



CULTIVATION OF REISHI MUSHROOM (*GANODERMA LUCIDUM*) ON DIFFERENT LOCAL SUBSTATES IN KURDISTAN REGION, IRAQ

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Abstract

In this investigation, the impact of five locally sourced sawdust types and additional substances on crop yield and chemical contents of *Ganoderma lucidum* and three levels of spawning rates (5, 10 and 15%) were studied. The organic materials used were as follows: The sawdust of Wheat straw, Rice straw, Populus Canadensis, Salix Alba and Cardboard, some supplemented substrates compare with raw organic Substrates led to an increase in fruits bodies fresh weight by 194 G bag⁻¹ compared to the raw substrates by 180 G bag⁻¹ and with significant differences. Otherwise the cap width also had significant differences by 6.3 cm in raw substrates while supplemented substrates were 5.5 cm, While there were some variations in other characteristics examined, such as the weight, length, and diameter of the cap and stipe, there were no noteworthy differences in the levels of protein, nitrogen, carbohydrates, and vitamin C in the mushrooms grown on different organic substrates, while there were significant differences between substrates with ash and fiber, the best substrates were Carolina poplar sawdust by 7.57% for ash and 31.26% for fibers and white willow sawdust 7.76% for ash and the lowest value for ash 6.77% for wheat straw. 30.93% for fibers with Rice straw substrate, there were clear significant differences in most of the studied traits of fungal inoculum levels, and the best level was 15%, which led to a proven increase in most traits.

Keywords: *Ganoderma lucidum*, Organic substrates, Supplements, Fruits bodies chemical components, Yield.

زراعة الفطر الريشي (*Ganoderma lucidum*) على بيئات عضويه محليه مختلفة في إقليم كوردستان العراق

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الخلاصة

اجريت هذه الدراسة لبحث تأثير خمسة أنواع مختلفة من المواد العضوية ومن مصادر محلية واطافة بعض محسنات النمو على إنتاجية انتاج الفطر الريشي ومحتويات الفطر من بعض المواد الكيميائية داخل الثمار وبثلاثة مستويات من اللقاح الفطري (5، 10 و15%) وكانت المواد العضوية المستخدمة كالاتي: نشارة قش القمح، قش الأرز، الحور الكندي، الصفصاف الابيض والكرتون، ادت اوساط الزراعة التي تم اضافة بعض المحسنات اليها الى زيادة في الوزن الطازج للأجسام الثمرية بمقدار 194 غم كغم⁻¹ مقارنة مع الاوساط الخام نفسها والتي كانت 180 غم كغم⁻¹ ومع وجود اختلافات كبيرة. فضلا عن عرض القبعة إذ كان هناك فروق معنوية كبيرة بمقدار 6.3 سم. في الاوساط الخام مقارنة مع المواد العضوية المضاف اليها المحسنات بمقدار 5.5 سم، على الرغم من أن الخصائص الأخرى التي تمت دراستها، كانت هناك اختلافات، ولكن لم يوجد فروق معنوية لكل من الوزن الجاف وطول القبعة وطول وقطر الساق، وايضا لم تكن هناك فروق معنوية لتأثير الركائز العضوية المختلفة على محتوى الفطر في كل من البروتين والنيتروجين والكربوهيدرات وفيتامين ج، بينما كانت هناك اختلافات معنوية بين المواد العضوية المختلفة مع الرماد والألياف، كانت أفضل الركائز هي نشارة خشب الحور الكندي بنسبة 7.57% للرماد و31.26% للألياف ونشارة خشب الصفصاف الأبيض 7.76% للرماد وأقل قيمة للرماد 6.77% لقش القمح. 30.93% للألياف مع وسط قش الأرز وايضا كان هناك فروق معنوية واضحة في معظم الصفات المدروسة لمستويات اللقاح الفطري وكان المستوى الأفضل هو 15% مما أدى الى زيادة مؤكدة في معظم الصفات.

كلمات مفتاحية: *Ganoderma lucidum*، مواد عضوية، إضافات، المكونات الكيميائية للأجسام الثمرية، المحصول.

Introduction

G. lucidum may be a part of parasitic bunch Basidiomycetes which has a place to Polyporaceae (Ganodermaceae) of Aphyllophorales. Its fruiting body is named as "Reishi" in Japanese and "Lingzhi" in Chinese (33). Commercial *G. lucidum* items are accessible in different shapes, such as powders, dietary supplements, and tea

which are cultivated from diverse parts of the mushroom, counting mycelia, spores, and natural product body (32). *G. lucidum* could be an expansive, varnish appearance and woody surface. The fruiting bodies have a yellow to reddish skin and an upper layer is smooth or frequently concentrically zoned and notched. Surface of the fruiting body is from time to time secured with brownish spore powder (17). Later ponders on lingzhi have illustrated various organic substances among this sort of mushroom, counting anti-tumor, anti-inflammatory, hepato-protective, anti-microbial, hypotensive, anti-diabetic and hypolipodemic impacts (3). Reishi or lingzhi too quality a few wellbeing benefits which basically include the control of blood glucose levels, balance of the resistant system, hepato-protection, and bacteriostatic. To meet the slowly increasing request for *G. lucidum* as a common pharmaceutical, commercial development of this mushroom has been started around the world, particularly within the tropical Asian nations (4). Yield of *G. lucidum* changed broadly depending on the kind of sawdust and supplements. In this manner, it is imperative to utilize the correct substrate for the commercial generation of *G. lucidum*. Diverse natural variables, oxygen level, and calcium particle rate, etc. are too imperative for the mushroom growing (25). They develop saprophytically or parasitically on dead natural matter (20). *G. lucidum* is competent of thriving within the subtropical and temperate climate zones, within the timberlands of Asia, Europe and North and South America as a saprophyte or facultative parasite. Owing to high medicinal esteem and its adaptability to different temperature ranges, there's developing intrigued among researchers, particularly within the tropical Asian nations (7). Reports appear that *G. lucidum* has been developed, accounts for 85% of fruiting body and 45% of mycelial dry weight by utilizing diverse substrates such as grain saw clean, broadleaf hardwood trees (commonly utilized species incorporate oak, pecan, senior, choke, cherry and plum), wood logs, tree stumps, agro buildups and sawdust sacks, *Acacia* sp., *Alnus* sp., *Carpinus* sp., *Dalbergia* sp., *Dipterocarpus* sp., *Fagus* sp., *Pinus* sp., *Populus* sp., *Quercus* sp. and *Swietenia* sp. (25). The Lingzhi contains bioactive compounds, including polysaccharides, peptidoglycans and triterpenes as well as high nutritional values such as amino acids, fiber, low fat, and vitamins B1, B2, B12, C, D, and E. (21). Diverse Mushrooms species prefer diverse substrates as development medium. For case, therapeutic mushrooms such as Shiitake, pearl and yellow clams develop superior on straws and gourmet. Reishi and Maitake develop superior on sawdust and logs, though *Agaricus* mushrooms are developed on manure with the likes of button mushrooms, Portobello mushrooms (12). The sawdust of *Alnus nepalensis*, *Shorea robusta* and *Dalbergia sissoo* and supplements of rice bran, wheat bran, corn flour and gram flour were utilized as substrates in *G. lucidum* development. *Alnus nepalensis* sawdust supplemented with gram flour appeared highest yield among all treatments (10) The impact of different wood sawdust substrates on the yield of *G. lucidum* was examined considering the shortage of rubber and mango wood as a sole source for substrate, the mixture of rubber and mango or rubber and *Lunumidella* can be suggested for cultivation of *G. lucidum*. Encourage investigate is recommended with the addition of nutrient supplements to the developing medium (17). Different supplements have been

