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Identification of a virulent isolate of the entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin, its mass multiplication and formulation for development into a mycoinsecticide for management of *Helicoverpa armigera* (Hübner)

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ABSTRACT: Two promising isolates of *Beauveria bassiana* – ITCC 4513 and HaBb DOR were multiplied on wheat bran supplemented with molasses and yeast extract in a polythene bag with sponge plugs for aeration. The yield of dry conidial powder per polythene bag was 18.25 ± 1.79 g and 10.03 ± 2.47 g for ITCC 4513 and HaBb DOR isolates, respectively. Laboratory bioassays with these conidial powders against 6 days old *Helicoverpa armigera* larvae resulted in LC_{50} values of 1.8×10^8 and 2.41×10^8 conidia ml^{-1} for the ITCC 4513 and HaBb DOR isolates, respectively, 5 days after treatment. Conidia of both the isolates were formulated as Suspension Concentrates (SC) in groundnut, sunflower and mineral oils. The formulations gave a clear suspension in water and could be sprayed with high volume knapsack sprayers. Performance of both isolates when formulated in oils was superior to unformulated conidia as reflected by the higher mortality of *H. armigera* larvae in laboratory bioassays and field trials on sunflower. The ITCC 4513 isolate was, however, superior to the HaBb DOR isolate in terms of conidial yield, efficacy as an SC formulation against *H. armigera* and safety to the egg parasitoid *Trichogramma chilonis*, thus proving to be an ideal candidate for development into a mycoinsecticide.

KEY WORDS: *Beauveria bassiana*, *Helicoverpa armigera*, mass multiplication, oil formulation, suspension concentrate, *Trichogramma*.

INTRODUCTION

With the ever-increasing awareness of the harmful effects of the chemical pesticides on man and environment, the immediate need for sustainable, eco-friendly pest management has been felt very strongly providing an impetus to research and development of microbial pesticides. Several entomopathogens have been found to play an important role in insect pest management and efforts have been stepped up by researchers world over to exploit their potential for large-scale field use. It is being increasingly recognized and accepted that effective formulations and appropriate delivery systems are keys to success of microbial control.

Entomopathogenic fungi like *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metchnikoff) Sorokin have been used successfully for the management of several insect pests in the temperate regions. However, large-scale exploitation of the fungi for insect pest management in tropical regions like India has been constrained by unfavourable environmental conditions, primarily high temperature and low humidity that lower the persistence of the fungal conidia. Effectiveness of entomofungal pathogens has been found to increase when formulated in oils (Bateman *et al*., 1993; Prior *et al*., 1988; Moore and Prior, 1993). These formulations have been developed for ULV application that is not feasible for adoption in developing countries like India where majority of the farmers are marginal farmers with small farm holdings using high and low volume sprayers. Given this scenario, there is a need to develop effective formulations of conidia for use in tropical countries like India.

The lipophilic conidia of *Beauveria* and *Metarhizium* spp. suspend readily in oils. Several vegetable oils such as sunflower, groundnut, safflower and cottonseed oils, petroleum based oils such as kerosene and diesel (Bateman *et al*., 1993; Nahar *et al*., 2003; Prior *et al*., 1988; Moore *et al*., 1995; Vimala Devi and Prasad, 1996) have been used for formulating conidia of the various entomopathogenic fungi. Formulations of *M. flavoviride* in cottonseed oil showed superior performance to water based suspensions (Bateman *et al*., 1993). Conidia of *B. bassiana* were 36 times more infective to the cocoa weevil

Pantorhytes plutus when formulated in coconut oil rather than water (Prior *et al*., 1988).

Specific requirements for development of fungal insect control agents as mycoinsecticides essentially include (a) identification of a fungal isolate for mass production with rapid growth, abundant sporulation and sufficiently high pathogenicity to the target pest, (b) amenability of the fungal isolate for mass production on a medium that is simple in composition, cheap in price and available in abundance, and (c) easy to run production procedure requiring minimum labour (Feng *et al*., 1994).

