Effects of Different Substrate Combinations on Mycelial Growth of *Pleurotus ostreatus*

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Abstract: Wastes of some lignocellulosic materials such as leaves of hazelnut, leaves of tilia, leaves of populus, needle of spruce, rice stalk, wheat straw, sawdust, waste paper, gross and bran were used for producing *Pleurotus ostreatus* culture mushroom. The effects of different substrate combinations and mixture ratios on the mycelial growth period were investigated. The most suitable combinations were leaves of tilia + bran (75 % + 25 %) and leaves of *Populus* + wheat straw (50 % + 50 %). In these combinations, the mycelial growth period was 19 days. Sawdust (100 %) gave the longest mycelial growth period (40 days).

Key words: Mycelial growth, *Pleurotus ostreatus,* mushroom

1 Introduction

Pleurotus species, the third largest commercially produced mushroom in the world, are found growing naturally on rotten wood material. The growing increase in the consumption of oyster mushroom is largely due to its taste, nutritional and medicinal properties.[1] Biologically active substances and especially polysaccharides isolated from *Pleurotus* ostreatus have shown antitumor, immunomodulating, antiviral, antibacterial and especially hypocholesterolemic activities because of the presence of lovastatin, which plays an important role in the inhibition of the most important enzyme in cholesterol metabolism. Fruit bodies of this species are rich in carbohydrates, dietary fiber, protein (10.5-30.4 % of dry weight), essential amino acids, vitamins (carotene, C, A, B2, B1, niacin, ascorbic acid) and minerals (K, P, Mg, Ca, Na, Fe, Se, Zn, Cu, Mn) [2].

Pleurous species are wood-inhabiting white-rot Basidiomycetes with important biotechnological and environmental applications. They are highly adaptable to grow and fruit on a wide variety of forest and agro-industrial lignocellulosic wastes because of their ability to synthesize the relevant hydrolytic and oxidative enzymes that convert the individual components of the substrate (cellulose, hemicellulose, lignin) into low-molecular weight compounds, which can be assimilated for fungal nutrition.[3] The fungus accomplishes enzymatic degradation of the lignocellulosic portion of substrates by using enzymes such as endoglucanase, β-glucosidase, xylanase, laminarinase, laccase and polyphenol oxidase that are involved in the degradation of lignocellulose.[1]

Despite the commercial importance of *Pleurotus* mushrooms, the biological potential of these white rot fungi is far from exploited. In addition to traditional bioconversion of organic wastes into edible protein and animal feedstock, there are other fields where the lignocellulolytic potential of oyster mushrooms may also achieve economic relevance. First, there is an increasing demand from agriculture, industry, and medicine for hydrolytic and oxidizing enzymes manufactured by inexpensive processes. Second, numerous data presented in the literature argue in favor of utilizing lignïnolytic Basidiomycetes as tools for the remediation of contaminated soils and industrial wastewaters. Among these Basidiomycetes, *Pleurotus* mushrooms are good candidates to use in bioremediation treatments of organopollutants.[3]

P. ostreatus, one of the most-produced species, is cultivated mainly on sawdust. The unavailability of sawdust, and the fact that felling of trees in most regions of the world is prohibited, makes it imperative that other sources

of substrates be utilized for its cultivation. In the tropics and sub-tropics, large volumes of unused lignocellulosic by-products can be found. These by-products are left to rot in the field or are disposed off through burning. Cultivation of mushrooms on these by-products may be one of the solutions to transforming these inedible wastes into accepted edible biomass of high market value.[1]

Previous research has shown great potential for using some lignocellulosic materials as raw material for the production of *P. ostreatus*.[4-11] However, every kind of lignocellulosic substance is likely be used as a substrate for *Pleurotus* sp. Cultivation, the main and co-substrate differ among countries and even regions on the basis of availability and cost.[12] Pleurotus species are widely accepted mushrooms cultivated in Turkey. Although large volumes of by-products are available in Turkey, their use as substrates for mushroom cultivation has not been fully exploited.

This paper reports on the comparative utilization of ten lignocellulosic materials as substrates on the mycelium growth of *P. ostreatus* (Jack. Ex. Fr) Kummer using the plastic bag method.

2 Materials and Methods

The substrates used in this study were agricultural and plant-based industry wastes that are usually burned or left in the field to rot. In order to determine suitable substrates and suitable ratios for the mycelial growth period, various wastes materials and combinations were tested.

