



The Effect of Ganoderma Strain Type, Growth Substrate, and Nitrogen Supplementation on The Concentration of Certain Biochemical Parameters in Fruiting Bodies

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Abstract

This study evaluated the efficiency of locally prepared media made from sawdust, Euphrates poplar, and crushed common reed supplemented with crushed alfalfa as a nitrogen source at 0%, 10%, 20%, and 30% levels in cultivating Chinese and local Iraqi *Ganoderma lucidum*. The prepared media were found to be effective in supporting the production of proteins, carbohydrates, minerals, and fatty acids (oleic acid). The Iraqi strain outperformed the Chinese strain in terms of metabolic compound production efficiency, with protein, carbohydrate, oleic acid, iron, and calcium levels of 17%, 24.548%, 2.406%, 8.11 mg kg⁻¹, and 40.55 mg kg⁻¹, respectively. The medium prepared from common reed showed higher efficiency compared to the others in cultivating both strains at 18.87%, 24.499%, 2.823%, 24.74 mg kg⁻¹, and 8.55 mg kg⁻¹, respectively for the same metabolic compounds. This shows that the protein, carbohydrate, mineral, and fatty acid content in *Ganoderma lucidum* varies significantly depending on the media, strain, and supplementation level.

Keywords: Reishi mushroom, Ggrowth substrate, Bagasse, Sycamore tree waste.

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Introduction

Basidiomycetes are distinguished by their remarkable ability to produce a wide range of bioactive compounds, including carbohydrates, proteins, nucleic acids, lipids, minerals, terpenoids, phenolic compounds, stimulants, lectins, and vitamins. Additionally, they have been shown to reduce cholesterol levels in the body. Due to the presence of these vital nutrients, mushrooms are considered a

superior food with high nutritional value. These compounds exhibit a broad spectrum of therapeutic effects and can act as immunomodulatory, anticancer agents, antiviral agents, antioxidants, and anti-inflammatory agents (4). Routine consumption of edible mushrooms can provide adequate protection due to the presence of these nutrients.

Recent studies in Europe and Japan have shown that large basidiomycetes from the

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Agaricaceae family and the *Aphylophorales* order, especially *Ganoderma* spp have significant therapeutic and pharmaceutical importance in treating tumors and immunodeficiency, in addition to their role as antioxidants. *G. lucidum*, with its woody texture, contains chemicals with numerous health benefits. It is a common treatment for conditions such as cancer, low and high blood pressure, diabetes, rheumatism, heart problems, paralysis, ulcers, arthritis, asthma, hepatitis A, B, and C, infertility, psoriasis, mumps, epilepsy, and alcohol addiction (4 and 1 ν). (5) mentioned that the nutritional value of fleshy fungi is influenced by several factors, including the type of fungus, environmental conditions, and the type of growth medium.

Generally, the moisture level in fleshy fungi is within the range of 80-90%, while protein content is between 12-35%. It has low-fat content with the absence of cholesterol and contains high levels of dietary fiber, approximately 8-10%. Fungi produce various metabolites such as sugars, peptidoglycan, and terpenoids, which are responsible for medicinal properties. Of late, the cultivation of *G. lucidum* has expanded due to its high global demand. Fruiting bodies are marketed in the form of dry powder or capsules, and annual global production is about 6000 tons. China ranks first with 50% of global production, and the price of one kilogram of *Ganoderma* fruiting body powder reaches \$600 (13).

There are various carbon sources used in the cultivation of edible fungi, generally for different purposes related either to the fungus itself or the cultivation medium. Some studies have indicated the possibility of using wheat straw, sawdust, and date palm fibers (21), Egyptian pea straw (*Sesbania Topterra*) (2 ν and 2 ξ). Additionally, palm fronds and grass clippings supplemented with urea, also in a mixture (1 ν), significantly affected productivity, biological efficiency, and the protein content of the biomass. Moreover, these media notably influenced the speed of mycelial colonization of the cultivation medium.

