J. Exp. Zool. India Vol. 19, No. 2, pp. 1099-1102, 2016 | www.connectjournals.com/jez | *ISSN 0972-0030*

GUT BACTERIA MEDIATED INSECTICIDE RESISTANCE IN *SPODOPTERA LITURA* (FAB.)

Hanamant Gadad and A. S. Vastrad

Department of Agricultural Entomology, University of Agricultural Sciences, Dharwad - 580 005, India.
e-mail: hsgadad@gmail.com

(Accepted 27 May 2016)

ABSTRACT : Gut bacteria mediated insecticide resistance was studied by antibiotic administration to test insect larvae to eliminate the gut bacteria. Bioassay was performed against lab reared and field collected larvae, results indicates that both lab and field population of test insects were more resistant in the presence of gut bacteria by recording higher LC_{50} values against test insecticides. Field population recorded LC_{50} values of 13.85, 18.28 and 68.54 ppm whereas lab population recorded LC_{50} values of 9.17, 4.32 and 27.88 ppm against flubendiamide, indoxocarb and chlorpyriphos, respectively. In the absence of gut bacteria both lab and field collected larvae were comparatively susceptible which recorded lower LC_{50} values. Field larval population recorded 10.85, 17.00 and 53.39 ppm of LC_{50} for fluebendiamide, indoxocarb and chlorpyriphos respectively in the absence of gut bacteria. Similarly against lab reared antibiotic treated larval population test insecticides flubendiamide, indoxocarb and chlorpyriphos recorded LC_{50} values of 3.55, 7.96 and 20.22 ppm, respectively.

Key words : Gut bacteria, *Spodoptera litura*, LC_{50}, antibiotics.

INTRODUCTION

Insect gut presents distinctive environments for microbial colonization and bacteria in the gut potentially provide many beneficial services to their hosts. Insects display a wide range in degree of dependence on gut bacteria for basic functions. Natural gut microflora of insects reflects the state of health of their host. Studies suggest that microorganisms provide essential nutrients or assist in important biochemical functions (Broderick *et al*, 2004). Loss of microorganisms often results in abnormal development and reduces survival of the insect host (Fukatsu and Hosokawa, 2002). Most insect guts contain relatively few microbial species compared to mammalian gut, but some insects harbour large gut communities of specialized bacteria. Others are colonized only opportunistically and sparsely by bacteria common in other environments. Insect digestive tracts vary extensively in morphology and physicochemical properties, factors that greatly influence microbial community structure (Engel and Moran, 2013).

Symbiotic microorganisms of insects have long been known to have played significant roles in host mating preference, resistance to parasitism, plant specialization, longevity and protection against pathogens, but insecticide resistance is a trait for which recent evidence has indicated the role of symbionts. The bean bug and related stinkbug species acquire bacteria of the genus *Burkholderia* from the soil which then replace the normal *Burkholderia* midgut symbiont, conferring resistance to the insecticide fenitrothion (Kikuchi *et al*, 2012). With this background present study was carried out to examine the influence on the susceptibility of *Spodoptera litura* (Fab) against selected synthetic insecticides.

MATERIALS AND METHODS

Insect collection and mass rearing

Spodoptera litura was mass cultured in the Department of Biotechnology in U.A.S. Dharwad laboratory. Field collected larvae were reared on castor leaves. The pupae obtained from these were kept in petriplate and placed in a cage of 25 cm^3 for adult emergence. When moths started emerging, fresh castor leaves were provided for oviposition. And kept in a plastic basin of 45 x 30 x 15 cm for larval emergence and third instar larvae were used for bioassay.

Antibiotic treatment

To study the influence of gut microflora on insecticide induced mortality, antibiotics treated and control larvae of test insects were used in the bioassay. Antibiotics concentration was determined prior to the bioassay in order to study the influence of gut bacteria on insecticide susceptibility. 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 dosage was 300 µg/ml each of streptomycin and rifampicin.

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* (2004). 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 showed no amplification of 16S rRNA gene from the DNA isolated from the antibiotic treated test insect (Plate 1). After standardization of antibiotics, treated insects and control insects (without antibiotic treatment) were subjected to bioassay to calculate LC_{50} against test insecticides.

Bioassay

Bioassay with three insecticides viz., Indoxocarb 15.8 EC, Flubendiamide 480 SC and chlorpyriphos 20 EC was performed against laboratory (Five generation old) and field collected larval population of *S. litura.* Apart from this of *S. litura* collected from soybean and groundnuts were used in the bioassay to know the host induced variability on the gut bacteria mediated susceptibility to insecticides. During the study leaf dip method of bioassay was followed with six different concentrations (selected based on the prior range finding test) of test insecticides. For each concentration three replications were used with ten larvae per replication and mortality was recorded 24 hrs after treatment and data was subjected to probit analysis.

