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ISOLATION AND IDENTIFICATION OF *Lentinula edodes* FROM THE IRAQI ENVIRONMENT AND STUDY OF THE OPTIMAL CONDITIONS FOR ITS GROWTH

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ABSTRACT

The recent research aims to collect fungal fruiting bodies from the Iraqi environment / Baghdad Governorate /Al-Salihiya and diagnose them morphologically and molecularly using the polymerase chain reaction (PCR) technique.The fruiting bodies obtained from wild mushrooms were subjected to quantitative and qualitative analysis using HPLC to detect biologically active phenolic compounds in the fungus. The effect of some physical factors and conditions on the growth rate of fungal mycelium and its density was also studied in terms of (temperature, pH, type and concentration of materials used in preparing the media in addition to the effect of light and darkness factors). The results of BLAST analyses showed that there is a 99% match between the genes of the local isolate and the fungal strains belonging to the edodes species. This local isolate is designated by the symbol (EkRu-Bagadad-1) and the sequence (OM432157). Regarding the phenolic compounds profile, the results showed that the mushroom contains 15 phenolic compounds, which included 6 phenolic acids (Vanillic, Chlorogenic, 4-hydroxyl benzoic, Caffeic, Gallic, Cinnamic,) and 9 flavonoids (catechol, cinnamaldehyde eugnol, quercetin, nigellone, rutin, Pyrogallo, lignans, kaempferol). The total phenolic compounds in the mushroom amounted to 661.64 mg/L, and 4-hydroxybenzoic acid was the most concentrated phenolic acid in the mushroom. The results also showed that the highest growth rate of radial mycelium of the local strain of Shiitake mushroom was achieved in potato peel medium at concentrations of 20 gl^{-1} , which reached (0.473 cm/day). The local strain under study recorded the highest growth rate in all culture media used at pH (6) and temperature 23 C. The results also showed that the growth rate in the dark was higher than the growth rate in the light in all natural media used.

Key words: ***Lentinula edodes*, molecular diagnosis, mother culture, phenolic compounds.**

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INTRODUCTION

In the past few decades, special attention has been paid to dietary supplements as sources of health improvement and a large proportion of attention has shifted towards food mushrooms based on their long history of use in nutrition and folk medicine (Rani, M.,*et al*. 2023). Edible mushrooms have been classified as "next generation food" due to their high nutritional value along with their biological and functional potential (Ayimbila F.& S. Keawsompong, 2023). Many reports have been published on the nutritional and therapeutic values of different edible mushroom species(Chaipoot, S.,*et al* .2023). Edible and medicinal mushrooms, especially those belonging to the phylum Basidiomycota, have witnessed an increasing growth rate in recent years (Vasilakis, G., *et al* 2023.), and are now being cultivated and consumed

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commercially worldwide (Farhan, E.M & R. A.Chechan. 2023).Mushroom cultivation is a cost-effective and environmentally friendly solution for converting lignocellulosic organic waste into food proteins. There are approximately 2,300 known species of edible mushrooms, of which about 80 species are accepted as food and 25 species are cultivated commercially worldwide. Shiitake mushrooms (*Lentinus edodes*) are the second largest edible mushrooms and have been experiencing increasing growth in production and use over the past few years. Shiitake mushrooms contain a wide range of nutrients and bioactive components, each with distinct biological properties and health-promoting benefits (Baptista F., *et al*. 2023 (Several studies indicate that mushrooms (as food or extracts) are an excellent source of proteins, fiber, vitamins, minerals, amino acids, and unsaturated fatty acids, which are important for healthy nutrition (Liuzzi, G.M., , *et al*. 2023). Moreover, mushrooms contain many active compounds such as polysaccharides, proteins, and phenolics that have important life-promoting activities, providing an essential substance for enhancing the immune system and preventing life-threatening diseases (Desisa, B. D., *et al*. 2023). Phenolic acids play a fundamental role in the biological activity of mushrooms, mainly due to their strong antioxidant activity and their ability to protect vital cellular structures, such as cell membranes, structural proteins, enzymes, membrane lipids, and nucleic acids (Rajesh, R.U .& D. A Sangeetha. 2023). Thus, it prevents cellular DNA mutations and reduces carcinogenic processes (Wang, M.& R .A. Zhao. 2023). Therefore, the main objective of this work was to isolate the most important types of fungi under the genus Lentinula from the Iraqi environment - Baghdad Governorate, purify them, identify them morphologically and molecularly, and then benefit from the agricultural waste available in the Iraqi environment to produce a pure mother culture with simple local capabilities to be an alternative to the mother culture imported from abroad with hard currency.

