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Original Article

Effects of organic, non organic fertilizers, and selected novel pesticides on survival of two *Entomopathogenic nematodes*

Efeitos de fertilizantes orgânicos e não orgânicos, e novos pesticidas selecionados na sobrevivência de dois *Nematoides entomopatogênicos*

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Abstract

In this study, the effect of six macro fertilizers i.e. urea, ammonium sulfate, ammonium nitrate, NPK, NP & DAP, and three micro elements i.e. Zn, Mn, and Fe at different concentrations as well as eight novel pesticides, thiamethoxam, spinetoram, azoxystrobin, chlorfenpyr, chlorantraniliprole, novaluron, lambda-cyhalothrin and avaunt at field recommended rate on two entomopathogenic nematodes (EPNs): *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* were determined under laboratory conditions. The obtained results reported that *S. carpocapsae* was more tolerant to NPK; NP and DAP macro fertilizers than *H. bacteriophora*, showed the lowest mortality percentages (12.63, 9.47and12.63%), (15.79, 13.68 and 31.58%) and (20.21, 30.85 and 34.04%) at three tested concentrations (1g/ liter water, 5g/liter water and10g/liter water), respectively. All tested micro fertilizers (Zn, Mn, and Fe) were lethal for two EPNs and induced 94.85, 100, 100 mortality % after five days of exposure. The morality percent increased as the concentration of fertilizers increased.

With respect to tested pesticides, the mortality percentages of two EPN were ranged between 2.67-23.67% for *S. carpocapsae* and 3.33-18.33% for *H. bacteriophora* after five days of exposure. The tested macro fertilizers such as DAP, NP and NPK can be used safely at tested concentrations (5-10 g/ liter water) with *Steinernema carpocapsae*. All tested macro fertilizers cannot be used with *Heterorhabditis bacteriophora*. All micro fertilizers could be used with two EPNs. All tested pesticides can be successfully used for integrated plant protection systems. EPNs are tolerant to the tested pesticides and the tank-mix application is possible in most compounds except Lambda cyhalothrin which significantly reduced the virulence of tested nematodes.

Keywords: pesticides, macro and micro fertilizers, EPNs, *Steinernema carpocapsae*, *Heterorhabditis bacteriophora.*

Resumo

Neste estudo, o efeito de seis macrofertilizantes, ou seja, ureia, sulfato de amônio, nitrato de amônio, NPK, NP e DAP, e três microelementos, ou seja, Zn, Mn e Fe, em diferentes concentrações, bem como oito novos pesticidas – tiametoxame, espinetoram, azoxistrobina, clorfenpir, clorantraniliprole, novaluron, lambda-cialotrina e avante – na taxa recomendada em campo, em dois nematoides entomopatogênicos (EPNs): *Steinernema carpocapsae* e *Heterorhabditis bacteriophora*, foi determinado em condições de laboratório. Os resultados obtidos relataram que *S. carpocapsae* foi mais tolerante ao NPK; com os macrofertilizantes NP e DAP, *H. bacteriophora* apresentou os menores percentuais de mortalidade (12,63, 9,47 e 12,63%), (15,79, 13,68 e 31,58%) e (20,21, 30,85 e 34,04%), nas três concentrações testadas (1g/litro de água, 5g/litro de água e 10g/litro de água), respectivamente. Todos os microfertilizantes testados (Zn, Mn e Fe) foram letais para duas EPNs e induziram 94, 85 e 100% de mortalidade após cinco dias de exposição. A percentagem de mortalidade aumentou à medida que a concentração de fertilizantes aumentou.

Com relação aos agrotóxicos testados, os percentuais de mortalidade dos dois EPNs variaram entre 2,67 e 23,67% para *S. carpocapsae* e 3,33 e 18, 33% para *H. bacteriophora* após cinco dias de exposição. Os macrofertilizantes testados, como DAP, NP e NPK, podem ser usados com segurança nas concentrações testadas (5-10 g/litro de água) com *Steinernema carpocapsae*. Todos os macrofertilizantes testados não podem ser usados com *Heterorhabditis bacteriophora*. Todos os microfertilizantes poderiam ser usados com os dois EPNs. Todos os pesticidas testados podem ser utilizados com sucesso em sistemas integrados de proteção fitossanitária. Os EPNs são tolerantes aos pesticidas testados e a aplicação em mistura em tanque é possível na maioria dos compostos, exceto lambda-cialotrina, que reduziu significativamente a virulência dos nematoides testados.

Palavras-chave: agrotóxicos, macro e microfertilizantes, EPNs, *Steinernema carpocapsae*, *Heterorhabditis bacteriophora.*

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1. Introduction

Entomopathogenic nematodes (EPNs) from the genera *Steinernema* and *Heterorhabditis* (Rhabditida: Steinernematidae and Heterorhabditidae) are one of entomopathogens that has been recognized as potential agents to target insects in the soil or in cryptic environments. These nematodes are the only ones associated with insects and are widely used for pest control. Furthermore, they have an unusual mutualistic association with the bacteria *Xenohabdus, Photorhabdus*, which invariably results in the rapid death of parasitized insects.

