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Research article

Efficacy of new formulations of *Bacillus thuringiensis* var. *kurstaki* (HD-1) against *Helicoverpa armigera* (Hübner)

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ABSTRACT: Bioassays were conducted under laboratory conditions at Division of Entomology, Indian Agricultural Research Institute, New Delhi during 2008, to assess the efficacy of new wettable powder (WP) formulations of *Bacillus thuringiensis* var. *kurstaki* (HD-1) against III[rd] instar larvae of *Helicoverpa armigera* (Hubner). Sixteen new WP formulations of *B. thuringiensis* var. *kurstaki* (HD-1) were developed and relative efficacy was calculated on the basis of LC_{50} values. The results revealed that LC_{50} values of recipe-2 (0.032%), recipe-7 (0.018%), recipe-9 (0.030) and recipe-15 (0.015%) were highly effective against III[rd] instar larvae of H. armigera *in* comparison to Dipel® 8L (0.037%) and Biolep® (0.046%), a commercial formulation of *B. thuringiensis* var. *kurstaki*. On the basis of laboratory performance, recipe-7 and recipe-15 were selected for field efficacy against *H. armigera* in pigeonpea crop. Recipe-15 was found significantly more effective than the Dipel® 8L under field conditions.

KEY WORDS: *Bacillus thuringiensis* var. *kurstaki,* bioassay, *Helicoverpa armigera,* pigeonpea, wettable powder formulations

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INTRODUCTION

Most of the insect pathogenic bacteria were recorded from the families Bacillaceae, Pseudomonadaceae, Enterobacteaceae and Streptococcaceae (Tanada and Kaya, 1993). Members of Bacillaceae, particularly *Bacillus* species have received maximum attention as microbial control agents, which occupy 90 per cent of the world biopesticides market and is pathogenic to more than 525 insect species belonging to various orders but, mainly to Lepidopteran, Dipteran, Coleopteran and Hymenopteran (Sundrababu, 1985). *B. thuringiensis* is a rod shaped, facultative, Gram positive, crystal bearing soil borne bacterium, which is highly pathogenic to insects. *B. thuringiensis* was first time discovered in Japan in 1901 from infected larvae of silk worm, *Bombyx mori* L. by Ishiwata, and later it was isolated and identified by Berliner in 1911 (Baum *et al.*, 1999). Spores and crystals formed by *B. thuringiensis* during the vegetative and reproductive phases are used as active ingredients in commercial formulations. The crystals produced by *B. thuringiensis* comprise of one or more δ-endotoxins. These δ-endotoxins are referred to as 'cry' proteins and vary among different *B. thuringiensis* strains. Based on Cry proteins *B. thuringiensis* was classified in to 4 main pathotypes, Cry 1 effective against Lepidoptera, Cry 2 effective against Lepidoptera and Diptera, Cry 3 effective against Coleoptera and Cry 4 effective against Diptera (Hofte and Whiteley, 1989).

Many authors have reported effectiveness of *B. thuringiensis* against *H. armigera* from India and elsewhere. Use of *B. thuringiensis* is also recommended for the management of insecticide resistance in *H. armigera* on cotton (Tang, 1992). *H. armigera* is a polyphagous pest which causes extensive losses in cotton, pulses, oilseeds and certain vegetable crops in India (Chari *et al*., 1990). Though, a large number of bacterial formulations are available in the market their efficacy always remain questionable because, of the poor storage and adverse environmental impact. In the present study the freshly prepared bacterial recipes were tested against *H. armigera* and compared with a commercial formulations (Dipel and Biolep).

MATERIALS AND METHODS

Insect Materials

Larvae of *H. armigera* were collected from IARI fields and were reared on the artificial diet. Adult moths were kept in jars for oviposition. Adults were provided with 10% honey solution fortified with multivitamins as food during oviposition. Eggs were washed with sodium hypoclorite (0.16%) followed by sodium thiosulphate (10%) to ensure

proper hatching. Pupae were treated with formaldehyde (10%) for proper adult emergence.

Insecticide Materials

Active ingredient: *Bacillus thuringiensis* var. *kurstaki* (HD-1) culture was obtained from insect pathology laboratory, Division of Entomology, IARI, New Delhi. This strain was used in the preparation of different formulations.

