Pakistan J. Zool., vol. 45(5), pp. 1415-1422, 2013

Biological Control Potential of Native Entomopathogenic Nematodes against the Potato Tuber Moth, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae) in Turkey

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Abstract.- The biocontrol efficiency of three Turkish isolates of the entomopathogenic nematodes *Steinernema carpocapsae, S. feltiae* and *Heterorhabditis bacteriophora* were evaluated against the last instar of potato tuber moth (PTM) *Phthorimaea operculella* under laboratory conditions. To determine optimum nematode application rate and temperature, the experiments were conducted with 100, 500 and 1000 IJs at 10, 15 and 25°C. Temperature and nematode concentration had a significant effect on *P. operculella* larval mortality. *S. carpocapsae* and *H. bacteriophora* species displayed generally increased virulence in parallel with rising temperature and the number of infective juveniles applied. At 25°C and 1000 IJs concentration, the larval mortality was 96 and 80% for *S. carpocapsae* and *H. bacteriophora*, respectively. However, *S. feltiae* did not exhibit more than 40% mortality at any temperature or concentration, except when the nematodes were applied in infected insect host cadavers. At 25°C, infected cadaver applications showed 97, 83 and 67% mortality for *S. carpocapsae*, *H. bacteriophora* and *S. feltiae*, respectively. Our results indicate that *P. operculella* larvae are quite susceptible to entomopathogenic nematode infection and, in particular, *S. carpocapsae* blacksea strain has a high level of potential to control this pest.

Key words: Entomopathogenic nematodes, potato tuber moth, *Phthorimaea operculella*, biological control, *Steinernema carpocapsae, Steinernema feltiae, Heterorhabditis bacteriophora.*

INTRODUCTION

Potato (*Solanum tuberosum* L.) is the most important vegetable crop in Turkey. Under field conditions, potato plants are under attack by a large number of insect pests such as aphids, leafhoppers, and lepidopterous pests. The potato tuber moth (PTM), *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae) is the most destructive pest. In addition to potato, *P. operculella* also attacks other solanaceous plants such as tomato, tobacco, eggplant and pepper in tropical and subtropical countries. In potato fields, the female *P. operculella* lays its eggs on the underside of the potato leaves or on the exposed tubers; in field that use a flooding irrigation system, eggs are laid mainly after irrigation is applied (Mandour, 1997). *P. operculella* larvae mine the foliage, stems, and tubers (Islam *et al.*, 1990; Mandour, 1997). The larvae of *P. operculella* also form blotches in leaves and fold leaves over for shelter during feeding and for pupation (Mandour, 1997). The new mines are always present in the upper part of the potato canopy. The old blotches, harboring the older larvae of *P. operculella*, are likely to be found the lower parts of the potato canopy particularly near the soil surface. Larvae may also bore into potato stems and petioles (Mandour, 1997). There is usually a 10% rate of tuber infestation by *P. operculella*, but when control methods are not used, the infestation rate may reach 100% (Shelton and Wayman, 1979; Sileshi and Teriessa, 2001). In potato storage facilities, infestation of *P. operculella* also causes partial or complete rotting by the subsequent infestation with fungi and/or bacteria, which renders the infested tubers unmarketable (Shelton and Wayman, 1979; Sarhan, 2004).

Until the last two decades, the control of *P. operculella* has relied upon the use of the traditional insecticides (Sarhan, 2004; Keasar and Sadeh, 2007). Recently, biological control of *P. operculella* using bioinsecticides and natural insect enemies has become important in potato protection, either in the field (Sileshi and Teriessa, 2001; Agamy, 2003) or

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0030-9923/2013/0005-1415 $ 8.00/0

in potato storage (Farrag, 1998; Moawad *et al.*, 1998; Mandour *et al.*, 2009), and has gained more credibility for controlling this pest. Increased research efforts have been taking place for integration using natural enemies like parasitoids, predators and entomopathogens (Mandour *et al.*, 2008).

