Efficacy of *Bacillus thuringiensis* var.galleriae Berliner and selected insecticides on cotton bollworm, *Earias vitella.*

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Abstract

Cotton, an important cash crop of tropical world, is attacked by many insects. *Earias vitella* is a major pest in winter crop.Bio-efficacy of *B.t,* var galleriae as Spicturin®, was evaluated in comparison with insecticides. Two field experimentswere conducted winter and summer seasons with the cotton cultivar LRA- 5166 to assess the efficacy of *B.t.* on *Earias* spp. in combination with the insecticides like endosulfan (0.035 %), quinalphos (0.025%), fenvalerate (0.01%) and diflubenzuron (0.075%),endosulfan (0.035%) in combination with *B.t.*g. @ 3 l/ha was found to be the best in reducingthe boll damage.The damage to the larva tissues is illustrated with thin sections of diseased larva after fixing in blackwax, microtomy sectioning and light microscopy. Cracks in gut lining, damage to gut lumen, epidermis and epithelialcells, basement membrane, musculosa , peritrophic membrane were observed and support the successful pathogenesisand mortality of treated larvae.

Key words: Cotton, boll worm, *Earias, Bacillus*, squares, pathogeneis

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Introduction

Earias vitella causes damage to shoots, squares, flowersand bolls resulting in significant loss both in quality andquantity of seed cotton in winter cotton. Over use andindiscriminate application of chemicals have beenidentified as the major causes for resurgence, resistance to insecticides(Rajmohan and Jayaraj. 1978;Manisegaran et al., 1991; Dhurua and Gujar,2011;Williams et al., 2011; Bruce et al., 2013; Wei et al.,2015; Fei et al., 2015), elimination of beneficial insectsand environmental pollution(Navon, and Meir Klein,1990). In tropical countries, *Bacillus thuringiensis* has been extensively used against the pests of vegetables, especially of cole crops (Jaques et al., 1981; Joshi et al., 1987; Lacey et al.,2001; Wan et al., 2012; Maninderand Brar,1987; Mensah et al., 2015; Jackson,2016). Role of endotoxin and CRY1A protein in pathogenicity of the bacterium has been discussed by many researchers (Duan et al., 2013;Huang et al., 2013; Paramasiva et al.,2014;Qiueta.l 2015).The bio-efficacy of *B.t,* var galleriae as Spicturin, a commercial product was assessed in comparison as well as in combination with insecticides.

Materials and Methods

Two field experiments were conducted with the cultivar LRA 5166 to assess the efficacy of *B.t.*var.galleriae alone and in combination with insecticides. Factorial Randomised Block Design was followed. Treatments were replicated thrice. Five insecticidesviz., carbaryl 50 WP(0.025%), endosulfan 35 EC (0.035 %), quinalphos 25 EC (0.025%), fenvalerate (0.01%) and diflubenzuron 25WP(0.075%), were sprayed with in combination with B.t. @ 2.0, 2.5 ,3.0 l/ha with an untreated check. Two rounds of sprays were given on 65 DAS and 95 DAS (days after sowing). Spraying operation was taken up in the evening hours using hand- operated high volume knapsack sprayer. Data were transformed and angular transformed values were analysed with ANOVA.

i) Square damage:The damage to squares was assessed at an interval of 10 days starting from 50 DAS. The fallen squares in each plot were collected, sorted out as 'infested' and 'uninfested' and counted. The symptoms were categorised as suggested by Manisegaran et al.,(1991) . The damage due to *Earias* was identified bythe presence of entry holes and partial feeding onsquares. Flower damage was also assessed.ii) Boll shedding: The damage due to *Earias* spp. was examined by the presence of soiled and irregular boreholes.iii)Damage to seed cotton:Seed cotton was picked out at weekly intervals from the whole plot leaving the borderrows. The total weight of both good and bad lint was separated to record its weight.iv) Yield: The total yield of seed cotton was taken as the 'plot yield' and computed to kg/hectare yield.

Microdissection, staining and microscopy

Larva was mounted, dorsoventrally, in black wax in a dissection dish. Black wax was gently melted with a warm spatula before inserting the insect tergite or sternite into the wax. The mounted, insects were covered with 200 µl of HEPES wash buffer. A small hole was made at the end of the abdomen of larva and an incision was made laterally contents in alimentary canal were removed gently without disturbing the natural location of the internal organs and stained staining the alimentary canal with 0.05% carmine stain. After staining, the alimentary canal was rinsed twice with HEPES wash buffer.Preparation of thin Transverse Sections for Light Microscopy was done after fixing the tissue in 2% glutaraldehyde and 2% paraformaldehyde in 0.1% cacodylate buffer.

