

CULTURE OF OYSTER MUSHROOM, PLEUROTUS FLABELLATUS ON AGRO WASTE – PADDY STRAW.

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Abstract

Mushroom culture is one of the simplest and excellent applications of biotechnology. Mushrooms are fungal origin and it now has universal focus and importance because of its nutritional value and therapeutic values which can be used for the remedy of various diseases and disorders. Mushroom cultivation uses agriculture waste products such as paddy straw, wheat straw sugar cane magesy, coconut pith etc., This present research is aimed to production of oyster mushroom *Pleurotus flabellatus* on agro waste paddy straw. The information will help the farmers for decision making for diversifying their cultivation as well generating extra income.

Key words: Agricultural waste, paddy straw, oyster mushroom, production

Introduction

Oyster mushroom (*Pleurotus flabellatus*) is an edible mushroom. It contains adequate amount of phosphorous, iron, protein, lipid, riboflavin and thiamine. Oyster mushroom fresh fruiting bodies indicates a high quantity of moisture (90.8%), where as dry as well as fresh oyster mushrooms are rich in carbohydrate (57.6%), protein (30.4%), fiber (8.7%), fat (2.2%) and ash (9.8%) with 345 kilocalories energy value on 100 g dry weight. Mushrooms are an excellent source of minerals and protein and also known as the vegetarian's meat [44]. The proteins of mushroom are considered to be intermediate between that of vegetables and animals [42]. The amino acids essential for human body are present in oyster mushroom [36]. *P. flabellatus* productivity is maximum in a short time providing more protein per unit area than any other crop [35]. The high content of nitrogen and protein in oyster mushroom increases the biological efficiency and yield while it reduces the growth period. *P. flabellatus* contains 18 essential amino acids such as methionine, isoleucine, lysine, glutamic acid, cysteine, aspartic acid phenylalanine, tyrosine, tryptophan, valine, arginine, histidine, alanine, glycine serine and proline [26]. The vitamins such as niacin, riboflavin and thiamin are found in oyster mushroom. The minerals such as ferrous sulfate, phosphorus, sodium and calcium are present in oyster mushroom [60]. It is used for both medicine and food [6]. According to Furlani and Godoy [32], mushrooms are considered as food with high nutritional value and delicious taste. Due to their low caloric value they are suitable for diets. The *P. flabellatus* produces metabolites of medicinal and pharmacological interest, such as antimicrobials, immunostimulants, antioxidants and antitumourals [28, 56, 38]. The substrate source, spawn quality, strain and compost affect the performance and growth of oyster

mushroom [77, 39]. The oyster mushrooms are known for anti-inflammatory and immune-modulator effects [48]. The cultivation is easy under both temperate and tropical climatic conditions and they are cultivated and harvested all over the year [7].

The present research was carried out to evaluate different agricultural substrates for growth and production of *Pleurotus flabellatus*, and to check quality of mushroom grown on paddy straw. The objectives of this research are to evaluate paddy straw substrate for the growth and production of oyster mushroom and to find the effect of substrate on the quality of oyster mushroom.

Materials and Methods

The culture was developed from the fruiting bodies growing at the Mushroom House. Malt Extract Agar (MEA) was employed as medium for the growth of pure culture of *Pleurotus flabellatus*. For one liter of the culture medium prepared, 20g Malt, 20g Dextrose, 20g Agar, 1g Peptone and distilled water were used to make the volume one liter. After autoclaving at 15 psi for 15 minutes, the pH of the medium was kept 6.5. Streptomycin was added in the sterilized medium @ 1g/ L when the medium was cooled to about 40°C to stop the bacterial contamination. The inoculation was made by cutting small pieces of the mushroom tissues from the cap to downwards with a sterile blade inside the laminar flow unit. A small piece of fruiting body was taken and inserted in to petridish. Plates were sealed with Parafilm and incubated at 25°C. After 3 to 4 days, the white mycelium has covered the agar surface. When the mycelium fully covered the plates, then they are kept in refrigerator for the preparation of spawn. The prepared cultures were multiplied and maintained through standard methods.