Heterorhabditis and *Steinernema* have been used in the biological control of insect pests of agricultural importance (Georgis et al., 2006). The extreme use of inorganic fertilizers induced a variety of environmental problems such as salinity of soil, accumulation of heavy metal, effect on greenhouse, and accumulation of nitrate (Sönmez et al., 2007). When EPNs exposed to soil salinity, the movement of EPNs is restricted and reduced their ability to find and recognize their host (Nielsen et al., 2011). (Şahin and Susurluk, 2018) reported that *Steinernema feltiae* (Tur-S3) was more resistant to inorganic fertilizers than *H. bacteriophora* HBH, and DAP, NPK and NP induced more adverse effects than the other fertilizers on both strains. The entomopathogenic nematodes (EPNs) are appropriate as ideal organisms for ecological research due to their high potential as biological control (Lewis et al., 2006; Stuart et al., 2006; Stock, 2015).

Chemical products used in agriculture are one of the main causes of environmental pollution and they threaten all life forms (Özdemir et al., 2021; Mamuk et al., 2023**)**. Alternative methods such as integrated pest management (IPM) and biological control are becoming increasingly important (Bhadani et al., 2022; Chatterjee and Kundu 2022; Cheng et al., 2023; Catani et al., 2023; Brewer and Elliott, 2023; Alam et al., 2023; Galli et al., 2024)

EPNs, which spend most of their life in the soil, are effective biological control agents (Hazir et al., 2004). The EPNs had no negative impacts on the environment, human health and non-target organisms compared with pesticides (Boemare et al., 1996; Ehlers, 1996**).** EPNs can be used in IPM and compatible with some pesticides (Ulu et al., 2016). There are many environmental factors in the soil that affect the viability, reproduction and efficacy of EPNs (Kaya, 1990; Susurluk, 2008; Aatif et al., 2015).

Neonicotinoid insecticides and oxadiazine are widely used for the control of lepidopterous pests of some vegetables and other crops (Palumbo and Castle, 2009). However, the excessive use of chemical pesticides has generated serious problems including selection for insecticide resistance (Nauen and Denholm, 2005), outbreaks of secondary pests (Szczepaniec et al., 2011), and environmental concerns (Lacey et al., 2001). In this respect, environmentally friendly alternatives such as microbial entomopathogens and entomopathogenic nematodes (EPN) including Steinernematids and Heterorhabditids have been suggested to ameliorate the negative consequences of these and other chemical pesticides (Zimmermann, 1993; Poprawski et al., 1998; McCoy et al., 2002; Ansari et al., 2007; Ebssa and Koppenhöfer, 2012). Many studies have evaluated the compatibility of EPN with chemical insecticides and assessed two key components: i) survival of the nematodes in tank mix, and ii) the effect of the nematode insecticide combination on the targeted pest (Rovesti et al., 1988; Vainio and Hokkanen, 1990; Koppenhöfer and Grewal, 2005).

The combinations of nematode and pesticide in tank-mixes are very effective alternative to integrated pest management (IPM) systems. However, before an ecologically integrated approach to pest management involving nematode-pesticide combinations in tank-mixes can be developed the compatibility of these nematodes with pesticides should be discovered, Grewal and Georgis (1998)**.** Negrisoli et al. (2010) observed that (chlorpyrifos, deltamethrin, lufenuron, deltaphos diflubenzuron, lambda-cyhalothrin, spinosad, cypermethrin, triflumuron, and permethrin) were compatible (class 1) with the three tested nematode species (*Heterorhabditis indica*, *Steinernema carpocapsae* and *Steinernema glaseri*) under laboratory conditions. Aioub et al. (2021) reported that the integrated pest management strategies are the compatibility of entomopathogenic nematodes with pesticide. Hara and Kaya (1983) concluded that most insecticides can be used at practical concentrations with *S. carpocapsae*. Radova (2011) reported that it is difficult to explain why EPNs react differently to different pesticides. *Steinernema* sp. (IJs) not affected and was more tolerant to insecticides at all tested concentrations Laznik and Trdan (2013)**.** EPNs viz., *H. bacteriophora* and *S. carpocapsae* were compatible with most of the pesticides tested against *Spodoptera littoralis* and therefore EPNs insecticide mixtures can be used in an integrated pest management system Atwa et al. (2017). Chavan et al. (2018) reported that *H. indica* showed compatible with all the tested agrochemicals.

In this study, the effects of eight novel pesticides i.e., bioinsecticide (Spinetoram), fungicide (Azoxystrobin), acaricide (Chlorfenpyr) and insecticides (Thiamethoxam, Chlorantraniliprole, Novaluron, Lambda-cyhalothrin and Avaunt) at field recommendation rate were evaluated on *S. carpocapsae* and *H. bacteriophora* EPNs under laboratory conditions. In addition, the effects of different organic and inorganic fertilizers, which are used extensively in agriculture, were investigated on the two species of the entomopathogenic nematodes.

