Research Article

Spawn and Spawning Strategies for the Cultivation of Pleurotus eous (Berkeley) Saccardo

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ABSTRACT
In present study, spawn developed on different cereals, viz., Barley (*Hordeum vulgare*), Maize (Zea mays), Oat (*Avena sativa*); millets, viz., Italian millet (*Setaria italica*), Pearl millet (*Pennisetum typhoides)*, Little millet (*Panicum miliare*) Sorghum (*Sorghum vulgaris*) and agricultural wastes like wheat straw, sugarcane bagasse, rice husk, mango saw dust and hulled corn cob were assessed against wheat grain spawn (*Triticum aestivum*) for enhancing yield and biological efficiency of *Pleurotus eous*. Three spawning techniques, viz., top, thorough and layer spawning were also evaluated in this study. The spawn developed on oat and barley grains were found most productive while hulled corn cob proved best alternative of grain substrate with 340 gm, 68% and 339 gm, 67.8% and 330 gm, 66.0 % yield and biological efficiency respectively. All the spawning techniques gave similar response though their magnitude varied among themselves.

Keywords: Biological efficiency, Grain spawn, and agro-waste spawn, Spawning technique.

INTRODUCTION
Mushroom is an unique horticultural crop. In contrast to the cultivation of higher plants, which started in pre-historical times, the culture of fungi is relatively of recent innovation. Historically, mushrooms were gathered from the wild for consumption and for medicinal use. The first commercial cultivation of edible mushrooms was developed in France in the 18th century since then it has traveled far a head. Over 200 species of mushrooms have been collected from the wild and utilized for various traditional and medical purposes mostly in the Far East. Till date, about 35 mushroom species have been cultivated commercially. Of these, about 20 are cultivated on industrial scale throughout the world due to their high nutritive and medicinal value which contributes to a healthy diet with rich source of vitamins, minerals and proteins (Garcha et.al., 1993). The spawn and spawn making has been primary concern in mushroom cultivation which is achieved by developing mushroom mycelia on supporting medium under controlled environmental conditions. In almost all cases the supporting matrix is sterilized grain which is preferred due to its bio-chemical properties and practical performance over others. Unfortunately, the ever increasing demand of food grains for human consumption leaves little scope for their use in spawn making. A number of other materials, mostly agricultural wastes, can be used to prepare mushroom spawn. The types of waste available varies from region to region. As spawn making, substrate inoculation is also a crucial phase in mushroom growing practices.

Pleurotus eous, a pink oyster mushroom, belonging to family Tricholomataceae of order Agaricales is less documented than other oyster species. Therefore, there is a vast scope of study on its cultivation. Aiming at the above, different attempts regarding to spawn and spawning techniques were made to find out their efficiency for the better production of this mushroom species.

MATERIALS AND METHODS
Micro-organism
The pure culture of *Pleurotus eous* (Berk) Sacc. was obtained from the mushroom section of Plant Pathology Department, Chandra Shekhar Azad University of Agriculture and Technology, Kanpur (U.P.) India. The culture was maintained and subcultured on potato dextrose agar (PDA) medium.

Spawn preparation
Various cereals, viz., wheat grains (*Triticum aestivum*), Barley (*Hordeum vulgare*), Maize (Zea mays), Oat (*Avena sativa*) and millets, viz., Italian millet (*Setaria italica*), Pearl millet

(*Pennisetum typhoides)*, Little millet (*Panicum miliare*) Sorghum (*Sorghum vulgaris*) were utilized as grain substrate for planting spawn. These were purchased from the seed market of Faizabad. Some locally available agricultural wastes like wheat straw, sugarcane bagasse, rice husk, mango saw dust and hulled corn cob were also utilized for this purpose. The mother spawn was prepared on traditionally used wheat grains (*Triticum aestivum*).
The spawns were prepared by following the standard method. The planting spawn, developed on wheat grains was later treated as control.

Substrate preparation
The substrate used for cultivation of *Pleurotus eous* was wheat straw which had been recommended as best substrate for mushroom cultivation (Singh and Singh, 1994). It was filled (500 gm) in polypropylene bags (42×30 cm size) and then washed separately in fresh water and then pasteurized in the solution of Formaldehyde (500ppm) and Bavistin (75ppm) for 18 hours as recommended by Vijay and Sohi (1987).

