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Mass Production of *Paecilomyces Fumosoroseus* from Agricultural Products and Waste Material

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ABSTRACT

Production of Paecilomyces fumosoroseus from solid agricultural waste and conidia growth was done using eight types of waste and agricultural products as substrate by solid state fermentation technology. Bajra, maize, rice, wheat bran, sugarcane bagasse, wheat, sorghum, and wheat straw were used in whole as well as crushed form. Sterile distilled water was used as moistening agent. Maize was found to be excellent substrate for maximum spore production

KEYWORDS agricultural waste product/substrate /conidial yield/biopesticide.

Introduction: Solid state technology is considered good for mass production of the beneficial fungi. It is economical, lesser time consuming and not harmful. Using this technology the Conidia production has been found to be high yielding and spores obtained are of better quality than those obtained through submerged culture and the cost of raw material is also economical.(kewat Harish,Mishra Prabhat ,Vikrant P,& Sandhu S.S(2002)Mass Production of *Trichoderma viride* (BCF-18) by solid state Fermentation, **J.Basic appl.Mycology 1(2):214-217)**

The fungus *Paecilomyces fumosoroseus* is found in many countries. It belongs to the Deuteromycotina, order Hyphomycetales and the section isariodea (Samson et al, 1988). It grows with white mycelium and forms brightly colored conidia, colors ranging from yellow to pink, with a size of 3.4×1.2µm. The fungus has been isolated from various infected insects of various orders belonging to its wide host range (Smith and Grula, 1982). It was first described as pathogenic against the green house white fly by Fang el al (1985) and against the sweet potato white fly by Osborne et al (1990). Having considered the importance of fungus as pathogenic to several plant pathogens,it was decided to explore the cheapest source for mass production of the *Paecilomces fumosoroseus.*

Materials and Methods

Identification and culture separation

The objective of this study was to isolate the fungus and identify the cheapest source of agricultural waste for mass production of *Paecilomces fumosoroseus.*. It was isolated from mummified insects. The fungal growth was examined microscopically after it was recovered from Potato Dextrose agar(PDA) media. After seven days, sub-culturing was done to obtain the pure culture. The plates were incubated for seven days at 27±1˚C and examined daily. When more than one species of fungus grew on the same plate, these were separated by sub-culturing .

Material used included glass slide, Petri dish, cotton and sterile distilled water. All steps were completed in inoculation chamber.

Preparation of substrate

Paecilomyces fumosoroseus maintained on Sabouraud's dextrose agar (SDA) slant solid agar media for fungus growth (what is slant) was used in eight different solid substrates viz. bajra, maize, rice, wheat bran ,sugarcane Bagasse, wheat, sorghum, and wheat straw. The substrates used were cheapest and available easily locally. All substrates were taken in two forms, one in original form where the particle size was >1 mm and second, all substrates as fine particles with size ~1mm. Growth and sporulation were done in both the types of substrates. The moistening agent used initially was basal salt solution (bss) and then bss + 1% yeast extract (w/v) for the whole substrate as well as the crushed substrate separately. To 25 gm of each substrate, moistening agent and sterile distilled water was added and mixed thoroughly in conical flask of 250ml capacity. The conical flask was plugged with cotton and autoclaved for obtaining solid substrate. In addition to the above, for the whole rice grain and for the crushed rice, saline in glass bottles and polypropylene bag of 500ml was used. In a bottle /polypropylene bag 50 gm of substrate was added and mixed thoroughly. Then these were autoclaved for 15 min at 15*ibs* pressure.

Inoculation and mass production: Spore suspension of five day old culture of *Paecilomyces fumosroseus* was inoculated through sterilized 5 ml disposable syringe. The spore suspension had count > 10^6 spores in per ml of suspension. All inoculated bottles/bags were incubated at 27±1˚Cf for 14-21 days. Content of the bottles and bags were gently shaken regularly for mixing of the new germinating conidia / spores, once a day. After 14 days, content of each bag / bottle became light brown due to sporulation of *Paecilomyces fumosoroseus*, which covered the whole content after 21days and appeared brown. Then the content of each cultured bag was pulverized 2-3 times in mixer grinder to obtain maximum spores in powder form. This dry spore powder was directly used for spore count. In some bags the moisture content was >30% and the fungal growth was in small clots form, hence these cultures were sieved through 0.5mm pore size after each grinding and this process was repeated 3-4 times.

