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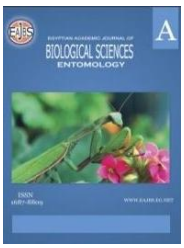

Evaluation of the Effect of Entomopathogenic Nematodes Against Two Instars of The Cotton Leaf Worm, *Spodoptera littoralis* (Lepidoptera: Noctuidae) Under Laboratory Conditions

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ABSTRACT

Spodoptera littoralis (Lepidoptera: Noctuidae) is one of the important insect pests in Egypt caused damage in cotton and other crops and vegetables. Many agents for controlling this insect pest exist, one of these agents is Entomopathogenic nematodes two families belong to EPNS use controlling that are Heterorhabditidae and steinermatides families, two species were tested *Hetrohabditis indica* and Steinernema *sp* under laboratory conditions to Determine their efficacy against the two instars of the cotton leafworm, the consternations of entomopathogenic nematodes were 1000 Ijs and 2000 Ijs for each one. Data indicate that on the 2nd instar high significance on the first, 3rd days 5th days and non-significance on the 7th in EPNs treatment, *H. indica* caused mortality of 85% for both concentrations on the contrary, Steinernema spp. caused 80% and 100% with concentrations 1000 IJs and 2000 IJs, respectively. On the other hand, the results on the 4th instar showed that mortality percentages were 90% caused by H. indica concentration 1000IJs 75% with concentration 2000 Ijs while *Steinernema sp.*

INTRODUCTION

Today, researchers are concerned with avoiding the serious problems resulting from the use of harmful insecticides, that cause residues, as well as the effect of the food chain, the polluting of natural enemies, and causing pest resistance **(**Baker *et al.*,2010). The recent trend is directed toward applying environmentally safe control. Entomopathogenic nematodes, one of the agents of biological control, are controlling various agricultural insect pests. The first nematode was tested, *successfully* that *Steinernema carpocapsae* to control insect pests from 30 years ago, and began in Australia. The first organization used the EPNs commercially that (CSIRO) The Commonwealth Organization for Scientific and Industrial Research, researchers used it against black vine weevil in ornamentals plants and tested against the moth of currant borer in black currants (Georgis, 2002). The pathogenic nematodes, *steinernema spp*, belong to steinermatides and *hetrohabditis bacteriophora belong* to hetrorhabditides affiliated with their two synergetic bacteria the first one is Xenorhabdus and the second photorhabdus (Gauglur& Kaya 1990). EPNs that, the first step, seek the host actively and then burst into the insect's natural openings,at the end move to the hemocoel and release synergetic bacterial cells. The bacteria augment and let go a number of harmful factors, working on complexes of toxins, hydrolytic enzymes, hemolysins, and

antimicrobial compounds, that settle the insect host usually within 48 h. (Eleftherianos *et al.*, 2010; ffrench-Constant & Bowen, 2000; ffrench-Constant *et al*., 2007). Included in an important insect pest is the cotton leaf worm, Spodoptera littoralis (Boisd.) (Lepidoptera: Noctuidae). This insect pest is considered the most destructive phytophagous insect in Egypt, causing damage to a lot of kinds of crops in fields (Shairra & Nouh, 2014). Accordingly, this paper aimed to study the impact of two species of Nematodes *Hetrohabditis indica* and Steinernema *sp.* aganist two instars of the cotton leaf worm *Spodoptera littoralis* under laboratory conditions.

MATERIALS AND METHODS

1. Laboratory Strain of The Cotton Leafworm:

Strain of the cotton leafworm was reread under laboratory conditions, that 27±2C°, photoperiod 14hrs. lights and 10hrs dark and R.H was 65±5%. For mass production, *S. littoralis* adults were placed in glass jars and then fed on sugar solution (Zhang *et al.*,2019). The egg masses were transferred to Patri dishes until emergence and were elected the larvae, the larvae were fed on castor leaves that were used for testing.

