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Beauveria bassiana suspension concentrate – A mycoinsecticide for the management of *Helicoverpa armigera* (Hübner)

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ABSTRACT: A local isolate of the entomopathogenic fungus, *Beauveria bassiana* (ITCC-4513) was formulated as a Suspension Concentrate (SC) using mineral oil as carrier. In laboratory bioassays against 5-day-old *Helicoverpa armigera* larvae, the formulation had an LC_{50} value of 61.22 mg l^{-1} at 3 days after treatment. Data on shelf-life of the formulation stored in HDPE bottles at room temperature (29±1°C) was generated for 24 months. The formulation was found to be effective at 200 mg l^{-1} in field trials against *H. armigera* on sunflower crop. There was no phytotoxic effect of the formulation.

KEY WORDS: *Beauveria bassiana*, *Helicoverpa armigera*, suspension concentrate, shelf-life

INTRODUCTION

Helicoverpa armigera (Hübner), popularly known as the American bollworm in cotton, pod borer in redgram, and head borer in sunflower, is a polyphagous pest of high economic importance. It is economically important on sunflower and its damage is most important at the button stage of the crop. The damage to the crop is more severe in situations where sunflower is cultivated adjacent to cotton, chillies and tomato. Estimation of avoidable loss by insect pests on sunflower in field-plot tests during 1976 showed that damage due to *H. armigera* could result in a yield loss of 120 kg ha^{-1} (Panchabhavi and Krishnamurthy, 1978). The economic injury level of *H. armigera* was reported to be 0.98 larvae/plant (Karuppuchamy *et al*., 1993).

The entomopathogenic fungus, *Beauveria bassiana* (Balsamo) Vuill. is a major pathogen infecting *H. armigera*. The criteria to justify a commercial mycoinsecticide formulation include ease of field application to target insects, long field persistence and extended shelf-life. *B. bassiana* is primarily available as wettable powder formulations (1.15%) in the Indian market with a short shelf-life of six months, *e.g*., Daman, TOXIN™, BBC, etc. There is only one SC formulation of *B. bassiana* (Mycojaal 10% SC) registered for use against the diamondback moth, *Plutella xylostella,* in India. Formulations of the lipophilic conidia of *B. bassiana* and *Metarhizium anisopliae* in oil are reported to increase the effectiveness (Prior *et al*., 1988; Batemen *et al*., 1993).

Studies initiated at the Directorate of Oilseeds Research, Hyderabad, resulted in the identification of a local isolate ITCC-4513 of *B. bassiana* as an ideal candidate for development into a mycoinsecticide. The fungus was formulated as a suspension concentrate (SC) using mineral oil, with the aim of increasing the effectiveness and shelf-life and was tested for the management of the sunflower capitulum borer, *H. armigera* (Vimala Devi and Hari, 2009). The formulation readily suspends in water and can be sprayed with conventional high volume knapsack sprayers. Commercial exploitation of this formulation can be possible only if the data are generated in conformity with the regulatory requirements laid down by the Central Insecticides Board (CIB), Government of India. Accordingly, data were generated with the SC formulation on aspects like bioefficacy in the laboratory and field, shelf-life, presence of human pathogens and other general contaminants, etc. The data generated are presented in this paper.

MATERIALS AND METHODS

The fungal isolate ITCC-4513 of *B. bassiana* purchased from the Indian Type Culture Collection (ITCC), Indian Agricultural Research Institute, New Delhi, was mass multiplied and formulated as a suspension concentrate (30%) in mineral oil (Vimala Devi and Hari, 2009). The formulation was prepared by blending 30g of pure dry conidia of the fungus with 66 g of mineral oil+polyoxyethylene sorbitan mono-oleate mixture (1:0.08 ratio), 3.0g of carboxymethylcellulose and 1.0g of talc and stored in HDPE bottles at room temperature (29±1°C) inside a wooden cupboard. Data generation for this formulation was done in accordance with the CIB guidelines for provisional registration of the entomopathogenic fungi.

Analytical Test Report

Analytical Test Report (ATR) of the SC formulation was generated on the various physicochemical and biological parameters, *viz*., form and appearance, composition, pH, colony forming units (CFU g^{-1}), biological contaminants/g, etc. Stock suspension for the studies was prepared by suspending 20 mg of the SC formulation in 100ml of sterile distilled water and used for determination of CFU, human pathogens and other bacterial/fungal contaminants.

