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Isolation of the Medicinal Mushroom *Ganoderma resinaceum* in Iraq, Morphological, Molecular Identification and Production of Basidiocarps on Novel Media

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Abstract

Ganoderma was isolated from an apple tree in the Tikrit region (Iraq), according to phenotypic characters and phylogenetic analyses based on internal transcribed spacer ITS 1 - 5.8S - ITS4 rDNA sequences, this isolate identified as *G. resinaceum* and registered with accession number MN448375 in the NCBI Database. We described a novel media for artificial cultivation of *G. resinaceum*, composed of wheat straw as basic substrate amended with premix (paper wastes, poplar sawdust and rice hulls at 1:1:1 (W:W:W) the most suitable medium was wheat straw amended with 20 % of premix, in this medium the highest yield (biological efficiency) of *G. resinaceum* basidiocarps was recorded. The cultural *G. resinaceum* characteristics grown in all tested media were also describted.

Keywords: Artificial cultivation, Novel media, Biological efficiency, Ganodermataceae

Introduction

Ganoderma spp (Reishi fungus) is one of the most famous medicinal mushrooms with a long tradition of usage and cultivation, especially in traditional medicine in China, Japan, Taiwan and other Asian countries [1].

In the last 20 years, researchers have shown that Reishi fungi have various biological activities and medicinal properties, such as antibacterial, antiviral, anticancer, antihypertensive, immunomodulatory activities and many other properties [2-5]. For this importance, huge demand for this mushroom and their products, according to Li *et al*. [6], the annual global turnover was more than 2.5 billion US dollars. *Ganoderma* was first cultivated on some trees logs than on substrates based on different sawdust or cereal straws with some amendments, *Ganoderma* sp. mostly, is grown on the sawdust of the broad-leaved trees, some of the agricultural and industrial wastes with or without amended of various cereal species, calcium sulphate (gypsum), calcium carbonate (Chalk powder), sugar and others [7].

In Iraq, several species of mushrooms such as many species of oyster mushroom, *Pleurotus* sp. [8,9], *Agaricus bisporus / bitorquis* [10], *Coprinus comatus* [11] successfully cultivated on a large number of agricultural wastes, while there is no study achieved in the taxonomy and cultivation of *Ganoderma* sp. The culture media of fungi are among the determining factors for the production of fruiting bodies, and the production of fungi is affected both quantitatively and qualitatively according to the type of the medium. In a study of Jandaik *et al*. [22], the productivity was different according to the type of the medium, the higher productivity of *Ganoderma lucidum* was 150 g/kg of mango sawdust medium inoculated with 3 % of spawn rate, while productivity was 100 g/kg of poplar sawdust medium at the same spawn rate.

The present study amid to isolation of pure culture of *Ganoderma* sp. from local Iraqi environment, identified morphologically and by the molecular method in addition to cultivated on a novel media.

Materials and methods

Samples collection

Basidiocarps of *Ganoderma resinaceum* collected by authors from decaying apple tree (*Malus domestica*) in Al-alam region, Salah Aldin Province, Iraq 34.7086267 ºN 43.6971042 ºE, collection date 3/11/2018.

Macroscopic and microscopic characterization

Macro-morphological characters were described based on fresh-basidiomata. Colour codes (e.g. 3A3) are from [12]. Micromorphological features (of sections cut with a razor blade, mounted in 5 % KOH) were recorded at the magnification of ×100×400×1000 with the aid of a light microscope (Olympus) camera (Canon type). Spore measurements are recorded based on that of thirty basidiospores with myxosporium. The cuticle sections were taken from the mature pileus portion and mounted in Melzer's reagent for observations.

The liters used in the description of the basidiospores:*n*:number of measured spores; *L*m: Mean spore length; *W*m: Spore width; *Q*: Length/width ratio *(L/W)* of a spore and *Q*m: Average *Q* of all spores measured. After recording the macromorphological characters in the base camp, basidiomata were dried in the sun and kept in the Department of Plant Protection, collage of the Agriculture University of Tikrit-Iraq.

DNA extraction, PCR, and sequencing

For Genomic DNA extraction, Samples were taken from fresh mycelial cultures (pure culture) which grown on PDA for 7 days at 25 °C. Genomic DNA was extracted using ZR Fungal/Bacterial/Yeast DNA MiniPrep™ / USA, Catalog No. D6005, according to the manufacturer's instructions.

