The Effect of some Insecticides on Two Entomopathogenic Nematodes and Evaluation of Its Virulence on the Greater Wax Moth *Galleria mellonella*

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Abstract: The toxicity effect of five insecticides (Dipel 2x, Radiant, Proclaim, Aphox and Coragen) on two species of entomopathogenic nematodes (EPNs) (*Heterorhabditis bacteriophora* BA1 and *Steinernema carpocapsae* BA2), were evaluated under laboratory conditions at different concentrations 100%, 50% and 25% of field rates (FR). Moreover, the virulence of insecticide-treated EPNs against the lst nymphal instar of the greater wax moth *Galleria mellonella* L. (Lepidoptera: Pyralidae) was also evaluated. Data indicated that all insecticides showed negative effect on the tested infective juveniles (IJs) of EPNs and the percentages of mortality were in order of *H. bacteriophora* BA1>*S. carpocapsae* BA2 and were concentration-dependent; significant differences existed between untreated (control) and insecticide treatments EPNs. Proclaim insecticide recorded the highest mortality rate in EPNs at all tested concentrations 24, 48 and 72 hours post treatment. Regarding the effect of tested insecticides on the virulence and the reproductive capacity of treated EPNs, data indicated that the ability of insecticide-treated EPNs to locate and infect larvae of *G. mellonella* decreased after exposure to insecticide and consequently reproductive capacity of EPNs was significantly reduced.

Keywords: Insecticides, *Steinernema carpocapsae*, *Heterorhabditis bacteriophora*, toxicity, *Galleria mellonella*, Virulence

INTRODUCTION

Generally, any integrated pest management (IPM) strategy is mainly accomplished by combining all available control methods to increase the degree of effectiveness and to obtain a long-term reliability. Entomopathogenic nematodes (EPNs) (Steinernematidae and Heterorhabditidae) and their symbiotically associated bacteria (*Xenorhabdus* sp. for steinernematids and *Photorhabdus* sp. for Heterorhabditids) are great values in the field of biological control (Kaya *et al.*, 1998).

The infective juveniles (IJs) of EPNs usually enter their hosts via body orifices (*e.g.* spiracles – Steinernematidae) and less often penetrate host cuticle (Heterorhabditidae). They do not kill their hosts directly; however certain mutualistic bacteria associated with EPNs release their toxins, metabolites or proteases that kill the host within 2-3 days (Poinar, 1990). EPNs are increasingly used to control a wide spectrum of insect species in different agricultural ecosystems (Kaya *et al.*, 2006; Mahmoud, 2016).

The interaction between EPNs and other bioagents is inevitable and may determine the degree of success for these biocontrol agents. This interaction may be an antagonistic or synergistic effect (Kaya and Burlando, 1989; Mahmoud, 2007).

The objective of the current work is to evaluate the toxicity effects of five bio- and conventional insecticides at different concentrations (100%, 50% and 25% FR) on two species of EPNs, together with evaluating its residual effects on the virulence and the reproductive capacity of the treated EPNs under laboratory conditions.

MATERIALS AND METHODS

Insecticides

The commercial products of the selected insecticides with their field rates, active ingredients, PHI (pre-harvest interval) and the target pests are listed in Table (1),. Theses insecticides include two bioinsecticides (Dipel 2x and Radiant) and three conventional insecticides (proclaim, Aphox and Coragen). The tested concentrations of insecticides in the present study were 100%, 50% and 25% FR, which prepared one hour prior to experiments.

Entomopathogenic nematodes

The preparations of the entomopathogenic nematodes (EPNs) of the families Heterorhabditidae and Steinernematidae (Order: Rhabditida) (*Heterorhabditis bacteriophora* BA1 and *Steinernema carpocapsae* BA2)), which were used in this study, were obtained during the experiments as a suspension product from Pests and Protection Department, Mass Production Unit, National Research Center (NRC), Giza, Egypt. All tested EPNs were reared *in vivo* according to the technique described by Glazer and Lewis (2000). Newly emerged infective juveniles (IJs) were collected and stored firstly at 5°C in order to evaluate their survival. Healthy laboratory emerging infective juveniles (IJs) were collected from White traps (White, 1927) and stored in distilled water until used in the experiments.

