

## Evaluation of Entomopathogenic Nematodes in Controlling Some Cabbage Pests

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**Abstract:** *P. rapae* tested larvae, appeared more susceptible than *S. littoralis* and *P. xylostella* larvae to all tested nematodes. For *P. rapae* larvae, *S. carpocapsae* All and *S. carpocapsae* S2 were more virulent to the 2<sup>nd</sup> larval instar than 5<sup>th</sup> one; but *H. indicus* SAA2 and *H. bacteriophora* HP88 were the most virulent heterorhabditids to 5<sup>th</sup> larval instar than 2<sup>nd</sup> one. As for *S. littoralis*, *S. carpocapsae* All and *S. carpocapsae* S2 were the most virulent and fastest in action especially against the younger instars larvae; while all *Heterorhabditis* sp. showed valuable efficiency in virulence and time required for killing the tested pest larvae as indicated by values of lethal mortality concentrations and lethal time required. As well, *S. carpocapsae* All and *S. carpocapsae* S2 showed more efficiency in virulence and faster in action to the 2<sup>nd</sup> and 3<sup>rd</sup> instar larvae of *P. xylostella*. On the other hand, the Egyptian heterorhabditids S1 and *H. indicus* SAA2 were more effective than *H. bacteriophora* HP88 to the 3<sup>rd</sup> and 4<sup>th</sup> instar larvae.

**Key words:** Cabbage, *Spodoptera littoralis*, *Plutella xylostella*, *Pieris rapae*, *Steinernema* spp., *Heterorhabditis* spp.

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### INTRODUCTION

Cabbage (*Brassica oleracea* var. *capitata* L.) is an economically important vegetable crop in Egypt and in many other countries. Wide areas of the newly reclaimed lands are grown every year by cabbage used for export and for local consumption. Cabbage in the field is attacked by many insect pests which cause great loss to the growers every year<sup>[1]</sup>. Among these pests, *Spodoptera littoralis* (Boisd.), *Plutella xylostella* (L.) and *Pieris rapae* (L.) are the most destructive agricultural pests which cause serious economic damage and reduce the commercial value of cabbage.

Synthetic chemical insecticides have been used for many years to control these pests on cabbage. The continuous application of such chemical insecticides resulted in numerous problematical situations including development of insect resistance, food hazards, ground water contamination and destruction of natural enemies. These disadvantages serve as a strong impetus for the development of alternative insect control measures. Particular attention, in recent years, has been focused on biological control agents; including certain entomopathogenic nematodes and bacteria.

Entomopathogenic nematodes in the families steinernematidae and heterorhabditidae are soil inhabiting insect pathogens that possess potential as

biological control agents<sup>[10,19,22,12,20]</sup>. Most biocontrol agents require days or weeks to kill their hosts, yet nematodes, working with their symbiotic bacteria, kill insects in 24-48 hr. The non-feeding infective Juvenile seeks out insect hosts; when a host has been located, the nematode penetrates into the insect body, usually through natural body openings (mouth, anus, spiracles) or areas of thin cuticle. Once in the body cavity, a symbiotic bacterium (*Xenorhabdus* for steinernematids and *Photorhabdus* for heterorhabditids) is released from the nematode, multiplies rapidly and causes rapid insect death. The nematodes feed upon the bacteria and liquefying insect; and mature into adults. Thus, entomopathogenic nematodes are a nematode-bacterium complex.

Steinernematid and heterorhabditid nematodes can parasitize thousands of insect species including many economic pests<sup>[21]</sup>. In spite of their broad host range and high virulence, these biocontrol agents show no mammalian pathogenicity<sup>[11]</sup>. United States Environmental Protection Agency (EPA) has subsequently exempted them from registration and regulation requirements<sup>[15]</sup>.