investigated for *G. lucidum* cultivation, such as wheat bran, rice bran, corn flour, gram flour, rice husks, coconut fiber, shelled nut frames, sorghum, sugarcane bagasse, sucrose, molasses, gluten feast, and tea waste, as added substances within the substrate to improve *Ganoderma* cultivation (9). It was anticipated that palm oil sludge contains a high nutrient substance useful as a supplement in mushroom culture, since particularly substrates rich in nitrogenous substrates provide high mushroom yields and quick mycelial growth (22). The ratio of carbon to nitrogen (C: N) plays an important part in spawn running, whereas the nitrogen substance is also a main factor for developing fruiting bodies on mushroom mycelia (27). Corn cobs can be utilized as a substrate in case supplemented with supplements to support development of the basidiocarp (31).

The purpose of this study is to evaluate the effects of five different local substrates in Kurdistan region, Iraq [wheat straw (WS), Rice straw (RS), Carolina poplar, White willow (WW) and Cardboard (C)] as raw through three rates of spawn (5, 10 and 15%) on the *G. lucidum* growth and yield.

Materials and Methods

The experiment was carried out during October 2021 to June 2022, in Grdarasha research station or in college of Agricultural Engineering Sciences / Salahaddin University, located at (Latitude 36°07'10.44"N Longitude 44°00'50.52" E), and evaluation of 421 meters above sea level. Reishi mushroom *Ganoderma lucidum* using five types of local organic Substrates were (Wheat straw, Rice straw, Carton, *Populus Canadensis* sawdust, and *Salix Alba* sawdust). All of them were collected from farming field and forestry at Ranya district to study cultivation of Reishi mushroom (*G. lucidum*). Mushroom mother culture was kindly obtained from Anbar University College of Agriculture, soil microbiology lab, then cultivated on different local substrates in plastic bags with 40 ×60 cm in size.

Preparation of organic substrates: The experiment was started in 24th of October 2021. The first steps, the local organic substrates were soaked for about 12 hours in water to maintain 65-75% moisture content then dropped nearly 1 hour after that put in the commercial sterilized heated steam tank. The substrate sterilized for about 8 hours by steaming. When the sterilization of the substrates was finished, they were allowed to cool prior to inoculation then added the supplements, CaSO₄ 2%, CaCO₃ 2% and wheat bran 10% (10 and 24).

Spawning: When the temperature of different organic substrates were cooled to the room temperature (about 18 ± 3°C in autumn), it was spread in one layer (15 cm thick) into plastic bags [40 x 60 cm]. The spawn material of *G. lucidum* was distributed over different substrates in three rates at the rate of (5, 10 and 15%) (w/w). Another layer of substrate (5 cm thick) was added over the spawn material to cover it. The total amount of the wet substrate used for *G. lucidum* cultivation was about 2kg for each bag and bag respectively as comparison between blanked substrate and organic substrate with supplements CaSO₄ 2%, CaCO₃ 2% and wheat bran 10% with 3 replicates for all organic substrates. (25) and three rate of spawn with

supplement and non-supplements. The spawn and organic substrate were closed tightly, and incubated at room temperature. Incubation period for six months in autumn and spring seasons

Mycelial growth and harvesting of *G. lucidum*: At the end of the incubation period, when the mycelial growth of the tested *G. lucidum* covered the organic substrate with mycelium, the bags were opened. Relative Humidity RH (about 80- 90%) was maintained daily by using a fog system and temperature recorded (data non-shown). After six month of cultivation, mushroom fruit samples were harvested periodically, and counted in each replicate. The fresh and dry weight of *G. lucidum* fruits was recorded. For chemical analysis, samples were taken, from fruits bodies (*G. lucidum*), for the determination of, nitrogen, carbon, fibers, Ashe, and Vitamin C contents.

Determination of Nitrogen and crude protein in the mushroom fruit: Determination of Nitrogen using Kjeldahl method (13) and detrained the crude protein according to (5 and 15).

Carbohydrate determination: Total percent carbohydrate contents of the samples were estimated according to (8).

Determination of crude fiber: Total crude fiber was determined utilizing the method of (20 and 23).

Ash determination Ash measurement in accordance with (14)

Determination of Vitamin C: The amount of vitamin C in the fruits ($\text{mg}100 \text{ ml}^{-1}$). It is measurements according to (26)

Experimental Design and Statistical analysis: The experimental design used was a $2 \times 5 \times 3$ equilibrated factorial plans (RCBD) with 3 replicates. Factor 1, substrates supplements and non-supplements and Factor 2, five types of local organic substrates. Factor 3 was three different level rates of spawn, each block was composed of two kg plastic bags filled with spawned organic substrates, a total of 90 plastic bags were placed on of $4 \times 5 \text{ m}^2$ as total area by using SPSS (Statistical Package for Social Sciences) (15). The data statistically analyzed using Statistical Analysis System (SAS) software. Duncan's Multiple Range Test at 0.05 for inter action between treatments

Results and Discussion

Effect of raw organic substrates, supplemented organic substrates, and spawn rate on some *G. lucidum* fruit bodies traits:

Effect of some supplements add to different organic substrates on some *G. lucidum* fruit bodies traits:

