

Bioconversion of Agro-Waste to Product of Bio-protein via Cultivation of Edible Oyster Mushroom *Pleurotus eryngii*

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Abstract

This study investigated the potentiality of utilizing some local Agro-wastes (lignocellulose) in Iraq as growing Substrate to product edible oyster mushroom *Pleurotus eryngii*. Biological process of cultivation and Production edible mushrooms on sold state fermentation its considering a biotechnology Product of Bio-protein or myco-protein, so we used nine substrate combinations (nine Treatment) from plant residues which were Three main substrate (Date palm wastes, Corn cobs and Common reed) and two type of enrichments Supplement (Rice husks and Corn seeds husks) and Without Supplement (control) included, this Experimental data were analyzed using One-way analysis of variance (ANOVA), according to a completely randomized design (CRD) and Tukey's test P 0.05. The less period of crop cycle (day) which in T7 and T8 (30.33, 31.67 day) when T2 (Corn cobs 100%), T1 (Date palm waste 100%), T7 (Date palm waste 80%+Corn husks 20%) and T8 (Corn cobs 80%+Corn husks 20%) gave the maximum mushroom yield reached 293.0 280.7, 268.7 and 264.0 g kg⁻¹ substrate Sequentially and this yield was significantly different to those found from the other substrate combinations. Bioeffeincy Significant different compared with other combination substrate.

1 Introduction

Mushroom which is a fleshy saprophytic fungus are found growing in nature on damp rotten log of wood trunks of trees, decaying organic matter and in damp soil rich in organic substances. Cultivation of mushroom can be viewed as an effective way to extract bio resources left behind in agricultural residues and environmental protection strategy (Chang and Miles 1992). The use of the residues in bioprocesses may be one–bioconversion solution of inedible biomass residue into nutrition protein rich food in form of edible mushroom. Cultivation of any type of mushroom implies principles of microbiology, environmental engineering and solid-state fermentation in the conversion of domestic agricultural, industrial, forestry wastes into food for humans (Sadh et al. 2018; Tsegaye and Tefera 2017).

The most cultivated edible mushroom worldwide is *Agaricus* spp, followed by *Lentinus edodes* and *Pleurotus* spp. (Samsudin and Abdullah 2019; Akbarirad et al. 2013) *Pleurotus* is a genus of edible mushrooms widely cultivated throughout the world in a variety of substrates and conditions (Obodai et al. 2003). This genus consists of more than 200 saprophytic species distributed worldwide in temperate and tropical environments. The most common species of *Pleurotus* genera (Oyster mushroom), are: *P. ostreatus*, *P. djamor*, *P. citrinopileatus* and *P. eryngii*, (Gomes-Correa et al. 2016; Patel et al. 2012).

Pleurotus eryngii, commonly known as the king oyster mushroom, has been used extensively in North Africa, Europe and Asia and therapeutic potential activities of edible Mushroom implement multiple bioactivities as an antioxidant, anticancer, antiviral, antimicrobial, anti-leukaemia, hypolipidemic, immuno-modulating and estrogen-like activity. These bioactive properties depend on its bioactive compounds such as polysaccharides, phenolic compounds, ergothioneine and triterpenoid (Al-Bahrani et al. 2017; Fu et al. 2016; Ghahremani-Majd and Dashti 2015)

Due to its remarkable flavor, high nutritional value, and numerous medicinal attributes, *P.eryngii* is commercially cultivated on various raw plant materials. Its efficacy in using nutrients from lignocellulose residues is based on occupation of a potent ligninolytic enzyme system, which successfully degrade different aromatic compounds (cyclic compound). Similarly, due to the ability of these enzymes, *P. eryngii* plays a very important role in many

biotechnological processes, such as food production (edible fungi), bio-transformation of raw plant materials to feed, bio-pulping and bio-bleaching of paper pulp, as well as bioremediation of soil and industrial waters (Adebayo and Martinez-Carrera 2015; Stajic et al. 2009)

