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Studies on agronomic supplementation for production of oyster mushroom (Pleurotus ostreatus)

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ABSTRACT
The current research experiment named " Studies on agronomic supplementation for production of oyster mushroom (Pleurotus ostreatus)" was carried out to investigate the cultivation of oyster mushroom with different agro-wastes. The main aim of this research work is to find out the best agronomic supplements for mushroom cultivation. Results regarding the time required for pinhead formation, total numbers of fruiting bodies per bed and total biological yield per bag on different supplements were showed. Mushroom appeared earlier on the bed with paddy straw and wheat bran (90:10). The highest number of fruiting bodies per bed was obtained from the bed with paddy straw and wheat bran (90:10). The highest biological yield of Pleurotus ostreatus (1271.87g) was also obtained from the bed with paddy straw and wheat bran (90:10) followed by the bed with paddy straw and wheat bran (95:5) where the biological yield was 1165.75g. Lowest number of fruiting bodies per bed and lowest yield (558.17g) was obtained from the bed with paddy straw and corn husk (85:15). The use of specific amount of wheat bran as supplement in mushroom cultivation enhances the biological yield and also helps in recycling of nutrients.

Keywords: Oyster mushroom, Supplements, Agro-wastes, Growth, Yield.

I. INTRODUCTION

Mushrooms are very delicious and an important food protein supplement, it can be a great replacement of meat for vegetarians. A mushroom is a fleshy, spore bearing reproductive structures of a fungus, typically produced above ground on soil or on its food sources (Rahul Aske et al. , 2020). They are classified in kingdom of Fungi, which is also called kingdom of Mycetae (Ammirati and Seidl,2007). These are the macro fungi, most of the edible mushrooms belongs to either Ascomycotina or Basidiomycotina. Mushrooms have been consumed since earliest history; ancient Greeks believed that mushrooms provided strength for warriors in battle, and the Romans perceived them as the "Food of the Gods" . " . Mushrooms are heterotrophic and depend upon the organic matter for nutrition and live saprophytically, parasitically or symbiotically on or with organisms. For a long time, wild edible mushrooms have played an important role as a human food and now-a-days is a leading food component. Mushrooms have high nutritional and functional value and are accepted as nutraceutical (Ergonul, Oboh G. , 2001).Mushrooms are of considerable interest because of their organoleptic merit, medicinal properties and economic significance. These are considered as an important food to address food and nutrition security and human health and climate change adaptation issues. Mushrooms are excellent source of protein and also known as vegetarian's meat. These are the good source of protein, vitamins and minerals(Khan et al., 1981). In addition to that mushroom has good quantity protein, no cholesterol, high fiber, low sodium, good quantity of vitamins and minerals, protein polysaccharides complexes that impart unique medicinal values like anti- cancer, anti-inflammatory and anti-viral properties (Nidhi Sinha and Chourasia , 2021). Mushroom contains about 85-95% water , 3% protein, 4% carbohydrates, 0.1% fats, 1% minerals and vitamins (Tewari, 1986). Mushroom also contains appreciable amount of potassium, phosphorus, copper and iron but low level of calcium (Anderson and Feller, 1942). Edible mushrooms are also rich in vitamins such as niacin, riboflavin, vitamin D, C, B1, B5 and B6 (A. A. Syed et al., 2009). Mushrooms are

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useful against diabetes, ulcer and lung diseases (Quimio, 1976).

The history of mushroom cultivation is traced to Roman. In early times, mushroom cultivation failed because the biology of fungi was not understood and this led to the initial problems for the cultivation (Oei, 1996; Stamets , 2000). However, empirical methods for mushroom cultivation are relatively recent (Martínez-Carrera, 2000). They were independently developed in China about 1,000 years ago for Auricularia spp. and Lentinula edodes .Cultivated mushrooms have higher protein contents and minerals, low in fat and rich in vitamin B, vitamin D, vitamin K and sometimes vitamin A and C (P. Manzi et al., 2001). Now a days China only produces 64% of all edible mushrooms in the world.

