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Joint Actions Between Entomopathogenic Nematodes and Abamectin for Controlling the Termites, *Psammotermes hypostoma* (Desn.), and *Anacanthotermes ochraceus* (Burm.)

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ABSTRACT

The two subterranean termites, *Psammotermes hypostoma* (Desn.), and *Anacanthotermes ochraceus* (Burm.) are widely distributed and cause serious damage to wooden structures in Egypt. The objective of the present study is an attempt to control these pests using entomopathogenic nematodes (EPNs) alone or combined with abamectin, which is applied at recommended and half recommended doses under laboratory conditions. The tested EPNs were *Steinernema carpocapsae* (All strain), *S. feltiae* (Filipjev strain), *S. glaseri* (NC strain), *Heterorhabditis bacteriophora* (HP88 strain) as well as the two local isolates of *H. bacteriophora* namely Ar-4 strain and Ht strain. The results showed that *S. feltiae* was the highest virulence species followed by *H. bacteriophora* and *S. glaseri*, while *S. carpocapsae* was the least infectious one. The Egyptian *H. bacteriophora* isolates showed a relatively lower effect compared to imported species. On the other hand, *P.hypostoma* was more susceptible to the tested EPN species compared to *A. ochraceus*. Application of EPNs with a half-recommended dose of abamectin gave the highest nematicidal effectiveness against the two termite species. For instance, a combination of half-recommended doses of abamectin with *H. bacteriophora* (HP88 strain) achieved percentages mortality reached 89.4 and 87.5% with *P.hypostoma* and *A.ochraceus,* respectively. On the other hand, the highest percentage of mortalities was 85.20 and 82.62% in local isolates with *H. bacteriophora* (Ar-4 strain) with the tested termites. Generally, our results emphasize the importance of applying a half-recommended dose of abamectin with EPNs to overwhelm incompatibility, particularly with heterorhabditid species.

INTRODUCTION

Two subterranean termite species, *Psammotermes hypostoma* (Desn.) and *Anacanthotermes ochraceus* (Burm.) have a wide distribution in arid and semi-arid locations and cause serious damage to constructional timbers or infested villages in Egypt (El-Sebay and Ahmed, 2006). Usually, they act as a major pest of human structures, decomposers, and herbivores feeding on a spacious variety of dead, rotten, or fresh plant material in tropic and sub-tropic climate zones (Traniello and Leuthold,2000 and Bignell and Eggleton, 2000). Unfortunately, termites occupy the same soil habitat as the nematode species but they are

not particularly susceptible to nematode infection under field conditions (Fujii, 1975) due to termite social behavior.

At present, using chemical termiticides to control termites is still the main option in controlling termites' species (Su and Scheffrahn,1988). However, the use of chemicals around villagers' homes or gaRCens causes contamination to humans and the environment such as groundwater (Krutmuang and Mekchay, 2005). Consequently, it has become essential to search for alternative strategies for termite control in the cultivated land as wells as in natural diverse habitats and avoid the use of these chemicals.

Therefore, biocontrol agents should be considered as a long-term research aim and many entomopathogens such as viruses, bacteria, protozoa and fungi were tested against termites to reduce using of chemicals, but each procedure has a changed success rate (Shahid *et al.*, 2012 and Sajap *et al.*, 2014). The infective juveniles (IJs) of two important families species, Heterorhabditidae Poinar, 1975 and Steinernematidae Travassos, 1927 belong to a group of entomopathogenic nematodes (EPNs) which are considered as one of the most successful examples of biological tools agents against subterranean termites in sub-tropic climate zones like Egypt and in a tropic zone in Asian countries (El-Sebay and El-Bishry, 1994; Shahid *et al.*, 2012; Khanum and Javed, 2020 and Bhairavi *et al.*,2021). Moreover, the safety of EPNs for humans, other vertebrates, and many non-target invertebrates (Georgis *et al*., 2006; Lacey and Georgis, 2012) but, their variable performance and persistence remain important factors restricting their adoption by pest managers (Georgis *et al*., 2006; Shapiro-Ilan *et al*., 2002). Some native EPN populations, adapted to a particular environment, have been shown to persist over multiple years when isolated, cultured, and applied to the soil (Koppenhöfer and Fuzy, 2009; Shields *et al*., 1999). *Heterorhabditis bacteriophora*, *Steinernema carpocapsae*, *S. glaseri* and *S. feltiae* were successfully used to control the subterranean termites under laboratory conditions (Epsky and Capinera, 1988 and Wang *et al*., 2002). On the other hand, Rich *et al.* (2006) revealed that susceptibility and behavioral response of termites to *S. carpocapsae* nematode can be increased if termites are first stressed with low concentrations of some insecticides such as imidacloprid (Boucias *et al.*, 1996) which used to control the third instar white grubs in the laboratory. Imidacloprid disturbs the nerve function of the grubs and becomes sluggish so that IJs of tested nematodes can easily infect them. Also, it was noticed that the degree of interaction in synergism varies with different species of nematodes (Koppenhofer *et al.*, 2000, El-Ashry *et al*.,2020 and El-Ashry and Ramadan, 2021). Manzoor, 2012 tested the combined effect of imidacloprid and EPNs for the control of eastern subterranean termite, *Reticulitermes flavipes*.

