International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 13, No. 1, p. 462-469, 2018

INNSPUB

RESEARCH PAPER ON ACCESS

Economic and potential impact of various substrates with agro-wastes on physiology and yield of *Pleurotus ostreatus-P2* production

Nasir Ahmad Khan[1], Javed Mureed[1], Imran Ul Haq[1], Muhammad Asif*[1], Muhammad Muzammil Jahangir[2], Abdul Qadus[1]

[1]*Department of Plant Pathology, University of Agriculture Faisalabad, Pakistan*

[2]*Institute of Horticultural Sciences, University of Agriculture Faisalabad, Pakistan*

Key words: Oyster mushroom, *Pleurotus ostreatus*, Agriculture waste compost, Banana leaves, Physiology, Mycelium and Pinhead formation, Yield performance.

http://dx.doi.org/10.12692/ijb/13.1.462-469 Article published on July 30, 2018

Abstract

Mushroom is the best recycler of agro-wastes. This experiment was carried out in mushroom cultivation Lab during 2013. Complete randomized design was implied to record the data. The investigations were done using different substrate alone or mixed with banana leaves were to examine the mycelia growth and yield performance of *Pleurotus ostreatus*. Data for the research was recorded in days and statistical analysis was performed. It was reported previously that *Pleurotus ostreatus* -P2 takes more than thirty-five days to complete its mycelial growth. It was inferred that mixture of Banana leaves (50%) with Cotton waste (50%) substrate decreased the number of days taken to complete it growth to 24 days. Moreover, 25%, 50% and 75% mycelium was developed in nine to twenty days on an average. This Mixture of compost with equal concentration was proven useful in converting waste product into healthy and useful protein-diet for human consumption within less number of days. However, among all the performance of 3rd treatment was very appraising in very short time for fruitification development. Similarly, Maximum total yield was obtained from similar treatment (T3) giving (113.5g) first flush and total of 282g as a whole. Similarly, yield potential of T2 and T4 was quite near to T3 respectively. However, among all treatments the performance of last treatment containing Banana leaves (50%) and Ccoconut corn waste (50%) was very lower than the potential of yield T3. It can be concluded that equal concentration of banana leaves mixed with equal amount of agro-wastes would be equally useful to convert them into healthy consumable product for mushroom industry and provide employment chances for layman.

* **Corresponding Author:** Muhammad Asif ✉ m.asifssa138@gmail.com

Introduction

The mushrooms have long ago drawn attention of human beings as a food, nutritionally and medicinally and now-a-days is a leading food component (Iqbal *et al.,* 2005). Edible fungi are natural recycler which converts lignocelluloses wastes into protein loaded healthy food. Mushrooms are rich in proteins, vitamins, minerals and called as the vegetarian's meat. Mushroom is rich in vitamin B and C complex whereas, protein content varies from (1.5 to 2.5) percent and minerals required by the human body. Mushrooms are the source of extra ordinary power and validity and are used in the preparation of many continental dishes and have medicinal properties like anti cancerous, anti-cholesterol. Mushrooms are useful against diabetes, ulcer and lungs diseases (Quimio, 1976). Oyster mushroom (*Pleurotus ostreatus*) cultivation carried out on various substrate for safe economical usage of waste. It belongs to class Basidiomycetes, and order Agaricales. *Pleurotus* species are considered nutritionally rich and ranks 2nd among 12 hundred cultivated mushroom species. It exhibits extraordinary flavor and relatively higher nutritional characteristics especially protein, carbohydrates and minerals.

It is medicinally important and have been used clinically for treatment of several diseases and disorders since ancient times. Oyster mushrooms have been broadly utilized as human foods for centuries and have been esteemed for texture and savor as well as some therapeutic and energizer attributes. These mushrooms are best sources of certain clinically important molecules which are considered pivotal in immune systems (Manzi *et al.,* 2001). Pakistan is a developing country and its economy is basically based on agriculture (Shukla and Biswas,2000).

