Journal homepage
www.ajas.uoanbar.edu.iq
Anbar Journal of Agricultural Sciences
(University of Anbar – College of Agriculture)

A BIOCHEMICAL STUDY TO EVALUATE THE EFFICIENCY OF SOME LOCAL CULTURE MEDIA IN THE GROWTH OF THE REISHI MUSHROOM

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Article info

Received: 2024-05-30
Accepted: 2024-07-31
Published: 2024-12-31

DOI-Crossref:
10.32649/ajas.2024.150282.1276

Cite as:
Mahmoud, A. T., Abed, I. A., and Almohammedi, O. H. M. (2024). A Biochemical study to evaluate the efficiency of some local culture media in the growth of the reishi mushroom. Anbar Journal of Agricultural Sciences, 22(2): 1273-1283.

Abstract

Despite the significant nutritional and medicinal value of Reishi mushrooms few studies have examined the effectiveness of local media in enhancing their productivity. This study evaluated the efficiency of various media derived from the abundant raw materials in the Iraqi environment. Organic compounds from the biomass of materials such as sugarcane bagasse, sycamore tree waste, and wood shavings were used to grow local and Chinese strains of the Reishi mushroom in the laboratory and their levels of amino acids, phenols, flavonoids, and vitamin D3 measured. The results show sugarcane bagasse as the most efficient in promoting the growth of both isolates for all parameters compared to the other media. The local strain was superior in growth terms for all media compared to the Chinese strain. The study concluded that levels of organic compounds in biomass varied significantly depending on the type of medium and the cultivated strain. Alanine had the highest concentration among all amino acids while arginine was the lowest, and phenols and flavonoids had higher concentrations in the Iraqi strain grown on sugarcane bagasse compared to the Chinese strain for all mediums. The results for vitamin D3 were also consistent with the

other organic compounds in terms of the superiority of the sugarcane bagasse medium in stimulating production in the Iraqi compared to the Chinese isolate, and for all media.

Keywords: Reishi mushroom, Culture media, Bagasse, Sycamore tree waste.

دراسة كيموحيوية لتقييم كفاءة بعض الاوساط الزرعية المحلية في تنمية الفطر الريشي

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الخلاصة

تعد زراعة الفطر الريشي من التطبيقات المهمة جدا لما لهذا الفطر من أهمية غذائية وطبية وبالنسبة لتحضير أوساط محلية تستعمل في تنميته، لا تتوفر دراسات كافية تحدد كفاءة الأوساط المحلية في انتاجية الفطر، لهذا تهدف الدراسة تحديد كفاءة بعض الأوساط المحضرة من مواد أولية وفيرة في البيئة العراقية. استخدمت مخلفات مجروش القصب ومجروش شجرة الغرب ومجروش مخلفات اخشاب الأثاث في تنمية السلالات المحلية والصينية للفطر الريشي مختبريا وقياس مستويات بعض المركبات العضوية في الكتلة الحيوية للفطر اذ تم فحص مستويات الاحماض الامينية والفينولات والفلافينويدات وفيتامين D3. تشير نتائج الدراسة الحالية الى ان وسط مجروش القصب كان أكثر كفاءة في تشجيع نمو العزلتين مقارنة مع بقية الأوساط ولجميع المعايير المدروسة بينما كان وسط مجروش شجرة الغرب هو الأقل كفاءة وأيضا تشير النتائج الى تفوق السلالة المحلية في النمو على جميع الأوساط مقارنة بنمو السلالة الصينية. خلصت هذه الدراسة أيضا الى ان مستويات المركبات العضوية في الكتلة الحيوية تباينت تباينا كبيرا تبعا لنوع الوسط والسلالة المزروعة فبالنسبة للاحماض الامينية كان الالنين هو الأعلى تركيزا بين جميع الاحماض الامينية اما الارجنين فكان اقلها، اما الفينولات والفلافينويدات فكانت تراكيزها اعلى في السلالة العراقية المنماة على مجروش القصب مقارنة مع السلالة الصينية وعلى جميع الأوساط. نتائج فيتامين D3 هي الأخرى جاءت متفقة مع بقية المركبات العضوية من حيث تفوق وسط مجروش القصب في تحفيز انتاجه في العزلة العراقية مقارنة بالعزلة الصينية ولجميع الأوساط.

