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Full Length Research Paper

Evaluation of agricultural wastes for the cultivation of *Pleurotus eryngii* (DC. ex Fr.) Quel. var*. ferulae* Lanzi

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This study investigated the possible use of local agricultural wastes for the cultivation of *Pleurotus eryngii* var. *ferulae*. For the propagation of the main culture, 2.0% malt-extract agar was used whereas wheat grains were used for the propagation of spawn. For the formation of basidiocarp, wheat straw (WS), cotton straw (CS), lentil straw (LS) and rice bran (RB) were used as culture media. Eight types of compost were prepared: a mixture of WS-CS, WS-CS + 10% RB, WS-CS + 20% RB, WS + 10% LS, WS + 20% LS, WS + 10% RB, WS + 20% RB and WS. The shortest mycelium growing period was determined as 9.2 days on WS-CS (1:1) + 20.0% RB and the longest period was 13.0 days on WS + 20.0% RB. The shortest primordium formation period was 97.4 days on WS-CS (1:1), while the longest period was 110.4 days on WS + 20.0% RB. The shortest harvest period was 117.2 days on WS-CS (1:1) + 10.0% RB, and the longest period was 125.8 days on WS + 20% RB. The highest biological efficiency (BE) was 77.2% on WS-CS (1:1) + 20.0% RB, while the lowest BE was 48.6% on WS-CS (1:1). The lowest yield per 100 g of material (70% moisture) was 14.6 g on WS-CS (1:1) and the highest yield was 23.2 g on WS-CS (1:1) + 20.0% RB. In conclusion, various local agricultural wastes can be used for the cultivation of *P. eryngii* var. *ferulae.*

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

INTRODUCTION

More than 2000 species of mushrooms exist in nature, but only approximately 22 species are intensively cultivated (Manzi et al., 2001). In most countries, there is a well-established consumer acceptance for cultivated mushrooms such as *Agaricus bisporus*, *Pleurotus* spp., *Lentinus edodes*, *Volvariella volvacea* and *Auricularia* spp*.* (Diez and Alvarez, 2001). *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. Since *Pleurotus* spp. can utilize many substrates, they may be cultivated on a large number of substrates, according to local availability in different regions of the world (Cohen et al., 2002). Therefore, many agricultural and industrial wastes can be utilized for production of *Pleurotus* spp*.* as a substrate. Mushroom cultivation is a simple, low cost and environmentally friendly technology for the utilisation of rural and agro-industrial residues.

Recently, mushroom culture has moved toward diversification. The culture of the following has been reported; *Agaricus* sp. (Dhar and Gupta, 1998), *Pleurotus* spp. (Ohga, 2000; Philippoussis et al., 2001; Ragunathan and Swaminathan, 2003; Ohga and Royse, 2004; Mandeel et al., 2005), *V. volvacea* (Ma and Buswell, 1998; Philippoussis et al., 2001), *Grifola frondosa* (Kirchhoff, 1996; Royse, 1997), *Chantarella cibarius* (Danell and Camacho, 1997), *Morchella esculenta* (Douxi and Yue, 1998), *Ganoderma lucidum* (Chen, 1998), *A. polytricha* and *Tremella fuciformis* (Pegler, 2001). Perhaps in the near future, it might be possible to cultivate at large scale species such as *Boletus reticulatus* (Yamanaka et al., 2000), *Tricholoma matsutake* (Vaario et al., 2002) and *Tuber melanosporium* (Olivier, 2000). Numerous past studies have indicated the need to examine the cultivation of other edible mushroom, and this is yet to be done for *Pleurotus eryngii* var. *ferulae*.

