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Influence of Supplementation on Productive and Qualitative Traits of Cultivation Two Mushrooms Species *Hypsizygus* Spp. in Iraq

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ABSTRACT

This study aimed to determine the optimal conditions for two mushrooms species, *Hypsizygus marmoreus* and *Hypsizygus tessulatus*, which first record cultivation in Iraq, utilizing locally available substrates such as fruit tree sawdust supplemented with olive waste, sesame meals, and coffee grounds. The experiment evaluated fungal mycelial growth, spawn running period, pinhead formation, yield, and biological efficiency (BE). Results indicated that the sesame meals-supplemented substrate outperformed others, exhibiting faster mycelial colonization, earlier pinhead initiation, and the highest yield and BE, reaching 121.8g, 27.6% for *H. marmoreus* and 97.2g, 22.06% for *H. tessulatus*, compared to in the control 76.2g, 19.7% and 82.9 g, 21.5% BE, respectively. Furthermore, the optimal growth conditions on Potato Dextrose Agar (PDA) were identified at 20°C and a pH range of 5-6. Analysis of the nutrient content of the harvested fruiting bodies showed a higher concentration of protein, fat, ash and fiber in the two mushrooms grown on olive waste compared to other supplements.

Key words: mushroom cultivation, mushroom substrate, Shimeji mushroom.

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INTRODUCTION

Mushrooms commonly referred to as such, are macroscopic fungal species whose fruiting bodies are perceptible without optical magnification and amenable to manual harvesting. These substantial fruiting bodies are characteristic of their respective fungal organisms and can develop either epigeously or hypogeously (Miles and Chang, 1997; Rashid et al., 2010; Hlail,2021). In addition to their distinctive flavor and high functional food, mushrooms are a popular and healthy food (Hlail, 2021). They are available in a variety of forms (fresh, pickled, dried, powdered, and canned). Mushrooms have a high water content (over 90%), low fat content (less than 1%), and are rich in B vitamins, copper, and selenium, with a low sodium content (Obodai et al., 2003). Uniquely, mushrooms are a natural source of vitamin D due to their ergosterol content, which is converted to vitamin D upon exposure to light (Sharma, 2015; Verma, 2023). The technology used in mushroom cultivation is environmentally friendly (Meshram et al, 2024). For cultivation mushrooms using materials like paddy, straw, cotton wastes, coffee grounds, water hyacinth, tree sawdust, sugar cane, bagasse, wild grasses, and various types of refuse and lignocellulose residues. Morphogenesis of mushroom fruit bodes are influenced by environmental factors (temperature, light, moisture, and pH) that act as inducers of fructification (Tinao et al., 2015). Shimeji mushrooms (*H. marmoreus* and *H. tessulatus*) are edible mushrooms found and consumed in East Asia (China, Japan, and North Korea). They are also cultivated in temperate climates, particularly in Europe, North America, and Australia (Yutaka et al., 2000; Wu et al., 2019; Vlasenko et al., 2021). Mushrooms have a crunchy texture and a

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sweet, crab-like flavor when cooked. In addition to their nutritional value, have demon strated bioactivities such as anti-tumor, anti-aging, cholesterol control, weight loss, and constipation (Shoji et al., 2000 ; Waites et al., 2001; Monira et al., 2012; Zhang et al., 2021). The cultivation of *Hypsizygus* spp. using bag or bottle technique, particularly in China. The entire cultivation cycle from inoculation to harvesting 140-150 days. This long period leads to higher production costs, inefficient use of cultivation facilities (Zhang et al., 2016).

MATERIALS AND METHODS

Experimental site: This is the first record of the cultivation of this mushroom in Iraq. The study was conducted in the mushroom farm belonging to the Department of Organic farming / Directorate of plant Protection / Ministry of Agriculture / Baghdad from early November 2024 to February 2025. The strains *Hypsizygus marmoreus* (White Beech LC) *and Hypsizygus tessulatus* (Brown Beech) obtained from the Prime Fungi Company of the United States of America.

