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## Compatibility of Entomopathogenic Nematodes with Neonicotinoids and Azadirachtin Insecticides for Controlling the Black Cutworm, *Agrotis ipsilon* (Hufnagel) in Canola Plants

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**Abstract:** Laboratory and greenhouse experiments were conducted to investigate the efficacy of the entomopathogenic nematodes (EPNs), *Steinernema carpocapsae*, *Heterorhabditis bacteriophora*, two insecticides based on Azadirachtin and two Neonicotinoid insecticides. These treatments were applied alone or in combination against the last instar larvae of the black cutworm, *Agrotis ipsilon* (Hufnagel). The survival rates of *S. carpocapsae* and *H. bacteriophora* in the two groups of insecticides ranged between 96.1 to 99.7 after 72 hours of exposure. Data clearly indicated that *S. carpocapsae* was more virulent against *A. ipsilon*, whereas *H. bacteriophora* was less virulent at all concentrations under laboratory conditions. Mortality percentage increased significantly with increasing IJs concentration and time of exposure, reaching up to 100% and 90% at 160 infective juveniles (IJs) concentration, after 72 hours of exposure in *S. carpocapsae* and *H. bacteriophora*, respectively. Combination between EPNs at the rate of 80 IJs concentration and either each of the both groups of insecticides (Azadirachtin and Neonicotinoid) increased the efficacy of EPNs and mortality rates of *A. ipsilon* significantly under the laboratory condition. Results proved that *S. carpocapsae* and *H. bacteriophora* were less efficient under greenhouse condition when applied in combination with any of the insecticides at 5000 IJs/ 25 ml. EPNs efficacy in combination with Azadirachtin has mostly increased in comparison to EPNs with neonicotinoid. This recommended that a mixture of Azadirachtin + EPNs could be applied for an efficient control of *A. ipsilon*.

**Keywords:** Entomopathogenic nematodes, Neonicotinoid, Azadirachtin, Compatibility, *Agrotis ipsilon*, canola plants

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### 1. INTRODUCTION

The black cutworm, *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae) is a serious pest of several agricultural crops in Egypt and globally [1, 2]. This noctuid insect almost polyphagously attacks a large number of vegetable crops. The control of this pest has become a serious challenge facing applied entomologists nowadays considering the widening circle of resistance and cross resistance to conventional insecticides such as organophosphates and carbamates. This results in increased demands for pest control methods that are both efficient and environmentally friendly.

In Egypt, canola crop has a bright future to contribute in reducing oil deficiency gap between production and consumption [3, 4]. Growing canola still faces many challenges; one of them is being heavily infested with various insect pests, causing poor growth and low yield [5]. In Egypt, the most serious insect pests of canola in the seedling phase are the black cutworms, *A. ipsilon*.

Botanical insecticides can be used as a tool for crop protection as one component of insect pest management [6]. Several researches confirmed that most of botanical insecticides are environment-friendly because they have less toxicity to human and other non-target organisms [6, 7]. The neonicotinoid group of insecticides is among the most broadly adopted, conventional management tools for insect pests of annual and perennial cropping systems [8]. Benefits of the neonicotinoid group of compounds include flexibility of application, diversity of active ingredients, and broad spectrum activity [9].

The use of entomopathogenic nematodes (EPNs) (Rhabditida: Steinernematidae and Heterorhabditidae) has been successfully used in many countries to control various crop pests, especially those that traverse the soil or inhabit cryptic environments [10]. EPNs of the *Steinernema*

and *Heterorhabditis* genera live in the soil and are natural parasites of insects found in this ecosystem, as well as having a symbiotic relationship with bacteria of the *Xenorhabdus* and *Photorhabdus* genera, respectively [11]. EPNs are now applied against wide range of foliar pests [12, 13, 14]. The black cut worm, *A. ipsilon* were reported to be susceptible to EPNs [15, 16].

