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Yield improvement of oyster mushroom (*Pleurotus ostreatus*) production using cotton seed cake with combination of wheat straw amended with rice bran cellulosic waste materials

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Key words: *Pleurotus ostreatus*, Wheat straw, Rice bran, Cotton seed cake, Mycelium, Pinhead, Yield.

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Abstract

In recent years agricultural and industrial waste bio-conversion into food has attracted the world attention but has received diminutive attention in Pakistan. *Pleurotus ostreatus* is a good source of protein, amino acid, vitamins and also contain less amount of fats cholesterol. The present study was conducted to check the yield production of oyster mushroom *Pleurotus ostreatus* by using different agriculture waste materials such as wheat straw and rice bran with cotton seed cake. Five treatments were prepared in different proportions to check the time span (number of days) for mycelium growth completion, formation of pinhead, total number of pinhead formed, formation of fruiting bodies and total number of mature fruiting bodies formation. Results described that highest number of days consumed for complete formation pinhead, total number of pinhead, fruiting bodies and total number of mature fruiting bodies were observed with T_2 treatment (cotton seed cake 100%) and was lowest in T_1 treatment (wheat straw 100%). Maximum yield production of oyster mushroom was observed T_1 treatment 407 gm as compared to other treatments. The highest mycelium growth of oyster mushroom was recorded with treatment T_5 (wheat straw 50% + cotton seed cake 25% + rice bran 25%) and the lowest was being with T_2. It was concluded that cotton seed cake along wheat straw and rice bran gave maximum mycelium growth of oyster mushroom while wheat straw produced maximum yield of oyster mushroom.

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Introduction

Mushrooms have protein, vitamin, calcium and different minerals such as copper, potassium, iron, and phosphorus. *Pleurotus ostreatus* is a rich source of minerals, vitamin, proteins and amino acids (Caglarirmak, 2007).

In detoxification of agro-industrial toxic waste compounds, lignocelluloses degrading mushroom species are used and also have medicinal attributes such as worked as an anti-diabetic, anti-carcinogenic, anti-inflammatory and immune modulating affects. Some edible mushrooms such as *Pleurotus ostreatus* have nutritional values, medicinal effects and abilities of biodegradation (Wasser, 2002). Mushroom is a white vegetable or vegetarian boneless meat with protein contents 20-35% as compared to other vegetables and fruits. Mushrooms are freshly spore bearing fungi, mostly grown in developing countries and edible in all communities throughout the universe.

Oyster mushroom (*Pleurotus ostreatus*) is cultivated mostly in temperate and subtropical regions for marketing purpose and in Pakistan mostly grown in wild logs and stumps of trees in the forests of Khyber Pakhtunkhwa, Azad Kashmir and in the plain areas of Sindh and Punjab. Dhingri mushroom is very famous in oyster mushrooms because of its oyster like shape. *Pleurotus ostreatus* is very good for human consumption having higher concentration of protein, fibers, carbohydrates, vitamins, minerals and low concentration of fat contents (Ibekwe *et al.,* 2008).

The genus *Pleurotus* has 40 species, 12 of them are cultivated in different areas of Pakistan. For the growth of *Pleurotus* species different substrates are used such as waste of cotton, seed cake of cotton, wheat straw, sawdust, cobs of corn, bran of rice, husk of coconut, pulp of woods, leaves of banana and different types of other waste materials mostly present in Pakistan (Thomas *et al.,* 1998). Pakistan economy mainly depends on agriculture due to agricultural base of Pakistan. GDP share of agriculture in Pakistan is 21% that's why agriculture is a backbone of Pakistan.

For the growth of mushrooms agricultural waste are used as a supplement (Shah*et al.,* 2004; Reguła and Siwulski, 2007). Currently, agricultural wastes are discarded, burned, neglected in millions of tons and have become a danger for humans by creating environmental pollution. For the cultivation of mushrooms farms waste material, plants waste ingredients and chemical industries remaining waste materials can be used as substrates for the mushrooms cultivation. Cotton waste, bagasse of sugarcane, bagasse of sisal, straw of wheat and rice, rice bran, corncobs, pineapple crown bracts, palm oil waste, grass, sawdust, pulp of coffee, bran of coffee, fiber of coconut and plans in water hyacinth are produced in abundance in tropics as agro-waste.