Effects of pH and temperature: Potato dextrose agar (PDA) was used as the primary mushroom growth. To study the appropriate pH, four different pH levels were used: 5, 6, 7, and 8. the required pH of the culture medium using solution of sodium hydroxide or hydrochloric acid used before sterilization and measured using a digital pH meter. After sterilization at 121°C (15 psi) for 20 min in a steam autoclave, the sterilized PDA medium was poured into Petri dishes (90 mm at 20 ml/dish). The dishes were inoculated with *H. marmoreus* & *H. tessulatus* cultures and incubated at 24 ± 1°C (Three replicates were used for each treatment). Radial growth and growth rate observations were recorded after the plate was fully grown (Jarial and Jarial, 2023). The growth rate was calculated by the equation below:

Growth Rate (cm/day) = x/y

x = Diameter of the Petri dish (usually 8.5 cm) this is the total distance the fungus covers

y = Time in days for the fungus to reach the dish's edge

To know the best temperature, the culture of *H. marmoreus & H. tessulatus* were inoculated into 9 mm diameter Petri dishes containing 20 ml of sterile potato dextrose agar medium under aseptic conditions and incubated at three different temperatures, 20, 25, and 30°C, for 10 days. The observations of radial growth and growth rate of vertical diagonals at the colony covered the full plate.

Effect of substrate supplemented on productivity traits: The sawdust(S) was as the main substrate and then supplemented with wheat bran (wb), sesame meal (sm), olive cake (oc) and coffee grounds (cg) the treatments were Table.1:

Table1. Essential components and ratios in mushroom growing substrates

Treatments	Components%
Swb	(S) 73% + (wb) 25 % + ($CaSO_4$) 2 %
Ssm	(S) 73% + (wb) 10% + (sm) 15% + ($CaSO_4$)2%
Soc	(S) 73% + (wb) 10% + (oc) 15% + ($CaSO_4$) 2%.
Scg	(S) 73% + (wb) 10% +(cg) 15% + ($CaSO_4$) 2%

Three replicates were used for each treatment, and each replicate represents an average of 10 experimental units (bags).

At first, all supplements were ground into small-sized (approximately 0.1–0.2 cm) (Abdulhadi and Hassan,2013). The moistened substrate was placed into a polypropylene bag (32 cm × 8 cm) and then sterilized via autoclaving at 121°C for 90 minutes (Abdulrazza et al., 2017). The bags were inoculated with mushroom spawn (20g/bag) in a sterile environment. The inoculated bags were incubated in a dark growth chamber maintained at a temperature of 20±1°C and a relative humidity of 70-80%, until mycelial growth was complete. The bags were transferred to the production room at 18 ± 2°C and a relative humidity of 80-90% to stimulate fruiting. The cotton plugs at the top of the plastic bags were removed. ventilation was sufficient to maintain carbon dioxide concentrations below 1,000 parts per million (using the device Extech instruments corporation, U.S.A TO control humidity and

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ventilation), and lighting was provided for 12 hours per day using fluorescent lamps (200 Lux). The mushrooms were harvested when they reached full size. The following parameters were assessed in the study: duration of mycelial colonization on the substrate (days), time to pinhead formation (days), total productivity (g) and Biological efficiency (%BE) using the equation below (Jaramillo Mejía and Albertó, 2019; Atíla, 2022).

$$\%\ BE = \frac{\text{Total weight of fruiting bodies}}{\text{Dry weight of the substrate}} \times 100$$

Evaluating the efficiency of different supplemented substrate on the qualitative traits: Chemical analysis of dried fruits bodes was conducted at the Department of Agricultural Research Laboratories, Ministry of Science and Technology the protein constituents was estimated by using the Kjeldahl method (Vlasenko et al.,2021), the percentages of fat, ash, and moisture were determined following the standard A.O.A.C methods (AOAC, 1995). Crude fiber content was estimated by digesting the defatted food components with sulfuric acid three replicates were used for each test (Balasubramanian and Sadasivam, 1987; Mccleary et al., 2015). Total Amino Acids prepared by Weigh out 50 g of the dry fruiting bodies and grind them using a mortar and pestle with a small amount of tartaric acid. For homogenization, add 5 to 10 ml of 80% ethanol. Centrifuge the mixture. Collect the supernatant from the centrifugation process and add a few drops of o-phthalaldehyde (OPA). Store the sample at 4°C for 30 minutes. Subsequently, take 0.1 ml of the extract and add 1 ml of ninhydrin. Make up the volume to 2 ml with distilled water. Heat the tube in a water bath for 20 minutes. Then, add 4 ml of a butanol-methanol mixture. After shaking for 20 seconds, measure the absorbance at a wavelength of 750 nm. A standard calibration curve is used to determine the concentration of the substance in mg/g (Misra et al., 1975; Theymoli and Sadasivam ,1987; Mccleary et al.,2015; Sharma, 2015).

