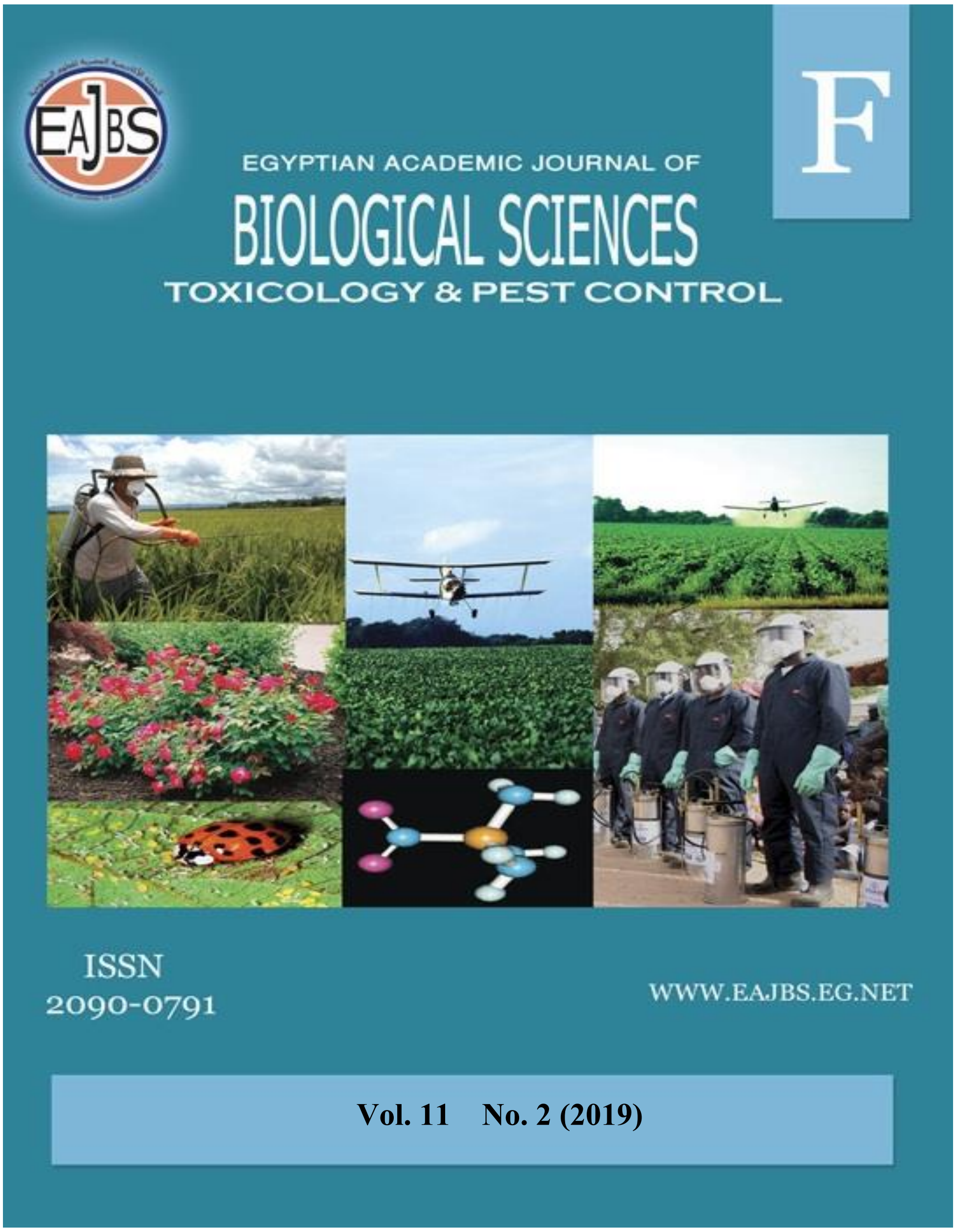

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Pathogenic and Lethal Effects of Some Entomopathogenic Nematodes Species against the Greater Wax Moth, *Galleria mellonella,* (L.) Larvae (Lepidoptera: *Galleridae*)

