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Survival, Pathogenicity and Propagation of Entomopathogenic Nematodes under Different Temperatures

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ABSTRACT

Survival of *Heterorhabditis* spp. was highest at temperatures from 15-30° C; with an optimal survival at 25° C followed by 30, 20 and 15° C. In contrast, *Steinernema* spp. survival was better at the lower temperatures than at the higher ones for 15 days. In all cases, temperatures above 30° C were less favored for tested nematode survival. In general, heterorhabditid nematodes pathogenicities were greater at temperatures form 20-30° C than at the lowest and highest temperatures (15 and 35° C). Optimal pathogenicities were obtained at 25° C by HP88 and at 30° C by SAA2 and S1. It was also noticed that *H. indicus* SAA2 could not induce larval mortality at temperature 15° C. Analysis of data revealed significant differences at 5% level of probability in pathogenicity among the tested nematodes at all tested temperatures as well as between the tested temperatures b each nematode species; except *S. carpocapsae* All and *S. carpocapsae S2* which showed no significant difference between each other in the pathogenicity at tested temperatures. Also, it could be concluded that the optimum temperature for steinernematids and heterorhabditids propagation (*i.e.* temperature at which maximum number of infective juveniles (IJs) were produced per host) was 30° C; except *H. bacteriophora* HP88 in 4th larval instar of *Spodoptera littoralis* and *S. carpocapsae* All in 3rd larval instar of *Plutella xylostella*, it was at 25° C.

Key Words: *Spodoptera littoralis*, *Plutella xylostella*, *Steinernema* spp. *Heterorhabditis* spp. Pathogenicity, Survival, Temperature

INTRODUCTION

Entomopathogenic nematodes from the familes Heterorhabditidae and Steinernematidae proved their usefulness as biological control agents of insect pests. Despite their recent success, nematode sensitivity to inactivation by environmental stresses prevents them from realizing their full biocontrol potential (Gaugler and Kaya, 1990). For example, infectivity and persistence of most species significantly reduced above 30° C (Kung *et al.*, 1991). Collection of novel indigenous isolates of entomopathogenic nematodes has merits to alleviate such problems. It may provide isolates more suitable for inundative release against local pest insects because of adaptation to their climate and population regulators (Bedding, 1990). In this respect, significant variation was found between two nematode isolates, recently recovered from Egyptian soils (Shamseldean and Abd-Elgawad, 1994) concerning their thermo tolerance under laboratory conditions (Shamseldean, 1994).

Experiments showed that Egyptian isolates of heterorhabditid nematodes could provide effective control of different insect pests (Shamseldean and Abd-Elgawad, 1993). However, the widespread natural occurrence in Egypt of these nematodes supported the possibility for choosing the most suitable native isolate(s) for future field applications; especially under stressed conditions.

The purpose of the present study was to test the tolerance of tested nematodes to different temperatures in order to gain some information on their survival, infectivity, propagation and extraction of the infective juveniles of steinernematied and heterorhabditid nematodes; indigenous nematodes compared to a well known species.

MATERIALS AND METHODS

1- Target insects

Original stock culture of the cotton leafworm, *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 Pflanzenschutzentrum (Plant Protection Centre) Manheim, Leverkusen, Entomology Lab, Germany. and both were maintained at the Applied Entomology Department Lab, Institute for Phytomedicine, Hohenheim University, Stuttgart, Germany.

2- Tested nematodes

Source of the tested entomopathogenic nematodeswere as follows; *Heterorhabditis bacteriophora* HP88, Utah, USA., Randy Gaugler, Rutgers University, New Brunswick, NJ, USA.; *H. indicus* SAA2, Salheia, Sharkyia Governorate, Egypt., Hussein (2004).; *Heterorhabditis* sp. S1, Ras Sadr, Sinai, Egypt.; *Steinernema carpocapsae* All, California, USA. Ramon Georgis, Biosys, Palo Alto, CA, USA.; *S. carpocapsae* S2, Sinai, Egypt., Shamseldean *et al.* (1996b).; *S. abbasi*, Salalah ,Sultanate of Oman., M. S. T. Abbas, Agric.,Res. Centre, Dokki, Egypt.and *S. riobravis,* Lower Rio GrandeValley, Texas, USA., Randy Gaugler, Rutgers University, New Brunswick, NJ, USA. All nematode species were maintained in the laboratory on *Galleria mellonella* last instar larvae according to the method adapted by Dutky *et al.* (1964).