Germination test: Germination of conidia was estimated by determining the length of the germ tube 10^6 after every 4h. For this, loop full of spore suspension was placed on a glass slide kept inside a moist chamber and incubated at 27±1˚C in a BOD Incubator.

Viability after storage /Formulation: From the harvested spore powder, 30 gm was mixed with 470gm of anhydrous silica gel with the 200 mg of uv reflecting agent T**enopal (Mfg.BASF obtained from local market)**. The formulated powder was stored at 4˚C for longer viability. After the required duration of storage, germination test was performed using 1gm of mixture and percent germination was calculated. Strain were sprayed onto two PDA plates, sealed and incu-

bated for 6-8 hours at 23 ± 2.0 °C to determine germination as described above. The number of viable spores per strain was determined by using the following formula: mean % germination x the number of spores Ml $^{-1.}$ Percent mortality of the growing fungus was determined using a dissecting microscope (10X) Pasco B. Avery, Monique S.

Results

Table 1 shows Solid State Mass Production of *Paecilomyces Fumosoroseus* on whole grain moistened with basal salt solution alone

It was observed that maize showed maximal conidial production on day 14 as well as day 21, the concentration being 8.96×10^{5},and1.43×10^{6} respectively. Rice was second suitable substrate for conidia production which was found to be 6×10^{5} and 1×10^{6} conidia /ml for day 14 and 21 respectively. Sugar cane bagasse showed least growth in 14 and 21 days at 0.5×10^{3} and 0.6×10^{4} .respectively.

Table 2,shows the Solid State mass production of *Paecilomyces fumosoroseus* on crushed substrate (~1mm) moistened with basal salt solution alone

Crushed Maize and Rice substrate showed good growth at 14 days while after 21 days,wheat showed the highest yield. Sugar cane bagasse in the crushed form also showed the least growth at 14 as well as 21 days.

Table 3 shows the solid state mass production of *Paecilomyces fumosoroseus* on whole grain using basal salt solution with 1% yeast extract as the moistening agent.

Rice showed maximum growth in basal salt solution with 1% yeast extract after 14 days(6.4×10^{5}). Bajra also showed good growth after 14 days (5.8×10^{5}) while Maize showed maximum growth after 21 days(1.60×10^{6}) . Though rice showed maximum growth after 14 days, at 21 days(1.2×10^{6}) it was slightly less than growth in Maize at 21 days. (1.60×10^{6})

Table 4 shows solid State mass production of Paecilomyces fumosoroseus on crushed substrate (~1mm) using basal salt solution with 1%yeast extract as moistening agent.

The results show that crushed rice at 14 days shows the maximum growth (6.5×10^{5})while at 21 days wheat shows the maximum growth(4.9×10^{6}). Bajra at 14 days and Maize at 21 days are next in producing growth.

Discussion

Large scale production of the ecofriendly biopesticide is a primary objective in the biocontrol programme for increasing agricultural output . Present investigation was carried out to assess the most economical locally available substrates for mass multiplication of **Paecilomyces fumosoroseus**

From our results it is seen that yeast extract plays a crucial role in increasing spore production .Mathivanan et al [4] have reported mass production of *Trichoderma viride* on molasses yeast medium with similar results. Beneficial effect of yeast extract in the mass production of fungal spore is also reported for different fungi by other researchers..

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Effect of different growing media on mass production of nomuraea rileyi

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Biomass Production of Paecilomyces Vairoti

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Influence of Additives on the Yield and Pathogenicity of Conidia Produced by Solid State Cultivation of an **Isaria javanica** Isolate

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Similar observation was also obtained by Devi[9] and Holdem and Klaohorst [10.]

We found that bajra and maize were good substrates to produce spores but were not useful to give many spores in shorter time. Wheat straw growth was very poor . Sugarcane bagasse growth was the least in both the whole and crushed form when BSS alone was used as the moistening agent.There was no improvement in spore growth on sugarcane bagasse even after addition of yeast to the moistening agent. From our results we find that when basal salt solution alone is used as moistening agent, crushed form of all grains was better than their whole form for the maximum spore production. It may be because all grains used here are sources of carbon in the form of starch and the maximum utilization /absorbance of starch depends upon its hydrolysis by the action of enzyme amylase .Crushing of grains increases the area for enzymatic/ hydrolyzing activity of amylase resulting in more spore production.

