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RESEARCH ARTICLE

Effect of spawn substrates on yield of *Pleurotus eous* (Berk.) Sacc.

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SUMMARY

Grains of cereals and pulses and crop residues (straws) were assessed to determine their suitability for production of spawn and sporophores of *Pleurotus eous* (Berk.) Sacc. For each replicate of the various treatments, the days from inoculation of the spawn substrates till total colonization were recorded. Mycelium of *P. eous* indicated marked preference for cereal grains over pulses and crop residues. Among the cereals, ragi grain colonized the best in only 6 days, followed insignificantly by maize, pearl millet, sorghum, wheat and paddy grains. Pulses did not allow growth of the fungal mycelium. Colonization of various straws was prolonged considerably. The cereal grain and straw spawns were used as inocula on wheat straw to compare their yield characteristics. Parameters assessed included the spawn run period, number of days from spawning till appearance of pinheads, the days from spawning to first flush and, fresh yield of sporophores. Biological efficiency was also determined. The results indicated that ragi, maize, pearl millet and sorghum grain spawns accelerated the spawn run, pinning and maturity of sporophores and gave higher sporophore yield.

Key Words : *Pleurotus eous,* Oyster mushroom, Spawn substrates

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Oyster mushroom (*Pleurotus* spp.) cultivation has increased tremendously throughout the world during the last few decades (Chang, 1999; Royse, 2002). Oyster mushroom accounted for 14.2 per cent of the total world production of edible mushrooms in 1997 (Chang, 1999). Gastronomically, oyster mushrooms are the only cultivated mushrooms classified among first quality mushrooms. Button mushrooms and shiitake are classified as mushrooms of second quality (Kohli, 1999). Above all, the wide choice of species available for cultivation under different climatic conditions has made this mushroom, a mushroom of a broad adaptability (Wani and Sawant, 1998). Among the various species of *Pleurotus, P. sajor-caju* is known to produce very high yields. However, consumer acceptability of *P. sajor-caju* has not been appreciable widely due to tough texture of stipe, brown to gray colour of the pileus and moderate aroma (Prabhu Dessai *et al.,* 1991). This calls for substitute to *P. sajor-caju* with a species having desirable attributes of consumer liking. *Pleurotus eous* (Berk.) Sacc. is a conspicuous species and because of its attractive bright pink sporophores, firm crisp and melting texture, it ranked higher in sensory evaluation *vis-à-vis P. sajor-caju. Pleurotus eous* also has yield potential comparable to that of *P. sajor-caju* and also it comes to yield earlier (Prabhu Dessai *et al.,* 1991). Proximate composition and nutritive constituents of this species are also similar to that of other species of *Pleurotus.* The protein content is 33.24 per cent on dry weight basis. It contains 13 free amino acids *viz.,* serine, glutamic acid, alanine, glycine, lysine, aspartic acid, arginine, tyrosine, methionine, valine, phenylalanine, isoleucine and leucine. In addition, the presence of cystine and threonine was also observed (Singh and Rajarathnam, 1977). *Pleurotus eous* contains moisture 93.73, protein 33.68, soluble carbohydrate 29.30, insoluble carbohydrate 26.10, crude fibre 2.16, fat 0.53 and ash 4.35 per cent on dry weight basis (Prabhu Dessai *et al.,* 1991). It is rich in iron (0.1972 g/100 g dry weight), aluminium (0.1558 g/100 g dry weight), potassium (2.98 g/100 g dry weight), calcium (0.3028 g/100 g dry weight) and magnesium (0.2137 g/ 100 g dry weight) (Gupta, 1998). It is likely that, *Pleurotus eous* mushroom has good future market. Yet there is a need for finding out the factors responsible for yield

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increase, since no many attempts have been made in this direction. Especially the vigour of spawn and medium used for spawn production influences the yield of mushrooms. Failure to achieve a satisfactory harvest may often be traced to unsatisfactory spawn used (Chang, 2009). This report seeks to compare the efficiency of grains of cereals, pulses and various straws for the production of spawn of *P. eous* and to assay the yield and biological efficiency of the mushroom when the spawns are used as inocula for cultivation of mushroom.

