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EXPLOITING THE BIOCONTROL POTENTIAL OF ENTOMOPATHOGENIC NEMATODES IN COMBINATION WITH CHEMICALS AGAINST GREATER WAX MOTH (*GALLERIA MELLONELLA* L.)

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ABSTRACT

Entomopathogenic nematodes as alternatives to chemical pesticides for management of insect pests of various crop is an environmentally safe approach. Compatibility of *Steinernema feltiae*, *S. asiaticum*, *Heterorhabditis bacteriophora* and *H. indica* with seven chemical and biopesticides (imidacloprid (0.60 ml/L), spinosad (0.45 g/L), azadirachtin (1.5 ml/L), abamectin (1.25 ml/L), emamectin (0.20 ml/L), lambda-cyhalothrin (0.15 ml/L) and radiant (1.5 g/L) against *Galleria mellonella* was evaluated in lab. *H. bacteriophora* survived best as compared to all other entomopathogenic nematodes (EPN) species in all tested chemicals. *S. feltiae* survival was higher in imidacloprid followed by lambda-cyhalothrin, while abamectin proved to be lethal for *S. feltiae*. Azadirachtin and lambda-cyhalothrin proved to be compatible with *H. bacteriophora* as its survival was higher as compared to the rest of chemicals while radiant proved to be less friendly for its survival. Emamectin followed by abamectin were more compatible with *S. asiaticum* whereas radiant was least compatible. Exposure of *H. indica* to spinosad and emamectin resulted in greater survival of nematodes while survival was lower when applied with lambda-cyhalothrin and radiant. Survival of *S. feltiae* and *H. bacteriophora* was higher when compared to *H. indica* and *S. asiaticum.* The infectivity of *S. feltiae* was the maximum when used with imidacloprid and lambda-cyhalothrin. *H. bacteriophora* proved to be more compatible with imidacloprid, azadirachtin, emamactin and lambda-cyhalothrin. *S. asiaticum* and *H. indica* were compatible with emamectin. Among all tested chemicals, EPN species were sensitive to abamectin. These results showed that EPN species. with better compatibility with evaluated pesticides may be included in Integrated Pest Management (IPM) Programs.

Key words: Compatibility, entomopathogenic nematodes, *Steinernema feltiae*, *S. asiaticum*, *Heterorhabditis bacteriophora*, *H. indica*, *Galleria mellonella*, chemicals, biopesticides.

INTRODUCTION

Entomopathogenic nematodes (EPNs) are soil inhabiting insect pathogens being successfully used in agricultural systems not only against insects but also against Root Knot Nematodes (Kaya and Gaugler, 1993; Javed *et al*., 2012; Aatif *et al*., 2015).They have beneficial relationship with bacteria as *Photorhabdus* spp. present inside *Heterorhabditis* and *Xenorhabdus* spp. live in *Steinernema* (Raheel *et al*., 2015, 2017). Due to their ability to survive without food, EPNs can be used for insect control purposes. Entomopathogenic nematodes have been proved the most effective against more than 300 species of insects (Shahina and Tabassum, 2010). Like other parasitoids or predators, EPNs have chemo-receptors and are motile in nature. They can be cultured both *in vitro* and *in vivo* conditions, and are extremely virulent to their hosts (Ehlers, 2001). There were also no difficulties in applying EPNs, as they are easily sprayed using standard equipment's including pressurized, mist, fan, electrostatic and aerial sprayers, and can be combined with almost all chemical control compounds (Georgis and Kaya, 1998; Ruhela, 2008; Radova, 2010, 2011). These were used against various insect pests both on foliage, and in the soil under cryptic habitats. As the best conditions for action and survival of EPNs were offered by the soil which is their natural habitat (Gaugler, 1988). Hence, opportunity to use EPNs is promising because a portion of the life circle of many insects (above 90%) is spent in the soil. Therefore, it is necessary to assess their compatibility with chemical and bio pesticides for insect management purposes.

