylenchida: Anguinidae) bitki örneklerinin %15'inde, toprak örneklerinin %61'inde bulunmuştur. Nematod sayıları 3 bitki başına 0-140 nematod, ortalama olarak 5 nematod, 100 g toprakta ise 0-165 arasında ortalama olarak 33 nematod olarak tespit edilmiştir. *Paratylenchus* (Micoletzky 1922) (Tylenchida: Paratylenchidae) (56%) ve *Tylenchus* (Bastian 1865) (Tylenchida: Tylenchidae) (49%) türleri yoğun olarak bulunan diğer bitki paraziti nematodlardır. Serbest yaşayan nematodlar içinde en yüksek %98 oranında bulunan bakterivor nematodlar içinde en fazla bulunan cinsler *Cephalobus* (Bastian 1863) (Rhabditida: Cephalobidae) ve *Eucephalobus*'(Steiner 1936) (Rhabditida: Cephalobidae) dur. *Aphelenchus* (Bastian 1865) (Aphelenchida: Aphelenchidae) ve *Aphelenchoides* (Fischer 1894) (Aphelenchida: Aphelenchoididae) cinslerine ait fungivor nematodlar örneklerin %86'sında bulunmuştur. Dorylaimida takımına ait omnivor nematodlar, örneklerin %23'ünde bulunmuştur. Bitki paraziti ve bakterivor nematodların dağılımı toprak mil içeriği ile ters ve kum içeriği ile doğrusal bir ilişki içerisindedir. Fungivor nematodlar ise toprak organik madde yoğunluğu ile negatif ilişki göstermiştir.

Anahtar sözcükler: Nematod, soğan, toprak fizikokimyasal özellikleri, Karaman

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Introduction

The onion is a vegetable, which plays an important role in human nutrition. Turkey is currently the seventh largest onion producer after China, India, USA, Iran, the Russian Federation and Egypt (Anonymous, 2014a). During 2012, the total area under onion cultivation in Turkey was 63 000 ha and total annual production was 1 819 000 tonnes (Anonymous, 2014a). Most production occurs in the Amasya province, followed by Ankara, Hatay, Tokat, Eskisehir, Corum, Adana and Bursa provinces in the Central Anatolian, East Mediterranean, Middle Black Sea and Trace Regions. Climatic conditions are favourable for onion production in Karaman province, with a production area of approximately 1000 ha providing 12 657 tonnes of onions during 2012 in Karaman (Anonymous, 2014b).

In addition to physiological disorders, diseases and pests compromise onion production. Plant-parasitic nematodes cause between 8.8 and 14.6% annual global crop losses (Nicol et al., 2011). The stem and bulb nematode, *Ditylenchus dipsaci* (Kühn 1857) (Tylenchida: Anguinidae) is a considerable constraint on onion production in Turkey. *D. dipsaci* was first isolated from onion plants in the Central Anatolian region by Yüksel (1958). Saltukoglu (1974) recorded *D. dipsaci* on onion plants in the Istanbul province. *Ditylenchus dipsaci* was recently recorded in the Konya, Karaman and Nevsehir onion-growing areas (Oztürk, 1990). *D. dipsaci* is currently on the European and Mediterranean Plant Protection Organization (EPPO) quarantine A2 list (No: 174), and has a local distribution in EPPO countries (Anonymous, 2014c).

Ditylenchus dipsaci results in stunting, discolouration and curving of leaves, local lesions and misshapen bulbs in plants and correspondingly, results in yield losses and reduction in market value (Anonymous, 2011a). Economic yield losses were calculated to occur at concentrations of the nematode of ten or more per 400 cm^3 soil (Seinhorst, 1956). Yield losses of 60–80% were recorded in soils heavily infested by *D. dipsaci* (Nickle, 1991). In addition, it has been shown that *D. dipsaci* infestation resulted in 5–100% yield loss of onion and bulbous plants in the Aegean region (Anonymous, 2011b). It has been reported that 54.09% of onion fields in the Suluova district of Amasya province were infected by *D. dipsaci,* and an average of 65% yield reduction was recorded (Mennan & Ecevit, 2002).

Ditylenchus dipsaci was reviewed to be distributed in both heavy and light soils, although a higher incidence of disease severity was associated with heavy soils (Seinhorst, 1956; Miyagawa & Lear, 1970; Elgin et al., 1975; Sikora & Fernandez, 2005). Movement and dispersal of *D. dipsaci* tend to be greater in sandy soils than in clay soils (Elgin et al., 1975). Gerasimow (1954) found that the activity of *D. dipsaci* juveniles was dependent on the soil texture and pH. Activity of the juveniles was found to be higher in sandy soils at pH 7 and in loamy soils at pH 5.

Information on the distribution of nematodes as well as the damage caused on host plants requires urgent revision. Therefore, the aim of this study was to investigate the distribution of nematodes in the onion growing area in Karaman, the potential damage they may cause to onion cultivation and their relationship with soil physicochemical characteristics.

Materials and Methods

Sampling

Plant and soil samples totalling 100 were collected from onion fields located 1–2 km apart in the central Karaman, Ayranci and Ermenek districts from June to July during 2012. Sampling locations were recorded by a global positioning system (GPS). Sampling was performed in a zigzag pattern by taking a soil core sample using a 2.5 cm diameter width auger of 20 cm depth every 15 footsteps in the field. An average of 2 kg (2 000 cc) of soil was collected in total from 15–20 cores taken from each field. Three onion plants were sampled from each field using the same pattern.

Soil physicochemical analysis

Soil samples were analysed for pH, electrical conductivity (EC), $CaCO_3$ and phosphorus (P) contents, organic matter and texture. PH and EC (µS/cm) analyses were conducted according to the 1:2.5 soil: water ratio methodology described by Richards (1954). The $CaCO_3$ content (%) was analysed using the Scheibler calcimeter (Caglar, 1949). The P content (mg/kg) of soil samples was determined using the Olsen methodology (Knudsen, 1975). Organic matter (%) was determined using the Walkley–Black method (Walkley, 1946). Sand, silt and clay contents (%) were fractionated using the Bouyoucos hydrometer methodology (Tüzüner, 1990).

Nematode extraction and identification

Nematodes were extracted from three plants and 100 g fresh soil from each sample using the 'Modified Baermann Funnel Technique' (Hooper, 1986a). Extracted nematodes were counted under a light microscope at 40× magnification in 50 µl nematode suspension. The total number of nematodes in a 1 ml whole sample was calculated by multiplying the number of nematodes in 50 µl by 20. The soil moisture of each sample was calculated from the weight difference between fresh soil and soil dried at 90°C for one night. Nematode numbers were presented in 100 g of dry soil considering the soil moisture of each sample. Nematode genera were grouped according to trophic habits as described by Yeates et al. (1993). Permanent slides of the plant parasitic nematodes were prepared from all available specimens according to Hooper (1986b). Identification of the specimens was performed according to morphological and morphometric characteristics.

Statistical analyses

Soil physicochemical characteristics and nematode numbers were analysed using distribution analysis. The mean, maximum and minimum values were calculated for all variables, and the frequency of the soil texture class was determined. ANOVA was used to determine the frequency and population densities of nematodes and the student's t-test was used to determine the statistical differences between the nematode genera and trophic groups. Regression relationships among soil physicochemical characteristics and soil nematode populations were investigated using multivariate correlation analysis.

Results and discussion

Physicochemical characteristics of soils

Soil pH of collected samples ranged between 6.69 and 7.88, with a mean of 7.60. EC of soils ranged from 70.70 to 274 µS/cm with a mean of 146.71 µS/cm. Soil physicochemical characteristics were evaluated according to standard values for soil fertility (Anonymous, 2013). The soil reaction changed the pH from neutral to weak alkaline. No problematic salinity levels were detected in any of the samples. Most samples had a high $CaCO_3$ content (range: 0.88–67.8%, mean: 24.07%). The soil P content was relatively high in all samples, with the lowest and highest being 3.23 mg/kg and 212.2 mg/kg, respectively (mean: 27.26 mg/kg). Most of the samples had low organic matter content, with the highest and lowest being 5.04% and 0.29%, respectively, in sampled soils. Mean organic matter content of the soil samples was 2.21%. The highest values of clay, silt and sand fractions were 55.29%, 55.29% and 65.69% and the lowest values were 16.11%, 13.67% and 13.67%, respectively. Mean clay, silt and sand contents were 35.45%, 33.13% and 31.23%, respectively. Most of the samples were clay loam (48%) and clay soil (34%). The frequency of sandy clay loam soil was relatively low (18%).

Frequency and population densities of nematodes

Nematodes obtained from the onion-planting areas were of the orders Tylenchida (Thorne 1949), Aphelenchida (Siddiqi 1980), Dorylaimida (Pearse 1942) and Rhabditida (Chitwood 1933). Identified trophic groups were plant-parasitic, hyphal-feeding, bacterial-feeding and omnivorous nematodes. Bacterial and plant-parasitic nematodes were at the highest frequency and population densities in sampled soils. Hyphal-feeding nematodes were grouped as having medium occurrence and population density. The lowest occurrence and population densities were obtained for omnivorous nematodes ($P < 0.05$).

Plant-parasitic nematodes were obtained from all samples. The mean population density of plant-parasitic nematodes was 196 nematodes/100 g of dry soil (range: 21–687 nematodes/100 g of dry soil). The most frequently obtained plant-parasitic nematodes were *D. dipsaci*, *Tylenchus* spp. and *Paratylenchus* spp.

The isolation frequency of *D. dipsaci* was 61%, which was significantly higher among plant-parasitic nematodes ($P < 0.05$). The population density of *D. dipsaci* varied between 0 and 165 nematodes per 100 g of dry soil (mean: 33 nematodes/100 g of dry soil). *D. dipsaci* was found in 15% of plant samples, with a population density of between 0 and 140 nematodes/three plants (mean: four nematodes/three plants). Figure 1 shows the distribution of *D. dipsaci* in soil of Karaman province according to population densities in sampling points. The population densities of *D. dipsaci* in soil samples were very high, with more than 100 nematodes/100 g of dry soil at the central villages of Morcali and Cukurbag. This area is irrigated and cultivated intensively for onion cultivation, and consequently, the higher *D. dipsaci* population densities were expected. In contrast, Damlapinar, Kizilyaka, Bozkondak and Baskisla are areas of non-irrigated onion production, where *D. dipsaci* was found at low frequency and population densities.

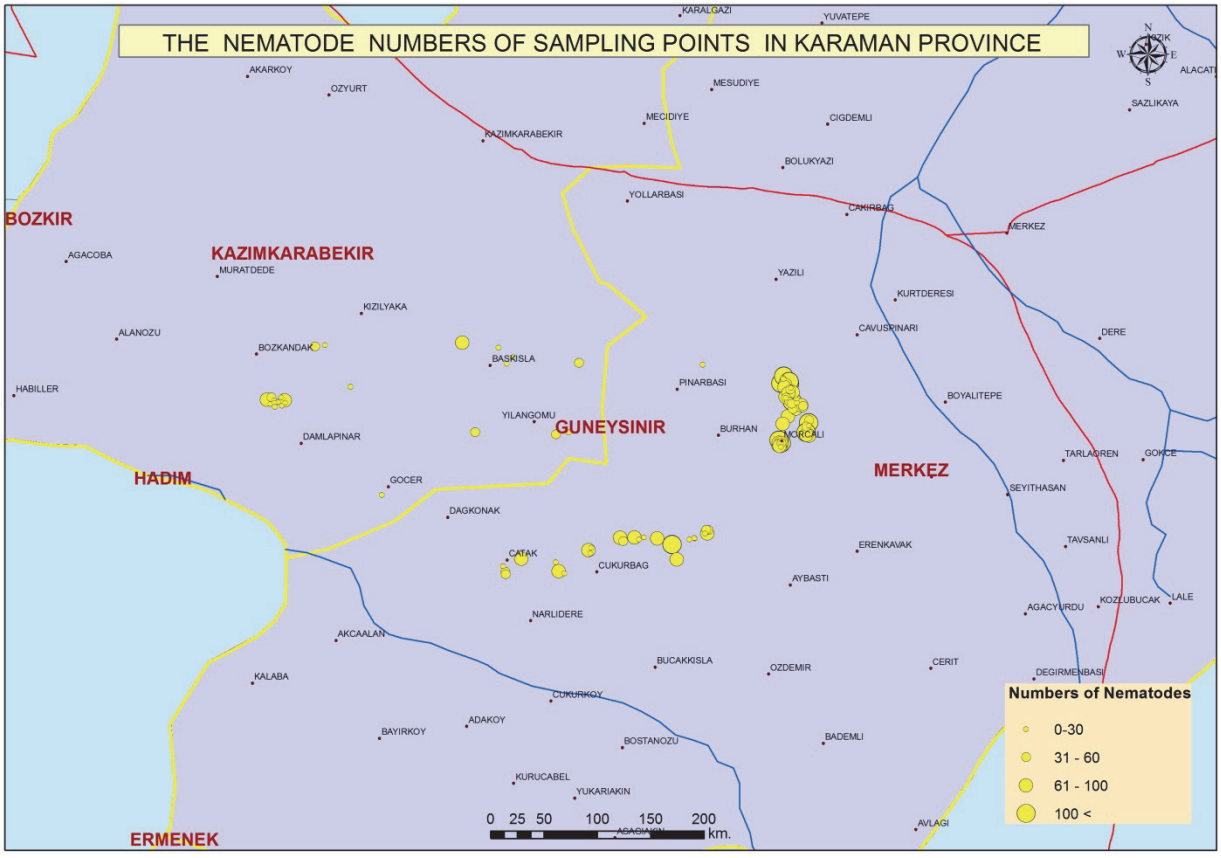

Figure 1. Distribution of *Ditylenchus dipsaci* based on population densities in soil in Karaman province during July 2012.

The second frequently identified genus belonging to plant parasitic nematodes was *Paratylenchus,* which was isolated from 56% of soil samples. The mean population density in sampled fields was 28 nematodes/100 g of dry soil, in a range of 0–163 nematodes/100 g of dry soil

Tylenchus spp. was found in 49% of collected soil samples. The population density of *Tylenchus* spp. ranged between 0–117 nematodes/100 g of dry soil (mean: 22 nematodes/100 g of dry soil).

Aphelenchus and *Aphelenchoides* are hyphal-feeding nematode genera that were found at a rate of occurrence of 74% and 76%, respectively, and their mean population densities were 65 (range: 0–355) and 79 (range: 0–335) nematodes/100 g of dry soil, respectively. There was no significant difference between population densities of hyphal-feeding nematode genera found. The occurrence of total hyphal-feeding nematodes was 86%, with a population range of 0–686 nematodes/100 g of dry soil (mean: 145 nematodes/100 g of dry soil).

The most abundant bacterial-feeding nematode genera was *Cephalobus* (86%; $P < 0.05$), followed by *Eucephalobus* (69%). The density of populations ranged between 0 and 446 nematodes/100 g of dry soil (mean: 81 nematodes/100 g of dry soil) and 0–2055 nematodes/100 g of dry soil (mean: 77 nematodes/100 g of dry soil) for *Cephalobus* and *Eucephalobus* genera, respectively. Total isolation frequency of bacterial-feeding nematodes was 98%, with population densities ranging between 0–2 105 nematodes/100 g of dry soil (mean: 223 nematodes/100 g of dry soil).

The frequency of omnivorous nematodes was 23% and populations ranged between 0 and 114 nematodes/100 g of dry soil (mean: 10 nematodes/100 g of dry soil).

Species identification of key plant-parasitic nematodes

Ditylenchus spp. includes migratory endoparasitic and hyphal-feeding nematodes (Yeates et al., 1993). Morphological identification of specimens obtained from plant samples showed that the specimens belonged to *D. dipsaci* species (Brezeski, 1991; Sturhan & Brzesky, 1991; Mollov et al., 2012). Morphometrical and allometrical measurements for the investigated specimens in comparison with the literature are presented in Table 1.

Table 1. Morphometric characteristics of *Ditylenchus dipsaci* collected from onion plant material. Measurements in µm and brackets indicate the mean ± standard error (range) (isolate K44)

Characteristics	*D. dipsaci* (K44)	*D. dipsaci* (Brzesky, 1991)	*D. dipsaci* (Sturhan & Brzesky, 1991)	*D. dipsaci* (Mollov et al., 2012)
n	6	-	-	-
Total body length	1 203 ± 49.41 (1156.8–1299.2)	-	- (1 000–2 200)	-
Stylet	11.3 ± 0.46 (11.2–12)	- (10–12)	- (10–13)	- (11.5–12.3)
Anterior end to median bulb	65.86 ± 7.53 (60.8–80)	-	-	-
Pharynx length	112.93 ± 22.77 (108.8–168)	-	-	-
V	0.80 ± 0.008 (0.8–0.82)	- (0.76–0.86)	- (0.76–0.86)	- (0.79–0.81)
Post vulval uterine sac	0.40 ± 0.02 (0.37–0.43)	- (0.40–0.70)	-	-
T	81.6 ± 1.75 (80–84.8)	-	-	- (95–105)
a	37.85 ± 4.48 (32–45.1)	-	- (36–64)	- (38–44)
b	9.72 ± 1.24 (7.73–10.92)	-	- (6.5–12)	- (5.8–8.0)
c	14.40 ± 0.97 (12.54–15.32)	- (11–20)	- (11–20)	- (14–17)
C'	3.99 ± 0.42 (3.57–4.81)	- (3–6)	- (3–6)	-

n: number of specimens, L: total body length, Stylet: stylet length, MB: median bulb length, OL: oesophagus length, V: (distance from anterior end of body to vulva)/body length, PUS: length of post vulval part of uterine sac in relation to vulva anus distance, T: tail length, a: body length/the largest width part of body, b: body length/distance from oesophagus intestine overlapping part to anterior end of body, c: body length/tail length, C': tail length/body width at anus.

Relationship between nematodes and soil characteristics

The total plant-parasitic and bacterial-feeding nematodes were negatively related to soil silt content (R = 0.21; 0.29, respectively; P < 0.05). Individual nematode genera of *Ditylenchus, Tylenchus* and *Paratylenchus* additionally showed a negative correlation with soil silt content (R = 0.20 for all; P < 0.05) (Fig. 2).

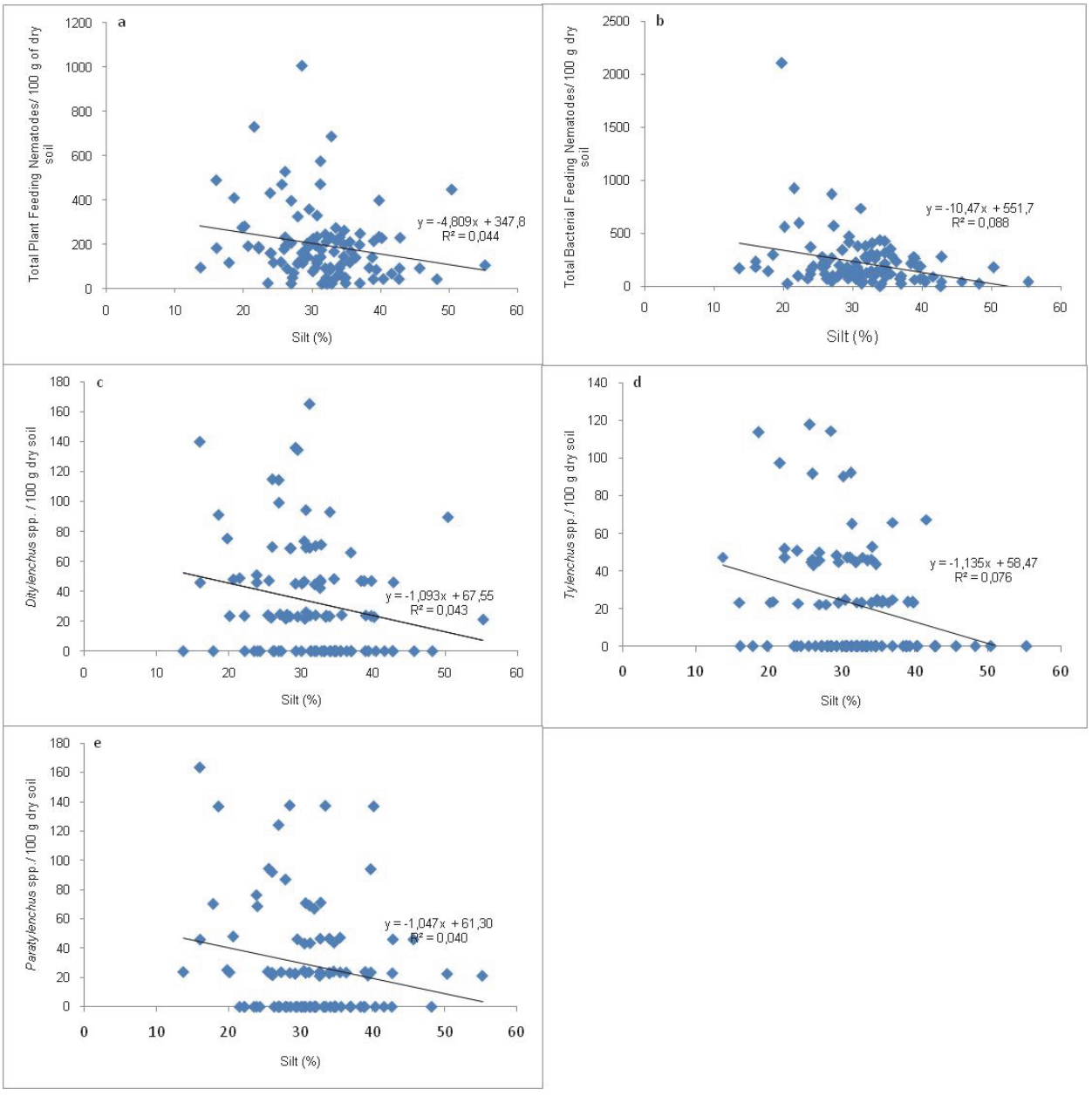

Figure 2. Relationship between soil silt content and plant- (a) and bacterial-feeding (b) nematode trophy groups, *Ditylenchus dipsaci* (c), *Tylenchus* spp. (d) and *Paratylenchus* spp. (e).

The total number of the bacterial-feeding nematodes was positively correlated to soil sand content (R = 0.34; P < 0.05). *D. dipsaci* and *Paratylenchus* spp. were positively correlated with soil sand content (R = 0.19 and R = 0.22, respectively; P < 0.05; Fig. 3). The distribution and survival of *D. dipsaci* in soil

has been found to be strictly dependent on soil texture and structure in past studies (Gerasimow, 1954; Miyagava & Lear, 1970; Elgin et al., 1975). Seinhorst (1954) reported that the distribution of *D. dipsaci* is circular and of a short distance in clay soils, whereas, in contrast, their distribution is irregular and of a relatively long distance in sandy soils, as presumably nematodes are carried by soil particles. The correlation analyses in the current study supported the previous findings for both *D. dipsaci* and plant-parasitic nematodes, with a positive relationship with soil sand content found. Even the effect of pH was not significantly related to nematode distribution in the current study, and *D. dipsaci* is known to be more active in alkaline and sandy soils as compared to acidic and loamy soils (Gerasimow, 1954). Miyagava & Lear (1970) had additionally noted that *D. dipsaci* had a longer infectivity in sandy soil containing 2.5% moisture.

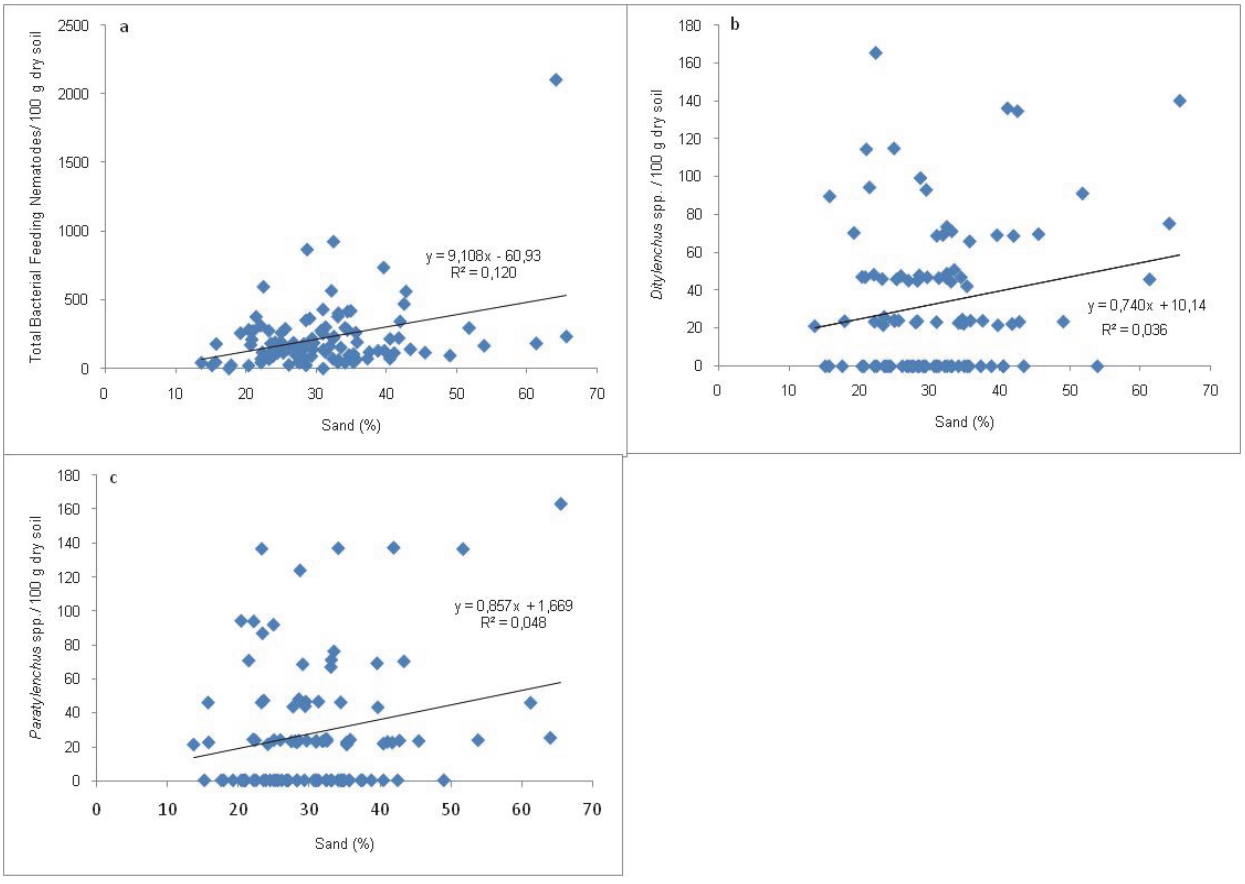

Figure 3. Relationship between soil sand content and bacterial-feeding nematodes (a), *Ditylenchus dipsaci* (b) and *Paratylenchus* spp. (c).

