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Cultural and morphological characterizations of *Beauveria bassiana*

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Abstract
An investigation entitled "Cultural and Morphological Characterizations of *Beauveria bassiana"* was carried out during 2018-2019. Effects of nutrition, pH, temperature and humidity were assessed on the basis of radial growth. The mummified bodies of lepidopteran pest larvae with symptoms of fungal infection were collected from field conditions from different places. Detailed morphological studies of effective isolate (Bb3) show that the fungus grew well on SDA (sabouraud dextrose agar). The mycelium on this medium, appeared white, dense and powdery. The colonies appeared raised in the center and developed radial furrows. The culture was turned yellowish white and powder type. Spore structures wasglobose to sub-globose. Two different semi synthetic media namely sabouraud dextrose agar and potato dextrose agar were evaluated for better growth of *B. bassiana*and it was observed that SDA medium (41.50 mm) support the growth of isolate. Significantly higher radial growth was however, seen in media on the basis of these results, pH value 6.0, temperature of 25 °C and 95 per cent relative humidty was considered as optimum for growth and sporulation of *B. bassiana.*

Keywords: Entomopathogenic fungi, *Beauveria bassiana*, radial growth

Introduction
Beauveria bassiana is an entomopathogenic deuteromycete which infect a variety of insects. Its hosts are predominantly members of the insect orders lepidoptera and coleoptera, including a number of economically important agricultural insects inhabiting soil or crop plants. The origins of the microbial pest control date back to the early nineteenth century, when the Italian scientist Agostino Bassi spent more than 30 years studying white muscardine disease in silkworms (*Bombyx mori* L.). He identified *Beauveria bassiana* (Bals. - Criv). Vuill., named in his honour, as the cause of the disease. The *Beauveria bassiana* is a parasite of many arthropods, including more than 200 species of insects and acaridae. The disease caused by the fungus is called white muscardine disease (Fargues *et al*. 1997) [4].
Approximately 750 species of fungi, two species such as *Beauveria bassiana* and *Metarhizium anisopliae are* mostly used for controlling harmful insects and as of 2007. A total of 58 biological preparations were produced by using fungus *Beauveria bassiana* are being broadly used for controlling harmful insects in rangelands, forests, crop fields and greenhouses (Marcos and Wraight, 2007) [6].
In culture *B. bassiana* grows as white mould. On most common culture media, it produces many dry, powdery conidia in distinctive white spore balls. Each spore ball is composed of a cluster of conidiogenous cells. The conidiogenous cells of *B. bassiana* are short, ovoid and terminate in a narrow apical extension called a rachis. The conidia is single celled, haploid and hydrophobic. Keeping all this in view the present investigation was undertaken on "Cultural and Morphological Characterization of *Beauveria bassiana".*

Materials and Methods
The present investigation on "Cultural and Morphological Characterizations of *Beauveria bassiana*" was conducted during 2018-2019 in the Laboratory of Plant Pathology Section and Department of Agricultural Entomology, College of Agriculture, Nagpur. In this experiment, various material were used and methods adopted during the entire course of investigation are described here.

Source of culture
The pure culture of fungus *Beauveria bassiana* was isolated from naturally infected larvae of lepidopteran pests and other insect pests on different field conditions from different places. Effects of nutrition, pH, temperature and humidity were assessed on the basis of radial growth

and spore density. Following methodology was adopted for assessment of these growth parameters.

Isolation of *B. bassiana* from infected larvae

The infected larvae of *Spodoptera litura* were collected from soybean field. The collected cadavers were surface sterilized with 0.1% mercuric chloride for five seconds and then rinsed three times with sterile distilled water to remove the traces of mercuric chloride. The surface sterilized specimen was cut into small bits in a sterile petri dish and a bit of infected tissue was transferred to a sterile petri dish containing potato dextrose agar media and kept in BOD at 25°C temperature and 90±5% relative humidity. After development of mycelial mat and sporulation, the fungus was under taken for initial identification. Identification of the selected isolates was again confirmed on the basis of morphological studies (Debnath, 2015) [3].

Method of inoculation

For inoculating both solid and liquid media as per requirement with equal amount of inoculation, a sterile corck borer of 5 mm diameter was used and discs were transferred with the help of sterile inoculating needle in an inverted position.

Measurement of growth and sporulation

Linear growth of the fungus colony on solid media was recorded by measuring the colony diameter along with the two marked direction at right angles to each other passing through the centre of the colony including the original inoculum disc of 5 mm diameter and average diameter of colony was recorded.For recording sporulation, a visual method of comparison to mention the density of sporulation was used as under.

Evaluation and characterization of *B. bassiana* on the basis of physiological and morphological parameters

Effects of nutrition, pH, temperature and humidity were assessed on the basis of radial growth. Following methodology was adopted for assessment of these growth parameters. Petriplates containing SDA medium were prepared by evenly spreading 0.5 ml of the inoculum as prepared above, over the agar surface. Agar plugs were cut after six days incubation at 25°C using a sterile 5 mm diameter cork borer. The control and treated plates were inoculated with these agar plugs in the centre. The mycelial growth was assessed periodically by measuring the colony diameter at right angles on each plate. For calculations, all the observations were recorded on 10th day of inoculation (Kotwal *et al.* 2006) [5].

The following parameters were evaluated

A. Media

Radial growth of different isolates of *B. bassiana* was estimated on different culture media. Fungal growth was assessed as colony diameter.

