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# Isolation and Solid Substrate Mass Production of *Paecilomces fumosoroseus* with the Help of Basal Salt Solution and Yeast Extract

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**A B S T R A C T**

Spore Production and Growth of *Paecilomyces fumosoroseus* was done 5 types of substrate through solid state fermentation technology viz. on maize, bajra, wheat, bran and mixture of wheat bran with fine rice granules in their whole form as well as in their crushed form. Crushed rice was found better substrate for maximum spore production. Similarly two moistening agent viz basal salt alone and basal solution with 1%yeast extract was used. It was observed with basal salt solution with yeast extract found suitable moistening agent for the growth of *Paecilomyces fumosoroseus.*

## Introduction

For the mass production of Infective conidia, blastospores and mycelium of biocontrol fungi many technologies are currently available[1,2,3] solid substrate fermentation is frequently used in many countries like Brazil and Colombia[2] with small local production facilities. This technology is a simple one in principle and continuously improve to produce large quantity of non contaminated and good quality fungal spores.

The teak leaf skeletonizer *Eutectona machaeralis* is also present in the teak plantation of India (Agarwal and rajak, 1985).

This caterpillar feeds on the leaves, leaving the major veins intact, hence the name skeletoniazer. Intachat (1998) identified the species in Indonesia, Malaysia and probably. Thailand as *Paliga qamastesalis*, as distinct from *E. machaeralis* present in India, although it has similar habitats. Also suggests that the correct nomenclature of *E. machaeralisis Paliga macheralis* in India out breaks of teak leaf skeletonizer occur mostly during the latter part of the growth season when leaves are old, and so its impact is negligible (Nair et al., 1986) and hence treated as minor pest. But other authors lists as a major pest of teak (Suratmo, 1996; Suharti and Sitepu, 1997).

The caterpillars were found to be infected with *Paecilomyces fumosoroseus* in central India (Agarwal &Rajak1985).

The fungus *Paecilomyces fumosoroseus* occurs in many countries. It belongs to the Deuteromycotina, order Hyphomycetales, and the section isariodea (Samson etal, 1988). It grows with white mycelium and forms brightly colored conidia. colors ranging from yellow to pinkish with a size of 3. 4×1. 2µm. The fungus can be found in the soil in very low densities and has frequently been isolated from various infected insects from many 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).

## Materials and Methods

### Survey and Collection

Systematic and periodical survey were conducted from the onset of monsoons till the availability of various teak pests in the forests of Mandla (M. P). The aim of these surveys in forest areas was to isolate insects infected with fungi, because these places were ecologically fit for the persistence of different insect species as well as different insect species as well as different fungal species which infect them. All cadavers of different insect hosts were collected on a purely random basis. If plants were found infested with insect pests. The insect colony was simply searched and cadavers were collected. Isolation the mummified insects or portions of insects with evidence of fungal growth were examined under a dissecting microscopes for evidence of mycelia and /or spores and if these were found to be infected with fungi, they were recovered frequently on 2% malt extract agar. if a newly dead insect, with no visible external growth, was collected, it was incubated for several days at high humidity, and observed intermittently for sporulation. The spore structure were mounted on a slide in water, or after specific staining with cotton blue and mounting in lacto phenol. Isolation of fungal pathogen from the insect cadavers with fresh external sporulation was done by taking spores with a fine, sterile needle. spores were streaked onto several different agar media with antibiotics. The insect cadavers not yet showing any sign of external vegetative growth, were surface sterilized in a 1% solution of sodium hypochlorite for 30 seconds and passed through a series of three washes in sterile water. The internal tissues were dissected with sterile scalpel to release hyphal bodies. For cadaver with sterile needle and transferred on to several different agar media with antibiotics like tap water, agar, potato dextrose agar supplemented with 0. 5%yeast extract and malt extract agar at ph6, 7and were incubated at 28±1˚C in a BOD incubator with a photoperiod of 16:8(Light :Dark ) Hours. All cultures were examined daily with a microscope.

