ARTICLE / INVESTIGACIÓN

Evolution of agricultural wastes for the cultivation of edible mushroom *Pleurotus eryngii*

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Abstract: This research studies the possible use of some of Iraq's local agricultural wastes as growth substrates for producing the edible oyster mushroom (*Pleurotus eryngii*). The biological process of growing and producing edible mushrooms is a bioconversion of organic wastes to bioprotein (mycoprotein). Therefore, this study aims to investigate nine different substrates from plant residues. The three primary substrates are typical viz date palm waste (*Phoenix dactylifera*), maize cob (*Zea mays*) and cane (*Phragmites communis*), with two types of additional enrichment, i.e., rice husks (*Oryza sativa*) and corn seeds husks (*Zea mays*), and without further enrichment (control). The experiment was randomly distributed according to Randomized Complete Design (RCD), with three replicates. The less crop cycle period (day) which in Treatments T7 and T8 (30.33, 31.67 days) when T2 (100%Corncobs), T1 (100%Waste of Date palm), T7 (80%Waste of the date palm with 20%Corn husks) and T8 (80% Corncobs with 20%Corn husks) provide the crest mushrooms yield arrived 293.0 280.7, 268.7 and 264.0 g kg^{-1} substrate respectively.

Key words: Bio protein, lignocelluloses, Mushroom substrates, Oyster mushrooms.

Introduction

The mushroom is a fleshy saprophytic fungus that grows naturally on tree trunks containing rotting natural matter and moist soil. Mushroom cultivation can be used as an efficient method of harvesting and as a strategy to protect the environment[1]. Using the residues in biological processes can consider as one of the biological transformations from non-edible biomass waste solution to nutrient-packed foods protein in the form of edible mushrooms. Production is the transformation of household agricultural or industrial waste into food for people. The concepts of microorganisms, environmental technology, and fermentation of solid-state cover every form of mushroom[2-4]. The genus and species of *Agaricus spp*, *Pleurotus spp* and *Lentinus edodes* Are the most common edible mushroom cultivated worldwide. However, *Pleurotus* is a widely cultivated type of edible mushroom across a globe at a range of pillars with surroundings[5]. That genus contains greater than 200 throwing organisms spreading in tropical regions worldwide. *P. ostreatus*, *P. citrinopileatus P. djamor*, *P. citrinopileatus* and *P. eryngii* are the most common species in the genus *Pleurotus* (oyster mushroom)[6]. In Asia, North Africa and Europe, *Pleurotus eryngii*, popularly referred to as the king oyster mushroom, is commonly used. Several primary functions, such as antioxidants (antioxidants, cancer, viruses, bacteria, and leukemia), hypolipidemia, immunity, and estrogen-like activities, include the potential therapeutic activities of edible mushrooms. Its biologically active compounds such as polysaccharides, phenolic molecules, ergothioneine and triterpenoids form the basis of this bioactive properties[7-9]. *P. eryngii* is commercially grown on several raw plant materials due to its excellent flavor, high nutritional value, and medicinal qualities. The tailings are based on the use of a powerful lignin degradation enzyme system that effectively breaks down various aromatic compounds and its usefulness in the nutrient use of lignocellulose. For the same purpose, and because of the potential in several biotechnology processes, *Pleurotus eryngii* plays a vital role in these enzymes, such as food preparation (edible fungi), biotransformation of raw plant material into forage, and biological pulp. As well as the biological treatment of soils and industrial water, pulp bleaching[10]. Oyster mushrooms have been found to grow quickly on various waste substrates (industrial, agricultural residues), such as grain straw, leaves (banana leaves), bagasse (cotton waste, coffee pulp and sugar cane), body (cottonseed husks), wood chips (sawdust and paper waste). Thus, most residues materials (lignocelluloses) containing hemicellulose, celluloses, and lignin can be used as a substrate for mushrooms[2]. Since organic carbon, nitrogen, and inorganic compounds are essential to produce mushrooms as a portion of the main food and nutrients are organic carbon origins like cellulose, hemicellulose and lignin, oyster mushrooms require less nitrogen more carbon origins. Mushroom cultivation requires an adequate balance of the carbon and nitrogen ratio in the substrate. The overall carbon quality, including refractory polysaccharides (cellulose and hemicellulose), represents the overall organic carbon (C/N) ratio[11]. This study aimed to find out the possibility of applying some of Iraq's local agricultural wastes as growing substrates for the production of the *Pleurotus eryngii* edible oyster mushroom.