It is an edible mushroom having outstanding flavor and taste. It can be cultured on various plant waste resources having cellulose, lignin and hemicellulose. Oyster mushroom contains 75-90% moisture level with up to 3% proteins, little fats, 4% carbohydrates, essential mineral, vitamins and essential amino acids. On the other hand when dried it contains almost up to 40% proteins (Bettin *et al.,* 2009). The reason of presence of sufficient nutritive components makes it high cost and essential food for patients as well as for common man, especially those who are health conscious.

Oyster mushroom being rich in proteins, essential as well as nonessential amino acids, macro as well as micro elements makes it useful in reduction of blood cholesterol levels to a minimum value. Proteins present in oyster mushroom equalized its rank and value to animal protein sources. Unsaturated types of fats make it good diet for people having obesity issues. Abundance of carbohydrates and high calorie value make it perfect food with low fiber contents. Higher mineral contents and vitamins (such as A, B, C, D and E) and more Iron amounts make it a good antioxidant agent. Reduced blood leakage from vessels and relaxation of muscles has also been reported by various scientists (Shukla *et al.,* 2005; Nataraja *et al.,* 2005). Folic acid present in mushrooms helps to cure anemia. Its current production is about (1.5) million tons in the world. It grows wild in the forests of hilly areas and is cultivated in temperate and subtropical regions of the world (Ibekwe *et al.*, 2008). Mushroom cultivation system is scattering now-a-days throughout the world because these are easy to grow. Besides the use of mushrooms as a food, the oyster mushroom is produced industrially for uses such as pulp and in the pharmaceutical industry (Kumari and varenyam, 2008).

Mushrooms are grown on agricultural waste and industrial waste by adopting boiling, pasteurization and fermentation process. Fermentation and decomposition is a great problem in developing countries like Pakistan which faces challenge of safe dumping of crop residues as an enormous problem. Pakistan is an agricultural country with 75% population depending on agriculture directly or indirectly. 44% of the country's work force is directly employed to agriculture profession. Every year after harvesting of required plant parts millions of tons of crop residue is wasted and burnt, which is a great loss to farmer imparting bad effects of overall country's economy (Islam *et al.,* 2009; Shah *et al.,* 2004; Royse, 2002). If such agricultural wastes are utilized by proper technologies they can create good revenue for country. These agricultural wastes contain a lot of lignin

content. Mushrooms have ability to degrade such lignocellulosic compounds. The cultivation of oyster mushroom is gaining importance in tropical and subtropical region due to its simple way of cultivation and high biological efficiency and useful for the degradation and recycling of bio-wastes (Oei, 2003; Koncsag and Kirwan, 2010). Currently, millions of tons of agricultural wastes are discarded, burned, neglected and has become a burden because of the destruction and by creating environmental pollution. Waste materials from farms, plantations or factories are used as substrates for the cultivation of the mushrooms. Agro-wastes, produced in abundance in the tropics are cotton waste, sugarcane bagasse, sisal bagasse wheat and rice straw, rice bran, corncobs, bracts of pineapple crown, oil palm waste, grass, sawdust, coffee pulp, coffee bran, coconut fiber water hyacinth plants, Banana leaves coconut husks, orange peel and wood logs mostly are the examples of organic waste used as substrates (Asif *et al.*, 2017). Otherwise all these materials are useless byproducts. That's why following study was carried out with following objectives to check the comparative growth and pattern of local strain (*Pleurotus ostreatus* - P2) pinhead, fruiting body and number of fruiting bodies formation and study the yield performance on different concentration of banana leaves composts with other bio substrates.

Material and methods

The present study was conducted in Mushroom laboratory of Department of Plant Pathology, University of Agriculture, Faisalabad during 2013. The study was mainly focused on determination different waste materials for yield production of *Pleurotus ostreatus P2*. The design of the study was completely randomized design.

