GERMINATION OF WHITE MUSCARDINE FUNGUS, *Beauveria bassiana* (Bals.) Vuill. ON DIFFERENT PLANT EXTRACTS

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Abstract

Laboratory experiment was conducted to evaluate the germination of white muscardine fungus *Beauveria bassiana* (Bals.) on different plant extract. *B. bassiana* was mass produced using unmilled rice as a substrate. Two-week-old culture of *B. bassiana* (GLH isolate) was used. Extracts from the leaves of test crops (rice, mungbean, peanut and soybean) was used and tested separately. Four serial dilutions (crude: 10,000 ppm; 1,000 ppm; 100 ppm) of the different extracts were made and tested separately. Germination of conidia was observed for 24 hours post-inoculation at room temperature.

Germination was observed in the extracts of all plant species tested 24 hours post-inoculation at room temperature. Highest germination rate of *B. bassiana* conidia was found in soybean (81.51%) followed by mungbean (51.91%), peanut (47.39%) and rice (36.94%). Germination of *B. bassiana* was also found to be significant among different levels of plant extracts. Highest germination was in 100 ppm (83.59%), 1,000 ppm (69.75%) extract concentration followed by 10,000 ppm (45.44%) and crude extract (18.97%). In general, germination rate was higher at lower extract concentration or germination rate was inversely proportional with level of plant extract.

Conidia of *B. bassiana* germinated in different plant species extracts and at various concentrations. It is concluded, that there is a high probability that these plants extracts contain toxic substances/compounds with inhibitory effects on the germination of *B. bassiana* conidia.

Key Words: *Germination, conidia, plant extract*

Introduction

The significance of biological control against insect pests offers an effective alternative component in the Integrated Pest Management program. Investigations on the potential of some entomopathogens including *Beauveria bassiana* (Balsamo) Vuillemin (Deuteromycotina: Hyphomycetes) have greatly contributed to their use as microbial control agents. Several studies reported that the fungus is virulent to a wide array of insects belonging to orders Lepidoptera, Homoptera, Coleoptera, Hemiptera and Diptera (Rombach et al., 1986; Reissig et al., 1987) both in the laboratory and under field conditions.

Among the major advantages of entomopathogens is by affecting other mortality agents less severely; they seldom induce outbreaks of secondary pests (Roberts and Yeldol, 1971; Fuxa, 1987; Rombach, 1986; Rombach et al., 1986a; Rombach et al., 1987; Wright, 1991; Wraight and Ramos, 2002).

One of the major constraints in the use of microbial insecticides is its vulnerability to environmental factors. In nature, there are ecological and behavioral barriers that inhibit them from causing infection such as the physical and chemical nature of leaf surface. Some studies have reported the inhibitory effects of some botanical materials to insect pests and fungal diseases in plants (Lapis and Dumancas, 1978; Quebral (1981); Agbola, 1984; and Franje, 1984).

Although none of the studies reported inhibitory effects of botanical materials against insect pathogens, it is possible that plant materials that control insects and plant fungal diseases may inhibit activity of entomogenous fungi. This study was conducted to determine the effects of plant extracts on the germination and survival of *B. bassiana.*

Methodology

Preparation of *Beauveria bassiana*

White Muscardine Fungus, *Beauveria bassiana* is mass produced in a substrate using unmilled rice (IR-42). Rice is boiled with sufficient water until it cracks; excess water is drained and the substrate is transferred to an autoclavable polyethylene bag. PVC ring is used to seal the bag, plugged with cotton and tied with a rubber band. The substrate is sterilized for one hour in an autoclave at 15 psi.

Conidia of *B. bassiana* cultures grown in SDAY slants are scraped off from the surface using wire loop and mixed with five ml of sterile Triton X-100 (0.02%). Inoculum of *B. bassiana* is then introduced into the polyethylene bags aseptically in a laminar flow hood. After inoculation, polyethylene bags are squeezed and shaken to spread the inoculums. Inoculated substrates are incubated at room temperature for three weeks.

Application of Fungal Spray

Two-week-old culture of *Beauveria bassiana* in SDAY slants are used to evaluate the germination of *B. bassiana* on different plant extracts. Conidia of *B. bassiana* (GLH isolate) in SDAY slants are scrapped using a wireloop and dislodged into the solution added with 5 ml of Triton X-100 (0.02%) per slant. Fungal suspension (at least 50 ml per isolate) is collected and kept in Erlenmeyer flask (250 ml cap).

Preparation and Application of Plant Extracts

Extracts from the leaves of test crops (rice, mungbean, peanut and soybean) are used to test the germination of *B. bassiana* in SDAY medium. Twenty-gram leaves of each test plant are prepared for its crude extracts. These are rinsed three times with sterilized distilled water, chopped, and pounded using sterile mortar and pestle at the ratio of 1:1 (leaves and water). The homogenous mixture is centrifuged at 500 rotations per minute (rpm) then filtered through a double layer aseptic mesh cloth. The supernatant, the crude extract, is mixed with 0.02% Triton X-100 and kept in a separate sterilized 250 ml capacity Erlenmeyer flask. Four serial dilutions (crude: 10,000 ppm; 1,000 ppm; 100 ppm) of the different extracts are made and tested separately. Germination of conidia was observed for 24 hours post-inoculation at room temperature in the extracts of all plant species.