Statistical analysis: The results were statistically analyzed using the analysis of variance (ANOVA). Tukey's post-hoc comparison (at a significance level of 5%) was applied to determine individual differences between the means. All analyses were performed using Genstat 15 version.

RESULTS AND DISCUSSION

Effect of pH: The result presented in Table (2) revealed that 0f the maximum mycelial growth *H. marmoreus* was seen 0.52 (cm/day) in pH 5 while the minimum growth was recorded 0.40 (cm/day) in pH 7, In *H. tessulatus* had maximum mycelial growth that was recorded 0.55 (cm/day) in pH 6 and minimum 0.36 (cm/day) in pH 8 (Figure 1). These results indicate a clear preference for moderately acidic conditions for the growth of both species, with a slight difference in the optimum pH between them. This result is also consistent with the results of (Verma, 2023) who reported that *H. tessulatus* achieved the highest growth rate in mycelium at pH 6, although growth was slightly lower at pH 5-7. The study with (Imtiaj et al., 2016) also confirmed that *H. marmoreus* achieved the highest growth rate at pH 7, while growth was lower at pH 5. The optimum pH for this mushroom was between 6 -8.

Effect of temperature: The mushrooms were incubated at 20, 25 and30°C for 10 day. The data presented in Table (3) indicate that mushrooms *H. marmoreus* and *H.tessulatus* had maximum mycelial growth at 20ºC 0.76 (cm/day),0.81 (cm/day) and minimum growth was seen at 30ºC 0.14 (cm/day) and 0.15 (cm/day) respectively. These results also indicated that temperature is a crucial environmental factor that significantly affects the growth rate of the mushroom under study, as both strains prefer relatively low temperatures 20°C for optimal growth (Figure 2). These results are in line with Stamets, (2011), Zhang et al.(2016) and Imtiaj et al.(2016) found that *H. marmoreus* and *H.tessulatus* are sensitive to environmental conditions, as their growth is restricted to a temperature range of 21-24°C. They did agree with the study of Verma, (2023) results showed that the appropriate growth rate is at a temperature range of 20-25°C.

Effect of supplementation on productivity *H. marmoreus* and *H. tessulatus:* The results showed the differences between the control

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substrate and the experimental supplements during the growth period of the sesame meal, olive cake and coffee grounds. The entire cropping period lasted 115 days. The *H. marmoreus* and *H. tessulatus* incubation duration ranged 67-81 days(Soc) 67-74 days, (Ssm)70-75 days both signfiulity. The (control) had the largest incubation 79 day *H. marmoreus*, 81 days *H. tessulatus* (Figure 3). The pinhead formation varied per supplements, taking 24-45 days on growing media prepared with (Ssm) 24days, 26 days for those prepared with Fruit tree sawdust. In all growing media, the *H. marmoreus* and *H. tessulatus* fruit body was harvested between 25-40th days 27-43 days of growing, approximately 14-17 days after pinheads appeared (Figure 4). Regarding the rate of fruit weight and biological efficiency (BE), the results In Table (4, 5) demonstrated a clear superiority of mushrooms on (Ssm) . The *H. marmoreus* recorded the highest values with a fruit weight121.8 (g/kg) of substrate and a BE of 27.6%,Similarly *H. tessulatus* cultivated on (Ssm) achieved a fruit weight of of 97.2 (g/kg) of substrate and a BE 22.06% ,outperforming the results obtained on olive and coffee residues, as well as the control treatment. In contrast, the lowest values in fruit weight and BE were recorded when using (Swb), reaching 76.2 g/kg of substrate with a BE of 19.7 % for *H. marmoreus*. As for *H. tessulatus* the lowest BE rate was observed in the (Soc) treatment registering 76.8 g/kg of substrate with a BE of 19.4%. Overall, the results indicate that all the used supports promoted the growth of the two fungal hyphae at various growth stages, from the appearance of pinheads and small button formation, the growth and elongation stages, and finally the fruiting body stage (Figure 6). These results are partially consistent with the results of Rashid et al. (2010), who demonstrated faster growth of *Hypsizygus* sp. on sawdust at room temperature, as well as with the results of Thong et al. (2018), who demonstrated similar growth on sawdust and rice bran. However, the results of this study contrast with those of Akavia et al. (2009), who found that the best medium for *H. marmoreus* in terms of biological efficiency was corn cobs with bran and olive sap, with a biological efficiency of 85.6%. This difference in the optimal performance of the different substrates can be explained by the chemical composition of the organic residues and the availability of nutrients that support fungal growth and production. The present results indicate that olive and sesame wastes represent promising substrates for the cultivation of the two studied mushrooms under local conditions.

