



E-ISSN: 2320-7078

P-ISSN: 2349-6800

www.entomoljournal.com

JEZS 2020; 8(4): 2343-2348

© 2020 JEZS

Received: 04-05-2020

Accepted: 19-06-2020

S Adithya

Department of Agricultural
Microbiology, GKVK,
University of Agricultural
Sciences, Bangalore, Karnataka,
India

MK Shivaprakash

Department of Agricultural
Microbiology, GKVK,
University of Agricultural
Sciences, Bangalore, Karnataka,
India

E Sowmya

Department of Entomology,
UAS, Raichur, Karnataka, India

Evaluation of insecticidal activity of entomopathogenic bacteria *Photorhabdus* and *Xenorhabdus* against shoot and fruit borer *Earias vittella* (Lepidoptera: Noctuidae) of vegetable crops

S Adithya, MK Shivaprakash and E Sowmya

DOI: <https://doi.org/10.22271/j.ento.2020.v8.i4aj.7468>

Abstract

The shoot and fruit borer, *Earias vittella* is one of the major polyphagous pest prefer various host plants attacking almost 38 plant species and causes yield loss of 15-45% in vegetable crops annually in India. In this study Entomopathogenic bacteria (EPB) were used for biocontrol larvae of *Earias vittella* under *in vitro* condition. Whole cell culture and cell supernatant of EPB was screened for insecticidal activity against the larvae and results has shown that the lethality of larvae started after 12-24h of treatment. The percent mortality treated with intact cell was significantly higher in larvae treated with EPB3 with 90% mortality at 72h followed by EPB1 and EPB3. The larvae treated with cell supernatant of EPB had shown lethality in the range of the mortality of larvae where in the range of 50-70% at 72h of treatment and there was no significant difference among the bacteria treated. The LD₅₀ value represent the concentration of cells required for the 50% population to be lethal and EPB3 had expressed significantly lower concentration of 4.28x10⁴ CFU / ml followed by EPB9 and EPB8. The LD₅₀ value for the larvae treated with cell supernatant were in the range of 10¹⁰ to 10¹² CFU / ml. The larvae treated with EPB3 cell culture has taken significantly less time for mortality of fifty percent of population with a media lethal time (LT₅₀) of 24h followed by EPB1 (30h) and EPB8 (32h). Among all the isolates EPB3 (*Xenorhabdus nematophila*) and EPB1 (*Photorhabdus luminescens*) had more virulence against the *Earias vittella* and can be further tested for biocontrol efficiency under greenhouse and field conditions.

Keywords: *Earias vittella*, entomopathogenic bacteria, insecticidal activity, mortality, LD₅₀ and LT₅₀

1. Introduction

Crop pests and diseases are major constraints in food production. Chemical insecticides proved to be effective means of control, but are costly and possess residual problems besides, resulting in development of resistance and resurgence in pests and diseases due to their repeated applications. Hence, there is a strong need for developing eco-friendly, cost effective, biodegradable & easily accessible and target specific alternate methods to control crop pests and diseases. Microbial pesticides are known to possess all these qualities and are now encouraged against several pests and diseases ^[1].

Natural enemies play an important role in pest management and can be used to specifically target unwanted host in an ecosystem hopefully without having adverse outcomes such as destroying crops themselves. These enemies can be parasites or predators both with the mission to attack and kill pests. Bacteria, fungi and nematodes can be used as natural enemies for control of insect pests ^[2]. Most of the insecticidal toxins used in agriculture come from a single bacterium *Bacillus thuringiensis* or 'Bt'. Yet, Dowling and Waterfield ^[3] reviewed the array of toxins produced by Entomopathogenic bacteria (EPBs) (*Photorhabdus* and *Xenorhabdus*) that are symbiotic with Entomopathogenic nematodes (EPNs) and discussed their potential for use in agriculture as alternatives to Bt. In this respect, numerous studies have recently shown the importance of the bacterium *Photorhabdus* and *Xenorhabdus* as a biological control agent against insect pests ^[4-6], mushroom mites ^[7, 8] and plant pathogens ^[9, 10]. Various toxins have been characterized in EPBs, which were classified into four major groups: the toxin complexes (Tcs), the 'makes caterpillars floppy' (Mcf) toxins, the *Photorhabdus* insect-related (Pir) proteins, and the *Photorhabdus* virulence cassettes (PVC) ^[11]. Tc toxins attracted attention from the fact that some of these toxin complexes are highly toxic to insects after oral feeding, suggesting potential as insecticides ^[12].

