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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, Helicovepa 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 LC_{50} values against B. thuringiensis subsp. kurstaki. Field population of test insects viz., S. litura, H. armigera, P. xylostella and C. binotalis recorded LC_{50} values of 0.62, 0.48, 0.77 and 0.44 g/l, whereas laboratory population recorded LC_{50} 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 LC_{50} values. Antibiotic treated field collected larval population of test insects viz., S. litura, H. armigera, P. xylostella and C. binotalis recorded LC_{50} 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 LC_{50} values of 0.49, 0.38, 0.57 and 0.43 g/l respectively.*

Keywords—Gut bacteria, Bacillus thuringiensis subsp. Kurstaki, Antibiotics and LC_{50}.

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.

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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 bacteria 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 prepared in distilled water and used. The 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 LC_{50} against commercial formulation of Bt insecticide.

Bioassay

Bioassay with commercial *B. thuringiensis* subsp. *kurstaki* formulation (Delfin WG) was performed against 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 larvae both in lab as well as field collected larval population. LC_{50} 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 LC_{50} 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 LC_{50} 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 LC_{50} value of 0.58 and 0.59 respectively in the presence of gut bacteria, whereas in the absence of gut bacteria the recorded LC_{50} values were 0.80 and 0.69 respectively for soybean and groundnut. Similarly, *H. armigera* collected from chickpea recorded comparatively lower LC_{50} 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 LC_{50} 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 LC_{95} 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

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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 *Lymantria 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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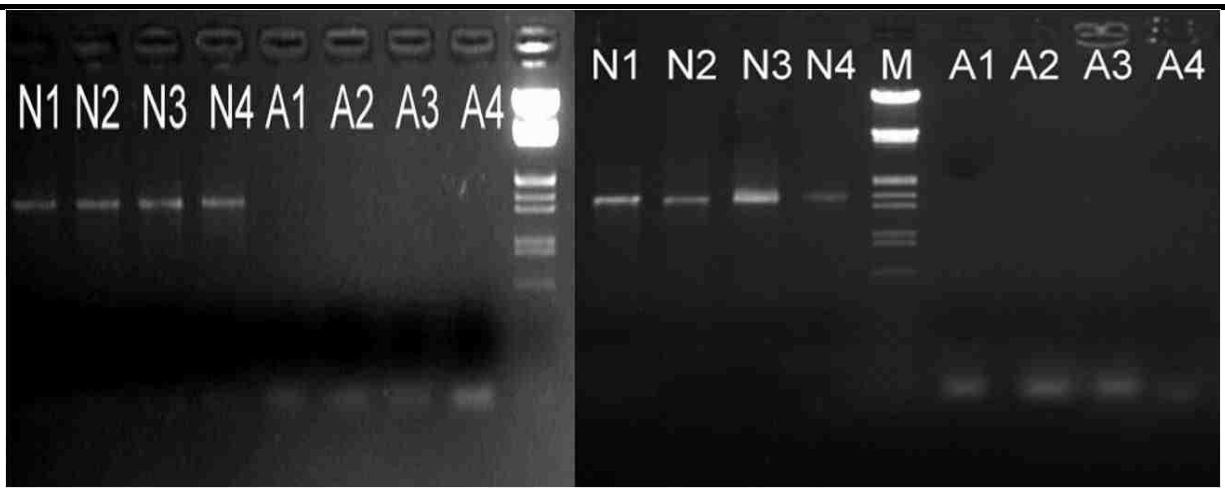

Fig.1: PCR Confirmation of reduced gut bacteria of Spodoptera litura and Helicoverpa armigera larvae

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

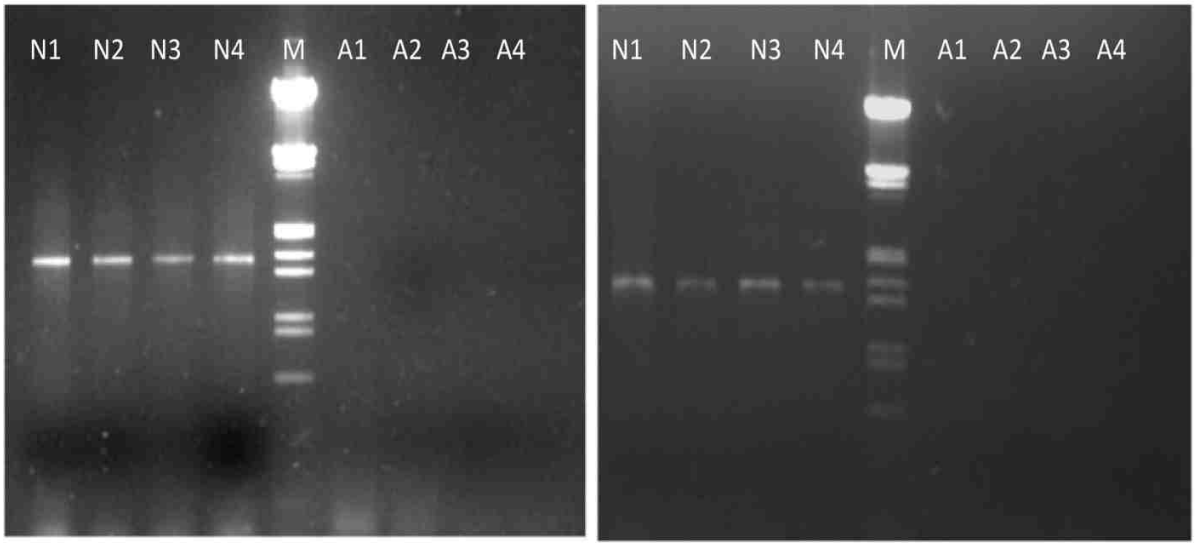

Fig.2: PCR Confirmation of reduced gut bacteria of Plutella xylostella and Crocidolomia binotalis larvae

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

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Table 1: Influence of gut bacteria on susceptibility of lepidopteran pests to the Bacillus thuringiensis subsp. kurstaki

	Field population (F1)						**Lab population (F_5)**				
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**	**LC_{50} (g/l)**	**Fiducial limit**	**LC_{95} (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