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Efficacy of different entomopathogenic nematode isolates, against the peach fruit fly, *Bactrocera zonata* (Saund.) (Diptera: Tephritidae)

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Abstract

Background The invasive peach fruit fly (PFF), *Bactrocera zonata* (Saunders) (Diptera: Tephritidae), is a native of South-east Asia. Entomopathogens like nematodes, bacteria, viruses and fungi have been shown to be effective as a biological control agent against *B. zonata*. Evaluation the efficacy of different entomopathogenic nematode isolates (EPNs) belonged to the two families (Steinernematidae and Heterorhabditidae); (*Steinernema carpocapsae* (All), *S. carpocapsae* (EGAZ10), *Heterorhabditis bacteriophora* (HP88) and *H. indica* (EGAZ2)) was carried out against the full-grown larvae of *B. zonata* under laboratory, semi-field and field conditions.

Results Data revealed that in all the tested nematode isolates succeeded to reduce the emerging of the PFF, *B. zonata* compared to controls with significant differences. The LC₅₀ values were 794.3, 1063.2, 1249.8 and 1446.8 IJs/ml, for *S. carpocapsae* (All), *S. carpocapsae* (EGAZ10), *H. bacteriophora* (HP88) and *H. indica* (EGAZ2), respectively, at 3 days post treatments. The strain, *S. carpocapsae* (All) was effective than *S. carpocapsae* (EGAZ10). Also, the strain *H. bacteriophora* (HP88) was more effective than *H. indica* (EGAZ2). Therefore, the combination between the effective two steinernematid strains (*S. carpocapsae* (All) & *S. carpocapsae* (EGAZ10)) and the two heterorhabditis strains (*H. bacteriophora* (HP88) & *H. indica* (EGAZ2)) was efficient in the semi-field experiment. In field condition, the combination of the two efficient strains *S. carpocapsae* (All) and *H. bacteriophora* (HP88) at the concentration of 3000 IJs/ml was more effective in controlling *B. zonata* causing mortality 97.5%. The Co-Toxicity factor values were – 67.6 for the combination of *S. carpocapsae* (All) with *S. carpocapsae* (EGAZ10) which recorded an antagonistic effect. Also, antagonistic effects were observed for the combined application of *H. bacteriophora* (HP88) with *H. indica* (EGAZ2) (– 66.6) in semi-field application; and the same effect was recorded for the combination of *S. carpocapsae* (All) with *H. bacteriophora* (HP88) (– 42.6) in field application.

Conclusion All EPNs' experiments showed that the efficacy of foreign nematodes than the local ones. So, combination of the two highly effective imported strains gave satisfied results, especially in the field experiment.

Keywords Peach fruit fly, *Bactrocera zonata*, Entomopathogenic nematodes, *Steinernema carpocapsae*, *Heterorhabditis bacteriophora*, *H. indica*, Efficacy

Background

The peach fruit fly (PFF), *Bactrocera zonata* (Saunders, 1841) (Diptera: Tephritidae), is an invasive pest species, native to Southeast Asia. It was first recognized as a new pest of guava and mango in the northern region of Egypt in 1998 (El-Minshawy et al. 1999). According to Hashem et al. (2007), *B. zonata* has a wide variety of hosts, including fruits and vegetables. It spreads quickly in great

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numbers throughout Egyptian Governorates. Due to the favorable climate in Egypt, the PFF lives in Egypt and developed into a significant pest over the past ten years and began infesting a variety of fruit hosts, including citrus, mango, peach, fig, guava, apricot, and apple. Additionally, it targets various foods as secondary hosts, including tomato, pepper, and eggplant (Ghanim 2009). Use of insecticides as an only method to control pests in fruits and vegetables has caused environmental pollution and hygienic problems that represent a risk for human and animals (Gallo 2007). In comparison with pesticides, biological management is less cost-effective and less harmful to the environment (Rizvi et al. 2009). According to Dias et al. (2018), natural enemies, parasitoids, predators, and pathogens are frequently used in biological management because they attack pests. Entomopathogens are natural microorganisms that can be found in a variety of ecosystems infecting different stages of the insect hosts. It has been demonstrated that pathogens like nematodes, bacteria, viruses and fungi are viable biological control agents against *B. zonata* (Bilal et al. 2021).

