The Pharma Innovation Journal 2023; SP-12(8): 1362-1365

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; SP-12(8): 1362-1365

www.thepharmajournal.com
Received: 14-05-2023
Accepted: 29-06-2023

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Safety studies of entomopathogenic nematode on natural enemies, larval predator *Chrysoperla carnea* and Parasitoid, *Trichogramma chilonis*

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Abstract
Potential of Entomopathogenic nematodes (EPNs) against the larval predator *Chrysoperla carnea* and parasitoid *Trichogramma chilonis* were studied. Pathogenicity of isolated EPNs at different concentrations (200, 400, 600, 800, 1000 and 1200 IJs/100 eggs or larva) were tested against different days after treatment. Isolated EPN strain *Steinernema* sp. found harmless to the different days of parasitized eggs of tricho-card at different concentrations with more than 70 percent adult emergence in all treatments. Additionally, *C. carnea* demonstrated that mortality was 93.22 percent at higher doses (1200 IJs/larva) compared to 65.35 percent at lower doses (200 IJs/larva). The study illustrated that at lower concentrations, EPNs were less harmful to the natural enemies.

Keywords: Pathogenicity, Entomopathogenic nematodes (EPNs), Steinernema sp., *Chrysoperla carnea*, *Trichogramma chilonis*, *Corcyra cephalonica*

Introduction
Entomopathogenic nematodes (EPNs) which are the members of Steinernematidae and Heterorhabditidae families in the order Rhabditida, have lately been employed as bio-control agents. (Dillman and Sternberg, 2011) [3]. Based on the laboratory and field evaluation studies, EPN showed a larger host adaptability against insect pests nearly 135 families under 17 orders (Ehlers and Peters, 1996) [4]. Furthermore, EPN having a symbiotic association with bacteria belongs to genera *Xenorhabdus* and *Photorhabdus* respectively. Entomopathogenic bacteria (EB) kills the host insect by the mechanism of septicaemia, ultimately leads to death within 48-72 h of infection (Georgis, 1992; Goodrich-Blair and Clarke, 2007) [6, 7]. Due to wider host range, serious concern about non-target pests as well as natural enemies and beneficial insects (Hazir *et al.*, 2003) [8]. In addition to insecticides, farmers now use bio-control agents, particularly a *Trichogramma* species and Green lacewing, *Chrysoperla carnea* (Neuroptera: Chrysopidae), is an active predator of soft scale insects, including mealybugs, aphids, thrips, whiteflies, mites as well as lepidopteran insect eggs, and *Trichogramma chilonis*, a parasitoid of lepidopteran eggs (Trichogrammatidae: Hymenoptera). This study aims to study the safety of natural enemies *C. carnea* and *T. chilonis*. against EPN.

Materials and Methods
Soil sampling and EPN isolation
Soil samples were collected from the maize-growing fields in the Tuticorin district of Tamil Nadu between the year 2022 and 2023. A total of 200 g of soil samples were taken from each location in a depth of 10-15 cm near cropping area (Kaya and Stock, 1997) [10]. A 10 final instar larvae of Rice Moth, *Corcyra cephalonica* Stain. were used to bait the samples in a plastic container, then maintained at room temperature (Bedding and Akhurst, 1975) [2]. After a week of incubation, dead cadavers were collected and arranged for White trap (White, 1927) [14]. After five days, IJs were start emerge out from the cadaver and collected at the bottom of the Petri plate filled with 0.01 percent formalin solution.

Harvesting and counting of EPN
Harvested EPNs were washed with distilled water for three times and supernatants were removed. Then the EPN suspensions were stored in a tissue culture flasks at 15 °C and aerated

two days once. IJs were counted by using nematode counting chamber (8 x 8 x 1.5 cm) under high resolution microscope.