Organic waste is also used as a substrate for example banana leaves coconut husks, orange peel and wood logs. On the other hand, if these materials are not used as a substrate is only waste (Salmones*et al.,* 1996; Ahmed*et al.,* 2013).

Mushroom growth on mycelium mainly depends on the substrate selections. Mycelium growth is faster on cotton seed wastes as compared to the other substrates (Royse, 2002). Environmental condition like temperature control is very necessary for the production of mushrooms. Mycelia growth is very good at room temperature such as between 20°C to 28°C temperature and for in vitro growth if temperature increases from 34°C causes inactivation of mycelium. For maximum fruiting bodies growth 16°C to 22°C temperature is more suitable(Nascimento and Eira, 2003).

Mycelium mushrooms (vegetative form) have capability of agricultural waste biodegradation in to products and in this way, environment remained good. Mushroom is environment friendly. From mushrooms types, oyster mushroom is used as a main source for the decomposition of vegetables and plants residues (Manzi*et al.,* 2001). Preparation of media is very important for the growth of mushroom.

Microorganism's growth or contamination from any source depends on the utilization of nutrients and physiological properties of the medium. In mushrooms, oyster mushroom is leading mushroom in terms of customer appealing and also very cost effective in terms of production (Murthy and Manonmani, 2008; Ukoima *et al.,* 2009).

Different additive likes mustard cake, gram flour, urea, cotton seed cake, soybean meal, ammonium sulphate and molasses are recommended as substrate supplements preceding from spawning to increase productivity and evaluated biology efficiency of oyster mushroom. Supplements substrate is sources of organic nitrogen which are essential for the growth of mycelium mass but may hinder with the productivity and biological efficiency of mushroom.

Rice bran is a most famous and easily available organic substrate and supplementing rice straw with cereal bran, oil seed cake and cotton seed cake are increase the development of oyster mushroom in Asia (Peng *et al.,* 2000). Mushrooms have average height of 38 cm with 5.5 gm of weight for each mushroom and were largest with 50% water addition in hyacinth plants to the straw of paddy (Nageswaran *et al.,* 2003).

Materials and methods

Recent study was accomplished in Mushroom Laboratory, Plant Pathology Department of Agriculture University, Faisalabad during years 2012-2014 for comprehensive estimation of different nitrogen organic materials of agriculture waste such as cotton seed cake, wheat straw and rice bran for the oyster mushroom cultivation under normal environmental conditions and steam pasteurization technique is used for the preparation of substrate, later on, inoculated oyster mushroom *Pleurotus ostreatus* (Jacq Fr.) with spawn of sorghum. Spawn of oyster mushroom *Pleurotus ostreatus* (Jacq Fr.)was collected from culture bank of Mushroom Laboratory, Department of Plant Pathology, University of Agriculture, Faisalabad.

This strain had huge potential to give superior yield under restricted environmental conditions. Differences nitrogen organic sources in experiments were used as a substrate due to their easier access in the market, their proper utilization for the cultivation of mushroom gives double benefits such as their management as well as source of income.

Cotton seed cake, rice bran and wheat straw are the raw material included in its preparation. These raw materials were purchased from the local market of Faisalabad and wheat straw was obtained from the field area of Agronomy Department.

The cotton seed cake was sun dried and chopped into tiny pieces. Seed cake for about 18-24 hours was soaked in water and water other than requirement was removed out. To moisten other waste materials from agricultural products such as straw from wheat and bran from rice were placed on clean floor or cemented floor after cleaning was soaked in water for 24 hrs. to get desired moisture of 70% by removing extra water from the substrate. To retain pH of substrate lime was added at the rate of 5%. Before filling in to the bags 5 days were given for the fermentation of substrate and covered with polythene sheath. In each polythene bag 500 gm of moist substrate was filled and were plugged by using plastic rings made from PVC pipe of 2.5 ×1 cm size.

