# EVALUATION ON THE STABILITY OF WHITE MUSCARDINE FUNGUS, *Beauveria bassiana* (BALSAMO) VUILL. CONIDIA UNDER EXPOSURE TO DIRECT SUNLIGHT

**Pio P. Tuan, PhD***
Department of Agricultural Sciences, College of Agriculture, Fisheries, and Natural Resources
University of Eastern Philippines, University Town, Catarman, Northern Samar, Philippines

*Email: pio.tuan@yahoo.com

## Abstract

In an effort to select potential mycoinsecticides for the biocontrol of Rice bug, *Leptocorisa oratorius* (Hemiptera : Alydidae), the effects of exposure to direct sunlight on the stability and viability of *Beauveria bassiana* conidia was evaluated under laboratory conditions. Green leafhopper (GLH) isolate of *B. bassiana* suspension was applied on filter paper and leaves of four test plants such as Peanut (*Arachis hypogaea* ), Mungbean (*Vigna radiata)*, Soybean (*Glycine max)* and Rice (*Oryza sativa*) at vegetative stage. The results showed that conidia of *B. bassiana* were substantially inactivated after an hour of exposure to direct sunlight. When time of exposure was extended to 2 hr an enlargement of conidia occurred. Total loss of viability was noted when conidia were exposed to direct sunlight for 3 hr.

**Key Words**: *Entomopathogenic fungi, B. bassiana, conidia, substrate, myco insecticide, stability and viability*

## INTRODUCTION

The White Muscardine fungus, *Beauveria bassiana* (Balsamo) Vuill. (Deuteromycotina: Hypomycetes) is one of the most important species of entomopathogenic fungi used in biological control of insect pests. The application of entomopathogenic fungi in biological control is increasing largely because of greater environmental awareness, food safety concerns and the failure of conventional chemicals due to an increasing number of insecticide resistant species (Rai et al., 2014).

. 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; Liu H., S. Bauer, L., 2006; Marannino et al., 2008; Quesada et al., ., 2006a; Quesada et al., 2006b; Stafford and Allan. 2010; Prasad, 2010; Tarasco et al., 2011; Monica et al., 2012; Mudrončeková, et al.,. 2013 ) 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; Shi and Feng, 2004; Scholte, et al., 2005 Blanford, et al., 2005; Achonduh and Tondje, 2008). An interesting feature of Beauveria sp. is the high host specificity of many isolates. Hosts of agricultural and forest significance include the Colorado potato beetle, the codling moth, and several genera of termites, American bollworm, Helicoverpa armigera (Thakur et al., 2010).

There are parameters other than its pathogenicity that must be considered in the development of a fungal isolate suitable for use as a mycoinsecticide. One of the major constraints in the use of microbial insecticides is its vulnerability to environmental factors. Environmental factors that influence the virulence of entomopathogens must be considered for the successful development of the fungus as a bio-control agent. In nature, there are ecological and behavioral barriers that inhibit them from causing infection such as the physical and chemical nature of leaf surface. The main abiotic factors that affect entomopathogenic fungi are humidity, temperature and exposure to ultra-violet (UV) radiation (Harty and McLeod , 1955; Getzin, 1961; Catwell and Franklin, 1966; Ferron, 1967; Catwell, 1967; David et al., 1`968; Walstad, et al., 1970; Frantz, 1971; Smirnoff, 1972; Ignoffo, et al., 1974; Ignoffo et al., 1976 Ferron 1978; Zimmermann, 1982; Fuxa, 1987; Fargues et al, 1996;; Glare, 2010; Rangel et al., 2005a; Rangel et al., 2006a Lazzarini, 2006; Meyling and Pell, 2006; Fargues et al., 2013; ). The solar radiation, which includes visible light, ultraviolet radiations, infrared rays and radio waves have been the dominant source in which all organisms evolved and adapted. In biological context, the UV radiations acclaim a special mention in terms of their impact on life (Bjorn, 2006). This study investigates the effects of direct exposure to sunlight of the stability and viability of *B. bassiana* (GLH isolate) conidia under laboratory conditions

## METHODOLOGY

Green leafhopper (GLH) isolate of *Beauveria bassiana* was used to evaluate the stability and viability of conidia under exposure to direct sunlight on filter paper and leaves (at vegetative stage) of test plants. Four test plants were used such as Peanut (*Arachis hypogaea* ), Mungbean (*Vigna radiata*), Soybean (*Glycine max)* and Rice (*Oryza sativa*). Leaves were washed with sterile distilled water and blotted dry with tissue paper. Conidial suspension ($1.0 \times 10^{13}$ conidia/ha), was sprayed on filter paper and on the upper leaf surface of the test plants using a 500 ml capacity hand atomizer. Spread droplets were allowed to evaporate completely before exposure to direct sunlight. Exposure was made between 9:00 in the morning and 4:00 in the afternoon. It was done progressively on an hourly basis (9:00 – 10:00; 9:00 – 11:00; 9:00 – 12:00; 9:00 – 1:00; 9:00 – 2:00; 9:00 – 3:00; 9:00 – 4:00). During the exposure, the filter paper (contained in petri dish) and plant leaves were oriented to face the sun. Care was taken to see to

it that even temporary shading was reduced to minimum. The same set of treatments were provided but kept under shade as control. Relative humidity (RH) and temperature was recorded at 68% and $28^0$C, respectively. After the required exposure, treated leaves were cut off from the plant and were taken to the laboratory for processing. Treated leaves were washed with 5 ml sterile distilled water with the use of a camel's hair brush. The suspensions were kept in a refrigerator for 12 hr before inoculation in SDAY medium.