The results in (Table 1) show that some supplemented of substrates compare with raw organic Substrates used for the cultivation of *G. lucidum* led to an increase in fruits bodies fresh weight by 194 G bag^{-1} compared to the raw Substrates by 180 g/ bag^{-1} with significant differences. Otherwise the cap width also had significant differences by 6.3 cm in raw substrates while supplemented substrates was 5.5cm, data in (Table 2) showed the interaction between raw organic substrates, supplemented organic substrates, different types of organic substrates on some *G. lucidum* fruit bodies traits,

the results showed that the interaction between type of substrates and supplements were significant differences in some of the studied traits, such as fresh and dry weight, length and width of the cap, meanwhile raw rice straw had the lowest weight value with a drive of 85.67 G bag⁻¹, the dry weight was also supplemented rice straw, followed by raw rice straw and Carolina poplar sawdust The best in terms with 39.33, 36.33 and 35.89 g respectively, and the lowest value was raw rice straw with 12.2g. Some additives that encourage and improve the growth properties of *G. lucidum* and other mushrooms. Adding supplements to the organic matter on which the *G. lucidum* fungus is grown can affect the fresh and dry weight as well as the cap width and length because the supplements provide additional nutrients for the fungus to grow and develop. These nutrients can influence the growth rate and overall size of the fungus, leading to changes in the fresh and dry weight and cap width and length. Additionally, certain supplements may also affect the overall health and vitality of the fungus, which can also impact its growth and development. This is why calcium sulphate can be employed to adjust pH, permeability, air flow, nitrogen fixation, and calcium and sulphur rates (6, 18 and 29). Vitamin B1, B6, and biotin are all growth factors involved in *G. lucidum* metabolism (25)

Effect of different organic substrates on some *G. lucidum* fruit bodies traits: The results shown in (Table 1) and (Fig. 1) the effect of the type of organic substrates on the studied characteristics was slight, and there were significant differences for the organic substrates on the features of fresh and dry weight and cap length with wheat straw 226.17, 37.83 g 9.6cm respectively while Carolina poplar sawdust in 233.78 g was the best results, whereas there were no significant differences in the length and diameter of the stipe and the width of the cap. In the interaction of two factors, the percentage of spawn rate and organic substrate, as shown in (Table 4), there are significant differences in each of the fresh weight, cap length and width, where the best organic substrate was Carolina poplar sawdust 15% spawn rate 302 G bag⁻¹ compared to the lowest value with rice straw rate 5% was 87.83 G bag⁻¹. Cap length, wheat straw 10 and 15%, white willow sawdust 15% and Carolina poplar sawdust 10% proportions 10.5, 9.83, 10 and 9.67 respectively, compared to the lowest value, which was rice straw 15% with a 6.17cm. The effect of organic matter on the fresh and dry weight as well as cap width and length of *G. lucidum* fungus is likely due to the fact that the fungus uses organic matter as a source of nutrients for growth and development. As well as, different types of organic matter may contain different levels of nutrients, which can affect the growth and development of the fungus in different ways. Additionally, the physical properties of the organic matter, such as its texture and water-holding capacity, can also affect the growth and development of the fungus (18 and 25).

Effect of different spawn rate on some *G. lucidum* fruit bodies traits: The results in (Table 1) showed that the level of spawn inoculum had a clear significant effect and difference on both of fresh weight and the dry weight at level 15% .215.79 G bag⁻¹ and 29.46 g respectively, while the rest of the rate levels were differences between them on traits, but not significant. Interaction exposed in (Table 3) between the level of the inoculation and the supplemented organic substrates that the 15% followed by

the 10% rate had a significant effect on both fresh weight, stipe length and cap, White willow sawdust 15% had the highest value in length and cap width by 10 and 7.33 cm, followed by Carolina poplar sawdust 10% 9.67 and 7.5 cm and the least of them is rice straw 15% with 6.17 and 4.5 cm. Typically, overlap between the three factors of supplements, the different types of organic substrates and the spawn inoculum level, there were significant differences on most of the studied traits, except for dry weight, as there were no significant differences. The fresh weight was the highest value for supplemented Carolina poplar sawdust with 15% and raw Carolina poplar sawdust with 10% with 333.3 and 321 G bag-1 respectively and the lowest value was with supplemented rice straw 5% 82.67 G bag-1. Cap length was 14cm with supplemented rice straw 10% and the least is raw rice straw 15% 5cm. While stipe length was supplemented rice straw 10% with 6 cm. The effect of certain additives on the morphological characteristics of mushrooms. They found that the addition of calcium carbonate (CaCO_3) to the substrate had a significant impact on the yield of black poplar mushrooms. The best results were obtained when the substrate was supplemented with 8 g/100 g of CaCO_3 . The main cap diameter of the carpophores and their average weight were also highest in this group. The addition of CaCO_3 also influenced the storability of the mushrooms, with the lowest weight loss observed when the substrate was supplemented with 4 g/100 g of CaCO_3 . Furthermore, the amount of CaCO_3 added affected the chemical composition of the black poplar mushroom carpophores. (16)

The spawn rate impacts the fresh and dry weight of *G. lucidum* mushrooms because it affects mycelial growth and substrate colonization, leading to larger and heavier mushrooms. A higher spawn rate also leads to better utilization of the substrate and nutrients for greater biomass production. However, temperature, humidity, and light also play a role in mushroom growth and development this results agree with (1)

Table 1 Effect of raw organic substrates, Raw organic substrates with supplements, and spawn rate on some *G. lucidum* fruit bodies traits.

Treatments Fruits bodies traits	Fresh weight (g)	Dry weight (g)	Cap length (cm)	Cap width (cm)	Stipe length (cm)	Stipe diameter (cm)
Raw organic substrates	180.72 ± 13.65 ^b	26.42 ± 2.66 ^a	8.92 ± 0.48 ^a	6.31 ± 0.39 ^a	2.81 ± 0.3 ^a	2.28 ± 0.19 ^a
Raw organic substrates with supplements	194.78 ± 12.68 ^a	28.67 ± 2.33 ^a	8.94 ± 0.48 ^a	5.53 ± 0.24 ^b	3.25 ± 0.27 ^a	2.25 ± 0.19 ^a
Wheat straw	226.17 ± 14.78 ^a	37.83 ± 4.03 ^a	9.61 ± 0.81 ^a	6.28 ± 0.48 ^a	2.94 ± 0.46 ^a	2.5 ± 0.22 ^a
Rice Straw	128.44 ± 12.54 ^c	33.56 ± 3.65 ^{ab}	9.44 ± 0.83 ^b	5.39 ± 0.34 ^a	2.83 ± 0.39 ^a	2.06 ± 0.21 ^a
Carolina poplar	233.78 ± 21.49 ^a	32 ± 3.56 ^b	9.44 ± 0.85 ^b	6.33 ± 0.47 ^a	2.61 ± 0.33 ^a	2.11 ± 0.23 ^a
White willow	162.61 ± 5.62 ^b	29.89 ± 1.67 ^b	9.56 ± 0.83 ^b	5.67 ± 0.52 ^a	3.72 ± 0.41 ^a	2.39 ± 0.2 ^a
Spawn rate 5%	160.88 ± 12.1 ^c	26.42 ± 2.77 ^b	8.67 ± 0.5 ^a	5.67 ± 0.33 ^a	3.13 ± 0.31 ^a	2.21 ± 0.26 ^a
Spawn rate 10%	186.58 ± 13.1 ^b	26.71 ± 2.77 ^b	9.5 ± 0.64 ^a	6 ± 0.39 ^a	3.25 ± 0.34 ^a	2 ± 0.17 ^a
Spawn rate 15%	215.79 ± 16.17 ^a	29.46 ± 2.77 ^a	8.63 ± 0.61 ^a	6.08 ± 0.47 ^a	2.71 ± 0.4 ^a	2.58 ± 0.24 ^a

* For each parameter, means not followed by the same letter are significantly different by Duncan's Multiple rang test ($P > 0.05$).

