Gut bacterial influence on susceptibility of lepidopteran pests to *Bacillus thuringiensis* subsp. *kurstaki*

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**Abstract**— Influence of gut bacteria on susceptibility of lepidopteran pests viz., *Spodoptera litura*, *Helicoverpa armigera*, *Plutella xylostella* and *Crocidolomia binotalis* to *Bacillus thuringiensis* subsp. *kurstaki* was studied by antibiotic mediated elimination of gut bacteria. Bioassay was performed against laboratory reared and field collected larvae of test insects to study the influence of gut bacteria on susceptibility of lepidopteran pests to *B. thuringiensis* subsp. *kurstaki*. Results indicated that both lab and field population of test insects were more susceptible in the presence of gut bacteria by recording lower LC₅₀ values against *B. thuringiensis* subsp. *kurstaki*. Field population of test insects viz., *S. litura*, *H. armigera*, *P. xylostella* and *C. binotalis* recorded LC₅₀ values of 0.62, 0.48, 0.77 and 0.44 g/l, whereas laboratory population recorded LC₅₀ values of 0.37, 0.32, 0.48 and 0.29 g/l respectively. In the absence of gut bacteria both laboratory reared and field collected larvae found less susceptible by recording higher LC₅₀ values. Antibiotic treated field collected larval population of test insects viz., *S. litura*, *H. armigera*, *P. xylostella* and *C. binotalis* recorded LC₅₀ values of 0.74, 0.54, 1.07 and 0.56 g/l respectively. Similarly antibiotic treated laboratory reared larval population of test insects recorded LC₅₀ values of 0.49, 0.38, 0.57 and 0.43 g/l respectively.

**Keywords**—Gut bacteria, *Bacillus thuringiensis* subsp. *Kurstaki*, Antibiotics and LC₅₀.

I. INTRODUCTION

Many insects harbor a robust complement of prokaryotes in their alimentary canals, which facilitate nutrient availability, utilization and detoxification of environmental toxins (Williams and Roans. 2006). Intestinal microorganisms play important role in the degradation of diet components of insects (Hayashi et al., 2007). The diversity of insecta is reflected in the large and varied microbial communities on the nutritional contributions of insects living on suboptimal diets. The indigenous gut bacteria, however also play a role in withstanding and colonization of the gut by non indigenous species including pathogens (Dillon and Dillon, 2004). The bacterial association with insects plays a significant role in the host insect morphogenesis, food digestion, nutrition, antifungal toxin production, pheromone production, regulation of pH, synthesis of vitamins, temperature tolerance, resistance against parasitoid development, and detoxification of noxious compounds (Genta et al. 2006).

*Bacillus thuringiensis* (Berliner) is the most widely used biological insecticide to manage insects that affect forestry and agriculture and transmit human and animal pathogens. This ubiquitous spore-forming bacterium kills insect larvae largely through the action of insecticidal crystal proteins. For decades, the mechanism of insect killing has been assumed to be toxin-mediated lysis of the gut epithelial cells leading to starvation or septicemia. But in recent years symbiotic relationships between insects and guts microflora have been studied extensively.

Studies on role of larval gut bacteria on susceptibility of *B. thuringiensis* and its interactions indicates that elimination of the gut microbial community by oral administration of antibiotics reduced *B. thuringiensis* insecticidal activity. Plus microbes residing in the insect gut play an important role in biological activity of *Bt* toxins against different insect pests (Broderick et al., 2006, Paramasiva et al., 2014). With this background present study was undertaken to know the influence of gut bacteria on *B. thuringiensis* induced mortality against selected lepidopteran pests.
II. MATERIAL AND METHODS

To study the influence of gut on susceptibility of lepidopteran pests to B. thuringiensis subsp. kurstaki gut bacteria were eliminated by antibiotic treatment and gut bacterial elimination was monitored by using PCR technique.

Antibiotic treatment

Antibiotics concentration is determined prior to the bioassay in order to study the influence of gut bacteria on the susceptibility of lepidopteran pests to the Bt insecticide. For the antibiotics treatment, a cocktail of streptomycin and rifampicin of different concentrations were used to eliminate the gut bacteria on the susceptibility of lepidopteran pests to the Bt insecticide. For the antibiotics treatment, each antibiotic solution was uniformly smeared on the leaves, dried under shade and fed to the test insect larvae from first to third instar. Standardized antibiotics dosages were 300 µg/ml each for S. litura, 400 µg/ml for H. armigera, 1mg/ml for P. xylostella and 300 µg/ml for C. binotalis. Subsequently these antibiotics dosages were used to eliminate the gut bacterial population prior to the bioassay.