One of the most successful groups of biological control agents against soil insect pests are the entomopathogenic nematodes (EPNs) in the families Steinernematidae and Heterorhabditidae. Nematodes in both families are obligate insect-parasitic organisms and mutualistically associated with bacteria from the genera *Photorhabdus* (heterorhabditids) and *Xenorhabdus* (steinernematids) that are carried within the nematode digestive tracts (Kaya and Gaugler, 1993). Infective juvenile (IJ) stages of the nematodes search an adequate host in the soil and enter the insect host through natural openings (mouth, anus, and spiracles) or through the cuticle. The symbiotic bacteria are released into the insect hemocoel when the nematode enters the target insect host (Dowds and Peters, 2002). The bacteria multiply and produce toxins in insect hemocoel. The nematodes also contribute to this procedure and insect host is killed within 48 h by septicemia and toxemia (Kaya and Stock, 1997; Duchaud *et al.*, 2003). Once nutrients exhausted in the insect cadaver, progeny nematodes develop into the IJ stage and emerge from the cadaver into the soil to search for another host (Griffin *et al.*, 2005).

A number of soil pests are potential candidates for biological control in Turkey using entomopathogenic nematodes (Hazir *et al.*, 2003; Kepenekci, 2012). One of them is the Potato tuber moth, *Phthorimaea operculella.*

In this paper, we evaluated the control potential of three Turkish nematodes against last instar larvae of *Phthorimaea operculella* in the laboratory: *Steinernema carpocapsae* Weiser (Blacksea isolate), *S. feltiae* Filipjev (Aydin isolate) (Rhabditida: Steinernematidae) and *Heterorhabditis bacteriophora* Poinar (Aydin isolate) (Rhabditida: Heterorhabditidae). Nematodes were applied to the soil in aqueous suspension as well as in infected insect cadavers. In addition, we determined the optimal temperature and nematode concentration for infection. These studies will determine which of the three native nematode species have the best potential for biological control of *P. operculella*. The use of native EPN species may increase the success of the application (due to adaptation to the local environment) and avoids the introduction of exotic species or strains that could affect the ecological equilibrium of native EPN populations (Akhurst and Smith, 2002).

MATERIALS AND METHODS

Nematode sources

Native entomopathogenic nematodes *Steinernema feltiae* (isolate 09-31) from a vegetable garden in Aydin, *S. carpocapsae* (Blacksea isolate) from grassland in Rize and *Heterorhabditis bacteriophora* (isolate 09-43) from peach orchard in Aydin were used in the experiments. The nematodes were cultured in last instar wax moth, *Galleria mellonella* (Pyralidae: Lepidoptera) larvae at room temperature (23–24^{o}C) using methods described by Kaya and Stock (1997). *G. mellonella*, was reared in the laboratory using an artificial medium containing 11% honey, 11% glycerol, 22% ground wheat, 22% ground maize, 11% milk powder, 5.5% yeast extract and 17.5% bee wax in a glass jar at 25±4°C (Han and Ehlers, 2000). *G. mellonella* larvae infected by the nematodes were placed on White traps (White, 1927), and the new IJs emerging from cadavers were harvested. Collected IJs were rinsed three times in sterile distilled water and each species kept separately in 1 L juice boxes (Gulcu and Hazir, 2012) before being stored at 10 °C. The harvested IJs were used within two weeks after emergence for the experiments.

Insect sources

The last instar larval stages of *P. operculella* were obtained from the laboratory colony maintained at the entomology division, Plant Protection Central Research Institute in Ankara, Turkey. The larvae were reared on potato tubers in a rearing room at 28±2°C, 14:10 L:D. The fourth instars were used within two hours in the experiments. *Tenebrio molitor* larvae were reared in laboratory conditions and the last instars were used in cadaver applications.

