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Compatibility of Bio-insecticide with Chemical Insecticide for Management of *Helicoverpa armigera* Huebner

Feeroza Khalique and Khalique Ahmed
Pulses Programme, Crop Sciences Institute, National Agricultural Research Centre, Islamabad, Pakistan

Abstract: The inter-specific compatibility between commercial preparation Costar® WP having *Bacillus thuringiensis kurstaki* Berliner (*Btk*) and Larvin® (Thiodocarb) were evaluated against chickpea pod borer, *Helicoverpa armigera* Huebner. The combination of low concentrations of Larvin® had reduced lethal concentration (that is, increased toxicity at LC_{50} and LC_{90}) of *Bacillus thuringiensis* (Costar® WP) when first instar larvae of chickpea pod borer, *Helicoverpa armigera* Huebner were fed on artificial diet treated with bacteria supplemented with low dosages of chemical insecticide. The combination treatment (Costar® WP+Larvin® LC_{10}) increased potency of Costar® WP from 77,600 to 1,04,000 IU mg^{-1}. Synergistic interaction indicate that Larvin® (Thiodocarb) can be used with *B. thuringiensis* for management of *H. armigera* in the field. Laboratory test of *B. thuringiensis* further in low dosages supplemented with same and other group of chemical insecticides against *H. armigera* are suggested.

Key words: *Bacillus thuringiensis kurstaki,* compatibility, *Helicoverpa armigera*, chemical insecticide

INTRODUCTION

The chickpea pod borer, *Helicoverpa armigera* (Hübn) is a regular economic pest of many important crops including chickpea in Pakistan. Every year, this insect pest infests chickpea causing sever economic losses in rice-based and other major chickpea growing areas[1]. The adverse effects of broad spectrum and persistent chemical insecticides on the agro-ecosystem are well documented. These insecticides are detrimental to the natural enemies like parasitoids and predators which otherwise regulate their abundance in the natural ecosystem[2].

Bacillus thuringiensis (Bt) is presently the most successful microbial insecticide with worldwide application for protection of crops, forests and human health[3]. Worldwide commercialization of *B. thuringiensis* subsp. *kurstaki* (*Btk*) was preceded by its development for management of many insect pests. The microbial (*Bacillus thuringiensis* based) insecticide can be used as part of Integrated Pest Management (IPM) approach to provide an environmentally safe and suitable alternative to generally hazardous, broad-spectrum chemical insecticides used against *Helicoverpa armigera* (Hübn). As for as environmental protection is concerned, there is pressing need for complementary use of microbial (*Bt* based) and botanical insecticides in support of the Integrated Insect Pest Management (IIPM) programme. Further more, the effective integration of insect pathogens with the low dosage of the synthetic chemical insecticides is one way of minimizing the use of hazardous insecticide. Published work stated that potentiation of *Bt* by addition of toxic and non-toxic compounds[4-6]. Microbial agent and chemical insecticide interaction has been reported by many researchers[2,7-10].

In the study reported here, the laboratory bioassay of Costar® WP (commercial preparation of *B. thuringiensis* Var. *Kurstaki*) in combination with Larvin® (Thiodocarb) was carried out to determine the compatibility and inter-specific relationship for synergism of two groups for management of *Helicoverpa armigera* (Hubn).

MATERIALS AND METHODS

Products tested: An exotic *Bacillus thuringiensis* Var. *kurstaki* based commercial preparation Costar® WP of the United States having 18% *Bacillus thuringiensis* Var. *kurstaki* active ingredient and 82% inert material obtained from Sandoz Inc. Des Plaines, Illinois, USA and a broad spectrum chemical insecticide Larvin® DF (80% a.i.) were used in the study.

Test insect: First instar larvae of *H. armigera* obtained from the rearing facility, established according to

Corresponding Author: Dr. Khalique Ahmed, Pulses Programme, Crop Sciences Institute, National Agricultural Research Centre, Islamabad, Pakistan Tel: +92-51-9255048 Fax: +92-51-9202968 E-mail: kahmed@apollo.net.pk

method of Ahmed *et al.*[11] and were 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-hydroxy-benzoate 10.0 g, vitamin mixture 5.0 mL, formaldehyde (10.0 %) 6.0 mL and tape water 3.50 L.

Preparation of Costar® and Larvin® concentrations: The initial suspension of bacterial toxin (spore-δ-endotoxin) of Costar® was made by adding 160.0 mg of the toxin to 50.0 mL of sodium phosphate buffer pH 7.00[7]. From this suspension 2.5, 5.0, 10.0, 20.0, 40.0 and 80.0 μg mL^{-1} diet dilutions of the toxin were prepared. The dilution of Larvin® (thiodocarb 80 DF) i.e. 2.5, 5. 0, 10.0, 20.0, 40.0 and 80.0 μg a.i. mL^{-1} diet were also prepared. For each dilution, required quantity of bacterial toxin (Costar®/chemical insecticide (Larvin® was added to the diet so as to make total volume of diet to 400.0 mL in a 1000 mL capacity sterilized beaker and vigorously shaken for 2.0 minute to achieve thorough mixing at 65.0°C. In order to evaluate the combinations effect of the Costar® WP (*BtK*) and chemical insecticide (Larvin® the calculated LC's of Larvin® (LC_0, LC_1, LC_{10} and LC_{25}) were separately combined with bacterial dilutions (six dilution) and blended with the diet.

