

Effect of cellulosic matter and container size on cultivation and yield of oyster mushroom *Pleurotus ostreatus*

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ABSTRACT

When cultivation the oyster mushrooms, we must put ability use locally low-cost wastes in this agriculture viz. houses and shops wastes. The ability of paper wastes (cardboard) and wheat straw were estimated in the oyster mushroom *Pleurotus ostreatus* production in plastic bottles of different volumes from wastes of houses in Hit city compare as using polyethylene bags. The results showed that the best significant ($P < 0.05$) crop reached to 143.8 g on bags that capacity 1.5 kg of wheat straw, followed by a rate 64.8 g on the same substrate in 0.55 kg plastic bottles. The larger number of flushes was 2.2 flushes/bag for the wheat straw in a bottle (0.55 kg), followed 2.0 flushes/bag by the cardboard in a bag of capacity 1.5 kg. Higher significant ($P < 0.05$) weight of the body was 120.4 g when grown in a bag 1.5 kg of wheat straw. The cardboard was showed the biological efficiency 32.5% compared with the wheat straw (control) 25.7% while the higher biological efficiency was 60.7% followed 45.9% for the wheat straw on bags that capacity 0.5 kg and bottle 0.1 kg, respectively.

KEY WORDS: Cardboard, paper wastes, plastic bottles, *Pleurotus ostreatus*, yield

INTRODUCTION

Oyster mushroom *Pleurotus ostreatus* belongs to the kingdom of fungi, phylum Basidiomycota. It is important for widespread in saprophyte or parasite living because analyst for wood and grows on different agro-wastes (Stamets and Chilton, 1983). *P. ostreatus* is one of twenty four edible mushrooms cultivated commercially in the special farm (Thomas and Schumann, 1993). For the first time, cultivation of *P. ostreatus* was succeeded in 1900, and increased the importance of the cultivation for its high nutritional value (Chang and Miles, 2004) and medicinal value (Owaïd, 2015a). *Pleurotus* species have high medicinal value due to possess significant anti-inflammatory, antiviruses (Carvalho *et al.*, 2007), antioxidant, anticancer (Kim *et al.*, 2009), anti-parasitic (David *et al.*, 2012), antifungal (Alheeti *et al.*, 2013b), anti-yeast (Owaïd *et al.*, 2015d), and anti-bacterial activities (Owaïd *et al.*, 2015a).

P. ostreatus is one of the most commercially important and successfully cultivated and is considered to be a delicacy

after *Agaricus bisporus* and *Lentinus edodes*. Like *P. ostreatus*, many of *Pleurotus* mushrooms are primary decomposers of hardwood trees found worldwide. Approximately, 70 species of *Pleurotus* were recorded and new species were discovered (Kong, 2004). This fungus is important to bio-convert cellulosic matters to a rich protein food (Owaïd, 2013; Owaïd *et al.*, 2015b).

What is produced from solid waste in Iraq alone is estimated at 5,446,000 tons/year, around is 12.3% is burned according to statistics of the year (UNSD, 2011). Oyster mushroom can be cultivated using different agro-wastes such rice straw, paper, coffee pulp, cotton waste, cotton seed hulls, corn cobs waste, bean straw, crushed bagasse, molasses wastes (Fan *et al.*, 2008), soybean straw, paddy straw, wheat straw (Ahmed *et al.*, 2009), blotting paper (Epogee, 2011), cardboard, cane (Al-Issawy, 2011), handmade paper wastes, industrial cardboard wastes (Owaïd *et al.*, 2015b), rice bran with sawdust (Owaïd *et al.*, 2015e) wheat straw, date palm wastes (Owaïd, 2013;

Alheeti *et al.*, 2013a; Owaid *et al.*, 2014; Owaid *et al.*, 2015c), and some their combination. Some researchers amended the substrates using phosphate rock to raise the nutritional value of date palm fiber, wheat straw and white sawdust (Owaid *et al.*, 2015c) because of mineral value of this fertilizer (Owaid and Abed, 2015).

Royse (2002) was cultivated oyster mushroom in polyethylene bags that capacity 9.1 kg and 13.6 kg, while Bao *et al.* (2004) used sawdust and rice bran mixture in plastic bottles 200 ml and 300 ml. In general, *P. ostreatus* was cultivated in bags, bottles and boxes in different sizes (Kong, 2004). While other mushrooms like *A. bisporus* were cultivated using boxes (Alheeti *et al.*, 2010; Muslat *et al.*, 2011).

