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Yield and Fruit Body Properties of *Pleurotus eryngii* Isolates Grown on Poplar Sawdust Supplemented with Different Additive Materials

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Abstract: The aim of this study was to investigate the possibility of using sunflower meal (SFM), grape pomace (GP) and green walnut husk (GWH) as new additive materials for substrate preparation in cultivation of *Pleurotus eryngii* (DC. ex Fr.) isolates collected from different regions of Turkey. In addition, effect of these agro-wastes on fruit body size of *P. eryngii* isolates was also determined in the study. Poplar sawdust (S) was used as a base medium and additive materials were added to sawdust in a ratio of 8:2 to prepare the substrates. It has been noticed that different additive materials affects on spawn run time (day), time of first harvest (day), total yield (g/kg), biological efficiency (BE), average of mushroom weight (g), mushroom pileus diameter (mm) and stipe width (mm), stipe length (mm) of *P. eryngii* isolates. Yield parameters and fruit body size were greatly affected by the substrates and isolates. S:SFM showed higher yield, biological efficiency and mushroom weight average than other substrates in K-16 and M-18 isolates, whereas these parameters were height in K-20 isolate grown on S:GP. The results revealed that all the three *P. eryngii* isolates can grow on poplar substrate with the supplement of any kind of the tested agricultural by-products. Although, an increase in spawn running time and cultivation period length of *P. eryngii* isolates was observed for S:GWH compared with the other substrates, utilization of green walnut husks for *Pleurotus eryngii* cultivation is promising and has potential commercial application in the mushroom industry.

Key words: grape pomace, green walnut husk, king oyster mushroom, sunflower meal

Farklı Katkı Maddeleri ile Takviye Edilmiş Kavak Talaşında Yetiştirilen *Pleurotus eryngii* İzolatlarının Verim ve Şapka Özellikleri

Öz: Çalışmanın amacı, Türkiye'nin farklı bölgelerinden izole edilen *Pleurotus eryngii* izolatlarının üretiminde, ayçiçeği küspesi (AK), üzüm posası (ÜP) ve yeşil ceviz kabuğu (YCK) gibi farklı katkı materyallerinin kullanım olanaklarının araştırılmasıdır. Yetiştirme ortamı hazırlığında, kavak talaşı ana materyal olarak kullanılır iken, bahsedilen katkı materyalleri yetiştirme ortamına 8:2 oranında eklenmiştir. Çalışma çerçevesinde, farklı katkı materyalleri kullanılarak hazırlanan bu yetiştirme ortamlarında üretilen *P. eryngii* izolatlarının misel gelişim süresi (gün), ilk hasada kadar geçen süre (gün), toplam verim (g/kg), biyolojik etkinlik (BE) ve bazı şapka özellikleri (şapka boyutları ve şapka rengi) belirlenmiştir. Sonuçlar, üretim parametreleri ve şapka özelliklerinin yetiştirme ortamı ve izolatlardan önemli oranda etkilendiğini ve çalışmada test edilen üç katkı materyalinin her üç *P. eryngii* izolatının üretiminde kullanılabileceğini ortaya koymuştur. T:AK ortamında yetişen K-16 ve M-18 izolatları daha yüksek verim, BE ve şapka iriliği sergiler iken, K-20 izolatında ise T:ÜP ortamında daha yüksek değerler elde edilmiştir. Diğer ortamlara kıyasla T:YCK ortamında yetişen *P. eryngii* izolatlarının üretim sürelerinde bir artış görülmesine rağmen, *P. eryngii* yetiştiriciliğinde katkı materyali olarak yeşil ceviz kabuğu kullanımının ticari olarak uygulama potansiyeline sahip olduğu gözlenmiştir.

Anahtar kelimeler: üzüm posası; yeşil ceviz kabuğu; kral istiridye mantarı; ayçiçeği atıkları

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Introduction

Lignocellulosic wastes include byproducts of agro and forest industry. Every year, thousands of tonnes of agricultural waste from industrial crops such as cereal grains, legume wastes and sugar beet, sunflower occur in the world. The destruction of these residues can be difficult due to their chemical and degradation properties. These residues are used as animal feed, and some of them are burned or mixed with soil. Mushroom production is one of the rare methods that can be used to evaluate these agricultural wastes. By the cultivation of mushroom, while these wastes are removed, a protein-rich nutrient is also obtained such as mushroom (Chang and Miles, 2004). In general, *Pleurotus* spp. is used as a natural food and drug at the same time (Fu et al., 2016; Majeed et al., 2017).