Table 2 Effect of interaction between raw organic substrates, Raw organic substrates with supplements, different types of organic substrates on some *G. lucidum* fruit bodies traits.

	Organic substrate	Fresh weight (g)	Dry weight (g)	Cap length (cm)	Cap width (cm)	Stipe Length (cm)	Stipe diameter (cm)
Raw organic substrates	Wheat straw	236.33 ± 10.64 ^{ab}	36.33 ± 7.12 ^a	9.67 ± 1.12 ^{ab}	7.22 ± 0.7 ^{ab}	2.56 ± 0.87 ^a	2.67 ± 0.33 ^a
	Rice straw	85.67 ± 6.56 ^e	12.22 ± 2.03 ^b	7 ± 0.76 ^c	4.89 ± 0.48 ^c	2.56 ± 0.29 ^a	2.11 ± 0.31 ^a
	Carolina poplar	252.67 ± 27.06 ^a	35.89 ± 9.72 ^a	10.33 ± 0.53 ^a	7.78 ± 0.52 ^a	2.44 ± 0.47 ^a	2.22 ± 0.4 ^a
	White willow	148.22 ± 8.73 ^d	21.22 ± 5.74 ^{ab}	8.67 ± 1.04 ^{abc}	5.33 ± 0.94 ^c	3.67 ± 0.65 ^a	2.11 ± 0.48 ^a
supplemented organic substrates	Wheat straw	216 ± 28.09 ^b	39.33 ± 4.2 ^a	9.56 ± 1.24 ^{ab}	5.33 ± 0.53 ^c	3.33 ± 0.33 ^a	2.33 ± 0.55 ^a
	Rice straw	171.22 ± 25.73 ^{cd}	26.89 ± 4.26 ^{ab}	8.89 ± 0.96 ^{abc}	5.89 ± 0.45 ^{bc}	3.11 ± 0.73 ^a	2 ± 0.29 ^a
	Carolina poplar	214.89 ± 33.79 ^b	22.33 ± 4.09 ^{ab}	7.67 ± 0.88 ^{bc}	4.89 ± 0.39 ^c	2.78 ± 0.49 ^a	2 ± 0.24 ^a
	White willow	177 ± 2.52 ^c	26.11 ± 4.65 ^{ab}	9.67 ± 0.75 ^{ab}	6 ± 0.5 ^{bc}	3.78 ± 0.55 ^a	2.67 ± 0.37 ^a

* For each parameter, means not followed by the same letter are significantly different by Duncan's Multiple rang test (P>0.05).

Table 3 Effect of interaction between raw organic substrates, spawn rate on some *G. lucidum* fruit bodies traits.

Organic substrate	Rate of spawn	Fresh weight (g)	Dry weight (g)	Cap length (cm)	Cap width (cm)	Stipe Length (cm)	Stipe diameter (cm)
Wheat straw	5%	276.67 ± 21.89 ^{ab}	41.67 ± 8.36 ^a	8.5 ± 0.85 ^{ab}	6.83 ± 0.5 ^{ab}	3.17 ± 0.54 ^a	3 ± 0.68 ^a
	10%	181.33 ± 20.28 ^d	29.17 ± 6.49 ^a	10.5 ± 1.88 ^a	5.5 ± 0.92 ^{abc}	2.83 ± 0.54 ^a	1.67 ± 0.33 ^a
	15%	220.5 ± 20.47 ^c	42.67 ± 5.55 ^a	9.83 ± 1.42 ^a	6.5 ± 1.02 ^{abc}	2.83 ± 1.25 ^a	2.83 ± 0.48 ^a
Rice straw	5%	78.83 ± 3.59 ^e	17.83 ± 1.6 ^a	8.33 ± 0.99 ^{ab}	5.5 ± 0.56 ^{abc}	2.33 ± 0.33 ^a	1.83 ± 0.48 ^a
	10%	140.5 ± 13.88 ^{ef}	23 ± 7.17 ^a	9.33 ± 1.26 ^a	6.17 ± 0.4a ^{bc}	3.83 ± 0.98 ^a	2.17 ± 0.17 ^a
	15%	166 ± 42.21 ^{def}	17.83 ± 5.3 ^a	6.17 ± 0.75 ^b	4.5 ± 0.67 ^c	2.33 ± 0.42 ^a	2.17 ± 0.4 ^a
Carolina poplar	5%	137.67 ± 25.12 ^f	26.5 ± 6.93 ^a	8.83 ± 0.83 ^{ab}	5.5 ± 0.72 ^{abc}	3 ± 0.63 ^a	1.83 ± 0.48 ^a
	10%	261.67 ± 29.54 ^b	30 ± 12.28 ^a	9.67 ± 1.12 ^a	7.5 ± 0.85 ^a	2.67 ± 0.67 ^a	2.33 ± 0.33 ^a
	15%	302 ± 16.53 ^a	30.83 ± 9.67 ^a	8.5 ± 1.23 ^{ab}	6 ± 0.77 ^{abc}	2.17 ± 0.48 ^a	2.17 ± 0.4 ^a
White willow	5%	150.33 ± 15.51 ^{def}	19.67 ± 6.77 ^a	9 ± 1.48 ^{ab}	4.83 ± 0.65 ^{bc}	4 ± 0.82 ^a	2.17 ± 0.4 ^a
	10%	162.83 ± 3.35 ^{def}	22.83 ± 6.23 ^a	8.5 ± 0.89 ^{ab}	4.83 ± 0.6 ^{bc}	3.67 ± 0.49 ^a	1.83 ± 0.48 ^a
	15%	174.67 ± 3.32 ^{de}	28.5 ± 6.46 ^a	10 ± 0.93 ^a	7.33 ± 1.09 ^a	3.5 ± 0.89 ^a	3.17 ± 0.6 ^a

* For each parameter, means not followed by the same letter are significantly different by Duncan's Multiple rang test (P>0.05).