كلمات مفتاحية: الفطر الريشي، الاوساط الزراعة، الكتلة الحيوية، فلافونويدات، فينولات ومخلفات نباتية.

Introduction

Basidiomycetes are among the most sophisticated and complex fungal groups with the ability to use cellulose and lignin as a source of energy from their secretions of large amounts of extracellular enzymes (12). Mushroom cultivation is one of the technologies for recycling lignocellulosic waste and may be the only process that combines healthy food production and waste disposal in an ideal way (23).

Fungi produce a wide range of chemical compounds during their metabolism some of which are important for their growth, and the formation of its cells and structures, including proteins, nucleic acids, and enzymes. Called primary metabolic compounds, many are used in the manufacture of many types of food and some medicines (25). After the growth of the fungus is stabilized, the substances accumulated from primary metabolism as well as intermediate compounds are transformed into other products called by-products of metabolism (6 and 24). It is known that they are not essential for fungal growth but are formed under special conditions as a result of secondary metabolic pathways. A large number of these compounds have much value to humans, the most important being antibiotics, which represent the secondary metabolism of fungi, as well as vitamins, organic acids, and pigments (20). There is an increasing demand for healthy foods and products related to their nutritional function, specifically functional foods, as well as dietary supplements and nutrients. In this context, mushrooms appear an excellent choice for their nutritional value, bioactive compounds, and health-promoting properties (8). They are considered a valuable health food, rich in proteins, vitamins, and minerals (13 and 22). Global mushroom production has increased significantly and its cultivation market is expected to reach $20.4 billion by 2025. The possibility of reusing agro-industrial waste as a substrate is another factor contributing to the increase in mushroom production (1, 27 and 29).

Studies have indicated the use of palm waste, wild reed, and cogongrass wastes in the preparation of edible fungi such as white and oyster mushrooms (2 and 19). Mushrooms have much medicinal value, are able to break down complex compounds, and can grow under the particular environmental conditions of the region (compared to commonly cultivated fungi). The possibility of using agricultural wastes such as from reeds, sycamore trees, and wood used for making furniture in the production of mushrooms allows this waste to be transformed from low-nutritional to high-value food materials while reducing the environmental impact of improper waste disposal. This study aimed to isolate, characterize, and refine a number of secondary metabolites formed in the liquid cultures of these media.

Materials and Methods

The experiment was conducted at the laboratories of the Faculty of Applied Sciences of Fallujah University from March to April 2023. Two strains of the fungus (*Ganoderma lucidum*) were used, namely the local strain from Anbar University/Faculty of Agriculture-Department of Plant Protection, and the Chinese strain from the Department of Plant Protection - Organic Agriculture Department/Ministry of Agriculture.

Mushroom isolation activation: Mycelium activation for both strains was performed by inoculating PDA media with a small piece of mycelium obtained for the Chinese and local strains (4). The PDA medium was prepared at a concentration of 40 g L^{-1} and the pH was set to 6.5, autoclaved at 121°C for 15 min, cooled to 55°C, and then distributed into 8.5 cm diameter Petri dishes (29).

Preparation of culture media extract: Liquid extracts were prepared from plant residues (sycamore tree waste, sugarcane bagasse and furniture wood shavings) by taking 25 g of chips from each treatment and soaked in 1 liter of boiling water for 15 minutes. They were then filtered with a soft cloth and filter paper, and transferred to a 1-liter glass flask to achieve a volume of 1000 ml (3). The extracts were transferred to 250 ml glass bottles, each filled with 50 ml of three replicates per treatment. The sterilization process was carried out in an autoclave for 30 min at 121°C and a pressure of 15 lbs. $inch^{-1}$.

After 24 h incubation the media were inoculated with 5 mm piece of mycelium for both the Chinese and local strains and the treatments coded as shown in Table 1. The vials were then incubated at 30±1°C and shaken every 4 days for mycelium growth, which was completed after 30 days from inoculation (19 and 34). The media were then filtered with filter paper and the biomass dried at 60°C for 24 hours (33) and used for the determination of amino acids, phenols, flavonoids, and vitamin D.

Table 1: Treatments of strains and their function codes.

