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EFFICACY OF SUNFLOWER OIL FORMULATION AND CONIDIAL SUSPENSION OF *BEAUVERIA BASSIANA* AGAINST *SPODOPTERA LITURA* (F.)

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ABSTRACT

Evaluation of oil formulation of entomopathogenic fungi *Beauveria bassiana* for its effect against tobacco caterpillar *Spodoptera litura* (F.) in six concentrations (1000x10^5, 700x10^5, 500x10^5, 250x10^5, 125 x10^{5}and 75x10^5) in the form of sunflower oil formulation and conidial suspension of *B. bassiana* has been evaluated in this study. All the five concentrations showed mortality and maximum mortality was observed at 108 hr after treatment at maximum concentration (1000x10^5); and the least mortality of 23.33% was observed at the lowest concentration of 75x10^5. No significant differences were observed between sunflower oil formulation and conidial suspension.

Key words: *Beauveria bassiana*, *Spodoptera litura*, entomopathogenic fungus, oil formulation, conidial suspension, sunflower, mortality, efficacy

Tobacco caterpillar *Spodoptera litura* (F.) (Lepidoptera: Noctuidae) is an important polyphagous pest causing serious damage. All over the world, it damages more than 389 species of cultivated crop plants belonging to 109 families (Lin et al., 2019; Shankara Murthy et al., 2006; Barman et al., 2019) of which 40 genera are cultivated in India (Basu, 1981; Muthukrishnanet al., 2005). The serious incidence of this pest normally occurs with a good rainfall after a long dry spell (Chelliah, 1985); and it can cause 10 to 30% economic losses based on different crop phase and its invasion level in the field (Cheng et al., 2017,). The widespread and indiscriminate use of insecticides against this pest has led to resistance in many insects (Samanta et al., 2020), and it constitutes a serious risk to crop protection (Rao and Dhingra, 2000). Also, use of pesticides is hazardous to human health, flora, fauna, and even to the atmosphere (Mahmoud et al., 2014). Hence, pest management involving biocontrol agents is assuming prominence as an important strategy. Entomopathogenic fungi (EPF) like *Beauveria bassiana* (Balsamo) Vuillemin, *Metarhizium anisopliae* (Metchnikoff) and *Paecilomyces fumosorosesus* (Wize) Brown and Smith are now recognized as important entomopathogens (Wanida and Poonsuk 2012; Shoaib et al., 2012; Meikle et al., 2005). Many commercial formulations of EPF have been developed for crop insect pest management. Among the 171 products of EPF developed, products based on *M. anisopliae* and *B. bassiana* represent 33.92% of total products, and *Beauveria brongniartii* and *Isaria fumosorosea* products represent 5.81 and 4.10, respectively (Moorhouse et al., 1992; De Faria and Wraight, 2007). This study explores the preparation of EPF formulations and an their oil formulations. Some selected oil formulations are also evaluated against *S. litura* larvae for their efficacy as conidia oil formulation.

MATERIALS AND METHODS

The diseased samples were collected from the Crop Research Centre (C R C) and Avenue Plantation, Pantnagar during autumn and winter season. The samples of cadaver were then subjected to series of washing with sodium hypochlorite solution and a series of distilled water. Then aseptic inoculation protocol was followed. After pure culture of the local isolate, identification of the fungus was done and maintenance of culture was done by doing subculturing of the isolate. For identification various slides were prepared with lactophenol and methyl blue, and examined done for the morphological characters under microscope (Olympus Cx33). The culture plates of 15 days old *B. bassiana* were taken to prepare stock suspension. For the preparation of sunflower oil-based formulation, surfactant mixed in oil phase with spore suspension in aqueous phase was used. Conidial count of sunflower oil formulation and conidial suspension was assessed

with Neubauer Haemocytometer, and the formulation and suspension were subjected to serial dilution to get six concentrations of 1000, 700, 500, 250, 125 and 75x10^5 conidia/ ml. In control only water and two drops of 0.05% Tween 80 was added. To set the fungus colony forming units/ ml (C F U), 1 ml of sunflower oil formulation and conidial suspension was suspended in 9 ml distilled water for the serial dilution. Prepared serial dilution was plated at 1 ml/ plate on PDA media. The plate was gently rotated for uniform spreading of spore suspension and incubated at 25± 2^{0}C and 70% RH, with three replications. The C F U count was recorded on 7th day after plating.

