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Full Length Research Paper

Use of local cellulosic wastes for the cultivation of *Pleurotus eryngii*

Mehmet Akyüz[1]* and Abdunnasır Yildiz[2]

[1]Department of Biology, Science Institute, Fırat University, TR 23119- Elazığ, TURKEY

[2]Department of Biology, Faculty of Science and Arts, Dicle University, TR 21280-Diyarbakir, TURKEY.

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This study investigated the possible use of local cellulosic wastes for the cultivation of *Pleurotus eryngii* (DC. ex Fr.) Quel. var. *ferulae* Lanzi and *Pleurotus eryngii* (DC. ex Fr.) Quel. For the propagation of the main culture, 2.0% malt-extract agar was used whereas barley grains were used for the propagation of spawn. For the formation of basidiocarp, wheat straw (WS), soybean straw (SS) and bran of rice (RB) were used as culture media. Three types of compost were prepared: a mixture of WS-SS (1:1), WS and SS. The three compost types were also supplemented with 5.0 and 10.0% of RB. The shortest mycelium growing period was determined as average 8 days on SS and the longest period was 17 days on WS + 10.0% RB. In addition, the shortest mycelium growing period for *P. eryngii* var. *ferulae* was determined as average 12 days on WS + 10.0% RB, while the longest period was 18 days on WS-SS (1:1) + 5.0% RB. The shortest primordium formation period for *P. eryngii* was determined as 36 days on + 10.0% RB, while the longest period was 95 days on WS + 5.0% RB. In addition, for *P. eryngii* var. *ferulae*, there was no basidiocarp formation on any trial after mycelium growth on the compost even after 108 days of culture. The first harvest period for *P. eryngii* was determined as average 48 days on SS + 10.0% RB, and the total harvest period was 108 days on WS + 5.0% and WS + 10.0% RB. The highest biological efficiency (BE) was 93% on WS-SS (1:1) + 5.0% RB and the lowest BE was 7% on WS + 10.0% RB. The lowest average yield per 100 g of material (70% moisture) was 2.0 g on WS + 10.0% RB, while the highest yield was 28.0 g on WS-SS (1:1) + 5.0% RB. In conclusion, various local agricultural wastes can be used for the cultivation of *P. eryngii*.

Key words: Cultivation, *Pleurotus eryngii*, *Pleurotus eryngii* var. *ferulae*, cellulosic wastes, yield.

INTRODUCTION

Pleurotus spp. represents the third largest group of cultivated edible mushrooms in the world, grown on a variety of plant residues, and they have been found to be nutritionally and gastronomically important. They may be cultivated on a large number of substrates, according to local availability in different regions of the world (Cohen et al., 2002). One of the advantages of the *Pleurotus* group is their ability to grow on lignocellulosic substrates without the need for a composting or casing layer. Pro-duction techniques for these basidiomycetes are well developed (Wood and Smith, 1987), and are relatively simple, compared with those used for the most commonly cultivated mushroom, *Agaricus bisporus*. Mushroom cultivation is a simple, low cost and environment friendly technology for the utilisation of rural and agro-industrial residues (Ragunathan et al., 1996). It is required there are numerous past studies indicating the need for the examination of other spesific edible mushroom which is not done in cultivation. More than 2000 species of mush-rooms exist in nature, but only approximately 22 species are intensively cultivated (Manzi et al., 2001). In most countries, there is a well-established consumer accep-tance for cultivated mushrooms such as *A. bisporus*, *Pleurotus* spp., *Lentinula edodes*, *Volvariella volvacea*, *Auricularia* spp. (Diez and Alvarez, 2001). The culture of *P. eryngii* var. *ferulae* is not done or taken in culture as

*Corresponding author. E-mail: mehmetaky210@mynet.com

little absent. The main reason for this is that the mycelium growth of this species is rather slow, more sensitive to pathogens, ecological and other factors such as light, temperature, dampness, CO_2, cultivation methods and tecniques etc., when compared with other culture mushrooms.

