Research Article

Investigation of the Effectiveness of Some Entomopathogenic Nematodes (*Steinernema feltiae*-Balıkesir İsolate and *Heterorhabditis bacteriophora*-Çanakkale İsolate) Against Potato Moth [*Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae) by Greenhouse-Potting Experiments

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Abstract | The potato tuber moth, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae) (PTM) is the most destructive pest. 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. This study assessed the effects of EPNs (Nematoda: Steinernematidae) species [*Steinernema feltiae* (Balıkesir isolates) and *Heterorhabditis bacteriophora* (Çanakkale isolates) (Nematoda: Heterorhabditidae)] detected in our country (Turkey) against the larvae and pupae of *P. operculella* in greenhouse-pot experiments. *S. feltiae* was the most effective killing 6.33±0.61 (63.30%) larvae, whereas *H. bacteriophora* only killed 3.67±0.56 (36.70%) dead larvae. 0.17±0.17 (1.70%) larvae died in control group after 10 days and 9.33±0.33 (93.30%) developed into the pupa. For pupal stages, *H. bacteriophora* was more effective causing 48.30% (4.83±0.60) pupal mortality whereas *S. feltiae* caused 35.00% (3.5±0.72) mortality. 1.00±0.36 (10.00%) dead pupa was detected at the end of 10 days in the control groups. Although *S. feltiae* was more effective than *H. bacteriophora* against potato moth larvae, *H. bacteriophora* was more effective than *S. feltiae* in applications against pupae. This research, which is the first study carried out in greenhouse-pot conditions on the use of EPNs in the control of *P. operculella* in Turkey, shows that more detail studies on the applications of EPNs with promising effects (*S.feltiae* Balıkesir isolate applications with a 63.30% mortality in larvae) under field conditions be conducted.

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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, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae) (PTM) 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. 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 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).

Entomopathogenic nematodes, fungi, viruses and bacteria are pathogenic organisms that kill insects, hence are used as microbial pesticides in the control of destructive pests in both field and storage conditions (Alcázar *et al*., 1992; BenSalah and Aalbu, 1992; Das *et al*., 1992; Raman, 1994; Kroschel *et al*., 1996; Lery *et al*., 1997; Roux *et al*., 1992; Setiawati *et al*., 1999). Although several researchers have demonstrated the potential use of microbial pesticides in the control of *P. operculella*, which is an important insect pest that feeds on potato crops, none has been developed and widely used as a commercial microbial pesticide (Lacey and Arthurs, 2005; Arthurs *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).

Soil-dwelling nematodes of the genera, *Steinernema* (Family: Steinernematidae) and *Heterorhabditis* (Family: Heterorhabditidae), are obligate pest of soil insects. These organisms are present in soil worldwide. The infective juvenile (IJ) stage is mass-produced and applied as biological control agents of harmful insects living in soil and those hiding in cryptic habitats in pest management programs. Several studies around the world have assessed the effects of these nematodes on insect pest (Gulcu *et al*., 2017), however not enough research on the effects of EPNs on pest groups of economic importance exists in Turkey, which has a high species diversity in its different region. In addition, it is extremely important to simulate promising results from laboratory experiments under greenhouse and natural conditions studies, and this study was carried out as greenhouse-pot studies.

Until mid-2011, no study was found on the use of EPNs in the fight against *P. operculella* in Turkey (Kepenekci, 2012, 2014). Subsequent to these studies, in *vitro* (laboratory) studies were conducted to assess the efficacy of local EPN isolates [*S. affine* (isolate-47), *S. carpocapsae* (Blacksea isolate), *S. carpocapsae* (isolate-1133), *S. feltiae* (Aydin isolate), *S. feltiae* (isolate-96), *H. bacteriophora* (Aydin isolate), *H. bacteriophora* (izolat-12)] against the last instar

of *P. operculella* and effective results were obtained (Kepenekci *et al*., 2013; Gözel *et al*., 2020).

Different studies have determined the activities of EPNs on *P. operculella* (Ivanova *et al*., 1994; Sweelam *et al*., 2010; Hassani-Kakhki *et al*., 2013; Abdelmonem *et al*., 2018; Moawad *et al*., 2018; Mhatre *et al*., 2020; Yan *et al*., 2020; Ebrahimi *et al*., 2021). Some studies also use EPNs to supplement chemical applications against *P. operculella* (Kary *et al*., 2018). Insects of the order Lepidoptera are highly sensitive hosts for *Steinernema* and *Heterorhabditidis* nematodes (Vashisth *et al*., 2013), therefore, many studies have been carried out on their effectiveness and use potential in both laboratory and natural conditions.