Mass production methods have been developed to the point that nematodes are competitive economically with many chemical insecticides and they often provide levels of insect control comparable to that of

chemicals<sup>[13]</sup>. No other biological control agents offers a comparable combination of attributes: broad host range, high virulence, inexpensive mass rearing and safety<sup>[23]</sup>. Despite this impressive list of attributes, the sensitivity of steinernematid and heterorhabditid infective juveniles to inactivation by extremes of the physical environment prevents them from reaching their full potential<sup>[12]</sup>. Therefore, the present study was carried out to evaluate the possibility of using entomopathogenic nematodes as biological control agents against the most destructive pests of cabbage; *Spodoptera littoralis*, *Plutella xylostella* and *Pieris rapae*.

## MATERIALS AND METHODS

**1-Target insects:** The original stock culture of *Spodoptera littoralis* (Boisd) (Lepidoptera: Noctuidae) was obtained from Federal Biological Research Centre for Agriculture and Forestry Institute for Biological Control in Darmstadt, Germany. The original stock culture of *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) was obtained from Bayer AG Pflanzenschutzzentrum (Plant Protection Centre) Monheim, Leverkusen, Entomology Lab, Germany and both were maintained at the Applied Entomology Department Lab, Institute for Phytomedicine, Hohenheim University, Stuttgart, Germany, *Pieris rapae* (L) (Lepidoptera: Pieridae) larvae needed for treatments were collected from cabbage fields in Stuttgart-Germany during July and maintained in the laboratory on cabbage leaves in plastic boxes measuring 18 × 13 × 6 cm until reached the desired larval stage.

**2- Tested nematodes:** Following is tested entomopathogenic nematodes, their geographical origin and their reference; *Heterorhabditis bacteriophora* HP88, Utah, USA., Randy Gaugler, Rutgers University, New Brunswick, NJ, USA.; *Heterorhabditis indicus* SAA2, Salheia, Sharkyia Governorate, Egypt., Hussein, Mona<sup>[17]</sup>. *Heterorhabditis indicus* EAS59, Benban Bahary, west of Aswan, Egypt., Shamseldean and Abd. Elgawad<sup>[25]</sup>. *Heterorhabditis* sp. S1, Ras Sidr, Sinai, Egypt.; *Steinernema carpocapsae* All, California, USA. Ramon Georgis, Biosys, Palo Alto, CA, USA.; *Steinernema carpocapsae* S2, Sinai, Egypt., Shamseldean *et al.*,<sup>[26]</sup>. *Steinernema abbasi*, Salalah, Sultanate of Oman., M.S.T. Abbas, Agric., Res. Centre, Dokki, Egypt. and *Steinernema riobravus*, Lower Rio Grande Valley, Texas, USA., Randy Gaugler, Rutgers University, New Brunswick, NJ, USA. All nematode species were maintained in the laboratory on *Galleria mellonella* 6<sup>th</sup> instar larvae according to the method adapted by Dutky *et al.*,<sup>[7]</sup>.

**3- Bioassay methodology:** Eight nematode species and strains were tested for their virulence to the most destructive cabbage pests: *Spodoptera littoralis*

(2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup> and 6<sup>th</sup> larval instars), *Plutella xylostella* (2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> larval instars) and *Pieris rapae* (2<sup>nd</sup> and 5<sup>th</sup> larval instars). The tested nematodes were suspended in distilled water to obtain the desired concentrations. Cabbage leaf disks (6 cm diameter) were cut from plants grown in the green house. They were placed in 9 cm diameter × 4.5 cm high plastic boxes lined with filter paper. Leaf disks were sprayed with 1 ml (0.5 ml/side) of different concentrations of a nematode suspension. A handheld aerosol sprayer was used to apply the spray. Sprayed cabbage leaves were left for several minutes to avoid water condensation.

From three to ten larvae (according to the test insect and larval instar) with uniform size of each test insect were placed on each leaf disk. From three to five replicates for each test were performed for each tested inoculum level. Insect larvae were allowed to feed on the sprayed leaves for 24 hr before they were transferred to untreated leaves. The untreated leaf disks (control) received 1 ml (0.5 ml/side) distilled water only. The treated and untreated replicates were incubated under constant conditions of 25±1°C and 65-70% RH.

