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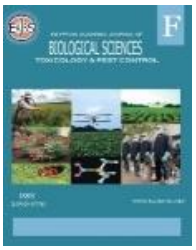

Combined Effect of Certain Entompathogenic Nematodes and Two Nematicides against Juveniles of *Eobania vermiculata* and *Monacha cartusinana* (Müller)

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ABSTRACT

Mortality of imported entomopathogenic nematodes, *Heterorhabditis bacteriophora* (HP88),*H. indica*, and *Steinernema carpocapsae* (All) compared with local EPNs isolates, *H. bacteriophora* (Ar-4), *H. bacteriophora* (Serag1), and *H. bacteriophora* (Ht) alone or combined with the recommended dose of abamectin and fenamiphos on juvenile mortality percentages of two land snail species, *Eobania vermiculata* (Müller) and *Monacha cartusinana* (Müller) have been studied in a series of laboratory experiments.

Results exhibited that, mortality percentages and combined effect in the two land snail species were obviously influenced by EPNs species/strains, concentrations and exposure time. Among EPNs, *H. bacteriophora* HP88, *H. indica*, and *H. bacteriophora* (Ar-4) achieved the highest means of mortality percentages (66.67 & 70.0, 65.33 & 68.67 and 54.67 & 62.0 %) after three weeks of exposure with *E. vermiculata* and *M. cartusinana*. Whereas, *S. carpocapsae* (All) achieved the least mortality means (46.0 & 49.33 %) respectively.

On the other hand, application of 500 IJs of EPNs conjunction with RD of abamectin and fenamiphos surpassed use EPNs or RD alone to reach (69.00, 70.00, 62.67 %), in fenamiphos and abamectin reached 71.33, 67.33 and 62.67 % in *E. vermiculata* with *H. bacteriophora* HP88, *H. indica* and *H. bacteriophora* (Ar-4), respectively.

While the parallel values with *M. cartusinana* were 81.33, 84.00, 76.00 % in fenamiphos treatments and 72.00, 46.67, 67.33 % with abamectin treatments. CF of the tested EPNs with nematicides and their response varied according to periods of exposures. Synergistic and additive effects were exposed with EPNs and tested nematicides after one week, whereas additive or antagonistic effects were recorded after two and three weeks with examined land snail species.

INTRODUCTION

Numerous species of land snails and slugs are important pests feeding on living plants in fields of vegetables and horticultural crops (Godan, 1983 and South, 1992). Their damage as a result of direct feeding as well as contamination of the harvested plants by faeces, slime, their eggs and bodies which play an important role in the deterioration of harvest, quality, financial loss, and its distribution in new areas which is exacerbated by

the fact that they are difficult to be controlled (Barker, 2002). For many decades the control of snails has relied predominantly on the application of chemical molluscicides by using bait pellets containing chelated iron phosphate, methiocarb, or metaldehyde which mixed with attractants and lead to stop feeding and ultimately die, but because of environmental contamination and the harm of non-target organisms, this control scheme is not maintainable (Bailey, 2002). So, many alternative methods were used to control slugs and snail pests.

In this respect, entomopathogenic nematodes (EPNs) of the genera *Steinernema* Chitwood and Chitwood (1937) and *Heterorhabditis* Poinar (1976) have shown great promise and have been successfully used in various countries to control insect pests that present at least one development stage in the soil (Grewal *et al*., 2001). Studies of the pathogenicity of entomopathogenic nematodes (EPNs) against terrestrial snails are relatively few. A study by Jaworska (1993) is one of few which described the susceptibility of *Deroceras agreste* (Linnaeus, 1758) and *Deroceras reticulatum* (Müller, 1774) to infection by three EPN species. Some of heterorhabditid and steinernematid were utilized as biological control agents against various insect pests. Nowadays, *Phasmarhabditis hermaphrodita*, isolated from gray garden slugs in England (Wilson *et al.*, 1993) has successfully been developed as a biological control agent under name of Nema Slug™ and show effectiveness against a wide range of economically important pest slugs and snail species (Coupland, 1995; Wilson and Gaugler, 2000).

Among the many approaches that have been investigated for controlling juveniles of land gastropod pests (snails and slugs) with relatively high susceptibility of the eggs and juveniles of slugs with some pesticides (Iglesias *et al.*, 2002). So, the aim of the present study was to evaluate the bioefficacy of imported EPNs with native isolates when applied lonely or combined with selected two nematicides against juveniles of certain land snail species, *Eobania vermiculata* and *Monacha cartusinana in vitro*.

MATERIALS AND METHODS

Pesticides Used:

The recommended dose of two commercial formulations of nematicides [(Tervigo 2% SC (abamectin), 3 liters/feddan and Dent 40% EC (fenamiphos), 6 liters/feddan] registered and available in the market used for controlling nematode pests in Egypt were obtained from the Central Laboratory of Pesticides, Dokki, Giza. And all current experiments were carried out in the Plant Protection Department, Faculty of Agriculture, Zagazig University.

Rearing of Tested Land Snail Species:

Immature stages (Juveniles), without reflexed white edge stripe, and less than 20 mm shell diameter in case of the brown garden snail, *Eobania vermiculata* (Müller). On the other hand, without chalky or white edge stripe and still glassy aperture ended moreover, it was less than 11 mm as a shell diameter in the case of the glassy clover snail, *Monacha cartusinana* (Müller) were collected from infested ornamental and field crops at Sharkia Governorate.

The tested snail species were kept in plastic containers filled with moist soil and daily fed on fresh leaves of lettuce (*Lactuca sativa* L.) to reared and laid in the laboratory from newly hatching to be reached their examined ages and kept the snails free from any infection by other parasites. Furthermore, Healthy snails only were lately used in the experiments.

Rearing of Entomopathogenic Nematodes (EPNs) on Greater Wax Moth, *Galleria mellonella* L.:

Last instar larvae of *Galleria mellonela* (Lepidoptera: Pyralidae) were used to isolate native nematode species belong to *Heterorhabditis* species from different areas of Sharkia and Ismalia governorates and El–Arish, Egypt with *G. mellonella* as trap insects (Schroeder *et al.* 1994), and modified after Akhurst and Bedding (1975). All nematodes were cultured *in vivo* at 24±2°C in *G. mellonella* larvae according to kaya and Stock, 1997. Fresh obtained infective juveniles from *G. mellonella* larvae were washed in three changes of distilled water and directly used in all experiments.

