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Effect of different spawn rates and substrate supplementation on yield of Indian Oyster mushroom, *Pleurotus pulmonarius* (Fr.) Quel.

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Abstract: A study was conducted to evaluate the effect of different spawn rates and substrate supplementation on yield of *Pleurotus pulmonarius* (Fr.) Quel. Among six spawn rates viz., 0.5%, 1%, 2%, 4%, 6% and 8%, respectively tried on wheat straw substrate, the spawn run was fastest (10.50 days) when spawn dose was 8%, followed by 6%, 4%, 2%, 1% and 0.5%, respectively. The pinheads appeared in 12.27 days by using spawn @ 8%, which proved to be the best spawn dose followed by 6%, 4%, 2%, 1% and 0.5%, respectively. Highest yield of 168.7 per 200g dry substrate was achieved @ 8% spawn rate. Lesser yields were recorded when spawn rate was reduced. The results also reveals the significantly highest biological efficiency of 84.33% at 8% spawn rate followed by 6%, 4%, 2%, 1% and 0.5%, respectively. It was concluded that spawn run was rapid at higher spawn rate but there was not much difference in yield when spawn dose was increased from 4 to 8%. Considering spawn cost and performance shown by different doses, 2-4% was found optimum dose for its cultivation.In case of substrate supplementation, wheat straw supplemented with cotton seed meal supported maximum mycelial growth (10.50 days of inoculation) and took minimum time for pinheads initiation (13.67 days). Similarly, maximum yield (155.3g) with biological efficiency of 77.65% was recorded on wheat straw supplemented with cotton seed meal followed by supplementation of saw dust, wheat straw (control), calcium ammonium nitrate (CAN), ammonium nitrateand urea, respectively. These studies will help to mushroom growers for selecting the most suitable spawn rate and also opens viable option of supplementation as wheat straw + cotton seed meal for better growth behaviour and optimum yield potential of *Pleurotuspulmonarius* as well as other oyster mushrooms cultivation.

Keywords: Biological efficiency, Spawn rates, Supplementation, Wheat straw

INTRODUCTION

The global food and nutritional security of growing population is a great challenge, which looks for new crop as a source of food and nutrition. In this context, mushroom cultivation helps to address the issue of nutritional security and also provides solution for proper recycling of agro-wastes. In addition to good quantity protein, no cholesterol, high fiber, low sodium, good quantity of vitamins and minerals, protein polysaccharide complexes that impart unique medicinal values like anti-cancer and anti-viral properties. Globally, China is the leading producer of mushrooms with more than 70% of the total global production which is attributed to community based farming as well as diversification of mushrooms. In India, owing to varied agro -climate and abundance of farm waste, different types of temperate, tropical and sub-tropical mushrooms are cultivated throughout the country.With ever increasing demand for quality food, mushroom cultivation is now emerging as an important activity in different parts of our country (Ambili and Nithya, 2014).

Mushroom cultivation can help in supporting the local economy by reducing vulnerability to poverty and strengthens livelihoods through generating additional employment and income through local, regional and national trade and offering opportunities for processing enterprises (Bose, 2016). In India, only 3 species, namely, *Agaricus bisporus*, *Pleurotussajorcaju* and *Volveriellavolvacea* are preferred for commercial cultivation. Of the three cultivated species, the white button mushrooms have the highest consumer preference and account for about 90 per cent of total mushroom production. Oyster mushroom (*Pleurotus*sp.) belonging to Class Basidiomycetes and Family Agaricaceae is popularly known as 'Dhingri' in India and grows naturally in the temperate and tropical forests on dead and decaying organic matters and wooden logs or sometimes on dying trunks of deciduous or coniferous woods (Randive, 2012). Cultivation of oyster mushroom (*Pleurotusostreatus*) has increased tremendously throughout the world because of their abilities to grow at a wide range of temperature and utilizing various agro-based residues (Sharma *et. al.,* 2013).

