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Effect of different liquid media on growth and sporulation of *Beauveria bassiana* in stationary culture

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Growth and sporulation of *Beauveria bassiana* was examined on four semi synthetic broths namely Jaggery-soy broth(JSB), Yeast peptone soybean oil broth(YPSB), yeast peptone dextrose broth(YPDB) and Corn meal broth(CMB) in stationary culture. 250 ml conical flasks with 100 ml broth was used and 25+1°C temperature and 85 + 5 R.H. was maintained for growth of the fungus. Highest fresh and dry biomass production was obtained from JSB (22.468 g and 1.858 g respectively) after 21 day of inoculation. But highest spore and CFU count was obtained from YPDB (3.29×10^8 spores/ml and 4.11×10^8 CFU/ml respectively) followed by YPSB (3.07×10^8 spores/ml and 4.01×10^8 CFU/ml respectively) after 21 days of inoculation. But there was no significant difference between YPDB and YPSB. The spore and CFU production obtained from JSB was also quite high (2.94×10^8 spores/ml and 3.54×10^8 CFU/ml respectively). But the performance of CMB was not at all promising in any respect.

Key words: *Beauveria bassiana*, liquid media, growth, sporulation

INTRODUCTION

Beauveria bassiana has been recognised as an important entomopathogenic fungus that attacks a wide range of agricultural pests of different orders. It is also an important tool for eco friendly pest management. On the other hand this entomopathogenic fungus has the capability to survive in soil for long time as saprophyte. Different methods can be employed for production of *B. bassiana* spores. Both solid and liquid media can be used for the small scale mass production of the bio control agent. But the mass multiplication method should be easier as well as cost effective. Liquid-culture production of *B. bassiana* is a suitable biotechnological process from the point of view of efficiency as well as profitability.

Several liquid media like molasses, carrot extract, potato extract, rice liquor, corn extract, yeast peptone dextrose broth or synthetic media can be used effectively. In liquid cultures, these fungi can be

grown in static liquid cultures or shake cultures or in fermenters using the appropriate media. The study of Thomas (1987) made with *B. bassiana* revealed that the carbon sources play an important role in spore production. Jackson *et al.* (1997) demonstrated that, the adequate sources of carbon and nitrogen in the culture media induce desiccation tolerance of blastospores of *Isaria fumosorosea* after air-dried conditions.

One experiment was carried out to assess the suitability of four semi synthetic broths namely Jaggery-Soy Broth (JSB), Yeast Peptone Soybean Oil Broth (YPSB), Yeast Peptone Dextrose Broth (YPDB) and Corn Meal Broth (CMB) on growth and sporulation of local isolate of *Beauveria bassiana* in stationary culture.

MATERIALS AND METHODS

Media composition

T1-Jaggery-soy broth (JSB): Jaggery-50g, Soy flour -10g, Water -1000 ml, T2- Yeast peptone

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Table 1: Effect of culture media on the weight of mycelial growth (g) of *Beauveria bassiana* under bench culture in 100 ml broth in 250 ml conical flask

	Days after inoculation					
	7 th day		14 th day		21 st day	
	FW(g)	DW(g)	FW(g)	DW(g)	FW(g)	DW(g)
YPDB	9.803	0.803	15.625	1.289	20.922	1.704
CMB	8.033	0.665	10.323	0.851	13.890	1.123
JSB	11.118	0.906	17.221	1.402	22.468	1.858
YPSB	9.687	0.796	15.138	1.229	20.789	1.694
SEm (±)	0.228	0.022	0.471	0.038	0.403	0.052
CD (5%)	0.839	0.082	1.731	0.138	1.481	0.190

soybean oil broth (YPSB): Dextrose- 40g, Peptone – 10g, Yeast extract – 5g, Soybean oil – 1ml, Water – 1000 ml, T3- Yeast peptone dextrose broth (YPDB): Dextrose- 40g, Peptone – 10g, Yeast extract – 5g, Water – 1000 ml, T4-Corn meal broth (CMB): Grind corn – 25g, Dextrose- 20g, Peptone – 10g, Water – 1000 ml. 250 ml conical flasks with 100 ml broth was taken for the experiment and 25+ 1°C temperature and 85 + 5 R.H. was main-

Table 2: Effect of culture media on the sporulation and CFU production of *Beauveria bassiana* under bench culture after 21st day of inoculation

	Spore production (10 ⁸ /ml)	CFU production (10 ⁸ /ml)
YPDB	3.29	4.11
CMB	2.38	2.69
JSB	2.94	3.54
YPSB	3.07	4.01
SEm (±)	0.117	0.212
CD (5%)	0.432	0.777

tained for growth of the fungus. Fresh weight and dry weight of the mycelia was measured after 7, 14 and 21 day of inoculation and spore and CFU counts were taken after 21 day of inoculation. For each treatment there were five replications. For each replication four conical flasks were prepared (1 for weight after 7th day, 1 for weight after 14th day, 1 for weight after 21st day, 1 for spore count and CFU count after 21st day).

