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From Waste to Utilization: Assessing the Feasibility of Using Post-Mushroom Substrate and Other Agro-Wastes for the Mass Production of Entomopathogenic Fungi

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ABSTRACT

Production of high-quality inoculum in sufficient quantities is crucial for biocontrol programs. Entomopathogenic Fungi (EPF) are highly suitable biocontrol agents due to their adaptability, mode of action, persistence, and wide host range. This study aimed to evaluate the suitability of agro-wastes, including sugarcane bagasse, paddy husk, Post Mushroom Substrate (PMS), and sorghum grains with and without 10% molasses fortification, for mass production of four EPF isolates (*Cordyceps fumosorosea*: MT997932, *Beauveria bassiana*: MT997933, *Akanthomyces lecanii*: MT997935, and *Hirsutella thompsonii*: MT997936) isolated from two agro-climatic zones in Karnataka, India. The study employed solid-state fermentation. Results showed that sorghum grains fortified with 10% molasses had the highest mycelial growth and spore production of all isolates, followed by PMS with 10% molasses (T_7). Fortification with molasses positively influenced the growth and spore production of EPF. The results indicated that while sorghum grains were the best choice for mass production, PMS fortified with molasses also had great potential as an alternative substrate.

Keywords: *Beauveria*, *Lecanicillium*, Biocontrol, Sorghum grains, Sugarcane bagasse.

INTRODUCTION

The effectiveness of using microorganisms to control insect pests is determined by not just their ability to cause disease, but also the successful mass production of these biocontrol agents (Sani *et al*., 2020). Despite its importance, the growth requirements of many entomopathogenic fungi remain poorly understood. To produce these fungi in large quantities, it is crucial to have a clear understanding of their nutritional requirements. The type of nutrients used will depend on the specific fungus being mass produced. These fungi need oxygen, water, an organic carbon and energy source, a source of inorganic or organic nitrogen, and other elements such as minerals and growth factors (Francisco *et al*., 2006). For an effective and successful integrated pest management program, it is important that entomopathogenic fungi (EPF) can be mass produced easily and inexpensively. The use of agro-industrial waste in Solid-State Fermentation (SSF) for producing EPF is particularly attractive due to its low cost and environmentally friendly nature (Mishra *et al*., 2016; Jaronski, 2023). The major substrates used for mass production of EPF in the tropics are rice and barley in the Northern Hemisphere (Jaronski, 2023). Researchers have been trying to identify low-cost agro byproducts and wastes as suitable substrates for mass production (as shown in Table-1). There are various methods of mass production based on the substrate, including solid-state fermentation (Krishna, 2005), liquid fermentation,

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Table 1. Various solid substrates evaluated for the mass production of the principal entomopathogenic fungi.

Sl. No.	Substrate/s	Organism/s	Reference
1	Green gram, sorghum	*Metarhizium anisopliae*	Agale *et al.,* 2018
2	Agricultural products	*Beauveria bassiana*	Bhadauria *et al.,* 2012
3	Apple Pomace (AP)	*L. lecanii*, *B. bassiana, P. fumosoroseus*	Reddy and shahotra, 2020
4	Broken rice grains, rice hulls	*B. bassiana Metarhizium anisopliae*	Bich *et al.,* 2018
5	Sorghum, rice, wheat, refuse potato chips and refuse banana chips	*Nomuraea rileyi*	Thakre *et al.,* 2011
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*	Prasad and Rishi, 2014.

submerged state fermentation, and biphasic culture system (Machado *et al*., 2010; Gouli *et al*., 2014), with solid-state fermentation emerging as the most appropriate technology.

After mushroom cultivation, partially degraded paddy or wheat straw, and other agricultural wastes, have been referred to as Spent Mushroom Substrate (SMS). However, the term "spent compost" or "spent mushroom substrate" has been recently replaced with the more appropriate term, "Post Mushroom Substrate" (PMS), as it is not 'spent' and is ready to be further attacked by a new set of microorganisms. It is estimated that the production of 1 kilogram of mushrooms generates approximately 5 kilograms of PMS. The mushroom industry must dispose of over 50 million tonnes of PMS each year. In some countries, such as China, which produces over 150 tonnes of PMS annually, the management of PMS presents many difficulties and, if not handled properly, can cause various environmental problems, including groundwater contamination and nuisance (Beyer, 1996). PMS typically 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, and other nutrients such as Mg, Ca, Al, and Fe (Chorover *et al*., 2000). Therefore, PMS can be used for the mass production of agriculturally important microorganisms, particularly EPF.

