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Biosafety of Different Entomopathogenic Nematodes Species on Some Insects Natural Enemies.

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ABSTRACT

Biosafety of ten entomopathogenic nematodes (EPNs)species were evaluated against three natural enemies; green lacewing, *Chrysoperla carnea* (Stephenus), seven spotted lady beetle, *Coccinella septempunctata* and minute pirate bug, *Orius albidipennis*.Three concentrations of each strain were used (500, 250 and 125 IJs/ml). The lowest lethal concentration for 50 % of each population (LC_{50},s) was 110.7, 109.2 and 730.7 IJs/ml for *C. carnea*, *C. septempunctata* and *O. albidipennis,* respectively. *Steinernema carpocapsae* (ALL) was the most effective entomopathogenic nematodes specie against all tested insects. The D1specie (*Heterorhabditis bacteriophora* Poinar) was the least effective against *C. carnea* and *C. septempunctata*. The $LC_{50,}$s were 214.9 and 163.3 IJs/ml, respectively. The S2 (*S. carpocapsae* Wiser) was the least effective specie against *O. albidipennis* (2099.1 IJs/ml). These results cleared that *O. albidipennis* not affected by all concentrations used. So, entomopathogenic nematodes can be used safely during the peak of *O. albidipennis*. The results recommended that avoiding using concentrations above 100 IJs/ml of entomopathogenic nematodes during the peak of *C. carnea* and *C. septempunctata*. The obtained results showed also, both *C. carnea* and *C. septempunctata* were more susceptible than *O. albidipennis* to all species of entomopathogenic nematodes.

Keywords: Entomopathogenic nematodes strains, Biosafety, *Chrysoperla carnea*, *Coccinella septempunctata* and *Orius albidipennis*

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INTRODUCTION

Entomopathogenic nematodes existed naturally in the soil and seek out their hosts in response to carbon dioxide, vibration and other chemical cues [1]. Both Heterorhabditidae and Steinernematidae species have been effectively used as biological control agent in pest management programs [2]. Entomopathogenic nematodes were suitable forthe integrated pest management (IPM) programs [3].Entomopathogenic nematodes use two search methods for their hosts: ambushers or cruisers [4]. Ambushers includes*Steinernema carpocapsae*,it have an energy-conserving approach and lie-in-wait to attack moving insects in the upper soil. Cruisers such as *Steinernema glaseri* and *Heterorhabditis bacteriophora*, which are highly active and generally subterranean, moving significant distances using volatile cues and other methods to find their host underground. So, these species were effective against less mobile pests such as white grubs (Scarab beetles). Other nematode species like *Steinernema feltiae* and *Steinernema riobrave* use an intermediate foraging method (combination of ambush and cruiser type) to find their host.

Green lacewing, *Chrysoperla carnea* (Stephenus) is an insect in the Chrysopidae family. It is spreads in many parts of the world. Although the adults of this insect feeds on nectar, pollen and aphidhoneydew, the larvae are considered an active predator and feed on aphids, lepidopteron eggs and other small insects. It has been used as abiological controlagent against many insect pests, such as mealybugs, aphids, thrips, whiteflies and mites [5 – 6 – 7].

Seven spotted lady beetles, *Coccinella septempunctata* is considered an important predator against many insect pests. Both adults and larvae are voracious predators of aphids, and are one of the gardener's greatest natural allies [8]. To use the seven spotted lady beetles as a biocontrol agent or in integrated pest management programmes, their susceptibility to the pesticides used must be taken into account [9]. So, there is a need to safe agent against insect pests to reserve the natural enemies.

Minute pirate bug, *Orius albidipennis* is a common insect predator that found in many agricultural crops.Nymphs and adults feed on a wide range of arthropods including aphids, chinch bugs, springtails, plant bugs, thrips, eggs and small larvae of corn earworms, whiteflies, spider mites.

MATERIAL AND METHODS

Entomopathogenic nematodes (EPNs)

Ten species of EPNs were used, three local strains and seven worldwide species:

The native species include:

- *Steinernema carpocapsae* (BA2), Sinai, Egypt [10]
- *Steinernema carpocapsae* (S2), Sinai, Egypt [11]
- *Heterorhabditis* sp. (D1),Dina Farmers

The worldwide species were imported from Kiel University, Germany, include:

- *Steinernema feltiae* (Filipjev) (SF),
- *S. carpocapsae* (All),
- *S. riobravae* (SR),
- *S. scabtarsci* (SS) ,
- *S. glasseri* (SG),
- *H. bacteriophora* (HP88)
- *H. marilatus* (MAR).