Hyphal-feeding and bacterial-feeding nematodes are indicators of soil microorganism abundance (Yeates et al., 1993; Bongers & Bongers, 1998). The increase in the population densities of *Aphelenchus* spp. and *Aphelenchoides* spp. are dependent on the mineralisation of organic matter in soil (Bongers & Bongers, 1998). Therefore, the results of the current study confirm this observation, with a significantly negative relationship between hyphal-feeding nematodes and soil organic matter content identified ($R = 0.26$; $P < 0.05$) (Fig. 4). High organic matter content, which is not degraded, caused lower multiplication of nematodes due to the lower microorganism abundance.

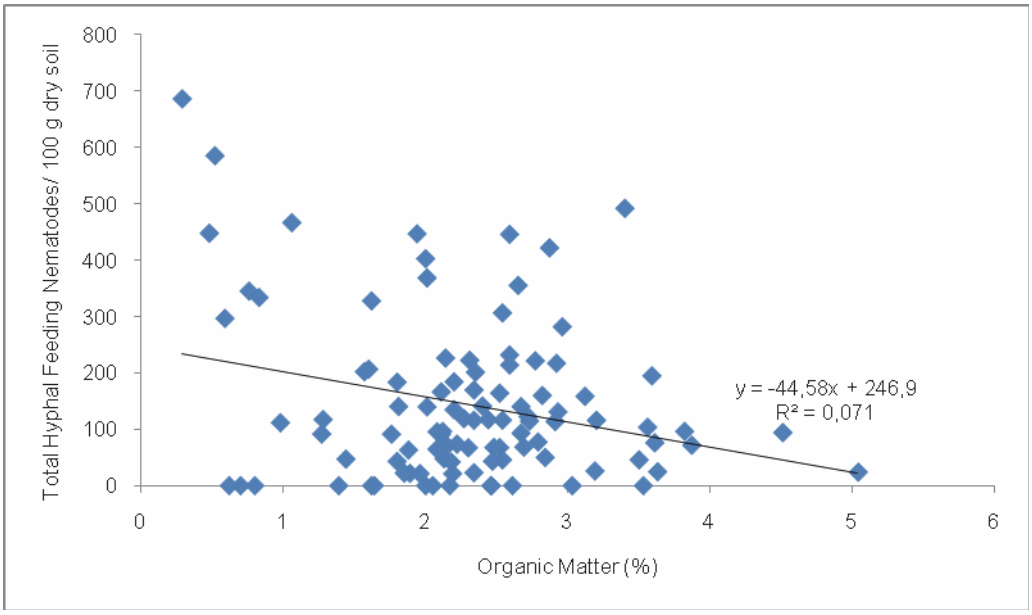

Figure 4. Relationship between soil organic matter content and hyphal-feeding nematodes.

The current study found that the main plant parasitic nematodes in onion-growing areas in Karaman were *D. dipsaci, Tylenchus* spp. and *Paratylenchus* spp. free living nematodes were in high incidence and abundance in the sampled areas. The distribution and abundance of nematodes was dependent on soil texture and mineralisation capacity. A wider nematode distribution and higher incidence was recorded in sandy and higher mineralised soils.

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Orijinal araştırma (Original article)

The toxic effects of *Perilla frutescens* essential oils in combination with CO_2-enriched modified atmospheres on the life stages of *Dermestes maculatus* Degeer (Coleoptera: Dermestidae)

CO_2 bakımından zengin değiştirilmiş atmosferler ile kombine edilmiş *Perilla frutescens* uçucu yağlarının *Dermestes maculatus* Degeer (Coleoptera: Dermestidae)' un biyolojik dönemleri üzerine toksik etkileri

Yang YE[1] Bo ZHANG[1*] Rui ZHANG[2] Kang WANG[2]

Summary

Fumigation with essential oils from plants is a popular, safe, and environment friendly alternative tool for pest control in stored products. To provide a range of choices and to reduce costs in a variety of contexts, more plant-derived pest management control agents from local plant species should be identified and more effective fumigant methods must be developed. This paper describes the toxic effects of the essential oils from *Perilla frutescens* (L.) Britt. (Lamiaceae) as a fumigant, either alone or in combination with CO_2-enriched modified atmospheres, for the control of *Dermestes maculatus* Degeer. The essential oils showed strong fumigant activities against this pest. After six hours of fumigation with the essential oils at a concentration of 0.10 µL/L, mortalities for each life stage was 73.0% (adult), 61.2% (larva), 39.6% (pupa) and 55.4% (egg). Furthermore, the effects were enhanced when CO_2 levels were increased. At an essential oil concentration of 0.08 µL/L in the treatment group with 60% CO_2, mortality rate for all life stages was 100%. We propose that CO_2 concentration plays a direct role during joint fumigation, as atmospheres with high CO_2 levels cause the permanent opening of insect spiracles, thereby increasing the uptake of plant essential oils.

Keywords: *Perilla frutescens*, *Dermestes maculatus*, pest control, fumigation, CO_2

Özet

Depolanmış ürün zararlılarının mücadelesinde bitkilerden elde edilen uçucu yağlar ile fumigasyon popüler, güvenli ve çevre dostu alternatif bir yöntemdir. Geniş bağlamda çeşitliliği arttırmak ve maliyetleri azaltmak için yerel bitki türlerinden bitki kökenli zararlı kontrolünde kullanılabilecek daha fazla maddeler tanımlanmalı ve daha etkili fumigasyon metotları geliştirilmelidir. Bu çalışma, *Perilla frutescens* (L.) Britt. (Lamiaceae)'dan elde edilen uçucu yağların, fumigant olarak, tek başına ya da CO_2 bakımından zengin değiştirilmiş atmosferler ile birlikte kullanımının, *Dermestes maculatus* Degeer. (Coleoptera: Dermestidae)'a karşı toksik etkisini açıklamaktadır. Uçucu yağlar bu zararlıya karşı kuvvetli fumigant etki göstermiştir. Uçucu yağın 0.10 µL/L konsantrasyonunda altı saatlik fumigasyondan sonra yaşam dönemlerinin yüzde ölüm oranları %73 (ergin), % 61.2 (larva), % 39.6 (pupa) ve %55.4 (yumurta) bulunmuştur. Ayrıca, CO_2 seviyesi arttığında fumigant etki de artmıştır. %60 CO_2 içeren ortamda uçucu yağın 0.08 µL/L konsantrasyon uygulaması tüm yaşam dönemlerinde %100 ölüme neden olmuştur. Yüksek CO_2 konsantrasyonu böcek stigmalarının sürekli açık kalmasına, dolayısıyla bitki uçucu yağlarının alımının artmasına neden olduğu için ortak fumigasyonda CO_2 konsantrasyonunun uçucu yağın fumigant etkisinde önemli bir rol aldığını düşünmekteyiz.

Anahtar sözcükler: *Perilla frutescens*, *Dermes tesmaculatus*, zararlı yönetimi, fumigasyon, CO_2

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Introduction

Dermestes maculatus Degeer (Coleoptera: Dermestidae) is a major cosmopolitan insect pest of dried fish and other products of animal origin, and it frequently causes severe damage (Lambkin & Khatoon, 1990). This insect attacks not only dried animal flesh, bones, hides, horns, and feathers, but also destroys other stored products, including dried blood, leather and cheese (Hiton, 1945; Macquillan & Shipp, 1976; Osuji, 1975; Islam et al., 2009). In addition to damaging stored products, tunneling larvae seeking to construct pupation chambers can severely damage wood, cork and other materials, including packaging (Khatoon & Heather, 1990). For these reasons, in surveys on pest control, *D. maculatus* has also been identified as a serious museum pest (Linnie, 1994, 1999). *D. maculatus* is a favorite experimental insect pest, as large numbers for each life stage can be readily reared in laboratory cultures (Linnie & Keatinge, 2000).

Conventional control methods for *D. maculatus* populations around the world are primarily based on the continued application of chemicals such as sodium arsenate, chlorinated hydrocarbons, and organophosphate insecticides (Wheatley, 1971; Macquillan & Shipp, 1976; Linnie & Keatinge, 2000). However, repeated use of these chemicals over decades has led to resistance in the target insects, undesirable effects on non-target organisms, and environmental and human health concerns (Kim et al., 2003). Increased concern over the adverse effects of pesticides highlights the need to develop alternative strategies for the control of *D. maculatus*, including the development of chemical substitutes, exploitation of controlled atmospheres, and integration of physical methods (Rajendran & Sriranjini, 2008).

Plant materials represent an attractive alternative to current insect control agents, as they are a rich source of bioactive chemicals. Plant materials are relatively inexpensive, widely available, generally safe, broad-spectrum in application, biodegradable, and environment friendly (Egwunyenga et al., 1998). In particular, some plant extracts and essential oils display effective insecticidal activities against the different life stages of *D. maculatus* (Rajendran & Sriranjini, 2008; Islam et al., 2009). For example, Fasakin & Abererjo (2002) showed that the pulverized powder of *Piper guineense* Schumach & Thonn and *Afromomum melegueta* K. Schum significantly inhibited egg hatching and adult emergence in *D. maculatus* when applied to smoked catfish. However, to provide a range of choices and to reduce costs in different contexts, more plant-derived pest management control agents from local plant species should be developed.

Perilla frutescens (L.) Britt is an annual herb of the Lamiaceae family that is traditionally grown in China, India, Japan, and other Asian countries. This herb has been in common use as a traditional Chinese medicine for over 1,000 years. Edible fresh leaves are typically used as vegetables, commonly used for seasoning pickles, or as a garnish for raw fish dishes in Japan. This plant is also a popular leafy vegetable in Korea that is generally consumed with a pickle or used for wrapping roasted meats (You et al., 2014). *P. frutescens* has significant anti-allergic, anti-inflammatory, and antitumor activities (Banno et al., 2004). Moreover, the essential oils of *P. frutescens* exhibit strong insecticidal and repellent activities against several pests of stored products, including *Lasioderma serricorne* (F.) and *Liposcelis bostrychophila* Badonnel (Hori, 2003, 2004; Zhao et al., 2012; You et al., 2014). Considering these previous findings, we decided to test whether the essential oils of *P. frutescens* also display toxicity against *D. maculatus*.

The aim of this study was to evaluate the effectiveness of the essential oils from the leaves of *P. frutescens* as a fumigant for the control of *D. maculatus*, either alone or in combination with CO_2-enriched modified atmospheres.

Materials and Methods

Extraction of the essential oils

Extraction of the *P. frutescens* essential oils was performed according to the method of Zhao et al. (2012). Fresh leaves of *P. frutescens* were obtained from the South China Botanical Garden,

Chinese Academy of Sciences. Fresh leaves were used to avoid volatilization. The leaves were subjected to hydro distillation using a modified Clevenger-type apparatus for 6 h and extracted with *n*-hexane. Anhydrous sodium sulfate was used to remove water following extraction. The essential oils were stored in airtight containers in a refrigerator at 4°C for subsequent experiments.

Gas chromatography and mass spectrometry analysis

Components of the essential oil were identified by gas chromatography-flame ionization detection (GC–FID) and gas chromatography-mass spectrometry (GC–MS) using an Agilent 7890A gas chromatograph hooked to an Agilent 5975C mass selective detector. The same column and analysis conditions were used for both GC–FID and GC–MS. They were equipped with a HP-5MS (50 m × 0.32 mm × 0.52 µm) capillary column. The oven temperature was programmed to increase from 50 ^{o}C to 250 ^{o}C at a rate of 5 ^{o}C/min and finally held for 10 min. The injector and detector temperatures were maintained at 250 ^{o}C. Helium was used as the carrier gas at a flow rate of 1.0 mL/min with a split ratio equal to 1/20. Spectra were scanned from 50 to 550 m/z. Most constituents were identified by comparison of their retention indices with those reported in the literature. The retention indices were determined in relation to a homologous series of *n*-alkanes (C_5–C_{36}) under the same operating conditions. Further identification was made by comparison of their mass spectra with those stored in NIST 10 and Wiley 275 libraries or with mass spectra from the literature (Adams, 2001). Relative percentages of the individual components of the essential oil were obtained by averaging the GC–FID peak area% reports.

Insect cultures

Unsexed *D. maculatus* adults were collected from naturally infested museum specimen of *Ctenopharyngodon idellus* Cuvier & Valenciennes at the Shenzhen Museum. The beetles were transferred into Kilner jars containing disinfested dried fish from *C. idellus* to initiate new colonies and to create a parent stock for experimental use. The culture jars were maintained at ambient conditions (28-32 °C and 60–65% RH). The Kilner jars and culture media were disinfested in a laboratory drying cabinet (Model LCON 53 CL) at 70 °C for 1 hour and then allowed to cool to room temperature prior to the introduction of the insects and culture media.

Preparation of gas treatment

The controlled atmosphere apparatus used in this experiment was modified as described by Hashem et al. (2014). Treatment with the gas mixtures was performed inside sealed, gastight wide-mouth bottles (1 L). Each bottle was tightly plugged with a customed glass stopper equipped with two lateral valves (inlet and outlet) leading into two vertical glass tubes. One of these tubes was long and reached near to the bottom of the bottle and acted as a gas inlet. The other tube was shorter and reached approximately one quarter of the way down the bottle and acted as a gas outlet. At the beginning of the treatment, the valves were opened until the desired gas concentration was reached, as indicated by an oxygen analyzer. Then, various doses of the essential oils were injected through the lid onto filter paper placed at the bottom of the bottle to reach the desired concentrations.

Fumigant activity

Different life stages (egg, larva, pupa, and adult) of *D. maculatus* were exposed to one normal atmosphere as a control (AIR) as well as two modified atmospheres, CA1 (25% CO_2, 15% O_2, and 60% N_2) and CA2 (60% CO_2, 8% O_2, and 32% N_2).

Five pairs (F_3 generation) of adults, ten pupae, ten third-instar larvae, or ten eggs were placed in small glass tubes (25–50 mm) with culture media, and the open ends were covered with muslin cloth. The tubes were hung in the geometric center of the bottles.

Fumigations were conducted at various concentrations (0.0, 0.02, 0.04, 0.06, 0.08, and 0.10 µL/L) for 6 hours with five replicates for the control and the treatments. After exposure, the insects were transferred to clean vials with the culture media, placed in incubators maintained at 30 ± 2 °C, and monitored daily for different numbers of days depending on the life stage. Survival of the adults and

larvae were determined by counts after two days of monitoring. Egg mortality was indirectly assessed by counting the hatching rate after seven days of monitoring. Pupal survival was measured by counting the number of F_1 adults that successfully emerged after 20 days of monitoring.

Differences in percentages of mortality for different life stages exposed to each of the experimental conditions were analyzed by analysis of variance using the SPSS software program (Anonymous, 1999). The percentage data were arcsine-transformed to meet the assumption of homogeneity and normality for analysis of variance. Tukey's HSD test was used to compare the means.

Results and Discussion

The essential oil was yellow with a yield of 0.07% (v/w) and density of 0.92 g/mL at 20 ℃. A total of 26 components of the essential oil were identified, account for 98.09% of the total oil (Table 1). The main compounds in the essential oil were perilla aldehyde (53.97%), caryophyllene (11.85%), limonene (9.13%) and trans-shisool (4.39%).

Table 1. Chemical composition of the essential oil from fresh leaves of *Perilla frutescens*

Compound	RI (min)	Relative content (%)
(Z)-3-Hexen-1-ol	12.122	0.10
Benzaldehyde	15.911	0.27
1-Octen-3-ol	16.178	1.18
(1S)-(1)-β-Pinene	16.579	0.15
3-Octanol	16.733	0.18
Limonene	18.226	9.13
Eucalyptol	18.395	0.11
β-Linalool	20.360	2.17
α-Terpineol	23.684	0.30
trans-Citral	25.981	0.19
trans-Shisool	26.233	4.39
Perillaldehyde	26.557	53.97
Perillol	27.039	2.92
Eugenol	28.697	0.20
α-Copaene	29.576	0.26
β-Elemene	29.898	0.42
Caryophyllene	31.021	11.85
β-Farnesene	31.221	0.35
α-Humulene	31.955	1.44
(Z,E)-α-Farnesene	32.203	2.41
β-Cubebene	32.646	3.33
δ-Cadinene	33.511	0.29
(Z)-β-Farnesene	34.135	0.33
Elemicin	34.329	0.22
Caryophyllene oxide	35.511	0.42
Asarone	36.970	1.51
Total		98.09

Mortality was significantly affected in each life stage by the CO_2 concentration (Figure 1). In the control group (AIR), in which the atmosphere was not modified, no deaths were observed when the essential oils were not added. However, CO_2 alone had some effect, especially on the adult stage. The highest mortality (37.4%) was observed for the adult stage in the CA2 treatment group with a CO_2 concentration of 60%.

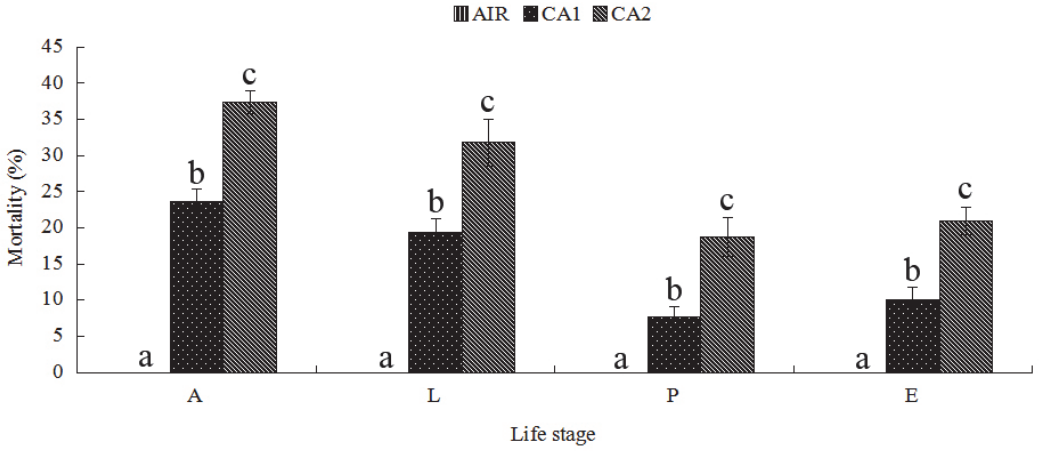

Figure 1. Total mortality (Mean ± SE) of adults (A), larvae (L), pupae (P), and eggs (E) of *Dermestes maculatus* exposed to different CO_2 concentrations (AIR, normal atmosphere; CA1, 25% CO_2; CA2, 60% CO_2) at 28–32 °C and 60–65% RH for 6 hours. Means indicated with the same letters were not significantly different ($P < 0.05$).

The essential oils showed strong fumigant activity against each of the life stages in the AIR control group (Figure 2). Furthermore, the toxicity of the fumigant progressively increased with increasing concentrations of the oil. At a concentration of 0.10 µL/L, mortality for each life stage was 73.0% (adult), 61.2% (larva), 39.6% (pupa), and 55.4% (egg).

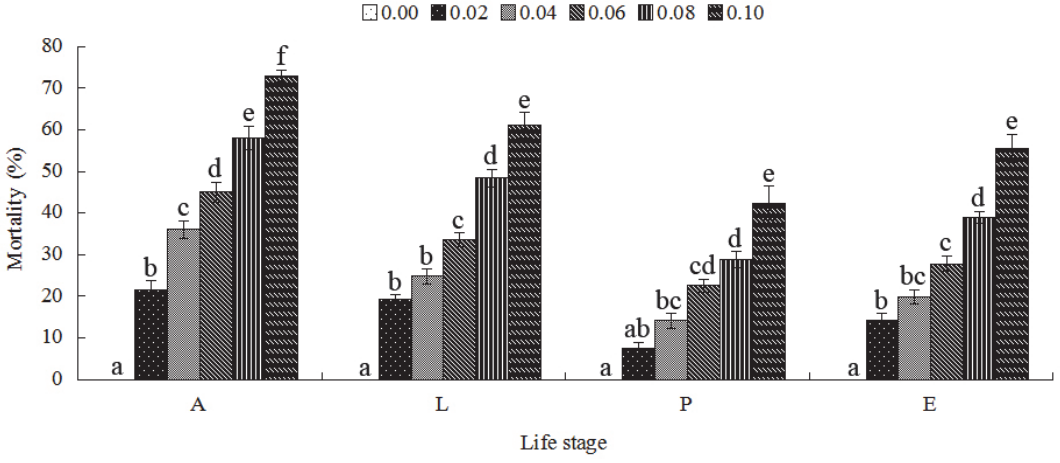

Figure 2. Total mortality (Mean ± SE) of adults (A), larvae (L), pupae (P), and eggs (E) of *Dermestes maculatus* exposed to different concentrations of essential oils from *Perilla frutescens* (0.0, 0.02, 0.04, 0.06, 0.08, and 0.10 µL/L) under a normal atmosphere at 28–32 °C and 60–65% RH for 6 hours. Means indicated with the same letters were not significantly different ($P < 0.05$).

The effects of the essential oils on *D. maculatus* also increased when the CO_2 concentration of the atmosphere was modified (Figure 3). Higher mortality was observed for each life stage in the CA1 and CA2 treatments compared with the AIR control group. Additionally, pest mortality in the CA2 treatment group with 60% CO_2 was higher than in the CA1 treatment group with 25% CO_2. The mortality of all life stages reached 100% in the CA2 treatment group at an essential oil concentration of 0.08 µL/L.

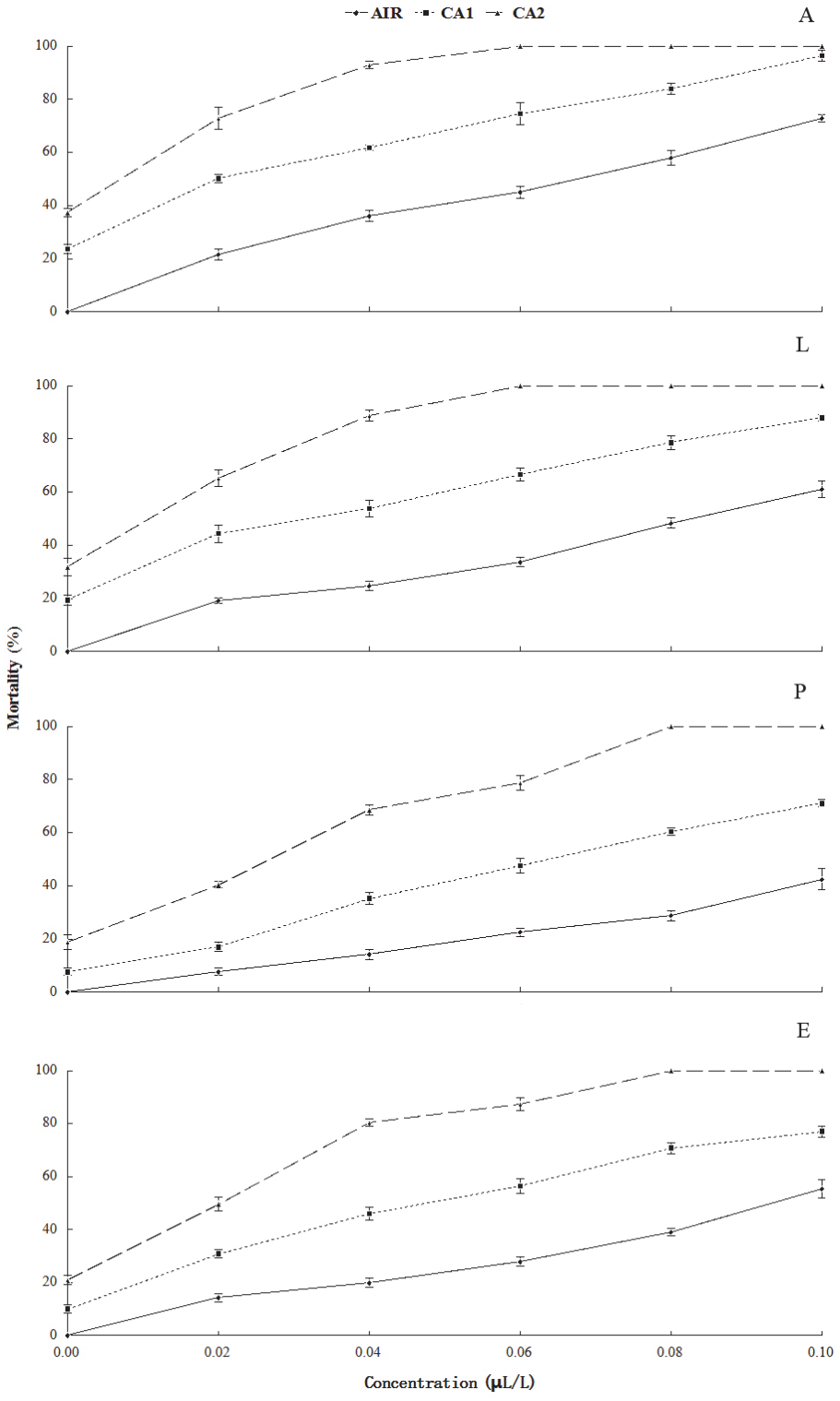

Figure 3. Total mortality (Mean ± SE) of adults (A), larvae (L), pupae (P), and eggs (E) of *Dermestes maculatus* exposed to different CO_2 concentrations (AIR, normal atmosphere; CA1, 25% CO_2; CA2, 60% CO_2) in combination with different concentrations of essential oils from *Perilla frutescens* (0.0, 0.02, 0.04, 0.06, 0.08, and 0.10 μL/L) at 28–32 °C and 60–65% RH for 6 hours.

The different life stages showed different degrees of susceptibility to the essential oils alone (AIR) or in combination with modified atmospheres (CA1 and CA2). Probit analyses showed that adults (LC_{50} = 0.06 µL/L) were the most susceptible, followed by larvae (LC_{50} = 0.09 µL/L), eggs (LC_{50} = 0.11 µL/L) and pupae (LC_{50} = 0.16 µL/L) in the AIR control groups (Table 2). Similar susceptibility trends were observed for the different life stages in the CA1 and CA2 treatments (Table 2).

Table 2. Fumigant toxicity of the essential oils from *Perilla frutescens* on the eggs (E), larvae (L), pupae (P), and adults (A) of *Dermestes maculatus*

Treatment	Life stages	LC_{50}	Slope ± SE	Chi-square (X^2)
AIR	A	0.06 (0.05–0.07)	1.89 ± 0.35	4.08
	L	0.09 (0.07–0.13)	1.65 ± 0.36	4.86
	P	0.16 (0.11–0.43)	1.68 ± 0.42	2.86
	E	0.11 (0.08–0.19)	1.70 ± 0.38	5.11
CA1	A	0.02 (0.02–0.03)	2.00 ± 0.36	8.91
	L	0.03 (0.02–0.03)	1.75 ± 0.34	6.06
	P	0.06 (0.05–0.07)	2.12 ± 0.36	2.25
	E	0.04 (0.03–0.05)	1.77 ± 0.34	3.03
CA2	A	0.01 (0.01–0.02)	3.79 ± 0.82	4.05
	L	0.02 (0.01–0.02)	3.88 ± 0.71	4.69
	P	0.03 (0.02–0.03)	3.33 ± 0.43	12.07
	E	0.02 (0.02–0.03)	3.29 ± 0.47	7.77

The chemical composition of the essential oil from *P. frutescens* in the present study was not same as that reported in previous studies. For example, carvone, perilla aldehyde, caryophyllene, and 2-furyl methyl ketone were the main volatile components of *P. frutescens* harvested from Beijing City, China (You et al., 2014). However, β-caryophyllene,2-hexanoylfuran, β-farnesene, 1,4,7-cycloundecatriene-1,5,9,9-tetramethyl-zzz, and 1-cyclohexane-1-carboxaldehyde were common constituents in *P. frutescens* collected from herb markets in China, and content and composition of the essential oil of *P. frutescens* were various in different parts of *P. frutescens* (Liu et al., 2013). These differences of chemical content and composition of the essential oils might have been due to harvest time and local, climatic and seasonal factors, storage duration of medicinal herbs as well as extraction method, and these differences may result in different biological activities (Huang et al., 2011; Liu et al., 2013; You et al., 2014).