So, the present study aimed to test the susceptibility of two subterranean termites, *Psammotermes hypostoma* (Desn.) and *Anacanthotermes ochraceus* (Burm.) to native and imported EPN species applied either alone or in combination with the recommended rate (RC) and half recommended dose(0.5 RC) of abamectin under laboratory conditions to understand how antagonistic or additive effect might vary across native and imported species of EPNs to build up proper IPM programs used in controlling termites.

MATERIALS AND METHODS

Test Insects:

The subterranean termites, *P. hypostoma* (Desn.) and *A.ochraceus* (Burm.), were collected from infested homes at Zarzaramoon village, Hehia and Elmesalamani village, Belbis, Sharqia governorate were conducted by using a modified trap.

Termites were collected by small brush from the traps and kept separately in Petri-dishes (1×9 cm height and diameter, respectively) and maintained in the laboratory in corrugated cardboard rolls in complete darkness at 22°C and 90% relative humidity (Yu *et al*.,2006).

After one week, the healthy insects were transferred to small plastic cups containing a source of cellulose feeding and humidity and used in the following experiments.

Entomopathogenic Nematodes (EPNs) Applied:

Entomopathogenic nematodes (EPNs), *Steinernema carpocapsae* (All strain), *S.feltiae* (Filipjev), *S. glaseri* (NC strain), *Heterorhabditis bacteriophora* (HP88 strain) were used in these experiments. Also, the three native species, *H.bacteriophora* (Ar-4 strain) and *H.bacteriophora* (Ht strain) isolated from Egypt (EL-Ashry *et al*., 2018).

Nematode species were reared in last-instar greater wax moth larvae, *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) at approximately 23±3°C according to procedures described in Kaya and Stock (1997). Infective juveniles (IJs) that emerged from insect cadavers into White traps (White, 1927) were stored in shallow water in transfer flasks at 15°C for up to 7 days prior to use.

Pesticides Used:

Recommended field concentration (RC) and half recommended field concentrations (0.5 RC) of abamectin (avermectin Gold) 1.8 E.C., insecticide, acaricide and nematicide as well as veterinary anthelmintic, macrocyclic lactone disaccharide isolated from the soil bacterium, Streptomyces avermitilis as a natural fermentation product were tested against the two species of white ants.

Bioassay Studies:

Laboratory experiments were carried out to determine the efficiency of certain native and imported strains of entomopathogenic nematodes (EPNs) with two applications (Petri dishes and soil analysis) methods against two species of white ants, *Psammotermes hypostoma* (Desn.) and *Anacanthotermes ochraceus* (Burm.).

A-Petri Dishes Method:

The first experiment was conducted in 9-cm Petri dishes filled with 10g of dry, autoclaved sand (0.3 to 0.5 mm particle size) according to Yu *et al.*, 2008. The imported entomopathogenic nematodes and local isolated species at three concentrations of each (approximately, 100 and 200 and 300 IJs/Petri dish was used in 1 ml) and each dish introduced 15 healthy (workers and nymphs) termites. Termites in control Petri dishes received only distilled water. Each treatment was replicated five times and the termite's mortality was checked daily for seven days until the experimental endpoint after 10 days. Lethal concentration [Lethal application rate (LC_{50})] and lethal Time (LT_{50}) of nematodes were estimated. Dead termites were dissected after mortality.

Dead termite percentages were calculated by the following formula:

Mortality (%) = (No. of Dead termites after incubation)/(Total number of termites at the beginning of treatment) × 100

B- Soil Analysis Method:

The second experiment was carried out in a soil medium for the reason that nematodes may act differently against termites with or without the presence of soil. To examine the effect of nematodes against termites with the presence of soil medium, 25 termites were put in each plastic container (9.2 cm diameter by 5.3 cm high) containing a mixture of sand (40 g), clay (30 g), peatmos (3 ml) and water (15 ml) with a buried block of a wooden structure. Nematodes were applied at 5 rates: 0, 200, 400 and 600 800 nematodes per termite and replicated three times. After 22 days, numbers of live and dead termites were checked and mean number of live termites from each rate was used for calculating LD_{50} of used EPNs against two termite species.

2- Combination (interaction) Treatments:

The mentioned plastic containers were filled with 25 g sand, two pieces of Whatmann No.1 filter paper and mixed well with 6 ml water, then provided with 25 termites (workers and nymphs). Each of the following treatments consists of five replicates. Termites were counted 1-2 days before treatment, any sluggish and unhealthy termites were removed and replaced with healthy termites which allowed foraging into the sand for one day before the treatment was applied.

