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USE OF LOCALLY AVAILABLE *BEAUVERIA BASSIANA* (BALSAMO) VUILLEMIN ISOLATE FOR THE CONTROL OF COFFEE BERRY BORER, *HYPOTHENEMUS HAMPEI* (FERRARI) (COLEOPTERA: SCOLYTIDAE)

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ABSTRACT

Coffee berry borer *Hypothenemus hampei* (Ferrari) is the most frequently occurring and destructive pest of coffee (*Coffea* spp.) in Sri Lanka. The fungus *Beauveria bassiana* (Balsamo) Vuillemin is found naturally infecting coffee berry borer in Sri Lanka. The possibility of using *B. bassiana* in management of *H. hampei* was studied under laboratory and in the field conditions. The estimated LC_{50} and LT_{50} of *B. bassiana* for *H. hampei* were $1.4x10^6$ conidia/ml and 2.6 days respectively. The mass production of *B. bassiana* was tested in different varieties of cooked rice namely, red rice, white rice and parboiled rice. Results showed that the conidia production in white rice was significantly higher than other two rice varieties. Conidia production in all rice varieties was increasing even after 35 days of inoculation of fungus. Application of *B. bassiana* conidia to bearing coffee trees in the field resulted in higher death of coffee berry borers with mycosis than that of untreated trees.

KEYWORDS: Coffee berry borer, *Hypothenemus hampei*, *Beauveria bassiana*, Mass propagation, Field application

INTRODUCTION

Sri Lanka has a high potential to develop coffee as an industry through commercial cultivation. However, coffee berry borer (*Hypothenemus hampei*) (Ferrari) (Figure:1) is the most destructive and the greatest economical threat to coffee industry in the world and also in Sri Lanka (Perera, 1983). Coffee berry borer (CBB) originated in East Africa, was reported for the first time in Sri Lanka in 1935 (Hutson, 1936).

There is a potential of 1000-1500 kg/ha coffee production in Sri Lanka (Anon. 1998). However, the coffee berry borer infestation could drop the yield by 50% and lower the quality of coffee (Perera, 1983). At severe infestation, 80% of berries could be attacked by the *H. hampei* (Le Pelley, 1968). Cultural and chemical control methods are currently recommended to control *H. hampei* in Sri Lanka (Dharmadasa, 2000). At present biological control methods are becoming more popular in many countries due to their environmental friendliness and the sustainable nature. Several species of predators, parasitoids and entomopathogens have been reported as natural enemies of *H. hampei* (Murphy and Moor, 1990; Vega *et al.*, 2006). Use of entomopathogens is widely practiced in the world due to its convenienct applicability. *Hirsutella eleutheratorum, Metarhizium anisopliae, Fusarium oxysporium,* and *Beauvaria bassiana* are the common entomopathogenic fungal species that have been tested against for the *H. hampei* in several coffee growing countries such as Brazil, Colombia and Mexico (Posada *et al.*, 2004).

Beauveria bassiana is the most promising and frequently used entomopathogenic fungus for biological control of *H. hampei* in the world, compared to other entomopathogenic fungi as it has high virulence towards *H. hampei* (Neves and Hirose, 2005). The fungus *B. bassiana* is naturally available in the soil through out the world (Groden, 1999).

For field application to control coffee berry borer, large scale availability of the fungal pathogen is a primary requirement. It is desirable if this could be achieved from an easy and economical method. Past studies have shown that *B. bassiana* was able to grow on a wide variety of agricultural products and byproducts of both solid and liquid state such as, rice bran, wheat bran, maize bran, carrot broth, potato broth, sugar mill effluent *etc.* (Sahayaraj and Namasivayam, 2008; Mondal and Bhattachary, 2004; Siwach and Jaipal, 2004). Rice can be used easily for mass production of the fungus, because it is freely available at any time of the year, cheap and has the ability of storing long periods with no spoilage. The common rice types available in Sri Lanka are red rice, white rice and parboiled rice.

The aim of this study was to investigate the possibility of using *B. bassiana* for the management of *H. hampei* by applying locally available

strain (MF-1). Experiments were conducted to investigate the virulence of *B. bassiana* isolated from CBB and to find out the most effective rice medium for mass production and the field efficacy of *B. bassiana* on CBB.

