DOI: 10.29050/harranziraat.1440438
Harran Tarım ve Gıda Bilimleri Derg. 2023, 28(2):235-247

Research Article/Araştırma Makalesi

Effects of cultivation substrate composition on biological productivity and quality parameters of *Ganoderma lucidum*

Yetiştirme substratı kompozisyonunun Ganoderma lucidum'un biyolojik verimliliği ve kalite parametreleri üzerine etkileri

Gülsüm İNCE[1], Mustafa Kemal SOYLU[2], Nuray ÇÖMLEKÇİOĞLU[3*]

[1]Eskişehir Osmangazi University, Institute of Science, Eskişehir, Türkiye
[2]Atatürk Horticultural Central Research Institute, Yalova /Türkiye
[3*]Eskişehir Osmangazi University, Faculty of Agriculture, Department of Horticulture, Eskişehir, Türkiye
[1]https://orcid.org/0000-0002-3596-6134; [2]https://orcid.org/0000-0003-3492-0043; [3]https://orcid.org/0000-0001-7189-613X

To cite this article:
İnce, G., Soylu, M. & Çömlekçioğlu, N. (2024). Effects of cultivation substrate composition on biological productivity and quality parameters of *Ganoderma Lucidum*. Harran Tarım ve Gıda Bilimleri Dergisi, 28(2):235-247

DOI: 10.29050/harranziraat.1440438

Address for Correspondence:
Nuray ÇÖMLEKÇİOĞLU
e-mail: ncomlekcioglu@ogu.edu.tr

Received Date:
20.02.2024
Accepted Date:
12.05.2024

ABSTRACT

A substrate composition experiment was carried out to utilize the waste parts of certain plants for cultivation of Ganoderma lucidum, a medicinal mushroom. Ganoderma mycelium (millet spawn) provided by the Atatürk Horticultural Central Research Institute (Yalova, Türkiye). Nine (9) different substrate recipes were used in this study; S1: 40% sawdust + 40% chickpea stalk + 20% bran, S2: 60% sawdust + 20% chickpea stalk + 20% bran, S3: 40% sawdust + 40% pea stalk + 20% bran, S4: 60% sawdust + 20% pea stalk + 20% bran, S5: 40% sawdust + 40% poppy stalk + 20% bran, S6: 60% sawdust + 20% poppy stalk + 20% bran, S7: 40% sawdust + 40% corn cob + 20% bran, S8: 60% sawdust + 20% corncob + 20% bran and S9 (Control): 80% sawdust + 20% bran. Mushroom fruiting bodies were obtained from all substrates. Biological efficiency (BE) was varied between 7.84 and 17.92%. BE in S2, S5, S6, S7, S8 and S9 was higher than S4, S3 and S1. The highest total yield was recorded in S6 with 88.38 g 1.5 kg bag-1. The earliest mushroom was harvested from corncob (S8) and sawdust (S9) recipes within 51 days. The protein content of fruiting bodies ranged from 31.51% to 51.4%. Nitrogen, potassium, calcium, magnesium and iron content of fruiting bodies were increased by mixing 40% corncob to substrate and zinc was enriched by adding 20% chick pea stalk. Adding corncob to the substrate may enrich the Ganoderma fruiting body protein and mineral content without decreasing the yield and biological efficiency.

Key Words: Medicinal mushroom, substrate, reishi, protein, biological efficiency

ÖZ

Tıbbi bir mantar olan Ganoderma lucidum'un yetiştirilmesi için bazı bitkilerin atık kısımlarını değerlendirmek amacıyla bir substrat bileşimi deneyi gerçekleştirilmiştir. Ganoderma tohumluk miselleri (darıya sardırılmış) Atatürk Bahçe Bitkileri Merkezi Araştırma Enstitüsü'nden (Yalova, Türkiye) sağlanmıştır. Bu çalışmada dokuz (9) farklı substrat reçetesi kullanılmıştır; S1: %40 talaş + %40 nohut sapı + %20 kepek, S2: %60 talaş + %20 nohut sapı + %20 kepek, S3: %40 talaş + %40 bezelye sapı + %20 kepek, S4: %60 talaş + %20 bezelye sapı + %20 kepek, S5: %40 talaş + %40 haşhaş sapı + %20 kepek, S6: %60 talaş + %20 haşhaş sapı + %20 kepek, S7: %40 talaş + %40 mısır koçanı + %20 kepek, S8: %60 talaş + %20 mısır koçanı + %20 kepek ve S9 (Kontrol): %80 talaş + %20 kepek. Tüm substratlardan mantar elde edilmiştir. Biyolojik verimlilik (BE) %7.84 ile %17.92 arasında değişmiştir. S2, S5, S6, S7, S8 ve S9'daki BE, S4, S3 ve S1'den daha yüksek bulunmuştur. En yüksek toplam verim 88.38 g 1.5 kg torba-1 ile S6'da kaydedilmiştir. En erken hasat edilen mantar 51 gün ile mısır koçanı (S8) ve talaş (S9) substratlarından elde edilmiştir. Mantarların protein içeriği %31.51 ile 51.4 arasında değişmiştir. Mantarların nitrojen, potasyum, kalsiyum, magnezyum ve demir içeriği

substrata %40 mısır koçanı karıştırılarak arttırılmış, %20 bezelye sapı eklenerek çinko zenginleştirilmiştir. Substrata mısır koçanı eklenmesi, verimi ve biyolojik etkinliği azaltmadan Ganoderma mantarının proteini ve mineral içeriğini zenginleştirmiştir.

Anahtar Kelimeler: Tıbbi mantar, substrat, reishi, protein, biyolojik etkinlik

Introduction

Ganoderma lucidum (W. Curt.: Fr.) P Karsten is a species belonging to the genus *Ganoderma*, family Ganodermataceae. *Ganoderma* species are found throughout the world, and different characteristics such as cap shape and color, host specificity, and geographical origin are used to identify individual members of the species. *G. lucidum* (reishi in Japan, Ling zhi in China) is a fungus with a distinctive appearance with a shiny, reddish-brown cap and woody texture. However, morphological characteristics are subject to variation resulting from differences in cultivation under different climatic conditions and in different geographical regions, and from the natural genetic evolution of individual species (Wachtel-Galor et al., 2011).

