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Research Article

Effect of carriers on the efficacy of *Bacillus thuringiensis* var. *kurstaki* (HD-1) formulations

DINESH SWAMI* and BISHWAJEET PAUL
Division of Entomology, Indian Agricultural Research Institute, New Delhi 110 012, India
** Corresponding author: E-mail: dswami6559@gmail.com*

ABSTRACT: Effect of carriers on the efficacy of new wettable powder (WP) formulations of *Bacillus thuringiensis* var. *kurstaki* (HD-1) was evaluated at Division of Entomology, Indian Agricultural Research Institute, New Delhi during 2006-2008. Sixteen wettable powder (WP) formulations were developed by using eight carriers *viz*., barium sulphate, bentonite, dolomite, fuller earth, kaoline, talc, precipitate of silica and pyrophyllite. Six physico-chemical properties i.e. bulk density, carrier reaction (pH), cation exchange capacity; moisture content, sorptivity and specific surface area of the carriers were studied. The formulations were evaluated against III[rd] instar larvae of *Helicoverpa armigera* (Hubner) and based on the LC_{50} values; formulation-7 (0.018%) and formulation-15 (0.015%) were found to be highly effective. The formulation-7 and formulation-15 contained precipitate of silica as carrier and we report that and it proved to be best carrier for preparation the WP formulation of *B. thuringiensis* var. *kurstaki.*

KEY WORDS: *Bacillus thuringiensis* var. *kurstaki,* carriers, *Helicoverpa armigera,* physico-chemical properties, wettable powder formulations.

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INTRODUCTION

The utilization of entomopathogens as biocontrol agents for insect pests is gaining much importance recently. The problems of stability of biopesticides both during storage and after application have stalled biopesticides development to a great extent (Tsuji, 1997). A typical formulation of bio-pesticide consists of a predetermined amount of biocide (insecticidal crystal protein, spores and fermentation solids) as active ingredient and certain adjuvants (carriers, wetting agents and sticking agents). The adjuvants are chemically and biologically active compounds that provide requisite physico-chemical properties for the formulation and confer the maximum bioefficacy of the product (Rodham *et al*., 1999). Carriers serve as vehicle for conveying the active ingredient to its place of action, is not truly inert but contribute towards the overall efficacy of the formulations due to its physic-chemical properties (Gooden, 1953). Choice of carriers is important as their physical and physico-chemical properties such as particle size, bulk density, pH, sorption capacity and electrical charges, affect the performance of toxic compounds in the formulations (Guenzi, 1974; Jepson, 1976). The important characteristics relating to application of clay minerals in the microbial formulations are particle size, surface properties, reaction, particle shape, surface area and other physical and chemical properties specific to a particular application (Murray, 1991). Stability, toxicity and compatibility of a toxicant in the formulation are affected by the pH, flowability and cation exchange capacity of the carriers (Van Valkenburg, 1969). Moisture play important role in the carrier insecticide compatibility, rate of toxicant decomposition is slower on clays containing more absorbed water (Fowkes *et al*., 1960). Sorption capacity and specific surface area are very important for any inert material to initially absorb the toxicant during production (Polon, 1973).

B. thuringiensis is a rod shaped, facultative, gram positive, crystal bearing soil borne bacterium, which is highly pathogenic to insects, and its growth has two phases; vegetative and reproductive. During reproductive phase it produces spore and one or more crystalline parasporal inclusions, known as crystal which is toxic to the insects. The crystals produced by *B. thuringiensis* comprise of one or more ä-endotoxins, these ä-endotoxins are referred to as cry proteins (Hofte and Whiteley, 1989). The spore crystal complex serves as active ingredient in the formulation. *H. armigera* (Hubner) is a polyphagous pest, causes extensive losses in cotton, pulses, oilseeds and certain vegetable crops in India (Chari *et al*., 1990). Present studies were undertaken to select most suitable carriers for preparation of WP formulations of *B. thuringiensis* var. *kurstaki* (HD-1) for use against Lepidopteran insects.