Preparation of waste materials: The substrate cotton waste was collected from Zulfiqar Cotton factory, District Faisalabad, wheat straw obtained from Agronomy farm, University of Agriculture Faisalabad. While, Coconut coir and Banana leaves were collected from local fruit market. The substrates were soaked in water. All those waste materials were spread over the floor, wetted thoroughly, piled up and enclosed with polythene sheets to avoid moisture escape. Wetted substrates were then allowed fermenting for 5-7 days. After fermentation moisture was maintained up to 70% on floor. The polypropylene bags of six multiply eight inches were packed with fermented substrates. 250g of banana leaves, cotton waste 300g and wheat straw 220g in each bag were filled in each bag, and bags were sealed with rubber bands. Bags comprising fermented substrates were sterilized in a country autoclave at 70°C for one hour. Heating and maintenance of temperature was carried out by providing constant flame using gas burner. Prepared spawn of oyster mushroom *P. ostreatus (*strain P2) was collected from mushroom laboratory, Department of Plant Pathology, University of Agriculture, Faisalabad. Sterilized bags containing fermented substrates were inoculated with 10 g spawn per bag. Once again, bags were sealed by rubber band and these bags were positioned on the shelves in well-ordered temperature for three weeks for spawn running.

Growth conditions: Pleurotus ostreatus (P2- strain) inoculated bags were kept in growth room under controlled temperature and moisture. Temperature and humidity were maintained at 25-30°C, relative humidity 80-90%. The aeration was provided by exhaust fan to increase fresh air for flushing in growth room. Initially spawned bags were kept in dark for enlargement and establishment of mycelium. Time was documented in days for the accomplishment of 25%, 50%, 75% and 100% spawn running on Banana leaves and with collective outcome of other agricultural waste materials. The following treatments were used in experiment:

T_1= Banana leaves 100%

T_2= Banana leaves 50% + Wheat straw 50%

T_3 = Banana leaves 50 %+ Cotton waste 50%

T_4 = Banana leaves50%+Coconut corn 50%

T_5 = Coconut corn 100%

Mycelial growth of Oyster mushroom *Pleurotus ostreatus* (P2- strain) on five substrates combinations was documented in days. In addition, Pinhead formation of oyster mushroom *Pleurotus ostreatus* (P2- strain) on such substrates was documented in

days taken to establish fruiting bodies in mushroom bags. Similarly Yield performance was recorded for the harvesting of mushroom in three flushes.

The first harvesting was done on maturity and subsequent flushes were recorded too. The weight of all fruiting bodies harvested was calculated from all three picking as total yield of mushroom.

Data Analysis: The recorded data was subjected to analysis of variance (ANOVA) and LSD test at 5% level of significance for comparing the difference among means.

Results and discussion

Days taken to complete 25%, 50%, 75% and 100% mycelial growth: Various composts were utilized to grow *Pleurotus ostreatus* -P2 strain p2 specie. Behavior of banana leaves compost used alone was quite average of treatments 2nd ,3rd and fourth. *Pleurotus ostreatus -P2* takes more than thirty days to complete its mycelial growth but these numbers of days were very reduced in treatment 3rd to the range of 24 days. In addition, in 2nd and fourth treatment mycelial growth was completed in 26 and 28 days respectively. It can be inferred that 2nd 3rd and 4th treatment (T2= Banana leaves 50% + Wheat straw 50%, T3= Banana leaves 50% + Cotton waste 50% and T4= Banana leaves 50% + Coconut corn 50%) substrates providing plenty of nourishing material for mycelium development. Moreover, such specie was taking deliberately less number of days to grow 25%, 50% and 75% mycelium within the range of nine days to twenty days on an average. Only last treatment in which Coconut corn (100%) compost was used took ample days to grow in control conditions. Mixture of different compost with equal concentration was proving useful in converting waste product into healthy and useful protein-ous diet for human consumption within less number of days (Table I, Fig. I).

Table 1. Comparison of mycelial growth completion on banana leaves along with agricultural waste materials.

