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In Vitro Compatibility and Combined Efficacy of Entomopathogenic Nematodes with Abamectin and Imidacloprid Against the White Grub, *Pentodon bispinosus* Kust.

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ABSTRACT

One of the challenges in mixing entomopathogenic nematodes (EPNs) with chemical control is low predictable compatible information; hence, pesticides are misused by farmers. Therefore, the study aimed to clarify the result of mixing abamectin and imidacloprid on the infectivity of EPNs against larvae of hard-black beetle *Pentodon bispinosus* Kust., besides, investigating the fluctuations of the joint action of EPN species and chemical pesticides during the study period. Recommended dose (RD) of imidacloprid (LT_{50}= 5.20 and 14.20 d) was more efficient comparing with abamectin (LT_{50}= 18.14 and 24.22 d) on the first third and instar larvae of white grubs. *Heterorhabditis bacteriophora* (Ar-4) showed the highest tolerance (LT_{50}=10.11 and 6.22 d) while *Steinernema feltiae* (Filipjev) was the most sensitive (LT_{50}=5.53 and 3.55 d) after exposure to abamectin and imidacloprid at RD, respectively. EPNs plus chemical insecticides combinations on first instar larvae recorded the higher median lethal concentration (LC_{50}) except *S. feltiae* (Filipjev) plus abamectin and all combinations of EPNs with imidacloprid. All combinations with EPNs showed lower LC_{50} values than EPN alone except abamectin with some tested *H. bacteriophora* with III instar larvae. Based on LC_{50} values, all EPNs combinations with imidacloprid surpassed EPN alone or abamectin combinations. Moreover, mortality reached 100% in the 1st instar of white grubs at concentration 250 IJs/larva *H. bacteriophora* (HP88 strain) compared with 92% in the 3rd instar larvae at the same concentration after 4 weeks of incubation. The potentiation effect was observed after one week in the 3rd instar larvae only when abamectin combined with *H. bacteriophora* (Ar-4 strain) and *S. carpocapsae* (All strain) while after 3 weeks with 1st instar larvae in combination between *S. feltiae* (Filipjev) and the RD of abamectin and imidacloprid at concentration 150 IJs/larva. No synergistic interaction was observed in combinations of EPNs with neither abamectin nor imidacloprid. During mixing chemical pesticides and bioagents, the chemical pesticide is the independent variable effect on EPNs viability and infectivity which is affected by combining period and species. The final interaction mainly depends on the chemical pesticide selectivity and toxicity to target insects (stage and instar).

INTRODUCTION

The common types of grubs are polyphagous insect attacks fruit trees, different field crops (Gentry, 1965), and severely affect green landscapes (Koppenhöfer and Fuzy, 2004) causing economic damage. The fields amended by large quantities of organic manure *(Crutchfield et al.,* 1995) are favored to scarab insects and their instar larvae or adult stages feed on the botanical parts beneath the ground surface, such as the stems and roots as well as the formed tubers and rhizomes.

The first instar larvae feed on the organic matter while the second and third instar larvae move downward into the soil surface to scarf out on roots or tubers. Their life cycle has 3 instar larvae and pupates in the soil (Frew *et al.,* 2016). The scarab insect has two generations per year. Hard-back scarab beetles mate in spring and dormant in the soil in winter (El-Metwally, 2003). The scarab beetles have the potential to cause serious damage to tubers and decreasing the quality of agricultural and horticultural crops, causing infection with many fungal and bacterial diseases in the soil, and reducing the number of tillers of infested plants (Alyokhin *et al.,* 2012; Giordanengo *et al.,* 2013; Radcliffe, 1982). Scarabs outbreaks may require rapid intervention with chemical control. Environmental, safety and dissipation efficacy of chemical insecticide concerns have raised the need to develop alternatives control methods involved in IPM. Nowadays, several non-chemical control methods included biocontrol agents *e.g.* fungi, bacteria, entomopathogenic nematodes (EPNs) do not always provide effective white grub control (Klein, 1993). These constraints make chemical pesticides at the forefront of control methods for white grub control.

Abamectin is avermectin derivatives showed activity as an insecticide, acaricide and nematicide with contact and stomach action. Abamectin target is the nervous system. It acts by stimulating the release of γ-aminobutyric acid (an inhibitory neurotransmitter) and activating chloride channels (Turner and Schaeffer, 1989). Imidacloprid is a neonicotinoid insecticide. It acts as an antagonist by binding to postsynaptic nicotinic receptors in the insect central nervous system. Imidacloprid showed potency against sap-sucking insects *e.g.* leaf- and planthoppers, aphids, thrips and whiteflies. Also effective against soil insects, termites and some species of biting insects (Buckingham *et al.,* 1997; Elbert *et al.,* 1991; Mullins, 1993).

The insecticide's potency declines with advancing white grub development, where, the first larval instar is the most susceptible to insecticides (Dotson, 1995). The third instar is responsible for typically causes visible damage (Coy *et al.,* 2019) and is therefore usually detected and treated, building up resistance to insecticides.

Species of EPN belong to the family Heterorhabditidae and Steinernematidae offer an environmentally safe and IPM compatible alternative to chemical pesticides for instance insecticides, nematicides and insecticides for the control of white grubs. The efficacy of EPNs to control the 3rd instar of scarabs can be improved by combining chemical insecticides with other control agents. Nematode combinations with commonly applied insecticides such as abamectin and imidacloprid may offer an applicable efficient alternative that is highly preferable over the use of conventional soil insecticides or widespread applications.

The present study aimed to determine the effect of the recommended application rate of abamectin and imidacloprid on first and third instar larvae of the white grubs of hard-black beetle *Pentodon bispinosus* (Coleoptera: Scarabaeidae) and the infective juveniles of local and imported species of EPNs *in vitro*. As well as the response of the1st and 3rd instar larvae of the white grubs to various concentrations of local and imported EPNs species in combined applications of abamectin and imidacloprid on larval mortality of the white grubs to understand how antagonistic or additive effect might vary across local

and imported species of EPNs to build up truthful IPM programs used in controlling white grubs.

MATERIALS AND METHODS

Source of Insects:

First and third instars of white grub, *Pentodon bispinosus* collected from citrus orchards in Waddi-Elmoulak district in Ismailia Governorate and fields of strawberry in different areas of Qaliuobia Governorate, Egypt in June 2018. Larvae were carried to wooden boxes contained soil and piece of sod from pasture as a food source from the collection sites. Boxes were stored in a cooler at 10°C until the start of the experiments.

Pesticides Used:

Two commercial formulations of imidacloprid **(**Avenue 70% WG, 120 g/feddan) and abamectin (Tervigo 2% SC, 3 L/feddan) were obtained from the Central Laboratory of Pesticides, ARC Dokki, Giza.

Propagation of EPNs on The Greater Wax Moth:

The last instar larvae of *Galleria mellonella* were used as a host for nematode multiplication (Kaya and Stock, 1997). The nematodes propagated obtained from infected larvae were reserved in aqueous suspension at 10 ± 1 °C and stored one week before the experiment. Three imported EPNs included (*Heterorhabditis bacteriophora* (HP88 strain), *Steinernema carpocapsae* (All strain) and *S. feltiae* (Filipjev), *S. glaseri* (NC strain) and two local strains [*H. bacteriophora* (Ar-4 strain) and *H. bacteriophora* (Ba-1 strain)] isolated previously by (EL-Ashry et al., 2018) from Belbies and El-Arish districts, Egypt using the modified baiting technique of *G. mellonella* by (Akhurst and Bedding, 1975) were used in all *in vitro* tests. The greater wax moth larvae infected with EPNs strains were used to harvest nematodes propagation (Woodring and Kaya, 1988) and the IJs were washed in three changes of distilled water (Dutky et al., 1964) and were stored at 10°C for one week before use in experiments.

