Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2018; 6(3): 622-627

Received: 18-03-2018
Accepted: 19-04-2018

Kelwatkar NM
Senior Research Assistant,
Abhinav Agriculture Polytechnic
Hingani, Wardha, Maharashtra,
India

Kankale MD
Development Executive,
Sudarshan Chemical Industries
Delhi, India

Das SB
Principal Scientist,
Department of Entomology,
Jawaharlal Nehru Krishi Vishwa
Vidyalaya, Jabalpur,
Madhya Pradesh, India

Correspondence
Kelwatkar NM
Senior Research Assistant,
Abhinav Agriculture Polytechnic
Hingani, Wardha, Maharashtra,
India

Identification and evaluation of economic substrate for mass multiplication of *Metarhizium anisopliae*

Kelwatkar NM, Kankale MD and Das SB

Abstract
The present investigation was carried out during *rabi* 2013-2014 at the Entomology laboratory, College of Agriculture JNKVV, Jabalpur at Completely Randomized Design (CRD) to select a suitable and economic substrate for mass multiplication of *Metarhizium anisopliae*. The experiment on mass production studies was undertaken on fourteen substrates for determining a suitable medium for growth and sporulation. The observations were recorded on 10th, 20th and 30th days after inoculation. Among the different substrates evaluated highest conidial count (4.47×10^7 spores/ml) was observed on broken rice media followed by wheat husk (3.83×10^7 spores/ml) and broken wheat (2.27×10^7 spores/ml). It was also clear that *M. anisopliae* is able to grow on a variety of cheap and easily available grains; hence they can be used for the mass multiplication of the fungus and produced in bulk and can be made available at the doorstep of the farmers.

Keywords: Mass multiplication, *Metarhizium anisopliae*, economic substrate, days after inoculation

Introduction
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 heterogeneous group of over 100 genera with approximately 750 species, notified from different insects. *Metarhizium anisopliae* (Metschnikoff) Sorokin, initially known under the name *Entomphthora anisopliae*, was first described near Odessa in Ukraine from infected larvae of the wheat cockchafer *Anisopliae austriaca* in 1879, and later on, *Cleonus punctiventis* by Metschnikoff. It was later renamed as *M. anisopliae* by Sorokin in 1883 [1]. *Metarhizium* causes a disease known as 'green muscardine' in insect hosts because of the green colour of its conidial cells. In 1883, Metschnikoff commenced mass culturing of fungus and carried out the first experiment with two beetle pests. *Metarhizium anisopliae* (Metschnikoff) Sorokin is the second most widely exploited entomopathogenic fungus in biocontrol trials. Species within the genus *Metarhizium* are pathogenic fungi having broad ranges of insect hosts. *M. anisopliae* was found to be a species complex composed of nine species based on multilocus phylogeny [2]. It is known to attack over 200 species of insects belonging to orders Coleoptera, Dermaptera, Homoptera, Lepidoptera and Orthoptera [3].

Mass production of the selected fungi is a necessary prerequisite for any large-scale field application. However, most of these novel systems have not yet been exploited in agricultural practice on a commercial scale. Hence lack of reliable substrates was found to be another major constraint in the mass production. Every living organism requires food for the growth and reproduction, fungi are not exception to it. Fungi secure food from the substrates upon which they live in. All media are not equally good for all fungi nor there can be a universal substrate, upon which all fungi grow. Faster and luxuriant growth of all the fungus can only be obtained when grown on suitable substrate. Locally available and agricultural wastes were found to be excellent substrates for on farm production of antagonists. This helps for large scale delivery to the infection court. Grains or seeds contain varying amount of proteins and carbohydrates, composition of seeds may probably influence the mass multiplication.Hence an attempt was made to determine the most suitable and locally available substrate use for the mass multiplication of the fungus.