Tsegaye and Tefera (2017) Oyster Mushrooms are reported to be easily grown on different substrates wastes (Agro-industrial residues) such as cereal straw (rice and wheat straw), leaves (banana leaves), bagasse (cotton waste, coffee pulps and sugarcane) hulls (cottonseed hulls), wood chips (sawdust) and waste paper. Thus, most organic matters (lignocelluloses) containing cellulose, hemi cellulose and lignin can be use as mushroom substrate. Mushroom cultivation requires carbon, nitrogen and inorganic compounds as their nutritional sources, and main nutrients are carbon sources such as cellulose, hemi cellulose and lignin, Oyster mushrooms require less nitrogen and more carbon source. Cultivation of mushrooms need to an appropriate balance in the substrate as the carbon and nitrogen ratio. The total carbon value in the C/N ratio represents the carbon contents, including intractable polysaccharides (cellulose and hemicellulose) (Ryu et al. 2015). Furthermore, the supplementation of the substrate with cereal brans or the use of new combinations may promote increased productivity and biological efficacy of the mushroom growth (Donini et al. 2009; Samuel and Eugene 2012).

2 Material And Methods

This Experiment was conducted in Department of Horticulture and landscape design - Agriculture College-Tikrit University (34° 36' 56.9" N., 43° 40' 43" E.) on 5th Nov 2018 to March 2019. the Oyster Mushroom, *Pleurotus eryngii* strain's name 008 was used for the cultivation. This experiment designed with two factors, first factor represented a substrate type included (three type) date palm waste, corn cobs and common reed, and the second addition represented a supplements type (20% Dry weight) which included (two type) rice husks, corn husks and without supplement,. then the experiment include 9 treatments as in Table 1. Some laboratory test was done to substrate and supplements to know its ingredients as set out in Table 2. Designed according to Completely Randomized Design (CRD) with five replicates for each treatment.

Table 1
Types of Agro-Waste (substrate and supplementation) and experiment treatments

No.	Main Substrate type (1st factor)	No.	Supplementation type (2nd factor)
1	Date palm waste	1	Without supplementation
2	Corn cobs	2	Rice husks
3	Common reed	3	Corn husks
Total Treatments (Combination of Substrate)			
Treatment	Substrate type	Supplementation type	
T1	Date palm waste 100%	Without supplementation	
T2	Corn cobs 100%	Without supplementation	
T3	Common reed 100%	Without supplementation	
T4	Date palm waste 80%	Rice husks 20%	
T5	Corn cobs 80%	Rice husks 20%	
T6	Common reed 80%	Rice husks 20%	
T7	Date palm waste 80%	Corn husks 20%	
T8	Corn cobs 80%	Corn husks 20%	
T9	Common reed 80%	Corn husks 20%	

Table 2

Analysis of some chemical and physical traits of the Agro-Waste (substrate and supplementation).

Substrate type	N%	Protein %	CHO %	Ash %	Fiber %	O.M*%	O.C*%	C:N* Ratio	Water content %	Porosity %
Date palm waste	0.58	2.57	22.5	5.316	11.49	94.68	54.91	93.38	120	69.42
Corn cobs	0.73	3.21	29.38	3.86	12.61	96.14	55.76	75.86	260	19.26
Common reed	0.83	3.64	13.15	5.03	11.55	94.96	55.07	66.11	180	60.74
Supplement type	N %	Protein %	Cho %	Ash %	Fiber %	O.M %	O.C %	C:N Ratio	Water content %	Porosity %
Rice husks	1.56	6.86	40.19	3.64	12.01	96.36	55.88	35.63	140	52.38
Corn husks	1.37	6.00	36.47	10.40	11.93	89.6	51.96	37.87	140	46.27
*organic matter (O.M) = 100 - ash % * Organic Carbon (O.C) = (O.M) × 0.58										
* Then calculated C:N Ratio (Alqaisi 2015; Chu et al. 2012; Chen 2000).										