Mortality counts were recorded daily for five days from the initiation of the experiment. Percent mortality was calculated for each concentration level. Probit analysis was used for determining the LC<sub>50</sub> and slope (b) values<sup>[9]</sup>, as well mortality data of inoculum level (250 Ijs/ml) were subjected to probit analysis; and then time-mortality relation was calculated by Linear regression using Excel 2000. The time taken to kill 50% of the insects was determined for each nematode on each tested insect species.

**4- Statistical analysis:** Mortality data were subjected to probit analysis in order to determine the LC<sub>50</sub> and slope (b) values<sup>[9]</sup>. Time-mortality relation was calculated by Linear regression, using Excel 2000; and the time taken to kill 50% of the insects (LT<sub>50</sub>) was determined.

## RESULTS AND DISCUSSIONS

**1- *S. littoralis*:** Comparing the virulence of the native *S. carpocapsae* S2 and *S. abbasi* to the world known, commercially produced *S. carpocapsae* All and *S. riobravus* to *S. littoralis* larvae, as indicated by values of lethal mortality concentrations and lethal time required (Tables 1 and 2), it might be concluded that *S. carpocapsae* All and *S. carpocapsae* S2 were more virulent and faster in killing *S. littoralis* larvae especially the younger instar larvae; followed by *S. abbasi* (abb) against 2<sup>nd</sup> and 3<sup>rd</sup> instar larvae, then *S. riobravus* (rio) against the older instar larvae (6<sup>th</sup> and

**Table 1:** Relative efficiency of Egyptian and foreign *Steinernema spp.* and *Heterorhabditis spp.* nematodes against *Spodoptera littoralis* larvae.

Host larval instar	<i>Steinernema spp.</i>								<i>Heterorhabditis spp.</i>							
	All		S2		abb		rio		HP88		SAA2		S1		EAS59	
	LC <sub>50</sub> (Ijs/ml)	(b)	LC <sub>50</sub> (Ijs/ml)	(b)	LC <sub>50</sub> (Ijs/ml)	(b)	LC <sub>50</sub> (Ijs/ml)	(b)	LC <sub>50</sub> (Ijs/ml)	(b)	LC <sub>50</sub> (Ijs/ml)	(b)	LC <sub>50</sub> (Ijs/ml)	(b)	LC <sub>50</sub> (Ijs/ml)	(b)
2nd.	<13	...	17.90	1.95 ± 0.48	32.18	0.90 ± 0.14	156.83	1.12 ± 0.17	110.69	2.20 ± 0.18	99.72	1.54 ± 0.15	403.37	2.65 ± 0.20	-	-
3rd.	<13	...	16.25	1.53 ± 0.30	85.87	1.21 ± 0.15	352.51	1.12 ± 0.10	176.93	1.29 ± 0.11	71.93	1.30 ± 0.15	443.27	2.22 ± 0.17	-	-
4th.	<13	...	27.35	1.47 ± 0.24	131.36	2.04 ± 0.16	329.05	1.04 ± 0.10	157.82	2.22 ± 0.17	210.78	1.30 ± 0.11	201.04	1.26 ± 0.11	-	-
5th.	13.36	1.87 ± 0.45	35.51	1.67 ± 0.24	200.52	1.92 ± 0.15	81.57	1.96 ± 0.16	72.92	2.04 ± 0.19	265.65	1.14 ± 0.11	171.67	1.19 ± 0.11	205.15	1.59 ± 0.11
6th.	23.68	2.55 ± 0.52	63.77	1.50 ± 0.16	430.91	1.75 ± 0.16	77.63	2.53 ± 0.25	86.76	1.74 ± 0.18	694.86	0.99 ± 0.11	155.94	0.99 ± 0.11	379.68	0.78 ± 0.11

All = *Steinernema carpocapsae* All; S2 = *Steinernema carpocapsae* S2; abb = *Steinernema abbasi*; rio = *Steinernema riobravus*. HP88 = *Heterorhabditis bacteriophora* HP88; SAA2 = *Heterorhabditis indicus* SAA2; S1 = *Heterorhabditis sp.* S1; EAS59 = *Heterorhabditis indicus* EAS59. (b) = Slope. (-) = not tested.