Sawa (1996) tested the suitability of 18 wood species for the culture of *P. eryngii* and found that *Cryptomeria japonica* was useful for sawdust-based cultivation. For fruit body formation in *P. eryngii*, *C. japonica*, *Acer mono* Maxim. and *Abies sachalinensis* Masters were suitable but not *Larix kaempferi* (Ohga, 2000). Sawdust and bamboo powder were also found to be potential sources of substrates for the cultivation of *P. eryngii* (Ohga, 1999). Utilization of *Cyperus alternifolius* for *P. eryngii* cultivation is also promising and has potential commercial application in the mushroom industry (Ohga and Royse, 2004). Little research has been done concerning the suitability of various lignocellulosic wastes for the cultivation of *P. eryngii*. Previous work had used wheat, cotton straw and peanut shell (Philippoussis et al., 2001), beech tree (*Fagus crenata Blume*) sawdust and a mixture of rice bran (Bao et al., 2004), *C. japonica* sawdust, corn cob, wheat and rice bran (Obatake et al., 2003), for the culture of *P. eryngii*. The substrates used in each region depend on the locally available agricultural wastes (Cohen et al., 2002). Those study have shown the need to examine other local agricultural wastes as alternative substrates for their cultivation.

The fungi selected for the present study were *P. eryngii* and *P. eryngii* var. *ferulae* which are edible, basidiomycetic and saprophytic, frequently consumed and distributed in the Mediterranean, Central Europe, Central Asia, North Africa (Lewinsohn et al., 1996) and Turkey. *P. eryngii* was found to be growing on the remains of roots of *Eryngium campestre* (Laessoe et al., 1996), but *P. eryngii* var. *ferulae* was found to be growing on the remains of *Ferulae* sp. Its better consistency, pleased aroma and culinary qualities would probably make it preferable to other species of *Pleurotus* (Zadrazil, 1978; Kong, 2004). Demand for the ‘‘king oyster mushroom’’, *P. eryngii*, is increasing rapidly because consumers prize its excellent texture and culinary value. Cultivation of *P. eryngii* on an industrial scale began in Japan in 1995. Current demand for the product indicates that production will continue its rapid growth for the foreseeable future (Ohga and Royse, 2004). This study investigated the possibility of using local cellulosic wastes for the cultivation of *P. eryngii*.

MATERIALS AND METHODS

Inoculum preparation

P. eryngii var. *ferulae* was derived from *in vitro* tissue culture grown naturally in the vicinity of Tunceli-Mazgirt/Turkey and the main culture of *P. eryngii* was obtained from Biology Departmant, Science Faculty, University of Hacettepe, Ankara-Turkey. For the propagation of the main culture, 2.0% Malt-Extract Agar (MEA) was used. MEA plates (90-mm diameter) were inoculated with a mycelium/agar plug (6-mm-diam.) of a young, actively growing margin of the colony. Prior to its use as an inoculum for grain spawn, a mycelium/agar plug was inoculated at the center of the plate and incubated at 25°C in the dark on average for ten days for *P. eryngii* and twenty three days for *P. eryngii* var. *ferulae*.

Spawn preparation

1 kg of barley grain was used in the production of spawn. The grains were cooked for 40 min, washed in flowing water and drained. The grains were supplemented with 2 g lime and 8 g gypsum and mixed manually. A 100 g grain sample was then placed in a 250 mL Erlenmayer flask and sterilized in autoclave at 121ºC for 15 min. After cooling, each flask was inoculated with two agar disks (6 mm diameter) containing the mycelium and incubated at 25ºC in total darkness for two weeks.

Conditions of cultivation

For the formation of basidiocarp, a mixture of wheat-soybean straw (WS-SS), soybean straw (SS) and rice brans (RB) were used as culture medium. Three types of compost were prepared: a mixture of WS-SS (1:1), WS and SS. In addition, wheat straw was used as the control treatment. One kilogram of material from each trial was placed in plastic buckets and kept for 48 h until the compost had reached a humidity of 70-75%. The compost was emptied into plastic bowels. To obtain the desired pH value (5.5-6.5), 35 g of lime and 35 g of gypsum were added to 1 kg compost (Zadrazil, 1978). The three composts were also supplemented with 5.0 and 10.0% of RB (for one kg dry matter). Each compost medium was mixed manually and steriliazed in an autoclave at 121ºC for 15 min. After cooling, the spawn grown on 100 g was used for one kg dried material as inoculation material. 300 g (for *P. eryngii* var. *ferulae*) and 400 g (for *P. eryngii*) of inoculated compost were placed in 1 litre glass jar and 20 x 30 diameter polyethylene bags. The lids of the bags and jars were tied up and taken into incubation room.