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ABSTRACT

Laboratory experiments were performed to evaluate the pathogenic and lethal effects of the genera entomopathogenic nematodes, (EPNs) (*Heterorhabditis bacteriophora Poinar* HP88) and Sc (*Steinernema carpocapsae* All strains, *(Steinernema carpocapsae*, *Steinernema. scapterisi* and *Steinernema. glaseri***)** on greater wax moth larvae, *Galleria mellonella*, at the three concentrations; 20IJs, 50IJs and100IJs (infective juveniles) after different times, 6, 12, 24 and 48hrs of exposure times. The mortality percentage of EPNs was determined on *G.mellonella* larvae for each concentration. It was found that the infectivity of nematode strains against *G. mellonella* larvae was concentration-dependent; i.e. the mortality percentage increased as the infective juvenile concentrations increased. The mortality percentages among *G. mellonella* larvae after 48hrs post-treatment by all EPNs strains were highly significant increased at 100 IJs/L for each concentration. The treatments at 20 IJ/L noticed that, LT_{25} was 10.17hrs for *G. mellonella* larvae treated with *S. carpocapsae* after 48hrs post-treatment. Also, results indicated *S. glaseri* gave the highest mortality rate for the tested larvae reached to 96.66 %, followed by *S. carpocapsae* achieved 96.16%, but the *H. bacteriophora* HP88 recorded 84.24 % mortality after four times of exposure. Meanwhile at 50IJ/L data cleared that, LT_{25} value was 7.36hrs for *G. mellonella* larvae treated with *S. carpocapsae* while *S. carpocapsae* treatment gave the highest significant mortality for the tested larvae reached to 94.45 % followed by 94.25% for *S. glaseri*, but *H. bacteriophora* recorded 86.01 % mortality. The results of the study noticed that, LT_{25} value was 3.26hrs for *G. mellonella* larvae treated with *S. scapterisi* also showed that *S. carpocapsae* treatment gave the highest significant mortality for the tested wax moth larvae recorded 98.18 % followed by *S. glaseri* achieved 98.09% and the *H. bacteriophora* recorded 92.77 % mortality at 100IJ/L after 48hrs of treatments. Also, data showed a strong coefficient correlation between the tested ENPs against *G. mellonella* larvae. Also, there were inverse relationship between the times of infection and concentrations. Our results suggest that *H. bacteriophora*, *S. carpocapsae*, *S. scapterisi* and *S. glaseri* can be used as valuable tools in biological control programs of last instar larvae of *G. mellonella*.

INTRODUCTION

Galleria mellonella was the pest of beehives feeding upon pollen and destroying the combs of weak or diseased hives. Wax contains many nutrients, pollen and honey, and is therefore attacked by various pests (Ebadi *et al*. 1980). Other researchers cleared that, the wax is one of the most useful products of honey bees and is used in the pharmaceutical industry, dentistry and cosmetics. The larvae caused severe damage in tropical and sub-tropical regions (Kwadha *et al.*, 2017). Larvae of the greater wax moth, *Galleria melonella* cause considerable damage to bees wax combs. Chemical control leads to a negative impact on the environment so there is a mass need to develop alternative means of control (El-Sinary, 2007). Also, other researcher stated that, *G. mellonella* wax worms caused economically damage in storage wax, chemicals which use in control caused bad effect on stored bee honey inside combs (Taha and Abdelmegeed. 2016). *G. mellonella* moth, larvae are known to be highly susceptible to the Entomopathogenic nematodes (Van Zyl *et al*., 2015). The effect of *S. carpocapsae* against the last instar larvae of *G. mellonella*, cleared that EPN strain was invasive ability and caused moderately infecting the host (Epsky and Capinera 1993). Other researchers, (Glazer 1992) stated that, *S. carpocapsae* all strains were less effective than *H. bacteriophora* HP88 when applied to different lepidopteron pests according to LD50 and LT 50 values. The EPNs in the genera *Steinernema* and *Heterorhabditis* have been used to control a wide range of agriculturally important insect pests (Kaya and Gaugler 1993). EPNs are presently used as bio-pesticides for controlling different pests (Lacey et al. 2006). EPN enter the hemocoel of its host via the intestinal tract and releases its symbiotic bacterium *Xenorhabdus nematophila*, which kills the insect in less than 48 hours (Louise *et al*., 2019). The effects of *S. feltiae* and *S. carpocapsae* on *G. mellonella* larvae were determined. The doses of nematodes caused moderately mortality rate of *G. mellonella* larvae (Gordon *et al*., 1996). *S. carpocapsae* caused attraction to *G. mellonella* cuticle. Also, reported that the highest mortality 100% scored for *Agrotis ipsilon* during *S. carpocapsae* infect its host. There were significant correlations between behavioural response and nematode-induced mortality at the lower dose and at the level of reproduction for *S. carpocapsae* (Lewis *et al*., 1996).

EPNs of Heterorhabditidae families are lethal endoparasites of insects. Their role in insect pathogenicity is aided by toxic secondary metabolites produced by symbiotic Photorhabdus bacterial species. *H. indica* is widely used in biological control of insect pests in agriculture (Brown *et al*. 2004, 2006) and (Ffrench *et al.*, 2007). (Soliman 2007b), (Toledo et al., 2006) used Hb nematode on *Anastrepha ludens* (*Diptera*: *Tephritidae*) and mentioned that Hb nematode caused high pathogenicity.

The tested *Steinernema* and *Heterorhabditis* caused mortality of *G. mellonella* larvae ranges from 12 to 96%. Also, *H. bacteriophora* caused 60% mortality at 5 infective juveniles per *G. mellonella* larvae (Stefanovska and Pidlishyuk.2008). The infective juveniles (IJs) of entomopathogenic nematodes (EPNs) are currently used as biopesticides for controlling various insect pests (Hom, 1994). *Heterorhabdit* is genus that has symbiotic bacteria belonging to Photorhabdus genus. Secondary metabolites produced by Photorhabdus have several bioactive properties that affect physiology and survival in several insect species. (Grewal *et al*., 2005), (Jung and Kim 2007) and Ullah *et al.*, (2014). The pathogenicity of the nematode-bacterium complex *S. feltiae*-*X. bovienii* to larvae of *G. mellonella* was investigated by injection juvenile nematodes. One axenic nematode caused kill 80% of *G. mellonella* in one day (Ehlers *et al*., 1997). Also, Ehlers (2001) reported, that ENPs are used to control pest insects. They are symbiotically associated with bacteria which are the major food source for the nematodes. *H.*

