***Original Research Article***

# Evaluating the stability of Post Mushroom Substrate (PMS) and other Agro-wastes for Mass Production of Entomopathogenic Fungi


## ABSTRACT

**Aim:** The study was undertaken to evaluate agro-wastes (Post mushroom substrate, Sugarcane bagasse, rice husk and sorghum grains) for mass production of entomopathogenic fungi like, *I. fumosoroseus*:MT997932, *B. bassiana*: MT997933, *L. lecanii*: MT997935 and *H. thompsonii*: MT997936 by solid stat fermentation.

**Place and duration of work:** The study was carried out in the Department of Agricultural Microbiology, GKVK, University of Agricultural Sciences, Bangalore during 2019-20.

**Methodology:** The substrates were dried, chopped and sieved through 2mm sieve. 100g of all substrates were sterilized and moistened to 60 % by adding sterile distilled water fallowed by addition of 5ml of spore suspension of fungal isolates. Treatments $T_5$. $T_6$, $T_7$, $T_8$ and $T_{10}$ were fortified with adding 10ml of molasses. Observation like, growth and spore production were taken at 7, 14 and 21 days after inoculation.

**Results:** The growth and spore production of entomopathogenic fungal isolates were observed to be increased accordance with the incubation period. Among four substrates maximum mycelial growth and spore production of all the isolates ($\times 10^9$ spores $g^{-1}$) was observed in sorghum grains ($T_4$:$T_8$) fallowed by treatment $T_7$ (SMS+10% molasses) on 21 DAI. Whereas, sugarcane bagasse and paddy husk substrates were not supported the satisfactory growth and spore production besides the addition of molasses ($\times 10^7$ spores $g^{-1}$). The addition of molasses has positively influenced the growth and spore production of entomogenus isolates in all treatments.

**Conclusion:** Based on results, it is evidenced that even though cereal grains are best option for mass production, SMS fortified with molasses will become a better substrate for mass production and reduce the load of using food grains as substrate.



*Keywords: Entomopathogenic fungi, Agro-wastes, Mass production, Spent mushroom substrate, B. bassiana, L. lecanii,*


## 1. INTRODUCTION

In modern agriculture, there is a decline in global crop losses due to various pests from 41.1 % during 1988-90 to 32.1 % during 2001-03 [31] because of extensive use approximately, 2.5 million tonnes of pesticides are annually [32] despite of the alarming problems like, development of resistance and resurgence of sucking pests [33] residual toxic effects to man, insect parasites, predators, animals and also the use will increase the cost of

production. In view of these side effects, it is necessary to find an alternative, sustainable and eco-friendly pest management technique is being largely felt in the recent times.

Entomopathogenic fungi are potentially the most diverse and versatile biological control agents, due to their wide host range that often results in natural Epizootics. These fungi have certain advantages in pest control programs over other insect pathogens because they infect all stages of insect, they directly infect pest through cuticle as other agents need ingestion hence these can even infect sucking and piercing pest also (Hajeck and Leger, 1994), high host specificity, negligible effect on non-target organisms and mass production techniques are simpler, easier, cheaper compare to the other microbial agents and their persistence nature.

The growth requirements of most entomopathogenic fungi have been poorly defined despite the fact that this information is essential for mass production. The choice of the nutrients will obviously be directly related to the nutritional requirements of the selected fungus. Entomopathogenic fungi require oxygen, water, an organic source of carbon and energy, a source of inorganic or organic nitrogen and additional elements including minerals and growth factors [8]. Production of adequate quantities of a good quality inoculum is an essential component of the biocontrol programme. A wide variety of organic materials have been evaluated as substrates for the mass production entomopathogenic fungi. Although rice and barley seem to be the major substrates used in the tropics and the Northern Hemisphere respectively [10], there has been considerable effort to identify low-cost agricultural materials, especially byproducts and waste products, as suitable substrates for mass production have taken by numerous researches from different countries (Table-1). There are different methods of mass production like, solid state fermentation [11,23], liquid fermentation, submerged stat fermentation and biphasic culture system [9,13] based on the type substrates, out of all, solid state fermentation has emerged as an appropriate technology.