Detection of Gene *ITS* by using PCR:

Detection of the *ITS* gene was conducted by using primers for amplification. A fragment of *ITS* was amplified using a forward primer, portions of the internal transcribed spacer region (ITS, ITS1 and ITS4, [13]) (*ITS1*F:5'-TCCGTAGGTGAACCTGCGG-3') and a reverse primer (*ITS4*R:5' TCCTCCGCTTATTGATATGC-3') (Primers set supplied by IDT (Integrated DNA Technologies company, Canada.). The PCR amplification was performed in a total volume of 25 µL containing 1.5 µL DNA (40 ng), 5 µL Taq PCR PreMix (Intron, Korea), 1 µL of each primer (10 pmol) then distilled water was added into a tube to a total volume of 25 µL.The thermal cycling conditions were done as follows: Denaturation at 94 °C for 3 min, followed by 35 cycles of 94 °C for 45 s 52 °C for 1 min and 72 °C for 1 min with final incubation at 72 °C for 7 min using a thermal Cycler (Gene Amp, PCR system 9,700; Applied Biosystem). The PCR products were separated by 1.5 % agarose gel electrophoresis and visualized by exposure to ultraviolet light (302 nm) after red stain staining (Intron Korea).

Sequencing and sequence alignment

Sequencing of the gene was performed by national instrumentation center for environmental management (nicem) online at (http://nicem.snu.ac.kr/main/?en_skin=index.html), biotechnology lab, machine is DNA sequencer 3,730XL, Applied Biosystem), Homology search was conducted using Basic Local Alignment Search Tool (BLAST) program which is available at the National Center Biotechnology Information (NCBI) online at (http:// www.ncbi.nlm.nih.gov) and Molecular Evolutionary Genetics Analysis (MEGA X program version 10.2.6) a computer software was used for conducting statistical analysis of molecular evolution and for constructing phylogenetic trees. The comparative sequences obtained in this study was deposited in GenBank (**Table 2**).

Pure culture

Pure culture of G. *resinaceum* was prepared by tissue culture technique, 1 cm^2 of internal basidiocarp parts transferred to Malt extract medium, incubated at 25 °C for 7days.

Spawn preparation

Spawn is made by growing G. *resinaceum* mycelium on sterilized wheat grains. The grains are initially pre-wetted by boiling them in water. Excess water was drained off and the grain was mixed with calcium sulphate (2 %) and calcium carbonate (8 %) by dry weight, then, the grain is filled into 1 L bottle

and autoclaved for 1 h at 121 °C and 15 p.s.i (pounds per square inch). The grain is allowed to cool, inoculated with mycelium, and incubated at 25 - 28 °C until the mycelium covered all the grains [9].

Preparation of premix

Premix prepared with 3 components including paper wastes, poplar sawdust and rice hulls. Paper wastes was chopped into the small piece (2 - 5 cm) with cutting paper machine, then washed very well with tap water, then paper wastes mixed with poplar sawdust and rice hulls at 1:1:1 (W:W:W).

Preparation of basidiocarps growth media

Fifteen combinations of media were prepared for the production of G. *resinaceum* basidiocarps (**Table 1**). wheat straw as a basic substrate was soaked overnight in tap water then amended with or without premix composed of the mixture of paper wastes: poplar saw dust: rice hulls (1:1:1) as showed in **Table 1**, all combination media-steamed at 80 °C for 12 h, cooled and mixed with spawn at a rate of 2 %, packed in polypropylene bags and incubated at 25 ºC for vegetative growth (mycelium growth) and 18 °C for reproduction growth (primordia and basidiocarps formation) with 85 % of relative humidity and light duration 1,500 lux for 12 h photoperiod. The parameters was included: percentage of biological efficiency (fresh weight of basidiocarps/Dry weight of medium) ×100), time of media colonization by mycelium (day), the time required for primordia formation (day), time required for basidiocarps formation (day), number of basidiocarps/bag (2 kg of wet medium), stipes and pileus length (cm), stipes and pileus diameter (cm) were recorded.

Table 1 Combinations of media used in this study.

Components of media	Code	Quantity (kg)	Percentage of premix (paper wastes, poplar sawdust and rice hulls at 1:1:1 / W:W:W)				
Wheat straw	WS	10	0	5	10	15	20
Wheat straw CaCO3 CaSO4	WSCC	10 0.2 0.2	0	5	10	15	20
Wheat straw CaCO3 CaSO4 Sucrose	WSCCS	10 0.2 0.2 0.2	0	5	10	15	20

Statistical analysis

The data statistically analyzed using Statistical Analysis System (SAS) software. Duncan's Multiple Range Test at 0.05 was used for significantly compare among means of biological efficiency, C:N ratio and cultural characteristics of *G. resinaceum*.