Material and Methods

The experiments were carried out during *rabi* 2013-2014 at the Entomology laboratory, College of Agriculture JNKVV, Jabalpur under Completely Randomized Design (CRD). There were fourteen substrates for determining a suitable medium for growth and sporulation of mass multiplication of *Metarhizium anisopliae*. (Table 1)

Media preparation:

Whole grain and Broken grains: Wheat, *Triticum aestivum* (L); rice, *Oryza sativa* (L); maize, *Zea mays* (L); sorghum, *Sorghum vulgare* Pers. grains were used for estimating the sporulation of *Metarhizium anisopliae*, at 25ºC. For the purpose, 100 g of each grain was washed and soaked in water overnight except rice which was soaked for 2 – 3 hours. The excess water was drained by decanting and shade drying them for half an hour to further remove the excess moisture. The grains were packed separately in 250 ml conical flask, with cotton plug and autoclaved at 15 psi for 30 minutes. After cooling, 5 mm fungal disc was inoculated into each flask under laminar air flow chamber. They were incubated in BOD incubator at 25ºC for 15 days. Two replications were maintained for each treatment. To avoid clumping, after 7 days of inoculation, the flasks were shaken vigorously to separate the grains and to break the mycelial mat.

Bran and Husk: To 100 g each of the bran and husk, 50 ml of sterile distilled water was added in a 250 ml conical flask. The substrates were sterilized in an autoclave at 15 psi for 30 minutes. After sterilization the substrates were artificially inoculated with 5 mm fungal disc under laminar air flow chamber. Each treatment was replicated two times. After inoculation, the flasks were incubated at 25 ^{0}C for 15 days. The conical flasks were shaken daily for the uniform growth of the fungus.

Effect of substrate on sporulation of *Metarhizium anisopliae:* The spore of the fungus grown on various substrates was estimated by using haemocytometer. For this purpose 10 g or ml homogenous grains or solutions sample was drawn from each replicate of uniformly sporulating flask and was transferred to 100 ml sterilized distilled water containing Tween 80 (0.05%) solution in 250 ml conical flask. The flasks were shaken in mechanical shaker for 10 minutes. 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 substrate [4]. Observations were taken on 10th, 20th and 30thday after inoculation of the fungus.

Table 1: Substrates used for mass multiplication of *M. anisopliae*

Treatments	Group	Substrates
I	**Solid Media**	
T_1	hole grains	Wheat, *Triticum aestivum* (L)
T_2		Rice, *Oryza sativa* (L)
T_3		Maize, *Zea mays* (L)
T_4		Sorghum, *Sorghum vulgare* Pers.
T_5	Broken grains	Wheat
T_6		Rice
T_7		Maize
T_8		Sorghum
T_9	Bran	Wheat
T_{10}		Rice
T_{11}	Husks	Wheat
T_{12}		Rice
II	**Liquid Media**	
T_{13}	Water soaked	Wheat
T_{14}		Rice

Statistical Analysis: All the data were subjected to statistical analysis after appropriate transformation as suggested by [5].

Results and Discussion

The experiment on mass production studies was undertaken on fourteen substrates for determining a suitable medium for growth and sporulation. During this period the maximum and minimum temperature of the laboratory were 39.55 ± 3.65 ^{0}C and 29.6 ± 6.4 ^{0}C, respectively while morning and evening relative humidity were 51 ± 19% and 24.5 ± 8.5%, respectively. The observations were recorded on 10th, 20thand 30th days after inoculation and the data presented in Table 2.

1 Spore count at different days after inoculation:

1.a Ten days after inoculation: Among the different substrates evaluated significantly highest conidial count (3.30 × 10^7 spores/ml) was recorded on broken rice media. This was followed by wheat husk (1.9 × 10^7 spores/ml), whole sorghum (1.6 × 10^7 spores/ml), broken wheat (1.6 × 10^7 spores/ml), water soaked rice (1.6 × 10^7 spores/ml), whole maize (1.4 × 10^7 spores/ml), wheat bran (1.4 × 10^7 spores/ml), rice bran (1.4 × 10^7 spores/ml), broken maize (1.3 × 10^7 spores/ml), broken sorghum (1.3 × 10^7 spores/ml), whole rice (1.2 × 10^7 spores/ml) and water soaked wheat (1.0 × 10^7 spores/ml), but all were at par with each other. On whole wheat (0.80 × 10^7 spores/ml) and least spore count was recorded in rice husk (0.2 × 10^7spores/ml), but both of them differed significantly from each other.

