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Utilization of Agricultural and Forestry by-Products in *Ganoderma lucidum* (Curt.: Fr.) P. Karst. Production

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Abstract: The aim of the study was to evaluate the use of some local agricultural and forestry by-products as *Ganoderma lucidum* growing subsrate. In the study, 8 different growing media prepared by mixing agricultural and forestry wastes such as oak sawdust (OS), poplar sawdust (PS), wheat straw (WS), wood chips (WCH), green almond hulls (AH) and green walnut hulls (GWH) alone or in different proportions, were used for *G. lucidum* cultivation and the effects of these substrates on spawn run time (SRT), days to pinhead initiation (DPI), days to the first harvest (DFH), yield (g/kg) and biological efficiency (BE%) were investigated. The results demonstrated that spawn run time (SRT) of *G. lucidum* was shorter in PS and WS:GWH12.5 (19.7 days and 20.8 days) as compared to other substrates and mixtures (22.2-28.5 days). The pinheads emerged in substrates between 43th- 49th days and the fruiting bodies were collected between 80.3 -93.5 days after the spawning. Total fresh mushroom yield and BE(%) varied from 71.39 to 110.10 g/kg of substrate and from 21.0% to 31.46, respectively. Moreover, PS substrate produced fruitbody also earlier compared with the other investigated substrates. The study revealed that although higher total yields were obtained by sawdust substrates, considering the difficulty of finding sawdust in some areas, WS and the mixture of WS and GHW (87.5:12.5%) could be also successfully used inof *G. lucidum* production.

Key words: Reishi, Sawdust, Agricultural wastes, Forestry wastes, Mushroom yield

Ganoderma lucidum (Curt.: Fr.) P. Karst. Üretiminde Tarım Ve Ormancılık Yan Ürünlerinin Kullanımı

Öz: Çalışmanın amacı, bazı yerel tarım ve orman yan ürünlerinin *Ganoderma lucidum* yetiştirme substratı olarak kullanımını değerlendirmektir. Çalışmada meşe talaşı (OS), kavak talaşı (PS), buğday samanı (WS), yonga (WCH), badem kabuğu (AH) ve yeşil ceviz kabuğu (GWH) gibi tarım ve orman atıklarının tek başına veya farklı oranlarda karıştırılmasıyla hazırlanan 8 farklı yetiştirme ortamı kullanılmıştır. *G. lucidum* yetiştiriciliği için kullanılmış ve bu substratların misel gelişim süresi (SRT), taslak oluşumuna kadar geçen gün sayısı (DPI), ilk hasata kadar geçen gün sayısı (DFH), verim (g/kg) ve biyolojik etkinliğe (BE%) etkileri araştırılmıştır. Sonuçlar, *G. lucidum*'un misel gelişim süresinin, PS ve WS:GWH12.5'te (19.7 gün ve 20.8 gün) diğer substratlara ve karışımlara (22.2-28.5 gün) kıyasla daha kısa olduğunu gösterdi. Misel ekiminden sonra 43-49. günlerde taslaklar oluştu ve *G. lucidum*'un şapkaları misel ekiminden 80.3-93.5 gün sonra hasat edildi. Toplam taze mantar verimi ve BE(%) sırasıyla 71.39 g/kg ila 110.10 g/kg substrat ve %21.0 ila %31.46 arasında değişmiştir. Ayrıca, PS substratında araştırılan diğer substratlarla karşılaştırıldığında hasat daha erken gerçekleşti. Çalışma, talaş substratlardan daha yüksek toplam verim elde edilmesine rağmen, bazı bölgelerde talaş bulmanın zorluğu göz önüne alındığında, WS'nin WS ve GHW karışımının da (%87.5:%12.5) *G. lucidum*'un yetiştirilmesi için başarıyla kullanılabileceğini ortaya koydu.

Anahtar kelimeler: Reishi, Talaş, Tarımsal atıklar, Orman atıkları, Mantar verimi

Introduction

Ganoderma lucidum (Curt.: Fr.) P. Karst. a species belonging to the class *Agaricomycetes*, the order *Polyporales* and the family *Ganodermataceae*. *G. lucidum*, commonly known as Ling zhi, Reishi or immortality mushroom, is one of the most popular medicinal mushrooms (Wachtel-Galor et al., 2011). *G lucidum*, which grows naturally in the flora of Turkey, is recognized as the mushroom of immortality (Sesli ve ark., 2020).

