



COMPARATIVE STUDY OF DIFFERENT GRAINS ON SPAWN DEVELOPMENT OF *PLEUROTUS FLORIDA*

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ABSTRACT

In recent year, there is great advancement in mushroom technology. Now commercially for food as well as medicinal purpose the production of mushroom has become important factor. In the present study, Mushroom seed (spawn) production involves high investment requiring laboratory and equipment with accessories three grain viz sorghum, rice, and wheat grain were tested for the production of *Pleurotus florida* for spawn, yield and bio efficiency. The result showed that rice grain (10days) taken for spawn, pinhead formation, stalk length, pileus diameter, pileus thickness, yield (383.81 ± 0.24) and bio efficiency (76.76%) were found to be the best substrate for spawn development of *Pleurotus florida* for cultivation paddy straw used as a substrate. Whereas sorghum (12days) yield (303.63 ± 0.01) and bio efficiency (60.72%) and wheat grain (13days) yield (283.21 ± 0.01) and bioefficiency (56.64%) took period of spawn development of *Pleurotus florida*.

Keywords: *Pleurotus florida*, grain spawn, spawn run time, bio efficiency

INTRODUCTION

Edible mushrooms are nutritionally rich (mostly Basidiomycetes) that grow on the trunks, leaves and roots of trees as well as decaying woody materials (Chang and Miles, 1992; Stamets, 2000; Lindequist *et al.*, 2005). These edible mushrooms include *Agaricus* spp. (Button mushrooms), *Volvariella volvacea* (Oil palm mushrooms), *Auricularia auricular* (wood ear mushroom), as well as *Pleurotus florida* (Oyster mushroom). Thus spawn comprises mycelium of the mushroom and a supporting medium which provides nutrition to the fungus during its growth. The propagating material used by the mushroom growers for planting beds is called spawn. The spawn is equivalent to vegetative seed of higher plants (Pathak *et al.*, 2000). In mushroom growing technology, the inoculums are known as the 'spawn'. Spawn is a medium that is impregnated with mycelium made from a pure culture of the chosen mushroom strain. Spawn production is a fermentation process in which the mushroom mycelium will be increased by growing through a solid organic matrix under controlled environmental condition. In almost all cases the organic matrix will be sterilized grain e.g. wheat, maize, sorghum etc (Jain and Vyas, 2005; Jain, 2005).

Growing medium of the mushroom is generally known as substrate. The substrates used for production of oyster mushroom are normally nitrogen deficient. An addition of organic and inorganic supplements to the substrate from outside to improve the yield of mushroom have therefore been recommended by many workers (Royse and Schisler, 1987a,1987b; Royse and Bahler, 1988; Royse, 2002; Madhusudhanan and Chandra Mohan, 2002; Jain and Vyas,2002; Jain and Vyas, 2005; Chaubey *et al.*, 2010). An attractive feature of oyster mushrooms is that they can utilize a large variety of agricultural waste products and transform the nature mushrooms use spores for generative multiplication and these are microscopic and difficult to handle (Oei, 1991). Alternatively, tissue cultures taken from cap-tissues may be used to prepare spawn. The fully colonized grain (spawn) is used to seed already prepared substrates such as agricultural and non-agricultural wastes for mushroom production. Grain spawn is in common use because of its ability to the substrate faster and ease of planting (Bahl, 1988).

Mushroom substrate has been defined as a ligno cellulose material which supports the growth, development and fruiting of mushroom (Chang and Miles, 1988). Vast quantities of renewable lignocellulose waste are generated every year in developing countries like India with economics, which are basically agricultural waste material left after animal consumption can be used as substrate for mushroom production. Mushroom spawn is the mushroom mycelium growing on a given substrate and serves as the planting material (seed) in mushroom production (Stanley and Awiwaadu 2010). They have immense abilities to utilize various lignocellulose substrates with the aid of extracellular enzymes capable of degrading complex organic material (Martinez-Carrera, 2002). Mushrooms play an important role in managing organic wastes whose disposal have become a problem and are causing massive pollution to the environment as a result of dumping of agricultural wastes (Das and Mukherjee, 2007; Akinmusire *et al.*, 2011). The wide range of plant waste that have been reported include sawdust, paddy straw, sugarcane baggage, cornstalk, corn cobs, waste

cotton, leaves and pseudo stem of banana, water hyacinth, duck weed, rice straw etc. and does not require costly processing method and enrichment material (Modal 2010; Stanley, 2011).