Sugarcane bagasse is a by-product generated during sugar production from sugarcane. On average, 140 kilograms of bagasse are produced for every ton of sugarcane processed, making it the most abundant lignocellulosic residue (Melati *et al*., 2017). Generally, bagasse contains approximately 47-52% cellulose, 25-28% hemicellulose, and 20-21% lignin (Dotaniya *et al*., 2016). The rice husk, also known as a rice hull, is the protective coating on a rice seed or grain. Each kilogram of milled white rice produces approximately 0.28 kilograms of rice husk as a by-product, with around 120 million tons produced each year. Rice husk is composed of 15% carbon, 18% ash, and 67% volatile matter (Lim *et al*., 2012). Adequate production of high-quality inoculum is a crucial component of a biocontrol program, and this study aimed to use these agro-wastes as a substrate for the mass production of promising entomopathogenic fungal agents.

MATERIALS AND METHODS

Entomopathogenic Fungal Isolates Used in the Study

Four entomopathogenic fungal isolates, Beauveria bassiana (Bals.-Criv.) Vuill, Akanthomyces lecanii (ZImm) [Formally known as Lecanicillium lecanii], Hirsutella

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thompsonii (Fisher) and Cordyceps fumosorosea (Wize) [Formally known as Isaria fumosorosea] were isolated from insect cadavers collected from two agro-climatic zones (Eastern dry zone and Southern dry zone) in Karnataka, India. The pure culture of isolates were preserved on PDA plates at 4±1°C (Ranadev et al., 2023). The EPF spore suspension was prepared by adding 10 mL of 0.5% sterile Tween 80-to10-day old cultures on Potato Dextrose Agar (PDA), and the concentration was adjusted to 10^8 conidia mL^{-1} using an improved Neubauer Haemocytometer.

Substrate Collection and Preparation

The agricultural waste materials, including sugarcane bagasse (collected from the VC Farm Mandya at the College of Agriculture, University of Agricultural Sciences, GKVK, Bangalore), paddy husk (procured from a paddy mill), and PMS (collected from the Mushroom Lab at the Department of Agricultural Microbiology, University of Agricultural Sciences, GKVK, Bangalore), underwent a preparation process. The materials were first shade dried for 5 days, then, chopped into small pieces using a chop cutter. The resulting substrates were ground and sieved through a 2 mm sieve. Finally, the processed substrates were packed in airtight polypropylene bags and stored at 25°C for further studies.

Solid State Fermentation

The Solid-State Fermentation (SSF) was performed to determine the best alternative substrate for mass production of entomopathogenic fungi other than cereals (sorghum). The study was conducted using 500 mL Erlenmeyer flasks, following the method described by Prakash *et al*. (2008). The experiment had 10 treatments and 3 replications; two sets of experiments were performed on all the substrates, one with fortification and the other without. Hundred grams of processed substrates were transferred into flasks and their moisture content was adjusted to 60% by adding distilled water. The flasks were sterilized at 121°C for 30 minutes. Five millilitres of different EPF conidial suspension (1×10^8 conidia mL^{-1}) was added to the flasks individually. In the second set of experiment, the substrates were fortified with 10% molasses before inoculation. The inoculated flasks were incubated at 25°C and were regularly mixed at 2 days interval during the incubation for oxygenation. The conidial count was determined at 7-day intervals using a haemocytometer by diluting 1 gram of substrate from each treatment in 10 mL of a water blank.

Statistical Analysis

The results of the conidia production of entomopathogenic fungi from various substrates were analysed using ANOVA (Analysis of Variance) with the help of the software Web Agri Stat Package 2.0 (https://ccari.icar.gov.in/wasp2.0/index.php, accessed on October 22, 2022), and the means were compared using post-hoc test (Duncan's multiple range test) at a 5% level.