2.1 Preparation of the Substrate

Substrate are compressed into the polyethylene bags and send for the heating treatment. the substrate put in, the bags were putted in metal tank contain tab water and immersed in hot water to ensure good sterilization for 60 minute with replace the water with each time to prevent interaction with each other. afterwards the Substrate let to cool and dispose excessive humidity. thereafter The addition of 2% calcium carbonate (lime) CaCO_3 to treatment according to wet weight to equivalent treatments acidity (Grace and Ayandele 2018). The substrates and supplements filled after cooling in polyethylene bags with dimensions 24 × 40 cm to include every one 1 kg of wet weight (Experimental unit).

2.2 Spawning (Inoculation)

Adding of mushroom spawning (inoculum) at 3% of substrate fresh weight, This is carried out in a aseptic conditions to avoid contamination. spawn is put down in the center of substrate bag afterwards close the bag orifice with thread tightly and hanged (Bernardi et al. 2013; Kwon and Kim 2004).

2.3 cultivation Room condition (Incubation & Fruiting)

Incubation room (aseptic conditions) according to design prepared for experiment between 25–27°C with darkness along the day with humidity 85–90% (Ahmed et al. 2013; Oei 2005). After mycelium run on most substrate the temperature decrease to 14–16°C to make cold shock with elevating humidity to 80–90% by spraying the water. Owaid et al. (2015) mentioned that ideal degree to induce oyster mushroom fruiting is 10–15°C with availability of light for 8–10 hours.day⁻¹ with intensity 400 lux.hours⁻¹ and decrease percentage of CO_2 and increase O_2 , thereafter make holes in bags at Pin-head generation at direction of light at cross shape

(+) by using sterile sharp scalpel at same number in all bags, harvesting fruit body after few days of initial primordia by twisting and pulling it gently to remove it from the base (Roksana et al. 2018). We continued this environment until end of first harvesting then returned back to incubation period again about 7–10 days.

2.4 Investigation parameters:

2.4.1 Period of initial growth characters (day):

1 - Period of spawn run (complete mycelium run):

Symbolized by number of days from inoculation until complete mycelium run on the substrate (Shah et al. 2004).

2 Period of Pin-head (generation of Primordia):

It Symbolized by number of days from the complete mycelium run to the starting of appearance of Pin-head or primordia.

3 Period of complete fruit body formation:

Number of days from primordia period until to be fruit body.

(Shah et al. 2004).

4 Production cycle:

Number of days from first harvesting to last one for each sac or repeater that contain 1gk of wet media (Al-Badrany 2010; Beyer 1996).

2.4.2 Yield Attributes:

1 Total production according to wet weight (Total wet weight):

It represent sum of all harvesting produced by one bags contain 1kg of wet substrate by g.kg^{-1} .

2 Number of fruit body:

Number of fruit body produced by one bag contain 1kg of wet substrate for all harvesting.

3 Mean of fruit body Weight (g):

It's calculated as following:

Weight of fruit body= sum of weight of fruit body produced by one bag/number of fruit bodies produce by same bag

4 Biological efficiency:

Biological Efficiency % = (wet weight of fruit body/dry weight of substrate) $\times 100$.

Statistical analysis:

The Experimental data were analyzed using One-way analysis of variance (ANOVA), according to a completely randomized design (CRD) and Tukey's multiple range test (Tukey's HSD test) was used to separate mean values at a significance level of $p \leq 0.05$. All data were analyzed using the Genstat 12th ed data analysis software system (Payne et al 2009).

3 Result And Discussion

The results of analysis of variance showed that the average Period of initial growth characters (spawn run, Pin-head, fruit body formation and Production cycle) of oyster mushroom *Pleurotus eryngii* is presented in Fig. 1. The spawn run of mycelium shows a statistically significant difference which was in T5, T6 and T8 required longer time to complete colonization period compare with less period in T1, T2 and T9. While the highest period of pin-head value was obtained in T8, T5 and T9 than lower Period to formation of pin head in T4, T1 and T3. Showed the significant difference in term of 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 Fig. 1. showed that the longer period of crop cycle (day) significantly was in T1 compare with other combination substrate which recoded less in T7, T8 and T6.