bacteriophora was tested on the armyworm, *Pseudaletia unipuncta*, which caused more effect against the 6th instar *P.unipuncta* larvae. Based on LC_{50} and LT_{50}, *H. bacteriophora* nematode was the most pathogenic effect (Simões and Rosa 1996). The three Egyptian isolates of entomopathogenic nematodes were evaluated against *R. ferrugineus*. A high mortality rate was recorded for 2nd and 6th instars larvae and adults of *R. ferrugineus* weevil, respectively (Shamseldean and Atwa 2004). Also, (Sankar *et al.*, 2009) cleared that, the tested *H. indica* nematode on *G. mellonella* larvae proved to be the most efficient causing 100% mortality in *G. mellonella* after 24 h of storage. The results proved that the interactions between entomopathogenic nematodes and other soil microorganisms may be the key to success in IPM programme. The natural history of many (EPNs) species were used as biological control agents on *G. mellonella*. The LC_{50} for F. *auricularia* was 226 *S. carpocapsae* (Hodson *et al.*, 2011).

(Andrew *et al.*, 2012) reported that, the nematodes Steinernema kraussei and S. carpocapsae provided excellent control with 100% mortality of larvae, *Aethina tumida*, is an invasive pest of honey bees. Reyad (2012) studied the infectivity of the four EPNs, *S. glaseri, S. carpocapsae, S. riobrave* and *S. scarptasci* on the earwig. *Labidura riparia* (Nymph and adult). *S. carpocapsae* exhibited a high virulence against the nymphs of L.riparia. On the other hand, *S. scarptasci* showed a higher mortality rate to the *L. riparia* adults. (Gokce *et al.*, 2013) cleared the tested of (EPNs) *steinernematid* against *G.mellonella*, the highest mortality rate was obtained within 7 d after inoculation. The results indicate that the new isolate is a highly promising biological control agent against *A. segetum*. In Egypt Nouh and Hussein, (2014) found that, the infectivity of nematodes genera *Heterorhabditis* and *Steinernema* against *G. mellonella* indicated the mortality percentage increased as the infective juvenile concentrations increased. Tested *S. Feltiae* isolated from larvae of *Bibio hortulanus* indicated high mortality on *G. mellonella* (Campos *et al.*, 2006). In addition, *S. carpocapsae* and *S. glaseri* species laboratory tested caused a high mortality rate to *G. mellonella* larvae (Archana *et al*., 2017).

S. feltiae, *S. carpocapsae*, and *H. bacteriophora* were tested against *Paranthrene diaphana* larvae. The tested nematodes strains caused significant effect larval The (LC_{50}) for each nematode species was a moderately effect. Also, results indicated expanded on the prospects for using EPN, especially *S. feltiae*, in managing *P. Diaphana* (Azarnia *et al.*, 2018). Other researchers tested (EPNs) against *G. Mellonella* and found it caused high mortalities for *G. mellonella* larval injected with *A. musiformis* (Bueno *et al*., 2018).

The aim of the present work was conducted to throw a spotlight on the efficacy of toxicological and virulence effects of the four EPNs, of *H. bacteriophora*, *S. carpocapsae*, *S. scapterisci*, *S. glaseri* against *G. mellonella* larvae.

MATERIALS AND METHODS

Target Insect: *Galleria mellonella* (Lepidoptera: *Galleridae*):

1. Rearing Technique:

The strain of the greater wax moth, *G. mellonella* larvae was obtained from the National Research center (NRC) and reared according to **Hussein (2004)**.The insect under study was reared on media developed from **(Wiesner, 1993)**. The larvae were reared on a semi-synthetic artificial diet as described by **(Ibrahim *et al.*, 1984)** then kept at laboratory constant conditions at 28± 2°c and 65± 5% R.H. in the insect rearing chamber. The larvae were originally obtained from bee hives and transferred to transparent plastic rearing jars, containing the previously prepared media, closed with a lid of muslin for aeration and incubated.

Nematoda Used:

Four species of EPNs were used for this study, *H. bacteriophora HP88, S. carpocapsae, S. scapterisi* and *S. glaseri* Table (1).

Table (1): Classification of Tested Entomopathogenic nematodes.