G. lucidum is a medicinal mushroom with a long history of use in traditional Chinese medicine (Zhou, 2017; Amiri-Sadeghan et al., 2022). Due to its potential health benefits, it is in great demand. It contains various bioactive compounds, including triterpenoids, polysaccharides, anti-inflammatory and antioxidants, which are believed to contribute to health benefits (De Silva et al., 2012; Bishop et al., 2015; Taofiq et al., 2017; Khatian and Aslam, 2018; Liu and Tie, 2019). In their detailed reviews, Cao et al. (2018) and Cör et al. (2018) reported that reishi contains triterpenoids and polysaccharides with high-grade biological activity, proven by animal and clinical studies, which enhance immunity and show antitumor, antimicrobial, anti-inflammatory and antioxidant activity. It also contains high amounts of various compounds such as proteins, lipids, phenols, sterols, etc.

G. lucidum is commercially cultivated in many countries, including Asia (China, Japan, and Korea) United States of America and Europe. It is the most popular and widely traded medicinal mushroom in the world. Many commercial products derived from *G. lucidum* mushrooms, mycelia or spores are sold in various (dried mushrooms, powdered extracts, tea, coffee, syrups, capsules, toothpastes, soapsn, a d lotions, and even as ingredients in some food and beverage products) forms (Atila, 2022).

Ganoderma species are saprophytic or parasitic fungi that feed on trees. They can grown on substrates sterilized sawdust and agricultural waste. Thus, they greatly help the cycling of plant and animal waste by converting plant waste into food (Hall et al., 2003). This is also very important for food sustainability.

During the process of cultivating reishi mushrooms, several researchers have added numerous additives (such as wheat and corn and rice bran, soybean meal, cottonseed meal, malt, sugarcane bagasse, sunflower meal or molasses) to corncobs and straw (Veena and Pandey, 2011; Rashad et al., 2019; Yuliana et al., 2020), sawdust of oak, mango, acacia, tuni, paddy straw, wheat straw, and soybean waste (Jandaik et al., 2013), sawdusts of sheesham, mango, and poplar (Mehta et al, 2014), sunflower seed husks (Gonzalez-Matute et al., 2002), paddy husk and plant waste (Yang et al., 2003; Rashad et al., 2019; Yuliana et al., 2020), tea waste (Peksen and Yakupoğlu, 2009), sawdust of various trees (Erkel, 2009; Nguyen et al., 2019; Atila, 2020; Adongbede and Atoyebi, 2021), olive plant residues (Koutrotsios et al., 2019), broad bean stalks, cotton stalks and wheat straw (Rashad et al., 2019; Atila, 2020), soybean straw and bean straw (Atila, 2020), hazelnut shells (Puliga et al., 2022) and found differences among the substrates. Growers generally prefer to use the best and cheapest substrate materials available locally (Özçelik and Pekşen, 2007). Researchers and producers have focused on using locally available agricultural waste materials and their economic

output to search for substrates for mushroom growing, to achieve more sustainable management and improve efficiency. Substrate selection and cultivation methods may vary depending on local conditions and available resources. Therefore, different substrate options should be investigated regionally for successful reishi cultivation.

Cultivation of *G. lucidum* has become increasingly widespread in Turkey in recent years, but its commercial production is extremely limited (Eren and Pekşen, 2019). For this reason, studies on substrate formulation using various plant wastes locally are also limited. This study aimed to evaluate some locally available agricultural wastes (chickpea, pea, corncob and poppy plant wastes) for formulation of the substrate in *Ganoderma* cultivation and to determine their effects on yield and quality characteristics.

Material and Methods

The millet spawn of *G. lucidum*, used in this experiment, was provided by Atatürk Horticultural Central Research Institute (Yalova, Türkiye). Pea (*Pisum sativum*) stalk, poppy (*Papaver somniferum*) stalk and chickpea (Cicer *arietinum*) stems, corncob (*Zea mays*), beech (*Fagus orientalis*) sawdust and wheat (*Triticum aestivum*) bran were used for substrate formulations. Nine (9) different substrates, including control, were compared in the experiment (Table 1).

The agricultural wastes used in the experiment were grounded to appropriate sizes (1-2 cm) by using a feed crushing machine. According to the substrate recipe, plant wastes were weighed, moistured and taken into containers. For pH measurement, 50 ml of distilled water was added to 20 g of substrate of each treatment, the mixture was filtered after 2-3 hours and measured with a pH meter. pH values were measured and lime/gypsum were added to bring the pH to 5.5-6.5 before sterilization. The mixed substrates as 1.5 kg was filled into the heat resistant polypropene bags. The plastic stick was placed in the middle of the bags for sowing and the bags were closed with plastic caps.

Table 1. Substrates composition (as substrate dry weight ratio)

Substrate	Formulation
S1	40% beech sawdust + 40% chickpea stalks + 20% wheat bran
S2	60% beech sawdust + 20% chickpea stalks + 20% wheat bran
S3	40% beech sawdust + 40% pea stalks + 20% wheat bran
S4	60% beech sawdust + 20% pea stalks + 20% wheat bran
S5	40% beech sawdust + 40% poppy stalks + 20% wheat bran
S6	60% beech sawdust + 20% poppy stalks + 20% wheat bran
S7	40% beech sawdust + 40% corncob + 20% wheat bran
S8	60% beech sawdust + 20% corncob + 20% wheat bran
S9 (control)	80% beech sawdust + 20% wheat bran

Many bacterial, fungal, and viral diseases and pests cause significant yield losses in mushroom cultivation. In cultivation, the substrate is the main source of some of the microbial diseases. Therefore, disinfection of the substrate at appropriate temperatures is extremely important (Öztürk et al., 2017; Öztürk et al., 2017b; Aydoğdu and Kurbetli, 2021). For these reasons, the prepared bags were sterilized at 121 °C and 1.2 atm pressure for 120 min. When the substrate temperature decreased to room temperature, the plastic stick was removed, and 75 g of spawn was inoculated in each bag and the bags were placed in the incubation room. The incubation room was set to 25-26 °C and humidity to 65-70%. After the substrates were completely covered with

mycelium, the bags were taken into the cultivation room and the cap of the bags opened. The growing room is set to 70-80% humidity, 25-26 °C temperature, and 400 lux for 10 hours a day lighting. The mushrooms were harvested once the white part on the edges turned red and reached the appropriate size.

In the dried and ground substrate samples, nitrogen (N) % according to Kjeldahl method (Kacar, 1972) moisture % and ash ratio were determined according to the modified (AOAC, 2019). Carbon (C) content was calculated as 50% of the organic matter by subtracting ash content from 100% (Cormican and Staunton, 1991). C:N ratio was calculated. The incubation period, pin formation, and fruiting body formation duration were determined in days.

The amount of product collected from two flashes was evaluated as total yield per bag (g bag^{-1}). Percent biological yield (% BE) was determined by dividing the fresh mushroom yield by the dry weight of the substrate (Bernabe-Gonzalez et al., 2015).