Evaluation of the efficiency of different agricultural supplements in improving the fruit quality of *H. marmoreus* and *H. tessulatus* : Considering the chemical analysis of the fruiting bodies of *H. marmoreus* and *H. tessulatus* grown on sawdust enriched with different materials, a clear variation in the proportions of components was observed. The results Table (6) showed that the addition of (Soc) resulted in the highest protein ,ash and Fiber content in both mushrooms, reaching 25.12% and 26.59% ,7.08% and 7.30% ,10.80% and 11.22%.exceeding the content in the control treatment, which reached 22.56% and 24.15% ,6.25% and 6.78 ,8.88% and 9.12% for the *H. marmoreus* and *H. tessulatus*, respectively. Regarding lipid content, (Ssm) recorded the highest percentage reaching 2.10% and 2.98%, while the control treatment recorded the lowest values, reaching 1.12% and 1.45%. On the other hand, the control treatment recorded the highest moisture and carbohydrate content in fruiting bodies, with moisture reaching 12.11% and 12.20%, 49.08% and 46.30%. The lowest recorded was (Soc), which amounted to 11.35%, 11.82%, 43.75%, and 40.18% *H. marmoreus* and *H. tessulatus*, respectively. The results presented in (Figure 5) revealed significant variations in the concentrations of certain total amino acid supplements. The highest content of these supplements was observed in (Soc), reaching approximately 90.22 and 93.07(mg/100g), thereby outperforming (Ssm) and (Scg). Conversely, the lowest level was recorded in (Swb), with a concentration of 80.98 and 83.15 (mg/100 g) for the *H. marmoreus* and *H. tessulatus*, respectively. These findings align with the report by Adebayo et al. (2014) indicating that fresh mushrooms contain a range of 2-21%.

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Furthermore, these results are consistent with the study by Azeez et al. (2020), which demonstrated a clear difference in the biochemical composition of brown and white isolates of *H. tessulatus*. The study showed that the brown isolate contained a higher proportion of both free amino acids 73.11 (µmol/g) and bound amino acids 169.50 (µmol/g) compared to the white isolate, which recorded lower levels of free amino acids 65.91 (µmol/g) and bound amino acids 165.19 (µmol/g).

Table 2. Mycelial growth (cm) of *H. marmoreus* and *H. tessulatus* on different pH on PDA at 24ºC

pH	*H. marmoreus*	*H. tessulatus*	Mean
5	0.52	0.41	0.47
6	0.48	0.55	0.52
7	0.40	0.47	0.44
8	0.41	0.36	0.39
L.S.D0.05	0.047	0.051	0.064

Table3. Mycelial growth (cm) of *H. marmoreus* and *H. tessulatus* on different temperature on normal PDA

T(°C)	*H. marmoreus*	*H. tessulatus*	Mean
20	0.76	0.81	0.78
52	0.48	0.53	0.50
30	0.14	0.15	0.14
L.S.D0.05	0.042	0.077	0.277

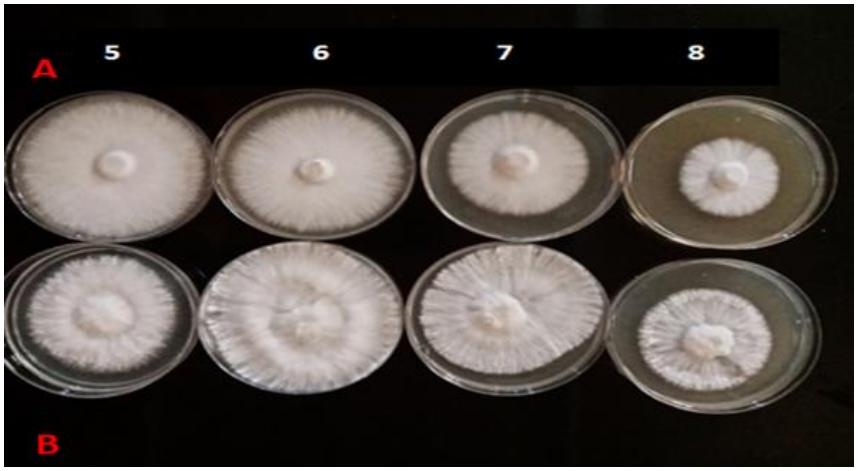

Figure 1. The effect of changing pH on the growth of mycelium of both mushrooms, represent (A) *H. marmoreus* and (B) *H. tessulatus*

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Figure 2. Effect of temperature on the mycelium growth for *H. marmoreus* (A) and *H. tessulatus* (B).