MATERIALS AND METHODS

Physico-chemical properties of the test carriers

The eight carries *viz.*, barium sulphate, bentonite, dolomite, fuller earth, kaolinite, talc, precipitate of silica and pyrophyllite were selected for preparing the WP formulations of *B. thuringiensis* var. *kurstaki* (HD-1) and six physico-chemical properties i.e. bulk density, carrier reaction (pH), cation exchange capacity, moisture content, sorptivity and specific surface area of the carrier were investigated for selection of the most suitable carrier.

Bulk Density

Bulk density before and after compaction of the carrier materials was determined as per ISI-IS 6940:1981.

$$\text{Bulk Density } \left(\frac{g}{100}\text{ cc}\right) \text{ (before compaction)}$$

$$\frac{\text{Weight of the carrier x 100}}{\text{Volume occupied by the same carrier before compact}}$$

$$\text{Bulk Density } \left(\frac{g}{100}\text{ cc}\right) \text{ (after compaction)}$$

$$\frac{\text{Weight of the carrier x 100}}{\text{Volume occupied by the same carrier before compact}}$$

Carrier Reaction (pH)

One per cent suspension of the carrier was prepared in fresh distilled water and pH of the suspension was measured with dynamic digital pH meter (CIPAC, 1970).

Cation Exchange Capacity (CEC)

Five grams carrier was weighed and transferred into centrifuge tube and 33 ml of 1N sodium acetate added, centrifuged for 5 minutes at 6000 rpm to settle the colloidal particles. Supernatant solution with replaced cations (replaced by Na ions) was removed. Then the material was centrifuged for 5 minutes at 6000 rpm with 35 ml of 95% ethyl alcohol to remove excess of sodium acetate solution. It was again centrifuged with 33 ml of ammonium acetate and supernatant liquid was collected in 100 ml volumetric flask. Volume in the flask was made up to the mark and exchangeable sodium was determined by flame photometer (Bower *et al*., 1952).

Moisture content

Determined by gravimetric method, a known weight of carrier was spread uniformly in a previously weighted aluminium cup and placed in an oven at 105 ± 1°C for 24 hrs, after which the cup was taken out and cooled in a dessicator containing fused calcium chloride. The cup with dry carrier was re-weighed till its constant weight and loss in weight was noted and per cent moisture content in the carrier calculated on the oven dry basis.

Sorptivity

Per cent sorptivity was determined by the ASTM rubout method (Anonymous, 1966). Known weight of carrier was taken and linseed oil was added drop by drop and mixes with spatula until the powder slipped freely from the tip of the spatula. The volume of the linseed oil absorbed by the carrier was noted and sorptivity was calculated as:

$$\text{Sorptivity (\%)} \quad \frac{\text{Ml of linseed oil required to slip the material from the spatula x 0.93 x 100}}{\text{Weight of the carrier taken}}$$

Specific Surface Area

Required amount of carrier were taken into small plastic boxes and dried over phosphorus pentoxide to its constant weight. Ethylene glycol monoethyl ether (EGME) was added to saturate the carrier and then dried to constant weight over phosphorus pentoxide (Carter *et al*., 1965) and specific surface area was calculated as:

$$\text{Specific Surface Area}\left(\frac{m^2}{g}\right)\frac{\text{Weight of EGME absorbed by carrier x 2.86 x 104}}{\text{Weight of the carrier taken}}$$

Preparation of WP formulations of *Bacillus thuringiensis* var. *kurstaki*

Sixteen WP formulations of *B. thuringiensis* var. *kurstaki* were prepared by mixing 10 per cent dry powder of *B. thuringiensis* var. *kurstaki* active ingredient**.** Barium sulphate, bentonite, dolomite, fuller earth, kaolin, pyrophyllite, precipitate of silica and talc were used as carriers. Gum acacia was used as sticking agent whereas sodium lauryl sulphate (SLS) and poly ethylene glycol (PEG) were used as wetting agents.

