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MASS PRODUCTION OF ENTOMOPATHOGENIC FUNGI, *BEAUVERIA BASSIANA* (BALSAMO) VUILLEMIN AND *METARHIZIUM ANISOPLIAE* (METCHNIKOFF) SOROKIN ON VEGETABLE BASED MEDIA

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ABSTRACT

A simple and cost effective technique for *in vitro* conidial mass production of *B. bassiana* and *M. anisopliae* has been developed by using different vegetable based liquid media. In case of *B. bassiana*, PDB was found to be the best media with biomass production of 1.20 g/100 ml, conidial count 14.1×10^7 and conidial viability 93 per cent. The least biomass production was recorded from *Ipomoea batata* with biomass production of 0.88 g., conidial count 4.30×10^7 and conidial viability 71 per cent, while in case of *M. anisopliae, Ipomoea batata* yielded highest biomass (1.31) with condial count of 11.43×10^7 and conidial viability of 92 per cent. Least biomass was recorded from *Dioscorea* sp. with biomass production of 0.08 g, conidial count 0.56×10^7 and conidial viability 80.33.

Keywords: Mass production, Entomopathogenic fungi, *Beauveria bassiana, Metarhizium anisopliae.*

The increased use of conventional chemical pesticides over the years has not only contributed to an increase in food production, but also caused adverse effects on the environment and non-target organisms. Therefore, the use of biocontrol agents like entomopathogenic fungi has gained attention as a potential substitute for chemical pesticide in recent years. To develop entomopathogenic fungi as a biopesticide, it is necessary to develop culture media and methods that not only maximize production, but do so at low cost. The present investigation was therefore done to test different growth media based on vegetable extracts, to find out their suitability for mass multiplication of the entomopathogenic fungi *B. bassiana* and *M. anisopliae*.

MATERIALS AND METHODS

Fungal strain: The strains used in the experiment were *Beauveria bassiana* (Balsamo) Vuillemin ITCC-6264 and *Metarhizium anisopliae* (Metchnikoff) ITCC-6265 ITCC New Delhi. The strains were screened for pathogenicity against some lepidopteran insects. For maintenance of fungal isolates potato dextrose media was used. The media was prepared using the standard method and autoclaved at 15 psi (121°C) for 15 minutes. The media was subsequently plated in sterile petriplates aseptically. The plates were then inoculated with the isolates.

Locally available vegetables like bean, carrot, sugarbeet, *Dioscorea* sp., *Chenopodium* sp., and spinach, were used in the study while (PDB), a conventional mycological medium was used as check.

Vegetable broths were prepared using 100 gram sample of all vegetables, separately washed and then cooked in 500 ml of tap water for 30 minutes. After staining through double muslin cloth, the extracts were distributed in 250 ml conical flasks at the rate of 100 ml per flask. The medium contained in the flasks were sterilized at 15 psi for 20 minutes in an autoclave. The flasks with media were then seeded with 5 mm disc cut from the edges of an actively growing colony of *B. bassiana* and *M. anisopliae* and incubated at 27±1°C and 95±5 per cent RH in an incubator for 15 days. Three replications for each medium were maintained. To estimate the biomass, the broth culture after 15 days of seeding was filtered through a pre-dried and weighted filter paper and the mat collected was dried at 100°C for 24 hr in the oven, and weighed. The difference in weight was recorded (Hall and Bell, 1961). The conidial count was taken with an improved Neubauer Weber Haemocytometer (Jones, 1962) and conidial viability was determined as suggested by Gillespie (1986).

RESULTS AND DISCUSSION

B. bassiana : The data presented in Table 1 indicate that among tested media the standard media *i.e.* Potato Dextrose Broth proved superior to all other media in respect of biomass production (1.20) g, conidial count (14.16×10^7) and conidial viability (93.00%). The biomass production, radial growth and conidial count on bean media were 0.32 g, 12.16 and 92.33 per cent, respectively. The biomass production was significantly lower in case of spinach but with good yield of spores 9.03×10^7 and 90.33 per cent viability. The spore production on *Dioscorea* sp. was at par with spinach. The biomass production was least in *Chenopodium* sp. (0.04gm) but conidial count was at par with spinach *i.e.* 8.13 and conidial viability 86.00 per cent, while the biomass on carrot was found to be 0.40 gm with spore count of 5.36 and conidial viability of 77.33 per cent. The least conidial production was found in *Ipomoea batata* (4.30) with least conidial viability (71.00%), but with good biomass of 0.88 g. Patel (1997) also tested carrot for mass multiplication and found 3.98×10^7 spores/ml with 91.25 per cent viability and 4.95×10^7 spores/ml with 94.87 per cent viability for *B. bassiana* and *M. anisopliae* respectively. Carrot and potato broths with dextrose allowed good fungal growth measuring a spore density of 4.30×10^7 and 4.26×10^7 conidia/ml (Sangle *et al.*, 2003).