Experiments

Effect of tested insecticides on EPN (IJs)

This experiment was conducted to evaluate the efficacy of five bio- and conventional insecticides at different rates (25, 50 and 100%FR) on two species of

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EPNs. The experiment was performed at 25°C and data were recorded after three different intervals (24, 48 and 72 hours). Untreated (control) experiment for the two studied EPNs species was also performed. Each treatment was replicated 4 times in a sterile tissue culture plate (24 well, 4ml capacity/well). Each replicate contained 1ml insecticide and 1ml EPNs IJs suspension (2000 IJs/ml), and then covered with hulls stretched plastic. Data were recorded after 24, 48 and 72 hours post treatment. Trypan blue coloring ($C_{34}H_{24}N_6Na_4O_{14}S_4$) was used to discriminate between the survived and the dead nematodes. The treated infective juveniles (IJs) survived after respective tested period were kept in Wassermann test tube (10 ml) with distilled water to evaluate their virulence.

Table (1): List of tested bio-and conventional insecticides including their active ingredients, recommended field rate (FR) and the target pests.

Tested Insecticides	Active ingredients	FR	Target pest	PHI* (day)
Dipel 2x 6.4% DF	*Bacillus thuringiensis* var. *kurstaki*	200 gm/fed 150 gm/ton	PTM in field PTM in storage	N/A**
Radiant 12% SC	Spinetoram	120 cm^3/fed 100 cm^3/fed	Aphids PTM	1
Proclaim oupty 5% WG	Emamectin benzoate	60 gm/fed	PTM in field	3
Aphox 50% SC	Primicarb	50 gm/100L	Aphids	15
Coragen 20% SC	Chlorantraniliprole	60 cm^3/fed	PTM in field	7

*** PHI =** (pre-harvest interval) the period in days after pesticide treatment until harvest, while the residue levels of the pesticide allowed in the harvested crop that degrade to a level that is acceptable by crop's growth, environmental conditions (such as rain or UV radiation) and by the microorganisms on the plants or in the soil **(**https://WWW.APC.gov.eg).
****N/A** = Not available.

Evaluation of the virulence of EPNs on full grown larvae of *Galleria mellonella*

This experiment was conducted to study the impact of different concentrations of each tested insecticide on the two tested EPNs and its first generation (F1) by using the full grown larvae (FGL) of *G. mellonella* as laboratory host. Untreated and insecticides-treated EPNs (IJs) were taken from the previous experiment, in which the effect of the tested insecticides at 100, 50 and 25% FR were studied. In insecticide-treated EPNs, IJs were taken after 72 hours of exposure to the insecticides. Each treatment was replicated ten times. The untreated and insecticide-treated EPNs IJs were placed with the host larvae in sterile tissue culture plate (24 wells, 4 ml capacity/well). 200 IJs (untreated or insecticide-treated IJs) as a suspension form was added to each larva of *G. mellonella* in each well and then covered with hulls stretched plastic. This experiment was performed under laboratory conditions of 25±2°C and 2:22 L/D photoperiod. Data were recorded for three days in terms of infected larvae with EPNs throughout the experimental period (3 days).

Evaluation of the offspring of EPNs

This experiment aimed to evaluate the offspring of insecticide-treated EPNs. The infected *G. mellonella* larvae (cadavers) were collected after 3 days and each cadaver was placed separately in a Petri dish in the form of White traps unit for 15 days. Then after, offspring of EPNs from the White traps unit was collected and conserved in clean Falcon tube (50 ml) (Mahmoud and Osman, 2007). The offspring obtained from this experiment was re-examined as second generation (F_2) against FGL of *G. mellonella*, the data were recorded as mentioned above in F_1 experiment.

Statistical analysis

All obtained data were analyzed using SAS package 11.0 v (SAS Institute Inc, 2005).When F values were significant, means were separated using Fisher's least significant differences (FLSD) at a 0.05 level of significance (SAS Institute Inc, 2005). Proportional data were transformed by arcsine square root (ARC Sine) before analysis.

RESULTS

1. Effect of tested insecticides on EPN (IJs)

1.1. Effect of tested bio- and conventional insecticides on *H. bacteriophora* BA1

Data showed that mortality percentage was higher in *H. bacteriophora* treated with conventional insecticides than bio-insecticides. Moreover, the

Proclaim insecticide caused the highest mortality percentage in nematode treated with 100% FR, 50% FR and 25% FR. Data showed that the Dipel 2x bioinsecticide at three different doses induced the lowest mortality percentage in treated nematodes. It was clear that the mortality percentage of *H. bacteriophora* increased significantly with increasing insecticide doses and time of exposure.

