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Determining the Effects of Container Types on Yield and Fruitbody Features of *Pleurotus eryngii* Strains

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ABSTRACT

The aim of this study was to determinate which of the substrate container is more suitable for yield and mushroom quality in cultivation of *Pleurotus eyngii* strains (M-18, K-16 and K-20) cultivation. For this purpose, three different substrate containers (polypropylene bags, trays, bottles) were compared with each other for all of these strains and determined spawn running time (day), yield (g/kg), biological efficiency (BE%), average weight of fruitbody(g), pileus diameter (mm), stipe length (mm) and width (mm). The experiment was carried out in complete randomized plot design with ten replications. M-18 strain was found to be the most productive strain in terms of yield, BE and mushroom quality. The shortest spawn running time was obtained from cultivation on trays, while the yield, BE and average mushroom weight were higher on the bag system for all of strains. K-16 and K-20 strains didn't produce fruitbody on the bottles and the 29.9% decrease in yield of M-18 strain was observed on the same container. The results revealed that polypropylene bags was most superior containers in cultivation of M-18, K-16 and K-20 strains.

Key words: Mushroom substrate containers, Polypropylene bags, Bottle system, Tray system, Mushroom yield, Mushroom size

INTRODUCTION

Pleurotus eryngii is belong to the *Pleurotaceae* family, a member of the *Pleurotus* genus. *P. eryngii* varieties can be found in pastures, meadows, gardens and seldom in grassy forest clearings and hilly areas (Szarvas et al, 2011). They grow as facultative parasitic and associated with numerous species of the *Apiaceae* (*Umbelliferae*) family (Lewinsohn et al., 2002; Rodriguez Estrada, 2008). Many strains of *P. eryngii* are available in the world. These species mainly occur in the subtropics region of the Mediterranean Sea, but they are present also in Central Europe, Russia, Ukraine, Central Asia and Iran (Szarvas et al., 2011). *P. eryngii* has also a considerable distribution in Turkey.

P. eryngii is commonly known as king oyster mushroom is considered to be one of the most commercially important species in the *Pleurotus* genus due to its excellent taste, flavor and longer shelf life. Production of the species started in the middle of the 1970's (Chang, 2005) and at the present time, Japan, China, South Korea and Italy are the major producers of this mushroom.

P. eryngii can be cultivated using different agrowastes such wheat straw, cotton straw, millet straw (Akyuz and Yildiz, 2007), sugarcane bagasse (Okano et al., 2007), soybean straw, cornstalk, rice bran (Kırbağ and Akyüz, 2008), sawdust, rice straw (Moonmoon et al., 2010), date-palm fiber (Owaid et al., 2016), *Ferula communis* plant waste (Kibar, 2016) and some their combinations. Various production systems have been reported for growing oyster mushrooms by Royse (2002), Bao et al. (2004), Rodriguez Estrada and Royse (2005) . Generally, cultivation is carried out on blocks, in bottles or in plastic bags. Visscher (1989), reported that different strains of king oyster mushroom response differently to different substrates and

cultivation conditions in mycelial growing, average yield and mushroom quality. Smith (1980) reported also that substrate containers have a positive influence on mushroom production. So, determination of the available containers will provide an advantage for cultivation of *P. eryngii* strains.

Although wild mushrooms are collected and sold fresh in the local markets, *P. eryngii* has not been widely cultivated commercially in Turkey yet. Determination of cultivation methods is required to increase production of this mushroom in the country. There are some studies about the using of different agro-wastes for the production of *P. eryngii*, but information on cultivation methods is lacking in Turkey. However, to get knowledge about cultivation attributions of these strains and define specific cultivation technology for the strains is necessary to enhance the biological efficiency by cultivation.

This research aims to determinate effects of types of bags, bottles and trays on yield and quality characters of some *P. eryngii* strains. For that purpose, 3 strains of *P. eryngii* collected from different locations of Turkey, namely K-16, K-20 and M18 were cultivated by using these container systems and the effects of container types on the mycelial growth, BE and mushroom quality were investigated.

MATERIALS AND METHODS

Experimental materials

Agricultural wastes were obtained from local markets (Kırşehir, Turkey). Three strains of *P. eryngii* (K-16, K-20, M-18) were provided from Atatürk Horticultural Central Research Institue in Yalova. The cultures were maintained in potato dextrose agar (PDA) medium and stored in a refrigerator at 4 ºC. The container type and sizes used in this investigation are given in Table 1. This study was conducted at the Mushroom Production Unit of Agriculture Faculty of Ahi Evran University in Kırşehir, Turkey.