Fruiting body weight and cap diameter, protein and nitrogen content, dry matter and ash content (AOAC, 2019) were determined. For mineral matter analysis, mushroom samples were dried in an oven at 65 °C until constant weight was reached and ground in a mill to a fineness of 20 mesh. For the analysis of phosphorus, potassium, calcium, magnesium, iron and zinc, 0.5 g samples were taken, 10 ml HNO3 (nitric acid) was added and wet digestion was carried out in a microwave oven under high temperature. These samples then transferred to a 50 ml container, the volume filled with deionized water and filtered through blue band filter paper. The amount of elements in the obtained filtrates measured by ICP-OES (Kacar and Inal, 2008).

The experiment was conducted according to the Randomized Plot Experiment Design with six replications. The data obtained was analyzed in Tarist (Açıkgöz et al., 2004) statistical program. Percentage values converted to angle values and analysis of variance applied. The means compared by the Least Significant Difference (LSD) test.

Results and Discussion

The chemical properties of the substrates are given in Table 2. pH value (5.67-6.30) of substrates was slightly acidic after sterilization. The pH of the substrate has a significant effect on fungal growth and development. Suitable pH management can directly affect mushroom yield and quality (Bellettini et al., 2019). Most mushroom species tend to grow best in the pH range of about 5.5 to 7. If the pH of the substrate is too low or too high, mycelial growth can be inhibited. This can lead to slow colonization and potential contamination problems (Erkel, 2009; Renu and Brij, 2015; Gurung et al., 2012; Kara et al., 2021; Atila, 2020; Carrasco et al., 2018; Nguyen et al., 2023).

Significant differences were also found in the moisture contents of the substrates (Table 2). The moisture contents of substrates were ranged from 63.74 to 58.14%. Suitable moisture levels are important for *Ganoderma* to form fruiting body. The moisture contents of the substrates should be kept relatively around 60-75% (Aghajani et al. 2018). Sufficient humidity prevents the mycelia from drying out and promotes fungal growth. Yakupoğlu and Pekşen (2011) reported the humidity range as 60.93-63.01% in substrates created with different plant wastes. Jeewanthi et al. (2017) adjusted the substrate moisture to 65%.

The highest C was 48.13% in S8 and the lowest value was 45.59% in S1 medium. According to the substrate composition, N content varied between 0.77% and 1.52%. The S3 medium, using pea stalks, had the highest N content, while the S9 medium (control) consisting of beech sawdust and wheat bran had the lowest N content. Accordingly, the C/N ratio was highest in S9 (62.12) and lowest in S3 (30.14).

The C contents of substrates were ranged from 45.59 to 48.13%. The C content of S8 formulation was the highest and S1 was the lowest. The N contents of substrates were varied between 0.77 to 1.52%. The calculated C/N ratios were ranged

from 30.14 to 62.19 (Table 2).

Table 2. pH, moisture, carbon, nitrogen, C/N and ash rate of substrates

Substrat	pH	Moisture (%)	Carbon-C (%)	Nitrogen- N (%)	C/N	Ash (%)
S1	5.75 f	58.14 ı	45.59 ı	1.18 b	38.63 d	8.82 a
S2	5.68 g	63.74 a	47.17 f	1.02 c	46.24 c	5.65 d
S3	6.30 a	62.10 d	45.79 h	1.52 a	30.14 e	8.41 b
S4	5.95 d	58.76 h	47.10 g	1.21 b	38.93 d	5.80 c
S5	5.94 d	63.24 b	47.24 e	1.06 c	44.62 c	5.53 e
S6	5.88 e	61.34 e	47.73 c	0.86 d	55.32 b	4.54 g
S7	6.16 b	62.74 c	47.66 d	1.02 c	46.71 c	4.69 f
S8	5.67 g	61.26 f	48.13 a	0.83 d	58.33 b	3.74 ı
S9	5.98 c	61.25 g	47.86 b	0.77 e	62.12 a	4.28 h
LSD p≤0.01	0.014	0.271	0.001	0.046	3.756	0.040

Mean values marked with a different letter in each column are statistically different from each other

Ganoderma mushrooms are lignin and cellulose-degrading fungi (Cheureuil et al., 2022). C and N are the two main macronutrients needed by fungi for their structural and energy requirements (Carrasco et al., 2018). The C content of fungal substrates plays an important role as a nutrient source in their growth and development. Carbon is essential for energy production and biomass synthesis. Nitrogen is important for protein synthesis and various enzymatic reactions. Carbon-rich substrates provide the necessary energy for efficient proliferation of mycelia and products (Hu et al., 2020). Lonardo et al. (2020) reported that the concentration and availability of N affect the activity and growth efficiency of saprotrophic fungi. When the N of substrates is scarce, growth efficiency may decrease. They found that low C:N ratio resulted in the highest biomass production as well as the highest growth efficiency. While carbon content is important, the overall composition and nutrient balance of the substrate, including nitrogen, minerals, and other organic compounds, also play important roles in Ganoderma mushroom cultivation (Atoji-Henrique et al., 2017). The C/N ratio of mushroom substrate is a critical factor in mushroom cultivation. A favorable C/N ratio provides the necessary nutrients to sustain strong mycelial growth and maintain healthy fungal metabolism. A balanced C/N ratio promotes the breakdown of organic compounds in the substrate such as lignin and cellulose. The ideal C/N ratio for Ganoderma mushrooms varies depending on the species, substrate composition and cultivation method. In general, a C/N ratio of approximately 20:1, 30:1, 40:1 to 60:1 is considered suitable for reishi cultivation (Atoji-Henrique et al., 2017; Atila, 2022; Amiri-Sadeghan et al., 2022; Bellettini et al., 2019; Fraga et al., 2014; Kumla et al., 2020). Hsieh and Yang (2004) reported that C/N ratio is a very important factor for mycelial growth rate and cap formation for reishi. They found that the fastest mycelial growth (16-18 days) in test tubes occurred in the medium with a C/N ratio of 70-80. The fungus formed only in the medium with a C/N ratio of 70 and 80.

Significant differences in ash ratios were determined depending on the substrate composition. The highest ash rate was determined as 8.82% in S1 (containing 40% chickpea stalks). Ash rate was 8.41% in S2 (containing 20% chickpea stalks). On the other

hand, the ash ratio was the lowest in S8 (containing 20% corncob) as 3.74%.

Mycelia, primordium, and mushroom formation duration are presented in Table 3. The fastest mycelial development was 18 days in S7, S8 and S9 substrates. The latest mycelial growth was 26.50 days in S1, 25.50 and 25.83 days in S2 and S3, respectively. According to the substrate content, mycelial development duration, pin and mushroom formation duration can give very different results (Atila, 2022; Gurung et al., 2012; Thiribhuvanamala and Krishnamoorthy, 2021). Yakupoğlu and Pekşen (2011), Jeewanthi et al. (2017) and Atila (2020) reported mycelial development duration as 55 to 59, 25.4 to 34.2 and 14.2 to 18.2 days, respectively.