Corresponding Author:**S Adithya**

Department of Agricultural
Microbiology, GKVK,
University of Agricultural
Sciences, Bangalore, Karnataka,
India

With this rationale the present study was conducted to test the insecticidal activity of entomopathogenic bacteria *Photorhabdus* and *Xenorhabdus* against shoot and fruit borer *Earias vittella*.

2. Materials and Methods

2.1. Bacterial strains

A total of six Entomopathogenic bacterial were used in this study to test the insecticidal activity against *Earias vittella*. Among the six bacteria five entomopathogenic bacterial isolates were isolated from two agro-climatic zones (zone 5 and zone 6) of Karnataka and were characterized and identified as *Photorhabdus luminescens* (EPB1, EPB4, EPB8 and EPB9) and *Xenorhabdus nematophila* (EPB3) (Adithya and Shivaprakash, unpublished). *Bacillus thuringiensis* culture was obtained from Biofertilizers lab, Department of Agricultural Microbiology, GKVK.

2.2. Preparation of Intact Cell culture and Cell supernatant

A single bacterial colony from the pure bacterial culture plates was transferred and grown in 25 ml LB broth and shaken at 180 rpm for 24h. The concentration of the intact cell culture was adjusted to 10^8 CFU/ml using phosphate buffered saline solution. To prepare cell supernatant, 1 ml whole cell suspension was centrifuged at 5000rpm for 5 min. The supernatant was then filtered using a 0.22- μ m filter. The flow-through was used as the cell-free supernatant [13].

2.3. Screening of Entomopathogenic bacteria for insecticidal activity against *Earias vittella*

Bioassay was carried out for EPBs against *Earias vittella* for screening of insecticidal activity as per the procedure detailed by Mahar, Munir [14]. The bioassay experiment was performed in Department of Agricultural Microbiology, GKVK, UASB, Bangalore and the larvae of *Earias vittella* for bioassay was procured from National Bureau of Agriculturally Insect Resources (NBAIR), Hebbala, Bangalore. The larvae were kept in capped plastic boxes having small holes in it for aeration. Okra pods were provided as diet in the boxes for their survival. Entomopathogenic bacteria were sub-cultured in Luria broth and incubated at 28 °C for 48h in a shaker cum BOD incubator at 180 rpm. A day old cultures were centrifuged for separation of cell pellet and supernatant. The cell pellet was taken and cells were dissolved in sterilized distilled water and similarly cell supernatant was diluted for treatment. A concentration of 1×10^8 bacterial cells (20ml) were mixed with the food and mortality of insects was recorded at regular interval of 6h, 12h, 18h, 24h, 36h, 48h, 72h and 96h. Percentage mortality is recorded from the average for the three replicates taken. The percentage mortality was calculated by using the formula:

$$\text{Percent mortality} = \frac{\text{Number of dead insects}}{\text{Number of insects tested}} \times 100$$

2.4. Virulence test

The virulence test was carried out in sterile polystyrene boxes under lab conditions. A day old cultures were centrifuged and the cell pellet was taken and cells were dissolved in sterilized distilled water. Cell count was done with the help of a haemocytometer and required bacterial concentrations were prepared by serial dilution. The bacterial cultures were transferred to the polystyrene boxed at different

concentrations ranging from 10^2 to 10^{10} cells/ml. After 15 minutes of adding bacterial culture, a single test larvae (*E. vittella*) was placed in each box. Each concentration of symbiotic bacterial isolate was replicated 10 times, and the experiment was independently repeated thrice. Similarly the experiment was also carried out with cell supernatant. The boxes were incubated at 28 °C and the larval mortality was recorded every 12h till 100% insect mortality or pupation, whichever was earlier. Mortality data were used to calculate median lethal dose (LD₅₀) and median lethal time (LT₅₀) values.

2.5. Statistical analysis

All the experiments were done in laboratory conditions in completely randomized design (CRD) and the values obtained from the study was analysed statistically using analysis of variance (ANOVA) [15]. The percent mortality values were arc-sine transformed and analysed. The treatment means were statically differentiated by performing Duncan's Multiple Range Test (DMRT) at $p < 0.05$ level. Statistically differentiated means were denoted by different alphabets. For all the above analysis, the software, DSAASTAT developed by Dr. A. Onofri, DSAA, Italy [16].