	25% Mycelial growth	50% Mycelial growth	75% Mycelial growth	100% Mycelial growth
Treatments	No. Of Days	No. Of Days	No. Of Days	No. Of Days
T1	12.50 bc	18.0 bc	24.5 b	30.5 bc
T2	9:50 d	15.0 c	20.5 c	26.0 cd
T3	8.00 cd	13.5 c	19.0 c	24.5 d
T4	11.50 b	16.5 b	22.5 b	28.0 b
T5	18.00 a	24.0 a	30.0 a	36.0 a

T1 = Banana leaves 100%, T2= Banana leaves 50% + Wheat straw 50%, T3= Banana leaves 50% + Cotton waste 50%, T4= Banana leaves 50% + Coconut corn 50%, T5= Coconut corn 100%.

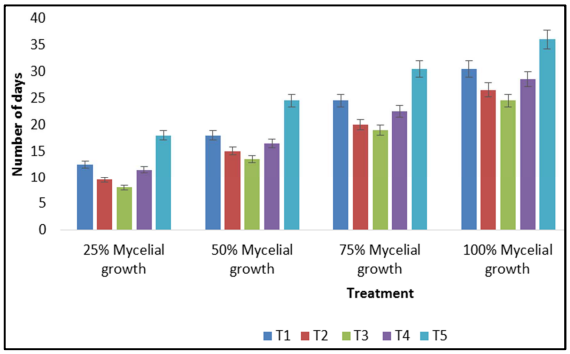

Fig. 1. Comparison of mycelial growth completion on banana leaves along with agricultural waste materials.

Treatment Means for Pattern of Pinhead Formation, Pattern of Fruiting Body, Pattern Total No. of Fruiting Body: Pleurotus ostreatus -P2 strain showed variation on different composts. When there was only banana leaves and coconut corn (100%) compost used, following strain taken maximum days to develop pinheads, develop pattern of fruiting body and to grow total fruiting bodies. Twenty two and 29 days were spent to show pinhead followed by 35 and 40 days for the development of total fruiting respectively. But the combined potential of other compost was very significant in taking very short number of days to develop pinheads and total fruiting bodies development. Minimum of 12 days were utilized by 3rd treatment containing Banana leaves 50% + Cotton waste 50% followed by fourteen and seventeen days by 2nd treatment Banana leaves 50% + Wheat straw 50% and 4th treatment Banana leaves 50% + Coconut corn 50% respectively. However, among all the performance of 3rd treatment having Banana leaves 50% + Cotton

waste 50% was very rewarding in the sense of taking very short time for fructification development (Table II, Fig. II).

Table 2. Comparison of pattern of pinhead formation, fruiting body and total no. Of fruiting body on banana leaves along with agricultural waste materials.

	Pinhead Formation	Pattern of Fruiting Body	Total No. of Fruiting Body
Treatments	No. of days	No. of days	No. of days
T1	22.0 B	29.0 A	35.0 B
T2	14.5 D	22.5 B	29.5 D
T3	12.0 E	19.0 B	27.0 E
T4	17.0 C	25.0 A	32.00C
T5	29.5 A	35.5 A	40.5 A

T1 = Banana leaves 100%, T2= Banana leaves 50% + Wheat straw 50%, T3= Banana leaves 50% + Cotton waste 50%, T4= Banana leaves 50% + Coconut corn 50%, T5= Coconut corn 100%.

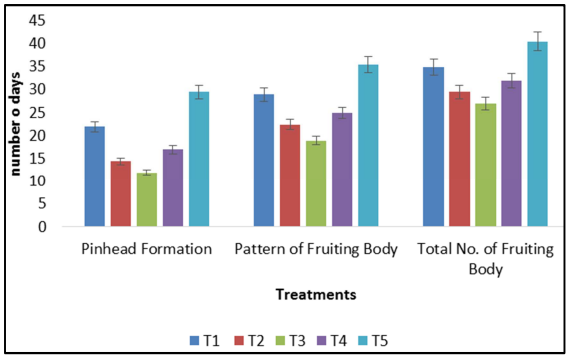

Fig. 2. Comparison of pattern of pinhead formation, fruiting body and total no. Of fruiting body on banana leaves along with agricultural waste materials.