The differences among the substrates in terms of primordium formation duration were found to be statistically significant. The shortest primordium formation duration was 36.66 days in S4 and the longest duration was 41.66 days in S3. Substrate composition significantly affected the duration of fruiting body formation. In S8 and S9, where mycelial development was the fastest, fruiting body formation was also the earliest with 51 days. The latest fruiting body formation duration was observed as 63 days in S2. Bernabe-Gonzalez et al. (2015) reported the mushroom formation duration as 70-72 days and Gurung et al. (2012) reported the harvest duration of different substrates as 60-66 days.

Among the substrates, the highest mushroom yield was obtained from substrates with a high C/N ratio. Yields were low in substrates with C/N ratio lower than 40/1 (S1, S3 and S4). The yield per bag was statistically in the same group in S2, S6, S7, S8 and S9 and varied between 88.38 g (S6) and 70.31 g. The lowest yield was 17.25 g bag^{-1} in S3 medium (Table 4).

Table 3. Mycelial growth, primordium and fruiting body formation duration according to substrate

Substrat	Mycelial development growth duration (days)	Primordium formation duration (days)	Fruiting body formation duration (days)
S1	26.50 a	40.33 abc	55.33 c
S2	25.50 a	39.50 bc	63.00 a
S3	25.83 a	41.66 a	55.00 c
S4	23.33 b	38.66 c	55.00 c
S5	22.50 b	41.50 a	60.33 b
S6	20.33 c	40.66 ab	56.00 c
S7	18.33 c	40.83 ab	55.33 c
S8	18.83 c	39.33 bc	51 d
S9	18.66 c	39.50 bc	51 d
LSD p≤0.01	2.010	1.746	2.069

Mean values marked with a different letter in each column are statistically different from each other

Atila (2020) reported the highest total yield of *Ganoderma lucidum* as 86.1 g kg^{-1} and the lowest as 28.6 g kg^{-1}. Baktemur et al. (2022) determined the highest yield as 53.90 g kg^{-1} from 2 groundnut + 1 wheat bran substrate and the lowest yield value as 14.63 g kg^{-1} from oak sawdust medium.

As seen from the Table 4 biological efficiency (BE) was the highest in S2 (17.61%), S5 (17.92%) and S9 (17.91%) and the lowest in S3 (7.84%) and S1 (11.01%). Yield and BE in *Ganoderma* cultivation can vary depending on several factors, including substrate composition, environmental conditions, strain selection and cultivation practices (Amiri-Sadeghan et al., 2022; Atila, 2022; Erkel 2009; Jeewanthi et al., 2017; Renu and Brij, 2015). The C content of the substrate can affect the yield and quality of *Ganoderma* mushrooms. The availability of sufficient carbon compounds in the substrate ensures that the mushrooms have sufficient nutrient supply for optimum growth. Insufficient C content can result in slower growth, reduced yield, and poor quality of harvested mushrooms. BE is an indicator of how effective the substrate is in mushroom production. A high

BE percentage indicates an efficient conversion process. In the study of Pekşen and Yakupoğlu (2009) on tea waste substrete, the BE range was between 31.0-34.9%. Atila (2020) reported that the BE calculated from reishi grown on substrate containing cottonseed meal and oak sawdust was changed from 8.9 to 24.2%. Increasing BE increases the profit in production. Thus, it can be considered as the first item examined in commercial production.

The composition of the growing medium significantly affected the average mushroom weight. The highest average mushroom weight was 71.08 g in S2 medium and the lowest was 17.25 g in S3 medium (Table 4). Yakupoğlu and Pekşen (2011) and Bernabe-Gonzalez et al. (2015) reported that the mushroom weight was in the range of 31.19 to 7.99 g and 40.9 to 47.9 g, respectively.

Table 4. Mushroom yield and certain quality parameters according to substrate formulation

Substrate	Yield (g bag^{-1})	Biological efficiency-BE (%)*	Mushroom Weight (g)	Fruiting body Diameter (mm)
S1	32.14c	11.01 d	32.09c	93.62b
S2	87.46 a	17.61 a	71.08a	122.29a
S3	17.25 d	7.84 e	17.25d	85.05d
S4	45.39b	13.11c	45.39b	91.70c
S5	75.65 a	16.40 b	56.78a	97.21b
S6	88.38a	17.92 a	67.75a	109.30a
S7	72.62 a	16.12 b	72.62a	116.47a
S8	70.31 a	16.24 b	70.39a	126.03a
S9	87.43a	17.91 a	62.22a	111.38a
LSD p≤0.01	26.229	0.326	16.012	23.241

Mean values marked with a different letter in each column are statistically different from each other

* Percentage values converted to angle values and analysis of variance applied.

There is a positive correlation between yield and C/N and C, and a negative correlation between N. The correlation between yield and moisture and pH was not significant (Table 5).

Table 5. Relationship between various parameters measured in substrates and yield

Variable	by Variable	Correlation	Signif Prob	Plot Corr
Yield	C/N	0,8259	P<0,0061	
Yield	C	0,8410	P<0,0045	
Yield	N	-0,8770	P<0,0019	
Yield	pH	-0,3925	0,2961	
Yield	Moisture	0,5088	0,1619	

The regression curve between yield and C/N, C and N is given in Figure 1 below.

Cap diameter differed significantly according to substrate composition. The highest value was recorded in S8 (126.03 mm) and the lowest in S3 (85.05 mm) (Table 4). Hal et al. (2021) recorded the cap diameter of reishi mushroom in the range of 50.63 - 45.23 mm. Veena and Pandey (2011) found cap diameter in the range of 73 to 93 mm. In a study comparing the reishi mushrooms, the average cap width of mushrooms collected from nature was 14.2 cm, while the average cap width of mushrooms cultivated on orange stumps was found to be 13.6 cm (Turfan et al., 2016). The size of the fruiting bodies of Ganoderma can vary depending on the species and environmental conditions. Typically, the upper part, the cup, is circular to semicircular, fan-shaped or kidney-shaped, 2-20 (35) cm wide (Vishwakarma et al., 2011).