MATERIALS AND METHODS

Sample Collection: The study was conducted on wild agricultural mushrooms belonging to the genus Lentinula. The mushrooms were collected from Al-Salihiya (Baghdad Governorate - Iraq) in January and February 2021**.**

Isolation and purification of Lentinula mushroom: The isolation and purification processes were carried out in the Fleshy Fungi Laboratory and the laboratories of the Department of Food Sciences - College of Agricultural Engineering Sciences using Potato Dextrose Agar (PDA) medium. Pieces of fruiting body tissue were planted in Petri dishes containing PDA medium and incubated at 25 °C. After growth and spread of the mycelium, pieces with a diameter of 5 mm were taken from it and planted in new dishes containing PDA medium to obtain pure isolates of Lentinula mushrooms. The pure isolates were stored at 4 °C until later use.

Molecular identification

The DNA of the fungus under study was extracted by using the DNA extraction kit - ZR Fungal/Bacterial/Yeast DNA MiniPrep™ (catalog No. D6005), according to the manufacturer. A sample of the filtrate representing the extracted DNA was taken and its purity was measured by using the Nanodrop devicea, and the following primers were used: forward primer (ITS1 F: 5'-TCCGTAGGTGAACCTGCGG -3'), reverse primer (ITS4 R: 5' TCCTCCGCTTATTGATATGC-3'.

To amplify the ITS1-ITS4 gene region, the amplification program included five minutes at 90°C for the activation step, one minute for the denaturation step at 94°C, one minute for the annealing step at 58°C, and two minutes for the extension step at 72°C. The gene amplification steps included 35 cycles (Spim, S.R.V., *et al*. 2021). The amplified ITS1-ITS gene products were sent to the Korean company to determine the nucleotide sequence of the amplified gene region and these sequences were compared with the sequences of fungal isolates in GenBank to determine the percentage of similarity with them.

Extraction and Analysis of Phenolic Compounds by HPLC: The fruiting bodies of

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the mushrooms were cleaned, washed with distilled water, air dried to constant weight and ground into a coarse powder using a laboratory grinder. Phenolics were extracted from the wild (dry) mushroom powder according to the method of (Çayan F.**,** *et al*. 2020) which involved mixing 50 g of dried and ground mushrooms with 250 ml of methanol (95% concentration) for 72 h in a cool and dark place accompanied by shaking and stirring from time to time with a magnetic stirrer. The extract was then filtered first using a piece of clean cotton material and finally through Whatman No. 1 filter paper. The filtrate was collected and the residue was extracted again using 100 ml of ethanol. The combined extracts were concentrated using a rotary evaporator under pickling pressure at 38 °C. The dried extract was filtered through a membrane filter with a diameter of 0.45 µm. The dried extracts are then stored in tightly sealed bottles at 4°C in the refrigerator to prevent oxidative damage until sample analysis by HPLC.Individual phenolic compounds were quantified by reversed-phase HPLC analysis, using a SYKAMN HPLC system equipped with a UV detector, Chemstation, and Zorbax-Eclipse Plus-C18-OSD. A column with a diameter of 250 mm, and a diameter of 4.6 mm. The temperature in the column was 30 °C, and a gradient extraction method was performed using emulsion A (methanol), plus emulsion B (1% formic acid in water v/v), as follows: initial 0-4 min, 40% B; 4-10 min, 50% B; and a flow rate of 0.7 ml/min. The volume of the injected samples was 100 µl, the standard was 100 µl and this was done automatically using an autosampler. Spectra were acquired at 280 nm.