Pleurotus eryngii, commonly known as king oyster mushroom, is an edible and saprophytic mushroom species belongs to *Pleurotus* genus (Lewinsohn et al., 2002). The commercial production began in the mid-1970s and is now being produced in more than 12 countries (Chang, 2005). This fungus has excellent texture and high nutritional value and medical properties. For that reason, the production of this mushroom has tripled between 1996 and 2000. Mane et al. (2007) reported that the widespread production of *Pleurotus* species in Asia and Europe could be identified with simple and low-cost production technology and higher biological efficiency. Recent years, there are serious increases in the production of *Pleurotus* species in Turkey (Eren and Pekşen, 2016).

P. eryngii is a white rot fungus and growing on lignocellulosic materials such as sawdust and straw. Sawdust and straw are rich in cellulose, hemicellulose and lignin, but they are poor regarding some micro and macro elements needed for fungal growth. In these substrates, since the majority of the nitrogen is used for mycelial growth, the amount of nitrogen in the substrate is reduced during the fruiting period. This cause limited mushroom yield. It was also expressed that materials such as wheat bran, soybean meal, and rice wastes

should be added to the substrates prepared with sawdust and straw to ensure good mycelial growth, yield and quality in previous studies (Khare et al., 2010; Carvalho et al., 2010; Onyango et al., 2011; Puri, 2012).

Different species of mushroom required different types of agricultural substrates for their development and was suggested that the nutritional needs of different isolates belong to same species can also be different (Shah et al., 2004). Due to the wide geographical spread of *P. eryngii* such as Mediterranean, Central Europe, Central Asia and North Africa, morphological, biochemical and genetic variations are seen in this taxon (Stajic et al., 2009). The different isolates of the king oyster mushroom grown on same substrates exhibit different properties in terms of spawn run time, average yield and quality (Visscher, 1989). Fruit body weight and size are also affected by the substrates (Mane et al., 2004; Royse et al., 2004; Nwanze et al., 2005; Oseni et al., 2012).

Eventhough sugi tree (*Cryptomeria japonica*) shavings supported by wheat bran are used commercially in the cultivation of *P. eryngii* in Japan (Ohga and Royse, 2004). It is essential that the materials used in the preparation of the substrates can be found easily and cheaply in the region where the production will be made. In the study, the effects of agricultural wastes which can be found abundantly and inexpensively which were investigated on yield parameters of *P. eryngii* isolates collected from different cities of Turkey.

Materials and methods

Materials

Three isolates of *P. eryngii* (K-16, K-20, M-18) which collected from different regions of Turkey (Table 1) were provided from Atatürk Horticultural Central Research Institute in Yalova. Pure cultures were maintained on malt extract agar (MEA) at 4°C. Agricultural wastes (sunflower meal, grape pomace and green walnut husks) were obtained from local farms of Kırşehir, Turkey and forest industry waste (poplar sawdust) was purchased from a lumber mill in the woodworking, Kırşehir, Turkey.

Table 1. Origins of *P. eryngii* isolates

Isolates	Species	Origin	Collection date
K-16	<i>P. eryngii</i> var. <i>eryngii</i>	Biga/Çanakkale	16.10.2009
K-20	<i>P. eryngii</i> var. <i>eryngii</i>	Biga/Çanakkale	16.10.2009
M-18	<i>P. eryngii</i> var. <i>ferulae</i>	Selçuk/İzmir	24.10.2009

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Spawn and substrates preparation

Spawn was prepared by boiled wheat grains in glass bottles and sterilized in an autoclave for 90 min at 121°C, cooled and inoculated bottles were incubated in the dark at 25°C until the completion of mycelial growth was done.

Poplar sawdust (S) was used as a base substrate and sunflower meal (SFM), grape waste (GP) and green walnut husk (GWH) were added to the sawdust at ratios of 8:2 to prepare the substrates (Table 2).

Table 2. Composition and ratio of substrates tested in this study

Substrates	Basal material	Additive material
S:SFM	Poplar sawdust (80%)	Sunflower meal (20%)
S:GP	Poplar sawdust (80%)	Grape pomace (20%)
S:GWH	Poplar sawdust (80%)	Green walnut husk (20%)

(S: poplar sawdust; SFM: sunflower meal; GP: grape pomace; GWH: green walnut husk)

Substrates were mixed and tap water was added until it was moistened to about 70%. Then, 1 kg (wet weight) of each substrate was packed into a polypropylene autoclavable bag and autoclaved at 121°C for 90 min.