**Table: 1 Solid Substrates Evaluated for Production of the Principal Entomopathogenic fungi.**

| Sl. No. | Substrate/s | Organism/s | Reference |
|---|---|---|---|
| **1** | Green gram, Sorghum | *Metarhizium anisopliae* | 1 |
| **2** | Agricultural products | *Beauveria bassiana* | 3 |
| **3** | Apple pomace (AP) | *Lecanicillium lecanii*, *Beauveria bassiana*, *Paecilomyces fumosoroseus* | 19 |
| **4** | Broken rice grains, Rice hulls | *B. bassiana namaste Metarhizium anisopliae* | 5 |
| **5** | Sorghum, Rice, Wheat, Refuse Potato Chips and Refuse Banana Chips | *Nomuraea rileyi* | 25,4 |
| **6** | FYM, Sugar industry Press mud, Sugarcane bagasse, *Corcyra* rearing waste (Maize) and Jawar grain + 1.0 g Dextrose | *Beauveria bassiana*, *Metarhizium anisopliae* and *Verticillium lecanii* | 18 |
| **7** | sugar cane, corn, barley, rice, millet and sorghum | *Beauveria bassiana* | 26 |

After mushroom cultivation, the partially degraded paddy or wheat straw and other agricultural wastes, which form as valuable by-products of edible mushroom cultivation have been termed as Spent Mushroom Substrate (SMS). Recently, the term spent compost or

spent mushroom substrate has been replaced by a more appropriate term, “post mushroom substrate” (PMS) because it is not ‘spent’ and is ready to be further attacked by a new set of microorganisms. Post mushroom substrate (PMS) normally contains 1.9:0.4:2.4%, N-P-K with a C: N ratio of 9 to 15: 1, pH- 5.8 - 7.7 along with other nutrients like Mg, Ca, Al and Fe [6], hence it can be used as a substrate for mass production of agriculturally important microorganisms, fungus in particular.

It is estimated that production of 1 kilogram of mushroom generates about 5 kg of PMS. Every year mushroom industry needs to dispose more than 50 million tonnes of PMS. In some countries (e.g., China produces more than 150 thousand tonne SMS per year) the management of spent mushroom substrates poses many difficulties and if not handled properly this may cause various environmental problems, including ground water contamination and nuisance [3]. Based on its nutrient contents it can be used as an alternative substrate for mass production of entomopathogenic fungi through solid stat fermentation [Table-2].

The other agriculture bioproducts or agro-wastes like, sugarcane bagasse produced during sugar production from sugarcane. Average of 140 kg of bagasse are produced for every ton of sugarcane processed thus, this is the most abundant lignocellulosic residue [14]. In general, bagasse composition consists of approximately 30-36% cellulose, 25-28% hemicellulose, and 20-21% lignin [7]. The rice husk, also called rice hull, is the coating on a seed or grain of rice, each kg of milled white rice results in roughly 0.28 kg of rice husk as a by-product of rice production during milling, approximately 120 million tons of rice husk is available each year after it has been removed from the whole rice paddy. Rice husk is composed of 15% carbon, 18% ash, and 67% volatile matter [12].

The success of microbial control of insects/pests depends not only on their pathogenicity, but also on the successful mass production of the microbial control agents. For a successful integrated pest management programme, the agents like the ENPF should be amenable to easy and cheap mass multiplication. The use of agro-industrial wastes in SSF for production of ENPF is of particular interest due to their availability and low cost, besides being an environment friendly alternative for their disposal [16]. Hence the present study was under taken to use this agro-wastes as a substrate for mass production of potential entomopathogenic fungal agents.

