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The Use of Phenolic-rich Agricultural Wastes for Hericium erinaceus and Lentinula edodes Cultivation*

Fenolik İçeriği Yüksek Tarımsal Atıkların Hericium erinaceus ve Lentinula edodes Mantarlarının Üretiminde Kullanımı

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ABSTRACT

Objective: This research investigated that the use of four selected agro-waste materials rich in phenolic content on cultivation of *Hericium erinaceus* and *Lentinula edodes* mushrooms.

Material and Method: For this purpose, these wastes were comparatively evaluated regarding their suitability for mycelial growth, yield and biological efficiency (BE), of these mushroom species. The oak sawdust (OS) basal medium was mixed with 20% grape pomace (GP), green walnut hull (GWH), olive press cake (OPC), and tea waste (TW) for the production of these species in factorial experiments based on a completely randomised design with ten replications.

Results: For both *H. erinaceus* and *L. edodes,* the shortest spawn running time (22.4 and 45.4 days, respectively) and the highest yield (225.0 g kg^{-1} and 282.9 g kg^{-1}, respectively) were found using GP. The BE of *H. erinaceus* and *L. edodes* were varied between 15.2-64.3% and 36.0-70.7% , respectively.

Conclusion: GP, TW and OPC were seen as promising alternative substrates for the cultivation of these species. Moreover, for the effective utilisation and profitable disposal of green walnut hulls, further research is needed. to test their performance for the cultivation of other mushroom species.

Anahtar Sözcükler:

Üzüm posası, yeşil ceviz kabuğu, aslan yelesi mantarı, zeytin prinası, shiitake, çay atığı

ÖZ

Amaç: Bu çalışmada fenolik içeriği yüksek olan dört farklı tarımsal atığın *Hericium erinaceus* and *Lentinula edodes* mantarlarının üretiminde kullanımı araştırılmıştır.

Materyal ve Metot: Bu amaçla, bu atıkların bahsedilen mantar türlerinin misel gelişimleri, verim ve biyolojik etkinlikleri (BE) karşılaştırmalı olarak değerlendirilmiştir. Meşe talaşı (OS) ana material olarak kullanılmış ve bu ortama %20 oranında üzüm posası (GP), yeşil ceviz kabuğu (GWH), zeytin pirinası (OPC) ve çay atıkları eklenerek mantar üretiminde kullanılmıştır. Denemeler 10 tekerrürlü olarak tesadüf parselleri deneme desenine göre planlanmıştır.

Bulgular: Hem *H. erinaceus* hemde *L. edodes*'de en kısa misel gelişim süresi (22.4 ve 45.4 gün, sırasıyla) ve en yüksek verim (225.0 g kg^{-1} and 282.9 g kg^{-1},sırasıyla) OS ve GP kombinasyonlarında gözlenmiştir. *H. erinaceus* ve *L. edodes* mantarlarının BE'leri ise sırası ile %15.2-64.3 ve %36.0-70.7 arasında değişmiştir.

Sonuçlar: Bu mantar türlerinin üretiminde GP, TW ve OPC alternatif katkı materyali olarak kullanılabilirler. Fakat mantar üretiminde yeşil ceviz kabuğundan etkili ve karlı bir şekilde yararlanabilmemiz için ek çalışmalar yapılması gerekmektedir.

INTRODUCTION

Tea (*Camellia sinensis*), walnut (*Juglans regia*), grape (*Vitis vinifera*) and olive (*Olea europaea* L.) are agricultural products grown in large quantities annually in Turkey. Their production generates huge amounts of green walnut hulls, olive press cake, grape pomace and tea waste. Generally, the utilisation of such agricultural waste is problematic due to the high lignocellulosic content. In addition, the varied chemical content of agricultural wastes makes them environmentally harmful. Green, black and yellow tea leaves (Kopjar et al., 2015) and grape pomace (Ramirez-Lopez et al., 2014) are rich in phenolic compounds. Regarding by-products of olive oil production, the considerable formation of phenolic compounds in the generated wastewater and solid residue are particularly toxic (Vlyssides et al., 2004). Similarly, green walnut hulls are also rich in juglone, another phenolic compound (Stampar et al., 2006). Several studies have carried out on the phytotoxicity of phenolic compounds on seed germination (D'Abrosca et al., 2004; Della Greca et al., 2001). Moreover, the high polyphenol content in these wastes is associated with environmental risk. The accumulation of these wastes not only threatens the environment but also requires considerable labour and space for its disposal. Conversion and utilization of these materials by different ways is a highly attractive idea.

