

Some lignocellulosic wastes used as raw material in cultivation of the *Pleurotus ostreatus* culture mushroom

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Abstract

Yield values, diameters and numbers of fruit bodies obtained from the cultivation of *Pleurotus ostreatus* mushrooms were determined and the effects of different substrate combinations on productivity were investigated. Wastes of some lignocellulosic materials such as leaves of hazelnut (LH), leaves of tilia (LT) and leaves of European aspen (LEA), wheat straw (WS), sawdust (S) waste paper (WP) were used for producing *P. ostreatus*. The best main material and the best substrate combination for mushroom productivity were WS and WS + WP (50% + 50%), respectively. Mixtures which involve WP generally produced higher yield values when compared to the other combinations. Mixtures which contained bran (25%) increased the risk of contamination. The lowest yield and the smallest fruit body diameters values were obtained from LT (100%) and LEA + S (50% + 50%). The greatest number of fruit body was obtained in the combination WS + LH + WP (30% + 50% + 20%). The largest diameter of fruit body was obtained from OT (100%), even though few fruit bodies were observed. © 2002 Published by Elsevier Science Ltd.

Keywords: *Pleurotus ostreatus*; Wheat straw; Sawdust; Yield; Fruit body

1. Introduction

All over the world and especially in developing countries, there is a problem of shortage of protein. Producing cultured mushrooms can be one suitable solution to this problem. Because of rapid industrialization, the amount of waste materials has increased and utilization of these wastes is very important for government economy and natural balance [1]. The ability of fungi to colonize wood and wood wastes and produce edible reproductive structures has been exploited for centuries in Asia for the production of mushrooms like Shiitake (*Lentinulus edodes*) and the oyster mushroom (*Pleurotus ostreatus*) [2,3]. *P. ostreatus* growing wild in nature, is well known for its culinary taste and flavour [4]. Because of their rich mineral contents and medicinal properties, short life cycle, reproducibility in the recycling of certain agricultural and industrial wastes and low demand on resources and technology, several

species of *Pleurotus* are cultivated commercially in different parts of the world. *P. ostreatus* is a prospective source of valuable food protein, and an organism with the ability to utilize various lignocellulosic materials [5]. In addition, the substrate used, following the harvesting of the mushrooms, is valuable as a fertilizer and a soil conditioner for the growth of plants [6]. Additionally, fermented residues could be used as animal feed after mushroom cultivation [7]. It is possible to provide additional income to people living in the rural areas particularly working on wheat, hazelnut and rice agriculture. The utilization of waste paper for the production of cultured mushroom can solve one of the most important problems in solid waste disposal. This will provide an economical gain and protect the environment while providing a nutritious food source such as mushrooms.

Previous research has shown great potential for using some lignocellulosic materials as raw material for the production of *P. ostreatus* [3,8–11,13–15]. However, every kind of lignocellulosic substances is likely to be used as substrate for *Pleurotus* sp. cultivation, the main and co-substrate differ among countries and even regions on the basis of availability and cost [16,17,19–23].

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This study dealt with the cultivation of *P. ostreatus* (Jack.ex.Fr.) Kummer on some available cheap and readily obtainable forest and agricultural wastes of the Eastern Black Sea Region of Turkey. This region has a suitable climate especially relative humidity and temperature for mushroom cultivation.

The objective of the study was to determine experimentally the effects of some lignocellulosic materials on the productivity of *P. ostreatus* culture mushroom.

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 cultivation of *P. ostreatus*, various wastes materials and combinations were tested.

In Trial 1, leaves of tilia (LT), leaves of European aspen (LEA), spruce needles (N), rice stalk (RC), and sawdust (S) were used as main materials; waste paper (WP), wheat straw (WS), bran (B), sawdust and grass (G) were used as additive materials. In Trial 2, sawdust and wheat straw were used as main materials while leaves of hazelnut (LH), gypsum (GY), grass and waste paper were used as additive materials.

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 Blacksea Region. Hazelnut leaves used were from *Corylus avellana*, *Corylus maxima*, *Corylus colurna* species. Sawdust was the remains of *Fagus orientalis* timber obtained from a timber mill in the Forest Industry Engineering Department, Karadeniz Technical University. Tilia leaves used were from *Tilia rubra* and *Tilia phylatiphyllos* species. European aspen leaves were obtained from *Populus tremula*. Similarly, spruce needles were obtained from *Picea orientalis*. Most of the leaf samples were collected on the campus of Karadeniz Technical University. Rice stalks were obtained from Samsun. Waste paper was obtained from the Environment Committee of Karadeniz Technical University. All samples were collected within the last 2 months prior to the analyses. Mycelium of *P. ostreatus* was obtained from Faculty of Agriculture of Ankara University.

