# PATHOGENICITY OF WHITE MUSCARDINE FUNGUS, *Beaveria bassiana* (Balsamo) Vuillemin AGAINST RICE BUG, *Leptocorisa oratorius* (F.) (HEMIPTERA: ALYDIDAE)

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## ABSTRACT

The pathogenecity of different isolates of *Beauveria bassiana* (Bals.) Vuill. to the rice bug, *Leptocorisa oratorius* (F.) was evaluated. All isolates tested were able to infect *L. oratorius* in the laboratory. GLH isolate was most potent with $LC_{50}$ of 4.65 x $10^9$; 2.22 x $10^9$; 3.68 x $10^9$ conidia/ml for third instar, fourth instar, fifth instar and adults, respectively. $LT_{50}$ was 7.95 days (third instar); 5.52 days (fourth instar); 3.37 days (fifth instar) and 2.89 (adults). The test also showed that early instars ($3^{rd}$ – $4^{th}$ instar) were more resistant to fungal infection than late instar ($5^{th}$) and adults. The results of the study revealed that *B. bassiana* is highly pathogenic to *L. oratorius* under laboratory conditions.

**Keywords**: Pathogenicity, *Beauveria bassiana*, *Leptocorisa oratorius*, conidia, Lethal Concentration ($LC_{50}$), Lethal Time ($LT_{50}$).

## INTRODUCTION

Rice bug, *Leptocorisa oratorius* (F.) is a major insect pest of rice at milking stage. Damage caused by the pest is serious in Eastern Visayas region in the Philippines.

Control strategies currently used against the pest is largely based on chemical insecticides. However, environmental awareness on the negative effects of chemical insecticide has stimulated renewed interest in the development of safe alternatives of insect pest management. The use of entomopathogenic fungus, *Beauveria bassiana* (Bals.) Vuill. is one of the potential alternatives reported to infect a variety of insect pest species and can be mass produced on cheap media such as bran, boiled rice and soybean (Rombach, 1986) without danger to humans.

In USSR, the fungus is applied against the Colorado potato beetle, *Leptinotarsa decemlineata*, the potato lady bug, *Epilachna sp.*, and several forest pests. In China, *B. bassina*

is mass produced at village level using simple technology as a regular component of their management effort against the corn borer, *Ostrinia nubilalis* (Rombach, et al., 1986).

In rice, *B. bassiana* was repeatedly reported on a number of insect pests including black bugs, *Scotinophora coarctata* and rice bug, *Leptocorisa spp*. (Reissig et al., 1987; Romback et al., 1986). In the laboratory, the fungus exhibited varying degree of pathogenicity (Tropago, 1993).

This study was aimed to evaluate the pathogenicity of *B. bassiana* on different life stages of *L. oratorius* and to identify the isolate of *B. bassiana* most virulent against *L. oratorius*.

## MATERIALS AND METHODS

### Source of Rice Host Plants

Seeds of UPL-Ri5, an upland rice variety, were sown in wooden seed boxes and transplanted in medium size clay pots. Plants were maintained outdoor until milking stage. Fertilizer at desired rate was applied and watering was done as often as necessary to ensure optimum growth. Host plants were cleaned and freed of other insects before they were introduced to the rearing cages for mass rearing of bugs.

### Mass Culture of *Leptocorisa oratorius* (F.)

The mass culture was initiated from adult *L. oratorius* collected from field population by a sweep net. Collected bugs were reared in a nylon mesh cage until they laid enough eggs. Leaves with rice bug eggs were cut from the plants and incubated in petri dishes until hatching. Nymphs that hatched within 24 hrs were collected and reared in separate nylon mesh cages until the desired nymphal stages were reached.