Samples were rinsed thrice in buffer, and post-fixedin 1% osmium tetroxide and rinsed for 20 min threetimes with ultrapure water. Tertiary fixation was donein 1%aqueous uranyl acetate, followed by as before.Dehydrated was done in an ethanol series and infiltrated with Epon resin. The resin was polymerizedat 55oC for2 d, after which the blocks were stored in a desiccator until sectioned. Semi-thin sections (µM)were cut with an ultra microtome (Reichert Ultra Cut S,Leica, Austria).Serial sections were placed on silane-coated slides and stained with 0.5% acid fuchsin and 0.5% carmine. The slides were air-dried at roomtemperature, mounted with Permount, and viewedwith an Olympus CX23biological microscope andimages were captured with a digital camera(Habibi etal., 2008; Salama and Sharaby,1985).

Results and discussion

Larvae of bollworm in cotton and other lepidopteran pests have been reported to be susceptible to variousstrains of B.t.(Salama et al.,1983; Jawahar and Gary1999; Amiri-BeSheli,2008; Chen et al., 2015; Qiu et al.,2015). Higher the dose of B.t.g, greater is the efficacyagainst the larvae of Earias spp. in avoiding squaresshedding. However, B.t. at various other doses, wasmoderately effective as it reduced the damage to squaresonly by 22.9 to 38.7 per cent as compared to unsprayedplots(Table 1). Endosulfan with high dose of B.t.g. wasthe most effective treatment as it reduced the damageto squares as much as by 83.3 per cent. When B.t.g was sprayed at 3.0 l/ha, 10.2 per cent of flowers was damaged against 23.4 per cent recorded in unprotected plots. High dose of B.t.g. in combination with endosulfan reduced flower damage to the extent of 83.6per cent as against 39.9 per cent in the untreated plots (Table 2).The boll shedding was significantly lower inB.t.g. sprayed plots (8.00 to 12.6%). High dose of B.t.g.reduced damage to bolls by 61.5 per cent. When mixed with insecticides, the effect of B.t.g increased significantly with the increase indose especially with endosulfan in reducing the boll shedding by as much as87.6 per cent at 3 l/ha (Table 3.).

