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Evaluation of different strains and substrates for cultivation of oyster (*Pleurotus* spp.) Mushroom

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Abstract

The present study was conducted in different substrates for Cultivation of Oyster (*Pleurotus* spp.) Five substrates viz., straw, sorghum straw, mustard straw, Pearl millet straw and wheat + sorghum straw were tested alone and in combination with supplements to compare their efficacy in the growth pattern, yield and yield related pattern of fruiting bodies of *Pleurotus* spp. The results indicated that S-01 strain observed minimum days (16.00) for complete spawn run, and minimum days (21.00) for pin head formation, (26.00) days of fruit initiation in wheat straw. In case strain S-02 recorded in minimum days (15.00) for complete spawn run, (19.00) for pin head formation and (24.00) days of fruit initiation in wheat straw, respectively. In S-03 observed minimum days (17.00) for complete spawn run and minimum days (22.00) for pin head formation, (27.00) days of fruit initiation in wheat straw, respectively. S-01 strain recorded maximum number of fruit bodies (30.00) on wheat straw whereas maximum average weight of fruit body (28.00) were observed in sorghum straw while minimum number of fruit bodies (22.00) was recorded on pearl millet straw. S-02 strain observed by maximum number of fruit bodies (94.33) on wheat straw whereas maximum average weight of fruit body (20.00) were observed in wheat straw followed by Minimum number of fruit bodies (77.67), and Average weight of fruit body (15.00g) was recorded on pearl millet straw. In case S-03 strain recorded by maximum number of fruit bodies (56.67) on wheat straw+ sorghum straw whereas maximum average weight of fruit body (18.00) were observed in wheat straw followed by minimum number of fruit bodies (42.00) in sorghum straw, while Average weight of fruit body (12.00g) was recorded on pearl millet straw respectively. In S-01 strain, wheat straw substrate gave highest total yield of 698.33g/1000g of dry substrate with corresponding 69.83 per cent biological efficiency while the least effective substrate was pearl millet substrate which produced a total yield of 576.69g/1000g of dry substrate. In case of S-02 strain recorded by maximum total yield of 787.12g/1000g of dry substrate with corresponding 78.71 per cent biological efficiency in wheat straw followed by minimum substrate was pearl millet which produced a total yield of 593.12 g/1000g dry substrate. In strain -03 observed by maximum total yield of 546.99g/1000g of dry substrate with corresponding 54.69 per cent biological efficiency in wheat straw+ sorghum straw followed by minimum substrate was sorghum straw which produced a total yield of 398.45 g/1000g dry substrate with 39.84 per cent biological efficiency.

Keywords: Biological efficiency, mycelia running, maturation, primordial initiation, spawn, yield

Introduction

Mushroom cultivation is followed due to their delicious flavour and low calorific value. It has been determined that more than 3000 mushroom species are edible, but only 10 of those are on an industrial scale. The most cultivated mushroom worldwide is *Agaricus bisporus*, followed by *Pleurotus* species. Oyster mushroom is regarded as one of the commercially important edible mushrooms throughout the world. It consists of a number of different species, those including *P. Sajor-caju*, *P. Eryngii*, *P. Sapedus*, *P. Ostreatus*, *P. Cystidiosus*, *P. Cornucopiae*, *P. Pulmonarius*, *P. Tuber-regium*, *P. Citrinopiliatus* and *P. Flabellatus* and the most commonly cultivating *Pleurotus* spp. in India is *P. Sajor-caju*, *P. eryngii*, *P. sapedus*, *P. Djamor*, *P. Citrinopileatus*, *P. Flabellatus*, and *P. Florida*. The potential of oyster mushrooms which possess the appropriate enzymatic mechanisms for the transformation of lignocellulosic wastes into simple compounds, have been exploited as the means for biodegradation of a wide range of litter due to their particular ability for selective delignification. Many species of *Pleurotus* possess pleasant flavour and are considered as delicacy by the connoisseurs of food. *Pleurotus* spp. contains various types of vitamins and amino acids, high content of fibre and protein and low-fat content. It has been reported with protein (17-42%), carbohydrate (37-48%), minerals (potassium, phosphorous, calcium, sodium) of about 4-10%, lipids (0.5-5%) and fibres (24-31%).