The experiment was carried out at laboratory temperature (22±3°C), in a negative (untreated termites with nematodes or pesticide and provided with distilled water only) and two positive control treatments, tested EPN species alone 6 nematode species at a concentration of 20 IJs/termite), or abamectin alone (recommended application rate, 3ml/1000ml (RC) and ½ RC with two species of tested termites). Also, combining the effect of the abovementioned EPNs and abamectin was conducted on 2 termite species. So, a total of 2700 termites were used in the experiments. Also, the termite could feed on the cellulose pad at the bottom of the container; their feeding behavior was also noted. Tested termites were observed daily for mortality up to seven days but only tables contain data of 1, 2, 3 and 4 days. Dead *P. hypostoma* and *A.ochraceus* (single worker/dish) were placed in the white trap for IJs production at 25±3 ºC for up to 21 days.

Analysis of the Interaction Data of Mixtures:

Interaction data for mixtures were estimated using Lempel's formula reported by Richer (2006) as follows:

$$\mathbf{E} = (\mathrm{X} + \mathrm{Y}) - (\mathrm{XY})/100$$

Where:

E: The expected additive effect of the mixture.

X: The effect due to component A alone.

Y: The effect is due to component B alone.

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

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

This factor was used to classify results into three categories. A positive factor of 20 or more is considered potentiation, a negative factor of 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 while, the greenhouse experiment used a completely randomized block design. 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.

The percent mortality data after corrections were subjected to probit analysis for calculating mean lethal concentrations (LC_{50} & LT_{50}). Results were corrected for control mortality by using Abbott's formula (Abbott, 1925).

RESULTS AND DISCUSSION

Assay in Petri Dishes:

Two Egyptian *Heterorhabditis* isolates (Ar-4 strain and Ht strain) and four imported species, was screened by Petri dish analysis against *Psammotermes hypostoma* (Desn.). Percentage mortality was used to compare between nematode species to announce the high virulence against tested termites (Table 1). LC_{50} and LT_{50} values, at 300 IJs/Petri imported *S. feltiae* (Filipjev) was the highest virulence species against *P.hypostoma* (Desn.) by recording 3.16 and 1.19 days, respectively. Whereas, *H. bacteriophora* HP88 strain recorded 2.99 and 1.38 followed by *S. glaseri* (NC) by recording 3.16 and 1.19, respectively. On the other hand, LC_{50} (3.36) and LT_{50} (1.87) values exposed that *S. carpocapsae* (All) was the least virulence one in imported EPN species. On the other hand, Egyptian *H. bacteriophora* isolates (Ar-4 & Ht) less effective against *P.hypostoma* (Desn.) when compared with imported species and *H.bacteriophora* (Ar-4) relatively more pathogenicity with LC_{50} (3.40) and LT_{50} (2.25) days than *H.bacteriophora* (Ht) with 3.43 & 2.74, respectively.

In general, *H. bacteriophora* (Ht strain) was the least effective nematode species with LC_{50} values 5.80, 5.16 and 3.77 with 100, 200 and 300 IJs/Petri dish against *Anacanthotermes ochraceus* (Burm.) followed by *H. bacteriophora* (Ar-4) with LC_{50} values 5.14, 4.75 and 3.82 with 100, 200 and 300 IJs/Petri dish, respectively. Based on LT_{50}, nematode species could be arranged as follows: *S. feltiae* (Filipjev), *H. bacteriophora* (HP88) and *S. glaseri* (NC) with 1.26, 1.50 and 1.65 days, respectively with *A.ochraceus*. Whereas, LC_{50} for these species revealed that *H. bacteriophora* (HP88) was the most effective one against *A. ochraceus* with 4.65, 3.99 and 3.33 with 100,200 and 300 IJs/Petri dish, respectively (Table 1).

Table1: Comparative infectivity between imported nematodes and native isolates of *H. bacteriophora* against *Psammotermes hypostoma* (Desn.) and *Anacanthotermes ochraceus* (Burm.) in Petri dish method by using LC_{50} and LT_{50}.

Nematode species	Strains	IJs/Petri dish	*P. hypostoma* (Desn.)		*A. ochraceus* (Burm.)	
			LC $_{50}$ (No. of IJs/termite)	LT_{50} (After 4 days)	LC $_{50}$ (No. of IJs/termite)	LT_{50} (After 4 days)
S. carpocapsae	(All strain)	100	4.5912		4.8938	
		200	3.7187		4.1177	
		300	3.3652	1,87	3.7842	2,05
S. feltiae	(Filipjev)	100	3.9912		4.3869	
		200	3.7648		4.1608	
		300	3.1644	1,19	3.5824	1,26
S. glaseri	(NC strain)	100	4.6162		4.8861	
		200	3.5045		3.9011	
		300	3.1646	1,50	3.5128	1,65
H. bacteriophora	(HP88 strain)	100	4.3779		4.6588	
		200	3.5893		3.9935	
		300	2.993	1,38	3.3318	1,50
H. bacteriophora	(Ar-4 strain)	100	4.9204		5.1465	
		200	4.3985		4.7518	
		300	3.4042	2,25	3.8232	2,38
H. bacteriophora	(Ht strain)	100	5.7058		5.8073	
		200	4.8983		5.1675	
		300	3.4335	2,71	3.7775	2,70

*Each value is a mean of five replicates with 15 termites.
**Tested termites were observed for seven days.