Virulence of EPNs Against First and Third Instar Larvae of White Grub:

The first and third instars of white grub were lodged individually in soil containing a small piece of strawberry plant and stored for 3-4 weeks at 10 °C. Before use in experiments, 1st and 3rd instar were kept at room temperature for 3-4 days; only healthy larvae were assayed as well as, grubs that did not enter soil within one day were replaced. Before application, nematodes were transferred from 10 °C to room temperature at 25±3 °C for 8 h for acclimation. A stock solution of 1000 IJs/ml was used. Cups of 50 ml volume containing 30 g soil (soil surface area: 6.5 cm^2). Each cup moistened with 2.5 ml distilled water first and then 1 ml IJs stock solution was pipetted as a soil drenching, followed by another 1 ml water to wash nematodes into the soil. A loamy sand soil (82.2% sand, 10.6% silt and 6.8% clay, with 4.5% organic matter and pH of 6.9) was used. The final soil moisture was adjusted to 15% (*w/w*).

The tested EPN species and strains were used to select their virulence against the 1st and 3rd instar of white grub. Six tested rates were 50, 100, 150, 200, 250 and 350 IJs/grub/cup used for each nematode strain. Grubs in this and following laboratory experiments were treated individually.

Cups were situated in trays and covered with lids punctured on each lid with a thumbtack to maintain the moisture and allow air exchange. Two small pieces of strawberry plants (local variety) were put on the soil surface replaced daily for 5 days of the experimental process until seeds of ryegrass were germinated to nourish grubs. The grub larvae mortality was checked weekly until the experimental endpoint after 28 days.

In Petri dishes (60 mm dia.) lined with moist filter paper, the dead larvae were incubated individually for observation later. The infection rate was observed under a microscope by examination inside grub cadavers searching for nematode activities according to the modified white traps method (White, 1927) to evaluate nematode infectivity and mortality.Each treatment was replicated five times, with 5 grubs per replicate. The treatments were incubated at light 12 h: dark 12 h, 25±3°C, with a relative humidity of 90%. Evaluations were made at one, two, three and four weeks after treatment to measure mortality rates. Larval mortality percent was calculated by the following equation: $\text{Mortality}(\%) = (\text{No. of Dead larvae})/(\text{Total number of larvae}) \times 100$

Bioassay of Tested Pesticides Against First and Third Instar Larvae of White Grub:

Prepared field application rate (concentration) of abamectin and imidacloprid was used against the 1st and 3rd instars of white grub. The healthy grub larvae were transferred to plastic cups containing 50 g sandy loam soil. Each plastic cup was sprayed with 5 ml of the prepared concentration of abamectin or imidacloprid on the grub's body and soil surface in cup soil. Each cup was provided daily with two small pieces of strawberry plants (local variety) as a fresh food source dipped for 10 sec of field application concentration and left to dry at room temperature then introduced to instar larvae. The cups were sealed tightly with punctuated covers. The grub larvae mortality was checked weekly until the experimental endpoint after 4 weeks. The dead larvae of the 1st and 3rd instars were counted in each treatment with five replicates. Percent of mortality were evaluated weekly for the end of the experiment. Larval mortality percent was calculated by the following equation:

$$\text{Mortality}(\%) = (\text{No. of Dead larvae})/(\text{Total number of larvae}) \times 100$$

Combining Effect of EPNs and Tested chemical Pesticides:

To investigate the combining effect of abamectin and imidacloprid and the tested EPNs strains on mortality percent of the 1st and 3rd larvae of grubs, healthy and active individuals of instar larvae of grubs were put individually in the plastic cups and let to enter soil within one day. Cups soil were kept at 15% (*w/w*) after adding 1ml of nematode suspension containing the required tested concentrations (50,100, 150, 200, 250, and 350 IJs/ml) and 5 ml of the recommended dose (RD) of abamectin and imidacloprid with a daily fresh food source. The cups with 10 holes punctured covers and sealed tightly were incubated under laboratory conditions at 24 ±3 C.

The weekly mortality observations were conducted at the same mentioned above technique. Each treatment was replicated five times, with 5 grubs per replicate. The interaction evaluations were continued for 4 weeks of treatment to measure observed mortality rates. Few larvae, whose color was not altered after nematode infection, were dissected to check the presence of nematodes.

Analysis of the Interaction Data of Mixtures:

The joint action was estimated using Richer, 1987 formula: $\mathbf{E} = (\mathrm{X} + \mathrm{Y}) - \frac{\mathrm{XY}}{100}$; where E: the expected effect of the combination as well X and Y: the mortality percentages resulted of X and Y, respectively.

The expected effect was compared with the actual effect obtained experimentally form the insecticides interaction mixture according to Mansour et al., 1966:

$$\textbf{Co} - \textbf{toxicity factor} = \frac{\text{Observed effect } (\%) - \text{Expected effect } (\%)}{\text{Expected effect } (\%)} \times 100$$

Based on the Co-toxicity factor results were classified into three categories. Co-toxicity factor ≥ +20 is considered potentiation, ≤ -20 is antagonism and -20: +20 indicated additive effect.

Statistical Analysis

A complete randomized design was implemented in all experiments. Data were subjected to ANOVA using CoStat version 6.45. Means were compared by Duncan's multiple range test at $P \leq 0.05$ probability. The median lethal time (LT_{50}) and median lethal concentrate (LC_{50}) values were calculated by probit analysis (Finney, 1971) using Analyst soft Biostat Pro V 5.8.4.3 Software.

RESULTS AND DISCUSSION

Laboratory Experiments:

Median Lethal Exposure Time Values in The First and Third Instar Larvae of White Grub, *Pentodon bispinosus:*

A laboratory experiment was conducted to determine the LT_{50} values for the first and third instar larvae of white grub, *P. bispinosus* treated with RD of abamectin and imidacloprid (Table 1). The periods to kill the two instar larvae of white grub are different in abamectin and imidacloprid as a result of significant differences between the toxicity of the two products at RD.

At the end of the experiment, imidacloprid was the most virulent pesticide against the 1st instar and 3rd instar larvae with LT_{50} values 5.20± 1.90 and 14.20± 1.77 days, respectively. Based on LT_{50} values with abamectin, the nematicide was less virulent against the 1st and 3rd instar larvae and the LT_{50} was 18.14± 2.09 and 24.22± 3.91 days, respectively.

Table 1. LT_{50} values of abamectin and imidacloprid at recommended doses on the first and third instar larvae of the white grub, *Pentodon bispinosus*.