Fungi play an important role in the breakdown of organic matter. Basidiomycetes are key organisms in the decomposition of the basic cell walls of plants (Naheed and Iqbal-Zafar, 1999). This supports the use of various agricultural and forest wastes in the production of mushrooms. Mushroom cultivation results in the conversion of environmentally hazardous agricultural wastes into sources of good-quality food with superior taste and nutritive value. In addition, mushrooms contain strong newly discovered pharmaceutical products. *Lentinula edodes* (Berk.) Pegler (shiitake) mushrooms contain many bioactive components such as antitumor polysaccharides (Zhang et al., 2010), antioxidant and antimicrobial substances (Kitzberger et al., 2007), antibacterial and antifungal compounds (Hearst et al., 2017), and cholesterol-lowering active ingredients (Fukushima et al.,2001). In addition, the extract of *Hericium erinaceus* (lion's mane mushroom) has been reported to exhibit antihyperglycemic (Chaiyasut and Sivamaruthi, 2017), anti-inflammatory (Mori et al., 2015), and anti-obesity activity (Hiraki et al., 2017), as well as neurological support (Spelman et al.,2017). Moreover, the production of mushrooms is one method of promoting employment and social development in rural areas (Celik and Peker, 2009).

H. erinaceus and *L. edodes* are white-rot fungi. White rot fungi are the most efficient lignin degraders in nature (Martinez et al., 2005). The principal substrate used for the commercial production of *H. erinaceus* is sawdust, previous studies have reported that different types of agricultural waste, such as different types of bran, Chinese cabbage, soybean powder, egg shell cotton seed hulls and olive press cake (Siwulski et al., 2005; Ehlers and Schnitzler, 2000; Ko et al., 2005) could be used as additive materials in the preparation of the growing media for *H. erinaceus* cultivation. Similarly, the use of several lignocellulosic by-products such as grape stalks (Nicolini et al., 1987), apple pomace (Worralli and Yang, 1992), white millet and wheat bran (Royse and Bahler, 1986) has been reported for the cultivation of *L. edodes* as well as tree logs (Boztok and Erkip, 2002). Limited data and reference texts are available on the use of tea waste, olive press cake and grape pomace in mushroom cultivation (Pardo et al., 2007; Yang et al., 2016; Atila, 2017). However, to the best of our knowledge, no study has been conducted to date on the use of green walnut hulls in the preparation of mushroom growing media. The high quantities of these waste materials indicate the need for their assessment as an economical substrate for mushroom cultivation.

With this approach in mind, four selected agro-waste materials rich in phenolic content were comparatively evaluated regarding their suitability for enhancing the production of *H. erinaceus* and *L. edodes*. These species were subjected to cultivation experiments and selected parameters for mycelial growth and fructification were recorded and analysed. The results were determined and reviewed with the view of establishing sustainable and economical production methodologies capable of utilising relevant hazardous agro-wastes.

MATERIAL and METHOD

Materials

The two species which were used in the present study were: (i) *Lentinula edodes* (provided by Sylvan) and (ii) *Hericium erinaceus* (provided by Agroma Ltd Co).

The pure cultures were maintained in potato dextrose agar (PDA, Merck) medium and stored at 4 °C. Spawn was prepared on wheat grain inoculated with actively growing mycelium of *L. edodes* and

H. erinaceus and incubated at 25 ± 2 ºC for mycelial growth until the mycelium fully covered the grains.

Experimental design

Five different growing media were tested for the cultivation of *L. edodes* and *H. erinaceus*. Oak sawdust (OS) was used as a base medium and supplemented with grape pomace (GP), green walnut hull (GWH), olive press cake (OPC), and tea waste (TW) in the ratio 8:2. (dry matter basis). The control medium was prepared with only OS.

The experiment was conducted in a randomised plot design with ten replications. The study was carried out at the Mushroom Production Unit of the Agriculture Faculty of Ahi Evran University in Kırşehir, Turkey.

Preparation of cultivation media

Agricultural wastes were obtained from local farmers. The sawdust and additive materials were thoroughly mixed, and water was added to raise the final moisture content to 60 ± 5%. One kilogram (wet weight) of each substrate was then packed into a 25 × 45 cm polypropylene autoclave bag, and the bag was stoppered with a cotton plug. The bags were autoclaved at 121 °C for 90 min. The sterilised substrates were then spawned using 3% grain spawn on a w/w wet weight basis. Ten replicates were performed for each growing medium formulation.

