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Biopesticidal Formulation of *Beauveria Bassiana* Effective against Larvae of *Helicoverpa Armigera*

Agarwal Ritu[1]*, Choudhary Anjali[1], Tripathi Nidhi[1], Patil Sheetal[2] and Bharti Deepak[1]

[1]*Assistant Professor, Department of Biotechnology and Biochemistry, Career College, Bhopal 462 023, M.P, India*
[2]*Trainee student, Department of Biotechnology and Biochemistry, Career College, Bhopal, 462 023, M.P, India*

Abstract

The present study was to emphasize entomopathogens in pest management of cash crops over chemical pesticides, optimization of media for growth of *Beauveria bassiana* and bioassay of different formulations for their efficacy as marketable and easily applicable biopesticide. *Beauveria bassiana* (Order: Hypocreales, Family: Cordycipitaceae) popularly known as white muscardine entomogenous fungi was isolated from soyabean fields of Misrod, Bhopal. Media for optimal growth of fungus were standardized. Its different formulations viz. carrier based powder formulation, oil based formulation and bentonite oil based formulation using homogenizers were prepared. These formulations were bioassayed against *Helicoverpa armigera* (Order: Lepidoptera, Family: Noctuidae), the most destructive pest in soyabean. Bentonite based liquid formulation was observed to be most effective as determined by measuring larval mortality as well as viability of fungal spores and ease of applicability.

Keywords: Biopesticide; *Beauveria bassiana; Helicoverpa armigera*; Formulations; Bentonite

Abbreviations

BOBLF: Bentonite Oil-Based liquid Formulation; OBLF: Oil-Based Liquid Formulation; CBPF: Carrier Based Powder Formulation

Introduction

Over reliance on broad spectrum pesticides has been severely condemned in different parts of the world after International Conference on Chemicals Management. Since then, an alternative eco friendly strategy for the management of noxious insect pests has been explored to trim down the harmful effects of chemical insecticides on humanity. Studies of biodiversity in agro ecosystems and the delivery of ecosystem services to agricultural products have usually ignored the contribution of entomopathogens in the regulation of pest populations (Tscharntke et al.).

In recent years, crop protection based on biological control of crop pests with microbial pathogens like virus, bacteria, fungi and nematodes has been recognized as a valuable tool in pest management [1,2]. The appropriate use of eco friendly microbial biopesticide can be engaged in recreation of sustainable organic crop production by providing a stable pest management program. In light of this understanding, extensive work has been done on various species of bacteria and fungi (especially entomopathogenic that parasitize insects) implicated as effective biocontrol agents.

Till date, various entomopathogenic fungi such as *Lecanicillium* sp. [3,4], *Beauveria bassiana* [5-7] and *Metarhizium anisopliae* [8-11] have been effectively used to control aphids, lepidopteron larvae and other pests.

Sixteen different mycotoxins have been analysed of which the cyclodepsipeptidic mycotoxin, beauvericin, produced by *Beauveria bassiana* has been documented to be most effective for its larvicidal properties. Sowjanya Sree and Padmaja [10] reported the ultrastructural effects of crude beauvericin on the salivary glands of 9-day-old *S. litura* larva after 24 h of treatment with the mycotoxin at a dosage of 0.147 µg/g body wt. (LD_{50}). *Helicoverpa armigera* (Hubner) is commonly known as the gram pod borer as it is a serious pest on pulses (Nahar et al., David and Ananthakrishnan). It has been reported that *M.anisopliae* is effective against *H. armigera* [12].

The present investigation was carried out to highlight the significance of entomopathogens in pest management of cash crops, optimization of media for growth of *Beauveria bassiana* and bioassay of different formulations for their efficacy as marketable and easily applicable biopesticide. The investigation established the role of *Beauveria bassiana* bentonite formulation as an effective biocontrol agent against the most destructive pest *Helicoverpa armigera.*

Material and Methods

Chemicals

Sabouraud-Dextrose media, Czapek Dox media, Yeast Extract and Bentonite were purchased from HiMedia Biosciences Mumbai, India. Corn oil, gum and glycerine were purchased from local market. Maize flour was prepared by grinding maize grains in laboratory. Ponds talcum powder was used as one of the carriers.