Cultivation of oyster mushrooms is a biotechnological process for lignocellulosic wastes recycling; can be produced at the level of the family in the home, garage, warehouse, and old houses, which provides an opportunity for the national income of the country (Yabraq *et al.*, 2010). It might be the only current process that combines the production of protein-rich food with the reduction of environmental pollution (Sanchez, 2009).

This research aims to use paper wastes (cardboard) alone compared with the wheat straw in the preparation of substrates, study its impact on a number of quality and quantity characters of the produced mushroom using by types of bags and plastic bottles instead of their burning; that will be ensure low cost and rid the Iraqi environment of houses and shops pollutants (plastic bottles and cardboard), which vital to increase biological efficiency in converting solid wastes into a useful fresh food.

MATERIALS AND METHODS

Spawn Preparation

Mycelium of *P. ostreatus* (grey) was obtained from Plant and Pathological Fungi Lab. Department of Biology in College of Science/University of Anbar, Iraq. 450 g of oyster mushroom's spawn was prepared using seeds of millet *Pennisetum americanum* in polypropylene bags that capacity 10 cm × 30 cm after sterilized the wet seeds in bags using autoclave at 121°C and 1.5 psi for 30 min, cooled, inoculated using 2 cm × 2 cm mycelium piece, incubated at 25°C for 2 weeks, and saved at 4°C until use. 3% of spawn was used on wet based of the substrate for inoculation these substrates (Singh and Singh, 2011).

Substrates Preparation and Pasteurization

From Hit city in Iraq, cardboard pieces 5 cm × 5 cm and wheat straw were obtained and used as substrates in

polyethylene bags and plastic bottles as shown in Table 1. Substrates were pasteurized, drained from exceed water, added 5% of CaSO₄ (based on dry matter), well-mixed and cooled for ready to inoculated by the spawn.

Cultivation and Yield Performance

Substrates were cultivated using layers in depth 5 cm, spawn added then another layer of substrate added until filling then closed. Incubation was achieved darkly at 24°C ± 1°C for 3 weeks until the complete growth of mycelia in the whole packet. 10°C for 48 h was carried out as cold shock to induce pin heads formation, packets opened with 24°C ± 1°C, 80-90% humidity, lighted using fluorescent light, and aerated twice per today so primordia form that developed to fruiting bodies, which harvested in best yield size (Epogee, 2011).

The measurement of yield was done after 2 weeks. The percentage of biological efficiency calculated using this formula; BE% = (Fresh yield g/dry weight of substrate in spawning phase g)*100. Furthermore, the average weight of fruiting bodies, number of flushes, the diameter of stipe (stem), the diameter of pileus (cap), and length of stipe (Beyer and Muthersbaugh, 1996).

Statistical Analysis

The experimental data were subjected to an analysis of variance by two-ways analysis (ANOVA) using GenStat Discovery Edition computer program version 7 DE3 (VSN International Ltd., UK) to determine the least significant difference among means at the level least of 0.05 (*P* < 0.05). Five replicates were examined in the experiments.

RESULTS

The productivity of grey oyster mushroom *P. ostreatus* was changeable on substrates *viz.* wheat straw and cardboard in polyethylene bags and plastic bottles as in Table 2. The best significant (*P* < 0.05) crop 116.2 g/packet using 1.5 kg wheat straw in bags based on wet weight as a comparison with 30.5 g/packet when use cardboard. Whereas 100 g of cardboard (Figure 1) in the bottle was gave 12.0 g/packet

Table 1: Cultivation containers and substrates

Cultivation packet	Wheat straw 100%		Cardboard 100%	
	Wet weight (g)	Dry weight (g)	Wet weight (g)	Dry weight (g)
Bag 50 cm×30 cm	1500	320.25	1500	568.5
Bag 25 cm×20 cm	500	106.75	500	189.5
Bottle 2.250 L	550	117.43	550	208.45
Bottle 0.500 L	100	21.35	100	37.9
Wet content (%)	78.65	0	62.1	0

of fresh mushroom compared as 9.6 g/packet with wheat straw in the same packet (Figure 2).

