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Synergistic Interaction Between *Bacillus thuringiensis* (Berliner) and Lambda-cyhalothrin (Pyrethroid) Against, Chickpea Pod Borer, *Helicoverpa armigera* (Huebner)

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Abstract: Compatibility and synergism of *Bacillus thuringiensis* commercial formulation and lambda-cyhalothrin were evaluated for the control of *Helicoverpa armigera* (Huebn.). Concentration-mortality response of 3rd instar *H. armigera* larvae due to *B. thuringiensis* Berliner subsp. *kurstaki* (Costar® WP) and lambda-cyhalothrin (Karate® 2.5 EC) alone and in combination with various concentrations incorporated in artificial diet was assessed. Combinations of low dosage of lambda-cyhalothrin and Costar (*B.t.k.*) formulations were found highly compatible and synergistic. In combination treatments (Costar+karate LC_0, Costar+karate LC_{10} and Costar+karate LC_{25}), the LC_{50s} of Costar underwent 1.3, 3.4 and 3.4 fold reductions respectively. Further laboratory test and field trials of *B. thuringiensis* var. *kurstaki* supplemented with other chemical insecticides against chickpea pod borer are warranted.

Key words: Lambda-cyhalothrin, *Bacillus thuringiensis* subsp. *kurstaki*, *Helicoverpa (Heliothis) armigera*

Introduction

Chickpea pod-borer, *Helicoverpa (Heliothis) armigera* is a major pest of chickpea. Earlier, this pest was confined to some hot spots in southern Pakistan but now has become a serious pest throughout Pakistan. Crop rotation, introduction of new varieties or crops, land reclamation, pest migration, use of irrigation and fertilizers have helped to increase the populations of polyphagous insect pests (Hariri, 1981; Elmosa, 1981; White, 1978). This insect attacks the chickpea crop at flowering stage and continues to damage green pods till maturity. Every year, chickpea yield is reduced (average around 25%) due to infestation of this pest but the reduction in yield may even exceed to 50 % in some years (Ahmed *et al.*, 1985).

Use of *B. thuringiensis* based microbial insecticides has become an integral part of IPM approaches in the developed world specially because, these preparations provide an environmentally suitable alternative to generally hazardous broad-spectrum chemical insecticides. Moreover, microbial insecticides besides sparing beneficial insects (parasitoids and predators) have the capability to target specific class of insects. Due to use of new strains of *B. thuringiensis* (*B.t.*) and improved commercial formulations, the insect pathogens are gaining international support against agricultural insect pests. Several reports indicated use of *B.t.* and its enhancement by incorporating suitable quantities of acids, salts, oils, (Ahmed *et al.*, 1998a, Chenzhu *et al.*, 1997, Rao & Krishnaw, 1996; Zhou, 1994), adjuvants, thuringiensin (β-exotoxin of *B.t.* (Liu *et al.*, 1997 and Morris *et al.*, 1995) and chemical insecticides (Welland *et al.*, 1997, Saleem *et al.*, 1995, Saleem & Shakoori, 1996; Gaffar & Kushwaha, 1994) against lepidopterous pests including *H. armigera* (Salama, 1984; Morris, 1988).

The present study was conducted to determine level of compatibility and synergistic interaction between spore-δ-endotoxin of *B. thuringiensis* var. *kurstaki* (Costar®, a commercial preparation) and lambda-cyhalothrin (Karate®, a chemical insecticide of pyrethroid group). These results can be helpful in promoting application of bio-pesticides as a component of Integrated Insect Pest Management (IPM) and minimizing the use of hazardous chemical insecticides in chickpea, vegetable, fruit trees and other high value crops in Pakistan.

Materials and Methods

Biological insecticides (Biopesticides) tested: Costar® (Sandoz biological insecticide) having 18.0% *B. thuringiensis* subspecies *kurstaki* active ingredient (a.i.) and 82.0% inert material (non-hazardous) obtained from Sandoz Agro, Inc. Des Plaines, Illinois, USA and Karate® (broad spectrum chemical insecticide produced by Imperial Chemical Industries (ICI) Ltd., Karachi, Pakistan containing 2.5% w/v (2.5 EC) lambda-cyhalothrin as active ingredient and 97.5 % other ingredients) were used.

Test insect (*Helicoverpa armigera*): Third instar larvae of *H. armigera* were obtained from the rearing facility, established according to method of Ahmed *et al.* (1998b) and reared on artificial diet containing agar 25.0 g, chickpea flour (*Cicer arietinum* L.) 600.00 g, ascorbic acid 7.0 g, sorbic acid 3.0 g, dried active yeast 20.0 g, methyl-para-hydroxybenzoate 10.0 g, vitamin mixture 5.0 ml, formaldehyde (10.00 %) 6.0 ml and tap water 3.50 liters.

