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Effect of various agro-residues on growing periods, yield and biological efficiency of *Pleurotus eryngii*

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Abstract

This study investigated the possible use of local cellulosic wastes for the cultivation of *P. eryngii*. 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 (W), soybean straw (S), corn stalk (C), bean stalk (B), millet straw (M), cotton stalk (P) and bran of rice (RB) were used as culture media. Six types of compost were prepared, consisting of a mixture of W, W-S (1:1), W-C (1:1), W-B (1:1), W-M (1:1) and W-P (1:1). The six compost types were also supplemented with 10.0 and 20.0% of rice bran (RB). The shortest mycelium growing period was 8.0 days on W-B (1:1) + 10.0% RB and the longest one 12.6 days on W-P (1:1) + 10.0% RB. The shortest period for the formation of primordium was 26.2 days on W and the longest one 44.2 days on W-C (1:1) + 20.0% RB. The shortest harvest period was 37.4 days on W, while the longest one was 54.8 days on W-C (1:1) + 20.0% RB. In the total yield, the lowest yield per 100 g of material (70% moisture) was 14.4 g on W, while the highest one was 25.6 g on W-M (1:1) + 10.0% RB. The highest biological efficiency (BE) was 85.2% on W-M (1:1) + 10.0% RB and the lowest one 48.0% on W. Based on the results, various cellulosic wastes can be used for the cultivation of *P. eryngii*, and also there was a relationship between yield and the materials used for growing mushroom. Therefore, mixed materials may be used in culture of mushrooms. According to data obtained, the main reason for different results is due to the use of different materials in culture medium.

Key words: Agricultural wastes, biodegradation, biological efficiency, cultivation, *P. eryngii.*

Introduction

Cultivation of oyster mushrooms has become a large-scale production in the last thirty years. In the world's mushroom production the oyster mushroom strains are in the third place after the white button mushroom and the shiitake [1]. *Pleurotus eryngii* belongs to the family of oyster mushrooms, which are edible, basidiomycetic and saprophytic and frequently consumed and distributed in the Mediterranean, Central Europe, Central Asia, North Africa [2] and Turkey. This would be a favourable situation for mushroom collectors, but unfortunately the numbers are getting less and less. In contrast to other oyster mushroom strains *P. eryngii* does not live of wood materials but takes its nutrients from organic materials in the soil [3]. It was found to be growing on the root remains of *Eryngium campestre* [4].

Industrial growing of this strain first happened in Hungary at the end of the 1950's on the mixture of composted hay and sawdust[5]. A few years later growing started on partially heat-treated and sterilized agricultural base materials [6]. Cailleux and Diop [7] used wheat straw with added oats on sterilized substrate. If the growing technology of *P. eryngii* will be developed successfully, that can make this strain most demanded out of all *Pleurotus* strains [8]. According to Szili [9], wheat should be prepared by dry heat treatment, addition of a nitrogen source is required and *P. eryngii* should not be grown in bags but in horizontal growing beds, for example in boxes, and covering soil should be used.

In Italy, which is the main mushroom producer in Europe, wheat straw and dry and wet heat treatments are used in substrate manufacturing according to the procedure described by Kószó[10]. As in the procedure used in Hungary, spawned substrate is filled into plastic bags and pressed into blocks. The bags are perforated and the fruit bodies will appear by the holes. The size and amount of perforations have an impact on the yield. The diameter of a perforation is on average 10 mm. The frequencies of the perforations are calculated on that the perforations are 5% of the total block area. Large hole size and too many perforations could result in the substrate drying out too soon. Also a small hole size and not enough perforations could obstruct the spawn run and have an impact on the fruit body formation and also on the size of the fruit bodies.

Some growers sink the blocks 25 cm deep into the soil, cut the foil of the top and cover it with casing material, 2–3 cm thick, used in white mushroom growing. The advantage of the use of casing soil is that it prevents the drying out of the substrate, so it can result in bigger yield than perforated bags or blocks. The reason for this is that the casing can be watered but not the bags and blocks. The disadvantage of this procedure is that soil residues can remain on the surface of the mushroom, and the product may not be favoured on the market. In Hungary [11] successful growing has been reported on sterilized substrate. Two Hungarian strains were used in the experiments. The results show that the yields of these strains were higher than those of any other oyster mushroom strain in cultivation.