Larval instar	LT_{50} (days)	
	Abamectin	Imidacloprid
1st	18.14±2.09	5.20±1.90
3rd	24.22± 3.91	14.20± 1.77

The values express median lethal time ± standard error

Toxicity of Abamectin and Imidacloprid Against Infective Juveniles of Certain EPNs:

Species of EPNs showed different responses after exposure to the RD of the two tested pesticides and the number of dead infective juveniles or speed of kill were exhibited in LT_{50} values (Table 2). LT_{50} values varied greatly according to the type of chemical pesticide and EPNs species at the end of exposure times. The highest values were detected with abamectin which exhibited more toxicity than imidacloprid to IJs of tested EPNs. On the other hand, the toxicity of the RD varied greatly according to EPNs species. LT_{50} values were 10.11± 0.54, 7.25± 0.34, 6.93± 0.36 and 6.66± 0.38 days with *H. bacteriophora* (Ar-4 strain), *H. bacteriophora* (HP88 strain), *S. glaseri* (NC strain) and *H. bacteriophora* (Ba-1 strain) in IJs treated with abamectin, while *S. feltiae* (Filipjev) was the most sensitive nematode species with LT_{50} value 5.53± 0.37 days. The parallel LT_{50} values in IJs treated with imidacloprid were 6.22± 0.32, 6.89± 0.34, 5.08± 0.28, 4.47± 0.26 and 3.55± 0.24 days with *H. bacteriophora* (Ar-4 strain), *H. bacteriophora* (HP88 strain), *S. glaseri* (NC strain), *H. bacteriophora* (Ba-1 strain) and *S. feltiae* (Filipjev), respectively. In general, *H. bacteriophora* (HP88 strain) and *S. glaseri* (NC strain) surpassed *S. carpocapsae* (All strain) and *S. feltiae* (Filipjev) intolerance of abamectin and imidacloprid toxicity. Concerning local nematode species, *H. bacteriophora* (Ar-4 strain) was tolerant as compared with *H. bacteriophora* (Ba-1 strain).

Table 2. LT_{50} values of IJs of different EPNs species exposed to recommended rates of abamectin and imidacloprid under laboratory conditions.

Nematode species	LT_{50} values in days*	
	Abamectin	Imidacloprid
H. bacteriophora (Ar-4 strain)	10.11± 0.54	6.22± 0.32
H. bacteriophora (Ba-1 strain)	6.66± 0.38	4.47± 0.26
H. bacteriophora (HP88 strain)	7.25± 0.34	6.89± 0.34
S. carpocapsae (All strain)	6.40± 0.35	5.24± 0.31
S. feltiae (Filipjev)	5.53± 0.37	3.55± 0.24
S. glaseri (NC strain)	6.93± 0.36	5.08± 0.28

The values express median lethal time ± standard error

After 4 weeks of treatment, *H. bacteriophora* (Ar-4 strain) alone killed the first instar larvae of white grub at the least LC_{50} value = 13.34± 7.63 followed by *S. carpocapsae* (All strain), *H. bacteriophora* (Ba-1 strain), *H. bacteriophora* (HP88 strain) and *S. glaseri* (NC strain) with LC_{50} values 18.53±6.88, 22.75± 19.20, 25.48± 9.98 and 36.36±6.79, respectively (Table 3). While LC_{50} value with *S. feltiae* (Filipjev) alone was 170.47± 21.68.

In combined treatments with EPNs species and abamectin in controlling the 1st instar larva of white grub, *P. bispinosus*, LC_{50} values increased gradually in all treatments to reach 44.015± 1.48 with *H. bacteriophora* (HP88 strain), 48.49± 1.42 with *S. carpocapsae* (All strain) and 50.05±1.93 with *H. bacteriophora* (Ar-4 strain). Consequently, imported EPNs species, *H. bacteriophora* (HP88 strain) and *S. carpocapsae* (All strain) were the most effective species against the 1st instar larvae of grub when combined with abamectin followed by local nematode species, *H. bacteriophora* (Ar-4 strain) while the imported species, *S. feltiae* (Filipjev) and local species, *H. bacteriophora* (Ba-1 strain) were the least effective against the 1st instar larvae of grub when combined with abamectin. When imidacloprid was used combined with EPN species, LC_{50} increased radically to high values and to reach 359.20± 19.31 and 155.00± 11.93 with *S. feltiae* (Filipjev) and *H. bacteriophora* (Ba-1 strain), respectively. On the other hand, the two imported species *H. bacteriophora* (HP88 strain) and *S. carpocapsae* (All strains) were the most effective species with LC_{50} values 80.08±6.38 and 76.61± 10.19, respectively.

At the end of bioassay against the 3rd instar larvae, the LC_{50} values used EPN species alone were less effective in killing the 3rd instar larvae with *S. carpocapsae* (All strain), 86.99± 7.57; *S. feltiae* (Filipjev), 436.54± 24.46; *S. glaseri* (NC strain), 134.94± 23.28 and *H. bacteriophora* (Ba-1 strain), 222.28± 21.90.

In combined treatments of EPNs species with abamectin and imidacloprid used for controlling the 3rd instar larvae of grub under laboratory conditions, the LC_{50} values decreased to73.52± 10.10 & 46.80± 8.57; 251.20± 15.68 & 204.272± 17.30 and 90.07± 15.58 & 79.92±11.24 with imported EPN species, *S. carpocapsae* (All strain, *S. feltiae* (Filipjev) and *S. glaseri* (NC strain) combined with abamectin and imidacloprid, respectively. On contrary, LC_{50} values in *H. bacteriophora* (Ar-4 strain) and *H. bacteriophora* (HP88 strain) alone was less (87.78± 8.84 &85.08± 10.07) than the combined with abamectin (90.18± 6.76& 87.11± 7.22) then reduced sharply to reach 53.82± 9.96 and 24.80± 9.72 in treatments of *H. bacteriophora* (Ar-4 strain) and *H. bacteriophora* (HP88 strain) combined with imidacloprid.

These results indicate that, compared with EPN alone, abamectin and imidacloprid combinations did not significantly improve the efficiency of IJs of EPN species in controlling the 1st instar larvae of white grub after 4 weeks of treatment and increased LC_{50}

values with all combination between tested nematodes and two pesticides except with *S. feltiae* (Filipjev). The vice versa was recorded with 3rd instar larvae, combined EPN species with imidacloprid only decreased LC_{50} with all EPN species while with abamectin reduced LC_{50} with *H. bacteriophora* (Ar-4 strain), *S. carpocapsae* (All strain, *S. feltiae* (Filipjev) and *S. glaseri* (NC strain) and increased only with *H. bacteriophora* (Ar-4 strain) and *H. bacteriophora* (HP88 strain).

Table 3. LC_{50} values of different EPNs species IJs used singly or mixed with recommended doses of abamectin and imidacloprid on the 1st and 3rd larval instars of while grubbing after 4 weeks incubation.

Treatments	Instar larvae					
	The first			The third		
	EPN alone	Abamectin +EPN	Imidacloprid + EPN	EPN alone	Abamectin +EPN	Imidacloprid + EPN
H. bacteriophora (Ar-4 strain)	13.34±7.63	50.05±1.93	95.01±6.62	87.78±8.84	90.18±6.76	53.82±9.96
H. bacteriophora (Ba-1 strain)	22.75±19.20	75.60±2.90	155.00±11.93	222.28±21.90	164.74±14.32	108.52±12.64
H. bacteriophora (HP88 strain)	25.48±9.98	44.015±1.48	80.08±6.38	85.08±10.07	87.11±7.22	24.80±9.72
S. carpocapsae (All strain)	18.53±6.88	48.49±1.42	76.61±10.19	86.99±7.57	73.52±10.10	46.80±8.57
S. feltiae (Filipjev)	170.47±21.68	99.50±4.34	359.20±19.31	436.54±24.46	251.20±15.68	204.272±17.30
S. glaseri (NC strain)	36.36±6.79	57.00±1.59	135.35±9.38	134.94±23.28	90.07± 15.58	79.92±11.24

The values express median lethal concentration ± standard error

Maximum Mortality of White Grub, *Pentodon bispinosus* Relatively Affected by Concentrations and Nematode Species:

Mortality percentage of the 1st and 3rd instar larvae of white grub, *P. bispinosus* relatively affected by serial of the tested concentrations (50, 100, 150, 200, 250 and 350 IJs/larvae) and EPN species (4 imported EPN species and 2 local isolated species) under laboratory conditions was studied and data present in Table (4).