Statistical analysis showed that significant differences among treatments in percentages of mortality at 100% FR after 24 hours ($F = 81.84$; $P < 0.0001$), 48 hours ($F = 163.42$; $P < 0.0001$) and 72 hours of treatment ($F = 298.81$; $P < 0.0001$, Table 2). Pertaining to 50% FR, statistical analysis showed that there were significant differences among treatments in mortality percentage after 24 hours ($F = 46.52$; $P < 0.0001$), after 48 hours ($F = 128.91$; $P < 0.0001$) and after 72 hours post treatment ($F = 170.39$; $P < 0.0001$, Table 2). As for 0.25% FR, it differed significantly among the tested insecticides after 24 hours ($F = 63.59$; $P < 0.0001$), 48 hours ($F = 182.38$; $P < 0.0001$) and 72 hours post treatment ($F = 245.82$; $P < 0.0001$).

1.2. Effect of tested bio- and conventional insecticides on *S. carpocapsae* BA2

The obtained data indicated that mortality percentages after 24, 48 and 72 hours in all treatments were higher than in control treatment, which gave mortality rates of 0.01, 0.03 and 0.04%, respectively (Table 3).

Statistical analysis revealed that significant differences were existed among tested treatments after 24 hours ($F= 63.64$; $P < 0.0001$), 48 hours ($F= 64.01$; $P < 0.0001$) and 72 hours of treatment ($F= 141.58$; $P < 0.0001$, Table 3). Apparently at 50% FR, statistical analysis indicated that significant differences were existed among treatments after 24 hours ($F = 65.46$; $P < 0.0001$), 48 hours ($F = 100.81$; $P < 0.0001$) and 48 hours of treatment ($F = 187.20$; $P < 0.0001$ Table 3). As for at 25% FR, statistical analysis indicated that the significant differences after 24 hours ($F = 98.06$; $P = 98.06$), 48 hours ($F = 87.02$; $P < 0.0001$) and 72 hours post treatment ($F = 60.00$; $P < 0.0001$).

2. Evaluation of the virulence of EPNs on full grown larvae of *G. mellonella*

2.1. Insecticide-treated *H. bacteriophora* BA1

2.1.1. Virulence of insecticide-treated *H. bacteriophora*

As shown in Table (4), the rate of mortality in FGL of *G. mellonella*, exposed to insecticide-treated IJs with 25% FR was greater than those recorded in the higher rates of insecticides (100 and 50% FR).

As the insecticide concentration increased to 100% FR, there was a significant effect in the percentage of mortality ($F = 3.68$; $P = 0.0061$) after 24 hours and ($F = 169.00$; $P < 0.0001$) after 48 and 72 hours. As for using 50% FR, the significant differences were ($F = 3.68$; $P = 0.0061$) after 24 hours and ($F = 53.35$; $P < 0.0001$) after 48 and 72 hours. The rate of mortality in *G. mellonella* FGL, exposed to insecticide-treated IJs with 25% FR recorded a significant differences and negative correlation with percentage of mortality ($F = 4.0$; $P = 0.0036$) after 24 hours and ($F = 27.04$; $P < 0.0001$, Table 4) after 48 and 72 hours of treatment.

2.1.2. Virulence of insecticide-treated *H. bacteriophora* (F1)

The mortality percentage of FGL of *G. mellonella* with F_1 of treating *H. bacteriophora* at 100, 50 and 25% FR, in control, Dipel 2x and Radiant treatments were 100% after 48 and 72 hours. No significant differences were observed among the studied insecticides in terms of mortality rates in *G. mellonella* FGL after respective exposure periods. However at 50% FR, significant differences were found among treatments in terms of mortality percentage after 24 hours ($F = 3.58$; $P = 0.0129$); 48 hours ($F = 39.24$; $P < 0.0001$) and 72 hours post treatment ($F = 12.80$; $P < 0.0001$, Table 5). Obviously, at 25% FR the significant differences among treatments after 24 hours ($F = 4.86$; $P = 0.0010$), 48 hours ($F = 15.14$; $P < 0.0001$) and 72 hours of treatment ($F = 5.40$; $P = 0.0004$, could be traced on Table 5).

2.2. Insecticide-treated *S. carpocapsae* BA2

2.2.1. Virulence of insecticide-treated *S. carpocapsae*

As shown in Table (6), the highest percentages of mortality of FGL of *G. mellonella* in the untreated (control) *S. carpocapsae* after 24, 48 and 72 hours post treatment were 70.00, 100 and 100%, respectively. The significant differences among larval mortality percentages at 100% FR after 24 hours ($F = 7.73$; $P < 0.0001$), 48 hours ($F = 79.20$; $P < 0.0001$) and 72 hours post treatment ($F = 23.01$; $P < 0.0001$). As for in 50% FR, significant differences after 24 hours ($F= 6.95$; $P < 0.0001$), 48 hours ($F= 36.00$; $P < 0.0001$) and 72 hours post treatment ($F= 7.22$; $P < 0.0001$, Table 6). Apparently at 25% FR, there was significant differences among larval mortality percentages after 24 hours ($F = 5.67$; $P= 0.0003$), 48 hours ($F = 12.60$; $P < 0.0001$) and 72 hours post treatment ($F = 3.26$; $P = 0.0121$, Table 6).

