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Effect of different solid media and liquid media on growth, sporulation and biomass of *Beauveria bassiana* (Bals. -Criv.) Vuill

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Abstract
The experiment on the effect of different solid and liquid media on the growth, sporulation and biomass of *Beauveria bassiana* (Bals. -Criv.) Vuill. was conducted at the Department of Entomology and Department of Plant Pathology, College of Agriculture, Navsari Agricultural University, Waghai, Gujarat, India during the year 2020-22. The result revealed that potato dextrose agar and sabouraud dextrose agar supported the higher radial growth and sporulation of *B. bassiana* followed by sorghum meal extract agar among the seven solid media. Among the seven liquid media tested for sporulation and biomass of *B. bassiana*, the significantly highest sporulation and dry mycelial biomass, was observed in potato dextrose broth, whereas the significantly lowest spore production and biomass was recorded in elliot's broth.

Keywords: Biomass, growth, *Beauveria bassiana*, solid media, liquid media, sporulation

Introduction
Biopesticides based on bacteria, viruses, entomopathogenic fungi (EPF) and nematodes are often considerable scope as plant protection agents against several insects (Noris *et al*., 2002) [7]. The use of entomopathogenic fungi as biological control agents for insect species has increased global attention during the last few decades. EPFs like *B. bassiana*, *Metarhizium anisopliae*, *Nomuraea rileyi*, *Lecanicillium lecanii* and *Paecillomyces* have been extensively studied among EPF for their bioefficacy, leading to the development of numerous commercial products (Faria and Wraight, 2007) [3]. Production of adequate quantities of a good quality inoculum is an essential component of the biocontrol programme. *B. bassiana* is the most important entomopathogenic fungus as a biological control agent and it is a cosmopolitan, naturally soil-inhabiting facultative necrotrophic pathogen of a broad host arthropod range spanning almost all orders of insects and extending to ticks and mites.
But now that new entomopathogenic fungi have been isolated and identified, it's critical to determine the best artificial growth conditions for laboratory culturing. This step is crucial for facilitating mass culturing, enabling further use in pest management. A culture medium is a mixture of different components in the right amounts that supply the vital nutrients required for fungus development and multiplication. The use of a variety of media types affects the growth, texture, pigmentation and sporulation of fungi, which are all influenced by the media's composition, pH, temperature and water contents (Northolt and Bullerman, 1982; Kumara and Raval, 2010) [8, 5]. On fungal growth, various culture mediums have variable impacts.
This study set out to determine the impact of various cultural media on the growth and sporulation of *B. bassiana*. By identifying suitable culture conditions, it becomes feasible to scale up the production of these fungi, providing a sustainable and environmentally friendly approach to managing insect pests in agriculture.

Materials and Methods
To find out the superior medium for the growth and sporulation of the fungus, five synthetic and two semi-synthetic media in solid and liquid states were compared.

Solid media test
All the agar media mentioned in Table 1 were prepared in the required quantity and autoclaved at 15 psi pressure and at 121 °C for 30 minutes and allowed for cooling. A trace amount of antibiotic streptocycline was added to the media when the media was warm (45º to 47 °C), to

inhibit the growth of bacteria and shaken well for uniform mixing. For solidification, 20 ml media was poured aseptically into a 90 mm diameter disposable Petri plate individually. The Petri plates were inoculated aseptically in the laminar airflow by placing a five mm diameter mycelial disc at a center which was cut aseptically with the help of a cork borer from the ten days pure culture of *B. bassiana* maintained on PDA medium. The Petri plates were incubated at 27±2 °C in the BOD incubator for 10 days.

The radial growth of the fungal colony (mm) was measured after 10 days. Each plate was examined critically for colour changes due to various media pigmentation. For conidial count, ten mycelial discs of five mm diameter were taken with the help of a cork borer from ten days old pure culture and suspended in 20 ml distilled water contained in 100 ml conical flasks added with 0.5 ml Tween-80 solution for assessment of sporulation. Flasks were shaken on a vortex mechanical shaker for 15 minutes. A clear conidial suspension was obtained by filtering the shaken biomass through a double layer of muslin cloth and conidial count per ml was counted by using Neubauer's hemocytometer. Three repetitions of each treatment were maintained. The data were analyzed using CRD design.