Mushroom offers for converting lignocellulosic residues from agricultural field, forest into protein rich biomass. Such processing of agro waste not only increase nutrient cycling in the environment but the by-product of mushroom cultivation is also a good source of manure, animal feeds and soil conditioner. Improved spawn production technology is necessary to increase the production of mushroom. Therefore, an attempt has been made to find out the best grain for spawn production.

MATERIALS AND METHODS

The grains viz., wheat, rice and sorghum were collected from the local market and screened to determine the most suitable grain spawn for better yield, biological efficiency and minimum spawn run time of *Pleurotus florid*. *Pleurotus floridia* pure culture (oyster mushroom) was collected from KVK Mushroom Foundation, Pondicherry.

Media and pure culture preparation:

The PDA was prepared by using 250g of dextrose 30g of agar and 250mg of chloramphenicol in one liter of water. About 5 ml of PDA mixture was poured in each test tube. The media was sterilized in autoclave for 15 minutes at 121°C. The sterilized PDA containing test tube was kept in a slanting position. The mushroom was thoroughly pre-washed with distilled water. Then it was cooled for 10 seconds. The cut was made length wise from the cap to downwards. Small pieces of the internal tissue of the mushroom was taken aseptically and then immediately inserted in to the test tube slant and the tissue laid on the agar surface. After 3 to 4 days, the tissue was covered with a white mycelium that was spread on the agar surface.

Preparation of mother spawn:

The mother culture substrates were prepared by using good quality of wheat, rice grain and sorghum. At first, 5kg of rice, wheat and sorghum were boiled for about 1 hour (30-45 min) until the three grains become brown and looked like succulent. Filtered rice, wheat and sorghum were spread out on a sterile polythene sheet placed on the floor. After draining out of excessive water, 1% CaCO₃ (50g) were mixed with rice straw manually and packed tightly in 5x12 inch polythene bag. Each bag containing 250g of rice, wheat, sorghum and plugged the neck with cotton. Then the bags were sterilized in an autoclave for an hour at 121°C. After sterilization the bags were allowed to cool for a day. Then a piece of pure culture was placed aseptically through the hole of the mother culture packet and again the packets were plugged with cotton and covered the brown paper with a rubber band. It was placed into the growth chamber at 25°C in dark place. After 15 to 21 days the packet of the mother culture become white due to the completion of the mycelium

running and then it was ready for inoculation in the spawn packet. The grain under aseptic condition and the pocket were incubated at 25°C for 15 day.

RESULTS

In the present work, three different grains were evaluated for spawn production of *P. florida*. The parameters assessed in the study, deals with the number of days taken for spawn production from the day of inoculation to the growth and development of mycelial net on the different grains showed in the Table 1 & 2. The spawn development was determined by the white mat of mycelia of *P. florida* on each grain. The sorghum (12 days) and wheat grain (13 days) took same period for spawn development. Rice grain recorded minimum (10 days) period for spawn development and it was suggested for spawn production by using *P. florida*. Irrespective of substrates used for production of *P. florida* under the condition of test mushroom utilize rice grain more efficiently in comparison to sorghum and wheat grain.

