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**Research Article**

# Intraguild predation and biosafety of entomopathogenic nematode, *Heterorhabditis bacteriophora* Poinar *et al.,* and its bacterial symbiont, *Photorhabdus luminescens,* to parasitoid, *Trichogramma chilonis* Ishii and predator *Chrysoperla zastrowi sillemi* (Esben, Petersen)

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**ABSTRACT**: Intraguild predation (IGP) appears to be pervasive among communities of biocontrol agents associated with nematode sharing the host with trophic interaction. Entomopathogenic nematode (*Heterorhabditis bacteriophora)* and its associated bacterium (*Photorhabdus luminescens*); an egg parasitiod, *Trichogramma chilonis* and a predator *Chrysoperla zastrowi sillemi* were selected for present study. There was no adverse effect of *H. bacteriophora* and *P. luminescens* observed on adult emergence of *T. chilonis.* Microscopic examination of eggs, larvae and adults of *T. chilonis* and *C. z. sillemi* treated with *H. bacteriophora, P. luminescens* and cell-free culture filtrates of *P. luminescens,* exhibited no deformity, discoloration or infection of organisms. Similarly, *H. bacteriophora, P. luminescens* or the cell-free culture filtrates exhibited no adverse activity on egg hatching or larvae of *C. z. sillemi* indicating that there was no intraguild competition under artificial epiphytotic conditions between the organisms under report, viz., *H. bacteriophora,* its associated bacterium (*P. luminescens*), *T. chilonis* and *C. z. sillemi*.

**KEY WORDS**: Intraguild predation, entomopathogenic nematode, *Heterorhabditis bacteriophora*, *Photorhabdus luminescens*, *Trichogramma chilonis*, *Chrysoperla zastrowi sillemi*

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## INTRODUCTION

Intraguild predation (IGP) occurs when two species that share a host or also engage in a trophic interaction with each other (parasitism or predation), different entomopathogenic nematode-bacterium complexes, biology, life cycle and vertebrate safety and related legislative issues, exchange of germplasm, commercial aspects, post application persistence, transgenic and defined the boundaries with pathogenic bacteria of medical, veterinary or agronomic importance and sustainability of wild and transgenic entomopathogenic nematode-bacterium complexes in the field were well studied (Jansson, 1993; Rosenheim *et al*., 1995; Richardson, 1996; Rizvi *et al*., 1996; Boemare *et al*., 1996, Ehlers, 1996; Smits, 1996; Gaugler *et al*. 1997). Kaya (1978) reported susceptibility of adults *Apanteles militaris* (Hymenoptera: Braconidae), parasitoid of the armyworm, *Pseudaletia unipuncta* and its larvae. Similar results were recorded during his study with the tachinid parasite *Compsilura concinnatau* (Diptera: Tachinidae) to *Neoaplectana carpocapsaeu* (Nematoda: Steinernematidae) and its associated bacterium, *Xenorhabdus nematphilus* in 1984. Haag & Boucias (1991) reported in their study to test the infectivity of the insect pathogens to weed control agent *Neochetina eichhorniea* that 2 strains of *Steinernema carpocapsae* resulted in 60–70% adult mortality.

Among several parasitoids and predators recorded as natural enemies of several insect pests, *Trichogramma* and *Chrysoperla* have the distinction of reaching commercial use against several lepidopteran pests. These parasitoids are most widely used for biological control in more than 30 countries, with use in recent years covering a total area of 32 million ha of agricultural and forestry land (Li, 1994). The chrysopid larvae are predaceous, feeding on the eggs and neonate larvae of lepidopterans, nymphs and adults of whiteflies, aphids and other homopterans. Among 69 species of chrysopids recorded in India, *Chrysoperla zastrowi sillemi* is the most common species (Jalali *et al*., 2003).

Beneficial organisms including entomopathogenic nematodes, their associated bacteria, chrysopid predator, trichogrammatid parasitoid share common insect species as hosts, although vary in infectivity/predation/parasitism to life-cycle stages of insect hosts. Hence, their field

success as biological control agents either individually or in combinations, depends also on their cross-infectivity and suppressivity. In other words, the biological control agents preferably must be non-inhibitory and minimal in IGP.