Mother culture production

The fruiting bodies of the wild mushroom Lentinula edodes from ErRu-Baghad strain were used to obtain a pure isolate using a tissue culture plate containing PDA medium. The plate was then transferred to an incubator at 25 ±1°C for three weeks with continuous monitoring to monitor the growth of the fungus and exclude the contaminated plate. After obtaining the pure isolate, it was stored at 4 °C for later use.

Preparation of Natural Media: Natural materials and agricultural wastes were obtained from local markets, which included potato peels, wheat flour, barley flour, corn flour and barley flour. These natural materials were subjected to a series of experiments to determine the optimum conditions for producing the mother culture of the fungus. The media were prepared by drying them at 60 °C, then grinding them and distributing them in plastic bags. PDA medium and natural media were prepared at a concentration of 40 g l^{-1} . Then agar was added to the natural medium at a concentration of 15 g/L. The components of each medium were mixed well; And sterilized in a pressure vessel at 121 °C for 15 minutes. Then the media were cooled to 55 °C and distributed in 8.5 cm diameter dishes. Then the prepared dishes were inoculated with a piece of activated fungal growth on PDA medium, incubated at 25 °C. (Rahman, M.A., *et al*. 2023).

Study of the optimum conditions for the growth of mycelium: The study included the effect of different parameters such as the type and concentration of the growth medium, temperature, pH, in addition to light and darkness. The growth of fungi was studied on four culture media (potato peel (PPA), wheat flour (WFA), Barley flour (BFA), Corn flour (CFA), and Shell flour (SFA) prepared at a concentration of 20, 30, and 40 g $l^{-1.}$. Additionally.testing the effect of three levels of pH (Baptista F., *et al*. 2023) the effect of three values of temperatures (Łysakowska, P., *et al*. 2023) C, and the effect of light and darkness, on the radial growth rate of the fungus on the mentioned media were studied. The radial growth of the fungus was recorded in each experiment.. Fruiting bodies of the diagnosed and registered wild fungus *Lentinula edodes*of the strain ErRu- Baghdad were used to produce a pure culture using tissue culture inplate containing the media PDA . After that, theplate were transferred to an incubator at 25^{o} ± 1 C for three weeks with continuous monitoring to observe the growth of mycelium and to exclude the contaminated plate. the fungal growth was monitored until it was fully grown in the dish. The growth rate was calculated as follows:

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Growth rate (cm/day) = x / y
X = diameter of the dish 8.5 cm
Y = time required for the fungus to grow to reach the edge of the dish or to fill the dish with growth (Morales, D., *et al*. 2023).

Statistical Analysis

The Statistical Analysis System -SAS (SAS. 2018) program was used to analyze the data to study the effect of different treatments on the studied traits according to a completely randomized design (CRD-Completely Randomized Design), in one direction or two directions according to the studied factors, and the significant differences between the averages were compared using the Least Significant Difference-LSD test.

Estimation of the mycelium density of *Lentinula edodes:* The media (PDB , Potato Peel (PPB) , Wheat Flour (WFB) , Barley Flour (BFB) , Corn Flour (CFB) and Shell Flour (SFB) were prepared without adding agar, at a concentration of 20 gl^{-1} and the pH adjusted to 6 and incubating them at 23°C .After the growth was completed in the aforementioned media separately. The mycelium was scraped from the media surfaces and transferred to a clean, dry, and pre-weighed Crucible to estimate the weight in a sensitive balance, which expresses the growth intensity as in the following equation:
Growth density (g /plate) = y – x
Note that: x = weight of the Crucible+ the weight of the Mycelium
Y = Weight of the empty Crucible ((Morales, D.,*et al*. 2023).

RESULTS AND DISCUSSION

Samples of fruiting bodies of the genus Lentinula growing wild were obtained during January and February 2021 from the Iraqi environment - Baghdad Governorate - Al-Salihiya area (**Figure** 1) The isolate was diagnosed morphologically based on some morphological characteristics as shown in the **(Table 1)**.