RESULTS

The results on evaluation of different agro-wastes for the mass production of Entomopathogenic Fungi (EPF) showed that each substrate had a distinct impact on the growth and conidia production of EPF. Out of 10 treatments, the treatment T_8 (sorghum grains fortified with 10% molasses) showed the highest spore count ($\times10^{10}$ conidia g^{-1}), 21 Days After Inoculation (DAI), followed by T_4 (sorghum grains without fortification) and T_{10} (25% rice husk+25% bagasse+25% PMS+25% sorghum grains + 10% molasses). The evaluation of different agro-wastes for mass production of *Beauveria bassiana* and *Akanthomyces lecanii* revealed that the highest conidial counts (8.43×10^4 and 8.30×10^4

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conidia g^{-1}, respectively) were recorded in T_8 (sorghum grains+10% molasses), 7 DAI, followed by T4 (1.33×10^4 and 1.33×10^4 conidia g^{-1}) and T_{10} (5.70×10^4 conidia g^{-1}). The lowest conidial count (300 and 700) was observed in treatment T_1 (only rice husk).

The spore production increased in all treatments over the incubation period. On the 14th DAI, the yield of conidia by *Beauveria bassiana* significantly increased due to increased growth. The highest spore count (84.6×10^6 conidia g^{-1}) was recorded from treatment T_8 (crushed sorghum grains + 10% Molasses), followed by T_4 (18.6×10^6). The lowest spore count (6.0×10^4) was recorded in treatments T_2 and T_3 inoculated with *Beauveria* isolates. At 21 DAI, there was a further increase in the conidial count of entomopathogenic isolates (from 10^6 to 10^9 conidia g^{-1} of the substrate) compared to the results of the 14th DAI. Treatment T_8 (crushed sorghum grains + 10% Molasses) showed a drastic increase in the spore count ($\times10^9$ conidia g^{-1} of the substrate), followed by treatment T_4 (crushed sorghum grains). The lowest spore count was recorded in treatments T_1 and T_2, with $\times10^6$ conidiospore per g (Table 2).

Akanthomyces lecanii, Hirsutella thompsonii, and *Cordyceps fumosorosea* produced the highest number of conidia per gram of substrate in treatment T_8, which consisted of sorghum grains and 10% molasses (Figure 1). Treatment T_4 was the second best followed by T_8 in conidia production, while the lowest conidia count was observed in T_1 and T_2. The production of spores was higher in all isolates, with 10^4 conidiospore per gram, compared to *B. bassiana* isolates, which produced 10^3 conidia per gram, on the 7 DAI. The results of evaluating different agro-wastes for mass-producing the entomopathogenic fungi *Hirsutella thompsonii* and *Cordyceps fumosorosea* are presented in Table 3.

The spore density of all isolates significantly increased from 7 to 21 DAI. Initially, treatments with sugarcane bagasse, paddy husk and PMS showed a lower spore count, but it gradually increased over a period of incubation. The conidial count of *Beauveria bassiana* in T_7 and T_3 (PMS with and without fortification with 10% molasses) was 1.33×10^3, 1.66×10^6, 0.30×10^9 and 0.30×10^3, 0.33×10^6, 0.06×10^9 conidia g^{-1}at 7th, 14th, and 21st DAI, respectively (Table 2). The conidial density of the other isolates (*Akanthomyces lecanii*, *H. thompsonii*, and *Cordyceps fumosorosea*) on PMS was also in the same range, except on the 7th day when these isolates produced 10 times more conidia compared to *B. bassiana* on PMS (Tables 2, and 3).

DISCUSSION

The Post-Mushroom Substrate (PMS) is a relatively good substrate for the mass production of entomopathogens after cereal grain (sorghum), with a spore count ranging from 10^6 to 10^7 conidia g^{-1} of substrate. This is due to its properties, such as a low C:N ratio (14:1), pre-decomposed organic substrates, and a 2-4% protein content, which enhance the growth and development of fungi compared to rice husk (10^4 conidia g^{-1}) that has a C:N ratio of 85:1 and contains complex carbohydrates like lignin, pectin, and hemicellulose. On the other hand, sugarcane bagasse contains approximately 47-52% cellulose, 25-28% hemicellulose, and 20-21% lignin, with a high C:N ratio (70-80:1). All these factors affected the fungi by limiting the availability of nutrients, resulting in poor growth and low spore production.