Table 1: Compositions of different agar media used for mass multiplication of *B. bassiana*

Agar media	Composition (g / litter)
T_1: Potato dextrose agar	Potato: 200, Dextrose: 20, Agar: 15
T_2: Sabouraud dextrose agar	Peptone:10, Dextrose: 40, Agar: 15
T_3: Richard's agar	Magnesium Sulphate: 2.50, Potassium nitrate: 10.00, Potassium dihydrogen orthophosphate: 5.00, Ferric chloride: 0.02, Sucrose: 50, Agar: 20
T_4: Czapek's dox dextrose agar	Sucrose: 30, Sodium nitrate: 2, Magnesium glycerophosphate: 0.5, Potassium chloride: 0.5, Dipotassium sulphate: 0.35, Ferrus sulphate: 0.01, Agar: 12
T_5: Elliot's agar	Sodium carbonate: 1.05, Magnesium Sulphate: 0.60, Asparagine: 3.00, Dextrose: 3.00, Potassium dihydrogen orthophosphate: 1.36, Agar: 20.00
T_6: Sorghum meal extract agar	Sorghum flour: 20, Dextrose: 20, Agar:15
T_7: Finger millet meal extract agar	Finger millet flour: 20, Dextrose: 20, Agar: 15

Liquid media test

All the liquid media mentioned in Table 1 were used as broth media with the same ingredients omitting agar. 100 ml of the liquid media was filled in each 250 ml conical flask. These flasks were plugged with non-absorbent cotton and sterilized at 15 psi pressure and 121 °C for 30 minutes in an autoclave. A trace amount of antibiotic streptocycline was added to the media. The flasks were inoculated aseptically in a laminar airflow by placing 5 mm diameter culture discs cut aseptically with cork borer from ten days old pure culture. The flasks were incubated at 27± 2 °C for 15 days in a BOD incubator.

After 15 days of incubation, mycelial mats were harvested on previously weighed oven-dried Whatman's filter paper no. 42. The filter papers with mycelia mats were oven-dried at 60 °C up to constant weight was obtained and the dry weight of mycelium was recorded by deducting the weight of the filter paper. For this purpose, 15 days old fungal biomass along with the broth in a conical flask was shaken well on a mechanical shaker for 15 minutes after adding 0.5 ml of Tween- 80 in each flask. A clear conidial suspension was obtained by filtering the shaken biomass through a double layer of muslin cloth and numbers of conidia per ml were counted using a Neubauer's hemocytometer. Three repetitions of each treatment were maintained. The data were analyzed using CRD design.

Results and Discussion

Solid media test

Seven different media in a solid state were tested for their suitability for growth and spore formation by *B. bassiana*. The data of the solid media test are analyzed and summarized in Table 2.

Radial growth

The radial growth of *B. bassiana* was measured after 10 days of inoculation in seven different solid media. The data presented in Table 2 revealed that the maximum growth of *B. bassiana* was obtained in potato dextrose agar (86.33 mm) which was at par with the sabouraud dextrose agar (83.67 mm). The next best media was sorghum meal extract agar (77.67 mm) which was followed by finger millet meal extract agar (72.67 mm) and czapek's dox dextrose agar (68.00 mm). The least area radial growth was recorded in richard's agar (57.67 mm) and elliot's agar (45.00 mm).

Sporulation

It is evident from the result presented in Table 2 that among the seven tested solid media, the excellent sporulation of *B. bassiana* was found in potato dextrose agar (23.90×10^7 conidia ml^{-1}) which was at par with the sabouraud dextrose agar (21.87×10^7 conidia ml^{-1}). The next in order of merit was sorghum meal extract agar (18.42×10^7 conidia ml^{-1}) followed by finger millet meal extract agar (16.07×10^7 conidia ml^{-1}) and czapek's dox dextrose agar (14.12×10^7 conidia ml^{-1}), while richard's agar showed poor sporulation (7.67×10^7 conidia ml^{-1}) which was followed by elliot's agar (6.20×10^7 conidia ml^{-1}).