Helicoverpa armigera (Hübner) is an economically important insect pest of polyphagous nature infesting oilseeds (sunflower, groundnut and safflower), pulses, vegetables, cotton, etc. The annual crop losses in India due to this insect pest are estimated around Rs. 2000 crore despite use of insecticides worth Rs. 500 crore. An outcome of the excessive use of insecticides for its management is the development of insecticide resistance resulting in control failures besides environmental and ecological imbalance (Lingappa *et al*., 2005). Biological control is, therefore, one of the most important eco-friendly alternatives for *H. armigera* management. *B. bassiana* is a potent pathogen infecting *H. armigera* (Abbaiah *et al*., 1998). In this paper, we present the results of studies conducted for the identification of a virulent isolate of *B. bassiana*, cost-effective multiplication and formulation for effective management of *H. armigera*. Our efforts in this direction focussed on formulation of conidia in a suitable oil that readily suspends in water to give a uniform spray suspension that could be sprayed with an ordinary knapsack or power sprayer regularly employed by the farmers.

MATERIALS AND METHODS

B. bassiana isolates

Fourteen isolates of *B. bassiana* from the Indian Type Culture Collection (ITCC), New Delhi, one isolate from the Project Directorate of Biological Control (PDBC), Bangalore, and one local isolate from *H. armigera* (HaBb DOR) were initially multiplied on potato dextrose agar (PDA) and screened against six days old larvae of *H. armigera* @ 10^7 conidia ml^{-1}.

Mass Multiplication of *B. bassiana* isolates

Two promising isolates ITCC 4513 and HaBb DOR were multiplied in polythene bags on a low cost medium comprising 250g wheat bran, 10g molasses, 1.25g yeast extract and 250ml single distilled water. The bag was sealed and two autoclavable sponge plugs, 45 mm in diameter, were inserted into small cut ends of both the corners for providing a continuous supply of sterile air for growth of the fungus. The bag containing the medium was autoclaved at 15 psi for 20min. After cooling, sponge plug from one end was removed, one ml of the conidial suspension of *B. bassiana* (10^8 conidia ml^{-1}) was inoculated aseptically into the medium and the sponge plug was replaced. The contents were shaken well and the bags were incubated in the dark at 25 ± 1°C. Mycelial growth initiated on the 2nd or 3rd day after inoculation and continued till 5 to 6 days. This was followed by sporulation that continued for 5-6 days. The substrate with the conidia was then dried at room temperature, sieved through a muslin cloth and the resulting conidial powder was used for preparation of the oil formulations. The conidial count was determined using an improved Neubauer's haemocytometer. Data from five bags was used for calculation of the conidial yield and related parameters.

Formulation of *B. bassiana* conidia as Suspension Concentrates (SC)

Conidia of *B. bassiana* isolates ITCC 4513 and HaBb DOR were formulated as suspension concentrates in three oils, *viz*., sunflower oil, groundnut oil and mineral oil. Oils were initially blended with Tween 80 in 9: 1 ratio and 45ml of each mixture was blended with 15g of conidia to get the corresponding SC formulation. The formulations readily suspended in water to give uniform spray suspensions.

Preparation of conidial suspensions for larval bioassays

Conidial suspensions for the larval bioassays with the SC formulations were prepared by suspending the required amount of the formulations in sterile water while unformulated conidia were suspended in 0.02% sterile Tween 80 in water.

Larval bioassays

Bioassays were conducted against six days old larvae of *H. armigera.* The insect culture was maintained in the laboratory on a semi-synthetic diet. The diet was poured into 50-well insect rearing trays, approximately at 4ml per well with a surface area of $4.4cm^{-2}$. Suspensions of the fungal conidia @ 100µl 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% R.H.

Semi-synthetic diet composition

Agar - 6.5g, yeast – 10g, ascorbic acid – 1.3g, methyl paraben – 1.0g, sorbic acid – 250mg, casein – 5.0g,

cholesterol – 55.0mg, streptomycin – 100mg, formaldehyde – 0.5ml, multivitamin capsule – 1 (400 mg), Vitamin E – 400mg, chick pea powder – 55.0g, distilled water- 360ml.