After one week of treatment by different concentrations of EPN species, percent mortality resulted from the imported nematode species *H. bacteriophora* (HP88 strain) was 44 at a concentration of 350 IJs/larva followed by the local species, *H. bacteriophora* (Ar-4 strain) and *S. carpocapsae* (All strain) with percent mortality of 40 (for each) at a concentration of 350 IJs/larva while the maximum mortality with *S. glaseri* (NC strain) reached 24% in the concentration of 250 IJs/larva and *S. feltiae* (Filipjev) gained the least mortality (12%) with 200 IJs/larva. With an increase of time exposure, mortality raised from 60 to 100% after two, three- and four-week intervals at concentrations of 350, 350 and 250 IJs/larva in the 1st instar larvae treated with local nematode species of *H. bacteriophora* (Ar-4 strain). Likewise, mortality increased from 64% to 100% after 2, 3 and 4 weeks in the 1st instar larvae inoculated with *H. bacteriophora* (HP88 strain) at concentrations of 350, 250 and 150 IJs/larva, respectively. The same trend was observed in 1st instar larvae treated with *S. carpocapsae* (All strain) with concentrations of 350, 350 and 150 IJs/larva. On the other hand, with the height concentration (350 IJs/larva), *S. feltiae* (Filipjev) gained the least mortality (28, 48 and 60%) after 2, 3 and 4 weeks of treatment, respectively.

The mortality percentage in the 3rd instar larvae increased gradually from one week to reach the maximum relatively mortality at the end of the experiment to reach 100% only with *S. glaseri* (NC strain) followed by 96, 92 and 92 % in the treatment of 350 IJs/larva

with *H. bacteriophora* (Ar-4 strain), *H. bacteriophora* (HP88 strain) and *S. carpocapsae* (All strain), respectively.

Table 4. The maximum mortality percentage resulted from different inoculum concentrations of EPNs infected the 1st and 3rd instar larvae of Pentodon bispinosus after 4 weeks of treatment under laboratory conditions.

Treatment	1st larval instar				3rd larval instar			
	Exposure periods (Weeks)							
	1	2	3	4	1	2	3	4
H. bacteriophora (Ba-1 strain)	24 (250)	44 (350)	68 (350)	76 (350)	28 (350)	44 (350)	52 (350)	60 (350)
H. bacteriophora (Ar-4 strain)	40 (350)	60 (350)	100 (350)	100 (250)	48 (350)	64 (350)	80 (350)	96 (350)
H. bacteriophora (HP88 strain)	44 (350)	64 (350)	100 (250)	100 (150)	52 (350)	72 (350)	92 (350)	92 (350)
S. carpocapsae (All strain)	40 (350)	64 (350)	100 (350)	100 (150)	44 (350)	68 (350)	92 (350)	92 (350)
S. feltiae (Filipjev)	12 (200)	28 (350)	48 (350)	60 (350)	16 (350)	28 (250)	36 (350)	40 (350)
S. glaseri (NC strain)	24 (250)	48 (350)	80 (350)	100 (250)	28 (350)	52 (350)	80 (350)	100 (350)

Numbers between parentheses refer to inoculum concentration (no. of IJs/ larva).

As shown in Fig.1, imidacloprid exhibited more toxic effects against infective juveniles of different tested strains of EPN species when compared with abamectin. The mean of LT_{50} values is used to confirm the number of dead IJs at different time intervals or to expose the speed of kill. IJs of *H. bacteriophora* (Ar-4 strain) followed by *H. bacteriophora* (HP88 strain) were the most tolerant EPN species to RD of abamectin and imidacloprid and gained high LT_{50} values while *S. feltiae* (Filipjev) and *H. bacteriophora* (Ba-1 strain) were the least EPN species and gained the lowest LT_{50} values when mixed with tested pesticides (Fig.2). Also, heterorhabditid species were the most compatible EPN species (high LT_{50} values) when combined with the two tested pesticides as compared with steinernematid species (low LT_{50} values) under laboratory conditions (Fig.3).

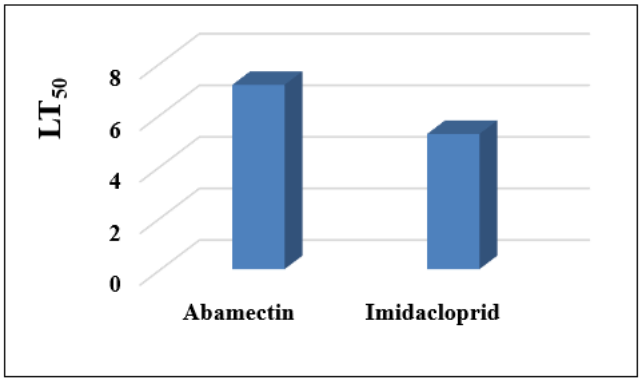

Fig.1. The general mean of LT_{50} values of abamectin and imidacloprid combined with tested strains of EPNs.

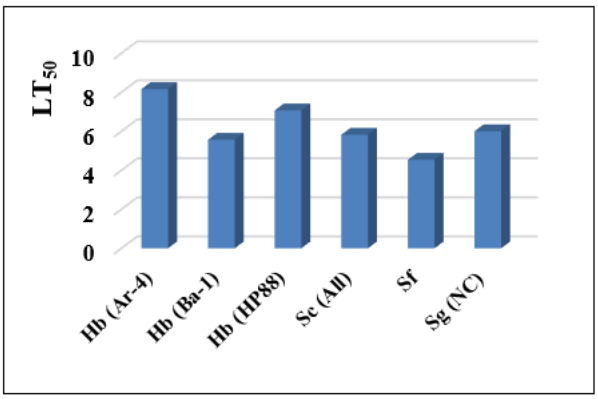

Fig. 2. The general mean of LT_{50} values of strains of EPNs combined with the tested chemical insecticides.

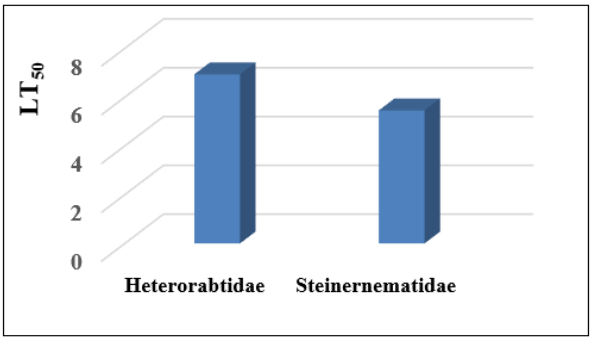

Fig. 3. The mean of LT_{50} values of Steinernematidae and Heterorhabtidae is combined with the chemical insecticides (abamectin and imidacloprid).