As a stored-product insect pest, *D. maculatus* is an ideal laboratory animal for the testing of biocontrol agents due to its vitality, ease of culturing, and high reproductive rate. In this study, adequate numbers of all life stages were obtained easily from laboratory cultures, simply by providing suitable living conditions (Wong-Corral et al., 2013). The ease with which an insect can be cultured is a primary consideration for such experiments, as large numbers of each life stage will be required to test many chemicals under different exposure conditions (Linnie & Keatinge, 2000). Moreover, in this study, no deaths were observed in the control groups over the nearly a month-long research period, confirming that the rearing conditions for *D. maculatus* were satisfactory.

The essential oils showed significant fumigant activity against all developmental stages of *D. maculatus*, causing significant mortality at higher concentrations. The essential oils used in this study contained different kinds of bioactive components, such as aldehydes, alcohols, terpenes, and esters. These chemical components have insecticidal, nematicidal, fungistatic, antimicrobial, and insect repellent properties (Hori, 2003; Choi et al., 2007; Dimri et al., 2008; Zhao et al., 2012; Tian et al., 2014). Our findings are consistent with other studies that have demonstrated the toxicity of these essential oils and their associated components against a variety of stored-product pests (Dimri et al., 2008; Zhao et al., 2012; You et al., 2014). For example, You et al. (2014) demonstrated that the components of the essential oils, including carvone, perilla aldehyde, 2- furyl methyl ketone, and β-caryophyllene, are highly toxic to *L. serricorne*.

In the present study, the modified atmospheres with high levels of CO_2 significantly reduced survival rate for the different life stages, and mortality observed following treatment with 60% CO_2 was significantly higher than with 25% CO_2. High levels of CO_2 are toxic to many stored-product insect species, such as *Sitophilus oryzae* (L.), *Sitotroga cerealella* (Olivier), *Stegobium paniceum* (L.) and *L. serricorne* (Annis & Morton, 1997, Gunasekaran and Rajendran, 2005, Hashem et al., 2014). Moreover, most stored-product insects eventually die under atmospheres containing more than 40% CO_2 (Navarro, 2006). The death of insects from high CO_2 concentrations is due to the combination of many effects. In insects, CO_2 poisoning directly affects the nervous, endocrine, respiratory and circulatory systems, as well as general metabolism (Wong-Corral et al., 2013). Furthermore, in many insects, high CO_2 induces permanent opening of the spiracles, leading to water loss and mortality (Wong-Corral et al., 2013). In addition, CO_2 can also have indirect effects on mortality, as Janmaat et al. (2001) demonstrated a strong narcotic and metabolic effects for high CO_2 concentrations, primarily due to changes in pH.

The mortality rate for all the life stages of *D. maculatus* fumigated with a combination of high CO_2 and essential oils was higher than in the groups where CO_2 or the essential oils were used alone. Therefore, the effects of the essential oils and CO_2 on mortality were synergistic when used in combination against *D. maculatus*. Similar synergistic toxic effects have been demonstrated for CO_2 in combination with other compounds. For example, adding CO_2 to fumigants such as sulfur dioxide, acrylonitrile, methyl bromide, phosphine, carbon disulfide, ethylene oxide, chloropicrin, methyl formate, and hydrogen cyanide increases their toxicity against insects and reduces the times required for treatment (Bond & Buckland, 1978; Riudavets et al., 2014). Moreover, the fumigant toxicity of essential oils from certain plants (*Allium sativum* L., *Citrus tangerina* Hort. ex Tanaka, *C. aurantium* L., *C. bergamia* R., *Pinus sylvestris* L., *Cupressus funebris* Endl, and *Eucalyptus citriodora* Hook) is also increased when combined with carbon dioxide for the control of stored-product insects (Wang et al., 2001; Isikber, 2010). In the present study, mortality was significantly higher in the treatment groups with higher CO_2 levels (60%) than in groups with lower CO_2 levels (25%). Therefore, it is likely that CO_2 concentration played a key role during the joint fumigations, as modified atmospheres with high CO_2 levels cause the permanent opening of insect spiracles, thereby increasing the uptake of plant essential oils (Nicolas & Sillans, 1989; Wang et al., 2001; Mitcham et al., 2006).

Our study revealed considerable variability in the susceptibility of the egg, larval, pupal, and adult stages to essential oil derived from *P. frutescens*. Notably, the susceptibility of each life stage was similar when exposed to CO_2, essential oils, or a combination of these factors. Generally, adults were the most sensitive, followed by larvae and eggs, whereas pupae were the least sensitive. Similar sensitivities to CO_2 during different life stages have been observed in other stored-product pest species, including *Callosobruchus maculatus* (F.), *Acanthoscelides obtectus* (Say), and *Zabrotes subfasciatus* (Boheman) (Wong-Corral et al., 2013). The effects of modified atmospheres with high CO_2 levels on pests could be related to differences in metabolic rates during different life stage. For example, pupae, which likely have the lowest oxygen demand, were most tolerant to the toxic effects of CO_2, whereas adults, with high metabolic rates, were the least tolerant (Mbata et al., 2000). Related to this, more essential oils are likely absorbed and accumulated in insects with higher metabolic rates, resulting in lower survival rates during fumigation with essential oils alone or in combination with CO_2.

The aim of this study was to determine the effectiveness of the essential oils from the leaves of *P. frutescens* as a fumigant for the control of *D. maculatus*, either alone or in combination with CO_2-enriched modified atmospheres. Results indicated that the essential oils showed strong fumigant activities against this pest under a normal atmosphere, with fumigant toxicity increasing with higher oil concentrations. Furthermore, the effects of the essential oils were enhanced as CO_2 levels were increased. At an essential oil concentration of 0.08 μL/L in the treatment group with 60% CO_2, the mortality rate for all life stages was 100%. It was suggested that the essential oils vapor and modified atmospheres could be used as an effective combined method to control the stored-product insects.

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Orijinal araştırma (Original article)

Comparison of different bioassay methods for determining insecticide resistance in European Grapevine Moth, *Lobesia botrana* (Denis & Schiffermüller) (Lepidoptera: Tortricidae)[1]

Salkım güvesi (*Lobesia botrana*)'nde insektisit direncinin belirlenmesinde farklı biyoassay yöntemlerin karşılaştırılması

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M.Oktay GÜRKAN[3] Graham MOORES[4]

Summary

The European Grapevine Moth [*Lobesia botrana* (Denis & Schiffermüller) (Lepidoptera: Tortricidae)] is a major pest in vineyards and insecticides are heavily used in its management. Currently, there is no IRAC approved bioassay method for *L. botrana,* so this study aimed to develop a suitable susceptibility test. For this purpose, 10 different populations were cultured from Manisa (Turkey) that province an important place in the world of viticulture. Diet-incorporation, topical application and diet covering methods selected as potential methods. Three different bioassay techniques (diet-incorporation, diet covering and topical application) were compared using indoxacarb, deltamethrin, spinosad and chlorpyrifos-ethyl on three different populations. Results revealed that the diet-incorporation by mixing was the most appropriate method for testing insecticide resistance against the European Grapevine Moth.

Keywords: Bioassay, European grape moth, insecticide resistance, diet incorporation, diet covering, topical application

Özet

Salkım güvesi [*Lobesia botrana* (Denis & Schiffermüller) (Lepidoptera: Tortricidae)] bağın ana zararlısıdır, salkım güvesi mücadelesinde yoğun olarak insektisit kullanılmaktadır. İnsektisit Direnç Eylem Komitesi (IRAC)'nde *Lobesia botrana* için onaylanmış bir yöntem olmadığından bu çalışma salkım güvesinde hassasiyet test metodu geliştirmek amacıyla gerçekleştirilmiştir. Bu amaçla, dünya bağcılığında önemli bir yeri olan Manisa (Türkiye) ilinden 10 farklı populasyon kültüre alınmıştır. İnsektisiti besine karıştırma, topikal uygulama ve besin kaplama yöntemleri kullanılması muhtemel metotlar olarak seçilmiştir. Indoxacarb, Deltamethin, Spinosad ve Chlorpyrifos ethyl aktif maddeleri, 3 farklı populasyonda 3 farklı yöntem karşılaştırmalı olarak kullanılmıştır. Sonuçlar, salkım güvesinde hassasiyet test metodu olarak insektisitin besine karıştırma yönteminin en uygun metod olduğunu göstermiştir.

Anahtar sözcükler: Biyoassay, Salkım güvesi, insektisit direnci, besine karıştırma, besin kaplama, topikal uygulama

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Introduction

Chemical insecticides are still considered the mainstay of agricultural pest control. Although development of resistance against insecticides is a common phenomenon, recent advances in research and technology has renewed interest in this subject and resistance risk assessments have been developed for many species using different assay methods.

In 1984 the Insecticide Resistance Action Committee (IRAC) was established for improved and systematic reporting of insecticide resistance. According to IRAC, resistance is a decrease of a pest population's susceptibility as the result of genetic changes, such that an insecticide is no longer effective when used at the prescribed dose. Short life span and high fecundity are among major factors that contribute to the onset of resistance to insecticides, when they are used extensively. The development of insecticide resistance is one of the most important problems in pest management, affecting agricultural production with an ultimate impact on yield. About 600 insect and mite species are reported to have developed resistance against insecticides (IRAC, 2014).

Reliable, quick and effective bioassay techniques to identify sensitive and resistant individuals are needed for effective resistance management (Brown, 1981; Gunning, 1993). One of the most important reasons to conduct resistance studies is to suggest more effective and safer insecticides to delay resistance development (Roush & Tabashnik, 1990).

Accuracy and a clear definition of the method of reporting insecticide resistance studies are critically important for comparison with results from other studies. IRAC has published 188 methods to determine the resistance of insects against insecticides (IRAC, 2014). The European Grapevine Moth (EGM) is multivoltine and an important pest in vineyards of Turkey and throughout Europe; as such, it is heavily sprayed during the year (Delen et al., 2004). However, the scarce literature on this pest is a major obstacle to finding a reliable and accurate method to manage resistance risk development in EGM.

There are several standard bioassay methods to determine insecticide resistance for important pest species; these methods are classified according to the technique of insecticide application (Ffrench-Constant & Roush, 1990). The methods can be grouped into four headings; dipping, residue (diet covering), topical application and feeding. The aim of the present study was to compare the last three methods; residue, topical application and feeding bioassays to select the best methods for monitoring insecticide resistance on the EGM. The outcome of this study would be useful for further studies with EGM.

Materials and Methods

Insects

The resistance related bioassay studies were conducted in the Entomology Laboratory, Department of Plant Protection, Faculty of Agriculture, Ege University, Turkey. EGM was collected from three different locations (Merkez 1, Merkez 2 and Yesilyurt region) in Manisa, Turkey. The insect colony was maintained at 25 ± 2º C, 60-65% RH in the laboratory with a 16:8 h light: dark photoperiod.

Insecticides

Insecticides used as active ingredients (%96-99 purity) in the bioassay were: chlorpyrifos-ethyl (Dow AgroSciences, Turkey), deltamethrin (Bayer Crop Science, Turkey), Indoxacarb (Hektaş, Turkey) and spinosad (Dow AgroSciences, Turkey).

Diet preparation

The diet was prepared as described in Rapagnani et al. (1990). According to this method, two autoclavable, screw capped 1 l glass bottles were used in diet preparation. The agar (20 g) and corn semolina (64 g) were added to one of these bottles with 500 ml pure water. Later, these two bottles (other bottle including only 500 ml pure water) were sterilized at 120 °C for 20 minutes in an autoclave. After that the diet media and other pure water were poured in a mixer at 70 °C. Other bottle of pure water was used

for the easier removal of the diet media. Wheat germ (66 g) and brewer's yeast (30 g) were added and mixed properly. When the mixture temperature dropped to 60 °C, ascorbic acid (5.7 g was solved in 2 ml pure water and nipagin was solved in % 96 alcohol (very little amount ~ 1 ml) and then they were added into the mixture. After mixing all the ingredients, the resulting 1 lt of artificial diet was placed in sterile plastic containers, and covered with aluminium foil, and left at room temperature for 24 hours. When the diet properly settle down, it was chopped into 1cm^3 (~1.3 g) cubes. The pure diet cubes were placed in a sixteen cell transparent containers and used for experiments of Topical bioassay & Diet covering methods (Bio-Assay Tray Lid – 16 cells, Bio-Serv Canada) (Fig.1). For diet incorporation method, diet cubes mixed with insecticide were used.

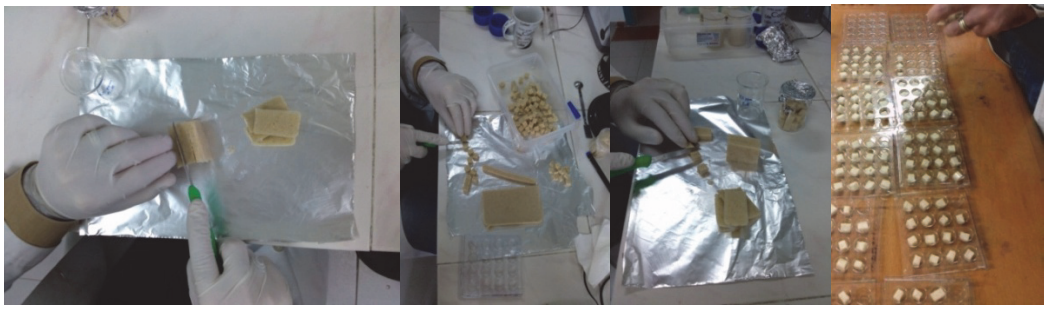

Figure 1. Dividing the diet which has been placed in storage boxes into 1cm^3 pieces and placing them on experiment containers.

Bioassays

Topical bioassay

A hand-operated micro-applicator (Burkard, England) was used to apply aliquots (1µL) on the thorax of individual 3rd stage larvae previously placed at 4º C for 4 minutes. They were then transferred into a 16-well polystyrene bioassay tray (Bio-Serve, Canada). Each cell contained a larva: The process was repeated for 30 individuals for each dose, with distilled water as the control. The bioassay tray was covered and incubated at 25 ± 2º C, 60-65% RH with a 16:8 h light: dark photoperiod. Mortality was assessed after 72 hours, and larvae were considered dead if unresponsive to gentle prodding with a fine brush. The observations were made and could not be observed pupae of these larvae. If moribund insect cannot be pupae, it was assessed as dead.

Diet covering

Two ml of insecticide were applied to a petri dish containing 10 cubes of diet using a Potter-Tower (Burkard, England) at a pressure of 1 bar. Two ml of sterile distilled water was used as a control. Each treatment was repeated three times. To be contacted with insecticide of the bottom surface of the diet cubes, diet cubes were transferred help of forceps sprayed surface of the petri dish immediately after application. The diet cubes were kept for 30 minutes at room temperature then transferred to individual cells of the 16-well polystyrene bioassay tray. A single 3rd stage larvae was placed into each cell and the tray covered and maintained at 25 ± 2º C, 60-65% RH with a 16:8 h light: dark photoperiod for 72 hours after which mortality was assessed as before.

Diet incorporation

Concentrations of insecticide were mixed with the prepared diet at a ratio of 1: 9 after waiting for the temperature of artificial diet to drop to 40 °C (the lowest temperature insecticides not lost integrity). The mixture was homogenised, left for 24 hrs at room temperature and then cut into 1 cm^3 pieces. Diet was mixed with sterile distilled water as a control. The cubes were placed into individual cells of the 16-well polystyrene bioassay tray. A single 3rd stage larvae was placed into each cell and the tray covered and kept at 25 ± 2º C, 60-65% RH with a 16:8 h light: dark photoperiod for 72 hours after which mortality was assessed as described above.

All three methods were repeated once a month intervals in the same way in three different dates.

Statistical analysis

The lethal concentration (LC) values were calculated by probit analysis using Polo Plus software version 2.0 (Le Ora Software, Berkeley, CA).“Natural response parameter” and “Convert doses to logarithms” options "yes" was selected.

Results and Discussion

The aim of our study was to determine the most suitable bioassay method for resistance risk assessment of EGM. The methods of topical application, diet covering, and diet incorporation methods were applied with a series of insecticide concentration with many pre-experiments. Three different EGM populations were used in the experiments. Factors like amount of the diet, application time, temperature, and humidity were kept constant throughout the experiments.

The topical application method was difficult due to small size and continuous movement of larvae. To overcome these difficulties, preliminary experiments were carried out to determine the incubation time of the larvae in the fridge to make them immobile. The larvae were placed in sterilized plastic petri dishes in groups of 10 and were kept for four minutes in the freezer (0ºC). It was observed that larvae kept in the fridge for 4 minutes became mobile after spending 1 minute in room temperature. That one minute time length was enough to do topical application to 10 larvae. The topical application was carried out after placing the larvae for 4 minutes in the freezer. Also, even if a drop of the solution is given without a problem, we observed another problem where the larvae became mobile before absorbing the liquid completely and transmitted the liquid to the area.

Before starting the experiments, as a preliminary experiment, water and methanol were applied to the larvae in order to analyse the side effects of methanol, used in dissolving the active ingredients. One µl methanol or water was applied to each larva. Water was used in 3 petri dishes containing 5 larvae each. Simultaneously methanol was applied to 15 larvae. Later, these larvae were left with diet and observed until turning into pupae. There was no difference observed between the applications of methanol and distilled water. In the topical application the active ingredient directly applied to the insect's body. Thus, using low doses in this method appeared to be successful.

After the preliminary experiments in the diet covering method, it was observed that the dose required complete death was quite high. Obtaining a homogeneous mixture and blockage of spray tower were observed as technical problems during application the insecticides. The dose series with effective results in this method was very high comparing with other methods. The method of diet incorporation was the only method in that the increase of death ratio was observed with increasing doses.

Although the same insecticides and the same insect were used the tested methods produced varying results depending on the application methods. The physical environments needed for a successful execution of the methods are different. For example, there was a need of good ventilation while using the spray tower. It was compulsory to place larvae in low temperature for the topical application. However, a specific temperature was required in mixing the insecticide with diet. While two methods other than the mixing method are immediately applicable after preparing insecticide solution, a period of time is required to expose the insects to insecticides.

There was a significant difference between the doses that produce 100% mortality. Therefore, it was necessary to determine a different series concentration for each method. In the mixing method, 100% mortality was observed at the maximum dose in all the insecticides and this method appeared to be more reliable than others. In the diet covering method, a higher concentration of insecticides was used to ensure covering all surface area the diet, which enable to deliver enough dose to produce mortality.

Results of the three bioassay methods are presented in Table 1. The LC_{50} values obtained in the diet incorporation method appeared to be more precise than for the other methods, i.e. the 95% confidence intervals (CI) were smaller. Heterogeneity values of the three methods for the different populations are also presented in Table 1. These values were 0.2 - 4.6 for the incorporation method, 0.7 – 10.4 for the covering method and 0.9 – 10.8 for the topical application method. Based on these values, the diet incorporation method delivered the results that can be interpreted by probit analysis.

Table 1. Results from three different bioassay methods on EGM populations

AI	M	P	N*	LC50 (ppm)	LC90 (ppm)	H	Slope±SE
Chlorpyriphos-ethyl	Diet incorporation	Merkez 1	450	4,47 (1,94-9,04)	38,42 (17,41-158,28)	2,56	1,37±0,10
		Yeşilyurt		16,44 (12,96-20,63)	102,80 (73,69-160,65)	0,24	1,61±0,14
		Merkez 2		4,09 (3,16-5,19)	21,69 (16,32-30,81)	0,42	1,76±0,14
	Diet covering	Merkez 1	540	658,26 (164,22-9447,60)	104988,11 (7962,15-938369909,32)	4,41	0,58±0,05
		Yeşilyurt		82,69 (13,12-361,08)	1370,74 (323,79-112270,25)	9,68	1,051±0,07
		Merkez 2		411,51 (119,78-2352,09)	17088,81 (2784,26-5429528,21)	5,36	0,79±0,06
	Topical application	Merkez 1	540	19,36 (2,24-84,14)	300,92 (72,57-66609,92)	10,74	1,07±0,08
		Yeşilyurt		51,39 (10,76-248,76)	873,21 (197,85-233486,85)	9,43	1,04±0,07
		Merkez 2		46,51 (12,23-167,69)	1083,63 (259,23-82236,44)	6,56	0,93±0,07
Spinosad	Diet incorporation	Merkez 1	630	0,66 (0,34-1,16)	3,72 (1,96-12,38)	4,43	1,71±0,12
		Yeşilyurt		0,85 (0,62-1,11)	3,92 (2,83-6,18)	1,02	1,94±0,17
		Merkez 2		1,00 (0,83-1,19)	4,11 (3,23-5,56)	0,23	2,08±0,15
	Diet covering	Merkez 1	540	114,11 (27,44-264,41)	1191,39 (474,55-11304,61)	4,61	1,25±0,11
		Yeşilyurt		36,23 (27,10-47,51)	328,11 (235,73-488,74)	0,73	1,339±0,09
		Merkez 2		39,88 (10,25-115,08)	656,15 (207,08-7248,94)	5,83	1,05±0,07
	Topical application	Merkez 1	540	10,69 (2,15-29,80)	295,26 (61,80-3446,68)	5,88	1,01±0,08
		Yeşilyurt		25,40 (8,62-61,97)	270,24 (100,16-2657,07)	5,81	1,24±0,09
		Merkez 2		19,81 (11,17-32,41)	144,63 (79,66-386,22)	2,37	1,48±0,11
Indoxacarb	Diet incorporation	Merkez 1	630	1,86 (0,93-3,33)	12,78 (6,53-43,39)	4,19	1,53±0,11
		Yeşilyurt		2,19 (0,85-4,31)	19,34 (8,93-89,96)	4,58	1,35±0,10
		Merkez 2		2,98 (2,25-3,81)	22,76 (16,80-33,60)	0,59	1,45±0,12
	Diet covering	Merkez 1	540	228,50 (43,92-939,79)	2485,00 (683,03-449106,97)	9,48	1,236±0,09
		Yeşilyurt		27,12 (3,80-105,83)	813,69 (186,54-34870,12)	7,24	0,86±0,06
		Merkez 2		65,38 (7,90-321,21)	2930,02 (515,14-894322,89)	7,78	0,77±0,06
	Topical application	Merkez 1	540	5,66 (0,79-15,26)	77,69 (28,06-745,85)	4,79	1,12±0,10
		Yeşilyurt		12,05 (3,82-25,09)	92,23 (42,19-454,65)	4,31	1,45±0,12
		Merkez 2		83,12 (38,88-191,75)	3171,67 (914,81-41855,01)	2,39	0,81±0,07
Deltamethrin	Diet incorporation	Merkez 1	450	3,73 (2,61-5,04)	29,67 (21,21-45,61)	0,97	1,420,13
		Yeşilyurt		7,93 (5,62-11,14)	191,20 (110,99-392,24)	0,48	0,92±0,07
		Merkez 2		6,03 (3,41-10,27)	88,70 (43,79-263,90)	1,16	1,09±0,08
	Diet covering	Merkez 1	540	57,14 (22,54-126,37)	1494,53 (553,52-8434,74)	2,90	0,9±0,06
		Yeşilyurt		101,74 (18,01-335,20)	615,50 (211,51-24278,71)	10,35	1,63±0,12
		Merkez 2		240,92 (74,57-738,83)	2878,73 (886,23-86752,22)	6,52	1,19±0,09
	Topical application	Merkez 1	540	11,46 (6,27-18,69)	2254,60 (965,02-7890,68)	0,92	0,55±0,06
		Yeşilyurt		6,12 (0,44-21,83)	86,79 (23,95-6195,33)	9,04	1,11±0,08
		Merkez 2		54,72 (31,18-96,14)	2688,90 (1030,03-12902,14)	1,18	0,75±0,06

AI = Active Ingredient, M = Method, P = Population, N = Number of larvae, H = Heterogeneity
LC_{50} = Concentration that confers 50% mortality (95% Confidence Intervals); LC_{90} = Concentration that confers 90% mortality (95% Confidence Intervals).
*Each population were tested with three different insecticides in 3 different dates with 30 insects per 6 (or 5&7) doses. Therefore, the number 540 (or 450&630) appeared individuals.

The Merkez 1 population had not been exposed to any insecticides for many years so that it was considered to be a susceptible population. The LC_{50} / LC_{90} values for this population were expected to be lower than for the other populations. The LC_{50} values obtained from the incorporation method for all insecticides were lower or equivalent to those of the other methods. In the other methods the lowest LC_{50} values were observed in different populations. For example, in the diet covering method the LC_{50} values for three insecticides in the Merkez 1 population were very high as opposed to expectations. In the topical application method, the lowest LC_{50} value for deltamethrin was found in the Yesilyurt population.

An increase in mortality should be directly proportional to increase in dosage of insecticide and this serves as another means of comparing methods. The ‘slope’ values that reflect this relationship are

presented in Table 1. These values were between 0.92 and 2.08 in the incorporation method as 0.58 to 1.63 in the diet covering method and 0.55 to 1.48 in the topical application method. The diet incorporation method seems to be the best method for comparison the dose-mortality relationship.

From the literature there are a limited number of studies on insecticide resistance and suitable bioassay methods with EGM. In the current study, testing four different insecticides against ten EGM populations from Manisa vineyards using diet incorporation showed that Ahmetli and Alasehir populations were potentially resistant to indoxacarb (Hatipoglu et al., 2015). A high resistance was also detected in Italy using the same bioassay method (Civolani et al., 2014). Perez et al. (1997) compared the leaf-dipping and diet incorporation methods to determine resistance of diamond back moth (*Plutella xylostella*) against *Bacillus thuringiensis*. The researchers stated that "if one wants to choose a concentration which kills 99% of a susceptible population the leaf-dipping bioassay method is a more efficient method to distinguish between resistant and susceptible individuals; because this method kills the more resistant individuals in a lower ratio compared to the bioassay using artificial diet." However, fFrench-Constant and Roush (1990) pointed out that an ideal bioassay method should be quick and efficient, thus insecticide incorporated into artificial diet would be the bioassay of choice where possible.

The major focus of this study was to test the suitability of various bioassay methods for measuring resistance of EGM against insecticides. The results confirmed that the diet incorporation method was the more suitable bioassay method in resistance studies against EGM.