2. Materials and Methods

2.1. Species and production of EPNs:

Two species of EPNs, *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* were obtained from pure culture reared on great wax larvae, *Galleria mellonella* in the Biological Control Laboratory of Dr M Sweelam in the Fac Agric. Menoufia University Egypt.

The obtained nematodes were identified according to the key organized by Lucskai (1999) and reared under laboratory conditions according to the method of White traps White (1927) for these experiments. Third stage juveniles (infective juveniles, IJs) which have the ability to infect hosts, were allowed to infect greater wax moth larvae (*Galleria mellonella*, Lepidoptera: Pyralidae). The last stage larval instars used in the study were obtained with the White trap method (White, 1927). Infective juveniles (IJs) of nematode species were produced on wax moth

instars at 21 ± 4 °C. Approximately 2 to 4 days old, newly released IJs were used in the experiments.

The experiments were conducted in the Biological Control Laboratory, Department of Economic Entomology and Agricultural Zoology, Faculty of Agriculture, Menoufia University to serve as source of required larvae to conduct the experiments.

2.2. Tested fertilizers:

2.2.1. Effects of some macro fertilizers on the entomopathogenic nematodes:

Six commonly used macro fertilizers were chosen. These fertilizers were as follow:

1. urea (NH_2-N), total N 46%),
2. ammonium sulfate (AS: $(NH_4)2SO_4$, total N 21%, total S 24%),
3. ammonium nitrate (AN: NH_4NO_3, total N 33%),
4. NPK (15-15-15+20SO_3+Zn, total N 15%, total P_2O_5 15%, total K_2O 15%, total SO_3 20%, total Zn 1%),
5. NP (20-20-0+5.5SO_3+Zn, total N 20%, total P_2O_5 20%, total SO_3 5.5%, total Zn 1%),
6. DAP Di-ammonium Phosphate: $(NH_4)2HPO_4$, total N 18%, total (P_2O_5 46%).

These experiments were conducted to determine the effect of the macro elements on the activity of the entomopathogenic nematodes, *S. carpocapsae,* and *H. bacteriophora* under laboratory conditions.

One hundred of third infective juveniles of each entomopathogenic nematode were placed in Petri dish 10 cm in 20 ml of aqueous fertilizer 1%, 5%, 10%.

Petri dishes were examined 1, 3, 5 days after treatment for the number of dead juveniles, meanwhile, Petri dish was left without fertilizers and applied only with distilled water was served as control.

2.2.2. Effects of some micro elements on the two Entomopathogenic nematodes:

Three micro elements i.e., Zinc Zn, Manganese Mn and Ferric Fe were evaluated against the entomopathogenic nematodes as well as their mixture. The determined doses of the fertilizers were dissolved in one liter of distilled water using a magnetic stirrer.

One hundred of the fresh third infective juveniles of each entomopathogenic nematode was placed in Petri dish 10 cm containing 20 ml of aqueous fertilizer 0.1%, 0.5% and 1.0% of each element under laboratory conditions of 21± 4 ºC and 65 ± 6%.

Petri dishes were examined 1, 3, 5 days after treatment for number of dead juveniles, one Petri dish was left without fertilizers and applied only with distilled water was served as control. One hundred IJ3s / Petri dish 10 cm was used. Each treatment was replicated six times.

The mortality rate of IJs was determined and subsequent observations were made on the mortality of live juveniles at 1, 3, 5 days after treatments.

2.2.3. Effects of some novel pesticides on two Entomopathogenic nematodes:

Eight pesticides with novel modes of action were selected for evaluation on selected EPN species. Further details about the recommended dose rates, trade name, and mode of action are provided in Table 1 i.e., bioinsecticide (Spinetoram), fungicide (Azoxystrobin), acaricide (Chlorfenpyr) and insecticides (Thiamethoxam, Chlorantraniliprole, Novaluron, Lambda-cyhalothrin and Avaunt). The test concentration of each pesticide in the present study was field recommended rate and each formulated pesticides were prepared in distilled water for using in all bioassay tests

Table 1. List of tested pesticides including their active ingredients, recommended field, and group, mode of action and rate of use.

Pesticides	Active ingredients	Group	Mode of action	Rate of use
Actara 25% WG	Thiamethoxam	neonicotinoid	Disrupting the nervous system of targeted pest either ingests or absorbs the poison into its body.	20 g/fed.
Radiant 12% SC	Spinetoram	spinosyns	Nicotinic acetylcholine receptor (Nachr) allosteric modulator	100 ml/fed.
Amistar 25% SC	Azoxystrobin	strobilurins	Respiration of fungi due to the disruption of electron transport chain, preventing ATP synthesis	50 ml/fed
Challenger super 24%SC	Chlorfenpyr	Pyrroles	Uncoupler of oxidative phosphorylation via disruption of the proton gradient	100 ml/ fed
Coragen®, Rynaxypyr®20% SC	Chlorantraniliprole	Ryanoid	Ryanodine receptor modulator	50 ml/ fed
Corvus 10%EC	Novaluron	insect growth regulator	Inhibitor of chitin synthesis	300 ml/ fed
Lambda®5% EC	Lambda-cyhalothrin	pyrethroids	Disrupt the normal functioning of the nervous system in an organism	20 g/fed.
Indoxacarb®15% SC	Avaunt	oxadiazine	Voltage-dependent sodium channel blocker	125 ml/ fed.

