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Mass multiplication of *Metarhizium anisopliae* on various substrates

Neelesh Raypuriya, SB Das, AK Bhowmick and Vibha

Abstract
During the 2016-17, for the mass multiplication of *Metarhizium anisopliae* (Metchnikoff) Sorokin, in the laboratory of the Department of Entomology (JNKVV, Jabalpur), many natural and liquid substrates (*i.e.* rice, maize, sorghum, green-gram, chickpea, wheat, potato dextrose broth, sabouraud's dextrose broth and rice starch) were used for mass multiplication of *M. anisopliae*, which found that the highest spore load of *M. anisopliae* (9.78 x 10^{10} cfu ml^{-1}) was harvested from rice followed by SDB (8.11 x 10^{10} cfu ml^{-1}) and chickpea (8.00 x 10^{10} cfu ml^{-1}) respectively. Whereas, the minimum number of spores (2.78 x 10^{10} cfu ml^{-1}) was obtained from wheat. Significantly lowest production cost was obtained in rice (Rs. 0.26 for 1 x 10^{10} spores' ml^{-1}). Therefore, rice was found to be the most appropriate and economical substrate for the mass multiplication of *M. anisopliae* followed SDB and Chickpea.

Keywords: Substrates, *M. anisopliae*, spore load and mass multiplication

1. Introduction
Biocontrol is the most promising way to manage insect pests with little aggravation to the environment. The insect cuticle is made of chitin, peritrophic matrix and other protein components that provide widely differing properties such as rigidity, elasticity and waterproofing protection and structure to the insect [6]. The entomopathogenic fungi (EPF) are living organisms that infect host insects by means of asexually produced conidia, which grow and penetrate the host exoskeleton under favorable ecological conditions. Among them, *Beauveria bassiana*, *M. anisopliae* and *Verticillium lecanii* the most studied insect pathogenic fungi species under Hyphomycetes that are the biocontrol agents [10]. Being facultative and amenable for easy multiplication on a large scale, they offer great scope to develop as dominant biopesticides [4]. The mass production of EPF is an essential component for their utilization in the IPM programme and there is a need to obtain an ideal cheap and high productive culture medium [5]. The major obstacle in the mass multiplication of *M. anisopliae* is its gentle growth rate and non-availability of an appropriate substrate. Eventually, the present study was undertaken to determine the most appropriate and locally available agricultural substrate and liquid media's for mass multiplication of *M. anisopliae*.

2. Materials and Methods
The experiment was conducted under an *in-vitro* condition at biocontrol research and production center, Department of Entomology, College of Agriculture, JNKVV, Jabalpur (MP). The experiment was laid out in completely randomized block (CRD) design with nine treatments which included six solid substrates and three liquid media, for the identification and standardization of an appropriate economical medium for the development and sporulation of *M. anisopliae*. The experimental details are presented in Table 1.

2.1 Solid substrates
Whole grains of rice (*Oryza sativa* L.), maize (*Zea mays* L.), sorghum (*Sorghum vulgaris* L.), green gram (*Vigna radiata* L.), chickpea (*Cicer arietinum* L.) and wheat (*Triticum aestivum* L.) which are generally used as dietary ingredients were assessed for their suitability as substrate/s to support the multiplication and development of *M. anisopliae*. A hundred grams of each substrate was washed and soaked in water for overnight, except rice which was soaked for 2-3 hrs before starting the experiment. The water was drained through decanting and further shade drying was done for half an hour to remove the excess moisture. Each substrate was packed separately in 250 ml conical flask, plugged with non-absorbent cotton and

autoclaved at 15 per square inch for 30 min. After cooling, 5 mm fungal disc was inoculated into each flask aseptically under laminar air flow chamber and incubated at 28^0C. To avoid clumping, after 7 days of inoculation, the flasks were shaken vigorously to separate the grains and to break the mycelial mat.