M. anisopliae : Data revealed that all the tested media supported the growth of fungus *M. anisopliae* (Table 2).

Table 1: Effect of different vegetable extracts on biological parameters of *B. bassiana*

S.No.	Media	Biomass (g)	Conidial count (10^7)	Spore Viability (%)
1.	PDB	1.20 (1.30)*	14.16 (3.82)*	93.00 (74.72)**
2.	Bean	0.32 (0.91)	12.16 (3.55)	92.33 (74.01)
3.	Spinach	0.07 (0.75)	9.03 (3.08)	90.33 (70.96)
4.	*Dioscorea* sp.	0.15 (0.81)	8.56 (3.00)	86.00 (68.16)
5.	*Chenopodium* sp.	0.04 (0.74)	8.13 (2.92)	77.33 (61.57)
6.	Carrot	0.40 (0.95)	5.36 (2.41)	71.00 (57.44)
7.	*Ipomoea batata*	0.88 (1.17)	4.30 (2.18)	1.50 (1.27)
	S.Em.±	0.08 (0.03)	0.84 (0.13)	1.50 (1.27)
	CD (5%)	0.26 (0.10)	2.56 (0.42)	4.55 (3.87)

* Parenthesis values are square root transformed $\sqrt{x+0.5}$

** Parenthesis values are angular transformed

Table 2: Effect of different vegetable extracts on biological parameters of *M. anisopliae*

S.No.	Media	Biomass (g)	Conidial count (10^7)	Spore Viability (%)
1.	*Ipomoea batata*	1.31 (1.34)	11.43 (3.45)*	92.00 (73.59)**
2.	Carrot	0.30 (0.89)	10.46 (3.31)	91.00 (72.67)
3.	Bean	0.24 (0.86)	9.36 (3.14)	89.66 (73.97)
4.	*Chenopodium* sp.	0.11 (0.78)	9.23 (3.11)	86.33 (68.44)
5.	Spinach	0.13 (0.79)	7.86 (2.89)	82.66 (65.42)
6.	PDB	0.33 (0.91)*	5.16 (2.37)	92.33 (65.42)
7.	*Dioscorea* sp.	0.08 (0.76)	0.56 (1.00)	80.33 (63.74)
	S.Em.±	0.04 (0.01)	0.40 (0.08)	1.53 (1.30)
	CD (5%)	0.14 (0.05)	1.21 (0.26)	4.66 (3.96)

* Parenthesis values are square root transformed $\sqrt{x+0.5}$

** Parenthesis values are angular transformed

The differences in the production of biomass due to different media used were statistically significant. The highest biomass (1.31), spores production (11.43) and conidial viability (92.00%) were recorded on the *Ipomoea batata*. Spore count on bean (9.36) was found to be at par with *Chenopodium* sp. (9.23), while on spinach it was 7.86. The spore count of check *i.e.* PDB was found to be 5.16. Among all the tested media lowest spores were recorded from *Dioscorea* sp. (0.56). The viability of the conidia in the tested media ranged between 80.33-92.00 per cent, highest on *Ipomoea batata* and lowest on *Dioscorea* sp. For *B. bassiana* the standard media PDB was the best media and the bean extract was found to be equivalent to PDB. In case of *M. anisopliae* all the vegetable extracts except *Dioscorea* sp. were superior than PDB. Gopalakrishnan *et al.* (1999) developed simple and cost effective technique for mass multiplication of conidia of *Paecilomyces farinosus* by using cereals, brans, pulses, vegetable, roots, seeds and synthetic media. Among potato, tapioca root, carrot and jackfruit seeds, carrot proved to be the best yielding 9.37×10^{12} spores/100g or ml.

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