Monitoring of reduced gut bacteria

The larvae (1st to 3rd instar) fed on antibiotic treated leaves were subjected to DNA isolation using the method described by Broderick et al. (2003). Each DNA extract was then used as a template for PCR amplification of 16S rRNA genes using universal primers. Larvae were considered to be deficient in gut bacteria if the amplicon could not be found during 16S rRNA gene amplification (Johnston and Crickmore, 2009). PCR results shown that there was no amplification of 16S rRNA gene from the DNA isolated from the antibiotic treated test insects (Fig. 1&2). After standardization of antibiotics treated insects and control insects (without antibiotic treatment) were subjected to bioassay to calculate LC50 against commercial formulation of Bt insecticide.

Bioassay

Bioassay with commercial B. thuringiensis subsp. kurstaki formulation (Delfin WG) was performed on laboratory and field collected larval population of test insects viz., S. litura, H. armigera, P. xylostella and C. binotalis. And also S. litura, H. armigera larvae collected from different host plants. To study the influence of gut microflora on B. thuringiensis induced mortality, antibiotics treated and control insects larvae of test insects were used in the bioassay. During the study leaf dip method of bioassay was followed with six different concentrations (selected based on the prior range finding test) of test insecticide. For each concentration three replications were used with ten larvae per replication and mortality was recorded 48 hrs after treatment and data was subjected to probit analysis.

III. RESULTS AND DISCUSSION

Results indicated that irrespective of test insects the antibiotic treated larvae were less susceptible to Bt insecticide than untreated larva both in lab as well as field collected larval population. LC50 values for B. thuringiensis subsp. kurstaki against field population of S. litura in the antibiotic treated and untreated larval population were 0.62 and 0.74 g/l respectively. Similar trend towards decreased susceptibility of antibiotic treated larvae was evident in the lab population (Table 1).

Similarly toxicity studies conducted against field and lab population of H. armigera revealed that larval population was more susceptible to B. thuringiensis subsp. kurstaki insecticide in the presence of gut bacteria. The observed LC50 values were 0.48 and 0.32 g/l against field and lab population respectively. In the absence of gut bacterial community both field and lab population of test insect recorded higher LC50 values of 0.54 g/l against field population and 0.38 g/l against lab population (Table 1).

The trend of influence of gut microflora on B. thuringiensis subsp. kurstaki induced mortality against antibiotics treated and control insects larvae of remaining two test insects viz., P. xylostella and C. binotalis remain similar (Table 1).

Influence of gut bacteria on susceptibility of S. litura and H. armigera collected from different host plants to B. thuringiensis subsp. kurstaki revealed that S. litura collected from soybean and groundnut recorded lower LC50 value of 0.58 and 0.59 respectively in the presence of gut bacteria, whereas in the absence of gut bacteria the recorded LC50 values were 0.80 and 0.69 respectively for soybean and groundnut. Similarly, H. armigera collected from chickpea recorded comparatively lower LC50 of 0.39 g/l in the presence of gut bacterial community whereas in the absence of gut bacteria it was 0.50 g/l. While H. armigera collected from cotton also showed similar response with LC50 of 0.42 g/l in the normal larval population and 0.51 g/l, the larval population in which the gut bacteria were eliminated by antibiotic treatment. Decreased trend towards susceptibility in the influence of gut microbiota was evident in the LC50 values also (Table 2).

Results present study revealed that decreased susceptibility of test insects to the B. thuringiensis subsp. kurstaki in the absence of gut microflora indicated this phenomena was common to all the test insects used in the study irrespective of laboratory and field collected larval population. Recent studies have shown that bacterial community present in the
gut of insect are known to produce various enzymes and these enzymes will be exploited by the host insects for effective digestion of food materials and some of the enzymes also helps for activation and degradation of insecticidal compounds by this way these gut microbes influences the susceptibility either it will be decrease or increase to particular compounds. Apart from this these gut bacteria are also pathogenic when they proliferates to the host hemolymph due to the lyses of midgut epithelial cells by the cry toxins. Present findings are in line with Broderick et al., (2006) reported that B. thuringiensis does not kill larvae of the gypsy moth in the absence of indigenous midgut bacteria. Elimination of the gut microbial community by oral administration of antibiotics abolished B. thuringiensis insecticidal activity. Broderick et al., 2009 studied the influence of gut bacteria on B. thuringiensis-induced mortality against Manduca sexta, Pieris rapae, Vanessa cardui, and Lymnantria dispar and reported that gut bacterial population is required for B. thuringiensis- induced mortality of lepidopteran larvae. Similarly Paramasiva et al., 2014 also suggested that gut microflora influences the toxicity of Bt towards H. armigera.

IV. CONCLUSION

Results of the present findings provides new insight in to the efficacy of B. thuringiensis subsp. kurstaki against lepidopteran pests and it can be deduced that resident gut bacterial community is playing certain role in the biological activity of the B. thuringiensis subsp. kurstaki so this information can be used for the better management of crop pests through Bt insecticide by exploiting the gut bacterial community of host insects

Authors’ contributions

Hanamant Gadad conducted an experiment and involved in taking observations, tabulation and writing article, A S Vastrad and Krishnaraj P. U involved in planning, constant monitoring of experiment, tabulation, data analysis and interpretation.

