Journal of Research in Biology

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Original Research Paper

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Studies on the suitability of certain culture media for *Beauveria bassiana* (Bals.) Vuill., and *Verticillium lecanii* (Zimm.) Viegas

Authors:
Karthikeyan A and Selvanarayanan V.

Institution:
Department of Entomology, Faculty of Agriculture, Annamalai University, Annamalainagar – 608 002, Tamilnadu, India.

Corresponding author:
Karthikeyan A.

Email:
karthi_ento@yahoo.co.in

ABSTRACT:

The entomopathogenic fungi, *Beauveria bassiana* (Bals.) Vuill. and *Verticillium lecanii* (Zimm.) Viegas are capable of infecting a wide range of insect pests and show promise in commercialization. This laboratory study was made at the Department of Entomology, Faculty of Agriculture, Annamalai University, Tamilnadu, India during 2008-2009 to evaluate the suitability of certain culture media for these fungi. Three synthetic media *viz.,* Potato Dextrose Agar (PDA), Czapek's Dox Agar (CDA), Rose Bengal Agar (RBA) and three natural substrates *viz.,* water hyacinth, rice bran and spent mushroom paddy straw were chosen. The suitability of the medium was assessed based on the specific parameters namely colony growth in diameter, spore density and biomass production. The maximum colony growth attained by *B. bassiana* (42.00 mm) and the highest spore density (4.52 ´ 10^7 spores/ml) and biomass production (466.33 mg) were obtained in the synthetic medium, PDA. Among natural substrates, rice bran amended medium achieved the highest colony growth (46.33 mm) and yielded more spore density of 4.86 ´ 10^7 spores/ml and the biomass of 485.00 mg. The yielded spores were bioassayed against *Spodoptera litura* Fab. which exerted 73.33 per cent mortality. *V. lecanii* achieved the maximum colony growth (47.67 mm) and spore density of 4.73 ´ 10^7 spores/ml and biomass of 484.67 mg on RBA and on rice bran, it is recorded as 48.67 mm, 5.06 ´ 10^7 spores/ml and 492.52 mg, respectively. The mortality of *Aphis gossypii* was 93.33 and 100.00 per cent when treated with *V. lecanii* grown on RBA and rice bran respectively.

Keywords:
Entomopathogenic fungi, *Beauveria bassiana, Verticillium lecanii,* Spores, Synthetic media, Natural substrates.

Web Address:
http://jresearchbiology.com/Documents/RA0132.pdf.

Article Citation:
Karthikeyan A and Selvanarayanan V.
Studies on the suitability of certain culture media for *Beauveria bassiana* (Bals.) Vuill., and *Verticillium lecanii* (Zimm.) Viegas.
Journal of research in Biology (2011) 7: 508-512

Dates:
Received: 20 Oct 2011 /**Accepted:** 27 Oct 2011 /**Published:** 07 Nov 2011

INTRODUCTION

India is bestowed with a rich biodiversity of entomopathogens, utilization of these natural and renewable resources is important in a successful biocontrol strategy. Among the biocontrol agents, especially entomopathogenic fungi (i.e. fungal pathogens which infect insects) plays a predominant role in pest control (Latge and Papierok 1988, Hajek and St. Leger 1994). Entomopathogenic fungi are the potential microbial alternatives to chemical insecticides and offer a number of benefits such as growth facility on a variety of substrates, high virulence, transcuticular penetration, broad host range, safety to human beings, animals and environment (McCoy, 1990). *B. bassiana* is an imperfect entomopathogenic fungus that attacks a wide range of agricultural pests (Feng et al, 1994) and also grows on soil as saprophyte (Bidochka et al,1998). *V. lecanii* has been recognized as high potential entomopathogen in biological control of aphids. Many isolates of this fungus demonstrate high pathogenicity to the several species of aphids (Kim et al, 2007, Derakhshan et al, 2008).

