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Evaluation of Most suitable Medium for the Mass Production of Entomopathogenic Fungi *Beauveria bassiana*

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ABSTRACT: *B. bassiana* is the most important and widely used entomopathogenic fungi for pest control but multiplication of adequate and good quality inoculum is important component in bio control approach. Solid media such as grains of rice, wheat, gram, pegion pea, vermicompost, *Corcyra* rearing waste and liquid media such as Potato Dextrose Broth (PDB) and Sabouraud's Dextrose Broth (SDB) were evaluated for the mass production of *B. bassiana*. Results showed that Rice supported maximum conidial count at all 10, 20 and 30 days after incubation. Among liquid media, SDB supported maximum conidial count. Highest mean dry weight was recorded on gram followed by SDB. The highest growth rate of conidia was recorded on pigeonpea at from 10 to 20 days.

Keywords: *B. bassiana*, entomopathogenic fungi, Solid media, liquid media, Conidia, Mass production.

INTRODUCTION

Microbial based formulations are the best approach to manage agricultural pests without harming to the environment. Microbial bio-pesticides comprised of microscopic living organisms *i.e.*, bacteria, fungi, virus, protozoa and nematodes or toxins produced by these organisms (Saxena *et al.*, 2020; Thakur *et al.*, 2020). The fungal formulations like *Beauveria bassiana*, *Paecilomyces*, *Metarhizium anisopliae*, *Verticillium lecanii*etc., have been used to control various insect pests. In India, total 970 bio-pesticide products are registered, amongthese the fungal bio-pesticide products contribute greatest (66%) followed by bacteria (29%), virus (4%) and other 1% (Rani & Saxena 2021). Among these bio-pesticides, fungi has fascinating lifestyles that can be exploited in biological control of pests and diseases. Unlike other microbial bio-pesticides like bacteria and virus that has specific routes of infection *i.e.* integument. Majority of entomopathogenic fungi infect insects by its unique way of action consequently it reaches insect haemocoel by penetrating the insect cuticle or by the buccal cavity, spiracles and other natural openings of the insect (Abbas, 2020). The infective units (spores) do not germinate in the insect digestive system as they are forced outside from the circulatory system with the excreta. The insect death might be considered due to concurrence of mechanical injury generated by cuticle damage, consequently leading to reduction in necessary growth elements and release of toxins (Bhadauria *et al.*, 2012).

Upon several entomopathogenic fungi, *B. bassiana* is the most important entomopathogenic fungi for pest control and also reducing the chances of development ofresistance in *H. armigera*, *Plutllax ylostella*, *S. Litura*and other insect pests. *Beauveria bassiana*, the anamorph stage of *Cordyceps bassiana*, is a facultative cosmopolitan entomopathogen with an extraordinarily large host range. First discovered by Agostino Bassi de Lodi (Keswani *et al.*, 2013) in larval silkworms, the fungus grows as a white (hyaline) mold producing single-celled, haploid, and hydrophobic conidia.

Multiplication of adequate and good quality inoculum is important component in bio control approach. Therefore, the present study was undertaken to investigate the solid media such as grains of rice, wheat, gram, pegion pea, vermicompost, *Corcyra* rearing waste and liquid media such as Potato Dextrose Broth (PDB) and Sabouraud's Dextrose Broth (SDB) for the mass production of *B. bassiana*.

MATERIALS AND METHODS

Beauveria bassiana culture was collected from the Bio-Control Lab, Department of Entomology, Sardar Vallabhbhai Patel University of Agriculture and