Substrates

Three types of substrates were used in this experimented (wheat straw, cotton seed cake and rice bran) for the oyster mushroom (Jacq Fr.) yield.

Treatments

Following treatments were designed using the prepared material in this study:

T_1 = Wheat straw 100%

T_2 = Cotton seed cake 100%

T_3 = Cotton seed cake 50% + Rice bran 50%

T_4 = Cotton seed cake 50% + Wheat straw 50%

T_5 = Wheat straw 50% + Cotton seed cake 25% + Rice bran 25%

Bags were sterilized in ordinary drums at 121 °C with pressure 15-20 (PSI) for killing the germs and pathogenic microbes which are harmful for mycelium growth edible mushroom. Edible mushroom mycelium when start growing on substrate, it appeared white in color. Depending upon the nature of raw material, it took several days to fill the bags completely. After filling the bags completely with mycelium, it took 3-4 days for the opening of the plug of the bags.

When mushroom mycelium entirely filled in the bags then primordial of small size that helps in producing fruiting bodies appeared. Primordial of all types did not help in the rising of fruiting bodies as some of them died earlier. After 4-5 days, oyster mushroom fruiting bodies reached at maturity stages. Maturity of fruiting bodies of oyster mushroom appeared in curing shape. After 3-4 days fruiting bodies appeared which varied in size. Fleshy parts of the mushroom were harvested by giving a slight twist at the base of the fruiting body. Days were counted for mycelial growth completions that were observed from the inoculation spawn when mycelium growth completed.

The bags impregnated in compact form. For primordial emergence number of days was counted, after completion of spawn running and also the days of spawning. After few days they produced the fruiting bodies when primordia appeared. Numbers of days were counted for its transformation to fruiting bodies from primordia. Fruiting bodies total numbers were counted for all treatments, which differed mostly among different treatments. From the substrates three different flushes were obtained and for each flush yield was recorded in grams for all the treatments. At the end cropping period mushroom total yield was calculated by adding the yield of all three flushes. Total yield was recorded by taking weight in fresh and dry form.

Biological efficiency

$$B.C = \frac{\text{Mushrooms fresh weight}}{\text{Substrate dry weight}} \times 100$$

Statistical analysis

In completely randomized design (CRD) experiments were laid out and the obtained data was analyzed statistically to determine the level of significance for experiments (McGraw-Hill, 2008). On the obtained data at the probability level of 5% LSD test was applied that given a reasonable and comprehensive comparison of the treatments to describe their effectiveness.

Results

Among all the treatments, treatment 4 (cotton seed cake 50% + wheat straw 50%), and treatment 5 (wheat straw 50%+ cotton seed cake 25%+ Rice bran 25%) showed significantly less number of days to run a spawn of *P. ostreatus* at each percentage level followed by treatment 1 (cotton seed cake 100%).

Remaining treatments taken comparatively a greater number of days for germination of spawn in usual days. Overall, very less number of days were recorded at treatment 5 when half percent wheat straw along with cotton seed cake and Rice bran was applied as substrate.

There were average six days in which such mushroom strain showed its pinhead and it taken around less then eighteen days. Similarly, T4 taken eight days to complete 25% to spawn run and twenty days to complete this process. Wheat straw alone and cotton seed cake mixed with rice bran (equal concentration) resulted in taking longer time period to complete spawn initiation and completion to 100%. Minimum 12 to 14 day were recorded for 25% and 24 to 28 days were spent to accomplish full *Pleurotus ostreatus* spawn running. It was inferred that only last treatment in which wheat straw, cotton seed cake and rice bran admixed are beneficial for quick and rapid production to meet challenging demand of mushroom production (Table 1).

Behavior of different treatments was vigorously different from each while were strikingly competing for less number of days taken to complete its seed germination.

