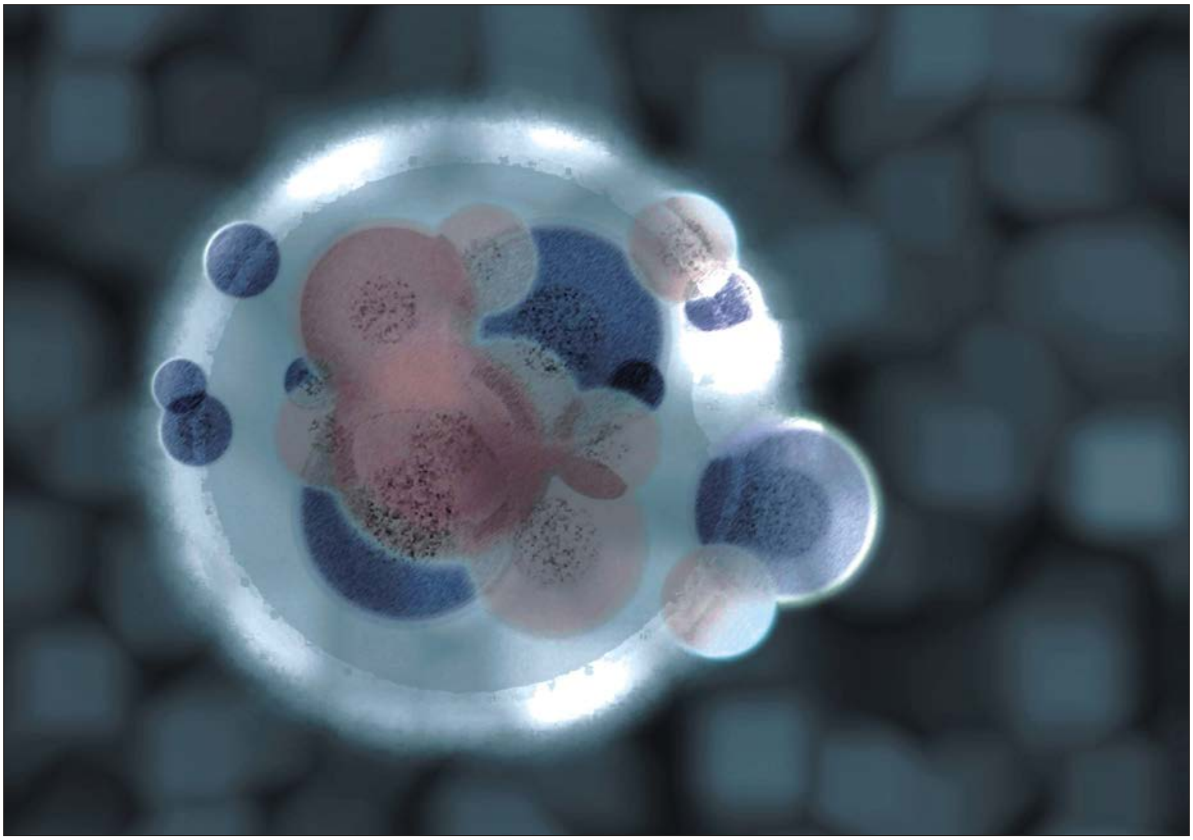

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Synergistic Effect of *Bacillus thuringiensis* subsp. *aizawai* with Synthetic Pyrethroids against Insecticide Resistant *Helicoverpa armigera* (Hubner)

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Abstract: Studies were carried out to find the effects of joint action between *Bacillus thuringiensis* subsp. *aizawai* (*Bta*) (Agree®) and synthetic pyrethroids against third instar larvae of *H. armigera* on different hosts. The joint action of *Bta*+pyrethroids at LC_{25}+LC_{25} revealed supplemental synergism as the combined application on different hosts showed 4.75-21.72 and 18.36-38.26% more than the expected mortality at LC_{50} of *Bta* alone and LC_{50} of pyrethroids alone, respectively.