Technology, (S.V.P.U.A.T.), Meerut, Uttar Pradesh, India. The collected culture was sub-cultured in a petri plate containing PDA media to obtain a pure culture of *B. bassiana*. Different solid and liquid media were evaluated for the identification of an appropriate medium for the development and sporulation of *B. bassiana*. Solid media contains Rice, Wheat, Gram, Pigeon pea, Vermicompost, *Corcyra* rearing waste and Liquid media contains Potato Dextrose Broth (PDB) and Sabouraud's Dextrose Broth (SDB). Hundred grams of grains were washed well and soaked for one day prior to use for estimating the best substrate for mass multiplication of *B. bassiana* at 25°C. Then shade dries them to remove excess moisture. Each treatment replicated thrice. Then these grain media were packed separately in individual 250 ml conical flask. They were plugged with cotton plugs and autoclaved at 15 psi for 20 minutes at 121° C. After cooling, 5mm of fungal culture was inoculated into each flask, separately. All these procedures were done under laminar air flow chamber and incubated in BOD at 25°C for 30 days. To avoid clumping, the flasks were shaken vigorously to separate the culture and to break the mycelia mat. Conidial count was determined according to the methods of (Sahayaraj and Namasivayam 2008; Rajanikanth *et al.*, 2010) were followed with little modification. Hundred ml of each liquid media was prepared according to the standard protocol and the media dispensed into 250 ml conical flask, plugged with non-absorbent cotton and autoclaved at 15 psi for 20 min at 121°C. Each flask was replicated three times. After that, inoculated with 5 mm fungal disc of *B. bassiana* aseptically under laminar air flow chamber and incubated at 25^0C for 30 days. Conical flasks were shaken daily for the uniform growth of the fungus. After that sufficient incubation of *B. bassiana* in both the substrates, sporulation was calculated by taking 1g of sample from each substrate and was transferred to 9 ml sterilized distilled water containing Tween 80 (0.001%) solution. Then the flasks were shaken in shaker for 10 minutes. The whole suspension was filtered through sterilized double layer muslin cloth. The spore suspension was dropped below the cover slip so as to fill it completely. The conidia concentration of the isolate was adjusted to 10^5 conidia/ml by adding measured quantity of sterilized distilled water. Average number of conidia per cell was calculated as a mean of conidia counting from the four corners and one central cell. The concentration of fungal suspension was calculated as per the formula

No. of conidia/ml of suspension = $X \times 10^5 \times D$

Where,

X = Average number of conidia per big square of haemocytometer

D = Dilution factor

RESULTS AND DISCUSSION

The present study is conducted to evaluate the best substrate for the mass multiplication of *B. bassiana*. Different solid and liquid media were evaluated for the mass production of *B. bassiana.* The observations were recorded on 10^{th}, 20^{th} and 30^{th} days after inoculation and the data presented in Table 1. The culture media plays major role in the multiplication of fungi (Kim *et al.,* 2010; Ying and Feng 2006). Among the different solid substrates evaluated, significantly highest mean conidial count (71.37×10^7 spores/ml) was recorded in rice media followed by gram (41.66×10^7 spores/ml) and wheat (10.68×10^7 spores/ml). Minimum conidial count (0.21×10^7 spores/ml) was observed in vermicompost followed by *Corcyra* rearing waste (5.23×10^7 spores/ml). The results are in closer proximity with the Ibrahim and Low (1993); Sharma *et al.* (2002); Pandey and Kanaujia (2010) who found rice was the best media for the mass culture of *B. bassiana*. Similar results were also found by Bhadauria *et al.* (2012); Karanja *et al.* (2013); Sahayaraj and Namasivayam (2008). The results showed that rice was best media for multiplication of *B. bassiana* which may be due to the presence of rich source of carbon and adequate source of nitrogen. It has been reported that the rice grain consists of 75-80% starch, 7% protein and sorghum contains 75% starch, 25% of amylase which are rich sources of carbon and adequate source of nitrogen that enhance the growth and sporulation (Oko *et al.*, 2012). Among liquid substrates, SDB yielded maximum conidial count (67.63×10^7 spores/ml). SDB was the best and results are in the agreement with the findings of Bhadauria *et al.* (2012); Karanja *et al.* (2013) who reported that SDB produced significantly higher spore production and biomass production of *B. bassiana*. Potato Dextrose Broth (PDB) also supported spore production of the fungus. The rate of increase in conidia of *B. bassiana* from 10 to 20 days and 20 to 30 days after inoculation among different substrates was recorded. The highest growth rate of increase in conidia from 10 to 20 days was recorded on pigeonpea (71.87%) and no growth rate of the fungus was recorded on vermicompost. The highest growth rate of increase in conidia from 20 to 30 days was recorded on vermicompost (78.26 %) and minimum growth rate (10.40%)of the fungus was recorded on rice. The mean dry weight was calculated after 15 days of incubation at 25^0C and presented in the Table 2. Highest mean dry weight (0.754 g) was recorded on gram followed by SDB (0.748 g), PDB (0.683 g). Lowest mean dry weight of fungus was recorded on vermicompost (0.001 g) followed by *Corcyra* rearing waste (0.13).