Cultivation conditions for mushroom production

The study was carried out in two different production room. For both species examined, colonization of the substrates took place at 25 ± 2 °C in dark production rooms. Depending on mushroom species, after spawn running period, environmental conditions were adjusted for basidiomata induction and maintained at the appropriate levels during the fructification period (Table 1).

Table 1. Environmental conditions of production rooms during fructification period

Species	Temperature (°C)	Humidity (%)	Aeration	Light intensity
L. edodes	15 ± 2	85-90	CO_2 level < 1200 ppm	1000 lux (8 h/day, fluorescent lamps)
H. erinaceus	18 ± 2	85-90	CO_2 level < 1200 ppm	1000 lux (8 h/day, fluorescent lamps)

Unlike *H. erinaceus*, after the full mycelial colonization, a dark brown-colored crust was formed in *L. edodes* cultivation. During the browning process, light was provided throughout the spawn running period for 4 h (Royse, 1997) and the bags were sliced substantially, but they were not removed thoroughly to protect moisture content of growing media. Moreover, the bags watered lightly once or twice per day to maintain continuous surface moisture.

Evaluation of cultivation parameters

The fruiting bodies of *L. edodes* were harvested from the substrates when the veil had broken and the gills were fully exposed, whereas fruitbodies of *H. erinaceus* were harvested when the spines of the fruitbodies had reached a length of 5 mm.

In order to test the suitability of the wastes as substrates for the cultivation of the mushroom species examined, a number of parameters were evaluated during the mushroom cultivation on different substrates. These included spawn running time (days), time to first primordia initiation (days), time to first harvest (days) yield (g kg^{-1}), biological efficiency (%) and average mushroom weight (g). Yields were expressed as grams of fresh mushrooms harvested at maturity per gram of wet substrate (w/w). Biological efficiency (BE) was calculated as the percentage ratio of the fresh weight of harvested mushroom per gram of dry substrate using the following formula: BE (%) = (fresh weight of harvested mushroom per bag / dry weight of substrate per bag) × 100.

Constituent analysis of substrates

Substrates were oven-dried at 60 ºC for 48 h and ground to pass through a 1-mm sieve. The ash, moisture content and pH were determined via standard procedure (Kacar and Inal, 2008). Carbon (C) content was assessed according to Royse and Sanchez (2007). The Kjeldhal method was used to determine the total nitrogen (N) content of each substrate and the carbon: nitrogen ratio of each substrate was then calculated.

Data analysis

The data obtained from the experiments were subjected to variance and means analyses. The Social Sciences (SPSS) version 16 was used to carry out Statistical analysis. Statistically significant differences among the means were determined by Tukey Test at a significance level of 5%.

RESULTS

Growing substrate composition

The main chemical properties of the growing substrates are presented in Table 2. The differences in ash, carbon, nitrogen and C:N ratios were statistically significant (P <0.01).

The ash content of OS:GP (4.01%) was the highest. The nitrogen (N) content of the substrates varied between 0.39% (control) and 0.77% (OS:GP), while the carbon (C) content of the substrates ranged between 53.32% and 53.5%. The C:N ratio of OS (138.6) was significantly higher than all other substrates whereas the OS:GP substrate had the lowest C:N ratio (69.4).

Effect of growing substrates on the mycelial growth

The effects of the different tested additive materials on the spawn running time, duration of the primordia induction period (earliness) and time to first harvest of *L. edodes* and *H. erinaceus* are presented as a sum of these three phases in Tables 3 and 4. Significant differences were observed among treatments in terms of times taken for spawn running period, days to first primordia initiation and days to first harvest of *H. erinaceus* (P<0.01).

Table 2. Chemical composition of different growing media used in the study

Growing media	Ash (%)	C (%)	N (%)	C:N
OS	3.71±0.04^{**c}	53.50±0.02$^{** a}$	0.39±0.02$^{** c}$	138.6±7.3$^{** a}$
OS:GP	4.01±0.14^{a}	53.32±0.08^{c}	0.77±0.04^{a}	69.4±3.3^{d}
OS:TW	3.71±0.05^{c}	53.50±0.03^{a}	0.43±0.02^{c}	124.6±5.1^{ab}
OS:OPC	3.77±0.06^{bc}	53.46±0.03^{ab}	0.54±0.04^{b}	98.7±6.4^{c}
OS:GWH	3.93±0.08^{ab}	53.37±0.04^{bc}	0.45±0.03^{c}	119.0±8.0^{b}

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 according to Tukey's test.