Substrate	DFSR	DFPA	Stalk length (cm)	Pileus diameter (cm)
Rice grain	10	4	4.6±2.4	7.7±2.5
Sorghum grain	12	6	3.8±2.1	4.3±2.2
Wheat grain	13	8	2.8±1.9	3.8±2.1

Table 1: Effective of different grain on spawn development of *Pleurotus florid*

DFSR =Days for spawn run, DFPA =Days for pin headed appearance

Mycelium running rate in spawn packet ranged from 10 to 12 days. The highest mycelium running rate was observed on rice grain with paddy straw substrate. The lowest mycelium running rate was recorded on wheat grain with paddy straw substrate (Table. 1). The presence of right proportion of alpha -cellulose, hemicellulose and light was the probable cause of higher rate of mycelium running rate in rice grain.

The minimum duration (4 days) for primordia initiation observed on rice grain spawn. The maximum duration for primordia initiation was found in sorghum grain spawn (6 days). The long duration for primordia initiation was found in wheat grain spawn (8 days). Shah *et al.*, 2004 found spawn heads appeared 6 days after the spawn running. There was a significant result in length of fruit body. Rice grain (4.6±2.4) showed maximum stalk length in sorghum (3.8±2.1) and lowest stalk length of fruit body was observed in (2.8±1.9) wheat grain.

Spawning	Yield (gm)			Total yield	Bio efficiency (%)
	I	II	III		
Rice grain	214.73±0.65	104.01±0.78	65.07±0.32	383.81±0.24	76.76
Sorghum grain	198.26±0.02	83.37±0.37	31.40±0.24	303.63±0.01	62.60
Wheat grain	175.82±0.65	73.97±0.21	24.02±0.45	283.21±0.01	54.76

Table 2: Yield and bio efficiency of *Pleurotus florida* in paddy straw substrate

The results revealed the yield, biological efficiency (B.E.) of the *P. florida*. Significantly maximum gram of yield of *P. florida* was obtained when it was cultivated on 0.5kg of paddy straw (383.81±0.24) rice grain and sorghum grain (303.63 ± 0.01) (Table. 2.). The maximum biological efficiency (BE), with 76.76% was observed in rice grain and sorghum grain (62.60 %). Whereas lowest gram of yield (283.21±0.01) and lowest B.E (54.76 %) was recorded in wheat grain (Table. 2). The crop of oyster mushroom was harvested in three flushes. Maximum yield was obtained in the first flush than the second and third flush.

DISCUSSION

Our present study clearly indicates that rice grains spawn are best for production of *P. florida* followed by sorghum grain spawn. Wheat grain spawn was comparatively less effective for production of *P. florida*. Selection of a suitable strain is the prime and important aspect in the spawn preparation. Second aspects of a quality spawn is selection of a suitable spawn substrate. The choice of grain depends on the availability of the same in a particular locality. Variations in spawn run rate and yield 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). Many workers worked on development of different grain spawns and their effect on yield (Elliot, 1985; Fritsche, 1988; Sharma, 2003; Chaurasia, 1997; Royse, 2002; Jain, 2005; Shah *et al.*, 2004; Arulnandhy and Gayathri, 2007; Pathmasini *et al.*, 2008). Pathmasini *et al.*, (2008) used locally available grains maize (broken), sorghum and paddy straw for spawn production. Chaubey, (2010) used wheat, maize and sorghum grain spawn for the cultivation of oyster mushroom. Thulasi *et al.* (2010) reported spawn production of oyster mushroom on different substrates. Khan *et al.* (2011) reported different spawning methods of oyster mushrooms on cotton waste.

Jiskani et al (2000) conducted experiments on the effect of different grain on spawn growth of oyster mushroom, *P. florida* and reported that the sorghum grain were found to be best medium for spawn growth followed by maize, wheat and millet grain, respectively. The observations are in agreement with the result of Kotwaliwale et al (1991) grains were suitable for spawn development of *Pleurotus* species (Sharma, 2003).