Trademark of SGC097.CF X.F Fitotron Sanyo Incubator was used for incubation. Temperature was fixed automatically to be 25ºC, and the rate of damp as 75 ± 10% during the development of mycelium. It has been clearly seen that the light is not necessary for mycelium growth of *Pleurotus* spp., however, it is essential at the phase of formation and progress of basidiocarp (Delmas and Mamoun, 1983). For this reason, the lightning was not made during mycelium growth. After mycelium had invaded medium of compost completely, the lids of bags and jars were opened. Temperature was brought and fixed at 17 ± 1ºC, and damp at 75 ± 10 fixed, during this phase (Obatake et al., 2003; Kong, 2004; Ohga and Royse, 2004). The lightning was provided at 1700 lux light intensity by keeping incubator’s fluorescence lamps open automaticly 12 h a day. The irrigation of culture was ensured by dampening the top of compost and spraying water once or twice a day. Incubator’s automatic system was operated for the culture environment to be scattered, aired and dampened.

Biological efficiency

Biological efficiency (BE) was calculated as the percentage yield of fresh mushroom fruiting bodies in relation to dry weight of the subsrate. BE was calculated because some substrates were denser than others.

$$\text{Biological efficiency \%} = \frac{\text{Weight of fresh mushroom fruiting bodies}}{\text{Weight of dry substrate}} \times 100$$

RESULTS

The time of mycelium growth of *P. eryngii* was determined as average 8-17 days, connected to the type of material that was used and the rate of additive matter (Table 1). The shortest mycelium growing period was determined as average 8 days on SS and the longest was 17 days on WS + 10.0% RB (Table 1). In addition, the shortest mycelium growing period for *P. eryngii* var. *ferulae* was determined as average 12 days on WS + 10.0% RB, while the longest was 18 days on WS-SS (1:1) + 5.0% RB (Table 2).

The shortest primordium formation period for *P. eryngii* was determined as 36 days on SS + 10.0% RB and the longest period was 95 days on WS + 5.0% RB as seen in Table 1. Primordium formation in the first phase was not observed in only one control group for WS-SS, but in two control groups for SS + 5.0% RB (Table 1). Primordium formation in the second phase was not observed in all the control groups of WS + 10.0% RB and SS + 5.0% RB, while it was observed in all the control groups of WS-SS (1:1) + 5.0% RB as seen in Table 1. It is also observed that formation time of primordium in the first phase shortened as RB ratios in WS-SS (1:1) and SS increased (Table 1). In addition, for *P. eryngii* var. *ferulae*, there was no basidiocarp formation on any trial after mycelium growth on the compost even after 108 days of culture (Table 2).

The first harvest period for *P. eryngii* was determined as average 48 days on SS + 10.0% RB, while the total harvest period was determined as average 108 days on WS + 5.0% and WS + 10.0% RB (Table 3). In the first harvest times, increase in RB ratios led to the shortening of time for WS-SS (1:1) and SS, but to the increase in the time for WS as shown in Table 3. When compared with other wastes, the mycelium growing days on WS, WS + 5.0%, WS-SS, WS-SS + 5.0%, WS-SS + 10.0% RB, SS, SS + 5.0% and SS + 10.0% RB were observed to be similar, but changeable on WS + 10.0% RB as seen in Table 1. Primordial initiation days and first harvesting periods were found to be changeable, depending on the biological structure of raw materials used and on the rice brans ratios as shown in Tables 1 and 3.

In the first harvest, the lowest average yield per 100 g of material (70% moisture) was 2.0 g on WS + 10.0% RB, while the highest yield was 10.0 g on SS as shown in Table 4. In the second harvest, the lowest average yield was 6.0 g on WS, WS + 5.0% RB, WS-SS (1:1) + 10.0% RB and SS, whereas the highest yield was 19.0 g on WS-SS (1:1) + 5.0% RB (Table 4). In the total yield, the lowest avarege yield was 2.0 g on WS + 10.0% RB, while the highest yield was 28.0 g on WS-SS (1:1) + 5.0% RB as seen in Table 4. Mushrooms grown on WS-SS (1:1) + 5.0% RB treatment had the highest biological efficiency (93%) and those grown on WS + 10.0% RB treatment had the lowest (7%). These results indicate that WS-SS (1:1) + 5.0% RB can be good materials for cultivation of *P. eryngii*. The total yield was found to be similar and also some value changeable, depending on the biological structure of raw materials used and on the rice brans ratios as shown in Table 4. In the second harvest, yield was not obtained in three control groups of WS + 10.0% RB and SS + 5.0% RB, while it was obtained in every three control groups of WS-SS (1:1) + 5.0% RB (Table 4).

DISCUSSION

In this study, the mycelium growing period of *P. eryngii* and *P. eryngii* var. *ferulae* were determined as average 8-17 days. These results were found to be compatible with those reported in some investigation (Ohga and Roys, 2004), but shorter than the time reported in other investigations (Ohga, 2000; Philippoussis et al., 2001; Obatake et al., 2003; Bao et al., 2004).