**Table 2:** Estimated time to kill 50 % (LT<sub>50</sub>) of *Spodoptera littoralis* larvae infected with Egyptian and foreign *Steinernema spp.* and *Heterorhabditis spp.* nematodes at concentration 250 Ijs/ml.

Host larval instar	LT <sub>50</sub> in hours								
	<i>Steinernema spp.</i>					<i>Heterorhabditis spp.</i>			
	All	S2	abb	rio	HP88	SAA2	S1	EAS59	
2nd.	<24.0	36.3	61.8	75.6	63.9	58.4	102.1	-	
3rd.	<24.0	36.3	64.9	82.1	68.4	64.8	158.0	-	
4th.	31.8	32.9	76.3	82.1	124.7	96.8	51.6	-	
5th.	31.8	32.9	110.1	61.8	92.3	122.5	51.6	98.6	
6th.	38.1	53.5	151.6	55.1	92.3	85.1	51.6	75.1	

All = *Steinernema carpocapsae* All; S2 = *Steinernema carpocapsae* S2; abb = *Steinernema abbasi*; rio = *Steinernema riobravus*. HP88 = *Heterorhabditis bacteriophora* HP88; SAA2 = *Heterorhabditis indicus* SAA2; S1 = *Heterorhabditis sp.* S1; EAS59 = *Heterorhabditis indicus* EAS59. (-) = not test

5<sup>th</sup> instars); while comparing the virulence of the Egyptian *H. indicua* SAA2, *Heterorhabditis sp.* S1 and *H. indicus* EAS59 to the world known, commercially produced *H. bacteriophora* HP88 to *S. littoralis* larvae (Tables 1 and 2), it could be concluded that *H. bacteriophora* HP88 was more virulent to 5<sup>th</sup> and 6<sup>th</sup> instar larvae but was more faster in effect to the 2<sup>nd</sup> and 3<sup>rd</sup> instar larvae. The Egyptian heterorhabditid S1 was more virulent and faster in effect to the 6<sup>th</sup> and 5<sup>th</sup> instar larvae; while *H. indicus* SAA2 was more virulent to the 3<sup>rd</sup> and 2<sup>nd</sup> instar larvae but it was more faster in effect to the 2<sup>nd</sup> than the 3<sup>rd</sup> instar larvae. Finally, *H. indicus* EAS59 was more virulent to the 5<sup>th</sup> than to the 6<sup>th</sup> instar larvae but it was more faster in effect to the 6<sup>th</sup> ones. These results are in accordance with those of El-Kifl<sup>[8]</sup> who obtained high mortality in larvae of *S. littoralis*; where 3<sup>rd</sup> and 5<sup>th</sup> larval instars were the most vulnerable to *S. carpocapsae*. Ahmed<sup>[4]</sup> found that 5<sup>th</sup> larval instar of *S. littoralis* was more susceptible than both 4<sup>th</sup> and 6<sup>th</sup> ones to *Neoaplectana carpocapsae*. Hussein<sup>[17]</sup> found that the Egyptian nematodes were as efficient as the imported ones and in some cases had higher efficacy in controlling *S. littoralis* and *Agrotis ipsilon*.

**2- P. xylostella:** In case of comparing the virulence of local *S. carpocapsae* S2 and *S. abbasi* to the world known, commercially produced *S. carpocapsae* All and *S. riobravus* to *P. xylostella* larvae, as indicated by values of LC<sub>50</sub> and LT<sub>50</sub> (Tables 3 and 4), it might be concluded that *S. carpocapsae* All was more virulent and faster in action to the 2<sup>nd</sup> instar larvae followed by *S. carpocapsae* S2 to the 3<sup>rd</sup> instar larvae, then *S. abbasi* and finally *S. riobravus* to the 3<sup>rd</sup> instar larvae as well as the 4<sup>th</sup> instar larvae. As well, comparing the virulence of Egyptian *H. indicus* SAA2 and *Heterorhabditis sp.* S1 to the world known, commercially produced *H. bacteriophora* HP88 to *P. xylostella* larvae, as indicated by values of lethal mortality concentrations and lethal time required (Tables 3 and 4), it could be concluded that the Egyptian heterorhabditids S1 and *H. indicus* SAA2 were more virulent than *H. bacteriophora* HP88 to the 3<sup>rd</sup> and 4<sup>th</sup> instar larvae, respectively. Meanwhile, the Egyptian heterorhabditid S1 was more virulent than *H. indicus* SAA2 and were comparable in time required for killing the 3<sup>rd</sup> instar larvae but *H. indicus* SAA2 was more virulent and faster in action than the Egyptian heterorhabditid S1 to the 4<sup>th</sup> instar larvae.