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Materials and methods

Pleurotus eryngii strain 008 was used in this study. The first factor Includes three types of substrates comprising three types) date palm waste (*Phoenix dactylifera*), corncobs (*Zea mays*) and common reed (*Phragmites communis*), and the second factor is the type of supplement (20 percent dry weight) comprising (2 classes) rice husks (*Oryza sativa*), corn husks (*Zea mays*) and without accompaniment. As in table 1, the experiment contains nine treatments. Table 2 shows the chemical analysis of the components of the agricultural wastes and supplementation. The experiment was laid out with five replicates according to Completely Randomized Design (CRD).

The substrate was put and in the polyethylene bags, then it sent for heating treatment for 60 minutes, after that it was put in a metal tank containing hot water, with the change the water. Then, the substrate was left to cool and get rid of excess moisture; after that, it was mixed with two percent calcium carbonate (lime) to the wet weighing procedure with an acidity equivalent to the acidity of the treatment[15]. It had been the addition of composites per 1 kg wet weight after cooling in 24*40 cm polyethylene bags (experimental unit).

Spawning was carried out at 3% of the substrate in the aseptic state to prevent contamination. After closing the bag's opening with a thread, the spawn was inserted in the middle of the substrate bag and hanging tightly[16].

The incubation place (aseptic conditions) was prepared by providing dark conditions, temperatures between 25-27°C with air humidity 85-90%[17-18]. The temperature decreases to 14-16oC after mycelium runs on the most substrate to create cold shock by spraying the water to increase humidity to 80-90 percent. Owaid *et al.* (2015)[19] show that it is 10-15°C with the light available for 8-10 hours. The first day with 400 lux. Level 1 is the ideal temperature for inducing the fruits of the oyster mushrooms and lowers carbon dioxide and O_2 increases. Holes were made in the bags in the direction of the light at the cross shape (+) when generating the pin-head with a sterile sharp scalpel in all bags with the same number[20]. The investigator retains this environment for about 7-10 days until the end of the first harvest and then returns to the incubation cycle again. The phases of fruiting growth in the Cultivation Room are shown in Figure 1.

Results and discussion

The results of the variance analysis show that the intermediate period stage of initial growth characters (spawn run, Pin-head, fruit body formation and Production cycle) of oyster mushroom *Pleurotus eryngii* is presented in Figure 2. The spawn run of mycelium shows a statistically significant difference in T5, T6 and T8 required a longer time to complete the colonization period compared with less period in T1, T2 and T9. While the highest period of pin-head value is obtained in T8, T5 and T9 than the less period to the formation of a pin-head in T4, T1 and T3. This shows the significant difference in the period of fruit body (fruiting). The earliest period was recorded from T1, T3 and T4 than the other substrate such as T2 and T7. The results in Figure 2 show that the considerable crop cycle period (day) significantly is in T1 compared with another combination substrate that records less in T7, T8 and T6.

The observational treatments take 44 to 74 days from spawn run (complete colonization) to the completion of the mushroom fruiting body. After that, mushrooms become ready to be picked from 89 to 32 days. This variation in periods may be due to the variation in chemical and physical characteristics of substrates (Nitrogen%, Protein %, CHO%, Ash %, Fiber %, O.M %, O.C %, C: N Ratio, water absorption % and Porosity %) Table 2.