EPN Species Interaction with Abamectin and Imidacloprid against the 1st and 3rd Instar Larvae of *Pentodon bispinosus*:

EPN Species Interaction with Abamectin and Imidacloprid against the 1st Instar:

The results illustrated graphically in Figure (4) clearly show the antagonistic effect was observed between tested EPN species (*H. bacteriophora* (Ba-1 strain), *H. bacteriophora* (Ar-4 strain), *H. bacteriophora* (HP88 strain), *S. carpocapsae* (All strain), *S. feltiae* (Filipjev) and *S. glaseri* (NC strain)) at concentration 150 IJs/larva and abamectin and imidacloprid after one week of application and additive interactions were observed only between *H. bacteriophora* (Ar-4 strain) and *H. bacteriophora* (HP88 strain) while other EPN species showed antagonistic effect one week at a concentration of 350 IJs/larva.

After two weeks, an antagonistic effect was observed with concentration 150 IJs/larva while an additive effect was observed between *H. bacteriophora* (HP88 strain) and *S. carpocapsae* (All strain) when combined with abamectin while combination between imidacloprid and *H. bacteriophora* (Ar-4 strain) showed additive effect at concentration 350 IJs/ larvae.

The only potentiation effect was observed after 3 weeks of the application when concentration 150 IJs/larva was used in combination between *S. feltiae* (Filipjev) and abamectin and imidacloprid as well as, *H. bacteriophora* (Ba-1 strain), *H. bacteriophora* (Ar-4 strain) and *S. glaseri* (NC strain) when combined with abamectin. Besides, *S. carpocapsae* (All strain) showed a synergistic effect when combined only with imidacloprid. After 4 weeks of application, an antagonistic effect was observed with all tested EPN species

at concentration 150 IJs/larva with the tested pesticides while *S. feltiae* (Filipjev) have a great antagonistic effect at concentration 350 IJs/larva.

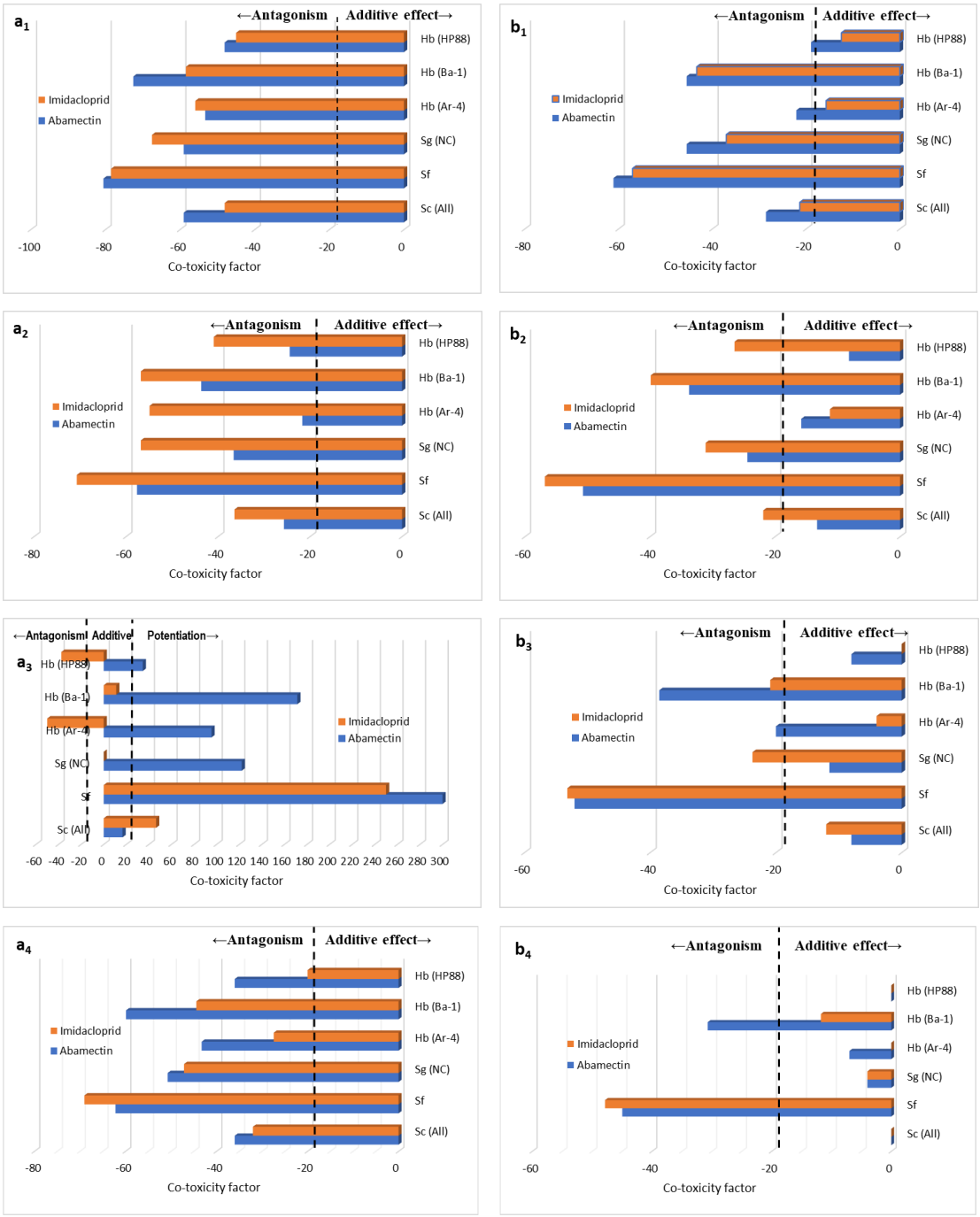

Fig. 4. Co-toxicity factor resulted from a combination of abamectin and imidacloprid with the tested EPNs (a: 150 IJs/larva; b: 350 IJs/larva; subscripted number refers to incubation period weekly) on mortality of the 1st instar of *P. bispinosus in vitro*.

EPN Species Interaction with Abamectin and Imidacloprid Against the 3rd Instar:

Additive and antagonistic effects were observed between tested EPN species and the two synthetic pesticides after one week of application at concentration 150 IJs/larva while potentiation, additive and antagonistic effects were observed between tested EPN species

and two pesticides after one week of application at concentration 350 IJs/larva. The potentiation effect was found in treatments of *H. bacteriophora* (Ar-4 strain) and *S. carpocapsae* (All strain) when combined only with the RD of abamectin (Fig. 5). After two weeks, an antagonistic effect was observed with all combination between EPN species and pesticides except with *H. bacteriophora* (HP88 strain) and *S. carpocapsae* (All strain) which showed additive effects when combined with imidacloprid at 150 IJs/larva as well as, an additive effect was observed in combinations between species of *H. bacteriophora* (Ba-1 strain), *H. bacteriophora* (Ar-4 strain), *H. bacteriophora* (HP88 strain) and *S. carpocapsae* (All strain) and imidacloprid at 350 IJs/ larva. The same trend was observed after 3 and 4 weeks between *H. bacteriophora* (Ar-4 strain), *H. bacteriophora* (HP88 strain) and *S. carpocapsae* (All strain) and the two tested insecticides when the additive effect was obtained from combinations at 350 IJs/larva concentration while the antagonistic effect was also observed after 3 weeks of combinations between all EPN species and the two tested chemical pesticides at 150 IJs/larva concentration.