The running culture of *S. litura* was maintained in the laboratory by standard rearing technique following Sabry and Khedr (2014) and Kumar and Srivastava (2016). From these, 3rd instar larvae were used for bioassay against *B. bassiana* developed conidial suspension and sunflower oil formulation. To evaluate the contact action or toxicity of formulation by larval atomization method (Thakur and Srivastava, 2019), different dilutions of respective myco-insecticide formulations were prepared in tap water by serial dilution method to get the six concentrations as given above. The virulence of *B. bassiana* was studied 12-120 hours after treatment (HAT) at every 12 hr interval. For each treatment there were 4 replications with 10 larvae/ replication. The observations on mortality obtained from bioassays were suitably analyzed by SPSS, the statistical probe and % mortality was subjected to DMRT analysis (Duncan, 1955).

RESULTS AND DISCUSSION

As given in Table 1, the sunflower oil formulation showed that none of the concentration caused any mortality in the treated larvae up to 24 HAT. At 36 HAT, the two concentrations i.e., 1000 and 700 x10^5 showed some mortality response (6.67%); and at 500x 10^5 it was 3.33%. The mortality increased with HAT, and at 48 HAT, mortality was observed in all except the lowest concentration of 75 x10^5, with maximum of 23.33% being at 1000 x10^5conc; at 60 HAT again no mortality was observed in the lowest concentration of 75 x10^5, but it was 13.33% at 72 HAT; at 72 HAT, maximum mortality was 70% at the highest concentration of 1000 x10^5, and complete (100%) mortality was observed at 108 HAT in this concentration of 1000x10^5 and at 120 HAT in 700x10^5. No other concentration could cause complete 100% mortality even up to 120 HAT. The lowest concentration could cause 23.33%

Table 1. Mortality of *S. litura* on hours basis for different concentrations of sunflower oil and conidial suspension formulation of *B. bassiana*

Concentration	Treatment	12 HAT	12 HAT	24 HAT	24 HAT	36 HAT	36 HAT	48 HAT	48 HAT	60 HAT	60 HAT	72 HAT	72 HAT	84 HAT	84 HAT	96 HAT	96 HAT	108 HAT	108 HAT	120 HAT	120 HAT
1000x10^5	T1	0± 0^a	0± 0^a	0± 0^a	0± 0^a	6.67± 5.77^a	6.67± 5.77^a	23.33± 5.77^c	16.67± 5.77^c	50± 10^e	46.67± 5.77^e	70± 17.32^d	66± 15.27^d	83.33± 5.77^e	80± 0^e	93.33± 5.77^e	93.33± 5.77^e	100± 0^e	100± 0^e	100± 0^f	100± 0^f
700x10^5	T2	0± 0^a	0± 0^a	0± 0^a	0± 0^a	6.67± 5.77^a	6.67± 5.77^a	13.33± 5.77^b	10± 0^{bc}	36.67± 5.77^d	33.33± 5.77^d	63.33± 5.77^{cd}	60± 10^{cd}	73.33± 5.77^{de}	70± 10de	86.67± 5.77^{de}	86.67± 5.77^{de}	93.33± 5.77^e	93.33± 5.77^e	100± 0^f	100± 0^f
500x10^5	T3	0± 0^a	0± 0^a	0± 0^a	0± 0^a	3.33± 5.77^a	3.33± 5.77^a	13.33± 5.77^b	6.67± 5.77^{ab}	30± 10^{cd}	26.67± 5.77^{cd}	50± 10^d	46.67± 11.54^e	63.33± 11.55^d	60± 10^d	73.33± 11.55^d	73.33± 11.55^d	80± 10^d	80± 10^d	86.67± 5.77^e	86.67± 5.77^e
250x10^5	T4	0± 0^a	0± 0^a	0± 0^a	0± 0^a	0.0± 0.0^a	0.0± 0.0^a	6.67± 5.77^{ab}	6.67± 5.77^{ab}	23.33± 5.77^{bc}	20± 0^{bc}	30± 10^b	26.67± 5.77^b	43.33± 5.77^c	40± 10^c	50± 10^c	50± 10^c	56.67± 11.55^c	56.67± 11.55^c	60± 10^d	60± 10^d
125x10^5	T5	0± 0^a	0± 0^a	0± 0^a	0± 0^a	0.0± 0.0^a	0.0± 0.0^a	3.33± 5.77^a	3.33± 5.77^{ab}	13.33± 5.77^b	13.33± 5.77^b	26.67± 5.77^b	26.67± 5.77^b	30± 10^{bc}	30± 10^{bc}	30± 10^b	30± 10^b	33.33± 5.77^b	33.33± 5.77^b	33.33± 5.77^c	33.33± 5.77^c
75x10^5	T6	0± 0^a	0± 0^a	0± 0^a	0± 0^a	0.0± 0.0^a	0.0± 0.0^a	0.0± 0.0^a	0.0± 0.0^a	0± 0^a	0± 0^a	13.33± 5.77^{ab}	13.33± 5.77^{ab}	20± 10^b	20± 10^b	20± 10^b	20± 10^b	23.33± 5.77^b	23.33± 5.77^b	23.33± 5.77^b	23.33± 5.77^b
Control (Untreated)	T7	0± 0^a	0± 0^a	0± 0^a	0± 0^a	0.0± 0.0^a	0.0± 0.0^a	0.0± 0.0^a	0.0± 0.0^a	0± 0^a	0± 0^a	0± 0a	0± 0^a	0± 0^a	0± 0^a	0± 0^a	0± 0^a	0± 0^a	0± 0^a	0± 0^a	0± 0^a