Mushroom cultivation

After the sterilization, the substrates were inoculated using 3% grain spawn (on a w/w wet weight basis) and incubated at 25±2°C with 80% relative humidity in a dark place for the mycelial colonization. After full colonization, the bags were transferred to the mushroom cultivation room at 15±2°C with a humidity of 80-90% and 8 hours of light daily to induce fructification. Sufficient air changes were maintained to hold the CO₂ concentration below 1000 ppm. 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. Mushroom production experiment was carried out in a completely randomized plot design, with ten replications.

Mushroom production was conducted at the Mushroom Production Unit of the Horticulture Department in the Faculty of Agriculture at Ahi Evran University in Kırşehir (Turkey).

Evaluation of the cultivation parameters

Several cultivation parameters were evaluated during cultivation period of *P. eryngii* isolates on different substrates. The following data were recorded; spawn running time (day), time of first primordia initiation (day), time of the first harvest (day), yield (g/kg), biological efficiency (BE%), average of mushroom weight (g),

mushroom pileus diameter (mm), stipe width (mm) and stipe length (mm). Total yield was determined as one flush grown on different substrates and expressed as grams of fresh mushrooms harvested per gram of wet substrate (w/w). The biological efficiency (BE%) defined as the percentage of the fresh weight of harvested mushroom to the dry substrate using the formula (Royse, 1985); BE (%)= (fresh weight of harvested mushroom per bag / dry weight of substrate per bag) x 100.

Statistical analysis

The data obtained from the experiment were subjected to variance analysis, and the statistical significance was compared by employing Tukey's test, using SPSS version 16.0 for Windows statistical computer program at a significance level 95% and 99% ($p < 0.05$) and ($p < 0.01$).

Results

Yield parameters of *P. eryngii* isolates grown on different substrates

The growth and yield pattern of *P. eryngii* isolates cultivated on different agrowastes is shown in Table 3. The differences between spawn running time of isolates and substrates are statistically significant ($p < 0.01$). When *P. eryngii* isolates were cultivated on S:SFM, the shortest spawn running time was observed for K-20 (14.8 days) which was significantly different from spawn running time of K-16 (15.6 days) and M-18 (17.4 days). Spawn running time of each isolate was slightly lower on S:GWH than other substrates. It was changed between 20.8 and 23.2 days depending on isolates.

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Table 3. Effect of substrates on yield parameters of *Pleurotus eryngii* isolates

Isolates	Substrates	Spawn running time (day)	Total yield (g/kg)	Biological efficiency (%)	Average of mushroom weight (g)
M-18	S:SFM	17.4 c**	206.5 a**	68.8 a**	88.6 a**
	S:GP	18.4 b	103.1 c	34.4 c	52.7 c
	S:GWH	23.2 a	136.4 b	45.5 b	70.7 b
Mean		19.8	148.67	49.55	70.68
K-16	S:SFM	15.6 c**	146.5 a**	48.8 a**	55.0 a**
	S:GP	16.2 b	118.2 b	39.4 b	40.4 b
	S:GWH	20.8 a	86.7 c	28.9 c	40.1 b
Mean		17.53	117.1	39.0	45.15
K-20	S:SFM	14.8 c**	128.1 b**	42.7 b**	41.0 ^{ns}
	S:GP	17.6 b	147.8 a	49.3 a	47.85
	S:GWH	21.8 a	99.8 c	33.3 c	39.50
Mean		18.06	125.23	41.74	42.77

Asterisks indicate significance at * $p < 0.05$, ** $p < 0.01$, ns: not significant; values within the same column followed by the same letter are not significantly different by Tukey's test. (S: poplar sawdust; SFM: sunflower meal; GP: grape pomace; GWH: green walnut husk)

As shown in Table 3, yield and BEs (%) of three *P. eryngii* isolates showed different preference to the agricultural by-products supplements tested. The yields obtained from *P. eryngii* isolates grown on different substrates ranged from 99.79 to 206.52 g/kg and BEs ranged from 33.3 to 68.8%. M-18 (*P. eryngii* var. *ferulae*) was determined as the most productive isolate with an average yield of 148.7 g/kg in different substrates. The highest yield was observed on S:SFM (206.5 g/kg) while the lowest yield (103.1 g/kg) was observed on S:GP unlike other isolates. The average of yield of K-20 isolate grown on different substrates was determined as 125.2 g/kg. Unlike the other isolates, the highest yield was obtained on S:GP (147.8 g/kg) in this isolate. The lowest average yield was exhibited by K-16 isolate (117.1 g/kg). The highest yield was obtained on S:SFM, the lowest yield was obtained on S:GWH in K-16 isolate.