2.2.2. Virulence of insecticide-treated *S. carpocapsae* first generation (F1)

Generally, rates of mortality in FGL of *G. mellonella* increased as the interval post exposure to the IJs increased. Also, the rates of mortality decreased as the rate of insecticides, in which the IJs were treated, increased from 25% FR to 100% FR. Moreover, statistical analysis showed no significant differences existed between treatments after48 and 72 hours (Table 7). At 50% FR, significant differences were found among treatments in mortality rates after 24 hours ($F = 3.49$; $P = 0.0144$), 48 hours ($F=6.75$; $P = 0.0002$) and 72 hours ($F = 4.50$; $P = 0.0038$, Table 7). Moreover, these differences were exited among treatments at 25% FR after 24 hours ($F = 2.68$; $P = 0.0311$), 48 hours ($F = 3.26$; $P = 0.0121$) and 72 hours of treatment ($F = 3.26$; $P = 0.0120$).

Table (2): Mortality percentages of *H. bacteriophora* (2000JIs/ml) treated by different concentrations of bio- and conventional insecticides

Treatment	Mortality (%)								
	100%FR			50% FR			25% FR		
	24h	48h	72h	24h	48h	72h	24h	48h	72h
Control	0.01e	0.01d	0.04d	0.01c	0.01c	0.04c	0.01d	0.01d	0.04d
Dipel 2x	0.65d	2.15c	3.84c	0.53b	1.83b	3.59b	0.25c	0.24c	2.56c
Radiant	0.81cd	2.55c	4.44c	0.80b	2.54b	4.58b	0.48c	1.59c	3.25c
Proclaim	1.84a	8.36a	19.45a	1.61a	6.44a	12.35a	2.25a	6.79a	13.13a
Aphox	0.94c	6.81b	15.98b	1.24a	5.63a	11.11a	1.16b	3.68b	7.41b
Coragen	1.41b	6.66b	17.03b	1.51a	6.30a	12.04a	1.68a	5.93a	11.70a
F	F =81.84;	F =163.42;	F =298.81;	F =46.52;	F =128.91;	F =170.39;	F =63.59;	F =182.38;	F =245.82;
P	P <0.0001	P <0.0001	P <0.0001	P <0.0001	P <0.0001	P <0.0001	P <0.0001	P <0.0001	P <0.0001
LSD	0.85	1.43	1.72	1.13	1.46	1.76	1.15	1.21	1.46

Means followed with the same letters (column wise) are not significantly different.
Obtained values of % mortality were transformed to ARC Sine before conducting ANOVA.
FR= field rate of insecticides.
Number of replicates for each treatment (4).

Table (3): Mortality percentages of *S. carpocapsae* (2000JIs/ml) treated by different concentrations of the tested bio- and conventional insecticides

Treatment	% Mortality (Mean)								
	100% FR			50% FR			25% FR		
	24h	48h	72h	24h	48h	72h	24h	48h	72h
Control	0.01c	0.03c	0.04d	0.01c	0.03c	0.04c	0.01d	0.03e	0.04e
Dipel 2x	0.49b	1.11b	2.36c	0.30b	1.08b	2.24b	0.05d	0.38d	0.88d
Radiant	0.54b	1.33b	2.60c	0.48b	1.43b	2.85b	0.24c	0.94c	2.16c
Proclaim	1.49a	4.79a	11.53a	1.68a	4.65a	8.75a	1.45a	4.01a	7.66a
Aphox	1.16a	3.99a	8.83b	1.45a	4.20a	7.75a	1.01b	2.66b	5.36b
Coragen	1.30a	4.28a	10.06ab	1.54a	4.43a	8.41a	1.24ab	3.69a	7.16ab
F	F =63.64;	F =64.01;	F =141.58;	F =65.46;	F =100.81;	F =187.20;	F =98.06;	F =87.02;	F =60.00;
P	P <0.0001	P <0.0001	P <0.0001	P <0.0001	P <0.0001	P <0.0001	P =98.06	P <0.0001	P <0.0001
LSD	0.93	1.73	1.85	1.04	1.39	1.40	0.85	1.40	2.33

Means followed with the same letters (column wise) are not significantly different.
Obtained values of % mortality were transformed to ARC Sine before conducting ANOVA.
*number of replicate for each treatment.
FR= field rate of insecticides.
Number of replicates for each treatment (4).