Table 1. Mean number of days taken for 25%, 50%, 75%, 100% spawn running of *Pleurotus ostreatus*.

Treatments	25%	50%	75%	100%
T1	10.60 c	11.40 d	13.60 d	20.60 d
T2	14.90 a	19.00 a	21.80 a	28.00 a
T3	12.10 b	15.00 b	18.00 b	24.80 b
T4	8.50 d	13.30 c	15.80 c	22.00 c
T5	6.10 e	9.50 e	12.40 e	17.80 e

T1= cotton seed cake 100%, T2= wheat straw 100%, T3 = cotton seed cake 50%+ Rice bran 50%, T4 = cotton seed cake 50% + wheat straw 50%, T5 = wheat straw 50%+ cotton seed cake 25%+ Rice bran 25%.

Treatment T_1 (wheat straw 100%) took minimum number of days for the initiation of pinhead while maximum days were taken by treatment T_2 (cotton seed cake 100%) as 17 days. Remaining treatments as treatment T_3 (cotton seed cake 50% and rice bran 50%) took 11 days, treatment T_4 (cotton seed cake 50% and wheat straw 50%) took 13.0 days and treatment T_5 (wheat straw 50% + cotton seed cake 25% and rice bran 25%) took 7.4 days for observation. It can be inferred that treatment T_1 is showing significant pinhead formation with compare to all other treatments and its combination with cotton seed cake is economically important to decrease the cost of production because minimum number of day for completion of mycelial growth are of primary importance. In addition, T_1 (wheat straw 100%) was recorded as taking maximum number of days (22 days) for pinhead formation and T_2 (cotton seed cake 100%) as 10 number of days for it. While all other treatments utilized more than 10 to 20 days to develops its pinheads. Treatment T_1wasfound most prominent in pinhead formation while comparing with all other treatments.

Table 2. Mean number of days taken for growth of pinhead formation, total No. of pinhead, fruiting body formation, total number of fruiting body.

Treatments	Pinhead formation	Total No. of pinheads	Fruiting body formation	Total number of fruiting bodies
T1	5.40 e	22.20 a	4.20 e	10.60 e
T2	17.00 a	10.00 e	15.40 a	22.00 a
T3	11.00 c	12.00 d	11.80 b	17.80 b
T4	13.00 b	17.40 c	9.00 c	15.20 c
T5	7.40 d	19.40 b	6.60 d	13.00 d

T1= cotton seed cake 100%, T2= wheat straw 100%, T3 = cotton seed cake 50%+ Rice bran 50%, T4 = cotton seed cake 50% + wheat straw 50%, T5 = wheat straw 50%+ cotton seed cake 25%+ Rice bran 25%.

The minimum number of days by treatment T_1 (wheat straw 100%) taken 4.2 days and the more number of days taken by a treatment T_2 15.4 days. Other treatments have different period following by 11.8 days for T_3, 9.0 days for T_4 and 6.6 days for T_5 respectively. From the Data found we can intensely conclude that treatment T_1 which is wheat straw (100%), for oyster mushroom fruiting bodies formation it took less number of days as compared to other treatments, T_2 treatment (cotton seed cake 100%), for spawn running and initiation of pin head and reaching to the oyster mushroom fruiting bodies completion took maximum number of days. Our findings were concluded that the cotton seed cake used in the substrate formation for the production of*P. ostreatus have* slower life cycle and to reach harvesting stage it takes longer period due to this it is not economically suitable. For the cultivation of oyster mushroom, due to its nitrogen rich nutritional contents wheat straw is very good that is easily available for mycelium growth and fulfills the nutritional demand of growing mushroom.

Table 3. Mean Number of days taken for 1st flush, 2nd flush, 3rd flush (grams) and total yield (grams).