3- Nematodes survival

To determine the survival of the nematode species and strains at different temperatures, they were used as suspensions in distilled water. One hundred infective juveniles (Ijs) of each nematode species and strains in 2 ml distilled water were pipetted into a small Petri-dish (55x15mm). The Petri-dishes were sealed tightly to avoid desiccation and placed in incubators at 15, 20, 25, 30 and 35° C (each ± 2° C) for an exposure period of 2, 5, 10 and 15 days intervals. The Ijs were then transferred to room temperature 25±1° C for about 2 hr. to ensure the reactivation of the nematodes. Number of survived infective juveniles was counted using a stereoscopic binocular microscope. Ijs were considered as alive when they were actively moving. Three replicates were used for each species and strain.

4- Nematodes pathogenicity

Fourth instar larvae of *S. littoralis* and third instar larvae of *P. xylostella* were fed on cabbage leaf disks 6 cm diameter treated with 500 Ijs/ml in one ml distilled water from each nematode species and strain separately. All nematode species and strains were incubated at respective temperatures for two hrs. for acclimatization of nematodes prior to the infection. Control treatments received one ml distilled water only. From three to five replicates five larvae each were used. The treatments were incubated at 15, 20, 25, 30 and 35° C (each ± 2° C). Larval mortality was recorded every 24 hours for up to five days.

5- Nematodes life span

The mean number of days taken from inoculation date to the first emergence of Ijs of different nematode species and strains was recorded and compared at 15, 20, 25, 30 and 35° C (each ± 2° C) for each tested insect species. Three replicates were used at each temperature degree; each one consisted of three cadavers.

6- Nematodes propagation

Dead larvae from each tested insect species with characteristic signs of nematode infection were transferred to White nematode trap (White, 1927) as modified by Dutky ***et al.*** (1964) for the recovery of the new generation of infective juveniles at 15, 20, 25, 30 and 35° C (each ± 2° C). Emerged nematodes (Ijs were counted every other day for one week after first day of emergence. Total number of Ijs/cadaver wa determined.

7. Statistical analysis

Obtained data were subjected to statistical analysis using One Way Anova (F-test) and the leas significant difference (L.S.D.) through the Computer program, Microstat ver. 2.0. Time-mortality relatio was calculated by linear regression, using Excel 2000; and the time taken to kill 50% of the insects (LT_{5} was determined.

RESULTS AND DISCUSSION

1- Nematodes survival

As shown in Table (1), analysis of variance showed significant differences at 5% level of probability i survival among the tested *Steinernema* spp. nematodes after 15 days of exposure at temperatures fron 20-30° C. At the lowest and highest temperature (15 & 35° C), no significant difference was detected. As well, statistical analysis evaluated significant differences at 5% level of probability in survival among the tested temperatures for the same nematode species. Thus, the rate of nematode survival was related to temperature used as well as to the exposure period. Survival of Ijs of *Steinernema* spp. was not affected by temperature ranging from 15-35° C for 5 days (Table 1). By increasing the exposure period, a reduction in the rate of survival was obtained; and was evident at 35° C after 15 days of exposure; where *S. carpocapsae* All, *S. carpocapsae* S2, *S. abbasi* and *S. riobravis* were recorded 49, 50, 52 and 55% survival, respectively. As showen in Table (2), the survivals of infective juveniles of *Heterorhabditis* spp. were not affected by

Table (1): Effect of temperature on the survival of *Steinernema* spp.* nematodes at different exposure periods.

Temp. °C	Survival % after the indicated days of exposure																F. value	L.S.D. at 5%
	All				S2				abb				rio					
	2 d.	5 d.	10 d.	15 d.	2 d.	5 d.	10 d.	15 d.	2 d.	5 d.	10 d.	15 d.	2 d.	5 d.	10 d.	15 d.		
15	100	100	98	91Aa	100	100	98	90Aa	100	100	100	95Aa	100	100	100	93Aa	1.00$^{N.S.}$	---
20	100	100	95	75BCb	100	100	98	70Cb	100	100	100	90Aa	100	100	100	77Bb	21.268**	6.03
25	100	100	90	78Ab	100	100	95	75Ab	100	100	100	70Bb	100	100	100	77Ab	6.609**	4.51
30	100	100	87	70Bc	100	100	82	70Bb	100	100	100	75Ab	100	100	100	70Bc	6.250**	3.26
35	100	100	75	49Ad	100	100	77	50Ac	100	100	90	52Ac	100	100	87	55Ad	0.857$^{N.S.}$	---
F. value		...		125.518**		...		33.424**		...		112.423**		...		38.270**	...	...
L.S.D. at 5%		...		4.31		...		7.80		...		5.08		...		6.99	...	...