Previously, Prasad and Rishi (2014) studied the mass production of various EPF on nine different agricultural and industrial wastes and found that the highest yield of conidia was 278.75×10^6, 171.75×10^6, and 185×10^6 per mL for *Beauveria bassiana*, *Metarhizium anisopliae* (Metschn.) Sorokin, and *Akanthomyces lecanii*, respectively, inoculated with farm yard manure, followed by Sabouraud dextrose broth (246.26×10^6, 157.25×10^6 and 180.00×10^6 conidia per mL) and significantly lower yields were obtained from sugarcane bagasse (65.25×10^6,

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Table 2. Evaluation of different agro-wastes for mass production of entomopathogenic fungi *Beauveria bassiana* and *Akanthomyces lecanii*.[a]

Treatments	Treatments details	*Beauveria bassiana* Spore density (Days After Inoculation)			*Akanthomyces lecanii* Spore density conidia g^{-1} (Days After Inoculation)		
		7 DAI×10^4	14 DAI×10^6	21 DAI×10^9	7 DAI×10^4	14 DAI×10^6	21 DAI×10^9
T_1	Rice husk	0.03 ± 0.02^{ef}	0.06 ± 0.03^{fg}	0.008 ± 0.03^{h}	0.07 ± 0.04^{ef}	0.06 ± 0.02^{g}	0.002 ± 0.10^{h}
T_2	Bagasse	0.06 ± 0.03^{ef}	0.06 ± 0.03^{fg}	0.009 ± 0.18^{gh}	0.07 ± 0.04^{ef}	0.03 ± 0.01^{g}	0.007 ± 0.17^{h}
T_3	Post Mushroom Substrate (PMS)	0.30 ± 0.05^{cd}	0.33 ± 0.07^{de}	0.06 ± 0.22^{fg}	0.13 ± 0.07^{d}	0.13 ± 0.05^{f}	0.30 ± 0.22^{e}
T_4	Crushed sorghum grains	1.33 ± 0.07^{b}	18.6 ± 0.47^{b}	1.3 ± 0.35^{b}	5.70 ± 0.09^{b}	18.7 ± 0.36^{b}	6.3 ± 0.27^{a}
T_5	Rice husk+10% molasses	0.30 ± 0.44^{cd}	0.63 ± 0.13^{d}	0.07 ± 0.66^{fg}	0.87 ± 0.48^{d}	0.23 ± 0.09^{de}	0.05 ± 0.51^{fg}
T_6	Bagasse+10% molasses	0.60 ± 0.49^{c}	0.70 ± 0.36^{d}	0.08 ± 1.12^{fg}	0.93 ± 0.51^{d}	0.67 ± 0.27^{e}	0.06 ± 0.91^{fg}
T_7	PMS+10% molasses	0.70 ± 0.53^{c}	1.33 ± 1.00^{cd}	0.50 ± 0.76^{cd}	1.37 ± 0.64^{c}	2.30 ± 0.87^{d}	0.72 ± 1.52^{c}
T_8	Crush Sorghum grains+10 % molasses	8.43 ± 0.73^{a}	84.6 ± 1.90^{a}	5.6 ± 1.88^{a}	8.30 ± 0.73^{a}	78.7 ± 1.42^{a}	6.6 ± 1.48^{a}
T_9	25% Rice husk+25% Bagasse+25% PMS+25% Crushed sorghum grains	0.66 ± 0.04^{e}	1.66 ± 0.42^{c}	0.16 ± 0.48^{e}	0.86 ± 0.04^{d}	0.37 ± 0.32^{de}	0.53 ± 0.45^{cd}
T_{10}	25% Rice husk+25% Bagasse+25% PMS+25% Crushed sorghum grains+10% molasses	1.33 ± 0.62^{b}	2.66 ± 1.06^{c}	0.60 ± 1.57^{c}	1.53 ± 0.53^{c}	9.63 ± 0.75^{c}	1.60 ± 0.82^{b}

[a] (a-h): The values represented by the same superscripts are statistically on par with each other according to Duncan's multiple range test (P> 0.05).