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Orijinal araştırma (Original article)

Storage studies of different stages of *Anthocoris minki* Dohrn (Hemiptera: Anthocoridae) under low temperatures

Düşük sıcaklıklarda *Anthocoris minki* Dohrn (Hemiptera: Anthocoridae)'nin farklı dönemlerinin depolanması

Ertan YANIK[1]* **Levent ÜNLÜ[2]**

Summary

The goal of this study was to evaluate the biological parameters of the predator *Anthocoris minki* Dohrn (Hemiptera: Anthocoridae) over various periods of growth at low temperatures. Storage studies were conducted for the following three stages: 1) 1-3 stage nymphs, 2) 4-5 stage nymphs, and 3) adult stages. All stages of the predator were stored at 7, 11, and 15 ± 1°C for 10, 20, 30, and 40 days under continuous scotophase. During storage, food eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) were added once a week. Following storage, the predator was transferred to long day periods (16:8 [L:D] h, 25 ±1 °C). A lower survival rate (7.33%) was determined for the 1-3 nymph stage following storage at 7°C for 40 days. The highest survival percentage (90.0-92.0%) was determined for 1-3 stage nymphs and adult stage stored at 11°C for 10-30 days. The largest quantity of eggs was obtained when *A. minki* was stored at 11°C. Overall, our results indicated that *A. minki* can be stored for up to 40 days at 11°C.

Keywords: *Anthocoris minki,* biological control, low temperature, mass rearing, storage

Özet

Bu çalışma ile farklı düşük sıcaklıkların ve farklı sürelerin predatör *Anthocoris minki* Dohrn (Hemiptera: Anthocoridae)'nin biyolojik özelliklerinin belirlenmesi amaçlanmıştır. Denemelerde *A. minki*'nin 1-3. nimf, 4-5. nimf ve ergin dönemleri kullanılmıştır. Her gruptaki bireyler 7, 11 ve 15±1°C sıcaklıklarda ve 10, 20, 30 ile 40 gün sürekli karanlıkta tutulmuştur. Düşük sıcaklıkta depolanan predatörlere haftada bir besin olarak *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) yumurtaları sunulmuştur. Depolama sonrası canlı kalan *A. minki* bireyleri uzun gün koşullarında (16:8 [A:K] h, 25±1°C) yetiştirilmeye alınmıştır. Depolama süresince en düşük canlı kalma oranı (%7.33), 7°C'de 1-3. nimf döneminde 40 gün depolandığında, en yüksek canlı kalma oranı (%90.0-92.0) ise 11°C'de 1-3. dönem nimf ve ergin döneminde 10-30 gün depolandığında gerçekleşmiştir. Depolama sonrası yetiştirilen bireylerde en fazla yumurta 11°C'de depolandığında elde edilmiştir. Sonuç olarak, *A. minki* 11°C'de 40 güne kadar depolanabilir.

Anahtar sözcükler: *Anthocoris minki*, biyolojik mücadele, düşük sıcaklık, depolama, kitle üretimi

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Introduction

Pistachio psylla, *Agonoscena pistaciae* Burckhardt and Lauterer (Hemiptera: Psyllidae) is an important pest that causes yield loss in pistachio trees (Mart et al., 1995; Mehrnejad, 2001; Souliotis et al., 2002). Due to increased resistance to insecticides, *A. pistaciae* is difficult to control (Mehrnejad, 2001). As a result of insecticide failure, biological controls are gaining prominence for controlling pistachio psylla. In Turkey, *Anthocoris minki* Dohrn (Hemiptera: Anthocoridae) has an important role as a natural enemy of pistachio psylla (Çelik, 1981; Mart et al., 1995; Yanik & Unlu, 2010). Based on data obtained from insect release studies in pistachio orchards, Yanık et al. (2007) reported that *A. minki* was effective for controlling pistachio psylla and was responsible for decreasing the pest population at the economic injury level. Detailed studies regarding the biology, ecology, and release of *A. minki* for controlling pistachio psylla are available in the scientific literature (Yanik & Unlu, 2010; 2011a, b; Yanık et al., 2007; 2009; 2011a, b; 2012).

In regards to the mass rearing of commercially produced biocontrol agents, low temperature storage is an important component. Synchronization, flexibility, and effectiveness is achieved in mass rearing operations when release demand has the highest biological control. Thanks to low temperature storage, standard stocks can be provided for long term ecologic, physiologic, or genetic research purposes (Leopold, 1998). However, parameters such as emergence rate, longevity, number of eggs laying and reproductive success can impact the number of beneficial insects that can be stored at low temperatures (Leopold, 1998). Therefore, knowledge of an effective storage period and the temperature required for the mass rearing of natural enemies significantly contributes to the sustainability of biocontrol agent production.

The anthocoridae family includes species than can be commercially reared for use as biocontrol agents (Anonymous, 2014a, b, c). Several studies regarding storage at low temperatures have been performed for Anthocorid species. Rudolf et al. (1993) stored *Orius majusculus* (Reuter) and *Orius laevigatus* (Fieber) (Hemiptera: Anthocoridae) adults at 13 and 9°C. The eggs of *Orius sauteri* (Poppius) were stored in the laboratory at 7.5°C and 12.5°C by Murai et al. (2001). Kim et al. (2009) stored *O. laevigatus* at 10°C and Bueno et al. (2014) reported that *Orius insidiosus* (Say) (Hemiptera: Anthocoridae) can be stored at 8°C for 10 days without a loss of quality. A large body of scientific research is also available on the low temperature storage of other natural enemies (e.g. Osman & Selman, 1993; Abdel-Salam & Abdel-Baky, 2000; Uçkan & Gülel, 2001; Bayram et al., 2005; Lo´pez & Botto, 2005; Coudron et al., 2007; Larentzaki et al., 2007; Luczynski et al., 2008; Tunca et al., 2014).

Based on the studies provided above, cold storage has been determined to be an important method in the mass production of biological control agents. However, to our knowledge, no studies regarding the storage of the Anthocorid predator *A. minki* at low temperatures are currently available in the scientific literature. The goal of this study was to determine the effects of cold storage on the survival rate, the potential reproductive rate, the longevity, and the fecundity of *A. minki*. Since cold storage temperature and the duration of temperature exposure are the two most important factors when defining a cold storage system for a natural enemy, these parameters were investigated in this study.

Materials and Methods

Insect stock cultures

The adult *A. minki* colony used for our study was collected from pistachio trees located in Şanlıurfa province in Turkey. Transparent plastic containers with a diameter of 12 cm and a height of 13 cm, covered with nylon tulle on two sides with a ventilation hole were used to rear adults and nymphs. Frozen *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs (0.1 gr), attached to black cardboard (5x5 cm) using water, were supplied as a food source. A green bean pod (*Phaseolus vulgaris* L.) was supplied as an egg laying site for predators and as a water source. *Ephestia kuehniella* eggs and green bean pods were replaced every two or three days. New cultures were formed by moving green bean pods containing *A. minki* eggs into separate plastic containers. To allow predator insects to hide and wander, the bottom of rearing containers was lined with paper towels. Adults and nymphs of *A. minki* were reared in a climate chamber at 25 ± 1^{o}C at a relative

humidity of 65 ± 5% for 16:8 hours of light:dark. *Ephestia kuehniella* was reared using methods described previously (Bulut & Kılınçer, 1987). *Anthocoris minki* used for tests were obtained from the stock culture.

Effects of cold storage on survival rate, longevity and fecundity of *Anthocoris minki*

The three biological stages of *A. minki* (1-3 stage nymphs, 4-5 stage nymphs, and the adult stage) were stored at constant temperatures of 7, 11, and 15 ± 1ºC for 10, 20, 30, and 40 days under constant scotophase. To enable nymphal stages to develop and to survive for a longer period, a food source was provided once per week. Fifty individuals of the same age from the *A. minki* population were randomly selected from the stock culture for each biological stage, each low temperature, and each storage period. Individuals were placed in plastic containers (12 cm in diameter x 13 cm in height) in three separate replicates (n = 3, a total of 150 individuals for each storage period; a total of 1,800 individuals for the four different storage periods at each temperature; and a total of 5,400 individuals in all combinations for the three temperatures examined). *Anthocoris minki* individuals were placed with *E. kuehniella* eggs. A creased paper towel was used so individuals could hide. No initial sex determination was performed for the adult stage individuals stored although Yanik & Unlu (2011a) reported that the sex ratio of *A. minki* was 1:1. Tests were performed in plastic containers having a diameter of 10 cm and a height of 10 cm with a ventilation hole, the lateral sides of which were covered with tulle. Following storage at low temperature for the stated periods, dead individuals in each container (replication) were recorded and the post-storage survival percentage was determined. Surviving individuals for each storage period were moved to an environment with a temperature of 25 ± 1ºC, a relative humidity of 65 ± 5%, and 16:8 hr light:dark. The study was continued by providing 0.2 grams of *E. kuehniella* eggs daily within the containers as a food source and by placing green bean pods in containers as egg laying material. In controls performed three times a week, male and female longevities were determined by performing a sex determination on dead individuals. The number of eggs lain was also recorded. The number of eggs laying, male and female longevity, and the pre-oviposition period starting date were determined, beginning from the day when adults surviving the various temperature and storage periods began to be reared at 25 ± 1ºC. The food source and green beans were replaced during these controls. For the control group, 50 *A. minki* individuals within the 0-24 hour population were collected from the stock culture and reared at a temperature of 25 ± 1ºC in three replicates. Male and female longevity and fecundity were determined. The number of eggs laying per female was determined by dividing the total number of eggs by the number of females contained in each replicate.

Determining ovarian development

Using the same method employed to determine survival rate and fecundity, *Anthocoris minki* were reared in combinations formed using various temperatures and storage times. *A. minki* that survived each low temperature and storage replicate were then reared at 25 ± 1ºC. Rearing at 25 ± 1ºC lasted for 10 days for previously cold-stored adult stages; 14 days for 4-5 stage previously cold-stored nymphs, and 18 days for 1-3 stage previously cold-stored nymphs in order to provide egg development in ovarioles. The rearing periods were several times longer than the normal pre-oviposition period required for the fecundity study. At the end of the reared periods, we recorded whether an oocyte was present in each female by dissecting *A. minki* individuals under a stereo-binocular microscope. For the procedure, female Anthocorids were placed into a drop of water on a dissection slide. Abdomen sclerites were removed using an insect pin and we carefully separated the ovaries from other internal material using a needle. If cold-stored individuals from the nymph stage were not able to reach the adult stage by the end of the storage periods, they continued to be reared at 25 ± 1ºC until they reached the adult stage. Individuals that were stored as 4-5 stage nymphs were reared for 14 days, while those that were stored as 1-3. stage nymphs were reared for 18 days. Adults were dissected at the end of storage. For the control group, new *A. minki* adults aged 0-24 hours obtained from the stock culture were dissected after being reared at 25 ± 1ºC for 10 days. Tests to determine the reproductive rate were performed using three replicates for each combination so that fifty randomly selected individuals of the desired stage were obtained from the stock culture. The percentage of females with developed ovaries was calculated. Thus, the potential reproductive rate was determined.

Statistical analysis

A two-way ANOVA analysis was used to evaluate the impact of various low temperatures and storage periods on male and female longevity, preoviposition periods, and the number of eggs laying. Tukey's multiple comparison test was used to determine the difference between averages.

Results and Discussion

The work presented is the first study on the effects of storage at low temperature on the biological parameters of *A. minki*. Significant differences were determined between the survival capacities of insects and mite species in cold storage. Therefore, it is not possible to generalize the tolerance of taxa, families, or even genera to cold storage (Leopold, 1998). Supplying food during the cold storage of insects is generally beneficial (Leopold, 1998). For example, Abdel-Salam & Abdel-Baky (2000) reported that feeding *Coccinella undecimpunctata* L. (Coleoptera: Coccinellidae) adults prior to cold storage significantly affected the survival percentage, the longevity, the fecundity, and prey consumption rates. In our study, food was supplied during cold storage. Food was especially necessary during the storage of nymph stages of *A. minki* so that development could be completed.

Table 1. The effects of cold storage on the survival rate of *Anthocoris minki* (%)

Temperature (°C)	Storage period (day)	1-3.stage nymph	4-5.stage nymph	Adult
7	10	28.66	58.00	84.00
	20	24.00	55.33	84.00
	30	21.33	35.33	84.66
	40	07.33	14.00	42.00
11	10	90.00	78.66	92.00
	20	91.33	75.33	92.00
	30	86.00	77.33	90.00
	40	80.66	64.66	88.33
15	10	84.00	82.00	87.33
	20	84.66	80.66	83.33
	30	89.33	84.00	86.00
	40	78.00	78.66	80.66

Our results indicated that the lowest survival rate of *A. minki* (7.33%) occurred for the 1-3 nymph stage at a temperature of 7ºC when stored for 40 days (Table 1). The highest survival rate (92.0-92.0%) was observed for the adult stage with a storage time of 10-20 days at 11ºC. The highest survival rate (88.33%) for a 40 day storage period occurred for the adult stage at 11ºC. Kim et al. (2009) reported that when stored at temperatures of 6, 8, 10, and 12°C, 10°C was the most suitable storage temperature for adults of *O. laevigatus*. These authors also reported a survival rate of 70% at this temperature at the end of 36 days. Rudolf et al. (1993) reported that *O. majusculus* adults had a survival rate of 50% for a storage time of 42 days, while *O. laevigatus* adults had a survival rate of 75-80% for a storage time of 40 days when stored at 9°C. Bueno et al. (2014) reported that the survival rate of *O. insidiosus* following storage at 10°C for 20 days was 61.4% for females and 50.4% for males. Based on the scientific literature, it is understood that the survival rate of different Anthocoridae species following storage at low temperatures is lower than the survival rates determined during our study. Such findings result indicates that the genera used for other studies have an adult longevity that is shorter than that of *A. minki*.

Since the post-storage death rate of *A. minki* stored at 7°C for the 1-3 and 4-5 nymph stages during various storage periods was very high, the potential reproductive rate could not be determined. Apart from these biological stages, the lowest potential reproductive rate (80.96%) was observed for storage at 15°C for 40 days for adult stages. The highest potential reproductive rate (98.22%) was observed for storage at 15°C for 40 days for the 1-3 nymph stage (Table 2). While *A. minki* had a potential reproductive rate of 95.91% for the control group, the potential reproductive rate after storage at 11°C was above 90% (90.33 - 95.84%) for all storage periods and biological stages.

Table 2. The effects of cold storage on the potential reproductive rate of *Anthocoris minki* (%)

Temperature (°C)	Storage period (day)	1-3.stage nymph	4-5.stage nymph	Adult
7*	10	0	0	88.24
	20	0	0	95.66
	30	0	0	90.90
	40	0	0	93.75
11	10	90.33	93.11	90.63
	20	92.31	90.91	92.18
	30	95.24	92.43	92.31
	40	92.59	95.84	91.38
15	10	92.10	91.30	97.30
	20	93.11	92.85	95.32
	30	94.65	92.46	87.50
	40	98.22	96.88	80.96
25 Control**	0	95.91		

* Since the post-storage death rate of *A. minki* stored at 7°C during the 1-3 and 4-5 nymph stages was very high, the potential reproductive rate could not be determined.
** Control group, not stored at a low temperature, were reared at a temperature of 25 ± 1ºC.

The post-storage female longevity for cold-stored adults and 1-3 stage nymphs of *A. minki* was significantly affected by temperature (adult, F=7.72, df=2, P=0.0026; 1-3 nymph, F=3.96, df=2, P=0.0327). However, the storage period and the interaction between the temperature-storage period was not statistically significant during all of the biological stages ($p>0.05$). The 1-3 stage nymphs stored at 7°C for 40 days displayed the longest post-storage female longevity (60.33 days) (Table 3). When storage periods were not taken into account, post-storage female longevity was statistically longer for the storage of 1-3 stage nymphs at 7 and 11°C, and for the storage of adults at 11 and 15°C as compared to other temperature values. However, no statistical difference between temperatures in terms of female longevity was determined for the 4-5 stage nymphs. Bueno et al., (2014) reported that female longevity of *O. insidiosus* at 25°C following storage at 10°C for 10 and 20 days lasted for 7.8 and 3.8 days, respectively. Kim et al. (2009) reported that female longevity for *O. laevigatus* lasted for 19.8 and 23.7 days at 25°C following storage at 10°C for 20 and 40 days, respectively. In our study, female *A. minki* stored at low temperatures at various stages displayed shorter longevity as compared to the control group (54.66 days) reared at 25°C without cold storage, suggesting that storage has a negative impact on adult longevity. Rudolf et al. (1993) reported that female *O. majusculus* lived for 25.9 and 19.8 days at 22°C following a 30 and 50 day storage, respectively, at 9°C, while Bahşi & Tunç (2012) reported that female *O. majusculus* lived for 45.0 days in *E. kuehniella* at 26°C without cold storage.

Table 3. Female longevity of various stage of *Anthocoris minki* (under 25°C, 16:8 L:D) after various storage periods at various low temperatures (day)

Temperature (°C)	Storage period (day)	1-3.stage nymph	Mean	4-5.stage nymph	Mean	Adult	Mean
7	10	41.91ab*	45.69a	25.34a	31.67a	17.74a	21.26b
	20	39.93ab		30.25a		22.51a	
	30	40.61ab		45.75a		24.14a	
	40	60.33a		25.33a		20.68a	
11	10	43.66ab	42.95ab	30.01a	30.66a	32.17a	30.81a
	20	44.47ab		31.83a		33.30a	
	30	45.66ab		29.32a		31.27a	
	40	38.01ab		31.47a		26.53a	
15	10	36.48ab	35.71b	30.17a	29.25a	28.40a	27.52a
	20	37.65ab		29.05a		25.92a	
	30	38.78ab		29.13a		28.09a	
	40	29.94b		28.63a		27.68a	
25 Control**	0	54.66					

*Means in the same column followed by a different letter are significantly different (Tukey test, P<0.05).
* *Control group, not stored at a low temperature, were reared at a temperature of 25 ± 1ºC.

Temperature, storage period duration, and temperature-storage period interaction for all biological stages stored did not have a statistically significant impact on post-storage male longevity (p>0.05). When storage periods are not taken into account, male longevity was found to be significantly longer for the storage of 1-3 stage nymphs at 11°C and for storage of adults at 15°C as compared to other temperature values (p<0.05). However, no statistically significant difference was determined between temperatures for the storage of 4-5 stage nymphs (p>0.05) (Table 4). Male longevity was shorter than the control group (64.54 days) for all temperature and storage periods.

Table 4. Male longevity of various stage of *Anthocoris minki* (under 25°C, 16:8 L:D) after various storage periods at various low temperatures (day)

Temperature (°C)	Storage period (day)	1-3.stage nymph	Mean	4-5.stage nymph	Mean	Adult	Mean
7	10	24.32a*	37.29b	27.59a	29.35a	20.80a	20.41b
	20	32.91a		28.94a		18.47a	
	30	43.66a		33.60a		22.06a	
	40	48.25a		26.88a		20.32a	
11	10	43.69a	44.50a	24.71a	26.25a	22.70a	23.99ab
	20	45.56a		26.65a		23.52a	
	30	45.50a		28.25a		24.32a	
	40	43.21a		29.25a		27.32a	
15	10	33.38 a	36.55b	27.43 a	27.89a	28.97 a	26.52a
	20	35.23 a		30.51 a		29.75 a	
	30	39.86a		28.26 a		24.79 a	
	40	37.75a		25.38a		22.58a	
25 Control**	0	64.54					

*Means in the same column followed by a different letter are significantly different (Tukey test, P<0.05).
** Control group, not stored at a low temperature, were reared at a temperature of 25 ± 1ºC.

The storage temperature was determined to have a significant impact on the pre-oviposition period for all cold-stored biological stages of *A. minki* (1-3 nymph, F=10.89, df=2, P=0.0004; 4-5 nymph, F=10.75, df=2, P=0.0005; adult, F=44.44, df=2, P=0.0001).

The interaction of the temperature-storage period was statistically significant for the storage of 1-3 stage nymphs and adult stages (1-3 nymph, *F*=2.70, df=6, *P*=0.0379; adult, *F*=7.11, df=6, *P*=0.0002), while storage periods were determined to be statistically significant only during the pre-oviposition period for stored adult stages (*F*=7.56, df=3, *P*=0.0010). Shortness of the pre-oviposition period during mass rearing is important for economic production. In our study, we determined that the post-storage, pre-oviposition period was shorter than that of control group that underwent no storage, excluding the storage of 1-3 and 4-5 stage nymphs of *A. minki* at 7°C (Table 5). Based on our results, cold storage has a positive impact on mass rearing in terms of shortening the pre-oviposition period of *A. minki*. The fact that the post-storage, pre-oviposition period of *A. minki* adults was shorter than the other stored stages could have resulted from mating during storage.

Table 5. Preoviposition period of various stage of *Anthocoris minki* (under 25°C, 16:8 L:D) after various storage periods at various low temperatures (day)

Temperature (°C)	Storage Period (day)	1-3.stage nymph	Mean	4-5.stage nymph	Mean	Adult	Mean
7	10	8.66ab*	11.08a	10.00ab	10.16a	2.66a	2.16a
	20	11.33ab		12.00ab		2.00a	
	30	16.33b		13.66b		2.00a	
	40	8.00ab		5.00ab		2.00a	
11	10	4.33a	4.66b	3.00a	5.08b	0.00b	0.50b
	20	4.66a		6.00ab		0.00b	
	30	2.66a		5.33ab		0.00b	
	40	7.00ab		6.00ab		2.00a	
15	10	8.66ab	6.75b	5.66ab	4.83b	3.33a	2.16a
	20	9.00ab		7.00ab		1.33ab	
	30	5.00a		3.66a		2.00a	
	40	4.33a		3.00a		2.00a	
25 Control**	0			10.76			

*Means in the same column followed by a different letter are significantly different (Tukey test, P<0.05).
** Control group, not stored at a low temperature, were reared at a temperature of 25 ± 1ºC.

The storage temperature was found to have a significant influence on the number of eggs laid per female for cold-stored 4-5 stage nymphs and adults of *A. minki* (4-5 nymph, *F*=7.81, df=2, *P*=0.0024; adult, *F*=16.44, df=2, *P*=0.0001). On the other hand, the storage period during all of the stored biological stages and the interaction between the temperature-storage period had no statistically significant impact on number of eggs lain per *A. minki* female (p>0.05). When storage periods were not taken into account, the storage of 4-5 stage nymphs (55.95 pcs/female) and adults (77.23 pcs/female) at 7°C yielded the lowest mean number of eggs laid per female (Table 6).

Considering all of the biological stages of *A. minki* tested, the mean number of eggs laid per female following storage at 11°C was closer to the control group values when compared to other temperature values. On the other hand, we determined that the 1-3 stage nymphs of *A. minki* stored at 15°C for 10 days laid quite a similar numbers of eggs as those of the control group that was not cold-stored. The fact that the mean number of eggs laid per *A. minki* female following cold storage was lower than those of the control group indicates a negative effect for cold storage in terms of the number of eggs laid. Kim et al. (2009) reported that following storage at 10°C for 20 and 40 days, females of *O. laevigatus* laid 109.2 and 69.2 eggs at 25°C, respectively, while the control group that was not stored cold laid 224.5 eggs. Rudolf

et al. (1993) reported that following storage at 9°C for 20 and 50 days, females of *O. laevigatus* laid 145 and 72 eggs, respectively, at 22°C, while the control group laid 190 eggs; females of *O. majusculus* laid 75 and 34 eggs while the control group laid 122 eggs. For mass rearing, the creation of a rearing environment that produces a high number of eggs is necessary to achieve economic viability. Considering that cold storage is an indispensable part of mass rearing, determining the most suitable storage condition for egg growth is important.

Table 6. Fecundity of various stage of *Anthocoris minki* (under 25°C, 16:8 L:D) after various storage periods at various low temperatures (number/female)

Temperature (°C)	Storage period (day)	1-3.stage nymph	Mean	4-5.stage nymph	Mean	Adult	Mean
7	10	131.91a*	121.03a	39.31a	55.95b	54.90b	77.23c
	20	114.81a		47.88a		69.59bc	
	30	121.68a		81.04a		96.82abc	
	40	108.83a		55.55a		87.61abc	
11	10	174.95a	155.43a	164.49a	148.83a	155.85a	141.54a
	20	168.02a		152.01a		144.55ac	
	30	157.16a		143.82a		141.22ac	
	40	121.56a		135.02a		124.54abc	
15	10	194.99a	163.02a	133.26a	120.55a	112.13abc	106.47b
	20	175.04a		132.72a		109.98abc	
	30	155.64a		118.26a		101.24abc	
	40	126.43a		98.10a		102.53abc	
25 Control**	0			207.51			

*Means in the same column followed by a different letter are significantly different (Tukey test, P<0.05).
** Control group, not stored at a low temperature, were reared at a temperature of 25 ± 1ºC.

Based on our experiments on *A. minki*, performed at various low temperatures and storage periods we suggest that this predator can be stored for up to 40 days at 11°C. Cold storage for the predator *A. minki* may be a valuable tool for insectaries, allowing them to store insects for prolonged periods rather than continuously rearing colonies during the off-season when demands for them are low. Additionally, cold-storage has the added benefit of making standardized cultures available for research and provides flexibility and efficiency for mass production, therefore, synchronizing the desired stage of development for crop release as well as facilitating the availability of insects to users. Determining low cost and effective storage methods for insect rearing is especially important for obtaining a high quality of biological control agents. Furthermore, understanding suitable storage conditions for obtaining higher numbers of natural enemy insects ready for crop release during intensive release periods is of great importance for mass rearing.

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Orijinal araştırma (Original article)

Description of a new species of *Luzulaspis* Cockerell (Hemiptera: Coccomorpha: Coccidae) from Turkey

Luzulaspis Cockerell (Hemiptera: Coccomorpha: Coccidae) cinsine ait yeni bir tür tanımlanması

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Selma ÜLGENTÜRK[3] **M. Rifat ULUSOY[1]**

Summary

A new soft scale insect species, *Luzulaspis filizae* Kaydan *sp. nov.* (Hemiptera: Coccomorpha: Coccidae), is described and illustrated based on the adult female and first-instar nymph. The new species was collected on *Carex* sp. (Cyperaceae) in Adana, Turkey.

Keywords: Eriopeltinae, *Scotica* group, woolly ovisac, *Hadzibejliaspis, Poaspis*

Özet

Bu çalışmada Adana'da (Türkiye) *Carex* sp. (Cyperaceae) üzerinden toplanan bir yumuşak kabuklubit (Hemiptera: Coccomorpha: Coccidae) türü *Luzulaspis filizae* Kaydan *sp. nov.*'un ergin dişisi ve birinci dönem nimfi tanımlanarak çizimleri verilmiştir.

Anahtar sözcükler: Eriopeltinae, *Scotica* grup, yünümsü yumurta kesesi, *Hadzibejliaspis, Poaspis*

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Introduction

Coccidae family (Sternorrhyncha: Hemiptera: Coccomorpha) is the third largest scale insect family worldwide, with over 1157 species in 160 genera (Ben-Dov et al., 2015). In the Palaearctic zoogeographical region 318 species in 63 genera are known (Ben-Dov et al., 2015). The family members are always covered by a soft, waxy covering which varies considerably in both texture and structure between the families (Ben-Dov, 1997). Ten subfamilies are recognized in the Coccidae based on the morphology of the adult females (Hodgson, 1997).