The field evaluations proved the insecticidal virulence of the *Photorhabdus luminescens* bacterium against the cabbage white butterfly, *Pieris brassicae* (Linnaeus) (Mohan *et al*., 2003), mango mealybug, *Drosicha mangiferae* (Green) (Mohan *et al*., 2004) and the pupae of the diamond-back moth, *Plutella xylostella* (Linnaeus) (Razek-Abdel, 2003). The bacterium is reported to be non-toxic to humans and mammals and differs genetically from the human clinical isolate *P. asymbiotica* (Fischer-Le Saux *et al*., 1999). Subsequently, Mohan and Sabir (2005) reported that *P. luminescens* from *H. bacteriophora* adversely affected trichogrammatids. The results suggest conflicting report of its safety. Therefore, in the present study laboratory screening of *P. luminescens* against *T. chilonis* and *C. z. sillemi* to examine the toxicity and biosafety in pure culture, its culture filtrate and in natural association with its nematode host, using two protocols for comparison – Standard IOBC protocol and in comparison method adopted by Mohan and Sabir (2005) in order to avoid experimental differences.

## MATERIALS AND METHODS

### Nematode

Monoxenic infective juveniles (IJs) of *Heterorhabditis bacteriophora* (strain PDBC Hbb1) were established by collecting freshly emerged infective juveniles from *Galleria mellonella* cadavers and washing them 5 times in sterile $dH_2O$, followed by surface sterilizing with 0.1% Hymine (methyl benzothionium chloride) solution and several rinses with sterile distilled water.

### Bacterial cultures and insect infection

### Isolation of symbiotic bacterium, *P. luminescens*

Pure culture of *P. luminescens* was isolated from haemolymph of *G. mellonella* cadavers infected with *H. bacteriophora* on Mac Conkey medium as per Akhurst (1980). Five healthy and robust 5$^{th}$ instar larvae of *G. mellonella* were inoculated with 100 monoxenic infective juveniles (IJs) of *H. bacteriophora* by moist filter paper method in sterile Petri plates and incubated at 28°C. After 72h of inoculation, the cadavers of *G. mellonella* were surface-sterilized with 70% ethanol for 1 min., ignited and plunged in sterile $dH_2O$. The surface-sterilized cadavers of *G. mellonella* were punctured with a sterile needle and the haemolymph was streaked out onto NBTA medium (Akhurst, 1980). Colonies of *P. luminescens* were identified by their cell and colony morphology and matched with primary phase characteristics as described by Akhurst (1980). Single cell colonies of the bacterium were then transferred to autoclaved 2% proteose peptone medium ($PP_3$) and incubated for 48 hours at 28°C on a rotary shaker (Sciegenics Make) at 90 rpm in dark. Bacterial cells of *P. luminescens* from 48 hours-old proteose peptone medium ($PP_3$) were obtained separately by spinning at 4,000 x g for 5mins. The bacterial cells were re-suspended in phosphate buffered saline (PBS), washed thrice before finally making a stock of bacterial suspension in phosphate buffered saline (PBS) and used for further experimental treatments. In another set, cell free culture filtrates were obtained by ultra filtration using 0.23µm filter paper and then using the culture filtrate for treatments.

### Natural enemy selection and maintenance

Two freshly collected and identified species of natural enemies *T. chilonis* and *C. z. sillemi* used for testing against bacterium were reared on *Corcyra cephalonica* Stainton eggs in the laboratory for the past 20 years and were designated as susceptible. Both species were maintained at 26±1ºC and 65±5% relative humidity.

### Bacterium and its preparation

### Testing protocol

Six different treatments were screened against *T. chilonis* and *C. z. sillemi* in the present study. Two methods were employed to test the effect on adult emergence, adult mortality and parasitism by female of *T. chilonis*; egg hatching, larval and adult survivability of *C. z. sellimi*. In the first method IOBC protocol was followed as suggested by Hassan *et al.* (1985). The treatments imposed were:

$T_1$ No treatment

$T_2$ Dry filter paper

$T_3$ Freshly emerged *H. bacteriophora* NBAIIHbb1 infective juveniles (5000 IJs)

$T_4$ Nutrient broth

$T_5$ *P. luminescens* cell suspension

$T_6$ Cell-free cuture filtrate of *P. luminescens*

$T_7$ Sterile distilled water (SDW)

To test the effect of treatments on immature stages (pupal stage of *T. chilonis* and egg and larval stages of *Chrysoperla*) and adults, a clear plastic container (6 x 6 x 2cm$^3$) was modified into a testing unit. One window on four sides was cut, and fine brass wire-mesh (80 mesh size) was heat-sealed across them to provide aeration. A layer of foam was fixed on all sides of the lid to make the testing unit insect escape-proof. The area of the testing unit was calculated 72cm$^2$ and the prepared solution (0.05 ml) was sprayed with an atomizer over *C. cephalonica* eggs parasitized by *T. chilonis* 1, 2, 3, 4, 5, 6 and 7 days after parasitisation. Sample card containing 100 parasitized eggs of each day was considered per replication. The egg cards were kept in the testing units sprayed with various treatments. The testing units containing sprayed egg cards were sealed tightly and kept in incubator maintained at 28°C.

