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Valorization of hazelnut branch pruning wastes to medicinal mushroom (Reishi-*Ganoderma lucidum*) cultivation and nutritional quality properties

Fındık budama atıklarının tıbbi mantar (Reishi-*Ganoderma lucidum*) üretiminde değerlendirilmesi ve besinsel kalite özellikleri

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Abstract

In this study, *Ganoderma lucidum* (Fr.) Karst (Reishi) was cultivated with hazelnut branches (HB) for the first time. Yield, biological activity, mushroom quality characteristics, spawn running time, and total harvesting time were determined with harvested mushroom fruiting bodies. To assess the degradation properties of HB due to *G. lucidum*, chemical analysis (holocellulose, α-cellulose, lignin, extractives, ash contents, and pH) of raw materials and cultivated mushroom composts was carried out. The changes that occurred in the structure of hazelnut branches examined according to their initial amounts. According to findings obtained from the current study, a 57.11 g/kg yield and 10.72% biological efficiency (BE) were achieved. Mean spawn running time (12.33 days), mean earliness (48.1 days), and mean total harvest time (95.1 days) were recorded. K element was the richest in mushroom fruiting body and HB. Nutritional quality properties were found to be similar with literature. After cultivation, holocellulose and pH values decreased while lignin, extractive, and ash contents proportionately increased, but α-cellulose content was not changed significantly in the study. These findings obtained from the study that hazelnut branch wastes could be usefull for the cultivation of *G. lucidum*.

Keywords: Hazelnut pruning waste, *Ganoderma lucidum* cultivation, Reishi, Waste, Biodegradation.

Özet

Bu çalışmada, *Ganoderma lucidum* ((Fr.) Karst (Reishi) mantarı fındık budama atıklarında ilk defa yetiştirilmiştir. Verim, biyolojik aktivite, mantar kalite karakteristikleri, sarım zamanı ve toplam hasat süresi mantarların meyvelerinden belirlenmiştir. Ayrıca, mantar yetiştirilmesinin öncesi ve sonrasında *G. lucidum*'dan kaynaklı fındık budama atıklarının degredasyon özellikleri, fındık budama atıklarının kimyasal analizleri (holoselüloz, α-selüloz, lignin, ekstraktifler, kül içeriği ve pH) yapılmıştır. Fındık budama atıklarının başlangıç miktarına oranla yapısında meydana gelen değişimler tespit edilmiştir. Bu çalışmada elde edilen bulgulara göre 57.11 g/kg verim ve 10.72% biyolojik etkinlik belirlenmiştir. Ortalama sarım süresi (12.33 gün), ortalama toplam hasat zamanı (95.1 gün) kaydedilmiştir. K elementi en yüksek mantar meyvesinde ve HB'de görülmüştür. Besinsel kalite özellikleri literatür ile benzer bulunmuştur. Kültivasyondan sonra holoselüloz ve pH değerleri azalırken lignin, ekstraktif vekül değerleri oransal olarak artmıştır. Fakat, α-selüloz içeriği belirgin bir şekilde değişmemiştir. Bu çalışmadan elde edilen bulgular fındık budama atıklarının *G. lucidum* yetiştiriciliğinde başarılı bir şekilde kullanılabilir olduğunu göstermektedir.

Anahtar Kelimeler: Fındık budama atığı, *Ganoderma lucidum* yetiştiriciliği, Reishi, Atık, Biyodegredasyon.

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1. Introduction

Hazelnut (*Corylus avellana* L.) is one of the most important plants for many fields of the food industry (Schiavi et al., 2022). Türkiye and Italy are responsible for 80% of the world's total hazelnut production. In 2019, Türkiye produced 776.046 tons of hazelnuts, Italy 98.536 tons, and Azerbaijan 53.793 tons, respectively. Shells and branch waste resulting from hazelnut production are seen as a significant source of concern (Puliga et al., 2022). It has been stated that 3.585×10^6 tons of pruning waste was released into nature in Italy, and 1.50×10^6 tons of it was burned in the fields (Moliner et al., 2021; Acampora et al., 2021). It has been stated that 1.7-2.1 million tons of hazelnut pruning waste were occurred into nature in Türkiye (Akcay et al., 2023; Ceylan, 2024). However, hazelnut branch pruning waste is not used as raw material in any industrial sector. Burning in the area causes environmental problems such as air pollution (Puliga et al., 2022; Guney, 2013).