Table (4): Mortality percentages of FGL of *G. mellonella* exposed to insecticide-treated *H. bacteriophora* at different concentrations

Treatment	% Mortality (Mean)								
	100% FR			50% FR			25% FR		
	24h	48h	72h	24h	48h	72h	24h	48h	72h
Control	50.00a	100a	100a	50.00a	100a	100a	50.00a	100a	100a
Dipel 2x	30.00ab	100a	100a	30.00ab	100a	100a	40.00a	100a	100a
Radiant	30.00ab	100a	100a	30.00ab	100a	100a	30.00ab	100a	100a
Proclaim	0b	0b	0b	0b	0c	0c	0b	10.00b	10.00b
Aphox	0b	10.00b	10.00b	0b	20.00b	20.00b	0b	20.00b	20.00b
Coragen	0b	0b	0b	0b	10.00bc	10.00bc	0b	20.00b	20.00b
F	F =3.68;	F =169.00;	F =169.00;	F =3.68;	F =53.35;	F =53.35;	F =4.01;	F =27.04;	F =27.04;
P	P =0.0061	P <0.0001	P <0.0001	P =0.0061	P <0.0001	P <0.0001	P =0.0036	P <0.0001	P <0.0001
LSD	28.42	10.42	10.42	28.42	17.36	17.36	29.05	22.24	22.24

Means followed with the same letters (column wise) are not significantly different.
Obtained values of % mortality were transformed to ARC Sine before conducting ANOVA.
Number of replicates for each treatment (10).
FR= field rate of insecticides.
FGL= full grown larvae of *G. mellonella*.

Table (5): Mortality percentages of FGL of *G. mellonella* exposed to F_1 of insecticide-treated *H. bacteriophora* at different concentrations

Treatment	% Mortality (Mean)								
	100% FR			50% FR			25% FR		
	24h	48h	72h	24h	48h	72h	24h	48h	72h
Control	50.00a	100a	100a	50.00a	100a	100a	50.00a	100a	100a
Dipel 2x	40.00a	100a	100a	40.00a	100a	100a	40.00a	100a	100a
Radiant	40.00a	100a	100a	40.00a	100a	100a	50.00a	100a	100a
Proclaim	–	–	–	–	–	–	0b	30.00b	50.00b
Aphox	–	–	–	0b	20.00b	40.00b	0b	20.00b	50.00b
Coragen	–	–	–	0b	10.00b	30.00b	0b	30.00b	50.00b
F	F =0.12;	F =0;	F =0;	F =3.58;	F =39.24;	F =12.80;	F =4.86;	F =15.14; P<0.0001	F =5.40;
P	P =0.8845	P =0	P =0	P =0.0129	P <0.0001	P <0.0001	P =0.0010		P =0.0004
LSD	42.94	–	–	32.65	19.11	25.64	29.87	26.45	30.07

Means followed with the same letters (column wise) are not significantly different.
Obtained values of % mortality were transformed to ARC Sine before conducting ANOVA.
Number of replicates for each treatment (10).
– The treatments were excluded during statistical analysis.
FR= field rate of insecticides.
FGL= full grown larvae of *G. mellonella*.

Table (6): Mortality percentages of FGL of *G. mellonella* exposed to insecticide-treated *S. carpocapsae* at different concentrations

Treatment	% Mortality (Mean)								
	100% FR			50% FR			25% FR		
	24h	48h	72h	24h	48h	72h	24h	48h	72h
Control	70.00a	100a	100a	70.00a	100a	100a	70.00a	100a	100a
Dipel 2x	50.00a	100a	100a	50.00a	100a	100a	70.00a	100a	100a
Radiant	50.00a	100a	100a	60.00a	100a	100a	60.00a	100a	100a
Proclaim	0b	0b	10.00b	10.00b	20.00b	40.00b	10.00b	30.00b	60.00b
Aphox	0b	10.00b	30.00b	0b	10.00b	40.00b	10.00b	30.00b	70.00ab
Coragen	0b	0b	20.00b	0b	10.00b	50.00b	10.00b	30.00b	60.00b
F	F =7.73;	F =79.20;	F =23.01;	F =6.95;	F =36.00;	F =7.22;	F =5.67;	F =12.60;	F =3.26;
P	P <0.0001	P <0.0001	P <0.0001	P <0.0001	P <0.0001	P <0.0001	P =0.0003	P <0.0001	P =0.0121
LSD	29.26	14.73	23.55	30.87	20.25	29.67	33.49	27.56	28.85

Means followed with the same letters (column wise) are not significantly different.
Obtained values of % mortality were transformed to ARC Sine before conducting ANOVA.
Number of replicates for each treatment (10).
FR= field rate of insecticides.
FGL= full grown larvae of *G. mellonella*.