Culture

Beauveria bassiana employed in this study was isolated from soil collected from soyabean field of Misrod, Bhopal (Rice technology bulletin, 2003 [13,14]. *Beauveria bassiana* was grown on Czapek Dox media modified with maize flour as per findings of Ramle et al. [15] and on Sabouraud-dextrose media with yeast extract [16] for growth optimization of the entomopathogenic fungus. Once the fungus was identified [17], pure cultures were obtained followed by flask cultures to develop the fungal mat.

***Corresponding author:** Ritu Agarwal, Department of Biotechnology, Career College, Bhopal 462 023, M.P, India, Ph: +91-9893835225; E-mail: ritu.agarwal@rediffmail.com

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Formulations

For preparing the formulations viz. carrier based powder formulation (CBPF) using talcum powder, glycerine and gum, oil-based liquid formulation (OBLF) using corn oil, gum and glycerin, bentonite oil-based liquid formulation (BOBLF) using corn oil, gum, glycerin and bentonite [6] using a knife mill grindomix (Remi, India). Fungal spore suspensions in different types of formulations were prepared from the fungal mats.

Viability of spores was assessed before and after preparation of formulations by serial dilution test in modified Czapek-Dox broth and after six months, viability of spores was again carried out in the prepared formulations as per Maheshwari [14]. Spore count of each formulation was done using Neubaeur chamber [18]. A range of concentration from 10% to 100% of each formulation was also prepared using distilled water and assayed at laboratory scale in triplicate and the optimal concentration for effective infection was screened for assay at the levels of pot culture and field assay. Reproducibility of each assay was established in triplicate.

Laboratory Bioassay

The fungal formulations of *Beauveria bassiana* were assayed against larvae of *Helicoverpa armigera* in the laboratory. Eggs of *Helicoverpa armigera* collected from the fields and were kept in optimum condition maintained at 23 ± 2°C and 35 ± 5% relative humidity under a 16:8 (L:D) cycle in a growth chamber. The larvae obtained from these eggs were fed with soya beans and sugar beet roots and leaves. The three days old larvae were divided into four groups of control and treated with different formulations and subsequently examined for symptoms of infection and mortality at regular intervals of 24 hours (Table 1).

Pot culture assay

In pot culture experiments, *Beauveria bassiana* formulations were evaluated against first and third instar larvae at 60% concentration of formulations with dosage equivalent to 2.1×10^4 spores ml^{-1}, calibrated on the surface area of the pot. As a control, contents of formulation were sprayed excluding *Beauveria bassiana*. The larvae were released in pots pre planted with sugar beet plants and allowed to settle for 24 hrs. In the test against larvae, different formulations carrying the fungal spores were applied to pots in replicates of four with seven larvae each. The pots were watered immediately. Larvae were examined for infection due to *Beauveria bassiana* at weekly intervals [19]. The experiments were repeated three times.

Field evaluation

Beauveria bassiana was tested at 2.1×10^4 spores ml^{-1} in a randomized block design (RBD) and plot size of 24 sq. m. in triplicate in soyabean fields of Misrod, Bhopal having four month old stalks. Spray method was used in the toxicity assays and as a control, contents of formulation was sprayed excluding *Metarhizium anisopliae*. First instar larvae were collected from the experimental plots about a month later by completely uprooting the plants or digging deep on either side of the rows. These larvae were brought to the laboratory in individual boxes filled with moist soil and reared on sugar beet and soya bean roots and leaves, which were changed every week. The proportion of larvae that showed symptoms of fungal attack after treatments with optimal concentration of all three formulations, were recorded as per protocol of Samuel et al. [20].

Statistical analysis

Each assay was established in triplicate for each parameter. The statistical analysis was performed using mean as a base for central tendency followed by calculation of deviation using standard error. Statistical significance was drawn by comparing the p value from students "*t*" test table. Significantly different from the control if $p<0.05$, significant if $p<0.01$, highly significantly if $p<0.001$.

Results and Discussion

The fungus, *Beauveria bassiana* showed fast growth on a medium consisting of yeast extract i.e Sabouraud-dextrose-yeast extract media which exhibited a shorter fungal mat development period of 15 days as compared to 20 days on Czapek Dox media.