Table 3. Showed a statistically significant difference in characteristics of *Pleurotus eryngii* yield (Number of the fruit body, weight of fruit body, number of flashes, Total yield and Bio-efficiency). Total yield, is one of the essential objectives of mushroom producers; the yield as recorded in T2 (100% Corn cobs), T1 (100% Date palm waste), T7 (80% waste of date palm with 20% Corn husks) and T8 (80% Corn cobs with 20% Corn husks) Had the highest values mushroom yield reached (293.00 280.70, 268.70 and 264.00 gk g^{-1} substrate). This yield is significantly different from those found in the other substrate combinations. Bio-efficiency is Significantly different compared with another combination substrate, in general, substrates of Corncobs and give the largest yield and give the height value of Bio-efficiency. Whereas the mushroom yield of wastes of date palm and common reed substrate comes in second and the Bio-efficiency. The supplementation of the substrate did not lead

Combination of Substrate		
NO. Treatment	**Main Substrate type (1st factor) %**	**Supplementation type (2nd factor) %**
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1	Date palm waste	lack of supplement
2	Corncobs	lack of supplement
3	Common reed	lack of supplement
4	80% (Date palm waste)	20% (Rice husks)
5	80% (Corncobs)	20% (Rice husks)
6	80% (Common reed)	20% (Rice husks)
7	80% (Date palm waste)	20% (Corn husks)
8	80% (Corncobs)	20% (Corn husks)

Table 1. Types of agricultural wastes (substrate and supplementation) and experiment treatments.