2. Entomopathogenic Nematodes:

Entomopathogenic nematodes *H. indica* and *Steinernema* sp. were provided at Plant Protection Research Institute Agricultural Research Center (ARC) Dokki, Giza Egypt. Contagious juveniles of nematode were reared in laboratory using the last instar of the greater wax moth *Galleria mellonella* that was used as the host insect for vivo culture following the methods described by kaya and Stok (1997).

3. Larvicidal Activity of EPNS, (Experimental Technique):

Evaluation of ENPS against *S. littoralis* was required the 2nd and 4th instars were tested, two nematode concentrations 1000Js and 2000Js of each spice. Five Patri dishes replicated each concentration for two spice, ten larvae individual of each replicate, Petri dishes containing moist filter paper with castor leaves discs, in order to analyze the two pathogenic nematodes *Heterorhabditis bacteriophora* and *Steinernema spp.* against *S. littoralis*, the treatments were evaluated in Petri dishes with 1000 and 2000 juveniles at the pair level. The mortality percentages were examined after one, three, five, and seven days of treatment and compared to the control.

4. Statistical Analysis:

The data were calculated to the analysis of difference program (ANOVA) (Gomez &Gomez1984) followed by multiple range test to compare means (Duncan1955).

RESULTS

Effect of Different Entomopathogenic Nematode on 2nd Instar *Spodoptera littoralis*.

Data showed in Table (1), that the mean mortality of the cotton leafworm in the 2nd instar when applying two concentrations of entomopathogenic nematode *H. indica and Steinernema* sp., *1000* and 2000 juveniles. The highest mortality number for concentration for *Steinernema* sp. 2000, and 1000 juveniles after treatment on the third and first days. The mean mortality values were 2.5, 2, 2, and 1.25, respectively. The highest mortality number for concentration for *H. indica* was achieved by 1000 and 2000 juveniles after treatment for five days. The mean mortality values were 2.5 and 1.25, respectively. On the other hand, the first day was significantly higher than on the 7th day, which was non-significant, while the mean mortality of the 2nd instar on the 5th day was significantly higher than on the 3rd day.

Table 1: Mean mortality of the 2nd instar larvae of *S. littoralis* larvae.

Treatments		Mean mortality of the 2nd instar larvae of *S. littoralis* larvae (in days) ± SEM			
		1 day	3 days	5 days	7 days
H. indica	1000IJs	0.25±0.25 cd	1.25±0.25 ab	2.5±0.5 a	0.25±0.25 a
	2000 IJs	0.75±0.25 cd	1.75±0.63 a	1.25±0.75 b	0.5±0.29 a
Steinernema sp	1000IJs	1.25±0.25 bc	2±0.41 a	0.75±0.25 bc	0 a
	2000 IJs	2±0.71 b	2.5±0.87 a	0.25±0.25 bc	0.25±0.25 a
Indoxacarb		3.25±0.25 a	1.25±0.25 ab	0.5±0.29 bc	0 a
Control		0 d	0 b	0 c	0 a
LSD		1.05	1.45	1.23	0.55
F-value		11.8	3.07	4.81	1.2
P-value		0.000***	0.0355*	0.0057**	0.349ns

The mortality percentage in Table (2), declared that entomopathogenic nematode *Steinernema* sp. was mor effective than entomopathogenic nematode *H. indica* with one concentration 2000IJs/1mL seen that, mortality percentage of entomopathogenic nematode *H. indica* on 1 day, 3 days, 5 days and 7 days were 5%,25%,50%and 5%with concentration 1000Ijs respectively while concentration 2000Ijs was 15%35%25% and 10 on tested days. On the contrary of entomopathogenic nematode *Steinernema sp.* concentration 2000Ijs caused a mortality percentage higher than concentration 1000IJs, that 25%, 40%,15% and 0% on 1day, 3days and 7 days respectively, while 40%,50%,5% and 5% on tested days.