The oyster mushroom (*Pleurotus*sp.) is a very popular mushroom due to its tremendous stability of cap and stem, cooking qualities and longer shelf life. Among the consumers, where *Pleurotus* mushroom is very trendy, but currently this mushroom is not cultivated in large scale. The oyster mushroom gained importance during the last decade and now several species such as *P. sajor-caju*, *P. florida*, *P. sapidus*, *P. eryngii*, *P. columbinus*, *P. cornucopiae*, *P. flabellatus*, *P.platypus*, *P. opuntiae*, *P. citrinopileatus*of *Pleurotus* are available for commercial production. It is now being cultivated in many countries in the subtropical and temperate zones. *Pleurotus*have the ability to excrete hydrolyzing and oxidizing enzymes which have capable of utilizing complex organic compounds that occurred agricultural wastes and industrial by-products with broad adaptability varied agro-climatic conditions (Bhattacharjya *et al.,* 2014). They possess extensive and efficient enzyme systems to degrade successfully a wide variety of inexpensive substrates such as lignin, cellulose, hemicelluloses, pectin and other industrial wastes resulting in the cheapest method of waste disposal as well as production of protein rich food (Subramanian and Shanmugasundaram, 2015).

Oyster mushroom can be grown on various substrates including paddy straw, maize stalks/cobs, vegetable plant residues, bagasse etc. However, an ideal substrate should contain nitrogen (supplement) and carbohydrates for rapid mushroom growth (Ashraf *et al.,* 2013). The mushroom mycelia requires specific nutrients for its growth and the addition of supplements increases mushroom yield by providing specific nutrients for the mycelium growth. Hence good growth and better yield of mushroom can be achieved when different substrates are supplemented (Josephine, 2015).

Among various cultivated Oyster species, *Pleurotuspulmonarius* (Fr.) Quel, being saprophytic can easily be introduced in any agroclimatic zone of the world. It is one the most appreciated mushroom which has been gaining popularity recent years especially because of its very good taste, attractive fruit body, high nutritional and medicinal value and capacity to grow under sub-tropical conditions, promises its cultivation as commercial species in India. It is warm weather variety and is mostly cultivated in Europe and the Northern America. Its mycelium and fruiting body can grow under a wide range of temperatures, from 10 to 31 ◦C, which signifies that its fruiting body iscapable of withstanding high ambient temperatures(Zhang *et al*., 2005; Yingyne*et al.,* 2014).

In India, not much work has been done on the cultivation technology of this mushroom. It is in the light of these facts,the aim of current work was to evaluate different spawn rates and to determine the influence of different substrate supplementation materials on the growth parameters and yields of *Pleurotuspulmonarius* cultivation. For successful cultivation of *P. pulmonarius*, it is, therefore, necessary to understand its appropriate spawn rate and appropriate substrate supplementation besides, considering spawn cost.

MATERIALS AND METHODS

The present investigation was carried out during 2012-14 at the Mushroom Research Laboratory, Department of Plant Pathology, Dr YS Parmar University of Horticulture and Forestry, Nauni, Solan (H.P). The culture of *P. pulmonarius*(Fr.) Quel, was procured from Directorate of Mushroom Research,Chambaghat,Solan and was maintained for further study on Potato Dextrose Agar (PDA) medium.

The chopped straw substrate was treated with a solution of 75 ppm carbendazim + 500 ppm formaldehyde for 18 hours. Excess water was drained in shade to retain 65-70% moisture with pH of 6.5. Gypsum ($CaSO_4$) @0.5 per cent and lime ($CaCO_{-3}$) @ 2% were added on dry weight basis of substrate. The substrate was then mixed thoroughly with hands and filled in polypropylene bags (one kilogram/bag) and tightly plugged with non-absorbent cotton. Sterilization was carried out at 22psi for two hours. Spawning was done aseptically by inoculating pasteurized substrate bags with wheat grain spawn @ 0.5%, 1%, 2%, 4%, 6% and 8% on w/w basis of straw by thoroughly mixing. Before spawning, cropping bags were thoroughly sterilized with 2 per cent formalin solution to avoid any surface contamination. In case of substrate supplementation, different supplemented material viz., wheat straw + saw dust (2:1), wheat straw + ammonium nitrate (5g/kg of wet weight of straw), wheat straw + cotton seed meal (5g/kg of wet weight of straw), wheat straw + CAN (5g/kg of wet weight of straw), Wheat straw + urea (2.5g/kg of wet weight of substrate) and were thoroughly mixed with wheat straw at the time of mixing. These were tried to see the ability of test fungus to colonize, pinheads initiation, yield performance and biological efficiency. Wheat straw alone was used as control.After complete mycelia run, the bags were shifted to cropping room. The temperature and relative humidity were maintained at 25^0C and 85 per cent respectively. The bags were sprayed with tap water once or twice a day as and when required for maintaining a relative humidity of 85-90 per cent. Yield was calculated as weight (kg) of mushrooms produced per kilogram of substrate.