Spawn dose
Inoculation of substrate was made with planting spawn of *P. eous* @ 5% w/w on dry weight basis under aseptic conditions.

Spawning techniques
Three methods of spawning were employed for substrate inoculation.

a) Top spawning
In this method, spawn was planted just above the surface of substrate. It was then covered by a thin layer of substrate.

b) Thorough spawning
It was achieved by thoroughly mixing of spawn with the substrate while filling the bags.

c) Layer spawning
The spawn was planted in the substrate in multilayered (3) manner. This mode of spawning was later treated as control and uniformly used in all the experiments.

Method of cultivation
Plastic bag technology was used in this experiment. The beds were prepared from pasteurized substrate following the procedure adopted by Bano (1971). These were incubated in cultivation room at 22-30°C temperature for spawn run. When mycelia had completely covered the beds, the polythene covering were turned off and relative humidity was maintained 85-95 per cent with the help of humidifier.

Presentation of data and evaluation of biological efficiency of mushroom
The data recorded in respect of yield parameters were time lapsed in spawn run, pin head initiation and maturity of fruit bodies, number of flushes, total yield, number and weight per sporocarp on different kinds of spawn and spawning techniques. The biological efficiency of mushroom was worked out as percentage yield of fresh mushroom in relation to dry weight of the substrate as suggested by Chang and Miles (1989).

$$\textbf{Biological efficiency} = \frac{\textbf{Yield of fresh mushroom (gm)}}{\textbf{Total weight of dry substrate used (gm)}} \times 100$$

Statistical analysis
Completely Randomized Design (CRD) was employed for all the experiments. All the data were statistically analysed. The critical difference (CD) was worked out at five per cent probability level.

RESULTS AND DISCUSSION
The results regarding various parameter of mushroom production are presented in Tables 1-3.
Effect of grain spawn on cultivation of *Pleurotus eous*
The spawn was developed on different kinds of cereals and millets. The grain substrates were inoculated with mother spawn of mushroom and incubated for mycelial run. The incubation period varied among themselves which ranged 13-16 days (Table.1). For the spawn development, Oat and barley grains registered least incubation time (13 days each) which was equal to control while long spawn run period was noted for Italian and little millets (16 days). Once spawn was prepared, it was planted in to the substrate where they showed variable effect on duration of spawn run, primordial initiation and fruit body maturation which ranged from 13-18 days, 16-22 days and 20-26 days, respectively. A faster rate of cropping was noted in Italian and little

millet spawn while maize spawn showed delayed cropping than control (Table.1). The yield and biological efficiency of mushroom were found to be significantly higher when beds were introduced with oat and barley spawns which were recorded 340 gm, 68% and 339 gm, 67.8% respectively. These values were at par to each other. The sorghum spawn was considered as third best spawn in comparison to control with 310 gm, 62.0% yield and biological efficiency which was found at par to control. After final harvest, all the grain spawns showed almost similar response among themselves in respect of number and weight per sporocarp. (Table.1)

Effect of agro-waste spawn on cultivation of *Pleurotus eous*

Agricultural wastes are generally constituted of lignocellulosic materials and as a spawn substrate they took longer incubation period for impregnation and development of mushroom mycelium than grain spawn (Control). Out of five agricultural wastes under taken in this study, corn cob was found superior over control in respect of better mycelial growth. After planting the spawn into the substrate, delayed spawn run, primordial initiation and fructification of mushroom was epitomized (Table.2). The yield data represents that only cob spawn produced significantly higher yield and biological efficiency (330 gm, 66%) than control while remaining treatments gave poor response in above respect. The influence of different agricultural waste spawn on number and weight per fruit body was at par among themselves. In this experiment, rice husk failed to bear mycelia over its surface. (Table.2)

Effect of spawning techniques on cultivation of *Pleurotus eous*

Amongst various techniques employed, the days required for colonization of substrate (14), initiation (16) and maturation of fruit bodies (20) were comparatively less in thorough spawning than in other types of spawning (Table.3). No much difference in yield and biological efficiency was observed in respect of different spawning techniques. However, there was a tendency for better yield through thorough spawning but statistically it was not significant. Similarly, regarding the number and weight per fruit body there was nearly similar response in respect of spawning techniques. (Table.3)