Preparation of Spawn

Spawn was prepared in jam bottles. The method of Jain (2005) was followed with some modifications. The paddy grains were boiled for 20-30 min. After boiling, excess water was drained off by spreading the grains on a wire mesh. Chalk powder (calcium carbonate) and gypsum (calcium sulphate) were added to the grains @ of 2% and 0.5%, respectively on dry weight basis. The jam bottles containing paddy grains were sterilized in an autoclave for one hour at 121°C. The grains were allowed to cool at room temperature and inoculated with actively growing mycelium of *Pleurotus flabellatus* from malt extract slants inside the laminar flow unit and incubated at (27 ± 2°C) for mycelial growth without any light for 10-15 days until the mycelium fully covered the grains. Paddy straw substrate was sundried and broken into small pieces and soaked in water to maintain 65-75% moisture content. The soaked substrate was piled up after and covered with polythene sheet. Substrate was allowed to ferment for hours and spread on floor for evaporation of excess moisture. The experiment was laid by using Completely Randomized design having ten replications.

Sterilization and Incubation

The paddy straw substrate was filled in 30 glass bags (12×18 inch) and bag mouth was loosely tied with fiber thread. Bags were sterilized in an air tight container at 15 psi for one hour. The sterilized bags cooled for 2-3 hours were inoculated with spawn @ 25 gm per kg bag [21]. The sterilized bags were then kept in mushroom house at ambient temperature. These bags after inoculation were then incubated for spawn running in a mushroom house under darkness at ambient temperature.

Cropping

When mycelia fully covered the substrates after 15-16 days, the bags were torn apart to open the substrates. The compact substrates were irrigated at least twice a day by sprinkling fresh water. After 7–8 days of the opening of bags small size pin heads (4-5 cm in diameter) appeared on all sides of the bags. These pinheads attained the full size in about 2-3 days and when fruiting body fully matured then they were harvested. The pin heads appearance time was also recorded. The oxygen needed for the fruiting bodies development was fulfilled by running fan several times daily. The experiment was laid out in a complete randomized design (CRD) having 5 treatments and ten replications. Data were recorded on the following parameters. Data on spawn running was recorded in days on substrate.

Appearance and Maturation of pinhead

After the completion of spawn running the pinheads appearance of *Pleurotus flabellatus* was observed. The data was recorded in days taken from spawning to the appearance of pinheads in of the substrate. When the pinheads reached to maximum size then time period was recorded in days from appearance of pinheads to maturation of pinhead treatment.

Yield

The data on the weight of mushroom in gram was recorded for the harvesting of mushroom. The first and respective harvesting done at maturity of fruiting bodies was noted. The total yield of basidiocarp was measured.

Physical, chemical and biochemical determination

Mushroom sample used in the study was oven dried at 105 ± 5 °C for 24 hours till constant weight was achieved. Moisture, ash content, crude fiber content, protein and crude fat contents were tested according the procedure followed by Raghuramulu *et al.*, 2003. Vitamin C, Phosphorous, Iron, Zinc and Nitrogen free extract content were estimated according standard procedure.

Results

The result shown in Table 1 indicates that fastest spawn running took place on paddy straw and it takes 26 days. Result of the experiment about the appearance of pinheads and their maturity are presented in Table 1. It was observed that time taken for first appearance of pinhead after spawning of the substrates was fastest (27 days) in paddy straw.

The paddy straw took 38 days from appearance of pinheads to the maturity of pinheads. In response to maturity of pinheads, the statistical analysis shows that paddy straw is highly significant. The average yield of the substrate from first flush was 430 gram, respectively. The total yield was determined by taking the weight of mushroom obtained after 1st, 2nd and 3rd flush. The moisture content of mature fruiting bodies of *Pleurotus flabellatus* cultivated on paddy straw is shown in Table 2. The Table indicates that higher moisture content (87%) was observed. The total Ash content of mature fruiting bodies of *Pleurotus flabellatus* grown on different agricultural waste paddy straw is shown in table 2. The result indicates that the ash content of sorghum straw 0.41% level.

The protein content of mature fruiting bodies of *Pleurotus flabellatus* cultivated on paddy straw and the result indicates that high protein content 7.5 gram level. From the Table it was observed that the paddy straw have the medium crude fat and crude fiber content 4 % and 2.50 % level. The vitamin C contents of mature fruiting bodies of *Pleurotus flabellatus* cultivated on paddy straw is presented in table 3. The result indicates that paddy straw exhibit vitamin C content of 12.17mg/ 100gram. From the result it was evident that paddy straw has phosphorous content of 0.59 mg/100 gram. Iron content was observed that paddy straw have 0.009 mg/ 100 gram. The zinc and nitrogen free extract of mature fruiting bodies of *Pleurotus flabellatus* cultivated on paddy straw was observed that 0.003 and 3.78 gram/100 gram.