Amount of moistening agent also played very important role for the growth of fungus. Excess amount of moistening agent caused clumping of the substrate particles, which created difficulty for fungus penetration, thus hindering the optimal utilization of substrate. On the other hand less amount of moistening agent caused dried and non soft condition of the grain and hence fungus could not grow and sporulate

From the different substrates, conidia were obtained and subjected to germination test to see the difference in the inherent dormant energy acquired by the spores. The faster a spore germinates the better the chance it carries to infect target host in the least possible time. Our results showed that length of germ tube after 4h was maximal in spores from rice grains followed by those from maize and wheat bran. But after 8 h, the germ tube length of spores produced on crushed rice with yeast extract exceeded all other spores grown on other substrates. proving it to be better medium . Our finding is dissimilar to Hasan et al[11] who have reported that the wheat bran is superior medium than rice and maize in case of *M.anisopliae*. and .Gopalakrishnan et al. (1999) who have . reported that sorghum was the ideal cereal for the mass production of *Paecilomyces farinosus*.

It is evident from our results that all natural substrates used by us are good for mass production and they support the principle of solid state fermentation in low cost settings. The size of the grains plays an important role in the mass production of spores. Presence of growth supplement (1% yeast extract), type of moistening agent (basal salt solution) and its ratio in solid substrate, optimal temperature and incubation period

also play an important role in the present investigations.

No doubt that the market price of rice is higher than bajra and maize, but in view of its faster, better and maximal spore production efficiency we propose that rice is a good solid substrate for the mass production of spores through the industry.

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Table 1: Solid State Mass Production of *Paecilomyces Fumosoroseus* **on whole grain**
(Moistening agent –Basal salt solution alone)

Substrate	**Paecilomyces fumosoroseus (Conidia /ml)**	
	14 th day	21st day
Bajra	5.55×10^5	0.9×10^6
Maize	8.96×10^5	1.43×10^6
Rice	6.00×10^5	1.0×10^6
Wheat Bran	0.3×10^5	0.85×10^6
Wheat straw	0.6×10^5	0.80×10^6
Wheat	4.00×10^4	5.0×10^5
Sorghum	4.3×10^5	5.3×10^5
Sugarcane baggase	0.5×10^3	0.6×10^4

Table 2: Solid State mass production of *Paecilomyces fumosoroseus* **on crushed substrate (~1mm) (Moistening agent –Basal salt solution alone)**

Substrate	Paecilomyces fumosoroseus	
	14thday	21th day
Bajra	5.6×10^5	0.91×10^6
Maize	8.99×10^5	1.45×10^6
Rice	6.3×10^5	1.1×10^6
Wheat	2.5×10^5	3.56×10^6
Wheat bran	5.1×10^5	0.81×10^6
Wheat straw	0.61×10^5	0.85×10^6
Sorghum	4.5×10^5	4.7×10^5
Sugarcane baggase	0.4×10^3	0.42×10^3

Table 3: Solid State mass production of *Paecilomyces fumosoroseus* **on whole grain**
(Moistening agent –Basal salt solution with 1% yeast extract)

Substrate	Paecilomyces fumosoroseus	
	14th day	21th day
Bajra	5.8×10^5	0.93×10^6
Maize	0.9×10^5	1.60×10^6
Rice	6.4×10^5	1.2×10^6
Wheat	5.7×10^5	5.0×10^6
Wheat bran	5.3×10^5	0.88×10^6
Wheat straw	0.5×10^2	0.54×10^2
Sorghum	5.0×10^5	5.4×10^5
Sugar cane baggase	0.4×10^3	0.41×10^4

Table 4: Solid State mass production of Paecilomyces fumosoroseus on crushed substrate (~1mm) (Moistening agent –Basal salt solution with 1%yeast extract)

Substrate	Paecilomyces fumosoroseus	
	14th Day	21st Day
Bajra	5.9×10^5	0.95×10^6
Maize	0.93×10^5	1.66×10^6
Rice	6.5×10^5	1.3×10^6
Wheat	3.8×10^5	4.9×10^6
Wheat bran	0.5×10^5	0.5×10^6
Wheat straw	0.5×10^3	0.52×10^3
Sorghum	5.0×10^5	5.2×10^5
Sugar cane baggase	0.4×10^3	0.42×10^3

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