*All = *Steinernema carpocapsae* All; S2 = *Steinernema carpocapsae* S2; abb = *Steinernema abbasi*; rio = *Steinernema riobravis*.

Means within a row followed by the same capital letter or within a column followed by the same small letter are not significantly different at 5 %.

** = Highly significant at 1% level; N.S. = Not significant at 5% level.

Table (2): Effect of temperature on the survival of *Heterorhabditis* spp.* nematodes at different exposure periods.

Temp.°C	Survival % after the indicated days of exposure												F. value	L.S.D. at 5%
	HP88				SAA2				S1					
	2 d.	5 d.	10 d.	15 d.	2 d.	5 d.	10 d.	15 d.	2 d.	5 d.	10 d.	15 d.		
15	100	95	80	77Ab	100	90	80	75Aa	100	100	69	55Ba	18.00**	9.92
20	100	95	80	78Ab	100	90	80	76Ab	100	100	70	56Ba	25.615**	8.32
25	100	96	96	93Aa	100	100	88	86Aa	100	100	70	60Ba	32.012**	10.63
30	100	95	95	81Ab	100	100	85	85 Aa	100	94	65	59Ba	37.532**	7.91
35	70	40	20	0Cc	74	50	48	5Bc	80	77	30	10Ab	22.500**	3.65
F. value		...		402.029**		...		187.952**		...		55.549**	...	...
L.S.D. at 5%		...		5.87		...		7.85		...		9.02	...	...

*HP88 = *Heterorhabditis bacteriophora* HP88; SAA2 = *Heterorhabditis indicus* SAA2; S1 = *Heterorhabditis sp.* S1.

Means within a row followed by the same capital letter or within a column followed by the same small letter are not significantly different at 5 %. ** = Higly significant at 1% level.

temperatures ranging from 15-30° C for 48 hours; but decreased to 70, 74 and 80% for HP88, SAA2 and S1 as the temperature increased to 35° C, respectively. A 100% mortality was recorded for *H. bacteriophora* PH88 at the highest tested temperature (35° C) after 15 days; while the other two heterorhabditids SAA2 and S1 recorded 95 and 90% mortality, respectively. From the aforementioned results, the results reveal that survival of *Heterorhabditis* spp. were greater at temperatures from 20-30° C, with optimal survival achieved at 25° C; while *Steinernema* spp. survival was greater at lower temperatures than at the higher ones throughout the 15 days of the experiment. Steinernematids are more capable to survive at low temperature than heterorhabditids (Molyneux, 1985). These differences may be attributed to the climatic origins. The original climatic localities tended to determine the temperature at which the Ijs of nematodes became inactive (Molyneux, 1985). This support the hypothesis that steinernematids are favored in cool and temperate regions; whereas heterorhabditids thrive in warm and tropical climates (Hara *et al.*, 1991).

Data also showed that the nematode survival declined as the temperature exceeded 30° C. However, some nematode survived at 35° C. These results agree to other studies suggesting that high temperatures accounted for a rapid decline in nematode survival; such as, full mortality of four novel heterorhabditid nematode isolates occurred after 48 hours exposure to 37 and 40° C (Shamseldean, 1994), poor persistence of *S. carpocapsae* at the highest temperature (35° C) tested (Kung *et al.*, 1991). Moreover, Gray and Johnson (1983) reported a sharp decline in survival of *S. feltiae* when soil temperatures exceeded 30° C.

2- Nematodes pathogenicity

S. carpocapsae All and *S. carpocapsae* S2 achieved 100% mortality of 4th larval instar of *S. littoralis* at all tested temperatures; while the pathogenicity of *S. abbasi* and *S. riobravis* increased as the incubation temperature increased form 15-35° C. On the other hand, the pathogenicity of heterorhabditids (HP88, SAA2 and S1) increased as the incubation temperature increased form 15-30° C and declined at the highest temperature (35° C). Pathogenicity of *S. abbasi* and S. *riobravis* was greater at higher temperature ranges (20-35° C) than at the lowest tested temperature (15° C); where they induced larval mortality 0.0 and 33.3%, respectively (Table 3). In general, heterorhabditid nematodes pathogenicities were greater at temperatures

form 20-30° C than at the lowest and highest temperatures (15 and 35° C). Optimal pathogenicities were obtained at 25° C by HP88 and at 30°C by SAA2 and S1. As also shown in Table (3) that, *H. indicus* SAA2 could not induce larval mortality at temperature 15°C. Analysis of data revealed significant differences at 5% level in pathogenicity among the tested nematodes at all tested temperatures as well among the tested temperatures for each nematode species; except *S. carpocapsae* All and *S. carpocapsae* S2 which showed no significant difference between each other.