Experiments

Pure conidia of ITCC 4513 isolate and HaBb DOR isolate were bioassayed at four concentrations, *viz*., 5 x 10^5, 5 x 10^6, 5 x $10^7$and 5 x 10^8 conidia ml^{-1} against six days old *H. armigera* larvae.

SC formulations of ITCC 4513 and HaBb DOR isolates were bioassayed against six days old *H. armigera* larvae @ 10^7 conidia ml^{-1}.

Larval mortality was recorded at 24h intervals for nine days after treatment (DAT). The data was subjected to Analysis of Variance (ANOVA) using the statistical package MSTATC while probit analysis was carried out using the statistical package SPSS 8.0.

Field testing of *B. bassiana* oil formulations

The experiment was conducted in sunflower: pigeon pea cropping system (2: 1) in 6 x 4 m plots, 60 x 30 cm spacing with sunflower var. Morden and pigeon pea var. ICPL 87 in the DOR farm at Rajendranagar, Hyderabad. Pigeon pea was sown in the first week of July, 2004 followed by sowing of sunflower after 30 days. The experiment was undertaken in a randomized block design with nine treatments and three replications. The treatments included the three suspension concentrates of two isolates of *B. bassiana* – ITCC 4513 and HaBb DOR @ 10^{10} conidia L^{-1}, one locally registered commercial formulation of *B. bassiana* (TOXIN™ 1.15% W.P.) @ 10 g L^{-1}, one insecticidal check (0.05% monocrotophos @ 1.5ml L^{-1}) and one water sprayed control. The formulations were suspended in water and sprayed with high volume knapsack sprayers. Incidence of *H. armigera* larvae was recorded before spraying and at 7 and 15 days after spraying from 5 tagged plants from each replication. Data were subjected to ANOVA (RCBD Factor 1) using the statistical package MSTATC.

Safety to the egg parasitoid, *Trichogramma chilonis*

The groundnut oil and mineral oil based SC formulations of ITCC 4513 isolate were tested for safety to the egg parasitoid, *Trichogramma chilonis* @ 2.5 x 10^{10} conidia L^{-1}, *i.e.*, 2.5 times higher than the dose effective against *H. armigera*. Spray suspensions were sprayed on four-day-old *Tricho* cards. Three cards containing approximately 300 eggs each were used for each treatment. The cards were sprayed uniformly with the conidial suspension and dried in a laminar flow. The cards were then placed in glass vials and plugged with non-absorbent cotton wrapped in tissue paper. The vials were then incubated at 27 ± 1°C. Observation of adult emergence from the *Tricho* cards was recorded at 24 h intervals from 2 to 5 DAT.

RESULTS AND DISCUSSION

Screening of *B. bassiana* isolates

Mycoinsecticide development begins with collection of fungal isolates and screening for virulence to the target pest (Jenkins *et al*., 1998). Accordingly, we obtained 16 isolates of *B. bassiana* and screened them through laboratory bioassays against six days old *H. armigera* larvae @ 10^7 conidia ml^{-1}. Only two isolates ITCC 4513 and HaBb DOR caused mortality of *H. armigera* larvae and hence were used for further studies.

Bioassays with pure conidia of ITCC 4513 and HaBb DOR isolates of *B. bassiana* against 6 days old *H. armigera* larvae

Bioassays were conducted with four concentrations of the two promising isolates ITCC 4513 and HaBb DOR of *B. bassiana* against six days old *H. armigera* larvae using conidia obtained through multiplication on potato dextrose agar (PDA) medium. Mortality of larvae due to ITCC 4513 isolate started from 3 DAT and showed a dose related response. The maximum cumulative mortality ranged 83.3–100% at 9 DAT in all the four doses. Mortality of larvae due to HaBb DOR isolate also showed a dose related response but was lower in comparison with the ITCC 4513 isolate (66.7% at 9 DAT at the dose of 5 x 10^5 conidia ml^{-1} and 80.0% at 9 DAT at 5 x 10^6 and 5 x 10^7 conidia ml^{-1}). The highest cumulative mortality of 100 % was observed at the dose of 5 x 10^8 conidia ml^{-1} at 8 DAT (Table 1a).