Rashad et al. (2015) suggested that EPNs and fungi can be used as alternative tools of pesticides for controlling *B. zonata* after validating protocols of field conditions. Some tephritid species have been observed to be mortally affected by steinernematid and heterorhabditis species. Different phases of *B. zonata* may be infected by EPNs, according to (Mahmoud et al. 2016).

The objective of this study was to evaluate the efficacy of different EPN isolates: *Steinernema carpocapsae* (AII), *S. carpocapsae* (EGAZ10), *Heterorhabditis bacteriophora* (HP88) and *H. indica* (EGAZ2) belonged to two families (Steinernematidae and Heterorhabditidae) against the full-grown *B. zonata* larvae under laboratory, semi-field and field conditions. Also, to evaluate the influence of combination for whether applying two EPN strains at (semi-field and field experiment); it was synergistic, additive or antagonistic effects.

Methods

Insect culture

The stock colony of PFF used in the present study was reared in cages measured (35×30×30 cm) at the Biological Control Department, Plant Protection Research Institute, Agricultural Research Center (ARC)—Giza, Egypt. Rearing was carried out under greenhouse conditions of 25±2 °C and 55–65% R.H. and the photoperiod ranged 14–16:8–10 L: D. on artificial diet according to (Hosni et al. 2011 and Rashad et al. 2015).

Entomopathogenic Nematodes' cultures

Source: The Egyptian nematode strains of *S. carpocapsae* (EGAZ10) and *H. Indica* (EGAZ2) and the imported

nematode species, *S. carpocapsae* (AII) and *H. bacteriophora* (HP88) were obtained from Biological Control Department (BCD), Plant Protection Research Institute (PPRI), Agricultural Research Center (ARC), Giza, Egypt.

For mass culturing of the tested nematode species, *S. carpocapsae* (AII); *S. carpocapsae* (EGAZ10); *H. bacteriophora* (HP88) and *H. indica* (EGAZ2), strains were propagated at the Physiology Laboratory, of PPRI, Giza, Egypt in vivo using larvae of the greater wax moth, *Galleria mellonella* (Linnaeus, 1758) as a host according to (Shamseldean et al. 2009). Rearing of *G. mellonella* was acquired from infested bee hives and reared in jars according to Metwally et al. (2012). Fifty last instar larvae of *G. mellonella* were placed in Petri-dishes; each contains two filter papers moistened with water suspension of nematodes at concentration of (30.000 IJs/2 ml. of distilled water). After 10–15 days of infection, the host cadavers were transferred to nematode-collecting dishes according to White traps method (White 1927) to collect the infective juveniles (IJs). The emerging juveniles of *S. carpocapsae* (at day 10 post-infection), and, *H. bacteriophora* (at day 15 post-infection) were harvested daily and then stored in plastic bottles (15×15×10 cm) containing distilled water at 8–10 and 15 °C until used.

Pathogenicity of the EPN species against *B. zonata* full-grown larvae under laboratory conditions

Three different species (four isolates) of EPNs: *S. carpocapsae* (AII), *S. carpocapsae* (EGAZ10), *H. bacteriophora* (HP88) and *H. Indica* (EGAZ2) were suspended in distilled water (d. w.) at the concentrations of 250, 500, 1000, 1500 and 2000 IJs/ml d.w. against *B. zonata* full-grown larvae. Plastic cups measuring (5.5×4 cm) filled with 25 gm. mixed soil (clay 50% + beach sand 50%), supplied with ten *B. zonata* full-grown larvae, treated with a suspension of nematode species, and then covered with a borer plastic lid. Control experiment was conducted by placing larvae on soil moistened with 1 ml. distilled water only. Four replicates (10 larvae/replicate) were used for each treatment. The mortality was estimated three days post treatments. The experiments were carried out at 25±2 °C and R. H. (60–70% and 20% soil moisture).