Significant differences in pathogenicity on 3rd larval instar of *P. xylostella* were observed among the tested nematodes at all tested temperatures. As well, statistical analysis showed significant differences at 5% among the tested temperatures for the same nematode species (Table 4). Pathogenicity of steinernematids and the Egyptian heterorhabditid S1 increased as the incubation temperature increased form 15-30° C and declined at the highest temperature (35° C); while the pathogenicity of the other two heterorhabditids (HP88 and SAA2) increased as the incubation temperature increased from 15-25°C and declined at the higher temperatures 30 and 35° C.

Optimal pathogenicities were obtained at 25-30° C by *S. carpocapsae* All, *S. carpocapsae* S2 and *H.* sp S1, at 25° C by *S. abbasi*, HP88 and SAA2 and at 30° C by *S. riobravis*. It was also indicated from (Table 4) that *S. abbasi*, *S. riobravis* and *H. indicus* SAA2 could not induce larval mortality at temperature 15°C. This could be attributed to the speed of bacterial multiplication, which in turn is affected by temperature. Poinar and Thomas (1965) reported that the best growth of the bacterium *Achromobacter nematophilus* was between 25 and 35° C, poor growth at 37° C and no growth at 43° C. Poinar (1966) added that the time needed to insect death depends on temperature. This confirms the present results. The obtained results also indicated that *S. abbasi*, *S. riobravis* and *H. indicus* SAA2 nematodes could not induce larval mortality at 15°C. This may be due to lower nematode survival at this temperature. Klein (1990) stated that low soil temperatures restrained nematode activity. In general, at the highest temperature (35° C), the pathogenicity of steinernematids to both 4th and 3rd larval instars of *S. littoralis* and *P. xylostella*, respectively, was greater than that of heterorhabditids; *H. bacteriophora* are generally found deeper in the soil, and thus, are buffered from temperature extremes than steinernematid nematodes which may tolerate high temperatures (Campbell and Gaugler, 1994).

Concerning LT_{50}, data presented in Table (3) indicated that the rate of 4th larval instar of *S. littoralis* mortality increased as the incubation temperature increased from 15-30° C. Effect of temperature on LT_{50} of the larvae differed due to the incubated temperature degree. It was found that at 30° C, all tested nematode species killed the host most rapidly; LT_{50} value was <24 hours for steinernematids; while it was 34.3, 36.0 and 25.5 hours for HP88, SAA2 and S1, respectively. It increased to 31.8, 37.8, 67.9 and 70.0 hours for steinernematids (All, S1, *S. abbasi* and *S. riobravis)* and 59.6, 36.6 and 42.5 hours for heterorhabditids (HP88, SAA2 and S1) at 25°C, respectively. As well, effect of temperature on LT_{50} of the 3rd larval instar of *P. xylostella* differed according to the incubating temperature degree. The results proved that at 30° C, all tested steinernematids (All, S2, *S. abbasi* and *S. riobravis)* and *H. sp.* S1 killed the larvae most rapidly; LT_{50} values were 2.7, 2.7, 14.8, 9.9 and 18.8 *H. indicus* SAA2 killed the host most rapidly at 25° C; LT_{50} recorded 36.8 and 25.0 hours (Table 4), respectively. Results comparable to those obtained in the present work are recorded by many authors such as, Dutky (1959) who exposed larvae of wax moth to *S. carpocapsae* on moist filter paper and found that the death time was 16 hours at 30° C, 24 hours at 25° C, 44 hours at 19° C, 120 hours at 15° C and 312 hours at 9° C. Dunphy and Webster (1986) reported that LC_{50} and LT_{50} values of *H. bacteriophora,* and *S. carpocapsae* declined as the temperature increased from 15 to 25° C and *H. bacteriophora* had a faster rate of killing insects than *S. carpocapsae*. Blackshaw and Newell (1987) demonstrated that *H. bacteriophora* infected the wax moth larvae *G. mellonella*, at a temperature range of 11-32.3°C and the optimum temperature was 28° C. Rate of larval mortality increased by increasing the tmperature from 14.5 to 31° C, larvae died after 72 hours between 20.5 and 31° C, while it took 96 hours at 14.5° C. Grewal *et al.* (1994) observed that the rate of insect mortality by the entomopathogenic nematodes, *H. bacteriophora* (HP88) and *S. glaseri* (NC) increased as the incubation temperature increased from 12-30° C.