Table 3. Effect of different additive substrates on yield parameters of *Hericium erinaceus*

Growing media	Spawn running time (days)	Time to primordia initiation (days)	Time to first harvest (days)	Yield		Total Yield (g/kg)	BE (%)	Average mushroom weight (g)
				Flush 1 (g)	Flush 2 (g)			
Control	25.2±1.30^{**c}	30.6±0.89^{**c}	34.6±0.89$^{** d}$	58.3	38.9	97.2±9.0^{**c}	27.8±2.3^{**c}	40.0±1.0$^{** d}$
OS:GWH	32.8±0.84^{a}	41±0.70^{a}	47.6±0.55^{a}	30.2	23.1	53.3±8.1^{d}	15.2±2.0^{d}	30.4±3.5^{e}
OS:TW	25.4±0.89^{c}	31.2±0.84^{c}	37.8±0.45^{c}	68.4	62.9	131.3±7.4^{b}	37.5±2.4^{b}	50.4±3.3^{c}
OS:GP	22.4±0.55^{d}	26±0.70^{d}	29.6±0.89^{e}	118.7	106.3	225.0±11.7^{a}	64.3±4.1^{a}	84.2±6.1^{a}
OS:OPC	30.2±0.84^{b}	36.8±0.83^{b}	40.4±0.89^{b}	63.7	71.0	134.7±11.9^{b}	38.5±3.0^{b}	66.6±2.2^{b}

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 according to Tukey's test.

Table 4. Effect of different additive substrates on yield parameters of *Lentinula edodes*

Growing media	Spawn running time (days)	Time to primordia initiation (days)	Time to first harvest (days)	Yield			Total Yield (g/kg)	BE (%)	Average mushroom weight (g)
				Flush 1 (g)	Flush 1 (g)	Flush 1 (g)			
Control	47.2±0.84^{**c}	60.2±0.84^{**c}	72.8±0.84^{**c}	62.2	68.9	25.3	156.7±10.6^{**b}	38.2±2.6^{**b}	13.1±0.76^{**b}
OS:GWH	No data	No data	No data	No data	No data	No data	No data	No data	No data
OS:TW	49.8±0.45^{b}	63.4±0.55^{b}	76.0±1.0^{b}	62.7	58.7	15.3	136.7±10.0^{c}	36.0±2.6^{b}	11.2±0.69^{c}
OS:GP	45.4±0.89^{d}	54.6±0.89^{d}	68.8±0.45^{d}	104.1	135.3	44.2	282.9±10.1^{a}	70.7±2.5^{a}	15.8±0.98^{a}
OS:OPC	51.8±0.84^{a}	68.2±0.84^{a}	80.2±0.44^{a}	64.1	65.8	18.7	148.4±7.9^{bc}	39.1±2.1^{b}	15.1±0.74^{a}
Control	47.2±0.84^{**c}	60.2±0.84^{**c}	72.8±0.84^{**c}	62.2	68.9	25.3	156.7±10.6^{**b}	38.2±2.6^{**b}	13.1±0.76^{**b}

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 according to Tukey's test.

Spawn running time of *H. erinaceus* grown on substrates supplemented with different additive material ranged between 22.4 - 32.8 days. GP was more precocious than other substrates, forming first primordia after 26.0 days of incubation in *H. erinaceus* grown on OS:GP medium. OS: GWH medium promoted slower colonization than the other growing media, forming first primordia in this substrate was also induced later (41.0 day). The first flush started after 47.6 days of incubation on OS:GWH substrate, while it started 29.6 days after incubation on OS:GP substrate.

Significant differences (P<0.01) among the substrates was also noted for the spawn running time, days to first primordia initiation and days to first harvest for *L. edodes*. The mycelial growth of *L. edodes* was slower than that of *H. erinaceus*, needing between 45.4 days (OS:GP) and 51.8 days (OS:OPC) to colonise the growing media. The time up to the appearance of the primordia varied for the substrates from 54.6 days to 68.2 days in *L. edodes*. The GWH failed to support mushroom production, whereas basidiomata formation was comparatively high on OS:GP in this species. The first flush started 68.8 days (OS:GP) – 80.2 days (OS:OPC) after the incubation, depending on the substrate.

