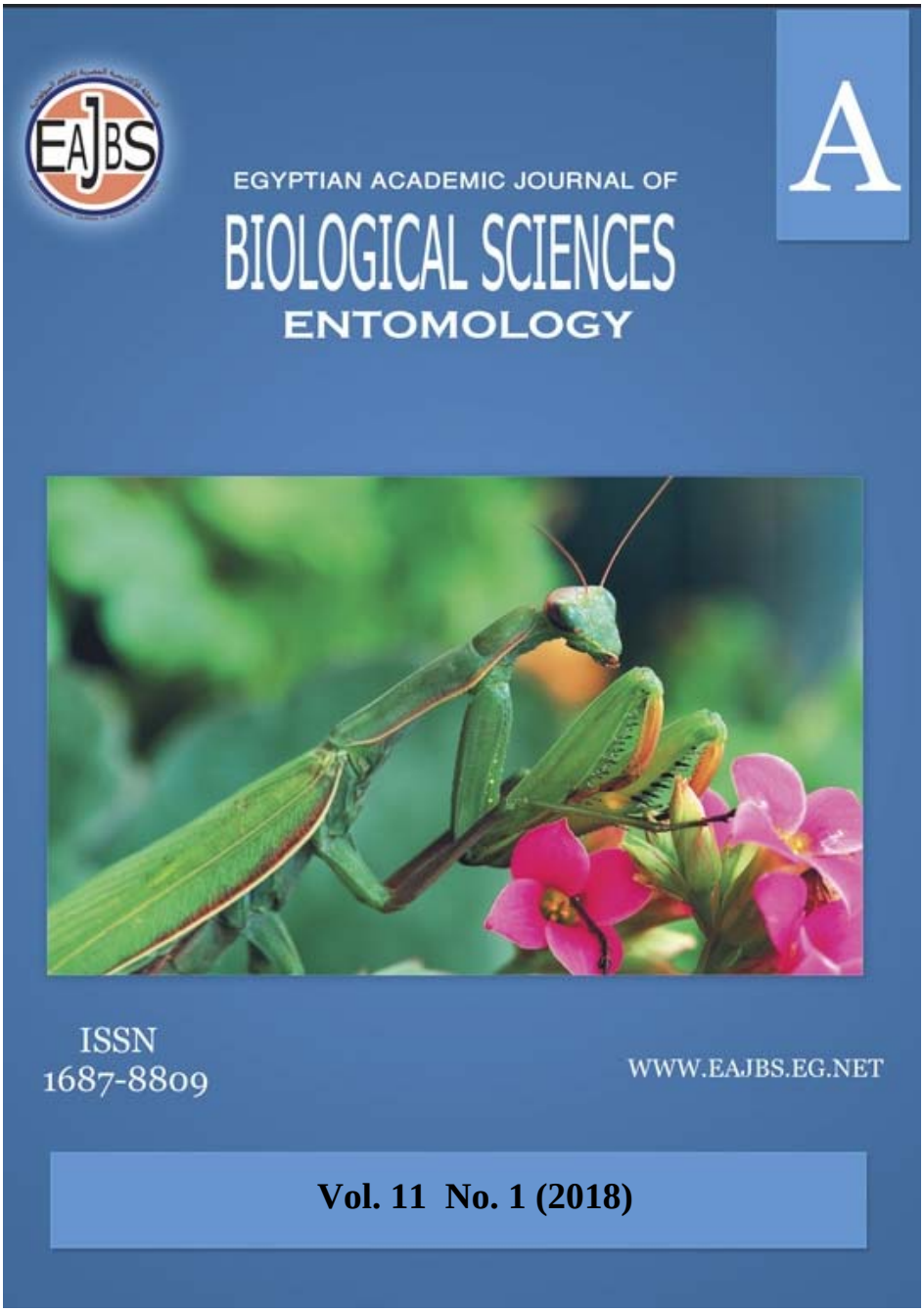

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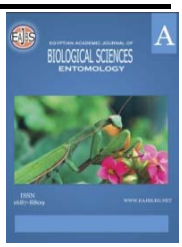

Evaluation of Four Entomopathogenic Nematodes as Biological Control Agents Against The Housefly, *Musca domestica* L. (Diptera: Muscidae)

