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RESEARCH NOTE

Evaluation of different cereal and millet based media for large-scale production of entomopathogenic fungus, *Metarhizium anisopliae* (Metchnikoff) Sorokin

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ABSTRACT: Six different media consisting of rice, bajra, sorghum, maize, wheat, and foxtail millet, were assessed for their potential in the mass-scale cultivation of the entomopathogenic fungus, *Metarhizium anisopliae*. Among these grains, crushed rice with 1% yeast extract demonstrated the highest spore count (27.56 x 10^8 conidia/g), on the 25th day for the fungus. This was followed by crushed bajra with 1% yeast extract (22.89 x 10^8 conidia/g), crushed sorghum with 1% yeast extract (12.54 x 10^8 conidia/g), and crushed maize with 1% yeast extract (7.57 x 10^8 conidia/g). In contrast, Crushed fox tail millet with 1% yeast extract yielded the lowest conidial production on the 25th day (5.86 x 10^8 conidia/g). Conidial production showed an increasing trend from the 6th to the 25th day and remained relatively stable thereafter across all treatments. This study underscores the potential use of alternative nutrient sources derived from various agricultural products for the large-scale cultivation of entomopathogenic fungus *M. anisopliae*. Such findings hold promise for biopesticide development and sustainable pest control practices.

Keywords: Fungus, maize, millet, rice, sorghum, yeast

Entomopathogenic fungi are highly versatile biological control agents, owing to their broad host range, often leading to natural epizootics. One appealing aspect of these fungi is their mode of infectivity, primarily through contact and penetration (Nadeau *et al.,* 1996). This fungal group comprises a diverse assemblage of more than 100 genera, encompassing around 750 species known to infect various insect hosts. Many of these species hold significant promise in pest management strategies. Notably, key fungal pathogens in this context include *Metarhizium spp*., *Beauveria spp*., *Nomuraea rileyi*, *Verticillium lecanii*, and *Hirsutella* spp. The paramount requirement for promoting the widespread use of any microbial bioagent is the cost-effective production of inoculums suitable for field-level application. While Sabouraud's maltose agar with yeast extract (SMAY) is a globally accepted standard medium that facilitates maximum growth of *M. anisopliae*, it is associated with high production costs, necessitating the exploration of alternative mass multiplication media to reduce expenses. Numerous efforts have been undertaken to propagate the fungus using semi-synthetic media and solid substrates. Notably, *M. anisopliae* has been successfully mass-produced using crushed sorghum with the addition of 1% yeast extract (Mamta *et al.* 2011). Carrot and rice was identified as the most economical and well-suited media for the large-scale multiplication of the green muscardine fungus (Gopalakrishnan and Mohan, 2000). Recognizing the significance of producing inoculums at a competitive cost, various substrates have been explored for the mass multiplication of *M. anisopliae*, with the aim of achieving cost-efficiency and adaptability using locally available indigenous substrates.

The fungal isolate used in this study was obtained from Sugarcane breeding institute (SBI), Coimbatore, Tamil Nadu. The culture was originally isolated from field collected *M. anisopliae* infected white grub on sugarcane crops in experimental plot.

Mycelial discs of *M. anisopliae* were inoculated in SMAY broth supplemented with 1% yeast extract and incubated at 26ºC for 48 h with shaking at 180 rev min-1. The fungal spores were harvested in 25 ml of sterilized distilled water (SDW) containing 0.05% Tween 20 (Polyoxyethylene sorbitan monolaurate) and the spore count of this stock suspension was estimated with an improved Neubaur haemocytometer. The number of spores was adjusted to 1x10^8 spores/ml and this suspension was used as inoculums for further experiments.

Six food grains *viz.,* Sorghum, bajra, foxtail millet, maize, rice and wheat with 1 per cent yeast extract were assessed for their suitability as substrates for mass production of *M. anisopliae*. To each of food grains, sterile distilled water was added in order to bring the moisture content to50 per cent. After thorough mixing, the bottles were plugged with cotton and autoclaved at 15psi and 121°C for 30 minutes. Circular agar discs of

5 mm diameter were taken from the 8ht day old fungal culture grown on SMAY plates. One disc was inoculated to each bottle and mixed with it to disperse the inoculums. The bottles were incubated in BOD incubator at 25±1°C and RH of 85±1%. Four replications were maintained for each treatment. The spores were harvested from 6th day onwards at definite intervals of upto 25 days by sampling 1 g of the digested material. The spore suspension of each sample was made by dispersing the inoculums in 10ml sterile water blank with one drop of 0.02 per cent Tween-80, serially diluted and the spore count estimated using a haemocytometer. Different food grains were evaluated for their suitability to support the conidial production of the mycopathogens based on the time taken to initiate mycelial growth, sporulation and conidial yield at 6, 8, 15, 20 and 25 days after inoculation (DAI)