In *Pleurotus* spp. the primordial initiation was generally observed on the 22-27 days (Ragunathan et al., 1996), 20-23 days (Yildiz and Karakaplan, 2003) and 24-30 days (Khanna et al., 1992). The shortest primordium formation period for *P. eryngii* was determined as 36 days on SS + 10.0% RB, while the longest primordium formation period was determined as 95 days on WS + 5.0% RB as seen in Table 1. When compared with other *Pleurotus* spp. (Khanna et al., 1992; Ragunathan et al., 1996; Yildiz and Karakaplan, 2003), the primordial initiation days were found to be changeable as seen in Table 1.

In *P. eryngii* the total harvest period was generally observed on the 50-55 days (Bao et al., 2004; Ohga and Royse, 2004), 56-59 days (Obatake et al., 2003), 30-72 days (Philippoussis et al., 2001) and 70 days (Ohga, 2000). These results (48-108 days) were found to be consistent with the time reported clearly in some investigations (Ohga, 2000; Philippoussis et al., 2001; Obatake et al., 2003; Bao et al., 2004; Ohga and Royse, 2004).

In addition, 28.0 g on WS-SS (1:1) + 5.0% RB obtained in study is higher than those reported in the literature (Zadrazil, 1978; Yildiz et al., 1998; Yildiz and Karakaplan, 2003), when comparated to other *Pleurotus* species. In addition, it seems that the mycelium growing days, primordium initiation days, harvesting periods and amounts of yields are changeable to those reported by other researchers (Sawa, 1996; Ohga, 1999; Ohga, 2000; Philippoussis et al., 2001; Obatake et al., 2003; Bao et al., 2004; Ohga and Royse, 2004), which may arise from the biological structure of substrat used for the culture of *P. eryngii*.

Table 1. The effect of local cellulosic wastes and addition material on the growing periods of *P. eryngii* (day).

Materials (1:1)	Growing mycelium (days)												Formation of primordium (first)												Formation of primordium (second)											
	Bran of rice ratio (%)												Bran of rice ratio (%)												Bran of rice ratio (%)											
	0				5				10				0				5				10				0				5				10			
	1	2	3	$\overline{X}$	1	2	3	$\overline{X}$	1	2	3	$\overline{X}$	1	2	3	$\overline{X}$	1	2	3	$\overline{X}$	1	2	3	$\overline{X}$	1	2	3	$\overline{X}$	1	2	3	$\overline{X}$	1	2	3	$\overline{X}$
WS	8	8	10	**9**	10	8	10	**9**	10	19	23	**17**	44	58	57	**53**	60	44	47	**50**	98	77	98	**91**	*	*	74	**74**	*	95	*	**95**	*	*	*	*
WS-SS	10	*	12	**11**	8	10	8	**9**	10	10	10	**10**	57	*	59	**58**	44	50	54	**49**	54	47	44	**48**	86	*	*	**86**	89	85	95	**90**	*	66	93	**80**
SS	8	8	8	**8**	12	8	8	**9**	8	10	8	**9**	39	87	52	**59**	*	*	40	**40**	39	29	39	**36**	89	*	*	**89**	*	*	*	*	*	93	87	**90**

*: Not Obtained, $\overline{X}$: Mean, 1-3: Experimental Groups, WS: Wheat Straw, SS: Soybean Straw, WS**: control group.

Table 2. The effect of local cellulosic wastes and addition material on the growing periods of *P. eryngii* var. *ferulae* (day).

Materials (1:1)	Growing mycelium (days)																		Formation of primordium (first)																	
	Bran of rice ratio (%)																		Bran of rice ratio (%)																	
	0						5						10						0						5						10					
	1	2	3	4	5	$\overline{X}$	1	2	3	4	5	$\overline{X}$	1	2	3	4	5	$\overline{X}$	1	2	3	4	5	$\overline{X}$	1	2	3	4	5	$\overline{X}$	1	2	3	4	5	$\overline{X}$
WS	14	14	8	14	8	**12**	19	14	8	14	15	**14**	14	15	8	15	8	**12**	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
WS-SS	19	14	14	15	14	**15**	17	22	22	14	14	**18**	17	17	20	14	14	**16**	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
SS	14	14	14	15	12	**14**	14	14	15	8	12	**13**	17	14	14	12	8	**13**	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*

*: Not Obtained, $\overline{X}$: Mean, 1-5: Experimental Groups, WS: Wheat Straw, SS: Soybean Straw, WS**: control group.

