Isolation of *Brauveria Bassiaana* from white Grubs and Comparing their Growth on Natural and Artificial Medium

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Abstract

Biopesticide formulation based on Beauveria bassiana, a wide host range insect pathogenic fungus are being marketed and used in insect pest management. These entomopathogenic hyphomycetes fungi have great potential as biological control agents against insects and in an important component within integrated pest management systems. They are being developed worldwide for the control of many pests of agricultural importance. Conodium of spore is infectious stage which attacked to the insect cuticles, germination of conodium and penetration of insect cuticles by a germ from the conodium. The insect disease caused by fungus is called muscadine disease, when the microscopic spore of fungus comes into contact with body of an insect host, they germinate, penetrate the cuticles and grow inside killing the insect within a matter of days afterwards a white mold emerges from the cadaver and produce new spores. In general 10-30% damage due to whitegrub, infestation in sugarcane crop was recorded however it is severely infested field, complex crop failure was observed, the maximum damage was observed in variety COS 88230. In the present study we have isolated *Brauveria bassaiana* from white grub. Conodial growth colonies were observed when grown over cadaver of white grub on Yeast Peptone Dextrose agar plate and pure culture was
obtained. We found that mean spore weight of Brauveria was more on rice than sorghum and solid state YPD agar.

**Keywords:** Entomopathogenic hyphomycetes; Brauveria bassiaana; White grub; Conodial growth.

1. **Introduction**
A chemical pesticides is used to protect crops and to kill pests. The use of chemical pesticides have many drawbacks including pest resistance, resurgence of pest, emergence of secondary pest due to loss of activity of parasitoids and predator effect on non-target organism, contamination of environment (soil, water, air) and presence of chemical residues on agricultural produce these drawbacks forced to the more selective and compatible method which may be safer to environment. Biological control is one of the most promising method of insect control and constitute an eco friendly alternative strategy. Biopesticide formulations based on *Beauveria bassiana* (Balsamo), a wide host range insect pathogenic fungus are being marked and used in insect pest management. These entomopathogenic fungi are being used worldwide for the control of many pests of agricultural importance. *Beauveria bassiana* is fungus naturally grown in soil ,conidium or spores is infectious stages of *Beauveria bassiana*. when this microscopic spore of fungus come into contact with body of an insect host, They germinate ,penetrate the cuticles and grow inside killing the insect within a matter of days.

India has always been an agricultural country and sugarcane is the second most important industrial crop of India after cotton. Unfortunately sugarcane roots form parts of small proportion of living tissue consumed by grub .white grubs are grub worm that commonly attack the roof of turf grasses and ornamental plants. White grub life cycle consists of four stages:
- Egg
- Larval instars
- Pupa
- Adult stage

The grub are the immature offspring of Japanese Beetles, June Beetles( May beetles). These immature stages of beetles that feed on the root system of lawn are called White Grubs. In recent times white grub has become a problem for growers and sugar mills of Western Uttar Pradesh. A team of scientist from IISR conducted a field survey to assess the white grub problems in the command area of Triveni Engineering and Industrial Ltd., Sugar unit Deoband, Saharanpur and Titawi Sugar Complex, Titwai, Muzaifarnagar. The affected villages are Jakhwala, Mathura, Bhala, Niamu, Charthwal, Akabargarh, Sikanderpur, Badgaon, Khudda, Shimbhalki in the Muzaifarnagar District; the white grub infection was recorded in about 200 ha sugarcane area of which 17 ha was severely infested.
2. Methodology

2.1. Collection of the White Grub
For the study of this research work, we have collected the white grubs from the sugarcane field of village Khudda, District Muzaffarnagar.

2.2. Inoculation of the White Grub
For the inoculation of white grub, Yeast Peptone Dextrose Agar medium was used. In the Yeast Peptone Dextrose Agar medium, the concentrations of yeast extracts was 2.0g/l, peptone concentration was 10.0g/l and dextrose concentration was 40.0 g/l and agar- agar was taken 16g/l, all the constituents were mixed in distilled water. For the sterilization of the YPD medium, the medium container was sterilized in hot steam autoclave chamber at 121°C, and 15 psi pressure for 20 minutes. The sterilized medium was poured in the petriplates and solidifies in the aseptic conditions. The isolated strain of white grub was incubated on the solidify Petri plate of sterilized Yeast Peptone Dextrose Agar artificial medium under aseptic conditions in the Laminar Air Flow Chamber and incubated the plates at 30°C for 2-3 days.

2.3. Preparation of inoculums and the solid substrates
Yeast broth was made by mixing 2g of yeast extract, 10g of peptone and 40g of sucrose in one liter of water. The mixture was dispensed into 250ml conical flasks then plugged loosely with a bung of non-absorbent cotton wool and autoclaved at 121°C, 15 psi for 40 minutes. After cooling, each flask was inoculated with a loop full of spores from cultures of Beauveria Bassiana. Two flasks were used for each isolate and were then incubated at room temperature (25± 5°C), on a rotary shaker revolving at 150 rpm for 72 hours as described (Jenkins et al. 1998) for production of blast spores. 250g of rice was weighed and boiled until the grains were just soft but not cooked. A sample of the boiled rice was sterilized by autoclaving at 121°C, 15 psi for 30 minutes. The procedure was repeated for sorghum. Sorghum were only washed and dispensed into the conical flasks.

3. Result and Discussion
From the microscopic study the isolated cultures Beauveria bassiana was pure. There was significant difference in the mean weight of spores of the isolates of Beauveria Bassiana on the different substrates. The Beauveria isolates had the highest mean spores weight of 8.38g on rice followed by 6.68g on sorghum and 7.46 on the solid state YPD agar and rice..In the study we used rice and sorghum as potential growing substrates for mass production of Entomopathogenic fungi and testing of germination are important steps in successful utilization of entomopathogenic fungi of Beauveria bassiana. These substrates are easily available low price where white grub pest is a problem in the Uttar Pradesh. Mass production of entomopathogenic fungi and testing of germination are important step in successful utilization of entomopathogenic fungi as biocontrol agents.
Our results partly agree with observations of Nelson et al. (1996) that demonstrated maximum yield was achieved when fungi were grown on rice for three weeks at 23°C. There is a need to determine yield to confirm whether the substrate with the highest quantity of spores has a maximum yield of spores per gram of substrates.

**Figure 1**: Isolated cadavers of white grub

**Figure 2**: Impure incubated colonies of the *Beauveria bassiana*

**Figure 3**: Solid state fermentation of natural media (sorghum and rice)

**Figure 4**: Growth of fungus *Beauveria bassiana* in sorghum solid state
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Figure 5: Pure incubated colonies of Beauveria bassiana in YPD media

Figure 6: Microscopic examination of Beauveria bassiana conodium

4. Conclusion
These finding imply that all the substrates supported growth of the Entomopathogenic fungi. The spore germinated irrespective of the treatment. Rice and YPD medium were found to have an overall higher amount of spores on rice. Least production was on sorghum. Sorghum and rice are relatively cheap and normally found in abundance in Uttar Pradesh, India.

In this paper, we used rice and sorghum as potential growing substrates for mass production of entomopathogenic fungi of Beauveria bassiana. These substrates are easily available at a relatively low price where white grub pest is problem in the Uttar Pradesh.

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