All these species are known to have potent activities against several insect species. The tested nematode species were maintained as in vivo cultures on *Galleria mellonella* at 27^{o}C. One hundred of last instars larvae of *G. mellonella* were placed on a Petri dish (20 cm diameter) padded with two filter paper discs. About 5000 infective juveniles (IJs) in5 ml distilled water were moistened but not wetted. The insect larvae

were dead within 48 hours after infection. The cadavers were placed on White traps White [12] as described by Kaya and Stock (1997) [13]. After 10 days, the IJs emerged from the cadavers and migrated into the water, from which they were collected.

Tested insects

- Green lacewing, *Chrysoperla carnea* (Neuroptera :Chrysopidae). The second instar larvae were used.
- Seven spotted lady beetles, *Coccinella septempunctata* (Coleoptera: Coccinellidae). The second instar larvae were used.
- Minute pirate bug, *Orius albidipennis* (Hemiptera : Anthocoridae). The immature and adults were used.

All tested insects were obtained from Plant Protection Research Institute, Dokki, Giza.

Experimental method

The basic experimental method developed under laboratory conditions for EPNs assessment against studied predators was carried out in glass Petri dishes (9 cm in diameter) according to Trdan et al. (2009)[14]. Nematode species was performed at three different concentrations (500, 250, 125IJs /ml) and each concentration replicated four timeand each replicate has 10 healthy insects at 27^{o}C. Other four replicates were treated by water only as a control with all tested insects. Equivalent controls were processed similarly except that the nematode to be assayed was omitted (just water was added). Pathogenecity was evaluated by counting dead larvae three days after application. The percentages of mortality in each treatments were counted after 24, 48, 72 hrs. The $LC_{50,}$ s for all treatments were calculated using Proban Program.

Statistical analysis

Data were subjected to the analysis of variance test (ANOVA) via Randomized Complete Block Design (RCBD) (F. test) and analysis of variance (one ways classification ANOVA) followed by a least significant difference (LSD) at 5% (Costat Statistical Software, 1990)[15]

RESULTS AND DISCUSSION

Entomopathogenic nematode (EPN) as an important biocontrol agent may be has a side effect on some insects natural enemies

Effect of some entomopathogenic nematodes species on green lacewing, *Chrysoperla carnea*

Table 1. Efficacy of different strains of entomopathogenic nematodes on the second instar larvae of green lacewing, *Chrysoperla carnea*

Strains	Percent of mortality			Slope	LC_{50} and confidence limits
	500 IJs/ml	250 IJs/ml	125 IJs/ml		
SR	77.5 ±5.0^{b}	62.5±5.0^{bcd}	37.5±9.7^{bc}	1.8± 0.3	182.8(141.6-220.5)
SS	100± 0.0^{a}	65± 5.8^{abc}	32.5± 5.0^{c}	2.8 ± 0.3	179.9(153.9–204.9)
SF	95.0 ± 5.8^{a}	77.5± 5.0^{a}	50.0±8.2^{ab}	2.7± 0.4	126.2(98.5 –149.2)
S2	95.0 ± 5.8^{a}	62.5± 5.0^{bcd}	57.5± 5.0^{a}	3.1± 0.4	171.1(147.1-193.6)
SG	80.0±8.2^{b}	50± 8.2^{d}	37.5±9.6^{bc}	1.9±0.3	205.9(166.1-246.1)
All	87.5 ± 9.6^{ab}	72.5± 15.0^{ab}	55.0± 5.8^{a}	1.7±0.3	110.7(66.3 – 144.9)
BA2	95.0 ± 4.1^{a}	75± 5.8^{ab}	55.0± 5.8^{a}	2.4±0.4	116.2(84.6-141.6)
HP_{88}	75.0± 5.8^{b}	55± 5.8^{cd}	45.0±10.0^{abc}	1.3±0.3	169.5(108.3-219.6)
MAR	77.5 ± 9.6^{b}	67.5± 5.0^{abc}	42.5±5.0^{abc}	1.6±0.3	155.2(108.1-193.8)
D1	72.5 ±9.6^{b}	50± 0.0^{d}	37.5 ± 5.0^{bc}	1.6±0.3	214.9(163.4-268.9)
control	22.5 ± 9.6^{c}	20± 0.0^{e}	15.0± 10.0^{d}		
F values	34.1***	233***	10.9***		
LSD	10.4	9.6	10.8		

*Means in each column followed with the same letter are not significantly at 0.05 probability level.