Results and discussion

Macroscopic and microscopic characterization

The following description concerned on the collected basidiocarps in natural habitat *Basidiocarp* annual, usually stipitate sometimes, in some fruitbodies-sessile; with a distinctly contracted base. Pileus (**Figure 1A**), 18 - 19.5 cm in diameter, with the thickness of 1.2 - 2.2 cm, imbricate, semicircular to dimidiate, laterally fused, corky; abhymenial surface laccate, sulcate, zonate, margin; blunt to truncate and greyish orange. Lower surface (**Figure 1B**); light brown. Reddish-brown when fresh, dark brown in full growth or drying; pile surface with a thick, dull cuticle or shiny with a cuticle of clavate end cells. Hymenial surface poroid, greyish orange when fresh, greyish brown with age or dry, pores initially greyish brown, bruising brown, pores, reached to 4 - 6 per mm, circular or sub-circular (**Figure 1G**), pore tubes reached to 0.5 - 0.7 cm long, greyish brown to brown; context up to 7 mm thick, usually light brown; margins wavy to irregular, obtuse, concolorous on the abhymenial side and sterile up to 2 - 4 mm, reddish brown on the hymenial side. Stipe *Hymenophore* up to 1.5×2.5 cm^2 long, torulose, solid, violet brown, laccate, fused laterally.

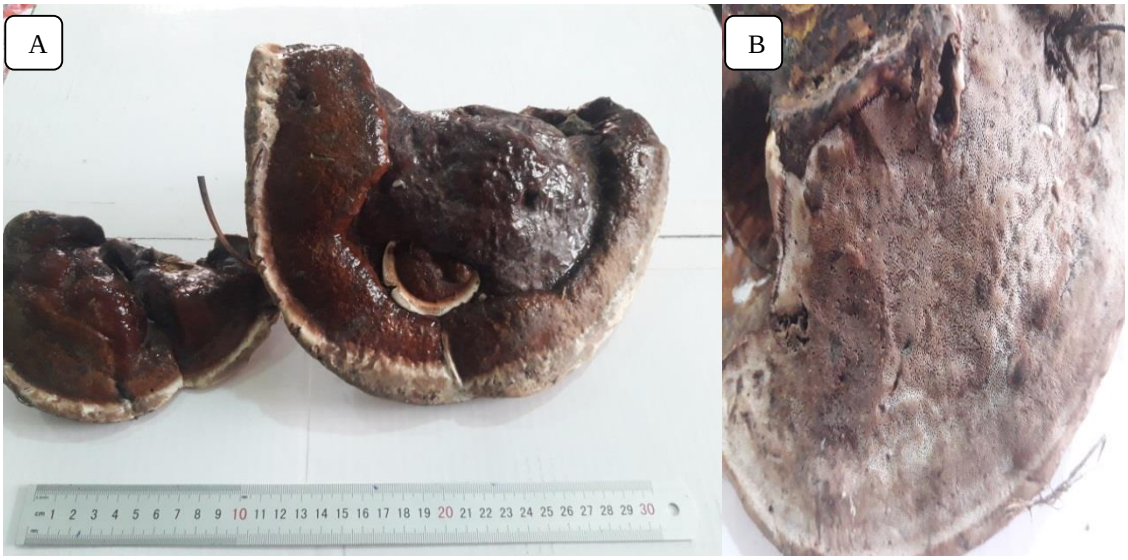
Hyphal system trimitic: binding hyphae about 2.4 µm wide, light brown, usually branched, non-septate, thick-walled, sinuous., nearly solid (**Figure 1C**), generative hyphae2.5 - 3.5 µm wide, branched, septate, clamped, thin-walled (**Figure 1D**), skeletal hyphae up to 5.3 µm wide, sometimes branched, greyish brown, non-septate, thick-walled (**Figure 1E**) Clamydiospores (**Figure 1F**) Ovate to obpyriform, lightly longer and wider than basidiospores, 9.5 - 14.6×5.7 - 8.8 µm

Basidiospores (**Figure 1H**), 9.2 - 13.3×5.5 - 7.2 µm, ovoid to ellipsoid, truncate at the distal end; brownish orange to light brown with a brown eusporium bearing fine, short, and distinct echinulae, overlaid by a smooth hyaline myxosporium. *Pileipellis* a hymeniderm, light brown to orange, clavate like cells, dextrinoid. *Context* up to 4 mm thick, duplex, dry; the lower layer of context usually is reddish brown, fibrous and composed of coarse loose fibrils; while the upper layer usually is reddish brown with the corky to woody texture. Habitat-On a decaying apple tree. Specimens examined - Iraq, Salah Aldin Province, Al-Alam region, 34.7086267 °N 43.6971042 °E, collection date 3/11/2018.