Kingdom	Phylum	Class	Family	Order	Scientific classification
A nimals	Nematoda	Secernentea	Heterorhabditidae	Rhabditida	*Heterorhabditis bacteriophora*
			Steinernematidae		*Steinernema scapterisci*
					Steinernema carpocapsae
		Chromadorea			*Steinernema glaseri*

1. Entomopathogenic Nematode Source:

The entomopathogenic nematode (EPNs) was obtained from a stock culture maintained for several generations in the laboratory of the Department of Insect Physiology, Plant Protection Research Institute, Agricultural Research Center, Dokky, Giza, Egypt**.**

2. Mass Production of ENPs:

The last instars larvae of *G. mellonella* were used to multiply the IJs (Infective juvenile) nematodes. The cultured nematodes from *G. mellonella* larvae kept at room temperature at 23–24ºC using methods described by (Kaya and Stock 1997). *G. mellonella* larvae infected by the nematodes were placed on White traps (White, 1929), and the new IJs emerging from cadavers were harvested. Collected IJs were rinsed three times in sterile distilled water and each species kept separately in one Litter juice boxes (Gulcu and Hazir2012) before being stored at 10 °C. The harvested IJs were used within two weeks after emergence for the experiments. Suspensions were stored in 25 - 30 ml of sterilized distilled water at a concentration of 2000 IJ's /ml and stored at 9˚C for no more than two weeks before they were used.

Bioassay Experiments:

Nematodes of each tested species were prepared at three concentrations 20, 50, 100 IJs/300µl of water. Nematodes were bioassayed against the last instar larvae of *G. mellonella. The Infection* of *G. mellonella* larvae was carried out in perforated eppindorf lined with filter paper (tissue paper). Each concentration was replicated five times (5replicates / concentration). The last tested larvae were topically inoculated and confined, individually, with IJs using Using an eppindorf and micropipette, One larva was used for each eppindorf and subsequently incubated. All experiments were carried out in a conditioned laboratory at 25 ±2 °C and 50 – 60 % R.H. The tested larvae were inspected after four exposure times as follow 6, 12, 24 and 48hrs post-treatment and percentage mortality recorded. A control treatment was carried out using distilled water.

Statistical Analysis:

The mortality percentages *G. mellonella* were corrected according to (Abbott's, 1925) formula. The LC_{25s} and LC_{50s} and the slope values were determined according to (Finney, 1971). Toxicity index (T.I) at LC_{25}, LC_{50} levels were determined using (Sun, 1950) equation. Relative potency levels of the tested compounds are expressed as the number of folds was measured according to the method described by (Zidan and Abdel-

Maged 1988). The proper "F" and $LSD_{0.05}$ value of various treatments was evaluated by range test ($P \leq 0.05$) was calculated as described by (Fisher, 1950) and (Snedecor, 1970).

RESULTS AND DISCUSSION

Toxicological Studies:

1. Mortality Percentage of EPNs on *G.mellonella* Larvae:

Data presented in Tables (2), (3), (4) and (5) showed mortality percentages among *G. mellonella* larvae 48hrs post-treatment by *H. bacteriophora Poinar* HP88 at different concentrations. These data showed that the percentage mortality increased as the concentration of IJs increased.

The mortality percentage was significant increase as the concentration of IJs increased up to 100 IJs. Percentage mortalities by strain *H. bacteriophora* reached 39.09, 52.21,73.38 %, for a density of one larva / eppindorf at concentrations of 20, 50 and 100 IJs / eppindorf, respectively. The mortality percentage was more toxic virulence on *G. mellonella* larvae reached 12.93, 37.99, 54.37 and 73.38 %, at, 6, 12, 24 and 48hr after times of exposer, respectively compared with control. While for *S. carpocapsae* species reached 21.79, 54.86, 70.91 and 96.65 %, respectively after the same exposer times at treatment with 100 IJs/ concentrations. Percentage mortalities by *S. carpocapsae* species were significant increased reached 49.71, 57.63 and 96.65 at the same previous concentrations, respectively. Also, Table (5) showed mortality percentages increased to 16.05, 41.45, 58.71 and 86.98 % after four times of exposer at 100IJs/L The percentage mortalities recorded 38.98, 49.65 and 86.98% at 48hrs from treatments respectively, for *S. scarabaei*. Results indicated that, mortality percentage by *S. glaseri* reached 18.35, 50.38, 65.33 and 88.60 %, respectively after four times post-treatment. Percentage mortalities by *S. glaseri* species were significant increased reached 44.57, 53.34 and 88.60 % compared with control.

Table (2): Mean Percentage mortality by different concentration of *Heterorhabditis bacteriophora* strain, (HP88) against larvae of *Galleria mellonella* after different times.

Con. (IJs/L)	Mortality% hrs.			
	6 hrs.	12 hrs.	24 hrs.	48 hrs.
Control	0.00c	0.00d	0.00d	0.00c
20	1.55c	6.83c	32.72c	39.09b
50	9.92b	27.79b	39.84b	52.21b
100	12.93a	37.99a	54.37a	73.38a
P	0.00	0.00	0.00	0.00
$LC_{0.05}$	2.99	6.36	6.36	7.15

IJs=Infective juvenile L=larva of Galleria mellonella. hrs. = hours P=Probability Within the same column and source data followed by the same letter are not significantly different (P>0.05; LSD mean separately

Table (3): Mean mortality percentages of last instars larvae of *G. mellonella* treated with *S. carpocapsae* after different times.

Con. IJs/L	Mortality% / hrs			
	6hrs	12hrs	24hrs	48hrs
Control	0.00c	0.00d	0.00d	0.00c
20	4.74b	13.06e	34.04c	49.71b
50	19.62a	35.01b	50.51b	57.63b
100	21.79a	54.86a	70.91a	96.65a
P	0.000	0.000	0.000	0.000
$LC_{0.05}$	4.62	6.19	6.23	9.43

IJs=Infective juvenile L=larvae hrs. = hours P= Probability Within the same column and source data followed by the same letter are not significantly different (P>0.05; LSD mean separately.