Three imported entomopathogenic nematodes, *Heterorhabditis bacteriophora* (HP88 strain), *Heterorhabditis indica* and *Steinernema carpocapsae* (All strain), and three local strains, *H. bacteriophora* (Ar-4 strain), *H. bacteriophora* (Serag1 strain), and *H. bacteriophora* (Ht strain) isolated by El-Ashry *et al.*,2018 with baiting technique of *G. mellonella*. Juveniles of, *E. vermiculata* and *M. cartusinana*, were separated as groups into plastic boxes (12×24×14 cm) for experimental definition. Small plastic boxes (9 x14 x 6 cm) were lined on the inside walls with tapes to prevent snails from moving and still in contact with nematode species. Ten small ventilation holes were made in the lids, and the plastic cages were lined with filter paper (Whatmann No.1). Stock suspension of infective juveniles (IJs) of tested EPNs was adjusted to 2000 IJs/ml to prepare five used concentrations. Infection was induced by exposure of juveniles to five aqueous suspensions adjusted to 250, 500, 750, 1000, and 1250 IJs applied directly in 1.5 ml of water to moist filter paper.

Tested juveniles were fed on lettuce leaf disk and observed daily for mortality up to 4 weeks but only tables contain data of one, two, and three weeks. Each screening or treatment consisted of five replicates consisting of 10 juveniles for each nematode species or strain. The control treatment was prepared using distilled water only and all treatments were incubated at 25±3˚C. Snails cadavers were placed in White traps (White, 1927) to confirm symptoms of infections and recover of infective juveniles and verification of completion of the cycle of EPNs in the snail. Mortality percentages were calculated according to the following formula:

$$\text{Mortality (\%)} = \frac{\text{Number of Dead Juveniles}}{\text{Total Number of Tested snails}} \times 100$$

Statistical Analysis:

The experiments were carried out in a completely randomized design with 3 replications for each treatment. Data were subjected to analysis of variance (ANOVA) using MSTAT version4 (1987). Means were compared by Duncan's multiple range test (Duncan, 1955) at $P \leq 0.05$.

Combining Effect of Entomopathogenic Nematodes (EPNs) and Two Nematicides:

The two nematicides Tervigo 2% SC and Dent 40 % EC were tested for their effectiveness against juveniles of, *E. vermiculata* and *M. cartusinana* as concomitant treatment (leaf dipping + soil incorporation) (Genena and Mostafa, 2008). Mentioned small plastic cages provided with immature juvenile snails treated with 500 IJs of EPNs/juvenile snail feed on similar leaf discs of fresh lettuce leaves which immersed for 10 seconds in recommended dose (RD) of the tested nematicides then left to air dry before application. Control treatment individuals were received fresh untreated leaves and without any of the tested EPNs infective juveniles. Each treatment was replicated five times. After 24 hrs. of treatment, the treated leaves were replaced daily with fresh untreated lettuce leaves for three weeks. Snails cadavers were placed in white traps (White, 1927) to recover any of IJs of EPNs. The tested snails were examined daily and mortality percentages were calculated after one, two, and three weeks of treatment

according to the following formula:

$$\text{Mortality (\%)} = \frac{\text{Number of Dead Juveniles}}{\text{Total Number of Tested snails}} \times 100$$

Analysis of Interaction Data of Mixtures between Tested Nematodes and Nematicides:

Interaction data for mixtures between tested nematodes and nematicides were assessed using Limpel's formula reported by Richer (2006) as follows:

$$E = (X + Y) - (XY)/100$$

Where:

E: The expected additive effect of the mixture.

X: The effect due to component A alone.

Y: The effect due to component B alone.

The expected effect was compared with the actual effect obtained experimentally from the mixture of component A alone and component B alone to determine the additive, antagonistic or synergistic effects, according to the equation given by Mansour *et al*., 1966 as follows:

$$\text{Co - Toxicity Factor} = \frac{\text{Observed effect (\%)} - \text{Expected effect (\%)}}{\text{Expected effect (\%)}} \times 100$$

The equation was used to categorize results into three classes. A positive factor 20 or more is considered potentiation, a negative factor 20 or more means antagonism and intermediate values between -20 and +20 indicate only additive effect.

Statistical Analysis:

The experiments were carried out in a completely randomized design in the laboratory. Data were subjected to analysis of variance (ANOVA) one way or two-way using MSTAT version 4 (1987). Means were compared by Duncan's multiple range test at $P \leq 0.05$ probability.

RESULTS

All mortality percentages of the tested entomopathogenic nematodes (EPNs) were obtained between one and three weeks after the starting of the treatments. Recovery of infected juveniles for snail species as dead was never observed.

Susceptibility of the Brown Garden Snail, *Eobania vermiculata* Müller:

Laboratory studies were conducted to evaluate the effectiveness of different entomopathogenic nematode species against juveniles of *E. vermiculata* Müller. A series of bioassays were done to test the susceptibility of juveniles to six species of imported and native (EPNs) *Heterorhabditis indica*,*H. bacteriophora* (HP88), *Steinernema carpocapsae* (All), *H. bacteriophora* (Ar-4),*H. bacteriophora* (Serag1), and *H. bacteriophora* (Ht) *in vitro*.

Results of the bioassay showed that the tested nematodes were infective with five nematode concentrations and strains causing significant mortality ($P \leq 0.05$) greater than in untreated Juveniles snail (Table 1). After one week of exposure, at inoculum levels of 750 and 1000IJs, *H. indica* gained mortality percentage 16.0 and 18.0 % followed by *H.bacteriophora* (HP88), *H. bacteriophora* (Ar-4) and *H. bacteriophora* (Serag1), with values of (10.0 & 18.0 %), (10.0 & 18.0 %) and (10.0 & 16.0 %), respectively. Whereas, *H. bacteriophora* (Ht) and *S. carpocapsae* (All) gave the least mortality percentages with values 10 & 12 % and 4.0 & 10.0 %, respectively. Mortality percentages were increased by increasing inoculum levels and exposure time to reach the maximum mortality percentages after three weeks of exposure.