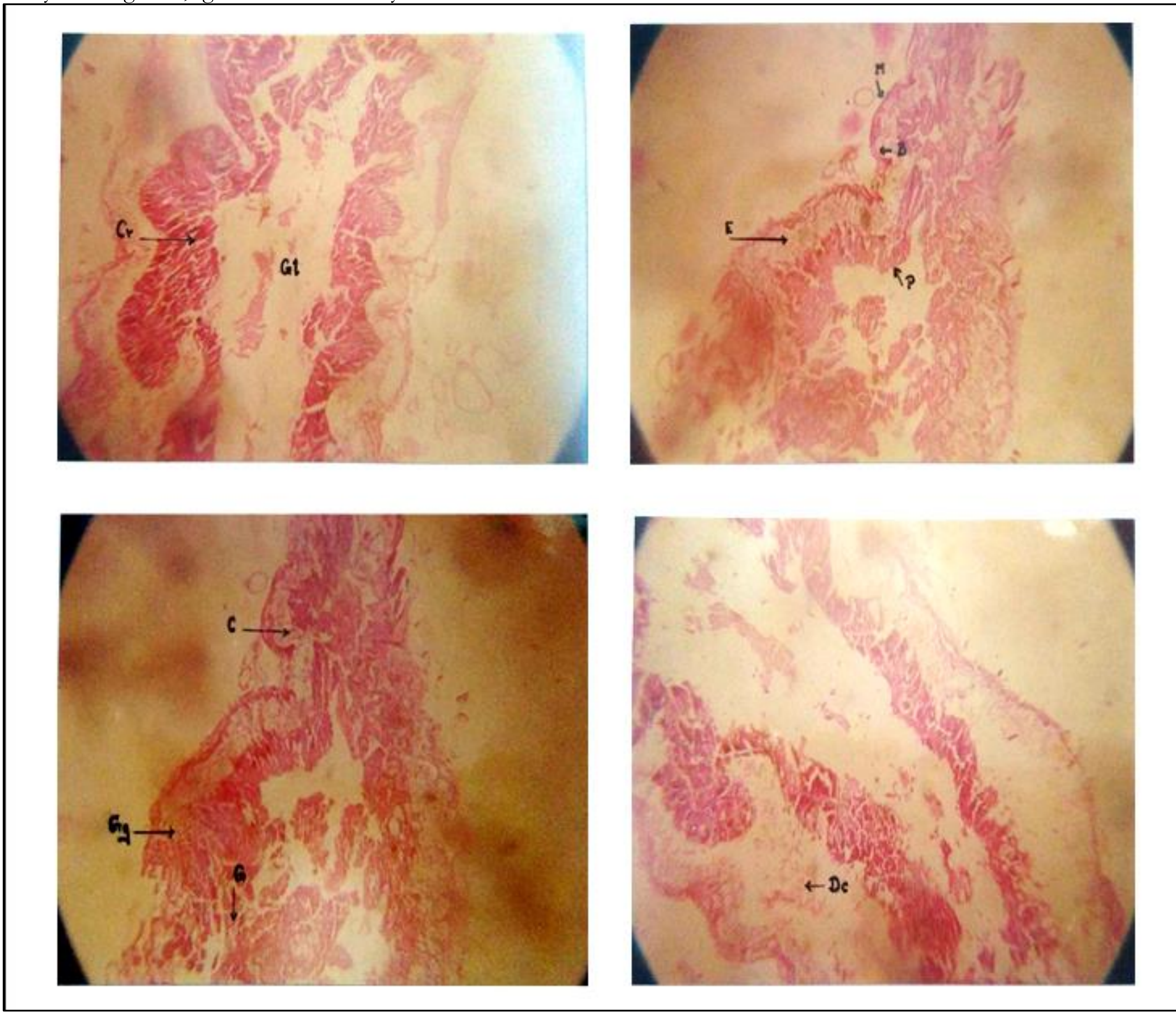
Spraying cotton plants with pesticides and B.t.g. had marked influence on seed cotton yield (Table 4.).The yield was the highest (1093 kg/ha) when high dose of B.t.g. was combined with endosulfan , the increase was as high as 748 kg over the yield from unsprayed plots (345 kg). However, B.t.g. at lesser doses was rather moderately effective against *Earias* spp. Variable B.t.g.doses reduced the *Earias* caused square shedding by 229to 387 per cent, flower shedding by 10.2 to 14.3 per centand boll shedding by 8.0 to12.6 per cent. Earlier,laboratory results of workers had indicated that standard concentration of many chemical insecticides and antibiotics applied on crops would not significantly inhibit the growth of B.t.g (Sundara babu, 1972; Gorashi et al., 2014;). Therefore, it is presumable that growth ofB.t.g. was notaffected as the concentration of insecticides usedwas sub-lethal as observed in the fieldconditions.Chemical insecticides act as stressors,making thelarvae more susceptible to the action of *B.t.* microbial toxins (Barnes and Ware, 1965; Ocelot etal., 2015; Shi et al., 2016). Susceptibility of bollworms vary to the B.t. strains and isolates from various geographical collections(Wan et al., 2012; Tabashnik etal., 2012; Gorashi et al., 2014).It is obvious that B.t.g.dose is one of the important factors as the number ofspores increases with the dose per unit area. However,the limitation of cost of higher doses of B.t.g could not be overcome by adding sublethal doses of conventional insecticides to low dose B.t.g.

Histologically, when ingested B.t. affects the alimentary canal, fat body and hypodermis of lepidopteran larvae viz., Helicoverpa armigera, *Spodoptera littoralis* and *S.litura*(Abdallah ,1985; Abdel Megeed et al., 1986; Dowd, and Sparks. 1987). The damage to the cells is illustrated with thin sections of diseased larva after fixing in black wax, microtomy sectioning and light microscopy. Cracks in gut lining , damage to gut lumen, epidermis and epithelial cells, basement membrane, musculosa, peritrophic membrane were observed and support the successful pathogenesis and mortality of larvae.

Endosulfan is metabolised oxidatively in insects(El-Zemaity and El-Refai. 1987). Hence the additive effects observed. Both B.t.g. and insecticides are needed to increase the susceptibility of the larvae. Efficacious additivity would probably be supplemental or synergistic which needs more research for confirmation. Results highlight the additive effects of B.t.g. and endosulfan. The rapid action of endosulfan predisposing the larvae to pathogen could well be the reason for this result as already reported by Joshi and Bhardwaj(1987). Dowd and Sparks(1987)postulated that the activity of those enzymes responsible for the breakdown of the insecticides ,could be suppressed by *B.t.g.* leading to an increased susceptibility of the insects to the insecticides.