The purification process was carried out by transferring the fungal spores onto several plates containing PDA media to obtain a pure isolate (Kobayashi, T., *et al*. 2020) **(Figure 2)**

Table 1. Morphological description of wild mushrooms

Scientific name	Fruit bodies Colour	Cap diameter	Stalk length	Stalk diameter
Lentinula edode	**Red to brown**	**2-5 cm**	**2-3 cm**	**0.5-1.2 cm**

Figure 1. Wild shiitake mushroom isolated from the Iraqi environment -Baghdad Governorate

Figure 2 . Shows a pure isolate of the shiitake mushroom under study

Molecular diagnosis of the fungus under study: The genetic material DNA was extracted from the mycelium of the fungus under study and its purity was measured using the Nanodrop device. The results showed that the purity percentage was 1.7% **.**The rRNA

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gene amplification reaction was performed for the 18S Internal Transcribed Spacer (ITS) region using a forward primer to amplify the ITS1 gene and a reverse primer to amplify the ITS4 gene. The ITS1 and ITS4 intergenic region is usually used to distinguish different types of fungi and gives decisive results in diagnosis. The separation was then carried out by electrophoresis on a 1.5% agarose gel at 5 V/cm 21x TBE for 1:30 hours (Garcia, B.L.,*et al*. 2020). The results of the migration on the agarose gel after examination using ultraviolet light showed the presence of a clear band, and the molecular size of this band was estimated by comparing it with the size index of the DNA migrated on the same gel and under the same conditions as shown in (Fig. 3).

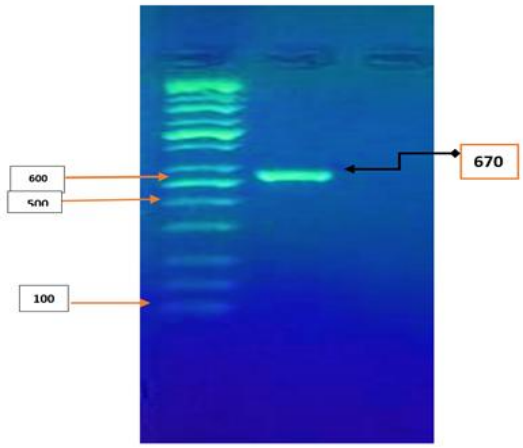

Figure 3. The resulting band size is 670bp after electrophoresis of the PCR products on a 1.5% agarose gel

Nitrogenous base analysis

The amplification products of the ITS region were sent to Macrogen, Korea. The genetic identity was studied with the National Center for Biotechnology Information (NCBI) by BLAST program. The results showed that there was 99% identity between the local isolate and the fungal strains belonging to the edodes species, designated as (EkRu-Bagadad-1) and sequence **(**OM432157).

Quantitative and qualitative estimation of phenolic compounds in the fruiting body of shiitake mushroom: 15 phenolic compounds were identified as shown in Table (1). The total phenolic compounds in the mushroom were 661.64 mg/L, and our results were higher than those recorded in a previous study (Elhusseiny, S.M.,*et al*. 2021). The variation in qualitative and quantitative composition in different studies can be attributed to environmental conditions, maturity stages, and genetic differences between species (Kumla, J.,*et al*. 2020). The mushroom recorded the highest percentage of the phenolic compound 4-hydroxy benzoic acid with a concentration of 224.3 mg/L and constituted 33.90% of the total phenolic compounds, followed by catechol with a concentration of 140.5 mg/L and constituted 21.24% of the total phenolic compounds, then vanillic with a concentration of 90.18 mg/L and constituted 13.62% of the total phenolic compounds. While the mushroom recorded the lowest concentration of gallic acid, which reached 0.07 mg/L and constituted 0.011% of the total phenolic compounds. Phenolic compounds in wild mushrooms can be classified in descending order from highest to lowest content as follows 4 :-hydroxybenzoic acid > catechol > vanillic acid >cinnamic > cinnamaldehyde > chlorogenic > eugnol > qurctin > nigellone > Caffeic acid > rutin > lignan > Pyrogallo > kaempferol > Gallic acid. In the current study, 6 phenolic acids were identified (Vanillic, Chlorogenic) Caffeic, Gallic, Cinnamic, 4-hydroxy benzoic), and the total phenolic acids in mushrooms reached 233.35 mg/L and constituted 66.93% of the total phenolic compounds, and 4-hydroxy benzoic acid was the most concentrated phenolic acid significantly in both species, as its percentage reached 96% of the total phenolic acids. 9 flavonoid compounds were also detected, and the total content of flavonoids in the mushroom was 424.27 mg/L, accounting for 35.34% of the total phenolic compounds. The phenolic compound catechol was the dominant compound, accounting for 33% of the total flavonoids, respectively. Our results were higher than those recorded in previous studies (Elhusseiny, S.M., *et al*. 2021).

