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Productivity of *Pleurotus ostreatus* under solid state fermentation on lignocellulosic substrates

***Zahida Nasreen[1], Rukshana Bajwa[2], Tasnim Kausar[1], Shajhan Baig[1], N. Habib[1] and M.B. Bhatti[1]**

[1]*Biotechnology and Food Research Centre, PCSIR Labs Complex, Lahore 54600, Pakistan*

[2]*Institute of Mycology and Plant Pathology, Punjab University Lahore*

**E. mail: zahidanasreen_pcsr@yahoo.com*

Abstract

Three lignocellulosic substrates (*Cedrus deodara, Abies pindrow* and *Populous ciliata*) were used for the cultivation of *Pleurotus ostreatus* and 2-3 flushes were obtained from these substrates. *C. deodara* and *A. pindrow* accelerated the mushroom growing processes. The mycelial completed colonization, primordium initiation and fruiting bodies formation were found within 28, 40 and 44 days respectively. The *C. deodara* sawdust gave the maximum mushrooms yield that was significantly different to those obtained from *A. pindrow* and *P. ciliata*. Likewise, influence of nitrogen source on mycelial growth and fruiting bodies of *P. ostreatus* was determined, forming the basis for the assessment of loss or gain of organic matter. The mycelium extension rates, number of fruiting bodies and organic matter loss was found to be the highest in 4% chick pea supplemented *C. deodara A. pindrow* and *P. ciliata* sawdust as compared to control as well as 1 and 2% supplemented substrates. Similarly, 4% ammonium and nitrate salts supported fast growth of *P. ostreatus* whereas urea was proved unsuitable for *P. ostreatus*. Supplemented *P. ciliata* sawdust was poor substrate to support *P. ostreatus* growth out of the three substrates tested.

Key words: Fruiting bodies, nitrogen sources, *P. ostreatus*.

Introduction

Mushrooms, a highly priced delicacy for more than two thousand years, are now consumed by many people in Pakistan. It has high nutritive and medicinal value, and contributes to a healthy diet because of its rich source of vitamins, minerals and proteins (Shah, 2004). In Pakistan, wild mushrooms are traditionally collected from the forest regions during the wet season from March to September. With the introduction of the plastic bag method in 1990, edible and medicinal mushrooms could be easily produced all year round on different agro wastes.

Mushrooms cultivation is profitable agribusiness and *P. ostreatus* is an edible mushroom having excellent flavor and taste. Mushroom production is small but important industry in many countries. Mushrooms could become and important additions to farmers looking for a value added product and way to supplement farm income while making use by products or co products from other crops. Wood saw dust; hulls, straw, stalk and paper will all support mushroom growth. The growths of diverse type of mushrooms require different type of substrates and availability of varied type of materials may dictate which type is used.

Since mushrooms can be grown on nearly any type of agricultural residues, they are an ideal crop for rural areas with large amounts of cultivated acreage and residue from field crops. Numerous types of mushrooms exist, but the most commonly consumed are *Pleurotus* and *Agaricus* (Burden, 2006). In the Northern areas of Pakistan *P. ostreatus*, *Agaricus bisporus*, chanterelles, morels and straw mushrooms grow in the wild and can be gathered and sold at farmers, markets or retail stores (Shah, 2004). In world mushroom production, *P. ostreatus* rate second, after *A. bisporus*. In 1986, *Pleurotus* sp. production accounted for approximately 7% of the total world production of edible mushroom; by 1990, production of *Pleurotus* sp. reached one million metric tones and accounted for 24% of the total mushroom production (Royse, 1992). Unlike other mushrooms, *Pleurotus* sp. has much diversity in their adaptation to the varying agro climatic condition as the locality available lignocellulosic substrates (Baysal, *et al.*, 2003). Commercial cultivation of *Pleurotus* sp. has not been performed in Pakistan. The cultivation of *Pleurotus* has been evaluated in the Laboratory.

In Sawat area it locally known as Dhengri, a relatively abundant timber species therefore, sawdust has a short composting period for *P. ostreatus* growing (Shah, 2004).Oyster mushrooms

which have a wide range of temperature adaptability (Chang, 1999) and substrate utilization (Cliquet and Jackson, 2005; Poppe, 2000) have been accepted by the Pakistani populace for their taste, nutritional and medicinal properties (Shah, 2004). Although various workers have studied the growth conditions and substrate utilization of various mushrooms in many countries, there has not been any attempt to study the liner mycelial growth and organic matters loss or gain of *Pleurotus* species under indigenous environmental conditions. This paper, therefore reports on the performance of *P. ostreatus* species grown on composted sawdust of two soft wood (*C. deodara* and *A. pindrow*) and one hard wood tree species (*P. ciliata*). The study also compares the results after amending the substrate with various N-supplements.