Fenvalerate and carbaryl were also significantly effective in improving the action of B.t. though second only to endosulfan. In several studies, B.t. plus fenvalerate proved highly effective against Spodopteraspp (Samraj, and Jesudasan. 1989; Jawahar and Gary, 1999; Ricardo et al., 2000). Carbaryl and fenvalerate each interactive with high dose of B.t.g. decreased square shedding by 69.2 to 74.4 per cent (Table 1). Flower damage was lower by 80.0 per cent when fenvalerate was added to moderate dose of B.t.g. (Table 4). Chen et al. (2015) tested several organophosphorus, carbamate insecticides in combination with B.t.g. against H. virescens and found that carbaryl was synergistic with B.t.g. when mortality was generally supplemented or additive at low dosage of chemical -insecticide combination and often less than additive at higher dosage.

Though diflubenzuron had earlier resulted in 97.0 per cent mortality of the larvae of E.vitella at 500 ppm concentration(Amiri-BeSheli,2008).In this study, diflubenzuron was not impressively effective against *Earia*s spp. However, in combination with B.t.g., it resulted in significant reduction in damage to squares, flowers and bolls though far inferior to other insecticides.The results indicate that few days is the maximum period of efficacy for all tested insecticides.In conclusion, the present study showed that under heavy infestation, use of synthetic insecticides or repeated application of B.t. product is necessary to prevent re-infestation. Nowadays, pest management methods, solely or together, get the satisfactory control of bollworms in cotton. Evaluation of the strains of B.t and initial frequency of resistance are useful strategies to sustain the effectiveness of *B.t.* against bollworms in cotton.As an alternative to chemicals, *B. thuringiensis* formulation tested could be employed for control of *E. vitella* larvae and to reduce the impact on beneficial insects in cotton ecosystem.



Transverse section of *E.vitella* larva

LEGEND:

Fig.1a & c.: L.S. of midgut of *B.t* treated larva

Fig.1b & d.: L.S. of midgut of *B.t* untreated larva(control)

Cr: cracks in gut lining
Gl: gut lumen
E: epidermis
B: basement membrane
M: musculosa
P: peritrophic membrane
G: goblet
C: columnar cells
Dc: damaged epithelial cells

Table 1. Data on larval population in cotton field (Trial I)

Insecticide	first spray					second spray					mean					% REDUCTION
	B.t. dose (l/ha)					*B.t.* dose (l/ha)										
	0.0	2.0	2.5	3.0	mean	0.0	2.0	2.5	3.0	mean	0.0	2.0	2.5	3.0	mean	
endosulfan (0.035 %)	10.4	6.6	4.2	2.8	6.0	11.6	7.3	7.0	6.3	8.1	11.0	7.0	5.6	4.5	7.0	62.1
carbaryl (0.025%)	11.9	8.3	6.2	4.5	7.7	11.6	10.2	11.5	10.1	10.8	11.7	9.3	8.9	7.3	9.3	49.9
quinalphos (0.025%)	12.5	10.1	8.1	6.3	9.2	17.6	16.9	15.0	14.7	16.1	15.1	13.5	10.7	10.5	12.4	32.9
fenvalerate (0.01%)	10.63	8.1	6.3	4.1	7.28	13.7	14.1	13.6	12.7	13.5	12.2	11.1	10.0	84.0	10.4	43.8
Difluben(.075)	16.2	12.8	10.6	8.6	12.1	18.2	17.7	17.4	16.5	17.5	17.2	15.3	14.0	12.6	14.8	20.3
untreated check	32.5	16.0	14.7	12.7	19.0	22.0	17.3	16.7	16.5	18.1	27.2	16.6	15.7	14.7	18.5	
mean	15.7	10.3	8.4	6.5	10.2	15.8	13.9	13.5	12.8	140	15.7	12.1	10.8	9.7	12.1	
% reduction from control	__	34.2	46.7	58.4	__	__	11.7	14.2	19.0	__	__	22.9	31.3	38..7	__	

	Insecticide	*B.t.*	period	chemical x *B.t.*	*B.t.* x period	chemical x period	chemical x *B.t* x period
SE	0.31	0.25	0.18	0.62	0.36	0.44	0.87
CD(p=0.05)	0.61	0.49	0.35	1.22	0.71	0.87*	1.73N.S.