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Table 2 . Phenolic compounds content in the fruiting body of the local strain of wild shiitake mushroom

Phenolic compounds	Shiitake mushroom mg/L
4- hydroxy benzoic acid	224.3395
Vanillic acid	90.1825
Cinnamic acid	79.22784
Chlorogenic acid	19.95040
Caffeic acid	10.5283
Gallic acid	0.077481
catechol	140.5766
cinnamaldehyde	55.46588
eugnol	12.85635
quercetin	12.19705
nigellone	11.61509
rutin	0.761048
Pyrogallo	0.205128
kaempferol	0.169445
lignans	3.494570
Total flavonoids	424.27
Total phenolic acids	233.35
Total	661.56

The effect of media concentration on the growth rate of *Lentinula edodes*: The results shown in the (table 3). showed that the growth rates of the local strain of Shiitake mushroom under study were affected by the type and concentration of the media used for growth. Three concentrations were used for each media 40, 20, 10 gl^{-1}. All media recorded the best growth rate for the local strain at the concentration of 20 gl^{-1} compared to the growth of mycelium in the standard media (PDA). The highest growth rate for the local strain was achieved in the PPA, which reached (0.473 cm/day). While the growth rates of the local strain in the media ; BFA, WFA, CFA and SFA reached (0.463 cm/day, 0.448 cm/day, 0.452 cm/day, 0.4515 cm/day) respectively. This represented a significant difference $P \leq 0.05$ when compared with the growth rate of the strain in PDA at the same concentration, which was (0.341 cm/day). The local strain did not record a significant difference in growth rates at a concentration of 40 gl^{-1} in all culture media compared to the control media PDA. It was also shown that the local strain of shiitake mushroom recorded the lowest growth rate at a concentration of 10 gl^{-1} in all culture media except for the solid potato peel media, which was an exception from the rest of the media, and the growth rate value was (0.447 cm/day). The better performance of mycelium on natural media compared to standard media can be attributed to the diversity of natural nutrients which are very essential for the growth of fungal cells such as carbohydrates, sugars, dietary fiber, protein, lipids, vitamins and minerals (Kalaw, S.P.,*et al*. 2021). According to Desisa et al., (Desisa, B. D., *et al*. 2023), the nutritional content of the culture media affects the growth of mycelium. In addition, the nutritional composition of the organic matter of the substrates affects the growth of fungi. The data showed that the solid potato peel media was the best culture media among the culture media used, which recorded the highest fungal growth rate at the lowest concentrations of 10 and 20 gl^{-1} . The superiority of the media is attributed to the components of potato peels, which consist of highly concentrated nutrients that contribute to increasing the growth of food fungi, namely cellulose 35%, hemicellulose 5%, and lignin 4% of the dry weight. Ajiboye and Said (Ajiboye, A . E .& R.O. Said. 2023) indicated that potato peels contain moisture (13.37%), ash (2.51%), fiber (41.71%), protein (1.65%), fat (0.59%), and

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carbohydrates (40.179%) of the dry weight. In addition, the percentage of total solids was (86.64%), energy (172.63%), and pH (6.01). The superiority of potato peels over other media is also explained by the percentage of carbon to Nitrogen C/N, which is 25:1, is one of the closely related factors in influencing the growth rates of fungi of different species, which gives it an advantage in use over other media in addition to its economic feasibility.

