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Virulence of Entomopathogenic Nematodes on *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) under Laboratory Conditions

Hadeer S. A. Rashed; Moamen A. Elbath; Naglaa F. Abd-Elhameid and Maha S. Khalil
Plant Protection Dept., Faculty of Agriculture, Benha Univ., Egypt.
*__Email__: moemen.elbath@fagr.bu.edu.eg

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ABSTRACT

The invasive fall armyworm *S. frugiperda* is considered nowadays the most important dangerous insect pest on the main crops especially in Egypt as it caused many losses to the Egyptian felid crops, this pest needs more alternative control methods to avoid chemical control harms, in this study, we have to estimate the virulence of two entomopathogenic nematodes on larval and pupal stages of *S. frugiperda* under laboratory conditions. Results indicated that mortality percentages of *S. frugiperda* were very satisfied with using the two entomopathogenic nematodes *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* in controlling FAW larvae and pupae. As *S. carpocapsae* and *H. bacteriophora* caused high mortality percentages against the 4 larval instars of FAW, results indicate that mortality percentages reached 100% after (48-120 h) post-treatment with five concentrations of *S. carpocapsae* (500, 1000, 1500, 2000 and 2500 IJs) against small larval instars (3rd and 4th) and after (72-120 h) against older instars (5th and 6th) exposed to the same five concentrations. Also, results indicated that *S. carpocapsae* caused faster mortality than *H. bacteriophora* under laboratory conditions. After calculation of LC_{50} of the two entomopathogenic nematodes against FAW larval instars; 3rd, 4th, 5th and 6th after 72 h, LC_{50} were; 454.183, 546.029, 600.777 and 908.079 for *S. carpocapsae* against the 4 larval instars, respectively, and 474.456, 552.126, 753.022 and 976.908 for the *H. bacteriophora* against the same four instars, this results cleared that the statistical analysis showed that there were slight or non-significant differences between the efficiency of the two EPNs in combating the fall armyworm larvae under laboratory conditions. Also, both EPNs were highly efficient in combating FAW pupae under laboratory conditions.

INTRODUCTION

The invasive fall armyworm, *Spodoptera frugiperda* is considered one of the different economic insect pests on many crops, which motivated the researchers to develop an effective alternative biological control method at the global level. FAW is a polyphagouse pest with high fecundity (100 to 1000 eggs) in clusters covered with scales. Like all the lepidopterans, it has four stages, *i.e.*, egg to adult and six larval instars, the oldest larval instars causing greater damage reaching over 70% of the damage in corn. Fully developed

larvae pupate in the soil at a depth of 3–10 cm, and adults hide in whorl during that time and lay eggs on leaves (Assefa 2018 & Mohamed and Shairra 2023). This pest has chewing mouth parts that feed on plant tissue, called "Whorl worm", as its larval stage prefers the leaves and tender shoots, mostly buds. Being polyphagous with a high capacity for dispersal and adaptation, this pest holds the title of a potential pest (Casmuz *et al*., 2010, Montezano *et al*., 2018). Major factors in its growth and proliferation have been attributed to its cannibalistic character and competitive displacement skills (Divya *et al*., 2021 and Ratnakala *et al*., 2023).

Various Control methods have been employed to eliminate this pest; however, farmers continue to depend on both traditional and modern chemical insecticides, which are regarded as the main management strategy by the majority of farmers. Frequent and continuous uses of the major classes of these chemicals and pesticides have developed high resistance against them. Chemical insecticides have harmful effects on the environment and human health; improving alternative environmentally friendly control against this pest is desperately required. (Wan *et al*., 2021 & Mohamed and Shairra 2023). Furthermore, chemical control has not provided a long-term solution for these pest damages due to the adverse effect on natural enemies, pollinators and biodiversity, environmental pollution, minor pests' resurgence and human health, especially the applicator (Akhtar and Farooq 2019). So, among the eco-friendly management strategies and methods, biological control by natural enemies is highly recommended for this pest. In this concept, entomopathogenic nematodes (EPNs) belonging to the Steinernematidae and Heterorhabditidae families have been widely used as promising bio-control agents in the last few decades on various economic insect pests (Thakur *et al.*, 2022). Various nematode families have been tested as potential bio-control agents for insect pests, species belonging to Steinernematidae and Heterorhabditidae families have the ability to be effective bio-control agents for insect pest management (Kaya and Gaugler, 1993; Grewal *et al.*, 2005 and Koppenho¨fer, 2007) and have been utilized as classical, conservational, and augmentative bio-control agents. The vast majority of applied research focused on their potential as inundatively applied augmentative bio-control agents (Grewal *et al*., 2005). Rhabditida (Heterorhabditidae and Steinernematidae) are characterized by their exclusive pathogenicity to insects as they killed hosts during a short period of time (24 to 48 h) due to their mutualistic association with bacteria (Akhurst and Smith 2002; Griffin *et al*., 2005 and Gozel and Gozel 2016). Divya *et al*., 2021 and Ratnakala *et al*., 2023 as the larval stage of FAW remains in maize whorl, that gave the placement of entomopathogenic nematodes (EPNs) opens up a wide opportunity for biological control of this dangerous invasive insect pest. Therefore, this study was designed to evaluate the bio-efficacy of two EPNs species, *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* (All), on various developmental FAW larval instars, ranging from the third to the sixth instar, in relation to differing nematode concentrations and exposure times.