Strain type and Treatment	Treatment code	Strain type and Treatment	Treatment Code
Chinese strain		Local strain	
Wood shavings	T1.S1	Wood shavings	T1.S2
Sugarcane bagasse	T2.S1	Sugarcane bagasse	T2.S2
Sycamore tree waste	T3.S1	Sycamore tree waste	T3.S2

Amino acids were extracted according to the method presented by (9). Samples of the acids weighing 3 g were placed in a 25 ml volumetric bottle and 25 ml of hydrochloric acid (1M) was added at a temperature 55°C for 3 hours. The sample was then dried using a rotary evaporator and 5 ml of sodium citrate pH 2.2 was added. The sample was filtered using a 0.45 um plastic filter and taken to the apparatus for injecting.

Two hundred µl of orthophthalene dihydrate (5%) (OPA) were added to 1 ml of the extracted sample and shaken for 2 min. Then, 100 µl of the final mixture was injected into the amino acid analysis device (9).

The test was conducted at the laboratories of the Ministry of Science and Technology/Department of Environment and Water using an amino acid analyzer (Korean origin). The method presented by (31) used the carrier phase consisting of methanol, acetonitrile, and 5% formic acid in 20:60:20 proportions at a flow rate of 1 ml min^{-1}. A C18-NH2 column (250 mm * 4.6 mm) was used to separate the amino acids, while a fluorescence detector was used to detect amino acids with wavelengths of Ex = 445 nm and Em = 465 nm, Clarity 2015 software was used to analyze the amino acids.

A 0.1 g mixture of high purity amino acids (99.9%) was dissolved in nonionic water and transferred to a 250 ml conical flask till the volume of the concentration

reached 250 ppm. Concentrations of the calibration curve were injected into the apparatus (31) using the dilution law.

The total flavonoid content of the crude extract was determined by the aluminum chloride colorimetric method. Fifty µl of the crude extract (1 mg ml^{-1} ethanol) was prepared with 1 ml methanol mixed with 4 ml of distilled water, and 0.3 ml of 5% NaNO2 solution and 0.3 ml of 10% AlCl3 solution added after 5 min. The mixture was left for 6 min, after which 2 ml of 1 mol. L^{-1} sodium hydroxide solution was added. The final volume of the mixture was completed to 10 mL by adding distilled water, and the mixture left for 15 min. The absorbance was then measured at 510 nm and the total flavonoid content calculated from the calibration curve. The result was expressed as mg rutin equivalents per gram of dry weight (14).

The total amount of phenolic compounds in the ethanolic extract was determined using a standard Folin-Ciocalteu reagent. The reaction mixture contained 100 µl of the extract, 500 µl of Folin-Ciocalteu reagent (Folin-Ciocalteu Merck, Germany) and 1.5 ml of 20% sodium carbonate. The sample was then mixed on a vortex mixer and diluted with distilled water to a final volume of 10 mL. After a 2-hour reaction, the absorbance was determined at 765 nm and used to estimate the phenolic content using a calibration curve made with galic acid (Sigma-Aldrich, Germany). The total amount of phenolic compounds was expressed as galic acid equivalent per gram of dry weight (16).

The biomass was first ground with a blender and dried. Then, 1.0 g of the ground powder was dissolved in 5 mL of methanol in a A15 mL falcon tube for 2 h in the dark with continuous shaking. The solid was separated from the liquid methanol using Whitman 1 filter paper, and vitamin D separated from the methanol by slowly mixing three volumes of hexane (3 × 2 mL) in 60s intervals. For all sample types, phase separation by centrifugation (4000 rpm for 15 min) and the upper 4 mL organic phase was transferred to a beaker and dried under liquid nitrogen gas. The dried extract was dissolved in methanol. A UV absorbance reading was taken for each sample and the baseline continuously monitored during this process. Standard vitamin D3 was analyzed using an absorbance value of 210 nm (28).

Data was analyzed using SAS software to detect the effect of the source of variation in the treatments, and LSD test 0.05 (analysis of variance-ANOVA) was used to compare the study factors.

Results and Discussion

In this study, 17 of the 20 amino and bioactive amino acids were found in the two strains. As Table 2 shows, Alanine was the most abundant amino acid in the two cultivated strains with 42.14 µg g^{-1} in T2S2 and 40.25 µg g^{-1} in T2S1 while the lowest value was for arginine at 0.8% of the total free amino acids (µg g^{-1}). The result also indicates that the T1S1 treatment gave the lowest percentage of the amino acid cysteine at 9.58 µg g^{-1}. As shown in table 2, the difference in amino acid ratios and their variation for the two isolates may be attributed to the varying chemical composition of Ganoderma due to factors such as strain, growing conditions, and the development media used (29).