MATERIALS AND METHODS

Isolation and culturing of fungus

The experiments were carried out at the Department of Export Agriculture, Matale during the period March 2007 to December 2009. Coffee research field in the Export Agriculture Research Station, Matale was selected to collect *H. hampei* and to isolate *B. bassiana.* Borer infested coffee berries were collected from coffee fields and fungal infected berry borers were obtained from those berries. Infected beetles were placed on Petri dishes with Potato Dextrose Agar (PDA) media. Streptomycin sulfate was added to the media as an antibiotic and incubated at 25 ± 2°C. *Beauveria bassiana* conidia were isolated from the 7-14 days old culture plates and then sub cultured for purification. Fungal conidia were scraped from the surface of 14 -21 days old sub cultures and conidial suspension was prepared in 0.1% Triton X- 100. All the activities were carried out under sterile condition in a class II biohazard safety cabinet (Esco, Airstream E-Series Class II Biohazard safety cabinet). Conidia concentration was estimated by using a Haemocytometer and the required conidia concentrations were prepared by adding 0.1% Triton X-100 solutions.

Rearing of coffee berry borer

Coffee berry borer infested berries were collected from the field and were reared in the laboratory keeping them in glass containers (20x16 cm dia) until new generation adults were emerged. These adults are ready to fly away and find new coffee berries to lay eggs (Le Pelly, 1968). Three to four days old adults were used to estimate the virulence of the fungus.

Virulence of *Beauveria bassiana* for Coffee berry borer

Estimation of LC_{50} value (Lethal Concentration - 50)

Five concentrations of fungal suspensions, $1x10^4$, $1x10^5$, $1x10^6$, $1x10^7$ and $1x10^8$ conidia/ml were prepared in 0.1 % Triton X-100 solution. Insects were

kept in Petri plates and each fungal suspension was applied to 15 living adult beetles by an atomizer. In the control test insects were treated only with 0.1% Triton X-100. The experiment was laid out in Complete Randomized Design (CRD) with four replicates. Treated insects were provided coffee berries as food. Number of dead adult beetles was recorded daily up to seven days. Dead insects were placed on sterile microscopic slides and those slides were placed in Petri dishes lined with wet filter paper. Those insects were then observed daily for the sporulation. Number of dead insects with sporulation (Figure 2) was considered as infected insects and these data were used to estimate of LC_{50}.

Estimation of LT_{50} value (Lethal Time -50)

Fungal suspension at the concentration of $2.4x10^6$ conidia/ml (LC_{90} value estimated from the previous experiment), was applied to 15 living adult beetles placed in Petri dishes. Control insects were treated only with 0.1% Triton X-100. The experiment was laid in a CRD and replicated four times. Treated insects were provided with coffee berries as food. Mortality data were collected daily and the dead insects were placed in Petri dishes lined with moisturened filter paper. Number of dead insects with sporulation was used to estimate the LT_{50} value.