In the last decade, zero waste policies have been adopted, and more environmentally friendly approaches have come to the fore for sustainable circular economies (Recepoğlu and Yüksel, 2021). For this reason, scientists are encouraged to benefit from by-products resulting from agricultural activities (Puliga et al., 2022). There are many studies previously published about the valorization of wastes generated as a result of hazelnut production and their transformation into value-added products. It has been stated that hazelnut shells are especially suitable raw materials for the creation of new MDF and chipboard materials (Çöpür et al., 2008). There are also more studies such as the production of ethanol, hydrogen, and pellets (Acampora et al., 2021; Hosgün et al., 2017; Midilli et al., 2000) from hazelnut wastes.

Mushrooms are an important food source and bioactive compounds with medicinal properties. Mushrooms such as Shiitake, Oyster and Reishi, which grow on lignocellulosic materials, can be grown in all seasons of the year. While Shiitake and oyster mushrooms are grown for culinary value, Reishi mushrooms are grown for their medicinal properties. Many lignocellulosic materials have been reported in the cultivation of Reishi mushrooms to date, but no study has been found on the valorization of hazelnut pruning wastes for the cultivation of Reishi. In our previous study, we also reported that hazelnut pruning wastes could be utilized for the production of oyster mushroom cultivation (Akçay et al., 2023).

The nutritional characteristics of mushrooms are dependent on the substrates used in the cultivation. Various factors can influence the chemical composition of mushrooms such

as organic content and biochemical constituents, pH value and mineral contents of substrates, decomposition activity of mushroom species (Garcia et al., 1998).

Ganoderma lucidum (Fr.) Karst is a basidiomycete, known as Linghzi or Reishi in China and Japan, belonging to the Polyporaceae family. In complementary medicine, *G. lucidum* has traditionally been known as a popular mushroom used to treat human diseases. There are some medicinally active ingredients in *G. lucidum* that have many properties for human health, such as anti-bacterial, anti-inflammatory, anti-tumor, anti-parasitic, anti-viral, nerve tonic, blood pressure regulation, cardiovascular disorders, kidney tonic, hepatoprotective, sexual potentiator, and chronic bronchitis (Wasser et al., 1999).

The purpose of this study was the cultivation of value-added medicinal mushroom *G. lucidum* from hazelnut branches as well as the determination of quality properties of the produced mushroom. In addition, degradation properties of HB due to *G. lucidum*, raw materials, and cultivated mushroom composts were carried out for the chemical analysis of hazelnut pruning waste (holocellulose, α-cellulose, lignin, extractives, ash contents, and pH). Chemical changes that occurred in the hazelnut pruning waste structure were examined.

2. Material and Method

2.1. Preparation of Compost Materials

Mushroom production was completed in the Mushroom Cultivation Laboratory, Application and Research Center for Recycling of Agricultural Wastes to Industry (DUTAGAM), Düzce University, Türkiye. Hazelnut branches were collected from hazelnut gardens after hazelnut harvesting. Hazelnut branches (*Corylus avellana* L.) were ground to a size of 1 cm in the Willey mill and used as the main compost material. Ground hazelnut branches were moisturized with water until the humidity reached 60%. 1% of $CaCO_3$ was added to the composts. Compost mixtures were filled into temperature-resistant bags and autoclaved at 90 ºC for 1.5 hours. Ten replicates were used for the hazelnut brunch composts. The composts were cooled after autoclaving, and *G. lucidum* mushroom spawn were inoculated into the composts at the rate of 2% according to the dry compost weight. All inoculations were carried out in the air chamber featured with UV. Compost bags were tied with plastic clips and transferred to a dark incubation room controlled automatically. *G. lucidum* spawn was purchased from Yalova Mushroom Company, Yalova, Türkiye.