Determination of CFU

CFU determination was carried out on potato dextrose agar (PDA) medium. Plates were prepared by pouring 20ml of the medium in each plate. Stock suspensions of the mother culture (pure dry conidia) and the formulation @20 mg/100 ml were serially diluted. One ml suspensions from 10^9 and 10^{10} dilutions were added to the PDA medium in plates when lukewarm and still molten, mixed well and allowed to solidify. The plates were incubated at 25±1°C for three days and the number of colonies was counted. The CFU g^{-1} of the formulation was calculated using the following formula: N x 100 x 50 x dilution, where –

N = number of colonies / plate, 100 is the volume of stock suspension and 50 is the multiplication factor for 20 mg (to arrive at the CFU / 1000 mg of mother culture or SC formulation).

Determining count of biological contaminants - human pathogens and other microorganisms

For determining the count of human pathogens, specific media from Hi-Media, *viz*., Simmons citrate agar (M099) for *E. coli*, *Salmonella* differential agar (M1078) for *Salmonella*, *Shigella* broth base (M1326) for *Shigella* and *Vibrio* agar (M820) for *Vibrio* were prepared and poured into sterile petri plates. For the determination of other microbial contaminants, plating was done on nutrient agar (NA) to check for the presence of bacterial contaminants and on potato dextrose agar (PDA) to check for the presence of fungal contaminants (yeasts, moulds, mesophiles, etc.). One ml of the stock suspension (20 mg/100 ml) was added to each plate when the medium was lukewarm and still molten, mixed well and allowed to solidify. The plates were incubated at 37±1°C for three days to check for the presence of any colonies of the human pathogens, at 30±1°C for 2 days for bacterial contaminants and at 25±1°C for 7 days for fungal contaminants. The contaminants/g was calculated using the following formula: N x 100 x 50, where N = number of colonies / plate, 100 is the volume of stock suspension and 50 is the multiplication factor for 20 mg (to arrive at the CFU / 1000 mg of mother culture or SC formulation).

Moisture content determination

For the determination of moisture content, the device Equitensiometer (thetaprobe sensor) (Delta-T Devices, Cambridge, England) to measure matrix potential (range: 0 – 1000 kpa) was used. The medium of sample was set as organic, FC = 0.600 m^3/m^3. The measurement of moisture was through HH2 moisture meter.

pH determination

For determination of the pH, 10 g of the technical / formulation was added to 25 ml of distilled water. The suspension was prepared and the pH was measured at room temperature using an Elico-L1-610 pH meter.

Shelf-life studies

The data pertaining to shelf-life of the SC formulation were generated on the following aspects:

Analytical Test Report (ATR)

ATR was generated at 3-month intervals for a period of 24 months as a replicated trial with three replicates on the various physicochemical and biological parameters, *viz*., form and appearance, composition, pH, colony forming units (CFU)/g, biological contaminants/g employing protocols as detailed earlier.

Laboratory bio-efficacy

Laboratory bio-efficacy was studied through larval bioassays against 2nd instar *H. armigera* through diet surface treatment as a replicated trial with three replicates. The test suspension contained 107conidia/ml.

Bio-efficacy

Laboratory bioassays for determination of LC_{50} value

The larvae of *H. armigera* were reared on a semi-synthetic diet. Larval bioassays were conducted against 2nd instar larvae by diet surface treatment method (Vimala Devi and Hari, 2009). The bioassay was conducted with the formulation at five doses - 250, 25, 2.5, 0.25 and 0.025 mg l^{-1} that resulted in spore suspensions of 10^{10}, 10^9, 10^8, 10^7 and 10^6 conidia l^{-1}, respectively. Respective doses of the formulation were suspended in one litre of sterile (single distilled) water to get the required test suspensions.

The diet was poured into 12-well tissue culture plates (Tarsons make), approximately at 4ml per well with a surface area of 38 sq.mm. Test suspensions of the fungal conidia @ 80µl were overlaid on the diet surface in each well for all concentrations. One larva was released in each well. A total of 30 larvae were used for each concentration @10 larvae / replication. The trays were incubated at 27±1°C at 70% RH.