Since ancient times, *G. lucidum* was collected from the wild and consumed by local people, but wild *G. lucidum* is difficult to collect and to maintain its quality. For that reason, today, artificial cultivation of *G. lucidum* has became widespread to meet the increasing demand of this mushroom. The first successful cultivation of this mushroom was performed on inoculated natural logs in China in 1969 (Yu and Shen 2003). Since then, the production of this mushroom has been popular espeacially in Far East countries (Li et al. 2016). *G. lucidum* production and consumption has been also growing fast in other countries.

G. lucidum has been used in Far East culture for thousands of years because of its medicinal properties (Cilerdzic et al., 2017). Also today, the hypoglycemic (Liu and Tie, 2019), lipid-lowering (Chan et al., 2021), antitumour, antimicrobial, antioxidant (Cör et al., 2018), anticancer (Sohretoglu and Huang, 2018), antidepressant (Zhao et al., 2021), anti-inflammatory (Xu et al., 2021), immunomodulatory effects (Ren et al., 2021) of this mushroom have been proven in many animal and clinical studies. Several commercial products of *G. lucidum* obtained from fruiting bodies, mycelia or spores, are sold in the form of tea, coffee, syrups, spore powder, slices, supplements, drinks, tooth pastes, soaps and lotions. China, which realizes 70% of the world's reishi production by itself, is the main country that producer and exporter in the world (Li et al. 2016).

Mushroom cultivation could be useful ecological and environmental and economic way to reuse or disposal of this kind of solid organic wastes. It has received especially important in most developing countries where millions of tons of lignocellulose-rich wastes. *G. lucidum*, a wood-decay fungi, is one of the most effective lignin degradater, and play a major role in the organic matter cycling. The substrates which use in mushroom cultivation may influence the yield of mushroom based on its potential to induce enzyme secretion (Sadeghan et al., 2022). Sawdust is one of the best basal substrates in the preparation of growing media for *G. lucidum* cultivation. However, compared to sawdust, most of the people who live in the different rural areas of the world have more access to wheat straw, and other agricultral wastes. Mushroom growers should select abundant and the cheapest locally available substrate materials to reduce production costs. In addition, the use of locally available agricultural and forest wastes in mushroom production is worth further research as it will also mediate the disposal of these wastes. Today, forest industry wastes are insufficient in many regions of the world and the pursuit of alternative substrates has been accelerating in the mushroom industry.

Various growing media formulations are used in the world, dependent on locally available agricultural and forest wastes (Mehta et al. 2013; Atila, 2020), beech sawdust and olive by-products (Koutrotsios et al., 2019), mixtures of sawdust and tea waste levels (Pekşen and Yakupoğlu, 2009), pecan wastes (Ozcariz-Fermoselle et al., 2018), sugarcane bagasse, banana skin, coffee grounds, eggshell, tea waste and diaper wastes (Khoo et al., 2022). However, for sustainable growth of the mushroom industry in different countries where have different forestry and agricultural residues potential, several studies directed toward reduced time to cultivation cycle, improving yield and quality are needed.

Material and Metod

Materials

Pure culture of the GL-52 strain maintained on Malt-Extract Agar (MEA, Merck) at 4 °C. Pure culture was used as the inoculum for spawn preparation on wheat grains as previously described by Atila (2017).

The agricultural waste materials were collected from local farms in Kırşehir, Turkey. Poplar and oak sawdusts were supplied from a lumber mill, Kırşehir, Turkey. The growing media examined in the study were presented in Table 1.

Experimental Design

Several agricultural and forest wastes and different mixed of these wastes were tested as *Ganoderma lucidum* growing media to determine the best substrate and optimal mixed ratio of substrates.

Mushroom cultivation was carried out in the Mushroom Production Unit in the Faculty of Agriculture at Ahi Evran University, Kirsehir (Turkey). The experiment was done in ten replicates for each growing medium using completely randomized plot design.