The results are closely related with the findings of Bhadauria *et al.* (2012) who reported the maximum dry weight of fungus on chickpea and SDB. The results were also correlated with the findings of Sahayaraj and Namasivayam (2008); Posada-Florez (2008); Prakash *et al.* (2021); Singh *et al.* (2019) also revealed that SDB was proved best substrate for producing highest spores of *B. bassiana.* Thus discussion confirms the results of the present investigation.

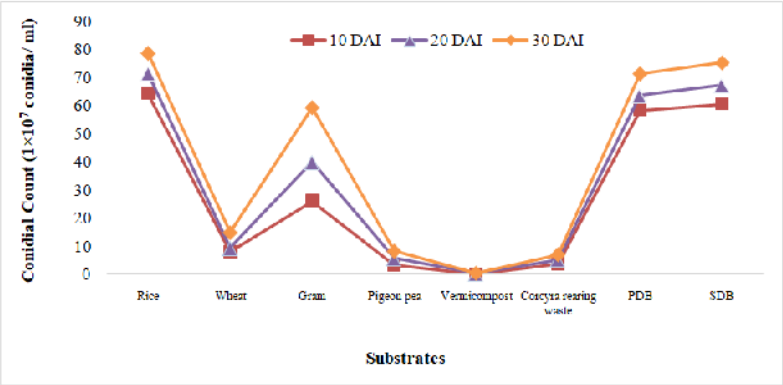

Fig. 1. Mass production of *B. bassiana* on different substrates.

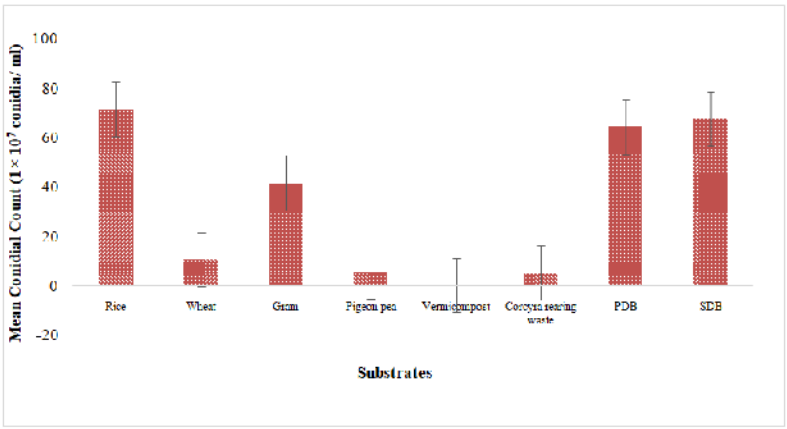

Fig. 2. Mean conidial count of *B. bassiana* on different substrates.

Table 1: Mass production of *B. bassiana* on different substrates.