MATERIALS AND METHODS

Mass Rearing of The Fall Armyworm:

FAW larvae were collected from Syngenta Company, which were collected from infested maize fields in the Egyptian governorates. *S. frugiperda* mass rearing technique was conducted according to Rashed 2023. *S. frugiperda* larvae were put into plastic containers (30 cm diameter × 20 cm height) and fed on fresh maize leaves until pupation. Maize plants were planted in the biological control greenhouse in the faculty of agriculture at Benha University. Pupae were collected daily and placed in glass jars until adult moths' emergence. Moths were fed on (10%) honey solution hanged the glass jar and changed daily. The newly

laid eggs were collected daily and served until hatching and the neonate larvae were reared on fresh maize leaves until pupation. Pupae were transferred to new jars until adult emergence and egg-laying. *S. frugiperda* was reared under laboratory conditions (25 ± 2 °C) for 4 generations for beginning the desired study.

Maintenance and Mass Rearing of EPNs:

The two entomopathogenic nematode species, *S. carpocapsae* (All and *Heterorhabditis bacteriophora* 88 were tested against different 4 larval instars and pupal stages of *S. frugiperda* to study their pathogenicity *on S. frugiperda*. EPN species were maintained from Plant Protection Research Institute, Agricultural Research Center, Giza, Egypt, by Dr. Ahmed Azazy, the two EPNs were cultured on the 6th larval instar of the GWM, *Galleria mellonella* Linnaeus. The Agricultural Research Centre (ARC), Giza, Egypt's Dr Ahmed Azazy provided the EPN species, which were then reared in vivo on fully developed larvae of the greater wax moth, *G. mellonella* Linnaeus (Lepidoptera: Pyralidae). Using a modified White trap by Kaya and Stock (1997), the emerging infective juveniles (IJs) from *G. mellonella* larval cadavers were collected in sterile distilled water (d. w.) upon inoculation. These were then preserved at 10 °C for *S. carpocapsae* and 15–20 °C for *H. indica* until needed. Before being used in the tests, freshly emerged IJs nematodes were maintained at room temperature for at least five hours.

Virulence and Bioassay of PNs on FAW Instars:

FAW larval instars from 3rd to 6th and newly formed pupae were exposed to different serial concentrations (500, 1000, 1500, 2000 and 2500 IJs/5 larvae) and were tested for each nematode species separately. Five larvae from each instar/concentration/ EPN species were placed into plastic cups (3 cm height × 5cm diameter) lined with a piece of tissue paper and provided with daily maize leaves as food for larvae. Three replicates for each concentration were conducted per each larval instar for nematode species and control was included in the bioassay experiment. Data recorded after 48, 72, 96 and 120 h post-treatment, mortality percentages of larvae and pupae, deformation %. Probit analysis was performed for calculating LC_{50} and LC_{90} after 72 hours post-treatment according to Finney (1971). Pupae were put on plastic cubs and placed in 10 gm. of a sterilized mixture of soil and sand and pupae placed on it and then treated with three concentrations of the two EPNs separately (500, 1000, 2500 IJs), this experiment was replicated three times and each replicate were 5 cups, each cup contains 5 pupae.

Statistical Analysis:

Probit analysis was performed for calculating LC50, and LC90 according to Finney (1971). All data were expressed as standard error and statistically analyzed using SAS (Statistical Analysis Software) SAS (1999). The statistical significance of differences among different study groups was evaluated by one-way analysis of variance (Danken) ($P \leq 0.05$). Duncan's multiple range tests were used to differentiate between means to determine differences between means of treatments at significance rates of ($P \leq 0.05$).

RESULTS

Effect of the Two EPNs on Larval Instars of *S. frugiperda*:

After treatment, the 3rd, 4th, 5th and 6th larval instars of *S. frugiperda* with *S. carpocapsae* and *H. bacteriophora* with five concentrations 500, 1000, 1500, 2000 and 2500 IJs / 5 larvae mortality and deformation percentages were recorded after 48, 72, 96 and 120 h post-treatment and the followed data indicated this results;

Pathogenicity of *S. carpocapsae:*

Data in Figure (1a) demonstrated the mortality percentages of the 3rd larval instar with *S. carpocapsae* as it caused 100 % mortality after 48, 72, 96, and 120 h for

concentrations 1000, 1500, 2000 and 2500 IJs, respectively, and for the concentration of 500 IJs caused the highest mortality percentage after 120 h. post-treatment as it recorded 93.33% with a deformation percentage of 6.66 %, (Fig. 3) for the emerged pupae, which died after the shot time of emergence. For the 4th larval instar, *S. carpocapsae* caused 100% mortality after 96 and 120 h post-treatment, with 1500, 2000 and 2500 IJs, and for lower concentrations 500 and 1000 caused the highest mortality percentages (86.66 and 93.33%) after 120 h post-treatment with deformation percentages (13.33 and 6.66 %), (Fig. 3) for the emerged adults which died after emergence, (Fig, 1b).

On the other hand the mortality percentages of the 5th larval instar which were treated with the same five concentrations; results showed that the concentrations 1500, 2000 and 2500 IJs caused 100% mortality after 72 h exposure time, while the mortality reached 80% post-exposure to concentration 500 IJs after 96 h, and 93.33 % mortuary after 120 h of treatment with 1000 IJs. Also, after treatment of the 5th larval instar with the same five concentrations the high concentrations caused mortality (100%) after exposure to 2500 IJs and 100% after 72 h. from treatment with 2000 IJs, while for low concentrations caused mortality 80 % after 96h. with deformation percentage 20% of emerged adults and 93.33 mortality after 120h. with 6.66 % deformation of adults, (Fig. 1c). On the same trend for the 6th instar, *S. carpocapsae* caused 100% mortality after 96 h post-treatment for concentrations 2000 and 2500 IJs and after 120 after treatment with 1500 IJs, while for lower concentrations 500 and 1000 IJs caused 80 and 86.66 % mortality with 20 and 13.33 % deformation of emerged adults, respectively, after 120 h post-treatment (Fig. 1d).