Hence, it is concluded that rice grain is best the grain spawn for production of oyster mushroom, *Pleurotus florida* preparing a suitable spawn. Therefore it is of great economical important and it also help to overcome the protein malnutrition in the world. These variations are mainly related to spawn rate, fungal species used and supplement added to the substrate (Mane *et al.*, 2007). Some of the elevated B.E. of *Pleurotus* spp. on commonly used substrates rice straw 85.5% (Mehta *et al.*, 1990), leguminous plants 103.8% (Sharma and Madan, 1993). *Pleurotus florida* mushrooms, commonly known as oyster mushrooms, grow in the wild in tropical, subtropical and temperate regions and are easily artificially cultivated (Akindahunsi and Oyetayo, 2006; Chirinang and Intarapichet, 2009). According to Chang and Miles (1982), BE of *P. florida* can be increased to nearly 100% depending on the composition of the substrate.

Costanin and Matruchot (1894) the two Frenchmen from Pasteur Institute, France germinated spores, made culture and used it for making spawn after sterilizing horse manure (Manure Spawn). These *Pleurotus sajor-caju* significantly on different amount of substrate with different amount of spawn. Kibar and Peksen (2008) recorded effect of temperature and light intensity on the development and yield of different *Pleurotus* species. About similar result was found by Pandey *et al.*, 2008 in their experiment maximum yield of *Pleurotus sajor-caju* was recorded on paddy straw.

Commercial production of oyster mushrooms is largely determined by the availability and utilization of cheap materials of which agricultural lingo-cellulosic waste represents the ideal and most promising substrates for cultivation. The substrates used in this study can be considered practical and economically feasible due to their availability throughout the year at little or no cost in large quantities. Utilization of these agro-wastes for the production of oyster mushrooms could be more economically and ecologically practical.

CONCLUSION

This study has demonstrated that locally available organic substrate is potentially suitable for use in the production of mushrooms. The suitability of different spawn substrate for mushroom cultivation was also confirmed by the average biological efficiency which was variable among the substrates. Rice grain shows the highest yield and biological efficiency. It is clear that rice grain in combination with paddy straw substrate is best substrate for oyster mushroom cultivation.

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REFERENCES

1. Chang ST and Chiu SW. Mushroom production an economic measure in maintenance of food security. In DaSilva, E. J., Ratledge, C. and Sasson, A. (Eds). *Biotechnology: Economic and social Aspects*, p 110-141. USA: Cambridge University Press.1992.
2. Lindequist U, Niedermeyer THJ and Julich W. The pharmacological potentials of mushrooms. *eCAM*. 2005. 2: 285-29.
3. Stamets P. *Growing Gourmet and Medicinal Mushrooms*.3rd edn. California, Berkley:Ten Speed Pres 9.2000.
4. Jain AK and Vyas D. Supplementation of Soybean choker: Enhances the growth and yield of *P. sajor-cajugrown* in lignocellulosic waste. *Journal of Basic and Applied Mycology*. 2005. 3&4: 88-90.
5. Jain AK. Thesis on Mushroom Cultivation with special reference to *Pleurotus florida* and their Marketing potential in Sagar Region.2005.
6. Jain AK and Vyas D. Yield response of *Pleurotus florida* on wheat straw in combination with other substrate. *Mushroom Research*. 2002. 11: 19-20.
7. Jain AK and Vyas D. Comparative study on the yield of three *Pleurotus* sp. grown in several lignocelluloses By- products. *Journal of Basic and Applied Mycology*. 2005. 4:155-157.
8. Royse DJ and Schisler LC. Yield and size of *Pleurotus ostreatus* and *Pleurotus sajor-caju* as effected by delayed release nutrient supplementation. *Applied Microbial and Biotechnology*. 1987. 26: 191-194.
9. Royse DJ and Schisler LC. Influence of benomyl on yield response of *Pleurotus sajor-caju* to delayed release nutrient supplementation. *Horticulture Science*. 1987. 22: 60-62.
10. Chaubey A. Studies on cultivation technology of medicinal mushrooms with special reference to marketing potential in Bundelkhand region. Ph. D. Thesis. Dr. H.S.Gour, University, Sagar, M.P. 2010.
11. Arulnandhy V and Gayathri T. Identification of suitable and efficient mushroom Undergraduate research report, Department of Agricultural Biology Eastern University, Sri Lanka. 2007.
12. Mane VJ, Patil SS, Syed AA and Baig MMV. Bioconversion of low quality lignocellulosic agricultural wastes into edible protein *Pleurotus sajor-caju* (Fr.) Singer J. Zhejiang. *Universal Science Biology*. 2007. 8: 745-751.
13. Oei P . *Manual on mushroom cultivation technique,species and opportunities for commercial applications in developing countries (first edition)*. Tool Foundation, Amsterdam. 1991. 249
14. Pandey RS. and SK Ghosh. *A Handbook on Mushroom Cultivation*. Emkay publications, Delhi. pp. 2008. 134.
15. Royse DJ and Schisler LC. Yield and size of *Pleurotus ostreatus* and *Pleurotus sajor-caju* as effected by delayed release nutrient supplementation. *Applied Microbial and Biotechnol*. 1987. 26: 191-194.
16. Shah ZA, Asar M and Ishtiaq. Comparative study on cultivation and yield performance of oyster mushroom on different substrates (wheat straw, leaves,saw dust). *Pakistan J. Nutrition* 2004. 3: 159-160.
17. Sharma BB. Effect of different substrates on spawn growth and yield of pink oyster mushroom *Pleurotus*