Semi-field experiment

The semi-field experiment was performed under trees (uncontrolled conditions) of citrus orchard at ARC-Giza, Egypt, as a preliminary test to examine the effect of the combination of EPN species. The experimental design consisted of six trees for two EPNs treatments and one another tree for control using a total of seven trees. The experiment was applied under trees canopies. The combined nematodes suspension concentrations (combination between the two tested steinernematids or

between those species of heterorhabditis) occurred for 1000, 2000, and 4000 IJs/ml. at maximum temperature (Max. T.) (28.85 °C); minimum temperatures (Min. T.) (19.71 °C) and R.H. (81.71%). Climatic conditions data were obtained from (Cairo International Airport Station) weather underground site (<https://www.wunderground.com>).

Four replicates were conducted for each concentration and distributed under every selected tree of the trial orchards; the accumulative mortality rate was estimated after ten days post treatments. The soil sample was taken using a spoon. The amount of the orchard soil was weighted at a rate of 25 gm and placed in a piece of muslin (to prevent larvae from escaping and attacking insects in the soil, especially ants) supplied with 20 full-grown larvae/replicate. The pieces of muslin were tied

with a piece of thread (Fig. 1). The time recorded during the experiment was 5 p.m., the muslin piece containing infected PFF full-grown larvae was buried 5 cm in the soil under the tree canopy and the control treatment was buried without nematodes. The experiment was irrigated every two days by a water hand sprayer in the 0.5 m² area surrounding each treatment.

Field experiment

Based on the initial screening of the previous four nematode strains, the most efficient steinernematid and heterorhabditis strains were selected for additional evaluations. The experiment was carried out in plastic cans measured (19×14×8 cm) (Fig. 2) filled with 200 gm soil (moisten soil) under four citrus trees canopies which maximum temperature (30.85 °C), minimum



Fig. 1 A piece of muslin contained infected full-grown larvae of *Bactrocera zonata*



Fig. 2 Cans contained *Bactrocera zonata* full-grown larvae in the field experiment at ARC, Giza Governorate

temperature (18.14 °C) and R.H. (88.00%), at the ARC, Giza, Egypt. The combined EPN species (*S. carpocapsae* (AII) + *H. bacteriophora* (HP88)) were treated at a concentration of 3000 IJs/ml. The EPNs applied for each test mixed with soil and water, which always kept at $55 \pm 5\%$ of the soil weight. In this experiment, every 20 full-grown larvae/replicate were placed in the plastic cans

Combined actions of the nematodes, *S. carpocapsae* (AII); *S. carpocapsae* (EGAZ10); *H. bacteriophora* (HP88); *H. indica* (EGAZ2); combined the two steinerematid species or heterorhabditis species, and combined *S. carpocapsae* (AII) + *H. bacteriophora* (HP88) on *B. zonata* full-grown larvae were used.

The combined action of the mixtures was expressed as a Co-toxicity factor, estimated by the equation of Mansour et al. (1966) as follows:

$$\text{Co - Toxicity factor} = \frac{\text{Observed \% mortality} - \text{Expected \% mortality}}{\text{Expected \% mortality}} \times 100$$

and covered with stockings to prevent escaping larvae and attacking by ants then tied with rubber bands. Part of the bottom of the cans was buried in the soil. Four replicates were used where each treatment placed next to its control. The numbers of dead larvae were recorded after 48 h, and the emergence of adults was recorded after 8 days post treatment.

The dead larvae (Fig. 3) and pupae for laboratory, semi-field and field experiments were incubated in white trap for collecting the infective juveniles of EPNs.