3- Nematodes life span and reproduction

Results showed that the life span of *Steinernema* spp. and *Heterorhabditis* spp. at inoculum level (500 Ijs/ml) in the 4th and 3rd instar larvae of *S.littoralis* and *P. xylostella*, respectively, declined significantly as the incubation temperature increased from 20-30° C (Tables 5 and 6). At 30° C, tested nematode species had significantly shorter life span in tested larvae. Infective juveniles of *S. carpocapsae* All, *S. carpocapsae* S2,

Table (3): Infectivity of Egyptian and foreign *Steinernema* spp. and *Heterorhabditis* spp. nematodes (500 Ijs/ml) to the 4th instar larvae of *Spodoptera littoralis* at different temperatures.

Temp.°C	Mortality % and LT_{50} in hours														F. value	L.S.D. at 5%
	All		S2		abb		rio		HP88		SAA2		S1			
	Mor.%	LT_{50}	Mor.%	LT_{50}	Mor.%	LT_{50}	Mor.%	LT_{50}	Mor.%	LT_{50}	Mor.%	LT_{50}	Mor.%	LT_{50}		
15	100Aa	71.5	100^{Aa}	73.5	0.0^{Dc}	...	33.3^{BCb}	>120	53.3^{Ba}	≤120	0.0^{Db}	...	20.0^{CDb}	150.5	26.061^{**}	25.35
20	100Aa	37.8	100^{Aa}	41.0	73.3^{ABCb}	71.7	46.7^{Cb}	73.9	86.7^{ABa}	65.2	53.3^{Ca}	65.9	60.0^{BCab}	60.1	3.956^{**}	29.60
25	100 Aa	31.8	100^{Aa}	37.8	80.0^{ABCb}	67.9	53.3^{Cb}	70.0	93.3^{ABa}	59.6	60.0^{Ca}	36.6	66.7^{BCab}	42.5	3.956^{**}	29.60
30	100 Aa	<24	100^{Aa}	<24	100^{Aa}	<24	100^{Aa}	<24	86.7^{Aa}	34.3	66.7^{Ba}	36.0	100^{Aa}	25.5	5.133^{**}	17.09
35	100 Aa	7.8	100^{Aa}	13.8	100^{Aa}	13.8	100^{Aa}	<24	53.3^{Ba}	65.9	$66.7A^{Bc}$	42.5	66.7^{ABab}	36.0	2.848^{*}	35.85
F. value	$1.000^{N.S}$	...	$0.75^{N.S}$	...	191.500^{**}	...	18.333^{**}	...	1.673	...	14.833^{**}	...	3.519^{*}	...	...	...
L.S.D. at 5%	...	...	...	...	9.39	...	23.01	...	...	...	23.01	...	47.90	...	...	...

All = *Steinernema carpocapsae* All; S2 = *Steinernema carpocapsae* S2; abb= *Steinernema abbasi*; rio = *Steinernema riobravis*. HP88= *Heterorhabditis bacteriophora* HP88; SAA2 = *Heterorhabditis indicus* SAA2; S1 = *Heterorhabditis* sp. S1.

Means within a row followed by the same capital letter or within a column followed by the same small letter are not significantly different at 5%.

Control mortality was zero% throughout the period of experiment.

*= Significant at 5% level. **= Highly significant at 1% level. N.S. = Not significant at 5% level.

Table (4): Infectivity of Egyptian and foreign *Steinernema spp.* and *Heterorhabditis* spp. nematodes (500 IJs/ml) to the 3rd instar larvae of *Plutella xylostella* at different temperatures.

Temp.°C	Mortality % and LT_{50} in hours														F. value	L.S.D. at 5%
	All		S2		abb		rio		HP88		SAA2		S1			
	Mor.%	LT_{50}	Mor.%	LT_{50}	Mor.%	LT_{50}	Mor.%	LT_{50}	Mor.%	LT_{50}	Mor.%	LT_{50}	Mor.%	LT_{50}		
15	88.0^{Aa}	71.1	52.0^{Bc}	110.4	0.0^{Dc}	...	0.0^{Dc}	...	4.0^{Dc}	>120	0.0^{Dc}	...	32.0^{Cb}	139.2	39.615^{**}	15.79
20	92.0^{Aa}	48.8	88.0^{ABab}	52.2	84.0^{ABab}	48.4	72.0^{BCb}	90.7	60.0^{Ca}	52.0	80.0^{ABa}	51.6	80.0^{ABa}	53.5	2.439^{*}	19.83
25	100^{Aa}	23.0	100^{ABa}	22.8	92.0^{ABa}	44.2	84.0^{Bb}	60.7	68.0^{Ca}	36.8	88.0^{ABa}	25.0	92.0^{ABa}	24.8	3.667^{**}	15.79
30	100^{Aa}	2.7	100^{Aa}	2.7	72.0^{Bc}	14.8	100^{Aa}	9.9	32.0^{Cb}	82.9	40.0^{Cb}	87.3	92.0^{Aa}	18.8	22.431^{**}	18.06
35	72.0^{Ab}	41.5	72.0^{Ab}	52.0	52.0^{Bc}	68.0	80.0^{Ab}	40.7	20.0^{Cbc}	204.8	24.0^{Cb}	100.9	20.0^{Cb}	204.8	17.556^{**}	18.58
F. value	5.929^{**}	...	11.57^{**}	...	32.500^{**}	...	63.133^{**}	...	11.895^{**}	...	34.720^{**}	...	31.125^{**}	...	...	...
L.S.D.at 5%	13.96	...	17.10	...	19.03	...	14.45	...	23.00	...	18.66	...	18.28	...	...	...