Table 4 Effect of interaction between organic substrates and spawn rate on some *G. lucidum* fruit bodies traits

	Rate of spawn	Fresh weight (g)	Dry weight (g)	Cap length (cm)	Cap width (cm)	Stipe Length (cm)	Stipe diameter (cm)
Raw organic substrates	5%	148.83 ± 21.67 ^d	27.5 ± 5.77 ^a	9.08 ± 0.66 ^{ab}	6.33 ± 0.45 ^a	3 ± 0.49 ^{ab}	1.92 ± 0.26 ^a
	10%	203.42 ± 24.15 ^b	21.08 ± 7.19 ^a	8.08 ± 0.86 ^b	6.08 ± 0.76 ^a	2.42 ± 0.3 ^{8b}	2.08 ± 0.26 ^a
	15%	189.92 ± 24.04 ^{bc}	30.67 ± 6.21 ^a	9.58 ± 0.95 ^{ab}	6.5 ± 0.8 ^a	3 ± 0.69 ^{ab}	2.83 ± 0.41 ^a
supplemented organic substrates	5%	172.92 ± 27.55 ^{cd}	25.33 ± 4.5 ^a	8.25 ± 0.77 ^b	5 ± 0.41 ^a	3.25 ± 0.39 ^{ab}	2.5 ± 0.45 ^a
	10%	169.75 ± 9.1 ^{cd}	31.42 ± 3.15 ^a	10.92 ± 0.78 ^a	5.92 ± 0.26 ^a	4.08 ± 0.47 ^a	1.92 ± 0.23 ^a
	15%	241.67 ± 19.83 ^a	29.25 ± 4.43 ^a	7.67 ± 0.7 ^b	5.67 ± 0.51 ^a	2.42 ± 0.43 ^b	2.33 ± 0.26 ^a

* For each parameter, means not followed by the same letter are significantly different by Duncan's Multiple rang test (P>0.05).

**Figure 1 Growth of *Ganoderma lucidum* on different organic substrates.**

- a- Growth of *Ganoderma lucidum* on wheat straw
- b- Growth of *Ganoderma lucidum* on rice straw
- c- Growth of *Ganoderma lucidum* on Carolina poplar sawdust
- d- Growth of *Ganoderma lucidum* on white willow sawdust

Effect of raw organic substrates, supplemented organic substrates, and spawn rate on some *G. lucidum* fruit bodies chemical components:

Effect of some supplemented organic substrates on some *G. lucidum* fruit bodies chemical components: Some chemical contents were measured inside *G. lucidum* fruit bodies, were shown in the (Table 5, 6, 7, and 8) the effect of the Supplements on different organic substrates in the (Table 5) show Significant differences only in ash and fiber in the percentage of 7.73 and 31.11% respectively, while there were no significant differences between the other traits, which were protein, nitrogen, carbohydrates and vitamin C. The overlap interactions between the supplements and the type of organic substrates shown in the (Table 6) there were no significant difference between the interventions in each of protein, nitrogen and vitamin C, while significant differences were found in each of the ash and fibers with the highest value with 9.71, 31.3% respectively with raw White willow sawdust, and the lowest value for ash was 5.37% in fruits bodies which cultivated on raw wheat straw and fibers as well 31.38 % in raw Carolina poplar sawdust and there is no difference between raw Carolina poplar sawdust and raw White willow sawdust, the lowest value for fiber was 30.93 % supplemented rice straw. Carbohydrates have the highest value 38.58% with raw rice straw and the lowest value with raw wheat straw and raw rice straw in 37.57, 37.22% respectively. The interaction between Supplemented raw substrates and spawn rates shown in (Table 7). There were significant differences in the interaction between them. The best treatment ever was the raw substrates with spawn rate 15%, the results it was 21.35, 3.42, 7.88, 31.3, 39.33 % for crude protein, nitrogen, ash, fiber and carbohydrates, respectively, Vitamin C was 4.7 mg100 ml⁻¹. The lowest values were with the supplemented substrates with spawn rate 5% 8.67, 1.39, 5.83, 30.93, 36.68% for each of crude protein, nitrogen, ash, fiber and carbohydrates, respectively and vitamin 2.97 mg100 ml⁻¹. The nature of the organic materials affects the extent to which the material is retained by the material, and there is a close correlation between the percentage of water in the fruits and the percentage of water in the materials used in agriculture, and also affects the percentage of components within the fruiting bodies (28). The method of cultivation also has an effect on the percentage of sugars and carbohydrates within the fruiting bodies of *G. lucidum* strains (11). Some supplements have an effect on the activation of cellulose- and lignin-degrading enzymes, such as manganese sulfate, thus improving the mycelium growth and effective contents of the fruiting bodies. (2, 19 and 30) investigated the use of organic substrates and supplements improve most quantitative and qualitative traits of *G. lucidum* strains.

Effect of different organic substrates on some *G. lucidum* fruit bodies chemical components: The results showed in a (Table 5) that there were no significant differences for the effect of different organic substrates on mushrooms contents of in each of protein, nitrogen, carbohydrates and vitamin C, while there were significant differences between substrates with ash and fiber, the best substrates were Carolina poplar sawdust by 7.57% for ash and 31.26% for fibers and white willow sawdust 7.76% for ash and the lowest value for ash 6.77% for wheat straw 30.93% for fibers with Rice straw substrate. The interaction between supplements substrates and the type of organic substrates shown in the (Table 7) the data it has been mentioned previously. Data in (Table 8) shows the results of the interaction between the organic

substrates with spawn rate, and there were clear significant differences between the measurements, and the substrates, white willow sawdust with spawn rate 15% was heist value in each of protein, nitrogen and vitamin C in the ratio of 22.41% and 3.59 % and 5.37 mg100 ml-1 respectively

Rice straw with spawn rate 15% was the best results with a percentage of 21.75% and 3.48% for protein and nitrogen and 39.44% for carbohydrates, the lowest value was with rice straw 5% spawn rate was 8.82, 1.41, 5.75 and 36.5% for protein, nitrogen, ash and carbohydrates.

Effect of different spawn rate on some *G. lucidum* fruit bodies chemical components: The results showed in a (Table 5) the presence of clear moral differences between the different spawn rates, where the spawn rate 15% was the best rates in all the entertainment characteristics, Protein, nitrogen, ash, fiber, carbohydrate, and vitamin c, with 20.6, 3.21, 7.56, 31.14, 38.76 %respectively and measuring vitamin c 4.62 mg100 ml-1 and the lowest results were observed with 5% spawn rate and interaction with the others factors mentioned previously and the best interaction were the cedar supplemented rice straw with spawn rate 15%. As it led to an increase in protein, nitrogen and carbohydrates fruit bodies contents and the highest value of ash with white willow 11% And the same treatment is vitamin c in a percentage 5.87 mg100 ml-1 the fiber was 31.53 % with supplemented Carolina poplar sawdust with spawn rate 5%.

