Effect of the entomopathogenic bacterium *Photorhabdus luminescens* to *Aphis gossypii* Glover

G. P. Uma*, A. Prabhuraj and Vimala

ABSTRACT

The *Aphis gossypii* Glover is an important pest of Bt cotton. We determined the toxicity of the primary and secondary forms of *Photorhabdus luminescens* (Thomas and Poinar) isolated from entomopathogenic nematode *Heterorhabditis indica* against *A. gossypii*. The treatment included different concentration (10^1 to 10^{10}cells/ CFS/ml) cells and cells free supernatant which was obtained by centrifugation (4000 rpm for 20 min) of broth culture. The treatment were imposed through potter's tower on aphid colony containing of 20 per leaf/ replication.observation of aphid mortality was recorded at 24, 48 hr and the data was subjected to ANOVA and probit analysis. The results revealed that both the forms were toxic to *A. gossypii* resulting in significant nymphal mortality within 24-48 hr. The CFS of both primary and secondary forms at the highest concentration of 10^{10} CFS were significantly more toxic than the cells, resulting in 100 per cent mortality within 48 hr. At the same concentration and exposure time the primary form cells recorded 77.50 per cent mortality and secondary form cells has recorded 83.75 per cent mortality. However, the per cent mortality decreased with the decrease in the cells and CFS concentration. The LC_{50} varied for cells and CFS of both the forms and with the exposure time. At 48 hr, primary form cells and CFS recorded the LC_{50} of 1.00×10^5 cells/ml and 2.02×10^2 CFS against aphid, respectively. Whereas, secondary form cells and CFS has recorded LC_{50} of 1.67×10^5 cells/ml and 2.27×10^3 CFS respectively. These results indicate the possibility of using *P. luminescens* in the integrated management of *A.gossypi* Bt cotton.

Key words: *Heterorhabditis indica, Photorhabdus luminescens, Aphis gossypii,* Crop pest, primary and secondary forms cells

INTRODUCTION

Aphis gossypii Glover is a serious pest on cotton. It is also known to occur in several other crops like cucumber, okra, watermelon, eggplant, citrus *etc*. Both adult and nymphs confine to undersurface of leaves, on tender shoots and desap the plants. Their feeding leads to chloratic patches, curling and distortion of leaves. Severe incidence results in stunted growth and death of the plant. Hence development of best plant protection technologies like non chemical or biological pest management strategy has become imperative as these technologies are easily biodegradable and harmless to beneficial organisms.

Photorhabdus luminescens (Thomas and Poinar), a symbiotic bacterium of entomopathogenic nematode *Hetereorhabditis indica* (Poinar) is a non-spore forming, motile, bioluminescent, gram-negative, entomodic and facultative anaerobic bacterium belonging to family enterobacteriaceae in the gamma subdivision of the purple bacteria. The *P. luminescens* occurs in two forms which differ with morphological, biochemical and antibiotic properties (Akhrust, 1980). This symbiotic bacterium can be isolated and cultured in the absence of its symbiont. Many authors have reported the use of *P. luminescens* against insects of different orders like *Lepidoptera, Coleoptera* and *Dictyoptera* (Bowen *et al.*, 1998; Fabiana Consolo *et al.*, 2010). *P. luminescens* produces an array of putative insecticidal toxins. Majorly three classes of toxins have been well known. The members of the first class, the toxin complexes (Tc) are orally toxic to caterpillar pests (Bowen *et al.*, 1998) and it is recently used to create insect-resistance transgenic plants (Liu *et al.*, 2003). The second class "Makes caterpillars floppy" toxins (Mcf1 and Mcf2) are active upon injection (Dabron *et al.*, 2002). Third and most recently, the "*Photorhabdus* insect related" proteins (Pir AB) have shown binary toxin with both oral (Duchaud *et al*, 2003) and injectable (Waterfield *et al.*, 2005) activities in some insects.