The mushroom growing process were accomplished in the Experimental Mushroom House of the Forest Industry Engineering Department, Karadeniz Technical University in which the temperature, ventilation and relative humidity could be controlled. The following procedures for growing *P. ostreatus* were based on those of Stamets and Chilton, Kurtzman [24,25].

Substrates were cut in to 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 × 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 then 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 maintained the same as for the primordium formation period [26,27].

Mushroom yield (g) was calculated by division of fresh weight of fruit bodies obtained from each one bag to dry weight of 1-kg compost. Biological efficiencies was defined as the percentage ratio of the fresh weight of harvested mushrooms over the dry weight of substrate as explained by Zevakis and Balis study [28].

Results were evaluated by analysis of variance (ANOVA) and Duncan tests to build up homogeneity groups which show significance among differences at a 95% level.

Substrate types and mixed ratios were prepared for as shown in Table 1.

3. Results

The effects of different substrates and mixture ratios on productivity were determined. Morphological properties and yield values of harvested fruit bodies were demonstrated for the first trial in Table 2 and for the second trial in Table 3.

In trial 1, (Table 2), the most suitable combination for high yield was LEA + WP (50% + 50%) followed by LEA + WS (50% + 50%), S + WS (50% + 50%), RS + WP (50% + 50%), LL + WP (50% + 50%), the corresponding biological efficiencies being 82.1, 68.9, 64.3, 59.0 and 52.9%, respectively. RS + WS (50% + 50%), N + WS (50% + 50%), S + G (50% + 50%) and S + WP (50% + 50%) were not significantly different from each other in terms of yield performance. The yield was significantly decreased in S (100%), N + S (50% + 50%), RS + S (50% + 50%), LEA + S (50% + 50%) and LT (100%) combinations with biological efficiencies of 8.6, 7.3, 6.7, 2.7 and 2.7%, respectively.

The greatest number of sporophores was seen in the combinations where the yield was the highest. Similarly,

Table 1
Substrates and mixture ratios for Trial 1 and 2

Trial 1			Trial 2		
Substrates	Ratio mixtures by weight (%)	pH	Substrates	Ratio mixtures by weight (%)	pH
LT	100	6.10	S+LH	80+20	6.40
LT+WS	50+50	6.14	S+LH	50+50	6.86
LT+S	50+50	6.71	S+LH	20+80	6.74
LT+B	75+25	6.53	S+GY	95+5	6.68
LT+WP	50+50	5.76	S+WS+WP	80+15+5	6.83
LEA	100	6.45	LH	100	7.50
LEA+WS	50+50	6.31	WS	100	7.00
LEA+S	50+50	6.89	WS+G	80+20	7.50
LEA+B	75+25	6.73	WS+LH	80+20	7.10
LEA+WP	50+50	5.94	WS+LH	50+50	7.27
N	100	5.87	WS+LH	20+80	7.40
N+WS	50+50	6.02	WS+S	80+20	6.80
N+S	50+50	6.60	WS+S	20+80	6.50
N+B	75+25	6.15	WS+WP	50+50	6.83
N+WP	50+50	5.65	WS+G+LH+WP	40+30+20+10	7.11
RS	100	6.50	WS+LH+WP	30+50+20	6.96
RS+WS	50+50	6.34			
RS+S	50+50	6.91			
RS+B	75+25	6.83			
RS+WP	50+50	5.96			
S	100	6.50			
S+WS	50+50	6.34			
S+G	50+50	6.91			
S+B	75+25	6.71			
S+WP	50+50	5.98			

LT, leaves of tilia; LEA, leaves of European aspen; N, spruce needles; RS, rice stalk; S, sawdust; WP, waste paper; WS, wheat straw; B, bran; G, grass; LH, leaves of hazelnut; GY, gypsum.

Table 2
Morphological properties and yield values of *Pleurotus ostreatus* on selected substrates in Trial 1

