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Research Article

Potential of Local Entomopathogenic Nematode Isolates to Control the Adults of the Scarab Beetle, *Epicometis hirta* (Coleoptera: Scarabaeidae)*

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Abstract. The scarab beetle, *Epicometis* (*Tropinota*) *hirta* (Poda) (Coleoptera: Scarabaeidae), is a serious pest that feeds and destroys developing and blossoming flowers of apple plants. The pest has recently been reported more frequently from apple orchards in Turkey. The control of the adults of *E. hirta* is challenging due to the restrictions on the application of the chemicals used against the adults during the flowering period of apple orchards in Turkey. Other control methods fail to achieve desired results in the control of the adults. Therefore, there is an increasing need for more sustainable and environmentally-friendly control methods against the adults of *E. hirta*. In the present study, the pathogenicity of Turkish entomopathogenic nematode (EPN) isolates was evaluated using adults of this pest under laboratory conditions, toward developing an EPN-based integrated *E. hirta* control plan. Virulence of local EPN isolates against *E. hirta* was tested in 12-well bioassay plates containing sterile soil treated with different EPN concentrations (0, 190 and 380 IJs adult^{-1}) and then incubated at 25 or 30 ºC. All the EPNs isolates caused high mortality ranging from 45 to 100% at 25 ºC, or 60 to 100% at 30ºC, respectively. The local *Steinernema carpocapsae* (Weiser) (Nematoda: Rhabditida) isolate E76-S from Turkey was the most efficient, achieving the maximum mortality rate (100%) at both temperatures. The evidence of this study gave promising results for the control of the adults of *E. hirta* using local EPN isolates but further studies should be carried out in field conditions to determine the field performance of EPNs.

Yerel Entomopatojen Nematod İzolatlarının, Bakla Zınnı, *Epicometis hirta* (Coleoptera: Scarabaeidae) Erginlerini Kontrol Etme Potansiyeli

Anahtar kelimeler:
Bakla zınnı, *Steinernema* spp., *Heterorhabditis* spp., faydalı nematodlar, biyolojik mücadele

Özet. Bakla Zınnı, *Epicometis* (*Tropinota*) *hirta* (Poda) (Coleoptera: Scarabaeidae), elma ağaçlarının tomurcuk ve açan çiçeklerinde beslenen ve ciddi tahribatlara neden olan önemli bir zararlıdır ve son zamanlarda Türkiye'deki elma bahçelerindeki artan bir sıklıkta bildirilmektedir. Türkiye'deki elma bahçelerinin çiçeklenme döneminde bu zararlıya karşı kullanılan kimyasalların uygulanmasına ilişkin kısıtlamalar nedeniyle *E. hirta* erginlerinin kontrolü oldukça zordur. Bu zararlıya karşı diğer mücadele yöntemleri kullanarak, *E. hirta* erginlerinin kontrolünde istenen sonuçları elde edilememektedir. Bu nedenle, *E. hirta*'nın erginlerine karşı daha sürdürülebilir ve çevre dostu kontrol yöntemlerine ihtiyaç artmaktadır. Bu çalışmada entomopatojen nematodların da içerisinde yer aldığı entegre bir mücadele programı oluşturulmasına yönelik, yerel entomopatojen nematod (EPN) izolatlarının *E. hirta* erginleri üzerindeki patojenisitesi laboratuvar koşulları altında değerlendirilmiştir. Yerel EPN izolatlarının *E. hirta* erginleri üzerindeki virülensi, farklı EPN konsantrasyonlarda (0, 190 ve 380 IJ ergin^{-1}) ve sıcaklıkta (25 veya 30 °C'de) içerisinde steril toprak bulunan (12) well-platelerde test edilmiştir. Test edilen EPN izolatları, 25° C'de %45-100 ve 30°C'de %60-100 arasında değişen ölümlere neden olmuştur. *Steinernema carpocapsae* (Weiser) (Nematoda: Rhabditida) E76-S izolatı her iki sıcaklıkta da maksimum ölüm oranı (%100) meydana getirerek en etkili izolat olmuştur. Bu çalışmanın sonuçları, yerel EPN izolatları kullanarak *E. hirta* erginlerinin kontrolü için umut verici sonuçlar vermiştir, ancak EPN'lerin arazi koşullarındaki etkinliğini belirlemek için arazi koşullarında çalışmalar yapılmasına ihtiyaç vardır.