The highest BE was found for M-18 isolate (68.8%) on S:SFM. BEs (%) of K-16 and K-20 isolates which grown in the same substrate were determined as 48.8% and 42.7%, respectively. Similar trend was observed on S:GWH (45.5% for M-18, 28.9% for K-16 and 33.3% for K-20). Highest BE was found in case of K-20 (49.3%) on S:GP, followed by S:SFM. As in the yield, the BE of K-16 and K-20 isolates was also lower when GWH was used as substrate.

Average mushroom weight, pileus diameter, stipe length and width of *P. eryngii* isolates grown on different substrates

The highest average mushroom weight was obtained in M-18 grown on S:SFM, whereas the lowest mushroom weight was determined in K-20 grown on S:GWH (Figure 1).

The isolate that has the highest average fruit body weight was identified as M-18 isolate. The average fruit body weight of M-18 isolate grown in different substrates ranged from 52.67 to 88.64 g. The K-16 (45.15 g) and K-20 (42.77 g) isolates were statistically in the same group in terms of fruit body weights. For the K-16 isolate, the average mushroom weight varied between 40.1 and 55.0 g depending on the substrates. The highest mushrooms was obtained in K-16 grown on S:SFM, while the smallest mushrooms was determined in S:GWH. There was no statistically significant difference was not determined in average mushroom weights of K-20 grown in different substrates.

The average mushroom weight, pileus diameter, stipe length and width of the fruiting bodies were affected by the different isolates and substrates as seen in Table 4.

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Figure 1. Fruit bodies of *Pleurotus eryngii* isolates grown on different substratesTable 4. Effect of substrates on fruit body size of *Pleurotus eryngii* isolates

Isolates	Substrates	Pileus diameter (mm)	Stipe length (mm)	Stipe diameter (mm)
M-18	S:SFM	114.3 a*	29.0 a**	23.4 a*
	S:GP	82.9 b	18.8 b	15.1 b
	S:GWH	105.9 ab	25.6 a	19.2 ab
Mean		101.02 a	24.45 b	19.22 b
K-16	S:SFM	92.1 a**	31.3 b**	27.8 a*
	S:GP	74.0 b	50.0 a	24.7 ab
	S:GWH	49.4 c	39.5 b	19.4 b
Mean		74.83 c	40.24 a	22.36 a
K-20	S:SFM	96.6 a**	39.5 b**	23.4** b
	S:GP	76.8 b	45.2 a	26.2 a
	S:GWH	79.2 b	42.8 ab	17.5 c
Mean		84.20 b	42.49 a	23.95 a

Asterisks indicate significance at * $p < 0.05$. ** $p < 0.01$. ns: not significant; values within the same column followed by the same letter are not significantly different by Tukey's test. (S: poplar sawdust; SFM: sunflower meal; GP: grape pomace; GWH: green walnut husk)

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The average of pileus diameter was higher on fruit bodies grown on S:SFM than other substrates on all isolates, which reach to 114.3 mm, 96.6 mm and 92.1 mm on M-18, K-20 and K-16 isolates, respectively. The highest stipe width (27.8 mm) occurred in K-16 grown on S:SFM. The lowest stem length was determined 17.5 in K-20 grown on S:GWH, significantly ($p<0.01$), similarly the longest stem length was obtained 42.8 cm in K-20 grown S:GWH.

Discussion

Poplar sawdust, grape pomace, sunflower meal and green walnut husk are agricultural wastes that can be easily found in Turkey. For that reason, these forest and agricultural wastes were selected in this study to investigate their usability in the cultivation of three different isolates of *P. eryngii*.

Spawn run time varies depending on mushroom genotype (Philippoussis et al., 2002) and materials used in the substrates (Ayodele and Akpaja, 2007). Jeznabadi et al. (2016) informed that spawn run time of *P. eryngii* varied between 11.25-18.25 days in different substrates. It was observed that the spawn running time in the substrates containing sunflower meal and grape pomace were consistent with the literature, while spawn running time was longer than that in the green walnut shell added as a substrates. The length of spawn running time is also influenced by the *P. eryngii* isolate tested, K-20 was determined as the isolate with the fastest mycelial growth. Thin and poor mycelium development was observed in K-16 and K-20 grown on S:GWH.