A combination of reed peat, palm roots, charcoal, limestone, dolomite, algae, spent mushroom substrate, river sand and algae were considered for producing white food mushrooms as it affected their cap diameter and total productivity in addition to the biological efficiency and average stem diameter. The reed plant (*Phragmites australis*) was used as an economic alternative to wheat straw, as well as actinomycetes *Streptomyces* spp bacteria as an auxiliary factor in the decomposition of the medium. This affected the yield and the quality and size of the fruiting body of the mushroom (2 \bullet). This research thus investigated the use of local media in developing two strains of Reishi mushrooms supported by crushed jet as a source of nitrogen and its effect on the percentage and concentration of active substances in their fruiting body.

Materials and Methods

This study investigated the ability of two local strains of *G. lucidum* (S1) and an imported strain from China (S2) to grow and produce protein, carbohydrates, and other compounds. Three local media were prepared from carbon sources with crushed sage (M1), sawdust (M2), and crushed reed (M3) and supplemented with crushed sage at 0% (N0), 10% (N1), 20% (N2), and 30% (N3) of the dry weight of the media as a nitrogen source.

The treatments were based on the factorial experiment system with three factors of equal importance and three replicates for each treatment. The media were filled with sterilizable plastic bags at a rate of 12 bags for each treatment and with three replicates. They were moistened by adding 1:1 water and calcium carbonate and calcium sulphate were added at a rate of 1.3% sequentially of the dry weight of the treatment (2 \circ), and mixed homogeneously. The mouths of the bags were reduced in size with a plastic ring and closed with a filling. The bags were transferred to an autoclave and sterilized for 20 minutes at 121 °C and 1.5 atmospheric pressure and then allowed to cool (12). After the media were cool, inoculation was carried out with locally

propagated fungal inoculum at a rate of 2% based on the dry weight of the medium (13). The bags were shaken after inoculation to homogeneously mix the inoculum in all corners of the medium (26).

The media were transferred to an incubation and production room, which was specially constructed for this purpose from an iron frame covered with insulating foam panels. The media were incubated without exposure to light at a temperature of 30 ± 1 °C and relative humidity of 75-85% and without ventilation to raise the concentration of carbon dioxide inside the incubation room. This process continued until the completion of fungal growth in most of the media (13). When the mycelium invaded most of the culture medium, the cotton pads were removed, the bags opened and the incubation process continued at 30 ± 1 °C with other environmental conditions controlled to stimulate the formation of fruiting bodies. The culture media were exposed to lighting at an intensity of 150–200 lux through a 13-watt LED lamp.

The relative humidity of the incubation chamber was raised to approximately 90–95% and the oxygen concentration increased by operating the air extractors and leaving the doors open for 30 minutes three times a day (13). Frequent humidification in the form of a spray was done to maintain high relative humidity (9). Fruiting bodies were formed within different periods and according to the type of culture medium. The fruiting bodies were harvested when the cap became completely red and the white margin disappeared by pulling and cutting them with a knife at the surface level of the culture medium. They were continuously exposed to conditions that stimulate the formation of fruiting bodies until the second harvest cycle (2^v). The protein content of the fruiting bodies was estimated after drying and grinding using the Kjeldahl method based on that mentioned by Van Dijk et al. (2000). The total carbohydrate content of the fruiting bodies was estimated by the method used by (10), while the oleic acid was estimated according to the method described by (2) after drying. The fat percentage was calculated using the equation:

$$\text{Oleic acid \%} = \frac{\text{Weight of flask before extraction} - \text{Weight of flask after extraction}}{\text{Weight of sample}} \times 100$$

As for the mineral elements, they were estimated according to the APHA method (3) after digestion, so the sample became ready for analysis. Then the absorbance of the elements in these samples was measured using the atomic absorption device.

Results and Discussion

The results shown in Table 1 indicate significant differences in protein content of the fruiting bodies of the two strains with the S1 at 17.00% significantly outperforming the S2's 14.90%. The M3 medium was superior, recording 18.87%, followed by M2 at 16.71%, while the M1 recorded the lowest value of 12.84%. As for supplementation levels, the N3 treatment achieved 18.87% compared to the control treatment of 12.06%. The interaction

between the S2 strain and M3 medium provided the highest value at 18.57%, followed by S1 and M3 (18.03%), and the lowest, at 10.16%, recorded for the S2 and M1 interaction.