In petri dish analysis, data showed that the entomopathogenic nematode species gave the highest percentage of mortality with the three concentrations of 100, 200 and 300 IJs/dish

as compared to soil analysis (Table 2). After 48 and 96 hrs, percentage mortality in the two termite species (*A. ochraceus* and *P. hypostoma*) treated with steinernematid species were (90.20,94.8;93.80, 98.90), (89.80,93.20;94.80,98.20) and (91.00,96.5;93.20,99.50) at concentration 300 IJs/dish with *S. carpocapsae* (All), *S. feltiae* (Filipjev) and *S. glaseri* (NC), respectively.

Percentage of mortality achieved by heterorhabditid species were: 81.50, 89.70;88.50,92.6& 90.00, 94.40; 95.10,98.70 and 86.20,90.3;96.9, 98.80 with *H. bacteriophora* (Ht) , *H.bacteriophora* (Ar-4) and *H.bacteriophora* (HP88) with *A.ochraceus* and *P. hypostoma* at concentration 300 IJs/Petri , and *H.bacteriophora* (Ar-4) was the most virulence one of them. Statistical analysis showed significant differences between all tested concentrations and nematode species ($P \leq 0.05$).

Table 2: Percentages mortality of Subterranean termites P. *hypostoma* (Desn.) and *A. ochraceus* (Burm.) after 48 and 96 hours of exposure to nematode species by using Petri dish method.

Nematode species	Strains	Concentrations (IJs/Petri dish)	Percentages of mortality (%) of Subterranean termite species					
			A. ochraceus			*P. hypostoma*		
			48 hr	96 hr	General mean	48 hr	96 hr	General mean
S. carpocapsae	(All strain)	100	24.6^{g}	50.4^{hi}	**37.50**	29.5^{i}	56.4^{f}	**42.95**
		200	60.4^{d}	84.6^{d}	**72.50**	62.3^{ef}	89.8^{b}	**76.05**
		300	90.2^{a}	94.8^{a}	**92.50**	93.8^{a}	98.9^{a}	**96.35**
S. feltiae	(Filipjev)	100	30.3^{fg}	42.8^{j}	**36.55**	36.2^{h}	42.8^{h}	**39.50**
		200	54.5^{d}	70.9^{f}	**62.70**	66.5^{de}	76.9^{d}	**71.70**
		300	89.8^{a}	93.2^{abc}	**91.50**	94.8^{a}	98.2^{a}	**96.50**
S. glaseri	(NC strain)	100	34.4^{f}	48.1^{i}	**41.25**	46.4^{g}	54.2^{f}	**50.30**
		200	60.4^{d}	79.3^{e}	**69.85**	70.8^{d}	84.6^{c}	**77.70**
		300	91.0^{a}	96.5^{a}	**93.75**	93.2^{a}	99.5^{a}	**96.35**
H. bacteriophora	(HP88 strain)	100	42.7^{e}	53.8^{h}	**48.25**	46.9^{g}	55.6^{f}	**51.25**
		200	69.5^{c}	84.2^{d}	**76.85**	80.8^{c}	89.1^{b}	**84.95**
		300	86.2^{ab}	90.3^{bc}	**88.25**	96.9^{a}	98.8^{a}	**97.85**
H. bacteriophora	(Ar-4strain)	100	30.5^{fg}	48.5^{i}	**39.5**	34.8^{h}	48.5^{g}	**41.65**
		200	56.6^{d}	73.7^{f}	**65.15**	76.5^{c}	78.4^{d}	**77.45**
		300	90.0^{a}	94.4^{ab}	**92.2**	95.1^{a}	98.7^{a}	**96.9**
H. bacteriophora	(Ht strain)	100	32.4^{f}	41.8^{j}	**37.10**	32.4^{h}	41.8^{h}	**37.10**
		200	45.6^{e}	60.3^{g}	**52.95**	56.6^{f}	69.2^{e}	**62.90**
		300	81.5^{b}	89.7^{c}	**85.60**	88.5^{b}	92.6^{b}	**90.55**

*Reported numbers represent the means of five replicates.

**Different letters in the same column represent significant differences ($P < 0.05$) among different nematode strains according to Duncan's multiple range tests.