Besides nutritional attributes of *Pleurotus* spp. the health benefitting effects like anticancer, antihyperlipidemic, antioxidant, hepatoprotective, antitumorigenic, and antimicrobial activities makes them a health food. The production of mushroom in India has been estimated as 200,000 tonnes per annum and Haryana is the leading state followed by Punjab, Himachal Pradesh and other states. In India, the mushroom industry recorded an average growth rate of 4.3 % per annum. From the total mushroom produced, white Button mushroom shares 73 per cent followed by oyster mushroom 16 per cent, paddy straw mushroom 7 per cent and milky mushroom 4 percent. Indian mushroom industry generated revenue of Rs. 7,282.26 lacs by exporting 1,054 quintals of white button mushroom in canned and frozen in the year 2016-2017. Oyster is the second most popular mushroom after button all over the world (Banik and Nandi, 2004) [1]. Presently, in Rajasthan, the tropical and subtropical varieties of mushroom like oyster and milky are gaining popularity. The most important mushroom growing districts of Rajasthan are Udaipur, Jaipur, Ajmer, Bikaner, Bhilwara, Kota, Banswara, Sri Ganganagar, Jodhpur and Sirohi. Success of oyster mushroom cultivation in any locality depends on the substrates which are cheap and easily available as well as viable technology suitable for a particular agro-climatic region. Various agricultural by-products are being used as substrates for the cultivation of oyster mushroom. Though wheat straw is considered as the best substrate in terms of yield, it has become necessary to find cheap and alternate substrates due to the higher cost of paddy straw and its non-availability in certain situations. Oyster mushrooms grow on agricultural wastes which are rich in cellulose, hemicellulose and lignin. These residues are low in nitrogen content (0.5 to 0.8 per cent), which is an essential element for cellular functions. At the time of fructification, most of the nitrogen is utilized for mycelial growth, the depleted nitrogen in the substrate becomes inadequate and limits mushroom yield. Several workers have reported that addition of organic amendments could enhance the yield of mushrooms and supplementing the growth substrate with oil seed cakes greatly influenced the production of mushroom, to double the yield. This technology has a great impact on mushroom production, since, per unit area, without significant prolongation of spawn run period, the bioconversion efficiency of the species could increase two-fold.

Martial and Methods

Description of the study area

The study was conducted in Instructional Farm, College of Agriculture, Jodhpur (Rajasthan), India. During the year 2021-22. Geographically, it is located between 26° 33' N to 26° 35' North latitude and 73° 00' E to 73° 10' East longitude at an altitude of 231 meter above mean sea level. This region falls under agro-climatic zone IA (Arid Western Plains Zone) of Rajasthan.

Oyster mushroom cultivation techniques

Oyster mushroom cultivation was done according to (Randive 2012) [18]. Table 1 shows the compositions of substrates used as a treatment groups for the cultivation of oyster mushroom. Initially, wheat straw, sorghum straw, mustard straw and pearl millet straw were chopped using edge tool into small pieces (2 -2.5 cm long). The substrates

were soaked in water for 24 h to moisten it thoroughly and pasteurized using clean steel drums. First the water was heated at 60 °C. Then the substrate was added and allowed to remain in the water for 30 min. Finally, once pasteurized, it was stalked in sterile plastic sheet (2 m × 4 m) laying on the steep cemented floor so as to remove the excessive moisture from the substrates to get 65–75% moisture level. Then, the spawn prepared was mixed with substrate in the sterile plastic sheet (2 m × 4 m) laying on the floor. The mixture was filled into 5000 ml sterile plastic bags and placed in dark room. Holes were prepared for aeration in the plastic bag. Once the mycelia colonized substrates in plastic bags, the bags were transferred to the cropping room. The cropping room had a limited light with average temperature of 25 °C. The growing mycelia were watered twice a day (morning and afternoon) using watering pot to maintain water activity of the substrates in the plastic bags and humidity of cropping room.