Pleurotus spp. Commonly known as oyster mushroom are edible fungi cultivated worldwide especially in south east Asia, India, Europe and Africa (Mandrel Q, et al., 2005). China produces 85% of all oyster mushrooms all over the world (Chang S-T., 1999). The oyster mushrooms also called as 'Dhingri' or 'Abalone' is mainly reported in temperate along with tropical forests and also found especially on decaying organic matter (Fan L. & Pandey A et al., 2000; Gregori A et al., 2007). A high nutritional values of oyster mushroom has been reported with protein (25-50%), fat (2-5%), sugars (17-47%), mycocellulose (7-38%) and minerals (potassium, phosphorus, calcium, sodium) of about 8-12% (R.P Stanley, 2011). Naturally oyster mushroom grow on dead and decaying wooden logs or sometimes on dying trunk of deciduous or coniferous wood in both temperate and tropical forests. They also grow on decaying organic matter but due to changes in climatic patterns harvesting wild mushrooms has become difficult. The alternative is to grow mushroom domestically. The production of mushrooms with better flavour, appearance, texture, nutritional qualities and medicinal properties at a sustainable cost constituent a challenge for industry and independent farmers for operational reasons (Sanchez C., 2004). The substances where the mushrooms grow are called as substrates. Oyster mushrooms can be grown on various substrates including paddy straw, maize stalks/cobs, vegetable plant residues, bagasses etc. (S. Hassan et al., 2011). The substrates influence the mushroom growth, yield and composition. However, an ideal substrate should contain nitrogen and carbohydrates for rapid mushroom growth (K. B. Khare et al., 2010).

Mushroom supplementation is understood as a farming method based on the physical addition of nutritional amendments to compost, during the process of composting the mixture of raw materials at spawning or during casing (Estrada et al., 2009; Pardo-Giménez et al., 2012a, 2016). The practice of nutritionally supplementing compost for mushroom cultivation at the time of spawning or casing to maximize crop yield emerged in 1960s (Schisler and Sinden 1962; Lemke 1963) and is widely recognised and accepted, however it's use can be restricted in some sectors because of technical and economic factors. Supplements are commonly manufactured products containing defalled vegetable meal, such as soybean meal and other organic protein sources, among them cereal bran enriched with minerals or vitamins were frequently used (Zied et al., 2011). The correct timing and methods of application of supplements is an essential condition for obtaining the expected results, with several important culture aspects, such as the composting process, the control of temperature for mycelial growth before and after casing, the hygiene measure, the choice of supplement and it's application timing especially, the uniform distribution of the product used in substrate, all affecting subsequent yields (Desrumaux et al., 1999). The various supplements such as wheat bran, ammonium sulphate, gram flour, soybean meal, rice bran, mustard cakes, cotton seed cake and molasses are recommended as substrate supplements prior to spawning to enhance oyster mushrooms (Naraian et al., 2009).The objective of this study is to find out the best agronomic supplement for effective cultivation of oyster mushroom (Pleurotus ostreatus).

II. MATERIALS AND METHODS

Culture and Cultivation

Stock culture of Pleurotus spp. Were obtained from "Trinath Mushroom Farm" at Jagdalpur near Berhampur, Ganjam, Odisha. Pure culture lines during the period of study were maintained on Potato dextrose agar (PDA) medium and inoculated at an interval of fifteen days.

Spawn preparation

The spawn for Pleurotus ostreatus was prepared with wheat grains. The wheat grains were cleaned several times manually to remove inert matter and debris. The cleaned grains were soaked with tap water for overnight. In addition, the grains were boiled soon after soaking for spawn preparation. Thereafter, the boiled grains were drained and the excess water is removed. The boiled grains were filled in to bottles (made of

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brass) locally found. The bottles were covered using non- absorbentcotton tightly. The bottles with spawn materials were autoclaved (22lb psi) for 2hours and cooled for over 6 hours. The media bottles were immediately inoculated with mycelial culture of Pleurotus ostreatus. maintained on PDA. For successive production of spawn , the media bottles were inoculated and incubated at 25°C for mycelial growth without any light for 10-15 days until the mycelium fully covered the grains. The bottles were shaken every 4 days to distribute mycelium throughout the grain till the end of growing day.

Bed /Bag preparation

Various agricultural wastes can be used as substrate for oyster mushroom cultivation. In present study Paddy straw is used as substrate. The bedding of oyster mushroom was done with polythene bags (capacity 1Kg). The paddy straw were cut into small Pieces of straw bits of length about 5-8 cm. The straw bits were soaked in tap water overnight to moisten and make the substrate soft for colonization to oyster mushroom hyphae.

Spawning and addition of supplements

The filling of polythene bags was done following the usual procedure of mushroom farming. The sterilized straw bits were filled tightly layer wise inside the polythene bags of which one end was closed with a rubber tag. Spawning was done at the rate of 1-3% (dry weight basis) for three to four layers up to the mouth of the bag.