Assay in Soil:

Although in soil analysis, every plastic pot was received the double concentration of each in Petri dish analysis, data in Table (3) showed that entomopathogenic nematode species (EPNs) gave lower mortality percentages with the three concentrations 200,400 and 600 IJs/plastic pot. After 48 and 96 hrs , percentage mortality in steinernematid species were (80.20, 84.80 ; 82.10 ,86.90), (80.10,83.20;84.8,88.20) and 81.00,86.50;83.20,92.80) at concentration 300 IJs/dish with *S.carpocapsae* (All), *S.feltiae* (Filipjev) and *S.glaseri* (NC) with the two termite species (*A.ochraceus* and *P. hypostoma*), respectively. The equivalent values with heterorhabditid species were: 61.50, 69.70; 71.80, 75.90 & 70.00, 74.40; 75.10, 78.70 and 76.20, 80.30; 86.90, 88.20 with *H. bacteriophora* (Ht), *H. bacteriophora* (Ar-4) and *H.bacteriophora* (HP88) with *A.ochraceus* and *P. hypostoma* at concentration 300 IJs/Petri. Moreover, *H. bacteriophora* (HP88) was the most virulent one of them. Statistical analysis showed significant differences between all tested concentrations and nematode species ($P \leq 0.05$).

Table 3: Percentages mortality of Subterranean termites *P. hypostoma* (Desn.) and *A. ochraceus* (Burm.) after 24 and 48 hours of exposure to nematode species by using soil analysis method.

Nematode species	Strains	Concentrations (IJs/Plastic pot)	*A. ochraceus*		*P. hypostoma*	
			48 hr	96 hr	48 hr	96 hr
S. carpocapsae	(All strain)	200	17.6^{hi}	40.4^{h}	22.8^{h}	46.5^{fg}
		400	53.7^{d}	74.6^{c}	55.9^{d}	76.4^{c}
		600	80.2^{a}	84.8^{ab}	82.1^{a}	86.9^{b}
S. feltiae	(Filipjev)	200	20.3^{gh}	32.8^{j}	26.3^{h}	32.8^{i}
		400	44.5^{e}	60.9^{f}	56.5^{d}	66.9^{d}
		600	80.1^{a}	83.2^{ab}	84.8^{a}	88.2^{ab}
S. glaseri	(NC strain)	200	24.4^{g}	38.1^{hi}	36.4^{fg}	44.2^{gh}
		400	50.4^{d}	69.3^{e}	64.1^{c}	74.6^{c}
		600	81.0^{a}	86.5^{a}	83.2^{a}	92.8^{a}
H. bacteriophora	(HP88 strain)	200	32.7^{f}	33.8^{ij}	33.5^{g}	45.6^{fgh}
		400	59.5^{c}	74.2^{cd}	70.8^{b}	79.1^{c}
		600	76.2^{a}	80.3^{b}	86.9^{a}	88.2^{ab}
H. bacteriophora	(Ar-4strain)	200	14.5^{i}	23.8^{k}	15.8^{i}	24.5^{j}
		400	33.2^{f}	53.7^{g}	49.8^{e}	58.4^{e}
		600	70.0^{b}	74.4^{c}	75.1^{b}	78.7^{c}
H. bacteriophora	(Ht strain)	200	17.0^{hi}	22.4^{k}	32.4^{g}	41.4^{h}
		400	25.6^{g}	40.3^{h}	39.8^{f}	49.2^{f}
		600	61.5^{c}	69.7^{de}	71.8^{b}	75.9^{c}

* Reported numbers represent the means of five replicates.

** Different letters in the same column represent significant differences ($P < 0.05$) among different nematode strains according to Duncan's multiple range tests.

Interactions between chemical pesticide, Abamectin and entomopathogenic nematodes (EPNs):

1. Interactions between abamectin and nematodes in infectivity *P. hypostoma* (Desn.):

Results documented the compatibility or incompatibility effect between concentrations [(recommended and half recommended application rate (RC)] of abamectin with nematode species (*S.carpocapase* (All), *S.feltiae, S.glaseri* (NC) *H.bacteriophora* (HP88), *H.bacteriophora* (Ar-4) and *H.bacteriophora* (Ht)) against the two subterranean termites, *A.ochraceus* and *P. hypostoma* after different time exposures as shown in Tables 4&5. The mortality percentages in two subterranean termites were used as an indicator for compatibility/incompatibility effect noticed between two application rates, nematode species and exposure times.

In vitro interactions between chemical pesticides and different EPN species in controlling the two subterranean termites, *P. hypostoma* (Desn.) and *A. ochraceus* (Burm.) demonstrated in Table (4). Abamectin mixed with the tested EPNs different species showed additive and antagonism interactions with recommended application rate (RC) and half recommended application rate (½ RC) doses.