The results of the study in Table 2 show a difference in the concentrations of amino acids measured in the biomass of the two strains and the media used. A total of 9 non-essential amino acids were present, namely Alanine, Asparagine, Tyrosine, Proline, Serine, Arginine, Glutamine, Cysteine and aspartic acid together with 8 essential acids, namely Valine, Leucine, Isoleucine, Phenylalanine, Tryptophan, Lysine, Threonine and Methionine, this indicates that the non-essential amino acids exceeded the study parameters, while the concentrations of essential and non-essential amino acids varied according to the type of isolation and medium. This finding is the first of its kind in such a study as it aimed to measure the ratios and concentrations of amino acids in the biomass of Reishi mushrooms. The results are consistent with previous studies on the content of amino acids in the fruiting bodies of such mushrooms (11).

Table 2: The effect of the studied treatment on the level of amino acids (µg g^{-1}).

Composition	T1S1	T2S1	T3S1	T1S2	T2S2	T3S2	LSD
Alanine	33.25	40.25	36.25	34.15	42.14	39.58	4.027 *
Tryptophan	30.69	36.98	33.65	31.5	39.22	35.98	2.679 *
Asparagine	24.69	31.55	27.9	25.98	33.65	30.12	4.921 *
Isoleucine	24.56	30.52	27.98	25.98	33.65	29.85	4.978 *
Tyrosine	23.66	30.25	26.88	24.59	33.25	29.58	3.467 *
Valine	20.66	27.85	23.65	21.85	29.25	26.99	3.406 *
Methionine	20.14	28.9	24.59	21.25	30.25	27.85	4.129 *
Phenylalanine	20.14	27.85	23.65	21.22	29.25	26.99	3.602 *
Proline	19.85	26.55	22.66	20.14	29.55	25.49	3.706 *
Serine	18.79	25.99	21.59	19.00	26.99	24.65	3.194 *
Arginine	18.55	24.25	21.66	19.45	26.88	23.65	2.863 *
Aspartic acid	15.44	21.25	18.97	16.66	23.65	20.65	3.071 *
Glutamine	15.26	22.88	18.99	16.66	25.28	21.56	5.441 *
Lysine	13.65	19	15.98	14.59	21.45	18.97	2.937 *
Leucine	10.25	17.58	13.65	11.25	19.58	16.55	3.834 *
Threonine	10.24	10.08	13.88	11.55	20.15	17.45	2.904 *
Cysteine	9.58	15.08	12.8	10	17.8	14.59	2.351 *

*** ($P \leq 0.05$)**

The results in Table 3 show that T2S2, T3S2, and T2S1 were superior in phenolic content at 236.59, 216.59, and 215 mg 100 g^{-1}, respectively, while T1S1 recorded the lowest value at 184.56 mg 100 g^{-1}. As for flavonoids, T2S2 recorded the highest value of 108.98 mg 100 g^{-1} and T1S1 the lowest at 84.12 mg 100 g^{-1} for the same compound. Flavonoids are natural compounds with a phenolic structure and are known for their positive effects on health as they are good antioxidants, anti-mutagenic, anti-cancer, and anti-inflammatory in addition to their ability to modify the activity of cellular enzymes (17). Phenols are chemical compounds that contain aromatic and hydroxyl groups. These compounds are found as products of metabolic processes and are medically important for their antibacterial, antiviral, anti-inflammatory and antioxidant activities. Phenols can protect key cellular components from free radical damage, mainly due to their properties in activating antioxidant enzymes and relieving oxidative stress (18 and 23).

Various studies confirm the accumulation of secondary metabolic compounds in mushrooms, the most important of which are phenols and flavonoids, allowing them to be used as antioxidants and reductants (5, 7, 10 and 19). This study also indicates that Reishi mushrooms contain phenolic and flavonoid compounds in varying proportions depending on the type of strain and the medium used. Table 3 shows that the percentage of these compounds increased when grown in a sugarcane bagasse medium, while their lowest percentage obtained was when grown in sycamore tree waste medium. These findings are consistent with several studies including (15, 22, 26, 32 and 35) which indicated high concentrations of flavonoids in mushrooms and their effectiveness as antioxidants.