To find out if combining two EPN species would have an antagonistic, additive, or synergistic effect on *B. zonata* full-grown larvae, laboratory, semi-field and field experiments were carried out.

where: Observed % mortality is the mortality of individuals treated by the combination. Expected % mortality is the sum of mortalities of each material when used singly. These factors were used to differentiate the results into 3 categories. The interaction result was assessed as, 'synergism' when Co-toxicity factor values were greater than or equal to 20; 'addition' when Co-toxicity factor values were between -20 and $+20$; and 'antagonism' when Co-toxicity factor values were less than or equal to -20 .

Statistical analysis

Obtained data were analyzed according to Finney (1971). Percentage mortality was plotted versus the corresponding concentrations probit lines, and the median lethal concentration LC_{50} was determined for established

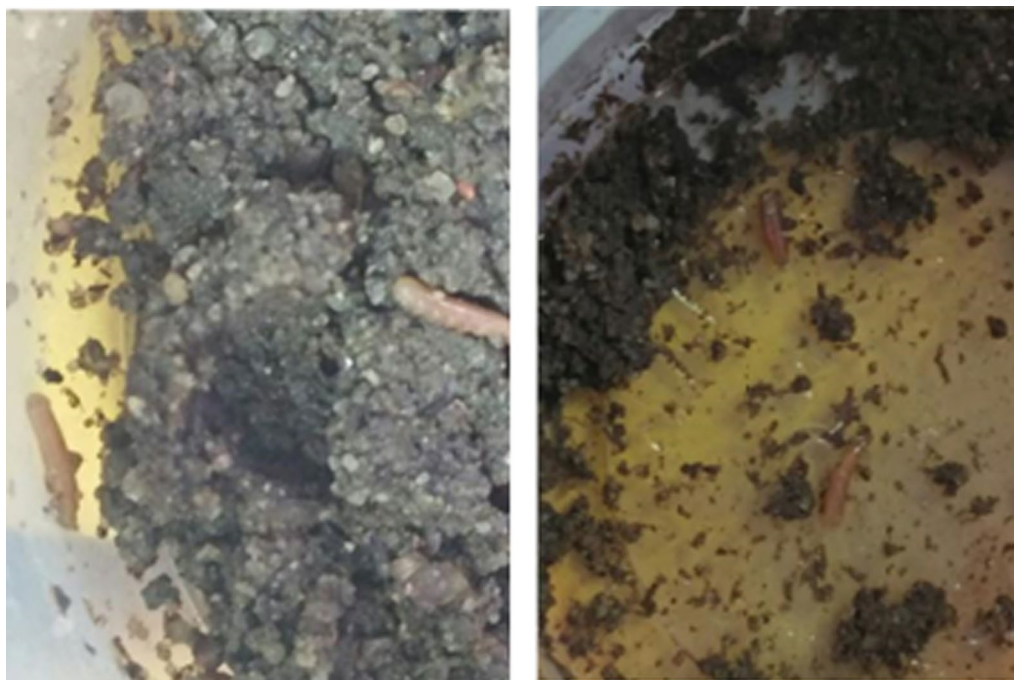


Fig. 3 Infected *Bactrocera zonata* full-grown larvae with entomopathogenic nematodes

regression lines using Ehab soft LDP line software to calculate probit analysis (www.Ehabsoft.com/LDP line). Lower and upper limits were determined when the (g) factor was less than 0.4. Data obtained for significant differences were analyzed using the Analysis of Variance one way (ANOVA) technique, and the means were separated using Fisher's least significant difference (LSD) test ($p=0.05$ level).

