Suitability of different synthetic and non synthetic media for mass culture of entomopathogenic fungi, *Beauveria bassiana* (Balsamo) Vuillemin

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ABSTRACT: The present investigation was done to test the different growth media; synthetic liquid substrate and non synthetic (agro waste) substrate to find out their suitability for mass multiplication of entomopathogenic fungi, *B. bassiana.* Among the 15 synthetic media tested maximum biomass (1.67g) was recorded in Czapek Dox Broth (CDB). However maximum sporulation and viability was observed in Potato Dextrose Yeast Broth (PDYB) (3.60×10^8 conidia ml^{-1}, 91.33 per cent) followed by Potato Dextrose Broth (PDB) (3.17×10^8 conidia ml^{-1}, 90.67 per cent) and Sabouraud's Dextrose Yeast Broth (SDYB) (2.87×10^8 conidia ml^{-1}, 89.67 per cent). Least suitable media with low spore count and low viability were Malt Extract Broth (MEB), Malt Extract Yeast Broth (MEBY) and Paris (P), while no growth, sporulation and conidial count was reported in JP(Jenkins Prior).Among different non synthetic media tested coconut water media was found best with respect to biomass (0.59 g), conidial count (2.00×10^8conidia ml^{-1}) and spore viability (86.33 per cent) followed by sugarcane molasses, rice cooked water, rice wash water and press mud. PDB as a control have maximum spore count (2.63×10^8 conidia ml^{-1}) and viability (93 per cent).

Key words: *B. bassiana,* non synthetic media, synthetic media,

Beauveria bassiana (Balsamo) Vuillemin (1912) is a ubiquitous entomopathogenic fungus which has been isolated from a wide variety of insects, soil, surface and interior of plants and air (Zimmermann 2007). It is most widely used against a wide variety of insect pests (Goettel *et al*., 2010). The choice of a suitable and economic medium which supports rapid growth of entomogenous fungi without loss of virulence for number of generations is one of the basic requirements in the mass production of fungi for microbial control of insect pests. *B. bassiana* is facultative pathogen and can be mass produced on various substrates. The present investigation were therefore, done to test the different growth media, synthetic liquid substrate and non synthetic (agro waste) substrate to find out their suitability for mass multiplication of locally isolated entomopathogenic fungi, *B. bassiana* from Kumaun region of Uttarakhand.

MATERIALS AND METHODS

B. bassiana was isolated from dead mycosed larvae of *Spilarctia obliqua* collected fromNainital district of Kumaon region of Uttarakhand in the year 2013-2014. To isolate the fungi, mycosed insects collected from the fields were surface sterilized with sodium hypochloride (3 %) and rinsed in plenty of sterile distilled water, then dried by filter paper. Surface sterilized cadavers were cut into small pieces and a small portion of infected part was transferred to a culture plate containing selective medium and kept under constant observation for the growth and development of microorganisms. Potato dextrose agar (PDA) was used for culture media, containing 0.25 mg/ml streptomycin to inhibit growth of bacteria and incubated at 27±2°C. Fungi was identified on the basis of morphological characters as per Humber (1997).

Different mycological broth culture media viz. Potato Dextrose Broth (PDB), Sabouraud's Dextrose Broth (SDB), Czapek Dox Broth (CDB), Malt Extract Broth (MEB) Jenkins Prior (JP), Paris (P), Sabouraud's Maltose Agar Yeast (SMAY) and chitin containing media with or without yeast extract were prepared and used in present investigation. The flasks containing media were sterilized under 15 lbs pressure (121°C) for 15 min in autoclave. To test the effect of yeast extract on growth and infectivity of entomogenous fungi, different media were fortified with 5g of yeast extract powder before autoclaving. For preparing non synthetic media, a 100