Petri-plate technique for larvae development in groups: This insect develops cannibalism from the late third to fifth instar. A technique was developed that allowed rearing of *H. armigera* in groups of 90 to 100 larvae per petri-plate (17.0 x 3.0 cm^2), with a thin layer of diet at the bottom and in the cover of the plate. After infestation of the petri-plate with about 110 first instar larvae, sterilized two tissue papers were folded and placed in petri-plate to soak up excessive diet moisture and allow the developing larvae to move in between tissue paper folds, thereby reducing chances of larval cannibalism. This technique enabled aseptic rearing up to the early third instar larvae for use in bioassays. This rearing technique significantly reduced the cost of development of third instar larvae as compared with individual rearing technique (one larva/container) (Ahmed *et al.*, 1998b).

Preparation of biopesticide (Costar®) and chemical insecticide (Karate®) concentrations: The initial suspension of bacterial toxin (spore-δ-endotoxin of costar®) was made by adding 320.0 mg of the toxin to 100.0 ml of sodium phosphate buffer (pH 7.00) (Ahmed, 1999). From this suspension 2.5, 5.0, 10.0, 20.0, 40.0 and 80.0 μg/ml diet dilutions of the toxin were prepared. The concentration of Karate® 3.125, 6.25, 12.5, 25.0, 50.0 &100.0 μg a.i./ml diet were also prepared. For each concentration, required quantity of bacterial toxin (costar®)/chemical insecticide (Karate® a.i.) was added to diet so as to make total volume of diet to 400.0 ml and thoroughly mixed for 2.0 minute at 65.0 ^{0}C. In order to evaluate the combination effect of the Costar® (*B.t.K*) and chemical insecticide (Karate®), the calculated LC_S of karate® (LC_0, LC_1, LC_{10}, LC_{25}) were separately combined with bacterial concentrations (six concentrations) and were blended with the diet.

Bioassay: The bioassay experiments using bacterial toxin and chemical insecticide (alone or in combination) were done by thorough mixing of the active ingredients in the insect diet to achieve uniform distribution. Four milliliter of this intoxicated liquid diet was poured into sterilized capsule vials. For each concentration, 100 capsule vials (25 vials/replicate) were filled with respective concentration of intoxicated diet. Each vial was infested with an early third instar larva of *H. armigera*. The vials were plugged with sterilized cotton wool. Four replications of each concentration along with their respective control were maintained. After seven days of incubation, the data were recorded in terms of the number of live and dead larvae. The bioassays were conducted at 25 ± 2 °C and relative humidity (RH) 65-70 %.

Data analysis: The LC_s, slopes and other statistical analysis of the data were done using probit analysis (Finey, 1964). The LC_1, LC_{10}, and LC_{25} of karate® were calculated by Programme D'analyse log probit (Raymond, 1985). The LC_0 was considered as the dosage of Karate® causing no larval mortality. The potencies and synergistic/antagonistic interactions of these combinations were also calculated using method of Dulmage *et al.* (1976); Ahmed *et al.* (1998a); Salama *et al.* (1984) and Morris (1988).

Results and Discussion

Salama *et al.* (1984) reported that pyrethroid insecticides were generally milder in their effect on sporulation titre as compared to other group of insecticides. They further reported that pyrethroid and most organophosphorus compounds potentiate the *B. thuringiensis* varieties against cotton leaf worm, *Spodoptera litoralis.* In present study, concentration-mortality response of third instar larvae (Table 1) of *H. armigera* exposed to different treatments of Costar® alone and in combinations with LC_0, LC_1, LC_{10} and LC_{25} of Karate® indicated that larval mortalities caused by the combination, Costar®+LC_0 of karate® were higher at all dose levels (except 2.5 µg/ml) as compared to the mortalities caused by costar® alone (Table 1). The lower larval mortalities were observed in all treatments of costar® + LC_1 of karate® (except at 80.0 µg/ml) in comparison with the mortalities in the respective treatment of costar alone. Therefore, the combination of costar® with LC_1 of karate® was not found to have increased the effectiveness of costar®. However, the combinations of costar® with LC_{10} and LC_{25} of karate® showed the highest mortality response at all treatments as compared to treatments of costar® alone (Table 1). Welland *et al.* (1997) observed that diflubenzuron (dimlin) in combination with *B.t.* cotton was more effective in reducing larval leaf feeding and increasing larval mortality of *Spodoptera exigua* than either diflubenzuron or *B.t.* alone (Table 1).