2.2 Liquid (artificial) substrates
100 ml of each media (*i.e.* potato dextrose broth, sabouraud's dextrose broth and rice starch) were dispensed into 250 ml conical flask, plugged with non-absorbent cotton and autoclaved at 15 psi for 30 min. Each flask was replicated three times. After that, inoculated with 5 mm fungal disc of *M. anisopliae* under laminar air flow chamber and incubated at 28^0C for 10 days. Conical flasks were shaken daily for the uniform growth of the fungus.

2.3 Observations recorded
Fungal growth and sporulation yield were observed on 10^{th}, 20^{th} and 30^{th} days after inoculation (Table 1 and fig. 1). Sporulation was estimated by suspending 1 gm or 1 ml colonized substrate from each replication. It was filtered through the two-fold layered muslin cloth. Spore counts were made after the serial dilution ($1x10^{10}$ spores ml^{-1}) of the suspension. 1 ml spore suspension was poured into petri dishes, which made 20 ml liquefied PDA unstable and was rotated gently for uniform spreading of the spore suspension and incubated at 28 ^{0}C in the BOD. Sporulation was observed as colony forming unit's (cfu $gram^{-1}$ or ml^{-1}) colonized substrate. The method proposed by [7, 11] were followed with some modifications.

3. Results and discussion
3.1 Overall mean
Data presented in Table 1 and depicted in fig.1, revealed that among the treatments, highest overall mean spore count was recorded on rice grain (9.78×10^{10} cfu ml^{-1}), followed by SDB (8.67×10^{10} cfu ml^{-1}) and chickpea (7.45×10^{10} cfu ml^{-1}) and were significantly superior than all the other substrates. They were followed by PDB (6.33×10^{10} cfu ml^{-1}) and green gram (6.00×10^{10} cfu ml^{-1}) but were at par with each other. The next substrate was sorghum which registered 4.55×10^{10} cfu ml^{-1} followed by rice starch (4.33×10^{10} cfu ml^{-1}) and maize (3.67×10^{10} cfu ml^{-1}), but they did not differ significantly from each other. Whereas the least spore count was recorded in wheat ($2.78x10^{10}$ cfu ml^{-1}). The cost of spore production on different substrates/media varied significantly and the minimum was obtained on rice (Rs. 0.26 of $1x10^{10}$ spores ml^{-1}). The present findings are in conformity with the findings of [3, 1, 12] as they also reported that rice grain was the best substrate for spore production of *M. anisopliae* strain. The amount of starch and amylase in rice and sorghum is high. Hydrolysis of starch in rice and sorghum resulted in glucose and maltose, which depends on clarification [9]. The starch hydrolysis present in the fungus stimulates the Maltose sporulation released by the action of the enzyme [2]. In the present findings, among liquid media SDB recorded maximum spore counts which are in accordance with the findings of [8].

4. Conclusion
From the above findings, it can be inferred that the highest spore count was recorded on rice followed by SDB and both emerged as the potential medium with the lowest production cost of Rs 0.26 and Rs 42.72 per $1x10^{10}$ spores ml^{-1}. However, the highest rate of fungal growth was recorded in green gram followed by SDB after 20 to 30 days. Therefore, the *M. anisopliae* was able to grow on a wide range of agriculture products of both solid and liquid stages and it can be effectively used for culture.

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Table 1: Mass multiplication of *M. anisopliae* on different substrates