Effect of temperature and nematode concentration on P. operculella *larval mortality*

Given that *P. operculella* larvae occur in the soil for at least 7-8 months from September to April (Çalışkaner *et al.,* 1989), we conducted temperature studies with different nematode concentrations. Plastic cups (6 cm deep and 6.5 cm diam) were used for the experiments. A hundred cm^3 (approximately 145 g) autoclaved and air-dried sandy soil (70% sand, 15% silt, 15% clay, pH = 6, organic matter = 2%) was placed into each cup. The soil was obtained from a potato growing area in Nevşehir, Turkey where *P. operculella* infestation was very high. A single last-instar was placed at the bottom of each cup. The cups were left overnight at the targeted temperature to equilibrate before nematodes were introduced. The soil moisture level was adjusted to 10% (w/w) by adding distilled water. Control cups were prepared as above except that water only was added to the cups. Four different nematode concentrations *viz.*, 0, 100, 500 or 1000 Ijs were applied to the cups, which were then placed in incubators at 10, 15 or 25°C. Ten days after nematode treatment, the soil in each cup was poured out and larval mortality was recorded. Ten replicates were used for each nematode concentration and each temperature regimes. The experiments were repeated three times on different dates.

Evaluation of insect cadavers containing IJs against P. operculella *larvae*

The same experimental design and temperature regimes were used for the nematode-infected cadaver applications. Instead of using free living IJs, insect cadavers containing nematodes were tested. Five *Tenebrio molitor* larvae were placed to 9 cm plastic petri dishes and exposed to 500 IJs for two days. Infected cadavers were transferred to White trap system and one 6-day-old *T. molitor* cadaver was buried in the soil at 2 cm depth for *Steinernema* species whereas, one 10-day-old insect cadaver was used for *H. bacteriophora* species. Ten replicates were used for each nematode species. The experiments were repeated three times on different dates.

Statistical analysis

One-way ANOVA was used to compare the mortality of *P. operculella*. Means were compared at the P=0.05 level, and Tukey's test was used to separate means (SPSS, 1999). Arcsine transformation was carried out on mortality (%) before analyses.

RESULTS

The data generally showed that all native nematodes were effective against the *P. operculella* larvae (Figs, 1, 2).

Effect of temperature and nematode density on P. operculella *larval mortality*

Temperature and nematode concentration had a significant effect on the larval mortality of *P. operculella*. *S. carpocapsae* and *H. bacteriophora* species displayed generally increased virulence in parallel with rising temperature and the number of applied IJs (Fig.1a,b,c). At 10^oC, *Steinernema* species showed significantly higher mortality than *Heterorhabditis bacteriophora*. At the lowest IJ concentration (100 IJs), *S. feltiae* showed the highest mortality followed by *S. carpocapsae* and *H. bacteriophora*. However, there was no significant difference between *S. feltiae* and *S. carpocapsae*. Significant difference was observed between *S. feltiae* and *H. bacteriophora* (F= 7.13; df= 3,8; P<0.05). *Steinernema* species produced significantly more mortality than control. However, no significant difference was observed between *H. bacteriophora* and control (Fig 1a). At the intermediate IJ concentration (500 IJs), *S. carpocapsae* and *S. feltiae* displayed the highest virulence. There was no significant difference between *S. carpocapsae* and *S. feltiae* species whereas, significantly higher mortality was observed between the steinernematids and *H. bacteriophora* and control (F= 58.74; df= 3,8; P<0.05). But no significant difference was observed between *H. bacteriophora* and control (Fig 1a). At the highest nematode concentration (1000 IJs), the lowest virulence was observed in *H. bacteriophora*. However, *S. carpocapsae* was the most virulent species and *S. feltiae* demonstrated an intermediate level of virulence (F= 79.67; df= 3,8; P<0.05). All nematode species produced significantly more mortality than control (Fig. 1a). Compared with