### Preparation and Application of Fungal Spray

Isolates of *B. bassiana* used in this study are presented in Table 1. Cultures of three fungal isolates were maintained on Sabouraud agar with 1.0% yeast extract (SDAY). Three-week old culture of each isolate was scraped with a plastic scraper. The mixture (conidia and culture medium) were suspended in sterile distilled water containing 2.0% Triton X-100. Fungal suspensions were passed through fine sterile mesh cloth. Conidial concentrations were determined using standard hemacytometer. Three serial dilutions ($10^7$; $10^8$; $10^9$) from each isolate plus control (2% Triton X-100 solution only) were prepared and tested separately.

Ten nymphs per instar ($3^{rd}$ to $5^{th}$ instar) including adults were treated in each conidial concentration in three replications. All test insects including those intended for the control were surface sterilized by dipping them in germicidal solution (10% Roccal solution) for three seconds.

All test insects were treated by dipping them into the conidial suspension. After exposure, the bugs (treated and untreated) were placed mylar cages, 8 cm diameter and 25 cm high with two fine nylon mesh windows (5 cm x 3 cm). Each cage was fitted and secured to rice panicles at milking stage and held in place by a bamboo stick pushed into the soil. Panicles were changed regularly by introducing fresh rice plants at 5-day interval. Test insects were observed daily for 10 days for mortality expressed in percentage. Lethal concentration ($LC_{50}$) and lethal time ($LT_{50}$) were determined by probit analysis.

Table 1. Isolates of *Beauveria bassiana* used in the study.

| ISOLATE | SOURCE | HOST | |
|---|---|---|---|
| | | Order | Species |
| AJ III-1 | NCPC[1] | Homoptera | *Nilaparvata lugens* |
| BPH | IRRI[2] | Homoptera | *N. lugens* |
| GLH | IRRI | Homoptera | *Nephotettix virescens* |

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*2-International Rice Research Institute*

## RESULTS AND DISCUSSION

All isolates of *Beauveria bassiana* tested were able to infect *L. oratorius* in the laboratory. The resulting $LC_{50}$ of each isolate on different stages of *L. oratorius* are shown in Table 2. Among the isolates, the one derived from Green Leafhopper (GLH) had the lowest $LC_{50}$ in all stages of the insect tested with $4.65 \times 10^9$; $2.22 \times 10^9$; $3.68 \times 10^9$ and $1.3 \times 10^9$ conidia/ml for third, fourth, fifth instars and adult of *L. oratorius*, respectively. Isolate AJ III-1 tended to display less virulence to the host than GLH with $8.21 \times 10^9$; $5.5 \times 10^{9;}$ $4.6 \times 10^9$ and $6.73 \times 10^9$ conidia/ml for third, fourth, fifth instars and adult, respectively. The BPH isolate was the least virulent in all stages of the test insect where it failed to induce 50% mortality at day 10 of exposure to the fungus. No mortality attributable to *B. bassiana* infection occurred in the control.

The time at which the treated bugs at different stages died of infection by *B. bassiana* varied considerably among the isolates tested (Table 3). The $LT_{50}$ induced by the highest concentration ($1 \times 10^9$ conidia/ml) on the different stages of *L. oratorius* occurred on day 8 (GLH isolate) and on day 11 (AJ III-1) for the third; day 5 (GLH) and day 9 (AJ III-1) for the fourth instars; day 3 (GLH) and day 7 (AJ III-1) for the 5$^{th}$ instars and on day (GLH) and day 6 (AJ III-1) for adults.

Table 2. Pathogenicity and regression coefficient of different isolates of *B. bassiana* on different life stages of *L. oratorius* (F.).