The Biological Efficiency (B.E.) was calculated by following the following the standard formula of Chang, 1981.

$$B.E. = \frac{\text{Fresh weight of mushroom}}{\text{Dry weight of substrate}} \times 100$$

The statistical analysis of the observations wasdone as per design of the experiment as suggested by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

In the present study, different spawn ratesand substrate supplementation material were evaluated to see their

Table 1. Effect of spawn dose on yield of *Pleurotuspulmonarius*(Fr.) Quel.

Treatments	Completion of spawn run (days)*	Primordia initiation stage (days)*	Yield (g/200g dry substrate)*	Biological efficiency (%)
0.5%	19.13	23.53	134.3	67.15
1%	17.40	22.07	145.0	72.50
2%	15.00	18.20	151.7	75.83
4%	13.63	14.70	157.3	78.65
6%	12.27	14.00	161.3	80.67
8%	10.50	12.27	168.7	84.33
Mean	14.65	17.46	153.05	
$C.D_{0.05}$	0.46	1.26	5.37	
S.E	0.21	0.58	2.46	

*Average of three replications

Table 2. Influence of substrate supplementation on yield of *Pleurotuspulmonarius*(Fr.) Quel

Treatments	Spawn run period (days)*	Primordia initiation stage (days)*	Yield (g/200g dry substrate)*	Biological efficiency (%)
Wheat straw + Saw dust (2:1)	11.47	15.53	121.7	60.85
Wheat straw + Ammonium nitrate (5g/kg of wet weight of substrate)	15.33	18.50	48.3	24.15
Wheat straw + Cotton seed meal (5g/kg of wet weight of substrate)	10.50	13.67	155.3	77.65
Wheat straw + CAN (5g/kg of wet weight of substrate)	14.67	19.13	63.7	31.85
Wheat straw + Urea (2.5g/ kg of wet weight of substrate)	18.50	23.43	0.0	0.0
Wheat straw control	12.23	15.30	84.3	42.15
Mean	13.78	17.59	78.88	
$C.D_{0.05}$	0.40	0.40	5.11	
S.E	0.18	0.18	2.34	

*Average of three replications

effect on spawn run, pinheads appearance, yield performance as well as on biological efficiency. In case of spawn rates, different spawn dose ranging from 0.5 to 8% were tried and it is clear from results given in Table 1 that the mean number of days taken for spawn run and primordia initiation of pink oyster from the date of spawning exhibited significant difference between spawn rates. The spawn run was fastest on (10.50 days) when spawn dose was 8%, followed by 6%, 4%, 2%, 1% and 0.5%, respectively. The results also reveals that the maximum yield (168.7g/ 200g dry substrate) with biological efficiency of 84. 33% was obtained by using spawn @ 8% followed by 6%, 4%, 2%, 1% and 0.5% with biological efficiency 80.67%, 78.65%, 75.83%, 72.50% and 67.15%, respectively. It was, therefore, found that, with the increase in spawn rates, there was corresponding increase in yield also.

Bhatti *et al.,* 2007 studied the effects of different spawn rates viz., 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 g spawn/kg substrate (dry weight) on *Pleurotusostreatus*. The spawn substrate was sorghum and the substrate wheat straw. The best yield was obtained with 70 g spawn/kg substrate (DW). The number of fruiting bodies was increasing with the amount of spawn and the fastest time to initial fruit body formation was obtained with 70g spawn/kg substrate. Also highest yield and biological efficiency with the increase in spawn doses (100, 200, 300, 400 and 500 g per kg dry substrate)was recorded in *Calocybeindica* (Pani, 2011).

Wallman (2015) also evaluated the effect of three different spawn doses viz., 45g, 90g and 145g on the mycelial growth, primordial initiation and yield of *Pleurotusostreatus*. The shortest time was 13 days and was obtained with 180g spawn and the longest time was 20 days and was obtained with 45g of spawn. The highest yields and shortest time for fruit body formation was obtained with the highest amount of spawn (180g). The yields were lower with lower amount of spawn.