Females in the family Eriopeltinae are characterized by producing a woolly ovisac which covers partly or entirely the body of adult female (Hodgson, 1994). The subfamily contains 13 genera, namely *Eriopeltis* Signoret*, Exaeretopus* Newstead, *Hadzibejliaspis* Koteja, *Lecanopsis* Targioni Tozzetti, *Luzulaspis* Cockerell, *Poaspis* Koteja, *Psilococcus* Borchsenius, *Scythia* Kiritshenko, *Vittacoccus* Borchsenius (Palearctic distribution), *Idiosaissetia* Brain, *Membranaria* Brain (African or Asiatic distribution) and *Symonicoccus* Koteja & Brookes and *Waricoccus* Brookes & Koteja (Australian distribution) (Hodgson, 1994). The genus *Luzulaspis* is characterized by the following features: (i) flattened (rarely convex) body; (ii) well developed 8-segmented antennae; (iii) legs well developed, slender; (iv) claw digitules each with an apical knob about as wide as base of claw; (v) dorsal setae spine-like or hair-like (vi) tubular ducts numerous on entire dorsum except head and finally (vii) two spiracular setae. The genus is most close to *Poaspis* in sharing the distribution of large ventral setae and the structure (sieve like) of the prevulvar pores. But, *Luzulaspis* differs from *Poaspis* in having lower numbers of ventral setae (especially interantennal setae) (much more in *Poaspis*), lower numbers of marginal setae which are also thinner (larger and more numerous in *Poaspis* (Koteja, 1979).

Seventeen *Luzulaspis* species are currently known worldwide of which 11 have a Palaearctic distribution, four are Nearctic, one is Afrotropical and one is Oriental in distribution (Ben-Dov et al., 2015). Koteja (1979) indicated that the genus *Luzulaspis* inhabited three centres of distribution, namely Europe, Far East and North America. According to earlier records, the genus occurs mainly in continental climates, with a few found in the Mediterranean region (except some records in France and the Turkish Republic of Northen Cyprus) up to now (Şişman & Ülgentürk, 2010). Like related genera such as *Exaeretopus*, *Poaspis* and *Hadzibejliaspis*, *Luzulaspis* adult females produce an ovisac which covers all the female body morever all members of these four genera infest generally monocotyledonous plants such as Poaceae, Juncaceae, Cyperaceae. *Luzulaspis* members were especially recorded on Juncaceae, Poaceae and Cyperaceae, with an exception of some Fagaceae records from Romania (Ben-Dov et al., 2015).

In Turkey, the family Coccidae contains 28 genera and 67 species with a Palaearctic distribution in general (Kaydan et al., 2013). Although there are some records on the genera; *Eriopeltis* (1 species)*, Exaeretopus* (3)*, Lecanopsis* (3)*, Scythia* (2)*, Vittacoccus* (1) belonging to the Eriopeltinae, up to now there are no records of any species from the genus *Luzulaspis* in Turkey.

In the present study we describe one new *Luzulaspis* species, collected in Turkey and provide a revised identification key to species in the genus. In addition, the first-instar nymph of the new species is described and illustrated.

Materials and Methods

Scale insect samples were collected in Turkey (Balcalı, Adana) in 2015. Specimens were taken from *Carex* sp. (Cyperacea) in natural areas. Collecting data, province, locality, date of collection, collector, host plant and collection number are given.

Specimens were slide-mounted for light microscopy using the method of Kosztarab & Kozár (1988). Morphological terminology that of Koteja (1979) and Hodgson (1994) for description of the new soft scale insect species were followed. Measurements and counts of the new species were taken from all available material.

Most part of the type material is deposited in the Coccoidea Collection in Çukurova University, Plant Protection Deparment, Balcalı, Adana, Turkey (KPCT) and one paratype is deposited in the Scale Insect Collection of Plant Protection Department in Ankara University, Agriculture Faculty, Ankara, Turkey.

Results

***Luzulaspis* Cockerell**

Signoretia Targioni Tozzetti, 1868. Homonym of *Signoretia* Stal, 1859, in Hemiptera.

Signorettia; Targioni Tozzetti, 1869. Misspelling of genus name.

Signoretia; Signoret, 1872.

Luzulaspis Cockerell, 1902. Replacement name for *Signoretia* Targioni Tozzetti, 1868

Type species: Aspidiotus luzulae Dufour, by monotypy. Homonym of *Signoretia* Stal, 1859, in Hemiptera.

Generic diagnosis. Adult female. Ovisac elongate, almost parallel sided, white, 3–6 mm long, covering the female entirely. Postreproductive female sclerotized, shrinking and falling out of ovisac, except when parasitized. Teneral female elongate, parallel sided, rounded or slightly tapered at both ends, dorsum slightly convex, venter almost flat; usually yellowish with two red dorsal stripes (Kosztarab & Kozár, 1988).

Venter. Antennae slender, 8-segmented. Labium cube shaped, with 5 pairs of setae, stylet loop about as long as labium. Legs slender, anterior legs always shorter, tibio-tarsal articulatory sclerosis present, claw digitules large, with expanded apical knob. Spiracular pore bands mostly of quinquelocular pores; 2 subequal spiracular setae in each group. Marginal setae spinelike or hair-like, usually intervals between setae about equal to length of setae or greater, body setae of various lengths, interantennal setae of various lengths, up to 150 µm long, numbering 10–25. Microducts normally form a marginal row, 1 or 2, rarely 3 campaniform pores, 2–3 µm in diameter at the base of each antennae. Multilocular pores usually with 8–10 loculi, forming transverse bands on abdominal sternites 6–8, rarely on anterior segments. Tubular ducts of various sizes. Microducts 1.0–1.5 µm in diameter, forming a subequal band, also present on medial area of head, thorax and in some species on abdomen (Kosztarab & Kozár, 1988).

Dorsum. Body setae of various shapes and sizes, from small hair-like to large conical. Minute simple pores 2 µm in diameter, scattered over entire surface. Discoidal pores with a sieve-like structure, 3–6 µm in diameter in a medial longitudinal band on thorax and abdomen. Tubular ducts numerous on body surface. Anal ring 45–80 µm in diameter, with 6 setae, each 110–180 µm long. Anal plates triangular, each with 4 apical setae (Kosztarab & Kozár, 1988).

Seventeen *Luzulaspis* species are currently known worldwide, namely; *Luzulaspis americana* Koteja & Howell, *L. bisetosa* Borchsenius, *L. borealis* Koteja & Howell, *L. caricicola* (Lindinger), *L. caricis* (Ehrhorn), *L. crassispina* Borchsenius, *L. dactylis* Green, *L. frontalis* Green, *L. grandis* Borchsenius, *L. kosztarabi* Koteja & Kozár, *L. luzulae* (Dufour), *L. macrospinus* Savescu, *L. minima* Koteja & Howell, *L. nemorosa* Koteja, *L. rajae* Kozár, *L. saueri* Lepage & Giannotti, *L. scotica* Green.

Koteja (1979), separated the genus *Luzulaspis* in five groups; the *Scotica*, *Luzulae*, *Bisetosa*, *Frontalis* and *Grandis* groups. He stated that the first three groups represented natural assembleges while the remaning two were recognized on the basis of some morphological similarity although this might have been artificial.

The new species described in this paper belongs to *Scotica* group which differs from the other *Luzulaspis* groups in having (i) strong, conical marginal setae situated in one row, in distances equal to or 2-3 times greater than the length of setae and (ii) small, conical or nearly parallel-sided subequal setae on the dorsal surface. Currently seven species referred to *Scotica* group are known worlwide namely; *Luzulaspis* **americana**, *L. minima*, *L. rajae*, **L. caricis**, *L. dactylis* Green, *L. borealis* Koteja & Howell, *L. scotica* Green.

In this study *Luzulaspis filizae* Kaydan, *sp. nov.* is described and illustrated based on the adult female and first-instar nymph.

Key to adult female *Luzulaspis* (*Scotica* group). The key is after Koteja (1979), Koteja & Howell (1979) and Kozstarab & Kozár (1988) with additions and changes.

1 – Antennae situated distinctly closer to apex of labrum than to anterior body margin; large marginal setae on head twice longer than those of lateral edge, long ventral setae absent from medial parts of thorax ..... *Luzulazpis americana* Koteja & Howell

- Antennae situated half way from anterior body margin to apex of labrum or slightly closer to latter; large marginal setae on head and lateral margin subequal, intervals between setae subequal to length of setae, occasionally greater 2

2- Setae on inner edge of tibia subequal to or shorter than tibia width, spiracular and lateral marginal setae subequal in length, sometimes the former shorter.....3

- Setae on inner edge of tibia at least twice as long as tibia width, spiracular setae longer than lateral marginal setae.....6

3- Marginal setae all subequal in legth, round, each 7–14 μm long; antennae 290–360 μm long .. .. *Luzulazpis minima* Koteja & Howell

- Marginal setae on head and anal lobes longer than other marginal setae, up to 25 μm long, antennae longer than 400 μm 4

4- Two apical marginal setae on head and apical setae of anal lobe thick, twice as wide at base as other marginal setae; interantennal setae less than 75 μm long…… ..... *Luzulaspis rajae* Kozár

- Two apical marginal setae on head and apical setae of anal lobe only slightly thicker than other marginal setae, but never larger than twice as wide at base as other marginal setae; interantennal setae longer than 75 μm long 5

5- Marginal setae between anterior and posterior spiracular setae numbering 16–21; 18–22 pores in spiracular pore band; spiracular setae and other marginal setae almost of the same length of other marginal setae..... *Luzulaspis caricis* (Ehrhorn)

- Marginal setae between anterior and posterior spiracular setae numbering 27–31; 42–52 pores in spiracular pore band; spiracular setae longer than other marginal setae..... ..... *Luzulaspis filizae* Kaydan *sp. nov.*

6- Antennae 370–460 μm long, ... *Luzulaspis dactylis* Green

- Antennae 460–550 μm long.....7

7- 10–14 interantennal setae; 22–27 marginal setae between anterior and posterior spiracular setae ..... *Luzulaspis borealis* Koteja & Howell

- 13–31 interantennal setae; 26–39 marginal setae between anterior and posterior spiracular setae ..... *Luzulaspis scotica* Green

Luzulaspis filizae* Kaydan *sp. nov. (Fig. 1, 2)

Type material. Holotype: adult female, Turkey, Adana, Balcalı, ex. *Carex* sp. (Cyperacea) 25 m., 07.v.2015, leg. A. F. Çalışkan, Paratypes: 5 adult females, 25 first instar nymphs same data as holotype,

Adult female

Living specimens. Oval, yellowish-pink, partly enclosed in a white, waxy, sub-spherical egg-sac (Fig. 2 a, b).

Mounted specimens. Body oval, 5.50–6.45 mm long, 2.2 mm (1.85–2.44) wide.

Venter. Derm membranous, dermal spinules present medially on thoracic and abdominal segments. Antennae 8 (rarely 7) segmented; length of segments in µm: I 62–85; II 70–75; III 150–180; IV 90–113; V 70–85; VI 45–50 (in 7 segmented antennae 70); VII 45 (in 7 segmented antennae 60–70) and VIII 45–55. Scape with 3 flagellate setae, segment II with 2 flagellate setae, III and IV each with 1 flagellate seta, V with 2 flagellate setae, VI with 1 fleshy seta, VII with 1 fleshy seta + 1 hair-like seta, and VIII with 3 fleshy setae + 5 hair-like setae. Legs well developed, narrow. Tibio-tarsal sclerosis present (Fig. 2 e). Measurements of hind leg: coxa 260–280 µm long; trochanter + femur 390–420 µm; tibia 390–420 long and 35–40 wide; tarsus 170–190 µm long; claw without a denticle, 30–40 µm; claw digitules longer than claw, as wide as half width of claw, slightly broadened apically (Fig. 2 e), tarsal digitules longer than claw, thin and with a small apical swelling, 70–80 µm long. Spiracles 80–105 µm long, 45–70 µm wide in peritreme. Spiracular disc pores, each 6–8 µm wide with 5 loculi (sometimes 7 or 8), forming a loose band of with 24–32 (anterior), 39–52 (posterior) pores from each spiracle to body margin,. Simple pores scattered. Pregenital disc pores mostly with 10–12 loculi (Fig. 2 f), each 8–10 µm wide, numerous around genital opening, becoming progressively less numerous across anterior abdominal segments. Tubular ducts of two sizes: large tubular ducts 14–17 µm long, 6–7 µm wide, with a long, thin inner ductule and small terminal gland; small ducts 15–17 µm long, 4.3–5.0 µm wide, with a long, thin inner ductule and small terminal gland. Larger tubular ducts present on body margin, small ducts in transverse rows on abdominal segments, present in mid area of thorax and head. Microducts particularly small, with small opening 2.0–2.5 µm in diameter, present sparsely on head and thorax, submarginally on abdomen,. Body setae very small, about 10–15 µm long, scattered; with 6 or 7 pairs of interantennal setae, 35–125 µm long; with 1 pair of pregenital setae present medially on last 4 or 5 abdominal sternites, each 80–125 µm long.

Margin. Marginal setae, each about 10–15 µm long on thorax and abdomen, setae on anal lobes and head apex up to 20–25 µm. Spiracular setae strong, wide, curved, each 30–35 µm long; with marginal setae between anterior and posterior spiracular setae numbering 29–39.

Dorsum. Derm membranous, with segmentation apparent on thorax and abdomen. Eyespots not seen. Preopercular pores 7–8 µm wide, forming a sparse, irregular band 3–4 pores wide from anal plates to metathoracic segment. Tubular ducts same shape and size as larger type those on on venter. Body setae short almost parallel side, about 7–10 µm long, sparse. Anal ring with 6 setae, each about 150–175 µm long. Anal plates subtriangular, each plate 70–125 µm wide, 90–135 µm long, with three apical or subapical setae, each 20–25 µm long.

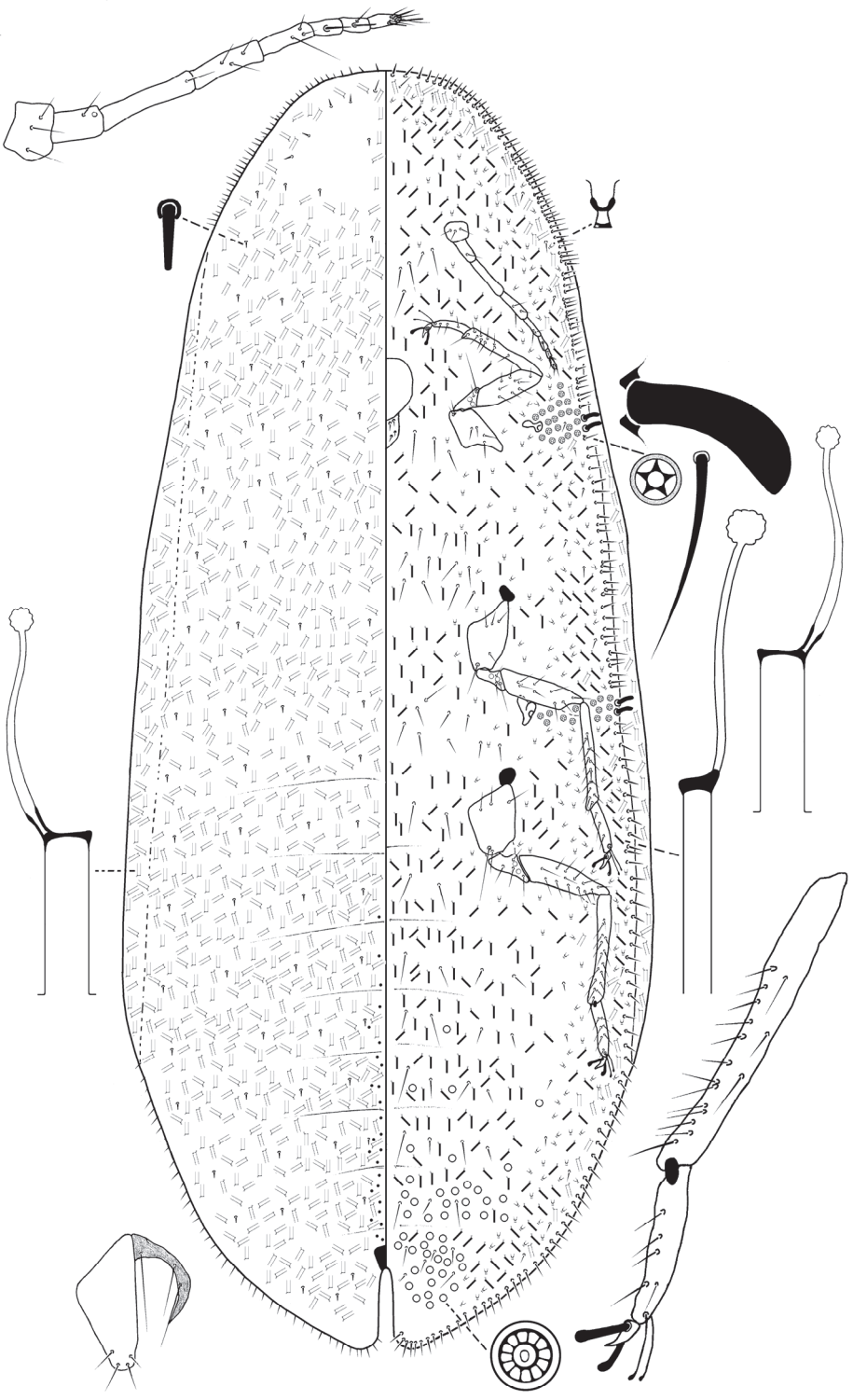

Figure 1. *Luzulaspis filizae* Kaydan *sp. nov.*, adult female.

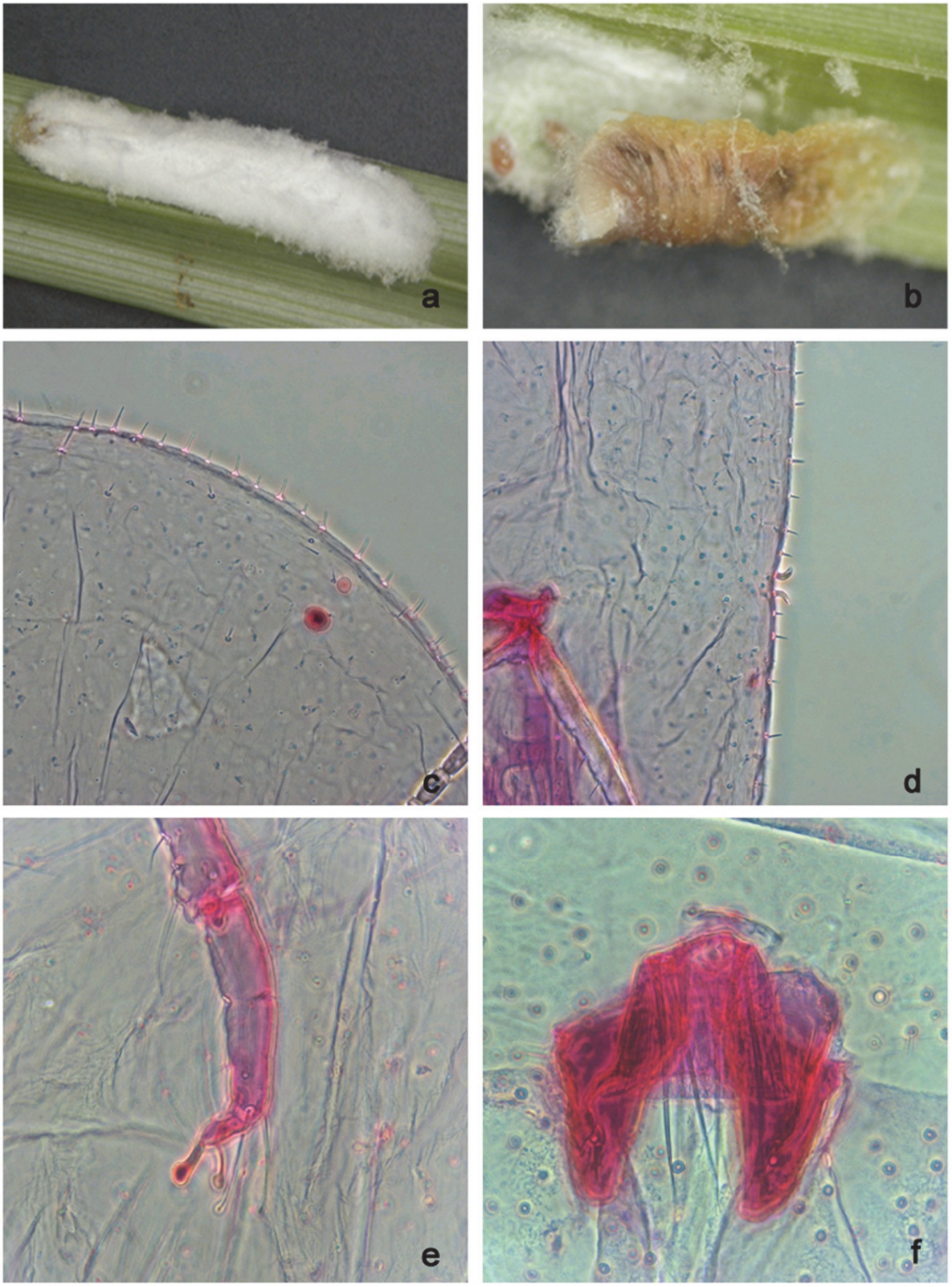

Figure 2. *Luzulaspis filizae* Kaydan *sp. nov*., adult female, a. ovisac, b. female body, c. marginal setae on apex of head, d. spiracular setae, e. hind leg with tibio-tarsal sclerosis and enlarged claw digitules, f. anal plates.

First-instar nymph (crawler) (Fig. 3)

Living specimens. Body yellowish, elongate oval and flattened. Legs and antennae well developed.

Mounted specimens. body elongate oval 550–590 µm long and 185–230 µm wide. Eyes situated dorso-marginally.

Venter. Antennae 6 segmented**,** 135–145 µm long, third segment slightly longer than others. With 1 pair of interantennal setae each 32–36 µm long and 3 pairs of hairlike setae on middle of adominal segments V-VII. Minute spinelike setae with 10 setae on abdominal segements in submarginal rows, 4 on thorax and 1 on head at the base of antennae. Legs subequal, well developed. Measurements of hind leg: coxa 45 µm long; trochanter + femur 80–85 µm; tibia 60 µm; tarsus 40–45 µm long; claw without a denticle, 15 µm long; claw digitules longer than claw, slightly broadened apically, tarsal digitules longer than claw, thin and with a small apical swelling 30–35 µm long. Spiracles 20–25 µm long, 6–8 µm wide in peritreme. Spiracular disc pores, each 4–6 µm in diameter, with 3–8 (generally 5) loculi, 2 or 3 pores in each row to spiracular setae. Loop of mouth stylets 100–120 µm long, reaching the mid thorax. Ventral microducts present submarginally, each 2 mm wide, with 2 between spiracles, and 6 between inner and outer submarginal setae in abdominal region.

Margin. Marginal setae spine-like, with 16 setae anteriorly between anterior spiracular setae, 3 between each anterior and posterior spiracular setae, 7 or 8 found between posterior spiracular setae and anal lobe on each side. Setae on head and thorax are larger than those on abdomen. Spiracular setae 1 pair in each atigmatic area, each setae 5 µm long, 3 µm wide, narrower at apex.

Dorsum. With minute simple pores present in submarginal area of abdomen. Anal plates well developed, each with an apical seta 162–200 µm long. Anal ring round, with simple pores and 6 short setae, each 50–55 µm long.

Etymology. The species is named after Dr. A. Filiz Çalışkan (Çukurova University Agriculture Faculty, Plant Protection Department, Balcalı, Adana, Turkey) who collected the species.

Host plant. *Carex* sp. (Cyperaceae).

Distribution. Turkey (Adana).

Comments. *Luzulaspis filizae* Kaydan *sp. nov.* can be distinguished from other *Luzulaspis* species by the combination of the following characters; (i) setae on inner edge of tibia subequal to or shorter than tibia width; (ii) marginal setae on head and anal lobes longer than those on lateral margin, up to 25 µm long; (iii) antennae longer than 400 µm; (iv) marginal setae between anterior and posterior spiracular setae numbering 29–39; (v) up to 52 pores in each spiracular pore band and (vi) spiracular setae at least two times longer than other marginal setae. *Luzulaspis filizae* is closest to *L. caracis* in having marginal setae on head and anal lobes longer than those on lateral margin, setae on inner edge of tibia subequal to or shorter than tibia width, but differs from *L. cariacis* in having: (i) marginal setae between anterior and posterior spiracular setae numbering 29–39; 42–52 pores in spiracular pore band (16–21 marginal setae; 18–22 pores in *L. caricis*) and spiracular setae longer than other marginal setae in length (almost the same size in *L. caricis*). *Luzulaspis filizae* is also close to *L. rajae* but differs from this species in having: (i) two apical marginal setae on head and apical setae of anal lobes slightly thicker than other marginal setae, but never larger than twice as wide at base as other marginal setae (thicker in *L. rajae*) and (ii) interantennal setae longer than 75 µm (shorter in *L. rajae*).