In comparison to the HaBb DOR isolate, the LC_{50} values were lower for the ITCC 4513 isolate with 3.4 and 1.8 x 10^8 conidia ml^{-1} at 4 and 5 DAT, respectively. The LC_{50} values for the HaBb DOR isolate, however, were higher with 4.7 and 2.41 x 10^8 conidia ml^{-1} at 4 and 5 DAT, respectively (Table 1b). Both isolates were highly effective, however, the ITCC isolate had lower LC_{50} values that are indicative of its higher virulence.

Mass multiplication of *B. bassiana* isolates

The two isolates of *B. bassiana* – ITCC 4513 and HaBb DOR were multiplied on wheat bran medium. The ITCC isolate gave significantly higher yield when compared to HaBb DOR. Mycelial growth initiated on the 2nd day with sporulation commencing on 5th day itself after inoculation for the ITCC 4513 isolate. In case of the HaBb DOR isolate, mycelial growth initiated on the 2nd day itself while sporulation commenced on the 7th day only.

Table 1a. Laboratory efficacy of two promising isolates of *B. bassiana* against 6 days old *H. armigera* larvae

Treatment	Dose (conidia ml^{-1})	Per cent cumulative mortality[#] at indicated days after treatment					
		4	5	6	7	8	9
ITCC 4513 isolate	5 x 10^5	16.7 (23.8)	20.0 (26.9)	23.3 (29.5)	53.3 (47.4)	76.7 (61.2)	83.3 (70.5)
	5 x 10^6	20.0 (26.9)	33.3 (35.0)	50.0 (45.0)	80.0 (63.6)	83.3 (66.2)	86.7 (66.2)
	5 x 10^7	36.7 (37.4)	53.3 (42.6)	76.7 (59.9)	83.3 (83.1)	96.7 (83.1)	100.0 (90.0)
	5 x 10^8	63.3 (52.2)	83.3 (66.2)	86.7 (68.6)	93.3 (76.2)	96.7 (83.1)	100.0 (90.0)
HaBb DOR isolate	5 x 10^5	0.0 (0.0)	20.0 (27.4)	30.0 (26.9)	46.7 (42.6)	56.7 (47.6)	66.7 (55.5)
	5 x 10^6	3.33 (6.9)	20.0 (26.4)	33.3 (32.1)	56.7 (49.8)	70.0 (57.9)	80.0 (63.1)
	5 x 10^7	8.3 (8.3)	43.3 (40.0)	46.7 (43.1)	66.7 (55.5)	80.0 (63.1)	80.0 (63.1)
	5 x 10^8	53.3 (47.4)	76.7 (66.2)	80.0 (69.3)	96.7 (83.1)	100.0 (90.0)	100.00 (90.0)
SEM±		2.9	3.4	3.4	3.3	3.7	3.2
CD (P = 0.05)		8.8	10.2	10.2	10.0	11.0	9.7

[#] Values in parentheses are angular transformed

Table 1b. Probit analysis of dose-mortality data for determination of the LC_{50} value of pure conidia of *B. bassiana* isolates

Isolate	Days after treatment	LC_{50} value (x 10^8 conidia ml^{-1})	**Confidence limits**	Regression equation
ITCC 4513 isolate	4	3.38	2.09 – 6.57	Y = -0.73 + 0.0002x
	5	1.83	0.97 – 2.99	Y = -0.73 + 0.0003x
HaBb DOR isolate	4	4.7	3.67 – 6.42	Y = -1.84 + 0.0004x
	5	2.41	1.42 – 4.02	Y = -0.66 + 0.0003x

Yield of dry conidial powder for the ITCC isolate averaged 18.25 ± 1.79 g per bag with 1.38 ± 0.35 x 10^{11} conidia $gram^{-1}$ of the conidial powder while it was 10.03 ± 2.47g bag^{-1} with 7.90 ± 1.15 x 10^{10} conidia g^{-1} of the conidial powder for the HaBb DOR isolate. The yield of conidia per gram of substrate averaged 1.0 ± 0.17 x 10^{10} with ITCC 4513 isolate and 3.23 ± 1.12 x 10^9 for HaBb DOR isolate (Table 2). The cost of 250g of wheat bran medium supplemented with molasses and yeast extract is only Rs.5/-. Hence, 18.25g of conidia of the ITCC 4513 isolate, *i.e.*, 2.51 x 10^{12} conidia produced at a meager expenditure of Rs.5/-would be sufficient for 250L spray fluid @ 10^{10} conidia L^{-1}.