Table (4): Mean mortality percentages of last instars larvae of *G. mellonella* treated with *S. scapterisci* after different times.

Con. IJs/L	Mortality% /hrs.			
	6hrs.	12hrs.	24hrs.	48hrs.
Control	0.00c	0.00d	0.00d	0.00d
20	2.82b	8.03c	33.73c	38.98c
50	16.22a	29.36b	42.65b	49.65b
100	16.05a	41.45a	58.71a	86.98a
P	0.000	0.000	0.000	0.000
$LC_{0.05}$	2.76	5.95	6.02	10.25

IJs= Infective juvenile L=larvae hrs. = hours P= Probability Within the same column and source data followed by the same letter are not significantly different (P>0.05; LSD mean separately.

Table (5): Mean mortality percentages of last instars larvae of *G. mellonella* larvae treated with *S. glaseri* after different times.

Con. (IJs/L)	Mortality% / hrs.			
	6hrs.	12hrs.	24hrs.	48hrs.
Control	0.00c	0.00d	0.00d	0.00c
20	3.20b	9.77c	36.69c	44.57b
50	17.45a	30.93b	48.24b	53.34b
100	18.35a	50.38a	65.33a	88.60a
P	0.000	0.000	0.000	0.000
$LC_{0.05}$	2.85	5.09	6.16	12.38

IJs= Infective juveniles L= larva hrs. = hours
P= Probability Within the same column and source data followed by the same letter are not significantly different (P>0.05; LSD mean separately.

2. Effect of EPN at Different Times of Exposure:

A. At 20 IJs:

The results in Table (6) showed that *S. carpocapsae* is the most effective species on *G. mellonella* larvae at LT_{25}, LT_{50} and LT_{90} were 10.17, 15.61 and 35.19 % at the slope value 3.63. The response rate for treated larvae with *H. bacteriophora* (HP88), *S. carpocapsae*, *S. scapterisi* and *S. glaseri* species achieved the highest values after 48 hours of treatment and reached to 84.24, 96.16, 76.27 and 96.66 %, respectively. The results showed that, the treatment of *S. glaseri* gave the highest mortality rate on *G. mellonella* larvae 96.66 % followed by the treatment of *S. carpocapsae* 96.16%, then the *H. bacteriophora* recorded 84.24 %. A strong coefficient correlation was found between the tested ENPs against *G. mellonella* larvae.

Table (6): The calculated (LT_{25}, LT_{50} and LT_{90}) lethal values and at concentration (20IJs/L) on *G. mellonella* larvae after different times response rate % of EPNs *H. bacteriophora* **,** S. *carpocapsae* , *S. scapterisci* and *S. glaseri*

Treatments	exposure times	LT_{25}	LT_{50}	LT_{90}	Slope ±S.E.	Correlation	Response%
H. bacteriophora	6hrs.	10.69	19.55	61.46	2.58± 0.23	0.97	9.33
	12hr.s						29.25
	24hrs.						59.07
	48hrs.						84.24
S. Carpocapsae	6hrs.	10.17	15.61	35.19	3.63± 0.29	0.99	6.61
	12hr.s						33.94
	24hrs.						75.12
	48hrs.						96.16
S. scapterisci	6hrs.	11.93	23.44	84.67	2.29± 0.23	0.88	8.70
	12hr.s						25.20
	24hrs.						50.93
	48hrs.						76.27
S. glaseri	6hrs.	12.68	18.02	35.13	4.42± 0.36	0.99	1.79
	12hrs.						14.66
	24hrs.						75.33
	48hrs.						96.66

B. At 50 IJs:

The results in Table (7) showed that, *S. carpocapsae* was more virulent to *G. mellonella* larvae. The values at LT_{25}, LT_{50} and LT_{90} were 7.36, 12.85 and 37.04 at slope value 2.79. Also, in the same Table showed that, the response rates for treated *G. mellonella* larvae with different ENPs recorded the highest value after 48 hours of treatment 86.01, 94.45, 80.35 and 94.25 %, *H. bacteriophora* (HP_{88}), *S. carpocapsae, S. scapterisi* and *S. glaseri* species,respectively. The results also showed that, the response rates in *G. mellonella* larvae differed significantly between the different concentrations of ENPs after four times of exposers.

C. At 100 IJs/Larvae:

The present results cleared that *S. scapterisci* was more virulent to *G. mellonella* larvae at LT_{25} was 3.26 and at LT_{50} and LT_{90} were 9.22 and 66.61 at the slope value 1.49. Meanwhile, *H. bacteriophora* (HP88) were 3.94, 8.69 and 38.97 at slope value 1.97, respectively. The results showed that, the response rates for the treatment on *G. mellonella* larvae with ENPs reached its highest value after 48 hours of treatment to 92.77, 98.18, 85.75 and 98.09 %, respectively. The results showed that, the treatment with *S. carpocapsae* gave the highest mortality rate on larvae of *G. mellonella* reached to 98.18 % followed by the treatment of *S. glaseri* 98.09%, and the *H. bacteriophora* recorded 92.77 % after four times of exposers. A strong correlation was found between the tested ENPs against *G. mellonella* larvae Table (8).

