



## Storage and Viability of Entomopathogenic fungus on agricultural and industrial waste

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### Abstract

A study conducted on storage and viability of Entomopathogenic fungus, *Beauveria bassiana*, *Metarhizium anisopliae* and *Verticillium lecanii* in the laboratory by using liquid and solid agricultural and industrial waste media. FYM liquid + Press mud liquid (1:1) + 1g Dextrose ( $43.62 \times 10^7$  spores/ml) followed by FYM ( $37.25 \times 10^7$  spores/ml) and Press mud ( $29.75 \times 10^7$  spores/ml) were the most suitable substrate with higher production of spores and viability of *B. bassiana* at 6 month of storage period. However, The FYM ( $25.37 \times 10^7$  spores/ml) produced maximum spores of *M. anisopliae* followed by FYM liquid + Press mud liquid (1:1) + 1g Dextrose ( $23.57 \times 10^7$  spores/ml) and Jowar grain + 1.0 g Dextrose ( $8.5 \times 10^7$  spores/ml) were the most suitable medium for spore production, viability and storage of *M. anisopliae*. While in case of *V. lecanii*, FYM ( $23.8 \times 10^7$  spores/ml), followed by FYM liquid + 1g Dextrose ( $11.5 \times 10^7$  spores/ml) and Sabouraud dextrose broth ( $4.75 \times 10^7$  spores/ml) were the most suitable media with higher growth of mycelium and production of large number of spores with viability of *V. lecanii*. However, sugarcane bagasse and crushed maize (*Corcyra* rearing waste) substrate were recorded no fungal spores. FYM was the best and suitable substrate for long time storage with viability of fungal spores.

**Keyword:** Storage, Viability of Entomopathogenic, Fungus, Agrowaste

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### Introduction

The indiscriminate use of insecticides over the years has not only to control crop pest besides, it is one of the main causes for secondary pest out breaks in many crop ecosystem, pesticide resistance, more stringent pesticide regulation and concern about human health, environmental quality and adverse effects on non-target organisms also. In view of these side effects, the necessity for sustainable crop production through eco-friendly pest management technique is being largely felt in the recent times. Biological control agents such as entomopathogenic fungi (EPF) can be used as a component of integrated pest management (IPM) of many insect pests. Under natural conditions, these pathogens are a frequent and often cause natural mortalities of insect populations. The main drivers behind the push for myco-insecticides are the need for more specific agents as components of IPM

programmes due to concerns over chemical residues on human health and the environment.

An attractive feature of these fungi is that infectivity is by contact and the action is through penetration (Nadeau *et al.*, 1996). These fungi comprise a heterogenous group of over 100 genera with approximately 750 species, reported from different insects. Many of these offer a great potential in pest management. The most important fungal pathogens are *Metarhizium* spp., *Beauveria* spp., *Nomuraea rileyi*, *Verticillium lecanii* and *Hirsutella* spp. Of the over 750 species of fungi known to be pathogenic to insects, six have been commercialized and the cosmopolitan pathogens, such as *B. bassiana*, *M. anisopliae* are the best known so far. Fungi often cause spectacular epizootics with large number of pathogenic insects showing visible fungal outgrowth (Hall, 1982). Conservation and periodic enhancement of efficacy of biological

control agents will help in crop protection and in producing agricultural commodities free from pesticide residues.

The major issues involved in mass production and utilization of mycopathogens are selection of effective strains, development of cost effective methods for mass rearing, development of effective methods for storage and shipment and creation of effective formulation. In this study we have evaluated different types of cultivation methods with different liquid and solid substrate for storage of different entomopathogenic fungi. It is therefore essential to determine the best substrate for spore production and their viability.

### Materials and Methods

The present study was carried out in Bio-control Laboratory, S.V.P. University of Agriculture & Technology, Meerut (India) in 2010. The experiment was conducted in laboratory to test the storage period for viability of entomopathogenic fungi on different agricultural and industrial waste (solid and liquid substrates).

**Substrates and *in vitro* production:** Nine mass production substrates were evaluated for the conidial production of *B. bassiana*, *M. anisopliae* and *V. lecanii* under temperature for period of six month. The substrates were (i) FYM, (ii) Sabouraud dextrose broth (SDB), (iii) Sugar industry Press mud, (iv) Sugarcane bagasse, (v) FYM liquid + 1.0g Dextrose, (vi) Pressmud liquid + 1.0g Dextrose, (vii) FYM liquid + Pressmud liquid (1:1) + 1.0g Dextrose, (viii) *Corcyra* rearing waste (Maize) and (ix) Jowar grain + 1.0 g Dextrose. The last six medium were supplemented with dextrose. There were nine treatments in three replications.

A quantity of 130 g dehydrated SDB was suspended in 2000 ml distilled water, heated to dissolve the medium (pH 5.6) and sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes. The lukewarm liquid media was poured in conical flask. Then *B. bassiana*, *M. anisopliae* and *V. lecanii* were inoculated aseptically and then conical flasks were incubated in BOD.