Most of the experimental treatments took between 44 to 74 day from spawn run (complete colonization) to maturation of mushroom fruiting body. After that, mushrooms became ready for picking from 89 to 32 day. This variation in time periods may be due to the variation in chemical and physical traits of substrates (N%, Protein %, Cho%, Ash %, Fiber %, O.M %, O.C %, C:N Ratio, Water content % and Porosity %) Table 2. However, the results of this study were similar to some previous studies, such as Hoa et al. (2015) and Mkhize et al. (2017).

Table 3. analysis shows a statistically significant difference in term of yield characters oyster mushroom (fruit number, fruit mean, number flash, mean flash, total yield, Bio efficiency) yield is one of the main purposes of mushroom cultivators, the yield as recorded in T2 (Corn cobs 100%), T1 (Date palm waste 100%), T7 (Date palm waste 80%+Corn husks 20%) and T8 (Corn cobs 80%+Corn husks 20%) the maximum mushroom yield reached (293.0 280.7, 268.7 and 264.0 g kg^{-1} substrate) and this yield was significantly different to those found from the other substrate combinations. Bioeffeincy Significant different compared with other combination substrate, In general, substrates of Corn cobs and gave the higher yield also gave the higher value of Bioeffeincy. Whereas, mushroom yield of Date palm waste and Common reed substrate, which came in second, as well as the Bioeffeincy. As for the supplementation of substrate did not produced promotion of yield, compared to substrate with out supplement.

Table 3
Yield attributes of mushroom *Pleurotus eryngii* grown on Agro-Waste substrates

Treatment	No. fruit	Mean. Flash g	No. flash	Mean. fruit g	Total. yeild g kg ⁻¹	Bio efficiency %
T1	4.0 a	93.56 a	3.00 a	70.17 a	280.7 a	61.68 bcd
T2	4.0 a	97.67 a	3.00 a	73.25 a	293.0 a	105.78 a
T3	4.0 a	116.25 a	2.00 ab	58.12 a	232.5 ab	65.10 bcd
T4	2.667 ab	86.56 a	2.667 ab	86.56 a	228.3 ab	51.04 cd
T5	3.0 a	100.44 a	2.333 ab	77.00 a	231.0 ab	75.60 abc
T6	1.333 b	87.00 a	1.667 b	118.17 a	139.3 b	37.77 d
T7	3.333 a	102.83 a	2.667 ab	81.50 a	268.7 a	60.06 bcd
T8	3.333 a	88.00 a	3.00 a	79.22 a	264.0 a	86.40 ab
T9	2.667 ab	94.72 a	2.333 ab	86.67 a	221.7 ab	60.08 bcd
C.V%	17.3	14.0	17.1	28.2	16.5	16.6
Means with the same columns followed by the same letters are not significantly different at $p \leq 0.05$ according to tukey's multiple range test.						

The three type of substrates (Date palm wastes, Corn cobs and Common reed) gave the approaching result between them, so that ability of oyster mushroom to grow successfully on the substrate of Date palm waste and Common reed substrate may be associated with the chemical composition and physical proprties of selected substrates that important for growth of this mushroom. The result was confirmed with the finding of Owaid et al. (2018) and in harmony with the value reported by Mkhize et al. (2016).

Conclusions

Agricultural waste materials is generally used for mushroom cultivations. Date palm wastes, Corn cobs, and Common reed and two type of enrichments Supplement (Rice husks and Corn seeds husks) possess suitable of physical and chemical properties (N%, Protein %, Cho%, Ash %, Fiber %,O.M %, O.C %, C:N Ratio, Water content % and Porosity %) and less cost compared to other plant residues. Based on the obtained results, Date palm wastes and Common reed with two type of enrichments Supplement (Rice husks and Corn seeds husks) with or mixture shortened the total growth compared to the Corn cobs. Date palm wastes as the substitution for Corn cobs as well as shortened number in crop cycle. The oyster mushrooms grown in the substrate with Rice husks and Corn seeds husks From these results, we concluded that Date palm wastes, Corn cobs and Common reed can be used as the base material for the oyster mushroom cultivation without supplements. In future, the investigation of Date palm wastes and anthers species of oyster mushroom (*Pleurotus* spp) could be applied in Iraq and other countries, in order to spread and expanded their usage.