Table 2: Mortality percentage of the 2nd instar larvae of *S. littoralis* larvae

Treatments		Mortality% of the 2nd instar larvae of *S. littoralis* larvae (in days)				
		1 day	3 days	5 days	7 days	Total mortality
H. indica	1000 IJs	5%	25%	50%	5%	85%
	2000IJs	15%	35%	25%	10%	85%
Steinernema sp.	1000IJs	25%	40%	15%	-	80%
	2000I Js	40%	50%	5%	5%	100%
Indoxacarb		65%	25%	10%	-	100%
Control		0%	0%	0%	0%	0%

As shown in Figure (1), total mortality percentage of the 2nd instar of cotton leafworm,1000IJs Concentration was caused 85%,80% in *H. indica* and *Steinernema* sp. Respectively, while concertation 2000IJs was 85% and 100% in *H. indica* and *Steinernema* sp.

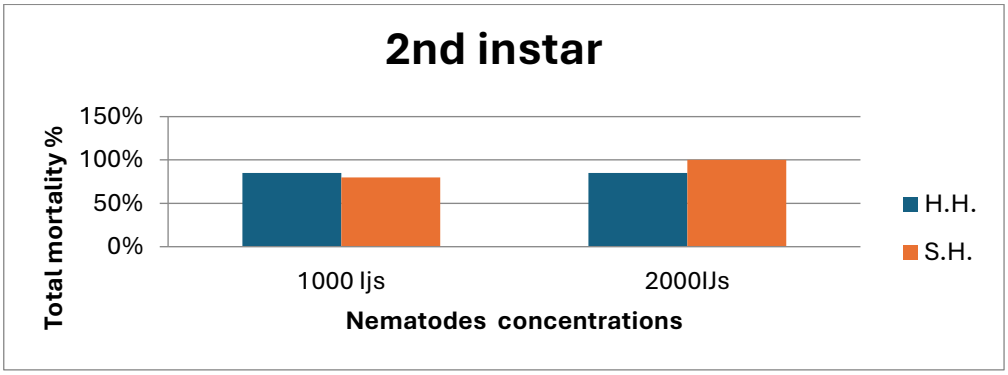

Fig. 1: Mortality total percentage of 2nd instar of cotton leaf worm

Effect of Different Entomopathogenic Nematodes on 4th **Instar *Spodoptera littoralis*.**

In Table (3), data declared that the mean mortality of the cotton leafworm 4th instar when applying two concentrations of entomopathogenic nematodes, *H. indica and Steinernema* sp., 1000 and 2000 juveniles. The highest mortality number for concentration for *Steinernema* sp., 2000 and 1000 juveniles after treatment on the third and first days. The mean mortality values were 2.5, 1.5, 1.25, and 1.0, respectively. The highest mortality number for concentration for *H. indica* was achieved by 1000 and 2000 juveniles after treatment for five days. The mean mortality values were 1.75 and 1.25, respectively. The mean mortality of cotton leafworm on the 4th instar on the first day was significantly higher, although the mean mortality of cotton leafworm attacked by entomopathogenic nematodes *H. indica and Steinernema* sp. 3, 5, and 7 days was non-significant.

Table 3: Mean mortality of 4th instar larvae of *S. littoralis* larvae (in days).

Treatments		**Mean mortality of the 4th instar larvae of *S. littoralis* larvae (in days) ± SEM**			
		1 day	**3 days**	**5 days**	**7 days**
H. indica	**1000 IJs**	**0.75±0.25 b**	**1.75±0.48 a**	**1.75±0.48 a**	**0.25±0.25 a**
	2000 IJs	**0.25±0.25 b**	**1.75±0.48 a**	**1.25±0.25 a**	**0.5±0.5 a**
Steinernema sp	**1000 IJs**	**1±0.71 b**	**1.5±0.87 a**	**1±0.41 ab**	**0.5±0.5 a**
	2000 IJs	**1.25±0.75 b**	**2.5±0.5 a**	**0.75±0.48 ab**	**0 a**
Indoxacarb		**2.75±0.25 a**	**1.25±0.25 ab**	**1±0.41 ab**	**0 a**
Control		**0 b**	**0 b**	**0 b**	**0 a**
LSD		**1.36**	**1.496**	**1.12**	**0.9097**
F-value		**4.56**	**2.704**	**2.36**	**0.64**
P-value		**0.0073 ****	**0.0542 ns**	**0.0822 ns**	**0.669 ns**