Use of entomopathogenic fungi as biocontrol agents against insect pests has received greater global attention during the last few decades. Unlike bacterial and viral insect pathogens, the mode of action of these fungi does not require ingestion by the target insects. The infective propagules are the conidia that contact and adhere to the insect cuticle through hydrophobic mechanism (Boucias et al, 1998). The conidia then germinate and the hyphae penetrate through the cuticle. These advantages show promise in commercial development of mycoinsecticides (Milner, 1997). For the commercial production of fungal spores, it is necessary to obtain an ideal, cheap and highly productive culture medium. The present study was therefore undertaken to evaluate the suitability of certain synthetic media and natural substrates as culture medium. To evaluate the pathogenicity of *B. Bassiana* spores obtained from each media were evaluated against Tobacco caterpillar, *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae) a polyphagous pest affecting several crops worldwide causing extensive loss in agricultural production. The spores of *V. lecanii* was bio-assayed against the cosmopolitan sucking insect pest *Aphis gossypii* (Glov.) (Hemiptera: Aphididae).

MATERIALS AND METHODS

The present investigation was carried out at the Department of Entomology, Faculty of Agriculture, Annamalai University during 2008 – 2009. Two entomopathogenic fungi *B. bassiana* and *V. lecanii* were obtained from Rom Vijay Biotech Pvt. Ltd., Pondicherry, India. Each fungus was grown on three commercially available media namely Potato Dextrose Agar (PDA), Czapek's Dox Agar (CDA) and Rose Bengal Agar (RBA) (M/S. Himedia Laboratories Pvt. Ltd., India). The depth of these agar media when poured into Petri dish was 3 mm. This was achieved by pouring approximately 15 ml of molten agar media into 95 mm ´ 15 mm Petri dishes (Borosil®).

In addition, certain natural substrates *viz.,* rice bran, water hyacinth leaf stalks (*Eichhornia crassipes*) and spent mushroom waste were also tested to assess the suitability of medium to the test fungi. 400 gm of each substrate *viz.,* overnight soaked and decanted rice bran, freshly collected water hyacinth leaf stalks and spent edible mushroom bed (paddy straw) were surface sterilized with 0.1% sodium hypochlorite and finely ground with blender. To each ground substrate, 300 ml of double distilled water (half of which was used to ground the substrate) was added and filtered with muslin cloth and collected into a 500 ml beaker. 15 gm of agar-agar was added to each and heated gently to dissolve the ingredients. The media contents were transferred to volumetric flask (1 lit.) and made up to the volume with sterile distilled water. The pH of all media were adjusted to pH 7 with sterile 1N, NaOH before autoclaving. The media were poured to petridishes upto 3 mm depth. All the petriplates were point inoculated at the centre of the medium with a 5 ml conidial suspension (10^7 condia ml^{-1}) and incubated at 25 ± 2°C with 70 ± 5 percent RH for 10 days. Each medium was replicated thrice.

The diameter growth circle of the fungal colony was measured as suggested by Daggupati Komala (1988). One cm diameter plug was taken exactly half way between the centre and edge of the colony from each pathogen on each of the substrates. The plugs were homogenized in 5 ml 0.01% Triton – X 100 in a 50 ml polyesterene tube for approximately 1 min. and the conidia were counted using a neubauer hemocytometer.

To assess the biomass production of test fungus, flame sterilized cork borer of 10 mm diameter was used to core out discs of the fungal

cultures grown on the respective medium in the petridishes so as to inoculate the same into 250 ml Erlenmeyer flasks containing the respective test broths. Three replicates of each broth were maintained. It was incubated for 14 days at 25 ± 2°C to attain maximum growth and sporulation. The mycelial mats were collected by suction filtering on preweighed filter papers (Whatman 100), and dried in hot air oven at 105°C for 24 hrs and weighed again. The difference in the weight loss gave the biomass produced (Hall and Bell, 1961).

To evaluate the pathogenicity, the spore suspensions prepared from the culture of *B. bassiana* and *V. lecanii* grown on different media were tested against *S. litura* and *A. gossypii* respectively at a spore load of 10^7 spores ml^{-1}. Three replications were maintained with a control sprayed with sterile distilled water alone. The mortality was recorded four days after treatment and the per cent mortality was calculated. The data were analysed using IRRISTAT analysis developed by IRRI, Philippines.

