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Research on the efficacy of three application techniques of entomopathogenic nematodes against the Colorado potato beetle [*Leptinotarsa decemlineata* (Say), (Coleoptera: Chrysomelidae)] under greenhouse conditions

Entomopatojen nematodların üç uygulama tekniği ile patates böceği [*Leptinotarsa decemlineata* (Say), (Coleoptera: Chrysomelidae)] üzerinde etkilerinin sera koşullarında araştırılması

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A B S T R A C T

Colorado Potato Beetle [*Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae)] (CPB) is one of the most destructive pests of potatoes. CPB is a polyphagous pest that damages every stage of the potato. Although entomopathogenic nematodes (EPNs) in the world have been demonstrated by many laboratories and field/garden studies activity against many harmful groups, very few studies have been conducted on CPB in our country (Türkiye). In the scope of work intended to be used EPNs against CPB. The main objective of the study is to reveal greenhouse-pot applications (soil, green limbs, and cadaver applications) of EPN [*Steinernema feltiae* (isolate 09-31) and *Heterorhabditis bacteriophora* (isolate 09-43)], which are present in our laboratories in our stocks. For the first time in Türkiye, greenhouse-pot applications studies have been carried out against this harmful group. There is also a lot of work in the world about the use of aqueous concentrations of EPNs against harmful effects. In recent years, efforts have been made to use EPNs in cadavers instead of aqueous concentrates. This application (cadaver applications) against CPB is the first working. The results revealed that EPNs performed better in soil applications, and the highest mortality rate was obtained from *S. feltiae* (65.23±4.45 and 77.33±2.59). Other applications (green limbs and cadaver applications) are seen to have a low level of efficacy. In trials, the mortality rate in cadaver applications did not exceed 40%, and the lowest mortality rate in *H.bacteriophora* was 37,40±8,88%. In the case of green devices, the mortality rate did not exceed 30%, and the highest mortality rate was 29.14±6.09 in *H.bacteriophora*. According to EPN greenhouse-pot experiments results, soil applications of *S. feltiae* (isolate 09-31) should be included within the scope of field trials.

INTRODUCTION

The potato (*Solanum tuberosum* L.) is the most important field crop both in Türkiye and the world. However, under current field conditions, potatoes are under attack by a large number of insect pests including aphids, beetles, leafhoppers, and lepidopterous pests. One such pest that negatively impacts potato production is the Colorado Potato Beetle (CPB) [*Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae)]. CPB is a polyphagous pest especially of potatoes that can damage all stages of potato development.

Pests such as the CPB are responsible for the important losses observed during potato production. *L. decemlineata* is a polyphagous pest that damages all above-ground organs of every stage of potato plants. The first and second larval stages consume the leaf epidermis of the plant whereas the third and fourth larval stages feed on the leaf. Importantly, the last instar transitions to a pupa libera after passing into the soil, where it overwinters as an adult.

Adult and all larval stages of CPB feed on the green portions of plants. Especially, last instar larvae cause lots of damage in the field. In the absence of pest control, up to 100% of the crop can be damaged. Additionally, CPB acts as a vector for bacterial ring rot, which can cause significant loss depending on climate conditions (Christie et al. 1991).

Chemical control of the CPB has led to high levels of resistance to insecticides (Forgash 1985, Mota-Sanchez et al. 2000, Stewart et al. 1997) and the contamination of groundwater with aldicarb, carbofuran, and oxamyl (Leach et al. 1986). Moreover, each year the control of CPB becomes more difficult because of the pest's cross-persistence against insecticides (Whalon et al. 2011). For that reason, alternative methods for the chemical control of CPB are required, and a number of investigations are being conducted worldwide. These investigations focused on plant extracts (Hough-Goldstein 1990, Scott et al. 2003, 2004) and biological control agents such as natural enemies including parasitoids, predators, and entomopathogens (Cantwell et al. 1985, Gelernter 1990, Grissel 1981, Lashomb et al. 1987, Obrycki et al. 1987, Özdemir et al. 2021, Toba et al. 1983, Welch 1958, Welch and Briand 1961, Zehnder and Gelernter 1989).

