



Laboratory culture and virulence of *Beauveria brongniarti* (Metschnikoff) isolates on sugarcane white grub, *Holotrichia serrata* F (Coleoptera : Scarabidae)

C. Thamarai chelvi¹, W. Richard Thilagaraj², R. Kandasamy³

ABSTRACT

The entomopathogenic fungus *Beauveria brongniarti* was cultured in seven different solid substrate viz. pearl millet, rice, maize, wheat, cow pea, rice bran and wheat bran for the growth and sporulation of the fungus. The production of conidia was significantly different among the different substrates. Conidia produced in all the substrates tested were pathogenicity to *Holotrichia serrata* at varying degrees. Cow pea was found to be the best solid substrate for the sporulation and virulence of the pest among the solid substrates. The conidia produced from cow pea showed high virulence against third instar larvae of *Holotrichia serrata* with LC₅₀ value of 1.30×10^9 conidia ml⁻¹ while conidia produced from rice was less virulent with value of LC₅₀ (5.56×10^9 conidia ml⁻¹).

Key words: *Beauveria brongniarti*, *Holotrichia serrata*, entomopathogenic fungus, solid state substrate, laboratory bioassays, virulence

INTRODUCTION

Sugarcane is an important commercial crop grown in India. Pests are known to inflict considerable losses in cane yield as well as sugar output. Among the pests, the subterranean pest white grub, *Holotrichia serrata* has potential to cause 100 per cent yield reduction. The grubs of *H. serrata* feed on the main roots and subsequently damage the underground portion of the stalk. Availability of abundant roots and adequate moisture for a longer time in sugarcane crop tend to increase the white grub build up markedly (David and Ananthanarayanan, 1986). This is further facilitated by prevalence of host trees for adult feeding on borders of sugarcane crop.

Indiscriminate use of pesticides to control insect pests of crops has upset the natural ecological balance. This has initiated biological control agent an important and alternate control practice. Among the microbial control agents, entomogenous fungi like *Beauveria bassiana* (Balsamo) Vuillemin and *Beauveria brongniarti* (Metschnikoff) Sorokin have proved excellent control against a large number of insect pests (Pandey and Kanaujia, 2005). It has a wide range of pathogenicity to insects belonging to different orders. Entomopathogenic fungi are important regulators of insect population. Unlike insect pathogenic bacteria and virus, the fungi invade hosts by directly penetrating cuticle, thus they can infect

non feeding stages of insects like eggs and pupae and are considered a promising group of microbial biocontrol agents against piercing and sucking type of pests as well as those with chewing mouthparts for ingestion. Fungi are dependent on the medium or the substrate for all the elements and compounds they require or utilize, except molecular oxygen and carbon dioxide which they obtain from the atmosphere (Tauro *et al.*, 1984). Knowledge of the effects of these nutrients may assist in understanding the population dynamics of the fungi under the influence of both biotic and abiotic factors in the soil and help in developing strategies for successful application of the fungi as biocontrol agents (Sharma *et al.*, 2002).

Mass production of entomogenous fungi and testing of virulence are important steps in successful utilization of entomogenous fungi. Latch and Falon (1976) have reported the use of cereal grains for mass production of several entomogenous fungi. Rao (1989) reported that rice grain was suitable for multiplication of *M. anisopliae*. Quintela (1994) reported that production of conidia of *M. anisopliae* on coarse grain was significantly greater than that on whole grain. The production of conidia and their viability dependent upon the culture medium and carbon source plays an important role in determining the quantity and the quality of conidia in terms of spore germination and infection (Pandey and Kanaujia, 2008). Different agricultural residues

cereals etc were reported to be used for the conidia production in solid state fermentation (Deshpande, 1999). The present investigation was carried out to study the effect of different grain media on sporulation and virulence of *Beauveria brongniarti*.