MATERIALS AND METHODS

The present investigation was conducted at the Department of Plant Pathology and Agricultural Microbiology, Mahatma Phule Krishi Vidyapeeth, Rahuri (M.S.). Pure culture of *Pleurotus eous* was isolated from sporophore which appeared on the straw bed inoculated with the spawn material from All India Coordinated Research Project on mushroom, College of Agriculture, Pune; by tissue culture technique and was maintained on potato dextrose agar. Cereal grains, pulses and dried straws were tested as substrates for spawn production and these were: 1) maize (*Zea mays* L.) grain, 2) paddy (*Oryza sativa* L.) grain, 3) pearl millet [*Pennisetum glaucum* (L.) R.Br.] grain, 4) ragi (*Eleusine coracana* Gaertn) grain, 5) sorghum [*Sorghum bicolor* (L.) Moench.] grain, 6) wheat (*Triticum aestivum*) grain, 7) blackgram [*Vigna mungo* (L.) Hepper] grain, 8) chickpea (*Cicer arietinum* L.) grain, 9) greengram [*Vigna radiata* (L.) Wilczek] grain, 10) lentil (*Lens culinaris* Medic) grain, 11) soybean [*Glycine max* (L.) Merrill] grain, 12) cotton (*Gossypium hirsutum* L.r. Hutch.) stalks, 13) paddy (*Oryza sativa* L.) straw, 14) pearl millet [*Pennisetum glaucum* (L.) R.Br.] stalks, 15) ragi (*Eleusine coracana* Gaertn) straw, 16) sorghum [*Sorghum bicolor* (L.) Moench] straw, 17) soybean [*Glycine max* (L.) Merrill] straw and, 18) wheat (*Triticum aestivum*) straw. Grain spawns were prepared by the method recommended by Garcha and Kalra (1979). For preparation of straw spawns the method described by Kathe *et al.* (1996) was employed. The treatments were replicated thrice. Observations on per cent colonization of spawn substrate by the mushroom mycelium were recorded at an interval of 2 days till all the substrates under study were fully colonized by the mycelium. Number of days required for full growth of mycelium on the spawn substrates were also recorded. Based on the results, cereal grain and straw based spawns were selected for further studies to evaluate their effect on the yield of sporophores.

Freshly harvested well-dried wheat straw was used for cultivation of *P. eous*. The cultivation substrate was prepared by hot water treatment as described by Singh and Dwivedi (1991). All the instruments were sterilized with dilute solution of potassium permanganate and alcohol. The spawning was done in pre-fumigated room (48 h with 2% formaldehyde). Pasteurized cultivation substrate was layer spawned in high density polythene bags of size 45 cm x 55 cm (100 gauge). Cereal grain spawns were inoculated at 2 per cent, whereas straw spawns were inoculated at 10 per cent of the wet weight of cultivation substrate. These bags had 25-30 pinhole perforations all over for ventilation of substrate. Few pinholes were also made at bottom of the bag for drainage. Each bag contained 3.5 kg wetted straw (one kg dry straw). Mouth of each bag was closed with a rubber band. Each treatment was laid out according to the completely randomized design with three replications. After spawning, the bags were incubated in dark at an ambient temperature of 24-28^{0}C in partially underground permanent mushroom house. No light and cross ventilation were allowed during spawn run. After the spawn had colonized the substrate fully with fungal mycelium, the polythene bags were removed to expose the substrate surface for initiation of sporophores. These were then kept on wooden racks in cropping room of the mushroom house for fruiting. Water was sprayed regularly (twice a day) on the compact cylinder mass of the substrate to keep them wet. The temperature of cropping room ranged from 22-28^{0}C during the experimental period. Humidity of the cropping room was maintained at 85-90 per cent by frequent sprays of water on the walls and floor. For cropping, 2-3 hours of light (with the help of fluorescent tube of 40 watts) and 2-3 hours of cross ventilation per 24 hours (by opening doors and windows) were provided. Watering was withheld a day before harvesting. Harvesting of sporophores was done before spore shedding. Fresh weight of mushroom was recorded immediately after harvesting. Small portion of straw all over the surface of bed was scrapped in order to obtain each next crop. Three flushes of the sporophores were harvested. Data recorded included the spawn run period *i.e.* the number of days from spawning to complete colonization of the cultivation substrate by the mycelia, number of days from spawning till appearance of pinheads, the days from spawning to first flush and fresh yield of sporophores. Biological efficiency (BE) was also determined using the relation (Chang *et al.,* 1981):