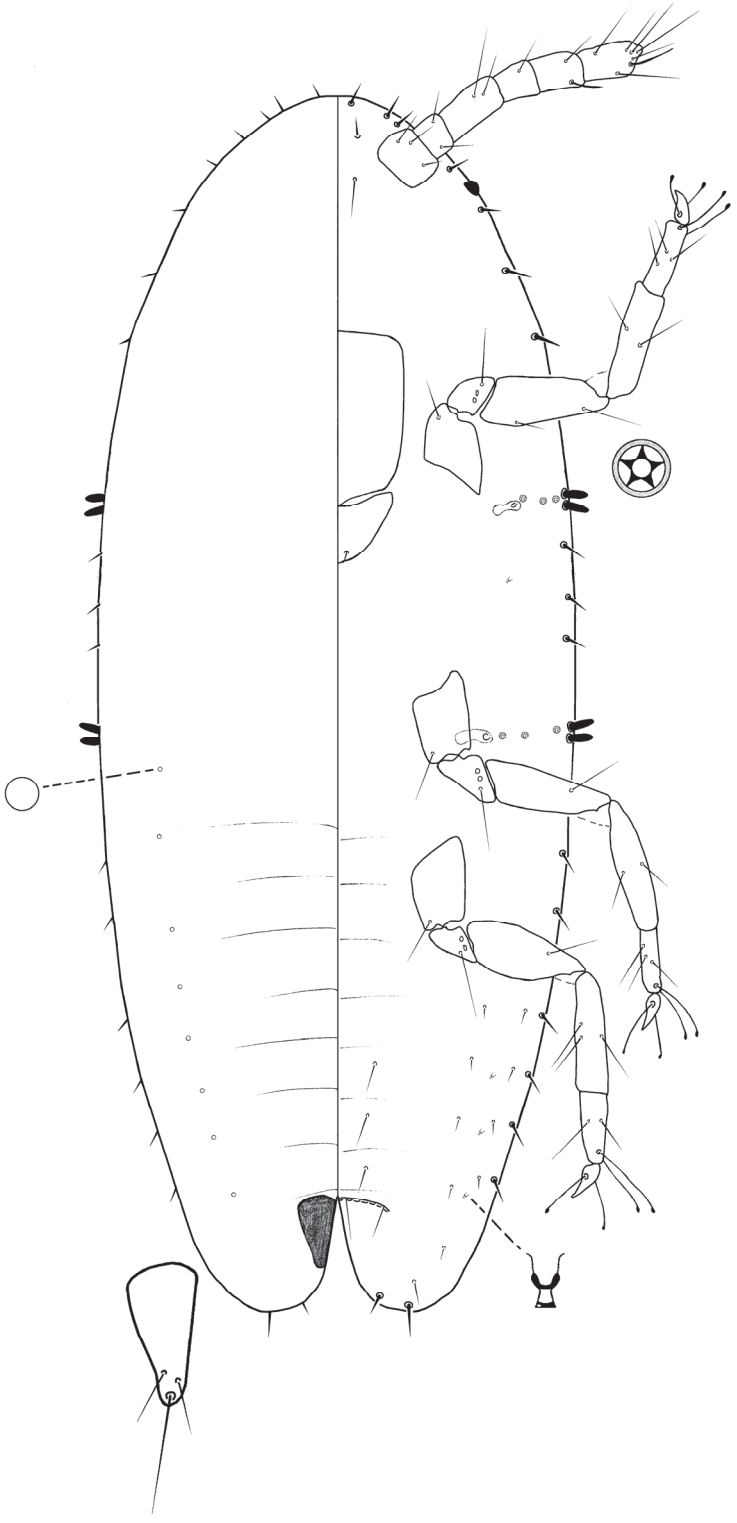

Figure 3. *Luzulaspis filizae* Kaydan *sp. nov.*, first instar nymph.

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Orijinal araştırma (Original article)

İzmir ve Manisa illerinde bağ alanlarında ekonomik öneme sahip bitki paraziti nematodların morfolojik ve moleküler yöntemlerle tanılanması [1]

Identification of the economically important plant parasitic nematodes in vineyards areas of Izmir and Manisa provinces by morphological and molecular techniques

İbrahim MISTANOĞLU[2] Galip KAŞKAVALCI[3*] Zübeyir DEVRAN[2]

Summary

In the study, economically important nematodes species found in vineyards of Izmir and Manisa provinces were identified using morphological and molecular techniques. A total of 188 plant roots and soil samples belonging to 147 samples from Manisa and 41 samples from Izmir provinces were analyzed. *Meloidogyne javanica* (Treub, 1885) Chitwood, 1919 (9.52%), *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949 (6.35%), *Meloidogyne incognita* (Kafoid & White, 1919) Chitwood, 1949 (3.18%) belonging to root-knot nematodes genus and *Xiphinema pachtaicum* (Tulaganov, 1938) Kirjanova, 1951 (87.30%), *Xiphinema index* Thorne & Allen, 1950 (19.05%) and *Xiphinema italiae* Meyl, 1953 (9.52%) belonging to dagger nematodes were identified by using morphological and molecular techniques. The prevalence and infestation ratio of these species were also high in the region. The results showed that the infestation ratio with root knot nematodes and dagger nematodes were found 34.01% and 31.71% in Manisa and Izmir vineyards, respectively. Our findings also indicated that heavily prevalence of plant parasitic nematodes were existed as 43.48% in the vineyard areas of Salihli district of Manisa and 66.67% in the vineyard area of Torbalı, Urla and Seferihisar districts of Izmir.

Keywords: Diagnostic**,** distribution, grape, *Meloidogyne* spp., *Xiphinema* spp.

Özet

Bu çalışmada, İzmir ve Manisa İlleri bağ alanlarında ekonomik olarak önemli olan nematod türleri morfolojik ve moleküler teknikler kullanılarak tanımlanmıştır. Araştırmanın materyalini, İzmir ve Manisa bağ alanlarından alınan, toprak örnekleri, bitki materyalleri ve nematodlar oluşturmuştur. Manisa ilinden 147, İzmir ilinden 41 olmak üzere toplam 188 adet bitki kökü ve toprak örneği analiz edilmiştir. Morfolojik ve moleküler teknikler kullanılarak, kök-ur nematodlarından *Meloidogyne javanica* Treub, 1885) Chitwood, 1919 (%9,52), *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949 (%6,35), *Meloidogyne incognita* (Kafoid & White, 1919) Chitwood, 1949 (%3,18) (Tylenchida : Heteroderidae) türleri ve kamalı nematodlardan ise *Xiphinema pachtaicum* (Tulaganov, 1938) Kirjanova, 1951 (%87,30), *Xiphinema index* Thorne & Allen, 1950 (%19,05) ve *Xiphinema italiae* Meyl, 1953 (%9,52) (Dorylaimida: Longidoridae) türleri belirlenmiştir. Ayrıca bu türlerin bölgedeki yaygınlıkları ve bulaşıklık oranları belirlenmiştir. Buna göre Kök-ur ve Kamalı nematodların İzmir ve Manisa bağ alanlarındaki bulaşıklık oranları sırasıyla %34,01 ve %31,71 olarak tespit edilmiştir. Bitki paraziti nematodların yoğun bulaşık olduğu bölgeler, %43,48 oranla Manisa İli Salihli ilçesi ve %66,67 oranla da İzmir İli Torbalı, Urla ve Seferihisar ilçeleri olduğu belirlenmiştir.

Anahtar sözcükler: Teşhis, yayılış, üzüm, *Meloidogyne* spp., *Xiphinema* spp.

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Giriş

Bağcılık, üzüm üretimini en yüksek kalite ve verimde gerçekleştirebilmek için uygun koşullarda yapılan tarımsal işlemler bütünüdür. Bağcılığın anavatanı Anadolu olmasına rağmen dünyanın birçok yöresine yayılma olanağı bulmuştur. Türkiye, dünyada üzüm üreten ülkeler arasında önemli bir yere sahiptir. Tarımsal üretim çeşitliliği açısından oldukça zengin olan Türkiye'de en önemli ürünlerden birini oluşturan üzüm, toplam meyve üretiminin yaklaşık % 25'ini oluşturmaktadır. Türkiye, 2011 yılı verilerine göre 477.786 ha bağ alanında 4.296.350 ton yaş üzüm üretimi gerçekleştirmiştir (Anonymous, 2011a).

Bitki paraziti nematodlar, bağ alanlarında doğrudan ya da dolaylı olarak zararlara yol açan önemli gruplardandır. Bağ alanlarındaki doğrudan zararlarıyla dikkat çeken Tylenchida takımının Meloidogynidae familyasına dahil nematod türlerinin omcalarda ölümlere neden oldukları (Lamberti et al., 1990) ve karışık popülasyonlar halinde bulundukları belirlenmiştir (Ertürk & Özkut, 1973; Ferris, 1976). Bağ alanlarında zararlara neden olan bir diğer nematod grubu ise Longidoridae ve Trichodoridae familyalarına bağlı nematod türleridir. Bu nematodlar doğrudan yapmış oldukları zararların yanında virüs vektörü olarak da dolaylı zararlara yol açmaktadırlar (Weischer, 1993). Tüm bitki paraziti nematodların bitkilerde beslenmelerine ve virüs partiküllerini vücutlarına almalarına karşın, virus vektörü olarak tespit edilen nematodlar, *Xiphinema, Longidorus*, *Trichodorus* ve *Paratrichodorus* cinslerine ait toplam 305 türden sadece 29 ile sınırlıdır (Brown & Trudgill, 1998). Dünyada bağ üretimi yapılan alanlarda *Xiphinema* cinsine dahil 10'un üzerinde tür bulunduğu belirlenmiştir (Raski, 1988). Farklı ülkelerde bağ alanlarında yapılan çalışmalarda *Xiphinema* cinsine bağlı nematod türlerinin yoğun olarak bulunduğu bildirilmiştir (Barbercheck & Heyns, 1986; Coiro et al., 1989; Arias & Fresno, 1994; Aballay et al., 2009).

Türkiye'de farklı bölgelerdeki bağ alanlarında değişik zamanlarda sürveyler yürütülmüş ve bazı bitki paraziti nematodlar belirlenmiştir. Ege Bölgesi tarım alanlarında *Xiphinema* cinsine bağlı yedi farklı nematod türü tanımlanmıştır (Arınç, 1982). Doğu Akdeniz Bölgesi'nde yapılan bir çalışmada bağ alanlarında en yaygın bulunan nematod türlerinin *Xiphinema pachtaicum* (Tulaganov, 1938) Kirjanova, 1951, *Xiphinema index* Thorne & Allen, 1950 ve *Xiphinema italiae* Meyl, 1953 (Dorylaimida: Longidoridae) olduğu tespit edilmiştir (Elekcioğlu & Uygun, 1994).

Bağ üretim verilerine göre, Ege Bölgesi 140.000 ha ve % 29'luk pay ile birinci sırada yer almıştır (Anonymous, 2011b). Bölgede İzmir ve Manisa illeri en önemli üretim alanlarını kapsamaktadır. Bununla birlikte, bu illerde bitki parazit nematodların belirlenmesi üzerine detaylı bir sürvey çalışması şu ana kadar yürütülmemiştir. Çalışmada, Manisa ve İzmir bağ alanlarında ekonomik öneme sahip *Xiphinema* spp. ve *Meloidogyne* spp. cinslerine ait nematodların türlerinin morfolojik ve moleküler yöntemler kullanılarak tanımlanması ve yaygınlıklarının belirlenmesi amaçlanmıştır.

Materyal ve Yöntem

Nematod sürveyi

Çalışma, 2011-2012 yıllarında İzmir ve Manisa illerindeki bağ alanlarında, maksimum örnekleme tarlası sayısı, Bora ve Karaca (1970)'dan değiştirilerek ilin toplam üretim alanının % 3-3,5'u olacak şekilde hesaplanmıştır. Buna göre 188 örnekleme alanından toprak ve bitki kök örnekleri alınmıştır (Çizelge 1).

Nematodların elde edilmesi

Kök-ur nematodlarının 2. dönem larvaları topraktan, geliştirilmiş Baermann Huni Yöntemi (Hooper, 1986) kullanılarak elde edilmiştir.

Morfolojik teşhislerde kullanılan kök-ur nematodu dişileri asma köklerinden elde edilmesi amacıyla "Blender-Elek-Santrifüj Metodu" (Coolen & D'Herde, 1972) kullanılmıştır. Ancak, hiçbir örnekten dişi Kök-ur nematodu bireyi elde edilememiştir. Bu bitkilere ait topraklardan elde edilen *Meloidogyne* cinsi

nematod larvaları steril topraklara şaşırtılmış olan hassas domates fidelerine (Şimşek F1, Bircan Tohum A.Ş.) bulaştırılmıştır. Bu bitkiler üzerinde gelişmelerini tamamlayıp, ur oluşturmuş olan Kök-ur nematodlarının dişileri stereo binoküler mikroskop altında domates köklerinden tek tek çıkartılarak, vulva ve anüs bölgelerine ait daimi preparatları "Perineal Örneklerin Preparasyon Yöntemi" ne göre hazırlanmıştır (Hartman & Sasser,1985).

Xiphinema ve *Longidorus* cinslerine ait bireyler topraktan Baermann Huni ve Cobb Elek Yöntemlerinin farklı bir kombinasyonu ile elde edilmiştir (Flegg, 1967). Elde edilen nematodların tür düzeyindeki morfolojik teşhisleri için Hooper (1986)'a göre daimi preparatları yapılmıştır.

Çizelge 1. İzmir ve Manisa illeri bağ alanlarında incelenen bahçe sayıları

İl	İlçe	Bağ Alanı (da)	Örnek Alınan Tarla Sayısı (adet)
İZMİR	Urla-Seferihisar	7,845	3
	Kemalpaşa	33,400	10
	Menderes	30,520	11
	Menemen	36,140	14
	Torbalı	12,310	3
İZMİR İLİ TOPLAMI		120,215	41
MANİSA	Alaşehir	185,960	35
	Merkez	83,455	22
	Salihli	97,224	23
	Sarıgöl	72,000	13
	Saruhanlı	76,465	22
	Turgutlu	82,490	32
MANİSA İLİ TOPLAMI		597,594	147
TOPLAM			188

Morfolojik ve allometrik yöntemlerle tanımlama

Morfolojik ve Allometrik teşhis çalışmalarında Leica DFC 295 mikroskop'a bağlı video kamera aracılığıyla Leica Application Suite (LAS) Software Version 4.1.0 yazılım programı kullanılmıştır. Kıvrık tüm yapılar "Curvimetre" ile ölçülmüştür.

Çalışmada saptanan Tylenchida takımına bağlı nematod türlerinin sınıflandırmaları ve sinonimleri Siddiqi (2000), Dorylaimida (Longidoridae familyası) takımına bağlı nematod türlerinin sınıflandırmaları ve sinonimleri Hunt (1993) ve Brown & Halbrendt (1997) esas alınarak verilmiştir. Tür teşhisleri standart formüllere göre teşhis anahtarları kullanılarak ilk önce tarafımızdan yapılmış ve daha sonra bu sonuçlar Prof. Dr. İ. Halil ELEKÇİOĞLU (Ç. Ü. Ziraat Fakültesi Bitki Koruma Bölümü, ADANA) tarafından kontrol edilmiştir.

Moleküler çalışmalar

DNA izolasyonu

Bitki paraziti nematodlara ait yumurta, larva veya ergin dönemlerinden DNA izolasyonu, "DNAeasy Tissue and Blood Kit" (Qiagen) kullanılarak yapılmıştır.

Türlere spesifik primerlerle PCR

Kök- ur ve Kamalı nematodların moleküler tanılanmasında türlere özgü PCR primerleri kullanılmıştır (Çizelge 2). Çalışmalar, 25 µl' lik (10XPCRBuffer, 0,2 mM dNTP, her bir primerden 0,4 µM, 2 mM $MgCl_2$,

20 ng DNA ve 1 ünite Taq DNA Polymerase) reaksiyon hacminde, DNA thermalcycler (Master cycler gradient, Biolab) cihazında gerçekleştirilmiştir. PCR ürünleri % 2,5'lik agaroz jelde 1X Tris-Acetate-EDTA (TAE) Buffer kullanılarak güç kaynağı yardımıyla yürütülmüş ve EtBr ile boyandıktan sonra jel görüntüleme sisteminde fotoğraflanmıştır. Real time PCR'da çalıştırılan XpaF / XpaR primerleri için Real time PCR programı kullanılmıştır.

Çizelge 2. Nematodların moleküler tanılanmasında kullanılan primerlerin adları, dizileri, fragment büyüklükleri ve referansları

Primer Adı	Nematod Türü	Band Uzunluğu (bp)	Primer Dizisi (5-3)	Kaynaklar
Inc-K14F	*M. incognita*	399	CCCGCTACACCCTCAACTTC	Randig et al., 2002
Inc-K14R			GGGATGTGTAAATGCTCCTG	
Fjav	*M. javanica*	670	GGTGCGCGATTGAACTGAGC	Zijlstra et al., 2000
Rjav			CAGGCCCTTCAGTGGAACTATAC	
Far	*M. arenaria*	420	TCGGCGATAGAGGTAAATGAC	Zijlstra et al., 2000
Rar			TCGGCGATAGACACTACAACT	
I27	*X. index*	340	GAGTCGTAACGTTTCTCGTCTATCAGG	Wang et al., 2003
ITA26	*X. italiae*	414	GAAATAAGAACCCTGAAAAAGATAGG	
XpaF	*X. pachtaicum*	80	CTCGCTTAGTAAATGACGGAGAGT	Bu çalışmada geliştirilmiştir
XpaR			TAATCGACCGAGCTATTAAACGA	

Araştırma Sonuçları ve Tartışma

Bu çalışmada İzmir ve Manisa illeri merkez ve ilçelerinde bulunan bağ alanlarındaki ekonomik önemi olan bitki paraziti nematod gruplarından Kök-ur ve Kamalı nematod türlerini belirlemek amacıyla bu alanlardan alınan 188 adet toprak-kök örneklerinin analizi yapılmıştır. Tylenchida ve Dorylaimida takımlarına bağlı 2 familya içerisinde sınıflandırılan toplam altı adet nematod türü morfolojik ve moleküler yöntemler kullanılarak tanımlanmıştır. Sürvey alanının söz konusu nematod türleriyle bulaşıklık durumu belirlenmiştir (Çizelge 3;4);

Çalışmada tespit edilen nematod türleri hakkında bilgiler aşağıda verilmiştir:

Cins: *Meloidogyne* Goeldi, 1892

Meloidogyne arenaria (Neal, 1889) Chitwood, 1949

Meloidogyne incognita (Kofoid & White, 1919) Chitwood, 1949

Meloidogyne javanica (Treub, 1885) Chitwood, 1949

Cins: *Xiphinema* Cobb, 1913

Xiphinema index Thorne & Allen, 1950

Xiphinema italiae Meyl, 1953

Xiphinema pachtaicum (Tulaganov, 1938) Kirjanova, 1951

Çizelge 3. *Meloidogyne* spp. ve *Xiphinema* spp. bitki paraziti nematod gruplarının bulaşıklık durumları

İl	İlçe	Sürvey yapılan bağ sayısı (Adet)	*Meloidogyne* spp. ve *Xiphinema* spp. ile bulaşık bağ oranı (%)	Temiz bağ oranı (%)
MANİSA	Merkez	22	36,36	63,64
	Saruhanlı	22	36,36	63,64
	Turgutlu	32	18,75	81,25
	Salihli	23	43,48	56,52
	Alaşehir	35	42,86	57,14
	Sarıgöl	13	23,10	76,90
	İL GENELİ	147	34,01	65,99
İZMİR	Kemalpaşa	10	30,00	70,00
	Torbalı	3	66,67	33,33
	Menderes	11	9,10	90,90
	Urla - Seferihisar	3	66,67	33,33
	Menemen	14	35,71	64,29
	İL GENELİ	41	31,71	68,29
BÖLGE GENELİ		188	33,51	66,49

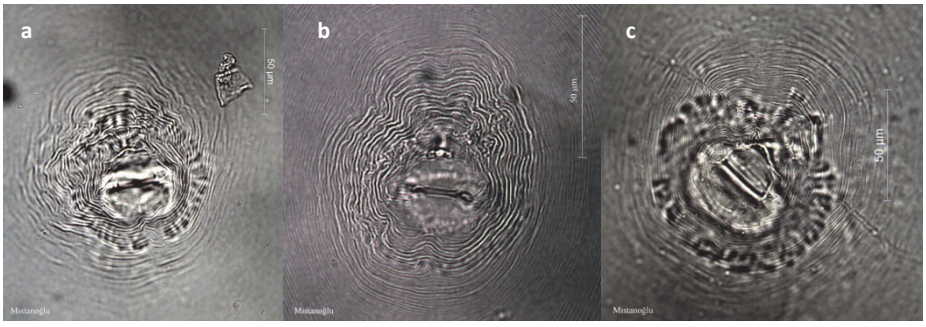
Çizelge 4. *Melodiogyne* ve *Xiphinema* cinslerine dahil türlerin yaygınlık oranları (%)

İl	İlçe	*M. arenaria*	*M. incognita*	*M. javanica*	*X. index*	*X. italiae*	*X. pachtaicum*
MANİSA	Merkez	-	-	-	37,50	-	75,00
	Saruhanlı	-	-	-	37,50	-	100,00
	Turgutlu	-	-	-	-	16,67	100,00
	Salihli	-	-	10,00	-	30,00	90,00
	Alaşehir	-	6,67	6,67	26,68	6,67	80,00
	Sarıgöl	33,33	-	33,33	66,67	-	100,00
	İL GENELİ	2,00	2,00	6,00	24,00	10,00	88,00
İZMİR	Kemalpaşa	-	-	-	-	-	100,00
	Torbalı	-	-	-	-	-	100,00
	Menderes	-	-	-	-	-	100,00
	Urla - Seferihisar	50,00	-	50,00	-	-	100,00
	Menemen	40,00	20,00	40,00	-	20,00	60,00
	İL GENELİ	23,08	7,69	23,08	-	7,69	84,62
BÖLGE GENELİ		6,35	3,18	9,52	19,05	9,52	87,30

Cins: *Meloidogyne* Goeldi, 1892

Morfolojik tanımlama

Kök-ur nematod türlerinin morfolojik tanımlaması perineal pattern' a göre yapılmıştır. *M. arenaria* perineal şeklin varyasyonu çok yüksektir. Anal kesitlerinde stria'lar dorsal ve lateral olarak dalgalıdan düze kadar değişen şekillerde olabilmektedir (Şekil 2a). "Dorsal arch" adı verilen sırta ait kemer yüksek olmayıp genellikle yuvarlaktır. Lateral alan belirgin değildir, ancak kutikula üzerindeki lateralde kırıklıklarla belirlenebilir. (Williams, 1975). Elde edilen perineal pattern sonuçları Eisenback et al. (1981) ve Williams (1975) çalışmalarına uygunluk göstermiştir.



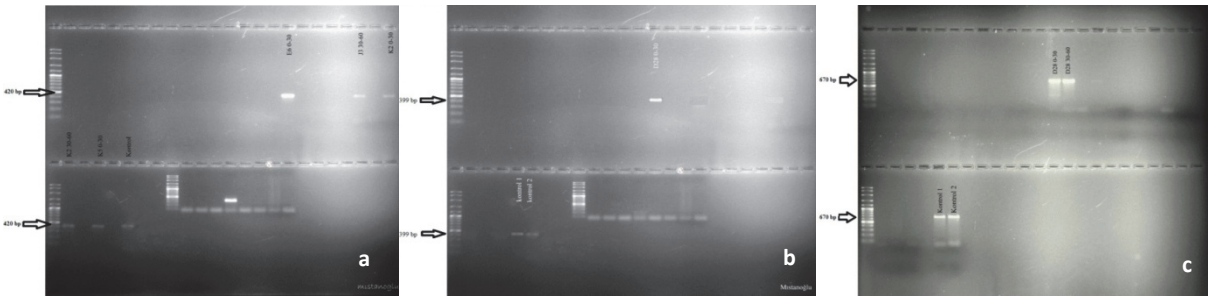
Şekil 2. İzmir ve Manisa illeri bağ alanlarında saptanan *Meloidogyne* spp.'ye ait anal kesitler: a) *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949; b) *Meloidogyne incognita* Kofoid & White, 1919,Chitwood, 1949; c) *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949.

Meloidogyne incognita anal kesitlerinde stria'lar çok yakın aralıklarla yerleşmiş, özellikle dorsal ve lateral olarak çok dalgalıdan düze kadar değişen şekillerde olup bazen zigzaglar çizmektedir (Şekil 2b). "Dorsal arch" adı verilen sırta ait kemer oldukça yüksek kare şeklinde olup, bazen yuvarlaklaşabilmektedir. Lateral alan belirgin değildir, ancak kütiküla üzerindeki çizgiler lateralde kırıklıklarla belirlenebilir. Genellikle bu uçlar çatallanabilmekte ve bu çizgiler vulval ağıza doğru yönelmektedir (Williams, 1973). Elde edilen perineal pattern sonuçları Eisenback et al. (1981) ve Williams (1973) çalışmaları ile paralellik göstermiştir.

Meloidogyne javanica anal kesitleri, genel olarak yuvarlaktan ovale kadar değişmekle beraber, bazen armut şeklinde de olabilir (Şekil 2c). Stria' lar düz veya dalgalı olabilmektedir. En tipik özelliği, lateral alanların her iki tarafından bir çift belirgin çizgiyle ayrılmış olmasıdır. Böylece oluşan anal kesit sırt ve karın bölgesi olarak belirgin bir şekilde ayırt edilebilmektedir. Lateral alan kuyruktan itibaren belirli bir uzaklığa kadar görülebilirse de boyun bölgesine kadar uzanamaz. Sırta ait kemer yuvarlak, hafifçe yüksek, bazen de sırt tarafına doğru basılmış ve yassılaşmış durumdadır. Phasmid'ler genellikle belirgindir (Williams, 1972). Elde edilen perineal pattern sonuçları Eisenback et al. (1981) ve Williams (1972) çalışmalarına benzer bulunmuştur.

Moleküler tanımlama

Kök-ur nematodlarının teşhisi türlere özgü primerlerle yapılmıştır. *M. arenaria* bireylerinin tanımlanmasında Far ve Rar primerleri kullanılmıştır. Bu primerlerin, pozitif olan örneklerde 420 bp'da bant oluşturdukları saptanmıştır (Şekil 3a). Zijlstra et al. (2000), dört farklı *M. arenaria* popülasyonu kullanarak yaptıkları çalışmada, tüm örneklerde 420 bp spesifik DNA bandını elde etmişlerdir. Benzer diğer bir çalışmada Kök-ur nematodlarının moleküler olarak tek bireyden tanımlanması için teşhis anahtarlarının oluşturulması amacıyla üç farklı *M. arenaria* popülasyonu kullanılmış ve bu örneklerde 420 bp DNA bandı elde edilmiştir (Adam et al., 2007). Devran & Söğüt (2009), Far / Rar primerleri ile yapmış oldukları moleküler çalışmada 7 farklı *M. arenaria* örneğinde 420 bp DNA bandını belirlemişlerdir. Bizim sonuçlarımız önceki çalışmalarla paralellik göstermiştir.



Şekil 3. İzmir ve Manisa illeri bağ alanlarında saptanan *Meloidogyne* spp.'ye ait DNA bant görüntüsü: a) *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949; b) *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949; c) *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949 (Kontrol örneği Devran & Söğüt (2009) çalışmasından alınmıştır).

Meloidogyne incognita'nın moleküler tanımlanmasında, Inc-K14F ve Inc-K14R primerleri kullanılmıştır. Bu primerlerin, pozitif olan örneklerde yaklaşık 400 bp uzunlukta DNA bandı oluşturdukları saptanmıştır (Şekil 3b). Randig et al. (2002), Inc-K14F ve Inc-K14R primerleri kullanarak dört farklı *M. incognita* popülasyonunda yaklaşık 400 bp DNA bandını elde etmişlerdir. Bir diğer çalışmada Devran & Söğüt (2009), 60 farklı *M. incognita* popülasyonu üzerinde yapılan çalışmada beklenen DNA bandını elde etmişlerdir. Bizim bulgularımız önceki araştırma sonuçlarıyla uyumluluk göstermiştir.