For strain and supplementation level interaction, S1N3 recorded the highest value at 29.16%, while the lowest was for S2N0 at 17.95%. For the interaction between medium and supplementation level, M3N3 recorded the highest value at 18.04% while the lowest value was recorded for M1N0 at 8.59%. In the three-way interaction between strain, medium, and supplementation level, S2M3N2 recorded the highest value (23.42%) compared to the lowest for S2M1N0 at 8.35%.

Table 1. The effect of *Ganoderma* strain and medium types, and nitrogen source supplementation on protein concentration in the fruiting bodies (%)

Strain	Media	Nitrogen supplementation level (% alfalfa residues)				S*M
		0 (N ₀)	10 (N ₁)	20 (N ₂)	30 (N ₃)	
S ₁	M ₁	8.83	17.00	16.37	19.9	15.52
	M ₂	16.21	17.65	18.29	17.69	17.46
	M ₃	17.48	16.69	12.04	25.90	18.03
S ₂	M ₁	8.35	10.44	13.00	8.83	10.16
	M ₂	16.83	17.25	13.71	20.06	15.96
	M ₃	12.67	17.35	23.42	20.85	18.57
S*N	S ₁	14.17	17.11	15.57	21.16	17.00
	S ₂	9.95	15.01	18.04	16.58	14.90
M*N	M ₁	8.59	13.72	14.69	14.36	12.84
	M ₂	12.52	17.45	18.00	18.87	16.71
	M ₃	15.07	17.02	17.73	23.38	18.87
Mean N		12.06	16.06	16.81	18.87	
LSD 5% S=1.907, M=2.336, N=2.697, SM=3.303, SN=3.815, MN=4.672, SMN=6.607						

The results also revealed significant differences in the carbohydrate content of the fruiting bodies (Table 2). Strain S1 was found to significantly outperform strain S2, with a carbohydrate concentration of 24.279% compared to 23.916%. With respect to supplementation level, the N3 treatment demonstrated superior results, at 24.601% compared to the control treatment's 23.427%. Medium M3 outperformed M1, with values of 24.499% and 23.562%, respectively.

Regarding the interactions, the S2M3 strain and medium interaction yielded the highest value at 24.548%, compared to the lowest for

S2M1 at 23.097%. The S1N3 interaction between strain and supplementation level recorded the highest value at 24.457%, while the lowest was for the S2N0 interaction (23.097%). Concerning the interaction between medium and supplementation level, the M3N3 recorded the highest value at 24.372%, while the lowest value was with the M1N0 interaction at 23.817%. As for the three-way interaction between strain, medium, and supplementation level, the S1M3N3 interaction recorded the highest value at 25.803%, while the lowest was for the S2M1N0 at 22.750%.

Table 2. The effect of *Ganoderma* strain and medium types, and nitrogen source supplementation on carbohydrate concentration in the fruiting bodies (%).

Strain	Media	Nitrogen supplementation level (% alfalfa residues)				S*M
		0 (N ₀)	10 (N ₁)	20 (N ₂)	30 (N ₃)	
S ₁	M ₁	22.883	22.883	24.280	24.173	24.773
	M ₂	24.147	24.147	24.390	24.500	24.400
	M ₃	24.363	24.363	24.227	23.407	25.803
S ₂	M ₁	22.750	22.750	23.160	23.593	24.883
	M ₂	22.883	22.883	24.323	24.400	24.803
	M ₃	23.537	23.537	24.340	25.377	24.940
S*N	S ₁	23.798	24.299	24.027	24.992	24.279
	S ₂	23.057	23.941	24.457	24.209	23.916
M*N	M ₁	23.817	24.720	23.883	23.828	23.562
	M ₂	23.515	24.357	24.450	24.602	24.231
	M ₃	23.950	24.283	24.392	24.372	24.499
Mean N		23.427	24.12	24.242	24.601	
LSD 5%, S=0.327, M=0.40, N=0.462, SM=0.566, SN=0.653, MN=NS, SMN=1.132						