**Table 3:** Relative efficiency of Egyptian and foreign *Steinernema spp.* and *Heterorhabditis spp.* nematodes against *Plutella xylostella* larvae.

Host larval instar	<i>Steinernema spp.</i>								<i>Heterorhabditis spp.</i>					
	All		S2		abb		rio		HP88		SAA2		S1	
	LC <sub>50</sub> (Ijs/ml)	(b)	LC <sub>50</sub> (Ijs/ml)	(b)	LC <sub>50</sub> (Ijs/ml)	(b)	LC <sub>50</sub> (Ijs/ml)	(b)	LC <sub>50</sub> (Ijs/ml)	(b)	LC <sub>50</sub> (Ijs/ml)	(b)	LC <sub>50</sub> (Ijs/ml)	(b)
2nd.	33.79	2.21 ± 0.39	-	-	-	-	-	-	114.84	1.20 ± 0.18	-	-	-	-
3rd.	44.33	2.67 ± 0.40	36.40	1.68 ± 0.28	66.06	1.66 ± 0.21	111.35	1.32 ± 0.18	254.61	0.10 ± 0.08	118.43	1.47 ± 0.15	59.45	1.42 ± 0.19
4th.	63.16	3.12 ± 0.38	64.74	1.42 ± 0.27	65.97	1.64 ± 0.20	114.47	1.2 ± 0.18	1091.02	1.15 ± 0.08	262.63	0.98 ± 0.08	882.64	1.12 ± 0.08

All = *Steinernema carpocapsae* All; S2 = *Steinernema carpocapsae* S2; abb = *Steinernema abbasi*; rio = *Steinernema riobravris*. HP88 = *Heterorhabditis bacteriophora* HP88; SAA2 = *Heterorhabditis indicus* SAA2; S1 = *Heterorhabditis sp.* S1. (b) = Slope. (-) = not tested.

**Table 4:** Estimated time to kill 50 % (LT<sub>50</sub>) of *Plutella xylostella* larvae infected with Egyptian an foreign *Steinernema spp.* and *Heterorhabditis spp.* nematodes at concentration 250 Ijs/ml.

Host larval instar	LT <sub>50</sub> in hours							
	<i>Steinernema spp.</i>				<i>Heterorhabditis spp.</i>			
	All	S2	abb	rio	HP88	SAA2	S1	
2nd.	20.5	-	-	-	56.3	-	-	
3rd.	23.0	25.9	52.2	73.1	74.2	36.8	37.8	
4th.	31.6	42.7	52.2	73.1	141.3	81.9	100.9	

All = *Steinernema carpocapsae* All; S2 = *Steinernema carpocapsae* S2; abb = *Steinernema abbasi*; rio = *Steinernema riobravris*. HP88 = *Heterorhabditis bacteriophora* HP88; SAA2 = *Heterorhabditis indicus* SAA2; S1 = *Heterorhabditis sp.* S1. (-) = not tested.

This finding is in agreement to that by Baur *et al.*,<sup>[5]</sup> who reported that mortality of late stage larvae of *P. xylostella* in a leaf disk ranged from <7% to >95% due to infection with *S. carpocapsae*, *S. riobravris* and *H. bacteriophora* Shinde and Singh<sup>[27]</sup> tested eight entomopathogenic nematode Species/strains against the final instar larvae of *P. xylostella*. They found that all nematodes were pathogenic. However, *H. bacteriophora* was the most pathogenic amongst the tested nematodes on the basis of LC<sub>50</sub> and LT<sub>50</sub>.