*Meloidogyne javanica'*nın moleküler teşhisinde Fjav ve Rjav primerleri kullanılmıştır. Bu primerlerin, pozitif olan örneklerde yaklaşık 670 bp'da bant oluşturdukları saptanmıştır (Şekil 3c). Fjav ve Rjav primerleri kullanılarak yapılan moleküler çalışmalarda *M. javanica*'ya ait popülasyonlarda 670 baz DNA bandı elde edilmiştir (Zijlstra et al., 2000; Tzortzakakis et al., 2005). Batı Akdeniz Bölgesi'nde Kök-ur nematodu türlerinin moleküler tanımlanmaları amacıyla yürütülen çalışmada *M. javanica'* ya ait 28 popülasyonun tamamında beklenen 670 bp DNA bandını elde etmişlerdir (Devran & Söğüt, 2009). Bizim sonuçlarımız önceki araştırma sonuçlarıyla benzerlik göstermiştir

Morfolojik ve moleküler tanımlamaların karşılaştırılması

Bu çalışmada türler, morfolojik ve moleküler yöntemler kullanılarak tanımlanmıştır. *M. arenaria'ya* ait dört popülasyonun dördü hem morfolojik hem de moleküler, *M. incognita*'ya ait iki popülasyonun, biri hem morfolojik hem de moleküler, diğeri ise yalnızca morfolojik; *M. javanica*'ya ait altı popülasyonun biri hem morfolojik hem moleküler, beşi ise yalnızca morfolojik yöntemler kullanılarak tanımlanmıştır.

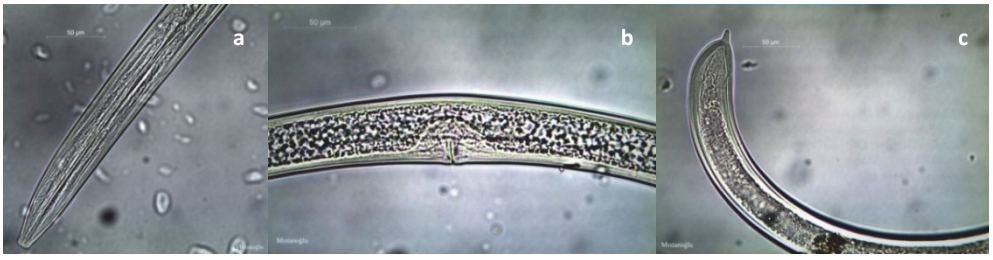
Cins: *Xiphinema* Cobb, 1913

Morfolojik tanımlama

Xiphinema cinsine bağlı türlerin morfolojik tanımlamaları, morfometrik ölçüm sonuçlarına göre yapılmıştır. Buna göre, *X. index* bireylerinde, vücut uzamış silindirik şekilde olup yaklaşık 3 mm uzunluğundadır. Dudak bölgesi yarım küre şeklindedir. Odontostylet güçlü, 126 µm uzunlukta ve iğne şeklindedir. Odontophore ortalama olarak 70µm uzunlukta ve 3 geniş basal yakaya sahiptir. "Basal ring" dudak başlangıcından 108 µm uzaklıktadır. Oesophagal bulb vücut genişliğinin yaklaşık 2,5 katı uzunluktadır. Vulva vücudun yaklaşık olarak %38–40'lık bölümünde yer almaktadır. Yumurtalıklar çifttir. Kuyruk meme şeklinde, anüs bölgesi vücut genişliğinin 1–1,3 katı kadar uzunlukta ve konveks şeklindedir (Heyns, 1971) (Şekil 4, Çizelge 5). Çalışmada elde edilen verilerin önceki yapılan Heyns (1971) ve Arınç (1982) çalışmalarıyla genellikle uyum gösterdiği saptanmıştır.

Çizelge 5. *Xiphinema index* Thorne & Allen, 1950'in farklı popülasyonlarına ait ergin dişilerinin morfometrik ölçüm değerlerinin karşılaştırılması

	Bu çalışmaya göre	Heyns (1971)	Arınç (1982)
n	11	10	18
L (mm)	3,03 (2,17–3,33)	3,25 (2,87 –3,58)	3,1 (2,6–3,6)
a	66,74 (61,9–74,28)	57 (54–61)	58,5 (43,9–71,3)
b	7,36 (5,32–10,62)	6,6 (6,2–8)	7 (6,2–7,9)
c	71,51 (48,2–86,93)	88 (72–98)	75 (60,8–90,9)
c'	1,24 (1,01–1,51)	0,9 (0,7–1,1)	1 (0,7–1,3)
Odontostylet (µm)	120,79 (86,11–145)	129 (123–134)	106,9 (91,8–145,8)
Odontophore (µm)	69,07 (53,5–86,7)	78 (74–81)	104,7 (72,9–148,5)
Kuyruk (µm)	40,26 (25–50,7)	-	42 (35,1–48,6)
V (%)	41,32 (33,96–48,81)	41 (40–42)	40,4 (38,3–43)



Şekil 4. *Xiphinema index* Thorne & Allen, 1950 'e ait a) baş ve özofagus bölgesi, b) vulva bölgesi, c) kuyruk bölgesi.

Xiphinema italiae bireylerinde vücut ince olup yaklaşık 3 mm uzunluğundadır. Dudak bölgesi hafif yuvarlaklaşmış ve 10–12 µm genişliğindedir. Odontostylet tabanı çatallanmış yaklaşık 95 µm uzunlukta ve iğne şeklindedir. Odontophore yaklaşık olarak 60 µm uzunlukta ve 8–11 µm genişlikte, 3 basal yakaya sahiptir. "Basal ring" dudak başlangıcından 81 µm uzaklıktadır. Vulva vücudun orta kısmının biraz ön bölümünde yer almakta, yumurtalıklar vulvadan iki yana yayılmaktadır. Kuyruk, anüs bölgesi vücut genişliğinin, 2,8–4,3 katı kadar uzunlukta ve konik şekilde uzamaktadır (Martelli et al., 1966) (Şekil 5, Çizelge 6). Çalışmada elde edilen verilerin önceki yapılan Martelli et al. (1966), Arınç (1982) ve Elekçioğlu (1992) çalışmalarıyla genellikle uyumluluk göstermiştir.

Çizelge 6. *Xiphinema italiae* Meyl, 1953'nin farklı popülasyonlarına ait ergin dişilerinin morfometrik ölçüm değerlerinin karşılaştırılması

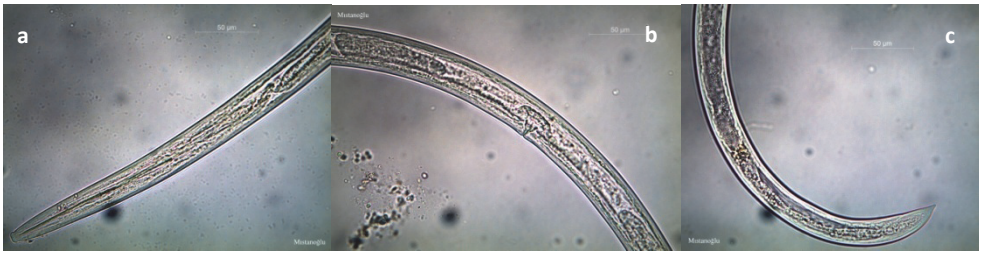
	Bu çalışmaya göre	Martelli et al. (1966)	Arınç (1982)	Elekçioğlu (1992)
n	7	12	16	1
L (mm)	3,1 (2,81–3,5)	3,04 (2,65–3,47)	2,8 (2,5–3,1)	2,86
a	102,1 (90,84–112,02)	97,0 (84–109)	87,7 (69,8–97,8)	97,0
b	7,46 (6,32–8,58)	8,1 (7,5–8,8)	7,6 (6,7–8,5)	7,8
c	33,29 (30,15–38,84)	42,0 (38–47)	35,5 (30,6–39,8)	37,0
c'	4,62 (3,81–5)	3,5 (3,2–3,9)	4 (3,1–4,7)	3,8
Odontostylet (µm)	97,94 (87,7–112,3)	94,0 (87–99)	89,2 (70,2–118,8)	96
Odontophore (µm)	63,39 (58,5–71,2)	57,0 (55–58)	67,6 (40,5–91,8)	51,0
Kuyruk (µm)	92,6 (84,1–100,3)	–	81,7 (75,6–89,1)	79,0
V (%)	45,92 (42,28–47,88)	45 (43–48)	45,2 (43,3–47,5)	46

Şekil 5. *Xiphinema italiae* Meyl, 1953*'* ye ait a) baş ve özofagus bölgesi, b) vulva bölgesi, c) kuyruk bölgesi.

Xiphinema pachtaicum bireylerinde vücut silindirik şekilde olup kademeli olarak incelmekte ve "C" şeklini alarak yaklaşık 2mm uzunluğa ulaşmaktadır. Dudak bölgesi düğme şeklinde olup 9,6 µ genişliktedir. Odontostylet güçlü, 83 µ uzunlukta, Odontophore ise basal yakayla birlikte 50 µ uzunluktadır. Vulva, yarık şeklinde ve vücudun ortasında yer almaktadır. "Basal ring" dudak başlangıcından 78 µ uzaklıktadır. Oesophagus, Basal bulbla birlikte tüm vücudun üçte birini kaplamaktadır. Yumurtalıklar bir çifttir. Kuyruk daralmış konik şekilli 26 µ uzunluğundadır (Siddiqi & Lamberti, 1977) (Şekil 6., Çizelge 7). Çalışmada elde edilen verilerin önceki yapılan Lamberti & Martelli (1971), Arınç (1982) ve Elekçioğlu (1992) çalışmalarıyla genellikle paralel olduğu belirlenmiştir.

Çizelge 7. *Xiphinema pachtaicum* (Tulaganov, 1938) Kirjanova, 1951'un farklı popülasyonlarına ait ergin dişilerinin morfometrik ölçüm değerlerinin karşılaştırılması

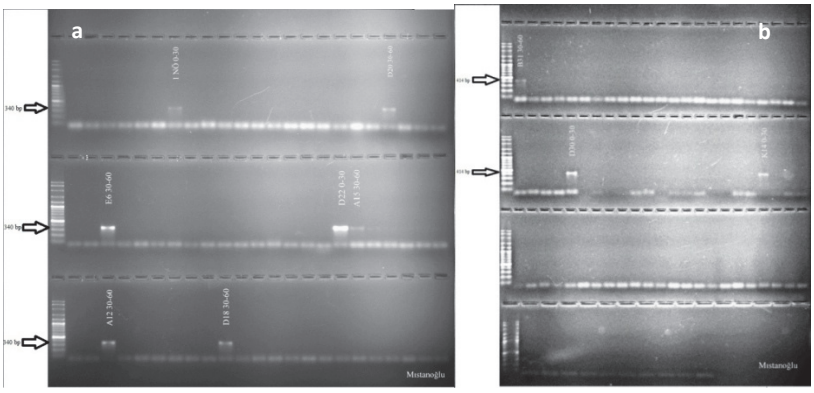
	Bu çalışmaya göre	Lamberti & Martelli (1971)	Arınç (1982)	Elekçioğlu (1992)
n	15	10	30	10
L (mm)	2,02 (1,41–2,3)	1,8 (1,5–2,1)	1,9 (1,6–2,2)	1,71 (1,55–1,85)
a	74,47 (52,1–114,26)	56 (50–63)	64,2 (46,6–73,1)	67 (61–79)
b	7,34 (4,71–9,26)	6,5 (4,7–7,5)	6,2 (5–8,4)	7,1 (5,6–8,3)
c	63,94 (42,22–78,09)	57 (47–67)	59,2 (46,4–78,2)	60 (54–67)
c'	1,82 (1,51–2,43)	1,7 (1,5–2,1)	1,7 (1,1–2,3)	1,6 (1,6–2,1)
Odontostylet (µm)	91,72 (73,7–114)	87 (70–99)	71,3 (59,4–94,5)	80 (70–88)
Odontophore (µm)	52,01 (41–69,3)	48 (42–51)	68,6 (43,2–81)	46 (37–50)
Kuyruk (µm)	31,98 (27,75–37)	–	33,9 (27–72,9)	–
V (%)	56,66 (53,62–60,8)	57 (55–58)	59,1 (54,9–62,1)	57 (55–59)



Şekil 6. *Xiphinema pachtaicum* (Tulaganov, 1938) Kirjanova, 1951'a ait a) baş ve özafagus bölgesi, b) vulva bölgesi, c) kuyruk bölgesi.

Moleküler tanımlama

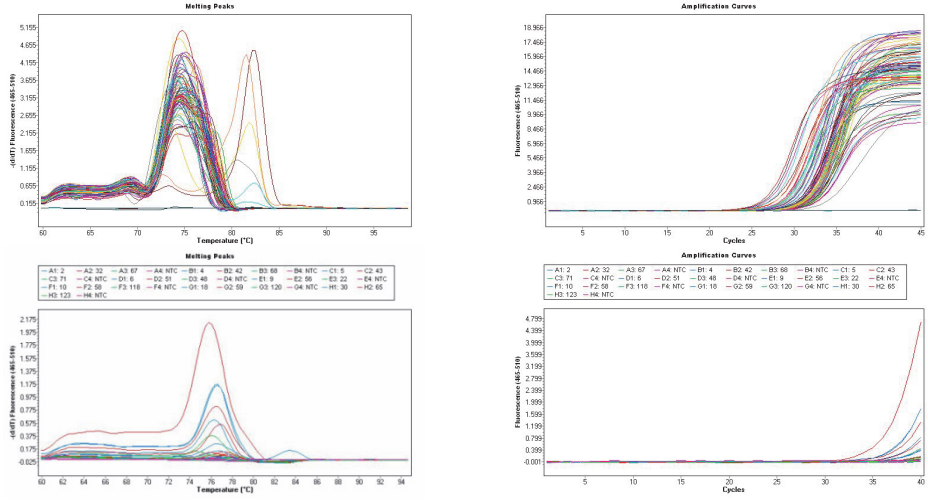
Xiphinema cinsine bağlı nematodların teşhisi türlere özgü primerlerle yapılmıştır. *X. index* türünün moleküler teşhisinde I27 ve A-ITS1 primerleri kullanılmıştır. Bu primerlerin, pozitif olan örneklerde 340 bp' da bant oluşturdukları saptanmıştır (Şekil 7a). Bu sonuç, *Xiphinema* cinsi nematodların moleküler olarak tanımlanması amacıyla yapılan ve yedi farklı *X. index* izolatıyla çalışılan Wang et al. (2003)'in çalışmasının bulgularıyla uyumluluk göstermiştir.



Şekil 7.İzmir ve Manisa illeri bağ alanlarında saptanan *Xiphinema* spp. bulunan örneklere ait DNA bant görüntüsü: a) *Xiphinema index* Thorne & Allen, 1950; b) *Xiphinema italiae* Meyl, 1953.

Xiphinema italiae türünün moleküler teşhisinde ITA26 ve A-ITS1 primerleri kullanılmıştır. Bu primerlerin, pozitif olan örneklerde 414 bp'da bant oluşturdukları saptanmıştır (Şekil 7 b). Bu bulgular, *Xiphinema* cinsi nematodların moleküler olarak tanımlanması amacıyla yürütülen çalışmada elde edilen sonuçlarla uyumluluk göstermiştir (Wang et al., 2003).

Xiphinema pachtaicum türünün moleküler olarak tanımlanması Real Time PCR yöntemiyle yapılmıştır. Türün teşhisinde XpaF ve XpaR primerleri kullanılmıştır. Bu primerler ilk kez bu çalışma kapsamında dizayn edilmiş ve pozitif örneklerde 80 bp'da DNA bandı oluşturdukları belirlenmiştir. Bu uzunluğu PCR'da net olarak gözlemlemenin güçlüğünden dolayı Real-time PCR'da SYBR GREEN Master Mix kullanılarak çalışılmıştır. Sonuçlar erime (melting) analiz eğrilerine göre değerlendirilmiştir (Şekil 8).



Şekil 8. *Xiphinema pachtaicum* (Tulaganov, 1938) Kirjanova, 1951 bulunan örneklere ait Real time PCR sonuç eğrileri.

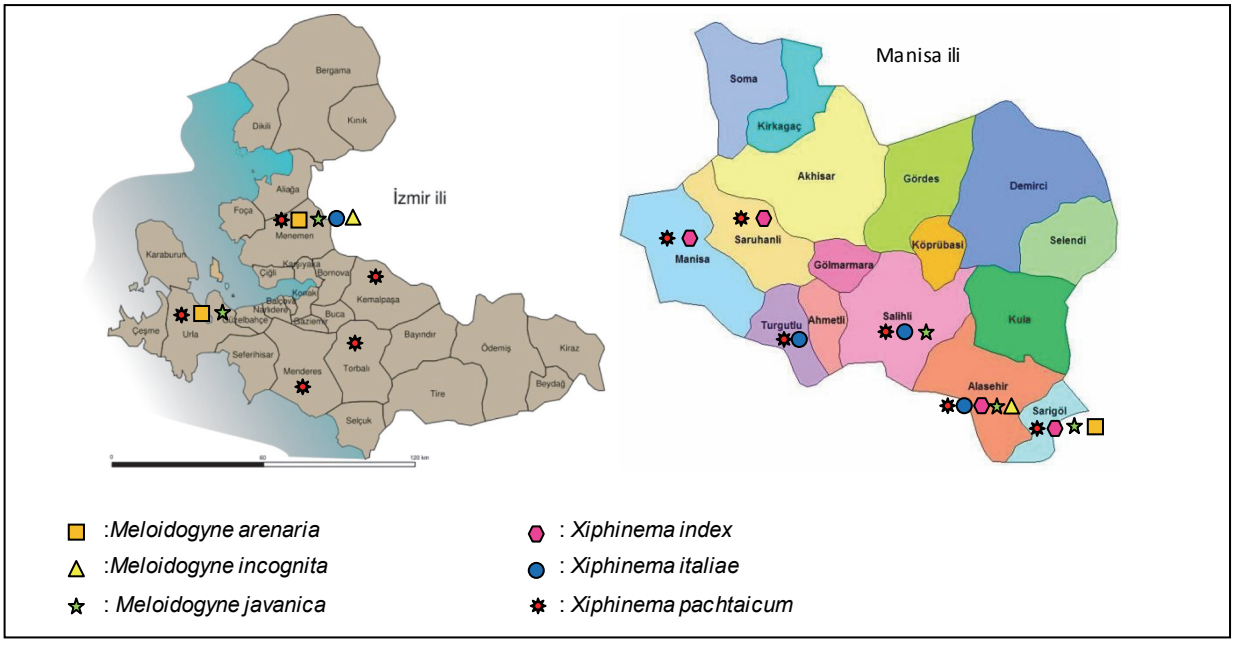
Morfolojik ve moleküler tanımlamaların karşılaştırılması

Bu çalışmada türler, morfolojik ve moleküler yöntemler kullanılarak tanımlanmıştır. *X. index*'e ait 12 populasyonun altısı hem morfolojik hem de moleküler, altısı ise yalnızca morfolojik; *X. italiae*'ya ait altı popülasyonun, üçü hem morfolojik hem de moleküler, üçü ise yalnızca morfolojik; *X. pachtaicum*'a ait 55 popülasyonun 48'i hem morfolojik hem moleküler, yedisi ise yalnızca morfolojik yöntemler kullanılarak tanımlanmıştır.

Meloidogyne ve *Xiphinema* türlerinin yaygınlıkları

Bu çalışmada, *M. arenaria*, Manisa ili Sarıgöl ilçesinde 1 adet, İzmir ili Urla ilçesinde bir adet ve Menemen ilçesinde iki adet olmak üzere sürvey yapılan toplam 188 bağ alanının dördünde tespit edilmiştir. *M. incognita,* Manisa ili, Alaşehir ilçesinde bir adet ve İzmir ili Menemen ilçesinde bir adet olmak üzere sürvey yapılan toplam 188 bağ alanının ikisinde belirlenmiştir. *M. javanica* ise, Manisa ili Salihli ilçesinde bir adet, Alaşehir ilçesinde bir adet Sarıgöl ilçesinde bir adet; İzmir ili Urla ilçesinde bir adet, Menemen ilçesinde iki adet olmak üzere sürvey yapılan toplam 188 bağ alanının altısında tespit edilmiştir (Şekil 9).

Bu çalışmada, *X. index,* Manisa ili, Merkez ilçesinde üç adet, Alaşehir ilçesinde dört adet, Sarıgöl ilçesinde iki adet ve Saruhanlı ilçesinde üç adet olmak üzere sürvey yapılan toplam 188 bağ alanının 12'sinde tespit edilmiştir. *X. italiae;* Manisa ili, Turgutlu ve Alaşehir ilçelerinde 1'er adet, Salihli ilçesinde üç adet; İzmir ili Menemen ilçesinde bir adet olmak üzere sürvey yapılan toplam 188 bağ alanının altısında belirlenmiştir. *X. pachtaicum* ise; Manisa ili, Merkez ilçesinde altı adet, Saruhanlı ilçesinde sekiz adet, Turgutlu ilçesinde altı adet, Salihli ilçesinde dokuz adet, Alaşehir ilçesinde 12 adet, Sarıgöl ilçesinde üç adet; İzmir ili, Kemalpaşa ilçesinde üç adet, Torbalı ilçesinde iki adet, Menderes ilçesinde bir adet, Urla ilçesinde iki adet ve Menemen ilçesinde üç adet olmak üzere sürvey yapılan toplam 188 bağ alanının 55'inde tespit edilmiştir (Şekil 9).



Şekil 9. İzmir ve Manisa illeri bağ alanlarının Kamalı ve Kök-ur nematod türleriyle bulaşıklık haritası.

Çalışmada bazı örneklerde morfolojik ve moleküler tanımlama sonuçları birbirlerinden farklılık göstermiştir. Bu farklılıklar, örnek alınan alanlarda tespit edilen türlerin karışık popülasyonlardan oluşmasından veya tanımlamaların saf kültür örnekleri üzerinde yürütülmemiş olmasından kaynaklanmış olabileceği düşünülmektedir. Kök-ur ve kamalı nematodlar ile bulaşıklığın görüldüğü bağ alanlarının sulak alanlara olan yakınlıkları ve bu alanlarda belirli dönemlerde görülen su baskınları, söz konusu nematodların bu sayede geniş alanlara yayılmış olabileceği olasılığını arttırmaktadır. Bölgede asma fidan üretimi, farklı özelliklere (geleneksel, modern vb.) sahip tesislerde yürütülmektedir. Bu tesisler, çoğunlukla nematodların tespit edildiği alanlarda (Salihli, Alaşehir vb.) faaliyet göstermektedir. Bu nedenle söz konusu tesislerde nematoddan ari fidan üretim kurallarına özen gösterilmesi gerekmektedir. Aksi takdirde nematod bulaşıklığı görülen fidanların temiz bağ alanlarında kullanılması nematodların geniş alanlara yayılma riskini artırabilecektir.

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Orijinal araştırma (Original article)

Entomopatojen fungus, *Purpureocillium lilacinum* TR1'in kök-ur nematodlarının (*Meloidogyne javanica*, *M. incognita* ve *M. arenaria*) mücadelesinde etkinliği[1]

Evaluation of entomopathogenic fungi, *Purpureocillium lilacinum* TR1 for the control of the Root-knot nematodes (*Meloidogyne javanica*, *M. incognita* and *M. arenaria)*

İlker KEPENEKÇİ[2] **Erçin OKSAL[3*]**

Summary

Root-knot nematodes (RKNs) (Nematoda: Meloidogynidae) are one of the major pests of the vegetables causing losses in crop production by forming knots on the roots. RKNs are generally seen in the greenhouse vegetable production areas of the coastal regions of Turkey. Because of commercial nematicides are highly toxic to environment and human health, alternative control strategies are needed. One of effective and environmental friendly methods is using entomopathogen fungi (EPFs) against nematodes. In this study, a potential biological control agents, Turkish isolate of EPF, *Purpureocillium lilacinum* TR1 (syn: *Paecilomyces lilacinus* (Thom) Luangsa-ard, Houbraken, Hywel-Jones & Samson) (Hypocreales: Ophiocordycipitaceae) was evaluated to control three species [*Meloidogyne arenaria* (Neal) Chitwood, *M. incognita* (Kofoid & White) Chitwood and *M. javanica* (Treub) Chitwood] of RKNs. Experiments were conducted in plastic pots, 1000 J2s and 3000 eegs of RKNs were applied to 2 cm deep holes in the pots. The serial dilutions of *P. lilacinum* conidia were prepared 10^6, 10^7 and 10^8 cfu ml^{-1} concentrations under haemocytometer. The two controls were pots with RKN eggs or J2s (positive control), and no RKNs (negative control). Bioassays were replicated (pots) five times for each treatment. Total number of egg masses for each plant, plant height, fresh and dry weight of the upper parts of plants and fresh and dry root weight were recorded. Numbers of nematodes were decreased by increasing the inoculum level of the entomopatogenic fungi. 10^8 cfu concentrations of *Purpureocillium lilacinum* TR1 were found more effective then other concentrations applied.

Keywords: Root-knot nematodes, *Purpureocillium lilacinum*, entomopathogen, biological control, vegetable

Özet

Kök-ur nematod (KUN) (Nematoda: Meloidogynidae)'ları sebzelerin en önemli zararlılarındandır. Türkiye'de daha çok kıyı kesimlerinde seralarda yetiştirilen sebzelerde daha yoğun olarak görülmektedir. Ticari nematisitler çevre ve insan sağlığına yüksek oranda toksik olduklarından alternatif mücadele yöntemlerine ihtiyaç vardır. Bu yöntemler arasında en etkili ve çevre dostu olan entomopatojen fungus (EPF)'ların kullanımı önemli bir yer tutmaktadır. Bu çalışmada KUN [*Meloidogyne arenaria* (Neal) Chitwood, *M. incognita* (Kofoid & White) Chitwood ve *M. javanica* (Treub) Chitwood]'lerin mücadelesinde *Purpureocillium lilacinum* TR1 (syn: *Paecilomyces lilacinus* (Thom) Luangsa-ard, Houbraken, Hywel-Jones & Samson) (Hypocreales: Ophiocordycipitaceae)'in Türk izolatının kullanım olanağı değerlendirilmiştir. Denemeler plastik saksılarda yürütülmüş, KUN'lerin 3000 yumurta ve 1000 L2 dönemleri toprağın 2 cm derinliğine uygulanmıştır. *P. ilacinum*, hemasitometrede 10^6, 10^7 ve 10^8 cfu ml^{-1} konsantrasyonda konidi sayımları yapılarak hazırlanmıştır. Pozitif kontrol KUN yumurtaları veya L2'leri içermekte, negatif kontrole nematod uygulanmamış sadece su verilmiştir. Denemeler her uygulama için 5 tekerrürlü olarak yürütülmüştür. Her bitkideki toplam yumurta sayısı, bitki uzunluğu, bitki üst kısımlarının kuru ve yaş ağırlığı ile kök yaş ve kuru ağırlığı kaydedilmiştir. Deneme sonuçlarına göre nematod sayısı inokulum yoğunluğunun artışına bağlı olarak azalmıştır. *Purpureocillium lilacinum* TR1'in 10^8 cfu konsantrasyonu diğer konsantrasyonlardan daha etkili bulunmuştur.