Oleic acid content of fruiting bodies also varied significantly (Table 3), with the S1 strain demonstrating superior performance, achieving 2.406%, whereas S2 recorded 2.210%. In terms of supplementation levels, the N3 treatment proved to be the most effective, at 3.946%, in contrast to the control treatment's 2.066%. As for the medium, M3 demonstrated the highest effectiveness (2.823%) compared to M1 (2.210%). In terms of strain and medium interaction, the S2M3 yielded the highest value at 2.892%, compared to the S2M1 which gave the lowest at 1.783%.

For strain and supplementation level interaction, S1N3 recorded the highest value at 6.247%, while the lowest was with the S2N0 at 2.382%. Concerning the interaction between medium and supplementation level, M3N3 registered the highest value at 2.823% while M1N0 at 1.693% had the lowest. In the three-way interaction of strain, medium, and supplementation level, S1M3N3 recorded the highest value at 3.620% while S2M1N0 recorded the lowest (1.500)%.

Table 3. The effect of Ganoderma strain and medium types, and nitrogen source supplementation on oleic acid concentrations in the fruiting bodies (%).

Strain	Media	Nitrogen supplementation level (% alfalfa residues)				S*M
		0 (N ₀)	10 (N ₁)	20 (N ₂)	30 (N ₃)	
S ₁	M ₁	1.887	1.677	2.593	3.397	2.638
	M ₂	2.523	1.757	2.840	2.723	2.711
	M ₃	2.737	2.637	2.023	3.620	2.754
S ₂	M ₁	1.500	2.837	2.163	1.630	1.783
	M ₂	1.630	2.707	2.767	3.067	2.543
	M ₃	5.117	2.720	3.493	3.237	2.892
S*N	S ₁	2.382	2.690	2.486	3.247	2.701
	S ₂	2.749	2.421	2.808	2.644	2.406
M*N	M ₁	1.693	2.257	2.378	2.513	2.210
	M ₂	2.077	2.732	2.803	2.895	2.627
	M ₃	2.427	2.678	2.758	3.428	2.823
Mean N		2.066	3.556	2.647	3.946	
LSD 5%, S=0.242, M=0.297, N=0.343, SM=0.42, SN=0.485, MN=NS, SMN=0.830						

The results presented in Table 4 indicate a variation and significant differences in the calcium content of the fruiting bodies. Specifically, the S1 strain exhibited the highest content, recording 40.55, compared to S2, which yielded 37.17. Regarding the fortification ratio, the N2 and N3 ratios showed superior performance, registering 40.30 and 43.62, respectively, in comparison to the

control treatment, which recorded 32.37. Concerning the growth medium, the results in the table demonstrated the superiority of media M2 and M3, which recorded 40.05 and 42.74, respectively, compared to medium M1, which recorded 33.79.

Table 4. The effect of Ganoderma strain and medium type, and nitrogen source supplementation on the calcium content of the fruiting bodies (mg kg⁻¹).

Strain	Media	Nitrogen supplementation level (% alfalfa residues)				S*M
		0 (N ₀)	10 (N ₁)	20 (N ₂)	30 (N ₃)	
S ₁	M ₁	27.34	40.70	39.65	45.35	38.26
	M ₂	38.75	41.70	42.77	41.28	41.12
	M ₃	41.45	40.15	32.37	55.12	42.27
S ₂	M ₁	25.72	30.02	34.13	27.38	29.31
	M ₂	27.38	41.08	41.80	45.63	38.97
	M ₃	33.60	41.23	51.08	46.93	43.21
S*N	S ₁	35.85	40.85	38.26	47.25	40.55
	S ₂	28.9	37.44	42.34	39.98	37.17
M*N	M ₁	26.53	35.36	36.89	36.37	33.79
	M ₂	33.07	41.39	42.28	43.46	40.05
	M ₃	37.52	40.69	41.72	51.02	42.74
Mean N		32.37	39.15	40.30	43.62	