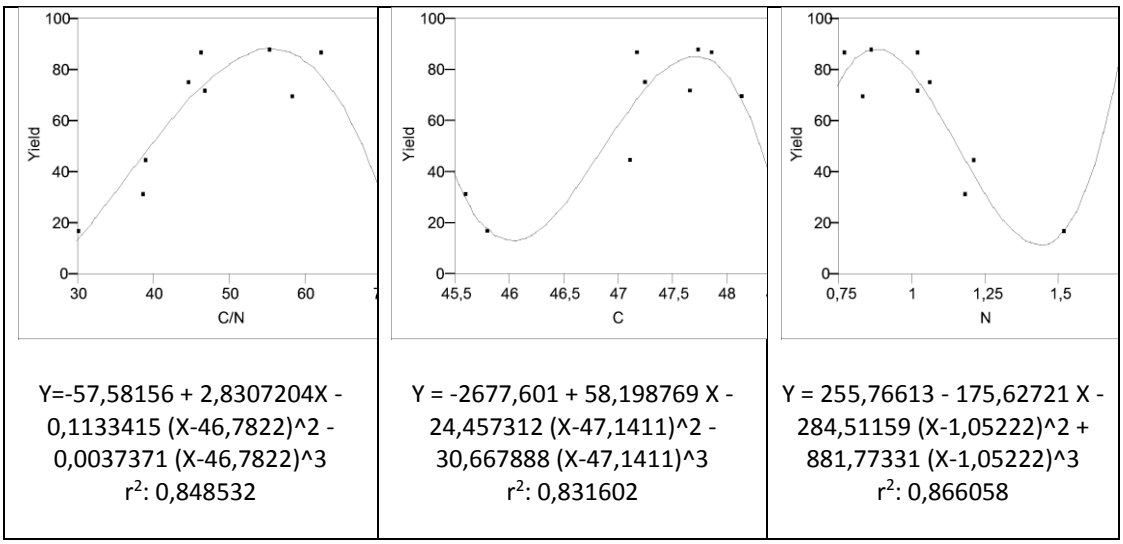

Figure 1. Regression graph between Yield-C/N, Yield-C and Yield-N

The protein and dry matter contents of mushrooms harvested from different substrates are given in Table 6. The highest protein content was 51.41% in S7 medium, the lowest value was 31.51% in S3 medium and the differences between the media were found to be significant. The protein content of *Ganoderma* mushrooms varies according to the substrate composition (Hsieh and Yang, 2004; Fraga, 2014; Osunde et al.; 2019; Hal et al. 2021). Although protein content is very important in most edible mushrooms, it loses its significance in medicinal mushrooms.

It was determined that the differences between the dry matter ratios were significant. The highest dry matter was calculated as 34.07% in S1 medium. The lowest value was found as 27.72% in S6 medium. Baktemur et al. (2022) measured the dry matter content of mushrooms harvested from different media between 20.29% to 17.97% and Hal et al. (2021) measured it between 25.24 to 22.13%.

Mineral matter contents of mushroom harvested from different substrates compositions are presented in Table 7. The mushrooms showed significant differences in terms of mineral matter content according to the substrate composition. S1 medium (chickpea stalk 40%) had the highest Zn content (10.75 mg 100 g dry mushroom^{-1}). S9 medium had the highest value with 0.42% P, followed by S7 with 0.40%. The mushrooms obtained from S7 medium, containing 40% corncobs, were higher than the mushrooms obtained from other substrates in terms of N, K, Mg, Fe and Ca.

Table 6. Some quality characteristics of harvested mushrooms according to substrate composition

Substrate	Protein Content %	Total Dry Matter %
S1	44.08 c	34.07a
S2	39.14 e	29.61b
S3	31.51 h	29.67b
S4	47.60 b	30.22b
S5	42.26 d	28.92b
S6	34.01 g	27.72e
S7	51.41 a	27.94c
S8	44.66 c	28.39b
S9	36.74 f	29.72b
LSD p≤0.01	1.303	1.968

Mean values marked with a different letter in each column are statistically different from each other.

Researchers often conduct studies on medicinal mushrooms mainly to analyze their bioactive components. Although the mineral contents of mushrooms are generally relatively low compared to other food sources, mushrooms in general are a good source of various minerals and can contribute to the diet. Specific mineral contents of mushrooms vary with strains, growing conditions, cultivation methods and substrate composition. The previously findings in terms of N, P, K, Ca, Mg, Zn and Fe contents were in agreement to each other (Hoa et al., 2015; Sharif et al., 2016; Chiu et al., 2000; Ahmad et al., 2021; Zinnah et al., 2020; Ijimbili and Adenipekun, 2023).

Table 7. Mineral matter contents of mushrooms according to the substrates used in production

Substrate	%					mg/kg	
	Nitrogen	Phosphorus	Potassium	Calcium	Magnesium	Iron	Zinc
S1	7,05 bc	0,25 ef	1,38 c	2,63 bc	0,85 bc	7,31 abc	10,75 a
S2	6,26 de	0,23 f	1,28 c	2,15 de	0,64def	6,77 abc	5,25 ab
S3	5,04 g	0,21 f	0,98 d	1,78 f	0,49 g	5,84 c	4,49 b
S4	7,62 b	0,33 bcd	1,63 b	2,86 ab	0,93 ab	7,89 ab	6,99 ab
S5	6,76 cd	0,31 cde	1,63 b	2,35 cd	0,70 cde	7,32 abc	7,45 ab
S6	5,44 fg	0,28 def	1,29 c	1,94 ef	0,53 fg	6,31 bc	6,55 ab
S7	8,23 a	0,40 ab	2,06 a	3,11 a	1,02 a	8,53 a	6,30 ab
S8	7,31 bc	0,37 abc	1,92 a	2,55 bc	0,77 cd	7,90 ab	7,09 ab
S9	5,88 ef	0,42 a	1,39 c	2,11 de	0,58 efg	6,82 abc	7,74 ab
LSD p≤0.01	0.576	0.072	0.212	0.325	0.144	1.772	5.884

Mean values marked with a different letter in each column are statistically different from each other.

Conclusion

The substrate plays a crucial role in the cultivation of *Ganoderma*. Nine different substrate formulations were to cultivate *Ganoderma lucidum* using available agricultural wastes. The quality and composition of the substrate, including moisture content and nutrient availability, C/N ratio of substrate significantly affected yield and quality characteristics. A balanced substrate that meets the nutritional requirements of the fungi will contribute to their successful cultivation. The evaluation of a substrate formulation should be evaluated by both the cost of the substrate and the yield and profitability obtained. Supplementing the corncob of 40% to sawdust substrate could be alternative material for Ganoderma growers since it enriched the nutrition components of fruitbody and high yield and biological efficiency and early harvesting time as well as sawdust.

Typically used for its medicinal properties, the active part of *Ganoderma lucidum* is the fruiting body or the mushroom itself. This part of the mushroom contains bioactive compounds believed to have various health benefits, including potential immune system support and anti-inflammatory effects. While more research is needed to understand its potential benefits and mechanisms of action, it remains a popular choice for those seeking natural remedies and wellness support.