The efficacy of EPNs in controlling numerous soil pests for long periods is one of their advantages when compared with chemical pesticides. The application of EPNs ensures that the control effect continues for a longer period on the target pests compared to chemical pesticides and their control effect disappeared quickly with active ingredient dissipation in the treated environment. Perhaps the effectiveness of EPNs on a wide range of insect pests encourages their use as a main or auxiliary control method besides chemical control. The broad host range of EPNs showed potency against insect pests of various insect orders (Lacey and Georgis, 2012) included scarab larvae (Coleoptera: Scarabaeidae) *e.g.* white grub causes damage in turfgrass, lawn, plant producing tubers, or rhizomes in addition to several economic plants for instance sugarcane.

Larval and adult stages of the scarab are feeding on the root system (oviposition) where white grubs are habitat and their injuries are classified as qualitative and quantitative according to the plant fed on it (Potter, 1998). The difficulty in deduction of the damage resulted from white grubs delayed the control decision and increased loss in infested crops Therefore, finding a safe and effective control method that has a long effect and compatible with chemical pesticides is a real challenge. Therefore, documented or detecting a worthy combination between different strains, isolates of EPNs and their response through synchronic admixed with abamectin and imidacloprid in controlling white grubs were evaluated.

Abamectin is avermectin derivatives targets the nerve by stimulating the gamma-aminobutyric acid (*GABA*) transmitter in end endings causes tremor/convulsion resulted from hyperpolarization of nerve/nerve or muscle cells (Food Safety Commission of Japan, 2016). The affected insect becomes paralyzed, stops feeding, and dies after a few days. Imidacloprid is belonging to neonicotinoids. It is an antagonist acting by binding to postsynaptic nicotinic receptors causing acetylcholine (ACh) accumulation. Hyperactive is a clear symptom resulting in the insect's paralysis and eventual death (Sone et al., 1994). Imidacloprid proved as contact and stomach poison (MacBean, 2012).

The imidacloprid approved efficiency as a soil insecticide against white grubs when applied as preventative treatments in spring during or immediately after egg-laying. Their efficacy sharply declines when the grubs reach the late-instar stage although it is relatively persistent (Grewal *et al.*, 2001; Rogers and Potter, 2003). Alternatively, on the soil surface, abamectin breaks down by photodegradation (DT_{50} = 1 week) and microbes in dark aerobic conditions (Wislocki *et al.*, 1989). Perhaps differences in the physicochemical properties of both insecticides as well as their behavior and stability in the soil are responsible for the potency of imidacloprid over abamectin. Moreover, laboratory bioassay verified the efficacy of imidacloprid on the 1st and 3rd instar larvae of white grub at the RD. This view is also

supported by the decrease of LT_{50} of imidacloprid at RD (5.20±1.90 and 14.20± 1.77 days) comparing with abamectin at RD (18.14± 2.09 and 24.22± 3.91 days) on the1st and 3rd instar larvae. Bearing in mind that, the short latency period of the chemical insecticide is essential for the efficiency in field applications as a result of the continuous and rapid deterioration of the pesticides in the environment. Consequently, the insecticide concentration gradually decreases less than the effective concentration.

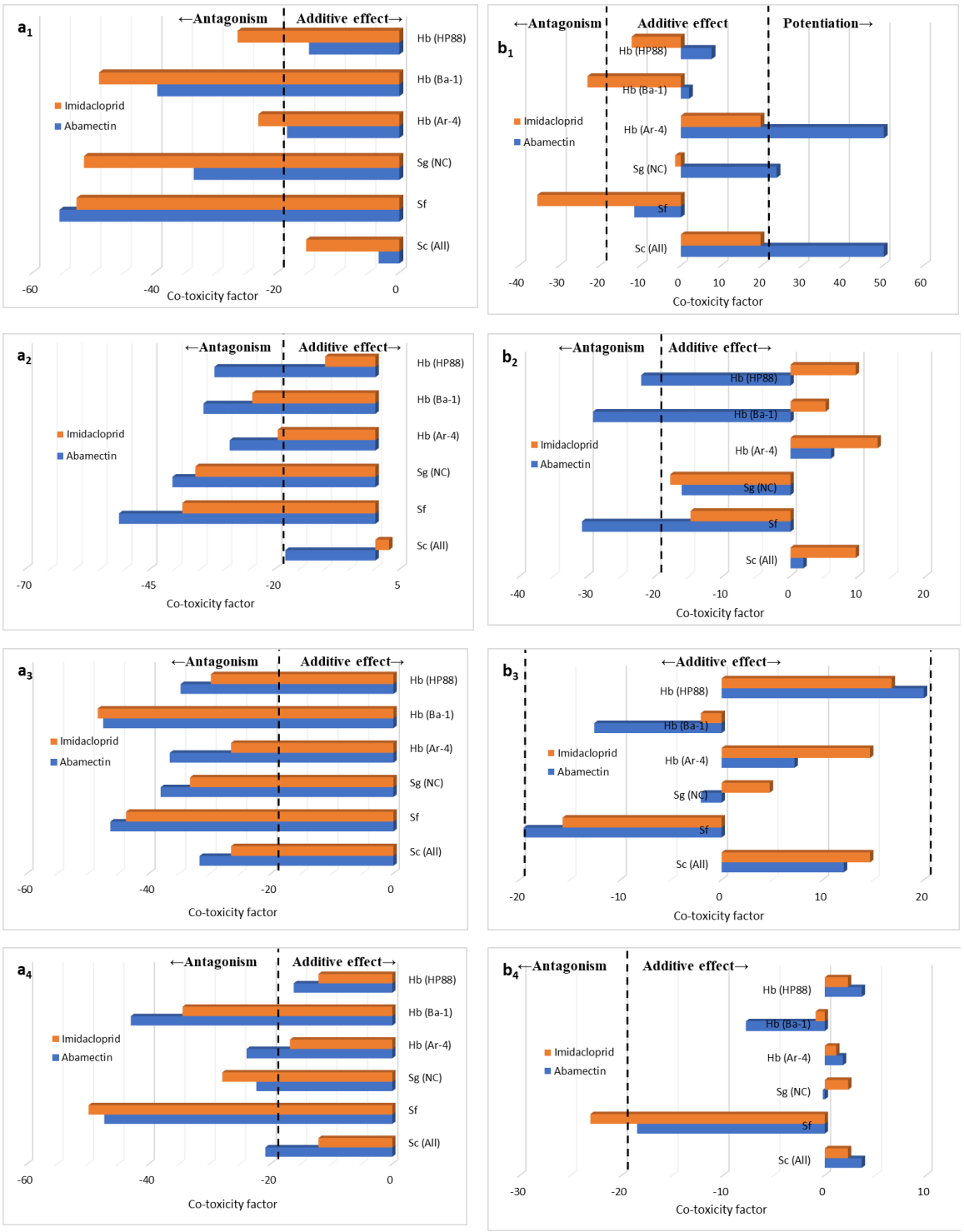

Fig. 5. Co-toxicity factor resulted from a combination of abamectin and imidacloprid on the tested EPNs (a: 150 IJs/larva; b: 350 IJs/larva; the number subscripted refer to incubation period weekly) on mortality of *P. bispinosus* 3rd instars *in vitro*.