2.2. Incubation and Harvest of Mushrooms

Composts were held in an air-conditioned room at 24 ± 2 °C and 65 ± 5 % (relative humidity). After the mycelium colonization was completed, the temperature in the room was increased to 28 ± 2 °C, and relative humidity was set up to 90% ± 5. Ventilation was provided for 6-7 hours for a day. 50-60 lux light was applied to the room to be able to promote the mushroom fruit body form. Fruit bodies of the mushrooms were harvested after growing processes were completed. Then their wet weights were saved on precision scales. A total of 3 mushroom harvesting periods were achieved in the current study. The following formula was used to determine the mushroom biological activities and yield of composts according to the weights of mushroom fruit bodies.

(Yield: Weight of fresh mushrooms harvested gr/ 1 kg substrate) 1

(Biological efficiency: Weight of fresh mushrooms harvested/ dry matter content of the substrate) × 100 2

In addition to yield and biological efficiency, spawn running time, first harvesting day (earliness), and total harvesting time were also determined.

2.3. Nutrient content analysis of cultivated mushrooms

Dry matter, ash, moisture, and nutrient analyses of the harvested mushrooms were carried out in Düzce University Scientific and Technological Research Application and Research Center (DÜBİT) laboratory, Düzce, Türkiye. Dry matter, ash, moisture, and nutrient element analysis of the *G. lucidum* mushrooms were determined according to procedure and explanations stated by Kacar (1994). Determination of fatty acids were carried out according to Turkish food codex regulation (2014), energy and carbohydrate FAO 77, fat NMKL 160, protein NMKL 60 (NMKL, 2024), total dietary fiber AOAC 991.43-1995 (AOAC, 1995), total sugar AOAC 968.20-1969 (AOAC, 1969), and salt Mohr's method (Korkmaz et al., 2001). These analyzes were carried out in Bilim Sağlık ve Laboratuvar Hizmetleri Tic. Ltd. Şti, Istanbul, Türkiye.

2.4. Measurement of total phenolic content (TPC) of the cultivated mushrooms

The total phenolic content of the *G. lucidum* was assessed according to the procedure reported by Singleton et al. (1999) and Demirci et al. (2021). First, 7.5 mL of distilled water was added to 100 µL of the mushroom extraction. Folin-Ciocalteu reagent and stock solution of Na_2CO_3 were then added to the mixture as 500 µL and 1 mL, respectively. After then,

the absorbance values were recorded at 720 nm. Finally, findings were obtained as mg GAE g^{-1}.

2.5. Chemical Analysis of Hazelnut Branch Wastes

2.5.1. Extractive content

The compost materials used before and after the cultivation were ground and sieved. Oven-dried samples (40 mesh) were prepared for the determination of extractives. 5 grams of control (undegraded) and degraded samples by *G. lucidum* were extracted by soxhlet with an acetone solution for 6 hours. Extracted samples were dried at 103 °C ±2 overnight. Extractive content of hazelnut branch control and degraded hazelnut branch was determined compared to the initial oven-dried weight. In this study, TAPPI T 204 cm-17 (TAPPI, 2017) standards with minor modification were used to determine the extractive content of the hazelnut branch.

2.5.2. Holocellulose content

Holocellulose contents for the hazelnut branch control and degraded samples were done according to the method of Wise and John (1952). Oven-dried and 40 mesh, 5 g of extractive-free samples were mixed with 160 mL of distilled water, 1.5 g of $NaClO_2$, and 0.5 mL of glacial acetic acid, and these mixtures were kept at 78°C in a water bath for a couple of hours. After 1 hour, the same amounts of $NaClO_2$ and glacial acetic acid were added into the mixture, and heating was continued for 1 hour. Four intervals were generated, and then the mixture was filtered by a vacuum through a por. no. 2 crucible. Acetone and cold distilled water were used for the washing of residue, and then it was dried in an oven at 103 ± 2 °C. The holocellulose of waste hazelnut branches before and after cultivation was then calculated relative to the initial dried extractive-free weight.