gram sample of each raw material (Sugarcane bagasse extract, Press mud, Sugarcane molasses) was taken separately, washed, soaked in water for 4 h and then cooked in 500 ml of tap water for 30 minutes. After straining through double muslin cloth, the extract was distributed in 250 ml conical flasks at the rate of 100 ml per flask. Another set of PDB and SDB was also prepared simultaneously. For evaluating suitability of non synthetic media for *B. bassiana* , Coconut water, Rice wash water and Rice cooked water, were used. The potentiality of these media was compared with potato dextrose broth and Sabouraud's dextrose broth for evaluating the growth and sporulation of fungi. The medium contained in the flasks were sterilized at 15 psi for 45 min in an autoclave. All the flasks containing synthetic and non synthetic media then seeded with 5 mm disc cut from the edges of an actively growing colony of *B. bassiana* and incubated at 27±1°C and 95±5 percent relative humidity in an incubator for 15 days. Four replication of each medium were maintained. To estimate the biomass, the broth culture after 15 days of seeding was filtered through a pre-dried and weighed filter paper and the mat collected was dried at 70°C for 24 hr and weighed (Im *et al.,* 1988). The difference in weight was recorded (Hall and Bell, 1961). The conidial count and conidial germination were also recorded.

RESULTS AND DISCUSSION

The observations with different mycological media with or without yeast extract on biomass, sporulation and viability of spores (Table 1 and Plate 1) showed that all the media except JP without yeast supported the growth and sporulation of *B. bassiana***.** Among fifteen synthetic media tested, maximum biomass (1.67g) was observed in CDB without yeast followed by SDYB (1.30g), PDYB (1.11g), JPY (0.82g), PDB (0.54g), PY(0.39g), SDB (0.29g), CDYB (0.28g), chitin with yeast (0.21g), MEBY (0.17g), P (0.17g), SMY (0.08g), Chitin containing media (0.07g) and MEB (0.04g). Whereas conidial count was reported maximum in PDYB (3.60×10^8 conidia ml^{-1}) followed by PDB (3.17×10^8 conidia ml^{-1}), SDYB (2.87×10^8 conidia ml^{-1}), CDYB (2.50×10^8 conidia ml^{-1}), SDB (2.43×10^8 conidia ml^{-1}), JPY (2.34×10^8 conidia ml^{-1}), CDB (2.00×10^8 conidia ml^{-1}), Chitin with yeast (1.70×10^8 conidia ml^{-1}), SMAY (1.60×10^8 conidia ml^{-1}), PY (1.60×10^8 conidia ml^{-1}), MEBY (1.53×10^8 conidia ml^{-1}), Chitin containing (1.30×10^8 conidia ml^{-1}) and MEB (1.13×10^8 conidia ml^{-1}) while there was no growth, sporulation and conidial germination reported in JP and it was found to be least suitable medium for *B.bassiana.* Spore viability was found maximum in PDYB (91.33 %) followed by PDB (90.67%),SDYB (89.67%), SDB (88.67%), CDYB (87.00%), CDB (86.33%), PY

Table.1: Effect of different Synthetic culture media on biological characteristics of *B.bassiana*

Sl.No.	Media	Biomass (gm)	Conidial Count (10^8) ml^{-1}	Spore Viability (%)
1.	PDB	0.54 (1.02) *	3.17 (1.91)*	90.67 (72.25)**
2.	PDYB	1.11 (1.27)	3.60 (2.02)	91.33 (72.90)
3.	SDB	0.29 (0.89)	2.43 (1.71)	88.67 (70.35)
4.	SDYB	1.30 (1.34)	2..87 (1.83)	89.67 (71.25)
5.	CDB	1.67 (0.82)	2.00 (1.58)	86.33 (68.35)
6.	CDYB	0.28 (0.88)	2.50 (1.73)	87.00 (68.93)
7.	JP	0.00 (0.71)	0.00 (0.71)	0.00 (0.00)
8.	JPY	0.82 (1.15)	2.34 (1.69)	75.33 (60.29)
9.	P	0.17 (0.82)	1.23 (1.31)	75.00 (60.00)
10.	PY	0.39 (0.94)	1.60 (1.45)	76.33 (60.91)
11.	Chitin containing media	0.07 (0.76)	1.30 (1.34)	75.33 (61.58)
12.	Chitin+Yeast	0.21 (0.84)	1.70 (1.48)	75.00 (60.11)
13	MEB	0.04 (0.74)	1.13 (1.28)	70.00 (56.84)
14.	MEBY	0.17 (0.82)	1.53 (1.43)	69.67 (56.59)
15.	SMY	0.08 (0.76)	1.60 (1.45)	71.33 (57.65)
	SEM±	0.06 (0.03)	0.07 (0.02)	1.68 (1.67)
	CD (5%)	0.17 (0.08)	0.21 (0.06)	4.84 (3.37)