Discussion

The study indicated that *Pleurotus flabellatus* can be successfully grown on almost all agricultural wastes but paddy straw gave better results. The structure of substrates is also important because it help in the penetration of mycelium. Paddy spawn is commonly used for mushroom cultivation because the growth of mycelium is faster on other grains. Present study revealed that the growth rate of mycelium is faster. Paddy straw has been used for the cultivation of oyster mushroom since the beginning of 19th century and it has been cultivated in many countries under natural condition [69].

Paddy straw is the natural substrate on which oyster mushrooms are cultivated leading to name the mushroom as delicious straw reported by Fasidi (1996) [31]. According to Balasubramanya and Kathe (1996) [8], the fungus *Penicillium* spp. and *Trichoderma* spp competed with *Pleurotus* spp. after pasteurisation with hot water (80°C for 2h) probably due to the partial breakdown of hemicellulose, cellulose and thus making them available to competitors. The successful mushroom cultivation depends on the purity and quality of spawn. Nita Bahl (1984) [58] reported that grain spawn is now almost universally used. The difference in days for full mycelial running on different substrates might be due to variation in their chemical composition and C: N ratio as reported by Bhatti et al. (1987) [11]. The results recorded on spawn running on different substrates were almost similar to the findings of Shah et al. (2004). They reported that the spawn running took 16-25 days after inoculation. Similar results were also reported by Tan (1981) [90] who reported 21 days for complete spawn running on cotton waste. Similar findings were also reported by Jiskani et al. (1999) [40]. The pinheads appeared earlier in paddy straw than other substrates.

Khan et al. (1981) [44] observed the appearance of 30 pin-heads of *P. ostreatus* (Strain,- 467) in 36 days, the same of *P. sajarcaju* and *P. ostreatus* in 40 and 46 days, after spawning. Tan (1981) [90] recorded 23-26 days for the appearance of pinheads. Ramzan (1982) [73] observed 20-40 days of five *P. ostreatus* strains on wheat and paddy straw. Patra and Pani (1995) [64] recorded 20-24 days on paddy straw.

Khan et al. (1981) [44] recorded 21-28 days for the maturity of pinheads in case of cotton boll locules. Khanna and Garcha (1981) [45] recorded 20-24 days for the maturity of pinheads on paddy straw and Tan (1981) [90] observed a month for the maturity of pinheads on cotton waste. There is difference in yield obtained from different substrates. The present finding indicates that highest yield was obtained in wheat straw followed by paddy straw. Bhatti et al. 1987 [13] observed the highest yields with the shortest incubation period in case of wheat straw. It was generalized from the data that first flush yield was highest in all treatments followed by second and third flush. Other scientists also recorded similar results. Zadrazil (1973) [95] got 2 flushes,

Tan (1981) [90] got three flushes, Ramzan (1982) [73] obtained 3-5 flushes from wheat and paddy straw and Bhatti (1984) [12] got 4-6 flushes from different substrates.

Kausar and Iqbal (1994) [43] investigated that B.E varied from 18.6 to 83.5% on the basis of different nitrogen supplements amended with straw. Jiskani et al., (1999) [40] obtained 24 and 7.6% fresh and dry yield on the basis of substrate dry weight by using wheat straw. Jiskani (1999) [40] reported that one kg of dry substrate can produce one kg of fresh mushroom which is the 100% substrate dry weight. According to Bughio (2001) [16] the maximum dry and fresh (wet) yield percentage on substrate dry weight basis (29.61 to 77.91 and 5.91 to 21.70) were obtained from wheat straw using in combination with sugarcane bagasse, paddy straw, cotton boll locules and sorghum leaves.

Graham and Clyde (1985) [33] recorded 80-120 percent biological efficiency of *P. sajor-caju* on cotton waste. Moonmoon et al., (2010) [55] studied *P. eryngii* King Oyster mushroom on paddy straw and saw dust in Bangladesh and found that saw dust. Nunez and Mendoza (2002) [59] reported that the biological efficiency of the studied substrates varied from 106.2 to 50.8% of *Pleurotus flabellatus*. The moisture content of the studied mushroom ranged from 88.20% to 93.44%. Moisture percentage in mushroom depends on the maturity of fruiting bodies, species and storage conditions during packaging or processing [34]. Present study revealed that the ash content of studied mushroom ranged between 0.33% - 1.006%. The amount of ash was higher in sorghum than other substrates. The amount of ash depends on salt content in substrates [68]. (Bonatti et al. (2004) [15] reported 0.5-0.6% of ash in dried *P. sajor* whereas Alam et al. (2008) [5] recorded 1.1 and 8.28 g/100g in fresh and dried *P. sajor-caju*, respectively. El-Kattan et al., (1991) [27] reported 8.00% and 6.60% ash content of *P. flabellatus* on soybean and paddy straw, respectively. The analysis of mushroom composition indicated that sugarcane bagasse gave high amount of proteins and amino acids from other substrates.