Data in Figure (1) indicates that the higher the concentrations the faster the death rate, especially at young instars, and progressively for older ones as they increased in age, as the concentration (2500 IJs) recorded the highest death rates in the shortest periods, while the concentration (500 IJs) recorded death rates after longer periods, also, the older treated larvae, the longer the death periods. After calculating the LC_{50} and LC_{90} in Table (1) data cleared that the LC_{50} of *S. carpocapsae* were 454.1546, 546,029, 600,777 and 908.079 IJs, respectively, for the 3rd, 4th, 5th and 6th larval instars. These results indicated that the 6th instar needs the highest concentrations to kill 50 % of the FAW population, in contrast to the 3rd instar which needs the lowest concentration to kill 50 % of the FAW population. Finally, this data shows that *S. carpocapsae* is an effective agent in combating this invasive insect pest *S. frugiperda* under laboratory conditions.

Table1. Pathogenicity of *Steinernema carpocapsae* against four different larval instars of FAW in different concentrations after 72 h of treatment.

Larval instar	LC_{50} (IJs/larva)	LC_{90} (IJs/larva)	Slope±SE	Coefcients (intercept)	R2
3rd	454.183 (278.452-740.816)	1630.341 (999.536-2659.243)	2.397±0.108	-1.363	0.734
4th	546.029 (356.717-835.809)	1705.707 (1114.327-2610.935)	2.712±0.094	-2.409	0.714
5th	600.777 (375.469-961.286)	2142.360 (1338.915-3427.929)	2.354±0.104	-1.538	0.851
6th	908.079 (659.452-1250.444)	2694.338 (1956.642-3710.160)	2.808±0.071	-3.310	0.843

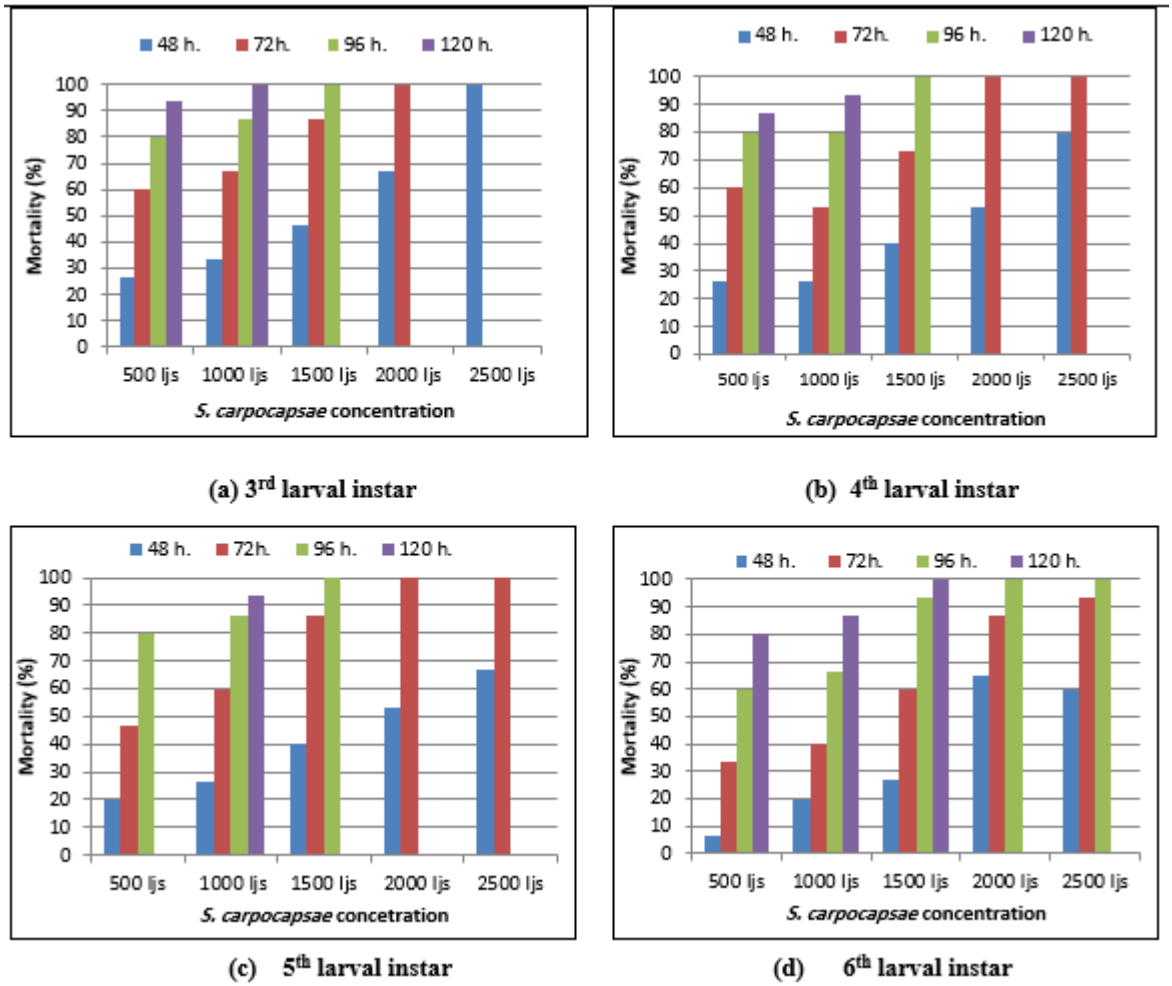


Fig. 1: ***S.*** *frugiperda* mortality percentages post-treatment period (48, 72, 96 and 120 hours) with *S. carpocapsae* in different concentrations under laboratory conditions.