| ISOLATE | FIDUCIAL LIMITS (conidia/ml) | | | |
|---|---|---|---|---|
| | $LC_{50}$ * | Lower | Upper | REGRESSION COEFFICIENT |
| | | **3RD INSTAR** | | |
| AJ III-1 | $8.21 \times 10^{9a}$ | $2.04 \times 10^{9}$ | $2.32 \times 10^{9}$ | $Y = 5.51 \times 10 + 4.70 \times 10^{10}_{x}$ |
| GLH | $4.65 \times 10^{9c}$ | - | - | $Y = 5.34 \times 10 + 2.57 \times 10^{10}_{x}$ |
| BPH | - | | | - |
| | | **4TH INSTAR** | | |
| AJ III-1 | $5.50 \times 10^{9a}$ | $1.18 \times 10^{9}$ | $2.51 \times 10^{13}$ | $Y = 5.50 \times 10 + 4.00 \times 10^{10}_{x}$ |
| GLH | $2.22 \times 10^{9b}$ | $6.60 \times 10^{8}$ | $1.83 \times 10^{10}$ | $Y = 5.88 \times 10 + 5.034 \times 10^{10}_{x}$ |
| BPH | - | - | - | - |
| | | **5TH INSTAR** | | |
| AJ III-1 | $4.66 \times 10^{9c}$ | $7.90 \times 10^{8}$ | $5.33 \times 10^{18}$ | $Y = 5.46 \times 10 + 3.49 \times 10^{10}_{x}$ |
| GLH | $3.68 \times 10^{9d}$ | $1.18 \times 10^{9}$ | $4.54 \times 10^{10}$ | $Y = 5.79 \times 10 + 5.54 \times 10^{10}_{x}$ |
| BPH | - | - | - | - |
| | | ADULTS | | |
| AJ III-1 | $6.73 \times 10^{9c}$ | $1.19 \times 10^{9}$ | $1.40 \times 10^{18}$ | $Y = 5.42 \times 10 + 3.55 \times 10^{10}_{x}$ |
| GLH | $1.30 \times 10^{9d}$ | $3.20 \times 10^{8}$ | $7.06 \times 10^{9}$ | $Y = 5.99 \times 10 + 5.25 \times 10^{10}_{x}$ |
| BPH | - | - | - | - |

** - Calculated using Probit Analysis based on 30 L. oratorius per dose (conidial concentration)*
*- - No analysis, mortality did not reach 50%*
*a- Mortality obtained from 10 days exposure*
*b- Mortality obtained from 7 days exposure*
*c- Mortality obtained from 8 days exposure*
*d- Mortality obtained from 4 days exposure*
*e- Mortality obtained from 6 days exposure*

Table 3. Lethal time and regression coefficient of different isolates of *B. bassiana* on different life stages of *L. oratorius*.

| ISOLATE | FIDUCIAL LIMITS (day) | | | |
|---|---|---|---|---|
| | $LT_{50}$ | Lower | Upper | REGRESSION COEFFICIENT |
| | | **3rd INSTAR** | | |
| AJ III-1 | 11.08 | 9.20 | 15.47 | Y = 1.9537 + 2.9165 X |
| GLH | 7.95 | 7.06 | 9.33 | Y = 1.9398 + 3.3979 X |
| BPH | - | - | - | - |
| | | **4TH INSTAR** | | |
| AJ III-1 | 8.55 | 7.54 | 10.26 | Y = 1.8666 + 3.3614 X |
| GLH | 5.42 | 4.88 | 5.98 | Y = 2.1296 + 3.9129 X |
| BPH | - | - | - | - |
| | | **5TH INSTAR** | | |
| AJ III-1 | 7.19 | 6.28 | 8.53 | Y = 2.6314 + 2.7652 X |
| GLH | 3.37 | 3.01 | 3.71 | Y = 2.7650 + 5.1799 X |
| BPH | - | - | - | - |
| | | **ADULT** | | |
| AJ III-1 | 5.49 | 4.79 | 6.32 | Y = 3.0034 + 2.6992 X |
| GLH | 2.89 | 2.55 | 3.20 | Y = 2.5969 + 5.2165 X |
| BPH | - | - | - | - |

*-No analysis, mortality less than 50% at day 10.*

Variation in pathogenicity of fungal strain towards an insect species had been pointed out by a number of workers (Feng and Johnson, 1990; Wright and Chandler, 1991; Khachatorians, 1992; Tropago, 1993 and Budeos, 1994). Such differences were attributed to one factor or a combination of factors such as variation in growth and sporulation of fungal strains, phylogenetic relationship between potential host, nutritive requirement of the fungal strain, enzymatic and physic-chemical interaction between hosts and the pathogen and other factors within the host insect.