The insect were also shaken up in 10 ml of distilled water to collect surface spores. The washings, together with 10 and 100 dilution in distilled water, were plated out onto either potato dextrose agar or Sabouraud dextrose agar containing streptomycin (100µl) to suppress bacterial growth. The cadavers were then surface sterilized in 0. 5% sodium hypochlorite solution for 1min and aqueous ethanol (70%) for 5 min, washed in sterile distilled water, and dissected using a flamed scalpel. Portions of cadavers with mycelium were placed on media already described. The plates incubated up to 7 days at 28±1˚C and examined daily. When more than one species of fungus grew on the same plate, they were separated by sub culturing to

prevent the more vigorous overwhelming growth of the other. For isolating pure culture, the germinating spores /small colony of interested fungus were removed from the agar plates by cutting round the tip of mycelia with the help of dummy cutter objective or cork borer and then the agar block was transferred to fresh desired media slants in tubes.

## Identification and Diagnosis

Slide culture technique was adopted for the identification of different isolates entomopathogens fungi on the basis of morphological characteristic. For this, sterilized moist chamber was prepared by keeping thin cotton pad, a wet filter paper and a slide inside a sterilized Petri plate. Saboraud dextrose agar medium was prepared and poured on a sterile glass plate in the form of a thin film (upto5mm). After solidification, this film was cut into small cubes with flamed scalpel. These cubes were placed on slide inside the moist chamber and inoculated with fungal spores separately. These slide cultures were incubated at 28±1˚Cin a BOD incubator. After sporulation, the slide were stained with cotton blue, mounted in lacto phenol and were observed for identification. For permanent mounts, stained fungal structure were mounted using DPX. Fungal identification was done on the basis of morpho taxonomic characters. Careful microscopic examinations were carried out to ensure that the cultures were not mixed.

*Paecilomyces fumosoroseus* maintained on PDA slant was used five different substrate viz, Bajra, Maize, Rice, wheat bran and rice with wheat bran(1:1w/w ratio) were select as solid substrate. All substrate were taken in 2 types of condition, first was their original form in which wheat bran was >1 mm in size and in second all substrate were in the form of fine particles which were size ~1mm. Growth and sporulation done in 2 type moistening agent first was basal salt solution (bss) alone and another was basal salt solution with1% yeast extract (w/v) and both moistening agent were use separately for the whole substrate as well as crushed substrate condition. 500 gm of each substrate was taken in polypropylene bag (30×45cm), to which moistening agent added (30% of substrate weight i. e. 150ml) and mixed thoroughly. Bags were closed by cotton plug having muslin cloth on it and were subjected to autoclave for 20-30 min.

Glass bottles of the saline water (500ml) were also used for the rice grain alone and for the crushed rice grains to compare with the polypropylene bags in different aspects. In a bottle of 500 ml only 50 gm of substrate and mixed thoroughly and in these bottles were autoclaved only for 15 min at 15*ibs* pressure.

Inoculation and mass production: Spore suspension of five days old culture of *Paecilomyces fumosroseus* was inoculated through sterilized 5 ml disposable syringe. The spore suspension having count more than $10^6$ spores in per ml of suspension. Then all inoculated bags were incubated at 28±1˚Cf for 12-24 days. Content of bags were gently shaken regularly for mixing of new germinating conidia or spores once a day. Similar steps were adopted in case of glass bottles.

After 14 days content of each bag and bottle became light brown due to sporulation of *Paecilomyces fumosoroseus,* which covered the whole content after 24 day and was appearing brown. Then content of each cultured bag were grind 2-3 times in mixer grinder to obtain maximum amount of spores in powder form. This dry spore powders was directly used for formulation.

In some bags moisture content was more than 30%thus the fungus growth was in small clots form, so these type of culture 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 four hours. For this loop full of spore suspension was placed on a glass slide kept inside a moist chamber and incubated at 28±1˚C in a BOD Incubator.