1.b Twenty days after inoculation: Among the different substrates evaluated significantly highest conidial count (4.8 × 10^7 spores/ml) was recorded on broken rice media followed by wheat husk (4.4 × 10^7 spores/ml), but they differed significantly from each other. The next substrate was broken wheat (2.5 × 10^7 spores/ml) followed by wheat bran (2.1 × 10^7 spores/ml) but they differed significantly from each other. The next substrates were whole sorghum (1.9 × 10^7 spores/ml), rice bran (1.9 × 10^7 spores/ml), water soaked rice (1.85 × 10^7 spores/ml) and whole rice (1.8 × 10^7 spore/ml), but all were at par with each other. The next group of substrates were broken sorghum (1.7 × 10^7 spores/ml), water soaked wheat (1.7 × 10^7 spores/ml), whole wheat (1.6 × 10^7

spores/ml), whole maize (1.6 × 10^7 spores/ml) and broken maize (1.6 × 10^7 spores/ml) however they did not differ significantly from each other. The least spore count was recorded in rice husk (0.5 × 10^7spores/ml).

1.c Thirty days after inoculation: Among the different substrates evaluated significantly highest conidial count (5.3 × 10^7 spores/ml) was recorded on broken rice media followed by wheat husk (5.2 × 10^7 spores/ml), but both were at par with each other. The next group of substrates included broken wheat (2.7 × 10^7 spores/ml), rice bran (2.5 × 10^7 spores/ml), wheat bran (2.45 × 10^7 spores/ml), whole sorghum (2.4 × 10^7 spores/ml), broken sorghum (2.4 × 10^7 spores/ml), whole maize (2.3 × 10^7 spores/ml) and water soaked wheat (2.3 × 10^7 spores/ml), but all were at par with each other. The next group of substrates were whole rice (2.2 × 10^7 spores/ml), water soaked rice (2.2 × 10^7 spores/ml), whole wheat (2.1 × 10^7 spores/ml) and broken maize (2 × 10^7 spores/ml), but they did not differ significantly from each other. The least spore count was recorded in rice husk (0.7 × 10^7 spores/ml).

1.d Mean: Among the different substrates evaluated highest conidial count (4.47 × 10^7 spores/ml) was recorded on broken rice media followed by wheat husk (3.83 × 10^7 spores/ml) and broken wheat (2.27 × 10^7 spores/ml) but they differed significantly from each other. The next group of substrates included wheat bran (1.98 × 10^7 spores/ml), whole sorghum (1.97 × 10^7 spores/ml), rice bran (1.93 × 10^7 spores/ml), water soaked rice (1.88 × 10^7 spores/ml), broken sorghum (1.80 × 10^7 spores/ml) and whole maize (1.77 × 10^7 spores/ml), but all were at par with each other. The next group of substrates were whole rice (1.73 × 10^7 spores/ml), water soaked wheat (1.67 × 10^7 spores/ml), broken maize (1.63 × 10^7 spores/ml), and whole wheat (1.50 × 10^7 spores/ml), but they did not differ significantly from each other. The least spore count was recorded in rice husk medium (0.47 × 10^7 spores/ml).

1.2 Rate of increase in growth: Rate of increase of growth of *M. anisopliae* was calculated and the data presented in Table 2 and depicted in Fig. 1.