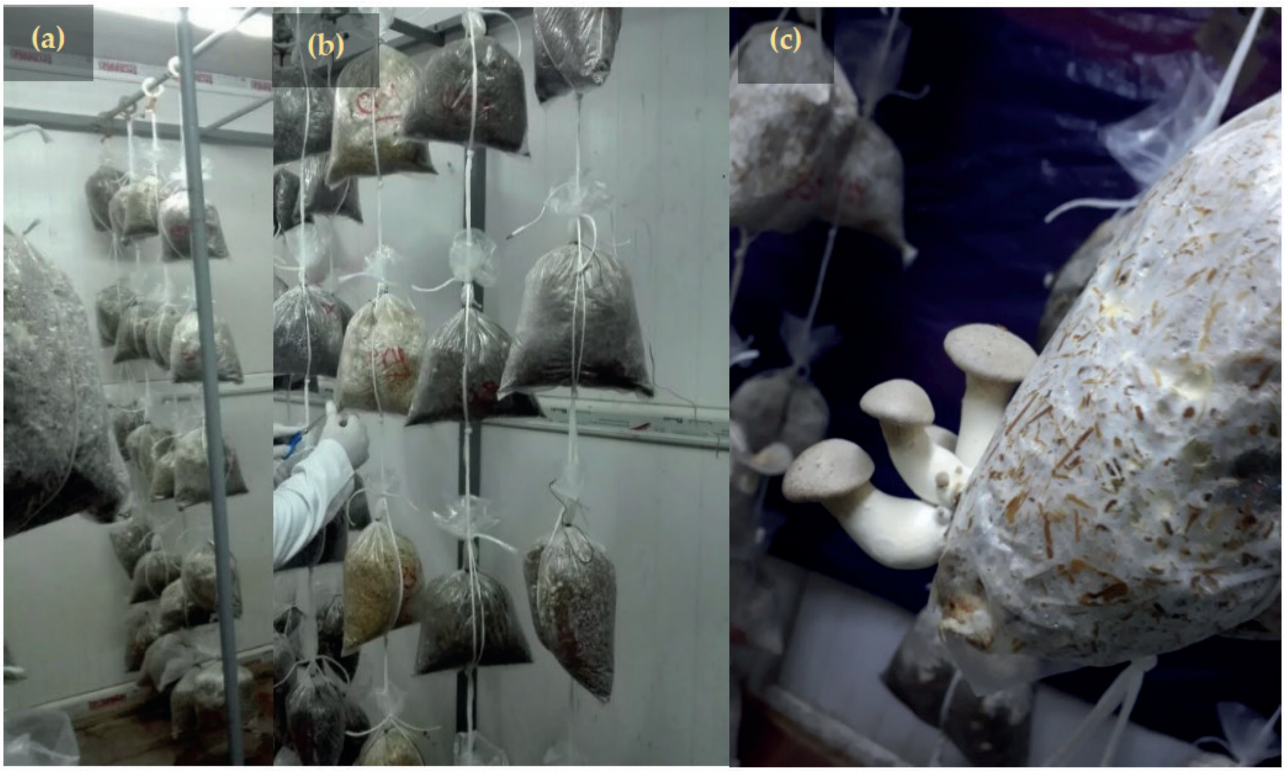

Figure 1. Fruit Body Developing from Mycelium in Cultivation Room of Oyster Mushroom *Pleurotus eryngii* (a) Post-spawning stage and mycelia growth began on the substrate; (b) Making holes in the bags for the exit of the fruiting bodies (c) The stage of formation of the fruiting bodies and their exit from the bags.

NO. Treatment	Number of fruit body	Mean of Flash. g	Number of flash	Mean of fruit body weight. g	Total yield g kg^{-1}	Bio-efficiency %
1	4.00a	93.56a	3.00a	70.17a	280.70a	61.68bcd
2	4.00a	97.67a	3.00a	73.25a	293.00a	105.78a
3	4.00a	116.25a	2.00ab	58.12a	232.50ab	65.10bcd
4	2.67ab	86.56a	2.667ab	86.56a	228.30ab	51.04cd
5	3.00 a	100.44a	2.333ab	77.00a	231.00ab	75.60abc
6	1.33b	87.00a	1.667b	118.17a	139.30b	37.77d

Different alphabets in the same column show significant differences using Turkey's HSD test.

Table 2. Analysis of chemical and physical qualities of the agricultural wastes for substrate and supplementation[1].

to the increment of profit compared to the substrates without supplements. The three types of primary substrates (wastes of date palm, Corncobs and Common reed) gave the highest values in yield the approaching result between them so that the efficiency of *Pleurotus eryngii* to grow successfully on the substrate of wastes of Date palm and Common reed substrate may be linked with the chemical composition and physical properties of selected substrates that important for the growth of this mushroom. The result is corroborative with the finding of Owaid *et al.* (2018)[19] and in harmony with the value reported by Kumar *et al.* (2020)[20] and Mkhize *et al.* (2016)[24].

The trend analysis shows that in the meantime for, the original attributes of development of the oyster mushroom *Pleurotus eryngii* (spawn running, pin-head, fruit body growth and production cycle) are shown in Figure 2. There is a statistically significant difference between ovulation. The period that takes longer to complete the T5 colonization cycle. And T6 and T8 compared to less time in T1, T2 and T9. Thus in T8, T5 and T9, the highest pin-head interval is obtained; in T4, T1 and T3, the lowest pin-head formation interval is obtained. Compared with other substrates, such as T2 and T7, the first period was recorded in T1, T3, and T4, indicating a large difference in the fruiting period of the body. The results from Figure 2 show that at T1, the crop cycle is much longer at T1 (day) compared to another stratum of the formulation, which is lower in T7, T8 and T6. Most proposed work operations take from 44 to 74 days of ovulation (complete colonization) to ripen the fruit body of the mushroom. Then from 89 to 32 days, mushrooms become ready for harvesting.

This change in cycles might be back to the concentration in the Physicochemical effects of the substrates percentage of Nitrogen (N), Protein, Total carbohydrates, Ash, Fiber, Organic matter (O.M), Organic carbon (C), C:N ratio, water absorption and porosity Table 2. However, the results of this analysis are close to some previous studies, such as Hoa *et al.* (2015)[21] and Mkhize *et al.* (2017)[19]. Table 3. The analysis shows that there is a statistically significant difference in the characteristics of the oyster mushroom yield (number of fruits, fruit body weight, flash number, average flash yield, total yield and bio-efficiency) yield, one of the main aims for mushroom producers, yield as is Listed in T2 (100 percent corn litter), T1 (100% palm waste), T7 (80% palm waste + 20% corn husks) and T8 (20% palm waste). In general, Corncob substrates give the most significant yield and provide the height value of Bio-efficiency compared to another mix substratum. While the mushroom yield, as well as the Bio-efficiency of palm waste timeline and mutual reed substratum, comes in second. As for the substrate supplementation, there is no yield promotion compared to the substratum without supplement.