Table 3: Effect of the studied treatment on phenolics and flavonoids.

Name	Total phenolic content (mg 100 g^{-1})	Total flavonoid content (mg 100 g^{-1})
T1.S1	184.56	84.12
T2.S1	215.00	99.12
T3.S1	198.74	92.65
T1.S2	191.48	95.66
T2.S2	236.59	108.98
T3.S2	216.59	100.22
LSD (P≤0.05).	17.601 *	8.902 *

The results in Table 4 indicate variations in the levels of vitamin D3 in the biomass of all isolates and on all media, with treatment T2.S2 outperforming the others in vitamin D content at 25.65 mg kg^{-1} while treatment T3.S1 had the lowest at 16.58 mg kg^{-1}.

The results of this study (Table 4) indicate that the isolates grown in the local media contained different concentrations of vitamin D3, with the treatment grown in the sugarcane bagasse medium outperforming the others. This finding is the first of its kind in local Iraqi media on the relationship between the type of isolation and the manufactured medium in inducing vitamin D3 production in the biomass of mushrooms. This accords with other studies on vitamin D3 content in mushrooms grown in the different media (21, 30 and 36)

Table 4: Effect of the studied treatment on vitamin D3.

Treatment	VIT D (mg kg^{-1})
T1.S1	19.11
T2.S1	21.49
T3.S1	16.58
T1.S2	21.45
T2.S2	25.65
T3.S2	17.99
LSD (P≤0.05).	4.029 *

Conclusions

This study shows that mushroom production can benefit from utilizing the abundant sources of local raw materials especially sugarcane bagasse which has been found to be more effective than the other media for that purpose.

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.

Funding:

This research received no external funding.

Institutional Review Board Statement:

The study was conducted according to the plan drawn up by the supervisor and what was approved by the Scientific Committee in the Scientific Department.

Informed Consent Statement:

No Informed Consent Statement.

Data Availability Statement:

No Data Availability Statement.

Conflicts of Interest:

The authors declare no conflict of interest.

Acknowledgments:

The researchers thank their supervisors for the assistance provided throughout the research period and are grateful to the Chairman and faculty members of the Horticulture Department for facilitating this study.

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References

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): p. 012060. DOI: 10.1088/1755-1315/1060/1/012060.
2. Abdullah, M. B., Al-Kobaisy, J. S., & Abed, I. A. (2023a). Effect of different local agro-residuals extract in growth parameters of Lentinula edodes in solid and liquid cultures. AIP Conference Proceedings, 2862(1), 020019.
3. Al-Qaisi, M. R. M. (2006). Evaluating the efficiency of some materials in the productivity of white mushrooms Agaricus bisporus (lange imbach) and their storage potential (Master's thesis, University of Baghdad).
4. Atoji-Henrique, K., Henrique, D. S., Glória, L. S., Mazaro, S. M., & Casagrande, M. (2017). Influence of substrate composition on beta-glucans production and growth of Ganoderma lucidum. Journal of Agricultural Science, 9(5), 190.
5. Al-Hadithe, A. H. , A., & M. A. Al-Hamdani, F. (2024). The Effect Of The Interaction Between Boron And Mannitol On The Content Of Some Nutrients In

Cauliflow. Anbar Journal Of Agricultural Sciences, 22(1), 584–595. https://doi.org/10.32649/ajas.2024.183759.