For immature stages of *C. z. sillemi*, 1, 2 and 3 days old eggs and 1$^{st}$, 2$^{nd}$ and 3$^{rd}$ instar stage larvae were tested in a similar manner as described for *T. chilonis* except for each stage 10 eggs or 10 larvae were used per replication. Toxicity to adults of *T. chilonis* and *C. z. sillemi* was tested as suggested in IOBC protocol (Hassan, 1980, 1985; Elzen, 1998). A Borosil glass tube opened both sides was used a the testing unit and was sprayed with the solutions and allowed to shade dry. One end of the dried tube was closed tightly with double layered black cloth and adults were allowed to move inside the tube from the other end. Movement of adequate number of adults was followed by closing of the end by double layered black cloth to permit the test organism in continuous surface contact with the treated surface and to avoid death of the adult due to suffocation. Fine streak of 50% diluted honey was provided. Hundred adults of *T. chilonis* and 10 adults of *C. z. sillemi* were introduced in each unit. Adult mortality was recorded after 24 h of constant exposure. Subsequently, observations on percentage parasitism, emergence and mortality of natural enemies on various treatments were recorded. Each treatment was replicated ten times. The evaluation categories for testing the effect of bio-pesticide were based on IOBC protocol as suggested by Hassan (1985).

In the second method, protocol as suggested by Mohan and Sabir (2005) was followed. The observations were similar to IOBC protocol. In the laboratory screening test, scores were assigned based on per cent mortality of *T. chilonis* and *C. z. sillemi,* after 24h of constant exposure (Table 1).

**Table 1: Scoring chart for screening the bio-safety of insecticidal pathogens**

| Mortality of test organism recorded (%) | Category | Score |
|---|---|---|
| <50 | Harmless | 1 |
| 50-79 | Slightly harmful | 2 |
| 80-99 | Moderately harmful | 3 |
| >99 | Harmful | 4 |

Data was transformed by arcsine transformation; subjected to ANOVA and drawn conclusions following the Scoring chart.

## RESULTS AND DISCUSSION

Systematic studies on the biosafety of *P. luminescens* (symbiotic bacterium associated with *H. bacteriophora* HIP) to the common beneficial insects which are commercialized, viz. *T. chilonis* and *C. z. sillemi* were carried out and the results are presented under different aspects.

### Emergence pattern of *T. chilonis* from parasitized eggs of *C. cephalonica* that received the treatments

The emergence pattern of *T. chilonis* adults from the parasitized eggs of *C. cephalonica* was recorded at 24 hours interval for 7 days in treated conditions. The percentage emergence of *T. chilonis* adults on first day ranged between 90.3 and 97.3 in different treatments as recorded by IOBC protocol, while the emergence ranged between 88.8 and 95.8 by the second protocol, with no significant differences among treatments (Table 4). The adult emergence pattern recorded on 2$^{nd}$, 3$^{rd}$, 4$^{th}$, 5$^{th}$, 6$^{th}$ and 7$^{th}$ days by both the protocols was more or less similar and statistically non-significant, clearly indicating that the treatments, including cell-free culture filtrates, *P. luminescens* cells and *H. bacteriophora,* had no discernable adverse effect on emergence of *T. chilonis* adults. However, Mohan and Sabir (2005) recorded significant reduction in the per cent adult emergence upto 84%.

### Adult mortality of *T. chilonis* and its parasitism as influenced by the treaments

A maximum adult mortality of (20.4%) in *T. chilonis* was recorded in nutrient broth (check), followed by

13.2% in *H. bacteriophora*, 9.4% in cell-free culture filtrate of *P. luminescens* treated condition and 6.7% in *P. luminescens* cells alone, while there was no mortality of adults recorded in untreated and sterile water tested conditions. Although adult mortality in *T. chilonis* was observed in 4 treated conditions, the respective values correspond to the score of 1 (i.e., <50% morality of the test organism) as per the mortality scoring chart of test organism, which accordingly come under the category of 'harmless'. Parasitism by *T. chilonis* ranged between 94.3 and 97.5% in treated conditions which was statistically on par with the untreated check (97.5%). Treatment with nutrient broth and cell-free culture filtrate of *P. luminescens* marginally reduced the parasitism of *T. chilonis* on the eggs of laboratory host, *C. cephalonica,* which were statistically not significant in comparison to the parasitism by *T. chilonis* in untreated and water treated control. These observations clearly indicated that there was no treatment effect on parasitism by *T. chilonis* or on adult mortality, thus showing that the *P. luminescens* associated with *H. bacteriophora* NBAII Hbb1 and its culture filtrate was biologically safe to *T. chilonis* (Table 2).