NS- Non-significant; * -significant at 5% level

Table 2. Data on larval population in cotton field (Trial II)

Treatment	first spray					second spray					mean					% reduction
	B.t. dose (l/ha)					*B.t.* dose (l/ha)					*B.t.* dose					
	0.0	2.0	2.5	3.0	mean	0.0	2.0	2.5	3.0	mean	0.0	2.0	2.5	3.0	mean	
endosulfan (0.035 %)	16.2	11.3	9.3	7.5	11.1	20.4	9.2	7.3	5.2	10.5	18.3	10.3	8.3	6.4	10.1	58.0
carbaryl 0.025%	17.3	13.2	11.3	9.11	12.7	21.5	11.2	9.4	7.2	12.3	19.4	12.2	10.4	8.1	12.5	51.3
quinalphos 0.025%	19.1	14.2	12.6	10.2	141	21.5	11.5	9.7	7.3	12.5	20.3	12.9	11.2	8.8	13.3	48.4
fenvalerate 0.01%	18.2	13.5	112	9.2	13.0	21.1	10.1	8.2	6.3	11.4	19.6	11.8	9.7	7.8	12.2	51.4
diflubenzuron 0.075%	21.4	16.5	14.2	12.1	16.1	26.5	14.1	12.3	10.0	15.7	24.0	15.3	13.3	11.1	15.9	38.1
untreated check	34.9	20.6	181	16.2	22.5	42.9	26.6	24.1	22.2	29.0	38.9	23.6	21.1	19.2	25.7	
mean	21.2	14.9	12.8	10.7	14.9	25.7	13.8	11.8	9.7	15.2	23.4	14.3	12.3	10.2	15.1	
% reduction from control	__	29.8	396	49.4	__	__	46.2	53.9	62.1	__	__	38.8	41.4	56.4		

	Treatment	*B.t.*	period	chemical x*B.t.*	*B.t.* x period	chemical x period	chemical x *B.t* x period
SE	0.07	0.06	0.04	0.14	0.08	0.10	0.20
CD(p=0.05)	0.14*	0.11*	0.08*	0.28*	0.16 *	0.20*	0.40*.

NS- Non-significant; * -significant at 5% level

Table.3. Data on boll shedding in cotton fields

Insecticide	first spray					second spray					mean					% REDUCTION
	B.t. dose (l/ha)					*B.t.* dose (l/ha)										
	0.0	2.0	2.5	3.0	mean	0.0	2.0	2.5	3.0	mean	0.0	2.0	2.5	3.0	mean	
endosulfan (0.035 %)	15.4	9.3	7.0	4.1	8.9	14.3	9.6	7.2	5.3	9.1	149	9.4	7.1	4.7	9.0	59.6
carbaryl 0.025%	17.5	10.4	8.3	5.1	10.3	15.5	10.2	8.5	6.2	10.1	16.5	10.3	8.4	5.6	10.2	54.3
quinalphos 0.025%	17.4	11.1	9.1	6.3	11.0	16.1	11.3	9.1	7.4	11.0	16.8	11.2	9.1	6.8	11.0	50.9
fenvalerate 0.01%	16.7	10.7	8.8	5.5	10.5	15.4	10.2	8.3	6.3	10.0	16.1	10.5	8.5	5.9	10.2	54.1
diflubenzuron 0.075%	25.5	16.2	14.3	11.0	16.8	19.3	13.3	11.6	9.4	13.4	22.4	14.7	12.9	10.2	15.1	32.5
untreated	43.3	23.2	19.7	16.9	25.8	32.4	16.2	14.3	12.5	18.9	37.9	19.7	17.0	14.7	22.3	
mean	22.6	13.5	11.2	8.1	13.9	18.9	11.8	9.8	7.8	12.1	20.8	12.6	10.6	8.0	13.0	
% reduction from control	__	40.5	50.5	64.1	__	__	37.5	47.9	58.4	__	__	39.1	49.1	61.5	__	

	Insecticide	*B.t.*	period	chemical x *B.t.*	*B.t.* x period	chemical x period	chemical x *B.t* x period
SE	0.18	0.15	0.11	0.36	0.21	0.26	0.51
CD(p=0.05)	0.36*	0.30*	0.21*	0.72*	0.42 *	0.51*	1.02NS.

NS- Non-significant; * -significant at 5% level

Table.4. Data on seedcotton yield

insecticide	*B.t.* dose (l/ha)				mean
	0.0	2.0	2.5	3.0	
endosulfan -0.035%	773	1018	1042	1093	981
carbaryl-0.025%	711	1006	1035	1071	956
quinalphos-0.025%	705	991	1024	1058	944
fenvalerate-0.001%	705	1001	1018	1063	949
diflubenzuran-0.075%	691	973	996	1011	918
untreated check	345	851	891	930	754
mean	655	973	1003	1038	917

	Insecticide	*B.t.*	chemical x *B.t.*
SE	1.89	1.54	3.78
CD (p=0.05)	3.81**	3.11**	7.61**

* significant at 5% level; ** significant at 1 % level

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