Determination of fresh weight (FW)

The material from a conical flask poured in a pre-weighted dried facial tissue paper, which was fitted on a fine strainer. After removal of excess media the tissue paper along with the mycelia put in a plastic tray and fan dried for two hours to dry up the excess media. Then the tissue paper along with the mycelium weighted in digital balance.

Fresh weight (FW) = Wt. of tissue paper along with mycelium - Wt. of tissue paper

Determination of dry weight (DW)

After taking the fresh weight the mycelia along with the tissue paper put in the incubator at 75°C temp. for 24 hours. Then the dried material weighted in digital balance

Dry weight (DW)

Wt. of tissue paper along with mycelia - Wt. of tissue paper

Determination of number of spores

The number of spores were determined by using haemocytometer.

Determination of CFU

The CFU was counted on 21st date of growth. Total content of a conical flask poured in a sterilised mixer and blended for 1 minute. Then 1 ml from this suspension added to 9 ml of distilled water in a sterile test tube. The suspension diluted up to 10⁻⁷. 1 ml from this diluted suspension was sprayed on a very thin PDA plate in a petri dish. The petri dish was incubated for 24 hours in 25°C temperature in BOD. After 24 hours it examined under stereoscopic microscope and total number of colonies counted.

Statistical analysis

Data analysis was performed using one-way ANOVA in CRD, for each parameter including growth, spore count and CFU count and the means were compared on each case using the least significance difference (LSD) method at 0.05 level.

Fresh weight and dry weight

The experimental result revealed that JSB supported production of 22.468 g fresh biomass and 1.858 g dry biomass after 21 day of inoculation which was highest among the four tested broths (Table 1). Biomass production of JSB was higher than biomass production of all other broths throughout the experiment. After 21 day of inoculation YPDB produced 20.922 g fresh biomass and YPSB produced 20.789 g fresh biomass which were statistically at par. JSB produced highest fresh and dried biomass after 7 (11.118 g and 0.906 g respectively) and 14 days (17.221 g and 1.402 g re-

spectively) of inoculation which were significantly higher than all other broths. But after 21 day of inoculation dry biomass production of JSB, YPDB and YPSB were at par with one another. In this experiment CMB exhibited the lowest fresh and dry biomass production of the fungi throughout the experimental period which were significantly lower than other three broths. This finding is in conformity with the findings of Rao (2007), where it was mentioned that highest biomass production was obtained from JSB both in stationary and shaker culture (12.5 and 20.0g/100ml) 10 days after inoculation. Easwaramoorthy *et al.* (2002) also reported that, mollasses at 3% concentration was found to be suitable for biomass and spore production of *B. bassiana*. Sharma *et al.* (2002) also reported that highest biomass production is supported by Molasses yeast broth.

Spore count and CFU count

But in case of spore production and CFU production the performance was totally different. Highest spore count and highest CFU count were obtained from YPDB followed by YPSB (Table 2). YPDB produced 3.29×10^8 spores/ml and 4.11×10^8 CFU/ml which was statistically at par with spore and CFU production of YPSB (3.07×10^8 spores/ml and 4.01×10^8 CFU/ml). Spore production and CFU production of JSB was also at par with YPDB and

YPSB. This finding was also supported by the experiment result of Rao (2007). The author reported highest CFU production (5.1×10^8 CFU/ml.) from YPSB in *B. bassiana* and Maximum CFU production of *M. anisopliae* 9.8×10^8 and 4.3×10^8 CFU/ml was observed with YPDB in shaker and stationary cultures respectively.

From the experiment it can be concluded that the Jaggery Soy Broth supports highest biomass production of *Beauveria bassiana* in stationary culture. Whereas highest spore production and CFU production obtained from Yeast Peptone Dextrose Broth which is slightly higher from Yeast Peptone Soybean Oil Broth.

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