The larval mortality data was subjected to probit analysis using SPSS software (Version 20.00) to estimate the median lethal dose (LD₅₀) of Entomopathogenic bacteria (Finney, 1971). The LT₅₀ values were calculated from the survival curves prepared by Kaplan-Meier survival analysis. The survival curves were compared using the log-rank test using GraphPad Prism 8.0 (GraphPad Software Inc., La Jolla, CA, USA) statistical software. The log-rank test calculates the chi-square value (χ^2) for each event time for each group and sums the results which are used to derive the ultimate chi-square to compare the full curves of each group [17].

3. Results and Discussion

The shoot and fruit borer, *Earias vittella* (Fab.) is noctuid pest causing more than 50% loss in vegetable crops especially in bhendi (69%) in various parts of India. This pest alone cause 13.8-41.6% net yield loss in bhendi alone annually [18]. The present study is aimed for finding out the virulent strain among the entomopathogenic bacteria for potential biocontrol of shoot and fruit borer of vegetable crops.

3.1. Screening of insecticidal activity of Entomopathogenic bacteria against larvae of *Earias vittella*

The insecticidal activity was recorded as percent larval mortality by taking regular mortality of larvae treated with entomopathogenic bacteria and the values were arc-sine transformed and presented in the Table 1 & 2 and Figure 1.

The larvae of shoot and fruit borer exhibited mortality that was treated with intact cells of entomopathogenic bacteria after 12h of exposure. Among the bacteria, EPB3 (*Xenorhabdus nematophila*) had expressed highest percent mortality of at all the time intervals recorded with reaching more than 50% lethality of larvae with 24h of treatment with intact cells which was significantly different from all other EPB used in the study. This was followed by EPB1 and EPB4 (*Photorhabdus luminescens*) reached 50% lethality of larvae at 36h of post treatment. Among the EPB, *Bacillus thuringiensis* had exhibited significantly less mortality value ranging from 20-70%.

The cell supernatant treated larvae also exhibited larval mortality at 12-24h of post treatment. The trend of larval

mortality were similar to the larvae treated with intact cells with considerably less percent mortality with a difference of 2-20%. EPB3 had expressed highest mortality (72%) at 72h followed by EPB1 (70%) and EPB3 (66%). There was no significant difference in percent mortality among the entomopathogenic bacteria after 48h of treatment with the cell supernatant. Among the EPB, least mortality was exhibited by *Bacillus thuringiensis*.

E. vittella has developed resistance against insecticides that are used to control them and to overcome this problem researchers has to concentrate on biological means like microbial agents for development of biopesticides for control of insect pests [19]. Biocontrol efficiency of *Bt* in combination with *Beauveria bassiana* against larvae of *Earias vittella* and recorded 100% mortality when treated with *Bt* and *B. bassiana* in the combination of 0.5µg /g and 1.58x10⁸ conidia / ml, respectively [20].

Pazhanisamy and Archunan [21] had used biological extracts like neem leaf extract (NLE) 5%, Prosopis leaf extract 5%, Calotropis leaf extract 5%, Pungam oil 3% Neem seed kernel extract (NSKE) 5% and panchagavya for biocontrol of *Earias vittella*. The larval mortality was higher in that are treated with panchagavya (3%) + NSKE (5%) with 84% lethality followed by panchagavya 3% + pungam oil 3% (67%).

3.2. Virulence test of Entomopathogenic bacteria against *Earias vittella*

Virulence test was carried out against *E. vittella* to find the best entomopathogenic bacteria that has the ability to control the larvae at low concentration within a short time. The larval mortality values were analysed and the LD₅₀, LD₉₀ and LT₅₀ was calculated and presented in the Table 3 & 4 and figure 2. Median lethal dose (LD₅₀) represents the dose required for the fifty percent of larval population to become lethal. In this study, EPB3 cell culture exhibited a LD₅₀ and LD₉₀ values of 4.28x10⁴ and 1.81x10⁹ CFU / ml respectively which was significantly different from the other bacterial cultures. Similarly the larvae treated with cell supernatant exhibited a LD₅₀ values in the range of 10⁵ to 10⁷ CFU/ ml and LD₉₀ in the range of 10¹⁰ to 10¹² CFU / ml. Among all the isolates, EPB3 had expressed LD₅₀ with significantly low

concentration of cells 3.41x10⁵ CFU / ml compared to other bacteria. *Bt* exhibited the LD₅₀ and LD₉₀ in the range of 10⁶ and 10¹¹ CFU/ml which had taken comparatively higher dose of cells for median lethality of larvae. EPB3 and EPB4 had exhibited LD₅₀ of 10⁵ CFU/ml and LD₉₀ of 10⁹ CFU/ml, respectively.