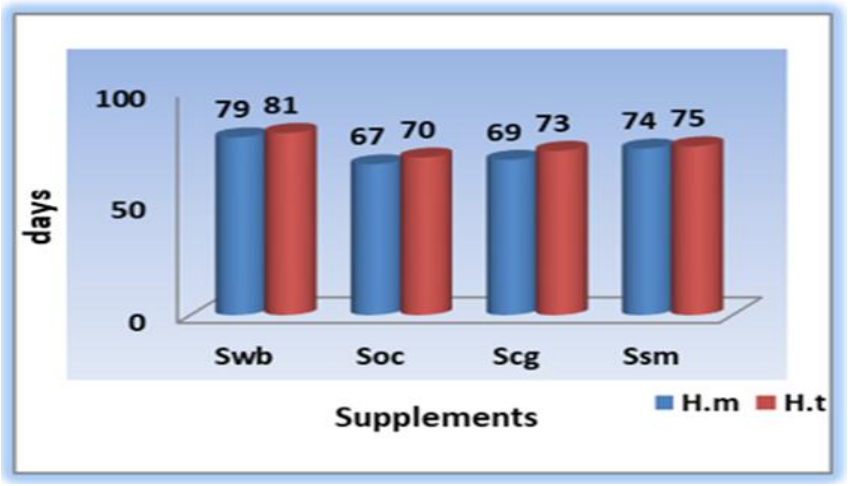

Figure 3. Effect of different supplements on mycelium growth period of *H. marmoreus* and *H. tessulatus*. (Swb) sawdust + wheat bran, (Soc) sawdust + olive cake, (Scg) sawdust +coffee grounds and (Ssm) sawdust + sesame meal. L.S.D0.05=3.85

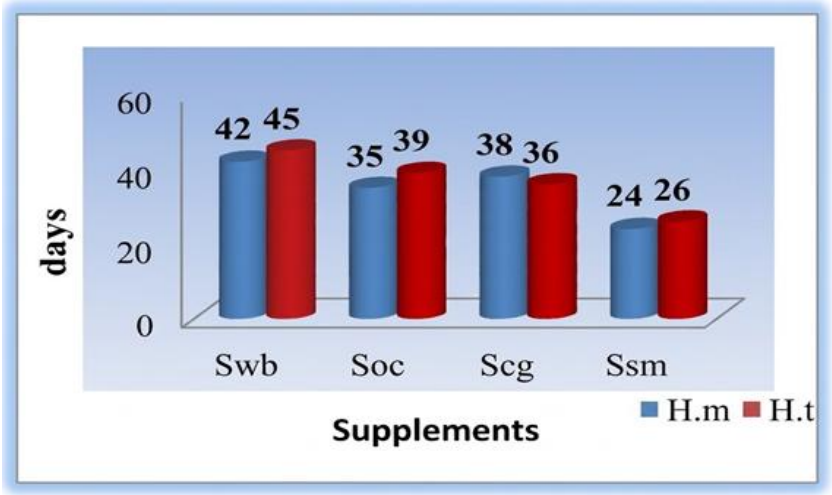

Figure 4. Effect of different supplements on pinhead formation period of *H. marmoreus* and *H. tessulatus* on different supplements. L.S.D0.05=6

Table 4. Mushroom productivity by using different supplements

substrate	*H. marmoreus*	*H. tessulatus*	Mean g

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Swb	76.2	82.9	79.5
Soc	91.7	76.8	84.3
Scg	83.3	81.5	82.4
Ssm	121.8	97.2	109.5
L.S.D0.05	8.79	4.51	2.067

