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Suitability of various spawn and different substrate on the sporophore yield of multispore isolates of *Pleurotus* spp.

P Renganathan, T Sivakumar, R Kannan, K Vinothini and KR Saravanan

Abstract
Mushrooms have achieved significant importance in many countries due to their high nutritive and genuine medicinal values as well as an income generative venture. Blessed with varied agro-climates, Indian weather is aptly suitable for the cultivation of edible mushrooms. Among the spawn substrates tested, "panivaragu" grains was not found suitable for the production of spawn as it recorded the lowest yield in all the isolates. With regard to various isolates tested, the spawn of the isolate *Pe x Po* prepared by using IFPg required minimum spawn run days (10.45) and produced the maximum yield (585.10 g/bed) and bio-efficiency (117.02 %) which was followed by *Pc x Pe, Pc x Pfl, Pf x Po* and *P. eous*(standard parent) in the decreasing order of merit. Among the isolates the minimum days for the first harvest (11.13 days) and maximum yield (635.00 g/bed) was observed with the isolate *Pe x Po* with paddy straw used as bed substrate. This was followed by paddy straw + sugarcane trash with a yield of 405.67 g/bed. It is evident from the result that there was considerable fluctuation in sporophore yield and biological efficiency with different isolates studied. The weight of sporophore was significantly low in other substrates. A similar trend was observed with other yield parameters tested.

Keywords: *Pleurotus* spp. multispore isolates, ill filled paddy grains, sorghum grains, paddy straw

Introduction
Mushrooms have achieved significant importance in many countries due to their high nutritive and genuine medicinal values as well as an income generative venture. Blessed with varied agro-climates, Indian weather is aptly suitable for the cultivation of edible mushrooms. The entire coastal belts of India running in to thousands of kilometers is a potent place to produce low cost specialty mushrooms which could supplement the protein deficiency and malnutrition, besides bringing in a sky – rocketing export market of a kind which is incomparable to any single cell protein (SCP) product (Kohlii, 2000) [16].

It is estimated that about 355 million tonnes of crop residue is generated annually and about 170 million is left out posing problems for disposal (Tewari and Pandey, 2002) [35]. Even if one per cent of this agricultural waste is used to produce mushrooms, India will soon become a major mushroom producing country in the world. Mushroom production is the only biotechnological means available to convert these agricultural wastes into highly valuable edible proteins. So far around 5658 species of mushroom in 230 genera have been recorded from all over the world; where as from India 850 species spread over 115 genera have been reported. Of this 850 species about 20 are being commercially cultivated (Saini and Atri, 1995) [26].

Among these, the White Button mushroom (*Agaricus bisporus*), oyster mushroom (*Pleurotus* spp.), Paddy straw mushroom (*Volvariella volvacea*) and Milky mushroom (*Calocybeindica*) are popular among the commercial growers in India as the techniques for their cultivation have been well developed (Vijaya Khader *et al.*, 1998) [37]. World mushroom production at present is estimated to be around 5 million tonnes/annum and is increasing @ 7 per cent/annum. The total mushroom production in India has increased from 4000 tonnes in 1955 to 30,000 tonnes in 1995 and it is estimated to be around 50,000 tonnes / annum (Tewari, 2004) [36].

Agaricus bisporus is highly temperature specific, and its cultivation is restricted to temperate regions. But oyster mushrooms can be cultivated easily in tropical and subtropical regions. Hence, it is rightly named as "the crop of the future". *Pleurotus* spp. has the ability to degrade most of the lignocellulosic agro wastes, thus the cultivation of this mushroom is an efficient means for the conversion of agricultural wastes in to valuable edible proteins (Deepika Sud and Sharma, 2005) [8].

The farmers and consumers have also developed preference towards *Pleurotus* spp. in recent years because of its advantages *viz.*, high nutritive value and easiness in cultivation using the farm wastes (Eswaran, 1998) [9].