Table 1. Container type and sizes used in the study

Container type	Container size	Substrate quantity (g)
Polypropylene bags	25×45 cm	1000
Bottles	1000 ml	1000
Trays	30x20x 6 cm	1000

Experimental design

Three different containers were tested for the cultivation of *P. eryngii* strains. Poplar sawdust (80%) was used as a base medium, and cotton seed hulls was added to this mixture at ratios of 20% to prepare the growing media. The experiment was conducted in a complete randomized plot design, with ten replications.

Experimental procedures

Spawn production

Spawn was prepared on wheat grains as described by Pieckenstain et al. (1999). Briefly, wheat grains was boiled and then glass bottles were filled with boiled wheat grains and 1% w/w $CaCO_3$. Afterwards, the bottles were sterilized for 1.5 h at 121 °C, cooled and inoculated with an agar plug (1 cm diam.) cut from the advancing margin of a 5 day old colony grown on PDA (potato dextrose agar). After inoculation, the bottles were incubated in the dark, at 25 °C until the completion of mycelial growth.

Preparation of cultivation media

Poplar sawdust and cotton seed hulls were thoroughly mixed, and distilled water was added to mixture until it was moistened to 70%. Then, 1 kg (wet weight) of each substrate was packed into a polypropylene autoclavable bags, bottles and trays. The

bags plugged with a cotton plug, bottle and trays were covered with a polypropylene sheet. Ten replicates were performed for each container type. The containers containing substrate were sterilized in an autoclave at 121 °C for 90 min and, after cooling, inoculated in a laminar flow chamber using 3% grain spawn (w/w wet weight basis).

Mushroom cultivation

Inoculated bags, bottles and trays were incubated at 25±2 ^{o}C with 80% relative humidity in the presence of light to be colonized by the mycelium. After full colonization, bags, bottles and trays were transferred to a production room at 15±2 ^{o}C with a humidity of 80–90% in order to induce fructification. The cotton plugs and polypropylene sheets were removed. Cool white fluorescent bulbs provided 8 h of light daily. Sufficient air changes were maintained.

Total mushroom yield (g kg^{-1} substrate) was obtained from one flush in a harvest period. Mushrooms were harvested as soon as the fruiting bodies developed and attained their full size above the substrate with a sharp knife from each treatment bag. The following data were recorded; weight of fruitbodies (g), mushroom pileus diameter (mm) and stipe width (mm), stipe length (mm), total mushroom yield (g/kg) and biological efficiency (BE%). The biological efficiency percentage (BE) was calculated as follows: ([weight of fresh mushrooms harvested/substrate dry matter content] × 100).

Statistical analysis

The data obtained from the experiment were subjected to variance analysis and the statistical significance was compared employing Duncan's multiple range test, using the SPSS 16.0 for Windows statistical computer program at a significance level of 5%.

RESULTS AND DISCUSSION

Spawn running time, yield and biological-efficiency of P. *eryngii* strains

Results show that there were significant differences on the strain, containers and their interactions on spawn running time, mushroom yield and BE ($p<0.01$). The time required for complete colonization on the different containers are given in Table 2. Among the different containers used in the present study, trays were found to be significantly superior for all strains in terms of spawn running time. The shortest spawn running time was obtained from cultivation on trays and the longest spawn running time was obtained from bottles for all strains. Correspondingly, large surface of trays may speed up the mycelial growth of *P. eryngii* strains. The opposite case was observed in the bottles (Figure 1.)

Figure 1. Mycelial growth of K-20 strain on 12th day

Mycelial growth was very slow at the bottom of the bottle and in general was weak. In *P. eryngii* mycelium growing durations were generally observed on the 10- 15 days (Ohga, 2000; Ragunathan and Swaminathan, 2003; Yildiz and Karakaplan,2003).

Table 2. Effect of different containers spawn running time, yield and BE of *Pleurotus eryngii* strains

Strain	Container	Spawn running time (day)	Yield (g/kg)	BE (%)
K-16	Bag	15.40^{b**}	175.87^{a**}	58.62^{a**}
	Tray	12.00^{c}	107.80^{b}	35.93^{b}
	Bottle	40.20^{a}	0.00^{c}	0.00^{c}
Mean		22.5^{b}	94.6^{b}	31.5^{b}
K-20	Bag	13.40^{b**}	124.16^{a**}	41.39^{a**}
	Tray	11.00^{c}	117.80^{a}	39.27^{b}
	Bottle	39.00^{a}	0.00^{b}	0.00^{c}
Mean		21.1^{c}	80.7^{c}	26.9^{c}
M-18	Bag	16.60^{b**}	237.07^{a**}	79.02^{a**}
	Tray	13.60^{c}	101.80^{c}	33.93^{c}
	Bottle	43.20^{a}	166.20^{b}	55.40^{b}
Mean		24.5^{a}	168.4^{a}	56.1^{a}
Strain		**	**	**
Container		**	**	**
Strain x container		*	**	**

ns=$P>$ 0.05, *P <0.05, **P <0.01; Mean values in the same column followed by the same letters are not significantly different by Duncan's multiple range test.