**Egg hatching, larval mortality and survival of adults of *C. z. sillemi* under treated conditions**

Biosafety of cells, cell-free culture filtrates of *P. luminescens* and the nematode, *H. bacteriophora,* to the beneficial insect, *C. z. sillemi*, was evaluated in term of their effect on egg hatching, larval mortality and survival of *C. z. sillemi at* 24 hours interval for 3days.

Percentage of hatching (95.2 – 100%) and survivability (80 – 100%) of 4 days old larvae of *C. z. sellimi* recorded after 1-3 days of spraying in different treatments were more or less similar between the two protocols (Table 5). Survivability of adults of *C. z. sellimi* ranged from 96 to

**Table 2: Effect of various treatments on adults of *Trichogramma chilonis* and its parasitizing ability**

| Treatments | Adult mortality (%) | Category | Parasitism (%) |
|---|---|---|---|
| No treatment | 0.0 (1.3)[b] | Harmless | 97.5 (81.7) |
| Filter paper with 100µl SDW | 0.0 (1.3)[b] | Harmless | 97.2 (81.0) |
| *Heterorhabditis bacteriophora* 5000 IJs (50µl) | 13.2 (18.5)[a] | Harmless | 95.7 (78.4) |
| Nutrient broth (50µl) | 20.4 (25.5)[a] | Harmless | 94.3 (76.6) |
| *Photorhabditis luminescens* cells alone (50 µl) | 6.7 (14.7)[a] | Harmless | 97.5 (81.3) |
| Cell- free culture filtrate of *P. luminescens* | 9.4 (16.3)[a] | Harmless | 96.5 (79.5) |
| Sterile distilled water | 0.0 (1.3)[b] | Harmless | 97.1 (81.0) |
| SEM± | 3.48 | – | 1.54 |
| CD at 5% | 10.2 | – | NS |
| **CD at 1%** | **13.8** | – | NS |

**Table 3: Effect of various treatments on survival of adults of *Chrysoperla zastrowi sillemi***

| Treatments | Adult survival (%) | Category |
|---|---|---|
| No treatment | 100.0 (90.0) | Harmless |
| Filter paper with 100µl sterile water | 100.0 (90.0) | Harmless |
| Freshly emerged *Heterorhabditis bacteriophora* 5000 IJs (50µl) | 96.0 (84.7) | Harmless |
| Nutrient broth (50µl) | 100.0 (90.0) | Harmless |
| *Photorhabditis luminescens* cells alone (50 µl) | 100.0 (90.0) | Harmless |
| *P. luminescens* supernatant cell-free culture filtrate of *P. luminescens* | 96.0 (84.7) | Harmless |
| Sterile distilled water | 100.0 (90.0) | Harmless |
| SEM± | 2.58 | – |
| CD at 5% | NS | – |
| CD at 1% | NS | – |

**Table 4: Emergence pattern of *Trichogramma chilonis* from treated parasitized eggs of *Corcyra cephalonica* in various treatments**

