



## Biomass Production of *Paecilomyces Vairoti*

### KEYWORDS

Mass production /Conidial yield /Substrate size/Moistening agents, *Paecilomyces vairoti*

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**ABSTRACT** Spore production and growth of *Paecilomyces vairoti* was observed on 5 different types of substrate through solid state fermentation technology viz . maize, bajra, rice, 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 to be a better substrate for maximum spore production. Similarly two moistening agent were used of which the basal salt solution with yeast extract was found most suitable for the growth of *P. vairoti*.

### Introduction

For the biomass production of infective conidia blastospores and mycelium of bio-control fungi, many technologies are currently available. Solid substrate fermentation is frequently used in many countries like Brazil and Colombia with small local production facilities. This technology is a simple one in principle and continuously improving to produce large quantity of non-contaminated and good quality fungal spores of *Paecilomyces vairoti*.

### Materials and Methods

*P. vairoti* maintained on PDA slant was used. Five different substrates viz. bajra, rice, wheat bran and rice with wheat bran (1:1w/w ratio) were selected as solid substrate for observing maximum biomass production and growth of *Paecilomyces vairoti*. All substrates were taken in 2 types of condition, first was their original form in which wheat bran was > 1mm in size and in second all substrate were in the form of fine particles which were size ~1 mm. Growth and sporulation was done in 2 types of moistening agent first was basal salt solution (BSS) alone and another was basal salt solution with 1% yeast extract (w/v) and both moistening agent were used separately for the whole substrate as well as crushed substrate condition. 500gm of each substrates was taken in polypropylene bag (30×45 cm) to which moistening agent was added (30% of substrate weight i.e., 150 ml) and mixed thoroughly. Bags were closed by cotton plug having muslin cloth on it and were subjected to autoclave for 20-30 min (Desgranges et al., 1993, Vega et al., 2003).

Glass bottles of the saline water (500ml) were also used for the rice grain alone and for 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 15lbs pressure.

Inoculation and mass production: Spore suspension of five days old culture of *Paecilomyces vairoti* was inoculated through sterilized 5 ml disposable syringe containing the spore suspension having count more than  $10^6$  spores per ml of suspension. Then all inoculated bags were incubated at  $28 \pm 1^\circ\text{C}$  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 (Antia-Londono et al. 1992).

After 12 days content of each bag and bottle became light brownish due to sporulation of *Paecilomyces vairoti*, which

covered the whole content after 24 day and was appearing brown. Then content of each cultured bag were ground 2-3 times in mixer grinder to obtain maximum amount of spores in powder form. This dry spore powders were directly used as formulation. In some bags moisture content was more than 30% thus the fungus growth was in small clots form, so these types of culture were sieved through 0.5mm pore size sieve 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 after every four hours. For this a loop full of spore suspension was placed on a glass slide kept inside a moist chamber and incubated at  $28 \pm 1^\circ\text{C}$  (Auld and Morin, 1995).

Viability after storage /Formulation: From harvested spore powder 30 gm was mixed carefully in 470 gm of anhydrous silica gel with the 200 mg of UV reflecting agent viz. tenopal. This formulated powder was stored at  $4^\circ\text{C}$  for long time viability. After the required duration of storage germination test was performed using 0.1 gm of mixture and percent germination was calculated (Vega et al., 1999).

### Results and Discussion

Table 1 depicts that whole rice alone, showing maximum conidial production ca.  $1.63 \times 10^6$  and ca.  $7.05 \times 10^6$  and ca.  $7.05 \times 10^6$  conidia/ml for 12<sup>th</sup> and 24<sup>th</sup> day respectively. Wheat bran alone is also good substrate for mass production of conidia which is ca.  $1.57 \times 10^6$  conidia/ml and ca.  $4.74 \times 10^6$  conidia /ml for 12<sup>th</sup> and 24<sup>th</sup> day respectively, but rice grain with wheat bran found second suitable substrate for maximum conidia production which is ca.  $1.55 \times 10^6$  conidia /ml and ca.  $4.81 \times 10^6$  conidia /ml and ca.  $4.81 \times 10^6$  conidia /ml for 12<sup>th</sup> and 24<sup>th</sup> day respectively.

Similar increase ratio of the data is observed in table 2, which is for crushed bajra, maize, rice, wheat bran and rice with wheat bran. Crushed wheat bran showing ca.  $4.27 \times 10^6$  conidia /ml for 12<sup>th</sup> day and ca.  $2.01 \times 10^7$  conidia/ml production for 24 day and crushed wheat bran with crushed rice showing better growth of conidia, which is ca.  $5.91 \times 10^6$  conidia/ml and ca.  $1.03 \times 10^7$  for 12<sup>th</sup> and 24<sup>th</sup> day respectively. Crushed rice showing maximum production of conidia ca.  $8.59 \times 10^6$  and ca.  $2.37 \times 10^7$  conidia/ml for 12<sup>th</sup> and 24<sup>th</sup> 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 the all grains is better than their whole form for the maximum spore production. It is because

all used grains are sources of carbon in the form of starch and for 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. For the mass production of the same fungal strain, solid state fermentation also has done by Singh et al. (2000) on sorghum grains and PDB (Potato Dextrose Broth). Using of crushed grains for this technology done by several scientist for different fungus. Vyas et al. (1991) showed crushed maize to be an ideal substrate for culturing. Aregger et al. (1992) obtained high conidial yield (ca.  $1 \times 10^8$  to ca.  $2 \times 10^9$  conidia/ml) for similar strain on Barely. Devi (1994) also reported intense sporulation of *Nomuraea rileyi* on crushed sorghum.