The pesticides, bioproducts and plant extracts (imidacloprid (0.60 ml/L), spinosad (0.45 g/L), azadirachtin (1.5 ml/L), abamectin (1.25 ml/L), emamectin (0.20 ml/L), lambda-cyhalothrin (0.15 ml/L) and radiant (1.5 g/L) were evaluated against the survival of four EPN species (*H. bacteriophora*, *H. indica*, *S. feltiae* and *S. asiaticum*).

MATERIALS AND METHODS

Nematodes: *Heterorhabditis bacteriophora*, *H. indica*, *Steinernema feltiae* and *S. asiaticum* were used for experimentation.

***G. mellonella*:** The larvae of *G. mellonella* were reared on cereal diet in laboratory (Wiesner, 1993).

EPNs culturing: Entomopathogenic nematodes were cultured on *G. mellonella* larvae. The freshly hatched EPNs were placed in shallow plastic containers having covers with suspension being no more than 1 cm in depth to confirm enough availability of oxygen at 10°C. The *in vivo* produced nematodes could be kept for 3 months. All stocked nematode cultures were again cultured after every 4 months. For current study fresh culture of EPNs (less than 2 weeks old) was used.

For the analysis of survival of these EPN species stock solutions of these chemicals were made in water at double strength of the necessitated dose. About 1500 IJs of all species in 0.5 mL of water were mixed with 0.5 ml of each stock solution in each well of the 24-well plates. These well plates were then kept at room temperature (20-25ºC). There were five replications of each treatment. In control treatment water was used. The products were examined in groups of four to eight chemicals with a control treatment included each time. This was carried out for comfort of handling.

Under a stereomicroscope the viability of IJs incubated in various chemicals was evaluated after 24h from each well of the 5 replicates. One hundred IJs were counted for each treatment and the control. Infective Juveniles which did not show any movement after prodding were considered dead (Elizabeth *et al*., 2003). The experimental units were arranged under CRD design and the data was analyzed statistically. The viable nematodes after 24 h exposure to pesticides/biopesticides (imidacloprid, spinosad, azadirachtin, abamectin, emamectin, lambda-cyhalothrin and radiant) were washed by using 400 mesh sized sieve and pouring distilled water on the IJs.

The infectivity of nematodes against larvae, after treatment with all the pesticides/biopesticides, was examined by using the 1:1 sand-well bioassay (Grewal *et al*., 1999) in 24-well plates. Sieved sand was washed and then allowed to dry. Sub samples from each treatment were removed from the wells, placed in 10 cm petri dishes, and diluted with water. Five IJs were picked with a micropipette in 90 mL of water under a stereomicroscope and were placed in each well containing 1.5 g of sterilized dry sand. One larva of *G. mellonella* was placed in each well and the plates were incubated at 22ºC and 60% RH. The mortality of *G. mellonella* larvae was evaluated after 6 days of incubation while the control treatment had no pesticides/biopesticides. There were three replications of each treatment and experimental units were arranged in completely randomized design. The data was analyzed statistically.

RESULTS

ANOVA for survival of EPNs at different soil moisture levels showed that effect of treatments and species was significant (p>0.05). Interaction effect of treatments and species was also significant. Maximum mortality was observed in *S. feltiae* (42%) when used with abamectin, followed by *S. asiaticum* (29.4%) with radiant. The mortality was recorded minimum in case of *S. feltiae* (0.2%) when used with imidacloprid. Hence, the survival of *S. feltiae* was significantly higher with imidacloprid followed by lambda-cyhalothrin and spinosad, while abamectin proved to be lethal for its survival (45% mortality). Effect of azadirachtin and lambda-cyhalothrin on survival of *H. bacteriophora* was significantly more friendly than rest of biopesticides used (as exhibited by least mortality of IJs) and radiant proved to be less friendly for its survival. Emamectin followed by abamectin were significantly more compatible with *S. asiaticum* whereas it was sensitive to radiant. Exposure of *H. indica* to spinosad and emamectin resulted in significantly higher survival while its survival was significantly lower against lambda-cyhalothrin and radiant. (Fig 1). *H. bacteriophora* showed best results against all biopesticides compared to other species.