This study investigated the activities of local EPN species [*Steinernema feltiae* (Balıkesir isolates) and *Heterorhabditis bacteriophora* (Çanakkale isolates) (Nematoda: Heterorhabditidae)] (two native nematode species) against *P. operculella* larvae and pupae under greenhouse (pot) conditions. It is the first study conducted in greenhouse (pot) conditions on the use of EPNs in the control of *P. operculella* in Turkey.

Materials and Methods

Nematode sources

Native Turkish entomopathogenic nematodes, *Steinernema feltiae* (Balıkesir isolate) from the pine forest in Balıkesir and *Heterorhabditis bacteriophora* (Çanakkale isolate) from the poplar planted areas in Çanakkale, Turkey were obtained and supplied by Prof. Dr. Uğur GÖZEL (Çanakkale Onsekiz Mart University, Çanakkale, Turkey). The nematodes were cultured in last instar wax moth, *Galleria mellonella* (Lepidoptera: Pyralidae) larvae at room temperature (23–24°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 infective juveniles (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 potato tuber moth, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae) (PTM) can always be produced under laboratory or greenhouse conditions. Generally, they are hard to find due to pesticide application done in commercial potato growing areas. Such production areas for commercial purposes are densely located in the province of Tokat (Turkey). In the study, PTM populations were collected from infested fields and brought to the Entomology laboratory at the Department Plant Protection, Faculty of Agriculture in Tokat Gaziosmanpaşa University (TOGÜ) (Tokat, Turkey). These insects were cultured at 14:10 L:D in insect breeding cabinet. Infected potato tubers brought from the field were placed in seven-liter plastic round jars which was covered with a net and blotting paper and the adults were allowed to eggs. Eggs left on blotting paper were separated and placed on new potato tubers in different jars; the hatched larvae were allowed to infect and develop on potato tubers until the fourth instar stage which were used within two hours in the experiments.

Cultivation of potato plant

Potato plants were grown in sterile soil in round pots (22×20) in TOGU greenhouse using potato tubers.

Greenhouse-pot activity studies

Cultured and produced PTM were infected with potato plants grown in pots under greenhouse conditions. For this purpose, 10 PTM last-stage larvae and pupae were added to pots with potato tuber buried in the soil in the storage containers. Trials were set up separately for late-stage larvae and pupae. The studies were carried out as pot trials in the TOGU greenhouse under controlled conditions.

Prior to use, the soils used in the experiments were taken from the potato field, sterilized and then moistened with water. One potato tuber was buried in the soil in each storage container filled to the rim with potting soil. The mouths of these storage containers (10×8 cm) were tightly covered with cheesecloth to prevent larvae or adult escape and buried in the soil. Routine watering of the potato plants in the large pots continued throughout the trials. In order to easily detect dead or live PTM larvae and pupae, the

soils used was sieved using a sieve with a suitable hole spacing. Each treatment had 3 pots with one potato plant and the experiment was conducted twice.

Three hundred ml sterile soil (approximately 445-480 g) was placed in a 500 ml storage container, one potato tuber (tubers weighing 75-84 g) and 10 PTM larvae (average weight of the larva 0.136 g) were added to these pots. EPN was applied by, considering the soil surface and adding EPNs at 25 infective juveniles (IJs) cm^{-2} concentration [80 cm^2 (soil surface) × 25 IJs cm^{-2} = 2000 IJs pot^{-1}] (200 IJs larvae or $pupa^{-1}$). Only water was used as a control.

After 10 days the cheesecloth on the container treated with EPNs in greenhouse conditions was removed. The soil in the containers was sieved and the live and dead PTM larvae, pupae and adults in the pots were counted and recorded. Finally, the potato tubers in the pots were visually inspected and, if necessary, they were crushed to see if the PTM larvae were in the tubers. Dead larvae (cadavers) were placed on the "White trap" system (White, 1927) in order to observe EPNs emergence. In the event that EPN IJs did not emerge from the cadavers, the cadavers were disintegrated to determine if nematodes were the cause of death. In the pupa applications, the adults in the containers were recorded first, and the procedures performed against the larvae were repeated for the immature pupae.