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Fig. 1: PCR Confirmation of reduced gut bacteria of *Spodoptera litura* and *Helicoverpa armigera* larvae

N1-N4 - Larvae reared normally without antibiotics
A1-A4 - Larvae reared with antibiotics incorporation in to the diet
M - Marker

Fig. 2: PCR Confirmation of reduced gut bacteria of *Plutella xylostella* and *Crocidolomia binotalis* larvae

N1-N4 - Larvae reared normally without antibiotics
A1-A4 - Larvae reared with antibiotics incorporation in to the diet
M - Marker
### Table 1: Influence of gut bacteria on susceptibility of lepidopteran pests to the Bacillus thuringiensis subsp. kurstaki

| Field population (F1) | Laboratory population (F5) | Test insects | Treatments | LC50 (g/l) | Fiducial limit | LC95 (g/l) | Regression equation | Chi Square | LC50 (g/l) | Fiducial limit | LC95 (g/l) | Regression equation | Chi Square |
|----------------------|-----------------------------|--------------|-------------|------------|----------------|------------|---------------------|------------|------------|----------------|------------|---------------------|------------|
| Spodoptera litura    |                             |              | Without antibiotics | 0.62  | 0.55-0.69 | 1.53 | Y=0.86+0.16x | 2.49 | 0.37 | 0.16-0.88 | 3.34 | Y=0.74+0.17x | 4.27 |
|                      |                             |              | With antibiotics   | 0.74  | 0.65-0.87 | 2.18 | Y=0.44+0.15x | 3.4  | 0.49 | 0.38-0.68 | 4.19 | Y=0.54+0.15x | 3.33 |
| Helicoverpa armigera  |                             |              | Without antibiotics | 0.48  | 0.42-0.54 | 1.1  | Y=1.32+0.23x | 1.98 | 0.32 | 0.26-0.39 | 1.66 | Y=1.13+0.23x | 0.46 |
|                      |                             |              | With antibiotics   | 0.54  | 0.47-0.62 | 1.69 | Y=0.88+0.21x | 0.17 | 0.38 | 0.31-0.47 | 1.76 | Y=1.03+0.23x | 1.94 |
| Plutella xylostella   |                             |              | Without antibiotics | 0.77  | 0.66-0.92 | 2.68 | Y=0.33+0.12x | 0.22 | 0.48 | 0.42-0.54 | 1.28 | Y=1.22+0.22x | 1.67 |
|                      |                             |              | With antibiotics   | 1.07  | 0.84-1.64 | 6.68 | Y=0.61+0.12x | 1.58 | 0.57 | 0.51-0.65 | 1.52 | Y=0.93+0.22x | 3.00 |
| Crocidolomia binotalis|                             |              | Without antibiotics | 0.44  | 0.37-0.49 | 1.21 | Y=1.31+0.23x | 0.84 | 0.29 | 0.23-0.36 | 0.79 | Y=1.18+0.22x | 1.15 |
|                      |                             |              | With antibiotics   | 0.56  | 0.49-0.59 | 1.59 | Y=0.96+0.28x | 0.40 | 0.43 | 0.36-0.58 | 1.17 | Y=0.83+0.23x | 1.08 |

### Table 2: Influence of gut bacteria on susceptibility of Spodoptera litura and Helicoverpa armigera collected from different host plants to Bacillus thuringiensis subsp. kurstaki

| Test insects          | Hosts   | Treatments | LC50 (g/l) | Fiducial limit | LC95 (g/l) | Regression equation | Chi Square |
|-----------------------|---------|------------|------------|----------------|------------|---------------------|------------|
| Spodoptera litura     | Soybean | Without antibiotics | 0.58  | 0.55-0.77 | 1.83 | Y=0.63+0.15x | 1.27 |
|                       |         | With antibiotics   | 0.80  | 0.71-0.92 | 1.97 | Y=0.40+0.15x | 1.36 |
| Groundnut             | Without antibiotics | 0.59  | 0.51-0.66 | 1.57 | Y=0.87+0.16x | 0.45 |
|                       | With antibiotics   | 0.69  | 0.62-0.77 | 1.65 | Y=0.69+0.16x | 1.96 |
| Helicoverpa armigera  | Chickpea | Without antibiotics | 0.39  | 0.45-0.55 | 1.11 | Y=1.42+0.341x | 2.27 |
|                       | With antibiotics   | 0.50  | 0.31-0.45 | 1.18 | Y=1.39+0.23x | 0.98 |
| Cotton                | Without antibiotics | 0.42  | 0.37-0.47 | 0.99 | Y=1.64+0.24x | 1.52 |
|                       | With antibiotics   | 0.51  | 0.44-0.57 | 1.43 | Y=1.07+0.12x | 0.86 |