Compared to previous findings, the spawn running time of *P. eryngii* strains cultivated on bottles is longer in our study. Mycelial growth reduction in bottles may be indirectly attributed to the possibility of excess heat development. Senthilmurugan and Krishnamoorthy (2015) reported that self heating of the container will inhibit with spawn running and primordial initiation. Completely colonization on the bottles was prolonged to 43.2 days in M-18 strain, whereas colonisation by mycelium of M-18 strain cultivated on tray was completed by day 13.6. In polypropylene bags, relative to trays complete colonization was delayed by 3 days. K-20 strain shows the shortest time, the strains needed to complete the colonization on the containers. Spawn running time was recorded as 11 days, 13.4 days and 39 days on trays, polypropylene bags and bottle, respectively for this strain. The spawn running time in K-16 strain took longer than in K-20, but it was shorter than M-18 strain. In K-16 strain, colonization was completed on tray, polypropylene bags and bottles respectively by days 12.0, 15.4 and 40.2.

Although two flushes of mushrooms were harvested from polypropylene bags treatments, one flush was harvested on tray and bottles. The primordia were deformed and did not form fruiting bodies during the second flush on trays. Among the different containers used in the present study, polypropylene bags were found to be significantly superior. Sivaprakasam et al.(1987) concluded that polybag containers would prevent evaporation and maintain the required levels of carbon dioxide inside the beds during spawn run and fruiting.

The yield was significantly influenced by mushroom strain. The highest mushroom yields (168.4 g/kg) and BE (56.1%) were produced by M-18 strain (Figure 2). There were significant differences in the case of yield and BE (%) between M-18, K-16 and K-20. Peng et al. (2000) also reported a biological efficiency of *P. eryngii* strains may be different. Yields (237.07 g/kg) and BE (79.02%) were highest in the interaction between M-18 strain and polypropylene bags. The biological yields of K-16 and K-20 and were recorded 175.87 g/kg, 124.16 g/kg on the same container.

Figure 2.Fruiting bodies of *M-18 strain* grown on different containers.

This result is similar to the findings of Kirbag and Akyuz (2008) who found the biological yield of king oyster mushroom to vary between 14.4 g and 19 g mushroom from 100 g of different substrates. BE (%) was accounted for 58.62%, 41.39% and 79.02% BE for K-16, K-20 and M-18 respectively, when they were grown wihin polypropylene bags. Moonmoon et al (2010) found that biological efficiency of different strains of *P. eryngii* was changed between 46.75 -73.5% on rice straw and sawdust. Our findings are similar with them.

Although, Senthilmurugan and Krishnamoorthy (2015) suggested that polypropylene bottles for cultivation of *P. oeus, P. florida* and *P. platysus*, there was a degree of elongation in time and reduction in yield with using of bottles on cultivation of *P. eryngii* strains. The statistically significant deleterious effects on colonisation time and no crop were recovered and that were noted when bottle was used in cultivation of K-16 and K-20 strains, and the 29.9% decrease in yield in cultivation of M-18 strain. Although K-16 and K-20 strains produced pinheads at the bottom of bottles, these strains didn't produce fruitbody on the bottles (Figure 3).

Figure 3. Pinheads of K-16 and K-20 strains grown on the bottles

Tray containers used in the experiment was displayed lower yield than polypropylene bags. It could be due to entrance of pathogens and excessive loss of moisture from the large bed surface of the tray. The container must not allow the excessive loss of moisture from the large bed surface. During cultivation on trays, approximately 80% of trays were found to be contaminated by green mould

Average mushroom weight, pileus diameter, stipe length and width

The average mushroom weight, pileus diameter, stipe length and width of the fruiting bodies were affected by the different strains and containers as seen in Table 3. The mean fruitbody weight ranged from 32.3 g to 54.7 g.

The highest average weight 54.7 g was recorded from M-18 strain grown on polypropylene bags and the lowest average weight 32.3 and 32.7 g was determined from K-16 and K-20 strains on the trays, respectively. Hassan et al. (2010) reported that average weight of *P. eryngii* grown on different substrates between 20-70 g. Our findings are confirmed in this study. Quality properties of oyster mushroom had been related with the fruit body size such as determination of pileus (cap) and stipe (Owaid et al., 2015). The characters of produced oyster mushroom caps on these containers were shown in Table 3.