Table 1: Concentration-mortality response of third instar larvae of *Helicoverpa armigera* exposed to *Bacillus thuringiensis* (Costar®, biological insecticide) and karate® (chemical insecticide) alone and combination.

Corrected Mortality (%)* + SE

Costar® WP** µg/ml of diet	Costar	Costar + karate(LC_0)	Costar + karate(LC_1)	Costar + karate(LC_{10})	Costar + karate(LC_{25})
2.5	13.0 ± 4.4	4.0 ± 1.9	9.5 ± 2.8	49.0 ± 4.9	32.9 ± 2.7
5.0	15.2 ± 0.9	26.0 ± 8.4	8.9 ± 5.2	54.5 ± 7.5	58.0 ± 4.1
10.0	29.3 ± 2.4	47.3 ± 5.9	18.7 ± 1.9	49.5 ± 3.1	71.6 ± 5.9
20.0	52.5 ± 3.5	63.8 ± 3.4	43.4 ± 2.6	82.0 ± 3.5	91.4 ± 1.7
40.0	88.9 ± 3.4	89.4 ± 1.3	77.1 ± 5.7	94.8 ± 1.2	89.7 ± 3.5
80.0	97.0 ± 1.9	98.9 ± 1.1	100.0 ±0.0	99.0 ± 1.1	100.0 ± 0.0

*Corrected for natural mortality by Abbott's (1925) formula ** Wettable powder

Table 2: Response of third instar larvae of *Helicoverpa armigera* to mixtures of Costar® (biological insecticide) and Karate® (chemical insecticide) in artificial diet.

Treatment costar® µg/ml + karate®	Observed mortality* (%)	Expected mortality (%)	X^2 value	Combined effect	Significance level
2.5 + LC_0	4.0	13.0	6.2	Synergistic	0.01
5.0 + LC_0	26.0	15.2	7.7	Synergistic	0.01
10.0 + LC_0	47.3	29.3	11.1	Synergistic	0.01
20.0 + LC_0	63.8	52.5	2.4	Antagonistic	---
40.0 + LC_0	89.4	88.9	0.003	Antagonistic	---
80.0 + LC_0	98.9	97.0	0.04	Antagonistic	---
2.5 + LC_1	9.5	13.9	1.4	Antagonistic	---
5.0 + LC_1	8.9	16.1	3.2	Antagonistic	---
10.0 + LC_1	18.7	30.0	4.3	Synergistic	0.05
20.0 + LC_1	43.4	53.0	1.7	Antagonistic	---
40.0 + LC_1	77.1	89.0	1.6	Antagonistic	---
80.0 + LC_1	100.0	97.0	0.1	Antagonistic	---
2.5 + LC_{10}	49.0	21.7	34.3	Synergistic	0.001
5.0 + LC_{10}	54.5	23.7	40.0	Synergistic	0.001
10.0 + LC_{10}	49.5	26.4	4.7	Synergistic	0.05
20.0 + LC_{10}	82.0	57.3	10.6	Synergistic	0.01
40.0 + LC_{10}	94.8	90.0	0.26	Antagonistic	---
80.0 + LC_{10}	99.0	97.3	0.03	Synergistic	---
2.5 + LC_{25}	32.9	34.8	0.1	Antagonistic	---
5.0 + LC_{25}	58.0	36.4	12.8	Synergistic	0.001
10.0 + LC_{25}	71.0	47.7	11.4	Synergistic	0.001
20.0 + LC_{25}	91.4	64.4	11.3	Synergistic	0.001
40.0 + LC_{25}	89.7	91.7	0.04	Antagonistic	----
80.0 + LC_{25}	100.0	97.8	0.05	Synergistic	-----

*Corrected for natural mortality by Abbott's (1925) formula

Table 3: Summary of regression analysis of probit mortality of *Helicoverpa armigera* third instar larvae against log concentrations for treatments in diet.

