Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2016; 5(3): 47-49
Received: 09-03-2016
Accepted: 10-04-2016

K Soundari
PG & Research Department of Botany & Microbiology, A.V.V.M Sri Pushpam College (Autonomous), Poondi, Thanjavur (Dt), Tamil Nadu, India

D Rekha
PG & Research Department of Botany & Microbiology, A.V.V.M Sri Pushpam College (Autonomous), Poondi, Thanjavur (Dt), Tamil Nadu, India

G Senthilkumar
PG & Research Department of Botany & Microbiology, A.V.V.M Sri Pushpam College (Autonomous), Poondi, Thanjavur (Dt), Tamil Nadu, India

A Panneerselvam
PG & Research Department of Botany & Microbiology, A.V.V.M Sri Pushpam College (Autonomous), Poondi, Thanjavur (Dt), Tamil Nadu, India

Correspondence
K Soundari
PG & Research Department of Botany & Microbiology, A.V.V.M Sri Pushpam College (Autonomous), Poondi, Thanjavur (Dt), Tamil Nadu, India

Biocontrol of coffee berry borer, *Hypothenemus hampe:* Current focus

K Soundari, D Rekha, G Senthilkumar and A Panneerselvam

Abstract
The early plantation in the Pulney hills were primarily coffee. It is one of the important crops of greater commerce. Today, coffee growing has become a challenge to the organized farmers because of the scarce of the coffee berry borer. Fungal endophytes can protect coffee berry from insect pests. In this study the soil sample from the coffee fields were collected and the fungal sample were isolated. The fungal samples were used for the bioassay. The bioassay was done with different fungal culture isolated from the soil to find the lethal effect of the coffee berry borer.

Keywords: Fungal endophytes, bioassay, coffee berry borer

Introduction
Coffee in India is invariably grown in hilly undulated terrain with gentle to steep slopes under mixed shade canopy. The Pulney hills of Western Ghats belong to Kodaikanal Taluk of Dindugul District, Tamil Nadu. The lower Pulney hills is experiencing a subtropical climate, with a temperature range of 15°C to 20°C (minimum) and a maximum of 20°C to 30°C. The North – East monsoon is predominant and nearly 43 to 46% rainfall received during the season. Farming is carried out under rain fed conditions. Under the lower Pulney hills conditions, coffee is cultivated as a primary crop under multitier cropping system. Coffee cultivation in Indian is unique and eco-friendly as it is grown under multi-tier shade in a stimulated micro-climate. Thus, coffee cultivation is instrumental for maintaining the forest cover and also in preserving rich biodiversity of flora and fauna in ecologically sensitive Western and Eastern hill ranges. In India, coffee is grown relatively under harsh environmental conditions with a long droughts period of nearly 150 days as against well distributed rain fall and rich volcanic soils of other major coffee growing countries. Among the two cultivated species, Arabica coffee is more susceptible to diseases than robusta. However, in India, coffee is free from the attack of viral and bacterial diseases. Ever since coffee was commercially cultivated in India, it has been subjected to attack of a range of diseases caused by the fungal pathogens. Among the various diseases in the coffee plant, the serious disease which reduces the production of coffee is caused by the pest, coffee berry borer (*Hypothenemus hampei*). The insect has been reported in many coffee growing countries producing challenge to the organic farmers (Bustillo *et al.*, 1998) [1].

The coffee berry borer (CBB) *Hypothenemus hampei* (Ferrari) is consider the most important coffee pest throughout the world. The biology of CBB presents formidable challenges for the implementation of pest management programs. CBB lives the greatest part of its life cycle inside the coffee berry, which involves egg laying followed by the emergence of adult females from the berry. The control of CBB still depends largely on the application of synthetic insecticides which show limitations due to potential adverse effects to human health and the environment, and the development of pesticide resistance by the coffee berry borer to endosulfan. Due to its cryptic life style, the use of biocontrol agents could be an effective alternative to chemical control. The entomopathogenic fungus, *Beauveria bassiana* (Balsamo) Vuillemin is the most prevalent fungus attacking CBB populations. The fungus is a widespread soil borne pathogen of insects (Butt *et al.*, 2001) [2]. A key advantage for microbial control agents is their potential to replicate and persists in the environment, offering continued suppression of insect pest populations. These pathogenic fungi infect insects by breaching the host cuticle. They secrete proteases, chitinase and lipases that degrade the major constituents of the cuticle (i.e., protein, chitin and lipids) and allow hyphal penetration (Wang *et al.*, 2005; Cho *et al.*, 2006) [8, 3]. The reported influence of ambient moisture on *B. bassiana* infectivity

for insect varies from no effect (Ferron, 1997). The biology of CBB presents formidable challenges for the implementation of pest management programs. Adult females bore into a coffee berry and lay their eggs in the endosperm, on which the larvae feed. Prior to emergence from their natal berry, adult females with male siblings and are thereby inseminated and ready to colonize another berry. In contrast, males are apterous and never leave the berry (Le Pelly, 1968; Bustillo *et al.*, 1998) [5, 1].