Several studies have been carried out evaluating the pathogenicity of *P. luminescens* against several crop pests in India. The *P. luminescens* (10^6 bacterial loads) sprayable formulation has given 90 per cent laraval mortality of *Plutella xylostella* (Rajgopal and Bhatnagar, 2002) and the *P. luminescens* isolated from *H. indica* IARI strain caused 100 per cent mortality of cabbage butterfly *Pieris*

© JBiopest. 106

brassica after 24 hr of foliar spray (Sharad Mohan *et al.*, 2003b).The supernatant of *P. luminescens* spp. Laumondii (1.0×10^6 cells/ml) resulted in 90-95 % mite mortality within 24-48 hr (Bussaman *et al.*, 2005). 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. The toxic effects of these bacteria have been attributed to different toxins and enzymes which inhibit the humoral immune system of insects and cause damage to the hemocytes (Au *et al.*, 2004). However, their study restricted to evaluation of crude bacterial culture on target insects, But it is not clear whether the death of the insect is due to bacterial cells (or) due to metabolites and the pathogenicity studies on the sucking pest is relative less Hence an attempt has been made to evaluate the bioefficacy of *P. luminescens* against *A. gossypii* in the Department of Agricultural Entomology, College of Agriculture, UAS, Raichur.

MATERIALS AND METHODS

Insect and nematode source

Nymphs of *A. gossypii* were collected from the cotton field of UAS, Raichur and reared on the cotton leaves till the treatment is imposed. The nematode was isolated from the soil obtained from the lawn College of Agriculture, Raichur using modified trapping technique (Prabhuraj *et al.*, 2000). By above stock culture *H.indica* was *in vivo* cultured (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*

P. luminescens was isolated from the haemolymph of infected hosts as described by Woodring and Kaya (1998) and maintained in monoxenic culture. Newly molted last instar larvae of the greater wax moth, *Galleria mellonella* (L.) were exposed to IJs at the rate of 100 IJs per larvae. 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 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 forms. Primary and secondary forms were grown in 150 ml of NA broth at 28^0 C for 24- 48 hr separately.

Preparation of bacterial cells and cells free supernatant

A loop full 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 *A. gossypii*

The fully grown cotton leaf was placed in petri dish with its stalk wrapped in wet cotton. Twenty 2^{nd} instar *A. gossypii* nymphs were released on the leaf and the plate containing aphid 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 replications. Each replication consists of 20 aphids. Solution with only distilled water with Paraffin liquid of 10 µl/ml containing sucrose @ 0.5 per cent served as control. Observation on mortality was recorded at 6 hour interval for up to 48 hr. The data obtained was subjected to ANOVA and probit analysis (Finney, 1971).

RESULTS AND DISCUSSION

Mortality response of *A. gossypii*

Per cent mortality of nymph due to primary and secondary form cells and cells free supernatant showed a cumulative increase in the mortality with increase in exposure time in both case. The mortality recorded at 24 and 48 hr are presented in the Table 1. The primary form cells recorded up to 75.00 per cent mortality from the treatment T1(10^{10} cells/ml) at 24 hr and slightly increased to 77.50 per cent at 48 hr, and superior to all other treatments but on par with the T2 (10^9 cells/ml), T3 (10^8 cells/ml) and T4 (10^7 cells/ml) recorded the mortality of 72.50, 71.25 and 67.50 per cent respectively, but in case of cells free supernatant at 24 hr itself T1(10^{10}CFS) and T2(10^9 CFS) have shown cent per cent mortality and even T4 (10^7 CFS) has also registered 100 per cent mortality at 48 hr. The secondary form cells have recorded up to 75.00 per cent mortality at 24 hr and increased to 83.75 per cent at 48 hr in the treatment T1(10^{10} cells/ml) but it was on par with T2(10^9 cells/ml) and T3 (10^8

Table1. Effect of different concentration of *P. luminescens* cells and cells free supernatant of primary and secondary forms against *A. gossypii*.