Entomopathogenic nematodes (EPNs) offer a good alternative to chemical insecticides for CPB control (Toba et al. 1983, Welch 1958, Welch and Briand 1961). EPNs, *Heterorhabditis* spp. and *Steinernema* spp., are soil-inhabiting parasites of insects. These nematodes carry symbiotic bacteria in the genera *Photorhabdus* and *Xenorhabdus* spp. (Fam: Morganellaceae) in their intestine; these bacteria help nematodes kill their host within 48 h by producing a copious set of virulence factors in host hemocoel. For years, these organisms have been mass-produced and used effectively as biological control agents against harmful pests, especially against larval stages, in pest management programs (Koppenhöfer et al. 2020, Shapiro-Ilan et al. 2020). This study determined the effects of EPNs [*Steinernema feltiae* (isolate 09-31) and *Heterorhabditis bacteriophora* (isolate 09-43)], which showed promising results in previous laboratory studies (Kepenekci et al. 2013a, Kepenekci et al. 2016), against different larval stages of the CPB (against 3rd larval stage in above-ground applications and last stage larvae in soil applications) in the greenhouse (pot) trials and soil, cadaver and above-ground applications. Many studies have previously assessed the effects of aqueous suspensions of EPNs against pests in the world (Yüksel et al. 2022). Recently, some studies have also applied EPNs in cadavers instead of aqueous concentrations (Shapiro-Ilan et al. 2003). Results from this study as well as information available in the literature will promote the use of EPNs against CPB, and reduce the use of chemical pesticides with negative effects on the environment. EPNs will serve as a sustainable and reliable method of *L. decemlineata* control.

MATERIALS AND METHODS

Nematode and insect sources

Native Turkish EPNs, *Steinernema feltiae* (isolate 09-31) and *Heterorhabditis bacteriophora* (isolate 09-43) were isolated from vegetable gardens and peach orchards in Aydın, Türkiye. Both nematodes were supplied by Prof. Dr. Selçuk Hazır (Adnan Menderes University, Aydın, Türkiye). The nematodes were cultured on the last instar larvae of the wax moth, *Galleria mellonella* (Linnaeus) (Lepidoptera: Pyralidae) at room temperature (23-24 ^{o}C) using methods described by Kaya and Stock (1997). The harvested infective juveniles (IJs) were used within two weeks after emergence in the experiments. The Colorado potato beetle (CPB) was also reared on potato plants. Because studies using populations obtained from the field (natural conditions) provide more practical results as compared to studies using laboratory-reared populations which are more sensitive. In this study, CPB populations were collected from the infested fields in potato cultivated areas and transferred to potato plants in a 1.5 decares area for field studies. CPB populations collected from the field were kept at 10±1 ^{o}C until used in greenhouse-pot studies.

Greenhouse-pot activity studies

Pot trial studies were carried out in the Tokat Gaziosmanpaşa University greenhouse. The plants used were all of the similar sizes in all applications. EPN was applied to the soil and the soil surface area (cm^2) was measured. Soils used in the pot experiment were collected from the potato field, sterilized, and then moistened with water. One potato tuber was planted in each before to use to eliminate dead or live larvae, pupae, and adults. EPNs were applied at a concentration of 25 infective juveniles (IJs) cm^{-2} to the soil. Tap water without nematodes was applied to the plants in the control group. After the applications, the pots were covered with gauze to prevent insects from escaping. Each treatment had 10 pots with one potato plant and the experiment was conducted twice. The 3rd larval stage was used in above-ground applications and the last stage larvae in soil applications. The third larval stage is the most destructive. The fourth stage only stays in the green parts for 2-3 days depending on the climate and nutrition and then descends to the ground.

Above-ground application

EPNs have sprayed at 25 IJs cm^{-2} concentration on the above-ground organs of the plants including the 3rd larval stage (10 insects in each pot). Before this, we made sure that nematodes could be passed through the sieve in the application tools and equipment by conducting a preliminary study. For this purpose, nematode suspensions were sprayed into a Petri dish and the contents were examined under a stereomicroscope. Only water was applied in the controls.

Soil applications

EPNs have applied at 25 IJs cm^{-2} concentration to the soil as in the greenhouse pot experiments. EPN applications were applied on the soil surface as the last stage larvae of the CPB migrate to the soil to pupate and may encounter with IJs in the treated soil. To obtain a concentration of 25 IJs cm^{-2} in pots with a ground surface radius of 8.5 cm, 5.670 IJs in 20 ml of water were applied to each pot (plant or soil surface applications). Only water was applied in the controls.

Cadaver applications

First *G. mellonella* last instar larvae were infected with 500 IJs in 9 cm Petri dishes (5 larvae per Petri dish) and incubated for 6 days for *Steinernema* spp., and 10 days for *Heterorhabditis* spp. Two of these *G. mellonella* larvae infected with EPNs were placed at a depth of 2-3 cm near each potato plant to each pot. Only water was applied in the controls (no cadaver).