Treatments	1st flush (grams)	2nd flush (grams)	3rd flush (grams)	Total yield (grams)
T1	153.20 a	137.40 a	116.40 a	407.00 a
T2	92.40 e	82.60 c	67.40 e	242.00 e
T3	119.40 d	99.80 d	84.60 d	303.80 d
T4	123.20 c	110.60 c	99.60 c	333.40 c
T5	133.40 b	118.00 b	111.20 b	362.60 b

T1= cotton seed cake 100%, T2= wheat straw 100%, T3 = cotton seed cake 50%+ Rice bran 50%, T4 = cotton seed cake 50% + wheat straw 50%, T5 = wheat straw 50%+ cotton seed cake 25%+ Rice bran 25%.

As treatment T_5 have fastest spawning and also have early development of fruiting bodies, that is why it produced more number of pinhead and fruiting bodies in less number of days. T_2 treatment on the other hand took maximum number of days for pinhead and fruiting bodies formation and showed slowest mycelia growth by taking maximum number of days for fruiting bodies formation as showed in (Table 2).

As shown in the table 3 maximum yield in first flush was obtained in T_1 treatment and the obtained value was 153.20 gm, while in T_2 treatment minimum yield was obtained (92.40 gm). Other treatments yield values were in range between them and their obtained values were recorded as 119.40, 123.20 and 133.40 gm for T_3, T_4 and T_5 treatment respectively. It is described that readings obtained from statistically analyzed data have highest yield in 1st flush than 2nd and successive flushes. From statistically analyzed data we can conclude that use of wheat straw and its combinations with cotton seed cake and rice bran in substrate formation are economically good in the cultivation of *Pleurotus ostreatus*.

Treatment T_1 (wheat straw 100%) have also given highest yield in 2nd flush (137.40 gm) but T_2 treatment (cotton seed cake 100%) have the lowest yield such as 82.60 gm of oyster mushroom fruiting bodies. Similarly, other values as yield were recorded as 99.80, 110.60 and 118.00 grams for T_3, T_4 and T_5 respectively. It can be inferred that wheat straw (100%) utilization as a substrate for economic cultivation of *Pleurotus ostreatus* is significant. T_2 treatment cotton seed cake (100%) by itself is not suitable economically but its combination with nitrogenous sources improves the conditions.

Treatment T_1 (wheat straw 100%) gave maximum overall yield 407.00 grams but treatment T_2 (cotton seed cake 100%) showed the minimum yield among all other treatments and its obtained value was 242.00 gm. In addition, other treatments have values in range such as 303.80, 333.40 and 362.60 gm for T_3, T_4 and T_5 respectively as shown in Table 3.

Discussion

Mushroom is a white vegetable or vegetarian boneless meat with protein contents 20-35% as compared to other vegetables and fruits. It was noted that the mycelium running rate might be due to the presence of different kind of polyphenolic substances in substrates (Hu *et al.,* 2009). Un-pretreated spent beer grains used as raw material for substrate successfully for the *P. ostreatus cultivation*. Effect of fruiting bodies were investigated to check the substrates density of packing on the yield and nutrition on the basis of spent grain types, additives, moisture contents. The cultivation results showed that Few fruiting bodies were formed on spent grain alone as shown from the results of cultivation and showed significantly high biological efficiency (19.1%) obtained with the addition of wheat bran (45%).

Our results are in lined with (Vetayasuporn *et al.,* 2006) four substrates of lignocelluloses (sawdust, peat of coconut husk, narrow leaf cattails and bagasse) were used for *P. ostreatus* cultivation, and

from these substrates 3-6 flushes were obtained. Mushroom growing process accelerated by using bagasse substrate. Colonization of mycelium completed, initiation of primordia and fruiting bodies formation were found within 28, 40 and 44 days respectively. Maximum mushroom yield (536.85 g per 1 kg substrate) was given by sawdust gave and was significantly different to the values found from bagasse (360.84 g), peat of coconut husk (278.78 g) and narrow leaf cattails (112.10 g) at a level of 95% confidence interval.