6. Blundell, R., Camilleri, E., Baral, B., Karpiński, T. M., Neza, E., and Atrooz, O. M. (2023). The phytochemistry of Ganoderma species and their medicinal potentials. The American Journal of Chinese Medicine, 51(04): 859-882. https://doi.org/10.1142/S0192415X23500404.
7. Buruleanu, L. C., Radulescu, C., Antonia Georgescu, A., Dulama, I. D., Nicolescu, C. M., Lucian Olteanu, R., and Stanescu, S. G. (2019). Chemometric assessment of the interactions between the metal contents, antioxidant activity, total phenolics, and flavonoids in mushrooms. Analytical Letters, 52(8): 1195-1214. https://doi.org/10.1080/00032719.2018.1528268.
8. Chechan, R. A., Mohyaddin, M. O., Abdul-Qader, Z. M., and Amar, M. M. (2017). Prepration of new national media for cultivation and effect of some enviromental factors on growth rate of Oyster Mushroom. The Iraqi Journal of Agricultural Science, 48(5): 1304-1312.
9. Dahl-Lassen, R., van Hecke, J., Jørgensen, H., Bukh, C., Andersen, B., and Schjoerring, J. K. (2018). High-throughput analysis of amino acids in plant materials by single quadrupole mass spectrometry. Plant Methods, 14: 1-9. https://doi.org/10.1186/s13007-018-0277-8.
10. De Menezes Filho, A. C. P., Ventura, M. V. A., Alves, I., Taques, A. S., Batista-Ventura, H. R. F., de Souza Castro, C. F., ... and Soares, F. A. L. (2022). Phytochemical prospection, total flavonoids and total phenolic and antioxidant activity of the mushroom extract Scleroderma verrucosum (Bull.) Pers. Brazilian Journal of Science, 1(1): 1-7. https://doi.org/10.14295/bjs.v1i1.2.
11. Farhan, E. M., and Chechan, R. A. (2023). Analysis of amino acids and fatty acids in the local strain of wild and cultivated food mushrooms. In IOP Conference Series: Earth and Environmental Science, 1158(11): p. 112019. DOI: 10.1088/1755-1315/1158/11/112019.
12. Gupta, A., Sharma, S., Saha, S., and Walia, S. (2013). Yield and nutritional content of Pleurotus sajor caju on wheat straw supplemented with raw and detoxified mahua cake. Food chemistry, 141(4): 4231-4239. https://doi.org/10.1016/j.foodchem.2013.06.126.
13. Gürgen, A., Sevindik, M., Yıldız, S., and Akgül, H. (2020). Determination of antioxidant and oxidant potentials of *Pleurotus citrinopileatus* mushroom cultivated on various substrates. KSU Journal of Agricultural Natural Science, 23(3), 586-591. DOI:10.18016/ksutarimdoga.vi.626803.
14. Habibatni, S., Zohra, A. F., Khalida, H., Anwar, S., Mansi, I., and Awadh Ali, N. A. (2017). In vitro antioxidant, Xanthine oxidase-inhibitory and in vivo Anti-inflammatory, analgesic, antipyretic activity of Onopordum acanthium. Int. J. Phytomed, 9(1): 92-100.
15. Kopylchuk, H., Voloshchuk, O., and Pasailiuk, M. (2023). Comparison of total amino acid compositions, total phenolic compounds, total flavonoid content, β-carotene content and hydroxyl radical scavenging activity in four wild edible mushrooms. Italian Journal of Mycology, 52: 112-125. https://doi.org/10.6092/issn.2531-7342/16457.