Results

Laboratory experiments

Data in Table 1 showed the mortality percentages of *B. zonata* full-grown larvae with *S. carpocapsae* (AII), *S. carpocapsae* (EGAZ10), *H. bacteriophora* (HP88) and *H. indica* (EGAZ2) strains at different concentrations (250, 500, 1000, 1500 and 2000), under laboratory conditions, at 25 ± 2 °C and R.H. (60–70% and 20% soil moisture). The lowest concentration of 250 IJs/ml of each tested strain (*S. carpocapsae* (AII), *S. carpocapsae* (EGAZ10), *H. bacteriophora* (HP88) and *H. indica* (EGAZ2)) resulted in mortality percentages of 15, 7.5, 10, and 7.5%, respectively, while mortality percentages reached 92.5, 85, 77.5 and 72.5% for the four mentioned EPN strains, respectively, at concentration 2000 IJs/ml. It was noticed that *S. carpocapsae* (AII) was effective than the other three strains. It is also clear that the mortality percentages increased when concentrations increased. The probit lines in Fig. 4 indicated the pathogenicity at different concentrations of steinernematid and heterorhabditis strains where the LC_{50} values were 794.3, 1063.2, 1249.8 and 1446.8 IJs/ml for *S. carpocapsae* (AII), *S. carpocapsae* (EGAZ10), *H. bacteriophora* (HP88) and *H. indica* (EGAZ2), respectively, at 3 days of treatments (Table 2).

Data pointed that steinernematid strains were more effective against *B. zonata* full-grown larvae than heterorhabditis strains at 25 ± 2 °C and R.H. (60–70% and 20% soil moisture). Also, Table 1 showed that there were

significant differences between mortality percentages at different concentrations.

Semi-field experiment

The combined concentrations of 1000, 2000 and 4000 IJs/ml for the two tested steinernematid (*S. carpocapsae* (AII) & *S. carpocapsae* (EGAZ10)) or those of heterorhabditis (*H. bacteriophora* (HP88) & *H. indica* (EGAZ2)) strains were used, in semi-field conditions at the ARC, Giza, Egypt. Data in Table 3 revealed that the combination of steinernematid strains was more effective than the combination of heterorhabditis strains. Direct relationship between the mortality percentages at different concentrations was recorded. Data in Table 4 revealed an antagonistic effect of the combined steinernematids, *S. carpocapsae* (AII) and *S. carpocapsae* (EGAZ10) where Co-toxicity factor value is (– 67.6). Also, *H. bacteriophora* (HP88) and *H. indica* (EGAZ2) in combination has a Co-Toxicity factor value (– 66.6). Despite the increase in the proportion of *B. zonata* death in the combined treatments for all the tested EPNs, the type of interaction was antagonism.

Field experiment

According to the previous trials, the field experiment was conducted. A nematode suspension was used at concentration of 3000 IJs/ml for the combined *S. carpocapsae* (AII) and *H. bacteriophora* (HP88) strains, under field conditions of maximum temperature (30.85 °C), minimum temperature (18.14 °C) and R.H. (88%). Results revealed that the combination of the two different strains of EPNs (*S. carpocapsae* (AII) + *H. bacteriophora* (HP88)) recorded a mortality percentage of 97.5%. On the other hand, the combined EPNs *S. carpocapsae* (AII) and *H. bacteriophora* (HP88) strains recorded an antagonistic effect, where the Co-toxicity factor value was (– 42.6) (Table 4).