RESULTS AND DISCUSSION

Results indicated that antibiotic treated test insect larvae were more susceptible to all the test insecticides than untreated larvae both in lab as well as field population collected from different hosts.

Influence of gut bacteria on insecticide susceptibility against field and lab population of *Spodoptera litura*

Bioassay carried out to know the influence of gut bacteria on insecticide susceptibility of field and lab population of *S. litura* revealed that both field and lab populations were resistant which recorded higher LC_{50} values against all the test insecticides in the presence of resident gut bacteria compare to the population where gut bacteria were eliminated by antibiotics administration. Among the three insecticides used for bioassay against field population of *S. litura*, fluebendiamide recorded LC_{50} of 13.85 and 10.85 ppm in the presence and absence of gut bacteria, respectively. Indoxcarb recorded LC_{50} of 18.28 ppm against the larval population where gut bacteria are retained without any antibiotics treatment, whereas

Table 1 : Influence of gut bacteria on insecticide susceptibility against field and lab population of *Spodoptera litura.*

Insecticides	Treatments	Field population					Lab population (F_5)				
		LC_{50} (ppm)	Fiducial limit	LC_{95} (ppm)	Regression equation	Chi Square	LC_{50} (ppm)	Fiducial limit	LC_{95} (ppm)	Regression equation	Chi Square
Indoxocarb	Without antibiotics	18.28	16.85-19.67	33.49	Y=7.89+1.61x	0.39	9.17	7.87-10.31	24.62	Y=3.69+0.73x	0.87
	With antibiotics	17.00	15.43-18.21	30.36	Y=5.64+1.16x	1.06	7.96	6.48-9.06	22.18	Y=3.33+0.72x	0.38
Flubendiamide	Without antibiotics	13.85	12.53-15.07	28.18	Y=6.08+1.17x	0.85	4.32	3.17-5.38	26.66	Y=1.32+0.31 x	0.85
	With antibiotics	10.85	10.94-13.58	25.28	Y=5.86+1.17x	0.83	3.55	2.41-4.51	22.6	Y=1.12+0.35x	1.00
Chlorpyriphos	Without antibiotics	68.54	55.19-87.37	410.25	Y=3.88+0.73x	3.93	27.88	19.6-40.86	501.88	Y=1.89+0.35x	4.09
	With antibiotics	53.39	41.13-66.65	343.35	Y=3.51+0.70x	3.90	20.22	11.83-31.88	363.98	Y=1.30+0.33x	3.77

Table 2 : Influence of gut bacteria on insecticide susceptibility against *Spodoptera litura* collected from soybean and groundnut crop.

Insecticides	Treatments	Larvae collected from soybean					Larvae collected from groundnut				
		LC_{50} (ppm)	Fiducial limit	LC_{95} (ppm)	Regression equation	Chi Square	LC_{50} (ppm)	Fiducial limit	LC_{95} (ppm)	Regression equation	Chi Square
Indoxocarb	Without antibiotics	17.30	15.65-18.62	32.46	Y=7.45+1.60x	0.51	15.91	14.27-17.05	27.11	Y=8.54+1.61x	0.51
	With antibiotics	16.48	14.63-17.76	30.82	Y=7.36+1.16x	0.60	14.78	12.95- 15.93	24.26	Y=8.94+1.68x	0.64
Flubendiamide	Without antibiotics	14.43	13.07-15.85	30.87	Y=5.78+1.16x	1.21	11.69	9.66-12.99	26.74	Y=4.89+1.15x	0.50
	With antibiotics	11.84	9.77-13.17	27.7	Y=4.78X1.15x	0.62	10.82	8.90-12.01	21.98	Y=5.52+1.22x	1.54
Chlorpyriphos	Without antibiotics	55.25	42.61-69.43	369.7	Y=3.47+0.70x	3.86	50.36	36.24-64.89	439.03	Y=2.97+0.68x	2.31
	With antibiotics	40.64	12.54-62.83	280.82	Y=3.51+0.68x	3.90	38.22	25.22-49.24	304.68	Y=2.88+0.67x	2.49