The *Corcyra* rearing waste (crushed maize grain) and sorghum grain were soaked overnight, cleaned with fresh water and 100 g of each was put in separate conical flasks (250-ml capacity) supplemented with dextrose (1.0 g), plugged with non-absorbent cotton and autoclaved. Upon cooling of media, *B. bassiana*, *M. anisopliae* and *V. lecanii* were inoculated aseptically and incubated in BOD incubator. The crushed jowar grain was collected from the Bio-control Laboratory as a waste material from *Corcyra* Rearing Unit. The crushed jowar grain (weight 50 g) was put in flask and 15 ml distilled water with 1 g dextrose was added. The sugarcane bagasse and Pressmud was procured from Modipuram, Meerut. The sugarcane bagasse was cut into small pieces and half-filled in flask with 15 ml distilled water.

**Spore counting:** A drop of conidial suspension of *B. bassiana*, *M. anisopliae* and *V. lecanii* (obtained from the growing media by filtering through muslin cloth) was placed on the hemocytometer. The cover glass was put over the grid carefully so that no air bubble entered between cover glass and slide. The conidia of entomopathogenic fungi were counted under Olympus BX41 phase contrast microscope at higher resolution in the middle square (V) of hemocytometer which contained 25 groups of 16 small squares, each group 0.2 mm square. This process was repeated every month for six times.

**Statistical analysis:** The conidial production of entomopathogenic fungi from different substrates were subjected to analysis of variance (ANOVA) using SPSS 10.0 for Windows software (SPSS, 1999). The means were separated using LSD and differences between treatments were considered significant at  $p < 0.05$ .

### Results and discussion

***Beauveria bassiana*:** Study on the viability of spores of *B. bassiana* at 6 month storage period on different agricultural and industrial waste (Table: 1). The results showed that the, (T<sub>7</sub>) FYM liquid + Press mud liquid (1:1) + 1g Dextrose ( $43.62 \times 10^7$  spores/ml) followed by (T<sub>1</sub>) FYM ( $37.25 \times 10^7$  spores/ml) and (T<sub>3</sub>) Press

mud ( $29.75 \times 10^7$  spores/ml) and ( $T_6$ ) pressmud liquid + 1 gram Dextrose ( $23.62 \times 10^7$  spores/ml) were the most suitable substrate with higher production of spores and viability at 6 month of storage period of *B. bassiana*. Treatment ( $T_2$ ) SDB, ( $T_4$ ) sugarcane bugasse and ( $T_9$ ) Jowar grain + 1.0 g Dextrose substrate have no fungal spores were recorded at the storage period of six month. These findings are in agreement with the findings of Nelson *et al.*, (1996) who found that for spore production of *B. bassiana* and *Metarhizium*, these fungi produced more spores on rice over other growing substrates. Pandey and Kanaujia (2008) found that the conidial production of EPFs is affected by the type of growing medium. This was also in agreement with Ibrahim and Low (1993) and Sharma *et al.*, (2002) who found rice to be the suitable media for the mass culture of *B. bassiana* and the cereal was also suitable for the mass production of other deuteromycete fungi. The substrates used in this experiment were relatively cheap, easily available and acted as nutritive media for mass production of the EPFs.

**Metarhizium:** The results showed that (Table 2) significant different among the media tested,

( $T_5$ ) FYM ( $25.37 \times 10^7$  spores/ml) followed by ( $T_7$ ) FYM liquid + Press mud liquid (1:1) + 1g Dextrose ( $23.57 \times 10^7$  spores/ml) and ( $T_9$ ) and Jowar grain + 1.0 g Dextrose ( $8.5 \times 10^7$  spores/ml) were the most suitable medium for spore production, viability and storage of *M. anisopliae*. However, treatment ( $T_2$ ) SDB, ( $T_4$ ) sugarcane bugasse and ( $T_8$ ) *Corcyra* rearing waste (crushed maize) substrate have no fungal spores were recorded at the period of six month of storage. The high amount of conidia in bajra and rice could be due to maltose released by the action of starch hydrolyzing enzymes present in the fungus inducing sporulation. Pressmud + 10 per cent molasses supported least yield  $0.42 \times 10^4$  spore/ g, where initiation of growth and sporulation resulted only after 20 days after inoculation (Bharati *et al.*, 2007). Nelson *et al.*, (1996) found that spore production of *B. bassiana* and *Metarhizium*, these fungi produced more spores on rice over other growing substrates. Pandey and Kanaujia (2008) found that the conidial production of EPFs is affected by the type of growing medium. The substrates used in this experiment were relatively cheap, easily available and acted as nutritive media for mass production of the EPFs.