Parenthesis values are square root transformed √x+0.5
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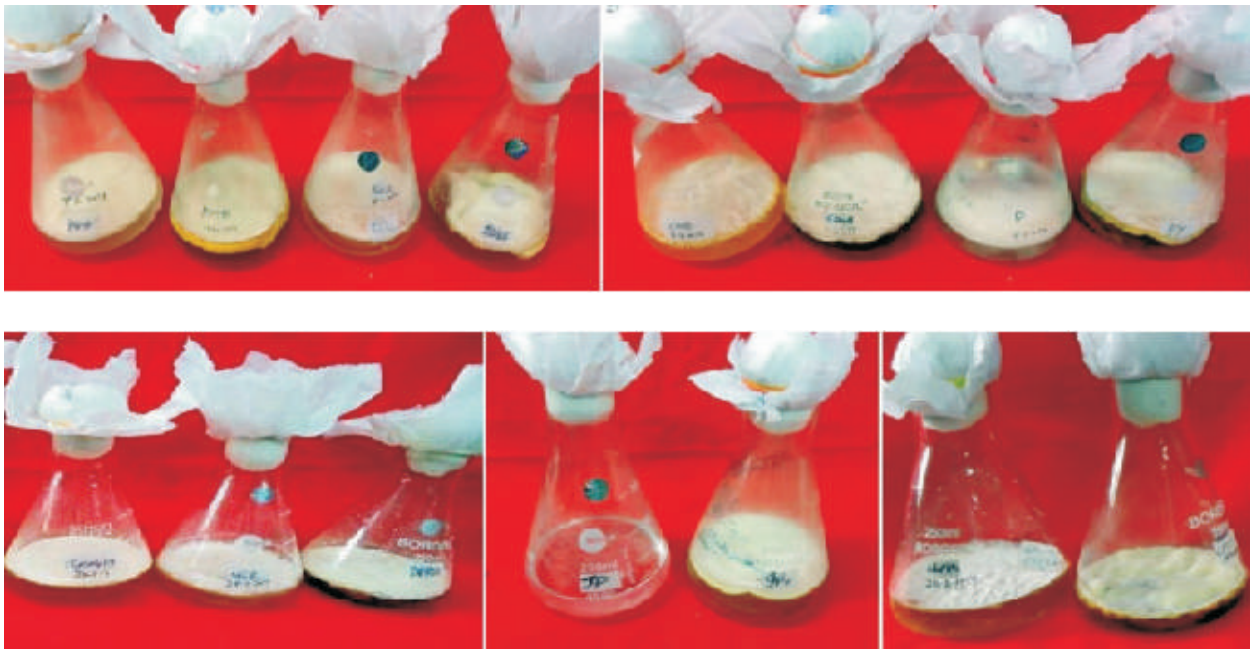

Plate 1 : Growth of *B. bassiana* on different synthetic broth media (a)PDB (b) PDYB (c) SDB (d) SDYB (e)CDB (f) CDYB (g) P (h) PY (i)SMY (j) MEB (k) MEBY (l) JP (m)JPY(n) Chitin containing media (o) Chitin+yeast

(76.33%), 75.00% for JPY, P, Chitin containing medium, chitin with yeast, SMY (71.33%), MEB (70.00%) and MEBY (69.67%). The standard medium PDB and SDB were taken as check media. Similar studies were conducted by Purwar and Sachan (2006) and reported that SDB with yeast extract was the best among different synthetic media for *B. bassiana.* Pandey and Kanaujia (2010) also reported that SDB media was significantly superior over all other media tested and supported maximum biomass (1.13g), conidial count (5.10×10^{7} conidia ml^{-1}) and spore viability(94.33%) for entomopathogenic fungus*B. bassiana*.