There are also no difficulties to apply EPNs via different techniques and they can be combined with almost all chemical control compounds [17, 18, 19]. The exposure of EPNs on foliage to extreme temperature, ultraviolet (UV), light and rapid fluctuation in moisture that causes desiccation reduces their potential as bio-control agents against foliage-feeding insect pests. Accordingly, EPNs applied to foliage must be protected from these detrimental environmental effects by avoiding high temperature and UV radiation with evening applications and desiccation by using adjuvants or anti desiccants. These additives are useful when nematodes are applied to waxy and glabrous leaves such as canola plants.

The objective of this study was to 1) determine the survival rates of EPNs *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* with selected insecticides. 2) to investigate their efficacy against the black cutworms, *A. ipsilon* when applied alone or in combination with two group of insecticides, azadirachtin and neonicotinoid in the laboratory and the greenhouse.

## 2. MATERIALS AND METHODS

### 2.1. The Black Cutworm Rearing

Eggs and newly larvae were collected from infested canola plants at the Experimental farm, Faculty of Agriculture, Suez Canal University, Ismailia. They were transferred to laboratory and supplied with castor bean leaves. (*Ricinus communis*) to feed the young larvae. To avoid cannibalism, the larvae were reared individually in a separate unit of plastic cell tray (10.8 X 22.7cm). The leaves were daily replaced with fresh ones. Newly formed pupae were collected and kept in glass jar containing moist saw dust and covered with muslin until adult emergence. Food was provided daily by using a cotton pad soaked in 10% honey solution, and then eggs were daily collected.

### 2.2. The Greater Wax Moth Maintenance

The greater wax moth larvae of *Galleria mellonella* L. were used to produce progeny of nematode species. The insect culture was reared in 1500 ml volume glass containers (11 cm diameter and 15 cm height) at  $25 \pm 2$  °C on an artificial medium reported by [20]. The glass containers were closed with filter paper and a metal screen. Mature females insects laid their eggs on the filter paper which was transferred to fresh artificial medium in the rearing container. The eggs hatched within 3-4 days and larvae were fed weekly. After 5-6 weeks, larvae reached the last instar and were collected to be used for nematode propagation.

### 2.3. Entomopathogenic Nematodes

Entomopathogenic nematodes, *Steinernema carpocapsae* (Wieser) All strain and *Heterorhabditis bacteriophora* (Poinar) BRT strain were used in this experiments to investigate their efficacy to larvae of the black cut worms, *A. ipsilon*. EPNs were propagated in the last instar larvae of *G. mellonella* using the method of [20]. The infective juveniles (IJs) were collected in modified White traps [21], and stored in sterilized distilled water at 5 °C for no more than two weeks before they were used.

### 2.4. Insecticides

Two groups of insecticides based on Neonicotinoid and Azadirachtin were used in this study. The first group was Imidacloprid: (Best 25% WP), applied at  $0.18 \text{ g}^{-1}$  water and the second one was Thiamethoxam: (Actara 25% WG) applied at  $0.5 \text{ g}^{-1}$  water. While the other group was NeemAzal T/S 1% at the rate 0.1% and Neemix 4.5% at the same rate of 0.1%. All insecticides were applied at low dose in compatible with EPNs.

### 2.5. Survival Rates of EPNs Combined with Insecticides

The concentration of 50 IJs/ 2 ml distilled water was prepared in 5 cm diameter Petri-dish, filled individually with 2 ml of each insecticides and distilled water (control) and kept for three days. The experiments were repeated twenty times and the dishes were kept at  $25 \pm 2$  °C in the laboratory. Number of alive IJs was counted after 24, 48 and 72 hours.

## **2.6. Laboratory Bioassay**

### *2.6.1. EPNs Alone*

Experiments with last instar larvae of *A. ipsilon* were conducted in Petri-dishes (90 mm diameter) lined with filter paper (Whatman No.1). Each dish was inoculated with one of the two nematode species at seven concentrations (0, 20, 40, 80 and 160 IJs/ml). The number of IJs were counted under a dissecting zoom microscope to check nematode viability. All plates were sealed and incubated at  $25 \pm 2^\circ \text{C}$ . There were twenty plates; one larva in a dish is an experimental unit. After 72 hours of contact period, dead larvae were individually transferred to modified white traps [21] to evaluate nematode infectivity, mortality and emergence of nematode progeny.