Table 5. Effect of biological efficiency on *H. marmoreus* and *H.tessulatus*

substrate	*H. marmoreus*	*H. tessulatus*	Mean
		%	
Swb	19.7	21.5	20.6
Soc	23.06	19.4	21.2
Scg	21.1	20.7	20.9
Ssm	27.6	22.06	24.8
L.S.D0.05	2.231	1.034	12.17

Table 6. Chemical analysis for *H. marmoreus* and *H. tessulatus*

Name	substrate	Protein %	Lipid %	Ash %	Moisture %	Fiber %	CHO %
H. marmoreus	Swb	22.56	1.12	6.25	12.11	8.88	49.08
	Ssm	24.08	2.10	6.89	11.98	9.11	45.84
	Scg	23.80	1.78	6.78	12.05	9.08	46.51
	Soc	25.12	1.90	7.08	11.35	10.80	43.75
H. tessulatus	Swb	24.15	1.45	6.78	12.20	9.12	46.30
	Ssm	25.88	2.98	7.11	11.90	9.35	42.78
	Scg	25.12	2.66	6.95	12.08	9.20	43.99
	Soc	26.59	2.89	7.30	11.82	11.22	40.18

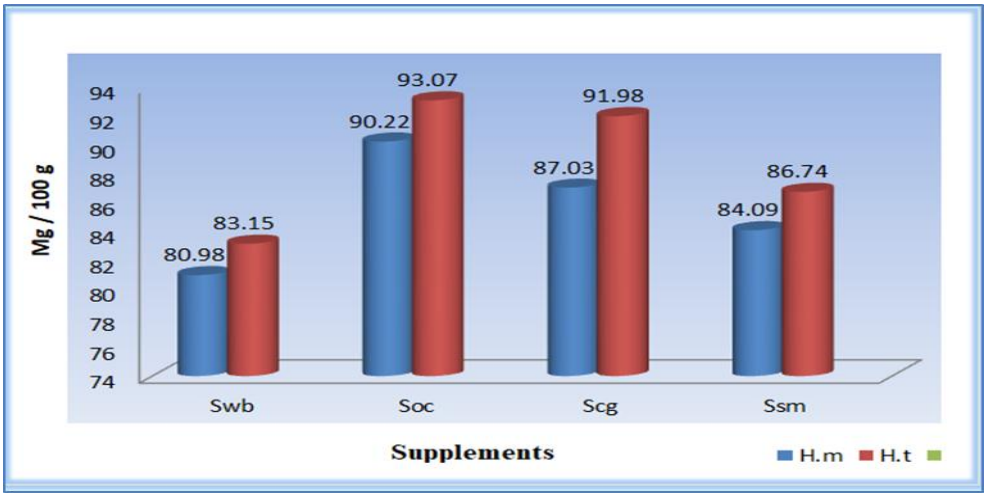

Figure 5. Total amino acid for *H. marmoreus* and *H. tessulatus*

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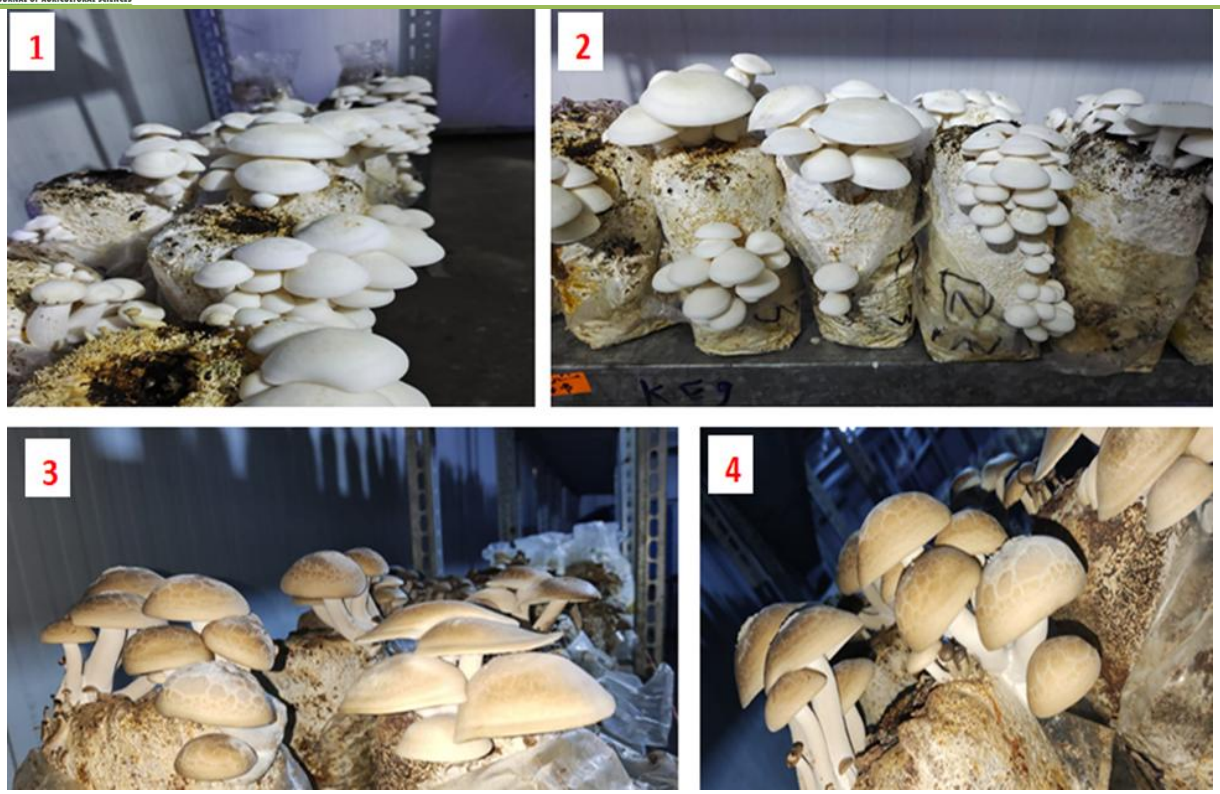