Pathogenicity of *H. bacteriophora:*

After exposure of the 3rd larval instar of *S. frugiperda* to 5 concentrations of *H. bacteriophora* the results indicated that *H. bacteriophora* caused 100% mortality after 72 h with exposure to the 3rd instar to 2000 and 2500 IJs. and after 96 h of treatment with 1500 IJs, and for concentrations (500 and 1000 IJs) it caused 86.33 and 83.33% mortality, respectively, after 120 h with 13.33 and 6.66 % deformation of the emerged adults for the same two concentrations, (Fig. 2a). The deformed emerged adults don't complete their life. For the 4th larval instar, the mortality percentage increased with increasing the concentration as after 96 h the percentage of mortality was 100% after being exposed to (1500, 2000 and 2500 IJs) and for the lowest concentrations (500 and 1000 IJs) were recorded (86.66 and 93.33 %), respectively, with 13.33 and 6.66 % deformation rates of the emerged adults, (Fig. 3) which died directly after emergence, (Fig. 2b).

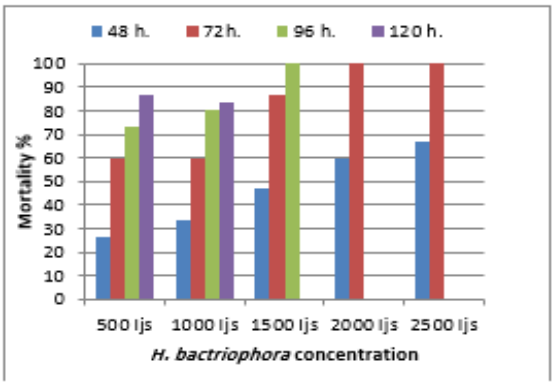
Data completed on the same trend for the 5th larval instar as *H. bacteriophora* recorded 100% mortality after 96 h after exposed to concentrations (1500, 2000 and 2500 IJs) and 80% after exposed to 500 IJs after 120 h with deformation percentage 20% of the emerged adults and 86.66 % mortality after 96 h of exposure to 1000 IJs with deformation percentage 13.33 %,(Fig, 2c). In the case of the 6th larval instar the highest concentrations (1500, 2000, 2500 IJs) caused 100% mortality after 96 h post-treatment and caused 73.33 and 80 % mortality, respectively, after 120 h from exposure to 500 and 1000 IJs with 26.66 % and 20% deformation percentage, (Fig. 3). From these results, it is clear that the higher the death rate and the faster the death periods, as the highest concentration of 2500 IJs recorded the highest death rates in the shortest periods, while the concentration of 500 recorded death rates after longer periods, also, the older treated larvae, the longer the death periods. And after calculation of LC_{50} for *H. bacteriophora* it was recorded 474.456, 552.126, 753.022 and 976.908 IJs,

respectively, for the 4 instars and that indicates the 6th instar needs the highest numbers of IJs for killing 50% from FAW 6th larval instar population, (Fig. 2d and Table 2).

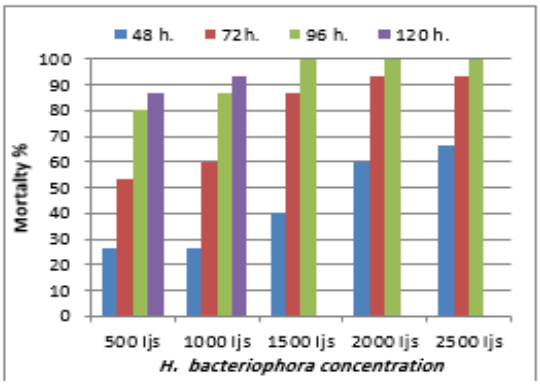
After statistical analysis of previous results, it was found that both species of EPNs are considered effective biocontrol agents in combating *S. frugiperda* larvae under laboratory conditions Table (3). The analysis also, demonstrated that there are no significant differences between *S. carpocapsae* and *H. bacteriophora* in controlling FAW larval instars, but *S. carpocapsae* was faster in killing FAW larval instars than *H. bacteriophora*, especially against the last instars.

Table 2: *H. bacteriophora* Pathogenicity against different larval instars of *FAW* at different concentrations (IJs/ml) after 72 h of exposure.

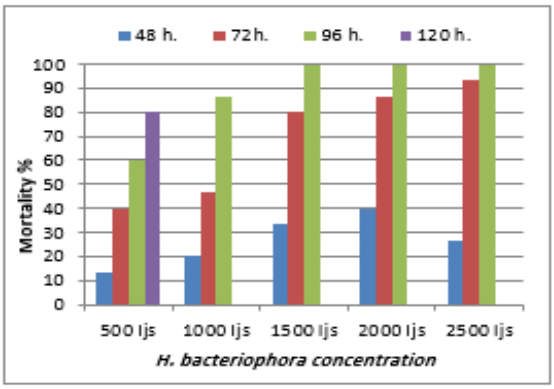
Larval instar	LC_{50} (IJs/larva)	LC_{90} (IJs/larva)	Slope ±SE	Coefcients (intercept)	R2
3rd	474.456 (289.208-778.360)	1816.784 (1107.436-2980.493)	2.334±0.110	-1.234	0.611
4th	552.126 (360.050-846.669)	1890.795 (1233.017-2899.479)	2.408±0.095	-1.594	0.857
5th	753.022 (530.695-1068.491)	2340.625 (1649.562-3321.199)	2.620±0.078	-2.538	0.900
6th	976.908 (671.459-1421.307)	3926.790 (2699.004-5713.100)	2.294±0.083	-1.846	0.733