Pigmentation

Differentiation of pigmentation was observed in tested media on the backside of various synthetic and semi-synthetic media (Table 2). White colour pigmentation was found in potato dextrose agar and sabouraud dextrose agar. Whereas dull yellow pigmentation was found in richard's agar. The creamy white color pigmentation was noticed in czapek's dox dextrose agar and elliot's agar. Sorghum meal extract agar and finger millet meal extract agar recorded dull white color pigmentation.

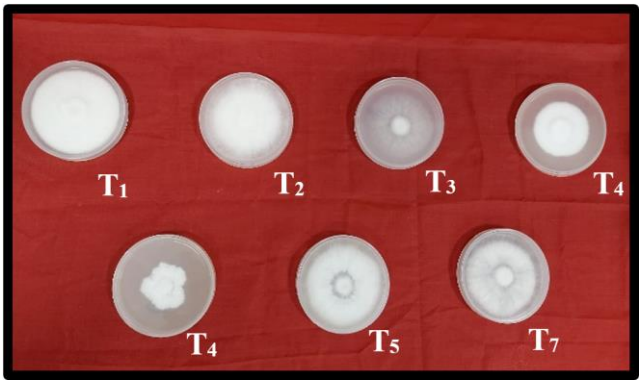

Plate 1: Growth of *B. bassiana* on different solid media

Table 2: Effect of various solid media on growth (radial diameter), sporulation and pigmentation of *B. bassiana*

Solid Media	Radial growth (mm)	Sporulation (×10^7 conidia ml^{-1})	Pigmentation colour
T_1: Potato dextrose agar	9.32^a (86.33)	4.94^a (23.90)	White
T_2: Sabouraud dextrose agar	9.17^a (83.67)	4.73^a (21.87)	White
T_3: Richard's agar	7.63^f (57.67)	2.85^e (7.67)	Dull yellow
T_4: Czapek's dox dextrose agar	8.28^d (68.00)	3.82^d (14.12)	Creamy white
T_5: Elliot's agar	6.74^e (45.00)	2.59^f (6.20)	Creamy white
T_6: Sorghum meal extract agar	8.84^b (77.67)	4.35^b (18.42)	Dull white
T_7: Finger millet meal extract agar	8.55^c (72.67)	4.07^c (16.07)	Dull white
SEm±	0.09	0.08	
CD at 5%	0.27	0.23	
CV (%)	1.81	3.39	

Figures in the parenthesis are original values those outside are √ X +0.5 transformed values, Means followed by a common letter in a column are not significantly different at P=0.05

Liquid media test

Seven different media in a liquid state were tested for their suitability for biomass production and spore formation of the *B. bassiana*. The data of the liquid media test are analyzed and summarized in Table 3.

Biomass

It is evident from the result presented in Table 3 that among the seven tested liquid media, the higher dry weight of the mycelial was observed in potato dextrose broth (2.70 g) followed by the sabouraud dextrose broth (2.23 g). The next in order of merit was sorghum meal extract broth (1.93 g), finger millet extract broth (1.70 g), czapek' dox dextrose broth (1.47 g) and richard's broth (1.28 g), while elliot's broth yielded poor mycelium dry weight (1.10 g).

Sporulation

Sporulation of *B. bassiana* was found to be significantly high in potato dextrose broth (24.78×10^8 conidia ml^{-1}) over the rest of the media tested. The next effective media was sabouraud dextrose broth (22.15×10^8 conidia ml^{-1}). The next best treatment was sorghum meal extract broth (18.58×10^8 conidia ml^{-1}) followed by finger millet meal extract broth (16.37×10^8 conidia ml^{-1}) and czapek's dox dextrose broth (14.23×10^8 conidia ml^{-1}), while richard's broth showed poor sporulation (8.08×10^8 conidia ml^{-1}) which was followed by elliot's broth (6.50×10^8 conidia ml^{-1}). In the present study, potato dextrose broth and sabouraud dextrose broth were found the best liquid media for biomass production and sporulation of *B. bassiana* which was in close agreement with the result of Karthikeyan *et al.* (2008) [4], Senthamizhlselvan *et al.* (2010) [11], Patel (2013) [8] and Leela (2020) [6], reported potato dextrose broth found superior in yield mycelium growth or sporulation. While, Bhadauria *et al.*, 2012 [2], Agale *et al.*, 2018 [1] and Rajni (2021) [10] observed that sabouraud dextrose broth gave the maximum growth and spore production of *B. bassiana.*