Key words: *Helicoverpa armigera,* insecticide resistance, *B. thuringiensis* subsp. *aizawai*, synergistic suppression

INTRODUCTION

The gram caterpillar or American bollworm *Helicoverpa armigera* (Hubner) is a polyphagous and multigeneration noctuid pest. *H. armigera* has a wide host range of over 200 weed plants, 25 crop plants including cotton, maize, sorghum, sunflower, tomato, okra and legumes like pigeonpea, chickpea etc. The damage by *H. armigera* is estimated at more than Rs. 2000 crores ($ 450 m) nationally with 15% decline in the cotton yield[1]. Continuous use of pesticides against this pest resulted in the development of resistance to these pesticides. Regular monitoring of field populations of *H. armigera* since 1993 confirmed very high level of resistance (>90%) to synthetic pyrethroids[2]. The prevalence of high level of resistance to synthetic pyrethroids indicates an urgent need for implementation of curative Insecticide Resistance Management (IRM) for *H. armigera.* IRM is a strategy of resistance containment or suppression using a variety of tactics including insecticides of different chemistries and other nonchemical control measures especially biocontrol agents[3]. The entomopathogenic infection viz., virus and bacterial infection was found to increase susceptibility of *H. armigera* to the insecticides[4,5]. Hence, a research was carried out to study the feasibility of using the *Bacillus thuringiensis* subsp. *aizawai* (*Bta*) in combination with synthetic pyrethroids.

MATERIALS AND METHODS

Cultures of *H. armigera* were maintained on semisynthetic diet[6]. The commercial *B. thuringiensis* subsp. *aizawai* (Agree® WP) formulation gifted by Margo Biocontrols Pvt. Ltd., Bangalore, India were used in the present investigations. To find out the median lethal concentration (LC_{50}) of synthetic pyrethroids viz., cypermethrin, fenvalerate, lambda-cyhalothrin and beta-cyfluthrin and *Bta*, five to seven concentrations were fixed at geometric progression for which the dilutions were prepared with distilled water. The bouquet bioassay (foliar residue/terminal bud bioassay) was used for the experiment[7]. Cotton leaves, squares, bolls, okra fruits and pigeonpea pods were surface sterilized with 0.5% sodium hypochlorite, rinsed in sterile water and shade dried. These plant parts were dipped in respective concentration of the chemicals/*Bta* for about 30 seconds and the excess fluid was drained off. The petiole stalk with cotton swab were immersed in water in a vial and allowed for shade drying. Each vial having the treated plant parts was kept separately in a small cup and covered with mylar film cage to which the third instar larva was released. The mouth of the cage was covered with plastic lid. The treatments were replicated three times with 20 larvae in each. An untreated check with third instar larvae was maintained as control. Mortality count was taken 48 h after treatment. The LC_{25} and LC_{50} values were determined through probit

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analysis[8]. To find out the joint action of synthetic pyrethroids and *Bta*, the sub lethal concentration of *Bta* at LC_{25} was combined with LC_{25} dose of the candidate insecticide. The percent mortality increase/decrease over LC_{50} of insecticides and LC_{50} of *Bta* was calculated.

RESULTS AND DISCUSSION

The LC_{50} values of cypermethrin, beta-cyfluthrin, lambda-cyhalothrin and fenvalerate were in the range of 3.66-4.36, 0.99-1.36, 1.22-1.65 and 3.80-5.80 μg/larva, respectively on different hosts (Table 1). Comparison of susceptibility of *H. armigera* on different hosts did not reveal critical differences as evidenced by overlapping fiducial limits.

The LC_{50} of *B. thuringiensis* subsp. *aizawai* recorded for third instar larvae of *H. armigera* varied from 1.02-1.46 g L^{-1} respectively on different hosts. The LC_{50} value of *Bta* on cotton squares and cotton bolls recorded the highest LC_{50} value of 1.46 g L^{-1}. *Bta* on cotton leaves showed the lowest LC_{50} value of 1.02 g L^{-1}. However, variation in the LC_{50} value of *Bta* on different hosts was not significant as indicated by overlapping fiducial limits (Table 1).