Treatments		Conidial count (1×10^7 conidia /ml)				Rate of increase (%)	
		10DAI	20DAI	30DAI	Mean	10-20 DAI	20-30 DAI
Solid substrates							
T_1	Rice, *Oryza sativa* (L.)	64.19 (53.24)	71.25 (57.60)	78.66 (62.58)	71.37 (57.68)	11.00 (19.35)	10.40 (18.79)
T_2	Wheat, *Triticumaestivum*(L.)	7.94 (16.35)	9.26 (17.70)	14.83 (22.63)	10.68 (19.05)	16.62 (24.03)	60.15 (50.84)
T_3	Gram, *Cicer arietinum* (L.)	25.93 (30.58)	39.76 (39.06)	59.3 (50.35)	41.66 (40.17)	53.34 (46.89)	49.14 (44.48)
T_4	Pigeon pea, *Cajanuscajan*(L.)	3.27 (10.41)	5.62 (13.69)	8.26 (16.68)	5.72 (13.82)	71.87 (58.00)	46.98 (43.24)
T_5	Vermicompost	0.00 (0.00)	0.23 (2.74)	0.41 (3.66)	0.21 (2.64)	0.00 (0.00)	78.26 (62.30)
T_6	*Corcyra* rearing waste	3.54 (10.83)	5.12 (13.07)	7.03 (15.36)	5.23 (13.21)	44.63 (41.89)	37.30 (37.62)
Liquid substrates							
T_7	Potato Dextrose Broth (PDB)	58.32 (49.77)	63.49 (52.82)	71.32 (57.65)	64.38 (53.36)	8.86 (17.30)	12.33 (20.54)
T_8	Sabouraud's Dextrose Broth (SDB)	60.54 (51.07)	67.11 (55.01)	75.25 (60.22)	67.63 (55.33)	10.85 (19.21)	12.13 (20.36)
SE(m)		**0.95**	**1.11**	**1.39**	**1.12**	**0.93**	**1.17**
CD (5%)		**2.87**	**3.37**	**4.20**	**3.41**	**2.82**	**3.56**

DAI= Days after Inoculation; Values in parentheses are angular transformed

Table 2: Dry weight of *B. bassiana* on different substrates.

Treatments		Dry weight (g)
Solid substrates		
T_1	Rice, *Oryza sativa* (L.)	0.597 ± 0.020
T_2	Wheat, *Triticum aestivum* (L.)	0.406 ± 0.014
T_3	Gram, *Cicer arietinum* (L.)	0.754± 0.025
T_4	Pigeon pea, *Cajanus cajan* (L.)	0.161 ± 0.005
T_5	Vermicompost	0.001 ± 0.000
T_6	*Corcyra* rearing waste	0.133 ± 0.004
Liquid substrates		
T_7	Potato Dextrose Broth (PDB)	0.683 ± 0.023
T_8	Sabouraud's Dextrose Broth (SDB)	0.748± 0.025
	SE(m)	**1.42**
	CD (5%)	**4.30**

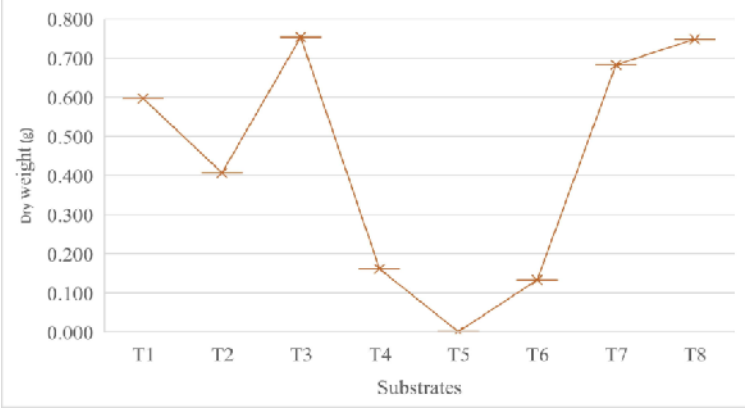

Fig. 3. Dry weight (g) of *B. bassiana* on different substrates.

CONCLUSION

These findings concluded that all the solid and liquid media used as substrate supported the growth of *B. bassiana*. Spores are germinated irrespective of the substrate used as a medium for the growth of fungus. However, highest quantity of conidia was produced in the Rice among solid media and SDB yielded maximum conidia among liquid media. Hence rice is the best media for the mass multiplication of *B.bassiana*. Research has to be done to commercialization of bio-pesticides and made them easily available to the farmers at reasonable price.

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Conflict of Interest. None.

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