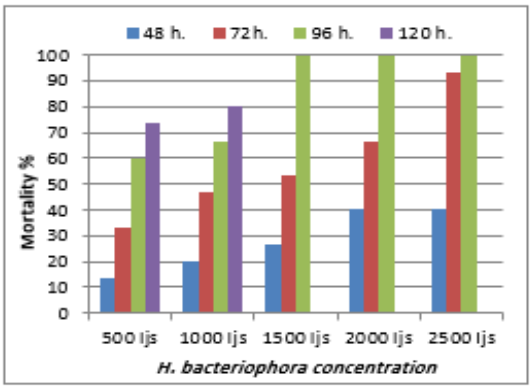
(a) 3rd larval instar



(b) 4th larval instar



(c) 5th larval instar



(d) 6th larval instar

Fig. 2: ***S. frugiperda*** mortality percentages after post-exposure period (48, 72, 96 and 120 hours) with *S. carpocapsae* in different concentrations under laboratory conditions.

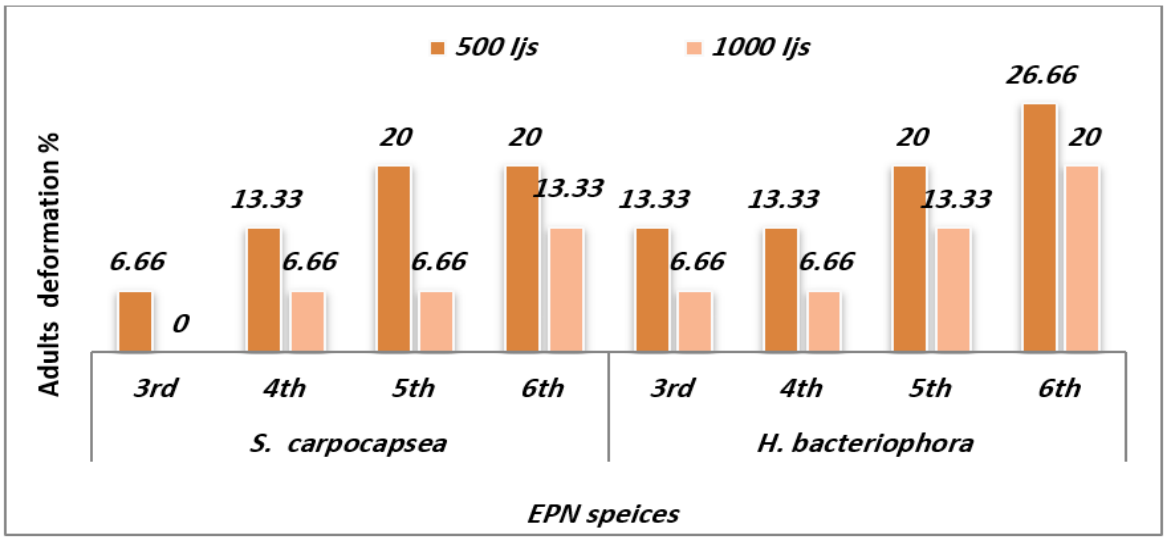


Fig 3: Deformation percentages of the resulting adults emerged from *S. frugiperda* pupae exposed to *S. carpocapsae* and *H. bacteriophora* with concentrations of 500 and 1000 IJs.

Table 3: Comparison of mortality percentages of *S. frugiperda* larvae exposed to five concentrations of *S. carpocapsae* and *H. bacteriophora* after 48, 72, 96 and 120 h of treatment.