2.5.3. alpha-cellulose content

TAPPI T 203cm-09 standard (TAPPI, 2009) was used to determine the alpha-cellulose content of hazelnut branches before and after mushroom cultivation according to holocellulose samples. 2 g of holocellulose materials were used as the initial. 10 mL of NaOH (17.5%) solution were added at 5-minute intervals. Afterwards, it was kept in a water bath at 20°C for 30 minutes, and then 33 mL of distilled water was added to the mixture and kept at 20°C for 60 minutes. Then the mixture was filtered through a por no 2 crucible. 100 mL of 8.3% NaOH, 15 mL of 10% acetic acid, and 250 mL of distilled water were used for washing the residue. After drying the residue at 105 ± 3 °C, α-cellulose (%) content of the

branch before and after mushroom cultivation was calculated according to the oven-dried holocellulose amount.

2.5.4. Lignin content

Theoretical lignin amount was accepted as lignin content after removing the content as holocellulose amount from the materials by chlorination.

3. Results and Discussion

Mushroom properties cultivated from hazelnut branch were shown in Table 1. According to data obtained from the current study, a mean 57.11 g/kg yield was achieved from the compost prepared with the hazelnut branch. When mean biological efficiency value (BE) was examined, it was found that mean BE was 10.77%. Mean spawn running time, mean earliness, and mean total harvest time were recorded as 12.33, 48.13, and 95.1 days, respectively.

Table 1. Mushroom properties cultivated from hazelnut branch.

Mean yield (g/kg)	57.11 (15.5)
Mean BE (%)	10.77 (2.5)
Mean spawn run time (day)	12.33 (1.36)
Mean earliness (day)	48.1 (6.83)
Mean total harvest time (day)	95.1 (6.83)

Note: The values in parentheses are standard deviations

When our study was compared with the previous studies of Pekşen and Yakupoglu (2009), yield values were similar while BE values were found lower. Pekşen and Yakupoglu (2009) used tea waste as a supplement and found yield values of *G. lucidum* between 42 and 87 g/kg and BE values between 16 and 34%. Regarding the BE value, Gurung et al. (2012) found BE between 7.81% and 22.62%. They used the sawdust of *Alnus nepalensis*, *Shorea robusta*, and *Dalbergia sissoo* trees and some supplements such as rice bran, wheat bran, corn flour, and gram flour as substrates in *G. lucidum* cultivation. When the current study is compared with the literature, BE values are well agreement with Gurung et al. (2012). However, very low BE values were also recorded in their study regarding *Shorea robusta* (BE: 0.00%). Differentiations between BE values of sawdust substrates may be due to tight packing, poor aeration, and low water-holding capacity of sawdust. Another reason for the difference between BE values may be due to the different nutritional quality of the substrates used. Kurd-Anjaraki et al. (2022) found spawn run time to be max (36.66 days) and min (21.11 days). Gurung et al. (2012) found between 30 and 35 days, depending on different substrates and supplement types. It seems that spawn of *G. lucidum* is growing and spreading

very fast on hazelnut branches used in the current study. Observing higher mycelium spawn run stages could be related to the amounts of nitrogen in the substrates. It was reported that plant materials with low C/N ratios are degraded more rapidly than those with high ratios, which is indicating that mycelium growth rate is related to nitrogen availability in the substrate used (Harith et al., 2014). The spawn run time of the mycelium also varies depending on the type of strain, substrate, and mineral elements, as well as the nitrogen contents in the substrate (Adenipekun and Gbolagade, 2006).

However, increasing the amount of N in substrates can cause a decrease in lignin degradation, which lead to reduce mycelium growth rate (Kurd-Anjaraki et al., 2022). Mean earliness was found between 99 and 65 days according to strain and substrate type in a study conducted by Kurd-Anjaraki et al. (2022). When our study results regarding earliness day (48.1 days) were compared with previous studies, our results showed shorter harvesting of the first fruiting body.