Cereal grains and millets are generally used as spawn substrate. They act as a reservoir of carbohydrates which offer sufficient nutrition for mycelial growth and provide vehicle for the eventual even distribution of mushroom inoculant. The major disadvantage of small grains is presence of less food material in their endosperm (Bahl, 1984) and greater surface area in a given amount as compared to larger grains. Due this reason, mycelium of *Pleurotus eous* took more time to establish and run over the surface of grains resulting delayed spawn development in Italian and little millets. After seeding the substrate, grain spawn allows a quick spreading of mycelium from a small propagation center because, small grains provide more point of inoculum per gram of spawn (Bahl, 1984). Obviously, the spawn prepared on small grains covers the substrate sooner as a result early spawn run, primordial development and fruit body maturation was noticed in case of Italian and little millets vice-versa maize spawn took longer period for this purpose. The significant yield and biological efficiency was observed in oat and barley spawns. It was either due to less grain density or presence of seed coat (Husk) which provided additional food material to fungal mycelium. This finding was somewhat similar to report of Suman et.al., (1988) who mentioned that wheat grains in addition to rice husk supported best mycelial growth and higher mushroom production. In our investigation, sorghum spawn was found third best spawn, equal to wheat grain spawn. This was confirmed by Kumar and Suman (1979) who recommended the use of sorghum grains for spawn preparation.

A number of materials, mostly agricultural wastes, can also be used to prepare planting spawn which are variously available in different region. In comparison to grain starch, lignocellulosic materials are chemically more complex. They are major constituents of agricultural wastes which limit the action of microbial enzymes resulting into long incubation period for spawn preparation. The delayed colonization of substrate and fruit body formation either might be due to agro-waste clumps which reduced total point of inoculum per gram of spawn or to their complex chemical nature. Among agro-waste spawn, only cob spawn produced higher yield and biological efficiency. This might be due to obvious reason(s).

As spawn making, the methods of spawning have also prime importance in mushroom cultivation. The proper and homogeneous mixing of spawn to the substrate enhances the growth of mushroom mycelium through the substrate more quickly. Due to this reason, thorough spawning took shorter period of spawn run and fructification of mushroom. Among the spawning techniques used, all of them showed equivalent response to each other in respect of mushroom yield. This may be due to similar cultural conditions e.g. quality and quantity of spawn, amount and nature of substrate used and cultivation method (bag culture), prevailing on mushroom beds and secondly, owing to the number of flushes (3) considered for mushroom yield. However, the overall scenario can be changed if harvesting period takes priority over number of flushes. (Figures.1&2)

CONCLUSION

Based on the investigations, mushroom growers are advised to develop their spawn on oat and barley grains. The sorghum grains can be used for some extent. To explore an alternative for grain substrate without detrimental effect on yield potential, hulled corn cob may be used as spawn substrate. The substrate should be spawned by thorough spawning followed by layer spawning techniques.

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Table 1: Effect of different grain spawns on yield performance and biological efficiency of *Pleurotus eous*

Spawn substrate (Grains)	Incubation Period for spawn development (Days)	Spawn run (Days)	Primordial development (Days)	First harvest (Days)	Total yield from three flushes [gm/500 gm dry substrate]	Biological Efficiency (%)	Average number of sporocarp	Weight per sporocarp (gm)
Maize	15	18	22	26	272	54.4	45	6.04
Oat	13	15	17	21	340	68.0	51	6.67
Barley	13	15	17	21	339	67.8	56	6.05
Sorghum	14	15	18	22	310	62.0	49	6.32
Pearl millet	14	15	18	22	298	59.6	47	6.34
Italian millet	16	13	16	20	270	54.0	43	6.27
Little millet	16	13	16	20	280	56.0	43	6.51
Control	13	15	18	22	301	60.2	44	6.84
SE					**15.71**	**3.14**	**7.78**	**0.88**
CD (P=0.05)					**33.32**	**6.66**	**16.51**	**1.86**