Table 2: Mass production of *Metarhizium anisopliae* on different substrates

Treatment no.	Media /Substrates	Spore count (1 ×10^7spores/ml) at different days after inoculation				Rate of increase in growth of *M. anisopliae* (%) (DAI)	
		10th day	20th day	30th day	Mean	10 to 20	20 to 30
I	**Solid media**						
Whole grains							
T_1	Wheat, *Triticumaestivum* (L)	0.80	1.60	2.10	1.50	50.79 (45.27)	23.18 (27.75)
T_2	Rice, *Oryzasativa* (L)	1.20	1.80	2.20	1.73	33.33 (35.26)	17.50 (24.22)
T_3	Maize, *Zeamays* (L)	1.40	1.60	2.30	1.77	12.50 (12.59)	30.30 (33.34)
T_4	Sorghum, *Sorghumbicolour* (L)	1.60	1.90	2.40	1.97	15.56 (23.42)	20.83 (27.02)
Broken grains							
T_5	Wheat	1.60	2.50	2.70	2.27	35.90 (37.07)	7.42 (15.73)
T_6	Rice	3.30	4.80	5.30	4.47	31.25 (34.26)	9.40 (17.73)
T_7	Maize	1.30	1.60	2.00	1.63	18.75 (25.35)	19.19 (25.45)
T_8	Sorghum	1.30	1.70	2.40	1.8	23.61 (29.06)	29.17 (32.62)
Bran							
T_9	Wheat	1.40	2.10	2.45	1.98	31.95 (34.14)	14.65 (22.32)
T_{10}	Rice	1.40	1.90	2.50	1.93	26.67 (30.90)	23.72 (28.85)
Husks							
T_{11}	Wheat	1.90	4.40	5.20	3.83	56.83 (48.91)	15.11 (22.27)
T_{12}	Rice	0.20	0.50	0.70	0.47	58.33 (49.67)	29.17 (32.62)
II	**Water soaked**						
T_{13}	Wheat	1.00	1.70	2.30	1.67	40.97 (39.77)	25.76 (30.21)
T_{14}	Rice	1.60	1.85	2.20	1.88	13.45 (21.40)	15.42 (22.79)
	SEm±	0.11	0.13	0.14	0.08	3.62	4.60
	CD at 5%	0.32	0.39	0.44	0.24	11.10	NS

Max. temp.39.55 ± 3.65 °C; Min. temp. 29.6 ± 6.4 °C; Max. RH(%) 51 ± 19 ; Evening RH(%) 24.5 ± 8.5
DAI-Days after inoculation
() = Figure in the parentheses are arcsin transformed values NS = Non significan

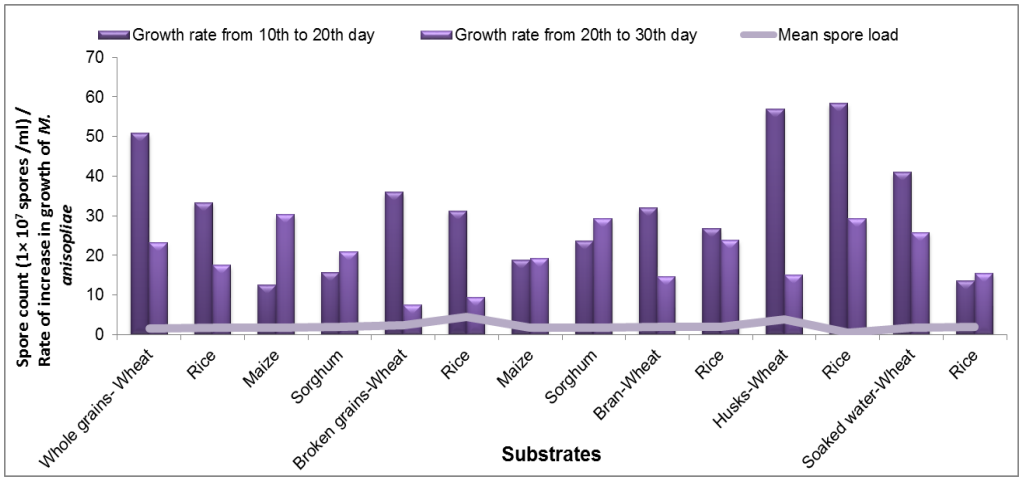

Fig 1: Summary of growth rate and spore production of *Metarhizium anisopliae* on / in different substrates

1.2.a 10 to 20 days after inoculation: The rate of increase in growth of *M. anisopliae* from 10th to 20th days after inoculation among different substrates was found significant. The highest rate of increase in growth of the fungus was recorded on rice husk (58.33%) followed by wheat husk (56.83%), whole wheat (50.79%) and water soaked wheat (40.97%), but all were at par with each other. The next group of substrates included broken wheat (35.90%), whole rice (33.33%), wheat bran (31.95%), broken rice (31.25%) and rice bran (26.67%), but they were at par with each other. The next group of substrates included broken sorghum (23.61%), broken maize (18.75%), whole sorghum (15.56%) and water soaked rice (13.45%), but they did not differ significantly from each other. However, least growth rate of the fungus was recorded on whole maize (12.50%).