Table (7): The calculated (LT_{25}, LT_{50} and LT_{90}) lethal values and response % of EPNs, *H. bacteriophora*, *S. carpocapsae* , *S. scapterisci* and *S. glaseri* at concentration (50 IJ/L) on *G. mellonella* larvae after different times.

Treatments	Time after treatment/hours	LT_{25}	LT_{50}	LT_{90}	Slope ± S.E.	Correlation	Response %
H. bacteriophora	6	10.51	18.84	57.09	2.66± 0.24	0.93	9.32
	12						30.12
	24						61.03
	48						86.01
S. carpocapsae	6	7.36	12.85	37.046 1	2.79± .22	0.99	17.85
	12						46.71
	24						77.52
	48						94.45
S. scapterisci	6	8.12	17.78	78.874 3	1.98± 0.21	0.94	17.52
	12						36.77
	24						60.19
	48						80.35
S. glaseri	6	9.94	15.93	39.029 6	3.29± 0.28	0.99	8.148
	12						34.29
	24						72.12
	48						94.52

Table (8): The calculated (LT_{25}, LT_{50} and LT_{90}) lethal values and response % of of EPNs, *H. bacteriophora*, *S. carpocapsae* , *S. scapterisci* and *S. glaseri* at concentration (100 IJ/L) on *G. mellonella* larvae after different times.

Treatments	Time after treatments /hours	LT_{25}	LT_{50}	LT_{90}	Slope ± S.E.	Correlation	Response %
H. bacteriophora	**6**	**3.94**	**8.69**	**38.97**	**1.97±0.23**	**0.97**	**37.60**
	12						**60.87**
	24						**80.72**
	48						**92.77**
S. carpocapsae	**6**	**5.16**	**8.87**	**24.83**	**2.87±0.28**	**0.97**	**31.35**
	12						**64.68**
	24						**89.23**
	48						**98.18**
S. scapterisci	**6**	**3.26**	**9.22**	**66.61**	**1.49± 0.21**	**0.82**	**39.03**
	12						**56.78**
	24						**73.24**
	48						**85.75**
S. glaseri	**6**	**4.82**	**8.46**	**24.66**	**2.76±0.28**	**0.93**	**34.03**
	12						**66.23**
	24						**89.41**
	48						**98.09**

Data are the means ±SE of the three replicate of immature stages.

