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ISOLATION, CULTIVATION, IDENTIFICATION AND SCREENING OF THE ENTOMOPATHOGENIC ACTIVITY OF *Beauveria bassiana* ON COFFEE BERRY BORER

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ABSTRACT

Economically important crops should be safeguard from residual pesticides. At present, application of microbial inoculants is extremely followed for the management of soil borne disease. This paper summarizes the progress and achievements of mass production, formulation and application technology of the entomopathogenic fungus, *Beauveria bassiana* (Balsamo). Data were presented on the production of proteins, phenols, enzymes like chitinase and pectinase to degrade the chitin and pectin present in the insect integuments, bioassay to detect the lethal effect of coffee Berry borer by *B.bassiana* at different concentration and field application of the fungus.

KEYWORDS: Entomopathogenic fungus, *Beauveria bassiana*, coffee plant, CBB, bioassay.

INTRODUCTION

Coffee is an important commercial plantation crop grown in South India in places from about 750-2000 meters above sea level. It requires an annual rainfall of about 150-200cm and an atmospheric temperature in the range of 13-30°C. The three main species of coffee in cultivation are *Coffea arabica*, *C.robusta* and *C. liberica*. (Bull. ex Hiern). The present area under coffee in India is above 4 lakh hectares, 47% of which is under the fine quality arabica species with most of the remaining area under robusta. There are more that 20 disease of coffee in India and other countries, only a few of which are severe. The productivity of crops is limited by several soil borne disease. So far this disease is controlled by plant sanitation method coupled with fungicide application. The Coffee Berry Borer, *Hypothenemus hampei*

(Ferrari) (Scolytidae: Coleopteran), a serious pest of coffee in many of the World's chief coffee producing countries, has caused great losses (Le-Pelly, 1968). It is considered the most important insect pest and the greatest economic threat to coffee (Baker 1984). It is prevalent in about 88% of the total coffee growing areas in the country (Kumar *et al.*, 1990). Infested coffee berries that fall to the soil are the main source of reinfection of the coffee plantations at the end of the harvest period (Chamorro *et al*., 1995). The use of synthetic pesticide against coffee berry borer leaves residue in the environment and it cause harm to the non target organism. These are very toxic substances and workers seldom use protective clothing, likelihood of poisoning is high. The white muscardine fungus, *Beauveria bassiana* (Balsamo) Vuillemin occurs throughout the world and it has the largest host range among the fungi imperfecti. *Beauveria bassiana* produce toxins, some of which become as novel insecticidal compounds. The toxins produced by *Beauveria bassiana* cause swelling and stiffening in insect larvae. Some toxins, which are high molecular proteases, either directly damage the principal functions of the haemolymph or cause damage indirectly by producing a toxin byproduct in the insect.

MATERIALS AND METHODS

Sample collection

The fields in Regional Coffee Research Station (RCRS), Thandikudi, Dindigul district were selected as the site of sample collection.

Preparation of inoculum

The infected coffee berries were taken to the laboratory. Sabourauds dextrose agar medium was prepared and sterilized with streptomycin as an antibiotic to avoid bacterial contamination. The media was poured into sterile petriplates and allowed to solidify. The plates were inoculated and incubated at room temperature.

Microscopic Observation

The microscopic observation of the fungus was done by cutting a small piece of sabouraud dextrose agar and placed on the slide using sterile forceps. Using inoculation loop the culture was taken and inoculated on the agar piece which was covered with sterile coverslip and incubated for 2 days at 23°C. After incubation the coverslip was flooded with lactophenol cotton blue and observed under microscope.

Mass production

About 250g of rice and 250ml of distilled water was taken in polythene bags whose lid was made by using a PVC pipe and was plugged with cotton. The bags were sterilized, cooled and inoculated in laminar air flow. The spores from the bags were extracted using water containing 0.2% oil emulsion. Two or three washings were done to remove most of the spores from the bags. The spores were extracted from the flask and the suspension was strained through a muslin cloth to remove the rice particles.

Chitinase Activity (Khachatourians *et al*., 1987)

Chitin agar was prepared and poured onto sterile petriplates. It was allowed to solidify. After solidification, the plates were inoculated with *Beauveria bassiana* and incubated for 4-5 days at 23°C. After incubation, the plates were observed for growth on it.

Test for Phenols

To the sample one drop of neutral ferric chloride solution was added. The presence of intense colour indicates the presence of phenolic group. The result was recorded.

Estimation of Total Phenol (Ainsworth *et al*., 2007)

The broth sample was sonicated and the sample was centrifuged. To 1ml of superntant, 1 ml of Folin Ciocalteau regent and 2 ml of 20% sodium carbonate were added. The tube was kept in a boiling water bath for one minute and cooled under running tap water. The obtained blue solution was observed at 650 nm. Total phenol was estimated from the standard graph made from catechol.