Abamectin showed additive effect after mixing RC with *S.carpocapase* (All) and *H. bacteriophora* (HP88) recorded -11.04 and -18.34, respectively. While, antagonistic effect showed when abamectin mixed with *S. feltiae* (-36.19); *S. glaseri* (NC), -42.06; *H. bacteriophora* (Ar-4), (-24.97) and *H. bacteriophora* (Ht), (-33.24) , with RC , respectively.

On the other hand, half recommended dose (½ RC) showed an additive effect with two local *Heterorhabditis* isolates (*H. bacteriophora* (Ar-4 strain) and *H. bacteriophora* (Ar-4 strain)). Also, additive effect showed with imported nematode species except for *S. feltiae.*

Table 4. Interactions between chemical pesticide, Abamectin and different EPNs strains on mortality of subterranean termites, *P. hypostoma* (Desn.) after 4 days of application.

Nematode Species	Concentrations	Mortality (%) (Nematodes + pesticides)		Co-toxicity factor (CF)	Response
		Observed	Expected		
Abamectin + Sc	RC	82.1	92.29	-11.04	additive
Abamectin + Sc	1/2 RC	85.3	86.76	-1.68	additive
Abamectin + Sf	RC	58.7	92.00	-36.19	antagonism
Abamectin + Sf	1/2 RC	59.9	86.24	-30.54	antagonism
Abamectin + Sg	RC	56.8	98.17	-42.06	antagonism
Abamectin + Sg	1/2 RC	78.3	86.02	-8.97	additive
Abamectin + HP 88	RC	74.8	91.61	-18.34	additive
Abamectin + HP 88	1/2 RC	89.4	85.58	4.46	additive
Abamectin + Hb Ar-4	RC	68.7	91.57	-24.97	antagonism
Abamectin + Hb Ar-4	1/2 RC	85.2	85.51	-0.36	additive
Abamectin + Hb Ht	RC	60.3	90.33	-33.24	antagonism
Abamectin + Hb Ht	1/2 RC	72.2	83.39	-13.41	additive

*Sc= *S.carpocapase* (All strain) , Sf = *S.feltiae* , Sg = *S.glaseri* (NC strain) , HP 88 = *H.bacteriophora* (HP88 strain), Hb Ar-4 = *H.bacteriophora* (Ar-4 strain) and Hb Ht = *H.bacteriophora* (Ht strain).

**RC = Recommended application rate and 1/2 RC = Half recommended application rate.

2. Interactions Between Abamectin and Nematodes in Infectivity *A. ochraceus* (Burm.):

When recommended application rate of abamectin mixed with EPN species used for controlling *A. ochraceus*, additive effect showed only in two imported species, *S.carpocapase* (All) and *H.bacteriophora* (HP88) and recorded -1.50 and -10.23, and recorded -19.25with *H.bacteriophora* (Ar-4) from the two native isolates. While, antagonistic effect showed when abamectin mixed with *S. feltiae*, *S. glaseri* and *H. bacteriophora* (Ht). On the other hand, half recommended application rate (½ RC) showed additive effect with *S.carpocapase* (All), *S.glaseri* (NC), *H.bacteriophora* (HP88). As well, two native *Heterorhabditis* isolates, *H. bacteriophora* (Ar-4) and *H. bacteriophora* (Ht) showed an additive effect.

Table 5. Interactions between chemical pesticide, Abamectin and different EPNs strains on mortality of subterranean termites, *A. ochraceus* (Burm.) after 4 days of application.

Nematode Species	Concentrations	Mortality (%) (Nematodes + pesticides)		Co-toxicity factor (CF)	Response
		Observed	Expected		
Abamectin + Sc	**RC**	78.99	80.20	-1.50	additive
Abamectin + Sc	**1/2 RC**	82.74	86.14	-3.94	additive
Abamectin + Sf	**RC**	51.52	79.44	-35.14	antagonism
Abamectin + Sf	**1/2 RC**	52.93	85.60	-38.16	antagonism
Abamectin + Sg	**RC**	49.29	79.11	-37.69	antagonism
Abamectin + Sg	**1/2 RC**	74.53	85.37	-12.69	additive
Abamectin + HP 88	**RC**	70.42	78.45	-10.23	additive
Abamectin + HP 88	**1/2 RC**	87.55	84.91	3.10	additive
Abamectin + Hb Ar-4	**RC**	63.26	78.35	-19.25	additive
Abamectin + Hb Ar-4	**1/2 RC**	82.62	84.84	-2.61	additive
Abamectin + Hb Ht	**RC**	53.40	75.50	-29.27	antagonism
Abamectin + Hb Ht	**1/2 RC**	67.37	82.58	-18.41	additive

*Sc= *S.carpocapase* (All strain) , Sf = *S.feltiae* , Sg = *S.glaseri* (NC strain) , HP 88 = *H.bacteriophora* (HP88 strain), Hb Ar-4 = *H.bacteriophora* (Ar-4 strain) and Hb Ht = *H.bacteriophora* (Ht strain).