Table 1. Analytical test report of SC formulation of ITCC-4513

Form and appearance	Brown coloured, free flowing liquid
pH	6.5
Composition	
i. CFU/g	10^{15}
ii. Per cent content of the bio-control organism in the formulation and nature of biomass	30.0 % w/w (biomass consists of pure dry conidia)
iii. Percentage of carrier	Mineral oil (62.0 % w/w)
iv. Percentage of wetting agent	Polyoxyethylene sorbitan mono-oleate (4.0 % w/w)
v. Percentage of dispersing agent	Talc (1.0 %)
vi. Percentage of thickening agent	Carboxymethylcellulose (3.0 %)
Moisture content	0.05 %
Suspensibility	The formulation forms a clear suspension in water when suspended @1.0 g/100 ml
Biological contaminants/g	
i. Human pathogens *(E. coli, Salmonella, Shigella and Vibrio)*	-NIL-
ii. Others – fungal and bacterial	-NIL-

The larval mortality was recorded at 24 h intervals till four days after treatment (DAT) and the data were subjected to analysis of variance (ANOVA) using the statistical package MSTATC while probit analysis for determination of LC_{50} value was carried out using the statistical package SPSS 8.0.

Field testing

The formulation was tested against *H. armigera* in farmers' fields at K. Nagulapuram Village in Kurnool district, Andhra Pradesh (agroclimatic zone ACR-3 of Andhra Pradesh) (Gedela, 2008) on sunflower crop (var. Sunbred-275, Syngenta) during November 2008. The experiment was undertaken in a randomized block design with seven treatments and three replications in 17 x 4 m plots with a row spacing of 65 cm. Based on the results from laboratory bioassays, the SC formulation was tested at 4 doses, *viz*., 200, 250, 300 and 350 mg l^{-1} ($1.0x10^{10}$, $1.25x10^{10}$, $1.5x10^{10}$ and $1.75x10^{10}$ conidia, respectively) along with one commercial formulation of *B. bassiana* (Mycojaal 10% SC @ 2 ml l^{-1}), one insecticidal check (endosulfan 0.07% @ 2ml l^{-1}) and a water sprayed control. The formulations were suspended in water and sprayed with a conventional high volume knapsack sprayer. Incidence of *H. armigera* larvae was recorded before spraying and at 7 days after spraying from 5 tagged plants from each replication. Observations on natural incidence of parasites/predators were also recorded. The data were subjected to analysis of variance ANOVA (RCBD Factor 1) using the statistical package MSTATC. Phytotoxicity of the formulation to the crop was assessed by recording observations pertaining to leaf injury (leaf tips and leaf surface), wilting, epinasty and hyponasty. The observations were recorded before spray, 15 and 30 days from the date of spray.

Shelf-life studies

There was no change in the consistency of the SC formulation even at the end of 24-month storage period. Freshly prepared formulation was found to contain 1.1 x 10^{15} CFU g^{-1}. CFU count showed only a slight decrease reaching a value of 3.0 x 10^{13} while there was no significant change in the pH and moisture content after 24 months of storage. However, there was no significant change in the efficacy of the formulation as reflected by the values of larval mortality. In bioassays against 5-day-old larvae of *H. armigera*, freshly prepared formulation caused a cumulative mortality of 93.33% while the 24-month-old formulation resulted in 83.33% mortality at 4 DAT. Larval mortality was over 90.00% by 6 DAT. No other bacterial or fungal contaminants and human pathogens were observed at the end of 24-month storage period through plating on the various specific media (Table 2).

Table 2. Shelf-life of SC formulation of ITCC 4513 stored in HDPE bottles stored at room temperature

Storage period at 29±1°C	Colour	pH	Moisture Content (%)	CFU g^{-1}	Bioefficacy* At 4 DAT	Other contaminants g^{-1}		Human pathogens g^{-1} (*E. coli*, *Salmonella*, *Shigella*, *Vibrio*)
						On PDA	On NA	
0 day	Brown	6.52	0.050	$1.1+0.1 \times 10^{15}$	93.3 + 2.7	-	-	-
3 months	Brown	6.51	0.052	$7.2+0.6 \times 10^{14}$	86.7 + 5.4	-	-	-
6 months	Brown	6.52	0.051	$4.8+0.2 \times 10^{14}$	83.3 + 2.7	-	-	-
9 months	Brown	6.50	0.055	$3.0+0.4 \times 10^{14}$	83.3 + 5.4	-	-	-
12 months	Brown	6.50	0.054	$2.8+0.2 \times 10^{14}$	80.0 + 8.2	-	-	-
15 months	Brown	6.49	0.055	$2.5+0.3 \times 10^{14}$	80.0 + 2.7	-	-	-
18 months	Brown	6.50	0.055	$1.0+0.1 \times 10^{14}$	83.3 + 2.7	-	-	-
21 months	Brown	6.50	0.052	$4.5+0.4 \times 10^{13}$	80.0 + 5.4	-	-	-
24 months	Brown	6.51	0.050	$3.0+0.2 \times 10^{13}$	83.3 + 5.4	-	-	-