This study aimed at the development of a sustainable pest control method against coffee berry borer that does not rely on chemical insecticides such as endosulfan, a product that has been banned in many countries and to which the insect has developed resistance. The first step in the process leading to commercialization is to conduct standard bioassay to select the more promising fungi.

Materials and methods

Isolates

The soil sample for the study was collected from Regional Coffee Research Station, Thandikudi near Kodaikanal. The soil samples were collected for four seasons. Monsoon (Jun – Sep), Post-monsoon (Oct – Dec), winter (Jan – Feb) and summer (Mar – May). The collected samples was serially diluted and plated on potato dextrose agar and rose Bengal agar and the fungal samples thus obtained was given in the results.

Collection of coffee berry borer

The infested coffee berries along with berry borer was collected from the field and taken aseptically to the laboratory from which adult coffee berry borer were selected for the bioassay. All adult infected used in the bioassays were about 1 month old. Before they were used the insects were washed in a 0.5% sodium hydrochloride solution with 0.01% Triton x-100 and gently shaken for two minutes. After wards, they were rinsed three times in sterile distilled water and dried in a container lined with sterile filter papers. Then they were used for the bioassay. No food was provided throughout the experiment to avoid problems with fungal contamination from the artificial diet and also because the insects burrow into the berries which would make it impossible to observed them. The insects can survive for extended periods of time without food (Posada, 1998) [7].

Bioassay (Posado, 2005) [6]

To produce the spores necessary to run the bioassay the single spore isolates stored in 10% glycerol at 80°C were grown in Sabouraud's dextrose agar and incubated at 25°C. All cultures were less than 30 days old when used in bioassays were collected with a spatula and placed in sterile tubes containing 100ml of sterile distilled water plus 0.01F triton X-100. The coffee berry borers assigned to the treatment were dipped in the 10ml of each fungal suspension. Insects were gently shaken for two minutes while dipped in their respective treatments and then placed in a sterile container from which they were taken individually with a paint brush and placed in a vial containing two pieces of sterile whatman filter paper (2.1cm diameter) moistened with 100µl of sterile distilled water was added to the filter paper as needed. All treatments were incublate in the dark at 25°C in a growth chamber. Insect mortality was assessed on a daily basis.

Field application (Regional Coffee Research Station)

To one barrel (200 litres), condia was harvested from one kg of rice culture (about 40 grams) and added to 100ml of non-ionic surfactants like APSA 80 or ACTIVE 80. It was mixed thoroughly to form a uniform paste (the conidia are not miscible with most of the ordinary wetting agent and hence they cannot be used). Small quantities of water was added to the bag and mixed thoroughly so that no lumps remain in the suspension. The suspension was filtered three or four times to extract maximum spores. It was make up to 200 litres and sprayed using a rocker sprayer fitted with solid cone or adjustable nozzle of 350 to 450 cc output per minute. Unlike chemical insecticides, *B. bassiana* has got very little persistence on the treated plant surface. Hence, for a pest like berry borer, direct contact of the formulation with insect cuticle is essential for effective control. The spray suspension should come in contact with all the berries.

Results

In this study we determined the pathogenicity of the fungus, *Beauveria bassiana* against the pest, coffee berry borer is the most important coffee pest worldwide. Seven different fungus were isolated from the soil collected in the coffee field for the four seasons. In that seven fungus some of the fungus was absent in some of the seasons. They were tabulated below.