Laboratory bioassay

Bioassay studies of sixteen WP formulations *Bacillus thuringiensis* var. *kurstaki* were carried out against third instar larvae of *Helicoverpa armigera.* The insect culture was maintained in the laboratory on a semi-synthetic diet. Five concentrations (10^{-1}, 10^{-3}, 10^{-5}, 10^{-7} and 10^{-10}) based on the 10% active ingredient of new WP formulations were prepared in distilled 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).

Statistical analysis

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

$$\text{Abbott's formula: Corrected percent mortality} = \frac{T - C \times 100}{100 - C}$$

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

Physico-chemical properties of the test carriers

Physic-chemical properties of the test carriers *viz,* bulk density, cation exchange capacity, carrier reaction, moisture content, sorptivity and specific surface area were carried out for screening the best carriers for preparation of WP formulations of *Bacillus thuringiensis* var. *kurstaki* (Table 1). Bulk density values (g/100 cc) were estimated before and after the compaction and it ranged from 9.20 to 126.50, 11.53 to 165.39 respectively. Bulk density of different carriers *viz.,* barium sulphate (63.15, 110.50), dolomite (126.50, 165.39), talc (64.50, 93.15), fuller earth (55.20, 79.10), bentonite (72.59, 95.85), pyrophyllite (58.55, 75.50), kaoline (51.10, 64.40) and precipitate of silica (9.20, 11.53) were recorded before and after the compaction. Precipitate of silica proved to be the best, because light carriers provide more dispersibility as compared to heavier carriers. Cation Exchange Capacity (CEC) (meq/100 gm) ranged from 0.82 to 18.50 for the carriers *viz,* bentonite (18.50), precipitate of silica (1.30), pyrophyllite (1.25), talc (1.15), kaoline (1.10), fuller earth (0.93), barium sulphate (0.85) and dolomite (0.82). Bentonite and precipitate of silica proved to be the best, because higher CEC more the buffering capacity. With regards to pH, precipitate of silica was found to be neutral (7.1) and was ideal, because neutral pH is not detrimental to biological material. Dolomite was highly alkaline (8.7) and fuller's earth highly acidic (4.2). pH of others were*,* kaolin (8.4), bentonite (8.2), talc (7.9), pyrophyllite (6.6) and barium sulphate (6.3). Highest moisture was recorded in dolomite (8.20%) and lowest in precipitate of silica (0.55%). Moisture content in other carriers *viz,* bentonite (5.40%), barium sulphate (1.60%), fuller earth (1.20%), kaoline

Table 1. Physico-chemical properties of the test carriers

Carrier	Bulk density (g/100 cc)		Carrier reaction (pH)	Cation exchange capacity (meq/100g)	Moisture content (%) (m^2/g)	Sorptivity (%)	Specific surface area
	Before	After					
Baruim sulphate	63.15	110.50	6.3	0.85	1.60	27.18	160.16
Bentonite	72.59	95.85	8.2	18.50	5.40	42.06	265.96
Dolomite	126.50	165.39	8.7	0.82	8.20	24.90	217.36
Fuller earth	55.20	79.10	4.2	0.93	1.20	79.98	214.64
Kaoline	51.10	64.40	8.4	1.10	1.15	38.78	223.08
Pyrophyllite	58.55	75.50	6.6	1.25	1.05	82.54	245.52
Precipitate of silica	9.20	11.53	7.1	1.30	0.55	104.86	1430.70
Talc	64.50	93.15	7.9	1.15	0.90	36.65	231.56

Table 2. Effect of different carriers on the efficacy of WP formulation of *B. thuringiensis* var. *kurstaki*