Sawa [12] tested the suitability of 18 wood species for the culture of *P. eryngii* and found that *Cryptomeria japonica* was useful for the sawdust-based cultivation. For fruit body formation in *P. eryngii*, *C. japonica*, *Acer pictum* Thunb.ex Murray subsp. *mono* (Maxim.) H. Ohashimono and *Abies sachalinensis* Masters were suitable but not *Larix kaempferi* [13]. Sawdust and bamboo powder were also found to be potential sources of substrates for the cultivation of *P. eryngii* [14]. Utilization of *Cyperus alternifolius* for *P. eryngii* cultivation is also promising and has a potential commercial application in the mushroom industry [15]. Oei [16] reported that on cereal straw with added additives *P. eryngii* strains need to be exposed to a special treatment before fruit body formation. This means for a week the spawn run blocks have to be kept at 5–18°C with a relative humidity of 50–70% and with the use of ambient lighting. After that the bags can be put on shelves. The foil has to be cut off from the top of the blocks.

Akyüz and Yildiz [17] tested the suitability of local agricultural wastes on *P. eryngii* culture and reported that wheat-cotton straw (1:1) + 15.0% rice brans was the most suitable culture medium for growing. Harvest period and yield were dependent on the biological structure of the substrates used and the rice-bran ratio. The differences in the time of mycelium development, the total harvest period, biological efficiency and yield may be due to the biological structure of the substrates used. Based on results, casing soil was not necessary in the formation of the fruiting body in *P. eryngii* and did not have any positive effect on the formation of basidiocarps. There was no relationship between mushroom growth and yield with the use of casing soil. However, there was a relationship between yield and the materials used for growing mushroom.

Report of Gyorfi and Hajdu [1] led to gathering of relevant information about the growing technology of *P. eryngii,* not determined in detail yet, and development of covering technology. New series of experiments with the use of new covering mixtures of different thicknesses were established to clarify the details of the technology. The substrates used were oak chips, sawdust, ground wheat straw and cooked rye. The results suggested that it is worth to cover the blocks of *P. eryngii* with casing because the yield was higher than on the uncovered blocks. The quality of the fruit bodies is also better on covered blocks. The possibility of using local agricultural wastes for the cultivation of *P. eryngii* was studied in the present investigation.

Materials and Methods

Inoculum preparation: The main culture of *Pleurotus eryngii* (DC ex Fr.) Quel. was obtained from Biology Department, 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 diameter) 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.

Spawn preparation: One kilogram 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 and mixed manually. A 120 g sample of grain was then placed in a 250 ml Erlenmayer flask and sterilized in an 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, wheat straw (W), soybean straw (S), corn stalk (C), bean stalk (B), millet straw (M), cotton stalk (P) and rice bran (RB) were used as culture medium. Six types of compost were prepared: W, W-S (1:1), W-C (1:1), W-B (1:1), W-M (1:1) and W-P (1:1). These local cellulosic wastes and rice brans were obtained from the vicinity of Diyarbakir and Elazig,Turkey. 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 reached a humidity of 70-75%. The compost was emptied into plastic bowls.

To obtain the desired pH (5.5-6.5), 35 g of lime and 35 g of gypsum were added to 1 kg of compost [17, 18]. The six types of compost were also supplemented with 10.0 and 20.0% of RB (for 1 kg of dry matter). Each compost medium was mixed manually and sterilized in autoclave at 121ºC for 15 min. After cooling, the spawn grown on 100 g was used for 1 kg dried material as inoculation material. Of inoculated composts 500 g samples were placed in polyethylene bags of 20 cm x 30 cm diameter. The lids of the bags were tied up and taken into incubation room at 25±1°C in the dark for 12 days.

Incubation was done in a room at 13±1°C for formation of basidiokarp. One air cooler was used 5 h daily for aeration to avoid the accumulation of CO_2. The culture room was provided with light from fluorescent bulbs with an intensity of 200 lux for 12 h a day. The culture room was constantly wet to maintain the required relative humidity (75±5%). The cultures were irrigated by spraying water once or twice a day.

Biological efficiency: Biological efficiency (BE) was calculated as percentage yield of fresh mushroom fruiting bodies in relation to dry weight of the substrate. It was necessary to calculate percentage BE because certain substrates were denser than others. Biological efficiency % = (Weight of fresh mushroom fruiting bodies/Weight of dry substrate) x 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. In tables means were followed with different small letters ''a-c'' based on statistical differences. In the case of means followed by 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 ($P<0.05$). Each value is expressed as mean ± SD of five replicates.