2.2.4. Effect of tested pesticides on EPN (IJs):

This experiment was conducted to evaluate the efficacy of eight novel pesticides at field recommended rate on two species of EPNs. Untreated (control) experiment for the EPNs species was also performed. Each treatment was replicated three times in a sterile tissue culture plate. Each replicate contained 1ml pesticide and 1ml EPNs IJs suspension (100 IJs/ml), and then covered with hulls stretched plastic. Data were recorded after three intervals (one, three and five days) and mortality % were calculated according to **Abbott's formula**.

2.3. Experimental design:

All experiments were arranged as randomized complete block design with six replicates for each treatment.

2.4. Statistical analysis:

All obtained data were subjected to ANOVA test using a computer program (CoHort Software, 2008) to determine Duncan's multiple range test and the LSD 5% (least significant difference). In addition, Abbott's formula was used to determine the Mortality percentages. Were counted according to **Abbott's formula** (Abbott, 1925).

$$Corrected\ mortality\% = \left(1 - \frac{no\ in\ Treatment\ after\ treatment}{no\ in\ Control\ after\ treatment}\right) * 100 \quad \text{Equation 1}$$

3. Results

3.1. Effects of different macro fertilizers (1, 5 and 10 g/liter water) on the activity of Steinernema carpocapsae one, three, five days after application.

The obtained data in Table 2 show that there were significant differences between the Urea, Ammonium sulfate and Ammonium nitrate in the number of total alive entomopathogenic nematode of *S. carpocapsae* after treated with 1g/liter, where there were no significant differences between NPK, NP, and DAP treatments and control.

As for the mortality percentages, the highest one was recorded with the treatment of Ammonium sulfate 95.79%,

Table 2. Effect of different macro fertilizers (1, 5, 10 g/liter water) on mortality % of *Steinernema carpocapsae* IJs one, three, five days after application.

Treatments	One day	Three days	Five days	Total alive	Mortality %
	Alive juveniles				
1g/liter water					
Urea	25	21	19	19 [c]	80.0
Ammonium sulfate	10	7	4	4 [d]	95.79
Ammonium nitrate	92	79	40	40 [b]	57.89
NPK	98	89	83	83 [a]	12.63
NP	98	88	86	86 [a]	9.47
DAP	93	85	83	83 [a]	12.63
Control	99	96	95	95 [a]	-
LSD 5%				13.72	
5g/liter water					
Urea	13	18	11	11 [c]	88.42
Ammonium sulfate	8	5	0	0 [d]	100
Ammonium nitrate	20	19	10	10 [c]	89.47
NPK	95	87	80	80 [a]	15.79
NP	94	85	82	82[a]	13.68
DAP	94	81	65	65 [b]	31.58
Control	99	95	95	95 [a]	-
LSD 5%				13.37	
10g/liter water					
Urea	14	9	2	2 [d]	97.87
Ammonium sulfate	5	2	0	0 [d]	100
Ammonium nitrate	45	38	34	34 [c]	63.83
NPK	81	80	75	75 [b]	20.21
NP	90	74	35	35 [c]	30.85
DAP	83	48	38	38 [c]	34.04
Control	99	95	94	94 [a]	-
LSD 5%				10.82	

Each Petri dish contains 100 Ijs. Values followed by different letters are the significant difference at 5%.

followed by the treatment of Urea 80%, and Ammonium nitrate 57.89%, while NP recorded the lowest one 9.47%.

The results of the concentration 5 g/liter water revealed that there were significant differences nearly between all treatments and control except with NPK and NP.

With respect to the mortality % the data in Table 2 revealed that Ammonium sulfate induced the highest mortality percentage 100% followed by the treatment of Ammonium nitrate 89.47% and Urea 88.42%, whereas, NPK recorded the lowest one 15.79%.

Regarding to the effect of different fertilizers at 10g/liter water on the activity of *S. carpocapsae*, it was clearly that the number of total alive EPNs were significantly differed compared with control where the obtained data revealed that Ammonium sulfate recorded the highest mortality percentage100%, while NPK recorded the lowest value 20.21%.

Generally, Ammonium sulfate, Urea and Ammonium nitrate recorded the highest EPN mortality percentages at three tested concentrations, whereas, DAP, NP and NPK recorded the lowest percentages nearly at the three tested concentrations. It was observed that morality percentages were increased as the concentration of macro fertilizers increased.