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ABSTRACT

The house fly, *Musca domestica* L. is considered as a notorious pest by medical, veterinary, and public health professionals worldwide. This study was carried out to evaluate the effect of different entomopathogenic nematode (EPNs) strains against the 3rd larval instar and pupal stage of the house fly. Two of them were native strains (*Heterorhabditis indica* and *Steinernema carpocapsae*) and the others were imported strains *Heterorhabditis bacteriophora* and *Steinernema glaseri*. The results indicated that the mortality of all tested stages of *M. domestica* were significantly influenced by the infective juveniles (IJs) concentrations and the exposure time where the maximum mortality was recorded 72 hrs. post-treatment and the larvae were more susceptible to nematodes pathogenicity than the pupae. Also, the *Heterorhabditis* nematodes *H. baceriophora* and *H. indica* appeared to be more effective than the *Steinernema* species, and *H. bacteriophora* (Hb88) was the most promising strain in management of both larvae and pupae causing the lower LC_{50} and LC_{95} (320.4 and 1987IJs/ml) for larvae and (1414.6 and 2664.4 IJs/ml) for pupae, respectively.

INTRODUCTION

The house fly, *Musca domestica* L. (Diptera: Muscidae), is considered as serious insect pest for animal and human worldwide. More than 100 animal diseases have been experimentally associated with house flies, including protozoan, bacterial, viral, and helminthic infections, and therefore they are a threat to humans and the poultry breeding industry (Khan *et al*., 2013; Forster *et al.*, 2007). It is a causative agent for the spread of various diseases like typhoid, dysentery, diphtheria, leprosy, tuberculosis and intestinal parasites in humans while diseases related to poultry and livestock includes fowl cholera and anthrax etc. Moreover, they are also vectors and intermediate hosts of horse nematodes and some cestodes of poultry (Merchant *et al*., 1987). So, it is necessary using of very safe management programs as using microbial insecticides.

Microbial insecticides are important biotic entities that aid in regulation of insect population and keeping it before the damaging level and certainly they have much to offer for insect control and it can be a safe alternative to chemicals.

The entomopathogenic nematodes are one of the most effective and safe bio-control agents and they are currently marketed worldwide for use in biological control. They are easy and relatively inexpensive to culture, live from several weeks up to months in different formulations or water and able to infect numerous insect species, occur in soil and have been recovered from all continents (Kaya and Gaugler, 1993). It belongs to two families Steinernematidae and Heterorhabditidae. The IJs of *Steinernema* and *Heterorhabditis* species used as active ingredient because they are symbiotically associated with toxic bacteria of the genera *Xenorhabdus* and *Photorhabdus*. An IJ carries between 1 and 2000 cells of its symbiotic bacterium in the anterior part of the intestine, once IJ penetrated the insect haemocoel, the bacterial symbionts are released from the nematode gut, septicemia becomes established and insect death occurs within 24-48 hrs.

Due to the harmful effects of chemical pesticides and presence of domestic house flies in the homes, animal houses and places of people's presence, it is necessary using of very safe management programs. So, this study aims to evaluate the ecofriendly management program for the first time in Egypt against House fly *M. demestica* by evaluating the susceptibility of different stages (3rd larval instar and pupal stage) to different species of entomopathogenic nematodes under laboratory conditions to keep this insect pest under threshold levels to avoid health hazards on both human and animals.

MATERIALS AND METHODS

Colony Establishment:

Adults of *M. domestica* were obtained from Medical Entomology Research Center, Egypt and then transferred to laboratory of medical entomology in the animal house, faculty of science, Al-Azhar university. Adults were reared under laboratory conditions (27±2^{o}C, 70±5 RH%, 12: 12 lights: dark). Adult house flies were maintained in (30×30×30) wooden cages covered by gauze. The emerged flies were fed on dry diet milk powder and sucrose solution (cotton pads soaked in 10% sucrose solution). Eggs could be collected from paper strips or from cotton pads of feeding, where they were deposited by females. Larvae were reared on an artificial diet (wheat bran, milkpowder and yeast; 200:100:5 gm.) per 200 ml distilled water according the method described by Busvin (1962).