Mortality of 3rd larval instars								
EPNs conc.	**After 48 H.**		**After 72 H.**		**After 96 H.**		**After 120 H.**	
	S.c	**H. b**	**S.c**	**H. b**	**S.c**	**H. b**	**S.c**	**H.b**
500 IJ	26.67±0.01^{a}	26.66±0.01^{a}	60.0±0.58^{a}	60.0±0.58^{a}	80±0.41^{a}	73.33±0.41^{b}	93±0.41^{a}	100±0.41^{a}
1000 IJ	33.33±1.65^{a}	33.33±1.65^{a}	66.66±0.04^{a}	60.0±0.04^{b}	86.66±0.01^{a}	80.0±0.01^{b}	83.33±0.41^{b}	86.33±0.41^{b}
1500 IJ	46.66±0.01^{a}	46.66±0.01^{a}	86.66±0.01^{a}	86.66±0.01^{a}	100±0.58^{a}	100±0.58^{a}	--	--
2000 IJ	66.67±0.04^{a}	60.0±0.04^{b}	100±0.58^{a}	100±0.58^{a}	--	--	--	--
2500 IJ	100±0.41^{a}	66.66±0.41^{b}	--	--	--	--	--	--
Mortality of 4Th larval instars								
	After 48 H.		**After 72 H.**		**After 96 H.**		**After 120 H.**	
	S.c	**H. b**	**S.c**	**H. b**	**S.c**	**H. b**	**S.c**	**H. b**
500 IJ	26.66±0.01^{a}	26.66±0.01^{a}	60±0.41^{a}	53.33±0.41^{b}	80±0.58^{a}	80±0.58^{a}	86.67±0.01^{a}	86.66±0.01^{a}
1000 IJ	26.66±0.01^{a}	26.66±0.01^{a}	53.33±0.41^{b}	60±0.41^{a}	80±0.01^{b}	86.66±0.01^{a}	93.33 ±0.01^{a}	93.33±0.01^{a}
1500 IJ	40±0.58^{a}	40±0.58^{a}	73.33±0.01^{b}	86.66±0.01^{a}	100±0.58^{a}	100±0.58^{a}	--	--
2000 IJ	53.34 ±0.41^{b}	60±0.41^{a}	100±0.41^{a}	93.33±0.41^{b}	--	--	--	--
2500 IJ	80±0.41^{a}	66.6±0.41^{b}	100±0.41^{a}	93.33±0.41^{b}	--	--	--	--
Mortality of 5Th larval instars								
	After 48 H.		**After 72 H.**		**After 96 H.**		**After 120 H.**	
	S.c	**H. b**	**S.c**	**H. b**	**S.c**	**H. b**	**S.c**	**H. b**
500 IJ	20±0.41^{a}	13.33±0.41^{b}	46.66±0.01^{a}	40±0.01^{b}	80±0.58^{a}	60±0.58^{b}	93.33±0.41^{a}	80±0.41^{b}
1000 IJ	26.66±0.41^{a}	20±0.41^{b}	60±0.41^{a}	46.66±0.41^{b}	86.67±0.01^{a}	86.66±0.01^{a}	100±0.41^{a}	93.33±0.41^{b}
1500 IJ	40±0.41^{a}	33.33±0.41^{b}	86.67±0.01^{a}	80±0.01^{b}	100±0.58^{a}	100±0.58^{a}	--	--
2000 IJ	53.34 ±0.41^{a}	40 ±0.41^{b}	100±0.41^{a}	86.66±0.41^{b}	--	--	--	--
2500 IJ	66.66±0.01^{a}	66.66±0.01^{a}	100±0.41^{a}	93.33±0.41^{b}	--	--	--	--
Mortality of 6Th larval instars								
	After 48 H.		**After 72 H.**		**After 96 H.**		**After 120 H.**	
	S.c	**H. b**	**S.c**	**H. b**	**S.c**	**H. b**	**S.c**	**H. b**
500 IJ	6.66±0.01^{a}	13.33±0.01^{a}	33.33±0.01^{a}	33.33±0.01^{a}	60±0.58^{a}	60±0.58^{a}	80 ±0.41^{a}	73.33±0.41^{b}
1000 IJ	20±0.58^{a}	20±0.58^{a}	40±0.41^{b}	46.66±0.41^{a}	66.66±0.01^{a}	66.66±0.01^{a}	86.66 ±0.01^{a}	80 ±0.01^{b}
1500 IJ	26.66±0.01^{a}	26.66±0.01^{a}	60±0.41^{a}	53.33±0.41^{b}	93.33±0.41^{b}	100±0.41^{a}	100±0.58^{a}	100±0.58^{a}
2000 IJ	64.66±0.41^{a}	40±0.41^{b}	86.66±0.01^{a}	66.66±0.01^{b}	--	--	--	--
2500 IJ	60±0.58^{a}	40±0.58^{b}	93.33±0.01^{a}	93.33±0.01^{a}	--	--	--	--

Pathogenicity of the two EPN species on the pupal stage

As presented in Figure (4), after exposing the fresh-formed pupae of *S. frugiperda* to the two EPNs *S. carpocapsae* and *H. bacteriophora* with three concentrations of 500, 1500, 2500 IJs/5 pupae, results indicated that after treatment the pupae with the three concentrations, that accumulative pupal mortality percentages were 52, 72, 80% after 120 h of treatment with *S. carpocapsae* and caused deformation % (50, 66 and 80 %) of the emerged adults which died after a short time of emergence from treated pupae, and after 120 h from treatment with *H. bacteriophora* infective juveniles it was recorded pupal mortality (48, 64, and 72 %) and the emerged adult individuals recorded deformation % (46, 62 and 66%). The remaining adults after treatment with *S. carpocapsae* and *H. bacteriophora* were unable to lay eggs and died shortly after treatment. These results indicate that pupae exposed to *S. carpocapsae* recorded higher mortality rates compared to *H. bacteriophora* which caused lower mortality and deformation percentages for the emerged adults. This experiment was conducted to facilitate future field experiments and to predict the ability of entomopathogenic nematodes to kill pupae in the soil.

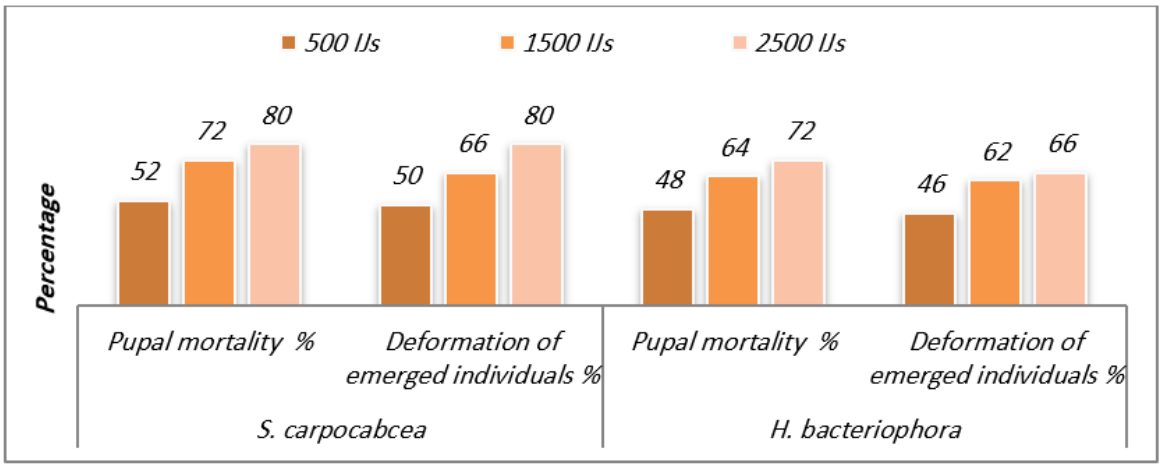


Fig. 4: Effect of the two EPN species on the pupal stage accumulative mortality and deformation percentages.