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INTRODUCTION

The adults of the scarab beetle *Epicometis* (*Tropinota*) *hirta* (Poda) (Coleoptera: Scarabaeidae) is considered to be a serious pest of fruit tree orchards and frequently have been reported to infest blossom, feeding on petals, staminae and stigmae of flowers (Ertop and Özpinar, 2011), especially in European countries such as Austria (Böhm, 1950), Hungary (Toth *et al*., 2003), Bulgaria (Kutinkova and Andreev, 2004), Croatia (Ražov *et al*., 2009) and Macedonia (Rozner and Rozner, 2009). Recently, the damage of the adults of *E. hirta* on the various fruit trees in Turkey appears to be increasing (Tezcan and Pehlivan, 2001; Öztürk and Ulusoy, 2003; Özkan, 2005; Özbek, 2008; Yaşar *et al*., 2013; Arslan and Aslan, 2015; Gezer and Özpınar, 2015; Kaçar and Koca, 2020).

Turkey is one of the most important fruit tree growers in the world and over 85 fruit tree species are cultivated successfully (Ercisli, 2004). The adults of these beetles may damage 70% of the flowers of fruit trees, if not efficiently suppressed (Kutinkova and Andreev, 2004; Ražov *et al*., 2009). Recent restrictions imposed on the insecticides in the flowering period of fruit tree plants to protect the non-target organisms and the waiting period of the insecticides, as well as the potential of the adults of *E. hirta* to develop resistance to insecticides have led researchers to search for new and effective biological control strategies (Schmera *et al*., 2004; Abdel-Razek, 2010; Abdel-Razek and Abd-Elgawad, 2013; Yaşar and Uysal, 2013; Al-Alawi, 2014; Atmaca *et al*., 2018; Özdemir and Gözel, 2018; Şahin *et al*., 2018; Aydin and Yaşar, 2019; Çolak *et al*., 2019; Özdemir *et al*., 2020; Şahin and Susurluk, 2020).

Entomopathogenic nematodes (EPNs) (*Steinernema* and *Heterorhabditis* sp.) have been receiving increasing attention in the last decades, due to their potential to control a wide range of pests of agricultural importance (Canhilal *et al*., 2007; Azizoglu *et al*., 2016; Canhilal *et al*., 2017; Kamali *et al*., 2017; Yuksel and Canhilal, 2018). The non-feeding stage of EPNs, infective juveniles (IJs), are capable of active host searching and killing the insect hosts within 24-72 h by the help of mutualistic bacteria mostly in the genera *Xenorhabdus* and *Photorhabdus* after penetrating host body (Poinar and Grewal, 2012; Stock *et al*., 2017Shan *et al*., 2019;). Although *Alcaligenes* sp. were isolated from *S. carpocapsae* there are still doubts about their symbiotic relationships with EPNs and pathogenicity against the insects (Boemare *et al*., 1996; Gouge and Snyder, 2016; Jiménez-Cortés *et al*., 2016; Fu and Lie, 2019; Shan *et al*., 2019; Özdemir *et al*., 2020).

Although the success of the foliar application of EPNs is limited compared to soil applications (Klein, 1990) they still possess a great potential to control foliage and cryptic pests. However, to be successful the right selection of application methods and time, species, isolate and temperature is important as these are among the most crucial factors affecting the efficacy of EPNs (Jacob and Mathew, 2016; Laleh *et al*., 2016; Begley *et al*., 2018; Hussein *et al*., 2019). Previous field studies showed that IJs of EPNs can remain alive and infective on the leaves and flowers of apple trees up to 4 days after application at dusk, which may be enough time-length for EPN-IJs to penetrate and kill large numbers of *E. hirta* pests in apple orchards (Bedding and Akhurst, 1975; Belair *et al*., 1998).

Laboratory evaluation of EPNs on the target pest is essential to indicate the control potential of the native species or isolates toward field studies. Thus, the efficacy of different EPN isolates (reported in Turkey) on the adults of *E. hirta* was established for varied IJ concentrations in EPN-treatments, dissimilar temperatures and increasing time-length of exposure of the adult *E. hirta* pests to the local EPN isolates under laboratory conditions. Our main objective was to predict at least a more promising local EPN isolate that may produce desirable results, if used, in field trials toward developing biocontrol strategy against the scarab beetle pest (*E. hirta*) in apple orchards in Turkey.