**3- *Pieris rapae*:** On the other hand, comparing the virulence of *Steinernema carpocapsae* S2, *Steinernema abbasi* and *Steinernema carpocapsae* A11 to *P. rapae* larvae, as indicated by values of LC<sub>50</sub> and LT<sub>50</sub> (Tables 5 and 6), it could be concluded that *S. carpocapsae* A11 was the most virulent to the 2<sup>nd</sup> and 5<sup>th</sup> instar larvae followed by *S. carpocapsae* S2 against 2<sup>nd</sup> instar larvae, then *S. abbasi* against the 5<sup>th</sup> instar larvae. In case of comparing the virulence of the Egyptian heterorhabditid S1 and *H. indicus* SAA2 to *H. bacteriophora* HP88 to *P. rapae* larvae as indicated by values of LC<sub>50</sub> and LT<sub>50</sub> (Tables 5 and 6), it might be concluded that *H. indicus* SAA2 was more virulent to the 5<sup>th</sup> instar larvae but was more faster in effect to the 2<sup>nd</sup> ones followed by *H. bacteriophora* HP88 and *H.*

*sp.* S1 against the 5<sup>th</sup> instar larvae. The sharp rapid effect of the nematodes in controlling the cabbage worm, *P. rapae* as proved by these results could be due to the high susceptibility of the pest to the nematode. It is worth mentioning here that during the isolation procedure, the larvae of *P. rapae* succeeded in trapping the nematode rather than the larvae of the common host *G. mellonella* Saleh<sup>[24]</sup>. In this respect, Jin<sup>[18]</sup> found that the larvae of *P. rapae* and *A. ipsilon* were very sensitive to the nematode; and both insects died 3-5 days after inoculation. Wu and Chow<sup>[28]</sup> reported that larvae of *P. rapae* infected with *S. feltiae* associated mortality 3 days after exposure ranging from 75 to 97.5%. Also, Saleh<sup>[24]</sup> found, in a bioassay test, that *H. tayserae* nematode at concentrations of 5-100 Ijs/larva of *P. rapae* induced 30-100% and 55-100% within 24 and 48 hours, respectively.

General comparison among steinernematid and heterorhabditid tested species through values of LC<sub>50</sub> and LT<sub>50</sub> indicated that nematodes of both genera gave valuable results in virulence and time required for killing the pest larvae. *P. rapae* tested larvae, appeared more susceptible than *S. littoralis* and *P. xylostella* larvae to all tested nematodes. For *P. rapae* larvae, *S. carpocapsae* All and *S. carpocapsae* S2 were more

**Table 5:** Relative efficiency of Egyptian and foreign *Steinernema spp.* and *Heterorhabditis spp.* nematodes against 2<sup>nd</sup> and 5<sup>th</sup> larval instars of *Pieris rapae*.

Host larval instar	<i>Steinernema spp.</i>						<i>Heterorhabditis spp.</i>					
	All		S2		abb		HP88		SAA2		S1	
	LC <sub>50</sub> (Ijs/ml)	(b)	LC <sub>50</sub> (Ijs/ml)	(b)	LC <sub>50</sub> (Ijs/ml)	(b)	LC <sub>50</sub> (Ijs/ml)	(b)	LC <sub>50</sub> (Ijs/ml)	(b)	LC <sub>50</sub> (Ijs/ml)	(b)
2nd.	13.36	1.87 ± 0.45	25.16	2.34 ± 0.50	-	-	42.28	1.90 ± 0.29	37.14	3.07 ± 0.54	-	-
5th.	31.65	2.65 ± 0.50	46.43	3.71 ± 0.57	33.01	1.24 ± 0.21	29.34	1.41 ± 0.22	25.17	2.34 ± 0.50	35.62	2.21 ± 0.38

All = *Steinernema carpocapsae* All; S2 = *Steinernema carpocapsae* S2; abb = *Steinernema abbasi* . HP88 = *Heterorhabditis bacteriophora* HP88; SAA2 = *Heterorhabditis indicus* SAA2; S1 = *Heterorhabditis sp.* S1. (b) = Slope. (-) = not tested.