In each layer the spawning is followed by the addition of supplements. Various agronomic supplements such as rice bran, wheat bran, corn husk and chickpea husk were used. Different beds were made by using different amounts such as 5% (50grams), 10%(100grams) and 15%(150grams) of supplement per 1kg of bed. Just after filling the substrates, spawn along with supplements; the upper loose end of the bag was closed tightly by tying with a rubber tag. Then the bags were taken into a dark room and hanged by means of plastic thread. During the process the room temperature was 26°C to 28°C to maintain the humidity.

Effect of pore size

After the filling of bags , different size of holes were made to evaluate the effect of aeration, contamination and moisture loss. Holes were made using circular cutter with different diameters.

Pinhead initiation

Light and temperature were used to initiate the formation of pinheads after the mycelium was fully grown on the substrate i.e after the substrate was fully turned into white color due to mycelium growth. The growing mycelium that was placed in dark was brought to a fully illuminated region.

Fruiting

When the substrate was completely covered by the mycelium, the cotton plug was removed . The plastic bags were partially removed the bags which contain primordia was sprayed with water two to three times per day to increase the relative humidity.

III. RESULTS AND DISCUSSION

The results regarding the time taken for pinhead formation, average numbers of fruiting bodies per bed , total biological yield of Pleurotus ostreatus on different amounts of various agronomic supplements were presented in table 1. Table 1 indicated the ability of wheat bran along with paddy straw facilitate faster growth of Pleurotus ostreatus.

The table also shows supplementation of wheat bran in substrate decreases the time for pinhead formation. Combination of paddy straw and wheat bran (95:5) takes 21 days, paddy straw and wheat bran (90:10) takes 19 days , paddy straw and wheat bran (85:15) takes 21 days for pinhead formation. Supplementation of cornhusk takes longer time for pinhead formation which is 29, 31 and 32 days for 95:5 , 90:10 and 85:15 of paddy straw and cornhusk combination respectively. Sher H et al. (2010) also stated that Pleurotus ostreatus completes spawn running in 17-20 days and time for pinhead formation is noted as 23-27 days. In our study it was found that wheat bran supplementation reduces the time taken for spawn running and pinhead formation while cornhusk increases the time for spawn running and pinhead formation.

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Table-1 Effect of different amounts of various supplements on growth and yield of Pleurotus ostreatus.

Supplement used	Amount of supplement	Pinhead emerge (in days)	No. of fruiting bodies	Total yield (1^{st} +2^{nd} +3^{rd} flush) in grams
Control	_	27	25	724.23
Rice bran	5%	23	30	1002.56
	10%	21	33	1146.09
	15%	22	27	935.87
Wheat bran	5%	21	29	1165.75
	10%	19	37	1271.87
	15%	21	32	1110.43
Corn husk	5%	29	24	653.06
	10%	31	22	611.73
	15%	32	21	558.17
Chickpea husk	5%	26	26	841.85
	10%	24	28	933.45
	15%	23	31	1004.39

Each value is mean of 3 replicates +_ SEM

According to the table average number of fruiting bodies from 1^{st} , 2^{nd} and 3^{rd}flushes also varies form bed to bed. Maximum number of fruiting bodies(37) was obtained from paddy straw and wheat bran (90:10) followed by paddy straw and rice bran (90:10) where the total number of fruitingbodies per bed is 33. Lowest number of fruiting bodies (21) per bed was obtained from paddy straw and cornhusk (85:15). Nidhi Sinha and Chourasia (2021) also recorded highest number of fruiting bodies (35) from paddy straw and wheat straw (50:50) combination and lowest number of fruiting bodies (23) from paddy straw and rice bran (90:10) combination. Rahul Aske et al. (2020) recorded highest number of fruiting bodies (68) from wheat straw and lowest number of fruiting bodies (41) from moong bean.

The yield of mushroom decreases with decrease in number of fruiting bodies per bed. The biological yield of mushroom is affected by the amounts of various supplements. Paddy straw and wheat bran (90:10) shows highest yield which is 1271.87g, followed by paddy straw and wheat bran (95:5) combination where the yield is 1165.75g. Lowest yield was obtained from paddy straw and cornhusk (85:15) combination which is 558.17g. Rahul Aske et al. (2020) reported highest yield (579.75g) from wheat straw substrate and lowest yield (403.50g) from moong bean straw. Nidhi Sinha and Chourasia (2021) reported their highest yield (1180g) from paddy straw and wheat straw (50:50) combination and lowest yield (630g) from paddy straw and rice bran (90:10) combination.