Materials and Methods

Establishing mycelial culture and spawn

The mycelial culture of *P. ostreatus* was prepared in test tube slant containing PDA medium. The mycelium from PDA slant was used for spawn production of test fungi.

Sorghum and barley grains were used for spawn formation, after socking, washing and drying this grain were impregnated by calcium carbonate; these coated grains were kept in a well cleaned and drained glass jar. The jars were covered with cotton plugged and latter autoclaved at 121 ^{o}C for 20 minutes after which jars were cooled at room temperature. The jars were then incubated at 25$\pm$1 ^{o}C in incubator for 8 to 12 days. Each jar was shaken thoroughly by hand periodically to distribute the mycelia to the grains.

Preparation of the substrates and their spawning

Cedrus deodara, *Abies pindrow*, *Populus ciliata* wood shaving and sawdust were soaked in water over night and boiled for 30 minutes. A moister content of the substrate was 60-70%. Sawdust was sterilized at 121 ^{o}C at 15 PSI for 15 to 40 minutes depending on volume.

A common recipe used for supplementation of sawdust was 76% sawdust, (2 parts of saw dust and 1 part of wood chips) 12% millet seed and 12% bran and 65% moisture. These recipes are based on dry weight of the ingredients because moister contents of the ingredients differ (Stamets, 2000).

Substrate was stuffed in (heat resistant) polythene bags (6.2"×2.4", 7"×9.5"and 12"×18"); with four replicate bags for each treatment. The bags were plugged with absorbent cotton by using PVC pipe and autoclaved. Sterilized bags were inoculated in the laminar flow cabinet and then shifted to spawn running room at 25 ^{o}C data was recorded after ramification and harvesting. After the completion of spawn running the bags were shifted to cropping room at 18$\pm$2 ^{o}C. The mouths of bags were opened and water was sprinkled thrice a day on these bags. At that time the humidity was 80%. The crop was obtained in three flushes.

Harvesting

Fruiting bodies of *P. ostreatus* were harvested when it completely mature (2-3 days of emerging). The substrates were incubated again under the same conditions for another 7 days after each harvesting for second and third flushes. Mushroom from different substrates and treatments were kept separately for fresh weight measurements. The biological efficiencies (B.E) were calculated according to method Miles and Chang (1997).

Analysis of substrate

Different important parameters like protein, soluble protein, crude fiber, lignin, cellulose and hemi cellulose of the substrates were analyzed before spawning and after harvesting.

All samples were dried for 24 h in an oven at 105 ^{o}C. They were then ground and sieved through 5 mm mesh. Each sample was stored separately in dry and clean bottle with airtight lid in a refrigerator until analyzed.

Substrates fiber was analyzed according to Goering and VanSoest (1970). The hemi cellulose content of the substrate then is obtained by subtracting ADF from NDF. Lignin and cellulose content of the substrate were determined according the methods of Kurschner and Hank (1930) and ASTM (1961). The total substrate nitrogen content was determined according to Markham (1942).

Statistical analysis. The Data of various biochemical parameters were analyzed by using the analysis of variance (ANOVA) and group means were compared by Duncan Multiple Range Test (DMR) (Steele and Torrie, 1996). Results were expressed as mean ±SD.

Results and Discussion

Mushrooms producing white rot fungi are able to colonize on wood chips and saw dust, because, of its metabolic system. *P. ostreatus* is one of the major edible and medicinal mushroom that cultivate on different lignocelluloses wastes. The present study describes utilization of wood chips and saw dust of *C. deodara*, *A. pindrow* and *P. ciliata* for the growth of *P. ostreatus*.

The different results obtained from the research work are shown in Fig 1a&d, 2a&d and 3a&d. The biodegradation of substrates, spawn

running, pin head formation and fruiting bodies formation are four important aspects in the cultivation of oyster mushroom on various saw dust media. As well as proper temperature and humidity of substrates were next two utmost factors for the formation of fruiting bodies. Temperature for spawn running was 18-20 ^{o}C for fruiting bodies presented good results.