Multiplication of entomopathogenic fungi through solid-state fermentation results in production of aerial conidia that are indistinguishable from those produced on the surface of insect cadavers in morphology and infectivity (Feng *et al*., 1994). Apart from the nutritional growth requirements, aeration is an important factor influencing growth and sporulation in mass production of entomopathogenic fungi. High conidial yield in our study could be attributed to the loose consistency of the medium and use of wheat bran that could have locked in more air providing a better condition for growth and sporulation of the fungus. Use of sponge plugs resulted in continuous aeration essential for good sporulation.

Table 2. Multiplication of *B. bassiana* isolates on wheat bran medium

Isolate	Yield (g) of dry conidial powder bag^{-1}	Conidia g^{-1} of dry conidial powder	Conidia g^{-1} of substrate
ITCC 4513	18.25 ± 1.79	1.38 ± 0.35 x 10^{11}	1.0 ± 0.17 x 10^{10}
HaBb DOR	10.03 ± 2.47	7.90 ± 1.15 x 10^{10}	3.23 ± 1.12 x 10^9

B. bassiana has been successfully mass produced on rice in autoclavable polypropylene bags yielding 3 kg of conidia per 300kg of substrate @ 2 x 10^{11} conidia g^{-1} of the

conidial powder in 12-15 days, *i..e.*, 2 x 10^{12} conidia kg^{-1} of medium (Alves and Pereira, 1989). Surface cultivation of *B. bassiana* for 10 days on a medium containing 70% wheat bran, 25% corn flour and 5% bean flour yielded 21kg of dry conidial powder from 320kg of medium @ 2.9 x 10^{10} conidia g^{-1} of the dry powder, i.e., 65.5g kg^{-1} of substrate and a conidial yield of 1.9 x 10^{12} conidia kg^{-1} of substrate (Tao *et al*., 1988). Puzari *et al*. (1997) reported conidial production of *B. bassiana* @ 1.96 x 10^{10} conidia kg^{-1} of medium (Rice hull + saw dust + rice bran 75: 25: 100) by 24 days after inoculation. In our study, multiplication of *B. bassiana* on wheat bran (alone) medium for 10-12 days resulted in 73.0g of conidia kg^{-1} of substrate (costing less than Rs.20/-, *i.e.*, wheat bran – Rs.6/- kg^{-1}, molasses 40g – 25 paise, yeast extract 5.0g – Rs.10/-) with a conidial yield of 10^{13} that is significantly higher than the results reported from earlier studies and sufficient for 1000L of spray suspension at the effective dose of 10^{10} conidia L^{-1}. The study shows that *B. bassiana* can be mass produced cost-effectively on wheat bran medium.

Bioassay with SC formulations of *B. bassiana* isolates

Bioassays were conducted with the SC formulations (employing sunflower, groundnut and mineral oils) of ITCC 4513 isolate and HaBb DOR at the dose of 10^{10} conidia L^{-1} against 6 days old *H. armigera* larvae. The test dose of 10^{10} conidia L^{-1} was selected based on results of the laboratory bioassays with pure conidia and earlier reports (Nahar *et al*., 2004; Pawar and Borikar, 2005; Prabhu *et al*., 2007). The cumulative larval mortality of ITCC isolate at the end of 9 DAT due to the groundnut oil (93.3%) and mineral oil (83.3%) formulations was significantly higher than the mortality caused by sunflower oil formulation (76.7%) and unformulated conidia (73.3%). In case of HaBb DOR isolate, cumulative larval mortality due to all the three oil formulations (83.3 - 86.67%) was significantly higher than that caused by unformulated conidia (60.0%). Observations of larval mortality from 2 to 9 DAT showed that both the *B. bassiana* isolates formulated in oils were equally effective against *H. armigera* larvae and superior in performance to unformulated conidia (Table 3).