Results and Discussion

The effect of some lignocellulosic wastes on the growing periods, biological efficiency and product yield of *P. eryngii* is shown in Table 1. The time of mycelium growth of *P. eryngii* was 8.0-12.6 days, depending on the type of material used and the rate of additive matter. The shortest mycelium growing period was 8.0 days on W-B (1:1) + 10.0% RB and the longest one 12.6 days on W-P (1:1) + 10.0% RB. Mycelium growing days increased as RB ratios in W, W-C (1:1) and W-M (1:1) increased. In the mycelium growing days, increase in RB ratios led to the change of time for W-P (1:1), W-S (1:1) and W-B (1:1) as seen in Table 1. These periods

(8.0-12.6 days) were shorter than in other investigations [13, 15, 17, 19, 21].

In *Pleurotus* spp. the primordial initiation was generally observed on Day 20-30 [22-25]. The time needed for primordial initiation of *P. eryngii* was 26.2-44.2 days, depending on the type of substrate used and the rate of additive matter (Table 1). The shortest primordium formation periods were 26.2 days on W and 27.6 days on W-S (1:1) + 20.0% RB. Length of periods increased as RB ratios in W and W-C (1:1) increased. In the primordium formation periods, increase in RB ratios led to the change of time for W-M (1:1), W-P (1:1) and W-B (1:1). Formation time of primordium was shorter as RB ratios in W-S (1:1) increased. When compared with other *Pleurotus* spp.[22-25] the primordial initiation period was similar.

The shortest harvest period was 37.4 days on W, while the longest period was 54.8 days on W-C (1:1) + 20.0% RB (Table 1). Increase in RB ratios led to the increase of period for W and W-C (1:1). Harvesting periods changed as RB ratios in W-M(1:1), W-P(1:1) and W-B (1:1) increased, and increase in RB ratios led to shorter time for W-S. The total harvest period in literature was between 50 and 70 days [13, 15, 17, 20-21]. These results (50.4 days on W-C (1:1) + 10.0% RB, 51.2 days on W-B + (1:1) 10.0% RB and 54.8 days on W-C (1:1) + 20.0% RB) were consistent with the time reported earlier in some investigations [15, 20-21]. The shortest harvest periods (from 37.4 (W) to 49.8 days (W-M (1:1) + 20.0% RB) were shorter than the time reported in other investigations [13, 15, 20-21] depending on the substrates used and the rice bran ratios.

The lowest yield per 100 g of material (70% moisture) was 14.4 g on W and the highest yields were 24.8 g on W-P (1:1) + 20.0% RB, 25.4 g on W-C (1:1) + 20.0% RB and 25.6 g on W-M (1:1) + 10.0% RB (Table 1). These materials can be good substrate for cultivation of *P. eryngii,* however, when compared with other wastes the yield was similar. Increase in RB ratios led to an increase in yield for W, W-C (1:1) and W-P (1:1). Yield changed as RB ratios in W-M (1:1), W-S (1:1) and W-B (1:1) increased, the yield being dependent on the substrates and the rate of additive matter. The amounts of yields were similar to those reported by other researchers [1, 12-15, 17-21]. The differences among the times of mycelium growth, the total times of harvest and the yield amounts may arise from the C and N amounts, the C/N ratio and the biological structure of substrates used.

For the cultivation of *Pleurotus* spp. the compost containing N 0.6-0.9% [26, 27] on dry weight basis was recommended. Hence, rice bran was used as an additive material in this study. The growing period and yield of mushroom varied according to nitrogen source and dose, C and N amounts, C/N ratio and substrate used. The differences among the values obtained may arise from the genotype of mushroom and biological structure of substrate as stated by Imbernon [28] and Oliver [29].

Biological efficiency of *P. eryngii* cultivated under controlled condition on various lignocellulosic residues using polyethylene bags is presented in Table 1. The cultivation was continued for 37-55 days. It was necessary to calculate percentage BE because certain substrates were denser than others. The conversion percentage from dry substrate weight to fresh mushroom weight (biological efficiency) was determined. The highest BE was 85.2% on W-M (1:1) + 10.0% RB and the lowest one 48.0% on W. Some BE values were different than those reported by other researchers[17, 30-32], and also when compared with other *Pleurotus* spp. [24] the BE of *P. eryngii* was higher (Table 1). The differences between the values may arise from the fact that the strain and culture media used were different.

In previous studies, different locally available agricultural wastes [1, 5-7, 9-21] were used as substrates for the culture of *P. eryngii*. Also mixed materials may be used in culture of mushrooms. Different results were obtained depending on the material in culture medium, structure of the compost and cultivation methods and techniques used for the culture.