Statistical analysis

In the laboratory, greenhouse, and field experiments data were calculated by Abbott formula (Abbott 1925). SPSS Packet Programme was used for data analysis with ANOVA and Duncan Multiple Comparison was used to determine differences in the means among the treatments.

RESULTS AND DISCUSSION

In the first repeat, the highest mortality rate was observed in the treatments with *S. feltiae* and *H. bacteriophora* (65.23 ±4.45% and 41.01±8.05%, respectively) for the soil application. This was followed by cadaver applications with *H. bacteriophora*. The effect of *S. feltiae* cadaver and above-ground application was found to be low [F (2.17) 20.18 P<0.05] (Table 1). In the 2nd repetition of the greenhouse trials, the highest mortality rate was obtained in *S. feltiae* soil application (77.33±2.59%). [F (2.17) 29.52 P<0.05]. The mortality rate in cadaver applications did not exceed 40% with the highest mortality rate of 37.40±8.88% exhibited by *H. bacteriophora*. In the above-ground applications, the mortality rate did not exceed 30% and the highest mortality rate was recorded as 29.14±6.09% in *H. bacteriophora* (Table 1).

Table 1. Mortality of *Leptinotarsa decemlineata* of soil, cadaver, and above-ground applications of *Steinernema feltiae* (isolate 09-31) and *Heterorhabditis bacteriophora* (isolate 09-43) isolates and controls over 10 days from treatment

Application	Mortality±SEM*(%)	
	First repeat	Second repeat
Steinernema feltiae Soil application	65.23 ±4.45 a**	77.33±2.59 a
Heterorhabditis bacteriophora Soil application	40.01±8.53 ab	37.81±5.47 b
S. feltiae Cadaver application	10.78±4.00 c	7.22±3.45 c
H. bacteriophora Cadaver application	37.40±8.88 b	28.14±4.24 b
Control (just water in soil no nematodes and no cadaver)	0.00±0.00 d	0.00±0.00 d
S. feltiae Above-ground application	2.11±1.41 d	6.43±3.15 c
H. bacteriophora Above-ground application	21.84±5.09 b	29.14±6.09 b
Control (just water above-ground application and no nematodes)	0.00±0.00 d	0.00±0.00 d

* SEM: Standard error of the mean;

** Means in a line followed by the same letter are not statistically significantly different (ANOVA P<0.05, Tukey's test).

This study assessed the performance of EPNs [*Steinernema feltiae* (isolate 09-31) and *Heterorhabditis bacteriophora* (isolate 09-43)] which were previously reported as effective in the laboratory studies (Kepenekci et al. 2013a, Kepenekci et al. 2016) against different stages of the CPB (*L. decemlineata*) (3rd larval stage in above-ground applications and last stage larvae in soil applications) in greenhouse conditions. Many studies have applied aqueous suspensions of EPNs against pests in the world. Recently some studies utilized EPN-infected cadavers instead of aqueous suspensions (Shapiro-Ilan et al. 2003). This is also the first report of the use of this approach against CPB under greenhouse (pots) conditions in the Türkiye (soil, cadaver, and above-ground applications).

Until mid-2011, no studies have been conducted on the use of EPNs in the control of CPB in Türkiye (Kepenekci 2012, Kepenekci 2014). Kepenekci et al. (2013a) conducted a preliminary study investigating the potential use EPNs, *Steinernema feltiae*, *S. carpocapsae*, and *Heterorhabditis bacteriophora*, detected in Türkiye against the last-stage larvae of CPB. Trials were conducted under laboratory conditions using two different EPN concentrations (1000 and 2000 IJs insect-1) at 25 °C. The authors observed that *H. bacteriophora* (97.63±6.99) showed the highest effect at 2000 IJs concentration, followed by *S. feltiae* (86.05±11.72%). *S. carpocapsae* was the least effective with 53.34±1.34%. Results obtained indicated that more detailed laboratory studies should be conducted especially under natural conditions. A detailed laboratory study carried out by Kepenekci et al. (2016) with *S. carpocapsae*, *H. bacteriophora*, and *S. feltiae* revealed that these nematodes were 52, 83, and 99% effective, respectively. In the same order, these nematodes applied as infected cadavers were 72, 80, and 99% effective. In another study, Kepenekci et al. (2018) investigate the efficacy of two isolates of *S. carpocapsae* isolates (GOP72 and GOP81) against CPB using infected cadavers trial, different substrates (filter paper and soil) with a single dose and different doses. Experiments were set up at different temperatures, concentrations, and time intervals, to determine the most suitable temperature, density, and time interval in the fight against CPB. In cadaver application, both isolates exhibited more than 60% mortality at 25 ºC which was the most suitable temperature. In dose-response trials, the most effective dose for *S. carpocapsae* GOP81 was 500 IJs application, and for *S. carpocapsae* GOP72 1000 IJs application. After the 7th day in a single-dose experiment with filter paper, the highest mortality rate was caused by *S. carpocapsae* GOP72 isolate. At the end of the 10th day, 100% mortality was detected in both isolates. In the filter paper trials, at the end of the 7th day, insect mortality was 100% at the doses. The effects of *S. carpocapsae* GOP81 isolate in the dose measurement with soil was dose-dependent increasing until the end of the 10th day. The authors stated that it was necessary to switch to greenhouse pot trials and then investigate the effectiveness of these EPNs under natural conditions.