Concentration (Cells/ CFSml)	Per cent mortality of *A. gossypii* 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}	75.00(60.05)^{a}	77.50(61.71)^{a}	100.00(90)^{a}	100.00(90)^{a}	75.00(60.05)^{a}	83.75(66.41)^{a}	100.00(90)^{a}	100.00(90)^{a}
T_2 -10^{9}	66.25(54.51)^{b}	72.50(58.39)^{a}	100.00(90)^{a}	100.00(90)^{a}	71.25(57.59)^{a}	80.00(63.52) ^{a}	97.50(83.53^{b}	98.75(86.76^{a}
T_3-10^{8}	66.25(54.51)^{b}	71.25(57.62)^{ab}	95.00(78.93)^{b}	100.00(90)^{a}	70.00(56.82)^{a}	76.25(60.91)^{ab}	88.75(70.47)^{c}	92.50(74.32)^{b}
T_4-10^{7}	60.00(50.78)^{b}	67.50(55.25)^{ab}	93.75(75.70)^{b}	98.75(86.76)^{a}	57.50(49.34)^{b}	65.00(53.75)^{c}	87.50(69.38)^{c}	92.50(74.32)^{b}
T_5-10^{6}	58.75(50.05)^{bc}	62.50(52.26)^{bc}	87.50(69.38)^{c}	90.00(71.85)^{b}	46.25(42.84)^{c}	50.00(45)^{d}	78.75(62.57)^{c}	81.25(64.46)^{c}
T_6-10^{5}	52.50(46.43)^{c}	56.25(48.59)^{c}	81.25(64.46)^{c}	81.25(64.46)^{c}	43.75(41.40)^{c}	47.50(43.56)^{d}	75.00(60.05)^{c}	75.00(60.05)^{c}
T_7-10^{4}	41.25(39.95)^{d}	50.00(45)^{cd}	72.50(58.39)^{d}	78.75(62.66)^{c}	32.50(34.74)^{d}	41.25(39.94)^{de}	56.25(48.60)^{e}	56.25(48.60)^{d}
T_8-10^{3}	26.25(30.80)^{e}	30.00(33.17)^{e}	50.00(45)^{e}	60.00(50.78)^{d}	23.75(29.14)^{e}	33.75(35.50)^{e}	41.25(39.95)^{f}	42.50(40.68)^{e}
T_9-10^{2}	17.50(24.67)^{f}	23.75(29.14)^{f}	43.75(41.39)^{e}	48.75(44.28)^{e}	16.25(23.73)^{f}	26.25(30.80)^{f}	26.25(30.80)^{g}	36.25(37.01)^{e}
T_{10}-10^{1}	12.50(20.61)^{f}	17.50(24.67)^{g}	21.25(27.42)^{f}	28.75(32.37)^{f}	8.75(17.05)^{g}	16.25(23.73)^{g}	13.75(21.69)^{h}	18.75(25.62)^{f}
T_{11} -control	0.00(0)^{g}	0.00(0)^{h}	0.00(0)^{g}	0.00(0)^{g}	0.00(0)^{h}	0.00(0)^{h}	0.00(0)^{i}	0.00(0)^{g}
S.Em±	1.113	1.104	1.547	1.505	1.112	1.157	1.471	1.473
CD at 1%	4.329	3.945	6.017	5.856	4.326	4.502	5.722	5.730
CV	5.645	4.791	5.312	4.849	5.930	5.499	5.609	5.386

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

cells/ml). In case of cells free supernatant only T1 (10^{10} CFS) has recorded 100 per cent mortality at 24 hr and only slight increase in mortality was observed at 48 hr where the mortality ranged from 75.00 to 100 per cent from the treatments T6 (10^{5} CFS) to T1 (10^{10} CFS).Over all T1 to T4 recorded significantly highest mortality compared to all other treatment. Except control an increase in the aphid mortality was noticed over a period of 48 hr.

The above results indicated that primary form of *P. luminescens* was more virulent compared to secondary form. The present results is in contradictory with results of Akhurst and Dunphy (1993), Steven Forst and Nealson (1996) and Abdel Razek (2002) who opined that both the primary and secondary forms are equally virulent against *G. mellonella*. The difference might be due to the variation in test insect. However, among the cells and cells free supernatant the latter was more virulent compared to former, where cumulative mortality was increased with increase in time at 48 hr. The primary form CFS recorded cent per cent mortality from T_1 (10^{10}CFS) to T_3 (10^{8}CFS) but secondary form cells supernatant recorded 100 per cent mortality only from the treatment T_1 (10^{10}CFS).Whereas, cells recorded mortality of 77.50 and 83.75 per cent, respectively. But the mortality decreased with decrease in the concentration in both forms of cells and cells supernatant.