## 2. MATERIAL AND METHODS

### 2.1 Entomopathogenic fungal isolates

Four virulent entomopathogenic fungal isolates *B. bassiana, L. lecanii, H. thompsonii and I. fumoroseous* isolated from two agro-climatic zones (eastern dry zoon and southern dry zone) of Karnataka, India. The spore suspension of isolates was prepared by adding 10ml 0.5% sterile tween 80 to 10 days old cultures and concentration was adjusted to $10^8$ conidia $ml^{-1}$ by using an improved Neubauer Hemocytometer.

### 2.2 Agro-waste substrates

The agricultural wastes like, Sugarcane bagasse (collected from VC farm Mandy, CoA, UAS, GKVK, Bangalore), Paddy husk (procured from paddy mill), Spent mushroom substrate (collected from mushroom lab, Dept. of Agril. Microbiology, UAS, GKVK, Bangalore) and sorghum grains were shade dried for 5 days to reduce the moisture content. The dried substrates were chopped in to small pieces using a chop cutter. The substrates were grinded to further reduce the particle size and were sieved through 2 mm sieve. Pre-processed substrates were packed air tight in polytene bags and stored at 25℃ for further studies.

**Table 2: Scenario of total production of different agro-wastes in India and world.**

| Sl. no | Substrate | India (MT/annum) | World (MT/annum) | Reference |
|---|---|---|---|---|
| 1 | Sugarcane Bagasse | 16 | 300 | 27 |
| 2 | Molasses | 20 | ** | 27 |
| 3 | Post mushroom substrate (PMS) | 7.5 | 50MT | 3 |
| 4 | Rice husk | 24 mt | 120 mt | 29, 30 |

### 2.3 Solid state fermentation

The SSF was performed to in order to select the best alternative substrate other than cereals (sorghum) for mass production of ENF. The study of SSF was carried out in Erlenmeyer flasks (500 ml). Two set of experiments were carried out for all substrates (with and without fortification). The dried substrates (100g) were weighed and transferred to flasks separately and initial moisture percentage was adjusted to 40-50 by adding sterile distilled water. The substrates were inoculated with 5ml of conidial suspension of all four isolates containing $1\times10^{8}$ conidia $ml^{-1}$ separately to all flasks contains different substrates. In second set of experiment the substrates were fortified with 10 percent molasses and inoculated with all isolates. The flasks were incubated for 21 days and spore count was checked for every 7 days interval using hemocytometer by diluting 1gm substrate from each treatment in 10ml water blank [28].

### 2.4 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 CRD and differences between treatments were considered significant at $p = 0.05$.

## 3. RESULTS AND DISCUSSION

The results on evaluating stability of different agro-wates for mass production of entomopathogenic fungal agents has revealed that, each substrate has influenced the growth and spore production of ENPF agents differently. Out of 10 treatments, treatments having sorghum grains fortified with 10 % molasses ($T_8$) and sorghum grains without fortification ($T_4$) have supported the good growth and spore production of all four isolates ($\times10^{10}$ conidia $g^{-1}$) in compare with other treatments. This is obvious because of easily digestible carbohydrates and other nutrients i. e. sorghum grains contain 8–15% protein, 5-15% sugar, and 32-57% starch and it is relatively rich in micronutrients (mg/kg) iron (41-127), zinc (14-35), phosphorus (1498-3797), Ca (207-447), K (1150-2569), Mn (10-24), Na (12-54), and Mg (750-1506) [22]. Since starch is a linear polysaccharide which can be easily digested by fungi in compare with other complex carbohydrates (lignin, cellulose, hemicellulose and pectins). The data was presented in the table 3.