The protein content usually ranges between 20–30% on a dry weight basis. The nitrogen present in substrate after spawn running enhances the mushroom yield and quality, in addition it help in bioconversion and bioaccumulation efficiency [63]. The fat content of *P. flabellatus* was 10% grown on maize straw being the highest followed by sorghum straw (2.50%). The percent content were similar as reported in earlier studies [63, 62]. The fat content ranged between 2.56% to 2.82% on dry weight basis. The fat content of dried *P. sajor-caju* was 5.26 and 4.99% on paddy straw and banana straw, respectively [15]. The highest crude fiber (3.5%) was obtained from mushroom on wheat straw followed by paddy straw (2.75%). Other agro wastes also yielded appreciable level of crude fiber. These results were confirmed with the findings of Singh et al. (2003) [83], Bonatti et al. (2004) [15], Kadlag et al., (1998) [41] and Sharma & Madan (1993) [81]. The amount of protein of *P. flabellatus* found in this study is near about similar to the results of Rai and Sohi (1988) [71] and Alam et al. (2007) [4]. But fiber and ash content are different from the report of Rai and Sohi (1988) [71], however relevant to Alam et al. (2007) [4].

Protein content in *P. flabellatus* were also similar to the findings of Banik and Nandi (2004) [9] as well as fat value of *P. flabellatus* is relevant to the findings of Shashirekha et al. (2005) [82]. The results indicated that not only the protein content of the substrate but also nature of protein in the substrate influences the protein content of the fruiting bodies [92]. The amount of vitamins C were maximum (11.35mg/100 gram) in paddy, maize and sorghum straw. Bano (1976) recorded 13.0 to 14.70 mg/100 g ascorbic acid in various mushroom species. Present study revealed that sugarcane bagasse have high phosphorous amount as compare to other

substrates. Similar results were observed by Chang et al. (1981) [20] and Alam et al. (2007) [4], but differ from [79] who recorded 0.97% phosphorus in oyster mushrooms grown on sawdust. The amount of phosphorous was maximum on soybean straw (920mg/100gm) whereas least was found on soybean straw and wheat straw (800 mg/100gm) [17]. Phosphorus content of *P. flabellatus* ranged from 790 – 1000 mg/100g [65].

Conclusion

Paddy straw was found most suitable substrate for mushroom cultivation. The spawn running, appearance and maturity of pinheads were fastest and also showed the highest flush wise yield, total yield and biological efficiency. Moisture, ash, nitrogen free extract and vitamin C were maximum in paddy straw. The highest protein content was obtained from paddy straw. Meanwhile the percentage of crude fat and iron were highest. Farmers are advised to use wheat straw for *Pleurotus flabellatus* cultivation for bumpy production. However as more nutrients have been found in mushroom grown on paddy straw, so they can also use paddy straw as substrate for the production of high quality mushrooms.

Table 1 Production of *Pleurotus flabellatus* on paddy straw substrate

S.No	Paramerters	Days
1	Days to spawn	26
2	Days of pin head appearance	38
3	Days to maturity	40
4	Yield (gm)	430

Number of replications n=8

Table 2 Percent moisture, ash, protein, crude fat and crude fiber in oyster mushroom (*Pleurotus flabellatus*) grown on paddy straw substrate

S.No	Paramerters	Percentage (%)
1	Moisture	87
2	Ash	0.41
3	Protein	7.5
4	Crude fat	4
5	Crude fiber	2.50

Number of replications n=8

Table 3 Vitamin C, Phosphorous, Iron, Zinc and Nitrogen free extract in *Pleurotus flabellatus* (mg/100 gram) grown on paddy straw substrate

S.No	Paramerters	mg/gram
1	Vitamin C	12.17
2	Phosphorous	0.59
3	Iron	0.009
4	Zinc	0.003
5	Nitrogen Free Extract	3.78

Number of replications n=8

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