### *2.6.2. EPNs in Combination with Insecticides*

The concentration of 80 IJs/ml distilled water was prepared in Petri-dishes (90 mm diameter) lined with filter paper (Whatman No.1), filled individually with 1 ml of each insecticides at the selected concentration, and kept for 72 hours. Distilled water was used as controls. The experiments were repeated 20 times and the dishes were kept at  $(25 \pm 2^\circ \text{C})$ . Dead larvae were individually transferred to modified white traps [21] to evaluate nematode infectivity, mortality and emergence of nematode progeny.

### *2.6.3. Greenhouse Bioassay*

Greenhouse study was conducted by spraying 25 ml nematodes suspension on canola plants with 5000 IJs/ 25 ml before flowering stage at evening hours 5.30 pm to prevent desiccation induced by sunlight. Five plants 45 days after sowing were selected and twenty five, healthy and active last instar larvae of *A. ipsilon* were released individually on the leaves of canola plants and then spraying 5000 IJs/ 25 ml of the two species of nematodes or 25 ml distilled water (control). The percent larval mortality was recorded after 72 hours of nematodes application. Scoring of larvae mortalities was achieved by recovering dead larvae to confirm mortality due to EPNs. Later, larva stages of *A. ipsilon* are photo-negative and tend to hide in the soil during the daytime hours. Also, larvae tend to be cannibalistic so mortality of larvae could be due to intrinsic behavior of the species

### *2.6.4. Statistical Analysis*

Data obtained in the present study was subjected to an analysis of variance (ANOVA) with the honestly significant difference value calculated as Tukey's statistic at  $P \leq 0.05$  [22].

## **3. RESULTS AND DISCUSSION**

The survival rates of *S. carpocapsae* and *H. bacteriophora* in the two group of insecticides have ranged between 99.0% to 99.7% in *S. carpocapsae* and 98.4% and 99.6% in *H. bacteriophora* after 24 hours, and 99.0% to 99.3% in *S. carpocapsae* and 98.2% to 99.3% in *H. bacteriophora* after 48 hours and 97.4% to 99.1% in *S. carpocapsae* and 96.1% to 99.1% in *H. bacteriophora* after 72 hours after application.

In appearance, the percentage of survival rates of nematodes has been compared between insecticides within species, by time point (column wise). Treatments of neemazal, neemix, imidacloprid and thiamethoxam did not show a significant difference of nematode survival rate with control (100%) at 24, 48 and 72 hours. However, all nematode survival rates in the three times points of exposure were higher than 96.1% (Table 1). The result in Table 1 suggests no significant difference between control and insecticide groups in later time points. Nematode survival rates in this study were similar to that reported by [23] who indicated that a recommended rates of pesticides directly applied with *S. carpocapsae* had no effect on *S. carpocapsae* virulence (more than 96% survival rate) at room temperature for 3 hours. [24] confirmed that the compatibility between nematodes and botanical insecticides or chemical insecticides at recommended doses had no effects on survival rates after 72 hours of exposure.