The $T_3$ and $T_7$ (SMS & SMS+10% molasses) relatively act as good substrate for mass production of entomopathogens after cereal grain (sorghum) with spore count varies between $\times10^{6}$ to $\times10^{7}$ conidia $g^{-1}$ of substrate, this is because of its properties like, low C:N ration (14:1), pre-decomposed organic substrates and 2-4 % protein content [2] may enhanced the growth and development of fungi in compare with rice husk ($\times10^{4}$ conidia $^{g-1}$) where, C/N ration is 85:1 along with it contains complex carbohydrates (lignin, pectin and hemicellulose). Whereas sugar cane bagasse consists of approximately, 47-52 % cellulose,

25-28 percent hemicellulose and 20-21 percent lignin with high C/N ration (70-80:1) [7], all these factors affect the fungus by limiting the nutrient availability resulted in under growth and spore production. Previously, Pal and Prasad, 2014, were studied on mass production of various ENPF on nine different agriculture and industrial wastes. The results revealed that maximum yield $278.75\times10^6$, $171.75\times10^6$ and $185\times10^6$ spores per ml of *B. bassiana, M. anisopliae, and V. lecanii* were obtained in FYM respectively, followed by Sabouraud dextrose broth ($246.26\times10^6$, $157.25\times10^6$ and $180.00\times10^6$ spores per ml) and lowest yield was obtained in sugarcane bagasse ($65.25\times10^6$, $34.25\times10^6$ and $39.00\times10^6$ spores per ml) [16]. similarly, Agale in 2018, were used ten different substrates (chickpea, pigeon pea, black gram, maize, sorghum, soybean, rice, wheat, ground nut and green gram) and two media like PDA and SDA for mass production of entomopathogenic fungi *M. anisopliae*. The result revealed that significantly highest conidial count ($67.6 \times 10^3$ spores/ml) was observed on green gram followed by sorghum in $10^3$ dilutions and Highest conidial count ($63.7 \times 10^3$ spores/ml) was observed on SDB media followed by PDB ($43.7 \times 10^3$ spores/ml) [1]. Similar findings were obtained by many other researchers [15, 17, 21, 24].
Among all the treatments, treatments fortified with 10 percent molasses were exhibited higher conidial count in compare with treatment without molasses, this is because molasses composed of roughly 55 percent sucrose and other sugars, 20 percent water, 15 percent organic non-sugars, and 10 percent ash [20]. These easily available sugars and nutrients were promoted the initial growth of fungus in compare with nonfortified treatments where in fungal isolates have to produce enzymes to convert complex carbohydrates in to sugars this process demands and consumes most of the energy generated by the organisms leads to early sporulation with low spore count.

The spore density of all the isolates were drastically increased from 7 DAI to 14 DAI and to 21 days after inoculation. Initially the treatments which received sugarcane bagasse, paddy husk and SMS were shown less spore count but it was gradually increased in compare with treatments which received sorghum grains and 10 % molasses. The spore count of *B. bassiana* in $T_7$ and $T_3$ (SMS with and without fortified with 10 % molasses) treatments was $1.33\ X10^3$, $1.66\times10^6$, $0.30\times10^9$ and $0.30\times10^3$, $0.33\times10^6$, $0.06\times10^9$ at $7^{th,}$ $14^{th}$ and 21 DAI respectively (Table:3). The spore density of other isolates (*L. lecanii, H. thompsonii* and *I. fumosorosea*) on SMS also were in the same range but on the $7^{th}$ day these isolates were produced 10 times more spores in compare to *B. bassiana* on SMS (Table:4).

**4 Conclusion**

Among the ten treatments which were used in the present experiment for mass production of entomopathogenic fungi reveled that treatment $T_8$ followed by $T_4$ has proved to be better substrate for mass production but the treatment $T_{10}$ and $T_7$ were also proved to be effective in compared with other treatments. Further study has to be conduct on use of other carbohydrate rich agro-waste in different rations along with SMS and also analyze the quality and pathogenicity of spores produced on these materials need to be evaluated. Spent mushroom substrate (post mushroom substrate) has a potentiality to become an alternative, sustainable and low-cost substrate for mass production of entomopathogenic fungal agents by fortifying with molasses, sugar beet wastes or just by supplementing with high carbohydrate containing agro-wastes will enhance the growth and spore production of entomopathogenic fungal agents.