Treatment Means for First Flush (gm), Second Flush (gm), Third Flush (gm), And Total Yield of Pleurotus ostreatus -P2: Pleurotus ostreatus -P2 strain demonstrated yield variability on different composts concentrations alone or combined with agricultural waste. There was very less yield obtained in first, second and third flush picking on coconut corn waste (T5) followed by banana leaves compost (T1). Obtained yield was 50 gram, 42g and 33g followed by 60g, 52 and 42g respectively in T5 and T1. Maximum total yield was recorded after usage of treatment 3 in which Banana leaves 50% + Cotton waste 50% was utilized, giving 113.5g first flush and total of 282g as a whole yield of oyster mushroom. While the yield potential of T2 (Banana leaves 50% + Wheat straw 50%) and T4 (Banana leaves 50% + Coconut corn 50%) was quite s=resembling to the treatment 3 respectively. In addition, first and 2nd flush production was 90g, 85g and 70g, 60g and the total yield was recorded 244 g, 209g respectively. However, among all the performance of 3rd treatment comprising Banana leaves 50% + Cotton waste 50% was very amazing in exhibiting for maximum potential of yield (Table III, Fig. III).

Table 3. Comparison of number of flushes and total yield (gm) on banana leaves along with agricultural waste materials.

Treatments	First flush (gm)	Second flush (gm)	Third Flush (gm)	Total Yield (gm)
T1	60.33d	52.33 b	42.50 d	155.16 d
T2	90.50 b	85.50 a	68.45 b	244.45 b
T3	113.50 a	90.24 a	79.12 a	282.86 a
T4	70.00 c	60.12 b	51.33 c	209.24 c
T5	50.33 e	42.66 c	33.66 e	126.65 e

T1 = Banana leaves 100%, T2= Banana leaves 50% + Wheat straw 50%, T3= Banana leaves 50% + Cotton waste 50%, T4= Banana leaves 50% + Coconut corn 50%, T5= Coconut corn 100%.

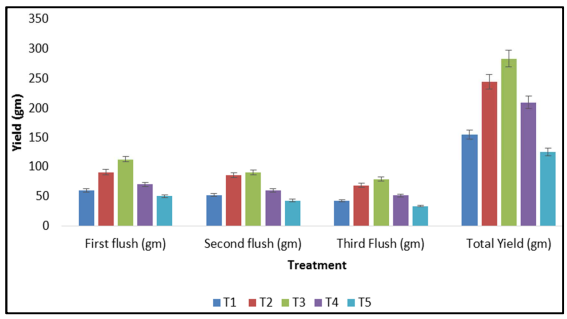

Fig. 3. Comparison of number of flushes and total yield (gm) on banana leaves along with agricultural waste materials.

Discussion

Agricultural wastes utilization is necessary to avoid environmental hazards and hideout of pests. It is necessary to explore the potential use of the abundant agro-wastes as additional substrates for mushroom production. All the different agricultural wastes used in this study supported growth of *P. osteratus* indicating efficient bio-conversion of these agricultural wastes. Jadhav *et al.,* (1996) who studied the effect of different substrate on the yield of oyster mushroom. Cotton stalks and leaves given effective

results whereas lowest yield was obtained on groundnut. Following results are in favor of this research. Khan *et al.,* (2001) studied the different aspect of oyster mushroom on industrial waste. highest yield of 277.67g was gained using Cotton waste while wheat straw gave 129.2g yield. In addition, current research results were somehow resembling to findings of Das and Mukherjee, (2007. They observed that the cultivation of oyster mushroom on dry weed plants as the substrate in mushroom fruiting body production. *P.ostreatus* spawn was inoculated on weeds mixed with rice straw. The main limitation of oyster mushroom cultivation on weed substrate was found to be low yield in the 2nd flush that was over-come by blending weed plants with rice straw.