On the other side, the best significant ($P < 0.05$) biological efficiency was 45.9% using bottle capacity of 100 g of wheat straw on wet weight as comparison as all containers, whereas the low biological efficiency was 5.36% on 1.5 kg cardboard in bags. The best significant ($P < 0.05$) number of flushes was 2.2 with bags that capacity 0.5 kg of wheat straw, whereas except bottle capacity of 0.55 kg of cardboard, the less flushes number was one.

In general, an average of fruiting bodies weight of produced mushroom was between 9.52 and 9.93 g. The 0.5 kg of cardboard bag has been the biggest weight (12.20 g) among weights of fruiting bodies in others containers.

While the specific characters of produced oyster mushroom on these substrates (Table 1) was shown in Table 3. The average of cap diameter was better on wheat straw than cardboard on all packets, which reach to 6.81-4.26 mm and 6.20-3.64 mm, respectively. Furthermore, the

diameter of stem has been same destination of the diameter of cap in positive correlation (Table 4). The less length of the stem was 1.66 using 100 g of wet wheat straw in bottles, significantly ($P < 0.05$), while the high value 4.26 using 500 g of wet cardboard in the bag.

DISCUSSION

The productivity and biological efficiency were increased in wheat straw as comparison with cardboard because of variation of capability these substrates to save and aid the nutritional and environmental requirements and difference of containing from cellulose, hemicellulose and lignin (Kuhad et al., 1997). Furthermore, biological efficiency



Figure 1: Agriculture of *Pleurotus ostreatus* in polyethylene bags



Figure 2: Agriculture of *Pleurotus ostreatus* in plastic bottles

Table 2: Quantity properties of oyster mushroom

Features	Substrates*	Bag 1.5 kg	Bag 0.5 kg	Bottle 0.55 kg	Bottle 0.1 kg
Productivity g/packet	Wheat straw	116.20	64.80	52.00	9.60
LSD ($P < 0.05$) 3.562	Cardboard	30.50	14.00	15.80	12.00
Biological efficiency %	Wheat straw	36.78	60.70	44.28	45.90
LSD ($P < 0.05$) 1.196	Cardboard	5.36	7.60	7.38	31.66
Number of flushes	Wheat straw	2.00	2.20	2.00	1.20
LSD ($P < 0.05$) 0.381	Cardboard	1.00	1.00	1.40	1.00
Fruiting body weight (g)	Wheat straw	10.50	10.00	11.31	7.90
LSD ($P < 0.05$) 1.014	Cardboard	10.17	12.20	6.49	9.20

*Cultivation packet (wet weight). LSD: Least significant difference

Table 3: Quality properties of oyster mushroom

Features	Substrates*	Bag 1.5 kg	Bag 0.5 kg	Bottle 0.55 kg	Bottle 0.1 kg
Diameter of cap (mm)	Wheat straw	6.81	6.37	5.88	4.26
LSD ($P < 0.05$) 0.383	Cardboard	6.20	5.00	4.60	3.64
Diameter of stem (mm)	Wheat straw	1.24	1.25	0.91	0.61
LSD ($P < 0.05$) 0.226	Cardboard	1.00	1.04	0.88	0.82
Length of stem (mm)	Wheat straw	2.74	2.57	1.92	1.66
LSD ($P < 0.05$) 0.341	Cardboard	2.50	4.26	2.00	2.14

*Cultivation packet (wet weight). LSD: Least significant difference

Table 4: Correlation among some characteristics

Correlation	Yield	Weight of fruiting body	Number of flushes	Biological efficiency	Diameter of cap	Diameter of stipe	Length of stipe
Yield	1.000						
Weight of fruiting body	0.325	1.000					
Number of flushes/bag	0.681	0.123	1.000				
Biological efficiency	0.420	0.045	0.605	1.000			
Diameter of cap	0.799	0.464	0.596	0.192	1.000		
Diameter of stipe	0.603	0.255	0.439	0.083	0.617	1.000	
Length of stipe	0.070	0.562	-0.124	-0.374	0.202	0.447	1.000

depends on the yield size (Beyer and Muthersbaugh, 1996), viz. a positive correlation between productivity and biological efficiency (Table 4), which affected by the dry weight of substrate that lead to increased biological efficiency in small packets (Table 2).

Small size of gradients of the substrate wheat straw has large influence on oyster mushroom growth, compared with pieces of cardboard, which lead to increasing decomposed wheat straw and big biomass of mycelia formed because of increasing the surface area this substrate, thus clusters were grew up on this medium (Aswad, 2005).