LSD 5%, S=3.05, M=3.74, N=4.32, SM=5.29, SN=6.11, MN=NS, SMN=10.58

Table 5 demonstrates substantial differences in iron levels across the fruiting bodies with strain S1 outperforming at 7.44 mg kg⁻¹ compared to 7.44 mg kg⁻¹ for S2. The N3 treatment surpassed the control with iron content values of 8.72 and 6.49 mg kg⁻¹, respectively. As for the medium, the results showed that M2 and M3 had higher iron content at 8.01 and 8.55 mg kg⁻¹, respectively, compared to M1 (6.77 mg kg⁻¹).

Interaction analysis revealed the highest value of 8.64 mg kg⁻¹ for the S2 and M3 strain/medium combination, while the lowest (5.88 mg kg⁻¹) was observed for S2 and M1.

For the strain and supplementation level interaction, S1N3 exhibited the highest value (9.45 mg kg⁻¹) followed by S2N2 and S1N1 (8.47 and 8.17 mg kg⁻¹, respectively). The lowest value of 5.81 mg kg⁻¹ was for the S2N0 combination. The interaction between medium and supplementation level was most pronounced in M3N3 at 10.20 mg kg⁻¹, while M1N0 yielded the lowest value at 5.35 mg kg⁻¹. For the three-way interaction, S1M3N3 exhibited the highest value (11.02 mg kg⁻¹), followed by S2M3N2 (10.22), whereas S2M1N0 recorded the lowest (5.22 mg kg⁻¹).

Table 5. The effect of Ganoderma strain and medium type, and nitrogen source supplementation on the iron content of the fruiting bodies (mg kg⁻¹).

Strain	Media	Nitrogen supplementation level (% alfalfa residues)				S*M
		0 (N ₀)	10 (N ₁)	20 (N ₂)	30 (N ₃)	
S ₁	M ₁	5.48	8.13	7.93	9.07	7.65
	M ₂	7.75	8.34	8.55	8.26	8.22
	M ₃	8.29	8.03	6.47	11.02	8.45
S ₂	M ₁	5.22	6.00	6.83	5.48	5.88
	M ₂	5.48	8.22	8.36	9.13	7.79
	M ₃	6.72	8.25	10.22	9.39	8.64
S*N	S ₁	7.17	8.17	7.65	9.45	8.64
	S ₂	5.81	7.49	8.47	8.00	8.11
M*N	M ₁	5.35	7.07	7.38	7.27	7.44
	M ₂	6.61	8.28	8.46	8.69	6.77
	M ₃	7.50	8.14	8.34	10.20	8.01
Mean N		6.49	7.83	8.06	8.72	

LSD 5%, S=0.61, M=0.75, N=0.86, SM=1.06, SN=1.22, MN=NS, SMN=2.11

The results of this study indicate that protein and carbohydrate levels vary significantly depending on the strain, with concentrations being higher in the Iraqi compared to the Chinese strain. This finding aligns with (14) that the levels of metabolic compounds such as proteins, carbohydrates, amino acids, and others differ in *Ganoderma* mushrooms depending on the strain. The results in Table 2 showed that carbohydrate levels varied significantly depending on the medium used and confirm the relationship between medium type and carbohydrates produced by the fungus, as the best carbohydrate production was on the cane medium. This is consistent with (8) that the components of the culture medium affect the production levels of metabolic compounds such as carbohydrates.

The same applies to the protein content of the fruiting body, as the ratios were significantly affected by the type of medium, with the highest protein production on the cane medium as well. This is what many studies have indicated about the effect of growth media on the levels of nutritional compounds for the Reishi mushroom and other fungi (19 and 31). The research aimed to extract some chemical compounds from the Reishi mushroom, noting that these fungi possess such extracts as distinctive chemical features and use them for their activities (7). The results of the study confirmed that the levels of oleic acid differed according to the strain and the type of medium used, as its concentration increased in the Iraqi compared to the Chinese strain, consistent with (14).

