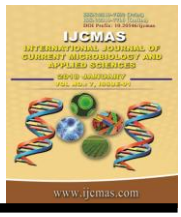
Int.J.Curr.Microbiol.App.Sci (2018) *7*(1): 2227-2232

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 7 Number 01 (2018)**
Journal homepage: http://www.ijcmas.com



Original Research Article https://doi.org/10.20546/ijcmas.2018.701.268

Mass Production of Entomopathogenic Fungi (*Metarhizium anisopliae*) using Different Grains as a Substrate

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A B S T R A C T

Investigations were carried out towards the "Mass production of entomopathogenic fungi (*Metarhizium anisopliae*) using different grains as a substrate" at International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Hyderabad during 2016-17. The accomplishment of microbial control of insect pests, the successful mass production of the microbial agents in the laboratory significant availability of the pathogen is a primary requirement in the biocontrol programme. *Metarhizium anisopliae* is an entomopathogenic fungus which is used against a number of insect pests' management and very successful biopesticides in integrated pest management practices to reduces the cost of production and minimize the environment and public health hazard. To develop a proficient method for the deployment of this fungus as a bio control agent, various grains and liquid media such as Potato Dextrose Broth and Sabouraud's Dextrose Broth were screened. Significantly highest conidial count (67.6 × 10^3 spores/ml) was observed on green gram followed by sorghum in 10^3 dilutions, the assessment fungus whereas SDB produced significantly higher spore production of the fungi. Highest conidial count (63.7 × 10^3 spores/ml) was observed on SDB media followed by PDB in 10^3.

Keywords

Metarhizium anisopliae, Grain substrates, Mass production

Article Info

Accepted:
16 December 2017
Available Online:
10 January 2018

Introduction

Biopesticides based on bacteria, viruses, entomopathogenic fungi and nematodes are often considerable scope as plant protection agents against several insects Noris *et al.*, 2002. *Metarhizium anisopliae* being a facultative fungal parasite, which can grow on non-living media under unnatural conditions, can infect and grow on natural insect host. The fungus readily sporulates on semi synthetic media like PDA, SMAY and carrot malt agar. Carbon and nitrogen are the most vital nutrients required for growth and sporulation Roberts, 1966 and Campbell, R.K. 1983. Microbial control has been considered an important tool in integrated pest management (IPM) and is an ecologically favorable strategy compared to conventional chemical control Barrancoflorido, *et al.*, 2002. In this

approach, entomopathogenic fungi are employed as bio-control agents reducing pest populations and, consequently, their damages in different agro- ecosystems Inglis, *et al.*, 2001. The conventional chemical pesticides has not only enhanced the food production, but also adversely affected the environment and non-target organisms. Due to the side-effects of chemical pesticides, the sustainable crop production through eco-friendly pest management is essentially required in recent scenario. Among the several micro-organisms viz., bacteria, fungi, virus, protozoans and entomopathogenic nematodes, a few have been systematically studied for their effective beneficial characteristics. The systematic study of these beneficial microorganisms can lead to gainful exploitation in microbial control programmes.

Entomopathogenic fungi are often reported as causing high levels of epizootics in nature and are the most versatile biological control agents, and are environmentally safe. An attractive feature of these fungi is that the virulence caused by contact and the action is through penetration. These fungi subsume a heterogenous group of over 100 genera with approximately 750 species, notified from different insects. Many of these are proved to be highly potential in pest management. The most considerable fungal species are *Metarhizium* spp., *Beauveria* spp., *Nomuraea rileyi*, *Verticillium lecanii* and *Hirsutella* spp in 1883, Metchnikoff commenced mass culturing of fungus and carried out the first experiment with two beetle pests. *Metarhizium anisopliae* (Metchnikoff) Sorokin is the second most widely exploited entomopathogenic fungus in biocontrol trials. It is known to attack over 200 species of insects belonging to orders Coleoptera, Dermoptera, Homoptera, Lepidoptera and Orthoptera. The present study was undertaken to evaluate grains such as rice, wheat, chickpea, pigeon pea, black gram, green gram, groundnut, sorghum, soybean, maize and solid media such as PDA and SDB for the mass production of *Metarhizium anisopliae.*

Materials and Methods

Metarhizium culture

Metarhizium anisopliae strain was available in the biocontrol laboratory at International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Hyderabad.

Different grain media

Ten whole grains viz., rice, wheat, chickpea, pigeon pea, black gram, green gram, groundnut, sorghum, soybean, maize were used for estimating the sporulation of *Metarhizium anisopliae* at 28ºC. 100g of each grain was washed and soaked in water overnight except rice which were soaked for 2 - 3h prior of starting the experiments. The excess water was drained by decanting and shade drying it for half an hour to further remove the intemperance moisture. The grains were packed separately in 500 ml conical flask, with cotton plug and auto calved at 15 psi for 20 min. After cooling, 1 ml of the spore suspension of fungal pathogen was inoculated into each flask under laminar air flow chamber. They were incubated in BOD incubator at 28ºC for 15 days. Three replications were maintained for each grain. To avoid clumping, after 7 days of inoculation, the flasks were shaken robustly to separate the grain and to break the mycelial mat. After 15 days of incubation, 10g homogenous grain sample drawn from each replicate of consistently sporulating flasks was transferred to 100 ml sterilized distilled water containing Tween 80 (0.05%) solution in 250 ml conical flasks. The flasks were shaken in mechanical shaker for 10 min. The suspension was filtered through double layered muslin cloth. Counting of spore's were made after the

serial dilution of the suspension using double ruled Neubauer haemocytometer for determining the number of conidia in 1 g of the cereal grains this methodology suggested by (Bhadauria, *et al.*,2012.)