Results and Discussions

Effects of Plant Extracts on the Germination *of Beauveria bassiana* Conidia

Highest germination rate of *B. bassiana* conidia was found in soybean (81.51%) followed by mungbean (51.91%), peanut (47.39%) and rice (36.94). However, there was no significant difference in germination between mungbean and peanut, (Tables 1).

Germination of *B. bassiana* was also found to be significant among different levels of plant extracts. Highest germination was in 100 ppm (83.59%), 1,000 ppm (69.75%) extract concentration followed by 10,000 ppm (45.44%) and crude extract (18.97%). Differences in germination between 100 and 1,000 ppm were not significant (Table 2).

Table 1. Germination of *B. bassiana* conidia as Influenced by Extract of Different Plant Species.

PLANT SPECIES	PERCENT GERMINATION*
Rice	36.49[c]
Mungbean	51.91[b]
Soybean	81.50[a]
Peanut	47.39[b]

**Means having the same letters are not significant at 5% level, DMRT.*

Table 2. Germination of *B. bassiana* Conidia as Influenced by Concentration of Plant Extract.

EXTRACT LEVEL (ppm)	PERCENT GERMINATION*
Crude extract	18.97[c]
10,000	45.44[b]
1,000	69.75[a]
100	83.59[a]

**Means having the same letters are not significant at 5% level, DMRT.*

Further, it was noted that plant species and extract level interaction had profound effect on conidial germination. Germination of *B. bassiana* conidia was found to be rapid and was the highest in all plant species at low plant extract levels (100 ppm). Although there was high germination rate at 100,000 ppm extract, but it was only found in soybean. While *B. bassiana* germinated in most plant species in reduced extract level of 10,000 ppm, i.e. in soybean with 81.36%, mungbean with 53.64%, peanut with 46.77%), but no germination was noted in rice. In general, result revealed that germination rate is inversely proportional to level of plant extract (Fig. 1).

The therapeutic values of some botanical materials against fungal plant diseases had long been recognized, but effect of plant extracts on entomopathogenic fungi has not been extensively investigated. In the present study, although it is premature to conclude and single out, that the inability of *B. bassiana* conidia to germinate in some of the plant extracts is due to toxic compounds present in these extracts (Taskeen-Un- Nisa et al., 2011). David et al. (1968) has reported the possibility that the observed inactivation of purified granulosis virus on the leaf surfaces might be due to toxic substances produced by the plants, and that these substances diffused into the virus affected its virulence. It poses a possibility that such is true in this case. Note that when crude extracts of rice, peanut and mungbean were diluted to 100 ppm, germination, occurred indicating that some substances with inhibitory effects could be present and have lost the ability to suppress germination when diluted to desired concentration.

Another possible explanation of the observed inactivation is that some substances present in the extract when decomposed could be toxic to the conidia. Such an effect due to

decomposing substances (allelopathy) has been reported in some plants (Garcia and Anderson, 1984). The identity of substances/compounds present especially in rice, mungbean and peanut is still unknown and warrant further investigation.

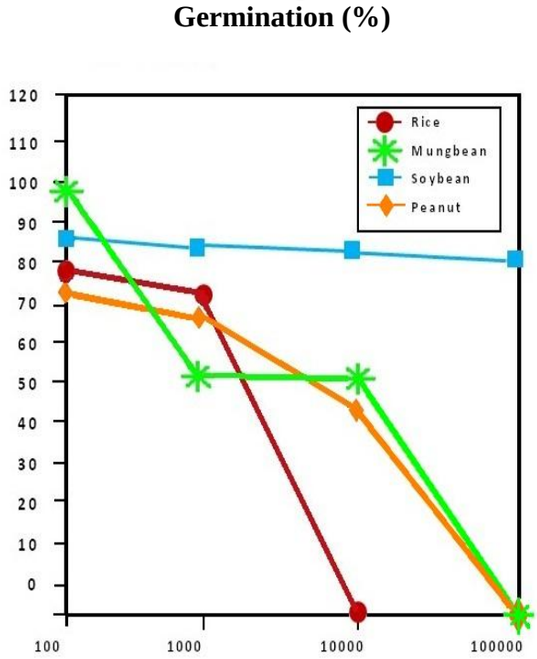

Figure 1: Germination of *B. bassiana* conidia on four plant extract concentration

CONCLUSION AND RECOMMENDATIONS

Conidia of *B. bassiana* germinated in different plant species extracts. Germination was also observed in all levels of concentration of plant extracts and was found out to be inversely proportional to plant extract concentration.

It is concluded, that there is a high probability that these plants extracts contain toxic substances/compounds with inhibitory effects on the germination of *B. bassiana* conidia. The identity of substances/compounds present especially in rice, mungbean and peanut is still unknown and warrant further investigation. On the other hand, the luxuriant germination of conidia on the crude extract of soybean also suggests that soybeans may contain substances/compounds that support germination of conidia. These substances/compounds might be absent or wanting on other crops (rice, mungbean and peanut).

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