Carriers: Eight carriers *viz*., barium sulphate, bentonite, dolomite, fuller earth, kaoline, pyrophyllite, precipate of silica and talc were used for the preparation of wettable powder (WP) formulation of *B. thuringiensis* var. *kurstaki* (HD-1).

Wetting agent: Two wetting agent *viz*., sodium lauryl sulphate (SLS) and poly ethylene glycol (PEG) were tested.

Sticking agent: One sticking agent, gum acacia (acacia powder) used.

Composition of newly developed WP formulation: Active ingredient- 10% *B. thuringiensis* var. *kurstaki* (HD-1), wetting agent- 1-10%, sticking agent- 1-10% and carrier- 50 to 90% were used.

Commercial formulation: Two commercial formulations Dipel® 8L and Biolep® were purchased from the market and used at different concentrations.

Bioassay Methods

Laboratory bioassay: Bioassay studies of WP formulations (different recipes) were carried out against third instar larvae of *H. armigera* under laboratory conditions (Table 1). A comparison was made with the Dipel® 8L and Biolep® under similar conditions. Five concentrations (10^{-1}, 10^{-3}, 10^{-5}, 10^{-7} and 10^{-10}) based on the 10% active ingredient of new WP formulations and commercially available formulations, (Dipel® 8L and Biolep®) were prepared in water and these solutions were mixed in known weight of artificial diet. Twenty IIIrd instar larvae of *H. armigera* were released in each replication and for each treatment three replications were maintained. Larvae released on untreated diet served as control. Mortality of the treated insects was recorded 24 hours onwards after treatment. Both moribund and dead larvae were counted as dead for the calculation of per cent mortality. LC_{50} values were calculated using maximum likelihood programme (Finney, 1971).

Field experiments

Field efficacy of two selected laboratory prepared formulations of *B. thuringiensis* var. *kurstaki* (HD-1) along with Dipel® 8L and Biolep® was evaluated against *H. armigera* on pigeonpea crop during *kharif* season of 2008. Crop was grown in IARI field in Randomized Block Design (RBD) and all the recommended agronomic practices were followed. UPAS-120 variety was used and a plot size of 20 sq. m. was maintained in all the three replicates. The commercial formulations (Dipel® 8L and Biolep®) along with laboratory prepared formulations were sprayed at the recommended dose (1.0 g/l of water). Ten plants were selected and tagged in each plot for observation. Observation was taken 1, 2, 4, 6, 8 and 10 days after spraying for the presence of *H. armigera* larvae. At harvest, the total

Table: 1. List of different recipes of WP formulations of *Bacillus thuringiensis* var. *kurstaki* (HD-1)

Formulation	Carrier	Wetting agent	Sticking agent
Recipe 1	Barium sulphate	SLS	Gum acacia
Recipe 2	Bentonite	SLS	Gum acacia
Recipe 3	Dolomite	SLS	Gum acacia
Recipe 4	Fuller's earth	SLS	Gum acacia
Recipe 5	Kaoline	SLS	Gum acacia
Recipe 6	Pyrophyllite	SLS	Gum acacia
Recipe 7	Precipitate of silica	SLS	Gum acacia
Recipe 8	Talc	SLS	Gum acacia
Recipe 9	Barium sulphate	PEG	Gum acacia
Recipe 10	Bentonite	PEG	Gum acacia
Recipe 11	Dolomite	PEG	Gum acacia
Recipe 12	Fuller's earth	PEG	Gum acacia
Recipe 13	Kaoline	PEG	Gum acacia
Recipe 14	Pyrophyllite	PEG	Gum acacia
Recipe 15	Precipitate of silica	PEG	Gum acacia
Recipe 16	Talc	PEG	Gum acacia

number of damaged and healthy pods was estimated and the damage percentage was calculated. Based on the data, field efficacy of the laboratory prepared formulations *vis a vis* commercial formulations was determined.