Table (7): Mortality percentages of FGL of *G. mellonella* exposed to $F_1$of insecticide-treated *S. carpocapsae* at different concentrations

Treatment	% Mortality (Mean)								
	100% FR			50% FR			25% FR		
	24h	48h	72h	24h	48h	72h	24h	48h	72h
Control	70.00a	100a	100a	70.00a	100a	100a	70.00a	100a	100a
Dipel 2x	60.00a	100a	100a	60.00ab	100a	100a	60.00ab	100a	100a
Radiant	60.00a	100a	100a	60.00ab	100a	100a	70.00a	100a	100a
Proclaim	–	–	–	–	–	–	20.00b	60.00b	60.00b
Aphox	–	–	–	10.00c	50.00b	60.00b	30.00ab	70.00ab	80.00ab
Coragen	–	–	–	20.00bc	50.00b	60.00b	20.00b	60.00b	60.00b
F	F =0.13;	F =0;	F =0;	F =3.49;	F=6.75;	F =4.50;	F =2.68;	F =3.26;	F =3.26;
P	P =0.8783	P =0	P =0	P =0.0144	P =0.0002	P =0.0038	P =0.0311	P =0.0121	P =0.0120
LSD	41.75	–	–	37.05	27.02	26.48	37.88	28.85	27.78

Means followed with the same letters (column wise) are not significantly different.
Obtained values of % mortality were transformed to ARC Sine before conducting ANOVA.
Number of replicates for each treatment (10).
– The treatments were excluded during statistical analysis.
FR= field rate of insecticides.
FGL= full grown larvae of *G. mellonella*.

3. Evaluation of the offspring of EPNs

3.1. Insecticide-treated *H. bacteriophora* BA1

3.1.1 First generation offspring of the insecticide-treated *H. bacteriophora*

The bioinsecticides potency of Emamectin benzoate represented as Proclaim had the least F_1 offspring of *H. bacteriophora* IJs in FGL of *G. mellonella* cadavers, and there were significant differences between the tested bio- and conventional insecticides at 100% FR (F= 266.72; P < 0.0001), 50% FR (F= 227.81; P <0.0001) and 25% FR (F= 285.42; P <0.0001, Fig 1a), post treatment.

3.1.2. Second generation offspring of the insecticide-treated *H. bacteriophora*

Generally, the F_2 IJs offspring of treated *H. bacteriophora* increased slightly with decreasing the rate of application of each tested insecticides. The obtained results indicated that all treatments had a profound significant effect on the F_2 offspring of the insecticide-treated *H. bacteriophora* at 100% FR (F = 4755.68; P <0.0001), 50% FR (F = 6672.72; P <0.0001) and 50% FR (F = 8021.37; P <0.0001, Fig 1b), after 15 days post treatment.

3.2. Insecticide-treated *S. carpocapsae* BA2

3.2.1. First generation offspring of the insecticide-treated *S. carpocapsae*

Pertaining to theF_1offspringof the insecticide-treated *S. carpocapsae* produced from FGL of *G. mellonella* cadavers in all tested concentrations was in the order of control > Dipel 2x > Radiant > Aphox > Coragen > Proclaim (Fig 2a). Significant differences were existed among the tested bio- and conventional insecticides after 15 days post treatment at 100% FR (F= 400.22; P <0.0001), 50% FR (F = 444.18; P <0.0001) and 25% FR (F= 407.15; P <0.0001). No significant differences were detected among Proclaim, Aphox and Coragen treatments (Fig 2a).

3.2.2. Second generation offspring of the insecticide-treated *S. carpocapsae*

The F_2 of the treated *S. carpocapsae* showed the greatest offspring rate in untreated (control), followed by Dipel 2x and Radiant treatments at all tested concentrations. Statistical analysis revealed that the tested insecticides caused significant differences in the F_2offspringof insecticide-treated *S. carpocapsae* at 100% FR (F= 51134.0; P <0.0001), 50% FR (F= 107028; P <0.0001) and 25% FR (F= 144675; P <0.0001) after 15 days post treatment (Fig 2b).