Statistical analysis

The % mortality values obtained in the studies were corrected using the Abbott formula (Abbott, 1925). Difference in the means of the data obtained was determined using ANOVA and means were separated using Duncan multiple comparison method.

Results and Discussion

In this study, the activities of EPN species [*Steinernema feltiae* (Balıkesir isolates) and *Heterorhabditis bacteriophora* (Çanakkale isolates) (Nematoda: Heterorhabditidae)] previously detected in Turkey and available in our laboratory stocks was assessed against larvae and pupa of the potato tuber moth, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae) (PTM) under greenhouse (pot experiments) conditions.

In the control group 0.17±0.17 (1.70%) *P. operculella* larvae died whereas 9.33±0.33 (93.30%) developed into pupal stage at the end of 10 days. In EPN treated groups, *S. feltiae* (Balıkesir isolate) was most effective; 6.33±0.61 (63.30%) dead larvae were found in these pots. With 3.67±0.56 (36.70%) dead larvae, *H. bacteriophora* (Çanakkale isolate) was considered the least effective. For the pupal stages, 1.00±0.36 (10.00%) dead pupae were detected at the end of 10 days in the control groups. In EPN applications, *H. bacteriophora* was found to be most effective with 4.83±0.60 (48.30%) dead pupae. In *S. feltiae*, the effect was low with 3.5±0.72 (35.00%) dead pupae (Figure 1).

Although *S. feltiae* was more effective than *H. bacteriophora* in applications against *P. operculella* larvae, it was less effective on pupa compared to *H. bacteriophora* was more (Figure 1).

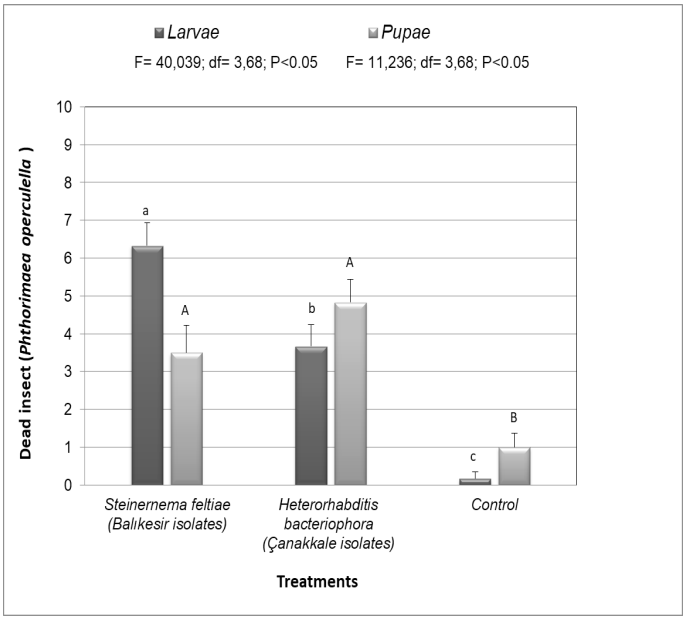

Figure 1: *Mortality of Phthorimaea operculella of soil applications of Steinernema feltiae (Balıkesir isolates) and Heterorhabditis bacteriophora (Çanakkale isolates) isolates and control over 10 days from treatment.*

In biological control studies entomopathogenic bacteria are the most studied against *Phthorimaea operculella* with much focus been on *Bacillus thuringiensis* subsp. *kurstaki*. Also, among viruses there are studies on the granulo-virus (PhopGV) (Arthurs *et al.*, 2008; Lacey *et al.*, 2010).

Several studies have determined the activities of entomopathogenic nematodes (EPNs) on *P. operculella* (Ivanova *et al.*, 1994; Sweelam *et al.*, 2010; Hassani-Kakhki *et al.*, 2013; Abdelmonem *et al.*, 2018; Moawad *et al.*, 2018; Mhatre *et al.*, 2020; Yan

et al., 2020; Ebrahimi *et al*., 2021). Some have used EPNs to supplement chemical applications against *P. operculella* (Kary *et al*., 2018).