Discussion

The various crop residues can be used in producing oyster mushrooms either as main substrates or in combinations with supplements. The mycelial growth in wet composts was improved when packed less densely, possibly because of improved aeration. Most rapid colonization occurs within the temperature range of 22-27°C. A wide range of organic waste materials have been proposed for mushroom cultivation. Wheat straw is one example and it's 25% supplementation with olive oil mill effluent gives economic mushroom yield (E. Kalmis et al., 2008).Oyster mushrooms have rich in fiber and low in fat contents this character is highly beneficial for heart patients. The study also indicates that the nutritional value of oyster mushroom differs but all species are healthful with abundant amount of fiber and protein and other essential nutrients. According to B.O Onyango (2011) the large sized fruit bodies are considered to be of good quality and rated highly in mushroom production but this as an

interior quality since such fruit bodies tend to break during packaging thereby reducing the quality.

The cultivation of edible mushrooms using agro-wastes such as paddy straw, rice bran, wheat bran, chickpea husk and cornhusk is a value added process to convert these materials, which are other wise considered as wastes, for mushroom production. Mostly in sub-tropical countries agricultural waste causing a significant role in causing environmental pollution due to waste disposal problem. Wastes are either brunt or dumped nearby water bodies creating a health hazard to human life. Some of the agro-wastes were currently being used for animal feeding also while human feeding is not indicated due to the presence of impurities and even aflatoxin (Fekete SG et al., 2004 ; Lee NA et al., 2004). In this sense, it is fundamental to search for practices that convert these wastes into food quality (closing the cycle of production) for the population, e.g., the bioconversion in fungi protein, known popularly as mushroom. Therefore, in our work, we proposed the use of various agro-wastes such as wheat bran, rice bran, chickpea husk and corn husk as supplements, not in the substrate formulation and we have observed a positive effect. Baysal (2003) investigated paper waste supplemented with rice husk, chicken manure and peat for Pleurotus ostreatus cultivation. Highest yield for fresh weight was recorded as 350.2 grams in substrate containing 20% rice husk.

Good control of humidity during cropping is very important for all types of mushroom. The moisture content of growing mushroom media is a very important factor; hence, the proper moisture content value encourages the growth, while higher or lower one had a negative effect on growth (Shah Z, et al., 2004). It is good to keep the humidity high (80-90%) by spraying water several times per day (Oei P et al., 2005). However, no water should be sprayed directly onto mushrooms that are ready for picking; their shelf life will decrease drastically if they become too wet (Dawit A et al., 1998).

The mushroom were harvested in 1^{st} , 2^{nd} and 3^{rd} flushes . However, the maximum yield was obtained in first flush than the second flush than third flush. In general, one crop of mushroom with four flushes is most economical. It is unwise to keep spawn running of substrate for longer than 90 days as the yield significantly decrease. The highest yield was obtained as 1165.75g, 1271.87g and 1110.43g from 5%,10% and 15% of wheat bran supplementation respectively. Second highest yield was obtained from 5%,10% and 15% of rice bran as supplement as 1002.56g, 1146.09g and 935.87g respectively. 5%,10% and 15% of chickpea husk as supplement yields 841.85g, 933.45g and 1004.39g respectively. Lowest yield was obtained from 5%,10% and 15% of cornhusk supplement which is 653.06g, 611.73g and 558.17g respectively. The results are in contrary to the report of Rahul Aske et al., (Rahul Aske, 2020) and Nidhi Sinha and Chourasia (2021) who also reported that addition of wheat straw and wheat bran increases the oyster mushroom quality and yield.

IV. CONCLUSION

The highest number of fruiting bodies was obtained from wheat bran supplementation and recommend commercially since supplementation of wheat bran in specific amount enhances the mushroom production. So the use of wheat bran as supplement appears to be most suitable due to improved growth and yield. We recommend paddy straw as substrate for mushroom bed for its cheapest quality and readily available in sub-tropical countries. India is an agricultural country and rich in agro-wastes, the cultivation of Pleurotus spp. on these agro-wastes decreases the environmental pollution and provide a sustainable means of adding value to the farmers. From our study we concluded that supplementation of 10% of wheat bran in substrate enhances the mushroom production. Further this data can be implemented in the farm house for commercialization of mushroom industry.

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