Bioefficacy of *Photorhabdus luminescens*, a symbiotic bacterium against *Thrips palmi* Karny (Thripidae: Thysanoptera)

G. P. Uma\textsuperscript{a}, A. Prabhuraj\textsuperscript{b} and Vimala\textsuperscript{c}

**ABSTRACT**

The primary and secondary forms of *Photorhabdus luminescens*, a symbiotic bacterium of an entomopathogenic nematode *Heterorhabditis indica* (RCR) was evaluated against *Thrips palmi* Karny, a serious pest on cotton. The treatment included different concentration (10\(^1\) to 10\(^10\) cells/ml) of cells and cell free supernatant which was obtained by centrifugation (4000 rpm for 20 min) of broth culture. The treatment was imposed through potter’s tower on aphid colony containing (20 per leaf/replication plants). Observation of aphid mortality was recorded at 24 and 48 hr. The results revealed that both the forms were pathogenic to *T. palmi* but the cells free supernatant (CFS) of both the forms was more virulent than cells causing high mortality within 24 to 48 hr. The cells of primary and secondary form recorded 67.50 and 72.50 per cent mortality whereas corresponding CFS recorded 85.00 and 80.00 per cent mortality, respectively at the concentration of 10\(^10\) cells/ml. The LC\(_{50}\) of both primary 2.38 \times 10\(^7\) cells/ml and secondary form cells 2.35 \times 10\(^7\) cells/ml and CFS of *P. luminescens* decreased with increase in exposure time from 24 to 48 hr. These results indicate the possibility of developing CFS as a potential biocontrol component against *T. palmi*.

**Keywords:** Bioefficacy, cell free supernatant, *Heterorhabditis indica*, LC\(_{50}\), *Photorhabdus luminescens*, primary and secondary form, *Thrips palmi*, crop pest.

**INTRODUCTION**

*Thrips palmi* Karny is one of the serious polyphagous pest occurring mainly on sunflower, groundnut, cotton etc. It lacerates the leaves leading to white patches affecting the plant growth. It is known to transmit sunflower necrosis in sunflower and peanut bud necrosis in groundnut. Indiscriminate use of pesticide against certain pest resulted in the secondary outbreak of thrips. The ill effects of the synthetic chemical pesticides resulted in the search for antagonistic for pest management by the researchers worldwide. In recent days *Photorhabdus luminescens* (Thomas and Poinar), a symbiotic bacterium of entomopathogenic nematode *Heterorhabditis indica* Poinar is gaining a considerable attention in pest management.

*Photorhabdus luminescens* is a non-spore forming, motile, bioluminescent, gram-negative bacterium belonging to the Enterobacteriaceae with in the gamma subdivision of purple bacteria (Akhurst, 1980). Infective juvenile (IJ) stage nematodes of the Heterorhabditidae carry this bacterium in their intestinal tract (Steven Frost and Kenneth Nealon, 1996), upon entering the hemolymph release the *P. luminescens* cells. The bacteria breakdown the fat tissue and make a way for nematode to feed inside insect host to grow and multiply, leading to death of the host with in 24-48 hr (Dunphy and Webster, 1991).

*Photorhabdus luminescens* is known to produce an array of toxins, antibiotic with antifungal and antibacterial compounds, hence it is gaining considerable attention due to commercial interest in insecticidal metabolites active against several insect by producing the symptoms similar to the *Bacillus thuringiensis* \(\delta\)-endotoxin (Blackburn et al., 1998) and the genus represent a possible alternative to Bt toxin genes for expression in transgenic plants (Guo et al., 1999). But the reports on the effectivity on sucking pest is relatively less hence the present work was carried out to know the bioefficacy of *P. luminescens* from indigenous isolates (Raichur) of *Heterorhabditis indica* (RCR).

**MATERIALS AND METHODS**

1. **Insect and nematode source**

   Nymphs of *T. palmi* were collected from the sunflower field of UAS, Raichur and reared on sunflower leaves till the treatment is imposed. The nematode was isolated from the soil obtained from the lawn of College of Agriculture, Raichur using modified trapping technique (Prabhuraj).
et al., 2000). From the stock culture, H. indica was cultured in vivo (Woodring and Kaya, 1988) in late instar, Galleria mellonella L. larvae at 25°C and emerged IJs were extracted by modified white traps (Prabhuraj, 1997) and stored in distilled water at 25°C.