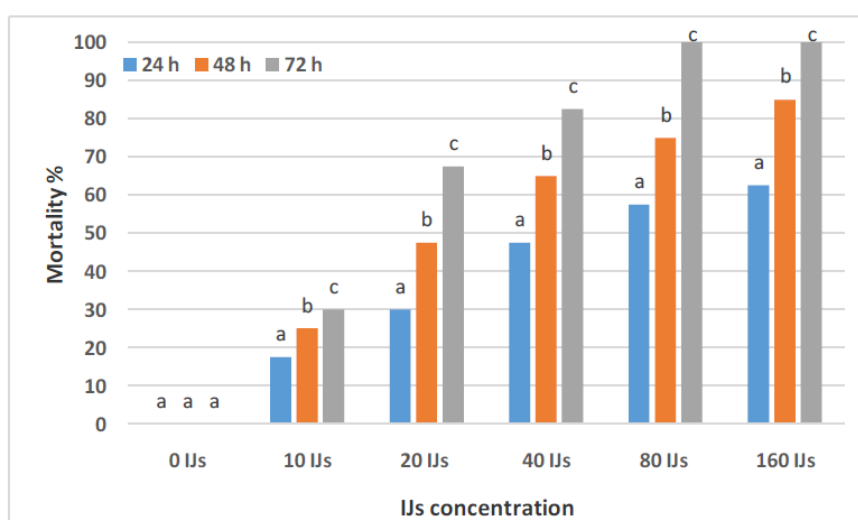
Efficacy of five concentrations of EPNs *S. carpocapsae* and *H. bacteriophora* against last instar larvae of *A. ipsilon* was estimated after 42, 48 and 72 hours of exposure (Figs 1 and 2). Significant differences were found in virulence of both species of EPNs against larvae of *A. ipsilon* in filter paper assay under laboratory conditions. Mortality rates increased significantly with increasing nematode concentrations in the tested species. Also, in both nematode treatments the mortality was significantly

higher than the mortality in control. Data clearly indicated that *S. carpocapsae* was virulent against the last instar larvae of *A. ipsilon*; whereas *H. bacteriophora* was less virulent at all concentrations and time of exposure.

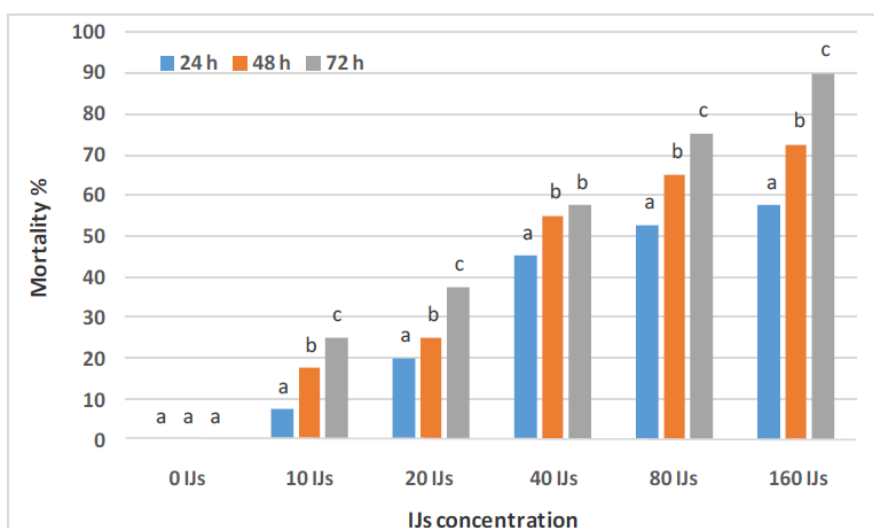
**Table1.** The survival rates of *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* which were soaked in insecticides for 24, 48 and 72 hours.

Treatments	Survival rate after,					
	24 h		48h		72 h	
	<i>S. carpocapsae</i>	<i>H. bacteriophora</i>	<i>S. carpocapsae</i>	<i>H. bacteriophora</i>	<i>S. carpocapsae</i>	<i>H. bacteriophora</i>
Control	100 a	100 a	100 a	100 a	100 a	100 a
Imidacloprid	99.0 a	98.4 a	99.0 a	98.2 a	98.3 a	98.1 a
Thiamethoxam	99.1 a	99.0 a	99.0 a	98.8 a	97.4 a	96.1 a
Neemazal	99.7 a	99.5 a	99.5 a	99.3 a	99.1 a	99.1 a
Neemix	99.7 a	99.6 a	99.3 a	99.2 a	99.0 a	99.1 a

Percentages followed with the same letters (column wise) are not significantly different (Tukey' HSD;  $P \leq 0.05$ )



**Figure1.** Efficacy of EPN *Steinernema carpocapsae* against the last instar larvae of *A. ipsilon* in the laboratory



**Figure2.** Efficacy of EPN *Heterorhabditis bacteriophora* against the last instar larvae of *A. ipsilon* in the laboratory