Biodiversity of soil fungus

S. No	Fungus	Monsoon (Jun-Sep)	Post-monsoon (Oct-Dec)	Winter (Jan-Feb)	Summer (Mar-May)
•	*Aspergillus flavus*	+	+	+	-
•	*Trichoderma atroviride*	+	+	+	+
•	*Penicillium simplicissimum*	-	+	+	+
•	*Aspergillus fischeri*	+	+	+	-
•	*Rhizopus stolonifer*	-	+	+	+
•	*Beauveria bassiana*	+	+	+	+
•	*Cladosporium oxysporum*	+	-	+	+

Bioassay of coffee berry borer was done with all the seven fungus of different seasons. Out of that fungus, all the four seasons of *Beauveria bassiana* shows the lethal effect of the coffee berry borer, which means that the controlling measures of the CBB can be done biologically by using the entomopathogenic fungus *B. bassiana*.

In vivo studies of the biocontrol of CBB by *B. bassiana* was done by the field application. Spraying method was used to

apply the fungus of the coffee plant. Like chemical insecticides, *B. bassiana* also gives better result in the treated plants. Hence, for a pest like berry borer, direct contact on the insect cuticle by the pesticide is essential for effective control. Thus the spraying of the formulation is implemented which will come in contact with all the berries. The infection of the fungus on the navel region of the coffee berries can be observed in the field in about 10 days. Almost 80% of the berries where recovered by the application of the fungus has achieved. The field application was done when the temperature is below 30°C and humidity above 70% during the period from June to September.

Discussion

The principal objective of this study was to infer the pathogenicity of *B. bassiana* against coffee berry borer. Superficially we wished to determine whether the ability by *B. bassiana* to infect CBB in all the four seasons study. Soil samples taken from the four seasons where plated to isolate the fungus present in it. Totally seven different fungus were isolated from the soil sample. There was the absence of some of the fungus in any one of the four seasons. Environmental factors may be the reason for the absences of some of the fungus in the four seasons. In the bioassay we come to conclude that out of the all fungus, *B. bassiana* shows the lethal effect which can be used as the biocontrol agent against coffee berry borer instead of the chemical insect ide. The field application of *B. bassiana* on the coffee plant show the good result against coffee berry borer which is highly useful to the farmers. The coffee berry borer is a serious insect pest of coffee crops worldwide. Historically, the strategies to overcome this pest have not been aligned with environmentally friendly schemes and problems such as resistant to chemical insecticide arrived soon as expected. An integrated pest management program has proved to maintain the coffee berry borer under the economic threshold. However more progress on less labor intensive strategies are needed. Biological control agents have been introduced, conserved and augmented in order to naturally control coffee berry borer. None, except the used of highly pathogenic fungi such as *B. bassiana*, has proved to be economically and biologically effective to control coffee berry borer in the field.

Acknowledgement

We are grateful to the Research and Extension centre of the Coffee Board of India at Thandikudi for their extensive support in the methodology. We specifically thank Dr. Anil kumar, Deputy Director, Dr. Stephen D. Samuel and Dr. Irulandi for their kindly help and support to start this work.

References

1. Bustillo AE, Cardenas R, Villababa DA, Benavides P, Orozco J, Posada FJ. *et al.* Manejo integrado de labroca del café *Hypothenemus hampei* (Ferrari) en Colombia, Cenicafe (Colombia), 1998, 134.
2. Butt TM, Goettel MS. Bioassays of entomogenous fungi. In: Bioassays of entomopathogenic microbes and nematodes. [Navon, A. and Ascher, K.R.S (ed.)], CABI Publishing, Wallingford, United Kingdom. 2001, 141-195.
3. Cho EM, Boucias D, Keyhani NO. EST analysis of cDNA libraries from the entomopathogenic fungus *Beauveria* (cordyceps) *bassiana* II. Fungal cells sporulating on chitin and producing oosporein. Microbiology, 2006; 152:2855-2864.
4. Ferron P. Influence of relative humidity on the development of fungal infection caused by *Beauveria bassiana* (Fungi Imperfecti, Moniliales) in imagines of Acanthoscelides obtectus (Col.: Bruchidae). *Entomophaga* 1977; 22:393-396.
5. Le Pelly RH. Pest of coffee. Longmans, London, 1968.
6. Posada F, Vega FE. A new method to evaluate the biocontrol potential of single spore isolates of fungal entomopathogens. Journal of insect Science. 2005; 5:37.
7. Posada FJ. Production, formulation and application of *Beauveria bassiana* for *Hypothenemus hampei* in Colombia. Ph.D thesis, University of London. 1998, 277.
8. Wang C, Hu G, St. Leger RJ. Differential gene expression by Metarhizium anisopliae growing in root exudates and host (Manduca sexta) Cuticle or hemolymph reveals mechanisms of physiological adaptation. Fungal Genet. Biol. 2005; 42:704-718.