Table 5 Effect of raw organic substrates, Raw organic substrates with supplements, and spawn rate on some *G. lucidum* chemical components.

Treatments chemical components	Crud Protein %	Nitrogen%	Ash%	Fiber%	Carbohydrate%	Vitamin C mg100 ml-1
raw organic substrates	16.53 ± 1.27 ^a	2.44 ± 0.18 ^a	6.89 ± 0.34 ^b	30.94 ± 0.05 ^b	37.58 ± 0.18 ^a	3.98 ± 0.21 ^a
supplement raw organic substrates	15.23 ± 1.11 ^a	2.64 ± 0.2 ^a	7.73 ± 0.4 ^a	31.11 ± 0.06 ^a	37.88 ± 0.25 ^a	3.81 ± 0.18 ^a
Wheat straw	14.91 ± 1.48 ^a	2.39 ± 0.24 ^a	6.77 ± 0.61 ^b	30.79 ± 0.08 ^d	37.54 ± 0.25 ^a	3.74 ± 0.23 ^a
Rice Straw	15.85 ± 1.78 ^a	2.54 ± 0.28 ^a	7.15 ± 0.48 ^{ab}	30.93 ± 0.07 ^c	37.9 ± 0.4 ^a	3.74 ± 0.25 ^a
Carolina poplar	15.17 ± 1.67 ^a	2.43 ± 0.27 ^a	7.57 ± 0.41 ^a	31.26 ± 0.05 ^a	37.7 ± 0.3 ^a	3.98 ± 0.27 ^a
White willow	17.59 ± 1.85 ^a	2.81 ± 0.3 ^a	7.76 ± 0.62 ^a	31.12 ± 0.06 ^b	37.78 ± 0.28 ^a	4.12 ± 0.36 ^a
Spawn rate 5%	9.51 ± 0.87 ^b	1.52 ± 0.14 ^b	6.68 ± 0.37 ^b	31.04 ± 0.07 ^b	36.63 ± 0.16 ^c	3.07 ± 0.2 ^c
Spawn rate 10%	18.06 ± 1.09 ^a	2.89 ± 0.17 ^a	7.69 ± 0.47 ^a	30.9 ± 0.06 ^c	37.8 ± 0.17 ^b	4 ± 0.2 ^b
Spawn rate 15%	20.06 ± 1.37 ^a	3.21 ± 0.22 ^a	7.56 ± 0.52 ^a	31.14 ± 0.06 ^a	38.76 ± 0.26 ^a	4.62 ± 0.23 ^a

* For each parameter, means not followed by the same letter are significantly different by Duncan's Multiple rang test (P>0.05).

Table 6 Effect of interaction between raw organic substrates, Raw organic substrates with supplements, different types of organic substrates on some *G. lucidum* fruit bodies traits.

	Organic substrate	Crud Protein %	Nitrogen %	Ash%	Fiber%	Carbohydrate%	Vitamin C mg100 ml ⁻¹
Raw organic substrates	Wheat straw	15.22 ± 2.23 ^a	2.44 ± 0.36 ^a	5.37 ± 0.63 ^e	30.81 ± 0.12 ^{cd}	37.52 ± 0.39 ^{ab}	3.59 ± 0.33 ^a
	Rice straw	15.94 ± 3.13 ^a	2.55 ± 0.5 ^a	7.45 ± 0.75 ^{bc}	30.93 ± 0.08 ^c	38.58 ± 0.64 ^a	3.47 ± 0.43 ^a
	Carolina poplar	16.54 ± 2.52 ^a	2.65 ± 0.4 ^a	8.39 ± 0.52 ^b	31.38 ± 0.08 ^a	37.53 ± 0.49 ^{ab}	4.45 ± 0.42 ^a
	White willow	18.41 ± 2.56 ^a	2.95 ± 0.41 ^a	9.71 ± 0.62 ^a	31.3 ± 0.06 ^a	37.88 ± 0.42 ^{ab}	4.43 ± 0.48 ^a
supplement raw organic substrates	Wheat straw	14.59 ± 2.07 ^a	2.33 ± 0.33 ^a	8.17 ± 0.83 ^b	30.77 ± 0.11 ^d	37.57 ± 0.33 ^b	3.89 ± 0.34 ^a
	Rice straw	15.75 ± 1.91 ^a	2.52 ± 0.31 ^a	6.84 ± 0.61 ^c	30.93 ± 0.11 ^c	37.22 ± 0.4 ^b	4.02 ± 0.26 ^a
	Carolina poplar	13.8 ± 2.24 ^a	2.21 ± 0.36 ^a	6.74 ± 0.53 ^{cd}	31.14 ± 0.06 ^b	37.86 ± 0.36 ^{ab}	3.52 ± 0.3 ^a
	White willow	16.77 ± 2.81 ^a	2.68 ± 0.45 ^a	5.8 ± 0.53 ^d	30.93 ± 0.05 ^c	37.68 ± 0.41 ^{ab}	3.81 ± 0.55 ^a

* For each parameter, means not followed by the same letter are significantly different by Duncan's Multiple rang test (P>0.05).

Table 7 Effect of interaction between raw organic substrates, spawn rate on some *G. lucidum* fruit bodies traits.

	Rate of spawn	Crud Protein %	Nitrogen%	Ash%	Fiber%	Carbohydrate rate%	Vitamin C mg100 ml ⁻¹
Raw organic substrates	5%	10.36 ± 1.44 ^b	1.66 ± 0.23 ^b	7.52 ± 0.6 ^a	31.14 ± 0.12 ^b	36.58 ± 0.22 ^c	3.18 ± 0.3 ^{bc}
	10%	17.89 ± 1.68 ^a	2.86 ± 0.27 ^a	7.79 ± 0.7 ^a	30.88 ± 0.09 ^c	37.73 ± 0.2 ^b	4.08 ± 0.34 ^a
	15%	21.35 ± 2.2 ^a	3.42 ± 0.35 ^a	7.88 ± 0.83 ^a	31.3 ± 0.04 ^a	39.33 ± 0.38 ^a	4.7 ± 0.36 ^a
supplemente d organic substrates	5%	8.67 ± 0.98 ^b	1.39 ± 0.16 ^b	5.83 ± 0.28 ^b	30.93 ± 0.08 ^c	36.68 ± 0.24 ^c	2.97 ± 0.27 ^c
	10%	18.24 ± 1.45 ^a	2.92 ± 0.23 ^a	7.59 ± 0.66 ^a	30.93 ± 0.09 ^c	37.87 ± 0.28 ^b	3.91 ± 0.23 ^{ab}
	15%	18.78 ± 1.66 ^a	3.01 ± 0.27 ^a	7.24 ± 0.64 ^a	30.98 ± 0.08 ^c	38.2 ± 0.27 ^b	4.54 ± 0.29 ^a

* For each parameter, means not followed by the same letter are significantly different by Duncan's Multiple rang test (P>0.05).