The efficacy of the nematode species [*S. carpocapsae* (Black Sea isolate), *S. feltiae* (Aydın isolate- isolate 09-31), and *H. bacteriophora* (Aydın isolate- isolate 09-43)] used in the study have been evaluated against other insect groups in Türkiye. In a study conducted against the forest pest, *Dendroctonus micans* (Kugelann), (Coleoptera: Scolytidae), *S. feltiae* (Aydin isolate- isolate 09-31), and *H. bacteriophora* (Aydin isolate- isolate 09-43) at 1000 IJ ml^{-1} concentration were 98.04% and 94.04% effective at 25 ºC, respectively. In the same study, *S. carpocapsae* (Black Sea isolate), however, was found to be ineffective as mortality caused did not exceed 40% (Kepenekci and Atay 2014). Atay et al. (2015) demonstrated that *S. carpocapsae* was more effective (89%) than *S. feltiae* and *H. bacteriophora* at different temperatures against *Acanthoscelides obtectus* (Say.) (Coleoptera: Bruchidae), which is an important storage pest. In this case, *S. feltiae* was considered to be ineffective with mortality not exceeding 60%. Likewise, in another study using these nematodes at 1000 IJ concentration against *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae), *S. carpocapsae* caused 96% whereas *H. bacteriophora* presented with 80% mortality at 25 ºC; *S. feltiae* was ineffective with mortality less than 40% (Kepenekci et al. 2013b). The study by Tülek et al. (2015) determined that *S. carpocapsae* caused the highest mortality rate (54%) of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae). They reported that *S. feltiae* and *H. bacteriophora* caused 28% and 12% larval mortality, respectively. Atay and Kepenekci (2015) demonstrated the effectiveness of *S. feltiae*, *S. carpocapsae*, and *H. bacteriophora* against *Holotrichapion pullum* (Gyllenhal) (Coleoptera, Apionidae), an important clover pest, under laboratory conditions. EPNs were applied at 3 concentrations (500, 1000, and 5000 IJs ml-1) and the setup was incubated at two different temperatures (15, 20 ºC). *S. carpocapsae* had the highest mortality rates (80%, 83%, 82%) at all concentrations at 20 ºC, followed by *S. feltiae* (30%, 41%, 35%), and *H. bacteriophora* (24%, 27%, 30%).

Armer et al (2004) reported that *H. marelata* was an effective biological control agent against CPB under laboratory and field experiments but stated that it was not commercially viable due to costs. Trdan et al. (2009) tested *S. feltiae*, *S. carpocapsae*, *H. bacteriophora*, and *H. megidis* against CPB larvae and adults at 15, 20, and 25 °C and at doses of 200, 1000, and 2000 IJs under laboratory conditions. All nematodes were found effective on CPB at 20, and 25 °C. Adel and Hussein (2010) assessed the effects of *S. feltiae* and *H. bacteriophora* against CPB and found that *H. bacteriophora* was more effective against the 4th stage larvae compared to *S. feltiae*. Kary et al. (2010), evaluated 4 different geographic isolates of *H. bacteriophora* and 3 different species of *Steinernema* (*S. bicornutum*, *S. carpocapsae* and *S. feltiae*) against CPB using 5 different concentrations and 3 different methods. The highest mortality was caused by *H. bacteriophora*. Laznik et al. (2010) tested a Slovenian strain of *S. feltiae*, commercial product Entonem (*S. feltiae*), and insecticide thiamethoxam against CPB under field conditions. At the end of the experiment both EPNs were found to be effective biological agents, especially in the larval stage of CPB. In our study, *S. feltiae* was more effective against CPB than *H. bacteriophora*.

