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# EVALUATION OF SUBSTRATES FOR MASS CULTURING OF METARHIZIUM ANISOPLIAE

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## ABSTRACT

**Entomopathogenic fungi are well known biological control agents and *Metarhizium* anisopliae is one among them. This study evaluates some substrates for mass production of this fungus. Both solid and liquid media were evaluated and it was observdd that maximum sporulation was obtained with Sabouraud dextrose broth (18.39 spores/ ml), a liquid media followed by sorghum grains (11.68 spores/ ml), while sugarcane bagasse medium gave the least (2.86 spores/ ml).**

**Key words:** *Metarhizium anisopliae,* sporulation, substrates, media, solid, liquid, sorghum, rice, FYM, vermicompost, vegetable waste, sugarcane bagasse, NSK, Sabourad dextrose, potato dextrose

Entomopathogenic fungi under the phylum Deuteromycota are widely distributed. (Zimmermann, 1986; Humber, 1997). There are around 200-250 such fungal isolates infecting Coleoptera, Dermoptera, Homoptera, Lepidoptera and Orthoptera (Moore et al., 1996). These provide biological control alternatives for insect control (Khachatourians et al., 2002). These include *Metarhizium* spp., *Beauveria* spp., *Nomuraea rileyi, Verticillium lecanii* and *Hirsutella* spp. In 1883, Metchinikoff mass cultured such pathogens for experiment with two beetle pests. The present study evaluates certain solid and liquid substrates viz., rice and sorghum grains, farm year manure, vermicompost, vegetable waste, sugarcane bagasse, and neem seed kernel along with with 1% yeast and liquid Sabouraud dextrose broth, and potato dextrose broth for mass culturing *Metarhizium anisopliae*.

## MATERIALS AND METHODS

The substrates evaluated include solid substrates viz., sorghum and rice grains, farm yard manure, vegetable waste, vermicompost, sugarcane bagasse and neem seed kernel. The study was done at 26± 2ºC with 100g of each substrate, which were washed and kept overnight for water absorption and cooked until became soft before shade dried. The culture was done in 250 ml culture flasks following standard procedures. The optimum temperature for mycelial growth at 20-25ºC, and pH 5-11 was followed (Sang-Myeong Lee et al., 1999). The substrates evaluated include: Solid media- sorghum and rice grains, Farm yard manure (FYM), vegetable waste, sugarcane bagasse, and neem seed kernel (NSK), all with 1% yeast extract (YE); and liquid media- Sabouraud and potato dextrose bath, prepared and cultures maintained as per standard protocol. Observations on spore counts were done in 15$^{th}$ and 25$^{th}$ days after inoculation using haemocytometer, and number of conidia in g/ ml of the substrate worked out.

## RESULTS AND DISCUSSION

The mycelia growth and spore yield obtained in the substrates evaluated is given in Table 1. The results revealed that *M. anisopliae* spore/ml production was significantly more vegetable waste+1% of yeast extract on 7$^{th}$ day fater inoculation and the least with sugarcane bagasse, while the liquid media viz., gave 9.83 and 7.68 spores/ ml (Sabouraud and potato dextrose broth, respectively). On 14$^{th}$ day spore production was significantly more with sorghum grain, vegetable waste, rice grain and farm yard manure, and with sugarcane bagasse it was the least; and Sabouraud and potato dextrose broths gave the maximum of all. Twenty one days after inoculation, spore production was significantly more with sorghum grain, vegetable waste, rice grain, and FYM, and sugarcane bagasse gave the least. Again, Sabouraud and potato dextrose were superior. Similar results were obtained after 28 days after inoculation. Overall it was observed that more