The mycelia covered the saw dust in about 15 days while colonization of the substrates was observed in 20 days. The total number of the fruiting bodies 48 in *C. deodara*, 46 in *A. pindrow* and 42 in *P. ciliata* and total weight of the fruits was 216.25 g, 197.56 g and 173.50 g. The yield observed this study agreed with the observation of Oei (2003) for similar mushroom. Biomass loss was in *C. deodara* 35.88, *A. pindrow* 34.85 and 34.26% in *P. ciliata* substrates, which shows that degradation and solubilization was intensive in the saw dust media. After complete ramification of mycelia on the substrates pinheads formation is the second stage of mycelial growth during cultivation of mushroom. Small pinheads like structures were observed, these pinheads were formed 5-8 days after the spawn running. These results are in agreement with Shah *et al.* (2004) who stated that *P. ostreatus* completed spawn in 17-20 days on different substrates and time of pinheads formation was noted as 23-27 days. Fruiting bodies formation is the final stage during the cultivation of mushroom. The fruiting bodies appeared 4-6 weeks after inoculation of spawn. These findings are in conformity with Quimio (1976, 1978) who reported that fruiting bodies 3-6 weeks after inoculation of spawn.

The crop of oyster mushroom was harvested in three flushes. The maximum yield was obtained in first flush than second and third flush. Maximum average yield was recorded in *C. deodara* (228.93 g) followed by *A. pindrow* (211.56 g) and *P. ciliata* (189.56 g). *C. deodara* produced more number of fruiting bodies then *A. pindrow* and *P. ciliata*.

Cellulose loss of 30.40 to 31.93% in *C. deodara*, 29.40% to 29.91% in *A. pindrow* and 26.65 to 28.24% in *P. ciliata* supplemented with 1, 2, 4 and 6% chick pea powder was significantly higher than without supplemented substrate. (Fig 2a & d, 1a & d and 3a 7d). Greatest growth was estimated on the *C. deodara* and *A. pindrow* substrates then *P. ciliata*. Therefore, it is recommended that *C. deodara* and *A. pindrow* sawdust as best substrates for the cultivation of oyster mushroom which is in agreement with the finding of Hami (1990) who studied the oyster mushroom cultivation on different agricultural wastes and found that *P. ostreatus* gave the maximum yields.

The reduction of hemi cellulose contents recorded for the fungus in supplemented sawdust were significantly higher 31.98-32.88% (in GP), 22.59-24.39% (SO_4) and 22.31-23.88% (NO_3) in *C. deodara* 28.43-29.83% (GP), 21.36-23.21% (SO_4) and 20.11-21.52% (NO_3) in *A. pindrow* and 27.99-28.91% (GP),18.36-19.19% (SO_4) and 17.59-19.04% (NO_3) in *P. ciliata* then the control samples (16.44-17.38%) (Fig 1, 2 and 3).The study revealed the potential of *C. deodara A. pindrow* and *P. ciliata* as good substrates for the cultivation of *P. ostreatus* and the spent substrates as a viable ingredient in ruminant feed. Supplementation of nitrogen increases the growth and biodegradation activities of the organism. There are few reports recorded on the effect of nitrogen in the deduction of such compounds (Stadler *et al*., 1994; Mandle and Vodickova 1994).

The fiber friction decreased appreciably in supplemented saw dust as compared to the non supplemented wastes. The decreased in fiber contents could be due to the production of various enzymes during the vegetative and reproductive phases with lignocellulose degrading properties (Jacqueline and Visser, 1996).

Similarly, the lignin contents removal in C. *deodara* 36.97-38.45% (GP), 31.05-32.42% (SO_4), 30.44-31.32% (NO_3) and 35.16-37.42% (GP), 29.35-32.01% (SO_4), 29.67-30.52% (NO_3) in *A. pindrow* and 27.48- 31.97% (GP), 26.93-29.39% (SO_4) and 26.89-29.19% (NO_3) in *P. ciliata.* The solubilization of the lignin occurs during the vegetative phase and enzymes like laccase, manganese peroxidase and lignin peroxidase are secreted while cellulose degrading enzymes is secreted during reproductive phase (Tamara, *et al*. 1995).

