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ROLE OF VARIOUS SUPPLEMENTARY MATERIALS WITH COTTON WASTE SUBSTRATE FOR PRODUCTION OF *PLEUROTUS OSTREATUS* AN OYSTER MUSHROOM

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Abstract

Pleurotusostreatus exhibit a lot of medicinal properties and mostly grown on different residual waste material in Asia. In past mushroom mostly grown on agricultural waste, but now a day to improve the composition of substrate different supplementary materials are used. Current study based on the impact of various supplementary materials on production of oyster mushroom. Cotton waste supplemented with 5 percent of wheat bran, rice bran, and gram flour was evaluated for spawn running, yield performance and biological efficiency of *Pleurotusostreatus*. Supplements have significant effect on spawn running, yield performance and biological efficiency. Wheat bran gave best results followed by rice bran and gram flour. Supplements improve the nutritional status of cotton waste substrate which resulted in better mushroom growth. Cotton is an important crop of Asia, so current research is a valuable contribution for utilization of its waste material in an efficient manner with addition of supplements to it. These outcomes will be helpful for further optimizing the best combination of cotton waste substrate with supplements.

Keywords: Spawn, Biological efficiency, Yield, Fruiting bodies.

Introduction

Mushrooms are rich in proteins, vitamins, and minerals and popularly called as the vegetarian's meat. Mushroom proteins are considered to be intermediate between that of animals and vegetables (Kurtzman, 1976) as it contains all the nine essential amino acids required for human body (Hayes & Haddad, 1976). Mushroom has high nutritional value and pro-biotic properties, therefore, recommended to be used in diet plan all over the world (Florczak *et al*., 2004; Rajewska & Balasinska, 2004; Khan *et al*., 2012). Mushrooms contain about 1.5-6.7% carbohydrate, 1.5-3% protein, 0.3-0.4% fats, and vitamins (Bernas *et al*., 2006; Haq *et al*., 2010). Furthermore, this fungus produces secondary metabolites with pharmaceutical applications and some proteins of potential industrial use (Wasser, 2010). Mushrooms are useful against diabetes, ulcer, lung diseases, and an excellent antitumor agent (Jose & Janardhanan, 2000). Mushrooms are widely used as food supplements, food additives, and in pharmaceutical industry due to their haematological, antibacterial, antiviral, and antioxidant activity (Yang *et al*., 2002; Ribeiro *et al*., 2006; Regula & Siwulski, 2007).

Pleurotus ostreatus is one of the best species among oyster mushroom and can be grown on plant waste materials lignin, cellulose and hemicelluloses, (Chang & Miles, 1989). Oyster mushroom (*Pleurotus* sp.) is commonly called as Dhengri in Pakistan because of its oyster like shape. *Pleurotus*is an efficient lignin- degrading mushroom and can grow well on different types of lingo cellulolosic materials. Cultivation of this mushroom is very simple and low cost production technology, profitable agribusiness which gives consistent growth with high biological efficiency (Khan *et al*., 2017). It provides both part time and full time employment to low income people of developing countries (Ferchak & Croucher, 2001). Mushroom consumption is increasing over period of time due to its significant benefits (Uddin *et al*., 2011). Different species of *Pleurotus* can grow well in variable temperature conditions; hence they are ideally suited for cultivation throughout the year in various regions of tropical country like Pakistan. Oyster mushroom are cultivated on substrates such as wheat straw, corn cobs, sawdust, sugarcane bagasse, wood pulp, cotton waste, banana leaves, coconut husks, poultry wastes, tree bark and leaves. These all substrates have proved their significance for effective cultivation of oyster mushroom. Cotton waste has been found best regarding commercial scale production of oyster mushroom. It is available in abundance in Pakistan especially in areas where cotton is cultivated in abundance. Substrates supplemented with wheat bran, rice bran gave significant results in mushroom yield, and biological efficiency (Jafarpour *et al*., 2011; Gurung *et al*., 2012). Cereals bran is a rich source of protein and added to mushroom substrate to improve mycelium growth and yield (Siddiqui *et al*., 1989; Kinge *et al*., 2016). Their nitrogen content may vary from 3-12% depending upon source (Pathak &Yadav, 1998). Supplements improve the nutritional status of substrate by providing easily degradable carbohydrates, more protein and nitrogen (Royse, 2002; Oseni *et al*., 2012).Cotton waste with some supplements such as wheat bran, rice bran and gram flour has been used for gaining higher yield. Therefore, current study is aimed to determine the efficiency of various supplementary materials including wheat bran, rice bran and gram flour in cotton waste for production of oyster mushroom to improve yield and overcome the gap of malnutrition as its cultivation for a cheap source of protein.