Nematode Bioassay:

Two different strains of *Heterorhabditis indica* and *Steinernema carpocapsae* were isolated from Egyptian fauna by Shehata (2010) and two imported strain *Heterorhabditis bacteriophora* (Hb-88) and *Steinernema glaseri* (Sg) were used. All tested nematodes were maintained on laboratory pests at Plant Protection Department, National Research Center, Dokki, Egypt using in vivo technique by *Galleria mellonella* larvae as described by Woodring and Kaya (1988).

The experiment was carried out according to Shehata (2010). Water suspensions of the tested nematodes were prepared at serial concentrations of 2500, 2000, 1500, 1000, 500 and 250 IJs/ml. The tested concentrations were prepared by dilution method according to Woodring and Kaya (1988).

The bioassay was conducted to evaluate the nematode's capability to reach the larvae and pupae so, for larvae, thirty individuals of 3rd larval instar were introduced into a Petri dish, 12 cm in diameter, furnished with filter paper. Two ml of each concentration was sprayed directly on the filter paper within the Petri dish. Four replicates were tested for each concentration, the dishes were incubated at 27±1˚C

with daily examination for larval mortality over 3 days. Samples of dead larvae were dissected to determine whether nematodes were present inside the cadaver. The efficacy of the nematodes was compared to the untreated control.

For pupae, sterilized plastic cups 120 ml were used, each containing 50 gm fine sterilized sand moistened with 3ml of pure water. Ten pupae (48 hours old)/cup were treated with 2 ml of each tested concentration using spray technique as mentioned above. The experiments were repeated four times for each concentration as replicates. All cups were incubated at 27±1˚C with daily examination for 3 days to calculate the percentage of pupal mortality.

Data Analysis:

Statistical analysis of the data was carried out according to the method of Lentner *et al*. (1982). LC_{50} was calculated using multiple linear regressions, Microsoft Excel 2010.

RESULTS

Pests management is facing economic and ecological challenge worldwide due to human and environmental hazards caused by majority of the synthetic pesticide chemicals. So, the obtained results were light spot on the ability of four EPNs in controlling of *M. deomestica* larvae and pupae to relatively protect human and animals against harmful effects of this pest and serving as an alternative to chemical insecticide.

Virulence of EPNs Against 3rd larval instar:

Data presented in (Table 1) showed the susceptibility of 3rd larval instar of *M. domestica* to different concentrations of the tested EPNs (two heterorhabditis strains and two steinernematiditis strains) using spray method.

The results referred to the absence of larval mortalities at treatments of all tested nematode concentrations at 24 hrs. pos-treatment. Meanwhile, increasing of the exposure time led to increasing the mortalities.

At 48 hrs.post-treatment; *H. bacteriophora* (Hb) caused mortality percentages 76.7, 70, 56.7, 40, 33.3 and 20 % at application of 2500, 2000, 1500, 1000, 500 and 250 IJs/ml, respectively. The local isolate. *H. indica* (Hi) caused 63.3, 60, 50, 46.7, 40 and 26.7% mortality after treatment of 2500, 2000, 1500, 1000, 500 and 250 IJs/ml, respectively 48 hrs. post-treatment. While, the larval mortalities after treatments with *S. glaseri* (Sg) reached 53.3, 46.7, 26.7, 20 and 10% at the mentioned concentrations, respectively.

While, *S. carpocapsae* appeared to be the lowest effective against larval instar after 48 hrs of treatments where the mortality recorded 43.3, 33.3, 23.3 and 16.7 at 48 hrs. post-treatment with 2500, 2000, 1500 and 1000 IJs/ml, respectively, while there is no significant mortalities in 500 and 250 IJs/ml. The maximum larval mortality percentages recorded at 72hrs. post-treatment where it reached to 100 % for both 2500 and 2000 IJs/ml and to 93.3, 76.7, 56.7 and 36.7% for 1500, 1000, 500 and 250 IJs/ml of *H. bacteriophora* (Hb), respectively. Data analysis showed that at 72 hrs the mortality significantly ($P<0.05$) increased with increasing the tested concentrations and with increasing exposure time. The lethal concentrations LC_{50} & LC_{95} values were 320.4 and 1987.0 IJs/ml, respectively at 72 hrs. post-treatment with slope value of 0.027±0.0873 (Table 3 and fig.1). While, treatment with *H. indica* (Hi) caused 100, 100, 83.3, 76.7, 50 and 40% mortality at concentrations of 2500, 2000, 1500, 1000, 500 and 250 IJs/ml, respectively. Highly significant differences were recorded ($P<0.05$) between the tested concentrations compared with the control after 72 hrs. The LC_{50} and LC_{95} values were 390.1 and 2014.7 Js/ml, respectively at 72 hrs. post-treatment and a slope value of 0.0277± 0.9212 (Table 3 and fig.1).