Preparation of Cultivation Substrate

All substrates were wetted in water for 24 h to increase the moisture content to about 60-65%. To assess effect of growing media and to find the best combination of substrates, they were mixed in different ratios as shown Table 1. Calcium sulphate and calcium carbonate were added in %1 ratio (w/w). 1 kg (wet weight) of the prepated growing media were placed in polypropylene bags (20×45 cm) and were plugged with cotton. Then the bags were autoclaved at 121°C for 90 minutes and spawned on 3% wet weight basis of growing media after cooling.

Table 1. Substrates types and their proportions used in the study

Substrate type	Substrate code	Ratio
Oak sawdust	OS	100
Poplar sawdust	PS	100
Wheat straw	WS	100
Wheat straw: Green walnut hulls	WS:GWH12.5	87.5:12.5
Wheat straw: Green walnut husk	WS:GWH25	75:25
Oak sawdust:Poplar chip	OS:PC	50:50
Oak sawdust: Almond hulls	OS:AH	50:50
Oak sawdust:Wheat straw	OS:WS	50:50

Mushroom Cultivation and Harvesting

The incubation period was conducted at 25°C and 70–80% of relative humidity without artificial lighting. After colonization, cotton plugs were taken to allow the development of fruiting bodies. The temperature and relative humidity were kept at 28 ± 2°C and 90–95% during the generative stage with light exposure (10 h, provided by white fluorescent bulbs). Adequate ventilation was provided to hold CO_2 concentration below 1000 ppm. Mushrooms were harvested when the caps became completely red and the white margin had disappeared. A total of three harvestings were performed between April and July, 2020.

Evaluation of the Cultivation Parameters

Cultivation parameters such as affects spawn run period (day), days to first primordia initiation (day), days to first harvest (day) yield (g/kg) and biological efficiency were evaluated on different growing media. Total yield were obtained from one, two or three flushes depending on growing media and expressed as grams of fresh mushrooms harvested at maturity per gram of wet substrate (w/w). The biological efficiency (BE%) was calculated by using the formula; BE (%)=(fresh weight of harvested fruitbody per bag / dry weight of growing medium per bag) x100.

Substrate Analysis

Samples to be analyzed were collected randomly from the substrates after the sterilization period and analyzed for moisture, pH, Ec, ash, N, lignin, hemicellulose and cellulose contents. After the samples was dried in an oven at 60 °C to a constant weight, they were stored at 4 °C till analysis. Moisture and ash content of substrates were determined by following standard procedure (AOAC, 2019). Total carbon was estimated from the ash following the methodology of Tiquia and Tam (2000). Total nitrogen (TN) was determined using micro-Kjeldahl method and C:N ratios were calculated of growing media. Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were analysed following the method of Van Soest et al. (1991). Cellulose, hemicellulose and lignin were calculated using NDF and ADF and the cellulose was determined using ADF and ADL (hemicellulose=NDF −ADF; cellulose=ADF −ADL) (Zadrazil and Brunnert, 1982). All analyses were conducted in triplicate in the Field Crop Laboratories of Ahi Evran University, Kirsehir, Turkey.

Statistical Analysis

Data were statistically analyzed using the analysis of variance (ANOVA). Differences between the means of individual groups were assessed via Tukey post hoc test at a significance level of 5%.

Results

Proximate Analysis of the Substrates

The physical and chemical properties of the substrates used in the growing media preparation are given in Table 2. Although, there were no significant differences among the moisture contents of substrates ($p > 0.05$), significant differences were found among the substrates regarding the pH, Ash, C, N contents and C:N ratio of the substrates ($p < 0.01$).

Moisture content of substrates varied between 65% and 67%. Pekşen and Yakupoğlu (2009) pointed out that the moisture content of the substrate is very important and reported that 70% moisture content was the critical value for G. lucidum. High moisture content of substrate limited the mycelium growth, while low moisture content could result in the death of the pinhead.

pH values of the substrates ranged from 4.3 to 6.7 (Table 1). Medicinal mushrooms require pH of 5.5 to 6.5 optimal growing (Gurung et al., 2012). Although the pH of the sawdust substrates is slightly lower than the recomended optimum pH range by Gurung et al (2012), these values did not adversely affect mycelium growth and mushroom yield. Lower values of electrical conductivity was determined (0.63-0.87 dS /m) for forestry wastes. Ec values of other substrates (1.07-2.23 dS/m) also below the threshold value which mycelium growth is inhibited (Venancio et al., 2017).