**Table 6:** Estimated time to kill 50 % (LT<sub>50</sub>) of 2<sup>nd</sup> and 5<sup>th</sup> larval instars of *Pieris rapae* infected with Egyptian and foreign *Steinernema spp.* and *Heterorhabditis spp* nematodes at concentration 250 Ijs/ml.

Host larval instar	LT <sub>50</sub> in hours					
	<i>Steinernema spp</i>			<i>Heterorhabditis spp.</i>		
	All	S2	abb	HP88	SAA2	S1
2nd.	<24	<24	-	31.8	17.7	-
5th.	<24	<24	17.6	31.1	41.7	36.3

All = *Steinernema carpocapsae* All; S2 = *Steinernema carpocapsae* S2; abb = *Steinernema abbasi* . HP88 = *Heterorhabditis bacteriophora* HP88; SAA2 = *Heterorhabditis indicus* SAA2; S1 = *Heterorhabditis sp.* S1. (-) = not tested

virulent to the 2<sup>nd</sup> larval instar than 5<sup>th</sup> one; but *H. indicus* SAA2 and *H. bacteriophora* HP88 were the most virulent heterorhabditids to 5<sup>th</sup> larval instar than 2<sup>nd</sup> one. As for *S. littorals*, *S. carpocapsae* All and *S. carpocapsae* S2 were the most virulent and fastest in action especially against the younger instars larvae; while all *Heterorhabditis sp.* showed valuable efficiency in virulence and time required for killing the tested pest larvae as indicated by values of lethal mortality concentrations and lethal time required. As well, *S. carpocapsae*. All and *S. carpocapsae* S2 showed more efficiency in virulence and faster in action to the 2<sup>nd</sup> and 3<sup>rd</sup> instar larvae of *P. xylostella*. On the other hand, the Egyptian heterorhabditids S1 and *H. indicus* SAA2 were more effective than *H. bacteriophora* HP88 to the 3<sup>rd</sup> and 4<sup>th</sup> instar larvae.

Data also indicated that the higher nematode inoculum levels; however, caused higher and faster mortality than the lower levels. The death of the treated insect larvae is caused mainly by the effect of the nematode associated bacteria. Larval death probably arises from the production of proteolytic enzymes which may explain the relative lack of resistance to bacteria<sup>[21]</sup>. Thus, it could be suggested that the higher concentrations of nematodes will elaborate much more bacteria which in turn multiply rapidly producing huge numbers of bacterial cells which in turn kill the insect larvae more rapidly. This may explain why the higher concentrations caused faster and higher mortality than the lower ones.

The present results also revealed that the youngest stages were the most susceptible ones and that susceptibility decreased with insect age. In other words,

LC<sub>50</sub> increased in proportion to age of insect larvae as indicated by values of lethal mortality concentrations. This may be attributed to differences in the rate of phagocytosis of parasitic infective juveniles between these host stages. In agreement with this finding, higher susceptibility of younger larvae has been reported for *Heliothis armigera* Hubner<sup>[14]</sup>. Herron and Baker<sup>[16]</sup> reported that the 1<sup>st</sup> and 2<sup>nd</sup> instars of acridid, *Chortoicetes terminifera* were more susceptible to infection with the nematode *Hexameris sp* than the 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> instar larvae and the adult. Obtained results also showed that the rate of tested larval mortality differed according to nematode species and strains. In this respect, Bedding *et al.*,<sup>[6]</sup> also reported that infectivity of the entomopathogenic nematodes varies widely according to nematode species and strains, also to insect species. As well, the fastest invasion rate was recorded with the smallest insect larvae, for all nematode strains. Thus, the LT<sub>50s</sub> increased in proportion to the size of insect larvae. Abdel-Kawy<sup>[2]</sup> reported that full grown larvae of *A. ipsilon* are to some extent, more resistant to nematode infection and a longer period to achieve a considerable mortality rate is required. Abu-Elmaged and El-Kifl<sup>[3]</sup> attributed this resistance to high rate of phagocytosis and the presence of anti-bacterial lytic factors.

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