Among the thirty eight species of *Pleurotus* existing in nature, only nine species are being cultivated under artificial condition (Jandaik, 1987) [14]. Every species has its own attributes and each is known for its yield, substrate utilization and wide temp. Adoption (Ravichandran, 2001) [25]. Inspite of its easy cultivation methods and adaptation to wide range of temp., the production of *Pleurotus* spp. is very less when compared to button mushroom production in India. Hence, a need was felt for up scaling the yield potential of *Pleurotus*spp. For large scale production.

Materials and Methods

Organism

The pure culture of *Pleurotus* spp. (*Pleurotus citrinopileatus* (Fr.) Singer, *P. djamor* (Rumph.) Boedijn, *P. eous* (Berk) Sacc, *P. flabellatus* (Berk and Br.) Sacc., *P. florida* (Eger) and *P. ostreatus* (Jacq.Fr.) Kummer) were obtained from National Centre for Mushroom Research (NCMR) Chambaghat, Solan, Himachal Pradesh. The sub cultures were maintained on oat meal agar (OMA) medium.

Pc- Pleurotus citrinopileatus
Pd- Pleurotusdjamor
Pe- Pleurotuseous
Pf- Pleurotus flabellatus
Pfl- Pleurotus florida
Po- Pleurotus ostreatus

Isolation and Purification

The mushroom tissue was cut at the junction of the pileus and stipe using a sterile scalpel and surface sterilized with 95 per cent ethyl alcohol for one min. These bits were placed on OMA in sterile Petri dishes and incubated at room temp. (28 ± 2° C) for seven days. The isolates were then purified by single hyphal tip method and maintained on OMA slants.

Preparation of Spawn

Sorghum grain spawn was prepared by adopting the method described by Sivaprakasam (1980) [32]. Sorghum grains were partially cooked in water for 40 min. After draining the excess water, the grains were mixed with calcium carbonate at two per cent (w/w) to prevent adhesion of the grains and for optimizing pH. The grains were filled up to two-third volume of glass glucose drip bottles plugged with non-absorbent cotton wool; the mouths were wrapped and sterilized at a pressure of 15 psi. for two h. The grains were inoculated with pure cultures of the fungus and incubated at room temp. (28 ± 2° C). All these were carried out under aseptic condition. The nature of the growth and time taken for complete colonization of the spawn were recorded.

Cultivation Trials

Preparation of Mushroom Bed

Cultivation of *Pleurotus* was carried out in transparent polythene bags of 60 x 30 cm size and thickness of 100 gauges. Cylindrical beds were prepared using 0.5 kg of paddy straw on dry weight basis, following the method described by Eswaran (1998) [9]. The unchopped whole straw was made into coils and used. A layer of coiled paddy straw was placed at the bottom of polythene bag. Over this, a layer of spawn was placed. In this manner five layers of coiled paddy straw and four layers of spawn were placed in the polythene bag and then the bag was tied at the top. The mushroom beds were hung from the ceiling by means of ropes ("Uri" method) instead of the usual method of keeping them in tiers made of bamboo or casuarina stacks. Two holes were made in the polythene bags and the beds were kept in cropping room, where the temp. was maintained between 23 to 28◦C and relative humidity between 80 to 90 per cent. Water was sprinkled regularly to maintain adequate moisture and relative humidity. The following yield parameters were studied in all the experiments.

Spawn Run

Number of days taken for 100 per cent colonization/ mycelial coverage on the substrate was recorded as spawn run period.

Time Taken For First Harvest

The number of days required for first harvest of the sporophores from the date of spawning of the bed was recorded.

Weight of Sporophores

The sporophores were weighted after harvest and yield per bed in g. was recorded.