| Treatments | Emergence (%) from treated parasitized eggs after days– IOBC Protocol | | | | | | | Emergence (%) from treated parasitized eggsdays – Mohan and Sabir, (2005) Protocol | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| No treatment | 92.3 (75.6) | 91.9 (74.0) | 83.6 (66.7) | 87.4 (70.0) | 92.1 (74.9)[a] | 84.9 (68.9) | 75.3 (59.9)[a] | 92.3 (76.3) | 92.3 (74.0) | 83.6 (66.7) | 87.4 (70.0) | 92.1 (74.9)[a] | 84.9 (68.8) | 74.6 (59.9) |
| Filter paper with 100ml sterile water | 93.3 (76.9) | 97.0 (83.8) | 88.3 (72.8) | 82.1 (65.2) | 78.8 (63.5)[b] | 81.3 (64.5) | 74.3 (59.4)[a] | 88.8 (77.3) | 90.3 (76.4) | 91.3 (73.8) | 87.0 (69.2) | 89.8 (72.2)[a] | 75.0 (60.2) | 74.4 (60.1) |
| Freshly emerged *H. bacteriophora* 5000 infective juveniles (50µl) | 90.3 (72.3) | 92.7 (76.1) | 83.7 (66.4) | 81.1 (65.1) | 74.4 (60.0)[b] | 78.1 (64.9) | 67.3 (54.9)[a] | 89.9 (76.3) | 93.3 (80.5) | 92.4 (77.8) | 81.8 (65.1) | 80.6 (64.4)[b] | 72.5 (58.6) | 73.2 (58.9) |
| Nutrient broth (50µl) | 90.3 (74.2) | 91.9 (75.6) | 93.2 (76.6) | 81.2 (64.7) | 84.4 (67.4)[a] | 73.2 (59.1) | 61.3 (51.6)[b] | 92.1 (80.3) | 95.3 (82.0) | 90.2 (74.0) | 75.8 (60.7) | 84.0 (67.1)[a] | 75.2 (60.4) | 73.6 (62.1) |
| *P. luminescens* cells alone (50 µl) | 94.3 (76.7) | 94.9 (81.8) | 87.8 (69.9) | 82.7 (68.2) | 75.8 (61.0)[b] | 81.6 (67.6) | 67.3 (54.8)[a] | 94.4 (78.3) | 96.3 (82.8) | 91.6 (73.4) | 87.4 (72.3) | 81.0 (64.5)[b] | 78.3 (63.1) | 85.1 (68.4) |
| *P. luminescens* supernatant cell free culture filtrate of *P. luminescens* | 90.3 (73.0) | 85.7 (68.5) | 91.2 (74.6) | 88.3 (72.8) | 75.9 (61.0)[b] | 76.5 (61.1) | 71.3 (57.5)[a] | 95.8 (81.3) | 87.3 (70.5) | 91.5 (75.1) | 84.4 (67.3) | 76.4 (61.1)[b] | 70.7 (57.7) | 78.2 (62.4) |
| Sterile distilled water | 97.3 (80.9) | 86.9 (71.5) | 86.1 (70.7) | 85.8 (68.3) | 75.7 (61.0)[b] | 74.9 (60.1) | 73.3 (58.6)[a] | 93.6 (76.3) | 87.3 (69.5) | 84.9 (67.5) | 80.8 (64.9) | 77.8 (62.5)[b] | 75.3 (60.5) | 74.8 (60.0) |
| SEM± | 3.29 | 4.28 | 3.90 | 4.04 | 3.05 | 4.09 | 1.81 | 5.09 | 4.45 | 3.57 | 3.34 | 2.89 | 3.16 | 3.83 |
| CD ($p = 0.05$) | NS | NS | NS | NS | 8.9 | NS | 5.3 | NS | NS | NS | NS | 8.4 | NS | NS |
| Category | Harm-less | Harm-less | Harm-less | Harm-less | Harm-less | Harm-less | Harm-less | Harm-less | Harm-less | Harm-less | Harm-less | Harm-less | Harm-less | Harm-less |

Figures in parentheses are arcsine transformed values, In the columns letter followed by different letter is significantly different at $p = 0.05$, NS = Non-significant.

100% at different treatments which was statistically not significant (Table 3). Microscopic examination of the treated eggs, adults and larvae exhibited no morphological or physical changes.

The effect of entomophilic nematodes on the natural enemies of some parasitoids and predators of insect pests was investigated in the laboratory and field in Poland (Jaworska *et al*., 1995). No effects of *Heterorhabditis bacteriophora* or *Steinernema carpocapsae* on Ichneumonidae or predatory Carabidae were recorded. In another study the effect of entomopathogenic nematodes on non-target arthropods in the laboratory, field soils, and a stream were assessed (Georgis *et al*., 1991). In the laboratory, adult predators were less susceptible to the nematodes, *S. carpocapsae* and *H. bacteriophora* than the immature stages. In field tests, entomopathogenic nematodes that had significantly suppressed pest populations (Japanese beetle) *Popillia japonica* Newman, *Scapteriscus vicinus* Scudder, tawny mole cricket, (black vine weevil) *Otiorhynchus sulcatus* (F.), (cabbage maggot), *Delia radicum* (L.) and (western corn rootworm) *Diabrotica virgifera* LeConte did not adversely affect the numbers of non-target soil arthropods in comparison with the untreated control.