The observations with different non synthetic agro waste and byproducts on biomass, sporulation and viability of spores (Table 2 and Plate 2) showed that all the non synthetic media taken in the study supported the growth, sporulation and viability of *B. bassiana.* There were no sporulation, conidial count and viability reported on sugarcane baggase. Coconut water media was found best regarding biomass (0.59g), conidial count (2.00×10^{8} conidia ml^{-1}) and spore viability (86.33) followed by Sugarcane molasses, Rice cooked water, Rice wash water and Pressmud. PDB media was taken as check and have biomass, conidial count and viability of 0.29g, 2.63×10^{8} conidia ml^{-1} and 93.00 per cent, respectively.

Table. 2: Effect of different Non Synthetic culture media on biological characteristics of *B.bassiana*

Sl.No	Media	Biomass (gm)	Conidial Count (10^{8}) ml^{-1}	Spore Viability (%)
1.	Rice wash water	0.32 (0.90)*	1.50 (1.41)*	71.00 (57.42)**
2.	Rice cooked water	0.44 (0.97)	1.63 (1.46)	82.00 (64.94)
3.	Sugarcane molasses	0.43 (0.96)	2.43 (1.71)	89.33 (70.94)
4.	Sugarcane baggase	0.17 (0.82)	0.00 (0.71)	0.00 (0.00)
5.	Pressmud	0.00 (0.71)	0.00 (0.71)	0.00 (0.00)
6.	Coconut water	0.59 (1.04)	2.00 (1.58)	86.33 (68.35)
7.	PDB	0.29 (0.89)	2.63 (1.77)	93.00 (74.68)
	SEM±	0.04 (0.02)	0.11 (0.04)	0.86 (0.70)
	CD (5%)	0.11 (0.05)	0.35 (0.11)	2.62 (2.12)

*Parenthesis values are square root transformed √x+0.5
**Parenthesis values are angular transformed

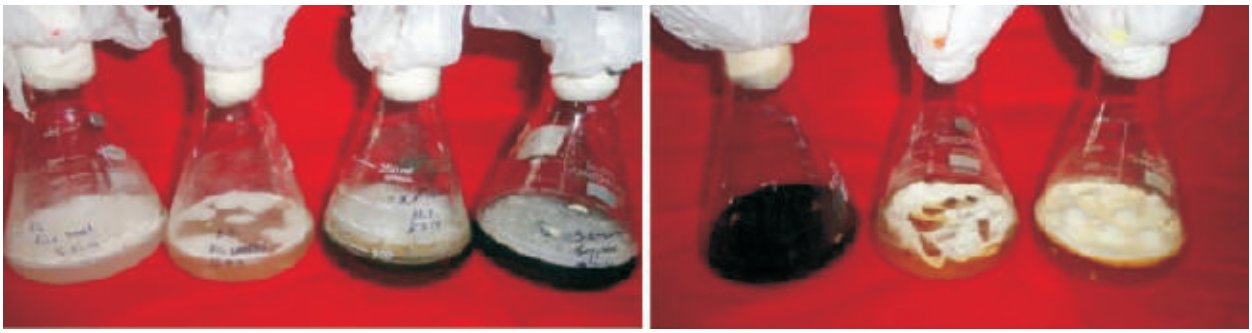

Plate 2: Growth of *B. bassiana* on different non-synthetic broth media (a) Rice wash water (b) Rice cooked water (c) Sugarcane molasses (d)Sugarcane baggase (e) Pressmud (f) Coconut water (g) PDB

Coconut water supported maximum growth and sporulation was also reported by Sahayaraj and Karthick (2008). From Table (no 2)it was clear that Pressmud and sugarcane baggase were found to be the least suitable media for *B. bassiana.* These findings were supported by Siwach & Jaipal (2004), who reported that inadequate amount of N in press mud (N 1.05-2.47%) might be possible cause of least fungal growth. Sharma *et al.* (2002) reported that rice was found to be the suitable media for the mass culture of *B. bassiana*. This cereal was also used for the mass production of other deuteromycete fungi. On the basis of present investigation it was concluded that among different synthetic media tested PDYB, PDB & SDYB were the best media. Among non synthetic media, coconut water is best media for growth and sporulation of *B. bassiana.*

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