16. Laouini, S. E., and Ouahrani, M. R. (2017). Phytochemical screening, in vitro antioxidant and antibacterial activity of Rumex vesicarius L. extract. Scientific Study and Research. Chemistry and Chemical Engineering, Biotechnology, Food Industry, 18(4): 367-376.
17. Liga, S., Paul, C., and Péter, F. (2023). Flavonoids: Overview of biosynthesis, biological activity, and current extraction techniques. Plants, 12(14): 2732. https://doi.org/10.3390/plants12142732.
18. Liu, W., Cui, X., Zhong, Y., Ma, R., Liu, B., and Xia, Y. (2023). Phenolic metabolites as therapeutic in inflammation and neoplasms: Molecular pathways explaining their efficacy. Pharmacological research, 193: 106812. https://doi.org/10.1016/j.phrs.2023.106812.
19. Mahmoud, A., and Khudair, M. Y. (2023). Evaluation Of Al Kabeer Al Shamali River's Water Suitability for Drinking, Based on Modeling and Pseudomonas Aeruginosa Detection in Syria. Journal of Life Science and Applied Research, 4(1): 9-29. https://doi.org/10.59807/jlsar.v4i1.60.
20. Mansoor, S. S., Al-Esawi, J. S., and Al-Falahi, M. N. (2023). Assessing The Efficiency of Cement Kiln Dust for Heavy Metals Removal from Simulated Polluted Water. Journal of Life Science and Applied Research, 4(1): 45-52. https://doi.org/10.59807/jlsar.v4i1.64.
21. Muhammed, A. H. , S., & M. Al-Joboory, W. (2024). Effect Of Levels And Methods Of Zinc Addition On The Growth And Yield Of Wheat Cultivated In Saline Soils. Anbar Journal Of Agricultural Sciences, 22(1), 343–357. https://doi.org/10.32649/ajas.2024.139484.1037.
22. Naji, H. F., and AL-Jabber, M. A. (2024). Genetic Diversity and Antibiotic Resistance Patterns of Pseudomonas aeruginosa Isolates from Iraqi Hospitals. Journal of Life Science and Applied Research, 5(1): 8-15.
23. Naji, H. F., and Hassan, A. A. (2023). Determining the occurrence of some virulence genes in proteus species isolates. Journal of Life Science and Applied Research, 4(2): 75-87. https://doi.org/10.59807/jlsar.v4i2.88.
24. Owaid, M. N., Muslat, M. M., and Abed, I. A. (2018). Mycodegradation of reed straw, Phragmites australis. Current Research in Environmental and Applied Mycology, 8(2): 290-297. DOI: 10.5943/cream/8/2/12.
25. Pathak, I., Saxena, M., and Pandey, S. (2022). A review on *ganoderma lucidum*: an important medicinal mushroom. International Journal on Biological Sciences, 1.
26. Radzki, W., Tutaj, K., Skrzypczak, K., Michalak-Majewska, M., and Gustaw, W. (2023). Ethanolic Extracts of Six Cultivated Mushrooms as a Source of Bioactive Compounds. Applied Sciences, 14(1): 66. https://doi.org/10.3390/app14010066.
27. Raghoonundon, B., Gonkhom, D., Phonemany, M., Luangharn, T., and Thongklang, N. (2021). Nutritional content, nutraceutical properties, cultivation methods and economical importance of Lentinula: a review. Fungal Biotec, 1: 88-100. http://dx.doi.org/10.5943/FunBiotec/1/2/6.
28. Rahman, A., Rahman, M. M., Hossain, M. S., Jahan, M. S., Jahan, N., and Bari, L. (2019). A simple and alternative UV spectrometric method for the estimation

of vitamin D3. Microb. Bioact, 2: 98-105. https://doi.org/10.25163/microbbioacts.212086A2127261219.

29. Rashid, H. M., Abed, I. A., and Owaid, M. N. (2018). Mycelia growth performance of Agaricus bisporus in culture media of composts supplemented with Sesbania sesban straw and phosphate rock. Current Research in Environmental and Applied Mycology, 8(3): 323-330. DOI: 10.5943/cream/8/3/4.
30. Rondanelli, M., Moroni, A., Zese, M., Gasparri, C., Riva, A., Petrangolini, G., ... and Mazzola, G. (2023). Vitamin D from UV-irradiated Mushrooms as a way for Vitamin D supplementation: a systematic review on classic and nonclassic effects in human and animal models. Antioxidants, 12(3): 736. https://doi.org/10.3390/antiox12030736.
31. Scriver, C. R., Beaudet, A., Sly, W., Valle, D., Stanbury, J. B., Wyngaarden, J. B., and Fredrickson, D. S. (2001). The metabolic and molecular bases of inherited.
32. Sevindik, M. (2024). Total phenolic, total flavonoid contents and antioxidant potential of the wild edible mushroom Clitocybe odora. Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi, 27(1): 75-81. https://doi.org/10.18016/ksutarimdoga.vi.1241327.
33. Srivastava, H. C., and Bano, Z. (1970). Nutrition requirements of Pleurotus flabellatus. Applied Microbiology, 19(1): 166-169. https://doi.org/10.1128/am.19.1.166-169.1970.
34. Thakur, R. (2013). Studies on genetic variability in ganoderma lucidum (curtis) p. karst. for identification of elite strains, (Doctoral dissertation, CSK Himachal Pradesh Krishi Vishavavidyalaya, Palampur).
35. Wang, S., Liu, Z., Wang, X., Liu, R., and Zou, L. (2022). Mushrooms do produce flavonoids: Metabolite profiling and transcriptome analysis of flavonoid synthesis in the medicinal mushroom Sanghuangporus baumii. Journal of fungi, 8(6): 582. https://doi.org/10.3390/jof8060582.
36. Zajac, I. T., Barnes, M., Cavuoto, P., Wittert, G., and Noakes, M. (2020). The effects of vitamin d-enriched mushrooms and vitamin d3 on cognitive performance and mood in healthy elderly adults: A randomised, double-blinded, placebo-controlled trial. Nutrients, 12(12): 3847. https://doi.org/10.3390/nu12123847.