LT₅₀ values represent the time taken for the lethality of fifty percent of larval population treated with EPB. Among the entomopathogenic bacteria, EPB3 cell culture treated larvae taken 24h for media lethality (LT₅₀) followed by EPB1, EPB9 and EPB4 with a LT₅₀ values of 30h, 32h and 33h, respectively. The larvae of *E. vittella* treated with cell supernatant of entomopathogenic bacteria recorded LT₅₀ in the range of 36-55h. Among the bacteria, larvae treated with cell supernatant of EPB3 (*Xenorhabdus nematophila*) had exhibited significantly lower median lethal time (LT₅₀) of 36h followed by EPB3 (*Photorhabdus luminescens*) with LT₅₀ of 40h.

Aziz and co-workers [22] used five methods namely biological control, cultural control, botanical control, mechanical control and chemical control for control of *Earias* spp. in Okra and the results had shown that the combination of treatment with biological control and botanical control had significant higher yield and 40-45% reduction in the fruit damage by the insect pest. And they also compared the cost benefit ratio for of the treatments with different control measures and found that CBR ratio was better in the treatment combination of biological control and chemical control compared to the other treatments.

The entomopathogenic bacteria shows insecticidal activity against the insect during the infection by suppressing the phospholipase A₂ [23] and also down regulate the gene expression of antibacterial peptides thereby inhibiting the humoral immunity of insect pest. This induces the apoptosis in haemocytes and causing septicaemia finally resulting in death [24]. Apart from this *Photorhabdus* and *Xenorhabdus* produce several insecticidal toxins like toxin complexes (Tc's) [25], the Making caterpillar floppy (Mcf) toxin [26], binary toxins PirAB [27], juvenile hormone esterase (JHE) [28] and finally the ureases displaying ureolysis-independent entomotoxic effect [29].

Table 1: Percent mortality of *Earias vitella* larvae treated with intact cells of Entomopathogenic bacteria

S. No	Bacteria	Percent Mortality					
		12h	24h	36h	48h	72hr	96h
1	EPB1	20.00 (26.57 ± 0.77) ^{ab}	53.33 (46.91 ± 1.35) ^{ab}	70.00 (56.79 ± 1.64) ^{ab}	80.00 (63.43 ± 1.83) ^{ab}	85.00 (67.21 ± 1.94) ^{ab}	90.00 (71.57 ± 1.85) ^{ab}
2	EPB3	23.33 (28.88 ± 0.83) ^a	60.00 (50.77 ± 1.47) ^a	76.67 (61.12 ± 1.76) ^a	83.33 (65.91 ± 1.90) ^a	90.00 (71.57 ± 1.83) ^a	93.33 (75.04 ± 1.72) ^a
3	EPB4	16.67 (24.09 ± 0.70) ^{abcd}	50.00 (45.00 ± 1.30) ^{bc}	63.33 (52.73 ± 1.52) ^{bc}	76.67 (61.12 ± 1.76) ^{abc}	82.00 (64.90 ± 1.87) ^{bc}	86.67 (68.58 ± 1.98) ^{bc}
4	EPB8	20.00 (26.57 ± 0.72) ^{abc}	46.67 (43.09 ± 1.24) ^{bcd}	60.00 (50.77 ± 1.47) ^{cd}	73.33 (58.91 ± 1.70) ^{bc}	80.00 (63.43 ± 1.57) ^{bc}	83.33 (65.91 ± 1.63) ^c
5	EPB9	13.33 (21.42 ± 0.77) ^{bde}	43.33 (41.17 ± 1.19) ^{cd}	56.67 (48.83 ± 1.41) ^{cd}	70.00 (56.79 ± 1.64) ^{cd}	76.00 (60.67 ± 1.75) ^{cd}	83.33 (65.91 ± 1.45) ^c
6	<i>Bt</i>	10.00 (18.43 ± 0.53) ^e	40.00 (39.23 ± 1.13) ^d	53.33 (46.91 ± 1.35) ^d	63.33 (52.73 ± 1.52) ^d	70.00 (56.79 ± 1.64) ^d	70.00 (56.79 ± 1.64) ^d
7	Control	0.00 (0 ± 0.00) ^f	0.00 (0 ± 0.00) ^e	0.00 (0 ± 0.00) ^e	0.00 (0 ± 0.00) ^e	0.00 (0 ± 0.00) ^e	10.00 (18.43 ± 0.83) ^e

Experiment was done in triplicates and the values are mentioned as means of triplicate studies. Values in the brackets are Arc-sine transformed ± SeM. Superscripted alphabet letters show statistical groups for that column. *Bt* – *Bacillus thuringiensis*.