Table 1: Substrate composition of the treatment groups used for the cultivation of oyster mushroom

Treatment group	composition of the substrate
T1	Wheat straw (100 %)
T2	Sorghum straw (100%)
T3	Mustard straw (100%)
T4	Pearl millet straw (100%)
T5	Wheat + sorghum straw (50 +50 %)

Harvesting and yield measures

Mature mushrooms were picked by clean hand without harming the substrate when they started to wrinkle-ripe. This was done for three subsequent flushes. Following the method of Iqbal *et al.* (2005) [9], the yield parameters were recorded with respect to time (days) taken for completion of spawn running, time taken for the first appearance of pinhead formation, time taken for maturity of fruit bodies, number of flushes, and yield of flushes on the treatment substrates (total weight of all the fruiting bodies harvested from all the three pickings were measured and considered as total yield of mushroom). The pileus diameter and the stipe length were measured with graduated transparent ruler. Mature mushrooms were weighed with analytical balance to determine the biological efficiency (BE) of mushrooms produced from substrates. The average Biological efficiency (BE) of harvests was computed (Peng *et al.* 2000) [25].

$$BE = \frac{\text{Weight of fresh mushroom harvested per bag}}{\text{Weight of dry substrate per bag before inoculation}} \times 100\%$$

Mycelium running—time required from inoculation to completion of mycelium running were recorded (days). Primordial initiation—time required for primordial initiation (days) were recorded. Maturity—time required from primordial initiation to harvest (days) were recorded. Number of flushes—the numbers of flushes were counted in each plastic bag. Average weight of individual fruiting body/plastic bag—average weight of individual fruiting body was calculated as:

$$\text{Weight} = \frac{\text{Total weight of fruiting body per plastic bag}}{\text{Total number of fruiting body}}$$

Pileus diameter was measured by using a string passing from one end of the pileus to the other through the center of

the pileus. It was obtained on three randomly picked mushrooms and then the average was calculated in millimeters (mm). Stipe length was measured by placing the string from one end, where it was attached to the substrate, to the point where the gills on the pileus start. The string was placed along a ruler to get the length in millimeters (mm). Yield of mushroom= total weight of all the fruiting bodies harvested from all the three pickings were measured as total yield of mushroom. The data on spawn running was recorded after complete colonization of substrate and pin head and fruit body formation were observed.

Data collection and data analysis

Data on the mycelium colonization period, pin head formation period, stalk length, BE, step length, pileus diameter were recorded and analyzed using SPSS version 20. Analysis of variance (ANOVA) was used to indicate significant mean differences at 95% confidence interval using Tukey's multiple comparisons method.

Results

The number of days taken for complete mycelial growth differs significantly ($p < 0.05$) Among the three different species (*P. Eryngii*, *P. Sajor-caju*, and *P. Sapidus*), one *P. Sajor-caju* based on their fast and vigorous mycelial growth were selected and consequently evaluated for the cultivation parameters of *Pleurotus* spp. Table (4.5). The minimum (earliest) days for spawn run (15.00 days) were observed with *P. Sajor-caju* on wheat straw substrate followed by mustard straw (17.00 days), However, maximum spawn run period of 22.00 days was observed in pearl millet substrate alone. In *P. Eryngii* the minimum earliest days for spawn run (16.00 days) was observed with wheat straw followed by mustard straw 18.00 days, Maximum spawn run period of 25.00 days in pearl millet alone. In *P. Sapidus*, the earliest mean spawn run was observed with wheat straw in 17.00 days followed by mustard straw (19.00 days). Maximum spawn run period of 23.00 days was observed with pearl millet straw alone.

Table 2: Effect of different substrates on cropping period of *Pleurotus* spp.