The morphological characteristics that recorded in the previous studies [14-17] included Basidiomes laccate, corky, Basidiomes sessile/or with a short stipe. Pileus thick at the base, sub-orbicular, rotund, upper surface; reddish brown to dark brown, concentrically sulcate zones with turberculate bumps and ridges and rivulose depressions, with irregularly ruptured crust overlying the pellis, margin; blunt to truncate, greyish orange, lower surface; brownish. Hymenophore up to 25 mm long, indistinctly stratose; pores initially light brown, pores circular or sub-circular, 5 - 6 per mm. Context up to 4 mm thick, duplex, dry; lower layer reddish brown, fibrous, composed of coarse loose fibrils; upper layer reddish brown, corky to woody. Basidiospores ellipsoid, light orange, brownish orange. Pileipellis a hymeniderm, brownish orange, clavate like cells, dextrinoid. Context dimitic; skeletal hyphae thick-walled, nearly solid, sometimes branched, greyish brown; binding hyphae, thick-walled, branched, nearly solid, light brown.

The tested mushroom identified as *G. resinaceum* according to the macroscopic and microscopic characterizations mentioned in the related literatures [14-17], in addition, Key to the *Ganoderma* species reported by Hapuarachchi *et al*. [16].

The pure culture of *Ganoderma resinaceum* and its dried fruit bodies was preserved in the in the Fungi Laboratory-Plant Protection Department-College of Agriculture-Tikrit University-Iraq