Table 2: Percent mortality of *Earias vitella* larvae treated with cell supernatant of Entomopathogenic bacteria

S. No	Bacteria	Percent Mortality					
		12h	24h	36h	48h	72hr	96h
1	EPB1	16.67 (24.09 ± 0.70) ^{ab}	43.33 (41.17 ± 1.19) ^{ab}	56.67 (48.83 ± 1.41) ^a	70.00 (56.79 ± 1.64) ^a	70.00 (56.79 ± 1.94) ^a	70.00 (56.79 ± 1.94) ^a
2	EPB3	20.00 (26.57 ± 0.83) ^a	46.67 (43.09 ± 1.24) ^a	60.00 (50.77 ± 1.47) ^a	72.00 (58.91 ± 1.70) ^a	72.00 (58.05 ± 1.68) ^a	72.00 (58.05 ± 1.68) ^a
3	EPB4	13.33 (21.42 ± 0.62) ^{bc}	40.00 (39.23 ± 1.13) ^{ab}	53.33 (46.91 ± 1.35) ^{ab}	66.00 (54.74 ± 1.58) ^a	66.00 (54.33 ± 1.57) ^a	66.00 (54.33 ± 1.57) ^a
4	EPB8	16.67 (24.09 ± 0.70) ^{ab}	43.33 (41.17 ± 1.19) ^{ab}	60.00 (50.77 ± 1.47) ^a	66.67 (54.74 ± 1.70) ^a	68.00 (55.55 ± 1.60) ^a	68.00 (55.55 ± 1.60) ^a
5	EPB9	16.67 (24.09 ± 0.57) ^{ab}	46.67 (43.09 ± 1.24) ^a	56.67 (48.83 ± 1.41) ^a	66.67 (54.74 ± 1.85) ^a	65.00 (53.33 ± 1.55) ^a	65.00 (53.33 ± 1.55) ^a
6	<i>Bt</i>	10.00 (18.43 ± 0.43) ^c	36.67 (37.27 ± 1.08) ^b	46.67 (43.09 ± 1.24) ^b	53.33 (46.91 ± 1.35) ^b	52.00 (46.15 ± 1.33) ^b	52.00 (46.15 ± 1.33) ^b
7	Control	0.00 (0 ± 0.00) ^d	0.00 (0 ± 0.00) ^c	0.00 (0 ± 0.00) ^c	0.00 (0 ± 0.00) ^c	0.00 (0 ± 0.00) ^c	6.67 (14.96 ± 0.83) ^c

Experiment was done in triplicates and the values are mentioned as means of triplicate studies. Values in the brackets are Arc-sine transformed ± SeM. Superscripted alphabet letters show statistical groups for that column. *Bt* – *Bacillus thuringiensis*.

Table 3: Median Lethal dose (LD₅₀) and LD₉₀ of Entomopathogenic bacteria against *Earias vitelli*

S. No	Symbiotic Bacterial isolates	Intact cells			Cell supernatant		
		LD ₅₀	LD ₉₀	χ ²	LD ₅₀	LD ₉₀	χ ²
1.	EPB1	6.25X10 ⁵	7.31X10 ⁹	1.106	5.25X10 ⁶	2.90X10 ¹⁰	9.026
2.	EPB3	4.28X10 ⁴	1.81X10 ⁹	1.284	3.41X10 ⁵	4.99X10 ¹⁰	3.798
3.	EPB4	4.31X10 ⁵	1.49X10 ¹⁰	1.234	5.43X10 ⁶	2.29X10 ¹²	5.184
4.	EPB8	9.24X10 ⁴	1.14X10 ¹⁰	2.749	7.12X10 ⁵	3.52X10 ¹¹	3.169
5.	EPB9	7.28X10 ⁴	5.60X10 ⁸	1.501	2.58X10 ⁶	1.71X10 ¹¹	5.435
6.	<i>Bt</i>	4.65X10 ⁵	5.45X10 ¹¹	3.46	8.42X10 ⁶	9.36X10 ¹²	4.83

Notes: The LC₅₀ and LC₉₀ values were calculated by probit analysis using IBM SPSS v 20.0. *Bt* – *Bacillus thuringiensis*.

