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

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ABSTRACT

The primary and secondary forms of *Photorhabdus luminescens* a symbitotic 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/ CFS/ml) of cells and cells 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 2.38×10^7 cells/ml and secondary form cells 2.35×10^7 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*.

Key words: 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 occuring 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. Indiscrimate 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 (Akhrust, 1980). Infective juvenile (IJs) stage nematodes of the Heterorhabditidae carry this bacterium in their intestinal tract (Steven Frost and Kenneth Nealson, 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* δ- 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. luminescenxs* from indigenous isolates (Raichur) of *Heterorhabditis indica* (RCR).

MATERIALS AND METHODS

Insect and nematode source

Nymphs of *T. palmi* were collected from the sun flower 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 25^0C and emerged IJs were extracted by modified white traps (Prabhuraj, 1997) and stored in distilled water at 25^0C.

Isolation of *P. luminescens* from the haemolymph

Photorhabdus 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 asceptically dissected with sterile forceps and a drop of hemolymph was streaked on to nutrient agar (NA) medium and incubated in BOD at 28^0 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 28^0 C, during which time many bacterial colonies produced secondary form. Primary and secondary forms were grown in 150 ml of NA broth at 28^0 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 loopfull 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^o 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 2^{nd} 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* and 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 10^{10} to 10^1 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 form, 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 (10^{10} 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 (10^{10} CFS) recording significantly higher mortality but was on par with T2 (10^9 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 (10^{10} cells/ml) recording the highest mortality but was on par with T2 (10^9cells/ml) at 24 hr and superior to all other treatment at 48 hr by recording 72.50 per cent. The secondary form CFS has recorded mortality ranging from 10.00 to 76.25 per cent again T1 (10^{10}CFS) recording the highest mortality but was on par with treatment T2 (10^9 CFS), T3 (10^8 CFS) at both 24 and 48 hr.

Dose mortality response of *T. palmi*

Data (48 hrs) subjected to concentration mortality (LC_{50}) studies revealed that the *T. palmi* has recorded lowest LC_{50} value from the CFS than compared to cells of the both forms. *The* CFS of primary form in *T. palmi* recorded an LC_{50} value of 1.28×10^4 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 ($R^2 = 0.985$). This was followed by CFS of secondary form with an LC_{50} of 7.53×10^4 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 ($R^2=0.986$). However, compared to CFS, the cells of primary and secondary forms recorded the highest LC_{50} of 2.38×10^7 and 2.35×10^7 cells/ml, respectively. Further,

Table 1. Effect of different dose of *P. luminescens* cells and CFS forms against *Thrips palmi*

Dose (Cells /CFS per ml)	Per cent mortality of *T.palmi* at different intervals							
	Primary form				Secondary form			
	Cells		CFS		Cells		CFS	
	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
T_1-10^{10}	57.50(49.31)^{a}	67.50(55.25)^{a}	83.75(66.41)^{a}	85.00(67.5)^{a}	56.25(48.60)^{a}	72.50(58.39)^{a}	68.75(56.02)^{a}	80.00(63.52)^{a}
T_2 -10^{9}	55.00(47.87)^{a}	58.75(50.05)^{b}	77.50(61.71)^{a}	80.00(63.52)^{a}	52.50(46.43)^{a}	65.00(53.75)^{b}	65.00(53.75)^{a}	71.25(57.59)^{b}
T_3-10^{8}	46.25(42.84)^{b}	52.50(46.43)^{c}	67.50(55.25)^{b}	72.50(58.45)^{b}	42.50(40.68)^{b}	56.25(48.59)^{c}	58.75(50.05)^{ab}	66.25(54.51)^{b}
T_4-10^{7}	40.00(39.21)^{c}	47.50(43.56)^{c}	61.25(51.52)^{b}	70.00(56.82)^{b}	36.25(37.01)^{c}	48.75(44.28)^{d}	52.50(46.44)^{b}	62.50(52.24)^{bc}
T_5-10^{6}	33.75(35.50)^{d}	40.00(39.21)^{d}	47.50(43.56)^{c}	58.75(50.05)^{c}	27.50(31.60)^{d}	36.25(36.98)^{e}	46.25(42.84)^{bc}	53.75(47.15)^{d}
T_6-10^{5}	27.50(31.60)^{e}	35.00(36.24)^{d}	43.75(41.39)^{c}	56.25(48.59)^{c}	23.75(29.14)^{d}	28.75(32.40)^{f}	43.75(41.40)^{c}	45.00(42.12)^{e}
T_7-10^{4}	25.00(29.94)^{e}	32.50(34.74)^{de}	41.25(39.94)^{c}	52.50(46.43)^{c}	22.50(28.28)^{d}	25.00(29.94)^{f}	35.00(36.24)^{d}	42.50(40.68)^{e}
T_8-10^{3}	22.50(28.28)^{e}	31.25(33.97)^{de}	40.00(39.21)^{c}	42.50(40.68)^{d}	18.75(25.62)^{de}	20.00(26.47)^{fg}	28.75(32.25)^{d}	40.00(39.21)^{e}
T_9-10^{2}	18.75(25.62)^{ef}	28.75(32.40)^{e}	31.25(33.93)^{d}	40.00(39.21)^{d}	17.50(24.67)^{e}	17.50(24.67)^{g}	27.50(31.60)^{de}	30.00(33.17)^{f}
T_{10}-10^{1}	15.00(22.78)^{f}	18.75(25.62)^{f}	22.50(28.28)^{e}	36.25(36.98)^{d}	8.75(17.05)^{f}	12.50(20.61)^{h}	18.75(25.62)^{f}	26.25(30.80)^{f}
T_{11}-control	0.00(0)^{g}	0.00(0)^{g}	0.00(0)^{f}	0.00(0)^{e}	0.00(0)^{g}	0.00(0)^{i}	0.00(0)^{g}	0.00(0)^{g}
S.Em.±	0.902	0.828	1.242	1.264	0.807	0.895	1.095	1.119
CD at 1%	3.508	3.222	4.830	4.916	3.138	3.480	4.260	4.352
C.V.	5.622	4.585	5.923	5.471	5.394	5.235	5.790	5.341