DISCUSSION

Patil *et al*., 2022, studied the virulence of two (EPNs) isolates under laboratory assay and field conditions along with comparing with the insecticide emamectin benzoate against fall armyworm. Results indicated that both *H. indica* 1 NBAIIH38 and *S. carpocapsae* NBAIRS59 caused 100% mortality in 3rd and 4th instar larvae of *S. frugiperda* and under field conditions the two species caused 85% and 72% mortality in pupae, respectively, pupae died after metamorphosis to malformed adults. Field trial results showed that *H. indica* significantly reduced the number of larvae and leaf damage scores compared to *S. carpocapsae*. Emamectin benzoate was more effective in reducing the larval population compared to EPN species. Also, in a similar study, Ratnakala *et al.*, 2023 evaluated the effect of *H. indica* (Poinar) and *S. carpocapsae* (Weiser) on all stages of *S. frugiperda* and results revealed that both EPNs had a high rate of ovicidal, larvicidal and pupicidal effects on *fall armyworm*, also significantly affected the resulting adults with deformation and death. Also, the authors studied the effect of the two EPNs application on *S. frugiperda* under semi-field conditions by pre-releasing third instar larvae to the whorl of maize plants. Application of *H. indica* and *S. carpocapsae* at 500 IJs mixed with five grams of sand was applied manually

to whorl leading to mortality of 86.67 and 83.33%, respectively. This study indicated that both the EPN species showed high effects as bio-control agents against *the invasive fall armyworm*, having the potential for its successful management. Mohamed and Shairra, 2023, also, studied the pathogenicity bioassays of the same previous two nematodes, *S. carpocapsae* (All) and *H. indica* (EGAZ2), on the 2nd, 3rd, 4th, 5th and 6th instars of *S. frugiperda* under laboratory conditions. Results indicated that *S. carpocapsae* application was more virulent and effective against all tested instar larvae than *H. indica*. Also, results indicated that *S. frugiperda* larval mortality % varied significantly related to nematode species, post 48–72 h post-treatment at different nematode concentrations (150-2400 IJs). However, *H. indica* caused 100% mortality in young instars only after 96 h, but late instars required a longer time extending to 120–188 h at tested concentrations. All recovery larvae post-infection died during the pupal stage or adults emerged with wing malformation. Finally**,** the two nematode species were virulent and effective against different FAW larval instars at different concentrations and exposure times and they are recommended as bio-control agents against this pest,

Conclusion

Because of the danger of fall armyworm in Egyptian fields planted with cereal crops, especially maize, and to avoid the extensive and repeated use of pesticides to control this pest, it is necessary to resort to alternative control strategies for this invasive pest, so, this study focused on evaluating entomopathogenic nematodes as one of the alternative and safe agents to control this pest under laboratory conditions, to be able to be used and applied in the future under field conditions. This study proved that the two types of nematodes evaluated in this study; *S. carpocapsae* (All) and *Heterorhabditis bacteriophora* 88 were very effective in combating the larval and pupal stages of this insect under laboratory conditions.

Declarations:

Ethical Approval: The Research Ethics Committee of the Faculty of Agriculture, Mashtohar, Benha University met and reviewed the following study protocol from an ethical perspective: Study number: REC-FOABU.14/00025

Competing interests: The authors declare that they have no duality of interest associated with this manuscript.

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REFERENCES

Akhtar, M. N. and Farooq A. 2019. Environmental impact of bollworms infestation on cotton *Gossypium hirsutum*. *Pakistan* Journal of *Zoology*. 51(6):2099–2106.

Akhurst, R. and Smith K. 2002. Regulation and safety. In: Gaugler R. (Ed.) Entomopathogenic. https://doi.org/10.1079/9780851995670.0311.

Assefa, F. 2018. Status of fall armyworm (*Spodoptera frugiperda*), biology and control measures on maize crop in Ethiopia: a review *International Journal* of *Entomology*. 06 (02):75-85.

Casmuz, A. *et al.* 2010. Review of the host plants of fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae) *Revista de la Sociedad Entomológica Argentina*.69:3-4.

Divya, J.; Kalleshwaraswamy, C. M.; Mallikarjuna, H. B. and Deshmukh S. 2021. Does recently invaded fall armyworm, *Spodoptera frugiperda* displace native lepidopteran pests of maize in India? *Current Science*, 120(8):25.

Gozel, U. and Gozel, C. 2016. Entomopathogenic Nematodes in Pest Management Integrated Pest Management (IPM): Environmentally Sound Pest. pp:55-69 http://dx.doi.org/10.5772/63894.

Grewal, P. S.; Ehlers, R.U. and Shapiro-Ilan D. I. 2005. Nematodes as biological control agents. Wallingford: CABI Publishing.

Griffin, C. T.; Boemare, N. E. and Lewis E. E. 2005. Biology and behavior. In: Grewal, P.S., Ehlers, R.U. & Shapiro-Ilan, D.I. (eds). Nematodes as biocontrol agents. CAB International, Wallingford. pp. 47 – 64. https://doi.org/10.1079/9780851990170.0047.

Rashed H. S. A. 2023. Biology, Host Selection Behavior and Growth Indices of Invasive Fall Armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) on Two Host Plants under Laboratory Conditions. *Journal of Plant Protection and Pathology.* 14 (6):181-186.