Table 4: Median Lethal time (LT₅₀) of Entomopathogenic bacteria against *Earias vitelli*

S. No	Symbiotic Bacterial isolates	Intact cells		Cell supernatant	
		LT ₅₀	χ ²	LT ₅₀	χ ²
1.	EPB1	30	17.89	40	5.18
2.	EPB3	24	17.45	36	4.65
3.	EPB4	33	17.95	45	8.95
4.	EPB8	36	20.71	42	7.35
5.	EPB9	32	17.93	43	8.57
6.	<i>Bt</i>	42	20.46	55	9.34

Notes: The LT₅₀ values were calculated from the Kaplan-Meier survival curves. The survival curves were compared using the log-rank test χ² (chi-square) value at *p* < 0.05. *Bt* – *Bacillus thuringiensis*.

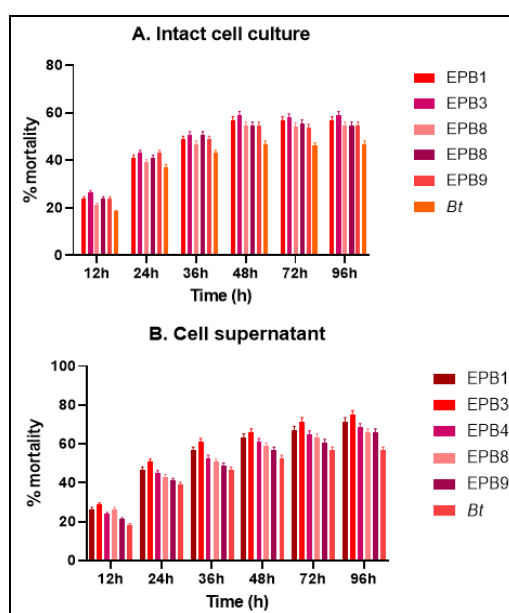


Fig 1: Mortality rates of *Earias vittella* treated with A) Intact cell culture and B) Cell supernatant of Entomopathogenic bacteria isolates with mortality recorded at 12h interval. Bars (mean ± SE) in the same time interval and dose with the same letter are not significantly different (*P* < 0.05) using DMRT test. *Bt* - *Bacillus thuringiensis*.