Acknowledgement

This study was prepared from Gülsüm İNCE's Master's Thesis. Scientific Research Projects Coordination Unit of Eskisehir Osmangazi University supported this study. Project no: FYL-2022-2274. Special thanks to Atatürk Horticultural Central Research Institute for supplying the *Ganoderma lucidum* spawn.

NÇ and MKS designed the study and set up the experiments, Gİ conducted the study, data collection, analysis, analyzed the data, and wrote the article. All authors contributed equally to the article.

References

Aghajani, H., Bahmani, M., Tajick Ghanbary, M. A., Zahedian, E., 2018. Influence of relative humidity and temperature on cultivation of Pleurotus

species. Maderas. Ciencia y tecnologia, 20(4) 571-578. Universidad del Bio-Bio.

A.O.A.C. (2019). Official Methods of Analysis of AOAC International [www Document]. 21stEd.https://www.aoac.org/aoac_prod_imis/AOAC/Publications/Official_Methods_of_Analysis/AOAC_Member/Pubs/OMA/AOAC_Official_Methods_of_Analysis.aspx

Açıkgöz, N., İlker, E., & Gökçöl, A. (2004). Biyolojik araştırmaların bilgisayarda değerlendirilmeleri, Ege Üniversitesi Tohum Teknolojisi Uyg. ve Araş. Merkezi yayınları No:2, Ege Üniversitesi Ziraat Fakültesi Ofset Atölyesi, Bornova-İzmir, 202s.

Adongbede, E.M., & Atoyebi, A.F. (2021). Isolation of *Ganoderma lucidum* (Curtis) P. Karst. From the wild in Lagos through tissue culture techniques and cultivation on sawdust of six Nigerian hardwoods. Jasem 25 (8), 1329–1335. https://doi.org/10.4314/jasem.v25i7.32

Ahmad, R., Riaz, M., Khan, A., Aljamea, A., Algheryafi, M., Sewaket, D., & Alqathama, A. (2021). *Ganoderma lucidum* (Reishi) an edible mushroom; a comprehensive and critical review of its nutritional, cosmeceutical, mycochemical, pharmacological, clinical, and toxicological properties. Phytotherapy Research, 35(11), 6030–6062. https://doi.org/10.1002/ptr.7215

Amiri-Sadeghan, A., Aftabi, Y., Arvanagh, R.A., Shokri, E., Khalili, M., Seyedrezazadeh, E. & Kuhar, F. (2022). A Review of substrates for solid-state fermentation of lingzhi or reishi medicinal mushroom, *Ganoderma lucidum* (Agaricomycetes), for basidiome production and effect on bioactive compounds. International Journal of Medicinal Mushrooms, 24(4): 15-29.

Atila, F. (2020). Comparative study on the mycelial growth and yield of *Ganoderma lucidum* (Curt.: Fr.) Karst. Acta Ecologica Sinica, 40:153-157. https://doi.org/10.1016/j.chnaes.2018.11.007

Atila, F. (2022). Utilization of agricultural and forestry by-products in *Ganoderma lucidum* (Curt.: Fr.) P. Karst production. The Journal of Fungus. 13 (Special issue)1-8, 3rd International Eurasian Mycology Congress.

Atoji-Henrique, K., Henrique, D.S., Gloria, L.S., Mazaro, S.M., & Casagrande, M. (2017). Influence of substrate composition on beta-glucans production and growth of *Ganoderma lucidum*. The Journal of Agricultural Science, 9, 190.

Aydoğdu, M., & Kurbetli, İ. (2021). Biotic interactions of cultivated mushroom and green mold disease in compost and casing soil. Harran Tarım ve Gıda Bilimleri Dergisi, 25(1), 13-22. https://doi.org/10.29050/harranziraat.795177

Baktemur, G., Kara E., Yarar M., Soylu, M.K., & Taşkın, H. (2022). Use of different agricultural wastes in *Ganoderma carnosum* Pat cultivation. Turkish Journal of Agriculture and Forestry: Vol. 46: No. 3, Article 8.

Bellettini, M. B., Fiorda, F. A, Maieves, H. A., Teixeira, G. L., Ávila, S., Hornung, P. S., Júnior, A. M., & Ribani, R. H. (2019). Factors affecting mushroom Pleurotus spp. Saudi Journal of Biological Sciences. 26, 633–646

Bernabe-Gonzalez, T., Cayetano-Catarino, M., Bernabe, G., Romero Flores, A., Angel-Rios, M.D., & Perez, P. (2015). Cultivation of *Ganoderma lucidum* on agricultural by-products in Mexico. Micologia Aplicada International, 27(2):25-30.

Bishop, K.S., Kao, C.H.J., Xu, Y., Glucina, M.P., Paterson, R.R.M., & Ferguson L.R. (2015) From 2000 years of *Ganoderma lucidum* to recent developments in nutraceuticals. Phytochemistry, 114: 56–65

Cao, Y., Xu, X., Liu, S., Huang, L. & Gu, J. (2018). Ganoderma: A Cancer immunotherapy review. Front. Pharmacol. 9:1217. https://doi.org/10.3389/fphar.2018.01217

Carrasco, J., Zied, D.C., Pardo, J.E. Preston G.M. & Pardo, A. (2018). Supplementation in mushroom crops and its impact on yield and quality. AMB Expr (2018) 8:146. https://doi.org/10.1186/s13568-018-0678-0

Cheureuil, L. R., Oliveira Junior, S. D. de, Vasconcelos, A. dos S., Binda, V. da S., Pessoa, V. A., Nascimento, L. B. de B. do, Gouvea, P. R. dos S., & Sales-Campos, C. (2022). Screening of ligninolytic enzymes produced by *Ganoderma lucidum* in solid residues from the Amazon. Research, Society and Development, 11(14). https://doi.org/10.33448/rsd-v11i14.36257

Chiu, S. W. Wang, Z. M. Leung T. M. & Moore D. (2000). Nutritional value of *Ganoderma* extract and assessment of its genotoxicity and anti-genotoxicity using comet assays of mouse lymphocytes. Food and Chemical Toxicology 38, 173-178.

Cormican, T., & Staunton, L. (1991). Factors in mushroom (*Agaricus bisporus*) compost productivity, In: Science and Cultivation of Edible Fungi, Maher (Ed.), Balkema, Rotterdam. 4: 221-224.