RESULTS AND DISCUSSION

In this study, extensive variability was observed in the growth of *B. bassiana* and *V. lecanii* when grown on different substrates. Among synthetic media for *B. bassiana,* PDA showed the maximum colony growth of 42.00 mm diameter with a conidial density of 4.52 ´ 10^7 conidia ml^{-1} and the biomass of 466.33 mg. The maximum pathogenicity against *S. litura* was 73.33 per cent. Whereas for *V. lecanii,* RBA achieved the best colony growth of 47.67 mm dia., conidial density of about 4.73 ´ 10^7 conidia ml^{-1} and the biomass production was 484.67 mg. The infectivity against *A. gossypii* was 93.33 per cent. Other media showed comparatively less of these parameters.

Among the natural substrates amended media, rice bran amended medium achieved the highest colony growth of 46.33 mm dia. for *B. bassiana* and 48.67 mm dia. for *V. lecanii.* The spore density attained was 4.86 and 5.06 ´ 10^7 conidia ml^{-1}; the biomass production was 484.67 mg and 492.52 mg for both *B. bassiana* and *V. lecanii* respectively. The infectivity of *B. bassiana* against *S. litura* was 80.00 per cent and *V. lecanii against A. gossypii* was 100.00 per cent mortality (**Table 1**).

Kamp and Bidochka (2002) studied different nutrient types in the agar media and observed variability in the number of conidia produced. In a study related to this investigation, Siwach and Jaipal (2004) documented that wheat bran and maize bran

Table 1. Suitability of certain culture media for the growth characteristics of *B. bassiana* and *V. lecanii*

	Media	*Beauveria bassiana*				*Verticillium lecanii*			
		Colony Dia. (mm)	Spore density (1×10^7 conidia/ ml)	Biomass (mg)	*S. litura* Mortal-ity* (%)	Colony Dia. (mm)	Spore density (1×10^7 co-nidia/ ml)	Biomass (mg)	*A. gossypii* Mortality* (%)
Synthetic Media	PDA	42.00	4.52	466.33	73.33 (59.21)	33.67	4.01	355.00	73.33 (59.21)
	CDA	29.33	2.98	274.33	46.67 (43.44)	30.33	3.01	291.67	53.33 (46.92)
	RBA	33.33	3.93	352.00	66.67 (54.99)	47.67	4.73	484.67	93.33 (78.44)
Natural Sub-strates	Hyacinth stalk	29.33	2.28	248.81	46.67 (43.44)	31.67	2.55	260.02	66.67 (54.99)
	Rice bran	46.33	4.86	484.67	80.00 (63.93)	48.67	5.06	492.52	100.00 (82.99)
	Paddy straw	18.00	0.55	173.64	26.67 (31.03)	19.33	0.76	171.37	46.67 (43.44)
	Control	–	–	–	0.00 (4.05)	–	–	–	0.00 (4.05)
	S.Ed.	1.20	0.04	2.15	3.03	1.15	0.10	1.31	4.48
	C.D. (P = 0.05)	2.40	0.11	5.97	6.75	2.35	0.28	3.66	9.98

*** Values mean of three replications**
Values in parentheses are arc sine transformed

allowed the maximum fungal sporulation with conidial count of 5.33 and 5.52 ´ 10^7 conidia/ml, respectively followed by rice bran (5.07 ´ 10^7 conidia/ml).

(Malarvannan et al, 2010) In a preliminary study on pathogenicity of *B. Bassiana* against *S. litura* the fecundity was completely arrested in the spore concentration of 2.4×10^7 conidia/ml. (Hanh Vu et al, 2007) reported that, among various strains of entomopathogenic fungi, *V. lecanii* 41185 recorded 100 percent mortality against *A. gossypii* and *Myzus persicae* on 2nd and 5th day after treatment respectively.

From the growth parameters of fungi studied, among the synthetic media, PDA proved its suitability to *B. bassiana* while RBA favoured *V. lecanii.* Among the natural substrates, rice bran amended medium was found to be the best for both entomopathogenic fungi.

ACKNOWLEDGEMENT

The authors are grateful to Romvijay Biootech Pvt. Ltd., Pondicherry, India, for providing entomopathogenic fungal formulations and financial support. They are grateful to the authorities of Annamalai University for providing necessary facilities for carrying out this research.

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