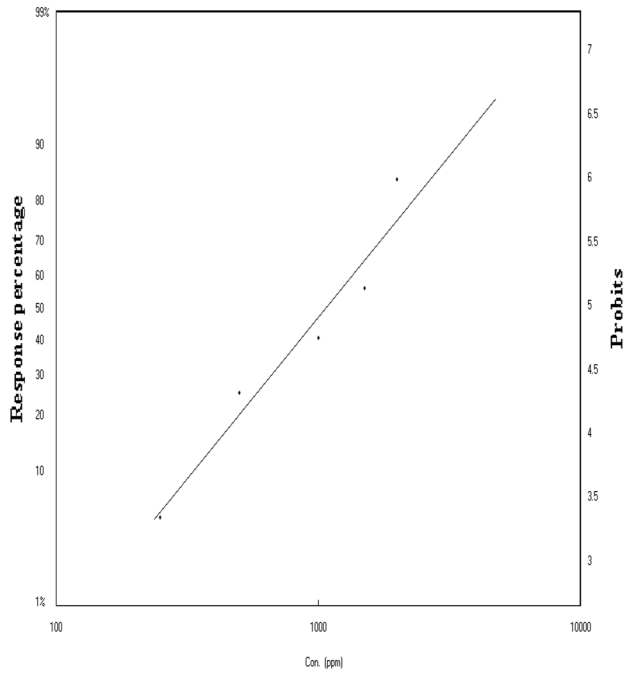
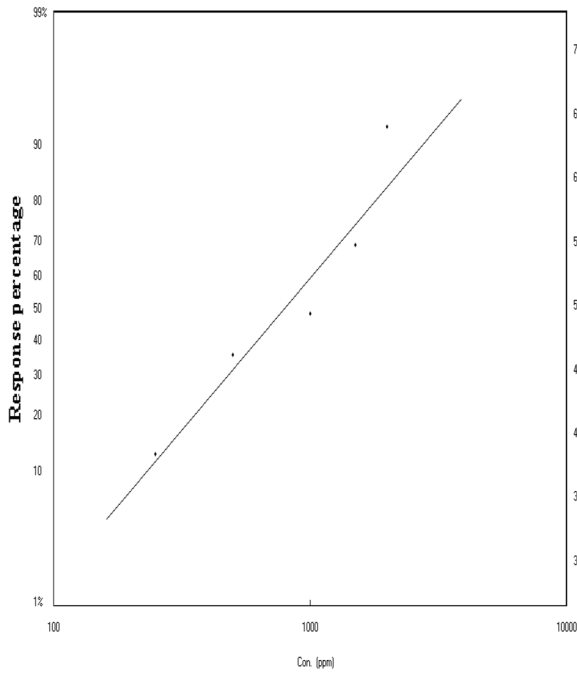
Table 1 Accumulative mortality percentages of *Bactrocera zonata* full-grown larvae with different concentrations under laboratory conditions at day 3 post experiment

Entomopathogenic Nematode strains (EPNs)	Control	Mortality %				
		250 IJs/ml	500 IJs/ml	1000 IJs/ml	1500 IJs/ml	2000 IJs/ml
<i>Steinernema carpocapsae</i> (AII)	2.5 f	15.0 e	37.5 d	50.0 c	70.0 b	92.5 a
<i>S. carpocapsae</i> (EGAZ10)	2.5 e	7.5 e	27.5 d	42.5 c	57.5 b	85.0 a
<i>Heterorhabditis bacteriophora</i> (HP88)	2.5 d	10.0 d	35.0 c	30.0 c	50.0 b	77.5 a
<i>H. indica</i> (EGAZ2)	2.5 d	7.5 d	22.5 c	27.5 c	47.5 b	72.5 a

Values followed by the same letter in the same row are not significantly different at $P=0.05$

Steinernema carpocapsae (AII)

S. carpocapsae (EGAZ10)



Heterorhabditis bacteriophora (HP88)