Pinhead initiations numbers are affected directly by following factors such as temperature, light, humidity, fructose, glucose and other substances present in the substrate. Late delay in fruiting bodies formation causes variation and also environmental conditions have great effect in the makeup of *Pleurotus spp*. used in the research work. Present study results were supported by Baysal *et al.* (2003) that pawn running, pinhead and development of fruiting bodies and oyster mushroom (*Pleurotus ostreatus*) yield with supplemented waste paper peat, manure of chicken and husk of rice (90 + 10; 80 + 20 w: w) were noticed under study.

Present study was conducted to check the water hyacinth suitability as an economic substrate for the nourishment of recently domesticated oyster mushroom specie *P. flabellatus*. A study was conducted to check the performance of mushroom under ambient temperature and relative humidity (RH) regimes of 18-25/27-29^{o}C and 55-85/78-93%, respectively. Mushroom growth cycle was completed in 40 days with three and four flushes respectively. At accelerated temperature and RH regime, faster growth of mushrooms occurred and the first flush after 13th days was harvested and substrate inoculation with a biological efficiency of 84% was obtained, while at lower temperature with relative humidity regime first harvesting was done on 19th day after inoculation with 53% biological efficiency. At the completion of growth cycle total fiber loss in the range of 31-40% was observed and most utilized fraction cellulose decreased up to 35-48%. During the growth of mushroom with the passage of time losses increased in the fiber rate and during the second and third flush growth was maximum, which was about 80% of the overall mushroom yield obtained. From water hyacinth shoots good quality substrate obtained which proved very good for the growth of local oyster mushroom specie at ambient environmental conditions (Tisdale *et al.,* 2006).

Agricultural wastes utilization is necessary to avoid environmental hazards and hideout of pests. For the cultivation of mushroom there is a need to explore the abundant agro-waste potential use as an additional substrate. All the agricultural waste materials used in this research to support the growth of *P. ostreatus* indicated that the bio-conversion of these agricultural wastes materials was efficient. According to Jadhav *et al.* (1996), who studied the effect of different substrates on the oyster mushroom yield and described that stalks and leaves of cotton have given effective results whereas lowest yield was obtained on groundnut. Following results are in favor of this research.

A study was conducted by Shah *et al.* (2004) to check the different aspects on industrial waste of oyster mushroom. Highest yield of 277.67 g was gained using Cotton waste while wheat straw gave 129.2 g yield. In addition, current research results were showed similarity to the findings of (Das and Mukherjee, 2007).

They described that the oyster mushroom cultivation on dry weed plants as the substrate in mushroom fruiting body production. *P. ostreatus* spawn was inoculated on weeds combined with rice straw. The limitations in the oyster mushroom cultivation on the substrate of weeds was noticed to be a little yield giving substrate in the 2nd flush that was controlled by mixing plant weeds and rice straw together.

Cotton waste gave the maximum amount of yield 198.67 g and wheat straw gave the minimum amount of yield 58.95 g from the cotton seed waste. His findings results are in line with the findings of Haq *et*

al. (2017) conducted a study and obtained maximum amount of yield from water hyacinth and waste of cotton seed was added at the rate of 1:1 ratio. Salmones *et al.* (1996) on rice straw obtained higher biological efficiency. Philippoussis *et al.* (2001) described that faster growth rate of *V. volvaceaon* obtained in the experiment when he utilized composted cotton seed cake waste as a substrate.

Akinyle and Akinyosoye (2005) reported higher mushroom growth rate on cotton waste which is similar to our findings. According to Zervakis *et al.* (2001) that faster growth rate and colonization in linear way have been noticed in cotton gin-trash. Obodai *et al.* (2003) reported that maximum production obtained when leaves of banana, peeling of palm oil and cocoyam oil pericarp used as a substrate.

Conclusion

T_1 treatment hasgiven the maximum yield in all three flushes (407 gm), while T_2 treatment has given the lowest value for yield (242.40 gm) during all the three flush productions. The wheat straw and combination of it with cotton seed cake and rice bran have been found more capable for substrates to grow mushroom on commercial point of view by farmer's community as a cheap source of protein.

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