MATERIAL AND METHOD

Obtaining Healthy Adults of E. hirta for Trial Under Laboratory Conditions

Pesticide application in Kayseri province was stopped in June 2017 to secure and obtain sufficient numbers of healthy adults of *E. hirta* from the infested apple orchards. The collected adults were put in plastic cages (20 x 20 x 25 cm^3) with the lid opened to allow air. Blossoming apple flowers from these orchards were also added to the cages to provide food to the adult beetles. Few beetles were later transferred from the cages to a 180-ml containers that lack food for beetle, and then examined for 24 hours under laboratory conditions at 25 ^{0}C and 60% relative humidity (R.H), to select the healthier adults for the experiments.

In-vivo Production of Local EPN Isolates for Experiment

Entomopathogenic nematode isolates (in Table 1) were produced using EPN-infected last instars of *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) under laboratory conditions (i.e., at 25±1 °C and 60% R.H.) (Kaya and

Gaugler, 1993; Canhilal, 2011; Yuksel *et al*., 2019). The IJ stock suspensions of each EPN isolate were rinsed into containers using distilled water and then stored at 7^0C for one week before being used for laboratory trials.

One hour after the stock suspensions of each isolate were adjusted to active 190 and 380 IJs/ml according to the recommended IJ concentrations by Stock (2009), the EPNs were applied against *E. hirta* in bioassays using a micropipette.

Table 1. Native entomopathogenic nematode (EPN) isolates in Turkey.
Çizelge 1. Türkiye'den izole edilmiş yerel entomopatojen nematod (EPN) izolatları.

Isolates	Districts/Province	References
Heterorhabditis indica 216-H	Afsin/Kahramanmaras	Canhilal *et al*., 2016
H. bacteriophora FLH-4-H	Felahiye/Kayseri	Canhilal *et al*., 2017
Steinernema bicornotum MGZ-4-S	Melikgazi/Kayseri	Canhilal *et al*., 2017
S. carpocapsae E76-S	Tomarza/Kayseri	Canhilal *et al*., 2017
S. feltiae KCS-4-S	Kocasinan/Kayseri	Canhilal *et al*., 2017

The Bioassay for the Laboratory Trial

A flat bottom 12-well-plate was used for the experiment. A well in a plate has a surface area of 3.8 cm2 with its depth being 1.7 cm. A 15 g sample of autoclaved air-dried sandy soil was added to each of the 12 wells per a plate. After that, a healthy-looking adult scarab beetle (*E. hirta*) was added in each well containing sandy soil. About a 5-g piece of apple flower as food was supplied to each beetle pest in each well before being treated with active IJs of the local EPN isolates.

The EPN Isolate-Treatments to E. hirta in the Bioassay

An adult beetle in each well was treated with 1 ml of 190 IJs, using a micropipette. After inoculation of the beetles in all the 12 wells per plate, Parafilm was used to cover the surface of the wells to maintain moisture in each well. Similar EPN-treatment was done to *E. hirta* at the concentration of 1 ml of 380 IJs/adult in each of the 12 wells per plate. One millilitre of tap-water only was applied to an adult beetle in each of the 12 wells in a plate. Four replicates of a treatment in a 12-well-plate were made for each of the IJ concentrations 0, 190 and 380 IJs/adult, and then incubated at 25±1 ^{0}C. Similarly, EPN-treatments at 0, 190 and 380 IJs adult^{-1} were made and then incubated at 30±1 ^{o}C. The mortality of the adult beetle pests was assessed on 4th, 8th and 12th days after treatment (DAT). A dead adult of *E. hirta* was retrieved from each well and the presence of IJs in the cadavers was done to confirm "mortality due to EPN" by examining a dissected cadaver under a microscope.