Tab 8 Effect of interaction between organic substrates and spawn rate on some *G. lucidum* fruit bodies traits.

Organic substrate	Rate of spawn	Crud Protein %	Nitrogen%	Ash%	Fiber%	Carbohydrate%	Vitamin C mg100 ml ⁻¹
Wheat straw	5%	10.14 ± 1.93 ^{bc}	1.62 ± 0.31 ^{bc}	6.51 ± 0.63 ^{def}	30.67 ± 0.08 ^e	36.6 ± 0.31 ^{de}	2.99 ± 0.26 ^d

	10%	19.31 ± 1.66 ^a	3.09 ± 0.27 ^a	6.54 ± 1.07 ^{def}	30.5 ± 0.05 ^f	37.81 ± 0.29 ^{bcd}	3.83 ± 0.27 ^{bcd}
	15%	15.28 ± 2.7 ^{abc}	2.45 ± 0.43 ^{abc}	7.26 ± 1.47 ^{cde}	31.2 ± 0.04 ^{ab}	38.23 ± 0.4 ^b	4.41 ± 0.46 ^{ab}
	5%	8.82 ± 2.13 ^c	1.41 ± 0.34 ^c	5.75 ± 0.46 ^f	30.95 ± 0.12 ^{cd}	36.5 ± 0.36 ^{de}	3.08 ± 0.54 ^{cd}
Rice straw	10%	16.97 ± 1.8 ^{ab}	2.72 ± 0.29 ^{ab}	9.48 ± 0.4 ^a	30.95 ± 0.11 ^{cd}	37.77 ± 0.29 ^{bcd}	3.94 ± 0.29 ^{bcd}
	15%	21.75 ± 2.71 ^a	3.48 ± 0.43 ^a	6.21 ± 0.52 ^{ef}	30.9 ± 0.14 ^d	39.44 ± 0.75 ^a	4.21 ± 0.39 ^{abcd}
	5%	8.86 ± 1.15 ^c	1.42 ± 0.18 ^c	6.11 ± 0.32 ^{ef}	31.23 ± 0.14 ^{ab}	36.58 ± 0.27 ^{de}	3.23 ± 0.18 ^{bcd}
Carolina poplar	10%	15.85 ± 2.46 ^{abc}	2.54 ± 0.39 ^{abc}	8.86 ± 0.43 ^{ab}	31.2 ± 0.06 ^{ab}	37.73 ± 0.42 ^{bcd}	4.22 ± 0.59 ^{abcd}
	15%	20.81 ± 2.57 ^a	3.33 ± 0.41 ^a	7.73 ± 0.81 ^{bcd}	31.35 ± 0.08 ^a	38.79 ± 0.4 ^{ab}	4.5 ± 0.45 ^{ab}
	5%	10.24 ± 2b ^c	1.64 ± 0.32 ^c	8.33 ± 1.01 ^{abc}	31.3 ± 0.1 ^a	36.84 ± 0.39 ^{cde}	2.99 ± 0.58 ^d
White willow	10%	20.13 ± 2.69 ^a	3.22 ± 0.43 ^a	5.89 ± 0.88 ^f	30.95 ± 0.08 ^{cd}	37.9 ± 0.42 ^{bc}	4 ± 0.47 ^{bcd}
	15%	22.41 ± 2.67 ^a	3.59 ± 0.43 ^a	9.05 ± 0.99 ^a	31.1 ± 0.1b ^c	38.61 ± 0.42 ^{ab}	5.37 ± 0.46 ^a

* For each parameter, means not followed by the same letter are significantly different by Duncan's Multiple rang test (P>0.05).

Conclusions: The effect of various kinds of sawdust and supplements on the yield of *G. lucidum* was investigated in this study, yield of *G. lucidum* varied widely depending on the kind of sawdust and supplements. The presence of clear moral differences between the different spawn rates, where the spawn rate 15% was the best rates in all the entertainment characteristics, Protein, nitrogen, ash, fiber, carbohydrate, and vitamin c, with 20.6, 3.21, 7.56, 31.14, 38.76 % respectively and measuring vitamin c 4.62 mg100 ml⁻¹. Therefore, it is important to use the proper substrate for production of *G. lucidum*. Supplemented Carolina poplar sawdust with (gypsum CaSO₄ 2%, lime CaCO₃ 2% and wheat bran10%) combined with 15% spawn level showed highest yield among all treatments.

Reference

1. Abdullah, M. B., Abed, I. A., and Alkobaisy, J. S. (2022). Effect of Different Substrates and Supplement with Three Types of Spawn on *Letinula Edodes* Parameters for First Production in Iraq. In IOP Conference Series: Earth and Environmental Science, 1060(1): 012060. IOP Publishing.
2. Abed, I., Hamad, H., Owaid, M., Hamdan, N., Lafi, A., and Mutlaq, H. (2021). Effect of using desert weeds (Chenopodiaceae) as supplements in substrates of *Pleurotus ostreatus* (oyster mushroom) production. Current Research in Environmental and Applied Mycology (Journal of Fungal Biology), 11(1): 185-196.
3. Azizi, M., Tavana, M., Farsi, M., and Oroojalian, F. (2012). Yield performance of Lingzhi or Reishi medicinal mushroom, *Ganoderma lucidum* (W. Curt.: Fr.) P. Karst.(higher Basidiomycetes), using different waste materials as substrates. International Journal of Medicinal Mushrooms, 14(5): 521-527.