Table 3. Evaluation of different agro-wastes for mass production of entomopathogenic fungi *H. thompsonii* and *Cordyceps fumosorosea*.[a]

Treatments	**Treatments details**	*Hirsutella thompsonii* Spore density (Days After Inoculation)			*Cordyceps fumosorosea* Spore density conidia g^{-1} (Days After Inoculation)		
		7 DAI×10^4	14 DAI×10^6	21 DAI×10^8	7 DAI×10^4	14 DAI×10^6	21 DAI×10^9
T_1	Rice husk	0.03 ± 0.02^{ef}	0.06 ± 0.10^{gh}	0.003 ± 0.12^{fg}	0.03 ± 0.02^{de}	0.06 ± 0.12^{ef}	0.002 ± 0.18^{f}
T_2	Bagasse	0.06 ± 0.04^{ef}	0.03 ± 0.12^{gh}	0.006 ± 0.18^{fg}	0.02 ± 0.02^{de}	0.03 ± 0.11^{ef}	0.003 ± 0.21^{f}
T_3	Post Mushroom Substrate (PMS)	0.13 ± 0.07^{cd}	0.60 ± 0.17^{e}	0.06 ± 0.19^{de}	0.21 ± 0.04^{bc}	0.23 ± 0.20^{d}	0.03 ± 0.25^{de}
T_4	Crushed Sorghum grains	1.66 ± 0.09^{b}	27.3 ± 0.21^{b}	1.30 ± 0.33^{b}	1.33 ± 0.07^{b}	31.3 ± 0.27^{b}	4.20 ± 0.41^{b}
T_5	Rice husk+10% molasses	0.26 ± 0.42^{c}	0.70 ± 0.57^{e}	0.03 ± 0.59^{de}	0.66 ± 0.42^{b}	0.27 ± 0.54^{d}	0.07 ± 0.66^{de}
T_6	Bagasse+10% molasses	0.31 ± 0.46^{bc}	0.20 ± 0.66^{ef}	0.07 ± 1.00^{de}	0.53 ± 0.51^{b}	0.63 ± 0.77^{d}	0.07 ± 1.09^{de}
T_7	PMS+10% molasses	0.66 ± 0.35^{bc}	2.30 ± 0.76^{cd}	0.60 ± 0.83^{c}	0.60 ± 0.33^{bc}	4.30 ± 0.79^{c}	0.40 ± 0.89^{c}
T_8	Crush Sorghum grains+10% molasses	4.30 ± 0.78^{a}	84.7 ± 1.00^{a}	9.70 ± 2.10^{a}	5.70 ± 0.84^{a}	79.6 ± 1.09^{a}	7.30 ± 2.27^{a}
T_9	25% Rice husk+25% Bagasse+25% PMS+25% Crushed sorghum grains	0.63 ± 0.04^{bc}	0.63 ± 0.18^{e}	0.08 ± 0.53^{de}	0.70 ± 0.04^{b}	0.63 ± 0.27^{d}	0.16 ± 0.64^{cd}
T_{10}	25% Rice husk+25% Bagasse+25% PMS+25% Crushed sorghum grains+10% molasses	0.98 ± 0.69^{bc}	6.30 ± 0.76^{c}	0.83 ± 1.57^{bc}	0.97 ± 0.64^{b}	4.70 ± 0.81^{c}	0.73 ± 1.71^{c}

[a] (a-h): The values represented by the same superscripts are statistically on par with each other according to Duncan's multiple range test (P> 0.05).