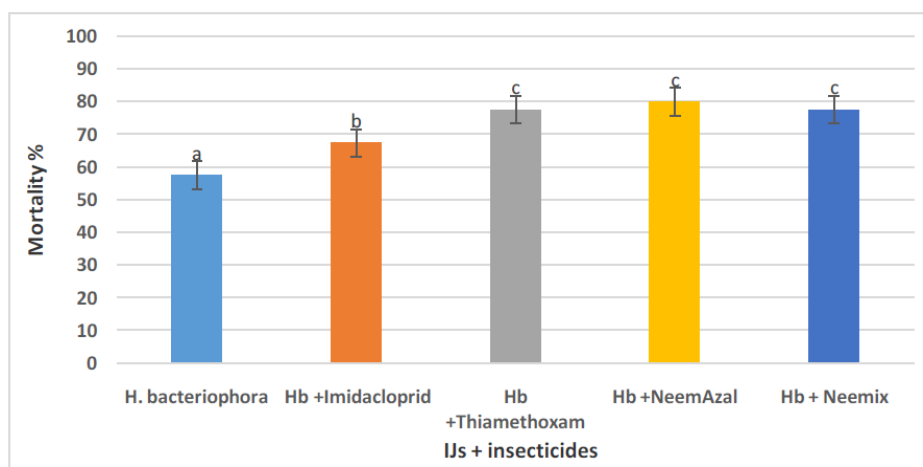
The highest larval mortality was observed in *S. carpocapsae* + neemix treatment (97.5%), followed by *S. carpocapsae* + neemazal treatment (95%). Nevertheless, neonicotinoid had lower larval mortalities (85%) in *S. carpocapsae* + imidacloprid and *S. carpocapsae* + thiamethoxam (Figure 3 and 4). Efficacy of *H. bacteriophora* in combination with both insecticides was lower than that of *S. carpocapsae* when mixed with the same insecticides. It was ranged between (67.5 to 80 % mortality).

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Results shows that both neonicotinoid insecticides in conjunction with EPNs did not produce higher mortality compared to *S. carpocapsae* alone.



**Figure3.** Efficacy of EPN *Steinernema carpocapsae* at 80 IJs/ larva in combination with insecticides against the last instar larvae of *A. ipsilon* in the laboratory



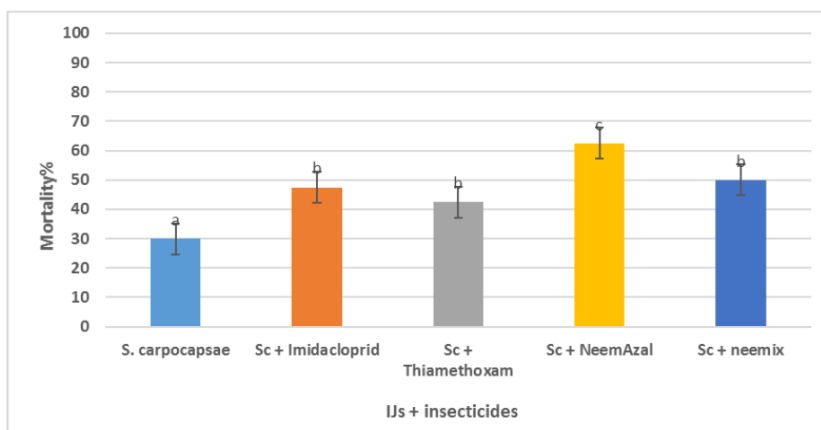
**Figure4.** Efficacy of EPN *Heterorhabditis bacteriophora* at 80 IJs/ larva in combination with insecticides against the last instar larvae of *A. ipsilon* in the laboratory

In the greenhouse tests, *S. carpocapsae* in combination with imidacloprid, thiamethoxam, neemazal and neemix caused 47.5, 42.5, 62.5 and 50% mortality, respectively against *A. ipsilon* compared to 30 % in control (*S. carpocapsae* alone). While, *H. bacteriophora* caused 30, 27.5, 45 and 50% mortality compared to 25% in control. Greenhouse experiment has confirmed the results of filter paper assay. Data revealed that *S. carpocapsae* was more virulence than *H. bacteriophora* when applied alone or in combination with insecticides (Figure 5 and 6). EPNs were capable of causing mortality in *A. ipsilon* in the tested conditions, demonstrating a potential for later tests under field conditions. [25] proved that EPN *Heterorhabditis indica* is a potential option for possible management, capable of nematode production with efficacy notable against cotton bollworm, *Helicoverpa armigera* and tobacco caterpillar, *Spodoptera litura* in soil and foliar spray in greenhouse.