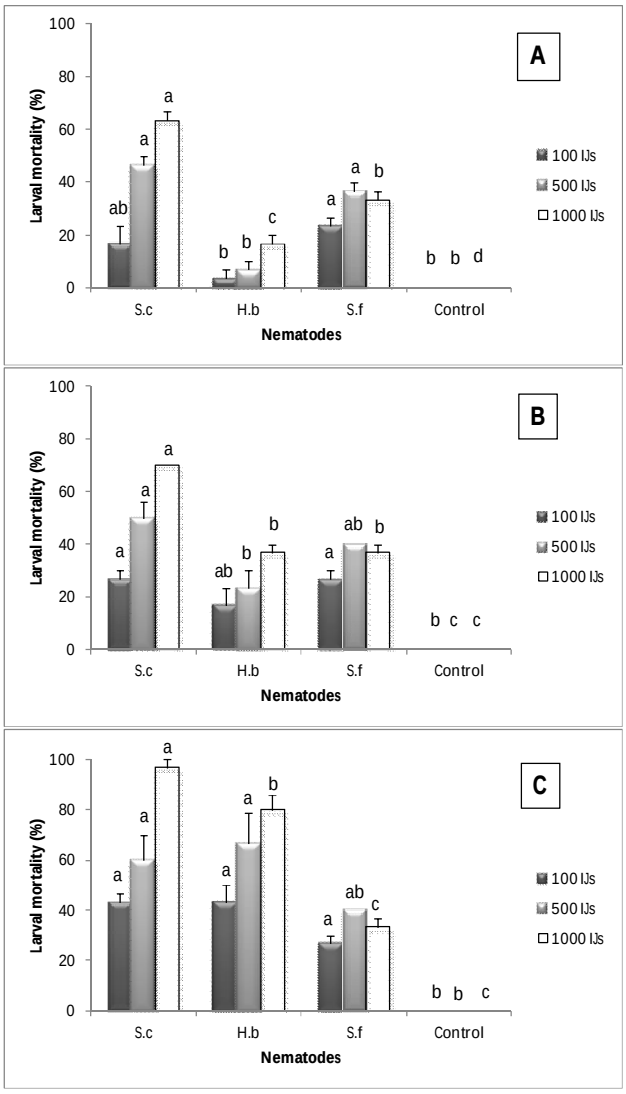

Fig. 1. Mortality (%) of *Phthorimaea operculella* larvae following application of entomopathogenic nematodes *Steinernema carpocapsae* (S.c), *S. feltiae* (S.f) and *Heterorhabditis bacteriophora* (H.b) (isolated from Turkey) at 10°C (a), 15°C (b) and 25°C (c). Data are expressed as mean±SEM. The same letter above the error bars indicates no significant difference (P>0.05; Tukey test).

10°C, higher larval mortality was obtained at 15°C for *H. bacteriophora* species. *S. carpocapsae* and *S. feltiae* produced slightly more mortality at 15°C. At the 100 IJ concentration, *S. carpocapsae* and *S. feltiae* showed numerically more mortality than *H. bacteriophora* but there was only significant difference between steinernematids and control (*F*=

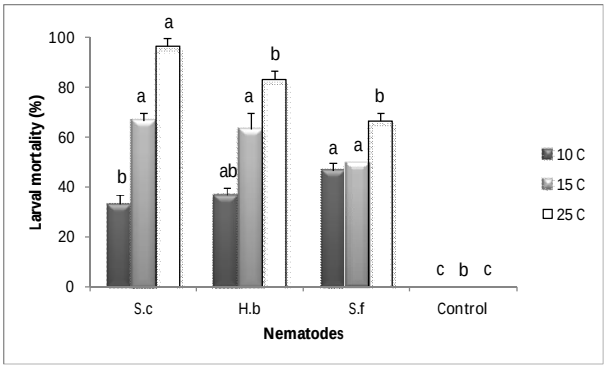

Fig. 2. Mortality (%) of *Phthorimaea operculella* larvae following application of insect cadavers infected with entomopathogenic nematodes *Steinernema carpocapsae* (S.c), *S. feltiae* (S.f) and *Heterorhabditis bacteriophora* (H.b) at different temperatures. Data are expressed as mean±SEM. The same letter above the error bars indicates no significant difference (P>0.05; Tukey test).

