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

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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 of *B. thuringiensis* var. *kurstaki* (HD-1) were developed using eight carriers namely barium sulphate, bentonite, dolomite, fuller's 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. Among the carrier tested, precipitate of silica was found to be the most suitable for preparation of WP formulation of HD-1. Efficacy of new formulations was evaluated. LC_{50} values of formulation-7 (0.018%) and formulation-15 (0.015%) were highly effective against III instar larvae of *H. armigera* (Hubner).

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

1. Introduction

The utilization of entomopathogens as biocontrol agents for insect pests is gaining much importance recently. The problems associated with stability of biopesticides both during storage and after application have stalled biopesticides development to a great extent [1]. The development of a suitable formulation is essential for successful utilization of bio-pesticides. A typical formulation of bacterial 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 can alter the physical properties of formulation, enhance its selectivity and reduce the dose of effective biopesticides required [2]. Adjuvants provide requisite physico-chemical properties of formulation and confer the maximum bioefficacy to the product. Carriers act like vehicle, a means of 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 [3]. Choice of carriers is important as their physico-chemical properties such as particle size, bulk density, pH, sorptive capacity and electrical charges, affect the performance of toxic compound in the formulations [4-5]. 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 [6]. Stability, toxicity and compatibility of a toxicant in the formulation are affected by the pH, flow ability and cation exchange capacity of the carriers [7]. Moisture also play important role in the carrier insecticide compatibility, rate of toxicant decomposition to be slower on clays containing more absorbed water [8]. Sorptive capacity and specific surface area are most important characteristic of any inert for initially absorbing the toxicant during production of the products [9].

B. thuringiensis is a rod shaped, facultative, gram positive, crystal bearing soil borne bacterium, which is highly pathogenic to insects and its develop in vegetative and reproductive phases. During reproductive phase, it produces spore and one or more crystalline parasporal inclusions known as crystal. The crystals produced by *B. thuringiensis* comprise of one or more δ-endotoxins referred as cry proteins. Based on Cry proteins *B. thuringiensis* was classified into 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 [10].

Helicoverpa armigera (Hubner) is a polyphagous pest, causes extensive losses in cotton, pulses, oilseeds and certain vegetable crops in India [11]. The present study was investigated for the selection of suitable carriers for the preparation of best WP formulations of *B. thuringiensis* var. *kurstaki* (HD-1).

2. Materials and methods

2.1 Physico-chemical properties of the test carriers

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. The eight carries namely barium sulphate, bentonite, dolomite, fuller earth, kaoline, talk, precipitate of silica and pyrophyllite were taken for preparing the WP formulations of *Bacillus 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 the best carriers.

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

$$\text{Bulk Density (g/100 cc)(before compaction)}: \frac{\text{Weight of the carrier} \times 100}{\text{Volume occupied by the same carrier before compaction}}$$

$$\text{Bulk Density (g/100 cc)(after compaction)}: \frac{\text{Weight of the carrier} \times 100}{\text{Volume occupied by the same carrier after compaction}}$$

2.3 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 [12].

2.4 Cation exchange capacity (CEC): Five grams carrier was weighted and transferred into centrifuge tube by adding 33 ml of 1N sodium acetate, centrifuged for 5 minutes at 6000 rpm to settle the colloidal particles at the bottom for clear supernatants solution. Supernatant solution with replaced cations (replaced by Na ions) was removed. Afterwards, material was centrifuged for 5 minutes at 6000 rpm with 35 ml of 95 per cent 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 [13].

2.5 Moisture content: It was 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 ºC for 24 hrs, after which the cup was taken out and cooled in a dessicat or containing fused calcium chloride. The cup with dry carrier was re-weighed till its constant weight and loss in weight was recorded and per cent moisture content was calculated.

2.6 Sorptivity: Per cent sorptivity was determined by the ASTM (American Society for Testing and Materials) rubout method [14]. Known weight of carrier was taken and linseed oil was added drop by drop and mixed 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:

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

2.7 Specific surface area: Required amount of carrier was taken in 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 [15] and specific surface area was calculated as:

$$\text{Specific Surface Area } (m^2/g): \frac{\text{Weight of EGME absorbed by carrier} \times 2.86 \times 10^4}{\text{Weight of the carrier taken}}$$

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

Sixteen WP formulations of *Bacillus 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, kaoline, pyrophyllite, precipitate of silica and talc were used as carriers. Gum acacia as a sticking agent and sodium lauryl sulphate (SLS) and poly ethylene glycol (PEG) were used as a wetting agents.