Exposure of the tested EPNs to abamectin and imidacloprid at the RD caused adverse effects and increased the mortality rate of EPNs depends on the incubation period with a toxic substance as indicated by LT_{50} values which defined the critical exposure periods for each EPN strain. *H. bacteriophora* (Ar-4 strain) was the most tolerance to abamectin (LT_{50} =10.11 days at RD) while, *H. bacteriophora* (HP88 strain) followed by *H. bacteriophora* (Ar-4 strain) were the most tolerance to imidacloprid (LT_{50}= 6.89 and 6.22 days at RD, respectively). LT_{50} value for the most sensitive EPNs, *S. feltiae* (Filipjev) was 5.53 and 3.55 days with abamectin and imidacloprid, respectively. The search results are in agreement with many research workers (Bajc *et al.,* 2017; Laznik and Trdan, 2017; Raheel *et al.,* 2017). Generally, imidacloprid was more toxic to the tested EPN species compared with abamectin based on the time required for killing 50% of the exposed EPN population and heterorhabditids species displayed more tolerance than steinernematid species to the tested insecticides.

The larger proportion of formulations in the insect/acar/nematicide group were harmful to IJs of *S. carpocapsae* and *S. feltiae* (Rovesti and Deseö, 1990). The sublethal effects may affect the nematode reproductive potential (Gutiérrez e*t al.,* 2008). Abamectin exhibited negative effects on *S. carpocapsae* IJs resulted in 70% mortality at 1000 µg/ml for 24 h. (Kary e*t al.,* 2018) and accelerate metabolic activity in both tested genus of EPN and releasing CO_2 without inhibition of symbiotic bacteria (Sabino e*t al.,* 2017) while, imidacloprid reduced the viability and the infectivity of *S. carpocapsae* (Negrisoli Junior *et al.,* 2008). Although, mixing imidacloprid (0.04:1.25%) caused mortality 3.5% (Patil *et al.,* 2015). So, decreasing the application rate of the combined chemical insecticides (imidacloprid) enhancing the efficacy of susceptible EPN (*S. feltiae* SN) by eliminating the toxic effect (Yan *et al.,* 2019). Perhaps the mortality of exposed IJs depends on the incubation period as observed with *H. bacteriophora* agitated in solutions of imidacloprid for 24 h, has no observed negative effect on nematode survival and infectivity (Koppenhofer and Kaya, 1998). Therefore, the variation in results of the chemical pesticides bioassay on EPNs and the development of toxic effects depends on species, exposure time, the active ingredient, concentration, and temperature (Bajc *et al.,* 2017; Laznik and Trdan, 2017; Patil et al., 2015). In addition to the adjuvants involved in every pesticide formulation of active ingredient (a.i.), which differ according to the manufacturer. These adjuvants may have a vital role in increasing or reducing the toxic effect of a.i. on nematodes besides their direct toxicity to egg and IJs of nematode (El-Ashry *et al.,* 2019; Krishnayyaand and Grewal, 2002) as well as, these adjuvants do not subject to registration during pesticide approval.

The inoculum potential is the inoculum amount of the pathogen to overcome host resistance and causing infection successfully (Garrett, 1960). Differences in EPNs virulence affected by factors such as host recognition and penetration ability, overcoming on host immune system, and host finding behavior including ambushing, cruising, and intermediate strategy (Grewal *et al.,* 2005). As, *H. bacteriophora* is a cruiser forager that actively searching for its victim (Ciche, 2007), while, *S. carpocapsae* has an ambushing strategy that waits for a potential host, and *S. feltiae* has an intermediate strategy (Lewis, 2002). On white grub *Polyphylla olivieri*, the LD_{50} value of *H. bacteriophora* (IRAN1) was 35 IJs/larva, 65 IJs/larva for *S. glaseri* (IRAN2) and LD_{50} for *Steinernema* sp. was >10000 IJs/larva causing only 16% mortality after 25 days (Karimi and Kharazi-pakdel, 2007) as well as, the pathogenicity of the symbiotic bacteria.

Scarabaeidae are a strong insect that includes many white grubs. It has an alert and responsive immune system that interacts with xenobiotics *H. bacteriophora* or *S. glaseri* can increase the immunocytes after 8-12 h after injection of the white grub larvae, *Polyphylla adspersa* (Alvandi *et al.,* 2014). Successful infection of EPNs required overcoming this weak

immune response (Alvandi *et al.*, 2017). Therefore, applied effective inoculum potential for controlling the white grub is relatively high, as 500, 1000 and 2000 IJs/100 g soil cause 68-93% mortality by *S. carpocapsae* and 39-71% mortality by *H. indica* after 7 days post-treatment (Sharma *et al.*, 2009). As always observed a relationship between inoculum potential and latency period (negative correlation) depended on the treated insect life stage and larval instar. The 2nd instar grubs of *L. lepidophora* were more susceptible to the EPN species tested than the 3rd instar and that the EPNs efficacy varies with species and isolates of a single species (Del Valle et al., 2017; Malinowski, 2011; Patil *et al.*, 2017). In addition to the effect of the insect host, *Spodoptera litura* was very sensitive to tested EPNs showed lower LC_{50} values comparing with white grubs (Kumar *et al.*, 2015).

Other factors that affect EPNs virulence and infectivity include soil temperature, soil moisture, clay content, root morphology and application methods(Cowles *et al.*, 2005). In the field, there are economic restrictions imposed on the amount of applied inoculum potential (recommended rate) to ensure the EPNs control efficiency and mass production cost called feasibility.

Toxicological interactions of chemical pesticides are the presence of other chemicals, at the same time, earlier, or later leading to affect the final toxicity of the mixture may: decrease (antagonism), add to toxicity (additivity) and increase toxicity (synergism or potentiation) of some chemicals. Perhaps the interactions between chemical pesticides are easy to study, and the combined result of the two chemical pesticides is stable but alters with the changing the treated biological systems (Zhu, 2008).

As for the mixing of chemical and microbial pesticides contain two types of interaction. The first is an internal reaction between the components of the mixture and the microbial response to chemical pesticides. The second is the final toxic effect and infectivity of the combination (chemical + microbial pesticides) on the target organism. The microbial bioagent exposure during the combining period is crucial to the final interaction output.
Most of the compatibility studies of chemical pesticides with approved EPN IJs can tolerate short-term exposure (2-24 h). This period enough to tank-mixed and applied together (Koppenhofer and Grewal, 2005). But practically, the EPNs exposure continued after pesticide application but with lower concentration diluted gradually. In addition to long latent periods of chemical on target insect especially with systemic insecticides with contact and stomach action as imidacloprid that required white grubs feed enough amount of treated plant to cause toxicity (hyperactivity, convulsion, and paralysis) ended with mortality. The abnormal behavior of insects may increase susceptibility to EPNs (Nishimatsu and Jackson, 1998).

In ecotoxicology, focused on a single endpoint (growth, reproduction, or mortality) at a consistent exposure time which chosen nevertheless of the properties of under testing chemicals, It should depend on the choice organism in combination with the compound (s) of interest (Baas *et al.*, 2010). Ascertaining toxicity data for a single compound exhibit clear patterns in time, it is expected that the effects of mixtures will also be strongly dependent on time. Therefore, long combining periods between EPNs and chemical insecticide will show different types of toxicological interactions with long (Koppenhofer *et al.*, 2000).