Studies on the efficacy of EPNs against *P. operculella* have indicated that while the larval and prepupal stages were susceptible, the pupal and adult stages are resistant to EPNs infection (Ivanova *et al*., 1994; Sweelam *et al*., 2010; Hassani-Kakhki *et al*., 2013). Our study revealed that *Steinernema feltiae* was more effective to larval stage with 63.30% mortality compared to *Heterorhabditis bacteriophora* with 35.0%. On the pupal stages, *H. bacteriophora* was more effective causing a higher larval mortality of 48.3%; *S. feltiae* presented with 35.0% efficacy. Hassani-Kakhki *et al*. (2013) showed that *S. carpocapsae* and *H. bacteriophora* isolate (commercial and FUM7) were more effective than *S. feltiae* and *S. glaseri* against the 4th instar of *P. operculella*.

In another study, *S. carpocapsae* and *H. bacteriophora* EPNs applied at 500 IJs cm^{-2} concentration caused 93.3% and 90% *P. operculella* larval mortality, respectively (Abdelmonem *et al*.,2018). At much lower concentrations of 5 and 10 IJs per cm^{-2}, *S. carpocapsae* and *H. bacteriophora* were highly effective on pre-adult stages, killing 98-100% of *P. operculella* larvae (Moawad *et al*., 2018). In contrast, *H. bacteriophora* applied at 25 IJs cm^{-2} in our study was not quite effective (36.70%) on *P. operculella* larvae. Difference in efficacy in these studies may be due to the different isolates belonging to the same EPN species used.

Until mid-2011, no study was found on use of EPNs in the control of *P. operculella* in Turkey (Kepenekci, 2012, 2014). In subsequent studies the effects of EPNs was investigated against the late stage larvae of *P. operculella* under *vitro* (laboratory) studies and effective results were obtained. These studies were conducted at three different temperatures (10, 15 and 25 °C) using three different concentrations (100, 500 and 1000 IJs larvae^{-1}) of local EPN species -*S. carpocapsae* (Blacksea isolate), *S. feltiae* (Aydin isolate) and *H. bacteriophora* (Aydin isolate)- against infect *P. operculella* larvae in laboratory-petri experiments. Temperature and nematode concentration had a significant effect on *P. operculella* larval mortality. *S. carpocapsae* and *H. bacteriophora* species displayed generally increased virulence with increase in temperature and infective juveniles concentration applied. At 25°C and 1000 IJs concentration, the larval mortality was 96 and 80% for *S. carpocapsae* and *H. bacteriophora,* respectively. *S. feltiae* did not exhibit more than 40% mortality at any temperature or concentration (Kepenekci *et al*., 2013). In contrast, efficiency of *S. feltiae* was 63.30% effective on larval stages of *P. operculella* in greenhouse-pot experiments conducted in our study.

In another study, Gözel *et al*. (2020) tested the effects of 4 local EPN species *S. affine* (isolate-47), *S. carpocapsae* (isolate-1133), *S. feltiae* (isolate-96) and *H. bacteriophora* (isolate-12) species at 50 IJs larva^{-1} against *P. operculella* larvae in laboratory-petri studies at 25 °C. Mortality rates of EPN species increased with time. On the 2nd day, *S. feltiae* caused 100% mortality. In our greenhouse-pot experiments, *S. feltiae* was the most effective EPN.

Conclusions and Recommendations

This research, which is the first study carried out in greenhouse-pot conditions on the use of EPNs in the control of *P. operculella* in Turkey, shows that more detail studies on the applications of EPNs with promising effects (*S. feltiae* Balıkesir isolate applications with a 63.30% mortality in larvae) under field conditions be conducted.

List of abbreviations

EPNs, Entomopathogenetic nematodes; IJs, Infective juveniles; PTM, The potato tuber moth; *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae); TOGU, Tokat Gaziosmanpaşa University, Tokat, Turkey.

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Novelty Statement

This research, which is the first study carried out in greenhouse-pot conditions on the use of ento-mopathogenic nematodes (EPN) in the control of

Phthorimaea operculella (Zeller) (Lepidoptera: Gelechiidae) in Turkey.

Author's Contribution

YYM rearing *P. operculella*, potato plants and nematodes (*S. feltiae* and *H. bacteriophora*) participated in experimental studies.
YYM and İK conceived and designed the research and analyzed the data.
YYM conducted the experiments.
İK applications and interpretation of data and corrected and revised the manuscript, corrected language mistakes and translation, and corrected references.
All authors read and approved the final manuscript.

Conflict of interest
The authors have declared no conflict of interest.

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