Bioassay: The bioassay experiments using bacterial toxin and chemical insecticide (alone and in combination) were done by thoroughly mixing 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 first 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 term of the number of live and dead larvae. The bioassays were conducted at 25.0±2.0°C and Relative Humidity (RH) 65-70%.

Data analysis: The LC_{50}, slopes and other statistical analysis of the data were done by probit analysis[12]. The LC_0, LC_1, LC_{10} and LC_{25} of Larvin® (were calculated by Programme D'analyse log probit[13]. The LC_0 was considered as the dosage of Larvin® (causing no larval mortality. The LC_{50}s, slopes, potencies and synergistic/antagonistic interactions of these combinations were also calculated[5,7,9]. The Synergistic Ratio (SR) or Relative Potency (RP) of each combination was calculated by the following formula[14]:

$$\text{Synergistic Ratio (SR)} = \frac{LC_{50} \text{ of the toxicant } (Bt)}{LC_{50} \text{ of the mixture } (Bt\text{+Chemical insecticide})}$$

The synergistic, antagonistic and additive effect was calculated according to the formula developed by Sarup *et al.*[14] stated that the value of joint action ratio>1.05 will indicate synergism and between 0 and 0.95, the antagonistic action and those between 0.95 and 1.05 will indicate additive effect.

RESULTS AND DISCUSSION

Sutter *et al.*[15] tested various chemical insecticide (including Carbamate) for compatibility with *Bacillus. thuringiensis in vitro* and observed that Carbamate group had no significant effect on growth. Altahtaway and Abaless[16] recommended Carbamate group (T58 and Sevin) for association with Thuricide.

Jaques and Morris[10] reported that most insecticides are compatible with *Bt* having little or no effect on spore germination and cell multiplication, they further reported that compatibility of *B. thuringiensis* and chemical insecticides at low concentrations of Carbamates and Organophosphates, did not effect bacterial growth but improved it, while others specially Chlorinated hydrocarbons inhibited growth.

In this study the concentration-mortality response (Table 1) of 1st instar larvae of *H. armigera* exposed to different treatments of Costar® WP and Larvin® alone and Costar® WP in combination with LC_0, LC_1, LC_{10} and LC_{25} of Larvin® revealed that at higher dosages (20.0,40.0 and 80 μg mL^{-1} of diet) of Costar® WP alone and in combination had 100.0% larval mortality. If we compare the corrected mortalities caused by Costar® WP, Larvin® alone and in combinations (LC_0, LC_1, LC_{10} and LC_{25} of Larvin®) at low dose levels (2.5,5.0 and 10.0 μg mL^{-1} of diet), the larval mortalities were found higher in all combination of treatments than Costar® WP and Larvin® alone except at 2.5 μg mL^{-1} of Costar® WP+LC_1 and LC_{25} of Larvin® cause lower mortality 32.4±8.41 and 26.4±3.86, respectively.

Chen *et al.*[17] studied the effects of organophosphate and carbamate insecticides on *Bacillus thuringiensis* preparation against tobacco budworm larvae, *Heliothis veriscens* (F). They reported that among Carbamate insecticide, the carbaryl was found most synergistic than all others (phosmet, methomyl and carbofuran). Salama *et al.*[9] reported that Carbamates, Diflubenzuran and Disa (combination of Methomyl and Diflubenzuran) have an additive effect when applied jointly with *Bacillus thuringiensis* varieties against *Spodoptera littoralis*.

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

Costar® WP* $\mu g\ mL^{-1}$ of diet	Corrected mortality (%) ±SE					
	Costar® WP	Larvin® DF	Costar®+ Larvin® (LC_0)	Costar®+ Larvin®(LC_1)	Costar®+ Larvin® (LC_1)	Costar®+ Larvin (LC_{25})
2.5	40.2±5.36	32.6±6.44	44.0±1.49	32.4±8.41	52.1± 6.34	26.4±3.86
5.0	59.1±8.78	37.4±9.79	72.6±3.23	78.4±4.92	71.8± 5.76	68.9±4.94
10.0	89.6±4.28	68.4±8.17	96.4±3.13	100.0±0.00	91.7 ±4.39	95.8±2.46
20.0	100.0±0.00	87.6±4.37	------------	-----------	-------------	-------------
40.0	100.0±0.00	98.0±1.15	------------	-----------	-------------	-------------
80.0	100.0±0.00	100.0±0.00	------------	-----------	-------------	-------------