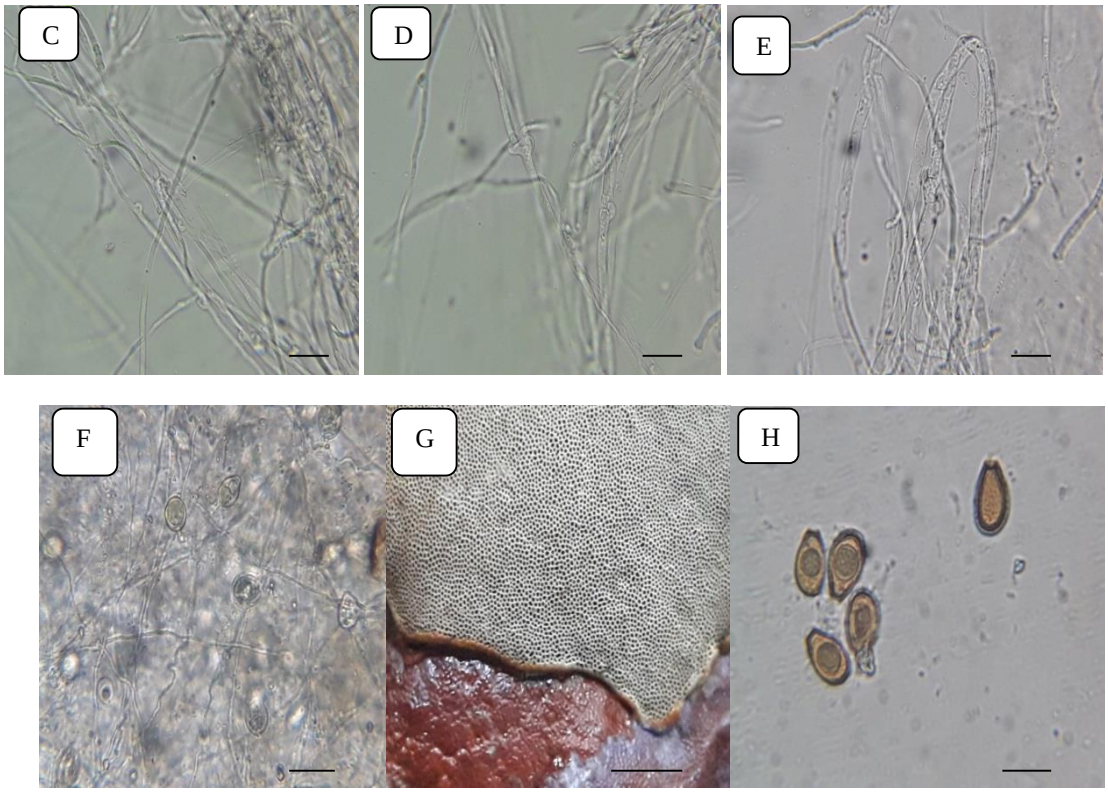


Figure 1 *Ganoderma resinaceum*: A) basidiocarp showing abhymenial surface (fresh), B) Sporocarp showing hymenial surface (in natural collected basidiocarp), C) Binding hyphae, D) Generative hyphae, E) Skeleto-binding hyphae, F) chlamydospores, G) pores, H) basidiospores.
Scale bars: C, D, E, F, H = 10 µm, G = 10 mm. *n* = 30 spores; *L*m = 9.2 - 13.3 µm; *W*m = 5.5 - 7.2 µm; *Q* = *L/E* = 1.67 - 1.85; *Q*m = 1.76.

Molecular analysis

According to Basic Local Alignment Search Tool (BLAST) analysis, the Iraqi *G. resinaceum* isolate (Accession Number MN448375) was similar to some recorded *G. resinaceum* from various sources in the GenBank. The highest similarities were 99.819 % with 4 Italian isolates followed by 99.666 % with 3 Chinese isolates, 99.656 % with Iranian isolate, 99.644 % with other Chinese isolates, 99.641 % with France isolate and 99.491 % with American isolate (**Table 2**) Based on ITS1 and ITS4 region sequences, Iraqi *G. resinaceum* isolate was more than 99 % homologous with many *G. resinaceum* isolates from GenBank and it can be observed that this isolate was phylogenetically very close and present *G. resinaceum sensu stricto* (**Figure 2**).

Table 2 Information on *Ganoderma resinaceum* species / isolates used in phylogenetic analysis.

Specie/Isolates	Source (country)	GenBank accession number (ITS1 and ITS4)	Probability similarity (%)	Overlap (%)
Ganoderma resinaceum isolate Gre5133	Italy	KP941447.1	99.819	92.487
Ganoderma resinaceum isolate PF280	Italy	JN176881.1	99.819	92.321
Ganoderma resinaceum isolate PF279	Italy	JN176880.1	99.819	92.321
Ganoderma resinaceum isolate PF281	Italy	JN176885.1	99.819	92.321

Specie/Isolates	Source (country)	GenBank accession number (ITS1 and ITS4)	Probability similarity (%)	Overlap (%)
Ganoderma resinaceum strain HB	China	KT921215.1	99.666	100
Ganoderma resinaceum strain G.l 0057	China	KT895429.1	99.666	100
Ganoderma resinaceum strain gutian	China	KT906370.1	99.666	100
Ganoderma resinaceum isolate HSBU2008100	Iran	KT343317.1	99.656	97.162
Ganoderma resinaceum strain BCRC 36147	China	KJ143916.1	99.644	93.823
Ganoderma resinaceum CIRM-BRFM 753	France	FJ805250.1	99.641	92.988
Ganoderma resinaceum	USA	AM269778.1	99.491	98.331

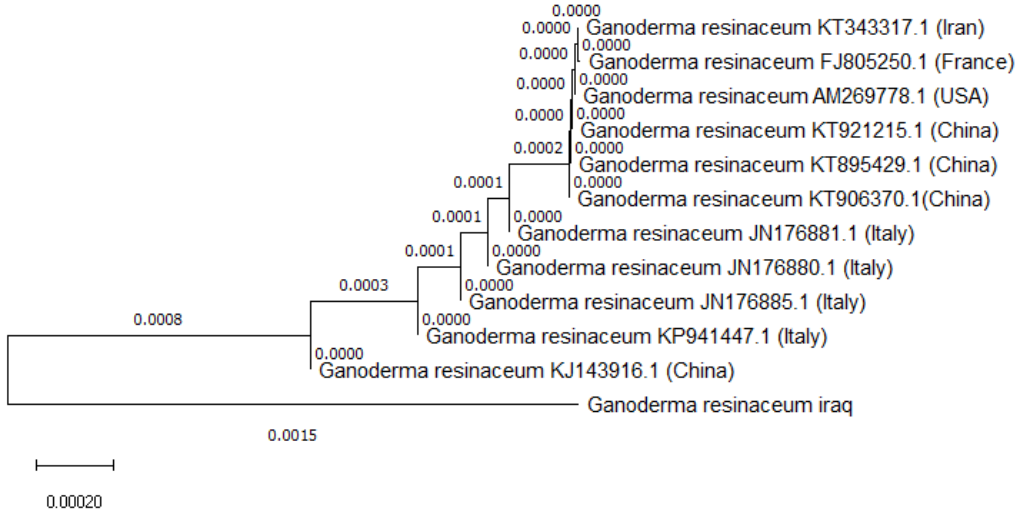


Figure 2 Maximum likelihood tree based on combined data set of ITS1 and ITS4 partial gene sequences made by MEGA X program version 10.2.6.