Field-testing of SC formulations of *B. bassiana* isolates

Three SC formulations, *viz*., sunflower, groundnut and mineral oil based formulations of *B. bassiana* ITCC 4513 isolate and HaBb DOR isolate were tested at the dose of 10^{10} conidia L^{-1} against the natural incidence of *H. armigera* larvae on sunflower crop in a sunflower-pigeon pea

Table 3. Laboratory efficacy of SC formulations of *B. bassiana* isolates against 6 days old *H. armigera* larvae at 10^{10} conidia L^{-1}

Treatment	Per cent cumulative mortality# at indicated days after treatment							
	2	3	4	5	6	7	8	9
ITCC 4513 isolate - Sunflower oil based SC	10.0 (15.0)	23.3 (32.2)	40.0 (38.9)	46.7 (43.1)	53.3 (47.0)	63.3 (52.8)	70.0 (56.8)	76.7 (61.2)
ITCC 4513 isolate - Groundnut oil based SC	23.3 (28.8)	30.0 (33.2)	30.0 (33.2)	43.3 (41.1)	46.7 (43.1)	66.7 (54.8)	83.3 (66.2)	93.3 (78.0)
ITCC 4513 isolate - Mineral oil based SC	16.7 (23.9)	20.0 (26.1)	36.7 (36.9)	40.0 (39.2)	56.7 (48.9)	56.7 (48.9)	63.3 (52.9)	83.3 (66.2)
ITCC 4513 - isolate unformulated conidia	6.7 (12.3)	10.0 (18.2)	26.7 (30.4)	30.0 (32.3)	50.0 (45.1)	63.3 (52.9)	70.0 (57.0)	73.3 (59.0)
DOR isolate - Sunflower oil based SC	13.3 (21.2)	23.3 (28.8)	33.3 (35.2)	43.3 (41.2)	56.7 (48.9)	70.0 (54.8)	76.7 (61.2)	86.7 (68.9)
HaBb DOR isolate - Groundnut oil based SC	13.3 (17.2)	16.7 (23.4)	20.0 (26.1)	26.7 (31.0)	46.7 (44.9)	60.0 (50.9)	70.0 (57.0)	83.3 (66.2)
HaBb DOR isolate - Mineral oil based SC	6.7 (11.0)	10.0 (15.0)	16.7 (23.4)	36.7 (37.1)	53.3 (46.9)	63.3 (53.1)	73.3 (59.7)	83.3 (70.0)
HaBb DOR isolate - unformulated conidia	6.7 (8.9)	13.3 (17.7)	20.0 (22.1)	26.7 (31.0)	30.0 (33.2)	43.3 (41.1)	56.7 (48.9)	60.0 (51.0)
SEM±	4.0	3.304	3.7	2.8	2.7	2.0	2.3	2.9
CD (P = 0.05)	NS	NS	NS	NS	NS	NS	NS	8. 9

Values in parentheses are angular transformed

cropping system for identifying the best formulation. One commercial formulation of *B. bassiana* (TOXIN™ 1.15% W.P.), one insecticidal check using monocrotophos and one water sprayed control were included for comparison. Incidence of the pest was observed at the star bud stage of the crop and ranged from 2-4 larvae (2nd to 3rd instar) per plant. The first spray was imposed at this stage. The decrease in larval incidence at 7 days after spraying was highest in plots sprayed with groundnut oil based formulation (90.0%) of ITCC 4513 isolate followed by mineral oil based formulation (85.02%) of ITCC 4513 isolate, groundnut oil based formulation (85.89%) of HaBb DOR isolate and sunflower oil based formulation (80.91%) of ITCC 4513 isolate. The larval incidence decreased by 75.0, 71.93 and 37.29 per cent in the plots sprayed with the commercial formulation of *B. bassiana*, monocrotophos and water, respectively (Table 4). The larval incidence was completely lowered in the fungus and insecticidal sprayed plots at 15 days after spraying while the larvae in the water sprayed control plots continued to feed, grew in size and pupated.