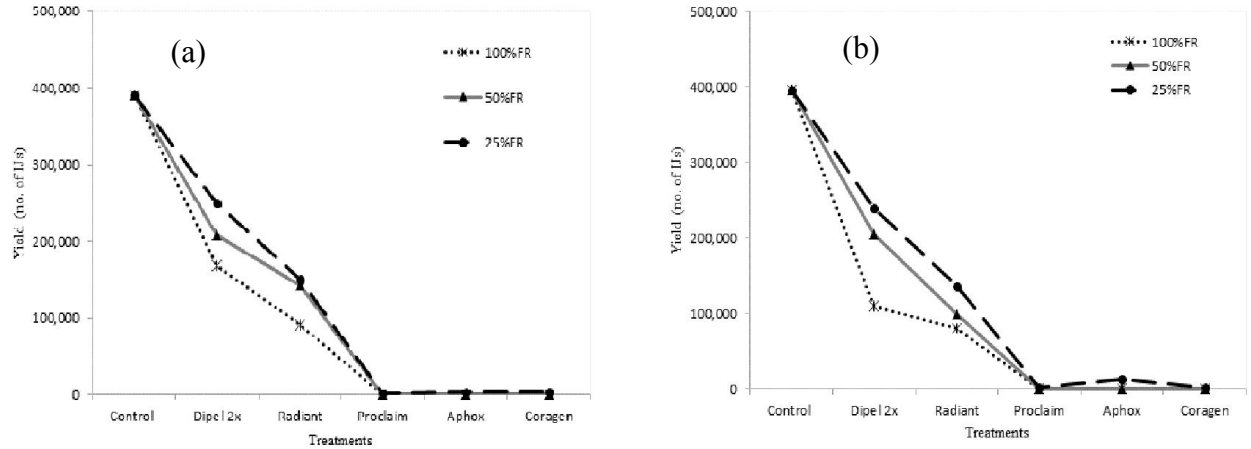

Fig (1): Number of F_1 (a) and F_2 (b) of the insecticide-treated *H. bacteriophora* (IJs) from FGL of *G. mellonella* cadavers after 15 days post treatment

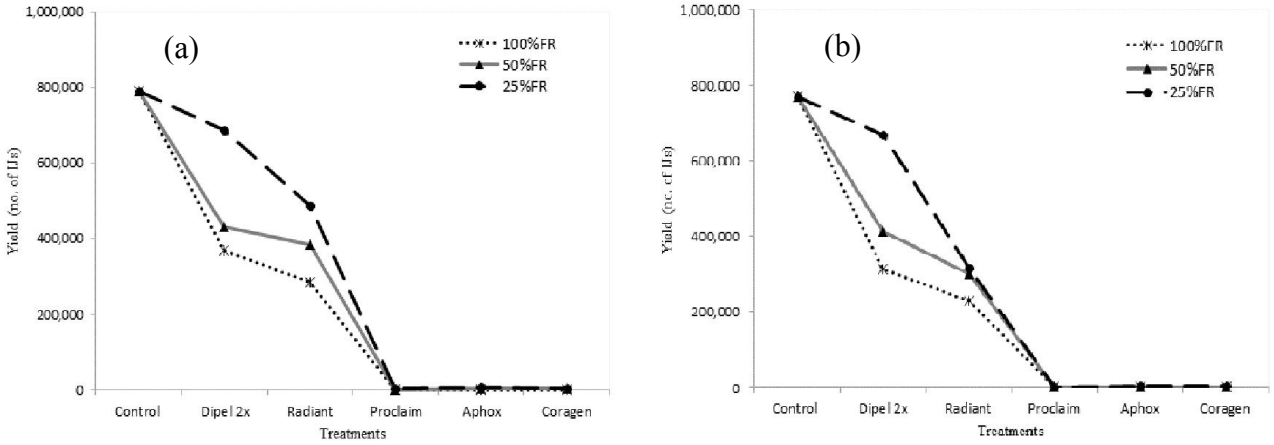

Fig (2): Number of F_1 (a) and F_2 (b) of the insecticide-treated *S. carpocapsae* (IJs) from FGL of *G. mellonella* cadavers after15 days post treatment.