**Table 1: Viability of Entomopathogenic fungi, *B. bassiana* on the different agricultural and industrial waste**

Viability periods (Months)	Treatments								
	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	T <sub>5</sub>	T <sub>6</sub>	T <sub>7</sub>	T <sub>8</sub>	T <sub>9</sub>
1 <sup>st</sup>	27.87	24.62	11.40	6.52	9.75	13.25	20.00	11.97	13.52
2 <sup>nd</sup>	29.21	26.46	13.84	4.62	10.06	15.75	23.81	11.08	8.31
3 <sup>rd</sup>	30.48	18.71	19.95	1.83	11.73	18.81	28.75	12.00	6.88
4 <sup>th</sup>	33.58	14.62	23.41	0.00	12.88	19.03	35.43	10.88	5.97
5 <sup>th</sup>	36.27	6.81	26.28	0.00	13.24	22.00	38.68	10.76	5.01
6 <sup>th</sup>	37.25	0.00	29.75	0.00	12.62	23.62	43.62	10.50	0.00
CD > 5%	6.041	4.395	6.021	1.749	N.S.	2.801	2.783	NS	1.820
SE (m)	1.939	1.411	1.933	0.561	0.849	0.899	0.893	0.944	0.584

\* mean of three replications

***V. lecanii*:** The results showed that significant difference among the media tested for viability (Table 3) of entomopathogenic fungi *V. lecanii*, T<sub>1</sub> or FYM ( $23.8 \times 10^7$  spores/ml), followed by (T<sub>5</sub>) FYM liquid + 1g Dextrose ( $11.5 \times 10^7$  spores/ml) and (T<sub>2</sub>) Sabouraud dextrose broth ( $4.75 \times 10^7$  spores/ml) were the most suitable media with higher growth of mycelium and production of large number of spores with

viability of *V. lecanii*. While the treatment (T<sub>4</sub>) sugarcane bagasse had no spores followed by (T<sub>6</sub>) Press mud liquid +1g Dextrose and (T<sub>7</sub>) FYM liquid + Press mud liquid (1:1) + 1g Dextrose at the six month of storage. The above finding indicated that the FYM was the best and suitable substrate for long time storage with viability of fungal spores.

**Table 2: Viability of Entomopathogenic fungi, *M. anisopliae* on the different agricultural and industrial waste**

Viability periods (Months)	Treatments								
	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	T <sub>5</sub>	T <sub>6</sub>	T <sub>7</sub>	T <sub>8</sub>	T <sub>9</sub>
1 <sup>st</sup>	17.17	15.72	4.57	3.42	5.55	6.35	8.32	7.87	5.70
2 <sup>nd</sup>	16.58	12.26	4.62	2.63	10.81	7.12	10.33	8.61	6.08
3 <sup>rd</sup>	11.37	7.19	4.67	1.88	13.06	8.34	14.67	6.77	6.91
4 <sup>th</sup>	8.05	5.08	4.84	0.86	18.51	6.67	19.33	3.63	7.43
5 <sup>th</sup>	6.87	2.16	4.99	0.00	21.73	5.33	21.81	1.02	8.00
6 <sup>th</sup>	4.52	0.00	5.33	0.00	25.37	4.70	23.57	0.00	8.50
CD > 0.05%	2.097	1.821	N.S.	0.510	1.557	1.530	2.325	1.258	1.304
SE (m)	0.673	0.584	0.626	0.164	0.500	0.491	0.746	0.404	0.419

\* mean of three replications

Lakshmi *et al.*, (2001) studied the mass culturing of *V. lecanii* on three grain media found that broken sorghum grain with a spore load  $1.5 \times 10^9$  spores<sup>-1</sup> was the best followed by pearl millet broken grain medium with  $1.3 \times 10^9$  spores<sup>-1</sup>. Pandey and Kanaujia (2008) found that the conidial production of EPFs is affected by the type of growing medium. Derakshan *et al.*, (2008) recorded spore count of *V. lecanii* on

rice, sorghum and corn were on par and significantly higher than rangi and wheat. It was found that viable spore's counts in different grains are not significantly different. According to Moore *et al.* (2000), fungal spores are living organisms and their viability diminishes with time depending on environmental conditions, in this case the storage temperature.

**Table 3: Viability of Entomopathogenic fungi, *V. lecanii* on the different agricultural and industrial waste**

Viability periods (Months)	Treatments								
	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	T <sub>5</sub>	T <sub>6</sub>	T <sub>7</sub>	T <sub>8</sub>	T <sub>9</sub>
1 <sup>st</sup>	18.50	18.00	4.75	3.90	5.65	8.05	11.75	11.07	4.95
2 <sup>nd</sup>	17.79	13.41	5.08	2.58	6.03	6.05	8.26	8.33	5.08
3 <sup>rd</sup>	20.58	11.36	6.00	1.06	7.84	4.83	6.81	3.67	5.61
4 <sup>th</sup>	21.06	6.59	6.16	0.61	8.93	3.61	5.76	2.33	4.08
5 <sup>th</sup>	22.48	5.44	4.02	0.00	9.07	1.03	4.22	1.67	3.66
6 <sup>th</sup>	23.80	4.75	3.70	0.00	11.50	0.00	3.62	0.00	3.25
CD > 0.05%	1.885	1.416	1.232	0.866	1.764	0.827	1.093	1.097	1.391
SE (m)	0.605	0.454	0.396	0.278	0.566	0.265	0.351	0.352	0.447

**Conclusion:** However, sugarcane bugasse and crushed maize (*Corcyra* rearing waste) substrate were recorded no fungal spores. FYM was the best and suitable substrate for long time storage with viability of fungal spores.

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