Ponmurugan *et al*., (2007) studied the cultivation of oyster mushroom on different biowastes such as paddy straw, sorghum straw, paper waste, saw dust and sugar cane molasses and reported that the most significant yield in form flushes was obtained due to paddy straw used as compost followed by sugar cane molasses and somehow least production was attained due to wood saw dust and paper waste. The maximum number of flushes obtained on paddy rice and minimum flushes obtained on paper waste. Ibekwe *et al.,* (2008) conducted experiments on oyster mushrooms using various carbohydrate substrates like millet, corn, rice and rye. Millet compost give the highest mycelia yield while rye gave the lowest yield. Manan, (2000) studied the cultivation of oyster mushrooms on cellulose material of paper waste, straw waste and cotton waste. He observed that cotton waste gave the highest yield 198.67g and wheat straw gave the minimum yield of 58.95g. His findings results are in line with our findings. Haq *et al.,* (2017) obtained highest yield from water hyacinth + cotton waste at the rate 1:1. Salmones *et al.,* (1996) recorded highest biological efficiency on rice straw. Philippoussis *et al.,* (2001) observed higher growth rate of *V. volvacea* on composted cotton waste substrate. Akinyele and Akinyosoye (2005) reported high fungal growth rate on cotton wastewhich is similar to our findings. Zervakis *et al.,* (2001) found on cotton gin-trash the highest linear growth and colonization rates. Obodai *et al.,* (2003) highest production on banana leaves, cocoyam peelings and oil-palm pericarp as substrates.

Conclusions

Pleurotus ostreatus P-2 cultivation proves to be a highly well-organized method for conversion of banana leaves and other waste materials as healthy producing protein rich food. The cotton waste along with banana leaves have been recommended one of the promising substrates on commercial basis for farmer's community other than to utilize banana leaves and coconut corn alone. It can also be concluded from this study that with little effort and proper management, we can produce more food by recycling the waste materials to feed the people and also get rid of these wastes without polluting the environment. Mushroom cultivation is fantastic approach to utilize crop residues and to make it more attractive and profitable business.

Conflict of Interest: The research was conducted at Department of Plant Pathology and evaluated by the Supervisory committee. Each author of this manuscript played the pivotal role during write up and did not possess any conflict of interest to publish this research in "International Journal of Biosciences".

References

Akinyle BJ, Akinyosoye FA. 2005. Effect of Volvariella volvacea cultivaton on the chemical composition of agrowastes. African journal of biotechnology **4(9).**

Asif M, Atiq M, Sahi ST, Ali S, Nawaz A, Ali Y, Subhani A Saleem A. 2017. Effective management of white rust (albugo candida) of rapeseed through commercially available fungicides. Pakistan journal of phytopathology **29(2),** 233-237.

Bettin F, Montanari Q, Calloni R, Gaio TA, Silveria MM, Dillon AJ. 2009. Production of laccases in submerged process by *Pleurotus sajor-caju* PS-2001 in relation to carbon and organic nitrogen sources, antifoams and tween 80. Journal of Indian Microbial Biotechnology **36(1),** 1-9.

Das N, Mukherjee M. 2007. Cultivation of *Pleurotus ostreatus* on weed plants. Bioresource Technology **98(14),** 2723-2726.

Haq IU, Ijaz S, Khan NA, Amrao L. 2017. Comparative study on the growth rate of exotic and local strains of *volvariella volvacea* fungus on different substrates. Pakistan Journal of Phytopathology. **29(2),** 227-32.

Ibekwe VI, Azubuike PI, Ezeji EU, Chinakwe EC. 2008. Effects of Nutrient Sources and Environmental Factors on the Cultivation and Yield of Oyster Mushroom (*Pleurotus ostreatus*). Pakistan Journal of Nutrition **7(2),** 349-351.