Tr. code	Substrates	Spore count ($1x10^{10}$ spores ml^{-1}) at different DAI*				Rate of increase in growth of M. anisopliae at different DAI#		Cost of substrate Rs per 100 g	Production cost of M. anisopliae $1x10^{10}$ spores ml^{-1} (Rs)
		10 DAI	20 DAI	30 DAI	Overall mean	10 to 20(%)	20 to 30(%)		
I Solid substrates									
T_1	Maize	2.33(1.68)	3.67(2.03)	5.00(2.35)	3.67(2.02)	55.56(48.25)	44.44(36.49)	2=00	0=60
T_2	Chickpea	5.00(2.34)	7.67(2.85)	9.67(3.19)	7.45(2.80)	32.38(34.62)	16.93(23.75)	6=00	0=87
T_3	Sorghum	3.00(1.86)	4.33(2.20)	6.33(2.61)	4.55(2.23)	23.33(28.86)	18.89(25.74)	3=00	0=72
T_4	Green gram	4.33(2.20)	6.00(2.54)	7.67(2.86)	6.00(2.54)	52.78(51.75)	69.44(61.75)	7=50	1=32
T_5	Rice	7.67(2.85)	9.67(3.19)	12.00(3.54)	9.78(3.19)	28.70(31.49)	24.44(29.46)	2=50	0=26
T_6	Wheat	1.67(1.46)	2.67(1.77)	4.00(2.12)	2.78(1.79)	66.67(60.00)	55.56(53.51)	4=00	1=63
II Liquid (artificial) substrates									
T_7	PDB	3.67(2.04)	6.00(2.54)	9.33(3.13)	6.33(2.58)	53.33(46.92)	26.39(25.15)	350=00	87.26
T_8	Rice starch	2.67(1.77)	4.33(2.20)	6.00(2.55)	4.33(2.18)	61.11(41.75)	40.00(53.86)	2=80	0=72
T_9	SDB	6.33(2.61)	8.33(2.97)	11.33(3.44)	8.67(3.01)	63.89(53.25)	63.49(53.34)	479=00	42.72
	SEm±	0.12	0.11	0.04	0.23	12.35	13.31	-	8.08
	CD (p=0.05)	0.35	0.33	0.12	0.67	NS	NS	-	24.00

NS=Non-significant; DAI-Days after inoculation; PDB = Potato dextrose broth; SDB; Sabouraud's dextrose broth

*Figures in parentheses are square root transformed values

Figures in parentheses are arcsin transformed values

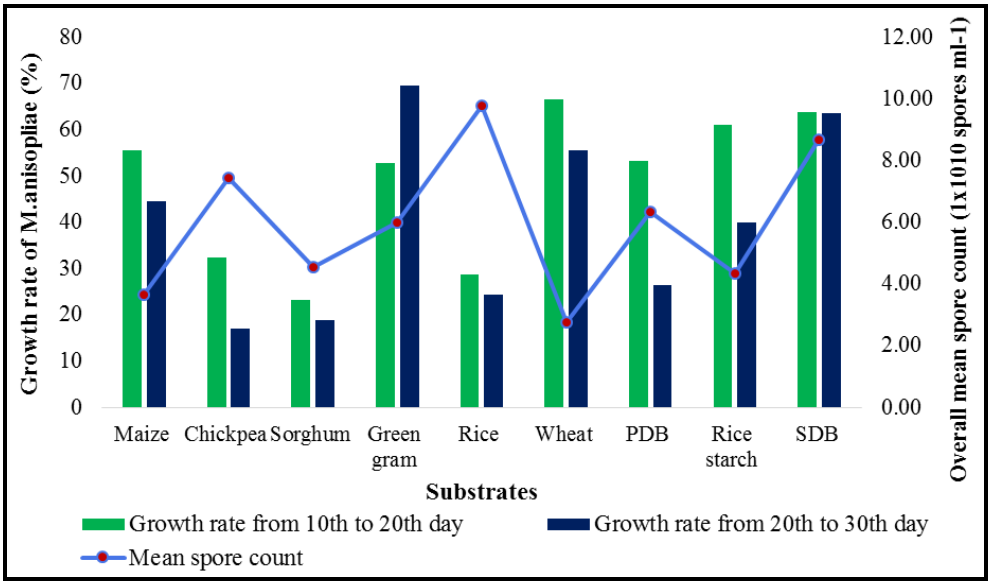

Fig 1: Impact of substrates on growth rate and overall mean spore count of *M. anisopliae*

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