The Pleurotus eryngii mushroom yield characteristics have been grown on Agro-Waste substrates. The converging result between the three substrate forms (palm waste timeline, corn cob and ordinary cane) gives oyster mushrooms the ability to grow successfully on date palm residues and Mutual reed substrates. To relate the Physicochemical effects of the selected substrates necessary for the growth of this fungus.

The results were similar to the results reported by Mkhize *et al.* (2016)[24] and Owaid *et al.* (2018)[25].

Conclusions

This study indicates that agricultural residues of date palms and corn cobs can give an efficient yield because these wastes appear as suitable substrates for the growth of oyster mushrooms (*Pleurotus spp.*) And a nutrient medium that provides these mushrooms with the necessary elements for forming fruiting bodies.

Author Contributions: Mustafa alqaisi designed and performed the experiments, derived the models and analyzed the data. Manaf K. M. Alabtan with Mazin A. Owine measurements and Mazin A. Owine helped carry out the Manaf K. M. Alabtan simulations. All authors read and approved the final manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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NO. Treatment	Number of fruit body	Mean of Flash. g	Number of flash	Mean of fruit body weight. g	Total yield g kg^{-1}	Bio-efficiency %
1	4.00a	93.56a	3.00a	70.17a	280.70a	61.68bcd
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3	4.00a	116.25a	2.00ab	58.12a	232.50ab	65.10bcd
4	2.67ab	86.56a	2.667ab	86.56a	228.30ab	51.04cd
5	3.00 a	100.44a	2.333ab	77.00a	231.00ab	75.60abc
6	1.33b	87.00a	1.667b	118.17a	139.30b	37.77d

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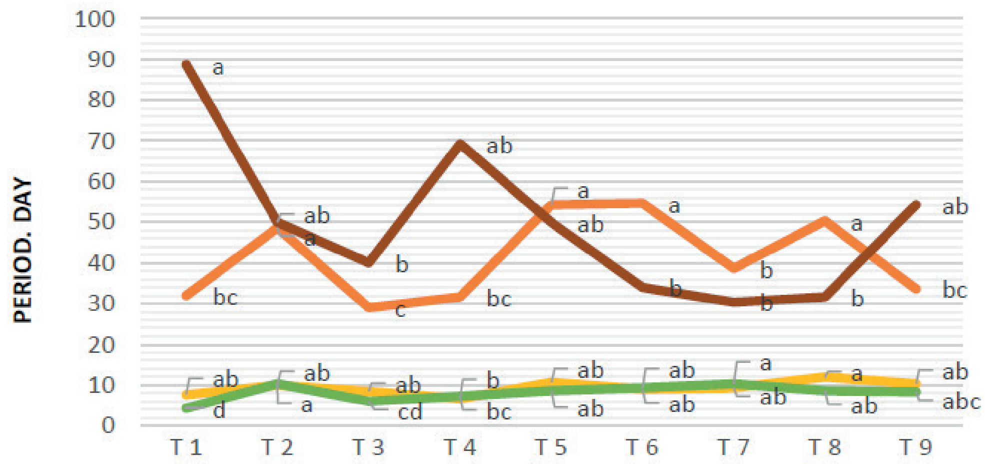
Table 3. Attributes of mushroom yield Pleurotus eryngii grown on agricultural wastes.



	T 1	T 2	T 3	T 4	T 5	T 6	T 7	T 8	T 9
Period of spawn run. day	32	48,67	29	31,67	54,33	54,67	38,67	50,33	33,67
Period of pin.day	7,667	10	8,5	6,667	10,667	9	9,333	12	10,333
Period of fruit body. day	4,333	10,333	6	7,333	8,667	9,333	10,333	8,667	8,333
crop cycle.day	88,67	50	40	69,33	50	34	30,33	31,67	54,33

Figure 2. Period stage of initial growth of oyster mushrooms (*Pleurotus eryngii*)

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