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Effect of different grains and alternate substrates on oyster mushroom (*Pleurotus ostreatus*) production

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The present investigation was undertaken by using various grains for spawn production, and waste paper, wood chips were used in comparison with wheat husk for mushroom production during 2010-2011 at Department of Botany, Singhania University, Rajasthan. The results of the analysis of variance showed that diameter of colony extension in various grains are different and were significantly affected by substrate type. The maximum and minimum growth rates were seen in the corn and millet substrates, respectively. It is concluded that wheat straw in combination with wood chips are best substrate for oyster mushroom cultivation.

Key words: Oyster mushroom, grains, substrate, wood chips.

INTRODUCTION

Mushroom is a unique horticultural crop. In contrast with 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 ahead. 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 (Siddhant et al., 2013). 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 vary from region to region. As

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spawn making, substrate inoculation is also a crucial phase in mushroom growing practices. Depending on the different process parameters the yield of mushroom varied from 74 - 204 g.

Optimum process parameters for maximum yield of *Pleurotus ostreatus* were found to be Bagasse 21.25 g/l, Wheat bran 3.5 g/l, Urea 5.0 g/l and Humic acid 4%. The process parameters also show significant effect on yield, productivity and biological efficiency. Mycelia colonization of compost bags and subsequent growth of oyster mushroom was faster in high Humic acid-based substrates. Hence they produced larger and firmer fruiting bodies (Prakash et al., 2010).

The main nutritional sources for oyster mushroom are ceIIulose, hemicellulose, Iignin and C/N ratio is important factor for optimal substrate composition for oyster mushroom. Oyster mushroom requires much carbon and less nitrogen source than button mushroom *(Agaricus bisporus)* but most of the main substrate materials such as cereal straw (Siddhant et al., 2013) cotton waste and sawdust need supplementation of nitrogen source such as wheat and rice bran to reach optimal C/N ratio for oyster mushroom. Therefore, any type of organic matters containing lignin and cellulose can be used for oyster mushroom substrates and this includes almost all agricultural wastes. Cultivation of mushroom on these solid residues can be viewed as an effective means to utilize bio-resource residue and simultaneously as a sound environmental protection strategy. Furthermore, the use of these residues in bioprocesses may be one of the solutions to bioconversion of inedible biomass residues into nutritious protein rich food in the form of edible mushrooms (Mshandete and Cuff, 2008).

The present study is aimed at using various grains for spawn production and use of waste paper and wood chips in comparison with wheat husk for mushroom production.

MATERIALS AND METHODS

Micro-organism

The pure culture of *P. oestreatus* fungi MTCC 1801 was from IMTECH Chandigarh (edible mushroom species). For the propagation of the main culture, 2.0% malt-extract agar (MEA) was used. MEA plates were inoculated with a mycelium/agar plug (5-mm-diameter) of a young, actively growing margin of the colony. Prior to its use as an inoculum for grain spawn, a mycelium/agar plug was inoculated at the center of the plate and incubated at 25°C in the dark for seven days.

Spawn preparation

Various cereals, viz., wheat grains (*Triticum aestivum*), barley (*Hordeum vulgare*), maize (Zea mays) and millets, (*Setaria italica*), were utilized as grain substrate for planting spawn. These were purchased from the seed market. Some locally available agricultural wastes like wheat straw, sugarcane bagasse, rice husk and 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*). 50 g grain was placed in polythene bags and held in place by rubber bands, it is sterilized at 121°C for 20 min. After cooling, each bag was inoculated with spawns and incubated at 25°C in full darkness for two weeks to enable the mycelia to permeate.

Substrate preparation

Waste paper

Waste paper was collected and cut in 0.5 cm width with different lengths.10 L of water was taken in a bucket and two cups of bleaching solutions were added to this water. Newspapers were added to this bleached water and, allowed to hydrate for about an hour then stirring was done. After an hour papers were taken out and were separated under shade so as to remove the extra water. Then loading of polythene bags was done.

About 3/4 of inch of paper layer was prepared then a coating of spawn on the top of each layer was added. Care is taken on the outer and top layer of news papers. Then bag was tied with the help of thread and holes were made around the bag. The bag was pushed down getting the air out of it and then the bag was kept in shade for a period 2-3 weeks.

Wheat straw and wood chips

Wood chips of 0.5 cm wide were collected. Then, equal quantity of wood chips was mixed with wheat straw. 10 L of water was taken in a bucket and two cups of bleaching solutions were added to the water. Wheat straw and wood chips were added to this bleached water and allowed/to hydrate for about an hour, then stirring was done. After an hour, wheat straw and wood chips were taken out and were separated under shade to remove the extra water. Then loading of polythene bags was done.