Formulations	Carriers	Wetting agent	Sticking agent	LC_{50} (%)	Heterogeneity dfχ^2 (5)	Regression equation Y= a + bx	Fiducial limits	
							Lower	Upper
Formulation-1	Barium sulphate	Sodium lauryl sulphate	Gum acacia	0.051	2.821	6.2582 + 0.9821x	0.041	0.067
Formulation-2	Bentonite	Sodium lauryl sulphate	Gum acacia	0.032	2.753	6.2755 + 08509x	0.022	0.042
Formulation-3	Dolomite	Sodium lauryl sulphate	Gum acacia	0.042	3.204	6.0955 + 0.7935x	0.029	0.056
Formulation-4	Fuller earth	Sodium lauryl sulphate	Gum acacia	0.052	0.907	6.2061 + 0.9406x	0.040	0.068
Formulation-5	Kaoline	Sodium lauryl sulphate	Gum acacia	0.063	5.434	6.1497 + 0.9550x	0.049	0.082
Formulation-6	Pyrophyllite	Sodium lauryl sulphate	Gum acacia	0.078	2.640	6.0740 + 0.9685x	0.061	0.103
Formulation-7	Precipitate of silica	Sodium lauryl sulphate	Gum acacia	0.018	2.018	6.9504 + 1.1174x	0.012	0.024
Formulation-8	Talc	Sodium lauryl sulphate	Gum acacia	0.036	0.867	6.7598 + 1.2153x	0.028	0.044
Formulation-9	Barium sulphate	Poly ethylene glycol	Gum acacia	0.030	6.169	6.9651 + 1.2924x	0.024	0.037
Formulation-10	Bentonite	Poly ethylene glycol	Gum acacia	0.053	1.221	6.1385 + 0.8873x	0.039	0.068
Formulation-11	Dolomite	Poly ethylene glycol	Gum acacia	0.037	5.580	6.3525 + 0.9474x	0.028	0.048
Formulation-12	Fuller earth	Poly ethylene glycol	Gum acacia	0.038	1.476	6.7213 + 1.2103x	0.030	0.046
Formulation-13	Kaoline	Poly ethylene glycol	Gum acacia	0.040	6.220	6.6690 + 1.1953x	0.032	0.049
Formulation-14	Pyrophyllite	Poly ethylene glycol	Gum acacia	0.061	4.461	6.0344 + 0.8531x	0.046	0.082
Formulation-15	Precipitate of silica	Poly ethylene glycol	Gum acacia	0.015	0.651	6.7144 + 0.9420x	0.009	0.021
Formulation-16	Talc	Poly ethylene glycol	Gum acacia	0.054	0.622	5.6893 + 0.5398x	0.032	0.084

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

(1.15%), pyrophyllite (1.05%) and talc (0.90%) were recorded. Precipitate of silica proved to be the best, because low moisture content carriers are free from anti caking properties. Highest per cent sorptivity was recorded for precipitate of silica (104.86%) followed by pyrophyllite (82.52%), fuller earth (79.98%), bentonite (42.06%), kaolin (38.78%), talc (36.65%), barium sulphate (27.18%) and dolomite (24.90%). Specific surface area SSA (m^2/ gm) ranged from 160.16m^2/gm to 1430.70 m^2/gm. SSA of different carriers *viz*, precipitate of silica (1430.70 m^2/gm), bentonite (265.96 m^2/gm), pyrophyllite (245.52 m^2/gm), talc (231.56 m^2/gm), kaolin (223.08 m^2/gm), dolomite (217.36 m^2/gm), fuller earth (214.64 m^2/gm) and barium sulphate (160.16 m^2/gm) were recorded. Precipitate of silica proved to be best carrier, because more specific area higher the absorption of toxin.