Substrates sorts and ratio mixtures by weight (%)	B.E (%)	Yield (%) / 570 g dry substrate			No. fruit bodies			Diameter of fruit bodies (cm)		
		Av ^a	S.D. ^b	Hg ^c	Av	S.D.	Hg	Av	S.D.	Hg
LT (100)	2.7	0.9	0.2	a	4.0	0.9	a	4.0	1.0	a
LT+WS (50+50)	21.8	7.1	2.7	abc	15.5	2.1	bc	5.0	2.9	ab
LT+WP (50+50)	52.9	17.2	2.5	de	25.5	4.9	de	6.0	3.2	b
LEA (100)	12.9	4.2	2.0	ab	12.0	0.0	bc	5.7	1.1	ab
LEA+WS (50+50)	68.9	22.4	4.3	ef	29.0	2.3	e	6.7	1.3	b
LEA+S (50+50)	2.7	0.9	0.1	a	2.0	0.0	a	4.0	0.0	a
LEA+WP (50+50)	82.1	26.7	5.8	f	42.5	3.4	f	5.3	0.8	ab
N+WS (50+50)	45.2	14.7	0.9	cde	22.2	0.5	cde	5.4	0.6	ab
N+S (50+50)	7.3	2.4	0.2	a	2.0	0.0	a	6.0	0.1	b
N+WP (50+50)	40.0	13.0	3.3	bcd	12.7	4.2	bc	4.9	0.0	ab
RS+WS (50+50)	46.4	15.1	2.4	cde	22.0	1.0	cde	6.1	0.1	b
RS+S (50+50)	6.7	2.2	1.0	a	4.0	3.0	a	5.0	0.1	ab
RS+WP (50+50)	59.0	19.2	2.9	def	22.5	2.4	de	5.5	0.2	ab
S (100)	8.6	2.8	0.3	a	2.0	2.0	a	10.0	0.4	c
S+WS (50+50)	64.3	20.9	1.7	def	29.0	3.8	e	5.4	0.1	ab
S+G (50+50)	43.7	14.2	4.9	cde	19.2	0.7	bcd	5.2	0.6	ab
S+WP (50+50)	40.6	13.2	2.3	cd	7.5	3.2	ab	8.8	1.3	c

^a Av, average.

^b S.D., standard deviation.

^c Hg, homogeneous groups; same letters denotes insignificant statistical differences ($P \leq 0.05$).

Table 3

Morphological properties and yield values of *Pleurotus ostreatus* on selected substrates in Trial 2

Substrates sorts and ratio mixtures by weight (%)	B.E (%)	Yield (%) /570 g dry substrate			No. fruit bodies			Diameter of fruit bodies (cm)		
		Av	S.D.	Hg	Av	S.D.	Hg	Av	S.D.	Hg
S+LH(80+20)	73.8	24.0	2.1	cdefg	22.6	1.0	def	7.2	0.6	fgh
S+LH(50+50)	102.1	33.2	2.5	fgh	36.0	4.0	g	7.2	0.3	fgh
S+LH(20+80)	27.0	8.8	1.7	abc	18.0	0.0	bcd	4.3	1.5	ad
S+GY(95+5)	27.7	9.0	0.0	abc	4.0	0.0	b	6.0	0.0	cde
S+WS+WP(80+15+5)	39.3	12.8	2.0	abcd	14.0	3.0	bcd	7.7	0.2	gh
LH(100)	9.2	3.0	0.0	a	8.0	0.0	bc	5.5	0.0	bcd
WS(100)	79.4	25.8	3.5	defgh	28	6.5	efg	8.1	0.9	h
WS+G(80+20)	34.4	11.2	0.0	abcd	16.0	0.0	bcde	6.0	0.0	cde
WS+LH(80+20)	25.2	8.2	1.3	abc	14.0	3.0	bcd	5.5	0.4	bcd
WS+LH(50+50)	35.0	11.4	0.8	abcd	22.0	5.0	def	5.2	0.7	abc
WS+LH(20+80)	27.0	8.8	0.0	abc	20.0	0.0	cdef	4.5	0.0	abd
WS+S(80+20)	46.7	15.2	0.0	abcde	38.0	5.0	g	5.5	0.7	bcd
WS+S(20+80)	16.0	5.2	1.6	ab	14.6	4.1	dc	4.8	0.8	abd
WS+WP(50+50)	121.2	39.4	5.5	h	32.0	7.5	fg	6.0	2.2	cde
WS+G+LH+WP(40+30+20+10)	59.7	19.4	5.8	bcdef	22.6	8.1	def	6.4	1.0	def
WS+LH+WP (30+50+20)	91.7	29.8	1.4	efgh	52.0	8.0	h	6.7	0.7	h

the least number of fruit body were seen in the combinations where the yield was the lowest.

The best results for diameters of fruit body were obtained from the combinations S (100%), S+WP (50%+50%), LEA+WS (50%+50%), RS+WS (50%+50%), N+S (50%+50%) and LT+WP (50%+50%). The other diameters were similar (Table 2).

In Trial 2, (Table 3), maximum yields were obtained from the mixtures WS+WP (50%+50%), S+LH (50%+50%), WS+LH+WP (30%+50%+20%), WS (100%) and S+LH (80%+20%), the corresponding biological efficiencies being 121.2, 102.1, 91.7, 79.4 and 73.8%, respectively. LH (100%), WS+S (20+80) and WS+LH (80%+20%) combinations proved to be poor substrates with remarkably reduced yield potentials. The biological efficiencies of these combinations were 9.2, 16.0 and 25.2%, respectively.

The WS+LH+WP (30%+50%+20%) combination has the highest number of the fruit body followed by WS+S (80%+20%), S+LH (50%+50%) and WS+WP (50%+50%). This order does not accord with the (best) yield results. The least number of sporophores was seen in S+GY (95%+5%) and LH (100%) combinations.