Experiments of Mrácekand and Spitzer (1983) revealed that *S. kraussei* was not a normal parasite of the predators (*Thereva* spp., *Rhagio* spp.) and parasitoids (Tachinidae: Ichneumonidae) of sawfly *Cephalcia abietis*; no reduction in the impact of the predators and parasitoids on *C. abietis* populations. Even though they recorded some invasion by *S. kraussei* in *Thereva handlirschi* and *Rhagio* spp., none occurred in the parasitoids. It was stated that

**Table 5. Effect of various treatments on eggs and larvae of *Chrysoperla zastrowi sillemi***

| Treatments | Egg hatching (%) after treatment after days – IOBC Protocol | | | Larval mortality (%) after treatment after instars – IOBC Protocol | | | Egg hatching (%) after treatment after days – Mohan and Sabir, (2005) Protocol | | | Larval mortality (%) after treatment after instars – Mohan and Sabir, (2005) Protocol | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 1st | 2nd | 3rd | 1 | 2 | 3 | 1st | 2nd | 3rd |
| No treatment | 98.0 (86.3) | 98.0 (86.3) | 100.0 (90.0)[a] | 92.0 (79.4) | 96.0 (84.7)[a] | 98.0 (68.9) | 98.0 (86.3) | 98.0 (86.3) | 100.0 (90.0) | 92.0 (79.4)[b] | 96.0 (84.7) | 98.0 (86.3) |
| Filter paper with 100µl sterile water | 96.0 (84.7) | 98.0 (86.3) | 100.0 (90.0)[a] | 100.0 (90.0) | 96.0 (84.7)[a] | 100.0 (64.5) | 96.0 (82.6) | 96.3 (83.0) | 100.0 (90.0) | 96.0 (84.7)[b] | 100.0 (90.0) | 100.0 (90.0) |
| Freshly emerged *H. bacteriophora* 5000 infective juveniles (50µl) Nutrient broth (50µl) | 96.6 (83.3) | 96.8 (83.4) | 95.2 (80.3)[b] | 80.0 (66.2) | 96.0 (84.7)[a] | 88.0 (64.9) | 96.5 (83.2) | 96.5 (83.1) | 95.1 (81.9) | 84.0 (68.8)[b] | 92.7 (79.9) | 92.0 (79.4) |
| *P. luminescens* cells alone (50 µl) | 96.2 (82.8) | 98.0 (86.3) | 94.7 (81.5)[b] | 83.0 (70.9) | 96.0 (84.7)[a] | 100.0 (67.6) | 98.0 (86.3) | 97.8 (86.1) | 98.0 (86.3) | 74.0 (59.6)[b] | 92.0 (79.4) | 92.0 (79.4) |
| *P. luminescens* supernatant cell free culture filtrate of *P. luminescens* | 98.0 (86.3) | 98.0 (86.3) | 100.0 (90.0)[a] | 89.0 (72.8) | 100.0 (90.0)[a] | 98.0 (61.1) | 96.0 (84.7) | 98.0 (86.3) | 98.0 (86.3) | 90.0 (75.7)[b] | 94.0 (81.0) | 94.0 (81.0) |
| Sterile distilled water | 96.0 (84.7) | 96.5 (83.2) | 100.0 (90.0)[a] | 92.0 (79.4) | 96.0 (84.7)[a] | 100.0 (60.1) | 98.0 (86.3) | 96.0 (82.6) | 98.0 (86.3) | 92.0 (79.4)[b] | 95.0 (84.0) | 100.0 (90.0) |
| SEM± | 4.57 | 3.23 | 2.57 | 5.28 | 4.77 | 4.09 | 3.97 | 3.96 | 3.58 | 5.16 | 5.60 | 5.23 |
| CD ($p = 0.05$) | NS | NS | 7.5 | NS | 8.9 | NS | NS | NS | NS | 15.1 | NS | NS |

Figures in brackets are arcsine transformed values, In the columns letter followed by different letter is significantly different at $p$ = 0.05%, NS = Non-significant.

neither the predators nor the parasitoids of *C. abietis* serve as supporting hosts for the development of *S. kraussei* in focuses of *C. abietis.*

Results of the present studies also revealed similar trend and are in concurrence with the reports of the above researchers.

## CONCLUSION

Results indicated that *P. luminescens*, its culture filtrate and *H. bacteriophora* NBAII Hbb1 did not cause any physical changes in eggs, larvae and adults of *T. chilonis* and *C. z. sellimi* and did not exhibit any significant reduction in egg hatching, emergence and parasitism by these natural enemies and was found to be safe without any intraguild effects.

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