Table 3 and 4 showed results of whole and crushed grain, which were supplemented with 1% yeast extract in moistening agent. Table 3 depicted that, whole rice repeated best growth agent for *Paecilomyces varioti* where ca.  $5.63 \times 10^7$  and ca.  $4.32 \times 10^8$  conidia/ml production for 12<sup>th</sup> and 24<sup>th</sup> day respectively. Then, mixture of whole rice, with wheat bran showed ca.  $5.11 \times 10^7$  and ca.  $4.20 \times 10^8$  conidia/ml production for 12<sup>th</sup> and 24<sup>th</sup> day respectively. Wheat bran alone is showing good growth of fungi that is ca.  $4.97 \times 10^7$  conidia /ml on 12<sup>th</sup> day and ca.  $4.03 \times 10^8$  conidia /ml on 24<sup>th</sup> day.

Table 4 is showing the effect of crushing of grains. Crushed rice granules showed maximum spore production for 12<sup>th</sup> and 24<sup>th</sup> day ca.  $7.93 \times 10^7$  and ca.  $4.09 \times 10^8$  conidia/ml respectively. In wheat bran conidial mass production was ca.  $6.82 \times 10^7$  (12<sup>th</sup> day) and ca.  $3.43 \times 10^8$  conidia /ml production was obtained in mixture of crushed wheat bran with crushed fine rice granules for 12<sup>th</sup> and 24<sup>th</sup> day respectively.

After comparing the data of table 3 and 4 with table 1 and 2, it was found that yeast extract play crucial role to increase spore production. Mathivanan et al. (2000) 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 in the same technology (Devi, 1994; Holdem and Klaohorst, 1986).

In our research work bajra and maize was also observed to be good substrate to produce spores but not much useful to produce spores in shorter time. Amount of moistening agent also noted to play very important role for the growth of fungus. Excess amount of moistening agent causes clumping of the substrate particles, which create difficulty for fungus 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.  $8.47 \times 10^6$  conidia /ml for 12<sup>th</sup> day and ca.  $2.28 \times 10^7$  conidia /ml for 24<sup>th</sup> 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.  $7.81 \times 10^7$  conidia /ml after 11 day and ca.  $4.08 \times 10^8$  conidia /ml after 23<sup>rd</sup> 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 4 hr was maximum in spores form 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 than wheat bran and other substrate where as Hassan et al. (2002) reported the wheat bran is superior medium than rice and maize in case of *M. anisopliae*.

It is evident from the above results that all natural substrate 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 substrates, optimum temperature and incubation period also play an important role in the present investigations. However market price 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 good solid substrate for the mass production of spores through the industry.

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**Table 1: Solid State mass production of *Paecilomyces varioti* on whole grain (Moistening agent –Basal salt Solution alone)**

S. No	Substrate	Paecilomyces varioti	
		12 <sup>th</sup> day	24 <sup>th</sup> day
1	Bajara	----	$2.30 \times 10^6$
2	Maize	$8.96 \times 10^5$	$1.43 \times 10^6$
3	Rice	$1.63 \times 10^6$	$7.05 \times 10^6$
4	Wheat bran	$1.57 \times 10^6$	$4.74 \times 10^6$
5	Rice+wheat bran	$1.55 \times 10^6$	$4.81 \times 10^6$

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

S. No	Substrate	Paecilomyces varioti	
		12 <sup>th</sup>	24 <sup>th</sup> day
1	Bajra	$5.29 \times 10^6$	$1.83 \times 10^7$
2	Maize	$6.03 \times 10^6$	$1.61 \times 10^7$
3	Rice	$8.59 \times 10^6$	$2.37 \times 10^7$
4	Wheat bran	$4.27 \times 10^6$	$2.01 \times 10^7$
5	Rice+wheat bran	$5.91 \times 10^6$	$1.03 \times 10^7$

**Table 3: Solid state mass production of *Paecilomyces varioti* on whole grain (Moistening agent –Basal salt Solution with 1% Yeast extract)**

S. No	Substrate	Paecilomyces varioti	
		12 <sup>th</sup>	24 <sup>th</sup>
1	Bajra	$1.89 \times 10^7$	$2.30 \times 10^7$
2	Maize	$1.59 \times 10^7$	$2.44 \times 10^7$
3	Rice	$5.63 \times 10^7$	$4.32 \times 10^8$
4	Wheat bran	$4.97 \times 10^7$	$4.03 \times 10^8$
5	Rice+wheat bran	$5.11 \times 10^7$	$4.20 \times 10^8$

**Table 4: Solid state mass production of *paecilomyces varioti* on crucial substrate (~1mm) (Moistening agent –Basal salt Solution with 1% Yeast extract)**

S.No	Substrate	Paecilomyces varioti	
		12 <sup>th</sup>	24 <sup>th</sup>
1	Bajara	$6.32 \times 10^7$	$1.38 \times 10^8$
2	Maize	$5.39 \times 10^7$	$1.47 \times 10^8$
3	Rice	$7.93 \times 10^7$	$4.09 \times 10^8$
4	Wheat	$6.82 \times 10^7$	$3.43 \times 10^8$
5	Rice+Wheat bran	$7.00 \times 10^7$	$3.81 \times 10^8$

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