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

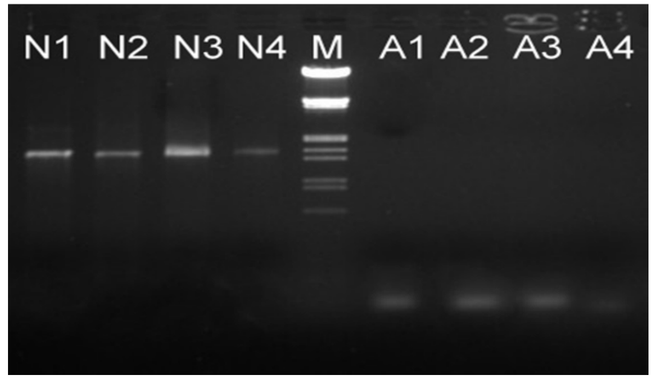

Fig. 1 : PCR Confirmation of reduced gut bacteria of *Spodoptera litura* larvae.

against larval populations, which have been treated with antibiotics recorded comparatively lower LC_{50} value of 17.00 ppm. Chlorpyriphos also showed similar trend with LC_{50} values of 68.54 ppm in the presence of gut bacteria and 53.39 ppm in absence of gut bacterial flora (Table 1).

Influence of gut bacteria on insecticide susceptibility against *Spodoptera litura* collected from soybean and groundnut

Bioassay studies against *S. litura* collected from soybean crop revealed that antibiotics treated larval population were more susceptible against all the test insecticides compare to the control. Among the different insecticides used in the bioassay fluebendiamide recorded LC_{50} of 11.84 ppm and 14.43 ppm in antibiotic treated and control larval population respectively. Similarly indoxocarb recorded the LC_{50} of 16.48 ppm against antibiotic treated larvae which was comparatively lower than the control larval population where LC_{50} was 17.30 ppm. Similar trend of increased susceptibility of antibiotics treated larvae was observed against chlorpyriphos (Table 2).

Larvae collected from groundnut showed similar trend of increased toxicity in antibiotic treated larvae. LC_{50} values of test insecticides *viz.,* fluebendiamide, indoxocarb and chlorpyriphos were 10.82, 14.78 and 38.22 ppm respectively against antibiotic treated larvae, whereas control larvae recorded LC_{50} values of 11.69, 15.91 and 50.36 ppm for fluebendiamide, indoxocarb and chlorpyriphos (Table 3).

Results of the present study revealed the increased susceptibility of *S. litura* in insecticides in the absence of gut microflora. A phenomenon which was common to both lab and field population from different host crops. 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 and some of the enzymes also helps for activation or degradation of insecticidal compounds which influences the susceptibility of larvae to insecticides Thakur *et al* (2005) reported gut bacteria mediated resistance in *Dicladispa armigera* (Olivler) against endosulfan, chlorpyriphos and quinalphos. Kikuchi *et al* (2012) also reported symbiont mediated mechanism of insecticide resistance against fenitrothion in case of the bean bug, which harbors mutualistic gut symbiotic bacteria. Similarly Madhusudan, 2015 observed a gradual decrease in the insecticide resistance in *Helicoverpa armigera* (Hubner) larvae treated with antibiotics to eliminate the gut. These results provide a new insight in to the enhanced efficacy of insecticides to manage crop pests where resistance to insecticides has been reported.

REFERENCES

Broderick N A, Goodman R M and Handelsman J (2004) Census of the bacterial community of the gypsy moth larval midgut by using culturing and culture–independent methods. *Appl. Environ. Microbiol.* **70**, 293-300.

Engel P and Moran N A (2013) The gut microbiota of insects – diversity in structure and function. *FEMS Microbiol. Rev.* **37**, 699-735.

Fukatsu T and Hosokawa T (2002) Capsule transmitted gut symbiotic bacterium of the Japanese common plataspid stinkbug, *Megacopta punctatissima*. *Appl. Environ. Microbiol.* **68**, 389-396.

Johnston P R and Neil Crickmore (2009) Gut Bacteria Are Not Required for the Insecticidal Activity of *Bacillus thuringiensis* toward the Tobacco Hornworm, *Manduca sexta Appl. Environ. Microbiol.* **75**(15), 5094-5099.

Kikuchi Y, Hayatsu M, Hosokawa T, Nagayama A and Tago K, (2012) Symbiont-mediated insecticide resistance. *Proc. Natl. Acad. Sci.* **109**(22), 8618-8622.

Madhusudhan S (2015) Influence of gut microflora on the susceptibility of *Helicoverpa armigera* (Hübner) and its parasitoids to insecticides. *Ph. D. Thesis,* Univ. of Mysore, Karnataka (India).

Thakur D, Bhuyan M, Majumdar S, Yadav A, Hazarika L K, Harman N, Baruah A A L H and Bora T C (2005) Isolation, characterization, in-vitro antibiotic susceptibility and pesticide tolerance of gut bacteria from rice hispa, *Dicladispa armigera* (Olivier). *Indian J. Microbiol*. **45**(3), 217-221.