After 3 weeks, mortality percentages reached to 100% with the two inoculum

levels 1000 IJs/juvenile and 1250 IJs/Juvenile with *H. indica*, followed by *H. bacteriophora* (HP88) with values 94.0 and 100 %. While, *S. carpocapsae* (All) showed the least values (68.0 and 70 %) According to the mortality percentage mean, at inoculum level 1250 IJs/juvenile, imported heterorhabditid species gave the best mortality mean % in relation to local species. Values of mortality percentage mean for *H. indica, H. bacteriophora* (HP88), *H. bacteriophora* (Ar-4), *H. bacteriophora* (Serag1) and *H. bacteriophora* (Ht) recorded 65.33, 66.67, 54.67, 48.67, and 48.67, respectively. Comparing the mortality percentage in *E. vermiculata* treated with *S. carpocapsae* (All) showed markedly the least percentage compared to heterorhabditid species.

Table 1: Mortality percentages in juveniles of *Eobania vermiculata* after exposure to five levels of imported and local entomopathogenic nematodes.

Nematode species	Inoculum levels	Mortality percentages (TM %) after			
		One week	Two weeks	Three weeks	Mortality mean %
H. indica	250IJs/Snail	0.0 g	40.0 gh	60.0 hi	33.33
	500IJs/Snail	6.0 ef	50.0 de	70.0 ef	44.67
	750IJs/Snail	16.0 abc	56.0 c	78.0 cd	50.00
	1000IJs/Snail	18.0 ab	76.0 a	100.0 a	64.67
	1250IJs/Snail	16..0 abc	80.0 a	100.0 a	65.33
H. bacteriophora (HP88)	250IJs/Snail	0.0 g	32.0 ij	48.0 1	26.67
	500IJs/Snail	4.0 fg	44.0 fg	62.0 hi	36.67
	750IJs/Snail	10.0 de	52.0 cd	74.0 de	45.33
	1000IJs/Snail	18.0 ab	76.0 a	94.0 b	62.67
	1250IJs/Snail	20.0 a	80.0 a	100.0 a	66.67
S. carpocapsae (All)	250IJs/Snail	0.0 g	26.0 k	40.0 n	22.0
	500IJs/Snail	0.0 g	30.0 jk	46.0 lm	25.33
	750IJs/Snail	4.0 fg	40.0 gh	58.0 ij	34.0
	1000IJs/Snail	10.0 de	46.0 ef	68.0 fg	41.33
	1250IJs/Snail	14.0 bcd	54.0 cd	70.0 ef	46.0
H. bacteriophora (Ar-4)	250IJs/Snail	0.0 g	20.0 1	42.0 mn	20.67
	500IJs/Snail	4.0 fg	36.0 hi	58.0 ij	32.67
	750IJs/Snail	10.0 de	46.0 ef	68.0 fg	41.33
	1000IJs/Snail	18.0 ab	52.0 cd	78.0 cd	49.33
	1250IJs/Snail	20.0 a	62.0 b	82.0 c	54.67
H. bacteriophora (Serag1)	250IJs/Snail	0.0 g	20.0 1	34.0 o	18.0
	500IJs/Snail	4.0 fg	32.0 ij	54.0 jk	30.0
	750IJs/Snail	10.0 de	40.0 gh	60.0 hi	36.67
	1000IJs/Snail	16.0 abc	46.0 ef	68.0 g	43.33
	1250IJs/Snail	18.0 ab	50.0 de	78.0 cd	48.67
H. bacteriophora (Ht)	250IJs/Snail	0.0 g	20.0 1	32.0 o	17.33
	500IJs/Snail	4.0 fg	32.0 ij	50.0 kl	28.67
	750IJs/Snail	10.0 de	42.0 fg	58.0 ij	36.67
	1000IJs/Snail	12.0 cd	50.0 de	64.0 gh	42.00
	1250IJs/Snail	14.0 bcd	56.0 c	76.0 d	48.67
Control (without nematode IJs)		**0.0 g**	**0.0 m**	**0.00 p**	**0.00**

Each value is a mean of five replicates.
Values followed by the same letter (s) in the same column are not different according to Duncan's multiple range test ($P \leq 0.05$).

Susceptibility of the Glassy Clover Snail, *Monacha cartusinana* Müller:

Results obtained from the tested EPNs against the glassy clover snail, *Monacha cartusinana* Müller were illustrated in Table (2). *M. cartusinana* juveniles showed the same trend in mortality percentage after one week when treated with five inoculum levels of EPNs. After one week of exposure, at inoculum levels of 500 and 750 IJs, percentage mortality did not differ between local heterorhabditid species *H. bacteriophora* (Ar-4), *H. bacteriophora* (Ht), and *H. bacteriophora* (Serag1). Whereas, *H. indica* gained the height mortality percentage (6.0 & 16.0%), after two weeks of exposure.

The mortality percentage of *M. cartusinana* juveniles treated with 1250 IJs/juvenile of *H. indica* and *H. bacteriophora* (HP88) increased to reach 90.0 % for each, followed by *H. bacteriophora* (Ar-4) with values of 72.0 % and *H. bacteriophora* (Serag 1) with a value of 62.0%, then *S. carpocapsa* (All) nematode with value 54.0 %. After 3 weeks, mortality percentages reached 100% only with the two inoculum levels 1000 IJs/ juvenile and 1250 IJs/Juvenile with *H. indica* and *H. bacteriophora* (HP88).