$$\textbf{B.E.(\%)} \left[\frac{\textbf{Weight of fresh mushrooms harvested}}{\textbf{dry weight to substrate}}\right] \textbf{x100}$$

Data were tested for their significance by employing completely randomized design.

RESULTS AND DISCUSSION

Growth of *Pleurotus eous* mycelium was found on all the substrates under study, barring the pulses. However, time taken by the mycelium to colonize the substrates completely showed varying responses (Table 1). Fungal mycelium promptly colonized the cereal grains in 6 to 8 days. Among the cereals, ragi grain supported *P. eous* for 100 per cent colonization in only 6 days, followed insignificantly by maize, pearl millet, sorghum, wheat and paddy grains, where it took 6.7, 7, 7.3, 8 and 8 days, respectively. Pulses did not support growth of the mycelium, which thus implies ineffectiveness of pulses as spawn substrate. Colonization of various straws with the mycelium was considerably prolonged, varying from 12.7 to 20.3 days. Among the straws, 100 per cent growth of *P. eous* mycelium reached on 12.7 days in cotton stalks and 20.3 days in pearl millet stalks. Thus, ragi, maize, pearl millet, sorghum, wheat and paddy grains were found to be the best and the pearl millet stalks to be inferior for spawn production of *P. eous*.

Chauhan and Pant (1988) also reported rapid mycelial growth rate of spawn in the grains than the straw substrates. Moreover, non-colonization of pulse substrates would indicate preference of this mushroom for starchy material and its inability to hydrolyze and metabolize proteinaceous substances for growth. Kotwaliwale *et al.* (1991) also reported infeasibility of pulses for the spawn production and use of maize grain as a good proposition for the spawn production of *Pleurotus* spp. Results of these workers are in agreement with the results reported in present investigation.

Since the pulses were found to be inferior for the spawn production, further study to assess the efficacy of various spawns was designed by using cereal grain spawns and straw spawns only and the results obtained from the investigation as well as relevant discussion have been presented under following heads:

Days taken for complete spawn run :

Different cereal grain and straw based spawns had significantly varied effect on the days required for complete spawn run (Table 2). Data revealed superiority of the grain spawns in general and ragi, pearl millet, sorghum and maize grain spawns in

Table 1 : Growth of *Pleurotus eous* mycelium on different spawn substrates

Spawn substrate	Per cent colonization of spawn substrate by *Pleurotus eous*											Days for full growth of mycelium
	2 DAI*	4 DAI	6 DAI	8 DAI	10 DAI	12 DAI	14 DAI	16 DAI	18 DAI	20 DAI	22 DAI	
Maize grain	26.7	56.7	76.7	100.0	-	-	-	-	-	-	-	6.7
Paddy grain	18.3	38.3	61.7	96.7	100.0	-	-	-	-	-	-	8.0
Pearl millet grain	23.3	48.3	73.3	100.0	-	-	-	-	-	-	-	7.0
Ragi grain	30.0	63.3	100.0	-	-	-	-	-	-	-	-	6.0
Sorghum grain	21.7	48.3	71.7	100.0	-	-	-	-	-	-	-	7.3
Wheat grain	18.3	40.0	66.7	100.0	-	-	-	-	-	-	-	8.0
Blackgram grain	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	-
Chickpea grain	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	-
Greengram grain	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	-
Lentil grain	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	-
Soybean grain	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	-
Cotton stalks	11.7	23.3	48.3	65.0	80.0	93.3	100.0	-	-	-	-	12.7
Paddy straw	5.0	11.7	33.3	51.7	63.3	75.0	86.7	100.0	-	-	-	15.7
Pearl millet stalks	NG	NG	X	10.0	25.0	45.0	61.7	76.7	85.0	93.3	100.0	20.3
Ragi straw	X	8.3	25.0	48.3	58.3	68.3	81.7	96.7	100.0	-	-	16.3
Sorghum stalks	NG	X	11.7	26.7	50.0	63.3	71.7	85.0	100.0	-	-	17.7
Soybean straw	8.3	18.3	40.0	61.7	71.7	86.7	100.0	-	-	-	-	14.0
Wheat straw	NG	X	8.3	23.3	43.3	56.7	65.0	80.0	100.0	-	-	18.3
S. E. ±												1.4
C.D. (P=0.05)												4.2