Average of three replications

Table 2: Effect of different agro-waste based spawns on yield performance and biological efficiency of *Pleurotus eous*

Spawn substrate (Grains)	Incubation Period for spawn development (Days)	Spawn run (Days)	Primordial development (Days)	First harvest (Days)	Total yield from three flushes [gm/500 gm dry substrate]	Biological Efficiency (%)	Average number of sporocarp	Weight per sporocarp (gm)
SB	19	22	27	31	235	47.0	50	4.70
MSD	24	26	30	35	270	54.0	47	5.74
CC	17	16	19	23	330	66.0	49	6.73
RH	-	-	-	-	-	-	-	-
WH	17	18	22	27	250	50.0	50	5.00
Control	13	15	18	22	301	60.2	44	6.84
SE					**12.02**	**2.40**	**5.05**	**1.09**
CD (P=0.05)					**26.80**	**5.36**	**11.28**	**2.43**

SB: Sugarcane bagasse; MSD: Mango saw dust; CC: Corn cob; RH: Rice husk; WH: Wheat straw
Average of three replications

Table 3: Effect of different type of spawning on yield performance and biological efficiency of *Pleurotus eous*

Spawning Techniques	Spawn run (Days)	Primordial development (Days)	First harvest (Days)	Total yield from three flushes [gm/500 gm dry substrate]	Biological Efficiency (%)	Average number of sporocarp	Weight per sporocarp (gm)
Top spawning	24	28	32	279	55.8	55	5.07
Thorough spawning	14	16	20	314	62.8	56	5.60
Layer spawning (Control)	16	18	22	301	60.2	44	6.84
SE				**14.44**	**2.84**	**6.94**	**0.64**
CD (P=0.05)				**35.39**	**6.96**	**17.02**	**1.58**

Average of three replications

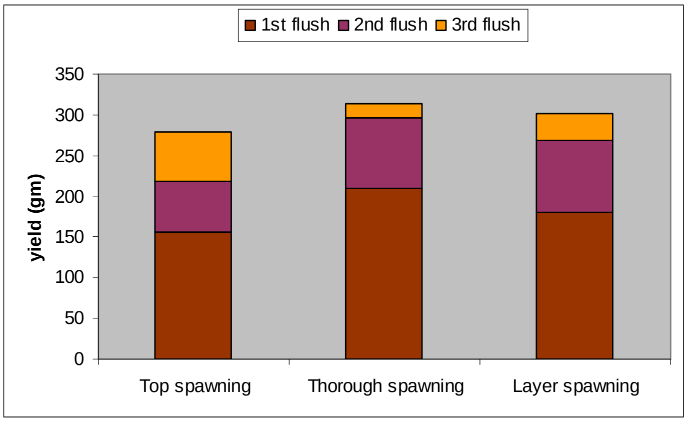

Fig. 1: Yield of *P. eous* after considering number of flushes

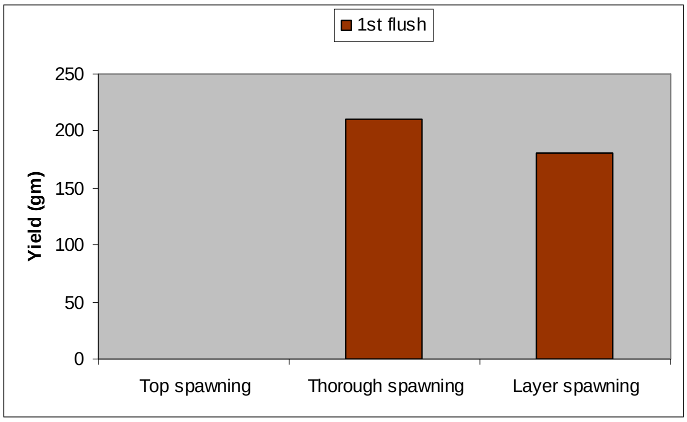

Fig. 2: Yield of *P. eous* on 22 days of harvest

(A) Thorough spawning

(B) Layer spawning

(C) Top spawning

Effect of spawning techniques on cropping of *P. eous*

Spawn developed on different grain substrate

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