Cör, D., Knez, Z., & Hrncic M.K. (2018). Antitumour, antimicrobial, antioxidant and antiacetylcholinesterase Effect of *Ganoderma lucidum* Terpenoids and Polysaccharides: A Review. Molecules. 23, 649; doi:10.3390/molecules23030649

De Silva, D.D., Rapior, S., Fons, F. Bahkali A. H. & Hyde K. D. (2012). Medicinal mushrooms in supportive cancer therapies: an approach to anti-cancer

effects and putative mechanisms of action. Fungal Diversity 55, 1–35. https://doi.org/10.1007/s13225-012-015

Eren, E., & Pekşen, A. (2019). Türkiye'de kültür mantarı üretimi ve teknolojik gelişmeler. Mantar Dergisi, 10(3), 225-233.

Erkel, I.E. (2009). Yield performance of *Ganoderma lucidum* (Fr.) Karst cultivation on substrates containing different protein and carbohydrate sources. African Journal of Agricultural Research, 4(11):1331-1333.

Fraga, I., Coutinho, J., Bezerra, R.M., Dias, A.A., Marques, G., & Nunes, F.M. (2014). Influence of culture medium growth variables on *Ganoderma lucidum* exopolysaccharides structural features, Carbohydrate Polymers, V:111, 936-946, https://doi.org/10.1016/j.carbpol.2014.05.047

Gonzalez-Matute, R., Figlas, D., Devalis, R., Delmastro, S., Curvetto, N. (2002). Sunflower seed hulls as a main nutrient source for cultivating *Ganoderma lucidum*. Micologia Aplicada International, 14(2):19-24.

Gurung, O.K., Budathoki, U. and Parajuli, G. (2012). Effect of different substrates on the production of *Ganoderma lucidum* (Curt: Fr) Karst. *Our Nature*, 10, 191- 198.

Hal, Y.B., Yarar, M., Kara, E., Baktemur, G., & Taşkın, H. (2021). Effect of Different Agricultural Wastes on Yield and Quality in Cultivation of *Ganoderma lucidum* (Reishi mushroom). Çukurova J. Agric. Food Sci. 36(1):275-288

Hall, I.R., Stephenson, S., Buchanan, P., Wang, Y. & Cole, A.L.J. (2003). Edible and poisonous mushrooms of the world. Timber Press, Portland. 371 p

Hoa, H. T., Wang, C. L. & Wang, C. H. (2015). The effects of different substrates on the growth, yield, and nutritional composition of two oyster mushrooms (Pleurotus ostreatus and Pleurotus cystidiosus). Mycobiology. 43:423–434.

Hsieh, C., & Yang, F. (2004). Reusing soy residue for the solid-state fermentation of *Ganoderma lucidum*. Bioresource Technology, 91:105-109.

Hu, Y., Xu, W., Hu, S., Lian, L., Zhu, J., Shi, L., Ren, A., & Zhao, M. (2020). In *Ganoderma lucidum*, Glsnf1 regulates cellulose degradation by inhibiting GlCreA during the utilization of cellulose. Environ Microbiol. 22(1):107-121. https://doi.org/10.1111/1462-2920.14826.

Ijimbili, S.B., & Adenipekun, O.C. (2022). Comparative study on growth parameters, proximate analysis and mineral composition of *Ganoderma lucidum* cultivated on different substrates. Advances in Food Sciences, Volume 44(1), 5-14.

Jandaik, S., Singh, R., & Sharma, M. (2013). Comparative growth characteristics and yield attributes of lingzhi or reishi medicinal mushroom, *Ganoderma lucidum* (Higher Basidiomycetes) on different substrates in India. International Journal of Medicinal Mushrooms, 15(5): 497–503 (2013)

Jeewanthi, L.A.M.N., Ratnayake, K., & Rajapakse, P. (2017). Growth and yield of reishi mushroom [*Ganoderma lucidum* (Curtis) P. Karst] in different sawdust substrates. Journal of Food and Agriculture, 10(1&2):8-16. https://doi.org/10.4038/jfa.v10i1-2.5208

Kacar, B. (1972). Bitki ve toprağın kimyasal analizleri, II. Bitki Analizleri. Ankara Üniversitesi Ziraat Fakültesi Yayınları, Ankara, No: 453. Uygulama kılavuzu 155, A.Ü.Basımevi, Ankara.

Kacar B., İnal A., 2008. Bitki Örneklerinin Alınması ve Analize Hazırlanması. Bitki Analizleri Nobel Yayın No: 1241 ISBN:978-605-395-036-3. s. 115-144, Ankara.

Kara, E., Baktemur, G., Yarar, M., & Taşkın, H. (2021). Farklı yetiştiricilik ortamlarının maitake mantarı (*Grifola frondosa*) yetiştiriciliğinde verim ve kalite üzerine etkisi. Akademik Ziraat Dergisi. 10(2): 201-218. http://dx.doi.org/10.29278/azd.831748

Khatian N, & Aslam M. (2018). A Review of *Ganoderma lucidum* (Reishi): A Miraculous Medicinal Mushroom. Inventi Rapid: Ethnopharmacology, 2018(4), 1-6.

Koutrotsios, G., Marianna P., Evdokia K. M., Georgios B., Maria K., Petros A. T., Vasiliki P., Adamantini K., & Georgios I. Z. (2019). Valorization of olive by-products as substrates for the cultivation of *Ganoderma lucidum* and *Pleurotus ostreatus* mushrooms with enhanced functional and prebiotic properties. Catalysts, 9(6): 537.

Kumla, J., Suwannarach, N., Sujarit, K., Penkhrue, W., Kakumyan, P., Jatuwong, K., Vadthanarat, S., & Lumyong, S. (2020). Cultivation of mushrooms and their lignocellulolytic enzyme production through the utilization of agro-industrial waste. Molecules, 25, 2811. https://doi.org/10.3390/molecules25122811

Liu, Q. & Tie, L. (2019). Preventive and therapeutic effect of ganoderma (lingzhi) on diabetes. In: Lin, Z., Yang, B. (eds) Ganoderma and health. Advances in Experimental Medicine and Biology, 1182. Springer, Singapore. https://doi.org/10.1007/978-981-32-9421-9_8

Lonardo D. P. Di, van der Wal, A. Harkes P. & de Boer W. (2020). Effect of nitrogen on fungal growth efficiency. Plant Biosystems - An International Journal Dealing with all Aspects of Plant Biology, 154:4, 433-437, https://doi.org/10.1080/11263504.2020.1779849

Mehta, S., Jandaik, S., & Gupta, D. (2014). Effect of cost-effective substrates on growth cycle and yield of

lingzhi or reishi medicinal mushroom, *Ganoderma lucidum* (Higher Basidiomycetes) from northwestern Himalaya (India). International Journal of Medicinal Mushrooms, 16(6): 585–591 (2014)