Effects of some macro elements (1,5 and 10g/liter water) on the activity of entomopathogenic nematode, *Heterorhabditis bacteriophora* one, three, five days after application:

The obtained data in Table 3 revealed that there were significant differences between all treatments in the number of total alive J3 of EPN and control after treated

Table 3. Effect of different macro fertilizers (1, 5, 10 g/liter water) on *Heterorhabditis bacteriophora* one, three, five days after application.

Treatments	One day	Three days	Five days	Total alive	Overall mortality %
	Alive juveniles				
1g/liter water					
Urea	21	16	15	15 [d]	84.21
Ammonium sulfate	23	19	11	11 [d]	88.42
Ammonium nitrate	85	80	65	35 [c]	63.16
NPK	94	14	68	68 [b]	28.42
NP	18	26	37	37 [c]	61.05
DAP	81	78	76	76 [b]	20
Control	98	96	95	95 [a]	-
LSD 5%				14.22	
5g/liter water					
Urea	19	15	9	9 [e]	90.63
Ammonium sulfate	18	11	7	7 [e]	92.71
Ammonium nitrate	89	74	48	48 [c]	50
NPK	90	79	66	66 [b]	31.25
NP	58	49	35	35 [d]	63.54
DAP	66	56	14	14 [e]	85.42
Control	98	95	96	96 [a]	-
LSD 5%				12.10	
10g/liter water					
Urea	11	8	2	2 [d]	97.89
Ammonium sulfate	11	8	0	0 [d]	100
Ammonium nitrate	75	65	30	30 [c]	68.42
NPK	88	80	54	54 [b]	43.16
NP	49	36	24	24 [c]	74.74
DAP	56	41	28	28 [c]	70.53
Control	98	96	95	95 [a]	-
LSD 5%				10.63	

Each Petri dish contains 100 IJs. Values followed by different letters are the significant difference at 5%.

with 1g/liter. The lowest alive numbers was recorded after treated with Ammonium sulfate as 11 J3, whereas the highest alive numbers was recorded after treated with DAP as 76 J3.

As for mortality percentages, the highest ones were recorded with the treatment of Ammonium sulfate 88.42% followed by the treatment of Urea 84.21%, Ammonium nitrate 63.16% and NP 61.05%, while DAP recorded the lowest one as 20%.

The results of the concentration of 5 g/liter water revealed that there were significant differences between all treatments and control in total alive numbers of *H. bacteriophora*.

With respect to mortality % the data in Table 3 revealed that all fertilizers induced higher *H. bacteriophora* and ranged between 50% - 92.71%.

Moreover, the effect of different fertilizers at 10 g/liter water on the activity of *H. bacteriophora* revealed that there were significant differences between all treatments and control in the total alive numbers of *H. bacteriophora* juveniles.

With respect to mortality %, the obtained data in Table 3 revealed that all fertilizers induced higher *H. bacteriophora* mortality % and ranged between 43.16% -100%.

Generally, the obtained data clearly revealed that all tested fertilizers recorded highest mortality % in *H. bacteriophora* at three concentrations. The morality percentages were increased as the concentration of fertilizers increased.

3.2. Effects of different concentrations of micro elements (0.1, 0.5 and 1.0 g / liter water) on Steinernema carpocapsae one, three, five days after application:

The effect of three micro elements at the concentration of 0.1 g / liter water) on the activity of the entomopathogenic nematode, *S. carpocapsae* IJs in Table 4 show that there were significant differences between all micro fertilizer treatments and control in the total alive EPN numbers.

With respect to mortality %, the obtained data in Table 4 revealed that all micro fertilizers induced higher *S. carpocapsae* mortality % ranged between 77.55% - 90.82%.

As for the effect of three micro elements at the concentration of 0.5 g / liter water on the activity of *S. carpocapsae* IJs, results in Table 4 show that there were significant differences between all micro fertilizer treatments and control in the total alive numbers of tested genus.

With respect to the mortality %, the obtained data in Table 4 revealed that all micro fertilizers induced higher *Steinernema carpocapsae* mortality percent and the mortality percent ranged between 70.72-100%.

Table 4. Effect of different micro elements (0.1, 0.5, 1.0 g / liter water) on *Steinernema carpocapsae* one, three, five days after application.

Treatments	One day	Three days	Five days	Total alive	Overall mortality %
	Alive juveniles				
0.1g/liter water					
Zn	41	16	22	12 [b]	87.76
Mn	27	23	10	10 [b]	89.80
Fe	27	23	9	9 [b]	90.82
Zn +Mn + Fe	19	14	11	11 [b]	88.78
Control	99	99	98	98 [a]	-
LSD 5%				8.65	
0.5 mg/liter water					
Zn	34	17	8	8 [b]	91.75
Mn	29	16	7	7 [b]	92.78
Fe	28	19	9	9 [b]	70.72
Zn +Mn + Fe	12	6	0	0 [b]	100
Control	99	98	97	97 [a]	-
LSD 5%				8.26	
1.0 g/liter water					
Zn	30	11	5	5 [b]	94.85
Mn	25	8	0	0 [b]	100
Fe	27	12	0	0 [b]	100
Zn +Mn + Fe	9	3	0	0 [b]	100
Control	98	98	96	96 [a]	-
LSD 5%				8.18	

Each Petri dish contains 100 Ijs. Values followed by different letters are the significant difference at 5%.