Evaluation of some combination media for production of *G.resinaceum* basidiocarps

The productivity of *G.resinaceum* which cultivated on 3 types of formulated media showed in **Table 3**. The highest biological efficiency (BE) recorded in WSCCS medium with premix at 20, 15 and 10 % resulting in 34.69, 31.12 and 24.14 %, respectively, followed by WSCC media supplemented with premix at 20 and 15 %, resulting in BE.32.04 and 28.88 %, respectively. The results also showed all premix rates superior to control. Significantly, WSCCS was the best medium at 20 % of premix that gives higher biological efficiency (**Table 3**).

Table 3 Evaluation of some media types on Biological efficiency (%) of Iraqi *Ganoderma resinaceum* isolate.

Components	code	Quantity (kg)	Percentage of premix (paper wastes, poplar sawdust and rice hulls at 1:1:1 / W:W:W)				
			0	**5**	**10**	**15**	**20**
Wheat straw	WS	100	8.43 Dc	13.33 Cc	15.69 Cc	21.24 Bc	24.27 Ac
Wheat straw CaCO3 CaSO4	WSCC	100 2 2	12.43 Ea	17.94 Db	22.55 Cb	28.88 Bb	32.04 Ab
Wheat straw CaCO3 CaSO4 Sucrose	WSCCS	100 2 2 2	12.76 Ea	21.37 Da	24.14 Ca	31.12 Ba	34.69 Aa

Different small letters indicated significant differences among the column data and different capital letters indicated significant differences among row data according to Duncan's Multiple Range Test at ($p < 0.05$). The values are the averages of 5 replicates for each treatment.

Table 4 shows the C:N ratio of the tested media in this study, the results showed that the lowest C:N ratio was recorded in all media free of premix-resulting in 97.56, 93.31 and 93.33 for the WS, WSCC and WSCCS media, respectively. This ratio increased with the increase in premix percentage and reached the highest C:N ratio at 20 % of premix resulting in 78.11 , 78.11 and 78.3 for the WS, WSCC and WSCCS media, respectively. The results in both **Tables 3** and **4** showed that there is a relationship between a low C:N ratio and higher biological efficiency.

Table 4 C:N ratio of tested media for cultivation of *Ganoderma resinaceum.*

Components	Code	Quantity (kg)	Percentage of premix (paper wastes, poplar sawdust and rice hulls at 1:1:1 / W:W:W)				
			0	**5**	**10**	**15**	**20**
Wheat straw	WS	100	97.56 Aa	90.41 Bb	84.66 Cc	81.05 CDd	78.11 De
Wheat straw CaCO3 CaSO4	WSCC	100 2 2	93.31 Aa	89.07 Bb	84.8 Cc	81.17 CDd	78.11 Ae
Wheat straw CaCO3 CaSO4 Sucrose	WSCCS	100 2 2 2	93.33 Aa	89.23 Bb	85.05 Cc	81.62 DEd	78.3 Ee

Different small letters indicated significant differences among the column data and different capital letters indicated significant differences among row data according to Duncan's Multiple Range Test at ($p < 0.05$). The values are the averages of 5 replicates for each treatment.

Cultural *G. resinaceum* characteristics

Table 5 - showed that the time required for full growth media with *G. resinaceum* mycelium was 47 days for both WSCC and WSCCS media. The earlier time required for primordial and basidiocarps formation was in WS medium resulting in 21 and 36 days, respectively, while the highest times were 24 and 38 days in WSCCS medium, respectively. WSCCS medium significantly superior on other media in a number of basidiocarps (3.3) compared with WS and WSCC media resulting in 2.33 and 1.33 basidiocaps, respectively. WSCCS also significantly superior on other media in length and diameter of stipes and diameter and thickness of pileus (**Table 5**). The basidiocarps were regular in shape when

grown lateral (**Figure A**, **B** and **D**), but sometimes the basidiocarps were irregular when they grow at the top of the bag containing the medium (**Figure C**).

Table 5 Cultural *G. resinaceum* characteristics grown in WS, WSCC and WSCCS media.