Viability after storage /Formulation : From harvested spore powder 30 grams was mixed carefully in 470gm of anhydrous silica gel with the 200 mg of uv reflecting agent viz ten opal. This formulated powder was stored 4˚C for long time viability. After the required duration of storage germination test was performed using 01g of mixture and percent germination was calculated.

## Results and Discussion

Table 1 depicts that, maize showing maximum conidial production ca. $8.96\times10^5$ and $1.43\times10^6$ conidia /ml for 14th and 21thday respectively. Wheat bran alone is also good substrate for mass production of conidia which is ca $5.03\times10^5$ and $0.8\times10^6$ From 12h and 24 day respectively, but rice second suitable substrate for maximum conidia production which is ca $6\times10^5$ and $1\times10^6$conidia /ml for 12th and 24th day respectively. Same increase ratio of the data seen, in table 2, which is for crushed bajra, maize, rice, wheat bran and rice with wheat bran. Crushed wheat bran showing ca conidia/ml for 12th day and ca Bajra $5.55\times10^5$ and $0.9\times10^6$ conidia/ml production for 24th day. and crushed wheat bran with crushed rice showing better growth of conidia, which is$5.\times10^5$and $0.8\times10^6$ conidia /ml and ca for 12th and 24th day respectively. In above both two cases moistening agent was basal salt solution alone. By comparing all the data of table 1 and table 2 where basal salt solution alone used as moistening agent, It is clear that crushed form of all grains is better than their whole form for the maximum spore production. It is because all used grains are source of carbon in the form of starch and the maximum utilization /absorbance of starch ; they depend upon its hydrolysis by the action of enzyme amylase. So crushing of grains increase the area for enzymatic/hydrolyzing activity of amylase.

Table 3 and 4 showing results of whole and crushed grains, which were supplemented with 1% yeast extract in moistening agent. Table 3 depict that, whole rice is again showing best growth of *Paecilomyces fumosoroseus*

Where ca. Conidia /ml production for 12th and 24th day respectively. Then, mixture of whole rice, wheat bran showing ca production for 12th and 24th day respectively. Wheat bran alone is showing good growth of fungi that is conidia /ml on 12th day and ca. Conidia /ml on 24th day.

Table 4 is showing the effect of crushing of grains. Crushed rice granules showing maximum spore production for 12th and 24th day ca. Conidia /ml respectively in wheat bran with crushed fine rice granules for 12th and 24th day respectively.

After comparing the data table 3and 4 with the data of table 1and 2, we find that yeast extract play crucial role to increase spore production. Mathivanan et al [4] also reported mass production of *Trichoderma viride* on molasses yeast medium with similar results. Effect of yeast extract in the mass production of fungal spore is also reported for different fungus is the same technology. similar observation was also obtained by Devi[9] and Holdem and Klaohorst [10.]

**Table.1** Solid State Mass Production of *Paecilomyces Fumosoroseus* on Whole Grain (Moistening Agent –Basal Salt Solution Alone)

| S No | Substrate | *Paecilomyces fumosoroseus*(Conidia /ml) | |
|---|---|---|---|
| | | 14 $^{th}$day | 21$^{th}$day |
| 1 | Bajra | 5. 55×10$^{5}$ | 0. 9×10$^{6}$ |
| 2 | Maize | 8. 96×10$^{5}$ | 1. 43×10$^{6}$ |
| 3 | Rice | 6. 00×10$^{5}$ | 1×10$^{6}$ |
| 4 | Wheat Bran | 5. 03×10$^{5}$ | 0. 85×10$^{6}$ |
| 5 | Sugar cane baggase | 5. 00×10$^{5}$ | 0. 80×10$^{6}$ |