The previous studies indicated that the larvae of the wax moth were highly susceptible to EPNs. From the obtained results we can conclude that the entomopathogenic nematodes: (*S. carpocapsae* All strains) and (*H. bacteriophora*HP88) were effective on *G.mellonella* larvae. The present results are consistent with those obtained by (Farag and Osman.2007*)* who reported that, the tested *S. carpocapsae* were more influences and caused a high mortality rate in the *G. mellonella* 2nd instar larvae. The efficiency of *S. feltiae* was tested against *Bactrocera zonata.* The LC_{20}, LC_{50} and LC_{90} and slope values were also estimated for 2nd and 3rd instar larvae. Also, the LC_{50} value of *S. carpocapsae* was estimated and found the *S. carpocapsae* strongly infective *G. mellonella* larvae (Hodson *et al*., 2011). The nematodes *Steinernema kraussei* and *S. carpocapsae* provided excellent control with 100% mortality of *Aethina tumida* larvae obtained and is relevant to the management of Small hive beetle by (Andrew*et al.,* 2012). (Fetoh *etal*., 2011) evaluate the pathogenic and lethal effects of the entomopathogenic nematodes Hb (Heterorhabditis bacteriophora Poinar HP88) and Sc (Steinernema carpocapsae All strains) on the full-grown larvae, newly formed pupae and seven days old adults of the peach fruit fly, *Bactrocera zonata* and the cucurbit fly, *Dacus ciliates,* Hb nematode was more virulent than Sc nematode and the larvae and adults of *B. zonata* and *D. ciliatus* were more susceptible to the nematodes infection than the pupae. Many studies on EPNs have been conducted throughout the world, the efficacy of EPNs against tomato leaf miner. In a similar study by (Batalla *et al*. 2010), the efficacy of the three nematode species application to potted tomato plants was evaluated under greenhouse conditions. They reported high larval mortality (78.6-100%). Other researchers stated that, *S. abbasi* and *H. indicus* were evaluated on larval and adult stages of the red palm weevil, *Rhynchophorus ferrugineus* and it caused highly mortalities by *S. abbas*. While *H. indicus* caused median mortality rate in 5th instar larvae. The toxicity of *H. indica* against *R. ferrugineus*. They found that *H. indica* caused a high mortality rate in larvae and adult stages (Abbas *et al.*, 2001). Saleh and Alheji (2003) studied the toxicity of *Heterorhabditis indicus* [*H. indica*] against *R. ferrugineus*. They found that *H. indicus* caused 70 and 75% mortality in larvae and adults, respectively in laboratory. Based on LC_{50} and LT_{50}, *H. bacteriophora* was the most pathogenic effect (Simões and Rosa 1996). A new strain of *S. feltiae* was isolated from larvae of *Bibio hortulanus*. A comparative morphometric was evaluated against *G. mellonella*. Larval mortality was higher ranged from 75.3: 50.00 % at 12.0 and at 11.25 hours (Campos *et al*., 2006). The effect of the initial infection densities (15, 100 IJs) was studied on the quality of the produced IJ's of two nematode species, *H. bacteriophora* (Abd El- Rahman and Hussein 2007). Other researcher tested *Steinernema* and *Heterorhabditis* against *G. mellonella* larvae and found it caused 12 to 96% mortality. Also, *H. bacteriophora* caused moderately mortality at 5IJs for insect host. Also, the LD_{50} values were also estimated (Stefanovska *et al* 2008). The effect of *Steinernema* and *Heterorhabditis* and their associated bacteria (*Xenorhabdus* spp. and *Photorhabdus* spp.) were evaluated against *G. mellonella* larvae The LC_{50} values were estimated (Noosidum *et al*., 2010). Bioassay the influence of *S. carpocapsae* and *S. scapterisci* on the last instar larvae of *G. mellonella* was conducted. Both of S. *carpocapsae* caused >60% larval mortality. While *S. scapterisci* caused 10% mortality. At 15 and 50 nematodes per larva, in *S. scapterisci* recoded moderately mortality rate whereas in *S. scapterisci* recorded minimal mortality at both concentrations. These results demonstrate that nematode infectivity could be strongly influenced by both the production and bioassay methods (Grewal *et al*., 1999). In addition, tested *H. bacteriophora* nematodes on the armyworm, *Pseudaletia unipuncta,* caused more effect against the 6th instar *Pseudaletia.unipuncta* larvae. Based on LC_{50} and LT_{50}, *H. bacteriophora* nematode was the most pathogenic effect from 44 to 62.9 hrs. (Rosa and Simões 2004). The three Egyptian isolates of entomopathogenic nematodes in the laboratory against *R. ferrugineus* were evaluated. High mortality rate ranged from 92: 100% for 2nd and 6th instars larval and adults of *R. ferrugineus* weevil, respectively (Shamseldean and Atwa 2004). Also, (Sankar *et al.*, 2009) cleared that, the tested of *H. indica* nematode on *Galleria mellonella* larva proved to be the most efficient causing 100% mortality in *G. mellonella* after 24 h of storage. The results proved that, the interactions between EPN and other soil microorganisms may be the key to success in IPM programme. The tested *steinernematid* against *G. mellonella* were evaluated. The highest mortality rate of 98% was obtained with 500 IJs within 7 days after inoculation (Gokce *et al.*, 2013). Results stated that the infectivity of nematode strains against *G. mellonella* larvae was dependent; i.e. the mortality percentage increased as the infective juvenile increased by (Nouh and Hussein, 2014). In addition bioassays of *S. carpocapsae* and *H. bacteriophora* were conducted on larvae of *G. mellonella.* At LC_{50}, LT_{50}, penetration ability of nematodes were estimated (Saleh *et al*., 2015).

(Sevgi and Kaşkavalc 2016) evaluated the effect of *H.bacteriophora*, *S. carpocapsae* and *S. feltiae* on the tomato leaf miner, *Tuta absoluta* under laboratory conditions. The mortality rates for *H. bacteriophora*, *S. carpocapsae* and *S. feltiae* were found between 21.2 - 74.2%, 28.8 - 99.4% and 17.5 - 95.2%, respectively. Also, (Veerle Van *et al*. 2016) evaluated *S. feltiae, S. carpocapsae* and *H. bacteriophora* against *Tuta absoluta*. Caused higher mortality in the later instars (e.g. all results cleared that *S. feltiae* and *S. carpocapsae* yielded better results than *H. bacteriophora. S. carpocapsae* and *H. bacteriophora* performed better at 25 °C (causing 55.3 and 97.4% mortality, respectively) than at 18 °C (causing 12.5 and 34.2% mortality, respectively), whereas *S. feltiae* caused 100% mortality at both temperatures. Other researchers found virulence of EPN, *S. glaseri* and *H. bacteriophora* were studied against 3rd, 4th, 5th and 6th instar larvae of *A. ipsilon*. The high observed mortality caused by the both tested nematodes at different time intervals (Hassan *et al.*, 2016). The survival of EPN species, (*S. feltiae*, *H. indica*, *S. carpocapsae*, *S. glaseri* and *S. abbasi*) nematodes cause high mortality rate against *G. mellonella* and. *H. indica* and *S. carpocapsae* caused minimal mortality where *S. feltiae, S. glaseri* and *S. abbasi* did not cause any mortality against *G. mellonella* (Archana *et al*., 2017). Lethal effect of *S. feltiae*, against pre pupae of *Helicoverpa armigera* at LC_{20}, LC_{50}, and LC_{80} were also estimated (Ebrahimi *et al*., 2018). Laboratory assay showed that *G. mellonella*, are susceptible to nematoda. The nematode spp is a new potential bio-control agent on insects (Ye *et al*., 2018). Also, (Bueno *et al*. 2018) tested the EPNs against *G. mellonella* are well known biological control. The tested effect of nematodes, immersion in conidial suspension, and injection of conidial suspension was tested in single, dual and triple species combinations, evaluating *G. mellonella* larval mortality and time to kill. *A. musiformis* was injected, it produced larval mortalities >70% in the same time span as EPN. *S. carpocapsae* used in biological control of agricultural pest insects such as *Spodoptera. frugiperda*. The mortality rates increased with increasing the nematode concentration and the period after treatment. Accumulative percentage mortality of the third instar *Culex pipiens* larvae infected by non-irradiated *Steinernema scapterisci* estimated by (Sayed 2018).