Liquid media

Potato Dextrose Broth and Sabouraud's Dextrose Broth were evaluated for the growth and sporulation of *Metarhizium anisopliae* fungi. 100 ml of each medium was poured in 250 mL capacity conical flasks and autoclaved at 15 psi pressure for 30 min. Five flasks of each medium was inoculated with 1 ml of spore suspension of fungi separately and incubated at 28ºC for 15 days. The spore suspension was subjected to spore counting and it was carried out as described in the previous section.

Statistical analysis

The ANOVA was used to analyses the significance of media on fungal sporulation using 'Genstat' software

Results and Discussion

In the current study, numerous naturally available substrates for liquid media were tested for mass multiplication of *M. anisopliae*.

The success of microbial control of insect pests depends not only on the isolation, characterisation and pathogenicity, but also on the successful mass production of the microbial agents in the laboratory. For a successful integrated pest management programme, the agents like the entomopathogenic fungi should be agreeable to easy and cheap mass multiplication. The entomopahogenic fungi, *Metarhizium anisopliae* was used for mass production studies to find out the appropriate media for sporulation on different grains and solid media. The experiment was conducted in Biocontrol laboratory at ICRISAT, Hyderabad

Among the different substrate evaluated, significantly highest conidial count (67.6 × 10^3 spores/ml) was observed on green gram media followed by maize in 10^3 dilution whereas lower spores was observed on green gram (34.5 × 10^4 spores/ml) followed by black gram in 10^4 dilution. In case of liquid media the highest conidial count (63.7 × 10^3 spores/ml) was observed in Sabouraud's Dextrose Broth (SDB) media followed by Potato Dextrose Broth (PDB) media in 10^3 dilution factor, whereas in 10^4 dilution lower spores was observed in PDB as compared to 10^3 dilution (Table 1 and Fig. 1). Significant differences in conidial count were observed on green gram, chickpea, sorghum and wheat. Whereas, no significant difference was observed on the fungal growth among rice, black gram, groundnut, maize, pigeon pea and soybean added media (Fig. 1). Inexpensive culture medium is required in order to increase the cost-benefit ratio. Hence, several sources were tested for mass multiplication. Low cost sources of nutrients like millets such as green gram and sorghum were assessed for their value in terms of conidial yield of the test fungus.

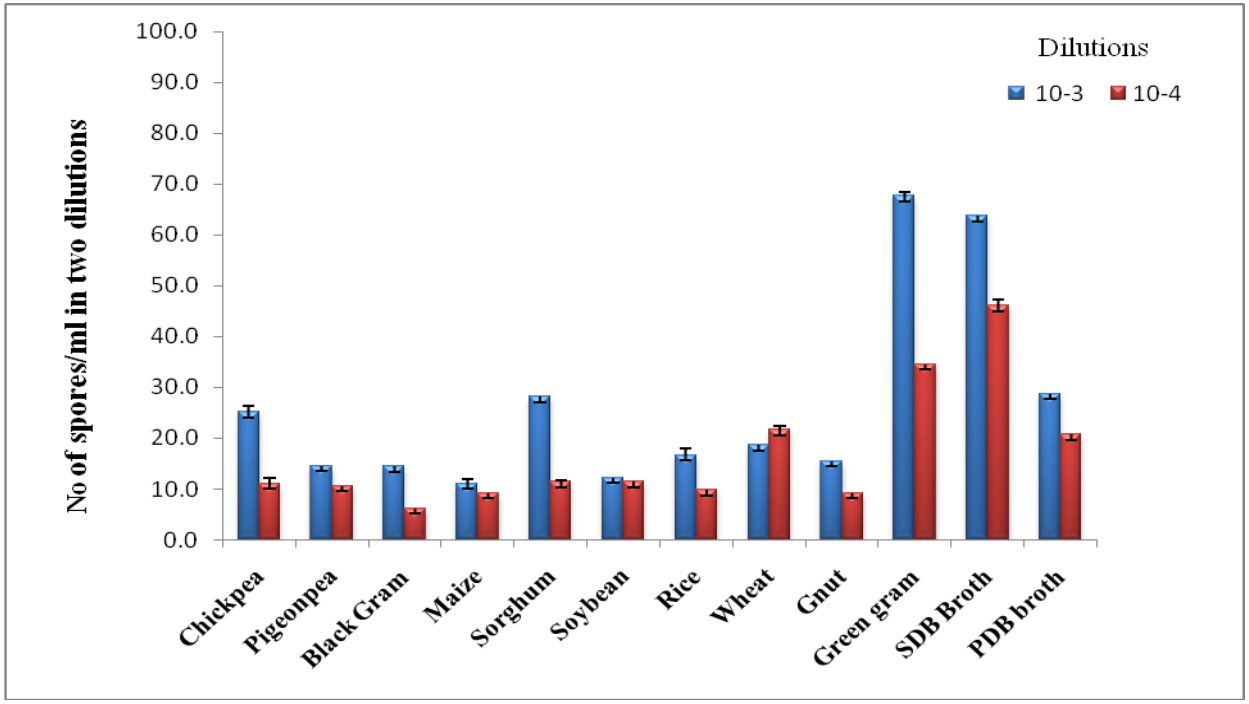
Some of these grains are inexpensive, easily available and act as greatest nutritive media for the mass multiplication of many micro and macro organisms (Ibrahim *et al.*, 1993 and Sharma *et al.*, 2002). Found rice as a suitable media for the mass culture of *B. bassiana.* The present study revealed that the highest spore couth were observed in case of grain media, green gram and in case of liquid media, Sabouraud's Dextrose Broth (SDB) media in 10^3 dilution factor respectively, however the 10^4 dilution shows least spore count in both the media as compare to 10^3 dilution.