The joint action of *Bta* and pyrethroids was studied at LC_{25}+LC_{25} in comparison with LC_{50} of *Bta* and pyrethroids. Irrespective of the host, *Bta*+pyrethroid had supplemental synergism. The increase in mortality was 4.75 to 21.72% more than the expected mortality at LC_{50} of *Bta* alone and 18.36 to 38.26% more than the expected mortality at LC_{50} of pyrethroids alone. The supplemental synergism of *Bta* and Synthetic Pyrethroids (SP) over *Bta* was high with fenvalerate > beta-cyfluthrin > cypermethrin > lambda-cyhalothrin. The effectiveness was maximum on pigeonpea pods > okra fruits > cotton leaves > cotton bolls > cotton squares. The supplemental synergism of *Bta* and Synthetic Pyrethroids (SP) over SP was high with fenvalerate > cypermethrin > lambda-cyhalothrin > beta-cyfluthrin. The effectiveness was maximum on pigeonpea pods > okra fruits > cotton squares > cotton leaves > cotton bolls (Table 2).

A synergistic effect was reported when *Bt* was applied with quinalphos, indoxacarb, carbosulfan, azadirachtin[9], carbaryl, endosulfan, malathion, monocrotophos[10] against *H. armigera*. The alimentary canal, fat body and hypodermis were affected by *Bt* in *H. armigera*[11]. Mixed function oxidases and esterases responsible for the breakdown of synthetic pyrethroids

Table 1: Concentration-mortality response of third instar larvae of *H. armigera* to synthetic pyrethroids and *Bacillus thuringiensis* subsp. *aizawai* (Agree®) determined by bouquet bioassay method

Hosts	Regression equation Y = a+bx	χ^2	LC_{25} (μg/larva)	Fiducial limits	LC_{50} (μg/larva)	Fiducial limits
Cypermethrin						
Cotton leaves	-1.57+2.46x	0.298	2.32	1.77-2.80	4.36	3.69-5.21
Cotton squares	-1.33+2.16x	1.609	2.02	1.46-2.51	4.15	3.45-5.07
Cotton bolls	-1.54+2.44x	0.776	2.28	1.73-2.76	4.30	3.64-5.14
Okra fruits	-1.15+2.04x	1.998	1.71	1.18-2.18	3.66	3.00-4.47
Pigeonpea pods	-1.57+2.46x	0.298	2.32	1.77-2.80	4.36	3.69-5.21
Beta-cyfluthrin						
Cotton leaves	-0.74+5.67x	0.079	1.02	0.93-1.11	1.35	1.25-1.49
Cotton squares	-0.42+6.25x	1.332	0.91	0.82-0.98	1.17	1.09-1.25
Cotton bolls	-0.70+5.22x	0.143	1.01	0.88-1.10	1.36	1.25-1.53
Okra fruits	-0.69+5.27x	2.922	0.74	0.64-0.81	0.99	0.91-1.07
Pigeonpea pods	-0.17+4.62x	1.025	0.78	0.66-0.86	1.09	1.00-1.19
Lamba-cyhalothrin						
Cotton leaves	-0.92+5.66x	1.721	1.10	0.99-1.19	1.45	1.36-1.56
Cotton squares	-1.32+7.35x	0.946	1.22	1.12-1.30	1.51	1.43-1.60
Cotton bolls	-1.56 +7.15x	0.141	1.33	1.17-1.42	1.65	1.55-1.77
Okra fruits	-0.50+5.67x	2.958	0.93	0.81-1.02	1.22	1.13-1.31
Pigeonpea pods	-0.62+5.89x	7.726	0.97	0.87-1.06	1.27	1.18-1.35
Fenvalerate						
Cotton leaves	-1.79+2.34x	3.602	2.99	2.08-3.74	5.80	4.84-6.88
Cotton squares	-2.30+3.04x	5.145	3.44	2.68-4.08	5.73	4.96 -6.56
Cotton bolls	-1.72+2.31 x	3.006	2.85	1.95-3.59	5.59	4.63-6.62
Okra fruits	-1.35+2.32x	6.020	1.95	1.21-2.59	3.80	2.95-4.57
Pigeonpea pods	-1.36+2.12x	6.078	2.12	1.27-2.83	4.41	3.45-5.31
Bacillus thuringiensis subsp. *aizawai* (Agree®)						
Cotton leaves	-0.01+1.98x	4.492	0.46	0.28-0.62	1.02	0.81-1.21
Cotton squares	-0.42+2.56x	3.547	0.79	0.61-0.94	1.46	1.27-1.69
Cotton bolls	-0.42+2.56x	3.547	0.79	0.61-0.94	1.46	1.27-1.69
Okra fruits	-0.22+1.90x	2.730	0.58	0.37-0.75	1.31	1.08-1.58
Pigeonpea pods	-0.27+1.90x	1.942	0.61	0.39-0.78	1.39	1.15-1.68