1.2.b 20 to 30 days after inoculation: The rate of increase in growth of *M. anisopliae* from 20th to 30th days after inoculation among different substrates were found to be non significant. The highest rate of increase in growth was recorded on whole maize (30.30%), followed by broken sorghum (29.17%),rice husk (29.17%), water soaked wheat (25.76%), rice bran (23.72%), whole wheat (23.18%), whole sorghum (20.83%), broken maize (19.19%), whole rice (17.50%), water soaked rice (15.42%), wheat husk (15.11%), wheat bran (14.65%), broken rice (9.40%) and least growth rate was recorded on broken wheat (7.42%).

Growth of *M. anisopliae* on different group of substrates:
Data on growth of *M. anisopliae* on different group of substrates are presented in Table 3.

Table 3: Mass production of *Metarhizium anisopliae* on different groups of substrates- at a glance

Media	Substrates	Mean spore count (1×10⁷spore/ml) at different days after inoculation on different groups of substrates			
		10 DAI	20 DAI	30 DAI	Mean
Solid	Whole grains	1.25	1.73	2.25	1.74
	Broken grains	1.88	2.65	3.10	2.54
	Bran	1.40	2.00	2.48	1.96
	Husks	1.05	2.45	2.95	2.15
	Mean	1.45	2.20	2.69	2.11
Liquid	Cereals	1.3	1.78	2.25	1.78

DAI = Days after inoculation

10 DAI: Among the solid and liquid substrates, the former recorded maximum spore load (1.45 ×10⁷ spores) while in the latter it was 1.30 × 10⁷ spores. However, among the different solid substrates, broken grains recorded maximum spore load (1.88 ×10⁷ spores) followed by bran (1.40 ×10⁷ spores), whole grains (1.25 ×10⁷ spores) and lowest on husks (1.05 ×10⁷ spores), respectively.

20 DAI: Among the solid and liquid substrates, the mean spore load was 2.20 ×10⁷ spores and 1.78 ×10⁷ spores, respectively. However among the different solid substrates, broken grains recorded maximum spore load (2.65 ×10⁷ spores) followed by husk (2.45 ×10⁷ spores), bran (2.00 ×10⁷ spores) and lowest on whole grains (1.73 ×10⁷ spores), respectively.

30 DAI: The mean spore load recorded on solid and liquid substrates were 2.69 ×10⁷ spores and 2.25 ×10⁷ spores, respectively. However, among the different solid substrates, broken grains recorded maximum spore load (3.10 ×10⁷ spores) followed by husk (2.95 ×10⁷ spores), bran (2.48 ×10⁷ spores) and lowest on whole grains (2.25 ×10⁷ spores), respectively.

Mean: The mean spore load recorded on solid and liquid substrates were 2.11 ×10⁷ spores and 1.78 ×10⁷ spores, respectively. However, among the different solid substrates, broken grains recorded maximum spore load (2.54 ×10⁷ spores) followed by husk (2.15 ×10⁷ spores), bran (1.96 ×10⁷ spores) and lowest on whole grains(1.74 ×107 spores), respectively.

Economics of mass production of *Metarhizium anisopliae* on different substrates:
Cost of production of 1×10⁷spores was calculated for all substrates and the data are presented in Table 4 and depicted on Fig 2.

Table 4: Economics of mass production of *Metarhizium anisopliae* on different substrate

Tret. Codes	Groups	Substrates	Mean spore count (1×10^7spore/ml)	Cost of substrate / 100g (Rs)	Cost of production of *M. anisopliae* 1×10^7spores/ml (Rs)
I	Solid Media				
T_1	Whole grains	Wheat, *Triticumaestivum* (L)	1.50	2=00	9=60
T_2		Rice, *Oryzasativa* (L)	1.73	4=00	9=48
T_3		Maize, *Zeamays* (L)	1.77	3=00	8=70
T_4		Sorghum, *Sorghumbicolour* (L)	1.97	2=50	7=56
T_5	Broken grains	Wheat, *Triticumaestivum* (L)	2.27	2=00	6=34
T_6		Rice, *Oryzasativa* (L)	4.47	4=00	3=67
T_7		Maize, *Zeamays* (L)	1.63	3=00	9=45
T_8		Sorghum, *Sorghumbicolour* (L)	1.80	2=50	8=28
T_9	Bran	Wheat	1.98	3=60	8=08
T_{10}		Rice	1.93	0=50	6=68
T_{11}	Husk	Wheat	3.83	1=50	3=63
T_{12}		Rice	0.47	0=60	27=65
II	Liqiud Media				
T_{13}	Water soaked	Wheat, *Triticumaestivum* (L)	1.67	2=00	8=62
T_{14}		Rice, *Oryzasativa* (L)	1.88	5=00	9=25
	SEm ±		0.08	--	1.12
	CD at 5%		0.24	--	3.44