MATERIALS AND METHODS

Conidia production

Seven different solid substrate like pearl millet, rice, wheat, maize, cow pea, rice bran and wheat bran were tested for the conidia production by *B. brongniarti*. Fifty grams of each of the solid substrates were soaked in distilled water for 1-2 hrs in Erlenmeyer flasks. The soaked grains were washed with fresh water and the flasks containing the medium were autoclaved at 1.08 Kg/cm² pressure for 40 minutes. After cooling, each flask was inoculated with five mm inoculation disc which were cut from seven day old actively growing cultures of *B. brongniarti* aseptically in a laminar flow. Flask containing SDA medium was also inoculated in a similar way as mentioned above which served as the control for comparison. There were three replications for each treatment. The flask containing different media were incubated at 25 ± 2° C and at 95 ± 5 per cent relative humidity for 15 days for conidia production. For conidial count, homogenous conidial suspension was prepared by adding 0.02 per cent Tween 80 as wetting agent. Then the flasks were shaken thoroughly to prepare proper suspension. The prepared suspensions were passed through double layered muslin cloth. The conidial count were made by serially diluted conidial suspensions using improved hemocytometer.

Effect of grain media on pathogenicity

To observe the virulence of conidia produced from different grain media, the spore suspension was prepared in 100ml distilled water containing 0.02 per cent Tween 80. From this stock solution 10⁵ – 10⁹ conidia ml⁻¹ suspension was prepared and used for bioassay test. Third instar larvae of *H. serrata* were collected from sugarcane fields in Bannari Amman Sugars, Sathyamangalam and Salem Cooperative Sugar Mill, Mohanur. Thirty larvae were treated in petridish (14.5 cm diam) for each concentration with 30 ml suspension for 2 min and transferred to moist soil placed in plastic rearing boxes and the remaining suspension after treatment were emptied into the plastic boxes. The grubs were fed with sugarcane roots which were changed every week. Each treatment was replicated thrice having thirty larvae per replication. The mortality due to mycosis was recorded

from 5th day onwards and continued upto 25 days and cumulative mortality data were subjected to probit analysis (Finney, 1964). Mortality at the concentration of 10⁹ conidia ml⁻¹ was used for calculation of LC₅₀ value.

RESULTS AND DISCUSSION

Effect of different solid substrate on the sporulation of *B. Brongniarti*

There was variation in quantity of spore produced from different grain media. Pearl millet produced conidia, rice produced conidia, wheat produced conidia, maize produced conidi, rice bran produced conidia. Among all the grain media cow pea produced highest conidia which was significantly higher than conidia produced from all the grain based media. Rice bran recorded the lowest conidia. Among all the test media SDA medium produced highest conidia. David *et al.* (2001) reported higher spore production of conidia on crushed sorghum grain media.

Effect of grain media on virulence

Pathogenicity tests of conidia produced from different solid substrate was carried out against third instar larvae of *Holotrichia serrata*. There was variation in virulence of *B. brongniarti* conidia produced from different substrate. The conidia produced from cow pea showed higher virulence to third instar larvae of *Holotrichia serrata* with LC₅₀ value of 1.30x10⁹ followed by Pearl millet with LC₅₀ value of 5.56x10⁹ conidia ml⁻¹ and lowest LC₅₀ value observed in Rice bran. However, conidia produced from SDA medium showed higher virulence against *H. serrata* larvae with LC₅₀ value of 1.24x10⁹ conidia ml⁻¹. Differences in virulence of conidia, produced from SDA and various grain media, may be due to presence of nutrient. Thus on the basis of data we may conclude that among the various solid substrate highest conidia produced with higher viability and virulence was recorded in conidia produced from cow

Table 1. LC₅₀ values of *Beauveria brongniarti* against third instar larvae of *Holotrichia serrata*

Grain media	Chi ² value	Regression equation Y = a + bx	LC ₅₀ (x 10 ⁹ conidia ml ⁻¹)
Pearl millet	0.23	Y= 1.9991+0.86278	3.478
Cow pea	0.26	Y=5.2771+0.23329	1.309
Rice	0.14	Y=1.3362+0.2270	4.815
Wheat	0.04	Y=1.8978+0.3859	4.182
Maize	0.05	Y=1.7221+0.3340	4.561
Rice bran	0.23	Y=1.3180+0.1838	5.56
SDA(Control)	0.28	Y=1.6479+0.6503	1.24

pea grain medium while lowest was in conidia produced from rice bran medium.

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**C. Thamarai chelvi¹, W. Richard Thilagaraj²
R. Kandasamy¹**

¹Entomologist, Main Biocontrol Research Laboratory, Venpakkam, Chengalpattu - 603111, Tamil Nadu, India, E-mail: thamarai_arasu@yahoo.com

²Department of Biotechnology, School of Bioengineering, S. R. M. University, Kattankulathur, Kancheepuram Dt, Tamil Nadu, India, Phone: Res (044) 26542262, 9003611419.