Among the three different formulations, it was found that the bentonite based liquid formulation exhibited the highest efficacy of infection against Helicoverpa armigera. A range of concentration from 10% to 100% of each formulation was assayed in the laboratory bioassay and it was found that a 60% concentration of each formulation showed optimum results (Table 1 and Figure 1).

Thereafter, for pot culture assay and field trial, the concentration of formulations was assayed at 60% as in laboratory assay the result obtained from 60% to 100% were nearby. Preparation of varied formulations did not hamper the viability of spores as was assessed before and after, by serial dilution test in modified Czapek-Dox broth and provided an easy applicability in the liquid bentonite formulation. After six months, the viability of spores was again assessed in the formulations and it was found that the spores were still viable, although better result was seen in bentonite formulation as compared to the other two.

Beauveria bassiana is cited to be highly active against more than hundreds of insect pests and highly selective in its parasitisation [21]. The fungus, *Beauveria bassiana* was cultured with excellent results on a medium consisting of yeast extract i.e Sabouraud-dextrose-yeast extract [15]. Among the three different formulations, it was found that the bentonite based liquid formulation had the highest efficacy.

S. No	Types of formulation used	Required days for infection on larvae
1.	BOBLF	05
2.	OBF	12
3.	CBPF	09

(BOBLF): bentonite oil-based liquid formulation
(OBLF): oil-based liquid formulation
(CBPF): carrier based powder formulation

Table 1: Infection rate of different formulations of *Beauveria bassiana* without dilution on larvae of *Helicoverpa armigera*.

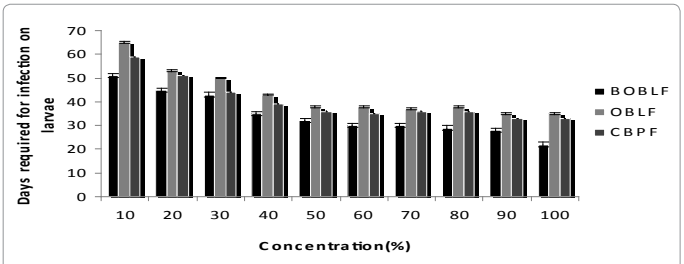

Figure 1: Showing number of days required by different concentration of formulations of *Beauveria bassiana* to infect larvae of *Helicoverpa armigera* in laboratory assay.

Anand et al. [1] has reported that the fungal pathogen *Beauveria bassiana* infects pupae of *Spodoptera litura* in a dose–dependent manner for each of the formulations investigated which corroborates the findings of the present investigation. They also reported that *Beauveria bassiana* was more infective and resulted in maximum average percent mortality among the three species viz. *Metarhizium anisopliae*, *Beauveria bassiana* and *Lecanicillium* sp. under investigation.

In the lab assay, 52% of larvae treated with 2.7×10^9 spores per ml of *Beauveria bassiana* developed infection in 7 days as against no infection in control, a finding also published by Chen et al. [22] during their work on *Metarhizium anisopliae*. Laboratory bioassay by Chandler and Davidson [8], documented higher mortality in *M. anisopliae* (ARSEF 7487) than *L. muscarium* (ARSEF 7037) in all soil based studies.

In pot culture assay, *Beauveria bassiana* caused low level of infection in larvae at the dosage equivalent to 2.1×10^4 spores ml^{-1} hectare since the fungus required 30-35 days incubation period to produce disease symptoms. In test, with a higher dosage range (1×10^6 – 10^8 spores per ml) observations made at monthly intervals, showed similar dosage dependent infection rates.

In the field trials with *Beauveria bassiana*, infection rates in larvae collected from the experimental plot were remarkably higher at the higher dosage of 2.1×10^4 spores ml^{-1} than at the lower dosage. The larvae collected 25 days after treatment developed infection in a maximum period of 32 days after collection from the field. This was in accordance with the field bioassay of Anand et al. [1].

Conclusion

Formulations of *Beauveria bassiana* can thus serve as an effective broad spectrum biocontrol agent for soyabean and various other cash crops. As also evident from the study, Bentonite is an effective carrier of the *Beauveria bassiana* in terms of being economical, maintaining the biological activity and increasing the ease of application. This carrier can also serve to be good medium for other fungal biopesticides for instance *Beauveria bassiana*, further studies on which are in progress.

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