Data in Table (4), showed the mortality percentage of the 4th instar larvae were attacked by entomopathogenic nematode *H. indica* with concentration 1000 IJs caused 15%, 35%, 35% and 5% on 1 days, 3days, 5days and 7 days. Moreover, 2000IJs concentration caused 5%, 35%, 25% and 10% mortality percentages. Entomopathogenic nematode *Steinernema sp.* concentration 1000IJs against the 4th instar that caused 20%,30%,25% and 10% mortality percentages while concentration 2000IJs caused 25%, 50%, 15% and 0% on 1 day, 3days,5days and 7 days respectively.

Table 4: Mortality of 4th instar larvae of *S. littoralis* larvae (in days).

Treatments		**Mortality% of the 4th instar larvae of *S. littoralis* larvae (in days)**				
		1 day	**3 days**	**5 days**	**7 days**	**Total mortality**
H. indica	**1000 IJs**	**15%**	**35%**	**35%**	**5%**	**90%**
	2000 IJs	**5%**	**35%**	**25%**	**10%**	**75%**
Steinernema sp	**1000 IJs**	**20%**	**30%**	**20%**	**10%**	**80%**
	2000 IJs	**25%**	**50%**	**15%**	**-**	**90%**
Indoxacarb		**55%**	**25%**	**20%**	**-**	**100%**
Control		**0%**	**0%**	**0%**	**0%**	**0%**

Figure (2), showed that, entomopathogenic nematode *H. indica* effective was higher than *Steinernema* sp. with a concentration 1000IJs. On the other hand, *Steinernema* sp. effective was higher than *H. indica* with concentration 2000IJs