The protein and soluble protein contents of the fungus supplemented sample was increased (10.18-12.11%, 10.39-11.52% and 10.93-11.49% and 0.56-1.09%, 0.57-1.09% and 0.57-0.71%) significantly than control (9.9% and 0.57%) due to the addition of fungal proteins during solubilization and degradation (Fig 1a to 3L). This agrees with the report of Farkas (1979) and Belewu and Lawal (2003) who reported that the extracellular enzymes secreted by the fungus contain amorphous homo and hetero polysaccharides which is often in association with protein production (fungal protein).

It was noted that *C. deodara* and *A. pindrow* provided to be a better substrate for cultivation of *P. ostreatus* as compared to *P. ciliata.*

During the fermentation of substrates by white rot fungi lignin, cellulose, hemicellulose and fiber degradation was comparatively retarded by the addition of urea in each case.

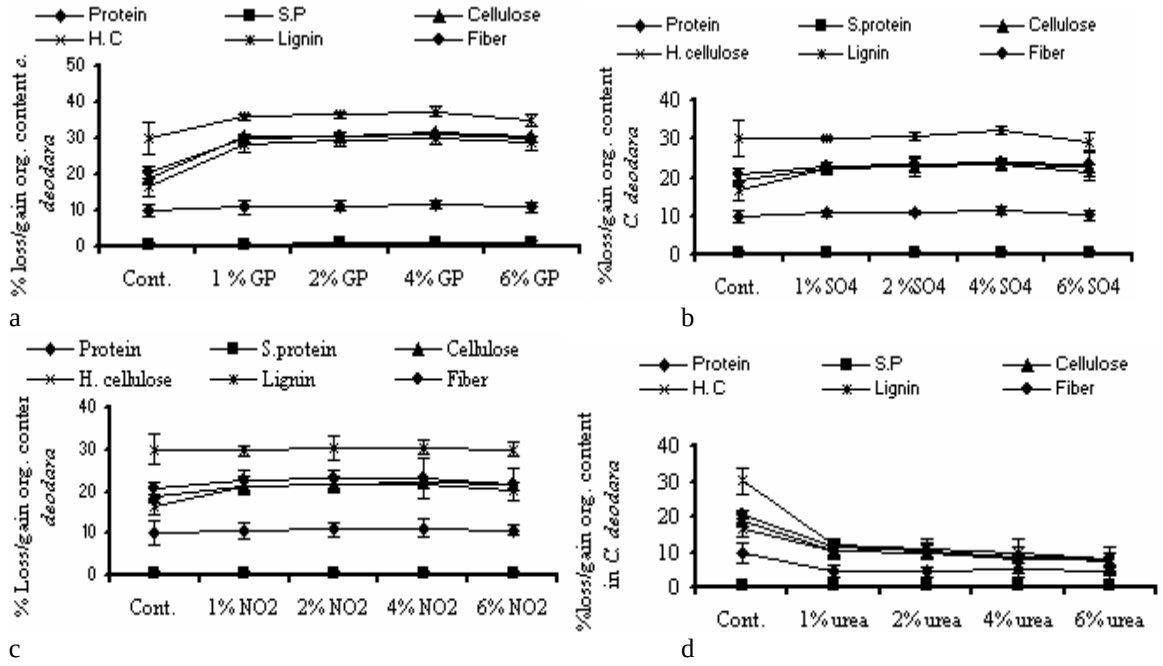

Fig.1: Mean with different letters in a column show significant difference (P= 0.05) as determined by DMR Test