Treatment	Days of spawn run			Pin head formation			Days of fruit initiation		
	Species			Species			Species		
	Sp.-01	Sp.- 02	SP.-03	Sp.-01	SP.-02	SP.-03	SP.-01	SP.-02	SP.-03
Wheat straw	16.00	15.00	17.00	21.00	19.00	22.00	26.00	24.00	27.00
Sorghum straw	21.00	20.00	22.00	26.00	24.00	26.00	29.00	28.00	30.00
Mustard straw	18.00	17.00	19.00	23.00	22.00	24.00	27.00	26.00	28.00
Pearl millet straw	25.00	22.00	23.00	28.00	26.00	27.00	31.00	30.00	32.00
Wheat + sorghum straw	23.00	19.00	21.00	27.00	24.00	25.00	31.00	28.00	29.00
SEm±	0.966	0.856	0.968	0.846	0.856	0.850	0.856	0.858	0.859
C.D ($p < 0.05$)	3.084	2.733	3.089	2.740	2.733	2.633	2.733	2.739	2.743

A number of investigators have reported different timing period for pinhead formation. Similar results in table 2. The minimum (earliest) days for pinhead formation (19.00 days) were observed with *P. Sajor-caju* on wheat straw substrate followed by mustard straw (22.00 days). However, maximum pinhead formation period of 26.00 days was observed in pearl millet substrate alone. In *P. Eryngii*, the minimum earliest days for pinhead formation (21 days) was observed with wheat straw followed by mustard straw 23.00 days. Maximum pinhead formation period of 28.00 days was observed on pearl millet straw alone. In *P. Sapidus*, the earliest mean pinhead formation was observed with wheat straw in 22 days followed by mustard straw (24.00 days). Maximum pinhead formation period of 27.00 days was observed with pearl millet straw alone.

A number of investigators have reported different timing period for fruiting bodies (maturity). Similar results in table 2. Minimum number of days recorded for fruit initiation by *P. Sajor-caju*, were (24.00 days) with wheat straw followed by 26.00 days in mustard straw. However, maximum days for fruit initiation (30.00 days) were observed with pearl millet substrate with *P. Sajor-caju*. In *P. Eryngii*, minimum days for fruit initiation (26.00 days) was recorded in wheat straw followed by 27.00 days in mustard straw. Maximum days for fruit initiation (32.00 days) were observed on pearl millet straw. It was noticed of *P. Sapidus*, Minimum number of days recorded for fruit initiation by (27.00 days) wheat straw followed by 28.00 days in mustard straw alone. However, maximum days for fruit initiation (32.00 days) were observed with pearl millet substrate with *P. Sapidus*.

Table 3: Effect of different substrates on cropping physiology of *Pleurotus* spp.

Substrate	Number of fruit bodies			Average weight of fruit body(g)		
	Species			Species		
	Sp.-01	Sp.-02	Sp.-03	Sp.-01	Sp.-02	Sp.-03
Wheat straw	30.00	94.33	52.67	25.33	20.00	18.00
Sorghum straw	23.33	80.67	42.00	28.00	18.00	16.00
Mustard straw	27.00	88.00	47.33	24.00	17.5	16.5
Pearl millet straw	22.00	77.67	44.33	20.00	15.00	12.00
Wheat + sorghum straw	26.00	90.00	56.67	21.33	16.00	14.50
SEm±	0.94	1.54	1.37	0.730	0.966	0.856
C.D ($p < 0.05$)	3.00	4.92	4.38	2.33	3.08	2.73

Table 3 indicates the effect of substrate on mushroom Number of fruit bodies (branch) in *P. Sajor-caju*, maximum number of fruit bodies 94.33 was recorded in wheat straw followed by 90.00 in wheat + sorghum straw respectively. Minimum number of fruit bodies of 77.67 per 1000g of dry

substrate was recorded in pearl millet straw alone. In case of *P. Eryngii*, maximum number of fruit bodies (30 per 1000g of dry substrates) was recorded in wheat straw followed by 27.00 in mustered straw. Minimum number of fruit bodies 22.00 per 1000g of dry substrates was recorded in pearl

millet straw. In *P. Sapidus* maximum number of fruit bodies (56.67 per 1000g of dry substrates) was recorded in wheat + sorghum straw followed by 52.67 in wheat straw whereas 47.33 and 44.33 in mustard straw and pearl millet straw respectively. Minimum number of fruit bodies 42.00 per 1000g of dry substrates was recorded in sorghum straw.