9.26; df= 3,8; *P*<0.05) (Fig. 1b). At the 500 IJ concentration, no significant difference was observed between *S. carpocapsae* and *S. feltiae*. However, percentage mortality was significantly different between *S. carpocapsae* and *H. bacteriophora*. All nematode species showed statistically more mortality than control (*F*= 22.98; df= 3,8; *P*<0.05) (Fig. 1b). At the 1000 IJ concentration, *S. carpocapsae* caused higher mortality than *S. feltiae* and *H. bacteriophora*, (*F*= 158.13; df= 2,5; *P*<0.05). No significant difference was observed between *S. feltiae* and *H. bacteriophora*. All nematode species produced more mortality than control (Fig. 1b). At the highest temperature (25°C), no significant differences were observed among the nematode species at 100 IJ concentration. There was a significant difference between nematode treatments and control (*F*= 22.85; df= 3,8; *P*<0.05) (Fig. 1c). At 500 IJ per insect, although numerically more mortality produced by *S. carpocapsae* and *H. bacteriophora* than *S. feltiae*, no significant difference was observed among the nematode species. Statistically more mortality was seen between the first group (*S. carpocpsae* and *H. bacteriophora*) and control (*F*= 9.55; df= 3,8; *P*<0.05) (Fig. 1c). At the highest nematode concentration, *S. carpocapsae* was the most virulent caused 96.6% mortality followed by

H. bacteriophora (80%) and then *S. feltiae* (33.3%). There was a significant difference among the nematode species (F= 47.10; df= 3,8; P<0.05), but no statistically difference was observed between *S. feltiae* and control (Fig. 1c).

Evaluation of insect cadavers containing IJs against P. operculella *larvae*

The insect cadaver application method also displayed a high level of virulence to *P. operculella* larvae. Percentage mortality was only significantly different between *Steinernema* species at 10°C (F=44.78; df:3,8; P<0.05). *H. bacteriophora* was intermediate and all nematode species produced more mortality than control (Fig. 2). At 15°C, significant difference was only observed between nematode treatments and control group (F= 50.50; df: 3,8; P<0.05) (Fig. 2). At 25°C, *S. carpocapsae* caused the highest mortality (97%) following with *H. bacteriophora* (83%) and *S. feltiae* (67%) (F= 49.58; df: 3,8; P<0.05). However, no statistically difference was observed between *H. bacteriophora* and *S. feltiae*. All nematode treatments produced more mortality than control (Fig. 2).

DİSCUSSİON

For biological control of *P. operculella,* research has mainly focused on the bacterium *Bacillus thuringiensis* subsp. *kurstaki* and granulovirus (PhopGV) (Arthurs *et al.,* 2008a, b; Lacey *et al.,* 2010). A number of studies have been conducted on the efficacy of entomopathogenic nematodes against *P. operculella* larvae, prepupa, pupa and adult stages. It was reported that the larva and prepupa were susceptible whereas, the pupae and adult stages were the resistant to nematode infection (Ivanova *et al.,* 1994; Sweelam *et al.,* 2010; Hassani-Kakhki *et al.,* 2013).

Our results indicate that fourth instar *P. operculella* are highly susceptible to some of the Turkish entomopathogenic nematode species with *S. carpocapsae* species being more virulent than *H. bacteriophora* and *S. feliae.* Although *S. carpocapsae* produced a higher level of mortality, *H. bacteriophora* was also effective against *P. operculella* larvae causing 80% mortality at 25°C and 1000 IJ per insect. Hassani-Kakhki *et al.* (2013) conducted a research in Iran and reported that *S. carpocapsae* (commercial strain provided by Koppert) and *H. bacteriophora* (commercial and FUM 7 Iranian strains) were more virulent than native strain of *S. feltiae* and *S. glaseri.* They also demonstrated that nematode strain and concentration had significant effects on the fourth instar mortality of *P. operculella* and *S. carpocapsae* exhibited higher mortality than *H. bacteriophora* in column assays.