Material and Methods

Substrate of cotton waste was collected and wetted thoroughly to retain its moisture level in mushroom laboratory. The wetted substrates were stacked on floor, rice bran, wheat bran and gram flour was added as supplements in cotton waste at the rate of 5%. Substrates were fermented for 4 to 5 d. A local method was used for determination of moisture by pressing handful of substrate mixture. If there was no water runoff and the material stayed in form it indicates that the moisture content was around 65 to 70%. The polypropylene bags of (4 × 6") size were filled with substrate 300 gm per bag and their mouth were plugged by rubber band. Bags were steamed sterilized in drum for (1) hr at 82 to 90ºC. Prepared spawn of *Pleurotus osteatus* was mixed in substrate (1.5g in each bag). Mouths of bags were again plugged with rubber band. Bags were kept at room temperature (25°C to 30°C) and relative humidity 80 to 90%. The bags were cut from bottom and water was sprayed for maintaining moisture up to the desired level in the form of fine mist with the help of a nozzle. The experiment was laid out according to completely randomized design (CRD) and each treatment was replicated.

$T_{1=}$ Cotton waste 95% + Wheat bran 5%
$T_{2=}$ Cotton waste 95% + Rice bran 5%
$T_{3=}$ Cotton waste 95% + Gram flour 5%
$T_{4=}$ Cotton waste 100%

Spawn running data was recorded on the basis of 25%, 50%, 75% and100% spawn growth. Numbers of days were calculated for appearance of primordia (pinheads) and numbers of fruiting bodies produced were calculated. The biological efficiency was calculated by the formula given by (Chang *et al*., 1981).

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

Statistical analysis: The experiment followed complete randomized design. The obtained data was subjected to DMR test. The least significant difference was used to compare means of treatment at 5% probability.

Results

Effect on spawn running of *Pleurotus ostreatus*: Spawn running on different substrates combination was recorded on the basis of 25%, 50%, 75%, and100% spawn growth. In all the four percentages (25%, 50%, 75% and100%) cotton waste substrate supplemented with wheat bran showed significantly effective results while least significant effect was observed in cotton waste substrate supplemented with gram flour. Minimum time for completion of 25%, 50%, 75%, and 100% spawn growth resulted with cotton waste substrate supplemented with wheat bran i.e., 10 days, 16 days, 24 days, and 32 days respectively. While maximum time for completion of 25%, 50%, 75%, and 100% spawn growth resulted with cotton waste supplemented with gram flour i.e., 17 days, 23 days, 30 days, and 55 days respectively. Results obtained in case of cotton waste supplemented with rice bran and simple cotton waste for 25%, 50%, 75%, and 100% spawn growth were 15 days, 20 days, 28 days, 52 days, and 16 days, 22 days, 29 days, 54 days, respectively (Table 1). Cotton waste substrate supplemented with wheat bran took minimum time for spawn running in polypropylene bags while in other treatments time required was significantly higher.