Figure 6. Morphological appearance of fruit bodies in bag cultivation of mushrooms on mixed supplements: (1,2) *H. marmoreus* and (3,4) *H. tessulatus*

CONCLUSIONS

In a pioneering step towards mushroom cultivation within the Iraqi environment, this study has unveiled the promising potential of *H. marmoreus* and *H. tessulatus*. mushrooms as a significant nutritional and medicinal resource. The trials demonstrated that utilizing sesame meals Supplement amendment provides the most conducive conditions for the mycelial growth of this mushroom's species, outperforming olive cake and coffee grounds. *H. marmoreus* and *H. tessulatus* is recognized for its high nutritional value, containing essential proteins, Carbohydrate, dietary fiber, lipid and minerals. For optimal fungal yield in Iraq, it is recommended to provide controlled environmental conditions encompassing an ideal temperature of approximately 20 °C and a pH range between 5 and 6. These findings encourage the broader adoption of *Hypsizygus* spp. cultivation in Iraq, recognizing its contribution to nutritional security and economic value.

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تأثير المدعمات في الصفات الانتاجية والنوعية لزراعة نوعين من الفطر *HYPSIZYGUS* SPP. في العراق

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المستخلص

هدفت هذه الدراسة إلى تحديد الظروف المثلى لنوعين من الفطر *H. marmoreus* و *H.tessulatus* والذين سُجِّلت زراعتهما لأول مرة في العراق باستخدام وسط محلي متوفر مثل (نشارة خشب أشجار الفاكهة) المدعم بمخلفات الزيتون، السمسم والقهوة وقد تضمنت الدراسة تقييم نمو الفطريات، وفترة الحضن، وتكوين رؤوس الدبابيس بالإضافة إلى قياس العائد والكفاءة البيولوجية. أظهرت النتائج تفوق مدعم مخلفات السمسم في سرعة انتشار الفطر وتكوين الرؤوس الدبوسية المبكرة وتحقيق أعلى عائد وكفاءة بيولوجية ، حيث بلغت 121.8غم و27.6% لفطر *H. marmoreus* و97.2غم و22.06% لفطر *H. tessulatus*، مقارنةً في معاملة المقارنة 76.2غم و19.7% و 82.9غم و21.5% على التوالي. علاوة على ذلك، تم تحديد الظروف المثلى لنمو الفطر على وسط PDA عند درجة حرارة 20 درجة مئوية ومدى حموضة 5-6 . أظهر تحليل محتوى العناصر الغذائية في الأجسام الثمرية المحصودة تركيزاً أعلى في البروتين والدهون والرماد والألياف في الفطرين النامين على مخلفات الزيتون مقارنة بالمدعمات الأخرى.

الكلمات المفتاحية: زراعة الفطر، وسط زراعة الفطر، فطر الزان.