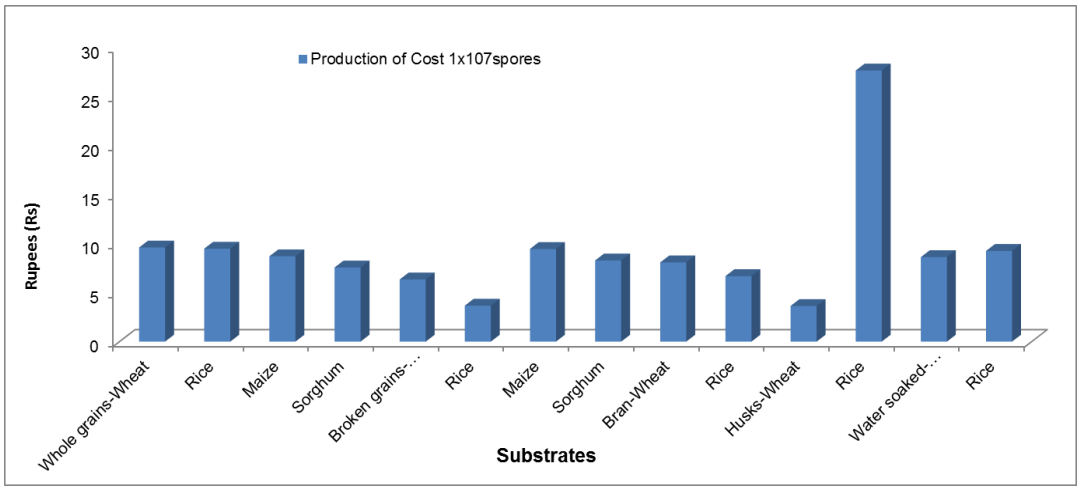

Fig 2: Economics of mass production of *Metarhizium anisopliae* on / in different substrates

The cost of production on different substrates significantly varied from each other. Significantly lowest production cost was recorded on wheat husk media (Rs 3=63), this was followed by broken rice (Rs 3=67), whole wheat grain (Rs 6=34) and rice bran (Rs 6=68), but all were at par with each other. The next substrate was whole sorghum grains (Rs 7=56), followed by wheat bran (Rs 8=08), broken sorghum grains (Rs 8=28), water soaked wheat (Rs 8=62), whole maize grains (Rs 8=70), water soaked rice (Rs 9=25), broken maize grains (Rs 9=45), whole rice grains (Rs 9=48) and whole wheat grains (Rs 9=60),but they did not differ significantly from each. Highest production cost was recorded on rice husk (Rs 27=65). In the present study, several naturally available substrates were tested for mass multiplication of *M. anisopliae*. The success of microbial control of insect pests depends not only on the isolation, characterization and pathogenicity, but also on the successful mass production of the microbial agents in the laboratory. Large-scale availability of the pathogen is a primary requirement in the bio-control programme. For a successful integrated pest management programme, the agents like the entomopathogenic fungi should be amenable to easy and cheap mass multiplication.

The fungus *M. anisopliae* was mass multiplied on locally available substrates like whole and broken grains of rice, sorghum, maize, wheat, bran and husks of wheat and rice and water soaked wheat and rice. The results indicated that the sporulation of the fungus differed significantly among different substrates. Highest sporulation was recorded on broken rice (4.47 x 10^7spores/ml) followed by wheat husk (3.83 x10^7 spores per ml). These two substrates differed significantly from each other with respect to sporulation. This was followed by broken wheat (2.27 x 10^7 spores /ml) and the lowest sporulation was recorded on rice husk (0.47 x 10^7 spores per ml). The present findings are in agreement with the findings of [6-13]. They also reported that rice was found to be the best solid substrate for spore production and their viability was also high but they further emphasized that the fungus also grows equally well on maize or other grains, SDB and yeast extract media. Rice and sorghum contain higher proportion of starch and amylase. Hydrolysis of starch in rice and sorghum resulted in release of glucose and maltose depending on clarification [14]. Maltose released by the action of starch hydrolysis enzymes present in the fungus induces sporulation [15].