Spawn dose

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

Spawning techniques

Three methods of spawning were employed for substrate inoculation.

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

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

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

Table 1. The results obtained from different grains.

Different grains	Diameter colony extension after 12 days of inoculation (mm)
Corn	38.60
Wheat	35.80
Barley	32.40
Millet	26.80

prepared from pasteurized substrate. pH of the substrate mixtures were maintained at 6.0. Compost medium was mixed manually. The mixture of varying compositions were packed in polythene bags and sterilized. After sterilization, the substrate is semi dried, inoculated with the spawns in alternate layers and incubated at 25°C in dark for 2 weeks for the ramification of mushroom mycelia. The culture rooms were damped by spraying the top of compost with water once a day. This maintains the relative humidity of 80%. After the development of mycelium on compost bags, they were torn and maintained at 28°C with adequate aeration; watering and high humidity to allow the fruiting bodies to emerge. The harvesting was done in 3 flushes of 1 week intervals. After the 2nd flush, the substrate was turned upside down and regularly watered to harvest the 3rd flush.

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).

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

Statistical analysis

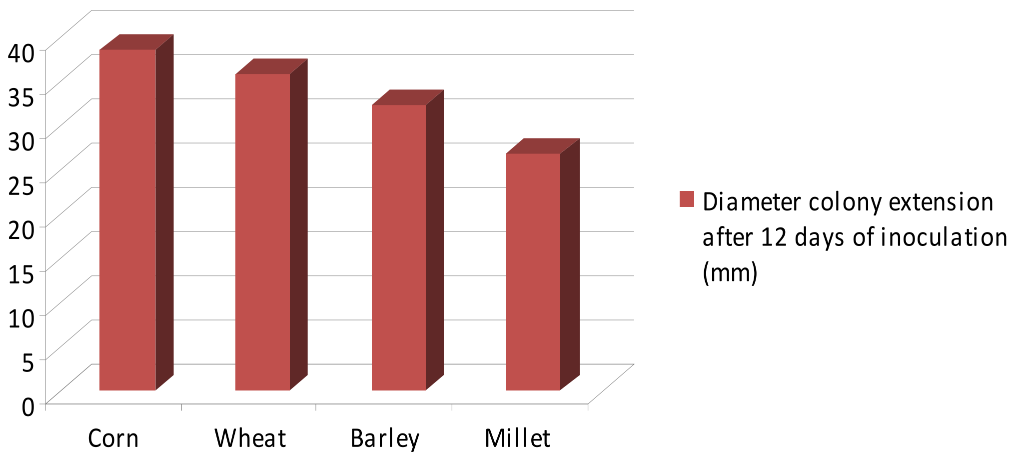
Completely randomized design (CRD) was employed for all the experiments. All the data were statistically analysed. The critical difference (CD) was worked out at five percent probability level.

RESULTS AND DISCUSSION

The results of the analysis of variance showed that diameter of colony extension in various grains are different and were significantly affected by substrate type. The maximum and minimum growth rate were seen in the corn (Table 1 and Figure 1A) and millet substrates, respectively. The larger surface area and pore of substrates are responsible for the more mycelium growth rate. For the reason that corn seeds size are larger than wheat and millet seeds size, consequently, corn seeds pore is also larger. As a result, it influences mycelia growth rate. Probably, the increased mycelium growth in corn is due to increased ventilation and O_2 concentration in corn substrate as O_2 is one of the most important environmental factors. It performs an important role in metabolism and essential for respiration mushroom. For maximum respiration there is active growth (or mycelia growth). Therefore at the lowest O_2 concentrations, respiration rate relate directly to O_2 concentration of substrate (Siddhant et al., 2013).

The crop of oyster mushroom was harvested in three flushers. Maximum yield was obtained in the first flush than the second and third flushes (Figures 2, 3 and 4). This study has demonstrated that locally available organic substrate is potentially suitable for use in the production of mushrooms. It means that the substrates contain lignin and cellulose which is utilized by the mushroom mycelium as a source of nutrition. The diverse range of substrates indicates that the mushrooms can be grown on almost all available organic wastes. The ability of mushrooms mycelia to degrade organic substrates can also be utilized in management of organic waste in the environment, which is otherwise left to decompose hence causing health hazards. It is one of the method that can help in recycling the organic wastes into profitable products. This is made possible by the ability of mushrooms (basidiomycetes) to secrete a wide range of hydrolyzing and oxidizing enzymes which breaks down natural lignocelluloses waste into simple soluble compounds.