*DAI : Days after inoculation
NG : No growth
X : Initiation of mycelial growth
Data are mean of three replications

particular, over straw spawns. This was evident from considerable reduction in the period required for complete colonization of cultivation substrate by the *Pleurotus eous* mycelium. Beds inoculated with ragi grain spawn were colonized completely in 7.7 days, closely followed by pearl millet, sorghum and maize grain spawns, which took 8, 8 and 8.3 days, respectively. In contrast, spawn run was much delayed with straw spawns in general and the latest with wheat straw and pearl millet stalk spawns which took 18.3 and 18.7 days, respectively. These observations substantiate that the spawn-running period with ragi, pearl millet, sorghum and maize grain spawns was abridged by 11, 10.7, 10.7 and 10.4 days, respectively in comparison to pearl millet straw spawn. The spawn-running period with various straw spawns was delayed by 6 to 11 days in comparison to ragi grain spawn.

Variation in spawn running period may be attributed to the size of the grains. Smaller grains have a greater number of inoculation points per kg than larger grains (Mamiro and Royse, 2008). This report is in conformity with the results of present study where smaller grain spawns *viz.,* ragi and pearl millet, although differed insignificantly from sorghum and maize grain spawns, numerically were superior from the latter in hastening the spawn run. Moreover, straw spawns have least number of inoculation points per kg than grain spawns and hence might had delayed the spawn running with them in present study. Thus, different types of spawn may influence growth and productivity. Chauhan and Pant (1988) also observed early spawn run with jowar, bajra and wheat grains than straw substrates. Pathmashini *et al*. (2008) found accelerated spawn running of *Pleurotus ostreatus* with kurakkan (*Eleusine coracana*) spawn. Results of the present investigation are in agreement with these workers.

Days taken for pinhead formation :

Significantly varied response of different spawn substrates to the period required to induce pinning was observed (Table 2). Among the various grains tested in the present investigation, the earliest pinning was observed in the beds inoculated with ragi, pearl millet, sorghum and maize grain spawns. Beds inoculated with these spawns induced pinning in 10, 10.3, 10.3 and 10.7 days, respectively. Much delayed pinning, varying from 16.3 to 21.7 days, was observed in the beds spawned with straw based spawns. Pearl millet stalks and wheat straw spawns, however, were the poor performers amongst the different straw substrates.

Reduction in spawn running period due to various grain spawns might have reflected in early pinning with them. Pathmashini *et al*. (2008) found earlier pinning of *Pleurotus ostreatus* with kurakkan (*Eleusine coracana*) spawn. This finding is in conformity with the present results.

Table 2 : Effect of different spawn substrates on number of days taken for spawn run, pinhead formation and first harvest and, yield and biological efficiency of *Pleurotus eous*