In *S. glaseri* (Sg) the mortality percentage after 72hrs. was 96.7, 90, 83.3, 70, 53.3 and 46.7% at the same before mentioned concentrations, respectively. The values of LC_{50} and LC_{95} were 494.1 and 2192.2 IJs/ml, respectively and the slope value was 0.0265±0.9162 (Table 3). Significant differences in mortality were recorded ($P<0.05$) between the tested concentrations compared with the control. Also, *S. carpocapsae* (Sc) appeared to be the lowest effective against the 3rd larval instar of *M. demestica.* The larval mortalities ranged between 90 to 40% when the tested concentration ranged 2500 to 250 IJs/ml. The LC_{50} & LC_{95} values were 407.5 and 2715.2 IJs/ml; respectively at 72hrs. post-treatment, and the slope value was 0.0195±0.9086 (Table 3 and fig.1). The significant differences in mortality were recorded ($P<0.05$) between the tested concentrations compared with the control after 72hrs.

Table 1. Virulence of different strains of EPNs against the 3rd larval instar of *Musca domestica* under laboratory conditions**.**

Conc. (IJs / ml)	Time post-treatment (hours)	% of accumulative larval mort.± SD after treatment with			
		Heterorhabditidae		Steinernematidae	
		H. bacteriophora (Hb)**	*H. indica* (Hi)*	*S. glaseri* (Sg)**	*S. carpocapsea* (Sc) *
Control	24	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}
	48	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}
	72	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}
2500	24	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}
	48	76.7 ± 15.3^{d}	63.3 ± 5.8^{d}	53.3 ± 11.5^{d}	43.3 ± 15.3^{d}
	72	100.0 ± 0.0^{d}	100.0 ± 0.0^{d}	96.7 ± 5.8^{d}	90.0 ± 10^{d}
2000	24	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}
	48	70.0 ± 10.0^{d}	60.0 ± 0.0^{d}	46.7 ± 5.8^{d}	33.3 ± 5.8^{d}
	72	100.0 ± 0.0^{d}	100.0 ± 0.0^{d}	90.0 ± 0.0^{d}	76.7 ± 11.5^{d}
1500	24	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}
	48	56.7 ± 15.3^{d}	50.0 ± 10.0^{d}	26.7 ± 5.8^{c}	23.3 ± 5.8^{b}
	72	93.3 ± 5.8^{d}	83.3 ± 11.5^{d}	83.3 ± 5.8^{d}	73.7 ± 15.3^{d}
1000	24	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}
	48	40.0 ± 10.0^{b}	46.7 ± 5.8^{d}	20.0 ± 0.0^{b}	16.7 ± 5.8^{a}
	72	76.7 ± 5.8^{d}	76.7 ± 11.5^{d}	70.0 ± 10.0^{d}	70.0 ± 10^{d}
500	24	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}
	48	33.3 ± 15.3^{a}	40.0 ± 10.0^{d}	16.7 ± 5.8^{a}	6.7 ± 5.8^{a}
	72	56.7 ± 5.8^{d}	50.0 ± 10.0^{d}	53.3 ± 5.8^{d}	53.3 ± 11.5^{d}
250	24	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}
	48	20.0 ± 10.0^{a}	26.7 ±11.5^{c}	10.0 ± 10.0^{a}	0.0 ± 0.0^{a}
	72	36.7 ± 5.8^{d}	40.0 ± 0.0^{d}	46.7 ± 5.8^{d}	40.0 ± 10.0^{c}

* Egyptian isolates. ** Imported isolates; Conc. = Concentration; IJs = infected juvenile per millilitre; mort. = mortality; h= hours; a = non-significant ($P>0.05$); b = significant ($P<0.05$); c = highly significant ($P<0.01$); d = very highly significant ($P<0.001$). Means in the same column followed by the same letter are statistically non-significant.