Pathogenicity test against second instar larvae of *C. carnea* Stephens

Virulence of isolated native strain were tested against *C. carnea* at a different dose of IJs with six treatments and one control. Due to their cannibalistic nature, a total of three replications were done with one larva per plate. A total of 10 larvae per replication, arranged by using a completely randomized (CRD) design. Larval mortality were assessed on 2, 3, 4, 5 and 6 days after treatment. Petri-plates were tightly sealed with parafilm, placed in a polythene cover and kept in a dark room at a temperature of 28 °C (Table 1).

Table 1: Various concentrations of EPN were tested against *C. carnea* larvae

S. No	Treatments
1.	200 IJs/larva/ml
2.	400 IJs/ larva/ml
3.	600 IJs/ larva /ml
4.	800 IJs/ larva /ml
5.	1000 IJs/ larva/ml
6.	1200 IJs/ larva /ml
7.	Control (1ml distilled water alone)

Pathogenicity test against eggs of *T. chilonis* Ishii

For *T. chilonis*, tricho-cards were used with 100 *Corcyra* parasitized eggs for each replication. Totally 3 replication were performed. Different dosage of EPN were sprayed on the strip of tricho-cards on different days parasitization (1, 2, 3, 4 and 5) and adult emergence were recorded (Table 2). To conserve moisture, Petri- plates were sealed with parafilm tape and kept in a polythene bags and placed in an optimum room temperature. (Lalitha *et al.*, 2012) [11].

Table 2: Different concentration of EPN strain against *T. chilonis* eggs

S. No	Treatments (for 100 eggs)
1.	200 IJs/50 µl
2.	400 IJs/50 µl
3.	600 IJs/50 µl
4.	800 IJs/50 µl
5.	1000 IJs/50 µl
6.	1200 IJs/50 µl
7.	Control (50 µl distilled water alone)

Statistical analysis

Using the Wasp 2.0 programme created by the ICAR, New Delhi, the analysis of variance (ANOVA) was calculated for evaluating the virulence of isolated EPN strain with checks.

Results and Discussion

EPN identification

Soil sample from Kayathar, Tuticorin, Tamil Nadu, India was identified as *Steinernema* sp. due to its white color of larval cadaver. Hussaini *et al.* (2001) [9] reported Steinernematids only in several locations of Tamil Nadu. Additionally, Ambika and Sivakumar (2002) [1] reported on Steinernematids only.

Pathogenicity of *Steinernema* sp. against second instar larvae of *C. carnea*: Pathogenicity of isolated strain *Steinernema* sp., against second instar larvae of *C. carnea* showed mortality percent were ranged from 4 to 93.22 percent at different concentrations of IJs during the six days of treatment. At higher dose of 1200 IJs/larva/ml mortality were ranged between 45 and 93.22 percent. From the results, it inferred that larva was more susceptible to isolated EPN. This findings are in line with the El – Mandarawy *et al.* (2018) [13] who reported 100 percent mortality at 800 and 1600 IJs/cup of EPN, *Heterorhabditis bacteriophora* after six days of treatment. Also Farag (2002) [5] observed larva was more susceptible than adults of *C. carnea* (Table 3) (Fig. 1)

Table 3: Safety study of *Steinerema* sp. against larvae of *Chrysoperla carnea*

Doses IJs/ml	Mean adult mortality (%)*				
	2 DAT	3 DAT	4 DAT	5 DAT	6 DAT
200	4.00 (11.54)^{f}	18.32 (25.34)^{f}	24.10 (29.39)^{e}	53.25 (46.87)^{e}	65.35 (53.94)^{d}
400	19.00 (25.84)^{e}	27.43 (31.58)^{e}	43.53 (41.28)^{d}	67.66 (55.34)^{d}	80.00 (63.48)^{c}
600	23.00 (28.66)^{d}	32.52 (34.77)^{d}	48.72 (44.27)^{c}	69.38 (56.41)^{cd}	85.72 (67.81)^{b}
800	37.00 (37.46)^{c}	43.00 (40.96)^{c}	64.68 (53.54)^{b}	71.23 (57.58)^{c}	88.53 (70.27)^{b}
1000	42.00 (40.40)^{b}	52.75 (46.58)^{b}	67.52 (55.26)^{a}	74.80 (59.87)^{b}	89.00 (70.69)^{b}
1200	45.00 (42.13)^{a}	57.80 (49.49)^{a}	69.73 (56.62)^{a}	78.00 (62.05)^{a}	93.22 (75.10)^{a}
Untreated control	0.00	0.00	0.00	0.00	0.00