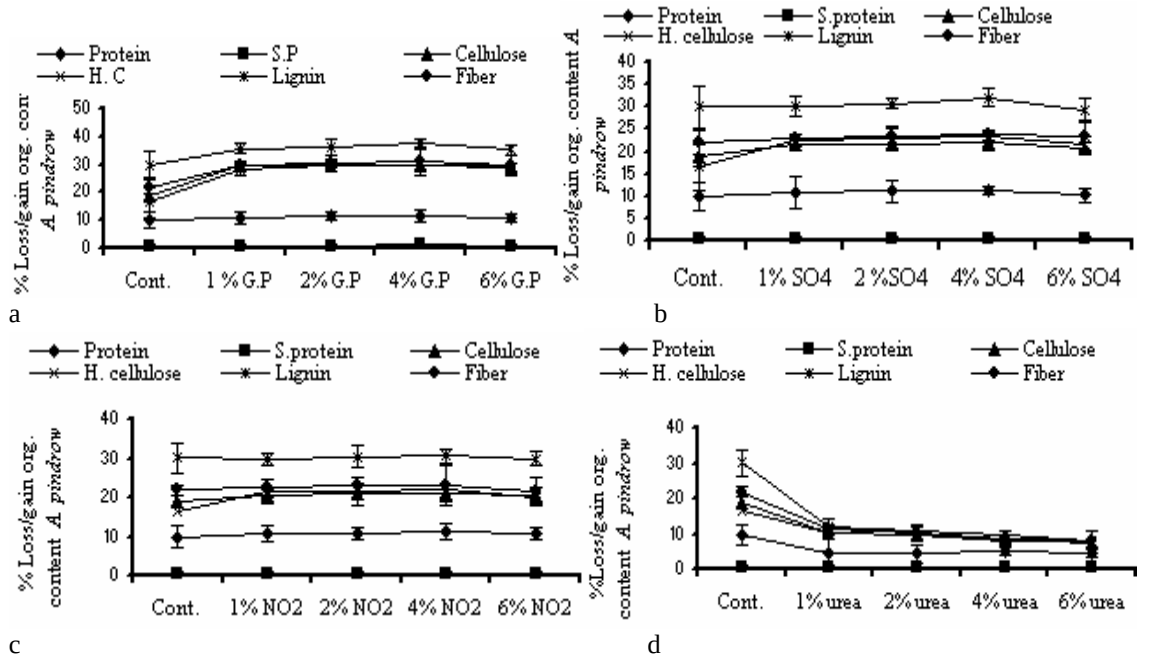

Fig.2: Mean with different letters in a column show significant difference (P= 0.05) as determined by DMR Test

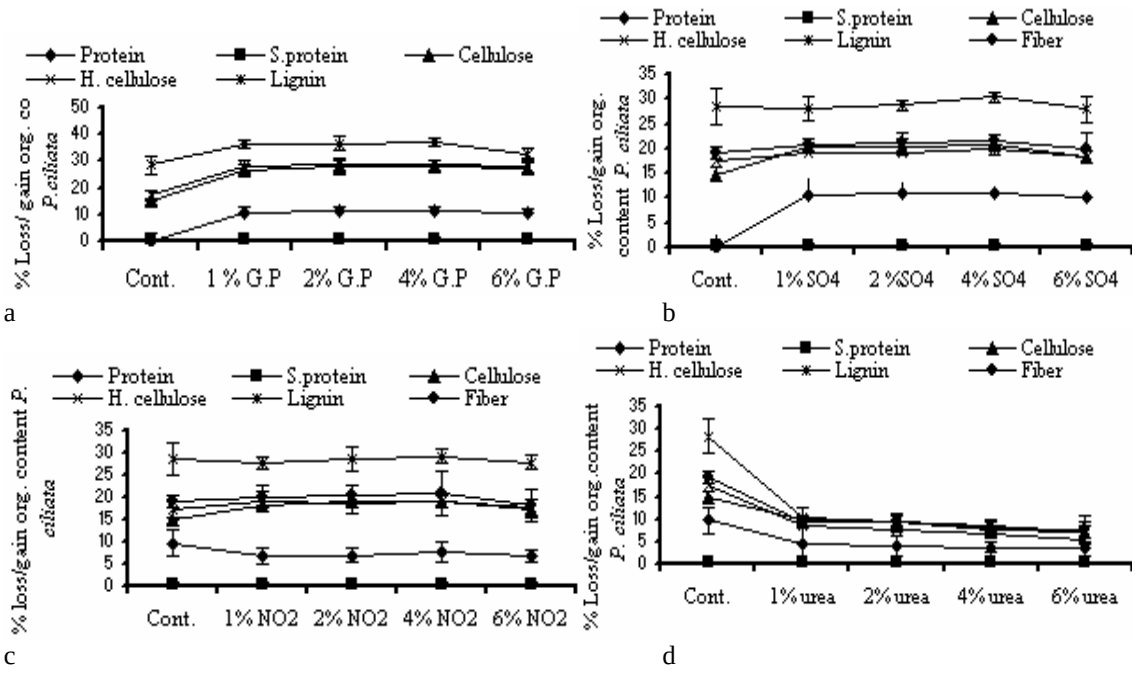

Fig.3: Mean with different letters in a column show significant difference (P= 0.05) as determined by DMR Test.

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