Kaya, H. K. and Gaugler, R. 1993. Entomopathogenic nematodes. *Annual Review of Entomology*, 38: 181–206

Kaya, H. K. and Stock, S. P. 1997. Techniques in insect nematology. Manual of techniques in insect pathology. Academic Press, London, 281–324.

Koppenho¨fer, A. M. 2007. Nematodes. Pp. 249–264 in L. A. Lacey and H. K. Kaya, eds. Field manual of techniques in invertebrate pathology: Application and evaluation of pathogens for control of insects and other invertebrate pests, second ed. Dordrecht: Springer.

Mohamed, H. O. and Shairra, S. A. 2023. Pathogenicity of entomopathogenic nematodes against the new invasive fall armyworm, *Spodoptera frugiperda* (J. E. Smith)Lepidoptera: Noctuidae. *Egyptian Journal of Biological Pest Control*.33:24, 10pp. https://doi.org/10.1186/s41938-023-00669-0.

Montezano, D.G.; Specht, A.; Sosa-Gómez, D.R. ; Roque-Specht, V.F. ; Sousa-Silva, J.C.; Paula-Moraes, S.V.; Peterson, J.A. and Hunt, T.E.2018. Host Plants of Spodoptera frugiperda (Lepidoptera: Noctuidae) in the Americas. *African Entomology*,26:286-300. https://doi.org/10.4001/003.026.0286.

Patil, J.; Linga, V.; Vijayakumar, R.; Subaharan, K.; Navik, O.; Bakthavatsalam, N. and Sekhard, P. H. M. J. 2022. Bio-control potential of entomopathogenic nematodes for the sustainable management of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in maize. *Pest Management Science*, 78: 2883–2895.

Ratnakala, B.; Kalleshwaraswamy, C. M.; Rajkumar, M. and Mallikarjuna, H. B. 2023. Bio-control potential of entomopathogenic nematodes against invasive fall armyworm, *Spodoptera frugiperda*. *Indian Biological Control*, 85(12):105304

SAS . 1999. SAS user's guide: Statical Analysis System Institute, SAS Inst. Cary NC, USA.

Thakur, N.; Tomar, P.; Sharma, S.; Kaur, S.; Sharma, S.; Yadav, A. N. and Hesham, A. E. 2022 Synergistic effect of entomopathogens against *Spodoptera litura* (Fabricius) under laboratory and greenhouse conditions. *Egyptian Journal of Biological Pest Control.* 32(1):1–10.

Wan, J.; Huang, C.; Li, C. Y.; Zhou, H. X.; Ren, Y. L.; Li, Z. Y.; Xing, L.S.; Zhang, B.; Qiao X.; Liu, B.; Liu, C.H.; Xi, Y, Liu, W.X.; Wang, W.K.; Qian, W.Q.; McKirdy, S. and Wan, F.H. 2021. Biology, invasion and management of the agricultural invader: fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *Journal of Integrative Agriculture.* 20:646–663.

ARABIC SUMMARY

ضراوة النيماتودا الممرضة للحشرات على دودة الحشد الخريفية ((Lepidoptera: Noctuidae تحت الظروف المختبرية

هدير شوقي عبدالله راشد و مؤمن احمد مصطفي محمد البطح و نجلاء فكرى عبدالحميد و مها سعيد خليل
قسم وقاية النبات, كلية الزراعة, جامعة بنها, مصر

تعتبر دودة الحشد الخريفية الغازية في الوقت الحاضر من أهم الآفات الحشرية الخطيرة على المحاصيل الرئيسية خاصة في مصر وتسببت في الكثير من الخسائر لمحاصيل الحقول المصرية، وتحتاج هذه الآفة إلى المزيد من طرق المكافحة البديلة لتجنب أضرار المكافحة الكيميائية، في هذه الدراسة تم تقييم تأثير نوعي نيماتودا ممرضة للحشرات *Steinernema carpocapsae* و*Heterorhabditis bacteriophora* والتي تم تقييمها على أطوار اليرقات والعذارى لدودة الحشد تحت الظروف المعملية. أشارت النتائج إلى أنه يوصى بشدة باستخدام النيماتودا الممرضة للحشرات *S.carpocapsae* و*H. bacteriophora* حيث أعطت نسب موت عالية في مكافحة يرقات وعذارى دودة الحشد الخريفية. حيث أن *S. carpocapsae* اعطت نسب موت لليرقات بنسبة 100% بعد (48-120 ساعة) للعمرين الثالث والرابع و(100%) بعد (72-120 ساعة) للعمرين الخامس والسادس. كما اثبتت النتائج أن النوع *S. carpocapsae* كان اسرع فاعلية من النوع *H. bacteriophora* ضد برقات وعذارى دودة الحشد الخريقية. وبعد حساب التركيز المميت لنصف التعداد LC50 لنوعي النيماتودا كان التركيز المميت ل 50% من يرقات كل عمر من الأعمار الأربعة ؛ الثالث, الربع, الخامس و السادس كالأتى (454,183، 546,029، 600,777 و908,079 طور معدي) لنوع النيماتودا *S. carpocapsae* علي التوالي، و474,456، 552,126، 753,022 976,908 طور معدى للنوع *H. bacteriophora* لنفس الاعمار الاربعة . كما أوضحت نتائج التحليل الإحصائي وجود فروق طفيفة أو غير معنوية بين كفاءة نوعي النيماتودا الممرضة للحشرات في مكافحة يرقات دودة الحشد الخريفية تحت الظروف المعملية .كما أثبتت النتائج أن كلا نوعي النيماتودا كانا فعالين للغاية في مكافحة عذاري دودة الحشد الخريفية تحت ظروف المعملية .