The present study also support the fact that among several naturally available substrates tested for mass multiplication of *M. anisopliae* on rice and wheat husks were most suitable for its growth and development and economically cheap. It was also clear that *M. anisopliae* is able to grow on variety of cheap and easily available grains so that these grains can be used for the mass multiplication of the fungus.

Conclusion

Broken rice was found to be the best substrate for mass production of *M. anisopliae* as it produced maximum spore production in minimum time followed by wheat husk and were found to be cheap substrates for mass production of *M. anisopliae*.

Acknowledgement

The authors express thanks to Head, Department of Entomology, College of Agriculture, JNKVV, Jabalpur (M. P.) for providing all the facilities to conduct work.

References

1. Tulloch M. The genus *Metarhizium anisopliae*. Transaction of the Britannic Mycology Society. 1976; 66:407-411.
2. Bischoff JF, Rehner SA, Humber RA. A multilocus phylogeny of *Metarhizium anisopliae* lineage. Mycologia. 2009; 101:512-530.
3. Sahayaraj K, Borgio JF. Tri-tropic interaction of cotton, red cotton bug and green muscardine fungi under in-vitro condition. Journal of Biopesticides. 2008; 1(1):41- 46.
4. Hokkanen H, Lynch JM. Biological control: Benefits and Risks. Cambridge University Press, UK, 1998, 304.
5. Snedecor GW, Cochran WG. Statistical Methods, Oxford and IBH Publishing Company, New Delhi, 1967, 1-292.
6. Sharma Shashi, Yadav RL. Selection of a suitable medium for mass multiplication of entomofungal pathogens. Indian Journal of Entomology. 2002; 64(3):254-261.
7. Kumar Mahesh, Choudhary Savita, Shahi SK, Shahi MP. Standardization of different substrates for the mass production of conidial yield of entomopathogenic fungus *Metarhizium anisopliae*. Plant Archives. 2007; 7(2):719-720.
8. Soundarapandian P, Chandra R. Mass production of Entomopathogenic fungus *Metarhizium anisopliae* (Deuteromycota; Hyphomycetes) in the laboratory. Research Journal of Microbiology. 2007; 2:690-695.
9. Babu CS, Venkatachalapathy CM, Anitha CN. Evaluation of locally available substrates for mass multiplication of entomopathogenic fungi, *Metarhizium anisopliae* (M etch.) Sorokin. Journal of Biopesticides. 2008; 1(2):146-147.
10. Vijayavani S, Reddy KRK, Jyothi G. Identification of virulent isolate of *Metarhizium anisopliae* (Metschin) Sorokin (Deuteromycotina: Hyphomycetes) for The management of *Helicoverpa armigera* (Hubner). Journal of Biopesticides. 2010; 3(3):556-558.
11. Mehta Jitendra, Dhaker Jayraj Kiran, Kavia Ambika, Sen Priya, Kaushal Neha, Datta Sakshi. Biomass production of Entomopathogenic fungi using various agro products in Kota Region, India. International Research Journal of Biological Sciences. 2012; 1(4):12-16.
12. Yadav Seema, Tandan Neeraj and Kumar Krishan. Mass production of entomopathogens *Beauveria bassiana* and *Metarhizium anisopliae* using rice as a substrate by diphasic liquid- solid fermentation technique. I.J.A.B.R. 2013; 3(3):331-335.
13. Prasad CS, Pal Rishi. Mass production and economics of Entomopathogenic fungus, *Beauveria bassiana*, *Metarhizium anisopliae* and *Verticillium lecanii* on agricultural and industrial waste. Sch J Agric Vet Science. 2014; 1(1):28-32.
14. Preen JC, Jong FD, Botes PJ, Lategon TM. Fermentation alcohol from grain sorghum starch. Biomass. 1985; 8:101-117.
15. Coudron TA, Kroha MJ, Sayed GN. A novel semi liquid for propagating entomopathogenic fungi. Journal of Invertebrate Pathology. 1985; 46:335-336.