Table 1. Mass production of *Metarhizium anisopliae* on substrates (DAI = Day after incubation, YE = Yeast)

| Treatment No. | Media/Substrates | Spore concentration | Increase in growth of M.anisopliae at different days after inoculation | | | | |
|---|---|---|---|---|---|---|---|
| | | | 7DAI | 14DAI | 21DAI | 28DAI | Over all Mean |
| | Solid Media | | | | | | |
| T1 | Sorghum grain +1% of YE | 1 ×10$^7$spore / ml | 5.35 | 10.50 | 14.52 | 16.35 | 11.68 |
| T2 | Rice grain +1% of YE | 1 ×10$^7$spore / ml | 4.69 | 9.63 | 13.40 | 13.55 | 10.31 |
| T3 | Farm yard manure (FYM) +1% of YE | 1 ×10$^7$spore / ml | 3.50 | 5.92 | 6.20 | 6.31 | 5.48 |
| T4 | Vegetable waste +1% of YE | 1 ×10$^7$spore / ml | 4.60 | 8.60 | 9.38 | 10.25 | 8.20 |
| T5 | Vermi compost +1% of YE | 1 ×10$^7$spore / ml | 2.93 | 3.18 | 3.35 | 3.40 | 3.21 |
| T6 | Sugarcane bagasse +1% of YE | 1 ×10$^7$spore / ml | 2.50 | 2.83 | 2.98 | 3.13 | 2.86 |
| T7 | Neem seed kernel (NSM) +1% of YE | 1 ×10$^7$spore / ml | 3.10 | 4.87 | 5.90 | 6.07 | 4.98 |
| | Liquid Media | | | | | | |
| T8 | Sabouraud dextrose borth | 1 ×10$^7$spore / ml | 9.83 | 18.33 | 20.26 | 22.14 | 16.89 |
| T9 | Potato dextrose Broth | 1 ×10$^7$spore / ml | 7.68 | 14.45 | 17.55 | 18.44 | 14.53 |
| | SEm± | | 0.14 | 0.10 | 0.11 | 0.09 | 0.11 |
| | CD at 1% | | 0.42 | 0.32 | 0.35 | 0.27 | 0.34 |

spores (16.89 spore/ ml) were on Sabouraud dextrose broth, and the least with sugarcane bagasse (2.86 spore/ ml). According to Gopalakrishnan and Mohan (2001), Gopala Krishnan et al. (1999) and Tincilley et al. (2000) reported that carrot was found cheapest and best suitable for large scale production of Deuteromycota fungi.

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**REFERENCES**

Gopalakrishnan C and Mohan K S. 2001. *Nomuraea rileyi* (Farlow): a potential entomopathogenic fungus for control of *Helicoverpa armigera* (Hub) on tomato. Journal of Entomology 26: 93-97.

Gopalakrishnan C, Anusuya D and Narayanan K. 1999. In vitro Production of Conidia of entomopathogenic Fungus Parcilomycesfarinosus. Entomology 24: 389-392.

Roberts D W and Humber R A. 1981. Entomogenous fungi. In: Biology of Conidial Fungi. Academic Press, New York, pp. 201-236.

Khachatourians G G, Valencia E and Miranpuri G S. 2002. Beauveria bassiana and other entomopathogenic fungi in the management of insect pests. In: Koul, O., Dhaliwal, G.S. (Eds.), *Microbial Biopesticides* Vol. 2. Harwood Academic Publishers, Reading, UK, pp. 239-275.

Moore D, Higgins P M and Lomer C J. 1996. The effects of simulated and natural sunlight on the viability of conidia of *Metarhizium flavoviride* Gams and Rozsypal and interactions with temperature. *Biocontrol Science Technology*, **7:** 87-94.

Sang-MyeongLee, Woon Hong Yeo, Hyeong - Jin Jee, Sang-Chul Shin and Kwnag-Sik Choi. 1999. Cultural characteristics of Entomopathogenic fungi isolated from southern parts of Korea. FRI. J. Fr. Sci. 62: 126-135.

Tincilley A, Easwaramoorthy G. and Santhanalakshmi G. (2000). Attempts on mass production of *Nomuraea rileyi* on various agricultural products and by products. Journal Biol Contro 18(1): 33-40.

Zimmermann G. 1986. The *Galleria* baits method for detection of entomopathogenic fungi in soil. Journal of Applied Entomology 102: 213-215.

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