As for the effect of three micro elements at 1 g / liter water on the activity of *S. carpocapsae* IJs, results in Table 4 show that there were significant differences between all micro fertilizer treatments and control in the total numbers of alive nematodes.

With respect to the mortality %, the obtained data in Table 4 revealed that all micro fertilizers induced higher mortality % of *S. carpocapsae* ranged between 94.85% - 100%.

Generally, all tested micro fertilizers induced higher mortality % of *S. carpocapsae* juveniles after three tested concentrations, as well as morality percentages were increased as the concentration of fertilizers increased.

3.3. Effects of different micro elements (0.1, 0.5 and 1.0 g / liter water) on Heterorhabditis bacteriophora one, three, five days after application.

The effect of three micro elements at the concentration of 0.1 g / liter water on the activity of *H. bacteriophora* (Table 5) show that there were significant differences between all micro fertilizer treatments and control in the total numbers of alive juveniles.

With respect to the mortality %, the obtained data in Table 5 revealed that all micro fertilizers induced higher mortality percentages of *H. bacteriophora* juveniles ranged between 87.76% - 90.82%.

As for the effect of three micro elements at the concentration of 0.5 g / liter water on the activity of *H. bacteriophora* IJs, results in Table 5 show that there were significant differences between all micro fertilizer treatments and control in the total numbers of alive juveniles.

With respect to mortality %, the obtained data in Table 5 revealed that all micro fertilizers induced higher mortality % of *H. bacteriophora* ranged between 70.72% -100%.

The effect of the tested micro elements at the concentration of 1 g / liter water on the activity of *H. bacteriophora* IJs, results in Table 5 show that there were significant differences between all micro fertilizer treatments and control in the total numbers of alive juveniles.

With respect to the mortality %, the obtained data in Table 5 revealed that all micro fertilizers induced higher mortality percentages of *H. bacteriophora* ranged between 94.85% -100%.

Generally, all tested micro fertilizers induced higher mortality % of *H. bacteriophora* exposed to the three tested concentrations, in addition morality percentages were increased as the concentration of fertilizers increased.

The obtained results are in harmony with those of Ebssa and Koppenhöfer (2012) who found that the tested

Table 5. Effect of different micro elements (0.1, 0.5, 1.0 g / liter water) on *Heterorhabditis bacteriophora* one, five, ten days after application.

Treatments	One day	Three days	Five days	Total alive	Overall mortality %
	Dead juveniles				
0.1 g/liter water					
Zn	39	24	10	10 [b]	89.58
Mn	35	18	8	8 [b]	91.67
Fe	49	25	11	11 [b]	88.54
Zn +Mn + Fe	23	21	9	9 [b]	90.63
Control	99	98	96	96 [a]	-
LSD 5%				8.65	
0.5 g/liter water					
Zn	22	18	8	8 [b]	96.67
Mn	20	20	3	3 [b]	96.88
Fe	73	19	6	6 [b]	93.75
Zn +Mn + Fe	13	9	3	3 [b]	96.88
Control	98	97	96	96 [a]	-
LSD 5%				8.29	
1.0 g/liter water					
Zn	19	16	4	4 [b]	95.83
Mn	14	8	0	0 [b]	100
Fe	25	15	0	0 [b]	100
Zn +Mn + Fe	7	6	0	0 [b]	100
Control	97	97	96	96 [a]	-
LSD 5%				8.18	

Each Petri dish contains 100 IJs. Values followed by different letters are the significant difference at 5%.

nematode recorded a high mortality as 90% of *A. ipsilon* larvae in laboratory. The mechanisms of insect larvae infection with nematode has been showed by Shapiro-Ilan et al. (2009) who stated that the nematodes enter the host once a host is located. Moreover, *H. bacteriophora* nematode enters through the insect body, it can continue the life cycle and induced insect's death, and this may be discussed in the variety between the larval mortality rates.

As for the effect of fertilizers on the tested nematode species, Şahin and Susurluk (2018) reported that, compared to control, all fertilizer solutions, except for urea doses, appeared to have a significant lethal effect on *H. bacteriophora* at the end of the first day. Furthermore, the same authors reported that, compared to control, only NPK (1 g/liter) and all doses of AN and AS did not show significant effect on *S. feltiae* after 1 day.