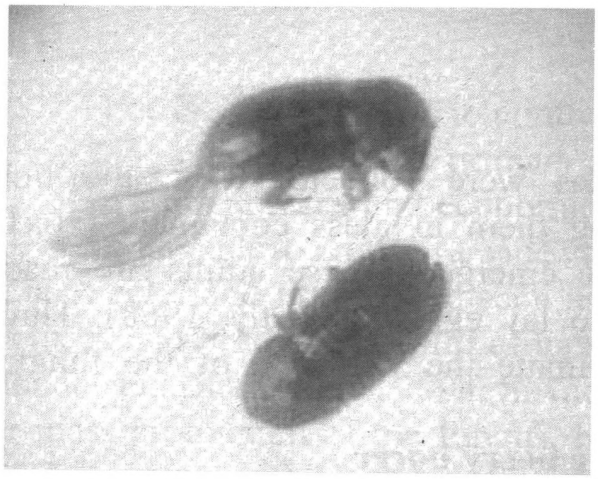


Figure 1. Healthy adult coffee berry borers

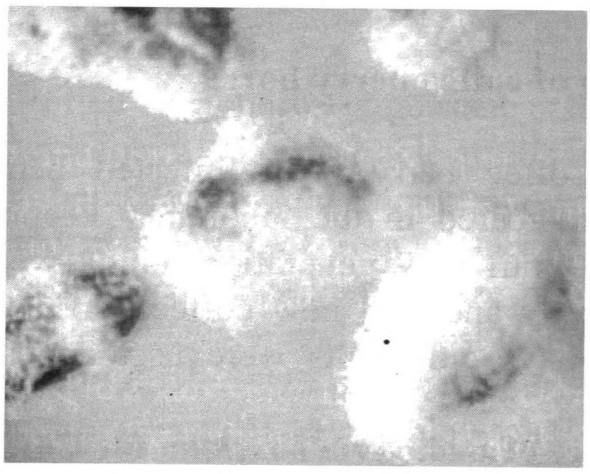


Figure 2. *Beauvera bassiana* infected adult coffee berry borers

Mass propagation of *Beauveria bassiana*

Common rice varieties available in the market (white rice, red rice, and parboiled rice) were used to find out the more suitable rice type for mass culturing. Impurities (stones, husk *etc.*) were removed manually from the rice samples. Hundred grams of each rice variety was measured and washed thoroughly with water. The washed rice samples were put in polypropylene bags (14x20 cm.) with 50 ml of distilled water and sealed with a cotton plug, covered the cotton plug with an aluminum foil and cooked using a pressure cooker at 121 °C for 15 minutes at 15 PSI. *Beauveria bassiana* fungal suspension at the concentration of 3 x 10^7 conidia/ml was prepared using 14 days old sub cultures in 0.1% Triton X-100 solution. Cooked rice in bags were inoculated with 10 ml of the prepared fungal suspension and incubated at 25 ± 2°C in the incubator.

Fungus extraction was done starting from 14 days old cultures and continued with 7 days intervals for 35 days. One bag from each rice variety was opened at a time. Rice mass was mixed with 1L of 0.1% Triton X-100 solution and filtered using a fine sterile cotton cloth. Ten milliliters from prepared solution were taken into a glass tube and a serial dilution was prepared to facilitate conidia counting. Number of conidia was estimated using a Haemocytometer. The experiment layout was completely randomized design (CRD). The treatments were three rice media and four different times of harvest. Each treatment combination was replicated three times.

Field testing of *Beauveria bassiana* against *Hypothenemus hampei*

Thirty bearing coffee trees, of five months after flowering were selected from 20 years old coffee plot at the Export Agriculture Research Station, Matale. The cultivated variety was Catimor. *Beauveria bassiana* conidia suspension was prepared by harvesting conidia from 28 days old rice culture and suspended in 0.1% Triton-X -100. The conidia suspension was applied at the rate of $1x10^7$ conidia/ml to fifteen trees by using a hand sprayer. Another fifteen trees (control) were sprayed only with 0.1% Triton X-100 solution. The second application of fungal suspension was carried out two weeks from the first application. Two bearing branches from each tree were selected for the data recording. Total number of berries, number of berries infested by CBB and number of CBB with fungal infection were recorded at weekly intervals until the harvest. Adult beetles which showed white colour fungal conidia were considered as infected beetles.

Analysis of data

Probit analysis was carried out with CBB mortality data after seven days by using Polo Plus statistical software package (Le Ora, Version 1.0) for the estimation of LC_{50} and LT_{90} values. The mean comparison of treatments were performed by analysis of variance (ANOVA) followed by LSD (SAS, 1999) in mass propagation experiment and the field experiment.

RESULTS AND DESCUSSION

Virulence of *Beauveria bassiana* for coffee berry borer

The mortality with sporulation of *H. hampei* was observed in an increase trend with the increase of conidia concentration. The lowest mortality was observed at the concentration of $1x10^4$ conidia/ml while the highest mortality percentage was observed at the concentration of $1x10^8$ conidia/ml (Table. 1). No significant difference in the mortality was observed between $1x10^7$ and $1x10^8$ conidia/ml Therefore, concentration of $1x10^7$ conidia/ml could be possible to use in the field to control CBB, but further experiments should be carried out before recommendation of application of *B. bassana* for the control of CBB in the field. Once *B. bassiana* is applied to the field it will be established in the environment and eventually it will control the CBB population (Posada *et al.*, 2004).

The estimated LC_{50} value was $1.4x10^6$ conidia/ml (limits $2.3x10^5$ to $3.6x10^6$) and the LC_{90} was $2.4x10^7$ conidia/ml (limits $9.9x10^6$ to $1.1x10^8$). The LC_{50} values of *B. bassiana* for CBB have been estimated in a few countries; In Central and South America the LC_{50} value for CBB has been recorded as $8.31x10^6$ conidia/ml (Rosa *et al.*, 2000 a), where as in Mexico it was $2.2x10^6$ conidia/ml (Rosa *et al.*, 1997). Also in Brazil it has been reported as $2.5x10^6$ conidia/ml (Neves and Hirose, 2005). The LC_{50} value of *B. bassaian* for CBB in Sri Lanka is different from that of Central and South America. Virulence of *B. bassiana* depends on many biotic and abiotic factors such as genetic diversity of the fungal strains (Cruz *et al.*, 2000; Rosa *et. al.*, 2000b; Samuels and Coracini, 2004) and environmental factors such as temperature, relative humidity and UV light (Walstad *et al.*, 1969; Zhang *et al.*, 2009).