The interaction results varied according to the insect host and imidacloprid did not affect the viability and infectivity of the EPNs treated with *Spodoptera frugiperda* compared with the control treatments without the insecticide (Souza *et al.*, 2012). Further insect host variations, great variability in pathogenicity and virulence against different white grubs have been observed among EPN species and isolates of a single species (Del Valle *et al.*, 2017). Moreover, life stage and instar (Kary *et al.*, 2018). Lesser instar larvae of white grubs are more susceptible EPNs species (Patil et al., 2017). In addition to the combining period (Mohankumar *et al.*, 2017), which showed high compatibility of imidacloprid with EPNs

after 96 h, after treatment (Kwizera and Susurluk, 2017) but long combining time will show the sublethal effect and the increased mortality on EPN and insect host. The final interaction will be affected by instar larvae which affect insecticide and EPN potency (Koppenhofer and Fuzy, 2008; Malinowski, 2011).

Imidacloprid interacts synergistically on the mortality of the 3rd white grubs. However, the degree of interaction varied with nematode species is a synergistic effect with *Steinernema glaseri* and *H. bacteriophora*, but the only additive with *S. kushidai* on the third-instar white grubs (Koppenhofer *et al.,* 2000).

The insecticide drastically reduced the activity of the grubs facilitate EPN attachment and penetration increasing infection resulted in the synergistic interaction *(Koppenhofer et al.,* 2000).

The role of insecticide in manipulating and/or affecting on nematode as well as the activity and behavior of the host leads to the invasion of the host by the nematode. The insecticide drastically reduced the activity of the grubs and facilitate the attachment and penetration of EPN species then increase infection resulted in the synergistic interaction (Koppenhofer *et al.,* 2000).

Other factors manipulate interaction, the combination application methods. Soil drenching with EPNs suspension increased mortality percentages of the third larval instars of white grub *P. bispinosos* (Ibrahim *et al.,* 2010). They used lower RD of chemical pesticides to avoid adverse effects on EPNs as optimize dosages (Gutiérrez *et al.,* 2008; Kary *et al.,* 2018).

There was no evidence for successful in-host survival or latent infection by the nematodes in endemic white grub populations (Elmowitz *et al.,* 2013). Subsequently, to ensure their sustainability, applied periodically to enhance their population. The need for knowing the short- or long-term adverse effects of chemical insecticides on EPNs for IPM designs (Vashisth *et al.,* 2013). Whether they combined or applied separately simultaneous or consecutive in outbreak case. The right application procedure will offer a cost-effective alternative to pest control (Vashisth *et al.,* 2013).

Conclusion

Fore become EPNs one of the most reliable biocontrol agents, many hypotheses need to be fully understood about compatibility and application rates *in vitro*. Our study revealed that local and imported isolates of EPNs (*H. bacteriophora* (Ar-4 strain) and *H. bacteriophora* (HP88 strain)) offer an effective alternative to synthetic insecticides (abamectin and imidacloprid) in controlling white grub, *P. bispinosus* when an appropriate concentration (250 IJs/larva) is used to achieve a stronger interaction with pesticides (potentiation) after a short time of combination to reduce their damage.

However, many factors can affect the optimum choice of nematode species/strains and targeted pest developmental stages. So, further experiments under greenhouse and fields have been needed to clarify the efficacy of EPNs species and strains with proper application rate on the speed of killing the developmental stages of *P. bispinosus*.

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ARABIC SUMMARY

التوافق والفعالية المشتركة للنيماتودا الممرضة للحشرات مع الأبامكتين والإيميداكلوبريد ضد يرقة الجعال ذو الظهر الجامد *Pentodon bispinosus* Kust. معمليا

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إحدى التحديات في خلط النيماتودا الممرضة للحشرات مع وسائل المكافحة الكيميائية هو ضعف المعلومات المتعلقة بالتنبؤ بمدى توافقهما معاً مما يترتب عنه سوء تطبيق المبيدات من قبل المزارعين. لذلك هدفت الدراسة توضيح نتيجة خلط الأبامكتين والإيميداكلوبريد مع النيماتودا الممرضة للحشرات (الموت والقدرة على الاصابة) ضد يرقات الجعل ذو الظهر الجامد (جعل القصب) *Pentodon bispinosus* بالإضافة إلى التحقق من تقلبات التأثير المشترك لأنواع النيماتودا الممرضة للحشرات والمبيدات الكيميائية أثناء فترة الدراسة. أظهرت النتائج أن الإيميداكلوبريد أكثر كفاءة (الوقت النصفي القاتل 2.20 و14.20 يوماً) مقارنة مع الأبامكتين (18.14 و24.22 يوماً) على يرقات العمر اليرقي الأول والثالث ليرقات الجعال المعاملة بالجرعة الموصي بها. أظهرت نيماتودا *Heterorhabditis bacteriophora* (Ar-4) أعلى درجة تحمل (الوقت النصفي القاتل 10.11 و6.22 يوماً) في حين كانت *Steinernema feltiae* أكثر حساسية (الوقت النصفي القاتل 5.53 و3.55يوماً) عند التعرض للأبامكتين والإيميداكلوبريد بالجرعة الموصي بها، على التوالي. وأظهرت المخاليط علي العمر اليرقي الأول ارتفاع قيم التركيز النصفي القاتل عدا مخلوط *S. feltiae* مع الأبامكتين. أما معاملات العمر اليرقي الثالث مع المبيدين الكيماويين خفضت من قيم التركيز النصفي القاتل عدا مخاليط الأبامكتين مع العزلات المختلفة من *H. bacteriophora* مع تفوق الإيميدكلوبريد على الابامكتين عند الخلط.

علاوة على ذلك بلغت نسبة الموت 100٪ في العمر اليرقي الأول المعامل بتركيز 250 يرقة معدية/ يرقة جعال، من نيماتودا *H. bacteriophora* (HP88 strain) مقارنة بـ 92٪ موت عند معاملة يرقات العمر الثالث بنفس التركيز بعد المعاملة لفترة استمرت 4 أسابيع. لوحظ وجود تأثير تنشيطى على يرقات العمر الأول بعد 3 أسابيع من المعاملة بتركيز 150 يرقة معدية/ يرقة جعال عند الخلط بين *S. feltiae* (Filipjev) والجرعة الموصي بها من الأبامكتين والإيميداكلوبريد، كما ظهر أيضا التأثير التنشيطى في معاملة يرقات العمر الثالث المعاملة بنوعي النيماتودا *H. bacteriophora* (Ar-4 strain) و *S. carpocapsae* (All strain) عند خلطهم مع الأبامكتين بعد أسبوع واحد. كما لم يظهر أي تفاعل تنشيطي في مخاليط النيماتودا الممرضة للحشرات سواء مع الأبامكتين ولا الإيميداكلوبريد.

عند خلط المبيدات الكيميائية بعوامل المكافحة الحيوية يعتبر المبيد الكيميائي هو المتغير المستقل في المخلوط مؤثرا على حيوية ومقدرة النيماتودا على الإصابة التي تتأثر بفترة الخلط. كما يعتمد التفاعل البيولوجي بشكل أساسي على اختيارية المبيد الكيميائي وسميته على الحشرة المستهدفة (طور الحشرة والعمر اليرقى).