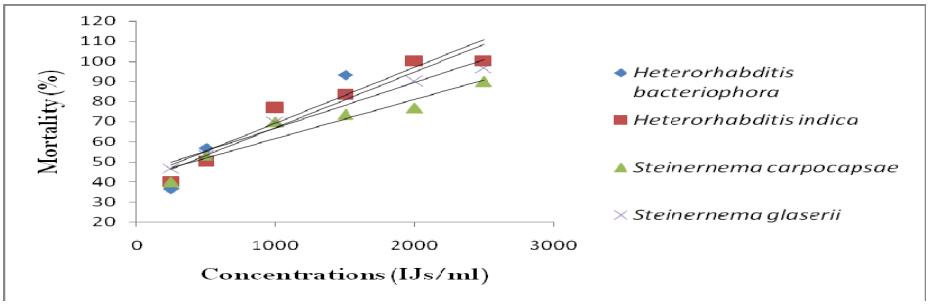

Fig. (1): Regression line of the house fly, *Musca domestica* larval Mortality as induced by different concentrations of entomopathogenic nematodes, *Heterorhabditis bacteriophora*, *Heterorhabditis indica*, *Steinernema carpocapsae* and *Steinernema glaseri*

Virulence of EPNs Against Pupal Stage:

The susceptibility of *M. domestica* pupae to different concentrations of EPNs were recorded in table (2) and the reported data showed that the pupae were less susceptible to EPNs than the larvae due to the life habits of the pupae.

All tested EPNs strains have no effects on pupal mortalities of 24 hrs post treatment at all concentrations. While, the susceptibility of the pupae to EPNs increased with increasing the exposure time to 48 hrs. The tested concentrations 2500 and 2000 IJs/ml caused pupal mortalities for all tested nematode strains, while the mortalities declined with decreasing the nematodes concentrations.

H. bacteriophora (Hb) caused mortalities of 43.3, 30 and 23.3% at application of 2500, 2000 and 1500 IJs/ml, respectively. The mortalities reduced to 33.3, 26.7 and 23.3 % at the same concentrations for *S. gleserri* nematodes, receptively.

Treatment with *H. indica* (Hi) caused 36.7 and 20% mortalities after application of 2500 and 2000 IJs/ml, respectively. The mortalities declined to 26.7 and 13.3% at the same concentrations of *S. carpocapsea* (Sc) with absence of mortalities after treatment with 1500 IJs/ml of both EPNs strains. All tested EPNs strains have no effects on pupal mortalities at application of 1000, 500 and 250 IJs/ml with negative significant effects compared with the control pupae.

The maximum mortalities were recorded at 96 hrs. after treatment. The heterorhabditid nematodes *H. bacteriophora* (Hb) and *H. indica* (Hi) were the best where the mortalities were 83.3, 70, 66.7, 23.3, 16.7 and 6.7%. These decreased to be 70, 66.7, 63.7, 50, 43.3 and 16.7% after treatment with 2500, 2000, 1500, 1000, 500 and 250 IJs/ml of both *H. bacteriophora* (Hb) and *H. indica* (Hi), respectively (Table 2).

The statistical analysis showed significant mortality ($P<0.05$) correlated to increasing of both the tested concentration and the exposure time. The lethal concentrations LC_{50} & LC_{95} values were 1414 and 2664.4 IJs/ml, respectively at 96 hrs. post-treatment with slope value 0.036±0.9261 (Table 3) for *H. bacteriophora* (Hb). For *H. indica* (Hi) caused significant differences at ($P<0.05$) where the LC_{50} and LC_{95} values were 1074.6 and 3547.1IJs/ml, respectively at 72 hrs. post-treatment and a slope value of 0.182±0.8623 (Table 3 and fig.2).