All = *Steinernema carpocapsae* All; S2 = *Steinernema carpocapsae* S2; abb = *Steinernema abbasi*; rio = *Steinernema riobravis*. HP88 = *Heterorhabditis bacteriophora* HP88; SAA2 = *Heterorhabditis indicus* SAA2; S1 = *Heterorhabditis* sp. S1.

Means within a row followed by the same capital letter or within a column followed by the same small letter are not significantly different at 5 %. Control mortality was zero % throughout the period of experiment.

* = Significant at 5% level ** = Highly significant at 1% level.

Table (5): Effect of temperature on the life span and reproductive potential of *Steinernema* spp.* and *Heterorhabditis* spp.* nematodes (500 Ijs/ml) in 4th instar larvae of *Spodoptera littoralis*.

Temp. (°C)	Mean no. of days taken to 1st. emergence ± S.E.							Mean no. of Ijs emerging /cadaver ±S.E.×10^2						
	All	S2	abb	rio	HP88	SAA2	S1	All	S2	abb	rio	HP88	SAA2	S1
15	17.0 ±0.58[a]	18.0 ±0.58[a]	---	0.0 ±0.0[e]	0.0 ±0.0[d]	---	0.0 ±0.0[e]	2.57 ±0.13[d]	1.90 ±0.56[c]	---	0.00 ±0.0[e]	0.00 ±0.0[d]	---	0.00 ±0.0[e]
20	16.0 ±0.58[a]	18.0 ±0.0[a]	15.0 ±1.0[a]	15.0 ±1.15[a]	16.0 ±1.15[a]	16.0 ±0.58[a]	16.3 ±0.33[a]	50.33 ±0.20[c]	29.77 ±0.23[b]	85.67 ±2.96[d]	51.90 ±1.56[d]	136.67 ±3.33[b]	4.97 ±0.03[bc]	15.77 ±0.77[d]
25	14.0 ±0.58[b]	14.0 ±0.58[b]	13.0 ±0.58[a]	14.0 ±0.0[a]	13.0 ±0.58[b]	13.3± 0.33[b]	13.0 ±0.0[b]	63.57 ±2.02[b]	33.57 ±1.79[b]	121.00 ±0.51[c]	63.43 ±1.72[c]	178.23 ±1.62[a]	6.00 ±0.17[b]	20.57 ±0.57[c]
30	6.0 ±0.58[c]	7.0 ±0.0[c]	5.3 ±0.88[b]	5.0 ±0.58[b]	7.7 ±0.33[c]	8.0± 0.58[d]	8.0 ±0.0[d]	203.67 ±2.33[a]	282.00 ±2.0[a]	185.57 ±2.89[b]	227.00 ±2.08[a]	141.67 ±1.67[b]	371.67 ±1.67[a]	161.67 ±1.67[a]
35	0.0 ±0.0[d]	0.0 ±0.0[d]	4.0 ±0.0[b]	4.0 ±0.0[b]	9.0 ±0.58[c]	10.0± 0.58[c]	12.0 ±0.58[c]	0.00 ±0.0[d]	0.00 ±0.0[c]	642.23 ±2.38[a]	151.10 ±1.10[b]	6.43 ±0.23[c]	3.40 ±0.26[c]	72.77 ±1.47[b]
F. value	201.750^{**}	456.000^{**}	94.553^{**}	129.900^{**}	87.447^{**}	328.142^{**}	441.625^{**}	359.236^{**}	985.275^{**}	138.01^{**}	361.248^{**}	212.283^{**}	470.685^{**}	373.211^{**}
L.S.D at 5%	1.63	1.15	2.05	1.82	2.05	1.15	0.94	4.36	3.80	6.77	4.66	5.69	2.39	3.41

*All = *Steinernema carpocapsae* All; S2 = *Steinernema carpocapsae* S2; abb = *Steinernema abbasi*; rio = *Steinernema riobravis*. HP88=*Heterorhabditis bacteriophora* HP88; SAA2=*Heterorhabditis indicus* SAA2; S1 = *Heterorhabditis* sp. S1.