The best results for diameters of fruit bodies were obtained from mixtures WS (100%) followed by S+WS+WP (80%+15%+5%), S+LH (80%+20%), S+LH (50%+50%). The smallest diameters of fruit body were seen in S+LH (20%+80%), WS+LH (20%+80%) and WS+S (20%+80%) combinations. The other diameters were similar.

4. Discussion

The results of Trial 2 were better than those of Trial 1. Wheat straw was found to be the best main material. A total of 79.4% of BE was attained when the wheat straw alone (i.e. 100%) was used. Wheat straw requires a shorter period of fermentation and fewer food supplements [29] because it contains 39–51% cellulose, 76% holocellulose, 18% lignin, 3.5–5% protein, 1.5–2% grease, 0.6% digestible protein, 6–10% ash, 2.64% silica and silicates [30]. This is in good agreement with previous studies in which straw was reported to be a good substrate for cultivation of *Pleurotus* species [31–33]. The best combination of the study for yield was WS+WP (50%+50%; 121.2% BE). This result can be explained by the easy digestion and fast decomposition of wheat straw [29]. The main function of wheat straw is to provide a reservoir of cellulose, hemicellulose and lignin, which is utilized during the growth of 'spawn' and during fructification. A variable quantity of nitrogen is also provided [34]. The role of nitrogen in building up the biomass was reported by Rajarathnam and Bano [35]. The best yields of different *Pleurotus* species on wheat straw and paddy straw have been stated by many researchers and our results are consistent with these findings [3,31,36–39].

The second highest yield value was obtained with the mixture S+LH (50%+50%; 102.1% BE). However, as can be seen in Tables 2 and 3, combinations which contained sawdust such as LEA+S (50%+50%), N+S

(50%+50%), RS+S (50%+50%), S+LH (20%+80%) and WS+S (20%+80%), gave unsatisfactory values for yield. This could be ascribed to the fact that 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 foods that are lacking, any mushroom that can grow on sawdust and produce a high yield [29]. It is also very surprising to note that although the natural substrates (woods on which *Pleurotus* species grow) are very poor in nitrogen content; nevertheless, the fruit bodies are produced [35]. Thus, the lack of nitrogen may also be factor affecting the overall yield.

The yield values decreased with increasing the ratio of leaf of hazelnut and the most suitable ratio for leaf of hazelnut was 50%. Therefore, it can be concluded that leaf of hazelnut is not very appropriate in ratios higher than 50%.

The mixtures which included waste paper generally produced high yields. This increase in yield can be attributed to the wide range of cellulose and hemicellulose in waste paper. The combinations which do not contain any additive materials such as LT (100%), LEA (100%), S (100%) resulted in the lowest yields. These materials are not very appropriate as sole substrate for cultivation. They should be used as substrate in a mixture with other agricultural or forest wastes.

The mixtures which contained bran (25%) increased the risk of bacterial diseases (contamination). Unfortunately, these mixtures were found to be useless in view of the poor yields produced. Ferri [40] reported on the occurrence and nature of loss in mushroom yield due to bacterial diseases on *Pleurotus* species. Bacterial diseases of *P. ostreatus* have 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 [35]. Problems with mycelial growth in the present study are not associated with pasteurization since contamination was seen after mycelium growth had been completed. According to the Kiran et al. [41] wheat bran attracted contaminants especially in the case of sparsely colonized substrates such as sawdust and wood shavings. Yield values of the study confirmed most of the conclusions reported in literature for various *Pleurotus* species on cultivated various substrates [28,42,43].

While the number of fruit bodies increased in WS+LH+WP (30%+50%+20%), in LEA+S (50%+50%), N+S (50%+50%) and S (100%) mixtures these decreased. However, S (100%) substrate showed the

greatest fruit body diameter. The smallest diameters of fruit bodies were seen in LT (100%) and LEA+S (50%+50%). If there is insufficient illumination while the mushroom is developing, the stalk will be long, the cap small and the yield rather low. The results obtained from this study regarding the diameter and number of fruit bodies showed a parallelism with the studies of Pettipher and Demirci [12,44].

The remaining degraded substrate has tremendous potentials for several applications including an up-graded form of ruminant feed, production of biogas, garden manure, or recycling for *Agaricus* cultivation, and production of saccharification enzymes [35]. Lignocellulosic wastes used in the study could be recycled for the cultivation of *Agaricus bisporus* mushroom. Bisht and Harsh proved that the stalk of the *Lantana camara* could be cultivated on waste paper for *P. ostreatus* mushroom and following the pasteurization. They also used the same compost for *Agaricus bisporus* cultivation [45].

Although activator materials (such as ammonium phosphate, ammonium citrate, urea), were not used growth performance in this study was found to be generally satisfactory when compared with the literature.

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