**Table.2** Solid State mass production of *PaecilomycesFumosoroseus* on Crushed Substrate (~1mm)(Moistening Agent –Basal Salt Solution Alone )

| S. No | Substrate | *Paecilomyces fumosoroseus* | |
|---|---|---|---|
| | | 12$^{th}$day | 24$^{th}$ day |
| 1 | Bajra | 5. 6×10$^{5}$ | 0. 91×10$^{6}$ |
| 2 | Maize | 8. 99×10$^{5}$ | 1. 45×10$^{6}$ |
| 3 | Rice | 6. 3×10$^{5}$ | 1. 1×10$^{6}$ |
| 4 | Wheat Bran | 5. 5×10$^{5}$ | 5. 56×10$^{6}$ |
| 5 | Rice+Wheat bran | 5. 1×10$^{5}$ | 0. 81×10$^{6}$ |

**Table.3** Solid State mass production of *Paecilomyces Fumosoroseus* on Whole Grain (Moistening Agent –Basal Salt Solution with 1% Yeast Extract)

| Serial Number | Substrate | *Paecilomyces fumosoroseus* | |
|---|---|---|---|
| | | 12$^{th}$ day | 21$^{st}$ day |
| 1 | Bajra | 5. 8 ×10$^{5}$ | 0. 93 ×10$^{6}$ |
| 2 | Maize | 0. 9 ×10$^{5}$ | 1. 60×10$^{6}$ |
| 3 | Rice | 6. 4 × 10$^{5}$ | 1. 2 ×10$^{6}$ |
| 4 | Wheat Bran | 5. 7 × 10$^{5}$ | 5 ×10$^{6}$ |
| 5 | Rice+wheat bran | 5. 3 × 10$^{5}$ | 0. 88 ×10$^{6}$ |

**Table.4** Solid State Mass Production of *Paecilomyces Fumosoroseus* on Crushed Substrate (~1mm)(Moistening Agent –Basal Salt Solution with 1%Yeast Extract)

| Serial No | Substrate | *Paecilomyces fumosoroseus* | |
|---|---|---|---|
| | | 12$^{th}$ | 21$^{th}$ |
| 1 | Bajra | 5. 9×10$^{5}$ | . 95×10$^{6}$ |
| 2 | Maize | . 93 ×10$^{5}$ | 1. 66 ×10$^{6}$ |
| 3 | Rice | 6. 5×10$^{5}$ | 1. 3 ×10$^{6}$ |
| 4 | Wheat Bran | 5. 8×10$^{5}$ | 5. 9 ×10$^{6}$ |
| 5 | Rice+wheat bran | 5. 5 ×10$^{5}$ | 5. 2×10$^{6}$ |

In our research work Bajara and Maize also found good substrate to produce spores but not useful to give much spores in shorter time. Amount of moistening agent also noted which play very important role for the growth of fungus. Excess amount of moistening agent causes clumping of the substrate particles, which create clumping of the substrate particles, which create difficulty for fungus for penetration. Thus the fungus could not consume whole substrate. On the other hand less amount of moistening agent causes dried and non soft condition of the grain and hence fungus could not grow and sporulated.

Fungal mass propagation in glass bottles yielded average of ca. conidia/ml for 12$^{th}$ day and ca conidia/ml for 24$^{th}$ day in crushed rice grain supplemented with the moistening agent, which was basal salt solution alone. Spore production increased when crushed fine rice granules were supplemented by 1% yeast extract in basal salt solution, it was ca. Conidia /ml after 23 day.

From the different substrate conidia were obtain 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. Results showed that length of germ tube after 4h was maximum in spores from rice grains followed by those from maize and wheat bran. But following 8 hours the germ tube length of spores produced on crushed rice with yeast extract was exceeding than all, proving it to be better medium wheat bran and other substrate where as hasan et al[11] reports the wheat bran is superior medium than rice and maize in case of *M. anisopliae*

It is evident from the above result that all natural substrates used are good for mass production and they support to principle of solid –state fermentation in low cost condition. 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, Optimum temperature and incubation period also play an important role in the present investigations.

However market prize of rice is little higher than the Bajra and Maize, but in view of its faster, better and maximum spore production efficiency, it can be consider as a good solid substrate for the mass production of spores through the industry.

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