At inoculum level 1250 IJs/ juvenile, mortality percentages with local EPNs species recorded 94.0, 88.0, and 84.0 % with *H. bacteriophora* (Ar-4), *H. bacteriophora* (Serag1), and *H. bacteriophora* (Ht), respectively. Steinernematid species, *S. carpocapsae* (All) was the least effective than heterorhabditid ones with a mortality percentage of 80.0 %. The mortality mean % at 1250 IJs/ juvenile were 68.67, 70.0, 49.33, 62.0, 56.0, and 54.67% for *H. indica*, *H. bacteriophora* (HP88), *S. carpocapsae* (All), *H. bacteriophora* (Ar-4), *H. bacteriophora* (Serag1) and *H. bacteriophora* (Ht)**,** respectively. Mortality mean percentages ranged from 42.67 to 68.67 % for *H. indica*, 33.33 to 70.0 % for *H. bacteriophora* (HP88), 27.33 to 49.33 % for *S. carpocapsae* (All), 35.0 to 62.0 % for *H. bacteriophora* (Ar-4), 23.33 to 56.0 % for *H. bacteriophora* (Serag1) and 23.33 to 54.67 % for *H. bacteriophora* (Ht), respectively. *S. carpocapsae* (All) exhibited the least mortality percentages against juvenile of the land snail, *M. cartusinana*. Also, *M. cartusinana* was less sensitive to tested entomopathogenic nematodes than the *E. vermiculata.*

Table 2: Mortality percentages in juveniles of *Monacha cartusinana* after exposure to five levels of imported and local entomopathogenic nematodes.

Nematode species	Inoculum levels	Mortality percentages (TM %) after			
		One week	Two weeks	Three weeks	Mortality mean %
H. indica	250IJs/Snail	0.00 e	52.0 efg	76.0 ef	42.67
	500IJs/Snail	6.0 de	62.0 cd	80.0 de	49.33
	750IJs/Snail	16.0 ab	66.0 bc	88.0 c	56.67
	1000IJs/Snail	18.0 a	86.0 a	100.0 a	68.0
	1250IJs/Snail	16.0 ab	90.0 a	100.0 a	68.67
H. bacteriophora (HP88)	250IJs/Snail	0.00 e	42.0 hi	58.0 k	33.33
	500IJs/Snail	4.0 de	54.0 ef	72.0 fg	43.33
	750IJs/Snail	10.0 bcd	62.0 cd	84.0 cd	52.0
	1000IJs/Snail	18.0 a	86.0 a	100.0 a	68.0
	1250IJs/Snail	20.0 a	90.0 a	100.0 a	70.0
S. carpocapsae (All)	250IJs/Snail	0.00 e	34.0 jk	48.0 l	27.33
	500IJs/Snail	0.00 e	36.0 ij	48.0 l	28.0
	750IJs/Snail	4.0 de	46.0 gh	64.0 ij	38.0
	1000IJs/Snail	10.0 bcd	48.0 fgh	78.0 e	45.33
	1250IJs/Snail	14.0 abc	54.0 ef	80.0 de	49.33
H. bacteriophora (Ar-4)	250IJs/Snail	8.0 cd	42.0 hi	55.0 k	35.0
	500IJs/Snail	4.0 de	52.0 efg	70.0 gh	42.0
	750IJs/Snail	10.0 bcd	56.0 de	76.0 ef	47.33
	1000IJs/Snail	18.0 a	68.0 bc	88.0 c	58.0
	1250IJs/Snail	20.0 a	72.0 b	94.0 b	62.0
H. bacteriophora (Serag1)	250IJs/Snail	0.00 e	28.0 k	42.0 m	23.33
	500IJs/Snail	4.0 de	42.0 hi	66.0 hi	37.33
	750IJs/Snail	10.0 bcd	46.0 gh	66.0 hi	40.67
	1000IJs/Snail	16.0 ab	58.0 de	78.0 e	50.67
	1250IJs/Snail	18.0 a	62.0 cd	88.0 c	56.0
H. bacteriophora (Ht strain)	250IJs/Snail	0.00 e	28.0 k	42.0 m	23.33
	500IJs/Snail	4.0 de	42.0 hi	60.0 jk	35.33
	750IJs/Snail	10.0 bcd	46.0 gh	66.0 hi	40.67
	1000IJs/Snail	16.0 ab	58.0 de	78.0 e	50.67
	1250IJs/Snail	18.0 a	62.0 cd	84.0 cd	54.67
Control (without nematode IJs)		**0.00 e**	**0.00 l**	**0.00 n**	**0.0**

Each value is a mean of five replicates.
Values followed by the same letter (s) in the same column are not different according to Duncan's multiple range test ($P \leq 0.05$).

Combination of Selected EPNs with Two Nematicides:

Mortality percentages of the two land snail species, *M. cartusinana* and *E. vermiculata* were stated after one week of exposure to 500 IJs/juvenile. Based on these results, the combination between inoculum level and recommended dose (RD) of two nematicides, abamectin, and fenamiphos were selected to increase mortality percentages in juveniles of the two land snail species *in vitro*.

A- Effectiveness against *M. cartusinana:*

Results in Table (3) demonstrated the mortality in juveniles of *Eobania vermiculata* after exposure to the recommended dose (RD) of two nematicides lonely or combined with imported and local entomopathogenic nematodes after one, two, and three weeks.

Results revealed that, after one week of treatment, mortality percentages resulted from RD of fenamiphos and abamectin were 26.0 % for each whereas, mortality percentage increased in land snail juvenile treated with mixed EPNs species with RD of fenamiphos to reach 50.0, 48.0, 33.0, 40.0, 32.0 and 38.0 % with *H. indica*, *H. bacteriophora* (HP88), *S. carpocapsae* (All), *H. bacteriophora* (Ar-4), *H. bacteriophora* (Serag1) and *H. bacteriophora* (Ht), respectively.

The parallel values with RD of abamectin after one week recorded 50.0, 52.0, 28.0, 40.0, 34.0, and 34.0 % with the abovementioned EPNs, respectively. After two weeks, the mortality percentage increased in femamiphos and abamectine alone to 50.0 and 54.0 % respectively. Moreover, an increase was observed in treatments of mixed nematicides and nematode species. In fenamiphos treatments, the highest significant mortality was obtained with fenamiphos + *H. indica*, fenamiphos + *H. bacteriophora* (HP88), and fenamiphos + *H. bacteriophora* (Ar-4) with values of 73.0, 70.0, and 63.0%. On the hand, in abamectin treatments, mortality percentages were 68.0, 72.0 and 66.0 % with abamectin + *H. indica*, abamectin + *H. bacteriophora* (HP88) and abamectin + *H. bacteriophora* (Ar-4), respectively. After three weeks of treatment, fenamiphos + *H. bacteriophora* (HP88) and abamectin + *H. bacteriophora* (HP88) recorded the highest significant mortality 89.0 and 90.0%, respectively.