*Corrected for natural mortality by Abbott's[19] formula

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

Treatment Costar® $\mu g\ mL^{-1}$+Larvin®	Observed mortality (%)*	Expected mortality (%)	χ^2 value	Combined effect	Significance level
2.5+LC_0	40.2	44.0	0.40	Antagonistic	NS
5.0+LC_0	59.1	59.1	3.10	Antagonistic	NS
10.0+LC_0	89.6	96.4	0.50	Antagonistic	NS
0.5+LC_1	40.8	32.4	1.70	Antagonistic	NS
5.0+LC_1	59.5	78.4	6.00	Synergistic	0.05
10.0+LC_1	89.7	100.0	1.20	Antagonistic	NS
2.5+LC_{10}	46.2	52.1	0.80	Antagonistic	NS
5.0+LC_{10}	63.2	71.8	1.20	------do-------	NS
10.0+LC_{10}	90.6	91.7	0.01	------do-------	NS
2.5+LC_{25}	55.2	26.4	15.00	Synergistic	0.01
5.0+LC_{25}	61.7	68.9	0.84	Antagonistic	NS
10.0+LC_{25}	92.2	95.8	0.14	Antagonistic	NS

*Corrected for natural mortality by Abbott's[19] formula

Table 3: Summary of regression analysis of probit mortality of *Helicoverpa armigera* first instar larvae against log concentrations for treatment in the diet

Treatments	LC_{50} (90% CL) ($\mu g\ mL^{-1}$ diet	LC_{90}(95%CL) ($\mu g\ mL^{-1}$ diet)	Slope	Potency IU mg^{-1}	S.R/RP*	Type of action
Larvin® 80DF	5.5(3.9-7.6)	22.5(14.5-36.6)	2.1	-	------	--------
Costar® WP	3.5(1.9-6.0)	10.1(5.0-23.0)	2.7	77,600	------	--------
Costar®+Larvin® (LC_0)	2.9(2.4-3.4)	7.4(6.3-9.3)	3.2	92,000	1.2	Synergism
Costar®+Larvin® (LC_1)	3.2(2.8-3.6)	6.0(5.3-7.3)	4.7	84,800	1.09	Synergism
Costar®+ Larvin® (LC_{10})	2.6(2.0-3.1)	8.5(7.1-11.0)	2.5	1,04,000	1.3	Synergism
Costar®+Larvin® (LC_{25})	3.7(3.3-4.1)	7.7(6.8-9.3)	3.9	73,400	0.95	Antagonism

*SR/RP = Synergistic Ratio/Relative Potency[14], Note: LC_0 = 0.21 $\mu g\ mL^{-1}$, LC_1=0.42 $\mu g\ mL^{-1}$, LC_{10}=1.33 $\mu g\ mL^{-1}$ and LC_{25}=2.60 $\mu g\ mL^{-1}$

Table 2 showed that the combined effect of Larvin® at LC_0, LC_1, LC_{10} and LC_{25} with Costar® WP (*Bt*) at low doses. The combined effect indicated that Chi-square (χ^2) values are <6.0 in most of the combinations showing slow interaction of *B. thuringiensis* toward killing the pest larvae. The insect response was significant at 5.0 $\mu g\ mL^{-1}$ of Costar® WP+LC_1 of Larvin® and 2.5 $\mu g\ mL^{-1}$ of Costar® +LC_{25} of Larvin® with higher response and χ^2 values 6.0 and 15.0, respectively.

Table 3 indicated that there was an increase in mortality of the insect thereby causing synergistic interaction at Costar® +Larvin® (LC_{10}) treatment which also showed maximum potency obtained at this treatment. Present results are in agreement with Dabi *et al.*[18] who reported that combination of low rate of Dipel and sub-lethal dose of insecticides viz., Carbaryl, Endosulfan, Malathion and Monochrotophos gave synergistic response against fifth instar larvae of *H. armigera* on gram (*Cicer arietinum*).

Demo and Solsoloy[19] determined the efficacy of *Bt* (commercial preparation) with different chemical insecticide at sub-lethal dosages for controlling *Helicoverpa armigera* at field condition and reported that *Bt*+½ Recommended Rate (RR) of Thiodicarb were as effective as Deltamethrin (the check insecticide) at five days after spraying.

Combination of *Bt* with chemical insecticides has not increased efficacy in all cases and at all level. There is a need to screen all group of chemical insecticides (Pyrethroid, Carbamate, Phosphate and Chlorinated hydrocarbon etc.) with *B. thuringiensis* for compatibility and better management of insect pests in the field. If we compare the potentiation of *Bt* (Costar® WP)+Larvin® Carbamate to *Bt* (Costar® WP)+Karate, Pyrethroid against the same insect, *H. armigera*, the studies[7] indicated that chemical insecticide (Karate, i.e. pyrethroid group) is more compatible than Larvin® Carbamate to *Bt* (Costar® WP).

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