Figure 1. Cultivated Reishi (*G. lucidum*) mushrooms from hazelnut branch pruning wastes.

When chemical analysis of hazelnut branches before cultivation was examined, 67% holocellulose, 57.4% α-cellulose, and 33% theoretical lignin was found in the cell wall component. On the other hand, 0.84% extractive content, 2.43% ash content, and 7.17 pH value were recorded before cultivation (Table 2). After cultivation, holocellulose, α-cellulose, and pH value decreased proportionately while lignin, extractives, and ash values increased. Similar results regarding increases in the rates of ash and extractive contents were also found in studies conducted by Akcay et al. (2023) and Zhang et al. (2002).

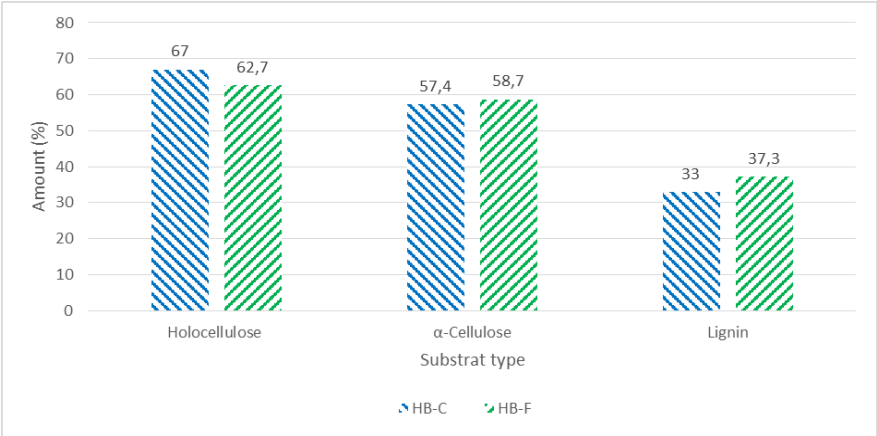

Figure 1. Chemical change of hazelnut branch after Reishi cultivation (HB-C: Hazelnut Branch Control/Not fungal degraded, HB-F: Hazelnut Branch Fungal Degraded /After fungal attack).

Gençer and Özgül (2016) conducted a study and they showed that holocellulose, alpha cellulose, lignin, ash, and extractive substance in the hazelnut branch control samples (undegraded) were found to be 82.07%, 41.33%, 15.89%, 0.72%, and 2.83%, respectively. The component values of the hazelnut branch were found to be lower than those of Gençer and Özgül (2016). According to findings obtained from the current study, *G. lucidum* affected the holocellulose component of the hazelnut branch. Cell wall component structure of lignocellulosic materials is an important factor in mushroom yield. It was reported that high cellulose and lignin content prolong the mushroom harvesting time compared to those with low content. Similar results were also obtained regarding the proportionately increase in extractive and ash content while decreasing pH in our previous study in which *Pleurotus ostreatus* mushrooms were cultivated in the various lignocellulosic materials (Akcay et al., 2023*)*. After cultivation, changes occurred in the components of the hazelnut branch that are attributed to secreting large amounts of enzymes by the fungi *G. lucidum*. During the cultivation, the enzymes secreted by *G. lucidum* cause the degradation of holocellulose, α-cellulose, and lignin, but the enzymes act selectively. Therefore, the components were degraded at different rates.

Table 2. Chemical changes in extractives, ash and pH after cultivation.

Substrate	Extractives (%)	Ash (%)	pH
HB (Before cultivation)	0.84 (0.11)	2.43 (0.23)	7.17
HB (After cultivation)	3.39 (1.67)	8.1 (2.70)	4.46

HB: Hazelnut Branch, *the values in parentheses are standard deviations

Fruit body size, mean total moisture content, and mean dry matter values of the mushrooms cultivated on hazelnut branches can be seen in Table 3. According to Table 3, mean mushroom fruit body dimensions were measured at 9x6 cm. Mean dry matter content was found to be 27.7%. These results were in agreement with Khoo et al. (2022).