However, abamectin mixed with *S. carpocapsae* (All) caused lower significant mortality (66.0%), followed by fenamiphos (73.0%). Mortality percentage showed insignificant differences ($P \leq 0.05$) between *H. indica* and *H. bacteriophora* (HP88) with the three-time exposure when mixed with fenamiphos while, significant differences were observed in treatments of abamectin after three weeks of exposure.

B- Effectiveness against *E. vermiculata:*

Results in Table (4) showed insignificant differences ($P \leq 0.05$) between *H. indica* and *H. bacteriophora* (HP88) after one, two, and three weeks of treatment. Mortality percentages resulted from RD of fenamiphos with *H. indica* and *H. bacteriophora* (HP88) recorded 66.0&62.0, 86.0&82.0, and 100&100% after one, two, and three weeks of exposure with mean mortality percentage, 84.0 and 81.33 %, respectively. The same trend was observed with RD of abamectin with *H. indica* and *H. bacteriophora* (HP88) and the parallel values were 54.0 & 52.0 and 72.0 &72.0 after one and two weeks of exposure. On contrarily, *H. bacteriophora* (HP88) gave higher percent mortality than *H. indica* when mortality mean percentages were 46.67 and 72.00 %, respectively. The two local heterorhabditid species *H. bacteriophora* (Ar-4), *H. bacteriophora* (Serag1) showed efficacy against juvenile of *E. vermiculata* with a percent mortality of 92.0 and 84.0% in mixed treatments with fenamiphos and 86.0 and 80.0 % in mixed treatments with abamectin. *S. carpocapsae* (All) when mixed with fenamiphos and abamectin exhibited the least mortality percentages against juvenile of the land snail, *E. vermiculata* after one, two, and three weeks (46.0

& 36.0 %), (66.0 & 56.0 %) and (86.0 & 74.0 %) with mean of mortality percentage 66.00 and 55.33 %, respectively.

Table 3: Mortality percentages in juveniles of *Eobania vermiculata* after exposure to the recommended dose (RD) of two nematicides lonely or combined with imported and local entomopathogenic nematodes**.**

Nematode species / nematicides	Treatment levels	Mortality percentages (TM %) after			Mortality mean %
		One week	Two weeks	Three weeks	
Fenamiphos	**RD alone**	**26 d**	**50 d**	**70 c**	**48.67**
Fenamiphos and *H.indica*	RD + 500 IJs/Snail	50 a	73 a	87 a	70.00
Fenamiphos and *H.bacteriophora* (HP88)	RD + 500 IJs/Snail	48 a	70 a	89 a	69.00
Fenamiphos and *S. carpocapsae* (All)	RD + 500 IJs/Snail	33 c	54 cd	73 bc	53.33
Fenamiphos and *H. bacteriophora* (Ar-4)	RD + 500 IJs/Snail	40 b	63 b	85 a	62.67
Fenamiphos and *H. bacteriophora* (Serag1)	RD + 500 IJs/Snail	32 c	55 cd	74 bc	53.67
Fenamiphos and *H. bacteriophora* (Ht)	RD + 500 IJs/Snail	38 b	58 bc	76 b	57.33
Mortality mean %		**38.14**	**60.42**	**79.14**	
Abamectin	**RD alone**	**26 d**	**54 d**	**72 c**	**50.67**
Abamectin and *H.indica*	RD + 500 IJs/Snail	50 a	68 ab	84 b	67.33
Abamectin and *H.bacteriophora* (HP88)	RD + 500 IJs/Snail	52 a	72 a	90 a	71.33
Abamectin and *S. carpocapsae* (All)	RD + 500 IJs/Snail	28 d	46 e	66 d	46.67
Abamectin and *H. bacteriophora* (Ar-4)	RD + 500 IJs/Snail	40 b	66 b	82 b	62.67
Abamectin and *H. bacteriophora* (Serag1)	RD + 500 IJs/Snail	34 c	60 c	76 c	56.67
Abamectin and *H. bacteriophora* (Ht)	RD + 500 IJs/Snail	34 c	52 d	72 c	52.67
Mortality mean %		**37.71**	**55.42**	**77.42**	

Each value is a mean of five replicates.
Values followed by the same letter (s) in the same column are not different according to Duncan's multiple range test ($P \leq 0.05$).

Table 4: Mortality percentages in juveniles of *Monacha cartusinana* after exposure to the recommended dose (RD) of two nematicides lonely or combined with imported and local entomopathogenic nematodes.

Nematode species / nematicides	Treatment levels	Total Mortality percentages (TM %) after			Mortality mean %
		One week	Two weeks	Three weeks	
Fenamiphos	**RD alone**	**36 d**	**56 f**	**78 d**	**56.67**
Fenamiphos and *H.indica*	RD + 500 IJs/Snail	66 a	86 a	100 a	84.00
Fenamiphos and *H. bacteriophora* (HP88)	RD + 500 IJs/Snail	62 ab	82 ab	100 a	81.33
Fenamiphos and *S. carpocapsae* (All)	RD + 500 IJs/Snail	46 c	66 de	86 c	66.00
Fenamiphos and *H. bacteriophora* (Ar-4)	RD + 500 IJs/Snail	58 b	78 bc	92 b	76.00
Fenamiphos and *H. bacteriophora* (Serag1)	RD + 500 IJs/Snail	44 c	64 e	84 c	64.00
Fenamiphos and *H. bacteriophora* (Ht)	RD + 500 IJs/Snail	46 c	72 cd	82 cd	66.67
Mortality mean %		**51.14**	**72.00**	**88.85**	
Abamectin	**RD alone**	**36 c**	**54 b**	**72 d**	**54.00**
Abamectin and *H.indica*	RD + 500 IJs/Snail	54 a	72 a	84 b	46.67
Abamectin and *H.bacteriophora* (HP88)	RD + 500 IJs/Snail	52 ab	72 a	92 a	72.00
Abamectin and *S. carpocapsae* (All)	RD + 500 IJs/Snail	36 c	56 b	74 cd	55.33
Abamectin and *H. bacteriophora* (Ar-4)	RD + 500 IJs/Snail	46 b	70 a	86 ab	67.33
Abamectin and *H. bacteriophora* (Serag1)	RD + 500 IJs/Snail	36 c	60 b	80 bc	58.67
Abamectin and *H. bacteriophora* (Ht)	RD + 500 IJs/Snail	36 c	56 b	72 d	44.33
Mortality mean %		**42.28**	**62.85**	**80.28**	