2.9 Laboratory bioassay

Bioassay studies of sixteen WP formulations of *B. thuringiensis* var. *kurstaki* were carried out against third instar larvae of *H. armigera* maintained in the laboratory on a semi-synthetic diet. Five concentrations (10^{-1}, 10^{-3}, 10^{-5}, 10^{-7} and 10^{-10}) of new WP formulations were prepared in distilled water and same were mixed in known weight of artificial diet. Twenty III instar larvae of *H. armigera* were released in each replication and for each treatment and three replications were maintained. Larvae released on untreated diet served as control. Larval mortality was recorded 24 hours after treatment. Both moribund and dead larvae were counted as dead. LC_{50} values were calculated using maximum likelihood programme [16].

2.10 Statistical analysis

The average per cent mortality of three replications was calculated for each concentration and was corrected by Abbott' formula (1925) [17].

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

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.

3. Results and discussion

3.1 Physico-chemical properties of the test carriers

Physico-chemical properties of the test carriers namely 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 the better WP formulations of *Bacillus thuringiensis* var. *kurstaki* (Table-1).

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 (%)	Sorptivity (%)	Specific surface area (m^2/g)
	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's 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

3.2 Bulk Density: Bulk density recorded before and after compaction ranged from 9.20 to 126.50, 11.53 to 165.39, respectively. Bulk density of different carriers namely barium sulphate (63.15, 110.50), dolomite (126.50, 165.39), talc (64.50, 93.15), fuller's 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.

3.3 Cation exchange capacity (CEC): CEC (meq/100 gm) ranged from 0.82 to 18.50. CEC of different carriers namely bentonite (18.50), precipitate of silica (1.30), pyrophyllite (1.25), talc (1.15), kaoline (1.10), fuller's earth (0.93), barium sulphate (0.85) and dolomite (0.82) were recorded. Bentonite and precipitate of silica proved to be the best because of higher CEC that result into more the buffering capacity.

3.4 Carrier reaction (pH): Precipitate of silica was found to be nearly neutral (7.1) and it's proved to be best, because neutral pH is not detrimental to biological material. Dolomite found highly alkaline (8.7) and fuller's earth highly acidic (4.2). Carrier reaction (pH) of other carriers namely kaoline (8.4), bentonite (8.2), talc (7.9), pyrophyllite (6.6) and barium sulphate (6.3) were analysed.

3.5 Moisture content: Highest moisture was recorded in dolomite (8.20%) and lowest in precipitate of silica (0.55%). Moisture content in other carriers namely bentonite (5.40%), barium sulphate (1.60%), fuller's earth (1.20%), kaoline (1.15%), pyrophyllite (1.05%) and talc (0.90%) were recorded. Precipitate of silica proved to be the best because of low moisture content and carriers were free from anti-caking properties.

3.6 Sorptivity: Highest per cent sorptivity was recorded for precipitate of silica (104.86%) followed by pyrophyllite (82.52%), fuller's earth (79.98%), bentonite (42.06%), kaoline (38.78%), talc (36.65%), barium sulphate (27.18%) and dolomite (24.90%).

3.7 Specific surface area (SSA): Specific surface area (m^2/ gm) ranged from 160.16 to 1430.70 m^2/gm. SSA of different carriers namely 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), kaoline (223.08 m^2/gm), dolomite (217.36 m^2/gm), fuller's earth (214.64 m^2/ gm) and barium sulphate (160.16 m^2/gm) were recorded. Precipitate of silica proved to be best carrier, having more specific area that resulted in higher absorption of toxin.

3.8 Effect of different carriers on the efficacy of WP formulations of *B. thuringiensis* var. *kurstaki* formulated with SLS as a wetting agent

Eight WP formulations of *Bacillus thuringiensis* var. *kurstaki* were prepared by combination of different carriers namely barium sulphate, bentonite, dolomite, fuller's earth, kaoline, talc, precipitate of silica and pyrophyllite with sodium lauryl sulphate (SLS) as a wetting agent and gum acacia as a sticking agent. Lowest LC_{50}values against III instar larvae of *H. armigera* were recorded in formulation-7 (0.018%) in comparison to the combination of the other carriers. LC_{50} values of different formulations ranged from 0.018 to 0.078 per cent. Highest LC_{50}values (0.078%) were recorded in 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 preparation the WP formulation of *B. thuringiensis* var. *kurstaki* (Table-2).