Cultural characteristics	Media (supplemented with 20 % of premix)		
	WS	**WSCC**	**WSCCS**
Time of media colonization by mycelium (day)	46b	47a	47a
Time required for primordia formation (day)	21c	23b	24a
Time required for basidiocarps formation (day)	36c	37b	38a
Number of fruitbodies (per bag)	1.33c	2.33b	3.33a
Stipes length (cm)	2.4c	3.0b	3.3a
Stipes diameter (cm)	1.8b	2.0b	2.4a
Pileus diameter (cm)	8.6c	10.5b	11.7a
Pileus thickness (cm)	1.2b	1.6b	2.4a

Different small letters indicated significant differences among row data according to Duncan's Multiple Range Test at ($p < 0.05$). The values are the averages of 5 replicates for each treatment.

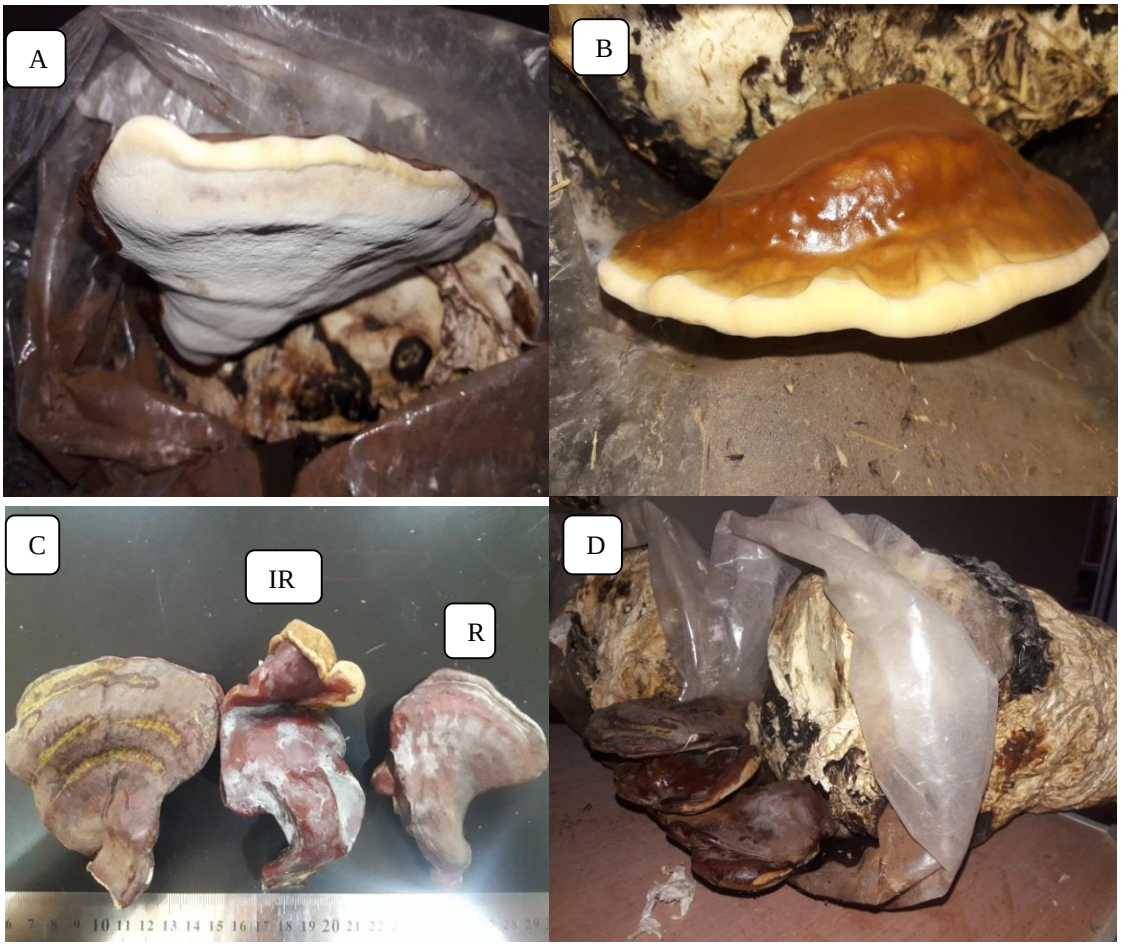


Figure 3 Artificial cultivation of *Ganoderma resinaceum* on WSCCS amended with premix (20 %), A) abhymenial surface, B) hymenial surface, C) basidiocarps grown on a medium, D) harvested basidiocarps; R = regular basidiocarp, IR = irregular basidiocarp.