Effect on primordia and no. of fruiting bodies of *Pleurotus ostreatus*: Data was recorded for the appearance of primordia (pin head formation) and shortest time was taken on cotton waste supplemented with wheat bran 14 d, followed by cotton waste supplemented with rice bran (17 days). Maximum number of days taken for primordia appearance on cotton waste substrate supplemented with gram flour (19 days) followed by simple cotton waste (18 days). Maximum numbers of fruiting bodies were observed in case of cotton waste supplemented with wheat bran (9 fruiting bodies), and minimum numbers of fruiting bodies were observed with simple cotton waste substrate (7 fruiting bodies). While no significant variation was detected for number of fruiting bodies formation in case of cotton waste supplemented with rice bran (8 fruiting bodies) and cotton waste supplemented with gram flour (8 fruiting bodies), (Table 2).

Table 1. Effect on spawn running of *Pleurotus ostreatus*.

Substrates	Phases of spawn running (in days)			
	25%	50%	75%	100%
Cotton waste + Wheat bran	10 a	16 a	24 a	32 a
Cotton waste + Rice bran	15 b	20 b	28 b	52 b
Cotton waste + Gram flour	17 d	23 d	30 d	55 d
Cotton waste (Control)	16 c	22 c	29 c	54 c

Treatments means with different letters differ significantly at p<5%

Table 2. Effect on pin head and fruiting bodies formation of *Pleurotus ostreatus*.

Substrates	Appearance of pinheads (in days)	No. of fruiting bodies
Cotton waste + Wheat bran	14 a	9 a
Cotton waste + Rice bran	17 b	8 b
Cotton waste + Gram flour	19 d	8 b
Cotton waste (Control)	18 c	7 c

Treatments means with different letters differ significantly at p<5%

Table 3. Effect on *Pleurotus ostreatus* yield.

Substrates	Yield in three flushes (gm)			Total yield (gm)	Standard error
	1st flush	2nd flush	3rd flush		
Cotton waste + Wheat bran	56 a	46 a	37 a	139 a	0.32
Cotton waste + Rice bran	45 b	32 b	34 b	111 b	0.18
Cotton waste + Gram flour	39 c	26 c	31 c	97 c	0.03
Cotton waste (Control)	36 d	24 d	28 d	90 d	0.60

Treatments means with different letters differ significantly at p<5%

Table 4. Effect on biological efficiency of *Pleurotus ostreatus*.

Substrates	Fresh weight of mushroom (gm)	Dry weight of substrate (gm)	Biological efficiency
Cotton waste + Wheat bran	139	300	46.33 a
Cotton waste + Rice bran	111	300	37.00 b
Cotton waste + Gram flour	97	300	32.33 c
Cotton waste (Control)	90	300	30.00 d

Treatments means with different letters differ significantly at p<5%

Effect on total yield of *Pleurotus ostreatus*: Harvesting of mushroom was done in three flushes and data was recorded. Harvesting was started on maturity and yield of subsequent flushes was also been recorded. After 1st flush harvesting cotton waste substrate supplemented with wheat bran produced (56 gm) of mushroom, followed by cotton waste substrate supplemented with rice bran (45 gm). Cotton waste substrate supplemented with gram flour produced (39 gm) and simple cotton waste substrate produced (36 gm) of mushroom. After 2nd flush harvesting cotton waste substrate supplemented with wheat bran produced (46 gm) of mushroom, followed by cotton waste substrate supplemented with rice bran (32 gm). Cotton waste substrate supplemented with gram flour produced (26 gm) and simple cotton waste substrate produced (24 gm) of mushroom. After 3rd flush harvesting cotton waste substrate supplemented with wheat bran produced (37 gm) of mushroom, followed by cotton waste substrate supplemented with rice bran (34 gm). Cotton waste substrate supplemented with gram flour produced (31 gm) and simple cotton waste substrate produced (28 gm) of mushroom. Total weight of all the fruiting bodies harvested from all the three pickings were measured as total yield of mushroom. Significant variation was observed in total yield of all treatments. Cotton waste substrate supplemented with wheat bran produced maximum yield (139 gm), followed by cotton waste supplemented with rice bran (111 gm). Yield of cotton waste supplemented with gram flour was (97 gm), whereas minimum yield was recorded in case of simple cotton waste substrate (90 gm), (Table 3).