Ash contents of the substrates ranged from 2.05% (OS) to 16.4% (WS:12.5GWH). Ash contents in WS supplemented with GWH were quite higher than that in other substrates. The amounts of C in the substrates were found between 48.5 and 56.81% . The highest C values were obtained from sawdust substrates. The highest N contents were obtained in WS supplemented with GWH in 12.5% and 25 ratios (0.71% and 0.75%, respectively). Total N contents of substrates prepared by the mixtures of oak sawdust supplement with WCH, WS and AH in 50% ratios ranged between 0.37%, 0.47% and 0.52% respectively. N contents in OS (0.29%) and PS (0.28%) were lower than that in other substrates. C:N ratio of the substrate has an important effect on mycelial growth and yield of G. lucidum (Atila, 2020) . C:N ratios varied from 65.3:1to 206.7:1 in the examinated substrates. Generally, C:N ratios of sawdust substrates were higher than that of the other substrates. The highest C:N ratio was determined in poplar sawdust, the lowest C:N ratio was found in WS:25GWH medium. There are different opinions about optimal C:N ratio for *G. lucidum* cultivation in the literature. According to Zhou and Deng (1996), *G. lucidum* requires an optimal C:N ratio of 30-40:1, but Hsieh and Yang (2004) reported that optimal C:N ratio of *G. lucidum* 70-80:1.

Table 2. Proximate analysis of growing substrates tested in the study

	Moisture (%)	pH	Ec (dS/m)	Ash (%)	N (%)	C (%)	C:N
OS	65.0	4.33**e	0.80**e	2.05** g	0.29**d	56.8**a	199.9**a
PS	65.0	4.97 cd	0.87 e	2.28 g	0.28 d	56.7 a	206.7 a
WS	67.0	5.57 b	1.28 c	12.33 c	0.41 c	50.9 e	123.2 c
WS87.5:GWH12.5	66.0	6.37 a	1.90 b	16.38 a	0.71 a	48.5 g	68.0 d
WS75:GWH25	66.0	6.70 a	2.23 a	15.71 b	0.75 a	48.9 f	65.3 d
OS:WCH	66.0	4.57 de	0.63 f	5.12 f	0.37 c	55.0 b	146.9 b
OS:AH	67.0	5.43 b	1.07 d	10.28 d	0.52 b	52.0 d	100.9 c
OS:WS	65.0	5.13 bc	1.07 d	7.91 e	0.47 b	53.4 c	113.0 c

OS:oak sawdust; PS: poplar sawdust; WS: wheat straw; GWH:gren walnut hulls; WCH:wood chips; AH: almond hulls. 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.

Significant differences (p<0.01) were found for lignocellulosic contents of the substrates. The cellulose, hemicellulose and lignin content of the substrates showed ranges of 30.6-50.8%, 2.1–23.1% and 9.43–29.5% respectively. The amount of lignin was similar for OS:WCH and OS:AH substrates and was significantly higher than that of other substrates. The maximum extent of hemicellulose content was observed in WS. The highest cellulose content was exhibited in PS, while the lowest concentration of cellulose was determined in OS:AH. Atila (2020) reported that *G. lucidum* had a preference for substrates having a high amount of cellulose and and lignin.

Table 3. Lignocellulosic content of growing substrates tested in the study.

	Hemicellulose (%)	Cellulose (%)	Lignin (%)
OS	14. 8**c	45.8**b	25.3**b
PS	13.8 c	50.8 a	22.5 c
WS	23.1 a	41.3 c	9.4 f
WS87.5:GWH12.5	9.7 de	45.8 b	10.6 f
WS75:GWH25	8.6 e	38.2 c	14.6 e
OS:WCH	10.4 d	40.1 c	28.4 a
OS:AH	2.1 f	30.6 d	29.5 a
OS:WS	17.2 b	41.1 c	19.9 d

OS:oak sawdust; PS: poplar sawdust; WS: wheat straw; GWH:gren walnut hulls; WCH:wood chips; AH: almond hulls. 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.