Abbreviations

O.M: organic matter, O.C: Organic Carbon, CHO: carbohydrate, N: Nitrogen, C:N: Ratio between Organic Carbon and Nitrogen.

Declarations

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Author contribution statements

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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Availability of data and materials

The data set (table and graphs) supporting this article's conclusion is available.

Ethics approval and consent to participate

All the authors have read and agreed the ethics for publishing the manuscript.

Consent for publication

The authors approved the consent for publishing the manuscript.

Competing interests

The authors declare that they have no competing interests.

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Figures

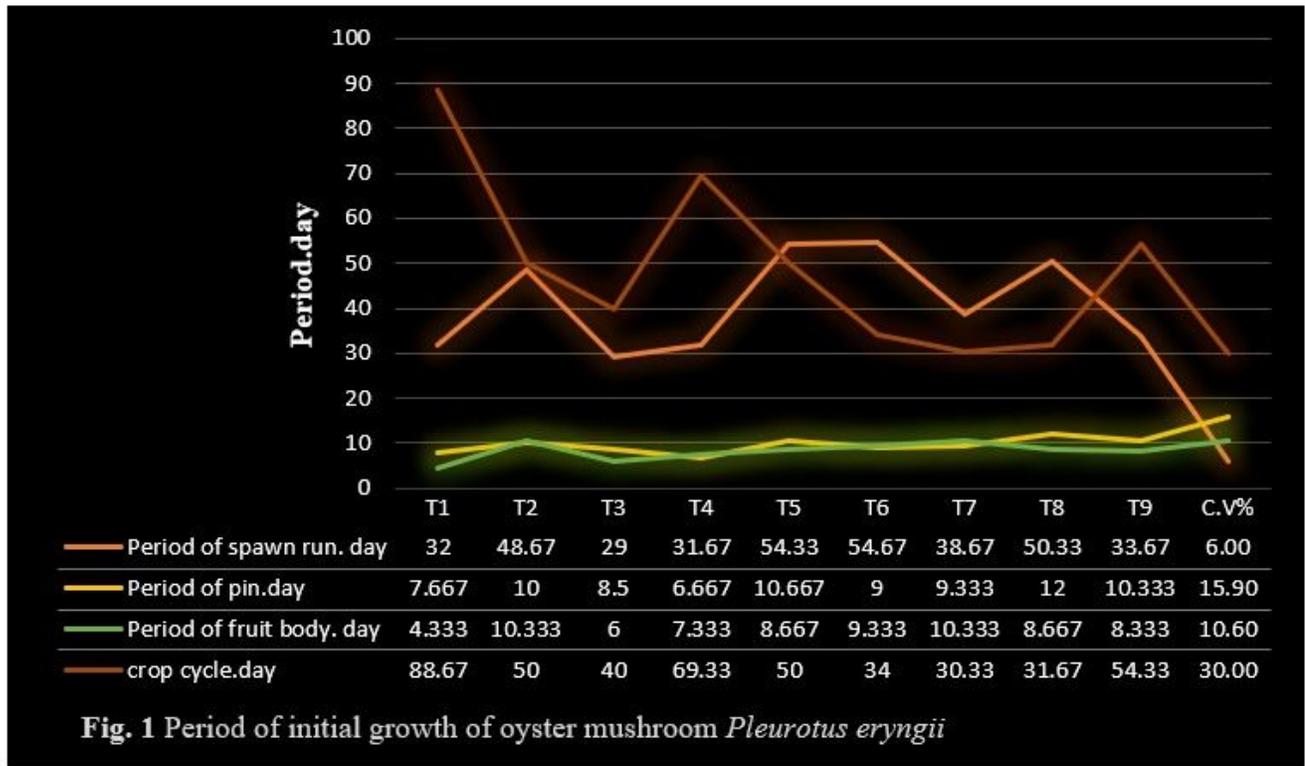


Figure 1

Period of initial growth of oyster mushroom *Pleurotus eryngii*

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