Concentration mortality response of *A. gossypii*

The cells of primary form at 24 hour recorded LC_{50} of 6.08 x 10^{5} cells/ ml and mortality was increased by 0.048 times with a unit increase of cells (Y = 0.048 x + 0.316) and at 48 hr LC_{50} decreased to 1.00 x 10^{5} cells/ ml with increase in mortality by 0.069 times by an unit increase of cells (Y = 0.069 x + 0.145) and in cells free supernatant, LC_{50} of 4.01 x 10^{2} CFS (Y = 0.048 x + 0.316) and 2.02 x 10^{2} CFS (Y = 0.076 x +0.368) was recorded at 24 and 48 hr after exposure, respectively. Similar trend was followed in secondary form , where cells at 24 hr, recorded the LC_{50} of 1.55 x 10^{6} cells/ ml and mortality was increase by 0.078 times with an increase of cells (Y = 0.078 + 0.016) and LC_{50} decreased to 1.67 x 10^{5} cells/ ml and mortality increased by 0.075 times with an unit increase of cells (Y = 0.075 x +0.103) and cells free supernatant, recorded an LC_{50} of 4.44 x 10^{3}CFS (Y = 0.097 x + 0.127) and 2.02 x 10^{2} CFS (Y = 0.092 x 0.184) was recorded at 24 and 48 hr of exposure, respectively .

On the basis of these results, the primary form was found more virulent than secondary form and CFS was more pathogenic than cells. similar results were quoted by Martin (2004) reported that *P. luminescens* has LC_{50} value of 6.4± 1.87×10^{7} cells for second instar laravae of Colorado potato beetle. The *P. luminescens* resulted in 60 per cent mortality with the LC_{50} value of 5.00× 10^{4} cells/ ml against pupae of diamond back moth *Plutella xylostella* (Abdel Razek, 2003).

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

Treatments	Chisquare $(\chi)^2$	D.F.	Regression Equation	LC_{50}	Fiducial limit at LC_{50} LL-UL	LC_{99}	Fiducial limit at LC_{99} LL-UL	R^2
Primary form Cells								
24 hours	139.63	2	Y = 0.048 x +0.316	6.08×10^5	$1.18 \times 10^4 - 1.02 \times 10^7$	8.40×10^{17}	$9.77 \times 10^{15} - 9.24 \times 10^{20}$	0.977
48 hours	134.41	2	Y = 0.069 x +0.145	1.00×10^5	$7.30 \times 10^2 - 2.82 \times 10^6$	2.68×10^{17}	$3.43 \times 10^{15} - 2.69 \times 10^{20}$	0.934
Primary form cells supernatant								
24 hours	296.32	2	Y = 0.084 x +0.279	4.01×10^2	$2.5 \times 10^1 - 3.04 \times 10^3$	1.94×10^9	$5.37 \times 10^8 - 1.87 \times 10^{10}$	0.884
48 hours	285.21	2	Y = 0.076 x +0.368	2.02×10^2	$0.17 - 5.28 \times 10^3$	8.06×10^7	$1.74 \times 10^7 - 8.14 \times 10^8$	0.853
Secondary form Cells								
24 hours	174.803	2	Y = 0.078 x +0.016	1.55×10^6	$5.80 \times 10^4 - 1.54 \times 10^7$	6.4×10^{16}	$1.29 \times 10^{15} - 3.57 \times 10^{19}$	0.983
48 hours	159.459	2	Y = 0.075 x +0.103	1.67×10^5	$7.6 \times 10^1 - 7.70 \times 10^6$	2.59×10^{16}	$3.72 \times 10^{14} - 1.51 \times 10^{19}$	0.83
Secondary form cells supernatant								
24 hours	327.358	2	Y = 0.097 x + 0.127	4.44×10^3	$3.94\ 10^2 - 2.69 \times 10^4$	3.04×10^{10}	$5.43 \times 10^9 - 3.00 \times 10^{11}$	0.931
48 hours	310.68	2	Y = 0.092 x +0.184	2.27×10^3	$1.3 \times 10^1 - 3.72 \times 10^4$	7.96×10^9	$1.48 \times 10^9 - 1.17 \times 10^{11}$	0.936