In case of evaluation of different substrate supplementation, it is clear from Table 2 that wheat straw supplemented with cotton seed meal (5g/kg of wet weight of substrate) supported maximum mycelia growth and colonized the entire substrate within 10.50 days of inoculation followed by mixture of wheat straw + saw dust (11.47 days), wheat straw (control), wheat straw supplemented with CAN (14.67 days), wheat straw supplemented with ammonium nitrate (15.33 days). However, the fungus took maximum days for spawn run on wheat straw supplemented with urea (18.50 days) followed by wheat straw supplemented with ammonium nitrate (15.33 days).Similarly, the data recorded for time taken for primordia initiation of *P. pulmonarius* revealed that minimum time for initiation of primordia (13.67days) was observed on mixture of

wheat straw supplemented with cotton seed meal (5g/ kg of wet weight of substrate) followed by wheat straw alone (15.30days) and mixture of wheat straw with saw dust (15.53days), respectively. Wheat straw supplemented with urea (2.5g/kg of wet weight of substrate) recorded maximum time of 23.43days for primordia initiation followed by wheat straw supplemented with CAN (19.13days) and wheat straw supplemented with ammonium nitrate(18.50days).

It is clear from the Table 2 that yield and biological efficiency of *P. pulmonarius* on different substrates combination viz., wheat straw supplemented with cotton seed meal (5g/kg of wet weight of substrate) recorded maximum yield (155.3g) with biological efficiency of 77.65% followed by wheat straw supplemented with saw dust, which gave yield of 121.7g with a biological efficiency of 60.85%. Wheat straw (control) gave yield of 84.3/200g, whereas, a combination of wheat straw with CAN (5g/kg of wet weight of substrate) gave yield of 63.7g/200g dry weight of substrate. Significantly much lower yield of 48.3g/200g dry weight of substrate was recorded on wheat straw supplemented with ammonium nitrate whereas, when wheat straw was supplemented with urea (2.5g/kg of wet weight of substrate), no yield was recorded.

Chauhan and Gupta (2015) also studied the effect of different forest and agricultural wastes in different combination of organic supplements. It was recorded that wheat straw+wheat bran (9:1) was colonized in minimum time of incubation (10.33 days) followed by mixture of wheat straw+cotton seed meal (9:1). Slowest spawn run was noticed with wheat straw+urea (1%). Maximum yield and biological efficiency was obtained from the treatment containing wheat straw+wheat bran (9:1) followed by wheat straw+cotton seed meal (9:1) whereas, lowest yield and biological efficiency was recorded with wheat straw+CAN (0.5%) .

Ashraf *et al.* (2013) compared the effect of different agricultural wastes on growth and yield of mushroom production, three species of *Pleurotus*viz. *P. sajor-caju* (V1), *P. ostreatus*(V2), and *P. djmor*(V3) were grown on three different substrates cotton waste (T1), wheat straw (T2) and paddy straw (T3). The fastest spawn running, primordial initiation, harvesting stage, maximum number of fruiting bodies and maximum yield was observed in cotton waste (T1).Sozbir*et al.,* 2015 also reported the potential of using cotton seed hulls (CSHs), walnut shells WSs) as new, essential substances for substrate preparation in the cultivation of *Pleurotusostreatus*. Substrates prepared with oak sawdust (OS) alone and with mixtures of OS, WSs and CSHs in different ratios were compared. The highest yields and highest biological efficiency were obtained with the mixtures of 25 OS: 75CSHs, indicating that the yield in substrates increased as amount of CSHs in the mixtures increased.

Conclusion

From the present study it can be concluded that the 8% spawn dose and supplementation of wheat straw with cotton seed meal resulted fastest spawn run and earlier primordial initiation on substrate. Moreover, the dose resulted highest yield 168.7g/200g dry substrate with maximum biological efficiency of 84.33% followed by 6%, 4% 2%, 1% and 0.5%, respectively. Also, 6% and 4% spawn doses also recorded good yield with the minimum period for maturation. Therefore, 8% spawn dose may be recommended for farmers to get attractive fruit body as well as higher yields in commercial production of *P. pulmonarius* and also for other oyster mushrooms.

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