EPNs are highly exposed to the effects of fertilizers due to excessive and prolonged use of inorganic fertilizers. EPNs were affected by inorganic fertilizer according to their ingredients and concentrations and the species of nematode (Bednarek and Gaugler, 1997). According to the present research, all inorganic fertilizers at different doses were lethal to *H. bacteriophora* (HBH. Similar results were reported by Bednarek and Gaugler (1997). Susurluk (2008) reported that *Steinernema* was not adversely affected when agricultural activities such as fertilization (organic and NPK) and herbicide (Trifluralin EC) were started together during a 2-year field study. In addition, Susurluk (2008) stated that prolonged exposure to high concentrations of inorganic NPK fertilizers inhibit the activities of *Heterorhabditis*.

3.4. Effects of some pesticides on Steinernema carpocapsae one, five, ten days after application:

The effects of tested pesticides on entomopathogenic nematode, *S. carpocapsae* shown in Table 6 revealed that, one day after exposure, there were no significant differences in their effects between tested pesticides and control except Lufenuron and Lambda-cyhalothrin which significantly differed than control.

Lambda-cyhalothrin revealed the highest mortality as 10.67% followed by 6.33%, whereas Spinetoram recorded the lowest mortality only 0.33%.

As for the effect of tested pesticides after 3 days the obtained data (Table 6) clearly revealed that mortality % significantly increased in pesticides treatment compared with control, and increased more than one day after treatment.

Lambda-cyhalothrin induced the highest mortality % of *S. carpocapsae juveniles as* 16.33%, followed by Lufenuron which recorded 10.33%, Avaunt 8.33%,Thiamethoxam 6.67%, Azoxystrobin 6.33%, and Chlorfenpyr 5.33% compared with control whereas, Spinetoram revealed the lowest mortality as 1.33%.

After five days of treatment, the obtained data (Table 6) revealed that the mortality percentages of *S. carpocapsae* juveniles were significantly differed than control, and increased compared with mortality % after one & three days.

Lambda-cyhalothrin revealed the highest mortality as 23.67%, whereas, Spinetoram achieved the lowest one as 2.67%.

With respect to overall mortality percentages of all tested pesticides results revealed significant increase ranged between 15.34% -50.67%. Lambda-cyhalothrin revealed the highest mortality as 50.67%, whereas Spinetoram revealed the lowest one as 4.33%.

Generally, mortality percentages were increased along the time after treatment increased. Nearly all tested pesticides increased mortality % except spinetoram which revealed the lowest mortality percentage.

3.5. Effects of some pesticides on Heterorhabditis bacteriophora one, three, five days after application:

The effects of tested pesticides on *H. bacteriophora* juveniles shown in Table 7, one day of treatment, revealed that there were no significant differences between all tested pesticides except lambda-cyhalothrin and Lufenuron which significantly differed than control and exhibited the highest mortality percent as 12.0% and 6.67%, respectively.

Table 6. Effect of different pesticides on mortality % of *Steinernema carpocapsae* IJs one, three, five days after application.

Treatments	One day	Three days	Five days	Overall mortality %
		Mortality %		
Thiamethoxam	2.33±0.19^{c}	6.67±0.58 ^{cd}	13.33±1.15^{b}	22.33±1.15^{cd}
Spinetoram	0.33±0.06^{c}	1.33±0.13^{e}	2.67±0.58^{c}	4.33±0.58^{e}
Azoxystrobin	3.0±0.58^{c}	6.33±0.58^{cd}	9.67±1.15^{b}	18.0±2.31^{cd}
Chlorfenpyr	2.0±0.58^{c}	5.33±0.58^{cd}	12.33±1.15^{b}	19.33±1.73^{cd}
Chlorantraniliprole	2.0±0.58^{c}	4.67±0.58^{d}	8.67±0.58^{b}	15.34±1.73^{d}
Lufenuron	6.33±0.58^{b}	10.33±1.15^{b}	12.67±1.15^{b}	29.33±2.31^{b}
Lambda-cyhalothrin	10.67±1.15^{a}	16.33±1.17^{a}	23.67±1.73^{a}	50.67±2.89^{a}
Avaunt	3.0±0.58^{c}	8.33±0.58^{bc}	13.0±1.15^{b}	24.33±2.31^{bc}
Control	1.0±0.58^{c}	0.0±0.0^{e}	1.0±0.0^{c}	2.0±0.58^{e}
LSD 5%	1.82	2.43	3.18	5.63

Each Petri dish contains 100 IJs. Values followed by different letters are the significant difference at 5%.

Table 7. Effect of different pesticides on *Heterorhabditis bacteriophora* one, three, five days after application.