3.9 Effect of different carriers on the efficacy of WP formulations of *B. thuringiensis* var. *kurstaki* formulated with PEG as a wetting agent

Eight WP formulations of *B. thuringiensis* var. *kurstaki* were prepared by combination of different carriers namely barium sulphate, bentonite, dolomite, fuller's earth, kaoline, talc, precipitate of silica and pyrophyllite with poly ethylene glycol (PEG) as a wetting agent and gum acacia as a sticking agent. Bioassay was conducted on III instar larvae of *H. armigera* by synthetic diet incorporation method in laboratory. Lowest LC_{50} values (0.015%) were recorded for formulation-15 than the combination of the other carriers. Precipitate of silica as a carrier was used for preparation the formulation-15, its proved to be the best carrier for preparation the WP formulation of *B. thuringiensis* var. *kurstaki*. LC_{50} values of different formulations ranged from 0.015 to 0.061%. Highest LC_{50} values (0.061%) were recorded in formulation-14 in which pyrophyllite used as a carrier (Table-2).

The survival of microbial load depends on the proper selection of carrier and carrier compatibility is of great significance in preparing of microbial formulations [18]. Findings of [19] are agreement with the present findings who reported that carriers having high specific surface area were better for the preparation of *B. thuringiensis* formulations. [20] reported 566m^2/g specific surface area of bentonite. Similarly, [21] reported that specific surface area of barium sulphate, dolomite, pyrophyllite and precipitate of silica was 139.82m^2/g, 215.27m^2/g, 232.10m^2/g and 1376.80m^2/g respectively and observed the formulations contain precipitate of silica as a carrier show better efficacy against *H. armigera* under laboratory in comparison to other carriers. Moisture play important role in the carrier insecticide compatibility, rate of toxicant decomposition is slower on clays containing more absorbed water [8]. Moisture content (%) values for fuller's earth, dolomite, barium sulphate and kaoline was 0.75, 7.0, 0.90 and 0.85 per cent, respectively and it was reported the lower moisture content in the carriers are better for preparation the WDP formulations of the *B. thuringiensis* [21]. [22] reported the bulk density of bentonite and talc before and after the compaction was 85.35 g/100, 103.88g/100 and 81.01g/100, 98.19g/100, respectively and reported the carrier having low bulk density are better for preparation the formulations in comparison to bulky carriers. Similarly, [21] reported the bulk density for precipitate of silica 8.00 g/100 cc and 9.31g/100 cc and for barium sulphate 78.00g/100 and 105.40g/100 before and after the compaction, respectively and reported the formulation contain precipitate of silica as a carrier show better efficacy against *H. armigera* in comparison to other carriers. Cation exchange capacity for kaoline (24.02 meq/100 gm) and fuller's earth (96.24meq/100 gm) were reported by the [23]. Similarly, [21] reported the CEC for fuller earth, pyrophyllite and talc as 0.82, 1.18 and 1.08 meq/100 gm, respectively and reported that the fuller's earth was found to be best for *B. thuringiensis* formulation. [22] reported the sorptivity (%) values for bentonite and talc was 67 and 63 per cent, respectively and observed the carriers having higher sorptivity are better than lower sorptivity carriers for preparation the formulation. [21] Reported the sorptivity of barium sulphate, fuller's earth, pyrophyllite and precipitate of silica was 22.32, 78.12, 70.60 and 92.92 per cent, respectively and observed the formulations contain precipitate of silica as a carrier show better efficacy against *H. armigera* under laboratory in comparison to other carriers.

This study thus related in the selection of suitable carrier for the development of best *B. thuringiensis* formulations. 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 above study fulfilled our objective of identifying a best carrier for effective WP formulations of *B. thuringiensis* for the management of *H. armigera.* The formulations thus developed and tested have immense scope for large scale development of WP formulations of *B. thuringiensis.*

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	Baruimsulphate	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's 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	Baruimsulphate	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's 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

4. Conclusion

In present study, it was concluded that the combination of carrier, wetting agent and sticking agent influence the efficacy of WP formulations of *B. thuringiensis.* It was found that the formulation prepared by the combination of precipitated of silica as a carrier and polyethylene glycol and sodium lignosulponate as wetting agent were found most effective against the management of *H. armigera.* The formulations thus developed and tested have immense scope for large scale development of WP formulations of *B. thuringiensis.*

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