Anahtar sözcükler: Kök-ur nematodları, *Purpureocillium lilacinum*, entomopatojen, biyolojik kontrol, sebze

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Giriş

Sebze ve meyve insan beslenmesinde çok önemli olan besinlerdir. Özellikle örtü altı yetiştiriciliğinde sebzelerin önemli zararlılarından biri köklerde urlar meydana getirerek ekonomik değerde ürün kayıplarına neden olan kök-ur nematodları (*Meloidogyne* spp.) (Nematoda: Meloidogynidae) (KUN)'dır. Kök-ur nematodları geniş konukçu dizisine sahip çok önemli zararlılar arasında yer almakta olup sebzelerde %10'un üzerinde ürün kaybına neden olmaktadır (Sahebani & Hadavi, 2008; Sikora & Fernandez, 2005; Topp et al., 1998; Whitehead, 1998). Dünya genelinde kök-ur nematodlarının 4 türü, *Meloidogyne javanica* (Treub) Chitwood, *M. arenaria* (Neal) Chitwood, *M. incognita* (Kofoid & White) Chitwood ve *M. hapla* (Kofoid & White) Chitwood yaygın olarak bulunmaktadır (Eisenback & Triantaphyllou, 1991).

Türkiye'de bu güne kadar yapılan çalışmalar sonucunda çeşitli konukçularda 8 kök-ur nematodu türü tespit edilmiştir. Bunlar; *M. acrita*, *M. arenaria*, *M. artiellia*, *M. exigua*, *M. hapla*, *M. incognita*, *M. javanica*, *M. thamesi*'dir (Kepenekci, 2012). Elekcioğlu & Uygun (1994), Doğu Akdeniz Bölgesi'nde muz ve birçok sebzenin köklerinde kök-ur nematod (*M. incognita, M.javanica, M. arenaria*)'larının yoğun olarak bulunduğunu önemli zararlar oluşturduğunu bildirmektedirler.

Cylindrocarpon, *Phoma*, *Fusarium*, *Gliocladium*, *Paecilomyces* ve *Pochonia* cinslerine ait funguslar bitki paraziti nematodların dişi ve yumurta paraziti olarak önemlidir ve bu funguslar toprak içerisinde bulunur (Siddiqui & Mahmood, 1995). Bu entomopatojen fungusların en önemli ve en iyi bilinen türleri *Pochonia* ve *Paecilomyces* cinsleri içinde yer alır. Bu fungus türleri içinde Sordariomycetes sınıfına ait toprak funguslarından olan *P. lilacinus* Luangsa-ard, Hywel-Jones, Houbraken & Samson (Hypocreales: Ophiocordycipitaceae) biyolojik mücadele etmenleri arasında yer alan en önemli fungus türü olup bitki paraziti nematodların biyolojik mücadelesinde kullanılan etmenlerdendir (Morgan-Jones et al., 1984; Jatala, 1986; Dube & Smart, 1987; Atkins et al., 2005; Khan et al., 2006). Entomopatojen fungus (EPF)'lar içinde, ürettiği sekonder ürünler ve enzimlerle etkili olan en önemli fungus *P. lilacinus* olup nematofagus (nematophagous) fungus olarak da bilinir (Park et al., 2004; Kiewnick et al., 2006).

Yapılan son çalışmalarla *Paecilomyces lilacinus*'un sistematik konumu değişmiş ve *Purpureocillium lilacinum* olmuştur (Luangsa-ard et al., 2011). *P. lilacinus* kök-ur ve kist nematodlarının önemli parazitidir (Cannayane & Sivakumar, 2001) ve önemli bir biyolojik mücadele etmenidir (Cabanillas & Barker, 1989; Oclarit & Cumagum, 2009; Hashem & Abo-Elyours, 2011; Udo et al., 2013). Khan et al. (2003) *P. lilacinus*'un ürettiği protease ve chitinase enzimleri sayesinde nematod yumurtalarına ve kütikulasına penetre olduğunu bildirmiştir. Cannayane & Sivakumar (2001) biyolojik mücadele etmeni olarak *P. lilacinus*'un kök-ur ve kist nematodlarına (özellikle patates kist nematodlarından *Globodera rostochiensis*) karşı başarılı sonuçların alındığı çok sayıda araştırmayı listelemiştir.

Türkiye'de nematodlara karşı ruhsat almış nematisitlerin büyük bir bölümü toksik olup KUN'lere karşı ruhsatlıdır. Bazı nematisitler nematodların mücadelesinde etkili olmasına rağmen, özellikle geniş spekturumlu bir etkiye sahip olduklarından yasaklanmış ya da kısıtlanmışlardır. Bu bağlamda alternatif mücadele yöntemlerinin geliştirilmesi ve bitki paraziti nematodların kontrolü için, kimyasal mücadeleyi tamamlayıcı ve bütünleyici yöntemlerin uygulamaya konulması kaçınılmaz hale gelmiştir. Bu mücadele yöntemleri içerisinde; biyolojik mücadele yöntemleri kapsamında entomopatojenlerin kullanımı önemli bir yer tutmaktadır. Biyopreparatlar içerisinde kök-ur nematodlarına karşı entomopatojen fungusların (özellikle *P. lilacinus*'un farklı izolatları) kullanımı dünyada son derece yaygın ve etkilidir. Bu çalışmada Türkiye'de daha önce yürütülen çalışmalarda elde edilmiş (Kepenekci et al., 2009) ve tanımlanmış (Kepenekci et al., 2015) *Purpureocillium lilacinum* (syn: *Paecilomyces lilacinus*)'un Türkiye izolatı'nın kök-ur nematodlarına karşı etkinliği ortaya konulmuştur.

Materyal ve Yöntem

Nematod kültürü ve üretimi

Kök-ur nematod (*Meloidogyne arenaria, M. incognita* ve *M. javanica*)'larına ait yumurtalar ve larvalar (L2); serada yetiştirilen domates (*Solanum lycopersicum* L.) (Solanaceae) (SC-2121 çeşidi) bitkilerinin urlu köklerinden elde edilmiştir. Nematod kültürleri Zirai Mücadele Merkez Araştırma Enstitüsü, Ankara (ZMMAE) Nematoloji laboratuvarında bulunmaktadır.

Urlu köklerden kök-ur nematodu yumurtalarının elde edilmesi için, kökler yıkanarak 1 cm boyunda kesilmiş ve %0.525 yoğunlukta NaOCl (sodyum hipoklorit) (çamaşır suyu) çözeltisi içinde 3-3.5 dakika çalkalanmıştır. Daha sonra bu çözelti 200 ve 500 mesh'lik eleklerden geçirilerek 500 mesh'lik elek üzerinde kalan nematod yumurtaları toplanmıştır (Hussey & Barker, 1973). Bu yumurtaların bir kısmı denemelerde kullanmak amacıyla, mikroskop altında sayım yapılarak 1 ml içinde 3000 yumurta olması sağlanmıştır (3000 yumurta ml^{-1}).

Daha sonra elde edilen nematod yumurtaları inkübasyona bırakılmış ve yumurtadan çıkan 2. dönem larvalar (L2) toplanmıştır. Mikroskop altında sayım yapılarak 1 ml içinde 1000 L2 olması sağlanmıştır (1000 L2 ml^{-1}). Elde edilen larvalar aynı gün denemelerde kullanılmıştır.

Fungus kültürü ve üretimi

Çalışmalarda kullanılan entomopatojen fungus, *P. lilacinum* TR1, ZMMAE kültür koleksiyonunda yer almakta olup, daha önce yürütülmüş olan ''Burdur, Isparta ve Eskişehir illerindeki örtüaltı sebze yetiştiriciliğinde sorun olan Kök-ur nematodları (*Meloidogyne* spp.)'nın fungal ve bakteriyel patojenlerinin belirlenmesi'' projesi kapsamında domates bitkisindeki kök-ur nematodları'ndan izole edilmiş (Kepenekci et al., 2009) ve tanımlanmıştır (Kepenekci et al., 2015). Fungus izolatı, -85°C' de %15 lik gliserol altında saklanmaktadır. Fungus Richard sıvı ortamında (Riker & Riker, 1936) 15 gün süreyle geliştirilmiştir. Bu süre sonunda, ortamdan fungus miselleri toplanarak ve destile su ile karıştırılarak 10 ml de 1 g misel olacak şekilde inokulum hazırlanmıştır.

Purpureocillium lilacinum TR1 üretimi için PDA (DifcoTM, Becton Dickinson and Company, USA) içeren petriler kullanılmış ve bu petrilere stok kültürden öze yardımıyla fungus aşılanmıştır. Petriler parafilm ile kapatılarak 12 saat ışık 12 saat karanlık içeren 25±1°C'ye ayarlı iklim dolabında 7-14 gün geliştirilmiştir. Sporulasyon olan petrilere bir miktar steril saf su eklenerek öze yardımıyla sporların suya geçmesi sağlanmıştır. Misel ve agar parçalarını elemine etmek için süspansiyon tül kullanılarak süzülmüştür. Spor süspansiyonuna %0.05 Tween-80 katılarak manyetik karıştırıcıda tamamen homojen olana kadar karıştırılmıştır (Wakil et al., 2012). 10^6, 10^7 ve 10^8 cfu ml^{-1} spor konsantrasyonlarını elde etmek amacıyla Thoma lamında sayımlar yapılmış ve steril saf su kullanılarak süspansiyondan seri dilüsyonlar yapılmıştır. Ayrıca hemasitometrede konidi sayımları yapılmıştır. Elde edilen spor konsantrasyonları kullanılana kadar +4°C'de buzdolabında muhafaza edilmiştir.

Sera-Saksı denemeleri

Sera-saksı denemelerinde; denemeler Ekim-Aralık 2012 [13.32-33.59 ºC (22.02 ±4.14 ºC) ve %23.40-77.10 (%36.34±9.25) orantılı nem]; Aralık 2012-Mayıs 2013 [16.7-39.6 ºC (25.04±4.18 ºC) ve %23.4-72.6 (%30.14±10.00 orantılı nem] ve Eylül-Kasım 2013 [12.93-38.77 ºC (25.92±5.61 ºC) ve %27.20-90.00 (%39.98±16.26 orantılı nem)] dönemlerinde 5 tekerrürlü olarak kurulmuştur. Denemeler boyunca sera içi sıcaklık ve nem değerleri HOBO (sıcaklık ve nem kaydedici) kullanılarak kaydedilmiştir.

Denemelerde 7×7cm (yaklaşık 340 ml veya 320 g toprak alan) ebatlarında içinde toprak kum karışımı (%80 kum, %15 toprak ve %5 kil) bulunan plastik saksılar kullanılmıştır. Hazırlanan toprak kum karışımı iki kere 121°C de 15 dakika otoklavda sterilize edilmiştir. Toprak karışımları saksılara konulmadan önce alt kısımlarına köklerin dışarı çıkmasını ve toprağın dökülmesini önlemek amacıyla kağıt tela yerleştirilmiştir. 23°C (±2)'de 16 saat aydınlık 8 saat karanlık olarak ayarlanan iklim odasında viyoller [45 gözlü (9x5) (en:5 cm, derinlik:6 cm)]'de yetiştirilen domates fideleri (SC-2121 domates çeşidi), 2-4 yapraklı döneme gelince (yaklaşık 10 cm boyda), her saksıya bir fide olacak şekilde şaşırtılmıştır.

Purpureocillium lilacinum TR1 3 farklı konsantrasyonda, 10^6, 10^7 ve 10^8 cfu (spor) ml^{-1} uygulanmıştır (Oclarit et al., 2009). Fideler şaşırtıldıktan sonra fidelerinin kökleri etrafına açılan 2 cm derinliğinde olan deliğe pipet yardımıyla fungus süspansiyonları verilmiştir. Denemelerinde, *M. arenaria, M. incognita* ve *M. javanica* uygulamalarında; fidelerinin kökleri etrafına açılan 2 cm derinliğinde olan deliğe pipet yardımıyla 3000 yumurta ml^{-1} veya 1000 L2 ml^{-1} olacak şekilde farklı zamanlarda 2 farklı uygulama yapılmıştır. *P. lilacinum* süspansiyonları nematodlarla birlikte aynı anda uygulanmıştır. Tüm denemelerde pozitif (+)

kontrol (sadece nematod yumurtası veya larvasının uygulandığı) ve negatif (-) kontrol (sadece su uygulanan, herhangi bir nematod uygulaması yapılmayan) olmak üzere 2 kontrol grubu bulunmaktadır.

Uygulamalardan 9 hafta sonra bitkiler saksılardan toprakları ile birlikte çıkarılarak musluk suyu altında kök sisteminin topraktan tam arındırılması için yıkanmışlardır. Yıkama işleminden sonra kökler phloxine B (0.15 g L su^{-1}) ile 15-20 dakika boyanmış (Daykin & Hussey, 1985) ve büyüteç (8×, Klipsli Işıklı Büyüteç) altında yumurta paketleri sayılmıştır. Her bir bitkiye ait üst aksamının uzunluğu (bitki boyu) ölçülmüştür. Daha sonra hassas terazide kökler ve bitki üst aksamı tartılarak kaydedilmiştir. Aynı işlem 70°C'de 48 saat kurutma (Mohammad et al., 2007) işlemi yapıldıktan sonra tekrarlanmıştır. Denemeler sonunda; her bir bitki kökündeki kök-ur nematodlarına ait yumurta paketi sayısı, bitkinin boyu (cm), bitkinin yaş ve kuru ağırlığı (g), kök yaş ve kuru ağırlığı (g) parametreleri istatistiki olarak değerlendirilmiştir. Elde edilen verilere varyans analizi uygulanmıştır. Etkiler kontrol gruplarına kıyaslanarak bulunmuştur. Gruplar arasındaki ayrım için Duncan testi kullanılmıştır (SPSS, 1999).

Araştırma Sonuçları ve Tartışma

Domates bitkilerinin köklerinde bulunan yumurta paketi sayıları değerlendirildiğinde; *P. lilacinum*'un yumurtaya etkisi açısından uygulamalarda en yüksek etkiler 10^8 cfu'de görülmüş, *M. arenaria, M. incognita* ve *M. javanica* için sırasıyla 3.8, 9.4 ve 4.6 yumurta paketi bitki^{-1} olarak kaydedilmiştir. En düşük etkilere yani en fazla yumurta paketi sayısına sahip uygulamalar 10^6 cfu'de görülmüştür (*M. arenaria, M. incognita* ve *M. javanica* için sırasıyla 102.4, 92.8 ve 102.1 yumurta paketi bitki^{-1}). Sadece nematod uygulanmış ve *P. lilacinum* uygulaması yapılmamış + kontrol gruplarında 91.6, 117.5 ve 104.2 yumurta paketi bitki^{-1} sayılmıştır (F= 22.40; df: 12.51; P<0.05) (Çizelge 1). Larvaya etki denemelerinde 10^8 cfu uygulamaları değerlendirildiğinde en yüksek etki *M. javanica*'nın kullanıldığı denemelerde görülmüştür, bunu *M. incognita* ve *M. arenaria* izlemiştir (42.2, 45.4 ve 51.6 adet yumurta paketi bitki^{-1}). 10^6 ve 10^7 cfu uygulamalarında yüksek sayıda yumurta paketi oluşumu dikkati çekmektedir (F= 33.47; df: 12.51; P<0.05) (Çizelge 1).

Bitki boyları açısından yapılan değerlendirmelerde, yumurtaya etki yönüyle, en yüksek boya sahip bitkiler *M. javanica* ve *M. incognita*'ya karşı 10^8 cfu uygulamalarında görülmüştür (46.7 ve 47.6 cm) (F= 3.65; df: 13.55; P<0.05) (Çizelge 1). Larvaya etki denemelerinde; *M. javanica* ve *M. incognita* 'ya karşı 10^8 cfu uygulamaları (46.4 ve 47.2 cm) hariç diğer tüm uygulamalar, sadece nematod uygulanan ve fungus uygulaması yapılmayan + kontrol gruplar (*M. arenaria, M. incognita* ve *M. javanica* için sırasıyla 44.5, 41.2 ve 44.4 cm)'ına ait bitkilerden daha kısa kalmıştır (F= 4.53; df: 13.55; P<0.05) (Çizelge 1).

Bitki üst aksam yaş ağırlığı açısından denemeler değerlendirildiğinde; yumurtaya etki bakımından en yüksek bitki ağırlığı *M. incognita*'nın 10^6 ve 10^8 cfu ile *M. arenaria*'nın 10^7 cfu uygulamalarının yapıldığı bitkilerde görülmüş (16.76, 16.65 ve 17.42 g) ve istatistiki olarak aynı grupta yer almıştır. Tüm uygulamalar + kontrol grupların (*M. arenaria, M. incognita* ve *M. javanica* için sırasıyla 13.75, 14.54 ve 13.10 g)'dan daha ağır bitkilere sahip bulunmuştur (F= 2.47; df: 13.55; P<0.05) (Çizelge 1). Larvaya etki denemelerinde istatistiki olarak fark bulunamamıştır (P>0.05).

Bitki üst aksam kuru ağırlığı değerlendirildiğinde, yumurtaya etki bakımından en yüksek etkiyi 10^8 cfu uygulamaları (*M. arenaria, M. incognita* ve *M. javanica* için 1.98, 1.96 ve 1.93 g) göstermiştir (F= 2.50; df: 13.55; P<0.05) (Çizelge 1). Larvaya etki denemelerinde de benzer sonuçlar elde edilmiştir (F= 2.54; df: 13.55; P<0.05) (Çizelge 1).

Bitki kök ağırlıkları değerlendirildiğinde; kök yaş ağırlığı açısından yumurtaya etki denemelerinde en yüksek bitki kök ağırlığına sahip bitkiler *M. arenaria*'nın 10^6 cfu uygulamalarında görülmüştür (16.74 g) (F= 2.61; df: 13.55; P<0.05) (Çizelge 1). Larvaya etki denemelerinde ise en yüksek bitki kök ağırlığına, istatistiki olarak aynı gruba giren *M. incognita* ve *M. arenaria*'nın 10^8 cfu uygulamalarının yapıldığı bitkiler sahiptir (15.54 ve 15.64 g). Fungus uygulamalarının tümü + kontrol gruplarından daha ağır bitki köklerine sahip bulunmuştur (F= 2.68; df: 13.55; P<0.05) (Çizelge 1).

Çizelge 1. Üç farklı kök-ur nematodu (*Meloidogyne arenaria, M. incognita* ve *M. javanica*) türünün yumurtalarına (Y) ve 2. dönem larvalarına (L2) karşı *Purpureocillium lilacinum* TR1'in üç farklı konsantrasyonunun (10^6, 10^7 ve 10^8 cfu ml-1) etkinliği (+ K: Sadece nematod uygulanan; - K: Sadece su uygulanan, nematod uygulanmamış)

Parametreler		Uygulamalar												
		Meloidogyne arenaria				*M. incognita*				*M. javanica*				- K
		+ K	10^6 cfu	10^7 cfu	10^8 cfu	+ K	10^6 cfu	10^7 cfu	10^8 cfu	+ K	10^6 cfu	10^7 cfu	10^8 cfu	
Yumurta paketi sayısı	L2	109.4±23.7* cd	97.6±26.2 e	101.6±50.3 d	51.6±12.4 e	234.7±30.1 a	238.6±78.6 a	158.2±45.8 b	45.4±4.5 e	150.1±18.2 bc	143.4±42.4 bcd	104.3±24.2 d	42.2±1.7 e	-
	Y	91.6±24.9 abc	102.4±26.8 ab	89.7±22.3 abc	3.8±2.6 d	117.5±37.4 a	92.8±20.7 abc	75.6±47.5 bc	9.4±5.7 d	104.2±11.5 ab	102.1±16.3 ab	68.8±34.5 c	4.6±4.2 d	-
Bitki boyu (cm)	L2	44.5±6.4 abcd	36.4±4.6 d	39.6±3.1 cde	36.2±3.7 d	41.2±5.6 bcde	39.8±7.4 cde	34.6±8.7 e	47.2±5.5 ab	44.4±2.6 abcd	37.1±6.5 de	39.2±4.5 cde	46.4±8.1 abc	50.3±3.8 a
	Y	42.7±12.2 abcd	38.8±4.1 cd	39.3±7.4 bcd	44.3±5.5 abcd	35.3±6.8 d	35.2±4.4 d	36.2±10.2 d	47.6±6.5 abc	34.6±5.6 d	34.4±4.4 d	38.9±3.3 cd	46.7±4.6 abc	50.5±6.4 a
Bitki üst aksam yaş ağırlığı (g)	L2	13.6±0.7 -	13.6±2.2 -	13±2.8 -	14.3±0.8 -	13.5±1.1 -	14.1±0.9 -	13.6±1.8 -	15±1.8 -	13.9±0.2 -	13.6±1 -	13.2±0.8 -	14.5±1.3 -	15.3±2 -
	Y	13.75±1.1 bcd	14.62±1.2 bc	17.42±1.3 a	14.95±1.4 abc	14.54±1.7 bc	16.76±2.2 a	15.44±2.5 abc	16.65±2.3 a	13.10±0.8 d	13.81±2.8 bcd	13.82±1.6 bcd	15.43±1.1 abc	15.92±1.2 ab
Bitki üst aksam kuru ağırlığı (g)	L2	1.74±0.2 abc	1.81±0.2 ab	1.81±0.4 ab	1.92±0.5 a	1.80±0.2 ab	1.79±0.2 ab	1.52±0.2 bc	1.94±0.6 a	1.73±0.3 abc	1.55±0.2 bc	1.72±0.4 abc	1.83±0.2 ab	1.86±0.2 ab
	Y	1.73±0.5 bc	1.52±0.4 cd	1.83±0.1 abc	1.98±0.2 ab	1.52±0.1 cd	1.66±0.2 bcd	1.78±0.3 bc	1.96±0.4 ab	1.82±0.4 abc	1.22±0.3 d	1.57±0.4 cd	1.93±0.4 ab	2.24±0.3 a
Kök yaş ağırlığı (g)	L2	12.94±2.5 d	13.92±0.7 d	14.22±1.2 cd	15.64±0.8 ab	13.84±1.4 d	14.31±0.6 cd	14.22±0.7 cd	15.54±0.4 ab	13.32±0.6 d	13.75±0.5 d	14.22±0.2 cd	14.35±0.5 cd	15.95±1.2 a
	Y	14.54±0.8 bcd	16.74±1.5 a	15.52±1.1 abc	14.16±1 cd	14.47±0.1 cd	13.68±1.3 d	14.04±1.3 cd	14.65±0.6 bcd	14.66±0.3 bcd	14.28±1.6 cd	13.68±1.4 d	14.66±0.8 bcd	16.26±0.7 ab
Kök kuru ağırlığı (g)	L2	1.55±0.4 bcd	1.54±0.6 bcd	1.24±0.2 cd	1.56±0.2 bcd	1.76±0.3 bc	1.54±0.4 bcd	1.88±0.4 abc	1.92±0.3 ab	1.85±0.2 abc	1.84±0.4 abc	1.76±0.1 bc	2.11±0.5 a	2.24±0.2 a
	Y	1.96±0.5 ab	1.14±0.2 d	1.77±0.2 bc	1.88±0.5 abc	1.65±0.6 bcd	1.66±0.3 bcd	1.78±0.1 bc	1.75±0.2 bc	1.67±0.5 bcd	1.55±0.1 bcd	1.22±0.4 cd	1.90±0.2 ab	2.14±0.3 a

* Her satır içerisinde farklı harfler önemli derecede farklıdır (P<0.05).

Kök kuru ağırlık açısından; yumurtaya etki denemelerinde en yüksek kuru kök ağırlıkları istatistiki olarak aynı grubu giren *M. javanica* ve *M. arenaria*'nın 10^8 cfu uygulamalarında bulunmuştur (1.90 ve 1.88 g). ($F= 2.76$; df: 13.55; $P<0.05$) (Çizelge 1). Larvaya etki denemelerinde ise en ağır bitki kökleri *M. incognita*'nın 10^8 cfu uygulamalarında ortaya konmuş ve bunu *M. javanica*'nın 10^8 cfu uygulamaları takip etmiştir (1.92 ve 2.11 g) ($F= 2.16$; df: 13.55; $P<0.05$) (Çizelge 1).

İlk olarak; Peru'da *M. incognita* yumurtalarında tespit edilen *P. lilacinus* son yıllarda üzerinde yoğun olarak çalışılan biyolojik mücadele ajanlarının başında gelmektedir. Bu fungus, *M. incognita* yumurtalarının fakültatif bir parazitidir ve diğer kök-ur nematodu türlerini de parazitleyebilmektedir. *P. lilacinus* izolatları *M. incognita* yumurtalarına bulaşıp yumurtadan larva çıkışını azaltmaktadır (Whitehead, 1998). Fungus tarafından, yumurta kabuğunu parçalayıcı enzimlerin üretilmesi ile kök-ur nematodu yumurtaları üzerinde enfeksiyon meydana gelmektedir. Fungusun salgıladığı serine proteaz enzimleri, nematodun yumurta kabuğunda yapısal değişikliklere neden olmaktadır. Kök-ur nematodu yumurtalarının *P. lilacinus*'un kitinaz enzimine maruz bırakılması sonucu yumurtadan larva çıkışında %60 azalma tespit edilmiştir ve sadece su uygulanmış kontrollerde ise larvaların yumurtadan çıkışında artış gözlenmiş olup çıkan larvaların sadece %9'u ölmüştür (Khan et al., 2005). Ahmad & Khan (2004)'nın yaptığı çalışmalarda; *P. lilacinus*'un toprağa uygulanması ile domates köklerindeki *M. incognita* populasyonu %67-77 oranında, köklerde meydana gelen urlar ise %30 oranında azalırken ikinci yılda elde edilen ürün üç misli artmıştır. Bu fungus, dikimden 10 gün önce ve dikim sırasında toprağa uygulandığında, domates bitkileri nematod saldırısından en iyi şekilde korunmaktadır. Ayrıca dikimden 40 gün sonra fungus toprağa uygulandığında birçok nematod yumurtası fungus tarafından enfekte edilmiştir. *P. lilacinus*'un en iyi izolatı "Biocon" ticari ismi ile Filipin'lerde pazarlanmıştır (Davide, 1990). *P. lilacinus* strain 251 etkili bir biyolojik mücadele etmeni olarak çok sayıda çalışma yapılmış ve etkili sonuçlar alınmıştır. Bu fungus preparat haline getirilmiş ve nematod mücadelesinde yaygın olarak kullanılmaktadır (Atkins et al., 2005; Kiewnick, 2004). Sharma et al. (2014) *P. lilacinus* 6029 izolatının nematolojik atktivitesi üzerinde çalışmışlar ve *M. incognita* üzerinde yaptıkları çalışmada yüksek etki bulmuşlardır (%98.2 ve %100). Bizim yaptığımız çalışmada da ülkemiz izolatı olan *P. lilacinum* TR1 tüm denemelerde etkili bulunmuştur. Denemeler sonucunda domates bitkilerinin köklerinde bulunan yumurta paketi sayıları değerlendirildiğinde; yumurta inokulasyonunda en yüksek etki 10^8 konsantrasyonunda görülmüştür. L2'lerinin kullanıldığı denemelerde de benzer sonuçlar alınmasına karşın domates köklerinde daha fazla yumurta paketi oluşumu dikkati çekmektedir. Diğer bitki parametreleri açısından da özellikle 10^8 uygulamalarının etkili olduğu görülmektedir.

Teşekkür

Bu çalışma, TÜBİTAK tarafından 111O784 nolu proje ile desteklenmiştir. Bu desteklerinden dolayı TÜBİTAK'a teşekkür ederiz.

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