Table 3. Effect of the type and concentration of the media on growth rate of mycelium of a shiitake mushroom

Media Type	Growth rate of mycelium (cm/day)			Average
	10	20	40	
PDA	0.303	0.341	0.4300	0.358
PPA	0.447	0.473	0.425	0.469
WFA	0.353	0.448	0.430	0.410
PFA	0.377	0.463	0.430	0.423
CFA	0.341	0.452	0.430	0.407
SFA	0.326	0.4515	0.430	0.402
LSD 5%		0.106*		------
Average	0.3578	0.438	0.429	0.0973*
LSD 5%		0.059*		

Effect of pH on the growth rate of *Lentinula edodes:* Three pH values (Carvajal E. S.S**.,** *et al*. 2012) were used for each culture media and compared with the same pH values in the standard PDA media. The results shown in (Table 4) indicate that the highest daily radial growth rate of the local strain under study in all the used culture media was achieved at pH (6), and the local strain recorded the highest growth rate in PPA, which reached (0.567 cm/day) compared to the growth rate in the standard PDA media (0.447 cm/day). While the growth rate of the strain in the BFA reached (0.531 cm/day). The local strain did not record a significant difference in the growth value in the CFA and the SFA, which reached (0.500 cm/day). While the lowest growth rate in the WFA reached (0.472 cm/day) compared to the daily growth rate of the local strain in other culture media. The strain also did not record any significan difference in the daily growth rate in the natural media used at pH (5), while its growth rate was higher than the growth rate in the standard PDA media. On the other hand, the lowest growth rate of the fungal spun was at pH (7) in all media used compared to other values of pH. The pH significantly affects the growth of mycelium through differentiation of cell membrane function, as well as influencing enzyme activity. It also affects the solubility of salts, absorption of essential nutrients from the media, and the mechanism of their transport into the cell (Morales, D., *et al*. 2023). The results shown in (Table 4) showed that the local strain of shiitake mushroom requires a slightly to neutral pH of the culture media to produce superior mycelial growth. The maximum mycelial growth of shiitake mushroom was observed to be achieved at pH (6). The results of the current study are consistent with the findings of Dulay et al. (Dulay R.M.R., *et al*. 2021a), who reported that the optimal fungal growth for most of the tested isolates of the genus *Lentinus* is in the pH range (5 to 7). In the same context, Kalaw et al. (Kalaw, S.P., *et al*. 2021) indicated that the genus *Lentinus* requires a slight to neutral pH of the culture media to produce high fungal growth.

Table 4 . Effect of pH on growth rate of mycelium of a shiitake mushroom

Media Type	Growth rate of mycelium (cm/day)			Average
	5	6	7	
PDA	0.344	0.477	0.339	0.386
PPA	0.446	0.567	0.426	0.479
WFA	0.423	0.472	0.360	0.418
PFA	0.446	0.531	0.402	0.459
CFA	0.446	0.500	0.355	0.433
SFA	0.446	0.500	0.341	0.429
L.S.D %5	0.431	0.051*	0.370	0.088*
Average		0.507		
L.S.D %5		0.078*		

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The effect of temperature on the growth rate of *Lentinula edodes:* The results shown in (Table 5) the effect of temperature on the growth rate of mycelium of shiitake mushroom under study in natural environments. The effect of three temperature values (Hajdú, P., *et al*. 2022) C was tested. The results indicate that the highest growth rate of mycelium was achieved at a temperature of (23 C) in all culture media used compared to growth in the standard PDA media. The local strain recorded the highest growth level in the PPA (0.708 cm/day), followed by the BFA (0.607 cm/day), while the growth rate was similar in the CFA and the SFA, which reached (0.567 cm/day). The local strain recorded the lowest growth rate in the WFA, which reached (0.531 cm/day), compared to the growth rate in the standard PDA media (0.500 cm/day). The results obtained in this study showed that the best temperature for the growth of mycelium was achieved at 23 °C, and it was noted during the study that the temperature of 25 °C gave a good growth rate in different media, and the local strain recorded the lowest growth rate at 21 °C in all culture media used in the study. Accordingly, the local strain of shiitake mushroom can be classified as mesophilic fungi that grow at temperatures ranging from 25-30 °C (Morales, D., *et al*. 2023). Our results were similar to the study of Trang et al. (Trang N., *et al*. 2023) reported that the optimum temperature for the growth of two shiitake strains (J1, J2) was 25 °C, and the growth of the strains was slow at 30 °C, possibly due to the denaturation of essential enzymes responsible for catalyzing metabolic processes (Łysakowska, P., *et al*. 2023).