The steinernematid nematodes *S. glaseri* (Sg) has median effects where the concentrations 250 and 500 IJs/ml caused low mortalities (10 and 26.7%, respectively). The mortality increased to be 33.3, 43.3, 53.3 and 66.7% when the concentrations increased to 1000, 1500, 2000 and 2500 IJs/ml, respectively with recording of significant differences compared with the control. The LC_{50} and LC_{95} values were 1737.3 and 3636 IJs/ml, respectively at 72 hrs. post-treatment and a slope value of 0.237±0.967 (Table 3 and Fig.2).

Steinernema carpocapsea (Sc) has the same line, where the mortality percentage increased from 6.7 to 70% when the concentrations increased from 500 to 2500 IJs/ml, respectively while application of 250 IJs/ml has no effects on tested pupae. The LC_{50} and LC_{95} values were 1718.1 and 3041.6 IJs/ml with the slope value of 0.034±0.9736 as in (Table 3 and fig.2). The mortality of pupae significantly ($P<0.05$) increased with increasing the concentrations. The statistical analysis indicated that the difference is significant (<0.05) for 2500, 2000, 1500 and 1000 IJs /ml as compared with the control, but the significance was negative at 500 and 250 IJs/ml compared with the control.

Table 2. Virulence of different strains of EPNs against pupae of *Musca domestica* under laboratory conditions.

Conc. (IJs / ml)	Time post-treatment (hours)	% of accumulative larval mortalities ± SD after treatment with			
		Heterorhabditidae		Steinernematidae	
		H. bacteriophora (Hb)**	*H. indica* (Hi)*	*S. glaseri* (Sg)**	*S. carpocapsea* (Sc) *
Control	48	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}
	72	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}
	96	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}
2500	48	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}
	72	43.3 ± 5.8^{d}	36.7 ± 5.8^{d}	33.3 ± 5.8^{d}	26.7 ± 5.8^{c}
	96	83.3 ± 11.5^{d}	70.0 ± 10.0^{d}	66.7 ± 5.8^{d}	70.0 ± 10.0^{d}
2000	48	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}
	72	30.0 ± 10.0^{d}	20.0 ± 10.0^{b}	26.7 ± 5.8^{c}	13.3 ± 5.8 ^{a}
	96	70.0 ± 10.0^{d}	66.7± 5.8^{d}	53.3 ± 15.3^{d}	66.7 ± 5.8^{d}
1500	48	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}
	72	23.3 ± 5.8^{d}	0.0 ± 0.0^{a}	23.3 ± 5.8^{b}	0.0 ± 0.0 ^{a}
	96	66.7 ± 5.8^{d}	63.7 ± 5.8^{d}	43.3 ± 11.5^{c}	46.7 ± 15.3^{d}
1000	48	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}
	72	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	6.7 ± 5.8^{a}	0.0 ± 0.0^{a}
	96	23.3 ± 5.8^{b}	50.0 ± 0.0^{d}	33.3 ± 5.8^{b}	23.3 ± 5.8^{b}
500	48	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}
	72	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}
	96	16.7 ± 5.8^{a}	43.3 ±5.8^{d}	26.7 ± 11.5^{a}	6.7 ± 5.8^{a}
250	48	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}
	72	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}
	96	6.7 ± 5.8^{a}	26.7 ± 5.8^{d}	10.0 ± 10.0^{a}	0.0 ± 0.0^{a}

*, **, Conc., IJs, mort., h, a, b, c, d,: See footnote of table (1).

Table 3. Lethal concentrations of different entomopathogenic nematodes against different stages of *M. domestica*.

EPNs strains		Third instar larvae			Pupae		
		LC_{50}	LC_{95}	Slope	LC_{50}	LC_{95}	Slope
		IJs/ml			IJs/ml		
H. bacteriophora	Imported strains	320.4	1987.0	0.027	1414.6	2664.4	0.036
S. glaseri		494.1	2192.2	0.0265	1737.3	3636	0.237
H. indica	Egyptian strains	390.1	2014.7	0.0277	1074.6	3547.1	0.182
S. carpocapsae		407.5	2715.2	0.0195	1718.1	3041.6	0.034