Estimation of Protein (Lowry *et al*., 1951)

The quantity of protein produced by the conidiospores was estimated by protein assay method.

Bioassay of Fungal Pathogen against CBB (Rosa *et al*., 2005)

The coffee berry borer was collected from the infested coffee berries by cutting the berries carefully and CBB were selected for the bioassay.

Efficacy of *Beauveria bassiana* on CBB

The isolated *Beauveria bassiana* was tested to determine the pathogenicity of fungal culture towards CBB. Conidial suspension was serially diluted as 10^{-1} -10^{-6}. Bioassay of the fungus was carried out by dipping the adult CBB in the each dilution of conidial suspension for 10

seconds. Then the CBB were transferred to sterile filter paper and then placed in the sterile beakers having the coffee bean, which was surface sterilized and was closed with sufficient aeration. The mortality rate was observed at regular intervals.

Field Application

The spores from the mass cultivation were extracted using water containing 0.2% oil emulsion. Two of three washings were done to remove most of the spores. The spore suspension was strained through a muslin cloth to remove the rice particles. The suspension was sprayed on berry clusters using rocker sprayer.

RESULT AND DISCUSSION

Isolation and identification of organism

The fungal strain was isolated from the berries from natural habitat. In the laboratory, after incubation, a fungus with white fluffy cottony growth with pale yellow edges was abundantly expressed on sabourads dextrose agar plates was identified as *Beauveria bassiana*.

Microscopic observation

When the agar piece was flooded with lactophenol cotton blue and observed under microscope abundant conidiospores arising from the vegetative hyphae, bearing groups of clustered conidiogenous cells which are clustered conidiogenous cells, branched to give rise to further conidiogenous cells; globose to flask-shaped was recorded. The above shows the identification *Beauveria bassiana*.

Mass production

The fungal culture was isolated inoculated in the rice and incubated. After 14 days of incubation, the appearance of white colour colonies shows the mass production of *Beauveria bassiana*. The produced fungus was used to apply in the field and the rice was also sieved. The filtrate was mixed with sterile talcum powder and stored at 4°C for long term storage.

Chitinase activity

The organism were streaked on the chitin agar plate and incubated. After incubation the growth of *Beauveria bassiana* represents the production of chitinase enzyme by the fungi to degrade the chitin which is present in the insect integument. The organisms enter the insects by degrading the cell wall. The insect cell wall is made up of chitin and pectin. To break down the cell wall the organism requires the enzymes. To know the presence of chitinase in

the organism, they were streaked in chitin agar plates. The growth observed represents the production of chitinase enzyme in the fungi to degrade the chitin in the cell wall of insect integuments.

Test for phenols

In the neutral ferric chloride test, the intense color was obtained which represents the presence of phenolic group in the suspension.

Estimation of total phenol

The total phenol content was estimated at 650nm. The catecol was taken as a standard. The phenol content was 2.933 mg/ml. Phenol is the alkaloid which is produced by the fungus to reduce the activity of the insect. The concentration of the phenol content is determined to know about the activity of the phenol produced by the fungus to kill the insect by reducing their activity.

Estimation of protein

The quantity of protein produced by the conidiospores was estimated by Lowry et al., (1951) method. Bovine Serum Albumin was used as a standard. The total protein produced was estimated as 0.090mg/ml. Enzymes are the protein which has the capacity to interfere in the synthesis of any biochemical reaction of the insects and this lead to the lethal effect of insects.

Bioassay of Fungal Pathogen against CBB

The mycosis effect of *Beauveria bassiana* against Coffee Berry Borer at various spore dilutions on 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, was detected by bioassay method. The mortality rate in the first day was observed in dilution, 10^{-1}, showed lethal effect and 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, the CBB was alive. In the second day the mortality rate was observed in 10^{-2}, 10^{-3}, and the CBB got inverted in 10^{-4}, 10^{-5}, 10^{-6}. During the third day the lethal effect was noticed in the remaining dilutions (10^{-4}, 10^{-5}, 10^{-6}.). The lethal effect can be caused by two methods, one is that it produces the various enzymes and degrade the cell wall and reduce the activity of the insect. Secondly, mycelium colonizes the gut of the insect and make lethal of the insect.

Field Application

Beauveria bassiana has no sexual life cycle, when it sprayed on coffee berries; it enters the infected berries. Insects are infected by conidia the asexual propagules, which attach to the

host cuticle and invade the haemocoel and precede various enzymatic activities in the insect integument which finally result in lethal effect on the coffee berry borer. After about two weeks, the infection will be manifested as the white powdery spots appearing at the borer entry point.

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