Biological Efficiency

The biological efficiency of *Pleurotus* spp. was calculated by

$$\text{Biological efficiency (\%)} = \frac{\text{Fresh weight of the mushrooms / bed}}{\text{Dry weight of the substrates / bed}} \times 100$$

Cultivation Technology

Suitability of Various Substrates for Spawn Production

To select a suitable substrate for spawn production of the selected high yielding isolates, seven cereal grains (Ill-filled paddy (*Oryza sativa* L.), maize (*Zea mays*), panivaragu (*Panicummiliare*), pearlmillet (*Pennisetum americanum*), samai (*Panicummilliaceum*), sorghum (*Sorghum vulgare*) and thenai (*Setariaitalica*)) were used for spawn preparation as per standard procedure (Munjal, 1973). The ill-filled paddy grains (IFPg) were separated using winnowing technique. The spawn substrates were taken up to two third of the saline bottles and sterilized. After sterilization, the bottles containing spawn substrates were inoculated with pure culture of the isolates and incubated at room temp. (28 ± 2◦ C) until complete colonization took place. The spawn thus prepared was further evaluated for its productivity using steam pasteurized paddy straw as bed substrate. The less expensive and effective IFPg spawn base identified in the present study was used for subsequent experiments.

Suitability of Various Substrates for the Bed Preparation

Various crop residues such as paddy straw, sawdust, sugarcane trash, groundnut haulm and their combination (1:1 w/w) with paddy straw were used to evaluate as bed substrates. The substrates were dried in sun and soaked overnight in water. The excess water was drained and then these were immersed in hot water for 60 min. After air drying in shade, beds were prepared using these substrates. Ill-filled paddy grain spawn (supplemented with horsegram @ 4%) of the various isolates *viz*., *Pc x Pe, Pc x Pfl, Pe x Po, Pf x Po* and standard parent *P. eous* was used and beds were prepared. The observations on yield attributes *viz*., days to first harvest, total sporophore yield and biological efficiency were recorded.

Effect of Various Spawn Substrates on the Sporophore Yield of Multispore Isolates of *Pleurotus* spp.

The results depicted in table 17 revealed that ill-filled paddy grains and sorghum grains as spawn substrates recorded higher sporophore yield and bio-efficiency than the other substrates tested in all the isolates. Among the spawn substrates tested, "panivaragu" grains was not found suitable for the production of spawn as it recorded the lowest yield in all the isolates. With regard to various isolates tested, the spawn of the isolate *Pe x Po* prepared by using IFPg required minimum spawn run days (10.45) and produced the maximum yield (585.10 g/bed) and bio-efficiency (117.02 %) which was followed by *Pc x Pe, Pc x Pfl, Pf x Po* and *P. eous*(standard parent) in the decreasing order of merit.

Suitability of bed substrates

Suitability of Substrates for the Cultivation of Multispore Isolates of *Pleurotus* spp.

The results on the suitability of various substrates for the cultivation of multispore isolates of *Pleurotus* spp. are furnished in table 2. Among all the substrates, paddy straw was found to be the best substrate for the cultivation of multispore isolates of *Pleurotus* spp. Paddy straw as bed substrate recorded minimum days for the first harvest and maximum yield in all the isolates tested. This was followed by paddy straw + sugarcane trash (1:1) and paddy straw + groundnut haulm (1:1). The least effect was observed with saw dust used as bed substrate. A similar trend was observed with biological efficiency

Among the isolates the minimum days for the first harvest (11.13 days) and maximum yield (635.00 g/bed) was observed with the isolate *Pe x Po* with paddy straw used as bed substrate. This was followed by paddy straw + sugarcane trash with a yield of 405.67 g/bed. It is evident from the result that there was considerable fluctuation in sporophore yield and biological efficiency with different isolates studied. The weight of sporophore was significantly low in other substrates. A similar trend was observed with other yield parameters tested.

Table 1: Effect of Various Spawn Substrates on the Sporophore Yield of Multispore Isolates of *Pleurotus* spp.