Conclusions:

The efficiency of infective juveniles, IJs of all EPNs after different exposer times against last instar larvae of *G. mellonella*, were found to be highly virulent against *G. mellonella* larvae, induced high mortality at the highest concentration within 48hours. In general, a pronounced gradient in the average mortality percentages increasing from *S. carpocapsae* to *Heterorhabditis* sp. the latter species was found to be the least virulent to

G. *mellonella* larvae. Using the nematode species that, considered as one of the biological control agents of the greater wax moth larvae in honey bee combs. The results suggested that, *H. bacteriophora, S. glaseri, S. carpocapsae* and *S. glaseri* can be used as valuable tools in biological control programs of *G. mellonella*, at last, instar larvae of *G. mellonella*.

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ARABIC SUMMARY

التاثيرات الممرضة والمميتة للنيماتودا الممرضية للحشرات على يرقات فراشة الشمع الكبري جاليريا ميلونيلا (حرشفيه الأجنحة : جاليريدى)

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أجريت تجارب معملية لتقدير التأثيرت الممرضة والمميتة لأربع أنواع من النيماتودا الممرضة للحشرات (هتيرورابيديتس بكتيريوفورا، شتينيرنيما كاربوكابسي، شتينيرنيما سكابتريسي وشتينيرنيما جلاسيري)على يرقات ديدان الشمع الكبرى بتركيزات ثلاثة؛ 20 و 50 و100 فرد معدى لكل يرقة بعد فترات زمنية مختلفة 6،12، 24 و 48 ساعة من أوقات التعرض ضد يرقات فراشة الشمع وذلك من خلال تسجيل نسب الموت لليرقات التى تم اصابتها بالنيماتودا المختبرة .وجد ان نسب موت اليرقات يتزايد بزيادة الأفراد المعدية من النيماتودا. أرتفعت النسب المئوية لموت اليرقات بعد 48 ساعة من المعاملة بجميع سلالات النيماتود معنويا عند التركيز 100 فرد معدى لكل يرقة. كما لوحظ ان المعامله بتركيز 20 فرد معدى لكل يرقة سجلت LT_{25} 10.17 ساعة ليرقات فراشة الشمع المعامله بـ شتينيرنيما كاربوكابسي بعد 48 ساعة. أيضا، أشارت النتائج إلى أن المعاملة بالسلاله شتينيرنيما جلاسيري أعطت نسب موت مرتفعه لليرقات المختبره والتي بلغت 96.66 ٪ تليها شتينيرنيما كاربوكابسي والتى اعطت 96.16 ٪ موت، ولكن السلاله هتيرورابيديتس بكتيريوفورا سجلت 84.24 ٪ موت بعد أربع فترات من اوقات التعرض. أما في حالة المعاملة 50فرد معدى/يرقه أوضحت البيانات أن قيمة LT_{25} كانت 7.36 ساعة بالنسبة ليرقات فراشة الشمع المعاملة بالسلاله شتينيرنيما كاربوكابسي وسجلت أعلى نسبة موت معنويا لليرقات المختبره وصلت إلى 94.45 ٪ تليها 94.25 ٪موت للسلاله شتينيرنيما جلاسيري ، ولكن السلاله هتيرو هابيديتز بكتيريوفورا سجلت 86.01 ٪ موت. اوضحت نتائج الدراسة أن قيمة LT_{25} كانت 3.26 ساعة بالنسبة لليرقات المعاملة بـ شتينيرنيما سكابتريسي كما أظهرت النتائج أن المعالجة بـ ستينيرنيما كاربوكابسي أعطت نسبة مرتفعه معنويا لليرقات المختبرة التي سجلت 98.18 ٪ يليها السلاله شتينيرنيما جاسيري وسجلت 98.09 ٪ موت و هتيرورابيديتس بكتيريوفورا أعطت 92.77 ٪ موت في التركيز100 فرد معدى لكل يرقه بعد 48 ساعة من المعاملة. أيضا ، أظهرت البيانات وجود علاقة ارتباط قوية بين سلالات النيماتودا المختبرة على يرقات فراشة الشمع. وأيضا وجد علاقة عكسية بين التركيزات واوقات العدوى . تشير نتائج تلك الدراسة إلى أن سلالات النيماتودا الاربعه المختبرة يمكن استخدامها كاحد عناصر المكافحة في برامج المكافحة البيولوجية ليرقات فراشة الشمع فى خلايا نحل العسل.