Each value is a mean of five replicates.
Values followed by the same letter (s) in the same column are not different according to Duncan's multiple range test ($P \leq 0.05$).

Analysis of Mixtures between Tested EPNs and Nematicides:

A- *In vitro* CF of EPNs with Nematicides and Response in Controlling of *E. vermiculata*:

After one week of treatment, all EPNs *H. indica*, *H. bacteriophora* (HP88), *S.carpocapsae* (All) *H. bacteriophora* (Ar-4), and *H. bacteriophora* (Serag1) and *H. bacteriophora* (Ht) exhibited synergistic interaction in treatments of fenamiphos. While, synergistic interaction showed with tested EPNs except for *S. carpocapsae* (All), and *H. bacteriophora* (Serag1) showed an additive effect with abamectin (Table 5). After two weeks, an additive effect was observed with all EPNs species in fenamiphos treatment and antagonism interaction was observed when *S. carpocapsae* (All) and *H. bacteriophora* (Serag1) mixed with abamectin in controlling juveniles of *E. vermiculata*.

After three weeks, the antagonistic effect was observed in all treatments of EPNs mixed with RD of fenamiphos and abamectin except *H. indica* with fenamiphos and *H. bacteriophora* (HP88) with abamectin which showed an additive effect.

Table 5: Interactions between fenamiphos and abamectin with different entomopathogenic nematodes strains on mortality of *Eobania vermiculata* under laboratory conditions.

Chemical pesticides	Nematode species	Co-toxicity factor (CF) and response after		
		One week	Two weeks	Three weeks
Fenamiphos	*H. indica*	+64.25 synergism	-2.66 additive	-19.78 additive
	H. bacteriophora (HP88)	+65.74 synergism	-16.92 additive	-20.99 antagonism
	S. carpocapsae (All)	+26.92 synergism	-16.92 additive	-20.99 antagonism
	H. bacteriophora (Ar-4)	+38.12 synergism	-7.35 additive	-35.56 antagonism
	H. bacteriophora (Serag1)	+10.49 additive	-16.66 additive	-36.19 antagonism
	H. bacteriophora (Ht)	+31.21 synergism	-12.12 additive	-31.76 antagonism
Abamectin	*H. indica*	+64.25 synergism	-9.33 additive	-26.09 antagonism
	H. bacteriophora (HP88)	+79.55 synergism	-6.49 additive	-19.42 additive
	S. carpocapsae (All)	+7.69 additive	-32.15 antagonism	-45.80 antagonism
	H. bacteriophora (Ar-4)	+38.12 synergism	-6.37 additive	-25.20 antagonism
	H. bacteriophora (Serag1)	+17.40 additive	-12.68 additive	-31.12 antagonism
	H. bacteriophora (Ht)	+3.76 synergism	-24.33 antagonism	-39.53 antagonism

Each value is a mean of five replicates.
Values followed by the same letter (s) in the same column are not different according to Duncan's multiple range test ($P \leq 0.05$).

B- *In vitro* CF of EPNs with Nematicides and Response in Controlling of *M. cartusinana*:

The same trend was observed after one week of treatment, tested EPNs exhibited synergistic interaction in treatments of fenamiphos but only *H. bacteriophora* (Serag1) showed an additive effect. While additive effect displayed with tested nematodes except with *H. indica* and *H. bacteriophora* (HP88) which showed synergistic interaction. After two weeks, an additive effect was found in all mixed RD of fenamiphos and abamectin except with *S. carpocapsae* (All), and *H. bacteriophora* (Ht)) treatments which showed anatagonistic effect. Also, after three weeks, an additive effect was observed with all EPNs species mixed with RD of fenamiphos and abamectin, and the only antagonistic effect was observed between *H. bacteriophora* (Ht) and abamectin.

The exposure to *H. indica* alone resulted in 70 and 80 % mortality in *E. vermiculata* and *M. cartusinana*, with the highest rate observed in the second week then

third weeks after exposure (Fig. 1 & 2). This percent mortality gradually decreased according to nematode species and strains. Changes in percent mortality of the infected snails occurred, characterized by a significant decrease in juveniles of *E. vermiculata* compared to *M. cartusinana.*

Variation in the effectiveness of nematode species was also observed after mixing with abamectin and fenamiphos nematicides. In this respect, the infection-induced a sharp increase with *H. indica* and *H. bacteriophora* (HP88) to reach 100 % with fenamiphos after 3 weeks of exposure in juveniles of *M. cartusinana*. As well as, juveniles of *E. vermiculata* showed less susceptibility to infect with nematode and mixed nematicides.

Additionally, least mortality percent was found in the two tested land snail species with *S.carpocapsae* (All) when used alone or mixed with abamectin and fenamiphos, and *H. bacteriophora* (Ar-4) exposed relatively high mortality percent as compared with *S.carpocapsae* (All) and nearby to *H. indica* in *E. vermiculata*.

Table 6: Interactions between fenamiphos and abamectin with different entomopathogenic nematode strains on mortality of *Monacha cartusinana* under laboratory conditions.