Recently, researchers have focused on the content of fatty acids in fruiting bodies due to their high importance in improving the production of some metabolites (30). One of the most important fatty acids found in edible mushrooms is oleic acid, which has an effective role in reducing blood fat levels as well as helping to mitigate arthritis (28). Various studies have also shown that edible fungi are rich in basic elements such as iron, magnesium, calcium, and others (31) as they can accumulate in the tissues of the fungus and

reach higher contents than the soil in which they grow (21).

The results of this study indicate that mineral and nutrient levels can differ and vary depending on the basic material and the medium on which the fungus grows. The concentrations of iron and calcium were high in the fruiting bodies formed on the reed medium and for the first strain, consistent with what was reached by (11). Their study noted that the effect of the medium on the content of the fruiting body of the fungus of essential minerals are effectively those elements that are indispensable for maintaining the proper performance of living organisms. This is due to their participation in many functions and biological changes in them, some of which the body needs in large quantities and others in very small amounts.

Conclusion

This study showed that the biochemical makeup of *Ganoderma lucidum* fruiting bodies is greatly influenced by the strain type, growing medium, and amount of supplementation, especially with regard to protein, carbohydrate, oleic acid, and vital mineral content. The Iraqi strain consistently outperformed the Chinese strain across various parameters, with the common reed-based medium showing superior results for nutrient accumulation. The findings underscore the importance of selecting appropriate strains and optimizing growth conditions to enhance the nutritional and therapeutic value of *G. lucidum*, offering valuable insights for improving mushroom cultivation practices.

Supplementary Materials

No Supplementary Materials.

Author Contributions

Author 1: Review and editing. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement

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Data Availability Statement

No Data Availability Statement.

Conflicts of Interest

The authors declare no conflict of interest.

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تأثير نوع السلالة Ganoderma نوع الوسط والتدعيم بمصدر النتروجين في تركيز بعض المعايير الكيموحيوية للأجسام الثمرية

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الخلاصة

أجريت هذه الدراسة في وحدة انتاج الفطر التابعة لكلية الزراعة/ جامعة الانبار لغرض معرفة كفاءة بعض الأوساط المحضرة محليا من نشارة الخشب والغرب ومجروش القصب المدعمة بمجروش الجت كمصدر للنتروجين وبنسب (٠،١٠،٢٠،٣٠) % في تنمية سلالتين من الفطر الريشي *G. lucidum* (الصينية والمحلية العراقية). حيث تشير نتائج الدراسة الحالية الى كفاءة الأوساط المحضرة في دعم انتاج البروتينات والكربوهيدرات والمعادن والاحماض الدهنية (Oleic acid) وان السلالة العراقية قد تفوقت على السلالة الصينية من حيث كفاءة الإنتاج للمركبات الايضية حيث كانت نسبة البروتين والكربوهيدرات وحامض الاوليك والحديد والكالسيوم (١٧%)، ٢٤.٥٤٨%، ٢.٤٠٦% و (٨.١١ ملغ كغم-١، ٤٠.٥٥ ملغ كغم-١) بالتتابع كما ان الوسط المحضر من القصب قد اظهر كفاءة اعلى مقارنة مع الأوساط الأخرى في تنمية السلالتين وكانت نسبة البروتين والكربوهيدرات وحامض الاوليك (١٨.٨٧، ٢٤.٤٩٩، ٢.٨٢٣%) والحديد والكالسيوم (٢٤.٧٤، ٨.٥٥ ملغ كغم-١) بالتتابع وهذا يوصلنا الى نتيجة ان الفطر الريشي محتواه من البروتين والكربوهيدرات والمعادن والاحماض الدهنية يختلف اختلافا معنويا باختلاف الوسط والسلالة ونسبة التدعيم.

كلمات مفتاحية: فطر الريشي، الأوساط الزراعية، مجروش القصب، نشارة شجرة الغرب.

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