4. Chang, S. T., and Buswell, J. A. (2008). Safety, quality control and regulational aspects relating to mushroom nutraceuticals. In Proc. 6th Intl. Conf. Mushroom biology and mushroom products, 5(6): 188-195.
5. Chen, S., Ma, D., Ge, W., and Buswell, J. A. (2003). Induction of laccase activity in the edible straw mushroom, *Volvariella volvacea*. FEMS Microbiology Letters, 218(1): 143-148.
6. Chen, Y., Sossah, F. L., Lv, Z., Lv, Y., Tian, L., Sun, X., ... and Li, Y. (2021). Effect of wheat bran and maize straw substrates on the agronomic traits and nutritional content of *Auricularia cornea* cv. Yu Muer. *Scientia Horticulturae*, 286: 110200.
7. Cosgrove, D. J. (1997). Assembly and enlargement of the primary cell wall in plants. *Annual review of cell and developmental biology*, 13(1): 171-201.
8. DuBois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. T., and Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical chemistry*, 28(3): 350-356.
9. Erkel, E. I. (2009). The effect of different substrate mediums on yield of *Ganoderma lucidum* (Fr.) Karst.
10. Gurung, O., Budathoki, U., and Parajuli, G. (2012). Effect of different substrates on the production of *Ganoderma lucidum* (Curt.: Fr.) Karst. *Our nature*, 10(1): 191-198.
11. Habijanac, J., Berovic, M., Boh, B., Wraber, B., and Petravic-Tominac, V. (2013). Production of biomass and polysaccharides of Lingzhi or Reishi medicinal mushroom, *Ganoderma lucidum* (W. Curt.: Fr.) P. Karst. (higher Basidiomycetes), by submerged cultivation. *International Journal of Medicinal Mushrooms*, 15(1): 81-90.
12. Haukongo, K. (2021). Effects of different substrates as medium for mushrooms cultivation.
13. Helrich, K. (1990). Official methods of analysis of the Association of Official Analytical Chemists. Association of official analytical chemists.
14. Iqbal, B., Khan, H., Saifullah, I. K., Shah, B., Naeem, A., Ullah, W., and Ahmed, N. (2016). Substrates evaluation for the quality, production and growth of oyster mushroom (*Pleurotus florida* Cetto). *Journal of Entomology and Zoology Studies*, 4(3): 98-107.
15. Jackson, D. N., and Messick, S. (1958). Content and style in personality assessment. *Psychological bulletin*, 55(4): 243.
16. Jasinska, A., and Siwulski, M. (2021). Impact of substrate supplemented with CaCO₃ on mycelial growth, yield, morphological features and storability of fruiting bodies of black poplar mushroom *Agrocybe cylindracea* (DC.) Marie. *International Journal of Horticultural Science*, 27: 76-86.
17. Jeewanthi, L. A. M. N., Ratnayake, K., and 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 and 2): 8-16.
18. Kumla, J., Suwannarach, N., Sujarit, K., Penkhruue, W., Kakumyan, P., Jatuwong, K., Vadthanarat, S., and Lumyong, S. (2020). Cultivation of mushrooms and

- their lignocellulolytic enzyme production through the utilization of agro-industrial waste. *Molecules*, 25(12): 2811.
19. Kurd-Anjaraki, S., Ramezan, D., Ramezani, S., Samzadeh-Kermani, A., Pirnia, M., and Shahi, B. Y. (2022). Potential of waste reduction of agro-biomasses through Reishi medicinal mushroom (*Ganoderma lucidum*) production using different substrates and techniques. *Acta Ecologica Sinica*, 42(1): 90-101.
 20. Miles, P. G., and Chang, S. T. (1997). *Mushroom biology: concise basics and current developments*. World Scientific.
 21. Nakagawa, T., Zhu, Q., Tamrakar, S., Amen, Y., Mori, Y., Suhara, H., ... and Shimizu, K. (2018). Changes in content of triterpenoids and polysaccharides in *Ganoderma lingzhi* at different growth stages. *Journal of natural medicines*, 72: 734-744.
 22. Pokhrel, C. P., Kalyan, N., Budathoki, U., and Yadav, R. K. P. (2013). Cultivation of *Pleurotus sajor-caju* using different agricultural residues. *International Journal of Agricultural Policy and Research*, 1(2): 19-23.
 23. Raghuramulu, N., Nair, M. K., and Kalyansundaram, S. (1983). *A manual of laboratory techniques*, National Institute of Nutrition, ICMR. Hyderabad, India, 53-328.
 24. Rasheed, H. M., Abed, I. A., and Hamod, J. H. (2020). Effect of spawn source and local organic substrates on duration of the crop *ganoderma lucidum* mushroom and biological efficiency. *Anbar Journal of Agricultural Sciences*, 18(2): 154-168.
 25. Roy, S., Jahan, M. A. A., Das, K. K., Munshi, S. K., and Noor, R. (2015). Artificial cultivation of *Ganoderma lucidum* (Reishi medicinal mushroom) using different sawdusts as substrates. *American Journal of BioScience*, 3(5): 178-182.
 26. Sadasivam, S., and Manickam, A. (2005). *Biochemical Methods Revised*. New Age International Publishers, New Delhi, 17-18.
 27. Seephueak, P., Preecha, C., and Seephueak, W. (2019). Effects of palm oil sludge as a supplement on *Ganoderma lucidum* (Fr.) Karst. cultivation. *Songklanakarin Journal of Science and Technology*, 41(2): 292-298.
 28. Simonic, J., Stajic, M., Glamoclija, J., Vukojević, J., Duletic-Lausevic, S., and Brceski, I. (2008). Optimization of submerged cultivation conditions for extra- and intracellular polysaccharide production by medicinal Ling Zhi or Reishi mushroom *Ganoderma lucidum* (W. Curt.: Fr.) P. Karst.(Aphyllorphomycetidae). *International Journal of Medicinal Mushrooms*, 10(4):351-360.
 29. Soh, E., Saeidi, N., Javadian, A., Hebel, D. E., and Le Ferrand, H. (2021). Effect of common foods as supplements for the mycelium growth of *Ganoderma lucidum* and *Pleurotus ostreatus* on solid substrates. *Plos one*, 16(11): e0260170.
 30. Stajić, M., Persky, L., Hadar, Y., Friesem, D., Duletić-Laušević, S., Wasser, S. P., and Nevo, E. (2006). Effect of copper and manganese ions on activities of laccase and peroxidases in three *Pleurotus* species grown on agricultural wastes. *Applied biochemistry and biotechnology*, 128(1): 87-96.

31. Ueitele, I. S. E., Kadhila-Muanding, N. P., and Matundu, N. (2014). Evaluating the production of Ganoderma mushroom on corn cobs. *African Journal of Biotechnology*, 13(22): 2215-2219.
32. Wachtel-Galor, S., Yuen, J., Buswell, J. A., and Benzie, I. F. (2011). *Ganoderma lucidum* (Lingzhi or Reishi). *Herbal Medicine: Biomolecular and Clinical Aspects*. 2nd edition.
33. Wagner, R., Mitchell, D. A., Lanzi Sasaki, G., Lopes de Almeida Amazonas, M. A., and Berovič, M. (2003). Current techniques for the cultivation of *Ganoderma lucidum* for the production of biomass, ganoderic acid and polysaccharides. *Food technology and biotechnology*, 41(4): 371-382.