Leaves of hazelnut (LH), tilia (LT) and poplar (LP), needles of spruce (NS), rice stalk (RS), wheat straw (WS), sawdust (S), waste paper (WP), grass (G) and bran (B) were used as raw materials for producing *P. ostreatus* mushroom. Wheat straw was obtained from Ankara, one of the leading cities in wheat straw production. Leaves of hazelnut were acquired from villages around Trabzon in the eastern Black Sea Region. Hazelnut leaves were from *Corylus avellana, C. maxima* and *C. colurna* species. Sawdust was the remains of Fagus orientalis timber obtained from a timber mill in the Forest Industry Engineering Department, Karadeniz Technical University. Poplar leaves were obtained from *Populus tremula.* Similarly, spruce needles were obtained from *Picea orientalis.* Tilia leaves were from *Tilia rubra* and *Tilia phylatiphyllos* species. Most of the leaf samples were collected on the campus of Karadeniz Technical University. Rice stalks were obtained from Samsun, in the center of the Black Sea Region. Waste paper was obtained from the Environment Committee of Karadeniz Technical University. All samples were collected within two months prior to the analyses. Mycelium of *P. ostreatus* was obtained from the Faculty of Agriculture of Ankara University. Procedures for growing *P. ostreatus* were based on those of Kurtzman[13] and Stamets & Chilton.[14]

Substrates were cut into 5-6 cm pieces and moistened with water until 70-80 % moisture content levels were attained and then placed in nylon bags of 1 kg (40 x 60 cm). Four replicate nylon bags were used for each substrate medium. The nylon bags were sterilized with direct steam at 65-70°C for 12 h. After cooling the substrates to 20°C, they were inoculated by spreading spawn on the surface of the substrate with a weight percentage of about 4 % of the wet weight of compost. Inoculated blocks were incubated at 25-28°C and illuminated for 12 h/day. After 15 days, the substrates were completely colonized by the mycelium. The blocks were than shocked at 4-5°C for 48 h to stimulate production of fruit bodies. The bags were then incubated at 12-15°C and 80-90 % relative humidity until fruit bodies developed. The room was ventilated with fresh air moving at 2 m/s and illuminated 9-12 h/day until primordia formed. Harvests were started 2 weeks after the first primordia emerged. Ecological conditions during the harvesting period were the same as those maintained during the primordium formation period.[15, 16]

Results were evaluated by analysis of variance (ANOVA) and Duncan test to build up homogeneity groups that show significance among differences at a 95 % level. Substrate types and mixed ratios were prepared for as shown in Table 1.

Table I. Substrate types and mix ratios

Substrate sorts	Ratio mixtures by weight (%)
LT	100
LT + WS	50+50
LT + S	50+50
LT +B	75+25
LT + WP	50+50
LP	100
LP + WS	50+50
LP + S	50+50
LP + B	75+25
LT + WP	50+50
NS	100
NS + WS	50+50
NS + S	50+50
NS + B	75+25
NS + WP	50+50
RS	100
RS + WS	50+50
RS + S	50+50
RS + B	75+25
RS + WP	50+50
S	100
S + WS	50+50
S + G	50+50
S + B	75+25
S + WP	50+50
S + LH	80 + 20
S + LH	50 + 50
S + LH	20 + 80
S + WS + WP	80 + 15 + 5
S + B + WP	80 +15 + 5

3 Results and Discussion

The effects of different substrates and mixture ratios on the mycelial growth period were investigated. Mycelial growth period (days) of *P. ostreatus* cultivated on different substrate combinations and statistical analysis are shown in Table 2.

The shortest period growth of the mycelium were recorded on leaves of tilia + bran (75 % + 25 %) and leaves of poplar + wheat straw (50% + 50%). In these combinations, the mycelium of the fungus totally colonized the substrates within a period of 19 days. Poplar + wheat straw (50 % + 50 %) mixture was also found to be one of the best combinations and showed maximum yield performance and biological efficiencies.[12] However, this did not correspond with yield, indicating that mycelial growth and yield of mushrooms have different requirements.[1] On the other hand, some authors reported that the yield of the mushroom is directly related to the spread of the mycelium within the substrate.[1] From the results, it is seen that the mixtures which involved wheat straw were generally colonized in a shorter period compared to the other combinations. Wheat straw requires a shorter period of fermentation and fewer food supplements.[17] The main function of wheat straw is to provide a reservoir of cellulose, hemicellulose and lignin, which is utilized during the growth of the spawn and

during fructification because it contains 39-51% cellulose, 76% holocellulose and 18% lignin.[18] A variable quantity of nitrogen is also provided.[19] The role of nitrogen in building up the biomass was reported by Rajarathnam & Bano.[20] This is in good agreement with previous studies in which wheat straw was reported to be a good substrate for cultivation of *Pleurotus* species.[21-23]