*Cumulative per cent mortality of 5 days old *H. armigera* larvae

Bio-efficacy

Laboratory bioassay

In bioassays conducted with five doses of the formulation against 2nd instar larvae of *H. armigera,* significant cumulative larval mortality of 80.0% was obtained by 4 DAT with doses of 25 and 250 mg l^{-1} containing 10^9 and 10^{10} conidia, respectively (Table 3a). Probit analysis of the data showed that the LC_{50} value of the formulation was 61.22 mg l^{-1} at 3 DAT (Table 3b). Thus for an LC_{50} value of 61.22 mg l^{-1}, the conidia ml^{-1} were 2.44 x 10^6 while conidia /sq.mm were 5.13 x 10^3.

Table 3a. Laboratory efficacy of mineral oil based SC formulation of *B. bassiana* isolate ITCC-4513 against 5 days old *H. armigera* larvae

Treatment – mg l^{-1}	Per cent cumulative mortality (days after treatment)	
	3	4
0.025	30.00 (33.21*)	60.00 (50.85)
0.25	50.00 (45.08)	60.00 (51.15)
2.5	53.33 (46.92)	63.33 (54.78)
25.0	60.00 (50.85)	80.00 (63.44)
250.00	66.66 (54.78)	80.00 (63.44)
S.E. Mean + CD (P=0.05)	3.80 12.36	3.44 11.18

* Figures in parentheses are angular transformed

Table 3b. Probit analysis of dose-mortality data for determination of the LC_{50} value of SC formulation of ITCC-4513 isolate

LC_{50} (mg/l) at 3 DAT	Fiducial limits	Regression equation
61.22*	-75.51 - 241.70	Y= -0.15 + 0.002 X

*For the LC_{50} value of 61.22 mg/l, the conidia/ml is 2.44 x 10^6

Field testing of the SC formulation for efficacy, safety and phytotoxicity

The SC formulation of *B*. *bassiana* isolate ITCC-4513 was tested against the natural incidence of the capitulum borer *H*. *armigera* on sunflower crop in farmers' fields near Kurnool along with a commercial *B*. *bassiana* formulation Mycojaal 10% SC, endosulfan and a water sprayed control. Incidence of the pest was observed at the bud opening stage of the crop and ranged from 8 to 18 larvae (2nd to 3rd instar) per 5 plants. Decrease in larval incidence at 7 days after spraying was highest in plots sprayed with the SC formulation of ITCC-4513 at all the four doses (91.0-98.0 %). The larval incidence decreased by 76.02, 93.26 and 27.03% in the plots sprayed with the commercial formulation of *B*. *bassiana*, endosulfan and water sprayed control, respectively. Decrease in larval incidence in the plots sprayed with all four doses of the SC formulation of *B*. *bassiana* was statistically on par with endosulfan and significantly higher in comparison to Mycojaal and unsprayed controls.

Only spiders were observed in the experimental plots. The incidence of spiders before spray ranged 1-2 per five plants and remained more or less same at seven days after spray in all the treatments, but in endosulfan check, the incidence was almost completely reduced (Table 4). Thus, the SC formulation was found safe to spiders, which are natural enemies of the sunflower capitulum borer.