IJs = infected juvenile per mil

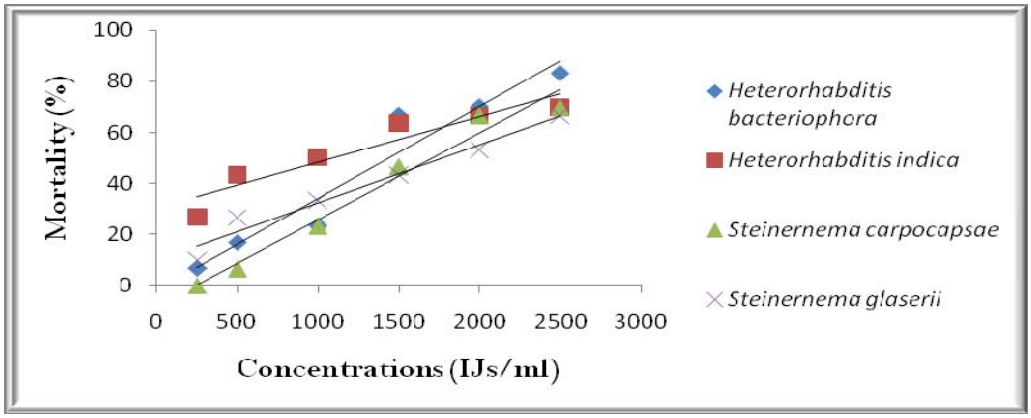

Fig. (2): Regression line of the house fly, *Musca domestica* pupal Mortality as induced by different concentrations of entomopathogenic nematodes, *Heterorhabditis bacteriophora*, *Heterorhabditis indica*, *Steinernema carpocapsae* and *Steinernema glaseri.*

The comparative study of the tested nematode strains

The lethal values of LC_{50} were computed through probit analysis program at 95% confidence limits at the end of the experiments 72hrs post-treatments and the tested nematode strains arranged as follow:

For larvae: *H. bacteriophora* (Hb) > *H. indica* (Hi) > *S. carpocapsea* (Sc) > *S. glaseri* (Sg). For pupae: *H. indica* (Hi) > *H. bacteriophora* (Hb) > *S. carpocapsea* (Sc) > *S. glaseri* (Sg).

DISCUSSION

Entomopathogenic nematodes of the genera *Steinernema* and *Heterorhabditis* serve as alternatives for conventional chemical pesticides (Saleh *et al.,* 2000; Saleh *et al.,* 2009). EPNs as bio-insecticides have been used worldwide in horticulture industry since 1964 (Dutky *et al.,* 1964). The active ingredient of these bio-insecticides is the infective juvenile (IJ). IJs occur naturally in soil and live in symbiosis with bacteria carried in their gut (Kaya and Gaugler, 1993; Kaya and Stock, 1997). The main reason in EPNs pathogenicity is the presence of special bacterial species. These bacteria are carried in an intestinal vesicle of the non-feeding infective stage of steinernematid

(Bird and Akhurst, 1983) and throughout the whole intestine of the infective juveniles of heterorhabditid nematodes (Endo and Nickle, 1994).

The nematodes release their bacterial symbionts into the haemocoel of the insects, where growth induces a lethal septicemia and contributes to the symbiotic relationship by providing nutrients required by nematode partners during reproduction in insect cadavers (Poinar and Thomas, 1966; Koppenhofer *et al*., 2007). All given nematode species is specifically associated with one bacterial species where all species in *Steinernema* harbor bacterial species in the genus, *Xenorhabdus*. While, those in *Heterorhabditis* harbor bacterial species in the genus, *Photorhabdus* (Kim *et al*., 2005).

Since the nematodes isolated from special soil are efficient for controlling pests of this soil (Hazier *et al.*, 2003). So, one target of this study was the comparison of 4 strains, two of them are imported strains (*H. baceriophora* and *S. glaseri*) while the others were local isolates (*H. indica* and *S. carpocapsae*), against different stages of *M. domestica* to evaluate the susceptibility of this pest.

Analysis of variance showed that, all tested stages of *M. domestica* mortality were significantly influenced by IJ concentrations and exposure time where the maximum mortalities were recorded 72 hrs post-treatment and the larvae were more susceptible to nematodes pathogenicity than the pupae.