B. pH

To study the effect of pH on growth of different isolates of *B. bassiana*, four pH levels (6.0 to 7.5) were tested. The pH of culture medium was adjusted by adding 1 N HCl and 1 N NaOH with the help of indicator paper prior to autoclaving. Fungal growth was assessed in the form of radial growth.

C. Temperature

For studying the effect of temperature on growth of *B. bassiana* isolates, four levels of temperatures (20, 25, 30, and 35°C) were tested. The petriplates containing the inoculated media were incubated at respective temperatures in an incubator. The fungal growth was assessed on the basis of colony diameter.

D. Effect of relative humidity

The inoculated petriplates in three replications were exposed to 80, 85, 90, and 95 per cent relative humidity levels in desiccators of uniform size. The humidity levels in desiccators were maintained by using concentrated sulphuric acid + water as per method suggested by Soloman (1951) [8].

Results and Discussion

Morphological characters of *B. bassiana*

Microscopic observations of conidia show that the different isolates cultured on SDA medium do not differ in shape. The hyaline, smooth walled conidia were globose to sub-globose in shape in all the 16 isolates studied. The size of conidia varied from 1.80 - 2.50 µm x 1.70 2.40 µm between different isolates. Variation was however also observed in size of conidia within the isolates. The detailed morphological studies of effective isolate (Bb3) showed that the fungus grew well on SDA. Mycelium on this medium, appeared white, dense and powdery. The colonies appeared raised in the center and developed radial furrows. The culture was turned yellowish white and powdery type. Spore structure was globose to sub-globose.

Cultural studies of *Beauveria bassiana*

Table 1: Different media tested for growth and sporulation of effective isolate of *Beauveria bassiana*

Sr. No.	Particulars of medium	Colony diameter after 10 days (mm)	Sporulation after 10 days	Remark
1	Sabouraud's Dextrose Agar (SDA) medium	41.50	++++	Abundant sporulation
2	Potato Dextrose Agar (PDA) medium	36.50	+++	Good sporulation

The growth and sporulation of virulent isolate of *Beauveria bassiana* (Bb3) was studied on two different solid media such as SDA and PDA on 10th days after incubation. Observation were recorded and result were presented in (table 1). The highest radial growth of *Beauveria bassiana* was observed in SDA media (41.50 mm) as abundant sporulation and the least growth was observed in PDA media (36.50 mm) as good sporulation. This was in consonance with Deb *et al.* (2017) [2] reported that the maximum colony diameter of 45.33 mm was observed in SDA followed by SDB (43.00 mm).

Effect of temperature

Table 2: Effect of different temperature regimes tested for growth and sporulation of *Beauveria bassiana*

Sr. No.	Temp.(°C)	Colony diameter after 10 days (mm)*	Sporulation after 10 days	Remark
1	20	35.50	+++	Good sporulation
2	25	41.50	++++	Abundant sporulation
3	30	38.50	+++	Good sporulation
4	35	32.00	++	Fair sporulation
SE(m)±		0.80		
CD (P=0.01)		3.52		

In general, *Beauveria bassiana* grew at a wide temperature range from 20°C to 35°C.The radial growth and sporulation were affected by temperature observation recorded and result were presented in (table 2). The maximum radial growth 41.50 mm and abundant sporulation was recorded at temperature 25° C. The least radial growth 32.00 mm and fair sporulation was at temperature 35° C. This was in consonance with Ahmad *et al.* (2016) [1] studied on the growth rate, spore production and spore germination of two different isolates of the fungus *B. bassiana*. The optimum temperature was 25°C without significant differences.

Effect of humidity levels

Table 3: Effect of different humidity levels tested for growth and sporulation of *Beauveria bassiana*

Sr. No.	Relative humidity levels %	Colony diameter after 10 days (mm)*	Sporulation after 10 days	Remark
1	80	31.38	+++	Good sporulation
2	85	38.83	++++	Abundant sporulation
3	90	40.00	++++	Abundant = sporulation
4	95	41.66	++++	Abundant sporulation
SE (m)±		0.47		
CD (P=0.01)		2.08		

At 95% relative humidity, highest radial growth of 41.66 mm was recorded till 10th day of incubation with abundant sporulation, result were presented in (table 3). Lowest radial growth of 31.38 mm and god sporulation was observed 10th day of incubation. Shipp *et al.* (2003) [7] investigated the influence of different humidity levels on per cent infection by *Beauveria bassiana* on greenhouse insect. In petridish trials, a humidity of 97.5 per cent relative humidity resulted in significantly higher percentage of infection (60-88.8 per cent)**.**

Effect of pH levels

Table 4: Effect of different pH levels tested for growth and sporulation of *Beauveria bassiana*

Sr. No.	pH	Colony diameter after 10 days (mm)*	Sporulation after 10 days	Remark
1	6.0	41.15	++++	Abundant sporulation
2	6.5	39.66	++++	Abundant sporulation
3	7.0	36.33	++	Fair sporulation
4	7.5	33.55	+	Moderate sporulation
SE(m)±		1.25		
CD (P=0.01)		5.52		

The effect of pH of medium on radial growth and sporulation was assessed using SDA as the basal medium and the results are presented (table 4). The highest radial growth of 41.15 mm was achieved after 10th days at pH of 6.0 with abundant sporulation. The lowest radial growth of 33.55 mm and sporulation were observed at pH 7.5.This was accordance with Ying and Feng (2006) [9] studied that conidia produced on media sabouraud dextrose medium at pH 4.0-8.0, the culture conditions were optimized as pH 5.0-6.0.

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