Statistical analysis

The average per cent mortality of three replications was calculated for each concentration and was corrected by Abbott' formula (1925).

$$\text{Abbott's formula Corrected per cent mortally} = \frac{T - C \times 100}{100 - C}$$

where- T: per cent mortality in treatment
C: per cent mortality in control

The data, thus, recorded were subjected to probit analysis (Finney, 1971) for calculating the LC_{50} values. Before analysis the percentage data were subjected to angular transformation.

RESULTS AND DISCUSSION

Sixteen WP formulations of *Bacillus thuringiensis* var. *kurstaki* were developed and bioassay was performed under laboratory against *Helicoverpa armigera* (Hubner) to find out the best formulation. The best formulation were selected and their bioefficacy was evaluated in comparison with Dipel® 8L and Biolep® under field conditions against *H. armigera* in pigeonpea crop.

Bioassay of laboratory prepared formulations under laboratory conditions

Bioassays were conducted with sixteen recipes of *B. thuringiensis* var. *kurstaki* formulations against third instar larvae of *H. armigera* and LC_{50} values were calculated. The LC_{50} values recorded ranged from 0.015% to 0.078% and lowest LC_{50} values were recorded in recipe-15 (0.015%) and recipe-7 (0.018%). The LC_{50} values (%) of different recipes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 and 16 were 0.051, 0.032, 0.042, 0.052, 0.063, 0.078, 0.018, 0.036, 0.030, 0.053, 0.037, 0.038, 0.040, 0.061, 0.015 and 0.054 respectively (Table-2). The less LC_{50} values of recipe-15 (0.015%) and recipe-7 (0.018%) indicated the high potential of these formulations against *H. armigera*. Hence, these two recipes were selected for further field evaluation against *H. armigera* on pigeon pea crop.

Bioassay of commercial formulation under laboratory conditions

The LC_{50} values of commercial formulation Dipel® 8L and Biolep® was calculated as 0.037% and 0.046% respectively (Table 2).

Table 2. LC_{50} values of different recipes of WP formulations of *Bacillus thuringiensis* var. *kurstaki* (HD-1)

Formulations	LC_{50} (%)	Eterogeneity dfχ2 (5)	Regression equation Y= a + bx	Slop ± S. Em.	Fiducial limits	
					Lower	Upper
Recipe 1	0.051	2.821	6.2582 + 0.9821x	0.982 ± 0.1226	0.041	0.067
Recipe 2	0.032	2.753	6.2755 + 08509x	0.850 ± 0.1207	0.022	0.042
Recipe 3	0.042	3.204	6.0955 + 0.7935x	0.793 ± 0.1191	0.029	0.056
Recipe 4	0.052	0.907	6.2061 + 0.9406x	0.940 ± 0.1217	0.040	0.068
Recipe 5	0.063	5.434	6.1497 + 0.9550x	0.955 ± 0.1221	0.049	0.082
Recipe 6	0.078	2.640	6.0740 + 0.9685x	0.968 ± 0.1229	0.061	0.103
Recipe 7	0.018	2.018	6.9504 + 1.1174x	1.117 ± 0.1318	0.012	0.024
Recipe 8	0.036	0.867	6.7598 + 1.2153x	1.215 ± 0.1292	0.028	0.044
Recipe 9	0.030	6.169	6.9651 + 1.2924x	1.292 ± 0.1326	0.024	0.037
Recipe 10	0.053	1.221	6.1385 + 0.8873x	0.887 ± 0.1207	0.039	0.068
Recipe 11	0.037	5.580	6.3525 + 0.9474x	0.947 ± 0.1223	0.028	0.048
Recipe 12	0.038	1.476	6.7213 + 1.2103x	1.210 ± 0.1288	0.030	0.046
Recipe 13	0.040	6.220	6.6690 + 1.1953x	1.195 ± 0.1281	0.032	0.049
Recipe 14	0.061	4.461	6.0344 + 0.8531x	0.853 ± 0.1201	0.046	0.082
Recipe 15	0.015	0.651	6.7144 + 0.9420x	0.942 ± 0.1275	0.009	0.021
Recipe 16	0.054	0.622	5.6893 + 0.5398x	0.539 ± 0.1155	0.032	0.084
Dipel® 8L	0.037	4.304	6.4688 + 1.0272x	1.027 ± 0.1241	0.028	0.047
Biolep®	0.046	3.987	6.5571 + 1.1613x	0.161 ± 0.1269	0.036	0.056

Y = Probit kill, X = Log concentration, LC_{50} = Concentration calculated to give 50 per cent mortality, a = Intercept, b = Slop.