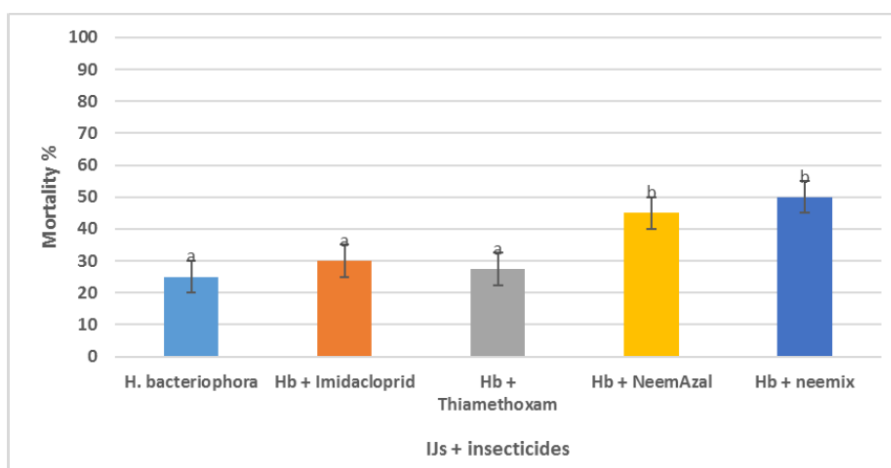
Our results suggest that integration of EPNs in IPM programs in combination with other biocontrol agents and pesticides is possible since they are compatible. [26] reported that the survival rates of nematode after combining with 0.01% confidor (imidacloprid), 0.025% actara (thiomethoxan), 0.05% agropest BT, 0.05% conserve (spinosad) and 2.00% neemglod (neem) were 97.2%, 83.2%, 93.6%, 87.2% and 92.4%, respectively.

*S. carpocapsae* combined with cypermethrin (0.025 L/ha), chlorpyrifos (0.300 L/ha) and lufenuron (0.150 L/ha) had high efficiency against *Spodoptera frugiperda* (Smith) for 82.0, 90.0% and 90.0%, respectively [27]. Furthermore, [28] indicated an interaction between *S. carpocapsae* with proclain (0.125mg/L) with additive effect, producing mortality rates of forth instar larvae of *Agrotis ipsilon* up

to 100%. Whereas, nematode alone (50 IJs) and proclaim (0.125mg/L) had killed *A. ipsilon* larvae at 85% and 89%, respectively. Nevertheless, this result also agreed with various researchers who reported that entomopathogenic nematode and pesticide have been compatible [29].



**Figure5.** Efficacy of EPN *Steinernema carpocapsae* at 5000 IJs/ 25 ml in combination with insecticides against the last instar larvae of *A. ipsilon* in the greenhouse



**Figure6.** Efficacy of EPN *Heterorhabditis bacteriophora* at 5000 IJs/ 25 ml in combination with insecticides against the last instar larvae of *A. ipsilon* in the greenhouse

#### 4. CONCLUSION

The study provides evidence that *S. carpocapsae* and *H. bacteriophora* under controlled laboratory conditions are able to exert an excellent degree of control over last instar larvae of *A. ipsilon*. Within the glasshouse situation a lower level of *A. ipsilon* mortality was obtained. However, the compatibility of *S. carpocapsae*, *H. bacteriophora* and neonctinoid, azadirachtin insecticides gives potential for the incorporation of EPNs into integrated pest management programmes for the control of *A. ipsilon* within canola greenhouse.

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