DISCUSSION

The present study evaluated the effect of five bio- and conventional insecticides at three different rates on two species of EPNs (IJs). Fortunately, the tested EPN (IJs) were rather compatible with tested bio- and conventional insecticides. Similar results were also reported by Laznik and Trdan (2013), who found that *Steinernema* sp. (IJs) was the most tolerant EPN to insecticides at all tested concentrations. Mahmoud *et al.* (2016) stated that combination between EPNs and insecticides such as Azadirachtin and Neonicotinoid increased the efficacy of EPNs toward host pests. Also, Bortoluzzi *et al.* (2013) found that insecticides had no effect on the symbiotic bacteria of EPNs, but they were harmful to EPNs infectivity. In addition, there is a unique phenomenon of "stretching" was observed in the survived insecticide-treated EPN (IJs) particularly at the highest rate 3 days post treatment. With respect to Dipel 2x® as a *B. thuringiensis* product, and Radiant® insecticide as a Spinetoram product, they were slightly toxic to the tested EPNs. This conclusion agreed to some extent with those obtained by Morales-Rodriguez and Peck (2009) who mentioned that the synergism integration between EPN (IJs) and Dipel 2x® yielded promising alternatives to control the host insects, which one of its life stages in the soil. However, this study revealed the effect on the reproductive capacity of treated EPNs female with previous insecticides as compared to untreated EPNs, especially on the EPNs first generation performance, that obtained from insecticide-treated EPNs, under the same conditions. The combination within EPNs and Dipel 2x® or Radiant® insecticides might be successful to control insect pests, as long as insecticide concentration is low. Primicarb (Aphox®) lead to reduction in the survival and infectivity of tested EPNs, but less than Proclaim® insecticide. It is likely that the effect on the mortality rate and biological properties could be happened at the long-term of application.

This study evaluated also the virulence of survived IJs obtained from insecticide treatment by using *G. mellonella* as insect host under laboratory conditions. Insecticide-treated EPN (IJs) at highest concentration (100% FR) showed no significant effect on the mortality of tested insect pests compared to insecticide-treated EPN (IJs) at lowest concentration (25% FR) or untreated control EPN (IJs). The present findings are consistent with those of Sznyk-Basalyga *et al*. (2002) and Sznyk-Basalyga and Bednarek (2003) who realized that the rise of insecticide concentrations led to decrease in EPNs activity. In addition, the results indicated that *H. bacteriophora* and *S. carpocapsae* had a high degree of infectivity and pathogenicity against *G. mellonella*. As for, the infected *G. mellonella* revealed variable aspects on the cadavers post exposure (IJs), such as the muscles that suffered destruction in the fibrillate with some fragments and fat body tissues that showed high vacillations (Nouh and Hussein, 2014).

In conclusion, the mortality percentage of EPNs (*H. bacteriophora* and *S. carpocapsae*) increased gradually with the increase in exposure period and concentration of the tested insecticides. Dipel 2x and Radiant insecticides have slight toxicity to tested EPNs. However, Proclaim, Aphox and Coragen insecticides are harmful to test EPNs under laboratory conditions. As for, Proclaim treatment resulted to the highest mortality of studied EPNs. Overall, Atwa *et al*. (2009) found that the pathogenicity of treated *H. bacteriophora* by Proclaim® insecticide was negatively affected; this result was in line with those reported for Proclaim® insecticide (Koppenhöfer and Kaya, 1998). Future research is in dire need to explore and exploit the role of EPNs as biological control elements without influenced by synthetic pesticides in IPM programs.

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تأثير بعض المبيدات على النيماتودا الممرضة للحشرات وتقييم مدى فاعليتها ضد دودة الشمع الكبرى تحت ظروف المعمل

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تم تقييم سمية بعض أنواع المبيدات Dipel 2x و Radiant و Proclaim و Aphox وCoragen في صورة تركيزات مختلفة (الجرعة الموصى بها، نصف الجرعة، ربع الجرعة) على الطور المعدي لنوعين من النيماتودا الممرضة للحشرات (*Heterorhabditis bacteriophora* BA1 و *Steinernema carpocapsae* BA2) والتي تعتبر من أهم الأعداء الحيوية المرتبطة بالآفات الحشرية. وقد أظهرت النتائج بعد معاملة النيماتودا بطريقة مباشرة للمبيدات أن نيماتودا *S. carpocapsae* BA2 كانت الأكثر تحملا لجميع أنواع المبيدات التي تم اختبارها عن*H. bacteriophora* BA1. كما أكدت النتائج أن مبيد Proclaim هو أكثر المبيدات له تأثير قاتل على أنواع النيماتودا التي تم اختبارها. أما بالنسبة لتقييم فاعلية النيماتودا المعاملة بالمبيدات على الطور الكامل ليرقات دودة الشمع الكبرى *Galleria mellonella*، أكدت النتائج أن نسبة موت يرقات دودة الشمع وصلت إلى ١٠٠% بعد العدوى بالنيماتودا التي لم يتم معاملتها (كنترول) أو حتى تلك المعاملة بمبيدى Dipel2x أو Radiant عند جميع التركيزات التي تم اختبارها بعد ٤٨ و٧٢ ساعة من المعاملة، على عكس النيماتودا التي تم معاملتها بكلا من Proclaim وAphox و Coragen. وبمتابعة باقي التجارب انعكس ذلك على معدل إنتاج الذرية لكل نوع من أنواع النيماتودا.