S.no	Spawn substrattes	*Pc x Pe*			*Pc x Pfl*			*Pe x Po*			*Pf x Po*			*P. eous*		
		Days for spawn run	Yield (g/ bed)	Bio efficiency (%)	Days for spawn run	Yield (g/ bed)	Bio efficiency (%)	Days for spawn run	Yield (g/ bed)	Bio efficiency (%)	Days for spawn run	Yield (g/ bed)	Bio efficiency (%)	Days for spawn run	Yield (g/bed)	Bio efficiency (%)
1	Ill filled paddy grain (*Oryzasativa* L.)	11.09	540.00	108.00	11.12	535.12	107.02	10.45	585.10	117.02	11.57	520.26	104.05	11.61	475.15	95.03
2	Sorghum grain (*Sorghum vulgare*)	11.76	529.25	105.85	11.82	532.00	106.40	11.67	576.00	115.20	11.90	495.15	99.03	11.94	465.00	93.00
3	Maize grain (*Zea mays*)	14.56	365.67	73.13	14.59	348.26	69.65	14.35	387.25	77.45	14.71	295.26	59.05	14.76	310.21	62.04
4	Pearl millet grain (*Pennisetum americanum*)	13.48	280.24	56.05	13.51	295.61	59.12	13.45	290.10	58.14	13.59	280.45	56.09	13.63	240.15	48.03
5	Panivaragu grain (*Panicummiliare*)	13.85	202.00	40.40	13.90	195.56	39.11	13.67	210.35	42.07	13.95	188.00	37.60	13.98	180.40	36.08
6	Samai grain (*Panicummilliaceum*)	12.55	235.20	47.04	12.59	225.20	45.04	12.45	240.12	48.02	12.63	210.57	42.11	12.68	270.15	54.03
7	Thenai grain (*Setariaitalica*)	12.76	212.10	42.42	12.80	208.00	41.60	12.69	225.22	45.04	12.85	196.80	39.36	12.90	256.47	51.29
S.Ed		0.22	4.49		0.22	4.39		0.45	3.59		0.22	4.49		0.09	3.37	
CD (P = 0.05)		0.45	9.02		0.44	8.84		0.90	7.21		0.45	9.02		0.18	6.77	

Table 2: Suitability of different substrates for cultivation of various Multispore isolates of *Pleurotus* spp.

S. No.	Substrates	*Pc x Pe*		*Pc x Pfl*		*Pe x Po*		*Pf x Po*		*P. eous*	
		DFH	Yield (g)	DFH	Yield (g)	DFH	Yield (g)	DFH	Yield (g)	DFH	Yield (g)
1.	Paddy straw	11.27	610.15	12.87	605.00	11.13	635.00	13.45	590.16	13.45	550.65
2.	Sugarcane trash	21.69	290.00	22.71	278.67	20.67	305.00	23.00	275.00	23.85	261.16
3.	Saw dust	25.37	145.13	25.87	135.33	24.35	164.67	26.13	115.00	26.55	96.00
4.	Groundnut haulm	22.05	198.50	23.15	186.00	21.05	205.67	24.00	186.33	24.60	175.00
5.	Paddy straw + Sugarcane trash (1:1)	19.42	395.20	20.43	384.33	18.37	405.67	21.15	381.37	21.87	368.67
6.	Paddy straw + Saw dust (1:1)	21.41	360.20	22.51	350.15	20.37	365.00	23.05	345.33	23.15	329.43
7.	Paddy straw + Groundnut haulm (1:1)	20.15	380.00	21.05	365.33	19.00	393.00	22.00	368.67	22.67	356.33
S.Ed		0.85	8.97	1.35	8.98	1.12	11.22	1.12	8.97	0.67	8.97
CD (P = 0.05)		1.71	18.04	2.71	18.04	2.26	22.55	2.26	18.04	1.35	18.04

DFH – Days to first harvest

Yield – Yield /bed/500 g substrate

Discussion

A wide variety of cereal grains were found suitable for spawn preparation and several workers have tried many other substrates or agricultural wastes because of their availability, accessibility and low cost (Kadiri, 1999; Nwanze *et al.*, 2005; Sharma and Puttoo, 2004) [5, 20, 29]. Spawn, the vegetative seed

material plays an important role in mushroom cultivation. The substrate on which the spawn is prepared, affects the mushroom production. In the present study, among the spawn base tried, ill-filled paddy grain (IFPg) spawn required the lowest period for complete colonization and gave the highest yield which was on par with sorghum grains (Table 1).

The variation in colonization of different substrates could be due to variation in the amount of moisture absorbed during boiling which is one of the critical factors responsible for mycelial growth (Mehta, 1985) [17]. The poor utilization of subabul (*Leucaenaleucocephala*) seed as a spawn substrate in the present study could be either due to less moisture absorbance, lack of aeration and space for the mycelial spread and colonization. Rangad and Jandaik (1977) [24] found maximum yield with sorghum spawn in different *Pleurotus*spp.