Discussion

In the modern studies, there are some differences between the natural collected and cultivated basidiocarps, this may due to the environmental factors and type of growth medium, thus problematic taxonomy of this mushroom is usually overcome by molecular analysis, which explains the variations among samples, the problems with the identification of *Ganoderma* species owing to the morphology characters which depend on environmental factors are variable in addition to some genetic modifications such as inter-hybridization phenomenon, for these reasons, taxonomy of *Ganoderma* required more than methods in addition to morphology, like chemical and molecular markers. The molecular analysis appears to more reliable in *Ganoderma* species taxonomy, especially ITS region, partial β-tubulin genes, sequences of partial large subunit rDNA and mitochondrial small subunit rDNA as that demonstrated by [18-20].

The biological efficiency (BE) of *Ganoderma* and also other cultivated mushrooms differ compared to other related studies because BE depends on different factors such as the basic substrate composition, supplementation and environmental conditions [7,9]. The used premix in this study encourage the *G. resinaceum* productivity, this may be due to it contain additional nutrients that make a suitable C:N ratio for *G. resinaceum* growth.

According to our observations in this study, we found the wheat straw as basic substrate alone not successfully suitable for cultivation of *G. resinaceum*, in which lowest BE was recorded, thus a large number of materials (available in Iraq) and combinations were tested for this purpose (data not showed), among them, *G. resinaceum* successfully cultivated in 3 media WS, WSCC and WSCCS based on the wheat straw with supplementation of premix.

WSCC showed as good media, namely some cultural *G.resinaceum* characteristics, besides biological efficiency was also significant.

As *G.resinaceum* mushrooms rare in nature and traditional cultivation on logs is not an ecologically and economically justified method, current study is finding of alternative media for production. Whether wheat straw, paper wastes, poplar trees and rice hulls, the most abundant materials in Iraq could be form (with CaCO3, CaSO4 and sucrose) a novel media for *G. resinaceum* cultivation, so not all components suitable for *G. resinaceum* cultivation i.e. oak sawdust, was less favorable for ligninolytic enzymes activities that are responsible for efficient medium degradation and mushroom biomass production [21].

The enrichment of basic substrates like sawdusts or cereals straw with additional nutrients sources led to further increase of the biological efficiency, according to [22], the highest biological efficiency (27.5 %) was recorded in *G. resinaceum* cultivated on paddy straw supplemented with wheat bran, which invariably resulted in significantly higher yield compared to the unsupplemented check(s) or other supplements. Biological efficiency in our study was higher than these in Ćilerdžić *et al*. [23] in which the biological efficiency of *Ganoderma lucidum* when cultivated on wheat straw substrates was from 6.0 ± 0.9 to 13.0 ± 0.9 (according to the strains), this may be due to the role of supplements in increasing mushroom productivity.

The cultural characters of *G. resinaceum* showed that WSCCS media (at 20 % premix) delayed in a day and 2 days for primordia and basidiocarps formation time compared with WSCC and WS media, this may due to the nature of the components in these media thus mycelium growth required additional time for degradation and absorption for the basidiocarps formation while WSCCS superior on other media in stipes and pileus diameter which reflected on the increase of BE.

The parameters of basidiocarps morphology depend on the cultivated media which included basic substrate type and the supplementation, thus the related studies record a variation in the parameters of basidiocarps in various cultivated media [24].

The most important factors that have an important role in the cultivation of *Ganoderma* spp. are the ratio of carbon to nitrogen C:N ratio. According to previous studies that confirmed that the range of 75 - 90 of this ratio is appropriate for the growth of this fungus [7], therefore it has been obtained to this C:N ratio range only in media that were invented in this study except for the medium of wheat straw without any additions without any proportion of the premix, therefore this medium gave the lowest number of mushrooms and the lowest productivity.

Conclusions

The isolated mushroom from decaying apple trees (*Malus domestica*) in Iraq classified as *G. resinaceum* depends on morphological and molecular properties. The best medium for artificial cultivation of this mushroom was carried out on the novel medium coded WCCS composed of Wheat straw (100 kg), CaCO3 (2 kg), CaSO4 (2 kg) and Sucrose (2 kg) supplemented with 20 % of premix (paper wastes, poplar saw dust and rice hulls at 1:1:1 / W:W:W), which give the highest biological efficiency in addition to improvement of some cultural characteristics. The production of *G. resinaceum* basidiocarps using agro-industrial wastes was one of the eco-friendly techniques that could potentially be applicable on the large scale.

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