The second instar larvae of green lacewing, *C. carnea* are treated by ten species of EPNs with three concentrations (500, 250 and 125 IJs/ ml). The percentages of mortality with the first concentration ranged between 100% (SS) and 72.5% (D1). The statistical analysis shows that there are significant differences between five species and other five. The first group includes SS, SF, S2, BA2 and All. The other group includes SR, SG, $HP_{88,}$ MAR and D1.The first group is more effective than the second one. These results occurred also in the second and third concentrations. Data in Table (1) show that the EPN specie*Steinernema carpocapsae* (All) is the most effective specie followed by *S. carpocapsae* Wiser (BA2). The LC_{50},s are 110.7 and 116.2 IJs/ml, respectively. *Heterorhabditis bacteriophora* Poinar (D1) is the least effective specie followed by SG, the LC_{50},s 214.9 and 205.9 IJs/ml, respectively. The highest concentration (500 IJs/ml) is the most effective against SS species, the percentage of mortality reached at 100 after three days of treatments. These results were not consistent with Rojht et al. (2009)[16]. The authors found that the concentration 500 was the lowest effective against the *C. carnea* larvae compared the other concentrations.The authors also found that *Steinernema feltiae*, *Steinernema carpocapsae* and *Heterorhabditis bacteriophora*were more effective especially with the high concentrations against *C. carnea* larvae.

Effect of some entomopathogenic nematodes species on seven spotted lady beetle, *Coccinella septempunctata* larvae.

The obtained results in Table (2) show that the susceptibility of *C. septempunctata* is higher than other predator, especially with the first concentration (500 IJs/ml). The percentages of mortalities reached at 100% in SR, SS, SF, ALL BA2 and HP_{88} species after three days of treatment with the first concentration. *Steinernema feltiae* (Filipjev) (SF) is the most effective species against the second instar larvae of *C. septempunctata* followed by SR and ALL species. The LC_{50},s are 109.2, 112.3 and 114.8 IJs/ml, respectively. *Heterorhabditis sp.* Poinar (D1) is the least effective species followed by S2 and MAR. The LC_{50},s are 163.3, 141.9 and 139.4 IJs/ml, respectively.

Table 2. Efficacy of different strains of entomopathogenic nematodes on the second instar larvae of seven spotted lady beetle, *Coccinella septempunctata*

Strains	Percent of mortality			Slope	LC_{50} and confidence limits
	500 IJs/ml	250 IJs/ml	125 IJs/ml		
SR	100 ± 0.0^{a}	80.0±0.0^{ab}	55.0± 5.8^{ab}	2.5±0.4	112.3(81.9-136.7)
SS	100 ± 0.0^{a}	75± 5.8^{bc}	52.5± 9.6^{ab}	2.5 ± 0.4	123.9(94.4-148.2)
SF	100 ± 0.0^{a}	77.5± 5.0^{bc}	57.5± 5.0^{ab}	2.3±0.4	109.2(76.7-135.1)
S2	90 ± 0.0^{b}	67.5± 5.0^{bc}	47.5± 5.0^{ab}	2.2 ± 0.3	141.9(107.9-170.4)
SG	82.5 ± 5.0^{c}	72.5± 9.6^{bc}	50.0± 11.5^{ab}	1.5 ± 0.3	118.4(68.6-156.2)
ALL	100 ± 0.0^{a}	87.2± 5.0^{a}	52.5± 5.0^{ab}	2.8 ± 0.4	114.8(87.9-136.6)
BA2	100 ± 0.0^{a}	72.5± 5.0^{bc}	50± 8.2^{ab}	2.6 ± 0.4	131.7(102.8-155.8)
HP_{88}	100 ± 0.0^{a}	72.5± 5.0^{bc}	60± 0.0^{a}	2.5 ± 0.4	133.6(104.4-158.1)
MAR	97.5 ± 5.0^{a}	75.0 ±5.8^{bc}	45± 10.0^{ab}	2.7± 0.4	139.4(111.6-163.1)
D1	90.0 ± 8.2^{b}	65.0± 5.8^{c}	40.0±8.2^{b}	2.5 ± 0.3	163.3(133.8-189.9)
control	15.0 ± 5.8^{d}	12.5± 5.0^{d}	20± 8.2^{c}		
F values	185.3***	49.6***	8.3***		
LSD	5.3	8.1	10.9		

*Means in each column followed with the same letter are not significantly at 0.05 probability level.