Means within a column followed by the same letter are not significantly different at 5 %.

** = Highly significant (Highly significant at 1%).

Table (6): Effect of temperature on the life span and reproductive potential of *Steinernema* spp.* and *Heterorhabditis* spp.* nematodes (500 Ijs/ml) in 3rd instar larvae of *Plutella xylostella.*

Temp. (°C)	Mean no. of days taken to 1st. emergence ± S.E.							Mean no. of Ijs emerging /cadaver ±S.E.×10²						
	All	S2	abb	rio	HP88	SAA2	S1	All	S2	abb	rio	HP88	SAA2	S1
15	19.7± 0.33a	0.0± 0.0d	...	...	0.0± 0.0d	...	0.0± 0.0d	0.66± 0.02d	0.00± 0.0d	...	...	0.00 0.0±c	...	0.00± 0.0c
20	16.7± 0.67b	18.0± 0.0a	14.3± 0.33a	15.3± 0.33a	16.0± 0.0a	16.3± 0.33a	16.3± 0.33a	5.50± 0.12b	6.40± 0.38c	2.56± 0.13b	5.00± 0.17c	8.56± 0.30b	6.37± 0.33c	3.00± 0.17b
25	14.0± 0.0c	14.3± 0.33b	13.0± 0.58b	14.3± 0.33b	12.0± 0.0b	13.3± 0.33b	12.7± 0.67b	6.50± 0.12a	7.80± 0.10b	2.80± 0.10b	6.33± 0.20b	9.67± 0.20b	7.50± 0.12b	3.37± 0.07b
30	9.3± 0.88d	8.3± 0.33c	5.7± 0.33c	5.3± 0.33c	8.0± 0.58c	8.0± 0.0c	8.0± 0.0c	4.83± 0.09c	17.00± 0.17a	14.77± 0.39a	14.33± 0.20a	49.00± 1.0a	34.33± 0.20a	51.33± 0.88a
35	0.0± 0.0e	0.0± 0.0d	0.0± 0.0d	0.0± 0.0d	0.0±0.0d	0.0± 0.0d	0.0± 0.0d	0.00± 0.0e	0.00± 0.0d	0.00± 0.0c	0.00± 0.0d	0.00± 0.0c	0.00± 0.0d	0.00± 0.0c
F. value	220.917**	150.825**	424.700**	840.000**	768.000**	126.450**	489.200**	125.773**	133.713**	103.865**	154.604**	184.226**	612.267**	306.195**
L.S.D. at 5%	1.63	0.66	1.05	0.81	0.81	0.66	1.05	0.26	0.60	0.60	0.47	1.50	0.57	1.27

*All = *Steinernema carpocapsae* All; S2 = *Steinernema carpocapsae* S2; abb = *Steinernema abbasi*; rio = *Steinernema riobravis* . HP88 = *Heterorhabditis bacteriophora* HP88; SAA2 = *Heterorhabditis indicus* SAA2; S1 = *Heterorhabditis* sp. S1.
Means within a column followed by the same letter are not significantly different at 5 %.
** = Highly significant (Highly significant at 1%).

S. abbasi, S. riobravis, H. bacteriophora HP88, *H. indicus* SAA2 and *H.* sp. S1 started to emerge from infected cadavers of *S. littoralis* after 6.0, 7.0, 5.3, 5.0, 7.7, 8.0 and 8.0 days of infection at 30° C, respectively; whereas, after 9.3, 8.3, 5.7, 5.3, 8.0, 8.0 and 8.0 days of exposure from infected cadavers of *P. xylostella*, respectively. As for the reproductively of tested nematodes, *S. carpocapsae* All and *S. carpocapsae* S2 could reproduce Ijs in the host cadavers (4th larval instar of *S. littoralis)* at 15° C. The time taken to 1st emergence was 17.0 and 18.0 days, respectively; but *S. riobravis, H. bacteriophora* HP88 and *H.* sp. S1 could not reproduce Ijs in the host cadavers, within 3 weeks (Table 5). On the other hand, at 35° C, *S. carpocapsae* All and *S. carpocapsae* S2 could not reproduce Ijs in the host cadavers; but *S. abbasi, S. riobravis, H. bacteriophora* HP88, *H.indicus* and *H. sp.* S1 could reproduce Ijs from the host cadavers, within 4.0, 4.0 , 9.0, 10.0 and 12.0 days, respectively. It was also noticed that at 35° C *S. abbasi* and *S. riobravis* were faster to complete reproduction in the host than at 30° C (Table 5). On the other hand, *S. carpocapsae* All was the only strain that could reproduce Ijs from the host cadavers (3rd larval instar of *P. xylostella*) at 15° C; time taken to1st emergence was 19.7 days (Table 6). Moreover, all the tested nematodes failed to reproduce in the dead larvae at 35°C. The number of emerging infective juveniles varied among the tested nematode species at tested temperatures (Table 5 and 6). The best reproductive potential was found at temperature 30° C in both 4th and 3rd larval instars for all tested nematodes except *H. bacteriophora* HP88 in 4th larval instar of *S. littoralis* and *S. carpocapsae* All in 3rd larval instar of *P. xylostella*, it was at 25° C. Significant differences were found at 5% level in reproductive potential among the tested temperatures for each tested nematode species in both 4th and 3rd instar larvae of *S. littoralis* and *P. xylostella,* respectively.