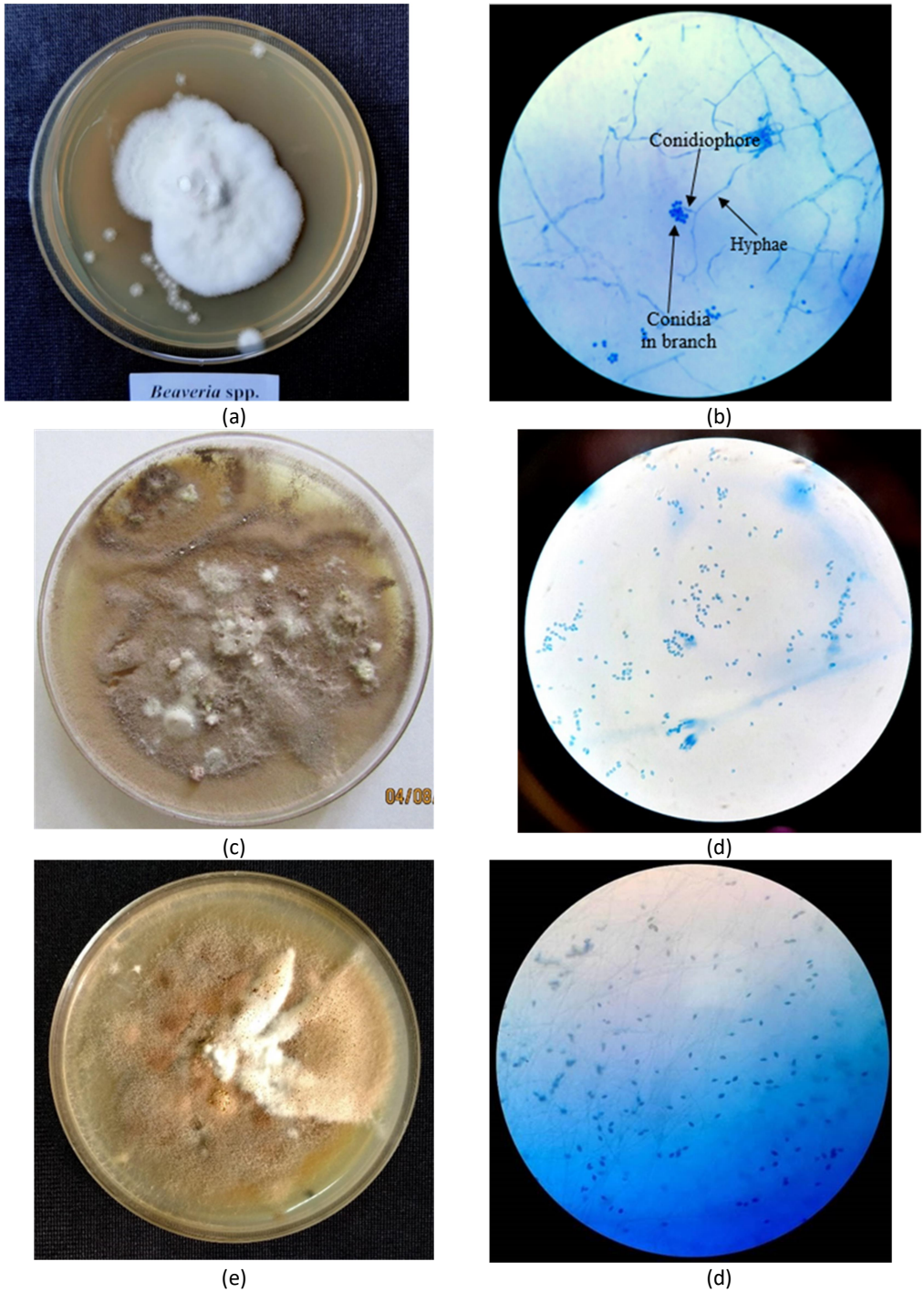

Figure 1. Growth of various entomopathogenic fungi viz. (a) *Beauveria bassiana*, (c) *Cordyceps fumosorosea,* and (e) *Hirsutella thompsonii* on potato dextrose agar and their conidia under light microscope at 40X.

34.25×10^6, and 39.00×10^6 conidia per mL). Similarly, Agale *et al*. (2018) used ten different substrates, including chickpea, pigeon pea, black gram, maize, sorghum, soybean, rice, wheat, groundnut, and green gram, as well as two media, PDA and SDA,

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for the mass production of the entomopathogenic fungus *Metarhizium anisopliae*. The results showed that the highest conidial count (67.6×10^3 conidia mL^{-1}) was observed on green gram, followed by sorghum, in 10^3 dilutions, and the highest conidial count (63.7×10^3 conidia mL^{-1}) was observed on SDA media, followed by PDA (43.7×10^3 conidia mL^{-1}). This was further confirmed by studies conducted by Pandey and Kanoujia (2005), Sahayaraj and Narasimayam (2008), Latifian *et al*. (2013), Mishra *et al*. (2016), and Sujatha *et al*. (2016).