Table 2. Mycelial growth period (day) and statistical analysis

		Mycelial Growth Period		
Substrates	**Ratio mixtures**	**Average (day)**	**Ss.***	**Hg****
LT	100	25	4.0	abc
LT + WS	50+50	25	4.0	abc
LT + S	50+50	21	5.7	a
LT +B	75+25	19	6.7	a
LT + WP	50+50	26	3.5	abc
LP	100	33	3.5	cde
LP + WS	50+50	19	3.5	a
LP + S	50+50	28	8.1	abc
LP + B	75+25	26	10.5	abc
LT + WP	50+50	23	3.5	ab
NS	100	28	8.1	abc
NS + WS	50+50	23	3.5	ab
NS + S	50+50	32	7.0	bcd
NS + B	75+25	21	0.0	a
NS + WP	50+50	25	7.0	abc
RS	100	Contamination	-	-
RS + WS	50+50	25	4.0	abc
RS + S	50+50	25	4.0	abc
RS + B	75+25	32	7.0	bcd
RS + WP	50+50	21	0.0	a
S	100	40	3.5	e
S + WS	50+50	21	0.0	a
S + G	50+50	32	4.0	bcd
S + B	75+25	37	3.5	de
S + WP	50+50	25	7.0	abc
S + LH	80 + 20	21	0.0	a
S + LH	50 + 50	21	0.0	a
S + LH	20 + 80	28	7.0	ab
S + WS + WP	80 + 15 + 5	28	7.0	ab
S + B + WP	80 + 15 + 5	35	0.0	b

*Standard deviation
**Homogeneous groups; same letters denote insignificant statistical differences (P≤0.05).

The longest period growth of the mycelium was recorded on S (100 %). The mycelia fully colonized the substrate in 40 days. The natural substrates (woods on which *Pleurotus species* grow) are very poor in nitrogen content; nevertheless, the fruit bodies are produced.[20] Thus, the lack of nitrogen may also be factor affecting the overall yield values. Combinations that do not contain any additive materials such as S (100 %), RS (100 %), LP (100 %) resulted in a longer colonization period. Also yield performance and biological efficiencies values decreased in the same variations.[12] These materials are not very appropriate as the sole substrate for cultivation. They should be used as substrate in a mixture with other agricultural or forest wastes. A mushroom

grown on fresh sawdust would develop a thin mycelium, produce low yield and have a long fruiting period, entailing a loss of time. Experiments have revealed that, if sawdust for mushroom culture was composted, microorganisms would help digest and turn the food there into a form available to mushrooms. Moreover, with the addition, where appropriate, of the nutrients that are lacking, any mushroom that can grow on sawdust would give a high yield.[17] In the study, the materials that give the best results with sawdust were waste paper, tilia leaves and hazelnut leaves. However, according to the results, leaves of hazelnut are not very appropriate in ratios higher than 50%.

RS (100%) prepared as sole substrate for cultivation was found to be useless due to the contamination. Ferri.[24] reported on the occurrence and nature of loss in the mushroom yield due to bacterial diseases on *Pleurotus species.* The bacterial disease of *P. ostreatus* has affected many mushroom farms, and the pathogen is known to cause at least the deformation of the fruit bodies. The exact reasons for the occurrence of the disease are not known except, possibly, the use of contaminated equipment or adoption of a poor pasteurization technique.[20] In other studies, successful utilization of rice stalk has been indicated. Obodai et al.[1] reported that among the different lignocellulosic by-products tested as substrates for the cultivation of *P. ostreatus,* composted sawdust and rice straw were found to best support growth of the fungus, with the mycelium fully colonising the substrates at 33 and 28 days, respectively.

As can be seen from Table 2, the mycelium growth period was extended with decreasing ratio of waste paper. Mixtures in which the waste paper ratio is 50% generally produced a shorter mycelium growth period. This situation can be attributed to the wide range of cellulose and hemicellulose in waste paper.[12] Cellulose-rich organic materials were reported to be good substrates for the cultivation of mushrooms.[1]

The substrate is the growth medium that provides physical structure (air and water capacity) and nutrients (micro- and macro elements and carbon source) needed for support mushrooms growth. Some ingredients are added in order to improve the nutrition and structure of the compost. During the lengthy growth period, changes in the chemical composition of the organic matter may take place; thus, a shift in quality and mushroom growth support may occur.[25] Although activators such as animal manures (horse and poultry manure) or chemical materials (ammonium phosphate, ammonium citrate, urea) were not used, mycelium growth performance in this study was found to be generally satisfactory.

References

[1] Obodai M, Cleland-Okine J, Vowotor KA. Comparative study on the growth and yield of *Pleurotus ostreatus* mushroom on different lignocellulosic by-products. J. Ind. Microbiol Biotechnol. 2003, 30:146-149.

[2] Stajic M, Milenkovic I, Brceski I, *et al.* Mycelial growth of edible and medicinal oyster mushroom *Pleurotus ostreatus* (Jack. Fr) Kumm. on selenium-enriched media. Int. J. Med. Mush. 2002, 4:241-244.