Table 4. Field efficacy of mineral oil based SC formulation of *B*. *bassiana* isolate ITCC-4513 against *H*. *armigera* on sunflower in farmers' fields at Kurnool

Treatment	Larval incidence / 5 plants#		% decrease in larval incidence	Spiders / 5 plants	
	before spray	7 days after spray		Before spray	7 days after spray
SC form. @ 200 mg l^{-1} (1 x 10^{10} conidia l^{-1})	16.33	1.33	97.77 (88.44*)	1.67	2.0
SC form. @ 250 mg l^{-1} (1.25 x 10^{10} conidia l^{-1})	12.33	1.66	94.44 (81.96)	2.0	2.0
SC form. @ 300 mg l^{-1} (1.5 x 10^{10} conidia l^{-1})	13.00	1.66	91.15 (82.61)	2.0	2.0
SC form. @ 350 mg l^{-1} (1.75 x 10^{10} conidia l^{-1})	9.66	1.66	96.66 (83.10)	1.67	1.67
Mycojaal 2 ml l^{-1}	9.66	3.00	76.02 (61.31)	1.33	1.67
Endosulfan 0.07 % @ 2 ml l^{-1}	10.33	1.66	93.26 (72.01)	1.67	0.0
Water sprayed control	13.33	10.00	27.03 (29.59)	2.0	2.0
S.E. Mean $\pm$ CD (P = 0.05)	0.46 1.41	0.29 0.89	3.76 11.55	NS	NS

values are n+1; * figures in parentheses are angular transformed

Observations pertaining to leaf injury (leaf tip and leaf surface), wilting, epinasty and hyponasty were undertaken before spray, at 15 and 30 days from the date of spraying. No such symptoms were recorded showing that the SC formulation was not phytotoxic to sunflower crop.

Success of mycoinsecticides is dependent on the identification of virulent isolates that are amenable to culture and able to endure the stresses of the environment in which they are used. Good storage characteristics for over a year without the need for storage at low temperature are crucial to the acceptance of a mycoinsecticide (Moore and Prior, 1993).

Our efforts towards the development of a mycoinsecticide based on *B*. *bassiana* for the management of *H*. *armigera* have addressed the above issues thereby leading to the development of an SC formulation with a virulent isolate ITCC-4513 promising for the management of *H*. *armigera* in the field coupled with a good shelf-life of 24 months at room temperature. The mother culture and SC formulation have been found to be safe in toxicological tests conducted as per CIB guidelines for 9(3b) registration at the International Institute of Toxicology and Biotechnology (IIBAT), Kancheepuram. Since the data have been generated on the quality parameters, toxicology, shelf-life and bioefficacy against *H*. *armigera* in the laboratory and field in accordance with the registration guidelines of the CIB, our SC formulation of *B*. *bassina* is an ideal mycoinsecticide for commercial exploitation and large scale field use. The formulation presents immense scope for the management of *H*. *armigera* on several crops through inclusion in the IPM modules as it is a polyphagous pest of economic importance.

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REFERENCES

Bateman, R. P., Carey, M., Moore, D. and Prior, C. 1993. The enhanced infectivity of *Metarhizium flavoviride* in oil formulations to desert locusts at low humidities. *Annals of Applied Biology*, **122**: 145–152.

Gedela, S. P. 2008. Factors responsible for agrarian crisis in Andhra Pradesh (A Logistic Regression analysis). *World Applied Sciences Journal*, **4**: 707–713.

Karuppuchamy, P., Balasubramanian, G. and Sundarababu, P. C. 1993. Economic injury level of gram pod borer (*Helicoverpa armigera*) in sunflower (*Helianthus annuus*). *Indian Journal of Agricultural Sciences,* **63**: 679–680.

Moore, D. and Prior, C. 1993. The potential of mycoinsecticides. *Biocontrol News and Information*, **14**: 31N–40N.

Panchabhavi, K. S. and Krishnamoorthy, P. N. 1978. Estimation of avoidable loss by insect pests on sunflower at Bangalore. *Indian Journal of Agricultural Sciences*, **48:** 264–265.

Prior, C., Jollands, P. and Le Patourel, G. 1988. Infectivity of water and oil formulations of *Beauveria bassiana* (Deuteromycotina: Hyphomycetes) to the cocoa weevil *Pantorhytes plutus* (Coleoptera: Curculionidae). *Journal of Invertebrate Pathology*, **52**: 66–72.

Vimala Devi, P. S. and Hari, P.P. 2009. Identification of a virulent isolate of the entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuill., its mass multiplication and formulation for development into a mycoinsecticide for management of *Helicoverpa armigera* (Hubner). *Journal of Biological Control*, **23**: 137–144.

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