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**Table 3: Evaluation of different agro-wastes for mass production of entomopathogenic fungi** *Beauveria bassiana* **and** *L. lecanii*

| Treatments | Treatments details | *Beauveria bassiana* | | | *Lecanicillium lecanii* | | |
|---|---|---|---|---|---|---|---|
| | | Spore density (Days after inoculation) | | | Spore density (Days after inoculation) | | |
| | | 7 DAI ($\times10^4$) | 14 DAI ($\times10^6$) | 21 DAI ($\times10^9$) | 7 DAI ($\times10^4$) | 14 DAI ($\times10^6$) | 21 DAI ($\times10^9$) |
| $T_1$ | Rice husk | $0.03\pm0.02^{b}$ | $0.06\pm0.03^{c}$ | $0.008\pm0.03^{b}$ | $0.07\pm0.04^{c}$ | $0.06\pm0.02^{d}$ | $0.002\pm0.10^{b}$ |
| $T_2$ | Sugarcane Bagasse | $0.06\pm0.03^{b}$ | $0.06\pm0.03^{c}$ | $0.009\pm0.18^{b}$ | $0.07\pm0.04^{c}$ | $0.03\pm0.01^{d}$ | $0.007\pm0.17^{b}$ |
| $T_3$ | Spent mushroom substrate (SMS) | $0.30\pm0.05^{b}$ | $0.33\pm0.07^{c}$ | $0.06\pm0.22^{b}$ | $0.13\pm0.07^{c}$ | $0.13\pm0.05^{d}$ | $0.30\pm0.22^{b}$ |
| $T_4$ | Crushed Sorghum grains | $1.33\pm0.07^{b}$ | $18.6\pm0.47^{b}$ | $1.3\pm0.35^{b}$ | $5.70\pm0.09^{b}$ | $18.7\pm0.36^{b}$ | $6.36\pm0.27^{ab}$ |
| $T_5$ | Rice husk + 10 % molasses | $0.30\pm0.44^{b}$ | $0.63\pm0.13^{c}$ | $0.07\pm0.66^{b}$ | $0.87\pm0.48^{c}$ | $0.23\pm0.09^{d}$ | $0.05\pm0.51^{b}$ |
| $T_6$ | Sugarcane Bagasse + 10 % molasses | $0.53\pm0.49^{b}$ | $0.70\pm0.36^{c}$ | $0.08\pm1.12^{b}$ | $0.93\pm0.51^{c}$ | $0.67\pm0.27^{d}$ | $0.06\pm0.91^{b}$ |
| $T_7$ | Spent mushroom substrate + 10 % molasses | $0.89\pm0.62^{b}$ | $1.66\pm1.06^{c}$ | $0.30\pm1.57^{b}$ | $1.37\pm0.64^{c}$ | $2.30\pm0.87^{d}$ | $0.72\pm1.52^{b}$ |
| $T_8$ | Crush Sorghum grains + 10 % molasses | $5.43\pm0.73^{a}$ | $84.6\pm1.90^{a}$ | $5.6\pm1.88^{a}$ | $8.30\pm0.73^{a}$ | $74.7\pm1.42^{a}$ | $9.6\pm1.48^{a}$ |
| $T_9$ | 25 % Rice husk + 25 % Sugarcane Bagasse + 25 % SMS + 25 % Crushed Sorghum grains | $0.66\pm0.04^{b}$ | $1.66\pm0.42^{c}$ | $0.16\pm0.48^{b}$ | $0.86\pm0.04^{c}$ | $0.37\pm0.32^{d}$ | $0.73\pm0.45^{b}$ |
| $T_{10}$ | 25 % Rice husk + 25 % Sugarcane Bagasse + 25 % SMS + 25 % Crushed Sorghum grains + 10 % molasses | $0.70\pm0.53^{b}$ | $2.33\pm1.00^{c}$ | $0.60\pm0.76^{b}$ | $1.53\pm0.53^{c}$ | $9.63\pm0.75^{c}$ | $0.60\pm0.82^{b}$ |

*Note: Mean values having same superscript letter are not significantly different at P<0.05*