Table 2: Joint action of *B. thuringiensis* subsp. *aizawai* (Agree®) and synthetic pyrethroids at sub lethal concentration on third instar larvae of *H. armigera*-mortality percent

					% increases over	
Hosts	Treatments	LC_{25}	LC_{50}	LC_{25}+ +LC_{25}	*Bta* at LC_{50}	Insecticide at LC_{50}
Cotton leaves	*Bta*	24.13	52.40	-	-	-
	Cypermethrin	23.07	46.40	60.50	15.45	30.38
	β-cyfluthrin	26.08	47.00	58.00	10.68	23.40
	λ- cyhalothrin	23.33	46.00	59.00	12.59	28.26
	Fenvalerate	22.91	47.50	62.44	19.16	31.45
Cotton squares	*Bta*	24.70	51.50	-	-	-
	Cypermethrin	25.45	46.50	57.35	11.35	23.33
	β-cyfluthrin	26.00	44.00	54.50	5.82	23.86
	λ- cyhalothrin	22.22	45.48	58.00	12.62	27.52
	Fenvalerate	25.00	43.50	57.50	11.65	32.18
Cotton bolls	*Bta*	21.05	46.30	-	-	-
	Cypermethrin	21.42	42.44	52.40	13.17	23.46
	β-cyfluthrin	25.00	43.50	54.84	18.44	26.60
	λ- cyhalothrin	21.81	40.74	48.50	4.75	19.04
	Fenvalerate	23.33	41.00	53.50	15.55	30.48
Okra fruits	*Bta*	27.27	53.74	-	-	-
	Cypermethrin	27.27	48.60	60.72	12.98	24.93
	β-cyfluthrin	26.66	49.00	58.00	7.92	18.36
	λ- cyhalothrin	23.21	47.44	60.50	12.57	27.52
	Fenvalerate	26.66	48.00	64.70	20.39	34.79
Pigeonpea pods	*Bta*	24.41	51.50	-	-	-
	Cypermethrin	22.00	46.25	60.40	17.28	30.59
	β-cyfluthrin	24.07	46.27	56.75	10.19	22.64
	λ- cyhalothrin	20.00	44.00	54.20	5.24	23.18
	Fenvalerate	21.81	45.34	62.69	21.72	38.26

have been found in the hypodermis, gut as well as fat bodies in the midgut in *H. virescens*[12,13]. It may be postulated that due to effect of *Bt* on the sites of action of the enzymes responsible for the breakdown of insecticides, the activity of these enzymes could have been suppressed leading to an increased susceptibility of the insects to the insecticides. By suitably introducing *Bt* in the integrated pest management system, we may delay, if not avoid the process of development of insecticide resistance in insect pests.

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