Table 1: Mean percentage mortality of *Hypothenemus hampei* at different concentrations of *Beuveria bassiana*

Concentration (conidia/ML)	Mean mortality percentage
$1x10^0$ (control)	23.33 ± 8.60 [d]
$1x10^4$	39.99 ± 14.40 [c]
$1x10^5$	39.99 ± 14.40 [c]
$1x10^6$	61.66 ± 20.63 [b]
$1x10^7$	91.66 ± 12.62 [a]
$1x10^8$	96.66 ± 3.85 [a]
LSD	14.48
CV	2.13

* Means with the same letter are not significantly different at 0.05 significant level

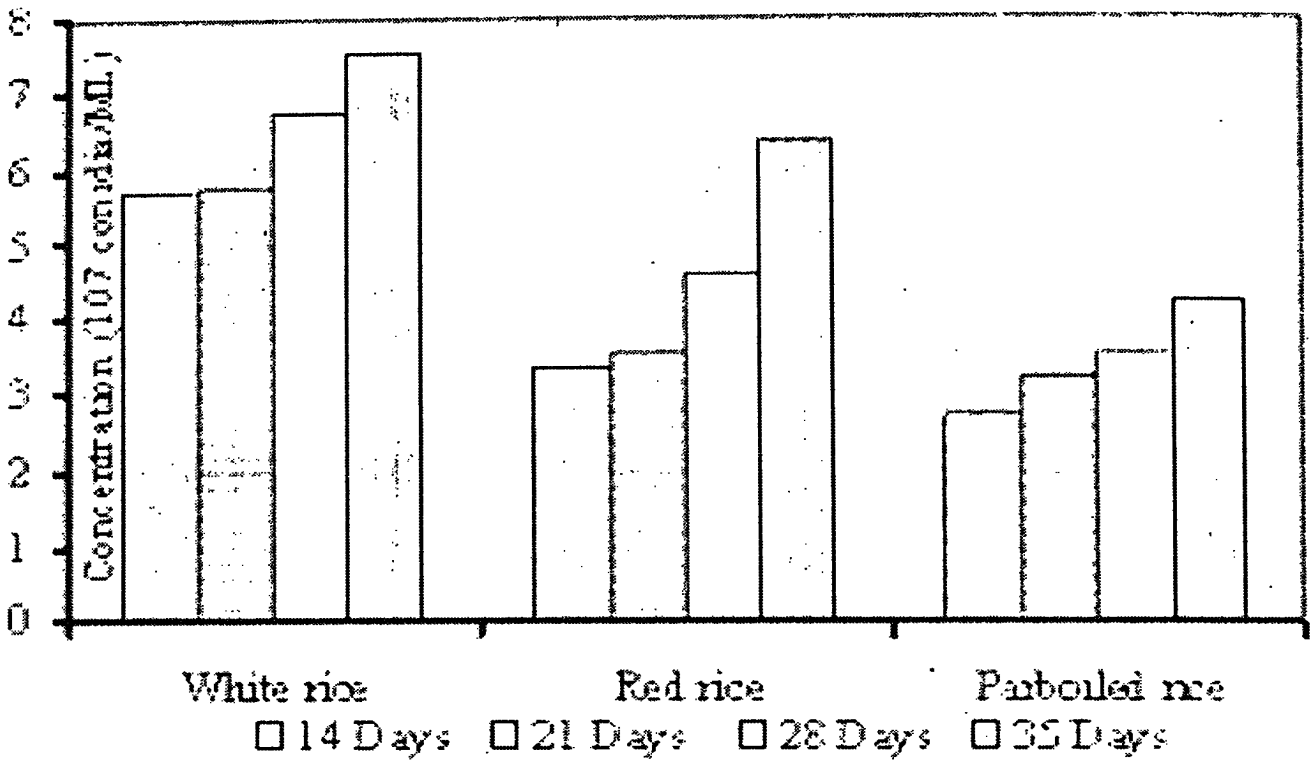
Sporulation on dead beetles observed for the first time two days after insect death in the treatment with $2.4x10^7$ (conidia/ml) (LC_{90}) and 100% of sporulated CBB were observed on the fifth day (Figure 2).