Effect on biological efficiency of *Pleurotusostreatus*: The biological efficiency was recorded against the dry weight of substrate. Addition of supplements to cotton waste substrate increases the biological efficiency. Significant differences were found in all treatments in relation to biological efficiency. Maximum biological efficiency (46.33%) was found in cotton waste supplemented with wheat bran, followed by cotton waste supplemented with rice bran (34.33%). Biological efficiency of cotton waste supplemented with gram flour was (32.33%), whereas minimum biological efficiency was observed in case of simple cotton waste substrate (30.00%), (Table 4).

Discussion

Pleurotus ostreatus was successfully grown on cotton waste substrate and cotton waste substrate supplemented with wheat bran, rice bran, and gram flour. Studies were conducted on spawn running, primordia appearance, fruiting bodies quantity, yield and biological efficiency of *Pleurotus ostreatus* in relation to different substrates. Addition of supplements to substrates having low protein and nitrogen contents significantly improves the growth and yield of mushroom, supplements provide extra nitrogen, protein and easily degradable carbohydrates (Royse, 2002; Oseni *et al*., 2012). In current study addition of supplements to cotton waste substrate is a pioneer attempt and spawn growth was significantly improve with the addition of wheat bran, and rice bran to cotton waste substrate during all the stages of spawn running (25%, 50%, 75%, and 100%). These outcomes showed the positive impact of supplements on mushroom mycelial growth and reduced the time required for spawn running. Different scientists have used the wheat and rice bran as a supplement in combination with wheat straw, sawdust, maize stalk, sugarcane bagasse, coffee leaves, and reported significant improvement in spawn running (Pokhrel *et al*., 2013; Petre *et al*., 2014; Hasan *et al*., 2015; Barshteyn & Krupodorova, 2016) and results of current study were in confirmation to above findings. Supplements not only affected the spawn running but also accelerated the primordia appearance and increases the number of fruiting bodies formation. Earliest primordia appearance and maximum number of fruiting bodies formation was observed on cotton waste substrate supplemented with wheat bran followed by cotton waste substrate supplemented with rice bran.

Supplements have also shown positive impact on the yield of mushroom both in case of individual flush as well as total yield. Maximum yield was recorded in case of cotton waste substrate supplemented with wheat bran followed by cotton waste substrate supplemented with rice bran and gram flour respectively. Current study findings were in confirmation to several scientist reports which showed significant improvement in mushroom yield due to addition of wheat bran, rice bran, gram flour (Murthy & Manonmani, 2008; Gizaw, 2010; Tripathy *et al*., 2011; Petre *et al*., 2014; Hasan *et al*., 2015). Highest yield in shortest time period is a most desirable factor for mushroom production, which could be achieved with the addition of above mentioned supplements. Biological efficiency is one of the important parameter during the mushroom studies. Biological efficiency of *Pleurotus ostreatus* also improved with the addition of supplements, maximum biological efficiency was recorded in cotton waste substrate supplemented with wheat bran, followed by cotton waste substrate supplemented with rice bran and gram flour respectively. Present results were in line with the findings of Gizaw (2010); Petre *et al*., 2014; Barshteyn & Krupodorova (2016); Buendia *et al*. (2016) that wheat and rice bran supplements to main substrate significantly improved the biomass and biological efficiency of mushroom.

A supplement like wheat bran provides vitamins and other growth factors which are significant importance for mushroom growth (Chang &Miles, 2004). Substrate supplemented with wheat bran increases its total content of nitrogen, protein and crude fat (Buendia *et al*., 2016). Significant results on addition of supplements have been reported by number of scientist (Erkel, 2009; Azizi *et al*., 2012; Gurung *et al*., 2012) therefore cotton waste substrate was evaluated for the first time and gave significant results. 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. The spawn running, yield performance and biological efficiency are best on cotton waste with supplementation, because due to the use of supplements (Rice bran, wheat bran, and gram flour) nutritional status of the substrate enhanced.

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