Table 5 . Effect of temperature on growth rate of mycelium of a shiitake mushroom in natural media

Media Type	Growth rate of mycelium (cm/day)			Average
	21	23	25	
PDA	0.386	0.500	0.447	0.444
PPA	0.4815	0.708	0.541	0.576
WFA	0.404	0.531	0.500	0.457
PFA	0.448	0.607	0.532	0.529
CFA	0.4315	0.567	0.5025	0.500
SFA	0.4315	0.567	0.501	0.499
LSD 5%		0.091*		0.0882*
Average	0.422	0.58	0.497	-----
LSD 5%		0.116 *		

Effect of light and darkness on mycelium growth rate of *Lentinula edodes:* The results shown in (Table 6) showed the effect of light and darkness on the growth rate of the local strain of shiitake mushroom. The results reached in this study indicated that the growth rate in the dark was higher than the growth rate in the light in all the natural media used. When comparing the growth rates in the culture media in the dark, we note that the highest growth rate of the local strain was recorded in the PPA, which reached (0.850 cm / day) compared to the growth rate of the local strain in the standard PDA media (0.607 cm / day). The local strain did not record any difference in the growth rates in the BFA and the CFA, which reached (0.772 cm / day). The local strain also recorded a similar growth value in the WFA and the SFA, which reached (0.710 cm / day). Lighting is an important environmental factor that regulates the growth and reproduction of fungi, which is necessary for their distribution and survival. It is either a growth stimulator or an inhibitor (Abdullah, S.N.F., *et al*. 2023). The strain recorded Shiitake mushrooms have high growth rates in all culture media used in the dark compared to the growth rate in the light as shown in (Table 6). This positive response of the local strain of Shiitake mushrooms in the dark is consistent with previous studies that indicated that darkness stimulates the growth of mycelium, while lighting leads to growth inhibition (2; 12). According to the researcher Tejedor-Calvo et al. (Tejedor-Calvo E., *et al* . 2020), the negative response of edible fungi in lighting is due to the decomposition of some light-sensitive materials such as pigments, some vitamins, and steroidal substances that enter into the composition of hormones.

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Table 6 . Effect of light and darkness on the daily growth rate of mycelium of a shiitake mushroom strain in natural media

Media Type	Growth rate of mycelium (cm/day)		LSD
	Dark	light	
PDA	0.607	0.500	0.116 NS
PPA	0.850	0.607	0.165 *
WFA	0.710	0.531	0.159 *
PFA	0.772	0.607	0.144 *
CFA	0.772	0.567	0.151 *
SFA	0.710	0.567	0.139 *
Average	0.171 *	0.136 NS	
LSD5%	0.735	0.5625	
	0.148 *		

Estimation of the mycelium density of *Lentinula edodes:* The results shown in (Table 7) indicate the effect of liquid media on the weight of biomass after 14 days of incubation for the local shiitake mushroom strain. The local strain recorded a significant difference in the average weight of biomass in all culture media compared to the standard liquid media PDB. Except for the SFB, which recorded the lowest average biomass (0.4015 g) and without any significant difference from the standard liquid media PDB (0.4005 g). The highest average biomass was recorded in the PPB, which reached (1.122 g), followed by the WFB which reached (1.0575 g), while the weight of biomass in CFB reached (0.988 g), and BFB (0.965 g). The effect of the media quality on the weight of the biomass is attributed to the efficiency of shiitake mushroom enzymes in analyzing the cellulosic and lignocellulosic materials that make up the agricultural waste media. This efficiency increases in liquid media as a result of the utilization of nutrients as they are dissolved in the media (Haro, A., *et al*. 2020).