Estimation of LT_{50} value (Lethal Time -50)

The LT_{50} value was estimated as 2.6 days (limits 2.3 to 3.1 days) at the concentration of $2.4x10^7$ conidia/ml while the estimated LT_{90} value was 4.5 days (limits 3.7 to 6.4) under laboratory conditions. The LT_{50} value for CBB was recorded as 3.5 days at $1x10^7$ conidia/ml in Brazil (Samuels *et al.*, 2002).

Mass propagation of *Beauveria bassiana*

The three rice varieties showed significant differences ($P \geq 0.05$) with respect to the production of *B. bassiana* conidia. Production of conidia in white rice was significantly higher ($P \geq 0.05$) than that of in other two varieties. Parboiled rice showed significantly lower ($P \geq 0.05$) conidia production than other two varieties of rice (Figure 3).



Means with the same letter are not significantly different at 0.05 significant level

Figure. 3 Variation in conidia production of *Beauveria bassiana* in three rice varieties with respect to the time

Conidia production of *B. bassiana* increased with time in all the rice varieties. Conidia production was significantly higher at 28 days after inoculation when compared with 14 days after inoculation, but with compared to 21 days after inoculation it was not significantly different. The highest conidia production was observed in all three rice varieties at 35 days after inoculation and this was significantly ($P \geq 0.05$) higher than the conidia production in 28 days after inoculation (Figure 3). For the maximum sporulation, a good surface area to volume ratio is essential. Individual substrate particles should remain separate after hydration and sterilization. Substrate particles clump together when water is added and reduce the surface area to volume ratio, limiting the space on which spores can produce. An ideal substrate should not only contain particles of correct

dimensions, but also maintain its structural integrity during preparation for the production process (Jenkins *et al.*, 1998). Red rice and white rice have similar amounts of calories, carbohydrates and protein, but several vitamins and dietary minerals are lost in removing bran and endosperm. Especially, magnesium (Mg) is not added back into white rice. One cup (195g) of cooked long grain red rice contains 84 mg of Mg while one cup of white rice contains 19 mg. Other key types of nutrition lost are fatty acid and fiber (Anon., 2009a). Therefore, white rice contains fewer amounts of Mg and fatty acid than red rice. Presence of either magnesium or phosphate suppressed fungal growth in acid hydrolyzed media in case of some fungi (Kaiser and Ekbal, 1972). Moslim *et al.* (2004) reported that some oils can completely inhibit the germination of *B. bassiana*. These may be the reasons for high conidia production in white rice than in red rice. Parboiled rice which had been boiled before dehusking is reported to remain only 80% of the nutrients of the original rice at the end of the parboiling process (Anon., 2009b). Therefore, it contains less amount of nutrient for growth of *B. bassiana* than other two rice types. This might have lead to provide compared significantly lower production of conidia in parboiled rice compared with other two rice varieties.

Field testing of *Beauveria bassiana* against *Hypothenemus hampei*

The experiments conducted in order to evaluate the field efficacy of *B. bassiana* showed no difference in percentage damage of CBB between *B. bassiana* treated berries (8.52%) and untreated berries (8.06%) at harvesting stage (within eight weeks after first application of the fungal suspension. However, it was observed a significantly higher ($P \geq 0.05$) percentage of dead CBB with fungal sporulation (13.59 ± 10.54%) where as untreated control treatments showed only 1.59 ± 1.67% of sporulation. This indicated that application of *B. bassiana* to the field increased the pathogenicity on CBB. Fungus infected the CBB adults died without producing their offspring (Posada, 1998). In similar field studies, Haraprasad *et al.* (2001) have observed higher mortality of CBB with the application of *B. bassiana* in the field. Furthermore, Rosa *et al.* (2000b) have recorded higher mortality in *B. bassiana* applied coffee fields and the mortality was increased with the increased altitude. Therefore, application of *B. bassiana* may lower the next generation population as a result of dying CBB adults before reproductions. However, further studies should be carried out in the field before recommend to use *B. bassiana* in the field for the management of CBB.

CONCLUSIONS

Results of the laboratory and field experiments carried out revealed that there is a high possibility of using *B. bassana* for the control of *H. hampei*. Mass production of *B. bassiana* could be done at domestic level with white rice in polypropylene bags. A pressure cooker could be used to sterilize the rice media prior to inoculate. For determination of the number of days required for optimum conidial harvest further experiments should be carried out.

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