**Table 4: Evaluation of different agro-wastes for mass production of entomopathogenic fungi *H. thompsonii* and *Isaria fumosoroseus***

| Treatments | Treatments details | *Hirsutella thompsonii* | | | *Isaria fumosoroseus* | | |
|---|---|---|---|---|---|---|---|
| | | Spore density (Days after inoculation) | | | Spore density (Days after inoculation) | | |
| | | 7 ($\times10^3$) | 14 ($\times10^6$) | 21 ($\times 10^8$) | 7 ($\times10^4$) | 14 ($\times10^6$) | 21 ($\times10^9$) |
| $T_1$ | Rice husk | $0.03\pm0.02^{g}$ | $0.06\pm0.10^{d}$ | $0.003\pm0.12^{d}$ | $0.03\pm0.02^{b}$ | $0.06\pm0.12^{d}$ | $0.002\pm0.18^{c}$ |
| $T_2$ | Sugarcane Bagasse | $0.06\pm0.04^{g}$ | $0.03\pm0.12^{d}$ | $0.006\pm0.18^{d}$ | $0.02\pm0.02^{b}$ | $0.03\pm0.11^{d}$ | $0.003\pm0.21^{c}$ |
| $T_3$ | Spent mushroom substrate (SMS) | $0.13\pm0.07^{fg}$ | $0.60\pm0.17^{d}$ | $0.06\pm0.19^{d}$ | $0.21\pm0.04^{b}$ | $0.23\pm0.20^{d}$ | $0.03\pm0.25^{c}$ |
| $T_4$ | Crushed Sorghum grains | $1.66\pm0.09^{b}$ | $27.3\pm0.21^{b}$ | $1.30\pm0.33^{b}$ | $1.33\pm0.07^{b}$ | $31.3\pm0.27^{b}$ | $4.20\pm0.41^{b}$ |
| $T_5$ | Rice husk + 10 % molasses | $0.46\pm0.42^{de}$ | $0.70\pm0.57^{d}$ | $0.03\pm0.59^{d}$ | $0.66\pm0.42^{b}$ | $0.27\pm0.54^{d}$ | $0.07\pm0.66^{c}$ |
| $T_6$ | Sugarcane Bagasse + 10 % molasses | $0.63\pm0.46^{d}$ | $0.20\pm0.66^{d}$ | $0.07\pm1.00^{d}$ | $0.53\pm0.51^{b}$ | $0.63\pm0.77^{d}$ | $0.07\pm1.09^{c}$ |
| $T_7$ | Spent mushroom substrate + 10 % molasses | $0.98\pm0.69^{c}$ | $6.30\pm0.76^{c}$ | $0.60\pm1.57^{c}$ | $0.97\pm0.64^{b}$ | $4.70\pm0.81^{c}$ | $0.33\pm1.71^{c}$ |
| $T_8$ | Crush Sorghum grains + 10 % molasses | $4.30\pm0.78^{a}$ | $84.7\pm1.00^{a}$ | $9.70\pm2.10^{a}$ | $5.70\pm0.84^{a}$ | $79.6\pm1.09^{a}$ | $7.30\pm2.27^{a}$ |
| $T_9$ | 25 % Rice husk + 25 % Sugarcane Bagasse + 25 % SMS + 25 % Crushed Sorghum grains | $0.33\pm0.04^{ef}$ | $0.63\pm0.18^{d}$ | $0.03\pm0.53^{d}$ | $0.70\pm0.04^{b}$ | $0.33\pm0.27^{d}$ | $0.06\pm0.64^{c}$ |
| $T_{10}$ | 25 % Rice husk + 25 % Sugarcane Bagasse + 25 % SMS + 25 % Crushed Sorghum grains + 10 % molasses | $0.66\pm0.35^{d}$ | $2.30\pm0.76^{d}$ | $0.70\pm0.83^{c}$ | $0.60\pm0.33^{b}$ | $4.30\pm0.79^{c}$ | $0.70\pm0.89^{c}$ |

*Note: Mean values having same superscript letter are not significantly different at P<0.05*