Effect of growing substrates on productivity parameters

The effect of growing substrates on the yield, BE and average mushroom weight was found to be statistically significant (P<0.01). The maximum yield (225.0 g kg^{-1}) and BE (64.3%) were obtained on the OS:GP substrate for H. erinaceus, which was distributed in two flushes. Mature fruiting bodies of *H. erinaceus* grown on all growing media are shown in Figure 1.

Although the OS:GWH appeared to be the worst performing substrate for *H. erinaceus* (53.3 g kg^{-1} yield

and BE of only 15.2%), the TW and OPC sustained satisfactory productivity by presenting an overall range of BE between 37.5% and 38.5%, respectively. Two flushes were harvested for *H. erinaceus* and the distribution of crop yield among individual flushes was not the same for the substrates. 57% of the total yield was obtained in the first flush on GWH, while on OPC, TW and GP 47.3%, 52% and 52.8% of the total yield, respectively, was obtained in the first flush with the second flush contributing almost equally to the remaining 50%. Approximately 60% of the total yield was harvested in the first flush on the control. The highest average mushroom weight (84.2 g) was obtained on OS:GP substrate. The TW (50.4 g) and OPC (66.6 g) substrates also presented heavier basidiomata for *H. erinaceus* than the control substrate (40.0 g). The lowest average mushroom weight value (30.4 g) was recorded on OS:GWH medium.

The yields, BEs and average mushroom weight of the five substrates were significantly different in *L. edodes* (P < 0.01). Concerning the production data of *L. edodes*, total yield of *L. edodes* varied between 136.7 g kg^{-1} and 282.9 g kg^{-1} wet substrate, while BEs varied between 36.0% and 70.7%. Three flushes were recorded in L. edodes in all growing media. Mature fruiting bodies of *L. edodes* grown on different growing media are shown in Figure 2.

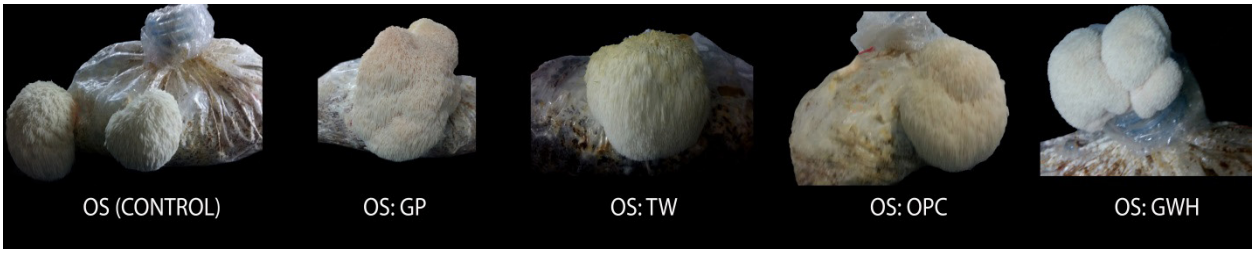

Figure 1. *Hericium erinaceus* grown on different growing media - OS (oak sawdust); GP (grape pomace); TW (tea waste); GWH (green walnut hull); OPC (olive press cake)

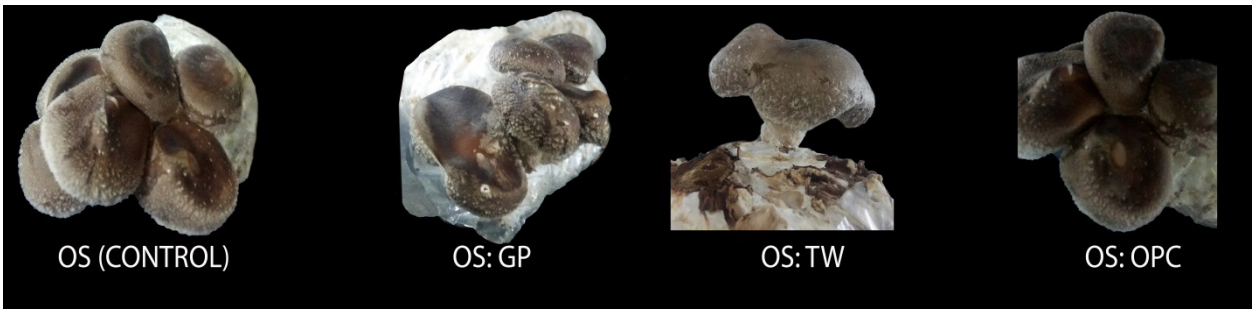

Figure 2. *Lentinula edodes* grown on different growing media OS (oak sawdust); GP (grape pomace); TW (tea waste); OPC (olive press cake)