**RC = Recommended application rate and 1/2 RC = Half recommended application rate.

DISCUSSION

Comparative infectivity between imported EPN species and native isolates of *H. bacteriophora* against two subterranean termites *P.hypostoma* (Desn.) and *A.ochraceus* (Burm.) not being mortality dose-dependent, it also dependents on strains. Imported nematodes were pathogenic than native isolates of *H. bacteriophora*. As well as, more virulence against *P. hypostoma* (Desn.) than *A.ochraceus* (Burm.) as shown from LD_{50} and LT_{50}. Moreover, in Petri dish analysis, LT_{50} were 1.19, 1.26, 1.38, 1.50 and 1.87, 2.05 days with *S. feltiae*, *H. bacteriophora* (HP88) and *S. carpocapsae* (All), with the two mentioned termites, respectively. In addition, half recommended application rate (½ RC) of abamectin showed additive when mixed with native and imported EPN species used in controlling two subterranean termites *in vitro*.

In Egypt, control of the two subterranean termites by EPNs nematodes revealed that *P. hypostoma was* more susceptible than *A.ochraceus* (EL-Bassiouny and Randa Abd El-Rahman, 2011). Moreover, the mortality increased as the nematodes concentrations increased and vice versa. El-Sebay and El-Bishry (1994) found that *S.carpocapsae* (All strain) when tested against *A.ochraceus* (Burm.) under laboratory conditions gave 100% mortality after 3 days of exposure. Azazy and Ahmed (2006) proved that EPNs were able to kill workers, nymphs, soldiers and alter casts of *P.hybostoma*. As well, Sanaa, Ibrahim and Abd El-Latif (2008) tested *Steinernema* spp. and *Heterorhabditis* spp. against *P.hybostoma* (Desn.) and mentioned to variation in their efficacy depending on nematode species besides; *H.bacteriophora* (HP88) and *S.carpocapsae* (All) were more effective against tested termite under laboratory conditions.

Steinernema (*S. feltiae*) is an ambushing nematode which readily and most effective at infecting highly mobile insects (Grewal *et al*., 1994). As well as, nematode ability to infect and multiply depending on behavioural, morphological and physiological defense strategies of insects (Razia *et al.*, 2012). Laboratory results of three EPNs, *S. siamkayai*, *S. pakistanense* and *H.indica* against subterranean termites, *Reticulitermes flavipes* and *Odontotermis* showed that *S. pakistanense* have a potential for control of termites and *S. pakistanense* required less number (5IJs/termites) and less time (20h) against *R. flavipes* and whereas *O. hornei*, required less number (5.84IJs/termites) and less time (15.5h). (Razia and Sivaramakrishnan,2016).

On the other hand, current results particularly agree with Changlu *et al*.,2002 who showed that the LD_{50} of *H. bacteriophora* against *R. flavipes* in Petri dishes was less than in containers with vermiculite/sand medium and *Heterorhabditis* spp. were more effective than *Steinernema* spp. against two subterranean termites, *Reticulitermes flavipes* (Kollar) and *Coptotermes formosanus* Shiraki.

Nath (2018) assessed the effectiveness of *H. bacteriophora* Poinar and *Steinernema* sp. against *Odontotermes obesus* (Ramb) at 100 IJs termite (worker/soldier) within 24-48 h under laboratory tests using Petri-dishes. Bhairavi *et al.*, (2021) However, *H. bacteriophora* was more virulent toward *O. obesus* and *A. ipsilon* than *S. aciari* under laboratory conditions.

A combination of nematodes with other chemicals may improve their control over termites. The large amount of variability between pesticides from the same chemical group in their compatibility with EPNs makes extrapolation of the data between products unreliable (Rovesti and Deseo,1990; thus, each candidate product for an IPM system should be tested individually with the specific nematode species (Krishnayya and Grewal, 2002).

Additive, antagonistic and synergistic interactions between EPNs and recommended dose of abamectin were observed. For instance, Manzoor (2012) mentioned that there was synergism between imidacloprid and nematodes species used against workers and nymphs of

Reticulitermes flavipes caused more than 50% termite mortality and selection of more infective nematode species.

From current results, a combination between *S.feltaie* and *S.carpocapsae* (All strain) and recommended application rate of abamectin showed an incompatibility effect. When half-recommended doses were used, percentage mortality in two termite species increased with all nematode species and a combination between *H. bacteriophora* (HP88 strain) and abamectin showed an ability to increase termites susceptibility as compared with steinernematid species followed by native isolated species, *H. bacteriophora* (Ar-4 strain). This finding is in agreement with Laznik and Trdan (2013) who revealed that compatibility is species-specific. Lazinik and Trdan (2013) assessed that *H. bacteriophora* is the species most tolerant to the tested insecticide, except abamectin. Moreover, the ingredients abamectin has a well-known nematicide effect, which may explain the relative incompatibility effect with *S. carpocapsae* or other steinernematid species proved to be the most sensitive species (Mahfooze *et al*.,2008). Also, when decreased the dose used with some nematode species, i.e. *H. bacteriophora* (HP88 strain and *H. bacteriophora* (Ar-4 strain) improved percentage mortalities in the two tested termite species (Lazinik and Trdan (2013).