mortality up to 120 HAT. With regard to conidial suspension, up to 24 HAT no mortality was observed in any concentration; at 36 HAT, 1000 and 700 $x10^5$ showed 6.67% mortality; and at 500 $x10^5$ only 3.33% mortality was observed. The mortality response showed an increasing trend with more HAT, at 48 HAT, mortality was observed irrespective of concentration except the lowest of 75 $x10^5$, maximum of 16.67% being with 1000 $x10^5$ concentration followed by 10% at $700x10^5$and lowest at $125x10^5$ i.e., 3.33%. At 60 HAT again no mortality was observed in the lowest concentration, but at 1000 $x10^5$ it was 46.67%. With 72 HAT in $75x10^5$ it was 13.33%, maximum being 66% with 1000 $x10^5$. Complete mortality was observed at 108 HAT with 1000 $x10^5$ and at 120 HAT in $700x10^5$. No other concentration could cause complete (100%) mortality even up to 120 HAT, with the least value being 23.33% up to 120 HAT. Comparison between the two formulations revealed no differences in the contact toxicity of the *B. bassiana.* However, oil formulation showed a little higher toxicity. At 48 HAT the mortality in case of conidial suspension was 16.67%, and 23.33% at highest concentration of $1000x10^5$ in case of oil formulation. In other concentrations at various HAT, oil formulation led to more toxicity as compared to conidial suspension (Table 1).

The efficacy of isolates of *B. bassiana* was evaluated against third instar of *S. litura* using the leaf spray method by Moorthi et al. (2011); these results revealed that at 96 HAT, 66.67, 73.33 and 80.0% mortality was obtained with the isolates Bb02, Bb09 and Bb10, respectively; LC_{50} values were $2.1x10^6$, $3.6x10^7$ and $1.2x10^7$ conidia/ ml for these, respectively, and the LT_{50} value for Bb02 and Bb09 was 4.8 days, whereas it was 4.0 days for Bb10 @10^8spore/ ml. Asi et al. (2013) by larval dip method, the results revealed that the LC_{50} value for 3rd instar larvae was $1.11x\ 10^7$ conidia/ ml for a local strain i.e., *B. bassiana* 25 at 10 days after treatment; and LT_{50} was 187 hours in *B. bassiana* 25 @ $1x\ 10^8$conidia/ ml. Thus, formulations of *B. bassiana* can thus serve as an effective broad spectrum biocontrol agents.

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