ANOVA for infectivity of EPNs with combination of different pesticides/biopesticides showed that effect of treatments and species was significant (p>0.05). Interaction effect of treatments and species was also significant. The pathogenicity/infectivity of *S. feltiae* and *H. bacteriophora* was significantly higher as compared to *H. indica* and *S. asiaticum.* The infectivity of *S. feltiae* was significantly higher when used with imidacloprid, spinosad, emamectin and lambda-cyhalothrin. Whereas, *H. bacteriophora* proved to be more compatible with imidacloprid, azadirachtin, emamectin and lambda-cyhalothrin. *Steinernema asiaticum* and *H. indica* were more compatible with emamectin. Out of 7 chemicals tested abamectin was most destructive as *S. feltiae* nematodes were not able to infect the larvae of *G. mellonella* it was followed by radiant in case of *S. feltiae* and *S. asiaticum* (Table 1). Overall *H. bacteriophora* showed best infectivity against larvae of *G. mellonella* when used with all biopesticides compared to other species.

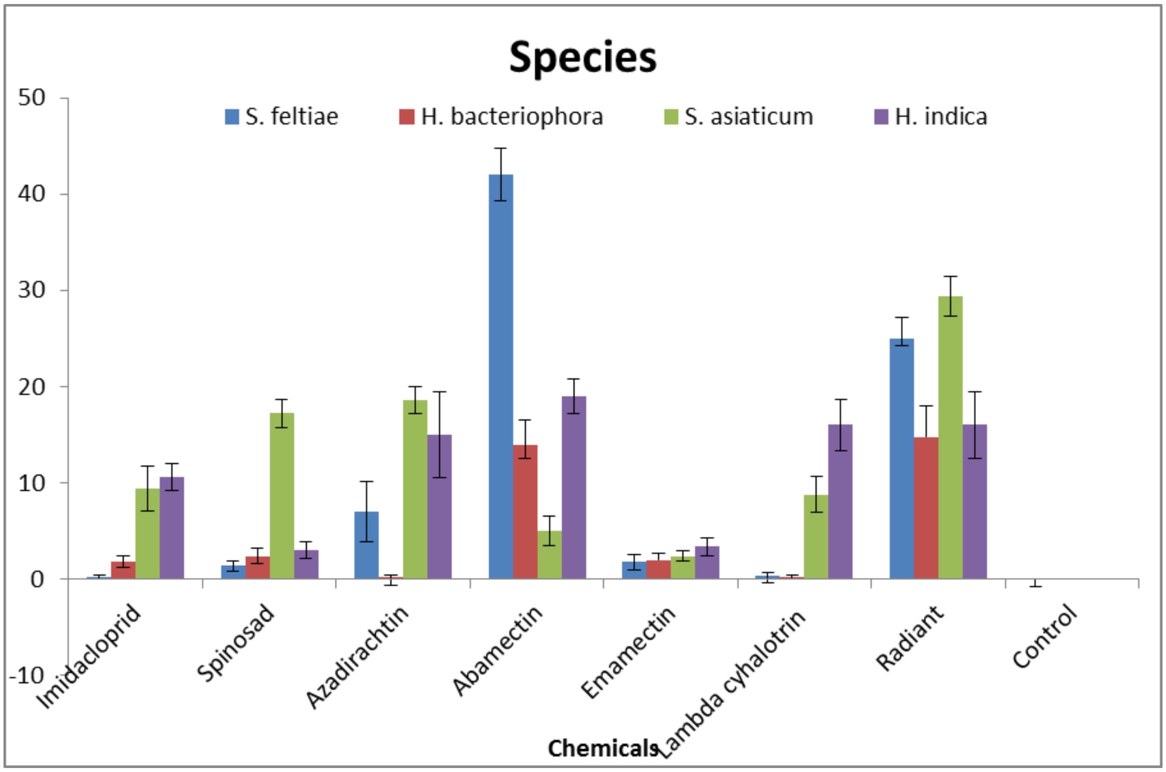

Figure 1. Effect of various pesticides on survival of infective juveniles of EPNs