All tested EPNs strains have no effects on larval and pupal mortalities 24 hrs post treatment at all concentrations. While, the susceptibility of the tested stages to EPNs increased with increasing of the exposure time to 48 and 72 hrs., the maximum larval mortalities ranged from 100% to about 40% for *Heterorhabditis* spp. (*H. bacteriophora* and *H. indica*). On the other hand, *Steinernema* spp. (*S. glaseri* and *S. carpocapsea*) were less effective where the mortality ranged from 96.7% to ca 40%. Susceptibility of larvae may be correlated to the movement behavior which increase the exposing of larvae body surface to infective juveniles where the nematodes penetrate the insect body through the natural opening as anus, mouth and/or respiratory pores located on the body surface. Our results coincide with Archana *et al*. (2017) who studied the effects of EPNs against eggs, larvae and pupae of house fly and Shehata (2010) who studied the local EPNs against *Galleria mellonella*, *Agrotis ipsilon* and *Tropionata sequalida*. Similarly, the larvae of *Tuta absoluta* appeared to be more susceptible to local and imported EPNs than the pupal stage (Shehata, 2015). In the same line, Taylor *et al.* (1998) reported that the EPNs *S. feltia* was the most promising species in management of house fly compared to other species of *Steinernema* and *Heterorhabditis*.

On the other hand, susceptibility of pupae to EPNs was varied and the highest mortality values were recorded after 72 hrs of treatment. The tested nematodes (*H. bacteriophora*, *S. glaseri* and *S. carpocapsea*) caused significant mortalities after treatment with 2500, 2000, 1500 and 1000 IJs/ml. While, *H. indica* caused significant mortalities at all concentrations where the mortality ranged from 70% to 26.7%. The pupae of some insects appear to be less susceptible to nematode infection than the larval stages (Kaya and Hara, 1980; Shehata, 2015). The only portal of entry to the insect puparia of *M. domestica* is via the spiracles, but the presence of spiracular slits within these openings may have prevented penetration (Bedding and Molyneux, 1982).

Generally, our results indicated that the *Heterorhabditis* nematodes, *H. baceriophora* and *H. indica*. appeared to be more effective against larvae and pupae than *Steinernema* spp.

Susceptibility of *M. domestica* to EPNs depends on the developmental stages of the insect and their effect varies with the nematode species. In the present study, among the EPN strains *H. bacteriophora* (Hb88) was the most promising strain showing a high virulence capacity compared with other species against larvae and pupae. Generally, EPN strains can be arranged as follow; *H. bacteriophora* (Hb88) > *H. indica* (Hi) > *S. carpocapsea* (Sc) > *S. glaseri* (Sg).

This activity of nematodes is varied due to their behavior where it reaches to the host by one of two ways. Firstly, it may move to the host place by the response to CO_2 which is released from the insect host. This species is called crusher (Lewis *et al*., 1992; Kaya and Gaugler, 1993), while the second may still stopped in the J form on the soil molecules and attach to the host when they pass through the soil. This species is called ambusher (Grewal *et al*., 1999). The first type lead to highly effect in all insect stages. Meanwhile, the latter form lead to highly effect in mobile insect stages (Kaya and Gaugler, 1993). Also, there are many factors affecting on the EPNs activity like environmental factors (Gaugler, 1999), the exposure time (Mason & Wright, 1997; Shameseldean *et al.*, 2008) temperature (Woodring & Kaya, 1988) and the behavioral strategies of the tested strains which is the main factor in nematodes activity (Kaya and Gaugler, 1993; Grewal *et al*., 1994; Lewis *et al*., 1995; Campbell & Gaugler, 1997). Also, substrate assay has a role in variation of nematodes efficacy as reported by (Abdel-Razek & Abdel-Gawad, 2007) and the difference in the bacterial symbionts they released as concluded by Forst *et al.* (1997) where the bacterial symbionts is the main reason in insect death as investigated by Eleftherianos *et al.* (2010) when studied the effect of *Heterorhabditis* carrying *Photorhabdus* nematodes free of bacteria (axenic nematodes) and bacteria alone against *M. sexta.*

Conclusion

This study concluded that the Entomopathogenic nematodes appeared to be promising for the control of *M. demestica* causing highly reduction in larval and pupal population, since it exhibits acute toxicity towards this insect, singly without any additives. So, it was recorded to be very effective, very safe and very economic in biological pests' management programs.

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