Table: 3. **Effectiveness of laboratory prepared *Bacillus thuringiensis* var. *kurstaki* (HD-1) WP formulations against *Helicoverpa armigera* on pigeonpea during *kharif* 2008 season.**

Formulation	Healthy pods%	Damaged pods%	Yield (Qt/ha)
Recipe - 7	87.97 (69.71)	12.03 (20.29)	11.43
Recipe -15	92.60 (74.21)	7.40 (15.79)	12.04
Dipel® 8L	78.31 (62.24)	21.69 (27.76)	10.18
Control	35.49 (36.57)	64.51 (53.43)	4.61
F test	Significant	Significant	
S. Ed	1.5925	2.6524	
C D ($P = 0.05$)	3.8969	6.4905	
C D ($P = 0.01$)	5.9043	9.8339	
CV%	3.18	11.13	

Figures in parenthesis are angular transformed values

Field testing of laboratory prepared WP formulations of *B. thuringiensis* var. *kurstaki*

Based on laboratory performance recipe-7 and recipe-15 were selected for field efficacy and trials were conducted with Dipel® 8L against the natural incidence of *H. armigera* larvae on pigeon pea crop. All the formulations were used @ 1 g/liter of water and control plots were sprayed with water. All the treatments proved to be significantly superior over control. Plots treated recipe-15 (92.62%) registered highest number of healthy pods followed by plots treated with recipe-7 (87.97%) and Dipel® 8L (78.31%) as compared to 35.49% in control plots. Highest yield was recorded with recipe-15 (12.04 Q/ha) followed by 11.43 Q/ha in recipe-7 and 10.18 Q/ha in Dipel treated plots as compared to 4.61 Q/ha in control plots (Table 3).

The freshly prepared laboratory formulations were found effective against *H. armigera* as compare to the commercial formulations. There are reports available about the better efficacy of HD-1 in comparison to HD-73 (Salama *et al.,* 1983). The commercial formulations are effective against different instars of *H. armigera*. Gaikwad *et. al.,* (1998) reported the LC_{50} of commercial formulation, Delfin® against IInd, IVth and Vth instar larvae of *H. armigera* as 2.17, 5.78 and 10.08 ml/lit after 5 days of application. Jeyakumar and Gupta (1999) used 0.04% Biobit® and observed 100% mortality of IInd instar larvae of *H. armigera* within 72 hrs of treatment. Chandra *et al.* (1999) evaluated the effectiveness of *B. thuringiensis* based products Biobit® Biolep® and Dipel® 8L against IIIrd instar larvae of *H. armigera* and reported the LC_{50} values as 0.114,0.21 and 0.213 respectively. Similarly, Reddy *et al.* (1997) reported that LC_{50} values for *B. thuringiensis* var. *kurstaki* against larvae of *H. armigera* was 0.023% when commercial formulation Delfin® was used. Formulations of strain HD-1 caused 56% mortality in *H. armigera*, which was marginally superior than chemical insecticide endosulfan (0.07 per cent) and performed superior than chemical insecticides (Gupta *et al*., 2000). Battu and Arora (1997) evaluated the Dipel® 8L and Biobit® in field against *Plutella xylostella* on mustard crop. Dipel® 8L and Biobit® treated plots were yielded 90.00% and 89.60% healthy pods as compared to 47.56% in control plots. Similarly, Gopalkrishnan and Gangavisalakshy (2005) tested Dipel® 8L, Delfin®, Halt® and Biobit® for field efficacy against *Papillio demoleusu* on citrus and observed that the applications at 1 kg/ha effectively controlled the larval population of *P. demoleus* on citrus.

The differences in observed values and literature values were due to the differences in the composition of commercial formulation, bioassay method and experimental conditions. The use of *B. thuringiensis* appeared to be the most appropriate proposition in the recent concept of integrated pest management due to its safety to human being and non-target organisms and easily grown on artificial media without loss of virulence and being spore formers are capable of enduring for years in storage.

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