The maximum sporophore yield and BE were obtained on the OS:GP. The yield of first flush was higher 23.1% and 67.3% than first and third flushes of OS:GP medium, respectively. The lowest yield and BE were determined in *L. edodes* grown on OS:TW medium. The highest yield was recorded in first flush by producing 62.7 g on OS:TW medium. On the other hand, only 15.3 g was obtained in *L. edodes* grown on same substrate in third flush.

The highest average mushroom weight (15.8 g) were obtained in *L. edodes* grown on OS:GP medium, followed by OS:OPC medium (15.1 g). The average mushroom weight was lower for the OS:TW (11.2 g) as compared to the control (13.1 g).

DISCUSSION

Wheat bran, millet bran, maize powder, sunflower seed hulls were popular additive materials investigated in previous studies for the cultivation of shiitake (Royse and Bahler, 1986; Moonmoon et al., 2011; Curvetto et al., 2005) and *H. erinaceus* (Siwulski et al., 2005; Ehlers and Schnitzler, 2000; Figlas et al., 2007) however, expensiveness of these substrates in many regions has brought up the necessity to find cheap alternatives. On the other hand, phenolic-rich agricultural wastes is limited in use and they could be harmfull for soil and water source. The present investigation was undertaken with the aim of providing data useful for the exploitation of some phenolic-rich agricultural waste as substrate for the cultivation of *L. edodes* and *H. erinaceus*.

Spawn run time varies depending on mushroom genotype and type of substrates (Philippoussis et al., 2003). Shorten spawn running period is important in mushroom cultivation because of high risk of contamination. The mycelium growth of *H.erinaceus* , was successful in all growing media used in the study. It was noted that although the incubation period appeared longer than the findings Staments (1993) who reported that the spawn run time of *H. erinaceus* grown on sawdust was ranged between 10-14 days, but shorter than the findings Hassan (2007). On the other hand, earliness values were in agreement with that reported by Atila et al. (2018). Regarding *L.edodes*, Ozcelik and Peksen (2007) reported that the spawn run time of *L. edodes* was ranged between 38.8- 59.8 days depending on growing media, while Philippoussis et al (2003) confirmed that this varied in *L. edodes* strains grown on different growing media, between 29.0 and 56.0 days. The spawn running times recorded here for *L. edodes* were comparable to those reported in recent relevant studies. On the other hand, mycelial growth was not observed in *L. edodes* grown on

OS:GHW. This negative effect of GWH was less pronounced in the case of *H. erinaceus*; however *H. erinaceus* cultivation on this media resulted in longer colonisation and basidiomata induction periods. On OS:GWH substrate, the first flush started 18 days later, than OS:GP substrate. It is reasonable to assume that this was due to the juglone content of the green walnut hulls in the substrate. Juglone is an organic compound naturally occurring in the leaves, roots, husks, and bark of plants in the *Juglandaceae* family (Ercisli and Turkkal, 2005). Several studies have addressed the inhibiting effect of juglone on the germination and growth of several plant species (Ercisli and Turkkal, 2005; Ercisli et al., 2005; Terzi, 2008; Zhang et al., 2008).

As far as crop production characters were concerned, the *H. erinaceus* and *L. edodes* species presented significantly faster mycelial growth, higher yields and BEs on OS:GP in comparison with the other substrates. It is reasonable to assume that the low C:N ratio in conjunction with the high nitrogen concentration exerted a positive effect on the fruiting of *L. edodes* and *H. erinaceus*. Zeid et al. (2009) reported that minimum, and maximum C:N ratios varied between 25 and 55, while the optimum ratio was 30-35 for *L. edodes*. Atila et al. (2017) stated that *Hericium americanum* cultivated on substrates with C:N ratios ranging between 51.7 and 82.7 showed increased mycelial growth. Morever, Balakrishnan and Nair (1995) reported that the highest yield was obtained from the substrate with 0.7–0.9% nitrogen content (dry weight) or the C:N ratio of the substrate was 50 or higher than 50 in *Pleurotus ostreatus*.