Table 1: Effect of EPNs treated with various chemical and Bio-pesticides on Greater Wax Moth Larvae

Treatments	Imidacloprid	Spinosad	Azadirachtin	Abamectin	Emamectin	Lambda-cyhalothrin	Radiant	Control
Species								
S. feltiae	56.67^{B}	55.33^{B}	43.33^{EF}	00.00^{I}	55.33^{B}	56.67^{B}	36.67^{G}	67.33^{A}
H. bacteriophora	55.33^{B}	52.67^{BC}	56.67^{B}	44.67^{DEF}	54.00^{B}	56.67^{B}	46.00^{DE}	67.33^{A}
S. asiaticum	46.00 ^{DE}	43.33^{EF}	43.33^{EF}	44.67^{DEF}	55.33^{B}	46.00^{DE}	31.33^{H}	68.67^{A}
H. indica	48.67^{CD}	47.33^{DE}	48.67^{CD}	48.67^{CD}	52.67^{BC}	46.00^{DE}	40.67^{FG}	68.67^{A}

Numbers followed by different letters are significantly different from each other at $p<0.05$. Data is mean of three replications.

DISCUSSION

Entomopathogenic Nematodes and chemical pesticides deal different but compatible tactics for management of insect population. EPNs may find out their host in unreachable parts where chemical pesticides may not perform. Therefore, efforts should be made to use EPNs in Integrated Pest Management (IPM). Though, compatibility of EPNs with chemicals was studied by invitro bioassays with direct exposure of EPNs to insecticides, still it is required to evaluate the harmful effects of these chemicals on EPNs, as the formulations of various insecticides may differ in toxicity to different nematode species due to the use of different surfactants. Furthermore, EPNs species of different origins may also vary in sensitivity to various formulations of same insecticides (Krishnayyaand and Grewal, 2002). In recent past studies have found that many species of EPNs were relatively not sensitive to many pesticides (Alumai and Grewal, 2004; Radova, 2010, 2011; Bortoluzzi *et al.*, 2013; Sabino *et al.*, 2014; Ulu *et al.*, 2016), although some carbamates and organophosphates proved to be lethal to IJs of *S. carpocapsae*. Moreover, few chemicals proved to be harmful to the virulence of nematodes (Radova, 2011; Bortoluzzi *et al.*, 2013).

The recent work evaluated the important chemical and bio insecticides commonly applied in IPM system against different insects in Pakistan. The findings of the recent work and those of previous studies (Krishnayyaand and Grewal, 2002; De Nardo and Grewal, 2003; Radova, 2011; Laznik *et al*., 2012) in which EPNs compatibility with plant protection products was accessed, showed that compatibility is species specific. Our results showed that azadirachtin did not affect the survival of *S. feltiae* and *H. bacteriophora* however, it did affect the survival of *H. indica* and *S. asiaticum*. Whereas, abamectin showed a nematicidal effect on EPN species which may explain the relatively high mortality values (Mahfooz *et al*., 2008). Moreover, it was resulted that *H. bacteriophora* was the most tolerant species as compared to *S. feltiae*, *S. asiaticum* and *H. indica*. Similar finding was reported (Radova, 2011; Laznik and Trdan, 2013). In recent study no significant difference was observed on the survival of IJs of *H. indica* against radiant and emamectin. These findings were in confirmation to other observations (Lalramliana and Yadav, 2008).

Therefore, it is concluded that only IJs of selected species of EPN exhibit compatibility with tested plant protective chemicals, some others may also show injurious effects to these EPNs. The EPNs with better compatibility may be considered as strong option for usage in IPM. The EPN species *S. asiaticum* has not been tested for its efficacy when used with various chemical pesticides and plant products.

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