[3] Elisashvili V, Chichua D, Kachlishvili E, *et al.* Lignocellulolytic enzyme activity during growth and fruiting of the edible and Medicinal mushroom *Pleurotus ostreatus* (Jacq.Fr.) Kumm. (Agaricomycetideae). Int. J. Med. Mush. 2003, 5:193-198.

[4] Zadrazil F. The ecology and industrial production of *Pleurotus ostreatus, Pleurotus florida, Pleurotus cornucopia,* or *Pleurotus eryngii.* Mush. Sci. 1974, 9:621-652.

[5] Zadrazil F. White-rot fungi and mushrooms grown on cereal straw: Aim of the process, Final products, Scope for the future. Essex UK:Elsevier, 1987, 165pp.

[6] Vissher HR. Experiments with oyster mushroom (Part I). Champignoncultuur, 1984, 28:55- 63.

[7] Vissher HR. Experiments with oyster mushroom (Part II). Champignoncultuur, 1984, 28:125-131.

[8] Pankow W. Outside culture of oyster mushrooms. Champignon, 1984, 276:20-33.

[9] Gapinski M, Ziombra M. The growth of *Pleurotus ostreatus* (Fr. Kumm.) and *Pleurotus florida* (Fr.Kumm.) in relation to temperature and type of substrate. Roczniki Akademii Polniczej -w- Poznaniv. 1988, 15:53-60.

[10] Bostanci S, Yalinkiliç MK. Preliminary studies on biodegradation of wheat straw (*Triticum aestivum* L.) by oyster mushroom (*Pleurotus ostreatus* Jack.) aimed at producing biopulp. TAPPI Pulping Conference, New Orleans. TAPPI Proceedings Book, 1988, 2:239-245.

[11] Zadrazil F, Grabbe K. Edible mushroom. Biotechnology, 1993, 3:145-187.

[12] Yildiz S, Yildiz ÜC, Gezer ED. Some lignocellulosic wastes used as raw material in cultivation of the *Pleurotus ostreatus* culture mushroom. Process Biochem. 2002, 38:301-306.

[13] Kurtzman RH. Metabolism and culture of *Pleurotus,* the oyster mushroom. Taiwan Mushrooms. 31, 979:1-11.

[14] Stamets P, Chilton JS. The mushroom cultivator: a practical guide to growing mushroom at home. Olympia, Washington: Agaricon Press, 1983, 415pp.

[15] Boztok K. Mantar Üretim Teknigi. Ege Üniversitesi, Ziraat Fakültesi. Yayin No 489, Izmir. 1990,168pp.
[16] Koçyigit AE. Türkiye için yeni bir yemeklik mantar türü olan *Pleurotus ostreatus*'un özellikleri ve yetistirme teknigi. Türkiye II. Mantar Kongresi. Yalova Bildiriler Kitabi, 1980, pp34-41.
[17] Anonymous. Growing mushrooms: Oyster mushroom, Jew's ear mushroom, straw mushroom. FAO Publication. Bangkok: Regional Office for Asia and the Pacific. 1983, 75:17-68.
[18] Eroglu H. O_2-NaOH Yöntemiyle bugday (*Triticum aeotivum* L.) saplarindan kagit hamuru elde etme olanaklarinin arastirilmasi (PhD Thesis). Trabzon: Karadeniz Teknik Üniversitesi, 1980, 174pp.
[19] Hayes WA. Nutrition, substrates, and principles of disease control. In: Chang ST, Hayes WA, eds. The Biology and Cultivation of Edible Mushrooms, USA:Academic Press, 1976, pp220-236.
[20] Rajarathnam S, Bano Z. *Pleurotus* mushrooms. Part IB. In vitro and in vivo growth requirements, and world status. Crit Rev Food Sci Nutri. 1988, 26:243-311.
[21] Jandaik CL, Kapoor JN. Studies on the cultivation of *Pleurotus sajor-caju* (Fr.) Sing. Mush. Sci. 1976, 9:667-72.
[22] Bano L, Nagaraja N, Rajarathnam S, *et al.* Cultivation of *Pleurotus* species in village model hut. Indian Food Packer, 1979, 33:19-25.
[23] Delmas J, Mamoun M. *Pleurotus cornucopiae* a mushroom which can now be grown in France. Revue Horticole, 1983, 240:39-46.
[24] Ferri F. Batteriosi in fungi del gen *Pleurotus*,. Mushroom Inf. 1984, 2:47.
[25] ChenY, Chefetz B, Heemst JDH, *et al.* Chemical Nature and Composition of Compost During Mushroom Growth. Compost Sci. Util. 2000, 8:347-360.