Chemical pesticides	Nematode species	Co-toxicity factor (CF) and response		
		One week	Two weeks	Three weeks
Fenamiphos	*H. indica*	+71.16 synergism	+15.46 additive	+8.08 additive
	H. bacteriophora (HP88)	+60.78 synergism	+2.80 additive	+9.17 additive
	S. carpocapsae (All)	+27.77 synergism	-8.12 additive	+1.89 additive
	H. bacteriophora (Ar-4)	+34.85 synergism	-1.11 additive	+10.98 additive
	H. bacteriophora (Serag1)	+14.10 additive	-14.07 additive	-6.45 additive
	H. bacteriophora (Ht)	+40.04 synergism	-9.72 additive	-10.48 additive
Abamectin	*H. indica*	+40.04 synergism	-1.80 additive	-7.16 additive
	H. bacteriophora (HP88)	+34.85 synergism	-8.67 additive	-0.17 additive
	S. carpocapsae (All)	+0.0 additive	-31.97 antagonism	-13.38 additive
	H. bacteriophora (Ar-4)	+19.29 additive	-10.16 additive	-6.11 additive
	H. bacteriophora (Serag1)	-6.63 additive	-18.16 additive	-11.58 additive
	H. bacteriophora (Ht)	-6.63 additive	-28.97 antagonism	-39.53 antagonism

Each value is a mean of five replicates.
Values followed by the same letter (s) in the same column are not different according to Duncan's multiple range test ($P \leq 0.05$).

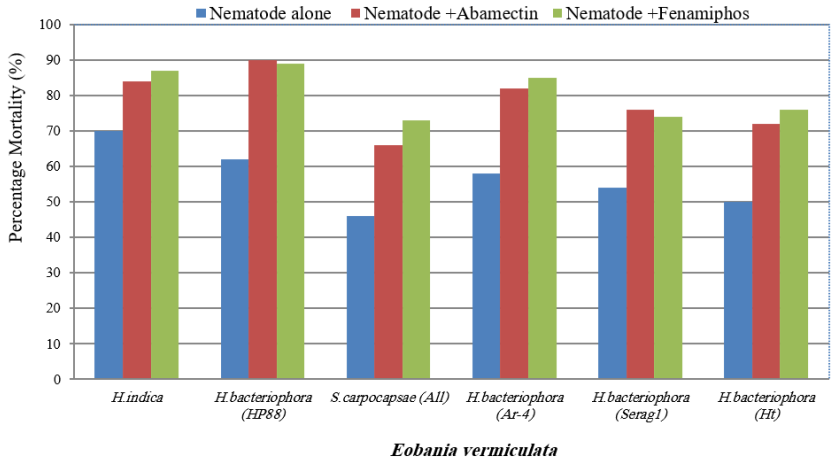

Fig.1: Mortality percentages from mixing chemical nematicides with imported and local entomopathogenic nematode species in controlling of *Eobania vermiculata.*

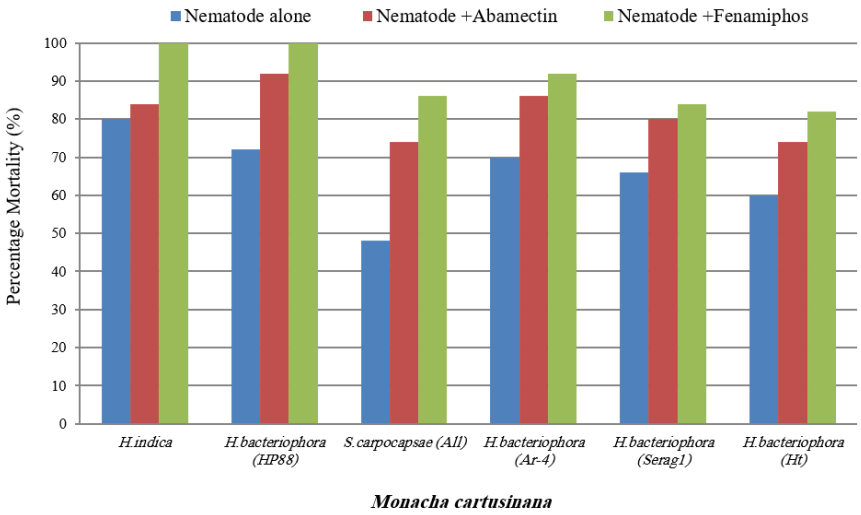

Fig. 2: Mortality percentages from mixing chemical nematicides with imported and local entomopathogenic nematode species in controlling of *Monacha cartusinana*.

DISCUSSION

The results of this study indicated that all examined juveniles of the brown garden snail, *E. vermiculata* Müller, and glassy clover snail, *M. cartusinana* Müller were infected by all the imported and local tested entomopathogenic nematodes alone or mixed with the recommended dose of abamectin and fenamiphos.

Two nematicides caused a fluctuated effect between decrease and increase for the two snail species. Moreover, *M. cartusinana* was the most susceptible to the five inoculum levels of tested EPNs than *E. vermiculata*. Additionally, the two imported EPNs, *H. indica, H. bacteriophora* (HP88) were the most effective ones followed by local nematodes *H. bacteriophora* (Ar-4). Whereas, *S. carpocapsae* (All) and *H. bacteriophora* (Ht) were the least effective against the two land snail species when used alone or mixed with RD of abamectin and fenamiphos. CF of the tested EPNs with nematicides and their response varied after the three periods of exposures. After one week of exposure, synergistic and additive effects were showed with abamectin and fenamiphos with all tested EPNs in juveniles of *E. vermiculata* and *M. cartusinana.* After two and three weeks of exposure, additive or antagonistic effects were obviously recorded.

The present results are agreement with Genena and Mostafa **(**2013) who reported that, heterrhabditid EPNs (*H. indica* & *H. bacteriophora* (HP88) achieved more percent mortality with *E. vermiculata* and *M. cartusinana* than *Steinernema* spp. (*S. carpocapsae* (All) and *M. cartusinana* was more sensitive to entomopathogenic nematodes than the *E. vermiculata.* Likewise, Georgis and Gaugler (1991) mentioned to the better performance of *Heterorhabditis* spp. than *Steinernema* spp. Likewise, suppressive effects of polish isolates of EPNs were confirmed on *Derocearas reticulatum* and *D. agresta* were able to reproduce within slug cadavers (Jaworska,1993) or against *D. reticulatum* or *Limax marginatus e.g. H. bacteriophora, H. marelatus, S. carpocapsae, S. glaseri, S. kushidai, S. longicaudum, S. oregonense, S. riobrave* and *S. Siamkayai* (Kaya, 2001).