Previous results [1, 9-10] showed that casing soil for the formation of *P. eryngii* was required and had a positive effect on the formation of basidiocarps. The results suggest that it is worth to cover the blocks of the *P. eryngii* with casing because the yield is higher than on uncovered blocks. The quality of the fruit bodies is also better on covered blocks [1]. Akyüz and Yildiz [17], Akyüz [18] and this study showed that casing soil was not required for the

Table 1. Growing periods (day), yield (g/100 g) and biological efficiency (%) of *P. eryngii* grown on various agro-residues.

Material	Mycelium growing days	Primordium initiation days	Harvesting periods	Yield (g/100 g)	Biological efficiency (%)
W**	9.0±0.0[ac]	26.2±4.1[a]	37.4±3.6[a]	14.4±3.6[a]	48.0±12.0[a]
W + 10% RB	11.4±0.9[abc]	31.8±2.8[ab]	42.2±2.4[ab]	19.6±7.1[ab]	65.4±23.9[ab]
W + 20% RB	12.4±1.3[ab]	34.0±4.5[ab]	44.2±4.6[ab]	22.0±3.3[ab]	73.2±11.2[ab]
W-C (1:1)	9.0±0.0[ac]	30.0±8.9[ab]	40.8±8.5[ab]	19.2±2.1[ab]	64.0±7.4[ab]
W-C (1:1)+10% RB	10.6±0.9[abc]	40.0±2.5[ab]	50.4±2.8[b]	22.2±4.5[ab]	74.0±14.9[ab]
W-C (1:1)+20% RB	11.2±1.8[abc]	44.2±7.7[b]	54.8±7.2[ab]	25.4±2.4[b]	84.6±8.1[b]
W-M (1:1)	9.4±0.9[abc]	38.8±8.2[ab]	49.6±8.3[ab]	22.0±1.6[ab]	73.4±5.2[ab]
W-M (1:1)+10% RB	9.4±0.9[abc]	37.4±8.4[ab]	48.0±8.2[ab]	25.6±2.9[b]	85.2±9.8[b]
W-M (1:1)+20% RB	10.8±1.1[abc]	39.4±9.6[ab]	49.8±9.1[ab]	20.8±2.7[ab]	69.4±8.9[ab]
W-P (1:1)	10.2±2.7[abc]	34.2±5.8[ab]	45.4±6.2[ab]	18.4±5.6[ab]	61.4±18.4[ab]
W-P (1:1)+10% RB	12.6±3.3[b]	31.6±4.9[ab]	41.8±4.6[ab]	20.8±3.1[ab]	69.4±10.5[ab]
W-P (1:1)+20% RB	11.8±3.0[ab]	32.4±5.7[ab]	42.4±5.6[ab]	24.8±4.8[b]	82.6±16.1[b]
W-S (1:1)	11.2±0.4[abc]	37.6±5.9[ab]	48.2±6.3[ab]	17.6±4.0[ab]	58.6±13.4[ab]
W-S (1:1)+10% RB	12.0±2.2[ab]	37.0±11.5[ab]	46.0±14.3[ab]	20.6±3.5[ab]	68.4±11.8[ab]
W-S (1:1)+20% RB	11.0±0.0[abc]	27.6±7.0[a]	38.4±7.4[a]	19.0±3.7[ab]	63.2±12.3[ab]
W-B (1:1)	8.2±0.4[c]	38.0±3.0[ab]	47.2±4.0[ab]	21.2±7.9[ab]	70.6±26.7[ab]
W-B (1:1)+10% RB	8.0±0.0[c]	41.2±4.5[ab]	51.2±4.5[ab]	23.0±6.3[ab]	76.8±20.8[ab]
W-B (1:1)+20% RB	9.2±1.3[abc]	38.2±4.0[ab]	48.6±3.5[ab]	19.6±1.5[ab]	65.2±5.0[ab]

W: Wheat straw, RB: Rice bran, C: Corn stalk, M: Millet straw, S: Soybean straw, B: Bean straw, P: Cotton stalk. Each value is expressed as mean ± SD of five replicates. Values with different small letters in the same column are significantly different at the level of 0.05 ($P<0.05$), **: control medium

formation of basidiocarps of *P. eryngii*. It is more prone to diseases and more sensitive to growing conditions. Hence, casing of soil provides a moisture reservoir for mushroom growth and protection against mushroom diseases, such as *Penicillium* sp., *Aspergillus* sp., *Rhizopus stolonifer* etc. The highest yield (25.6 g/100 g) was obtained on W-M (1:1) + 10.0% RB, and therefore this is the most convenient culture medium for *P. eryngii*, which is relatively new, edible and valuable mushroom species.

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