In our study, the nematodes had little effect on CPB when applied to aboveground parts of plants. *S. feltiae* was 2.11-6.63% effective, whereas was *H. bacteriophora* 21.84%-29.14% effective. The main reasons for the low success of EPNs in such applications are that nematode IJs are sensitive to drying (Lello et al. 1996), high temperatures (Grewal et al. 1994), and ultraviolet radiation (UV) (Gaugler et al. 1992, Gaugler and Boush 1978). Most EPNs are ineffective at temperatures exceeding 32 ºC. Tolerance to drying and UV also differs from different EPN species. According to Lello et al. (1996), the effect of sunlight can be minimized by applying nematodes at dusk. Although adjuvants generally increase nematode activity (Bauer et al. 1997, Broadbent and Olthof 1995, Eidt 1991, Glazer et al. 1992, MacVean et al. 1982), this level of increase is generally considered insufficient to be recommended green component applications (Bauer et al. 1997, Mason et al. 1998). However, other avenues to optimize the use of EPNs for more service in the biocontrol of such insect pests (Abd-Elgawad 2019) and exploit available opportunities for their effective applications (Askary and Abd-Elgawad 2021) should be sought.

According to the results, the highest mortality rate was obtained in *S. feltiae* (65.23 ±4.45% and 77.33±2.59%) when EPNs were applied to the soil. It was seen that the effect was lower than in other applications (above-ground and cadaver applications). According to the results of the EPN greenhouse-pot test, it would be appropriate to conduct field trials with *S. feltiae* (isolate 09-31).

Many studies have used aqueous concentrations of EPNs against harmful insects in the world. In recent years, efforts to use EPN-infected cadavers instead of aqueous concentrate are increased. This study was also used against CPB in the greenhouse (pots) studies different three applications (soil, cadaver, and above-ground applications) using EPNs [*Steinernema feltiae* (isolate 09-31) and *Heterorhabditis bacteriophora* (isolate 09-43)] for the first time in the world. According to EPN greenhouse-pot experiments results, field trials should be conducted with *S. feltiae*. EPNs can serve as a sustainable and reliable method of *L. decemlineata* control.

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ÖZET

Patates üretimini azaltan zararlı organizmalardan biri de patates böceği [*Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae)] (PB)'dir. Patates bitkisinin her dönemine zarar veren polifag bir zararlıdır. Dünya'da entomopatojen nematodların (EPN) birçok zararlı grubuna karşı etkinliği laboratuvar ve tarla/bahçe çalışmaları ile ortaya konulmuş olmasına karşın ülkemizde PB ile ilgili çok az çalışma yapılmıştır. Yapılan bu çalışma kapsamında PB ile mücadelede EPN'lerin kullanılması amaçlanmıştır. Çalışmanın ana hedefi, laboratuvar stoklarımızda mevcut olan EPN [*Steinernema feltiae* (izolat 09-31) and *Heterorhabditis bacteriophora* (izolat 09-43)]'lerin toprak, yeşil aksam ve kadavra uygulamaları şeklinde sera-saksı denemeleri oluşturmuştur. Türkiye'de ilk defa bu zararlı grubuna karşı sera-saksı çalışmaları yürütülmüştür. Ayrıca dünyada EPN'lerin sulu konsantrasyonlarının zararlılara karşı kullanımı ile ilgili çok çalışma bulunmaktadır. Son yıllarda sulu konsantrasyonların yerine kadavra içinde EPN'lerin kullanılması çalışmaları başlamıştır. PB'ye karşı bu uygulama (kadavra uygulamaları) ilk çalışma niteliğindedir. Araştırma sonucu elde edilen sonuçlara göre EPN'lerin toprağa yapılan uygulamalarda en yüksek ölüm oranı *S. feltiae* (65.23±4.45 ve 77.33±2.59)'da elde edilmiştir. Diğer uygulamalar (yeşil aksam ve kadavra uygulamaları)'da etkisinin düşük düzeyde olduğu görülmektedir. Deneme sonuçlarında, kadavra uygulamalarında ölüm oranı %40'ı geçmemiş ve en yüksek ölüm oranı *H. bacteriophora*'da %37.40±8,88 olarak tespit edilmiştir. Yeşil aksam uygulamalarında ise ölüm oranı %30'u geçmemiş ve en yük ölüm oranı *H. bacteriophora*'da 29.14±6.09 olarak tespit edilmiştir. EPN sera-saksı deneme sonuçlarına göre *S. feltiae* (izolat 09-31)'nın toprak uygulamalarının arazi denemeleri kapsamına alınması uygun olacaktır.

Anahtar kelimeler: entomopatojen nematodlar, patates böceği, *Leptinotarsa decemlineata*, biyolojik mücadele.

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