Isolation of P. luminescens from the haemolymph
Photobacterium luminescens was isolated as described by Woodring and Kaya (1998) and maintained in monoxenic culture. Newly molted last instar larvae of Galleria mellonella (L.) were exposed to IJs at the rate of 100 IJs per larva. After 72 hr, the dead larvae were surface disinfected by dipping them into 95 per cent ethanol and igniting them. The cadavers were aseptically dissected with sterile forceps and a drop of hemolymph was streaked to nutrient agar (NA) medium and incubated in BOD at 28°C. The resulting phase one (Boemare and Akhrust, 1988) was subcultured at 13-14 day interval on NA to keep it in primary form. To obtain the secondary form, the primary form was maintained without subculture for 20 days at 28°C, during which time many bacterial colonies produced secondary form. Primary and secondary forms were grown in 150 ml of NA broth at 28°C for 24–48 hr separately during which bacteria are known to produce maximum toxin secretion.

Preparation of bacterial cells and cells free supernatant
A loopful of symbiotic bacteria from NA plates was added to 150 ml of sterilized nutrient broth under aseptic conditions and kept in the incubator for 24–48 hours at 28°C. The broth was diluted to desired concentration by serial dilution and bacterial count taken by plate count technique. After reading the initial concentration the suspension was again serially diluted according to the treatment. Later the culture was centrifuged at 4000 rpm for 20 minutes. The supernatant and the cells were collected in separate test tubes and used for the study.

Bioefficacy of P. luminescens against T. palmi
The fully grown sunflower leaf was placed in petri dish with its stalk wrapped in wet cotton. Twenty 2nd instar T. palmi nymphs were released on the leaf and the plate containing thrips with leaf was kept on pneumatic spray table of potters tower and paraffin liquid of 10 µl/ml and sucrose @ 0.5 per cent were added to test tubes containing cells and cells free supernatant (CFS) of primary and secondary forms of P. luminescens. 1.5 ml of above solution, was pipetted out into liquid reservoir. The compressed air is then turned on, till all the fluid has been sprayed. The compressed air is turned off and sprayed Petri plates were removed and covered with the lid. Each experiment included 10 treatments with four replication. Each replication consists of 20 thrips. Solution with only distilled water with Paraffin liquid of 10 µl/ml and sucrose @ 0.5 per cent served as control. The 10 treatment includes concentration ranging from 10⁰ to 10⁵ cells/ml. Observation on mortality was recorded at 24 and 48 hr after treatment. The data obtained was subjected to ANOVA and probit analysis (Finney, 1971).

RESULTS AND DISCUSSION
Percent mortality response of T. palmi
A per cent mortality response study depicts that, P. luminescens found pathogenic to T. palmi. Both the forms were found to be equally virulent, but within each forms, CFS was more pathogenic than cells concentration and the results are presented in Table 1. The per cent mortality of nymph due to primary form cell ranged from 15.00 to 67.50 per cent. However, T1 (10⁸ cells/ml) recorded highest mortality at both 24 and 48 hr and superior over all other treatments. In case of primary form cells free supernatant (CFS) the mortality ranged from 22.50 to 85.00 per cent with T1 (10⁶ CFS) recording significantly higher mortality but was on par with T2 (10⁴ CFS) at both 24 and 48 hr. however the mortality increased with increase in time but decreased with decrease in concentration.

In case of secondary form cells the mortality ranges from 8.75 to 72.50 per cent with T1 (10⁸ cells/ml) recording the highest mortality but was on par with T2 (10⁴ CFS) at 24 hr and superior to all other treatment at 48 hr by recording 72.50 per cent. The secondary form CFS and an increase in mortality by 0.057 (Y=0.057x +0.215) indicated that a unit increase in CFS concentration resulted in nymphal mortality by 0.057 times. The level of significance 98.5 per cent (R²=0.985). However, compared to CFS, the cells of T. palmi recorded the highest mortality but was on par with T3 (10⁰ CFS) at both 24 and 48 hr.

Dose mortality response of T. palmi
Data (48 hrs) subjected to concentration mortality (LC₅₀) studies revealed that the T. palmi has recorded lowest LC₅₀ value from the CFS than compared to cells of the both forms. The CFS of primary form in T. palmi recorded an LC₅₀ value of 1.28 x 10⁸ CFS. The regression equation (Y=0.055x +0.289) indicated that a unit increase in CFS concentration resulted in nymphal mortality by 0.055 times. The level of significance 98.5 per cent (R²=0.985). This was followed by CFS of secondary form with an LC₅₀ of 7.53 x 10⁸ CFS and an increase in mortality by 0.057 times (Y = 0.057 x +0.215) for every unit increase in CFS concentration and the level of significance was 98.6 per cent (R²=0.986). However, compared to CFS, the cells of primary and secondary forms recorded the highest LC₅₀ of 2.38 x 10⁹ and 2.35 x 10⁸ cells/ml, respectively. Further,
Table 1. Effect of different dose of P. luminescens cells and CFSp forms against Thrips palmi