Although the GWH appeared to be the worst performing substrate for *H. erinaceus* (53.3 g kg^{-1} yield and BE of only 15.2%) the TW and OPC sustained satisfactory productivity by presenting an overall range of BE between 43.8% and 44.9%, which was significantly higher than those recorded by Ko et al (2005) on sawdust and rice bran (4:1). The BE of *H. erinaceus* on TW and OPC was close to the control substrate. Two flushes were harvested for *H. erinaceus* and the distribution of crop yield among individual flushes was not the same for the substrates. 57% of the total yield was obtained in the first flush on GWH, while on OPC, TW and GP 47.3%, 52% and 52.8% of the total yield, respectively, was obtained in the first flush with the second flush contributing almost equally to the remaining 50%. Approximately 60% of the total yield was harvested in the first flush on the control. The differences in yield distribution among flushes in different substrates were an indication that the nature of substrates influenced yield pattern as well Philippoussis et al (2001).

Unlike *H. erinaceus, L. edodes* did not grow or develop on the OS:GWH substrate. Although three flushes were harvested, more than 80% of the total yield was obtained in the first two flushes for *L. edodes* on all substrates tested. The GP proved to favour earliness in *L. edodes* since basidiomata formation was achieved four days sooner than on the control, while the OS:OPC substrate exhibited a later initiation. It has been reported in previous studies by Zervakis et al. (2013) and Atila (2017) that a high concentration of OPC had a negative effect on earliness. The *L. edodes* produced equally high yields of basidiomata in both the control and OPC, while the TW was the least efficient in terms of crop yield and average mushroom weight. For *H. erinaceus*, the OS:TW and OS:OPC media appeared to be more suitable than for *L. edodes*, especially regarding crop yield and BE. However, in previous studies on various substrates, BE values of *L. edodes* were between 25.12% and 54.17% Philippoussis et al (2003) , between 43.73% and 87.73% (Ozcelik and Peksen, 2007) or between 52% and 86% (Royse et al., 2004). In this context, GP supported very satisfactory yields, with overall BEs superior to those recorded for *L. edodes* in a previous work Philippoussis et al (2003), while it was similar to those reported in other works for *L. edodes* on different substrates (Ozcelik and Peksen, 2007; Royse et al., 2004).

The basal substrate and additive materials are important sources of variety for the fruitbody of the oyster mushroom Royse et al. (2004). The mushroom size was generally bigger for the OS:GP medium as compared to the control. The OS:TW and OS:OPC media also presented heavier basidiomata for *H. erinaceus* than the control substrate. The lowest average mushroom weight value was recorded on OS:GWH substrate. Regarding mushroom size, for *L. edodes,* the OPC produced fewer but heavier fruit bodies than the control and the large-sized fruit bodies were considered to be of good quality (Onyago et al., 2011). Philippoussis et al. (2007). reported that there was a positive correlation between BE and average mushroom weight. However, with *L. edodes,* although a BE value of 39.1% was recorded on the OS:OPC substrate, the basidiomata possessed high-quality properties in terms of size and shape. Ruiz-Rodriguez et al. (2010) also reported that the size of the *Pleurotus ostreatus* mushroom increased with the addition of olive press wastes to the mushroom growing media.

The results of the present study indicated that all of phenolic rich materials examined in the study, except green walnut hulls, can be disposed by *L. edodes* and *H. erinaceus* cultivation. *H. ernaceus* and *L. edodes* grown on sawdust supplemented with 20% grape pomace exhibited the highest productivity. Moreover, the growth of *L. edodes* and *H. erinaceus* on OS:TW and OS:OPC was well established and that these residues are promising alternative substrates for the cultivation of these species. Oak sawdust supplemented with %20 green walnut hulls failed to support mushroom production for *L. edodes*. Although, fruitbody was obtained *H. erinaceus* grown on OS:GWH, the process resulted in longer colonization and basidiomata induction periods and low yield. So, to ascertain the suitability of GWH for the cultivation of this species, additional experiments should be conducted using different proportions in the cultivation substrates. Moreover, for the effective utilisation and profitable disposal of green walnut hulls, further research is needed to test their performance for the cultivation of other mushroom species.

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