Safety of groundnut oil and mineral oil based SC formulations of ITCC 4513 isolate to *Trichogramma chilonis*

Based on the laboratory bioassays and field studies, SC formulations of ITCC 4513 isolate in groundnut and mineral oil were selected and tested for safety to the egg parasitoid *T. chilonis*. Emergence of *T. chilonis* adults from the *Tricho* cards sprayed with the groundnut oil and mineral oil based SC formulations of ITCC 4513 isolate was first observed on the 3rd day after spraying and continued till 6 days. Cumulative adult emergence at 6 DAT was similar in *Tricho* cards sprayed with the *B. bassiana* formulations as well as the unsprayed *Tricho* cards ranging 74.07 - 76.86%. The study shows that both the formulations are safe to *T. chilonis* (Table.5).

This study thus resulted in the identification of ITCC 4513 isolate as an ideal candidate for development into a mycoinsecticide. Groundnut oil and mineral oil based SC formulations of the isolate were consistently superior in performance to unformulated conidia both in lab and field and superior to monocrotophos and safe to the egg parasitoid *T. chilonis*. The study shows that ITCC 4513 isolate is more promising than the DOR isolate against *H. armigera* and SC formulations based on groundnut oil and mineral oil can be used effectively for the management of *H. armigera* in the field.

The above study fulfilled our objective of identifying a virulent isolate of *B. bassiana* amenable for cost-effective mass multiplication and its effective formulation for the management of *H. armigera*. The formulations thus developed and tested have immense

Table 4. Field efficacy of SC formulations of *B. bassiana* isolate ITCC 4513 against *H. armigera* on sunflower at DOR farm

Treatment	No. of larvae plant^{-1}*		% decrease in incidence by 7 days after spray
	Before spray	7 days after spray	
ITCC 4513 isolate - Sunflower oil based SC	3.27 (4.27)	0.33 (1.33)	80.91
ITCC 4513 isolate - Groundnut oil based SC	2.73 (3.73)	0.27 (1.27)	90.10
ITCC 4513 isolate - Mineral oil based SC	2.67 (3.67)	0.40 (1.40)	85.02
HaBb DOR isolate-Sunflower oil based SC	2.60 (3.60)	0.67 (1.67)	74.23
HaBb DOR isolate-Groundnut oil based SC	3.33 (4.33)	0.47 (1.47)	85.89
HaBb DOR isolate- Mineral oil based SC	2.80 (3.80)	0.77 (1.77)	72.50
Commercial formulation of *B. bassiana* - TOXIN™ 1.15% W.P.	3.20 (4.20)	0.80 (1.80)	75.00
Monocrotophos 0.05%	2.60 (3.60)	0.73 (1.73)	71.93
Unsprayed	2.87 (3.87)	1.80 (2.80)	37.29
SEM±	0.13	0.16	-
CD (P = 0.05)	0.39	0.48	-

*values in parentheses are n+1

Table 5. Safety of the oil based SC formulations of ITCC-4513 isolate to the egg parasitoid, *Trichogramma chilonis*

Treatment	Cumulative % adult emergence of *T. chilonis* at indicated days after spraying			
	3d	4d	5d	6d
Groundnut oil based SC	21.74 ± 1.26	66.08 ± 8.04	73.77 ± 12.05	76.70 ± 11.74
Mineral oil based SC	51.21± 13.80	60.36 ± 10.29	72.69 ± 8.06	76.51 ± 9.98
Control	45.31 ± 4.53	55.91 ± 4.66	68.88 ± 2.59	76.86 ± 2.03

scope for large-scale promotion in tropical regions since formulating fungal conidia in oils is expected to render protection from desiccation thereby resulting in longer persistence under field conditions (Moore and Prior, 1993). The SC formulations readily suspend in water to give spray suspensions that are uniform, clear and stable and can be sprayed with the regular high volume sprayers. These properties render the formulations highly suitable for use in India and other developing countries. Future studies are aimed at generating data in accordance with the regulatory requirements for registration that enable its promotion as a mycoinsecticide.

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