Spawn substrate	Number of days taken for			Average yield of fresh sporophores (g/kg dry straw)	Biological efficiency (%)
	Complete spawn run	Pinhead formation	First harvest		
Maize grain	8.3	10.7	13.0	982.3	98.2
Paddy grain	10.0	12.7	15.7	924.0	92.4
Pearl millet grain	8.0	10.3	13.0	978.0	97.8
Ragi grain	7.7	10.0	12.3	984.0	98.4
Sorghum grain	8.0	10.3	13.0	976.0	97.6
Wheat grain	9.7	12.3	15.3	928.0	92.8
Cotton stalks	13.7	16.3	19.0	862.3	86.2
Paddy straw	16.0	19.0	22.0	774.0	77.4
Pearl millet stalks	18.7	21.7	24.7	624.0	62.4
Ragi straw	16.0	19.0	22.0	768.0	76.8
Sorghum stalks	16.7	19.7	22.7	684.0	68.4
Soybean straw	15.7	18.3	21.3	826.3	82.6
Wheat straw	18.3	21.3	24.3	646.0	64.6
S. E. ±	0.4	0.5	0.5	9.5	-
C.D. (P=0.05)	1.1	1.4	1.6	27.7	

Data are mean of three replications

Days taken for first harvesting :

Crop duration is an economically important aspect in the cultivation of any crop. Various spawn substrates tried in the investigation varied significantly in number of days from spawning to first picking of sporophores (Table 2). In general, grain spawns showed excellent performance *vis-à-vis* straw spawns in hastening maturity of sporophores. Beds inoculated with ragi spawn were ready for first harvest in 12.3 days, followed insignificantly by maize, pearl millet and sorghum grain spawns, which took 13 days for the harvest. In contrast, first picking of sporophores was considerably delayed with straw spawns, which varied from 19 to 24.7 days, the latest being with pearl millet stalk spawn. Thus, the results clearly indicate that the period required for first harvest with ragi grain spawn was abridged by 12.4 days, whereas with maize, pearl millet and sorghum grain spawns it was abridged by 11.7 days in comparison to pearl millet stalk spawn.

Sivaprakasam and Kandaswamy (1981) also obtained the first crop earlier with sorghum, bajra and maize grain spawns. Pathmashini *et al.* (2008) found that the time taken for the first flush of *Pleurotus ostreatus* was longest in paddy spawn (43 ± 1 days) while sorghum, kurakkan (*Eleusine coracana*), and broken maize took 32 ± 1, 31 ± 1, and 38 ± 1 days, respectively.

Sporophore yield and biological efficiency :

Yield data were pooled from all the three harvests of each replication and expressed as g/kg dry straw. Effect of various spawn substrates on the yield of sporophores was significantly variable (Table 2). In general all the grain spawns, however, indicated their superiority over straw spawns by the way of substantial improvement in the yield of sporophores. Highest sporophore yield was obtained from the beds spawned with ragi, maize, pearl millet and sorghum grain spawns. Ragi grain spawn although differed insignificantly from the latter, it was found to be the best performer, which produced maximum (984.00 g) yield of sporophores and also supported the highest biological efficiency of 98.4 per cent. Sporophore yield and biological efficiency with various straw spawns ranged between 624.00 and 862.3 g and 62.4 and 86.2 per cent, respectively. Pearl millet stalk spawn was the poor performer. The increase in yield due to various grain spawns was to the tune of 48.1 to 57.7 per cent in comparison to pearl millet stalk spawn.

Significantly higher mushroom yields obtained with grain spawns *vis-à-vis* that obtained with straw spawns could be due to the fact that grains might have supplied nitrogen and minerals for fruiting as indicated earlier by Tshinyangu and Hennebert (1995). Munjal (1973) reported that the substrate on which spawn is made, affects the yield of mushroom. The present investigation reveals that, grains of ragi, maize, pearl millet and sorghum are equally good for commercial spawn production of *P. eous* depending on their cost and availability. Rangad and Jandaik (1977), Sivaprakasam and Kandaswamy (1981) and, Chauhan and Pant (1988) also found jowar and bajra grains as the best substrates for spawn production as evidenced from higher yields obtained by them with these spawns, followed by maize grain spawn. Pathmashini *et al.* (2008) obtained higher yield of *Pleurotus ostreatus* with sorghum (45.69 g), kurakkan (*Eleusine coracana*) (55.37 g) and maize (29.83 g) grain spawns and comparable yield for paddy (21.57 g) spawn. Straws have been reported by Rangad and Jandaik (1977) to be inferior as spawn bases for *Pleurotus* spp.

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