Note: LL – Lower limit, UL – Upper limit

Over all results of bioassay revealed that *P. luminescens* was found to be highly pathogenic to *A. gossypii*. No reports are available about the pathogenicity of *P. luminescens to A. gossypii*. Hence the present study forms the first report on *P. luminescens* pathogenicity against *A. gossypii*. Among the two forms, primary form was more virulent than secondary form. The primary form is known to produce different antibiotics in high concentration compared to secondary form (Bleakely and Nealson, 1988). Further, secondary form is nothing but the infinite form of *P. luminescens* as resultant of depletion of host nutrient. Hence the concentration of cells secretion might be lower than the primary form. Thus resulted in lower pathogenicity compared to primary form. Between the cells and CFS, the latter was more virulent than the former in both the forms. 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 *A. gossypii* 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* iinsect 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. In conclusion, our results suggest the possibility of developing the cell-free supernatant of *P. luminescens* forms for use in the management of *A. gossypii*. Further studies on the identification and mode of action of toxins from these bacteria are needed before large scale field evaluation or commercial production.

REFERENCES

Abdel-Razek, A. S. 2002. Pathogenic of bacteria symbiotically associated with insect pathogenic nematodes against the greater wax moth, *Galleria mellonella* moth (L.). *Archives of Phytopathological Plant Protection,* **35**(1): 53-60.

Akhurst, R. J. 1980. Morphological and functional dimorphism in *Xenorhabdus* spp. bacteria symbiotically associated with insect pathogenic nematodes *Neoaplectana* and *Heterorhabditis*. *Journal of General Microbiology*, **121**: 303–309.

Akhurst, R. J. and Dunphy, G. B. 1993.Tripartite interactions between symbiotically associated entomopathogenic bacteria *Xenorhabdus* spp. and nematodes (Steinernematidae and Heterorhabditidae) and their insect hosts. *Parasites and Pathogens of* Insects. Academic Press, New York, **2**: 1–23.

Au, C., Dean, P., Reynolds, S. E. and Ffrench-Constant, R. H. 2004.Effect of the insect pathogenic bacterium *Photorhabdus* on insect phagocytes.*Cellular Microbiology,* **6**: 89-95.

Bleakely, B. and Nealson, K. H. 1998, Characterization of primary and secondary form of *Xenorhabdus luminescens* strain Hm. *FEMS Microbiology and Ecology,* **53**: 241-250.

Boemare, N. E. and Akhurst, R. J. 1988.Biochemical and physiological characterization of colony form variants

in *Xenorhabdus* spp. (Enterobacteriaceae). *Journal of General Microbiology*, **134**: 751–761.

Bowen, D. and Ensign, J. C. 1998. Purification and charac terization of a high- molecular weight insecticidal protein complex produced by the Entomopathogenic bacterium *Photorhabdus luminescens. Applied and Environmental Microbiology,* **64**(8): 3029-3035.

Bussaman, P., Sermswan, R.W. and Grewal, P. S. 2005, Toxicity of the entomopathogenic bacteria *Photorhabdus* and *Xenorhabdus* to the mushroom mite (*Luciaphorus* sp.; Acari : Pygmephoridae). *Biocontrol Science and Technology,* **16** (3/4): 245-256.