Effect of different carriers on the efficacy of WP formulations of *Bacillus thuringiensis* var. *kurstaki* formulated with Sodium lauryl sulphate as a wetting agent

Eight WP formulations of *B. thuringiensis* var. *kurstaki* were prepared by combination of different carriers *viz*, barium sulphate, bentonite, dolomite, fuller earth, kaolin, talc, precipitate of silica and pyrophyllite with SLS as a wetting agent and gum acacia as a sticking agent. Lowest LC_{50} value against IIIrd instar larvae of *H. armigera* were recorded with the formulation-7 (0.018%). LC_{50} values of different formulations ranged from 0.018% to 0.078%. Highest LC_{50} value (0.078%) was recorded with formulation-6, in which pyrophyllite was used as a carrier. Precipitate of silica as a carrier was used for the preparation of formulation-7 and it proved to be best carrier for preparing the WP formulation of *B. thuringiensis* var. *kurstaki* (Table 2).

Effect of different carriers on the efficacy of WP formulations of *B. thuringiensis* var. *kurstaki* formulated with PEG as a wetting agent and gum acacia as a sticking agent was tested against IIIrd instar larvae of *H. armigera* by synthetic diet incorporation method in laboratory. Lowest LC_{50} (0.015%) was recorded with the formulation-15 where precipitate of silica was used as a carrier. LC_{50} values for other formulations ranged from 0.015% to 0.061% and least effective was pyrophyllite (formulation-14) which showed LC_{50} value of 0.061% (Table 2). The survival of microbes depends on the proper selection of carrier and carrier compatibility is of great significance while preparing microbial formulations (Farmery, 1976). Observations of Rengasamy and Parmar (1988) are in agreement with the present findings; they reported that carriers having high specific surface area were better for the preparation of *B. thuringiensis* formulations. Halder and Parmar (1984) reported 566 m^2/g specific surface area of bentonite, similarly, Prasad (2003) reported the specific surface area of barium sulphate, dolomite, pyrophyllite and precipitate of silica was 139.82 m^2/g, 215.27 m^2/g, 232.10 m^2/g and 1376.80 m^2/g respectively and observed that the formulations containing precipitate of silica as carrier showed better efficacy against *H. armigera.* Moisture also plays important role in the carrier insecticide compatibility and rate of toxicant decomposition will be slower on clays containing more absorbed water (Fowkes *et al*., 1960). Moisture content (%) values for fuller earth, dolomite, barium sulphate and kaolin was 0.75%, 7.0%, 0.90% and 0.85% respectively and lower moisture content in the carriers are better suited for preparation of WDP formulations of the *B. thuringiensis* (Prasad, 2003). Powar (1992) reported the bulk density of bentonite and talc before and after the compaction was 85.35 g/100, 103.88 g/100 and 81.01 g/100 , 98.19 g/100 respectively and opined that the carrier having low bulk density are better for preparation of formulations wheb composed to bulky carriers. Similarly, Prasad (2003) reported the bulk density for precipitate of silica as 8.00 g/100 cc and 9.31g/100 cc and for barium sulphate 78.00 g/100 and 105.40 g/100 before and after the compaction respectively and found that the formulations containing precipitate of silica as carrier showed better efficacy against *H. armigera.* Cation exchange capacity for kaolin (24.02 meq/100 gm) and fuller earth (96.24 meq/100 gm) were reported by Kumar and Parmar (1999). Similarly, Prasad (2003) reported the CEC for fuller earth, pyrophyllite and talc as 0.82, 1.18 and 1.08 meq/100 gm respectively and he opined that fuller earth was most suitable. Powar (1992) reported the sorptivity (%) values for bentonite and talc was 67% and 63% respectively and observed the carriers having higher sorptivity are better than lower sorptivity carriers for preparation of the formulation. Prasad (2003) reported the sorptivity of barium sulphate, fuller earth, pyrophyllite and precipitate of silica was 22.32%, 78.12%, 70.60% and 92.92% respectively and also observed that formulations containing precipitate of silica as a carrier show better efficacy against *H. armigera*.

We report that precipitates of silica based WP formulations of *B. thuringiensis* were consistently superior in performance against *H. armigera* larvae in laboratory. The Physico-chemical properties of each clay mineral determine to a large extent the applications of that particular clay. The formulations thus developed and tested have immense scope for large scale development of WP formulations of *B. thuringiensis.*

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