Dead *L. oratorius* infected with *B. bassiana* became mummified and their appendages turned brittle. When conditions (temperature and humidity) were favorable, whitish mycelia growth began to appear on the inter-segmental region and joints of the appendages of the cadaver one day after death. At advanced stage of infection, fungal growth was uniformly distributed on the insect body and dead insects usually stuck to the host plants.

One interesting result in this study is the relative susceptibility of the different developmental stages of *L. oratorius* to *B. bassiana* infection. The early instars (3rd - 4th) were more resistant to infection than adults (Table 3). This finding run counter to that of Burdeos (1994) who claimed that early instar ($1^{st}$ – $3^{rd}$) were more susceptible to *M. anisopliae* infection than adult *L. oratorius*.

The higher susceptibility of adults *L. oratorius* over the immature stages ($3^{rd}$ – $4^{th}$) can be attributed to the combined effects of environmental condition (micro-climate) and insect development during the conduct of the test. In the previous test carried out using whole rice plant enclosed in mylar cage, created a micro-climate inside the cage characterized by higher humidity and temperature due to higher evapotranspiration rate within the mylar cage in the presence of water or moist soil in the clay pot. In this study, the test was carried out using a single panicle enclosed in a mylar cage where the humidity and temperature were very much lower thereby subjecting the test insects to environment less favorable for fungal infection. Ferron (1978) reported that under less favorable conditions, death due to fungal infection may occur without external manifestation (fungal growth) of the disease. Veen (1968) also reported that outgrowth and conidiation from fungus-killed cadavers are very dependent on the prevailing humidity. In this study, immature insects inside the cage were hard and brittle without external fungal growth. External fungal growth resulted only when cadavers were incubated in petri dishes with moist filter paper. This indicated that the micro-climate inside the cage was less favorable for fungal infection of immature *L. oratorius* than that of adults. Excessive external growth of fungus in adults was observed inside the cage.

The findings of this study confirmed the report of Rothschild (1970) that *L. oratorius* passes through five nymphal instars which last for 2.5, 3.2, 3.3, 4.0 and 5 days, respectively. On the other hand, Getzin (1961) claimed that at least 48 hrs would be needed for infection of entomopathogenic fungus to begin. In relation to the disease development, the resistance of immature *L. oratorius* to fungal infection was the result of the shedding-off of germinating conidia during molting. This phenomenon was observed by Layaoen, (1979) on corn earworm, Helicoverpa armigera armigera; Rombach et al., (1986) on black bug, *Scotinophora coarctata* and Vey and Fargues, (1977) on Colorado potato beetle, *Leptinotarsa decemlneata*.

## CONCLUSION AND RECOMMENDATION

Among the three isolates of *Beauveria bassiana* tested, GLH isolate was most pathogenic to *Leptocorisa oratorius*. Fifth instars and adults were most susceptible to *B. bassiana* than the early instars ($3^{rd}$ – $4^{th}$).

The results of this study suggest that pathogenicity of *B. bassiana* against *L. oratorius* is influenced not only by different isolates and conidial concentration but also by the differences in developmental stages of the insect. It does not necessarily follow that bugs become less susceptible to fungal infection as they develop from instars to adults, subject to the prevailing environmental conditions. These observations indicated that conditions needed for fungal infection of adults are not necessarily the same for nymphs. A follow-up study is desired to investigate whether the resistance exhibited by early nymphal instars is mainly due to the shedding of conidia during molting. This is a major concern in the management of insect particularly *L. oratorius* where immature stages can inflict considerable damage to rice crop.

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