Good yield of *P. sajor-caju* was obtained with sorghum and pearlmillet spawns (Sivaprakasam and Kandaswamy, 1983; Sharma and Puttoo, 2004) [29]. Sharma (1984) [28] recorded equally good colonization of *P. eryngii* on wheat, barley, sorghum and pearlmillet grains. Beds laid out using half-filled grain spawn recorded good yield (Gokulapalan *et al.*, 1994) [12]. *Panicummiliare* grains showed shortest period of eight days for spawn development indicating its suitability for efficient spawn production and this duration was significantly less than the wheat grains (Bharat Bhooshan Sharma, 2003) [6].

Corn and millets spawn induced comparable carpophores wet weight which was superior to that induced by wheat spawn (Nwanze *et al*., 2005) [20]. These reports by the earlier workers lend support to the present findings. The favorable results with IFPg spawn could be due to increased surface area. The use of IFPg has an added advantage from the economic point of view as it is cheaper and gave more number of spawn bottles per unit weight as reported by Eswaran *et al.* (1998) [9].

The yield of *Pleurotus* spp. Depends on the nature of substrate used as bed material (Zadrazil, 1978) [38]. In the present study, the substrates such as paddy straw, paddy straw + sugarcane trash (1:1), paddy straw + groundnut haulm (1:1), paddy straw + saw dust (1:1) and sugarcane trash recorded lesser days for the first harvest and increased the sporophore yield in all the multispore isolates tested (Table 2).

Superiority of paddy straw for the cultivation of *Pleurotus* spp. had been reported by many workers (Nallathambi, 1991; Pardeepkumar *et al.*, 2000; Gunjan Gogoi and Adhikary, 2002; Periasamy and Natarajan, 2004) [18, 19, 21, 13, 23]. The yield of sporophores was positively correlated to the cellulose content and cellulose: lignin ratio (Sivaprakasam and Kandasamy, 1981c) [33]. Geetha and Sivaprakasam (1994) [11] also reported the maximum yield of oyster mushrooms in the case of paddy straw as a substrate with minimum days for the first harvest. Eyini *et al.* (1995) [10] reported higher yield of *P. ostreatus*in paddy straw pre treated with lime water.

Likewise, Bahukhandi and Munjal (1989) [1] reported that the maximum yield in *P. sajor-caju*, *P. sapidus*, *P. ostreatus*and *P. florida*were obtained when grown on groundnut shell and paddy straw. Increase in yield of *P. sajor-caju*wasobtained when these were grown on maize cob and paddy straw and combination of corn cob and saw dust (Dar *et al.*, 1989) [7]. Soybean residue was reported to be one of the best substrates for oyster mushroom production (Patil and Jadhav, 1991) [22]. The suitability of sugarcane trash for cultivation of the oyster mushroom was reported by several earlier workers (Singh *et al.*, 1992; Singh *et al.*, 1995) [31, 30].

The result of the present study is in agreement with Sangeetha (2006) [27] who obtained significantly higher yield of oyster mushroom with paddy straw substrate. Nallathambi (1991) [18, 19] claimed the superiority of legume straw over sugarcane bagasse and saw dust for the production of oyster mushroom.

Also, rice husk, sorghum stover, saw dust, cotton waste, banana leaves, cocoa bean shell and saw dust have been reported as suitable substrates for the cultivation of edible mushrooms (Belewu, 2001; Belewu and Ademilola, 2002; Belewu and Lawal, 2003; Oei, 2003; Belewu and Belewu, 2005) [2, 3, 5, 4]. All these earlier reports corroborates and lend support to the present findings.

It is probable that paddy straw substrate provided a more balanced supply of cellulose and nutrients to the fungus that resulted in the increased biological efficiency. Also this might be due to the production of more of lytic enzymes by the fungus which resulted in better utilization of the substrates as reported by Sivaprakasam (1980) [32].

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