The time taken by the mycelia to start pinning after ramification depends on the substrate used. Materials with high quality lignin and cellulose contents take a longer time to start pinning as compared to the substrates with low contents of the lignin and cellulose. As compared to the substrates with low nutrition values, the substrates with high nutrition value take a short time for full colonization and ramification. This is because the mycelia remains vegetative for a longer period hence the vigorous growth and late pinning. In turn, the highly colonized substrates exhibited low mycelia densities. Poor nutritional substrates exhibited low mycelia densities making them prone to contamination especially by the green mold. The suitability of different substrates for mushroom cultivation was also confirmed by the average biological efficiency which was variable among the substrates (Table 2 and Figure 1B). Wood chips and wheat straw shows highest yield (780 g) and biological efficiency (78%). It is clear that wheat straw in combination with wood chips are best substrate for oyster mushroom cultivation. Comparable results of *Pleurotus*



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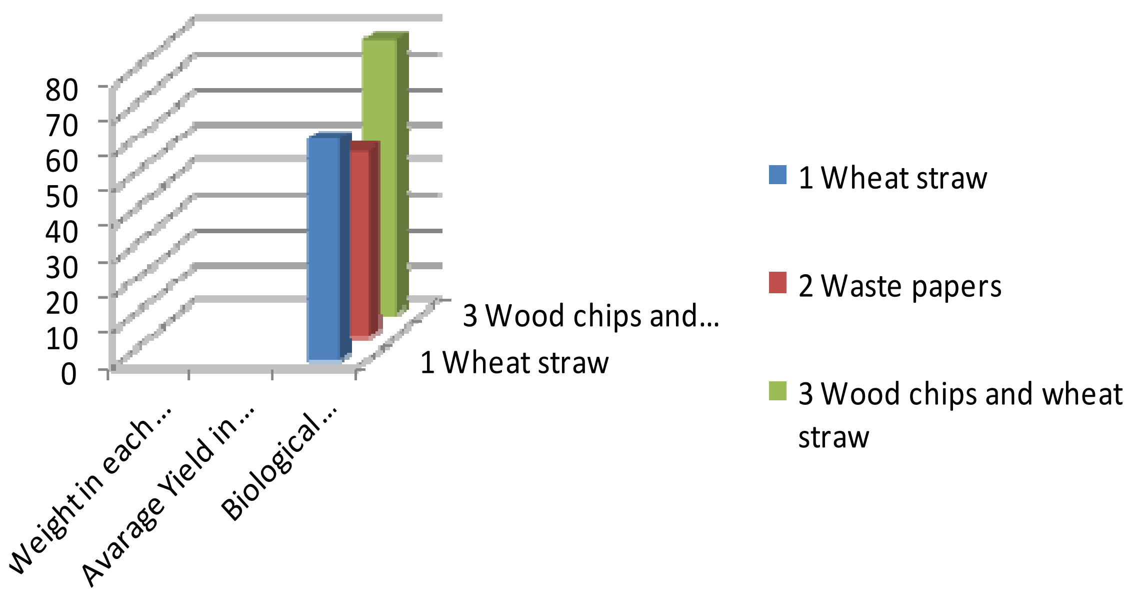

B

Figure 1. oyster mushroom harvested in three flushers.

Table 2. Showing the average yield and biological actions.

Substrate	Weight in each substrate (g)	Average Yield in three Flushes (g)	Biological efficiency (%)
Wheat straw	1000	630	63.00
Waste papers	1000	530	53.00
Wood chips and wheat straw	1000	780	78.00

species grown on different substrates has been earlier reported (Baysal et al., 2003; Shah et al., 2004; Mshandete, 2011). Mycelia of *Pleurotus* species are well known to colonize various lignocellulosic materials due to their extensive enzyme systems capable of utilizing complex organic compounds, which occurs in organic matter residues (Mane et al., 2007). Thus, a range of 12-14 days has been reported as time period of spawn running for various *Pleurotus* species on lignocelluloses substrates (Mane et al., 2007). Thus, the results from this

Figure 2. Substrate– waste paper.

Figure 3. Substrate– wheat straw.

Figure 4. Substrate– wood chips and wheat straw.

study concur with the previous spawn running periods reported by others on *Pleurotus* species.

Conclusion

Based on the investigations, mushroom growers are advised to develop their spawn on corn and wheat grains. It is evident that many organic substrates have high potential for utilization as substrates in mushroom cultivation. Mushrooms growers are advised to make the right choice of substrates based on the locally available materials. It is clear that wheat straw in combination with wood chips is best substrate for oyster mushroom cultivation.

Conflict of interest

The authors declare that they have no conflict of interest.

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