At 15° C, it was noticed that most steinernematids and heterorhabditids did not reproduce in both insects within three weeks. This could be attributed to the cessation of nematode activity at low temperatures. This finding is in agreement with that obtained by Molyneux (1986) who reported that *S. carpocapsae* does not reproduce below 15°C. Zervos *et al.* (1991) examined the effect of temperature on reproduction and development of *H. heliothidis* and *S. glaseri* in *G. mellonella* and reported that, no juveniles of the two nematode species were emerged at 5 or 10° C. At high temperature of 35° C, all tested nematodes failed to develop within 3rd larval instar of *P. sylostella* as well as *S. carpocapsae* All and *S. carpocapsae* S2 within 4th larval instar of *S. littoralis* within three weeks; although infective juveniles could infect and kill the host. This may be due to lower nematode survival and nematode infection. These results agree with other studies suggesting that high temperatures tend to inhibit nematode development in their hosts (Kaya, 1977 and Milstead, 1981); although Shamseldean *et al.* (1996a) stated that at 35° C infective juveniles of *H. bacteriophora* (EASD98) could not emerge from the cadavers. Shamseldean *et al.* (1998) found that *H. bacteriophora* EASD98 failed to reproduce at 35° C and concluded that entomopathogenic nematode species and/or strains are governed by the temperature range of their original localities.

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الملخص العربى

قدرة النيماتودا الممرضة للحشرات على المعيشة واحداث العدوى والتكاثر تحت تأثير درجات حرارة مختلفة

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وجد أن حياة النيتماتودا المختبرة تتأثر بدرجة الحرارة المعرضة لها ومدة التعرض. حيث كانت نسبة معيشة الأنواع النيماتودية من جنس *Heterorhabditis* أعلى عند درجات حرارة من ٢٠-٣٠°م وكانت درجة الحرارة ٢٥°م هى الدرجة المثلى لمعيشة ونشاط الأنواع التابعة لهذا الجنس. بينما كانت نسبة حياة الأنواع من جنس *Steinernema* أعلى عند درجات الحرارة الأقل. كانت نسب العدوى بأنواع النيماتودا التابعة لجنس *Heterorhabditis* أعلى عند درجات الحرارة ١٥-٣٠°م عنه عند درجة ٣٥°م وكانت درجة الحرارة المثلى للعدوى ٢٥°م فى حالة *H. bacteriophora* HP88 ، ٣٠°م فى حالة *H. sp.*S1 and *H. indicus* SAA2 ولم يحدث *H. indicus* SAA2 وفيات لدودة ورق القطن عند درجة حرارة ١٥°م. انخفضت فترة حياة النيماتودا المختبرة فى العمر اليرقى الرابع لدودة ورق القطن والعمر اليرقى الثالث لدودة الفراشة ذات الظهر الماسى انخفاضاً ملحوظا بارتفاع درجات الحرارة من ٢٠ إلى ٣٠°م. وعند درجة ٣٠°م كانت النيماتودا أسرع فى إتمام التكاثر فى يرقات العائل. كانت درجة الحرارة ٣٠°م هى الدرجة المثلى لإنتاج النيماتودا للأطوار المعدية، فيما عدا *H. bacteriophora* HP88 فى العمر اليرقى الرابع لدودة ورق القطن ، *S. carpocapsae* All فى العمر اليرقى الثالث لدودة الفراشة ذات الظهر الماسى، حيث كانت الدرجة المثلى ٢٥°م. ولم تنتج معظم النيماتودا أطوارا معدية عند درجة حرارة ١٥°م فى اليرقات المذكورة لمدة ثلاثة أسابيع.