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The fungi selected for the present study, *P. eryngii* var. *ferulae*, is edible, basidiomycetic and saprophytic. *P. eryngii* grows on the remains of *Eryngiium* sp. (Laessoe et al., 1996), but *P. eryngii* var. *ferulae* grows on *Ferulae* sp. (Guo et al., 2007). It is known that *P. eryngii* was once mistaken for *P. eryngii* var. *ferulae* and known to be compatible with *P. eryngii*, but they are different in appearance. *P. eryngii* is usually collected in Southern Europe, North Africa and Central Asia. It has many synonyms-subspecies, variety and similar taxa such as *P. eryngii* var. *ferulae, Pleurotus nebrodensis, Pleurotus hadamardii, Pleurotus fossulatus* (Kong, 2004). In Turkey and most other countries, cultivation of *P. eryngii* var. *ferulae* is not done. The main reason for this is that the mycelium growth of this variety is rather slow, more sensitive to pathogens, ecological and other factors such as light, temperature, dampness, CO_2 and also requires longer periods for formation of basidiocarp, when compared with other cultured mushrooms. It is determined that the optimal temperature range for fruit body development is relatively low. To find it wild in nature is extremely rare and this scarcity makes it rather precious. Hence, this study investigated the possibility of using local agricultural wastes for the cultivation of *P. eryngii* var. *ferulae*.

MATERIALS AND METHODS

Inoculum preparation

P. eryngii var. *ferulae* was derived from *in vitro* tissue culture grown naturally in the vicinity of İçme-Elazığ, 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 twenty three days.

Spawn preparation

1 kg of wheat 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 (Akyüz and Yildiz, 2007; Akyüz and Yildiz, 2008) 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 diam.) containing mycelium and incubated at 25ºC in total darkness for two weeks.

Conditions of cultivation

For the formation of basidiocarp, wheat straw (WS), cotton straw (CS), lentil straw (LS) and bran of rice (RB) were used as culture media. These local agricultural wastes, rice brans and lentil straw were obtained from Diyarbakir and Elazig, Turkey. Eight types of compost were prepared: a mixture of WS-CS (1:1), WS-CS (1:1) + 10% RB, WS-CS (1:1) + 20% RB, WS + 10% LS, WS + 20% LS, WS + 10% RB, WS + 20% RB and WS. In addition, WS 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 bowls. 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; Akyüz and Yildiz, 2007; Akyüz and Yildiz, 2008). Each compost medium was mixed manually, after which, 500 g of each compost was placed in 2-L glass jar, closed and sterilized in an autoclave at 121ºC for 15 min. After cooling, compost media in the jar were inoculated with 10% spawn per 1 kg of dried material and incubated at 25°C in the dark for 10-15 days. The jars were opened and the colonized substrate in each jar was covered with a 2-cm layer of cooled soil.

The incubation was performed in a room at 13±1°C. One air cooler was used 5 h daily for aeration to avoid the accumulation of CO_2. In order to supply a homogenous condition in the incubation room, a ventilator was used 5 h a day. The culture room was provided with light from fluorescent bulbs with an intensity of 200 lux for 12 h a day (Delmas and Mamoun, 1983). The culture was constantly wet to maintain the required relative humidity (75-90%). The cultures were irrigated by spraying water once or twice a day.

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.

$$BE\ (\%) = \frac{\text{Weight of fresh mushroom fruiting bodies}}{\text{Weight of dry substrate}} \times 100$$

Statistical analysis

The least significant differences among means were determined by Tukey HSD s multiple comparison test at the level of 0.05, with SPSS 12.0 computer programs. After multiple comparisons, the means were interpreted as follows: Tables were followed with diffferent small letters ‘’a-b’’ based on their values and statistical differences. In the case of means followed with the same letter (s), these means were not significantly different from each other. However, means with different letter were significantly different at the level of 0.05.

RESULTS

The time of mycelium growth of *P. eryngii* var. *ferulae* was determined as average 25 days on MEA medium, and the time of spawn growth was 15 days on wheat grain medium. The shortest mycelium growing period was determined as 9.2 days on WS-CS (1:1) + 20.0% RB and the longest period was 13.0 days on WS + 20.0% RB (Table 1).