Mushrooms grown on WS-SS (1:1) + 5.0% RB treatment had the highest biological efficiency (93%). These values are different from those reported by Yildiz et al. (2002), Hernandez et al. (2003) and Mandael et al. (2005), and compared with those reported by Ragunathan and Swaminathan (2003), the values were higher (Table 3). The differences may be due to the genotype of the mushroom and the biological structure of the compost. In our study, BE values varied depending on the biological structure of the raw materials used and on the rice bran ratio.

Previous work had used different local wastes (Sawa, 1996; Ohga, 1999; Ohga, 2000; Philippoussis et al., 2001; Obatake et al., 2003; Bao et al., 2004; Ohga and Royse, 2004) for the culture of *P. eryngii.* The substrates used in each region depend on the locally available agricultural wastes

Table 3. The effect of local cellulosic wastes and addition material on the harvest periods of *P. eryngii* (day).

Materials (1:1)	First harvest												Second harvest											
	Bran of rice ratio (%)												Bran of rice ratio (%)											
	0				5				10				0				5				10			
	1	2	3	$\overline{X}$	1	2	3	$\overline{X}$	1	2	3	$\overline{X}$	1	2	3	$\overline{X}$	1	2	3	$\overline{X}$	1	2	3	$\overline{X}$
WS	53	68	68	**63**	75	57	60	**64**	107	*	108	**108**	*	*	85	**85**	*	108	*	**108**	*	*	*	*
WS-SS	66	*	*	**66**	57	61	65	**61**	65	60	57	**61**	97	*	*	**97**	97	96	108	**100**	*	79	107	**93**
SS	49	98	62	**70**	*	*	57	**57**	53	40	52	**48**	103	*	*	**103**	*	*	*	*	*	108	105	**107**

*: Not Obtained, $\overline{X}$: Mean, 1-3: Experimental Groups, WS: Wheat Straw, SS: Soybean Straw, WS**: control group

Table 4. The effect of local cellulosic wastes on the product yield (g/100 g) and biological efficiency (%) of *P. eryngii*.

Materials (1:1)	First harvest												Second harvest												Total harvest			Biological efficiency (%)		
	Bran of rice ratio (%)												Bran of rice ratio (%)												Bran of rice ratio (%)			Bran of rice ratio (%)		
	0				5				10				0				5				10				0	5	10	0	5	10
	1	2	3	$\overline{X}$	1	2	3	$\overline{X}$	1	2	3	$\overline{X}$	1	2	3	$\overline{X}$	1	2	3	$\overline{X}$	1	2	3	$\overline{X}$	$\overline{X}$	$\overline{X}$	$\overline{X}$	$\overline{X}$	$\overline{X}$	$\overline{X}$
WS	6	3	3	**4**	5	9	7	**7**	1	*	2	**2**	*	*	6	**6**	*	6	*	**6**	*	*	*	*	**10**	**13**	**2**	**33**	**43**	**7**
WS-SS	9	*	*	**9**	10	10	7	**9**	12	4	8	**8**	18	*	*	**18**	19	31	7	**19**	*	9	3	**6**	**27**	**28**	**14**	**90**	**93**	**47**
SS	11	10	9	**10**	*	*	9	**9**	6	4	8	**6**	6	*	*	**6**	*	*	*	*	*	5	13	**9**	**16**	**9**	**15**	**53**	**30**	**50**

*: Not Obtained, $\overline{X}$: Mean, 1-3: Experimental Groups, 100 g of material 70% moisture, WS: Wheat Straw, SS: Soybean Straw, WS**: control group.

(Cohen et al., 2002). The values are diffent from those observed by other researchers (Sawa, 1996; Ohga, 1999; Ohga 1999; Ohga, 2000; Philippoussis et al., 2001; Obatake et al., 2003; Bao et al., 2004; Ohga and Royse, 2004) and may be due to the biological structure of the compost used. Our study shows that a wide range of local agricultural wastes can be used for the cultivation of *P. eryngii*.

In conclusion, mycelium of *P. eryngi* var. *ferulae*, % 2.0 malt-extract agar was used for the propagation of the main culture. Grains of barley were used for the propagation of spawn. However, basidiocarp was not obtained on the materials used in this study. Therefore, we think that we need to carry out further studies to get basidiocarp of this mushroom. Also, more researches need to be done to obtain regulary

and homogeneous product supply. The highest yield in total harvest was obtained as 28 g from WS-SS (1:1) + 5.0% RB for *P. eryngii*. We can suggest the producers that WS-SS (1:1) + 5.0% RB is the most convenient culture medium for *P. eryngii*, which is an edible and valuable mushroom species.

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