The increased conidia count in treatments T_8, T_4, and T_{10} was due to the increased availability of simple carbohydrates and other nutrients; for example, sorghum grains contain 32-57% starch, 8-15% protein, 5-15% sugar, and micronutrients (Fe 41-127; Zn 14-35; Ca 207-447; Mn 10-24; Na 12-54 and Mg 750-1506 mg kg^{-1}) (Shegro *et al*., 2012). Since starch is a linear polysaccharide that can be easily utilized compared to complex carbohydrates (lignin, cellulose, hemicellulose and pectin), the spore count in treatments T_7 and T_{10} was satisfactory ($\times10^8$) compared to treatments with sugarcane bagasse and rice husk, but statistically lower compared to sorghum grains. The use of 10% molasses along with 25% sorghum grains has positively affected the growth and spore production, and there is a possibility of using PMS (presumably "partially molasses substrate") as a substrate for mass production instead of sorghum grains alone. This will reduce cost and the burden of using food grains for mass production. A previous study conducted by Dakshayini and Mallesha (2018) on using SMS as a substrate for the mass production of plant growth-promoting microorganisms revealed that SMS had the potential to be used as a substrate for mass production of biopesticides, including *Trichoderma* and other beneficial microorganisms. Similar results were obtained by Agale *et al*. (2018) and Mishra *et al*. (2016). Among all the treatments, those fortified with 10% molasses exhibited a higher conidial count compared to treatments without molasses. This is because molasses is composed of roughly 55% sucrose and other sugars, 20% water, 15% organic non-sugars, and 10% ash (Rein and Attard, 2007). These easily available sugars and nutrients promote the initial growth of the fungus compared to non-fortified treatments, where fungal isolates have to produce enzymes to convert complex carbohydrates into sugars. This process demands and consumes most of the energy generated by the organisms, leading to early sporulation with a low spore count. Among the different concentrations of sugarcane molasses tested (0.5, 1, 2, 3, 4, 5 and 6 per cent), maximum radial growth (4.65 cm), biomass production (1.55 g 100 mL^{-1}), and spore production (5.0×10^{10} conidia per mL) of *Nomuraea rileyi* were observed on 6% molasses (Tincilley *et al*., 2010).

CONCLUSIONS

In conclusion, the spore density and growth of entomopathogenic fungi can be significantly influenced by the type of substrate and medium used for mass production. PMS fortified with 10% molasses was found to be more favourable for the growth and development of entomopathogenic fungi compared to other substrates like sugarcane bagasse and paddy husk. Addition of molasses in PMS provided the necessary nutrients and sugars for the initial growth of the fungi, leading to an increase in spore density. Further research is necessary to determine the optimal concentration and type of molasses for mass production of entomopathogenic fungi.

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کاربرد زباله: ارزیابی امکان‌سنجی استفاده از بستر "پس‌از قارچ " و دیگر ضایعات کشاورزی برای تولید انبوه قارچ‌های بیمارگر حشرات (Entomopathogenic)

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چکیده

تولید مایه تلقیح با کیفیت بالا در مقادیر کافی برای برنامه های کنترل‌زیستی بسیار مهم است. قارچ‌های بیمارگر حشرات (EPF) به دلیل سازگاری، نحوه عملکرد، ماندگاری و گستره وسیع میزبان، عوامل کنترل-زیستی بسیار مناسبی هستند. این پژوهش با هدف ارزیابی مناسب بودن ضایعات کشاورزی شامل باگاس نیشکر، پوست شلتوک، بستر "پس‌از قارچ " (PMS) و دانه سورگوم با و بدون غنی‌سازی ملاس ۱۰ درصد،

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برای تولید انبوه چهار جدایه EPF (*Cordyceps fumosorosea*: MT997932، *Beauveria bassiana*: MT997933، *Akanthomyces lecanii*: MT997935، و *Hirsutella thompsonii*: MT997936) جدا شده از دو منطقه کشاورزی-اقلیمی در کارناتاکا (Karnataka) هند انجام شد. این بررسی از تخمیر حالت جامد (solid-state fermentation) استفاده کرد. نتایج نشان داد که دانه های سورگوم غنی شده با ملاس ۱۰ درصد بیشترین رشد میسلیوم و تولید اسپور را در بین جدایه ها داشتند و پس از آن PMS با ملاس ۱۰ درصد (T_7) قرار داشت. غنی‌سازی با ملاس بر رشد و تولید اسپور EPF تأثیر مثبت گذاشت. نتایج نشان داد که در حالی که دانه‌های سورگوم بهترین انتخاب برای تولید انبوه است، PMS غنی شده با ملاس نیز پتانسیل بالایی به عنوان بستر جایگزین دارد.

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