Effect of Substrates on Cultivation Period of *Ganoderma lucidum*

The mycelial growth of *G. lucidum* was successful in all substrates used in the study. Spawn run time (SRT), days to primodia initial (DPI) and days to first harvest (DFH) were affected by the substrates (P< 0.01).

As regards the SRT, the PS substrate was fully colonized within a period of 19.67 days after inoculation, while the respective period was significantly longer on OS:WS (28.5 days). Although SRTs of the substrated examined in the present study appeared longer than the findings Mehta et al. (2013), the incubation periods were shorter than the findings Hal et al. (2021). However, SRT values were in agreement with Rashad et al (2019) who reported that the SRT of *G. lucidum* grown on different agro-wastes was varied from18 days to 30 days.

Atila (2020) found that the SRT was positively correlated with the nitrogen content of the substrates and negatively correlated with the cellulose and hemicellulose content of the substrate, but no correlation was identified between SRP of G. lucidum and the substrates content used in the present study.

The pinheads emerged in substrates between 43th- 49th days. Zhou (2017) reported that primordia initiation had taken place around 60-70 days after spawning. On the other hand, Mehta (2013) determined the formation of G. lucidum primordia to be after 21-24 d from substrate inoculation in different sawdusts.

The period between initial primordia formation and first harvest took between 33.5-48 days. This period was longer than the findings Zhou (2017) who reported that from primordia formation to fruiting body for harvest takes approximately 25–30 days. First harvests took place between 80.5 and 93.5 days after inoculation of bags in different growing media. The period for the completion of the entire cultivation cycle was longer in WS supplemented with different ratios of GWH (12.5% and 25%) (93.5 and 93.17 days) compared with other substrates. GWH contains some compounds such as juglone which has antagonistic effect on plants (Terzi, 2008). In contrast, shorter periods of cultivation were noted in PS and OS:WS substrates by 80.5 to 81.17 days, respectively. Karma and Bhatta (2013) reported that first harvest days of fruit bodies of G. lucidum was 92 days while Roy et al. (2015) confirmed that The average first harvest days of fruit bodies of G. lucidum grown on diffrent sawdusts supplemented with bran varied between 60-90 days. These results are consistent with our findings. Differences in the SRT, DPI and DFH reported by different researchers may vary depending on the substrates, strains and climatic condition of the growing room.

Effect of Substrates on Yield and Biological Efficiency of *Ganoderma lucidum*

The total yield (g) and BE (%) of *G. lucidum* cultivated on several agro-forestry wastes are presented in Table 3. Yield and BE (%) of the mushroom were affected by the growing substrates (P< 0.01).

Total fresh mushroom yield varied from 71.39 to 110.10 g/kg of substrate. As shown in Table 3, the highest yields and BEs were obtained from sawdust media (110.10 g and 107.37 g/kg of substrate, respectively). Also, the short growing time (80.5 days) in addition to the high yield in PS is a notable advantage. Atila (2020) reported that *G. lucidum* prefer substrates having high C:N ratio. The C:N ratio of sawdust substrates were considerably higher than other substrates. The findings obtained in the study are consistent with Peksen ve Yakupoğlu (2009) who determined a negative correlation between C:N ratio and yield of *G. lucidum*. The yields of WS, AH and WCH combinated with OS were not significantly different from each other (82.88 g/kg, 83.91 g/kg, 83.98 g/kg). It has been shown that combination of AH, WCH and WS with OS does not increase the yield. Differences between yields of substrates not only depend on the physical or chemical properties of substrates but also strain used. In the past studies, Peksen and Yakupoglu (2009) reported that there was a possitive correlation between N content of substrates and biological efficiency values of *G. lucidum*, while Atila (2020) recorded a negative correlation among N content of substrates and yield of *G lucidum*. When yield of WS alone (94.84 g/kg) compared with WS supplemented with different ratio of GWH, it was noticed that supplementation of WS with GWH in 12.5% ratio did not improve yield significantly. Increasing the supplement amount of GWH from 12.5% to 25% decreased the mushroom yield. The yield of sawdust substrate was higher than wheat straw substrate, and the addition of GWH (25%) to wheat straw decreased yield by 24.7%. Yield was significantly lower in WS+GWH25 substrate (71.39 g/kg of substrate). This result could be attributed to the high content of juglane in GHW that decrease mycelial growth and yield. Juglone is the major phenolic compound in walnut green hulls (Stampar et al. 2005) . This phytotoxic compound has an inhibitory effects on plants (Zhang et al., 2008; Terzi, 2008). Atila (2019) reported that when GWH was used in Hericium erinaceus cultivation , colonisation period was prolonged and yield of the mushroom was decreased. Moreover, the substrate containing GWH failed to support mycelial growth and mushroom production of shiitake in the same study.