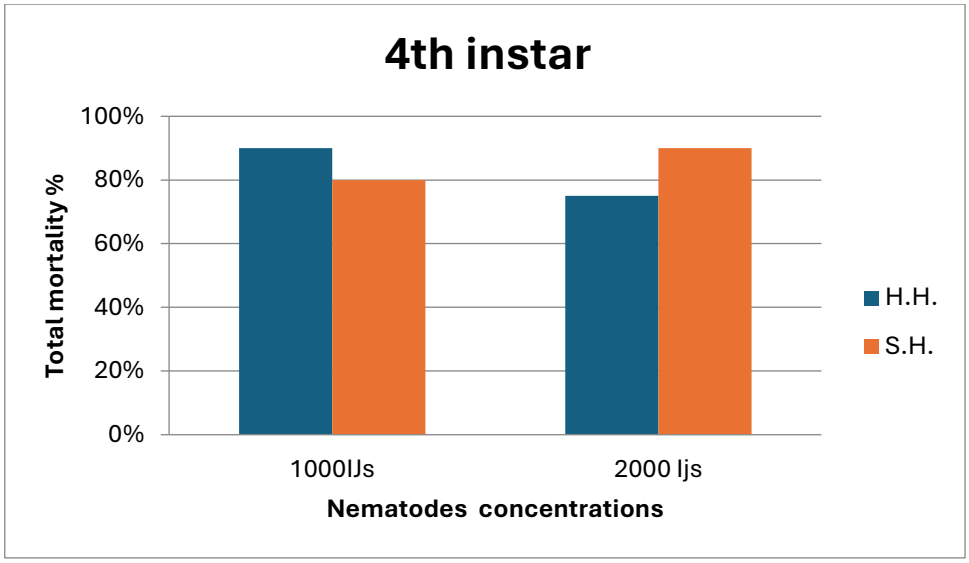

Fig. 2: Total mortality of 4th instar larvae of *S. littoralis.*

DISCUSSION

The previous stuies similar with Elawad *et al.,* (1997) who mentioned that the mortality of larvae caused by EPNS, maintained that, pathogenic nematode could be used as a biological control agent with LD_{50} value of 60.3 IJs/larva. Mogahed (1996) showed that two species of Heterorhabditidae multiplied with the increase of concentration and the duration after treatment of instars of the cotton leafworm. The obtained results agree with Sobhy *et al.,* (2020) who reported that the 2nd instar of *S. littoraslis* and *Agrotis ipsilon* were of higher susceptibility than the rest of instars were used in treatment. Results declared from Hassan *et al.,* (2016) who indicated that, the effect of *S. glaseri* and was greater than *H. bacteriophora*. The obtained results agree with Shairra & Nouh (2014) who reported that higher concentrations of nematodes made a high Mortality. In addition to Gally (1995) who mention that, the data was similar with who mention that, the grad of development of *S. feltiae* was faster, on the other hand the grad of reproduction was higher in the cotton leafworm. These results that findings agree with Sikora *et al. (*1979) who reported that the mortality percentages were positively correlated with ENPS density.

El-Bishery *et al.,* (2002), declared that, there are many factors affecting of penetration of three isolates of Heterorhabdits these factors are nematode dose, infective juvenile age, exposure period host species, host size, larva diet and starvation Hassan (2018), reported that the entomopathogenic nematode *S. carpocapsae* caused 92.5%, 85.0%, 80.0 % and 75.0 % mortality percentages to cotton leaf worm 5th larval instar, respectively. In spite of the entomopathogenic nematode *H. bacteriophora* caused 83.4%%, 77.2%, 71.0% and 60.6 % mortality percentages of the cotton leafworm *S. littoralis*, respectively. Shoukry *et al.,* (2019) mentioned that, the mortality percentages of larvae of *G. mellonella* after 48hrs after treatment by all EPNs strains were increased at 100 IJs for each concentration.Shairr(2000) and shamseldean *et al.*,(2008), demonstrated that mortality rate of larvae of *G. mellonella, S. littoralis and S. litura* was achived100% mortality percentages with 10,20,1000 IJs/caterpillar. (Sevgi & Galip 2016) declared the effect of three EPNs species *H. bacteriophora, S. carpocapsae* and *S. feltiae* on the tomato leaf miner, Tuta absoluta under an experimental setting. The mortality percentages caused with *H. bacteriophora, S. carpocapsae* and *S. feltiae* were found 21.2% - 74.2%, 28.8% - 99.4% and 17.5% - 95.2%, respectively.

Conversely, Saleh & Alheji (2003) studied the toxicity of *H. indica* against the palm weevil *Rhynchophorus ferrugineus*, they found that *H. indicus* caused mortality precentage 70% and 75% in larvae and adults,

Finally, Abdel-Aliem *et al.* (2024) tested *S. carpocapsae*, and *H. bacteriophora* against the second instar of *Spodoptera frugiperda*, the results showed that, the highest concentrations of mortality recorded the highest level of mortality in larvae of *Spodoptera frugiperda*. *S. carpocapsae* specially larvae in the second instar, in addition that, using *H. bacteriophora*, complete mortality after 5 days of treatment. Entomopathogenic nematodes *S. carpocapsae* and *H. bacteriophora* have the same effect against the fourth larval stage of *S. frugiperda*.

Conclusion

In this work, we study the effectiveness of the entomopathogenic nematodes against the cotton leafworm *Spodoptera littoralis* under an experimental setting for use it in the biocontrol programs. The data rely on density of EPNS on the 2nd instar of cotton leaf worm moreover, the effective of *Steinernema sp. Higher than H. indica*.

Declarations:

Ethical Approval: All experiments in this research were approved by the Ethics Committee of the Plant Protection Research Institute, Agriculture Research center, Dokki, Giza, Egypt (Approval code: ASU-SCI/ENTO/2024/5/1).

Authors Contributions: All authors contributed equally, and have read and agreed to the published version of the manuscript.

Conflicts Interests: The authors declare no conflict of interest.

Availability of Data and Materials: All datasets analysed and described during the present study are available from the corresponding author upon reasonable request.

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