Data Analysis

Because none of the adult beetles died in the control, mortality of *E. hirta* observed in EPN isolate-treatments was not corrected before analysis. The SPSS-software (Version 11.0) was used to compute all statistics. The multiple analysis of variance (MANOVA) was used to examine whether manipulating IJ concentration of an EPN isolate in EPN-treatments, the number of EPN isolates used in the trials and level of temperature may cause a significant variation in their observed mean (± standard error) values of the mortality of the scarab beetles at each DAT (Gotelli and Ellison, 2013). Tukey's multiple range tests at $P \leq 0.05$ were used to compare mean (±standard error) values of mortality (the response variables) among the predicting treatment factors.

RESULTS AND DISCUSSION

All native EPN isolates tested were capable of infecting and killing the adults of *E. hirta* with varying levels of pathogenicity at both temperatures tested (Tables 2 and 3). The mortality rates of the adults were significantly influenced by the nematode isolates and concentrations of IJs as main factors at all time-lengths after EPN-treatment, whereas the temperature was significantly different only on the 4th and 8th DAT (Table 2). The effect of the interactions of the main factors on the mean mortality rates was not significant for the majority of the DAT (Table 2). Increasing the nematode concentration and temperature yielded an increase in the mortality rates of E. hirta (Table 3). Similarly, the mortality of *E. hirta* in all the treatments tended to increase with extension of exposure time to the EPNs.

Table 2. MANOVA parameters for the main effects and associated interactions for the mortality rates of the adults of *Epicometis hirta*.

Çizelge 2. Epicometis hirta erginlerinin ölüm oranları için ana faktörler ve interaksiyonlarının çok değişkenli varyans analizi (MANOVA).

Source*	4th DAT			8th DAT			12th DAT		
	df	F	P	df	F	P	df	F	P
N	4	7.429	0.000	4	9.158	0.000	4	5.769	0.000
C	1	482.187	0.000	1	1.224.516	0.000	1	2.315.263	0.000
T	1	11.929	0.001	1	6.579	0.012	1	3.857	0.053
N*T	4	3.945	0.005	4	2.474	0.050	4	0.729	0.575
N*C	4	2.566	0.014	4	2.424	0.020	4	1.127	0.353
C*T	1	4.071	0.020	1	1.274	0.285	1	1.594	0.209
N*C*T	4	2.015	0.053	4	1.471	0.179	4	0.973	0.462

*DAT: Days after EPN-treatment, N: Number of local EPN isolates, C: Different EPN-IJ concentrations, T: Dissimilar temperatures, F: F-statistic, P: Probability for significant level and df: the degree of freedom. *Calculated P-value ≤ 0.05 indicates that the source of variation in mortality of adult *E. hirta* was significant.

On the 12th DAT, 100% mortality of adult beetles was achieved by the local *S. carpocapsae* isolate at both temperatures on the 12th DAT (Table 3), but the least efficient isolate was the native *S. feltiae* (in Tables 2 and 3) that caused 45% and 60% mortality at 25±1 and 30±1 °C, respectively. On the 4th DAT, the *S. carpocapsae* isolate (in Tables 2 and 3) had already caused 80% mortality at a concentration of 190 IJs at 25 °C, whereas the remaining EPN isolate-treatments with 190 IJs/adult at 25 °C could only achieve very low mortalities ranging from 45±10 to 57±17% (Table 3).

Table 3. The mortality rates (Mean±Se) of the adults of *Epicometis hirta* exposed to different species of entomopathogenic nematodes, at two different concentrations of infective juveniles (IJs) in the well plates at 25±1 ^{0}C or 30±1 ^{0}C.

Çizelge 3. Farklı entomopatojen nematode izolatlarının iki farklı sıcaklıkta (25±1 ^{0}C ve 30±1^{0}C) ve konsantrasyonda (190 ve 380 IJs/ergin) well-plate denemelerinde uygulamadan sonraki 4., 8 ve 12. günlerde Epicometis hirta erginleri üzerinde meydana getirdikleri ölüm oranları.