Iqbal SM, Rauf A, Iqbal M. 2005. Yield performance of Oyster mushroom on different substrates. International Journal of Agriculture and Biology **07,** 900-903.

Islam MZ, Rahmani MH, Hafiz F. 2009. Cultivation oyster mushroom (*Pleurotus ostreatus)* on different substrates. International Journal of Sustainable Crop Production **4(1),** 45-48.

Jadhav AB, Bagal PK, Jadhav SW. 1996. Effects of different substrates on yield of Oyster mushroom. Maharashtra Agriculture **3,** 424-426.

Khan AM, Khan SM, Khan SM. 2001. Study on cultivation of Oyster mushroom *Pleurotus ostreatus* on different substrates. Pakistan Journal of Botany **2,** 140-143.

Koncsag CI, Kirwan K. 2010. Heat and mass transfer study during wheat straw solid state substrate fermentation with *P. ostreatus*. Series of chemistry and environmental engineering **55(69),** 11-13.

Kumari D, Varenyam A. 2008. Effect of different substrates on the production and non-enzymatic antioxidant activity of *Pleurotus ostreatus*. Life Sciences Journal 73-76.

Manan, A. R. 2000. Cultivation of Oyster mushroom on paper waste. MSc. (Thesis), Department of Plant Pathology, University of Agriculture Faisalabad.

Manzi P, Aguzi P, Pizzoferrato L. 2001. Nutritional value of mushroom widely consumed in Italy. Food Chemistry **73,** 321-325.

Natarajan K, Kaviyarasan N, Marimuthu R. 2005.Cultivation of *Pleurotus citrinopileatus* on paddy straw. Mushroom Resources **2,** 65-78.

Obodai M, Cleland-Okine J, Johnson PN. 2003. Use of agricultural wastes as substrate for the mushroom Volvariella volvacea. Tropical science **43(3),** 121-4.

Oei P. 2003. Mushroom cultivation: appropriate technology for mushroom growers. Backhuys Publishers.

Philippoussis A, Zervakis G, Diamantopoulou P. 2001. Bioconversion of agricultural lignocellulosic wastes through the cultivation of the edible mushrooms *Agrocybe aegerita, Volvariella volvacea* and *Pleurotus* spp. World Journal of Microbiology and Biotechnology **17(2),** 191-200.

Ponmurugan P, Gopi R, Maripandi A. 2007. Studies on Actinomycetes diversity in Southern Indian tea soils for antifungal activity. Journal of Plant Crops **35,** 28-32.

Quimio TH. 1976. Culturing *ganoderma*. The *pleurotus* way. Mushroom Newsletter for Topics. **6(4),** 12-13.

Royse DJ. 2002. Influence of spawn rate and commercial delayed release nutrient level of *Pleurotus ostreats* (oyster mushroom) yield, size and time production. Applied Microbial Biotechnology **58,** 527-531.

Salmones D, Waliszewski KN, Guzmán G. 1996. Use of some agro-industrial lignocellulose by-products for edible mushroom Volvariella volvacea cultivation. Revista Internacional de Contaminación Ambiental **12(2),** 69-74.

Shah ZA, Ashraf M, Ishtiaq M. 2004. Comparative study on cultivation and yield performance of oyster mushroom (Pleurotus ostreatus) on different substrates (wheat straw, leaves, saw dust). Pakistan Journal of Nutrition **3(3),**158-60.

Shukla CS, Biswas MK. 2000. Evaluation of different techniques for oyster mushroom cultivation. Journal of Mycology and Plant Pathology **30,** 431-435.

Shukla K, Shetty KS, Krishnamoorthy V. 2005. Possibility of protein enrichment of paddy straw by mushroom spp. PAU, Ludhiana India. pp. 363-367.

Zervakis G, Philippoussis A, Ioannidou S, Diamantopoulou P. 2001. Mycelium growth kinetics and optimal temperature conditions for the cultivation of edible mushroom species on lignocellulosic substrates. Folia microbiologica **46(3), 231.**