Treatments	One day	Three days	Five days	Overall mortality %
	Mortality %			
Thiamethoxam	2.67±0.58^{c}	7.33±0.58^{c}	14.67±1.15^{bc}	24.67±1.73^{bc}
Spinetoram	0.67±0.17^{c}	1.67±0.17^{d}	3.33±0.58^{e}	5.67±0.58^{e}
Azoxystrobin	2.0±0.58^{c}	5.33±0.58^{c}	8.33±0.58^{d}	15.66±1.73^{d}
Chlorfenpyr	2.33±0.58^{c}	6.67±0.5^{c}	18.33±1.73^{b}	27.33±1.15^{b}
Chlorantraniliprole	2.33±0.58^{c}	5.67±0.58^{c}	11.67±1.15^{cd}	19.67±2.89^{cd}
Lufenuron	6.67±0.58^{b}	11.33±1.15^{b}	14.67±1.15^{bc}	32.67±2.89^{b}
Lambda-cyhalothrin	12.0±1.15^{a}	18.0±1.73^{a}	28.0±1.73^{a}	58.0±2.89^{a}
Avaunt	3.33±0.58^{c}	10±1.15^{b}	16.0±1.73^{bc}	28.33±2.31^{b}
Control	1.0±0.0^{c}	1.0±0.0^{d}	1.0± 0.0^{e}	3.0±0.33^{e}
LSD 5%	1.82	2.63	3.66	6.11

Each Petri dish contains 100 IJs. Values followed by different letters are the significant difference at 5%.

As for the effect of tested pesticides on *H. bacteriophora* after three days of treatment, the obtained data (Table 7) clearly show that there were significant differences between all tested pesticides except Spinetoram which recorded the lowest mortality percentage as 1.67%. Whereas, lambda-cyhalothrin achieved the highest mortality percentage followed by Lufenuron and Avaunt recording mortality percentages as 18.0%, 11.33% and 10%, respectively.

The data after five days of treatment of *H. bacteriophora* juveniles with tested pesticides (Table 7) indicated that the mortality percentages were the highest compared with one and three days after treatment. The mortality percentages in all pesticides were significantly increased compared with control, except spinetoram which recorded the lowest one as 3.33%. Lambda-cyhalothrin recorded the highest mortality percentage as 28.0%, followed by Chlorfenpyr, Avaunt, Lufenuron and Thiamethoxam which recorded18.33, 16.0, 14.67 and 14.67 mortality %, respectively.

With respect to overall mortality percentages of tested pesticides on *H. bacteriophora* juveniles, the data in Table 7 revealed that Spinetoram recorded the lowest total mortality percentage as 5.67%. On the other side, lambda-cyhalothrin showed the highest mortality percentage as 58% followed by Lufenuron, avaunt, chlorfenpyr and Thiamethoxam which recorded 32.67, 28.33, 27.33 and 24.67%, respectively.

Generally, all tested pesticides increased mortality percentages of *H bacteriophora* juveniles. Lambda-cyhalothrin induced the highest mortality percentage, whereas spinetoram recorded the lowest one.

The obtained results revealed that the two EPNs were tolerant to all tested pesticides, where the mortality percentages were ranged between 2.67% -23.67% in *S. carpocapsae* and 3.33% - 18.33% in *H. bacteriophora* after five days of treatment except Lambda cyhalothrin which recorded relatively the highest mortality percentages as 23.67 and 28.0% for *S. carpocapsae* and *H. bacteriophora*, respectively.

The obtained results are in agreement with Radová (2010) who exposed entomopathogenic nematode, *Steinernema feltiae* to 8 insecticides, 7 acaricides and 4 fungicides under laboratory conditions and found that *S. feltiae* was tolerant to all tested insecticides and fungicides, mortality during 72 hours varied from 2.26% to 18.68% and from 7.04% to 8.86%, respectively. Also, Atwa et al. (2017) evaluated the effect of (Dipel 2x, Radiant, Proclaim, Aphox and Coragen) on *Heterorhabditis bacteriophora* BA1 and *Steinernema carpocapsae* BA2 and found that all insecticides showed negative effect on the tested infective juveniles (IJs) of EPNs. In addition, Hassan and Ibrahim (2019) studied the effects of certain insecticides viz., Coragen, Nomolt, Ekio and Magic smart with *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* against cotton leaf worm, *Spodoptera littoralis* and found that all tested insecticides induced low effect on the tested entomopathogenic nematodes. El Roby et al. (2023) evaluated the activity of *Heterorhabditis bacteriophora* (HP88) and *Steinernema carpocapsae* (AT4), as well as their compatibility with two common insecticide formulations (lambda cyhalothrin and flubendiamide) and one insect growth regulator (lufenuron) at LC_{50} and LC_{25} against *Spodoptera frugiperda* (AT4) under laboratory conditions and found that all tested insecticides with the two doses were nontoxic to the two strains of EPN according to IOBC test, moreover, they concluded that the mixtures of insecticides with the two EPN can be used in integrated *S. frugiperda* management except Lufeuron with LC_{25}.

4. Conclusion

Tested macro fertilizers such as DAP; NP and NPK can be used safely at tested concentrations with *Steinernema carpocapsae*. All tested macro fertilizers cannot be used with *Heterorhabditis bacteriophora*. All micro fertilizers couldn't be used with two EPNs. All tested pesticides can be successfully used for integrated plant protection systems. EPNs are tolerant to the tested pesticides and

the tank-mix application is possible in most compounds except the Lambda cyhalothrin which significantly reduced the virulence of tested nematodes.

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