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

primary and secondary form cells recorded an increase in the mortality by 0.056 (Y = 0.056 x+0.084) and 0.068 (Y = 0.068x + 0.005) times, respectively with an every unit increase in cells concentration. The level of significance was 98.7 (R^2= 0.987) and 96.6 (R^2= 0.966) per cent in primary and secondary form cells respectively (Table 2).

Our study indicates the susceptibility of *T. palmi* but only two reports are available with respect to susceptibility of sap feeders where Bussaman *et al.* (2005) reported that the supernatant of *P. luminescens* spp. Laumondii (1.0 × 10^6 cells/ml) resulted in 90-95% mite mortality within 24-48 hr and Sharad Mohan *et al.* (2003a) reported that *P. luminescens* isolated from the *H. indica* IARI strain @ 1.477 ×10^6 cells/ml resulted in 92.50 per cent mortality of second instar nymph of mango mealy bug. Hence, the present study forms the first report on the susceptibility of *T. palmi* to *P. luminescens*. However, many authors have reported the use of *P. luminescens* against different orders including Lepidoptera, Coleopteran and Dictyoptera (Bowen *et al.*, 1998). The *P. luminescens* (10^6 bacterial loads) sprayable formulation has given 90 per cent laraval mortality of *Plutelle xylostella* (Rajgopal and Bhatnagar,2002) and the *P. luminescens* isolated from *H. indica* IARI strain caused 100 per cent mortality of

Table 2. LC_{50} and LC_{99} of primary and secondary form of cells and cells free supernatant of *P. luminescens* on *T.palmi*

Treat ments	Chisquare $(\chi)^2$	D.F.	Regression Equation	LC_{50}	Fiducial limit at LC_{50}	LC_{99}	Fiducial limit at LC_{99}	R^2
					LL-UL		LL-UL	
Primary form Cells								
48 hours	90.98	2	Y = 0.056 x +0.084	2.38 x 10^7	2.18 x 10^2 – 2.00x10^9	2.59 x10^{22}	3.5 x 10^{18} – 1.24 x 10^{34}	0.987
Primary form cells supernatant								
48 hours	89.30	2	Y = 0.055 x +0.289	1.28 x 10^4	0.00 – 3.16 x 10^6	1.0 x 10^{14}	5.8 x 10^{13} – 1.00 x 10^{20}	0.985
Secondary form Cells								
48 hours	140.298	2	Y = 0.068 + 0.005	2.35 x 10^7	6.25 x 10^5 – 1.63 x 10^8	8.35 s 10^{16}	4.32 x 10^{14} – 1.38 x 10^{22}	0.966
Secondary form cells supernatant								
48 hours	91.898	2	Y = 0.057 x +0.215	7.53 x 10^4	0.00 – 2.40 x 10^8	1.00 x 10^{20}	7.90 x 10^{16} – 2.91 x 10^{33}	0.986

Note: LL – Lower limit, UL – Upper limit

cabbage butterfly *Pieris brassica* 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 sucking pest. 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 (mcf_1 and mcf_2) are active at injection (Dabron, 2002). Third and most recently discovered "*Photorhabdus* insect related" proteins (Pir 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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