Table 3 indicates the effect of substrate on mushroom average fruit body weight on *P. Eryngii*, the maximum average weight of fruit bodies 28.00g was recorded in sorghum straw followed by 25.33g in wheat straw, 24.00g and 21.33g in mustard straw and wheat + sorghum straw respectively. Minimum average weight of fruit bodies 20.00g per 1000g of dry substrate was recorded in pearl

millet straw. In case of *P. Sajor-caju*, maximum average weight of fruit bodies (20.00g per 1000g of dry substrates) was recorded in wheat straw followed by 18.00g in sorghum straw whereas 17.5g and 16.00g in mustard straw and wheat + sorghum straw respectively. Minimum average weight of fruit bodies 15.00g per 1000g of dry substrates was recorded in pearl millet straw. In *P. Sapidus* maximum average weight of fruit bodies (18.00g per 1000g of dry substrates) was recorded in wheat straw followed by 16.5g in mustard straw whereas 16.00g and 14.50g in sorghum straw and wheat + sorghum straw respectively. Minimum average weight of fruit bodies 12.00g per 1000g of dry substrates was recorded in pearl millet straw.

Table 4: Total yield and biological efficiency of the *Pleurotus* spp on different substrates

Substrate	Total yield(g.) /1000 (g.) of substrates			Biological efficiency (%)		
	Species-1	Species-2	Species-3	Species-1	Species-2	Species-3
Wheat straw	698.33	787.12	505.46	69.83	78.71	50.54
Sorghum straw	606.12	623.00	398.45	60.61	62.30	39.84
Mustard straw	615.22	655.96	459.11	61.52	65.59	45.91
Pearl millet Straw	576.69	590.45	436.30	57.66	59.04	43.63
Wheat + Sorghum straw	603.45	593.12	546.99	60.34	59.31	54.69
C.D ($p < 0.05$)	23.19	19.23	21.07			
SEm±	7.26	6.02	6.60			

Table 4 indicates the effect of the treatment groups with varying substrate composition on yield (g). The highest total yield of 787.12 g /1000g of dry substrates in *P. Sajor-caju*, followed by mustard straw total yield of 655.96g, sorghum straw (623.00g), and pearl millet straw giving fruit body yield of 593.12g, The least effective substrate proved to be pearl millet straw with minimum total yield of 590.45 g/1000g of dry substrates. Similar trend was observed in case of *P. Eryngii* in which wheat straw proved to be best with maximum total yield of 698.33g/1000g of dry substrates whereas total yield of 615.22g, 606.12g, g and 603.45g were recorded in sorghum straw, mustard straw, and wheat + sorghum straw, respectively. The least effective substrate was pearl millet straw with minimum total yield of 576.69g/1000g of dry substrates. In *P. Sapidus*, maximum total yield of 546.99g /1000g of dry substrates was recorded in wheat + sorghum straw followed by 505.46g in wheat straw, 459.11g in mustard straw and 436.30g in pearl millet Straw respectively. Minimum yield of 398.45g per 1000g of dry substrate was recorded in pearl millet straw alone. Table 4 indicates the effect of the treatment groups with varying substrate composition on biological efficiency of *Pleurotus* spp. was significantly affected by the selected substrates. In *P. Sajor-caju*, maximum biological efficiency of 78.71 per cent was recorded on wheat straw, followed by 65.59, respectively. Minimum biological efficiency of 59.04 percent was recorded in pearl millet Straw. In case of *P. Eryngii*, maximum biological efficiency of 69.83 per-cent was recorded in wheat straw which was followed by 65.59 per-cent in mustard straw. Minimum biological efficiency of 57.66 percent was recorded in pearl millet straw. In *P. Sapidus*, maximum biological efficiency of 50.54 was recorded in wheat straw followed by 45.91 in mustard straw, respectively. Minimum biological efficiency of 43.63 was recorded in pearl millet straw alone.