H. indica (EGAZ2)

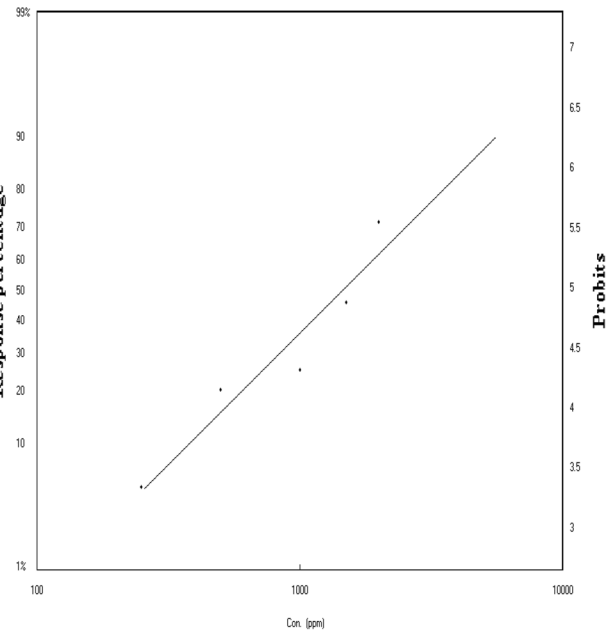
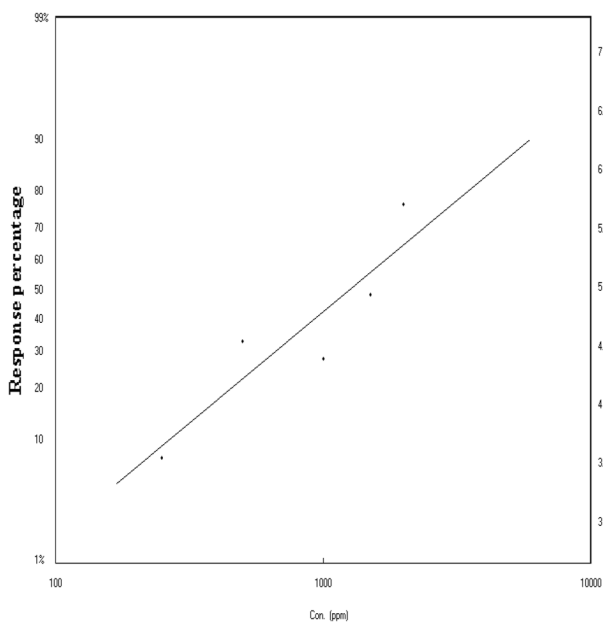


Fig. 4 Pathogenicity lines for different concentrations of steinernematid and heterorhabditis strains under laboratory conditions

Table 2 Toxicity of EPN strains against *Bactrocera zonata* full-grown larvae under laboratory conditions

Entomopathogenic Nematode strains	LC ₅₀	Lower limit	Upper limit	Slope
<i>Steinernema carpocapsae</i> (All)	794.3	463.3	1254.8	2.4
<i>S. carpocapsae</i> (EGAZ10)	1063.2	728.5	1662.2	2.5
<i>Heterorhabditis bacteriophora</i> (HP88)	1249.8	–	–	1.9
<i>H. indica</i> (EGAZ2)	1446.8	–	–	2.2

Table 3 Mortality percentages of *Bactrocera zonata* full-grown larvae infected with EPNs in semi-field experiment

Combination of EPN strains (Semi-Field experiment)	Mortality %		
	1000 IJs/ml	2000 IJs/ml	4000 IJs/ml
*steinernematid spp	40.0 de	57.5 c	92.5 a
**heterorhabditis spp	30.0 e	50.0 cd	77.5 b
Control	12.5 f	12.5 f	12.5 f

Values followed by the same letter in the same column are not significantly different at P=0.05

* *Steinernema carpocapsae* (All) + *S. carpocapsae* (EGAZ10)

** *Heterorhabditis bacteriophora* (HP88) + *H. indica* (EGAZ2)

Discussion

Results indicated that all tested nematodes had variable modes of bactericidal action in the *B. zonata* host, resulting in markedly antagonistic effects after strains' combination. The previous results revealed that steinernematid strains were more effective against *B. zonata* full-grown larvae than heterorhabditis strains. Also, there were significant differences between mortality percentages at different concentrations when EPNs were separately tested. Combination of the steinernematid or heterorhabditis

strains was more effective on the target pest. Data revealed the presence of an antagonistic effect due to the combinations of (*S. carpocapsae* (All) + *S. carpocapsae* (EGAZ10)) or (*H. bacteriophora* (HP88) + *H. indica* (EGAZ2)). In the field experiment, results revealed that the combination of the two different strains of EPNs (*S. carpocapsae* (All) + *H. bacteriophora* (HP88)) were more effective and recorded mortality reached 97.5%.