	(0.28)^{g}	(0.28)^{g}	(0.28)^{f}	(0.28)^{f}	(0.28)^{e}
SEd	0.32	0.62	0.98	0.99	1.55
CD (P=0.05)	0.69	1.33	1.45	2.14	3.32

*Mean of three replications
Figures in parentheses are *arc* sine transformed values
In a column, means followed by common letter(s) are not significantly different by LSD ($p \leq 0.05$)

Fig 1: Dead larva of *C. carnea* treated with EPN

Pathogenicity of *Steinernema* sp. against eggs of *T. chilonis*

Eggs of *T. chilonis* with different days of parasitization showed adult emergence were ranged from 74.40 to 97 percent during six days after treatment. On first day, adult emergence was higher (89-95%) than sixth day (74.40 - 83.60%). These findings were cope up with the Lalitha *et al.* (2012) [11] who reported adult emergence were ranged from 61.30 to 97.30 percent with seven days of parasitizied trichocard. Contrarily, Mohan and Sabir (2005) [12] reported 84 percent reduction in adult emergence by exposing the eggs with *H. indica* and their symbiotic bacteria (Table 4) (Fig 2).

Table 4: Efficacy of *Steinernema* sp. against eggs of *T. chilonis* under a laboratory condition

Doses IJs/ml	Emergence (%) of T. *chilonis* from Corcyra parasitized eggs Mean emergence (%)*				
	1 DAP	2 DAP	3 DAP	4 DAP	5 DAP
200	95.00 (77.39)^{b}	93.00 (74.72)^{b}	90.00 (71.65)^{b}	87.40 (69.21)^{b}	83.60 (67.14)^{a}
400	95.00 (77.22)^{ab}	96.00 (78.46)^{ab}	92.00 (73.62)^{ab}	85.00 (67.24)^{bc}	84.90 (59.61)^{c}
600	94.00 (75.85)^{ab}	94.20 (76.36)^{ab}	90.00 (71.73)^{b}	83.20 (65.88)^{c}	74.40 (60.20)^{bc}
800	93.50 (75.29)^{b}	94.00 (76.21)^{ab}	88.00 (69.82)^{bc}	83.70 (66.20)^{bc}	75.30 (62.11)^{b}
1000	92.00 (73.77)^{bc}	92.80 (74.48)^{b}	91.00 (72.62)^{ab}	84.40 (66.78)^{bc}	78.10 (59.62)^{c}
1200	89.00 (70.76)^{c}	83.00 (65.65)^{c}	82.00 (64.94)^{c}	76.50 (61.01)^{d}	74.40 (57.61)^{d}
Untreated control	97.00 (80.10)^{a}	96.80 (79.88)^{a}	95.00 (78.07)^{a}	92.10 (73.78)^{a}	83.60 (66.13)^{a}
SEd	2.09	2.01	2.64	1.41	0.92
CD (P=0.05)	4.48	4.30	5.67	3.03	1.97
Category	Harmless	Harmless	Harmless	Harmless	Harmless

*Mean of three replications
Figures in parentheses are *arc* sine transformed values
In a column, means followed by common letter(s) are not significantly different by LSD ($p \leq 0.05$)

Fig 2: Adult emergence from EPN treated eggs

Additionally, all of the treatments during six days were revealed harmless as per the protocol given by IOBC. It demonstrated that, that all of these treatments had an adult emergence rate of more than 70 percent.

Conclusion

The current study illustrated that isolated *Steinernema* sp. were safer and harmless to the eggs of *T. chilonis*. It was less pathogenic to both insects at lower EPN doses. Therefore, it should be safer to use in a fields at a lesser dosage to combat the pest insects.

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