Nguyen, L.T., Van Le, V., Nguyen, B.T.T., Nguyen, H.T.T., Tran, A.D., & Ngo, N.X. (2023). Optimization of mycelial growth and cultivation of wild *Ganoderma sinense*. BioTechnologia (Pozn). 104(1):65-74. https://doi.org/10.5114/bta.2023.125087

Nguyen, N.X.N., Van Le, V., Nguyen, L.T., Kana, R., & Nguyen, H.D. (2019). Optimal culture conditions for mycelial growth and fruiting body formation of Ling Zhi mushroom *Ganoderma lucidum* strain GA3. Bich Thuy Thi. 61(1), 62-67. https://doi.org/10.31276/VJSTE.61(1).62-67

Osunde, M.O., Olayinka, A., Fashina, C.D. & Torimiro, N. (2019). Effect of Carbon-Nitrogen Ratios of Lignocellulosic Substrates on the Yield of Mushroom (*Pleurotus pulmonarius*). Open Access Library Journal, 6: e5777. https://doi.org/10.4236/oalib.1105777

Özçelik, E., & Pekşen, A. (2007). Hazelnut husk as a substrate for the cultivation of shiitake mushroom (*Lentinula edodes*). Bioresource technology, 98(14), 2652-2658.

Öztürk, N., Basım, E., & Basım, H. (2017a). Yemeklik Kültür Mantarında (Agaricus bisporus (J. Lge) Imbach) Yaygın Görülen Mikrobiyal Hastalıklar. Harran Tarım ve Gıda Bilimleri Dergisi, 21(1), 112-125. https://doi.org/10.29050/harranziraat.303179

Öztürk, N., Basım, E., & Mamay, M. (2017b). Yemeklik Kültür Mantarı Üretim Alanlarında Görülen Genel Mantar Zararlıları ve Mücadelesi. Harran Tarım ve Gıda Bilimleri Dergisi, 21(4), 507-523. https://doi.org/10.29050/harranziraat.319808

Peksen, A., & Yakupoglu, G. (2009). Tea waste as a supplement for the cultivation of *Ganoderma lucidum*. World Journal of Microbiology and Biotechnology, 25(4):611-618.

Puliga, F., Pamela L., Francesco M., Alessandra Z., & Ornella F. (2022). Valorization of Hazelnut Shells as Growing Substrate for Edible and Medicinal Mushrooms. Horticulturae 8,3: 214.

Rashad, F. M., El Kattan, M. H., Fathy, H. M., Abd El-Fattah, D. A., El Tohamy, M., & Farahat, A.A. (2019). Recycling of agro-wastes for *Ganoderma lucidum* mushroom production and Ganoderma post mushroom substrate as soil amendment. Waste Management, 88, 147–159.

Renu, T. & Brij, M.S. (2015). Deployment of indigenous wild *Ganoderma lucidum* for better yield on different substrates. Afr J Agric Res. 2015;10(33):3338–41.

Sharif, S., Mustafa, G., Munir, H., Weaver, C.M., Jamil, Y. & Shahid, M. (2016). Proximate composition and micronutrient mineral profile of wild ganoderma lucidum and four commercial exotic mushrooms by ICP-OES and LIBS. Journal of Food and Nutrition Research, 4,(11), 703-708. https://doi.org/10.12691/jfnr-4-11-1

Taofiq, O., Heleno, S.A., Calhelha, R.C., Barros, L., Gonzalez-Paramas, A M., Barreiro, M.F., & Ferreira, I.C.F.R. (2017). The potential of *Ganoderma lucidum* extracts as bioactive ingredients in topical formulations, beyond its nutritional benefits. Food and Chemical Toxicology, 108, Part A, 139-147. https://doi.org/10.1016/j.fct.2017.07.051

Thiribhuvanamala G. & Krishnamoorthy A.S. (2021). Evaluation of different lignocellulosic substrates for cultivation of medicinal mushroom *Ganoderma lucidum*. J. Envinronmental Biology, 42, 1313-1319.

Turfan, N., Karadeniz, M., & Ünal, S. (2016). Turkish Journal of Agriculture - Food Science and Technology, 4(3): 158-162.

Veena, S.S., & Pandey, M. (2011). Paddy straw as a substrate for the cultivation of Lingzhi or Reishi medicinal mushroom, *Ganoderma lucidum* (W.Curt.:Fr.) P. Karst. in India. International Journal of Medicinal Mushrooms, 13(4):397- 400. https://doi.org/10.1615/intjmedmushr.v13.i4.100

Vishwakarma M.P., Bhatt, R.P., & Gairola, S. (2011). Some medicinal mushrooms of Garhwal Himalaya, Uttarakhand, India Int. J. Med. Arom. Plants, 1(1), 33-40

Wachtel-Galor S., Yuen, J., Buswell, J.A., & Benzie, I.F.F. (2011). *Ganoderma lucidum* (Lingzhi or Reishi): A Medicinal mushroom. In: Benzie IFF, Wachtel-Galor S, editors. Herbal Medicine: Biomolecular and Clinical Aspects. 2nd edition. Boca Raton (FL): CRC Press/Taylor & Francis; Chapter 9. Available from: https://www.ncbi.nlm.nih.gov/books/NBK92757/

Yakupoğlu G., & Pekşen A. (2011). Çay atığından hazırlanan farklı kompost ve partikül büyüklüğünün *Ganoderma lucidum* mantarının verimi ve bazı morfolojik özellikleri üzerine etkisi. Ekoloji 20 (78): 41- 47.

Yang, F., Chienyan, H., & Chen, H. (2003). Use of stillage grain from a rice-spirit distillery in the solid-state fermentation of *Ganoderma lucidum*. Process Biochemistry, 39: 21-26.

Yuliana, T., Putri, N.Z., & Komara, D.Z. (2020). Study of *Ganoderma lucidum* in laccase production using corncob and paddies straw substrates on submerged fermentation system. Pakistan Journal of Biological Sciences: PJBS. 23(8):1060-1065.

https://doi.org/10.3923/pjbs.2020.1060.1065

Zhou, X.W. (2017). Edible and medicinal mushrooms: Technology and application. First edition. Ed. Zied D.C. & Pardo-Gimenez, A. John Wiley & Sons Ltd. p. 385-409.

Zinnah, M. A., Shelly, N. J., Ahmed K. U. & Nuruddin M.M. (2020). Comperatıve study on the proximate composıtıon of reishi mushroom (*Ganoderma lucidum*) Grown on different sawdust substrates. Int J of Advance Research and Innovative Ideas in Education. 6(4), 1744-1754. ISSN(O)-2395-4396.