We confirmed that IJ concentration has a significant effect on the efficacy of tested nematodes against *P. operculella* larvae. There was a direct and positive relationship between the concentration of *S. carpocapsae* and *H. bacteriophora* IJs and mortality of *P. operculella* larvae. However, *S. feltiae* was an exception in that no differences were detected based on concentration. Sweelam *et al.* (2010) tested five different nematode concentrations and reported that *S. carpocapsae* species controlled larvae of *P. operculella* along the five days with 74 % mortality at 2000 infective juvenile individuals per 10 larvae. It was clear that the infectivity of nematodes against *P. operculella* larvae was related with temperature. We noted that the virulence of *S. carpocapsae* and *H. bacteriophora* increased, whereas the virulence of *S. feltiae* decreased or remained the same from low (10 and 15°C) to high (25°C) temperatures. In general, *Steinernema* species were more virulent than *H. bacteriophora* at 10 and 15°C whereas *H. bacteriophora* showed higher virulence than *S. feltiae* at 25°C. It is known that *S. carpocapsae* and *H. bacteriophora* species are more geared to moderate temperatures whereas, *S. feltiae* is a low temperature adapted species and active at lower temperature (Hominick *et al.,* 1996; Hazir *et al.,* 2001). However, at 1000 IJ concentration, *S. carpocapsae* also showed higher mortality than *S. feltiae* at 10 and 15°C.

Hassani-Kakhki *et al.* (2013) reported that *S. carpocapsae* and two *H. bacteriophora* strains (commercial and FUM7) were more virulent than *S. feltiae* and *S. glaseri* against the L4 stage of *P. operculella*.

S. carpocapsae is particularly effective against lepidopteran larvae including various webworms, cutworms, armyworms, girdlers, some

weevils, and wood-borers and *H. bacteriophora* species attacks mainly to lepidopterous and coleopterous larvae among other insects. *S. feltiae* is especially effective against immature dipterous insects, including mushroom flies, fungus gnats, and tipulids as well some lepidopterous larvae (Grewal *et al.*, 2005). Infectivity of nematode species to hosts has been correlated with their foraging behavior and responses to host cues (Grewal *et al.*, 1994; Lewis, 2002; Lewis *et al.*, 1992). *S. carpocapsae* is an "ambush" forager, standing on its tail in an upright position near the soil surface and attaching to passing hosts. *H. bacteriophora* is a “crusier” characterized by high motility and distribution throughout the soil profile. *S. feltiae* is situated between these two extremes, placing them as intermediate foraging strategists (Campbell and Gaugler, 1997). These intermediate strategists are adapted to infecting insects that occur just below the soil surface, such as prepupae of lepidopterous insects, fungus gnats, or weevil larvae (Hazir *et al.*, 2003).

From an applied standpoint, entomopathogenic nematodes are usually applied in aqueous suspension through a variety of agricultural spray equipment or irrigation systems. As an alternative approach to applying nematodes, recent studies showed that nematodes could be formulated and successfully applied as infected cadavers (Shapiro-Ilan *et al.*, 2001). Our results indicated that *T. molitor* cadavers containing IJs were an effective application method. Shapiro-Ilan *et al.* (2003) compared the efficacy of infected cadavers in greenhouse conditions. The diaprepes root weevil, *Diaprepes abbreviatus* (L.) and the black vine weevil, *Otiorhynchus sulcatus* (F.) were the targeted insect pests. Aqueous suspension and *T. molitor* cadavers containing IJs of *H. indica* (Hom 1 strain) and *H. bacteriophora* (Oswego strain) were used against *D. abbreviatu* and *O. sulcatus* respectively. The survival of both *D. abbreviatus* and *O. sulcatus* was lower in the infected cadaver treatment than the aqueous applications. This superior efficacy in the cadaver application was explained by additional physiological stress in aqueous application and/or some compounds in the infected host cadaver that could enhance nematode infectivity or dispersal (Shapiro-Ilan *et al.*, 2003).

In conclusion, our study indicates that *S. carpocapsae* (Blacksea isolate) has great potential to control of *P. operculella* larvae and future work is necessary to determine the efficacy and feasibility of *S. carpocapsae* (Blacksea isolate) in aqueous suspension and infected cadaver formulation against *P. operculella* larvae under the field condition.

ACKNOWLEDGMENT

We wish to thank Prof. Dr. David Shapiro-Ilan for critical reading and editing of the manuscript.

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(Received 25 July 2013, revised 7 August 2013)