In general, the mycelial growth and conidial production exhibited an upward trend with an increase in the number of days after inoculation (DAI). By the 25th DAI, the mycelial growth almost entirely covered the surface of the media. Notably, the source of grain significantly influenced the conidial yield at all observed intervals. *M. anisopliae* displayed notably higher growth on crushed rice supplemented with 1% yeast extract, producing a remarkable (27.56 x 10^8 conidia per gram). This was followed by crushed bajra with 1% yeast extract, yielding 22.89 x 10^8 conidia per gram, and crushed sorghum with 1% yeast extract, which produced 12.54 x 10^8 conidia per gram. Crushed maize with 1% yeast extract showed a moderate yield (7.57 x 10^8 conidia per gram) followed by Crushed wheat with 1% yeast extract (6.18 x 10^8 conidia per gram). On the other hand, foxtail millet with 1% yeast extract was the least productive, generating (5.86 x 10^8 conidia per gram). The conidial production exhibited a notable increase (Table 1.) from the 6th to the 25th day, after which it remained relatively constant across all treatment groups, highlighting their limited suitability as substrates for fungal productivity. The peak conidial load was reached after 25 DAI across all tested treatments.

Table 1. Showing the conidial yield of *M. anisopliae* at 6th, 8th, 15th, 20th and 25th day intervals on different agricultural products

	Number of spores x 10^8/g (Days After Inoculation)				
Treatment	**6 DAI**	**8 DAI**	**15 DAI**	**20 DAI**	**25 DAI**
(Crushed rice +1% Yeast extract)	21.35	24.05	25.85	26.32	27.56
(Crushed bajra +1% Yeast extract)	14.25	16.66	18.86	20.26	22.89
(Crushed sorghum +1% Yeast extract)	8.37	9.75	10.26	11.66	12.54
(Crushed maize +1% Yeast extract)	3.66	4.06	5.15	6.79	7.57
(Crushed wheat +1% Yeast extract)	2.07	3.05	4.19	5.56	6.18
(Crushed fox tail millet +1% Yeast extract)	2.90	3.10	3.85	4.56	5.86

Grains are not only cost-effective and readily available but also serve as excellent substrates for the growth and multiplication of microorganisms on a large scale. They offer a substantial surface area and serve as nutritious media for mass-scale cultivation of various microorganisms. Rice, in particular, contains a higher proportion of starch and amylase. Starch hydrolysis in rice leads to the release of glucose and maltose, primarily depending on the enzymatic actions involved (Preen *et al.,* 1985). Maltose, generated through starch hydrolysis by enzymes present in the fungus, plays a crucial role in inducing sporulation, a process (Coudron *et al.,* 1985). Furthermore, rice has consistently demonstrated its suitability as a substrate for the rapid and efficient mass multiplication of *N. rileyi* (Gopalakrishnan and Mohan, 2000 and Lingappa *et al.,* 2002).

The results of the present study align with these findings, confirming the effectiveness of rice as a natural medium for mass multiplication. This natural medium can be applied in future assessments of mass-scale cultivation *M. anisopliae* and other related fungi with potential applications in biological control. To achieve cost-effectiveness and high concentrations of viable fungal spores and to make the most of the abundant agricultural waste produced in fields; this study proposes exploring alternative nutrient sources for mass-scale cultivation of *M. anisopliae*. The results obtained in this study suggest the viability of using various grains for the mass-scale cultivation of entomopathogenic fungi. Further work is required to optimize the media, particularly with regard to substrate moisture content, to enhance the process.

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REFERENCES

Coudron, T. A., Kroha, M. J. and Sayed, G. N. 1985. A novel semi liquid for propagating entomopathogenic fungi. *Journal of Invertebrate Pathology*, **46**: 335-336.

Gopalkrishnan, C., and Mohan, K. S. 2000. A simple and cost effective *invitro* method for the mass production of conidia of *Nomuraearileyi*(Farlow) Samson. *Pest Management in Horticulture Ecosystems*, **6** (1), pp 36 – 39.

Lingappa, S., Patil, R. K., Rachappa,V., Hegde, R. and Navi, S. S. 2002. Large-scale demonstration of efficacy of *Nomuraea rileyi* (F) Samson on soybean. *In: Biological control of lepidopteran pests*. Proceedings of the Symposium of Biological Control of Lepidopteran Pests, (Tandon, P. L. ed.), Bangalore, India. 251–253 PP. *Journal of Invertebrate Pathology*, **46**: 335-336.

Mamta Thakre, Mahendra Thakur, Nagesh malik. and Suman Gangar. 2011. Mass scale cultivation of entomopathogenic fungus *Nomuraea rileyi* using agricultural products and agro wastes. *Journal of Biopesticides,* **4** *(2): 176-179.*

Nadeau, M.P., Dunphy, G. B. and Boisvert, J. L., 1996. Development of *Eryniaconica*(Zygomycetes :Entomophthorales) on the cuticle of the adult black flies *Simuliumrostratum*and *Simulium decorum* (Diptera : Simuliidae). *Journal of Invertebrate Pathology*, **68**: 50-58.

Preen, J. C., Jong, F. D., Botes, P. J. and Lategon, T. M. 1985. Fermentation alcohol from grain sorghum starch. *Biomass*, **8**: 101-117.

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