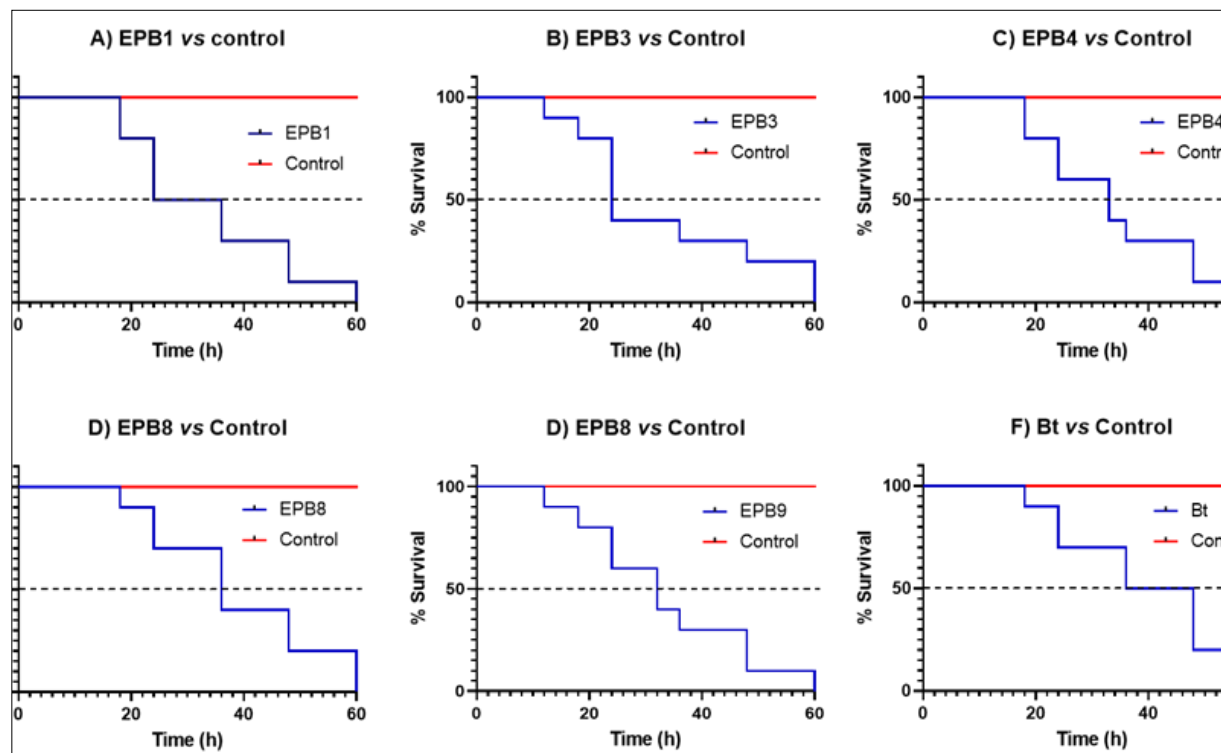


Fig 2: Kaplan-Meier survival curves of Entomopathogenic bacteria (A) EPB1 (B) EPB3 (C) EPB4 (D) EPB8 (E) EPB9 and (F) *Bacillus thuringiensis* against *Earias vittella*. Red line shows un-inoculated control, blue line shows the EPB treated. The survival curves were compared using log-rank test; all EPBs were significantly different from control ($P < 0.0001$). Three biological repeats with 10 larvae per replicate were used for survival analysis. For all graphs, X-axis – time in hours, Y-axis – % survival of the insect. *Bt* - *Bacillus thuringiensis*.

4. Conclusion

In conclusion, entomopathogenic bacteria *Photorhabdus* and *Xenorhabdus* used in the study had exhibited significant insecticidal activity against shoot and fruit borer, *Earias vittella*. Among the bacteria, EPB3 (*Xenorhabdus nematophila*) had exhibited 90% mortality at 72h with a median lethal dose (LD_{50}) of 10^4 and 10^5 CFU/ml of with LT_{50} value of 24h and 36h for both cell culture and cell supernatant, respectively. Entomopathogenic bacteria is one of best alternate to *Bt* toxins as they have wide range of insecticidal toxins making them more flexible to use in field conditions for biocontrol of wide range of insect pests.

5. Acknowledgements

Authors are thankful to The Director, NBAIR, Hebbala, Bangalore- 560 065 for providing larvae for bioassay studies.

6. References

1. Usta C. Microorganisms in biological pest control-a review (bacterial toxin application and effect of environmental factors). Current progress in biological research, 2013, 287-317.
2. Jagdale G, Kamoun S, Grewal P. Entomopathogenic nematodes induce components of systemic resistance in plants: biochemical and molecular evidence. Biological Control. 2009; 51(1):102-9.
3. Dowling A, Waterfield NR. Insecticidal toxins from *Photorhabdus* bacteria and their potential use in agriculture. Toxicon. 2007; 49(4):436-51.
4. Abdel-Razek A. Pathogenic effects of *Xenorhabdus nematophilus* and *Photorhabdus luminescens* (Enterobacteriaceae) against pupae of the Diamondback Moth, *Plutella xylostella* (L.). Anzeiger für Schädlingskunde. 2003; 76(4):108-11.
5. Mohan S, Raman R, Gaur H. Foliar application of *Photorhabdus luminescens*, symbiotic bacteria from entomopathogenic nematode *Heterorhabditis indica*, to kill cabbage butterfly *Pieris brassicae*. Current Science. 2003; 84(11):1397.
6. Shahina F, Tabassum K, Salma J, Mahreen G. Biopesticidal affect of *Photorhabdus luminescens* against *Galleria mellonella* larvae and subterranean termite (Termitidae: Macrotermis). Pakistan Journal of Nematology. 2011; 29(1):35-43.
7. Bussaman P, Sermswan R, Grewal P. Toxicity of the entomopathogenic bacteria *Photorhabdus* and *Xenorhabdus* to the mushroom mite (*Luciaphorus* sp.; Acari: Pygmephoridae). Biocontrol Science and Technology. 2006; 16(3):245-56.
8. Bussaman P, Sobanboa S, Grewal PS, Chandrapatya A. Pathogenicity of additional strains of *Photorhabdus* and *Xenorhabdus* (Enterobacteriaceae) to the mushroom mite *Luciaphorus perniciosus* (Acari: Pygmephoridae). Applied Entomology and Zoology. 2009; 44(2):293-9.
9. Shapiro-Ilan DI, Reilly CC, Hotchkiss MW. Suppressive effects of metabolites from *Photorhabdus* and *Xenorhabdus* spp. on phytopathogens of peach and pecan. Archives of Phytopathology and Plant Protection. 2009; 42(8):715-28.
10. Webster J, Chen G, Hu K, Li J. Bacterial metabolites In: Entomopathogenic Nematology (Gangler, R. (Ed.)), CABI Publishing, New York, 2002, 99-114.
11. Rodou A, Ankrah DO, Stathopoulos C. Toxins and secretion systems of *Photorhabdus luminescens*. Toxins. 2010; 2(6):1250-64.
12. Waterfield NR, Bowen DJ, Fetherston JD, Perry RD. The *tc* genes of *Photorhabdus*: a growing family. Trends in microbiology. 2001; 9(4):185-91.
13. Bussaman P, Sa-Uth C, Rattanasena P, Chandrapatya A. Acaricidal activities of whole cell suspension, cell-free