Table 7 . Dry weight of mycelium of shiitake mushroom strain Lentinula edodes in natural liquid media and comparison with PDA

Media Type	Growth density (g)
PDB	0.4005
PPB	1.122
WFB	1.0575
BFB	0.965
CFB	0.988
SFB	0.4015
LSD	0.437 *

CONCLUSION

The total phenolic compounds in the mushroom amounted to 661.64 mg/L, and 4-hydroxybenzoic acid was the most concentrated phenolic acid in the mushroom. The results also showed that the highest growth rate of radial mycelium of the local strain of Shiitake mushroom was achieved in potato peel medium at concentrations of 20 gl^{-1} , which reached (0.473 cm/day). The local strain under study recorded the highest growth rate in all culture media used at pH (6) and temperature 23 C. The results also showed that the growth rate in the dark was higher than the growth rate in the light in all natural media used.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR/S DECLARATION

Conflicts of Interest: None. - We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours, have been given the permission for re publication

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attached with the manuscript. - Ethical Clearance: The project was approved by the local ethical committee in University of Baghdad, Iraq.

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عزل وتشخيص فطر *Lentinula edodes* من البيئة العراقية ودراسة الظروف المثلى لنموه

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المستخلص

يهدف البحث الحالي الى جمع الاجسام الثمرية الفطرية من البيئة العراقية / محافظة بغداد/ الصالحية وتشخيصها مظهريا وجزيئيا بتقنية تفاعلات البلمرة المتسلسل (PCR)، خضعت الاجسام الثمرية المستحصل عليها من الفطر البري الى التحليل الكمي والنوعي بواسطة جهاز HPLC للكشف عن المركبات الفينولية النشطه بايلوجيا في الفطر. كما تم دراسة تأثير بعض العوامل والظروف الفيزيائية في معدل نمو الغزل الفطري وكثافته من ناحية (درجة حرارة ، رقم هيدروجيني، نوع وتركيز المواد المستخدمة في تحضيرالاوساط بالاضافة الى تأثيرعاملي الضوء والظلام). أظهرت نتائج تحليلات BLAST ان هناك تطابق بنسبة 99% بين جينات العزلة المحلية وبين سلالات الفطر العائدة للنوع dodes . ورمز لهذه العزلة المحلية بالرمز (EkRu-Bagadad-1) وبالتسلسل (OM432157). فيما يتعلق بملف المركبات الفينولية بينت النتائج أن الفطر يحتوي على 15 مركبا فينوليا والتي شملت ستة أحماض فينولية (Vanillic, Chlorogenic, 4- hydroxyl benzoic, catechol، eugnol cinnamaldehyde، quercetin، Gallic, Cinnamic, Caffeic) وتسعة فلافونيدات (kaempferol، Pyrogallo, lignans،rutin، nigellone) . بلغ مجموع المركبات الفينولية في الفطر 661.64 ملغم / لتر وكان حمض 4-هيدروكسي بنزويك هو أكثر الأحماض الفينولية تركيزاً في الفطر . كما بينت النتائج ان اعلى معدل نمو للغزل الفطري الشعاعي للسلالة المحلية لفطرالشيتاكي قد تحقق في وسط قشورالبطاطا عند التركيز 20غم/لتر والذي بلغ (0.473 سم / يوم)، وسجلت السلالة المحلية قيد الدراسة اعلى معدل نمو في جميع الاوساط الزرعية المستخدمة عند الرقم الهيدروجيني (6) و درجة الحرارة 23 م، كما اظهرت النتائج ان معدل النمو في الظلام اعلى من معدل النمو في الضوء في كافة الاوساط الطبيعية المستخدمة.

الكلمات المفتاحية: *Lentinula edodes*، التشخيص الجزيئي ، المركبات الفينولية ، المزرعة الام.