Table 3: Effect of various liquid media on biomass and sporulation of *B. bassiana*

Liquid Media	Biomass (g)	Sporulation (×10^8 conidia ml^{-1})
T_1: Potato dextrose broth	1.79^a (2.70)	5.03^a (24.78)
T_2: Sabouraud dextrose broth	1.65^b (2.23)	4.76^b (22.15)
T_3: Richard's broth	1.34^f (1.28)	2.93^f (8.08)
T_4: Czapek's dox dextrose broth	1.40^e (1.47)	3.84^e (14.23)
T_5: Elliot's broth	1.26^g (1.10)	2.64^g (6.50)
T_6: Sorghum meal extract broth	1.56^c (1.93)	4.37^c (18.58)
T_7: Finger millet meal extract broth	1.48^d (1.70)	4.11^d (16.37)
SEm±	0.02	0.08
CD at 5%	0.07	0.24
CV (%)	2.63	3.44

Figures in the parenthesis are original values those outside are √ X +0.5 transformed values, Means followed by a common letter in a column are not significantly different at P=0.05

Plate 2: Growth of *B. bassiana* on different liquid media

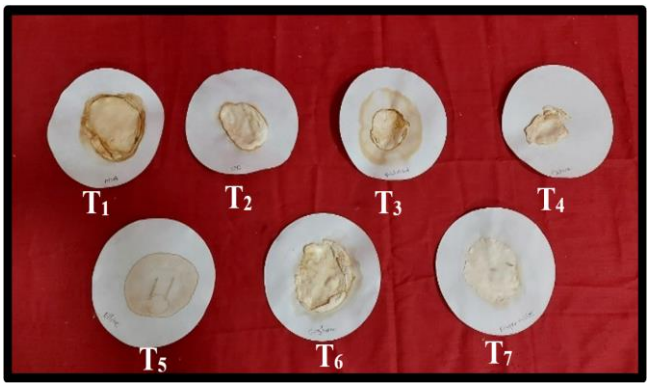

Plate 3: Dry mycelial mats of *B. bassiana* from different liquid media

The different media showed different responses in both cases *i.e.*, vegetative and reproductive growth. These differences in physiological parameters among different media might be due to differences in the content of nutrition among different media. In the case of sorghum meal extract broth and finger millet meal extract broth, no added sucrose and other nutrition sources like peptone, potassium, magnesium and iron were present, whereas other sources like glucose, sucrose and trace elements also varied in concentration from media to media or absent in some media. Here every media has a different set of composition of nutrients.

In the present study, for both semi-synthetic (solid and liquid) media, sorghum meal extract and finger millet meal extract gave good results. So, it may be used as alternative media for the laboratory studies of *B. bassiana*.

Conclusion

Among all the solid media and broth media potato dextrose and sabouraud dextrose supported the growth of *B. bassiana* which showed the highest sporulation. Sorghum meal extract and finger millet meal extract were also found good for the growth and sporulation of *B. bassiana*. So, it may be used as alternative media for the laboratory studies of *B. bassiana*.

The findings highlight the significance of creating artificial media adapted to particular fungi for mass culturing and field research on novel entomopathogenic fungi. Finding the ideal medium is essential for the effective culture of fungi because each one appears to have unique preferences for growing circumstances. In order to develop efficient pest management techniques, it is crucial to carefully choose the culture media that will support the growth and sporulation of entomopathogenic fungi. In order to achieve optimal growth and ultimately increase their potential use as biocontrol agents in pest formulation of management strategies in sustainable agriculture, further research is required to better understand the requirements of each entomopathogenic fungus.

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