<table>
<thead>
<tr>
<th>Dose (Cells/CFSp)</th>
<th>Primary form</th>
<th>Secondary form</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Per cent mortality of T. palmi at different intervals</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cells</td>
<td>CPS</td>
</tr>
<tr>
<td></td>
<td>24 hr</td>
<td>48 hr</td>
</tr>
<tr>
<td>T. -10^6</td>
<td>57.50(49.31)</td>
<td>67.50(55.25)</td>
</tr>
<tr>
<td>T. -10^7</td>
<td>55.00(48.77)</td>
<td>58.75(50.05)</td>
</tr>
<tr>
<td>T. -10^8</td>
<td>46.25(42.84)</td>
<td>52.50(46.43)</td>
</tr>
<tr>
<td>T. -10^9</td>
<td>40.00(39.21)</td>
<td>47.50(43.56)</td>
</tr>
<tr>
<td>T. -10^10</td>
<td>33.75(35.50)</td>
<td>40.00(39.21)</td>
</tr>
<tr>
<td>T. -10^11</td>
<td>27.50(31.60)</td>
<td>35.00(36.24)</td>
</tr>
<tr>
<td>T. -10^12</td>
<td>25.00(29.94)</td>
<td>32.50(34.74)</td>
</tr>
<tr>
<td>T. -10^13</td>
<td>22.50(28.28)</td>
<td>31.25(33.97)</td>
</tr>
<tr>
<td>T. -10^14</td>
<td>18.75(25.62)</td>
<td>28.75(32.40)</td>
</tr>
<tr>
<td>T. -10^15</td>
<td>15.00(22.78)</td>
<td>22.50(28.28)</td>
</tr>
</tbody>
</table>

NOTE: Figures in the parentheses are angular transformed values Means followed by same letters in a column are not significantly different (P=0.01) by DMRT.

Table 2. LC50 and LC90 of primary and secondary form of cells and cells free supernatant of P. luminescens on T. palmi

<table>
<thead>
<tr>
<th>Treatment</th>
<th>LC50 (g/l)</th>
<th>LC90 (g/l)</th>
<th>Fiducial limit at LC50</th>
<th>Fiducial limit at LC90</th>
<th>R2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary form</td>
<td>48 hours</td>
<td>2</td>
<td>90.98</td>
<td>2</td>
<td>0.056 x ± 0.084</td>
</tr>
<tr>
<td>Secondary form</td>
<td>48 hours</td>
<td>2</td>
<td>89.30</td>
<td>2</td>
<td>0.055 x ± 0.289</td>
</tr>
<tr>
<td>Primary form</td>
<td>48 hours</td>
<td>2</td>
<td>140.298</td>
<td>2</td>
<td>0.068 x ± 0.005</td>
</tr>
<tr>
<td>Secondary form</td>
<td>48 hours</td>
<td>2</td>
<td>91.898</td>
<td>2</td>
<td>0.057 x ± 0.215</td>
</tr>
</tbody>
</table>

Note: LL – Lower limit, UL – Upper limit.
cabbage butterfly Pieris brassicae after 24 hr of foliar spray (Sharad Mohan et al., 2003b).

Over all results of bioassay against the T. palmi clearly indicated increased mortality with increase in the cell concentrations irrespective of primary/secondary form. This might be due to higher concentration of the secreted toxins from the cells. But whether the mortality is due to oral or contact action remains uncertain. However, looking into above results mortality through contact action seems more applicable as T. palmi is a stinging bug. In both the primary and secondary forms the secretion of cells seems to play an important role in bringing the pest mortality indicating the role of toxin compound produced by cells of P. luminescens. Earlier reports suggest that P. luminescens produce three classes of toxin. Members of the first class, the toxin complexes (Tc) are orally toxic to caterpillar pests (Bowen, 1998). The second class “makes the caterpillar floppy” toxin (mcf1 and mcf2) are active at injection (Duchaud, 2002). Third and most recently discovered “Photorhabdus insect related” proteins (Pr AB) have shown binary toxin with both oral (Duchaud, 2003) and injectable (Waterfield, 2005) activity. The toxin group in our study might belong to any of the toxin class mentioned above and hence it is necessary to identify the exact nature and mode of action of the toxin group and reason for difference in the potency of cells and supernatant obtained from bacterial culture.

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Thrips management by symbiotic bacterium

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