Days to the first harvest found in this study (40.6-55.6 days) were similar to or longer than the values reported by Akyüz and Yildiz (2007) (53-72 days) and Moonmoon et al. (2010) (26.5-30.0 days). An increase in the cultivation period length of *P. eryngii* isolates was observed for S:GWH compared with the other substrates. The reason for the slow mycelial growth and more extended cultivation period in the substrate may be a substrate contained in large quantity in the green walnut husks, called juglone (Sun et al., 2006). Juglone is an example of the allelopathic compound, a substrate that is synthesized by one type of plant and affects the growth of another (Cosmulescu et al., 2011). This substrate is also toxic to many plants such as lettuce (Zhang et al., 2008), watermelon, tomato, alfalfa (Kocaçaliskan et al., 2008).

The yield of mushroom are related to environmental (temperature, humidity, light and ventilation), nutrients (carbohydrate, nitrogen and vitamins) and chemical factors (Ko et al., 2005). Although

temperature, humidity, and light are kept constant in trials, differences in yields of the same isolate grown in different substrates may be due to differences in nutritional and chemical properties of substrates. The influence of physical and chemical properties of the substrates on mushroom production characters and especially on mycelium growth, yield and BE has been reported in previous studies (Atila et al., 2017; Obodai et al., 2003).

P. eryngii isolates are different in terms of yield and biological activity. The most productive isolate was identified as M-18 isolate belonging to *P. eryngii* var. *ferulae* isolate. Another result obtained in the study is that the substrate preference of different isolates belonging to the same species may be different. The highest yield in M-18 and K-16 isolates were obtained in sunflower meal added media whereas the highest yield in K-20 isolate was taken from the medium containing grape pomace. Also, the lowest yield in K-16 and K-20 isolates was obtained in substrate supplemented with green walnut husks, while the lowest yield in M-18 isolate was determined in substrate supplemented with grape pomace. Peng et al. (2000) reported that two species of *P. eryngii* (originated from Netherlands and Czechoslovakia) needed rice in different quantities in order to reach maximum yield. Moonmoon et al. (2010) determined that three different isolates of *P. eryngii* prefer different substrates and exhibit difference in terms of yield when they are grown different substrates.

BE was significantly affected by the interaction between genotype, spawn run time, and substrate formulation (Royse and Bahler, 1986). The BE values obtained by Moonmoon et al. (2010) (46.75-73.5%), Kırbağ ve Akyüz (2008) (48.05%) and Kibar (2016) (53.3-81.3%). Rodriguez Estrada and Royse (2007) reported that BE values of *P. eryngii* were increased up to 35%, when the base formulation was supplemented with an additional 6% ground soybean. Jeznabadi et al. (2006) informed that the BEs of *P. eryngii* was between 67.23 and 236.32% in different substrates. The yield and BE values found in the present study were acceptable when compared with those mentioned above.

Obtained average mushroom weight values in the study are corroborated by the findings of Hassan et al. (2010) who reported that average weight of *P. eryngii* grown on different substrates between 20-70 g. There is a positive correlation between biological efficiency and size of fruit body. Beyer and Muthersbaugh, (1996) reported that biological efficiency depends on the yield size. Although, pileus diameters of *P. eryngii* isolates used in the study are similar by the findings of Moonmoon et al. (2010) who reported that diameter of pileus of

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different *P. eryngii* isolates between 5.25 and 8.2 cm, stalk length and stalk width were lower in the study when compared with results of the same study. The M-18 isolate was identified as the one with the largest size among the isolates used in the study. Owaid et al. (2015) reported that quality properties of oyster mushroom had been related with the fruit body size such as determination of pileus (cap) and stipe.

In conclusion, the results indicate all the three *P. eryngii* isolates can grow on poplar substrate with the supplement of any kind of the tested agricultural by-products. Moreover, utilization of green walnut husks for *Pleurotus eryngii* cultivation is promising and has potential commercial application in the mushroom industry. The green walnut shell contains toxic effects regarding soil and environment and the use of this waste

is limited. Apart from providing nutrient-rich food material such as mushroom, cultivation of *P. eryngii* isolates on substrate contained green walnut husk helps in efficient disposal of this wastes. But additional research is needed to optimize the cultivation formula for green walnut husk substrate that will further enhance yields and quality of this mushroom. Productive combinations of this material that would allow sustainable commercial cultivation of *P. eryngii*.

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