Table 3. Effect of different containers on average mushroom weight, pileus diameter and stipe length and width of *Pleurotus eryngii strains*

	Container	Weight of fruitbody (g)	Pileus diameter (mm)	Stipe lenght (mm)	Stipe width (mm)
K-16	Bag	43.6^{**a}	85.3^{**a}	31.3^{**a}	16.3^{**a}
	Tray	32.3^{b}	44.2^{b}	17.4^{b}	11.0^{b}
	Bottle	0.00^{c}	0.00^{c}	0.00^{c}	0.00^{c}
Mean		25.3^{b}	43.2^{b}	16.3^{b}	9.1^{b}
K-20	Bag	46.2^{**a}	70.9^{**a}	38.6^{**a}	18.6^{**a}
	Tray	32.7^{b}	31.5^{b}	16.7^{b}	11.^{b}
	Bottle	0.00^{c}	0.00^{c}	0.00^{c}	0.00^{c}
Mean		26.3^{b}	34.1^{c}	18.5^{b}	9.9^{b}
M-18	Bag	54.7^{**a}	101.8^{ns}	32.5^{ns}	22.8^{ns}
	Tray	43.7^{c}	83.78	34.7	25.4
	Bottle	48.5^{b}	91.7	32.5	20.3
Mean		49.0^{b}	92.4^{a}	33.2^{a}	22.8^{a}
Strain		**	**	**	**
Container		**	**	**	**
Strain x container		**	**	**	**

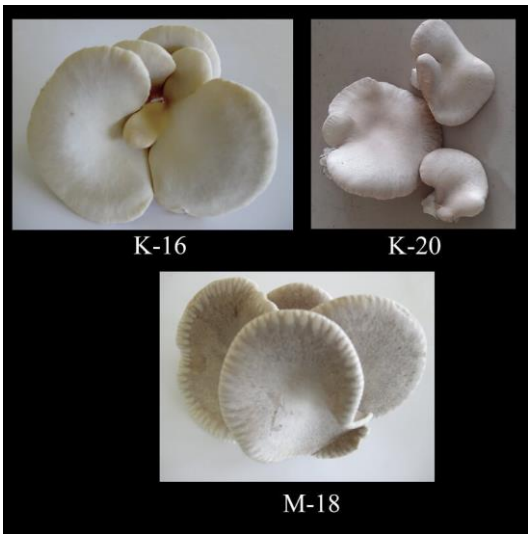
ns= *P*> 0.05, **P* <0.05, ***P* <0.01; Mean values in the same column followed by the same letters are not significantly different by Duncan's multiple range test

The average of pileus diameter was better on polypropylene bags than tray and bottles on all strains, which reach to 101.8 mm, 70.9 mm and 7.1 cm 85.3 mm on M-18, K-20 and K-16 strains, respectively (Figure 4). The highest stipe width (2.54 cm) occurred in M-18 produced on trays. Our results are corroborated by the findings of Moonmoon et al. (2010) who reported that length of stalk different *P. eryngii* strains between 2.7 and 3.2 cm.

The lowest stem length was determined 1.67 in K-20 grown in trays, significantly (P < 0.01), while the longest stems were obtained 3.86 cm in K-20 grown in the polypropylene bags.

Stipe length was lower in K-16, K-20 and M-18 strains when compared with previous studies (Moonmoon et al., 2010). Sizes of pileus and stipe of K-16 were not significantly (p>0.05) different from K-20 strain.

Figure 4. Fruiting bodies of *Pleurotus eryngii* strains



The means of the diameter of pileus of M-18 was higher in polypropylene bags, while stipe length and width were increased with tray system. On the other hand, pileus diameter, stipe length and width were higher in K-16 and K-20 strains grown on polypropylene bags than trays. According

to results in Tables 2 and 3, there is a positive correlations between biological efficiency and size of fruitbody. Beyer and Muthersbaugh, (1996) reported that biological efficiency depends on the yield size.

CONCLUSIONS

Many producers use different containers for the production of *P. eryngii* in different countries. Based on the results, it is concluded that polypropylene bags were determined the best substrate containers for M-18, K-16 and K-20 strains. It was observed that polypropylene bags, trays and bottles can be used successfully for M-18 strain, but bottle system was not recommended for the production of K-16 and K-20 strains. Although, the spawn run and primordial initiation were very quick on trays, the total mushroom yield and BE were substantially reduced. Casing soil was not necessary for the formation of the fruiting body in M-18, K-16 and K-20 strains, but casing soil may be increased mushroom yield and protection against mushroom diseases. However, more research were needs to obtain certain results.

We can suggest to producers that M-18 strain is the most productive strain in terms of yield, BE and mushroom quality. But it should be researched to determinate most suitable substrates.

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