These results are agreed with Farag (2002)[17]. The author found that the entomopathogenic nematodes *H. taysearae* Shamseldean and *S. carpocapsae* strain S2 caused a high mortality to the larvae of *C. undecimpunctata* under laboratory condition, so the author recommended that the entomopathogenic nematodes not suitable when the peak of this insect. [18]The authors cleared that the larvae of lady beetles as a nontarget insect were less susceptible to entomopathogenic nematodes *H. bacteriophora and S. carpocapsae* than the larvae of *Agrotis ipsilon* as a target insect.

Effect of some entomopathogenic nematodes species on minute pirate bug,*Oriusalbidipennis*

Data in Table (3) show that *O. albidipennis* individuals are less susceptible to all tested entomopathogenic nematodes species. The highest mortality percent not exceed 40% with the first concentration of *S. carpocapsae* (ALL). The obtained results show that the *S. carpocapsae* (ALL) is the most effective specie followed by *S. carpocapsae* Wiser (BA2). The $LC_{50,}$s are 730.7 and 759.3 IJs/ml, respectively. On the other hand, the *S. carpocapsae* Wiser (S2) is the least effective specie against the *O. albidipennis* (2099.1 IJs/ml).

Table 3. Efficacy of different strains of entomopathogenic nematodes on minute pirate bug, *Oriusalbidipennis*

Strains	Percent of mortality			Slope	LC_{50} and confidence limits
	500 IJs/ml	250 IJs/ml	125 IJs/ml		
SR	25.0±10^{b}	10.0±0.0^{abc}	2.5± 5.0^{ab}	2.0±0.4	1085.1(718.4 – 2883.9)
SS	20.0±0.0^{b}	7.5±5.0^{abc}	0.0^{b}	2.6±06	1004.1(710.3 – 2253.6)
SF	20.0±11.5^{b}	10.0±8.2^{abc}	2.5± 5.0^{ab}	1.7±0.4	1557.9(862.1 – 9235.1)
S2	20.0±8.2^{b}	7.5±5.0^{abc}	5.0±5.8^{ab}	1.4±0.4	2099.1(974.8 – 38107.3)
SG	17.5±5.0^{b}	10.0±0.0^{abc}	2.5± 5.0^{ab}	1.5±0.4	1912.4(946.3 – 22125.1)
ALL	40.0±8.2^{a}	17.5±5.0^{a}	10.0±0.0^{a}	1.8±0.4	730.7(529.7 – 1422.5)
BA2	32.5±9.6^{ab}	15.0±5.8^{ab}	2.5± 5.0^{ab}	2.3±0.4	759.3(575.2 – 1292.1)
HP_{88}	15.0±5.8^{b}	5.0±5.8^{bc}	2.5± 5.0^{ab}	1.8±0.5	1976.3(983.4 – 25011.4
MAR	27.5±9.6^{ab}	12.5±5.0^{ab}	2.5± 5.0^{ab}	2.1±0.4	933.4(654.1 – 2019.3
D1	15.0±5.8^{b}	7.5±5.0^{abc}	0.0^{b}	2.3±0.6	1334.7(825.8 – 5302.8)
Control	0.0^{c}	0.0^{c}	0.0^{b}		
F values	7.4***	3.9***	1.9***		
LSD	10.9	6.9	5.9		

*Means in each column followed with the same letter are not significantly at 0.05 probability level.

These results cleared that the *C. carnea* and *C. septempunctata* were more susceptible to all tested entomopathogenic species than *O. albidipennis. S. carpocapsae* (ALL) was the most effective entomopathogenic nematodes specie against all tested insects. *Heterorhabditis bacteriophora* Poinar (D1) was the least effective against both *C. carnea* and *C. septempunctata,* while *S. carpocapsae* Wiser (S2) was the least effective against *O. albidipennis.* Both *Heterorhabditis bacteriophora* Poinar (D1) and *S. carpocapsae* Wiser (S2) can be used safely when the peak of all tested insects. These results recommended that avoiding using of *S. carpocapsae* (ALL) during the peak of all tested insects.

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