Discussion

Mycelial growth provides suitable internal conditions for fruiting table 2. In this study, the fastest mycelia extension

was observed in T1 (16 days), T3, and T5 equally. Thus, outstanding growth of mycelium is a vital factor in mushroom cultivation (Pokhrel *et al.* 2009)^[17]. In this study, wheat straw, sorghum straw, mustered straw, wheat straw+ sorghum straw and pearl millet produced mycelium extension within short period of time which is similar with the control except the T2. This variance could be due to the variation in nutrient content, lignin and cellulose composition and moisture holding capacity of the substrate. Similar results were reported by Shah *et al.* (2004)^[21] who the growth of *Pleurotus* species on wheat straw, rice husk as well as saw dust took 2–3 weeks for mycelial growth after inoculation. Moreover, Kumari and Achal (2008)^[13] noted that colonization of the substrate with *P. Ostreatus* was completed within 20 days of inoculation. Conversely, the current study contradicts with the results of (Girmay *et al.* 2016)^[6] who reported that mycelia running on wheat straw took 16 days. The variation in mycelia extension might be due to the difference in condition of the environment and the nature of the substrate. *P. Ostreatus* grew quickly at 30 °C (Marino *et al.* 2003)^[14] and oyster yield decreases when the temperature decreases in different climatic zones (Zervakis *et al.* 2001)^[24]. Shah *et al.* (2004)^[21] indicated that relatively higher room temperature resulted primordial initiation ranged 27 to 34 days of incubation. Moreover Sharma *et al.* (2013)^[22] reported shorter pinning period (26.40–31.60 days of incubation) on various substrates. In the present study, all the treatments initiated the pin head within few days (ranged 9–16 ± 4). This study reveals that as the amount of waste paper increases the time taken for pinning increases (Table 3). Thus, the longer time taken for pinning might be due to the cellulose and lignin content of wheat straw. Different scholars (Shah *et al.* 2004; Sharma *et al.* 2013)^[21, 22] reported different pinning days. The variation in pin head formation might be due to the difference in room temperature of the cultivation room and nutrient availability of the substrate (Oei 2003; Shah *et al.* 2004; Sharma *et al.* 2013)^[15, 21, 22]. A number of investigators have reported different timing period for

fruiting bodies (maturity). Similar results (4 ± 0.7 days) for the maturation of fruit bodies were reported by Gume *et al.* (2013) [7]. Appendix 2 exhibits cultivated fruiting body of oyster mushroom in the present study. The current result is also comparable with Islam *et al.* (2009) [10] who reported that maturation period of *Pleurotus* species ranging from 3.29 to 4.33 growing on saw dusts of Mango, Shiris, Jackfruit, Kadom, Jam and Coconut. Moreover, higher (27–40) number of maturation days of *P. Ostreatus* mushroom cultivated on waste paper has been reported (Girmay *et al.* 2016) [6]. This variation in maturity of fruiting bodies could be owing to the difference in physiological requirements and the nature of the substrate (Girmay *et al.* 2016) [6]. Yield among the flushes of each treatment varied significantly ($p < 0.05$) for some of the treatments (Table 4). Besides, the yield of all the treatments did not varied significantly ($p > 0.05$). This indicates that wheat straw, sorghum straw, mustared straw, wheat straw and sorghum straw and pearl melt straw for cultivation of mushroom. In this research, higher yield was obtained compared to Sharma *et al.* (2013) [22] with 381.85 g yield of *Pleurotus ostreatus* growing on rice straw, rice straw + wheat straw, rice straw + paper, sugarcane bagasse and sawdust of alder Biological efficiencies (BE), the conversion efficiency of substrate in mushroom cultivation, was computed as the ratio of the fresh mushroom harvested per bag to the dry weight of each substrate. The maximum biological efficiency (64.64 ± 273.1) was recorded on T3, while the lowest ($17.92 \pm 81.95\%$) BE was obtained from T4. This is in line with the works of Holkar and Chandra (2016) [8] who reported that the biological efficiencies of *P. Ostreatus* growing on wheat straw ranged from 63.4 to 74. As per Gume *et al.* (2013) [7], substrates that gave over 40% BE could be recommended for oyster mushrooms cultivation. Thus, the current study reveals that all the treatments except 100% wheat straw (T4) gave higher BE (Table 4).

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