Treatment	LC_{50} (95%CL) (μg/ml diet)	LC_{90} (95%CL) (μgh/ml diet)	Slope	Potency IU/mg	NRR (%)
Karate® 2.5 EC	4.6 (4.0-5.2)	12.4 (10.6 - 15.3)	3.0	-	0.0
Costar ®WP	14.2 (12.4-16.3)	59.0 (47.7 - 76.9)	2.1	98,000	0.0
Costar® + Karate® (LC_0)	11.3 (9.8-12.9)	41.7 (34.3 - 53.2)	2.3	1,23,000	3.0
Costar® + Karate® (LC_1)	23.5 (20.8-26.2)	52.7 (45.5 - 64.6)	3.6	59,000	7.1
Costar® + Karate® (LC_{10})	4.2 (3.1-5.3)	37.1 (27.9 - 54.6)	1.4	3,31,000	0.0
Costar® + Karate® (LC_{25})	4.2 (3.3-5.1)	24.8 (19.7 - 33.6)	1.7	3,31,000	1.0

NRR = Natural response rate, Karate=2.5 EC (mg a.i./ml diet)

Note:- LC_0 = 0.38 μ g/ml, LC_1 = 0.76 μ g/ml, LC_{10} = 1.7 μ g/ml, & LC_{25} = 2.7 μg/ml CL= confidence limit

Table 2 indicates that in general, combination of Karate® at LC_0, LC_{10} and LC_{25} enhanced the action of Costar®+ at 2.5 μg to 20.0 μg/ml concentration in the diet than at 40.0 μg and 80.0 μg/ml concentration. The chi square (χ^2) values 6.2, 7.7 and 11.1 at 2.5+LC_0, 5.0+LC_0 and 10.0 +LC_0 μg/ml concentration respectively and 34.3, 40.0 and 10.6 at 2.5+LC_{10}, 5.0+LC_{10} and 20.0 +LC_{10} μg/ml concentration respectively were higher (Table 2) showing faster interaction of *B. thuringiensis* towards killing the test insect.

Our results have fallen in agreement with Jaques and Morris (1981), who stated that generally, enhancement was greatest among larvae fed low concentrations of chemicals and low to moderate concentrations of the pathogen. Creighton *et al.* (1974) reported that the efficacy of mixtures of *B. thuringiensis* + chlordimeform at lower rates resulted in 97% marketable heads of cabbage. In the present studies, response of the third instar larvae of *H. armigera* to the mixture of microbial-chemical pesticide at different concentrations differed significantly from the expected mortalities resulting into synergistic interaction at low dosage of Costar® (Table 2). Mohamed *et al.* (1983) reported that *Bacillus thuringiensis* var *kurstaki* (Dipel) in combination with methoprene (IGR), benomyl, chlorodimeform (chemical insecticide) and cyhexalin (acaricide) showed significantly higher mortalities of 1st instar larvae of *H. virescens* than expected mortalities at 7 days and at pupation.

The slope of concentration-mortality regression for Costar® + Karate® LC_0 and Costar® + Karate® LC_1 were steeper than the slopes for other LC_s (Table 3), which indicates higher response of 3rd instar larvae for these mixtures (combination treatments). The slopes for these treatments were also higher than the Costar alone (2.1). The LC_{50s} in combination treatments (Costar® + Karate® LC_0, Costar® + karate® LC_{10} and Costar® + karate® LC_{25}) underwent significant reduction indicating faster interaction towards killing the insect. Significant increase in potencies was also recorded in combination treatments (Costar® + Karate® LC_0, Costar® + karate® LC_{10} and Costar® + karate® LC_{25}) (Table 3).

Liu *et al.* (1997) studied the synergism of *B. thuringiensis* by ethylenediamine tetra-acetate (EDTA) + trypsin in susceptible and resistant larvae of diamondback moth, *Plutella xylostella*. They observed that the addition of EDTA+ trypsin reduced the LC_{50} of *B. thuringiensis* by 2 to 3 fold in neonates and 3rd instar for resistant larvae. Saleem *et al.* (1995) reported the toxicity of insecticides with reference to their effects on enzymes and other metabolites of sixth instar larvae of *Tribolium castaneum*. These were graded as *B. thuringiensis* +Cypermethrin > *B. thuringiensis* + Permetherine > *B. Thuringiensis*. Rao and Krishnayya (1996) observed highest larval (38.9 %) and pre-pupal (44.4 %) mortalities and lowest emergence of normal adults of *Spodoptera letura* when developed on the combination of diflubenzuron (0.0125 %) and *B. thuringiensis* var *kurstaki* (0.075 %).

The mortality response of chickpea pod borer *H. armigera* to mixtures of karate® (lambda-cyhalothrin) and costar® (*B. thuringiensis* var *kurstaki*) in 3 combinations (Costar® + Karate® LC_0, Costar® + karate® LC_{10} and Costar® + karate® LC_{25}) were synergistic and these combinations can be tested in the field for economical and effective control of this insect pest without causing environmental hazards.

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