The shortest primordium formation period was determined as 97.4 days on WS-CS (1:1), while the longest period was 110.4 days on WS + 20.0% RB (Table 1). It was also observed that primordium formation periods in-

Table 1. The effect of local agricultural wastes and addition materials on growing periods (days), yield (g/100 g) and biological efficiency (%) of *Pleurotus eryngii* var. *ferulae*.

Materials	Mycelium Growing Period	Formation of Primordium	Harvest period	Yield (g/100 g)	Biological efficiency (%)
WS**	10.0±0.0^{ab}	102.4±10.9^{a}	118.6±9.2^{a}	15.4±3.8^{a}	51.4±12.6^{a}
WS + 10% RB	11.6±2.3^{ab}	110.0±5.4^{a}	125.0±5.4^{a}	17.6±5.5^{ab}	58.6±18.1^{ab}
WS + 20% RB	13.0±2.7^{a}	110.4±4.9^{a}	125.8±4.9^{a}	17.2±3.1^{ab}	57.4±10.4^{ab}
WS + 10% LS	10.8±1.1^{ab}	110.0±3.8^{a}	125.0±3.8^{a}	16.6±2.1^{ab}	55.4±7.1^{ab}
WS + 20% LS	10.0±0.0^{ab}	103.2±7.9^{a}	118.6±7.2^{a}	16.4±3.4^{ab}	54.8±10.0^{ab}
WS-CS (1:1)	10.4±1.3^{ab}	97.4±14.5^{a}	120.4±14.5^{a}	14.6±3.4^{a}	48.6±11.6^{a}
WS-CS (1:1) + 10% RB	10.8±3.0^{ab}	102.2±5.9^{a}	117.2±5.9^{a}	20.8±2.9^{ab}	69.2±9.6^{ab}
WS-CS (1:1) + 20% RB	9.2±1.1^{b}	105.4±10.2^{a}	120.8±9.4^{a}	23.2±3.2^{b}	77.2±10.7^{b}

Each value is expressed as mean ± SD of five replicate analyses. Value with different small letters in the same column is significantly different at the level of 0.05 ($P<0.05$). Materials used are wheat straw (WS), cotton stalk (CS), rice brans (RB), lentil straw (LS).
**: Control group.

creased as RB ratios on WS and WS-CS (1:1) increased (Table 1). For primordium formation periods, increase in RB ratios led to the increase in the time for WS and WS-CS (1:1) as shown in Table 1. The shortest harvest period was determined as 117.2 days on WS-CS (1:1) + 10.0% RB and the longest period was 125.8 days on WS + 20% RB (Table 1). For harvest periods, increase in RB ratios led to the increase in the time for WS (Table 1).

The lowest yield per 100 g of material (70% moisture) was 14.6 g on WS-CS (1:1), and the highest yield was 23.2 g on WS-CS (1:1) + 20.0% RB (Table 1). For yield, increase in RB ratios led to the increase in yield for WS-CS (Table 1). The highest biological efficiency (BE) was 77.2% on WS-CS (1:1) + 20.0% RB, while the lowest BE was 48.6% on WS-CS (1:1) as seen in Table 1. Increase in RB ratios led to the increase in BE for WS-CS (Table 1).

There were, however, no significant differences in the primordium formation and harvest period for *P. eryngii* var. *ferulae* grown on different agricultural wastes. When compared with other wastes, the mycelium growing, primordium formation, harvest period, yield and BE were found to be similar and changeable statistically, depending on the raw materials used as shown in Table 1.

DISCUSSION

The time of mycelium growth of *P. eryngii* var. *ferulae* was determined as 9.2 - 13.0 days, depending on the type of materials that was used. In *Pleurotus* spp. mycelium growing days were generally observed on the 10 - 15 days (Ohga, 2000; Ragunathan and Swaminathan, 2003; Yildiz and Karakaplan, 2003). Our results (9 - 13 days) were found to be compatible with those reported in other investigations (Ohga, 2000; Ragunathan and Swaminathan, 2003; Yildiz and Karakaplan, 2003).