- supernatant, and crude cell extract of *Xenorhabdus stokiae* against mushroom mite (*Luciaphorus* sp.). Journal of Zhejiang University Science B. 2012; 13(4):261-6.
14. Mahar A, Munir M, Mahar A. Studies of different application methods of *Xenorhabdus* and *Photorhabdus* cells and their toxin in broth solution to control locust (*Schistocerca gregaria*). Asian Journal of Plant Sciences, 2004.
 15. Gomez KA, Gomez AA. Statistical procedures for agricultural research: John Wiley & Sons, 1984.
 16. Onofri A, Carbonell E, Piepho H, Mortimer A, Cousens R. Current statistical issues in Weed Research. Weed Research. 2010; 50(1):5-24.
 17. Rich JT, Neely JG, Paniello RC, Voelker CC, Nussenbaum B, Wang EW. A practical guide to understanding Kaplan-Meier curves. Otolaryngology-Head and Neck Surgery. 2010; 143(3):331-6.
 18. Kodandaram M, Rai A, Halder J. Novel insecticides for management of insect pests in vegetable crops: A review. Vegetable Science. 2010; 37(2):109-23.
 19. Gao Y, Reitz SR, Wang J, Tamez-Guerra P, Wang E, Xu X *et al.* Potential use of the fungus *Beauveria bassiana* against the western flower thrips *Frankliniella occidentalis* without reducing the effectiveness of its natural predator *Orius sauteri* (Hemiptera: Anthocoridae). Biocontrol Science and Technology. 2012; 22(7):803-12.
 20. Ali K, Wakil W, Zia K, Sahi ST. Control of *Earias vittella* (Lepidoptera: Noctuidae) by *Beauveria bassiana* along with *Bacillus thuringiensis*. International Journal of Agriculture and Biology, 2015, 17(4).
 21. Pazhanisamy M, Archunan K. Efficacy of botanical extracts and panchagavya against bhendi shoot and fruit borer, *Earias vittella* (Fab.). Journal of Biological Control. 2019; 33(4):372-7.
 22. Aziz MA, Hasan MU, Ali A, Iqbal J. Comparative efficacy of different strategies for management of spotted bollworms, *Earias* spp. on Okra, *Abelmoschus esculentus* (L). Moench. Pakistan Journal of Zoology. 2012; 1:44(5).
 23. Seo S, Lee S, Hong Y, Kim Y. Phospholipase A2 inhibitors synthesized by two entomopathogenic bacteria, *Xenorhabdus nematophila* and *Photorhabdus temperata* subsp. *temperata*. Applied and environmental microbiology. 2012; 78(11):3816-23.
 24. Crawford JM, Kontnik R, Clardy J. Regulating alternative lifestyles in entomopathogenic bacteria. Current Biology. 2010; 20(1):69-74.
 25. Ffrench-Constant R, Waterfield N. An ABC guide to the bacterial toxin, complexes. Advances in applied microbiology. 2006; 58:169-83.
 26. Daborn P, Waterfield N, Silva C, Au C, Sharma S. A single *Photorhabdus* gene, makes caterpillars floppy (mcf), allows *Escherichia coli* to persist within and kill insects. Proceedings of the National Academy of Sciences. 2002; 99(16):10742-7.
 27. Duchaud E, Rusniok C, Frangeul L, Buchrieser C, Givaudan A, Taourit S *et al.* The genome sequence of the entomopathogenic bacterium *Photorhabdus luminescens*. Nature biotechnology. 2003; 21(11):1307-13.
 28. Kamita SG, Hammock BD. Juvenile hormone esterase: biochemistry and structure. Journal of Pesticide Science. 2010; 35(3):265-74.
 29. Salvadori JDM, Defferrari MS, Ligabue-Braun R, Lau EY, Salvadori JR, Carlini CR. Characterization of entomopathogenic nematodes and symbiotic bacteria active against *Spodoptera frugiperda* (Lepidoptera: Noctuidae) and contribution of bacterial urease to the insecticidal effect. Biological control. 2012; 63(3):253-63.