Dabron, P. J., Waterfield. N., Au, C. P. Y., Sharma, S. and Ffrench-Constant, R. H. 2002. A single *Photorhabdus* gene, makes catertpillars floppy (*mcf*), allows *Escheria coli* to persist within and kill insects. *Proceedings of National Academic Science,* USA, **99**:10742-10747. www.pnas.org/doi/10.1073

Duchaud, E., Rusniok, C., Frangeul, L., Buchrieser, C., Givaudan, A., Taourit, S., Bocs, S., Boursaux-Eude, C., Chandler, M., Charles, J. F., Dassa, E., Derose, R., Derzelle, S., Freyssinet, G., Gaudriault, S., Medigue, C., Lanois, A., Powell, K., Siguier, P., Vincent, Wingate, R. V., Zouine, M., Glaser, P., Boemare, N., Danchin, A. and Kunst, F. 2003.The genome sequence of the entomopathogenic bacterium *Photorhabdus luminescens. Nature Biotechnology,* **21:** 1307-1313.

Fabiana Consolo, V., Viviana Mucci, Graciela L. Salerno and Corina M. Berón. 2010. Pathogenicity of bacterial isolates to *Cyclocephala signaticollis* (Coleoptera: Scarabaeidae). *Biocontrol Science and Technology,* **20** (5): 475 – 482.

Finney, D. J. 1971. Probit analysis III edition, Cambridge University Press, 333 **PP**.

Liu, D., Burton, S., Glancy, T., Li, Z. S., Hampton, R., T. Meade and Merlo, D. J. 2003, Insect resistance conferred by 283-kDa *Photorhabdus luminescens* protein TcdA in *Arabidopsis thaliana*. *Nature Biotechnology,* **21**:1222-1228

Martin, P. A. W., Blackburn, M. and Shropshire, A. D. S. 2004. Two new bacterial pathogens of Colorado potato beetle (Coleoptera; chrysomelidae). *Journal of Economic Entomology,* **97**(3): 774- 780.

Prabhuraj, A. 1997.Faunastic studies on entmopathogenic nematodes families Steinernematidae and Heterorhabditidae and their potential as bio-control agents of white grubs and caterpillar pests. *Ph.D. thesis.* University of Agricultural Sciences, Dharwad, Karnataka, India, 172 **PP**.

Prabhuraj, A., Viraktamath, C. A. and Kumar, A. R. V. 2000. Modified technique for the isolation of insect parasitic nematodes. *Journal of Biological Control,* **14**: 83-85.

Rajgopal, R. and Bhatnagar, R. K. 2002. Insecticidal toxic proteins produced by *Photorhabdus luminescens* akhurstii, a symbiont of *Heterorhabditis indica*. *Journal of Nematology,* **34** (1):23-27.

Sharad-Mohan, Anil-Sirohi and Gaur, H. S. 2003a. Successful management of mango mealy bug, *Drosicha mangiferae* by *Photorhabdus luminescens*,a symbiotic bacterium from entomopathogenic nematode *Heterorhabditis indica. International Journal of Nematology,* **14**(2):195-198.

Sharad-Mohan, Rajagopal-Raman and Gaur, H. S. 2003b. Foliar application of *Photorhabdus luminescens*, symbiotic bacteria from entomopathogenic nematode *Heterorhabditis indica*, to kill cabbage butterflies *Pieris brassicae. Current Science,* **84**(11): 1397.

Steven Forst and Kenneth Nealson,1996. Molecular biology of the symbiotic pathogenic bacteria *Xenorhabdus* spp. and *Photorhabdus* spp. *Microbiological Reveiw,* **60**: 21- 43.

Waterfield, N., Kamita, S. G., Hammock, B. D., and Ffrench-Constant, R. 2005. The *Photorhabdus* Pir toxins are similar to a developmentally regulated insect protein but show no juvenile hormone esterase activity. *FEMS Microbiological Letters,* **245:** 47-52.

Woodring, J. L. and Kaya, H. K. 1998. *Steinernematid* and *Heterorhabditid* nematodes: hand book of biology and techniques. South coop. Ser. Bull. Arkans. Agric. Exp. Stn., Fayetteville., **331**: 1-30.

G. P. Uma*, A. Prabhuraj and A. Vimala
Department of Entomology, College of Agriculture, UAS, Raichur 584 102, Karnataka, India, Phone: 09480318050, E-mail: umacftzp@gmail.com.