- djamar*. J. of Mycol. and Pl. Pathol. 2003. 33 (2):265-26.
18. Thulasi EP, Thomas D Ravichandran B and Madhusudhanan K. Mycelial culture and spawn production of two oyster Mushrooms, *Pleurotus florida* and *Pleurotus eous* on Different Substrates. International Journal of Biological Technology. 2010. 1(3): 39-42.
 19. Fritsche and Fritsche G. Spawn: properties and preparation, In: The Cultivation of Mushrooms. van Griensven, L.J.L.D. (Eds.), Darlington Mushroom Laboratories, Sussex. Pp.1988. 1–99.
 20. Elliot TJ. Spawn – making and Spawns. In: *The Biology and Technology of the Cultivated Mushrooms* P.B. Flegg, D.M. Spencer and D.A. Wood (Eds.), John Wiley & Sons Ltd. Pp.1985. 131– 139.
 21. Sharma S and Madan M. Microbial protein from leguminous and non-leguminous substrates. Acta Biotechnologica. 1993. 13: 131–139.
 22. Pathmashini L, Arulnandhy V and Wilson RS. Cultivation of oyster mushroom (*Pleurotus ostreatus*) on saw dust. Cey. J. Sci. Bio. Sci. 2008. 37 177-182.
 23. Pathak VN, Yadav N and Gour M. Mushroom Production and Processing Technology. Agrobios, India, 2000.
 24. Mehta V, Gupta JK and Kaushal SC. Cultivation of *Pleurotus florida* mushroom on rice straw and biogas production from the spent straw. World Journal of Microbiology and Biotechnology 1990. 6, 366–370.
 25. Chang, Miles Chang ST and Miles P.G. Edible Mushroom and their cultivation. CRC press, Inc. Boca Raton, Florida U.S.A. 1988.27:83-88.
 26. Chaurasia VK Studies on production technology of *Pleurotus columbines* at Raipur. M.Sc. Thesis submitted at I.G.A.U. Raipur (C.G.).
 27. Stanley HO and Awi-Waadu GD. Effect of substrates of spawn production on mycelial growth of Oyster mushroom species. Research Journal of Applied Sciences. 2010. 5: 161-164.
 28. Kibar B and Pekien A. Modelling. Modelling the Effects of temperature and light intensity on the development and yield of different *Pleurotus species* Agricultura Tropica ET Subtropica. 2008. 41: 68 -73.