Table 3. Some mushroom quality properties.

Compost formulation	Fruit body diameter (cm)	Mean total moisture (%)	Mean dry matter (%)
HB (Hazelnut branch)	9x6	72.3 (3.39)	27.7(3.39)

Table 4 shows elemental and ash results of MF (Mushroom fruitbody) and HB. According to obtained results, the highest element was found to be P by 6380.9 mg/kg, while the lowest was B element by 2.1 mg/kg in MF. When Table 4 investigated that K (4010.3 mg/kg) was found the highest while Cu was found as 3.8 mg/kg in HB. Peksen and Yakupoglu (2009) also found K as the highest element in mushroom fruit bodies cultivated from tea wastes. For this reason, the results obtained from the current study were well agreement with those reported by Peksen and Yakupoglu (2009). K was found to be the highest element in raw material in both their study and our current study.

Table 4. Some elemental contents of Mushroom MF and HB.

Material Type	mg/kg									
	P	K	Ca	Mg	Fe	Cu	Zn	Mn	B	Ash (%)
MF	6380.9	10617.5	226.6	1014.8	66.6	30.7	38.9	7.4	2.1	7.3
HB	1159.3	4010.3	1724.2	311.2	28.9	3.8	8.3	90.5	5.8	1.3

MF: Mushroom Fruitbody, HB: Hazelnut Branch Waste

Table 5 shows some important nutritional contents in 100 g of mushrooms cultivated from hazelnut branches in the study. Protein amount of mushrooms was found to be 17.39 g. Peksen and Yakupoglu (2009) found similar protein amounts (between 13.59% and 16.34%) in *G. lucidum* cultivated from tea waste. Total carbohydrate value (68.6 g/100 g) in the current study was found to be higher than carbohydrate value (26–28%) in the study conducted by Mau et al. (2001). They also found that *G. lucidum* contains 1.8% ash, 3–5% crude fat, 59% crude fiber, and 7-8% crude protein. Since total phenolic content (TPC) is important for the antimicrobial properties, it was determined in the study. 2.1 mg GA/g was found in the *G. lucidum* mushroom cultivated from HB. Demirci et al. (2021) determined TPC ranged from 2.35 to 10.46 mg GA/g in commercial Reishi mushroom products in powder form. Ćilerdžić et al. (2014) reported that TPC of *G. lucidum* cultivated on wheat

straw varied from 28.06 mg GAE/g to 52.15 mg GA/g. When our results were compared to other studies, lower TPC values were obtained. Raw material used in the mushroom cultivation and method for the determination of the TPC may affect the obtained different TPC values.

Table 5. Some important nutritional contents in 100 g mushroom cultivated from hazelnut branch wastes.

Crude fat (g/100g)	5.6
Saturated fatty acids (g/100g)	3.5
Mono unsaturated Fatty Acids (g/100g)	1.61
Poly unsaturated fatty acids (g/100g)	0.49
Trans fatty acids (g/100g)	nd
Total sugar (g/100g)	1.95
Total carbohydrate (g/100g)	68.6
Protein (g/100g)	17.59
Total dietary fiber (g/100g)	61.18
Salt (g/100g)	0.64
TPC (mg GA/g)	2.1
Energy (kcal/kj)	272/1141

nd: not detected, TPC: total phenolic content

4. Conclusion

In this study, hazelnut branch wastes were evaluated as compost material for *G. lucidum* mushroom cultivation. According to the current study, *G. lucidum* yield was obtained at 57.11 g/kg, which were similar results with previous studies conducted in the literature. Mean earliness time (48.1 days) has been found shorter compared with literature. K element was found the highest in cultivated mushrooms and HB materials. Total sugar, carbohydrate, protein, dietary fiber, salt in 100 g mushroom indicated that nutritional quality values were accordance with literature. After cultivation, holocellulose and pH values of substrates were proportionately decreased while lignin, extractives, and ash values increased. This study indicated for the first time the potential valorization of recycling hazelnut branch waste to generate *G. lucidum* mushroom growth, sustaining zero-waste production.

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