Present study results on mushroom yields were also similar to those of Mehta et al. (2013) which reported that the mushroom yields varied between 100 and 150 g in the sawdust substrates. However, a previous study using substrate prepared by adding 10% of wheat bran and crushed sugar cane into elephant grass and mango tree sawdust reported a yield of 160 g–360 g/kg and 32–72% BE in G lucidum (Rolim et al., 2014), which were considerably higher than those of the current study. However, the yields from the present study were higher than the results of Hal et al (2021) who declared that the yield of *G lucidum* grown on different substrate was ranged between 25.3 2 g- 49.55 g/kg. When compared to previous reports, all substrates examined in the study demonstrated satisfactory performances.

BE(%) of *G. lucidum* cultivated on several agro-forestry wastes ranged from 21.0% to 31.5% in the study. Considering the BEs from Table 4, BE of WS (28.74%%) was similar to MT (30.68%) and lower than PS (31.46%). Although, WS and WS:25 GWH substrates exhibited similar colonization times, the obtained BE of WS:25 GWH was quite lower (21%). This result is an evidence that rapid mycelium growth does not always lead to high yields.

Table 4. Effect of different substrates on cultivation cycle and yield of *Ganoderma lucidum*

	SRP (days)	DPI (days)	DPF (days)	Yield (g/kg)	BE(%)
OS	24.83**bc	47.50**ab	89.00**b	107.37**a	30.68**a
PS	19.67 d	44.17 c	80.50 d	110.10 a	31.46 ab
WS	25.50 b	43.50 c	83.83 c	94.84 b	28.74 bc
WS87.5:GWH12.5	20.83 d	44.67 c	93.50 a	92.49 bc	27.20 cd
WS75:GWH25	24.50 bc	47.17 b	93.17 a	71.39 e	21.00 f
OS:WCH	25.33 b	44.00 c	80.33 d	83.98 cd	25.45 de
OS:AH	22.17 cd	49.00 a	85.00 c	83.91 cd	23.97 e
OS:WS	28.50 a	43.83 c	81.17 d	82.88 d	24.38 e

OS:oak sawdust; PS: poplar sawdust; WS: wheat straw; GWH:gren walnut hulls; WCH:wood chips; AH: almond hulls. 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.

BE (%) previously reported for *G. lucidum* varied considerably depending on the substrate used, 13.19% to 39.62% in sawdust based substrates supplemented with tea waste at the different levels (Pekşen and Yakupoğlu, 2009), 27.57% to 42.86 % in various sawdusts, (Mehta et al. 2013), 4.54% and 61.24% in different mixtures of beech sawdust and olive by-products (Koutrotsios et al., 2019) and 8.9% to 24.7 in varios agricultural and forest wastes (Atila, 2020).

Conclusion

The results of the study demostrated that all of agriculture and forestry by-products examined in the study had a satisfactory for performances. *G. lucidum* cultivation. Although higher total yields were obtained by sawdust substrates, considering the difficulty of finding sawdust in some areas, wheat straw alone the mixture of wheat straw and green walnut hulls (87.5:12.5%) could be successfully used for cultivation of *G. lucidum*. On the other hand, since green walnut hulls has high phenolic content and antimicrobial properties, further research is recommended for pharmacological properties in *G. lucidum* mushroom grown on formulations with added green walnut hulls.

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