Nematode Isolates /temperature*	Days after treatment (DAT)					
	4th day		8th day		12th day	
	190 IJs/adult	380 IJs/adult	190 IJs/adult	380 IJs/adult	190 IJs/adult	380 IJs/adult
25°C						
MGZ-4S	47±20 Aa	60±13 Bab	70±11 Aab	82±17 Bb	87±15 Ab	92±9 Bb
KCS-4S	45±10 Aa	40±8 Aa	65±12 Ab	65±19 Ab	87±9 Ac	77±5 Abc
E-76-S	80±8 Ba	87±15 Cab	95±5 Bb	92±9 Bb	100±0 Bb	95±10 Bb
216-H	50±14 Aa	85±10 Cb	92±9 Bb	90±8 Bb	95±10 ABb	90±8 Bb
FLH-4-H	57±17 Aa	72±17 BCb	85±5 ABc	87±9 Bc	95±11 ABc	95±10 Bc
30°C						
MGZ-4S	77±9 Aa	82±9 Ca	95±5 Bb	90±11 Bb	95±5 ABb	97±5 Bb
KCS-4S	60±8 ABa	67±5 Ba	75±12 Aab	85±12 Bb	85±5 Ab	92±9 Bb
E-76-S	70±8 Ba	80±8 Cab	92±9 Bb	95±5 Bb	100±0 Bb	100±0 Bb
216-H	70±8 Ba	75±12 BCa	90±11 Bb	92±5 Bb	95±10 ABb	95±5 Bb
FLH-4-H	75±12 Ba	75±12 BCa	85±5 ABb	85±12 Bb	90±8 ABb	92±9 Bb

*Mean values followed by different lowercase letters in the same line and mean values followed by different uppercase letters in the same column are statistically different according to Tukey's test ($P \leq 0.05$). FLH-4-H: *Heterorhabditis bacteriophora*, 216-H: *H. indica*, E76-S: *Steinernema carpocapsae*, MGZ-4-S: *S. bicornotum,* KCS-4S: *S. feltiae.*

Mortalities of adult *E. hirta* beetles observed in the *S. feltiae* isolate-treatments at 190 or 380 IJs adult^{-1} mostly increased marginally with increasing DAT (Tables 2 and 3). Nonetheless, the 380 IJs adult^{-1} of *S. bicornotum*, *H. indica* and *H. bacteriophora* isolates in the treatments at 30±1 °C achieved high mortality rates of *E. hirta* pests, similar to those observed for the 190 IJs adult^{-1} of *S. carpocapsae* isolate in treatments at 25±1 °C (Tables 2 and 3).

This study showed that pathogenicity of EPNs to *E. hirta* varies depending on EPN species, the concentration of IJs, increasing time-length of exposing *E. hirta* to local EPN isolates, and temperature. The effectiveness of some species remained the same, or even decreased with the increasing concentration and temperature.

Steinernema carpocapsae (a native isolate from Tomarza/Kayseri Province of Turkey) was the most outperforming pathogenic isolate among other local virulent EPNs tested against the adults of *E. hirta*, in this study. The higher efficacy of *S. carpocapsae* E-76 isolate could be due to the insect-killing symbiotic bacteria associated with it and its ambushing-to-cruising foraging strategy which helps them to reach the target insect (Lacey and Georgis, 2012; Campos-Herrera, 2015). In general, all the EPN isolates caused higher mortality to the adults of *E. hirta* starting from the fourth day after EPN-treatments, suggesting the need to select the more promising local EPN isolate(s) observed in this study for field trials against *E. hirta* in apple orchards. Principles to use these local EPN isolates against *E. hirta* in Turkey must be established, perhaps, similar to the way they were examined for the control of an Anastrepha fruit fly pest in guava orchards (Heve *et al*., 2016; 2017; 2018).

This is primarily because pathogenicity of EPNs, which high efficiency of the species against the *E. hirta* pest on blossoming flowers of apple trees may vary between laboratory and field environments. Thus, additional studies are needed to reveal the potential of the more virulent local EPN isolate(s) in apple orchards toward making rational decisions, even though these native isolates have established in Turkey where they are better adapted to changing climatic conditions.

CONCLUSION

In the present study, all local EPN isolates caused between 45 and 80% mortality within the first 96 h after inoculation. Obtained results are promising for field studies of EPNs to control the adults of *E. hirta* considering their surviving ability on the apple trees for 4 days after application (Belair *et al*., 1998). Further studies are needed to reveal the field potential of EPNs in apple orchards. Biotechnical methods such as colour and attractant traps may be used to enhance the efficiency of EPNs and provide better control.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHORS' CONTRIBUTIONS

FA, EY and RC conceived and designed the research. FA and EY conducted the experiments. FA and EY analyzed the data and wrote the manuscript. All authors read and approved the manuscript.

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