The shortest primordium formation period was determined as 97.4 days on WS-CS (1:1), while the longest was 110.4 days on WS + 20.0% RB as seen in Table 1. The shortest harvest period was 117.2 days on WS-CS (1:1) + 10.0% RB, and the longest was 125.8 days on WS + 20% RB (Table 1). In *Pleurotus* spp. the primordial initiation days were generally observed on 20 - 30 days (Ragunathan and Swaminathan, 2003; Yildiz and Karakaplan, 2003; Khanna et al., 1992; Ragunathan et al., 1996), and the harvest periods were generally observed on 35 - 70 days (Ohga, 2000; Ragunathan and Swaminathan, 2003; Yildiz and Karakaplan, 2003; Khanna et al., 1992; Ragunathan et al., 1996).

For the cultivation of *Pleurotus* spp., the compost containing 0.6 - 0.9% N (Imbernoon et al., 1983; Laborde, 1987) on dry weight was recommended. The growing periods and yield of mushroom were found to vary according to nitrogen source, variety and dose (Delmas and Mamoun, 1983; Yıldız and Karakaplan, 2003; Rajarathnam and Banu, 1987). Hence, rice bran and lentil straw were used as additive materials in our study.

The yield (g/100 g) and BE (%) of *P. eryngii* var. *ferulae* cultivated under controlled conditions are shown in Table 1. Mushroom cultivation was continued for about 117.2 - 125.8 days, during which one crop was harvested. The lowest yield per 100 g of material (70% moisture) was 14.6 g on WS-CS (1:1), while the highest yield was 23.2 g on WS-CS (1:1) + 20.0% RB as shown in Table 1. Other researchers have reported various values for the yield (Khanna et al., 1992; Ragunathan et al., 1996; Ohga, 2000; Ragunathan and Swaminathan, 2003; Yildiz and Karakaplan, 2003), which may have arisen from the biological structure of substrate used for the culture. The differences may be due to the genotype of the mushroom and biological structure of substrate as stated by Imbernon (1990) and Olivier (1990). The highest BE was determined as 77.2% on WS-CS (1:1) + 20.0% RB, while the lowest BE was determined as 48.2% on WS-CS (1:1)

(Table 1). These values (77.2% on WS-CS (1:1) + 20.0% RB) are different from those reported by Yıldız et al. (2002), Hernandez et al. (2003), Mandeel et al. (2005), and Akyüz and Yildiz (2008), and comparable with those reported by Ragunathan and Swaminathan (2003), Akyüz and Yildiz (2007). BE values varied depending on the biological structure of the raw materials used and on the rice bran and lentil straw ratio as stated by Akyüz and Yildiz (2007) and Akyüz and Yildiz (2008).

Based on our results, casing soil (peat soil) was not necessary in the formation of the fruiting body in *P. eryngii* var. *ferulae*. It is more prone to diseases and more sensitive to growing conditions. Hence, casing soil provides a moisture reservoir for mushroom growth and protection against mushroom diseases. Also, it requires longer periods for formation of basidiocarp, when compared with other culture mushrooms (Delmas and Mamoun, 1983; Imbernoon et al., 1983; Laborde, 1987; Rajarathnam and Banu, 1987; Khanna et al., 1992; Ragunathan et al., 1996; Ohga, 2000; Ragunathan and Swaminathan, 2003; Yıldız and Karakaplan, 2003; Mandeel et al., 2005; Akyüz and Yildiz, 2007; Akyüz and Yildiz, 2008).

Basidiocarp of *P. eryngii* var. *ferulae* was obtained in this study. It was succesfully domesticated in Elazığ-Turkey in 2007 too and determined that the optimal temperature range for fruitbody development is relatively low (13ºC). The highest yield per 100 g of material (70% moisture) was 23.2 g on WS-CS (1:1) + 20.0% RB. We can suggest to producers that WS-CS (1:1) + 20.0% RB is the most suitable culture medium for growing *P. eryngii* var. *ferulae*, which is a relatively new, edible and valuable mushroom species. However, more research needs to be done to obtain regular and homogeneous supply of this mushroom.

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