Table.1 Mass production of *M. anisopliae* fungi using different grain, with two dilutions

Dilution	Chickpea	Pigeon pea	Black Gram	Maize	Sorghum	Soybean	Rice	Wheat	Groundnut	Green gram	SDB Broth	PDB broth
10^{-3}	25.1	14.5	14.4	11.0	28.1	12.3	16.7	18.6	15.5	67.6	63.7	28.7
10^{-4}	11.0	10.5	6.1	9.2	11.4	11.4	9.8	21.6	9.3	34.5	46.0	20.7
SE±	2.36*	0.49*	1.75^{NS}	0.94^{NS}	10.57^{NS}	0.60^{NS}	0.69*	0.29*	1.23^{NS}	4.43*	6.55^{NS}	1.64^{NS}
LSD (5%)	14.38	2.99	10.62	5.73	64.34	3.66	4.22	1.79	7.46	26.95	39.87	9.99
CV%	23	7	30	16	93	9	9	3	17	15	21	12

*= Statistically significant at 0.05, NS= Not significant

Fig.1 Mass production of *M. anisopliae* fungi using different grain, with two dilutions



Gopalakrishnan, *et al.,* (1999) reported that sorghum was the ideal cereal for the mass production of *Paecilomyces farinosus*, it also supported satisfactory growth and sporulation of *B. bassiana*. Dangar *et al.,* (1999) also observed similar result in *M. anisepliae.* Abundance of glucose and minerals in the culture media may enhance the growth and spore production of fungi. In 10^3 dilutions sorghum was found to be the ideal cereal for mass production, which is in substantiation with the findings of Lakshmi, *et al.,* 2001. The present study also supported this fact in which among a number of naturally available substrates tested for mass multiplication of *Metarhizium anisopliae* SDB and PDB broth in 10^3 dilution are most suitable for its growth and development (Table 1, Fig. 1) Moreover, *Metarhizium anisopliae* has wide host range and it incredibly effective for the pest management in agricultural crops. The present finding with Vimala Devi and Prasad, successfully used agro-wastes such as crushed maize cobs, wheat bran, rice bran, bagasse and press mud singly and along with supplementation for mass multiplication of *Metarhizium* and *Verticillium*. The use of different agricultural wastes is economical and also helps in their efficient utilization. For a successful integrated pest management programme, the agents like the entomopathogenic fungi should be amenable to easy and cheap mass multiplication. Puzari, *et al.,* 1996 Reported that rice husk supplemented with 2% dextrose solution recorded more sporulation of *M. anisopliae*. From the study it was clear that the tested fungi was able to grow on a wide variety of agriculture products and by products of both solid and liquid state and this can be useful to farmers to culture these fungi easily. From this study it was clear that the test fungus is able to mature on a variety of cheap and easily available grains. These grains can be used for the mass multiplication of the fungus and it may increase its efficiency as a biocontrol agent which is also economic and easily available. Refined experiments using other easily available nutrient sources like rice, wheat, chickpea, pigeon pea, black gram, green gram, groundnut, sorghum, soybean, maize and other agricultural wastes will perhaps provide more information on the utility of different agro wastes for production of *Metarhizium anisopliae.*

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How to cite this article:

Agale, S.V., S. Gopalakrishnan, K.G. Ambhure, Hemkant Chandravanshi, Rajeev Gupta and Wani, S.P. 2018. Mass Production of Entomopathogenic Fungi (*Metarhizium anisopliae*) using Different Grains as a Substrate. *Int.J.Curr.Microbiol.App.Sci.* 7(01): 2227-2232.
doi: https://doi.org/10.20546/ijcmas.2018.701.268