The results agree with those of Abd El-Motaal et al. (2021) who reported that the mortality percentage increased as IJs concentration increased. Rashad et al. (2015) demonstrated that both *S. carpocapsae* and *S. riobrave* were superior to *H. bacteriophora* in contact treatment against *B. zonata* full-grown larvae; and infectivity among those tested nematode strains was found significantly different. Steinernematid strains, also referred to as "ambushers," were more successful than heterorhabditis strains and more effective against soil-dwelling insects, as reported by Attalla and Eweis (2002). In field tests, *Steinernema* and *Heterorhabditis* species have been employed to control pest insects that emerge from fruit and burrow into the soil to pupate, with differing degrees of success. For example, fruit flies *Rhagoletis indifferens* and *Anastrepha ludens*

Table 4 The Combined activity of different EPN strains on mortality percentages of *Bactrocera zonata* full-grown larvae

Tested EPN strains	Mortality %	Expected mortality %	Observed mortality %	Co-Toxicity factor	Joint action category
* <i>S. carpocapsae</i> (All)	92.5	177.5	57.5	– 67.6	Antagonism
<i>S. carpocapsae</i> (EGAZ10)	85.0				
** Mix steinernematids	57.5				
*** <i>H. bacteriophora</i> (HP88)	77.5	150	50.0	– 66.6	Antagonism
<i>H. indica</i> (EGAZ2)	72.5				
****Mix heterorhabditis	50.0				
<i>S. carpocapsae</i> (All)	92.5	170	97.5	– 42.6	Antagonism
<i>H. bacteriophora</i> (HP88)	77.5				
*****Mix <i>S. carpocapsae</i> (All) + <i>H. bacteriophora</i> (HP88)	97.5				

* *Steinernema carpocapsae* (All)

** *Steinernema carpocapsae* (All) + *Steinernema carpocapsae* (EGAZ10)

*** *Heterorhabditis bacteriophora* (HP88)

**** *Heterorhabditis bacteriophora* (HP88) + *H. indica* (EGAZ2)

***** *Steinernema carpocapsae* (All) + *Heterorhabditis bacteriophora* (HP88)

(Diptera: Tephritidae) (Toledo et al. 2005) and several other species reviewed by Dolinski and Lacey (2007). Soil properties such as moisture level, pH, organic matter content, texture, and others, affect EPNs dispersal and potential to find a host (Stuart et al. 2015). Third instar larvae of several tephritid flies were reported to be susceptible to EPNs (Langford et al. 2014). Similarly, *S. carpocapsae* and *S. feltiae* were found to be more effective species under laboratory, semi-field, and field conditions against the European cherry fruit fly larvae where the mortalities were 88, 78, and 88%, respectively, while no mortality of pupae was observed (Köppler et al. 2004). On the other hand, at field temperature of 30 °C, *Heterorhabditis* species have a very strong ability to signal their maximal degree of pathogenicity according to El Khoury et al. (2018). These results are different from one insect to another may be due to some reasons such as insect behavior or insect physiological or/and the competition between the used two nematode strains or species. It was reported that combining nematodes species might be more effective in the field, because of the strongest concentration effect and highest propagation rate of the EPNs (O'Callaghan et al. 2014). The effect of mutualistic EPNs *Xenorhabdus* and *Photorhabdus* is gram-negative bacteria that can produce several secondary metabolites, including antimicrobial compounds. Otherwise, the nematodes or bacteria themselves make significant contributions to pathogenesis within the insect, poisoning effect and may be due to the presence of some physical or chemical reactions in the insect hemolymph (Lewis and Clarke 2012) and (Chang et al. 2019).

Conclusion

All EPNs experiments showed the efficacy of foreign nematodes than the local ones against *B. zonata* full-grown larvae and increasing mortality with concentration increase. Also, combination of the two highly effective imported strains gave satisfied results especially in the field experiment.

Acknowledgements

Not applicable.

Author contributions

All of the authors of this manuscript contributed equally to the design and execution of the experiments described in the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable—the study was conducted on insect species that are abundant in the ecosystem and does not require ethical approval.

Consent for publication

The manuscript has not been published in completely or in part elsewhere.

Competing interests

The authors declare that they have no competing interests.

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