In the laboratory, soil treatment with the recommended rate of *P. hermaphrodita* caused significant mortality only for the snail *M. cantiana* and the susceptible slug *Deroceras reticulatum* (Wilson *et al*., 2000). The local and imported EPNs (*S. carpocapsae* All strain, *H. bacteriophora* H88 strain, *H. bacteriophora* Serag1 strain, and

H. bacteriophora Ht strain) belonging to steinernematids and heterorhabditids were investigated against two terrestrial slugs, *D. reticulatum* than *D. leave* (El-Ashry and Abd El-Aal, 2019). However, Glen and Coupland (2017) mentioned that slug parasitic nematode, *P. hermaphrodita* was effective as a biocontrol agent.

The synergistic, additive, and negative effect of abamectin and fenamiphos rates on *S. carpocapsae* were confirmed (Kary *et al.,* 2018), the exposure time (after two and three weeks) with abamectin plays the vital role in interaction type which varied from additive to antagonistic effect with nematode species and strains. Previous studies revealed that the chemical pesticides showed a strong sublethal effect on *S. carpocapsae* and *H. indica* reproductive potential and limiting their possible recycling under field conditions (Devindrappa *et al*., 2017). Feasibility of combinations and integrated use of these nematode species and nematicides in plant protection could be managed (Rovesti and Deseö,1990) by optimizing the dosage of tested pesticides and concentration of IJs depend on the interaction results *in vitro* treatments (Gutiérrez *et al*., 2008, El-Ashry *et al*., 2020).

CONCLUSION

The mortality percentages of the two tested land snail species *E. vermiculata* and *M. cartusinana* varied according to EPNs species, strains, and concentrations. As well as, nematicides varied in their effect according to pesticides and time of exposure. Therefore, as a precaution, mixing IJs of EPNs with pesticides in controlling juveniles of land snail species can be included after one or two weeks of applying pesticides to avoid adverse effects and also ensure sustainability.

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ARABIC SUMMARY

التأثير المشترك لبعض أنواع النيماتودا المتطفلة على الحشرات واثنين من المبيدات النيماتودية على الأطوار غير البالغة للقوقعي الحدائق البني *Eobania vermiculata* والبرسيم الزجاجي *Monacha cartusinana*

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قورنت نسب الموت الناتجة عن استخدام النيماتودا المتطفلة على الحشرات (الأنواع المستوردة *Heterorhabditis bacteriophora* (HP88), *H. indica* and *Steinernema carpocapsae* (All)) مع تلك الناتجة من الأنواع المحلية *H. bacteriophora* (Ar-4), *H. bacteriophora* (Serag1) and *H. bacteriophora* (Ht) بمفردها أو مشتركة مع التركيز الموصى به للمبيدين النيماتودين الأبامكتين والفيناميفوس وذلك على الأطوار غير البالغة (الصغار) لنوعين من القواقع الأرضية هما قوقع الحدائق البني *Eobania vermiculata* (Müller) و قوقع البرسيم الزجاجي *Monacha cartusinana* (Müller) فى سلسلة من الاختبارات المعملية.

أظهرت النتائج تأثيرات معنوية علي كل من النسبة المئوية للموت والتأثير المشترك على نوعي القواقع الارضية المختبرة بنوع وسلالة وتركيزات نيماتودا الحشرات المستخدمة وفترة التعريض ، ولقد أظهرت الأنواع *H. bacteriophora* HP88 و *H. indica* و *H. bacteriophora* (Ar-4) أعلى متوسط لنسبة الموت علي القواقع المختبرة بعد ثلاثة أسابيع من التعريض ومقداره (66.67 & 70.0 و 65.33&68.67 و 54.67 & 62.0 %) لكلا النوعين *E. vermiculata* و*M. cartusinana* *علي* الترتيب ، بينما حققت النيماتودا *S. carpocapsae* (All) أقل نسبة مئوية للموت ومقدارها 46.0 & 49.33 % لكلاهما على التوالى.

ومن ناحية أخرى ، تفوقت النسبة المئوية للموت الناتجة من استخدم التركيز 500 طور معدى من النيماتودا المختبرة مع التركيز الموصى به لمبيدى الأبامكتين والفينامبفوس المختبرين علي النسبة المئوية للموت الناتجة لأى من المبيدين بمفردهما أو الناتجة من النيماتودا المتطفلة على الحشرات بمفردها لتصل فى المعاملات التى جمعت بين مبيد الفينامبفوس والأنواع *H. bacteriophora* HP88, *H. indica* and *H. bacteriophora* (Ar-4) على الأطوار غير البالغة لقوقع *E. vermiculata* الى 69.00 و 70.00 و 62.67 % على التوالى بينما كانت النسب المئوية للموت الناتجة من استخدام الأنواع السابقة مع التركيز الموصى به من مبيد الأبامكتين هى 71.33 و 67.33 و 62.67 % على التوالى. في حين كانت قيم النسب المئوية للموت الناتجة من التأثير المشترك لمبيد الفينامبفوس والأنواع السابقة من نيماتودا الحشرات ضد الأطوار غير البالغة لقوقع *M. cartusinana* هى 81.33 و 84.00 و 76.00 % ومع مبيد الأبامكتين 72.00 و46.67 و67.33 % على التوالى.

وقد اختلف معامل السمية الناتج من التأثير المشترك لاستخدام الأنواع المختبرة من النيماتودا المتطفلة على الحشرات مع المبيدين النيماتودين تبعا للفترة الزمنية للتعريض ، حيث ظهر تأثير التنشيط وتأثير الإضافة عند استخدام نيماتودا الحشرات والمبيدين المختبرين بعد الأسبوع الأول من المعاملة بينما سجل تأثير الإضافة وتأثير التضاد بعد أسبوعين وثلاثة أسابيع على نوعي القوقعين المختبرين.