Sterile glass slides containing the medium were inoculated with uniform volume (two drops) of conidial suspension. Inoculated slides covered with cover slips were incubated at room temperature. Germination was scored as the visible protruberance of the germ tube from the conidium. Counting commenced 12, 15, 20 and 24 hours post-inoculation. Five randomly selected microscopic fields from each glass slides were taken for calculation of the mean germination rate over time using the formula:

$$\% \text{ Conidial germination} = \frac{\text{Total no. of conidia germinated}}{\text{Total no. of conidia}} \times 100$$

Germination rate at 24hr post-inoculation was compared using Duncan's Multiple Range Test (DMRT).

## RESULTS AND DISCUSSION

The stability and viability of *B. bassiana* conidia in the laboratory was evaluated by exposing the conidia under direct sunlight. Results of the test are summarized in Table 1. Since there were very few germinating conidia, an arbitrary code was used to differentiate germinating from non-germinating conidia as follows: ++ germinated; + enlargement of conidia and – no germination. The results showed that conidia of *B. bassiana* were substantially inactivated after an hour of exposure to direct sunlight. When time of exposure was extended to 2 hr an enlargement of conidia occurred. Total loss of viability was noted when conidia were exposed to direct sunlight for 3 hr.

Conidia of *B. bassiana* applied on filter paper and leaves of test plants kept in a shaded condition were also inactivated. Conidia recovered from filter paper were totally inactivated in 3 hours; those on the leaves of the test plants in 4 hours. The result in this present study with *B. bassiana* was very much in line with those previously reported. For example, Roberts and Campbell (1977) earlier reported of the rapid decrease in the viability of spores exposed to direct sunlight; they suggested that the spore mortality was caused by UV radiation. Zimmermann (1982) also reported a striking decline in the viability of *M. anisopliae* spores between 6 and 12 minutes of exposure and a nearly total loss in viability at 24 minutes. He claimed that radiation might have retarded the germination process. Ignoffo et al., (1977) speculated that hydrogen peroxide produced by the near ultraviolet radiation of one or more amino acids, reduces both the

vitality and pathogenicity of microbiological control agents. In the present study, conidia of *B. bassiana* were totally inactivated in less than 3 hours exposure to sunlight.

Table 1. Viability of *B. bassiana* conidia at varying time of exposure to direct sunlight.

| | TIME OF EXPOSURE (HR) | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| | IN DOOR | | | |
| Filter paper | ++ | ++ | _ | _ |
| Rice leaves | ++ | ++ | _ | _ |
| Mungbean leaves | ++ | ++ | + | _ |
| Peanut leaves | ++ | ++ | + | _ |
| Soybean leaves | ++ | ++ | + | _ |
| | OUT DOOR | | | |
| Filter paper | ++ | + | _ | _ |
| Rice leaves | ++ | + | _ | _ |
| Mungbean leaves | ++ | + | _ | _ |
| Peanut leaves | ++ | + | _ | _ |
| Soybean leaves | ++ | + | _ | _ |

++ conidia germinated
+ conidia enlarged
_ no germination

## CONCLUSION AND RECOMMENDATION

It is difficult to single out that the inactivation of *B. bassiana* conidia applied on the leaves of test plants and filter paper was primarily due to direct effect of exposure to sunlight, since the amount of heat and/or ultraviolet radiation received by the microorganism was not accurately measured. In this study, viability of conidia was substantially and totally inactivated after one and three hours of exposure to direct bright sunlight, respectively. Conidia kept in shade were also substantially inactivated after three hours presumably due to the effects of high temperature and desiccation. The results however, highlight the importance of proper timing and placement of microbial insecticide when applying in the field.

In the field application of fungal insecticides, some conidia may lodge on the underside s of the leaves or in other places where they can be protected from direct sunlight. Nevertheless

much of the conidia are certain to be deposited on the upper surface of the leaves and as they are inactivated they will inevitably loss biological efficiency rapidly. More test (assay) and detailed experimentation are necessary to substantiate any conclusions and elucidate the influence of the important environmental factors such as the inactivation of conidia under exposure to direct sunlight should be critically and accurately evaluated including other factors that cannot be separated from radiation studies such as short and long wave radiation, temperature, humidity and probably wind movement

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