The synergistic interaction and the negative effect of higher abamectin rates on *S. carpocapsae* were confirmed in a greenhouse experiment on potato plants (Kary *et al.*,2018) but the interaction type relies on abamectin concentration, which showed a strong sub-lethal effect on *S. carpocapsae* and *H.indica* reproductive potential, limiting seriously their possible recycling in the field (Devindrappa *et al*,2017). General, results indicate the possibility of integrated use of EPN species and chemical pesticides in crop protection (Rovesti and Deseö,1990).

However, these chemicals showed a strong sublethal effect on the nematode reproductive potential, limiting seriously their possible recycling in the field (Gutiérrez *et al*.,2008). So, our results are valuable to optimize EPN species and pesticides application rates to estimate their field recycling (Gutiérrez *et al*.,2008).

With eusocial arthropod decomposers like termites, farmers should improve their cultural management practices by integrating traditional practice with recently developed approaches can be an effective strategy for termite management Farhan Ahmad *et al*.(2021)

CONCLUSION

It could be concluded that the interactions between EPN species and pesticides affected potential EPN performance and should be considered when studying the effectiveness of use recommended rate of test pesticides with EPN strains or with other control methods under field conditions. We suggest that utilization of half recommended application rate to avoid incompatibility between EPNs and pesticides. As well as, search and examine native EPN isolates/strains against termites to explore their efficacy against various soil insect pests under laboratory and field conditions.

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ARABIC SUMMARY

التأثيرات المشتركة بين النيماتودا الممرضه للحشرات مع مبيد الإبامكتين فى مكافحة نوعين من النمل الأبيض *Anacanthotermes ochraceus* (Burm.)و *Psammotermes hypostoma* (Desn.)

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ينتشر نوعين من النمل الأبيض تحت الأرضى وهما *Psammotermes hypostoma* (Desn.) و*Anacanthotermes ochraceus* (Burm.) على نطاق واسع والتى تسبب إضرار جسيمه للمنتجات الخشبيه فى مصر. لذلك أجريت تلك الدراسة لمحاولة السيطرة على تلك الأفات بإستخدام النيماتودا الممرضه للحشرات EPNs منفردة أو مشتركه مع مبيد الأبامكتين حيث تم تطبيق الجرعه الموصى بها ونصف المعدل الموصى به من هذا المبيد تحت ظروف المعمل. تم استخدام أنواع من النيماتودا الحشرية المختبرة هى *Steinernema carpocapsae* (All strain) و *S. feltiae* (Filipjev) و *S. glaseri (NC strain)* و *Heterorhabditis bacteriophora* (HP88 strain) بالإضافه إلى نوعين معزولين محليا وهما *H. bacteriophora Ar-4 strain* و *H. bacteriophora* Ht strain . كما أوضحت النتائج أن نيماتودا *S.feltiae (Filipjev)* هى أكثر الأنواع قدرة على إحداث الإصابة يليها كلا من نيماتودا *H.bacteriophora (HP88)* ونيماتودا *S.glaseri (NC)* بينما كانت نيماتودا *S.carpocapsae (All)* هى أقل الأنواع قدرة على إحداث الإصابة. وكانت النيماتودا المستوردة أكثر قدرة على الإصابة من عزلات النيماتودا المحلية التابعة لنيماتودا *H. bacteriophora* . من ناحيه أخرى كان هذا النوع من النمل *P.hypostoma* أكثر حساسيه للإصابه بإنواع النيماتودا الحشرية المختبرة مقارنه بالنوع الاخر *A. ochraceus* . المعاملة بإستخدام نصف الجرعه الموصى به من مبيد الأبامكتين وذلك عند خلطه مع كل من أنواع النيماتودا المتطفله الحشريه أعطت أعلى تأثير فاعليه ضد نوعين النمل الأبيض. على سبيل المثال، تطبيق خلط نصف الجرعة الموصى بها من مبيد أبامكتين مع سلاله نيماتودا *H. bacteriophora* HP88) حقق نسب موت وصلت إلى 89.4 و 87.5٪ مع نوعين النمل الأبيض *P.hypostoma* و*A. ochraceus* على التوالي. من ناحية أخرى، كانت أعلى نسب موت 85.20 و 82.62٪ في العزلات المحلية المصابة *H.bacteriophora (*Ar-4) مع كلا من نوعين النمل الأبيض المختبرة. بشكل عام، تؤكد النتائج على أهمية تطبيق نصف الجرعة الموصى بها من الأبامكتين مع EPN للتغلب على عدم التوافق خاصة مع الأنواع غير المتجانسة .