The decline of determinations of caps in small bags and bottles with all substrates may be return to get near fruiting bodies because of small area with small packets, which lead to small size of fruiting body in small packet. Whereas in the big containers; fruiting bodies have best chance to grow and extend that due to the big size of the container that gave big size of the fruiting body in big packet. The increasing means of weight of fruiting bodies (Table 2) and diameter of caps (Table 3) in wheat straw belong to available environment for big biomass with this substrate that hurry to fruiting bodies formation, while that was decreased with cardboard substrate (Aswad, 2005). Good explication of results in Tables 2 and 3 was given according to positive correlations (Table 4) between number of flushes and biological efficiency on the one hand and the diameter of cap and productivity from another hand.

REFERENCES

- Ahmed SA, Kadam JA, Mane VP, Patil SS, Baig MM. Biological efficiency and nutritional contents of *Pleurotus florida* (Mont.) Singer cultivated on different agro-wastes. Nat Sci 2009;7:44-8.
- Alheeti MN, Al-Assaffii IA, Muslat MM. Evaluation the efficiency of reed plant (*Phragmites australis*) wastes and aqueous extract of liquorices (*Glycyrrhiza glabra*) on the production of *Agaricus bisporus*. J Univ Anbar Pure Sci 2010;4:13-21.
- Alheeti MN, Al-Saeedi SS, Al-Assaffii IA. Evaluation of qualification of substrates containing date palm fibers (leef) and sawdust on quality and productivity of four *Pleurotus* spp. 2nd Conference of Desert Studies, Ramadi, Iraq; 2013a.
- Alheeti MN, Al-Saeedi SS, Al-Assaffii IA, Sabaratnam V. Antifungal activities of mycelia and culture filtrate of four oyster mushroom species (*Pleurotus* spp.) against pathogenic Fungi. The 7th International Medicinal Mushroom Conference, Beijing, China; 2013b.
- Al-Issawy KJ. Improve the local components of the substrate to increase the production Oyster mushroom *Pleurotus ostreatus*. M.Sc. Thesis. College of Agriculture, University of Anbar, Iraq; 2011. p. 84.
- Aswad HB. Effect of microbial biotechnological and media mixtures on production of Oyster Mushroom (*Pleurotus ostreatus*). M.Sc. Thesis. College of Agriculture, University of Anbar, Iraq; 2005. p. 78.
- Bao D, Kinugasa S, Kitamoto Y. The biological species of oyster mushrooms (*Pleurotus* spp.) from Asia based on mating compatibility tests. J Wood Sci 2004;50:162-8.
- Beyer DM, Muthersbaugh HR. Nutritional factors affecting later break yield of *Agaricus bisporus*. Can J Plant Sci 1966;76:835-40.
- Carvalho MP, Der Sand ST, Rosa EA, Germani JC, Ishikawa NK. Investigation of the antibacterial activity of basidiomycetes. Biociencias Porto Alegre 2007;15:173-9.
- Chang ST, Miles PG. Mushrooms Cultivation, Nutritional Value, Medicinal Effect and Environmental Impact. 2nd ed. USA: CRC Press LLC; 2004. p. 451.
- David OM, Fagbohun ED, Oluyege AO, Adegbuyi A. Antimicrobial activity and physicochemical properties of oils from tropical macrofungi. J. Yeast Fungal Res 2012;3:1-6.
- Epogee Ltd. How to Grow Oyster Mushroom. Sheet. United Kingdom: Mushroom Box Company, Epogee Ltd.; 2011. p. 5.
- Fan L, Soccol CR, Pandey A. Mushroom production. In: Current Developments in Solid-state Fermentation. India: Asiatech Publishers Inc.; 2008. p. 517.
- Kim JH, Kim SJ, Park HR, Choi JI, Ju YC, Nam KC, et al. The different antioxidant and anticancer activities depending on the color of oyster mushrooms. J Med Plants Res 2009;3:1016-20.
- Kong WS. Spawn. In: Mushroom Growers Handbook, Oyster Mushroom Cultivation, MushWorld. Korea: Aloha Medicinals Inc.; 2004. p. 54-61.
- Kuhad RC, Singh A, Eriksoon KE. Microorganisms and enzymes involved in the degradation of plant fiber cell walls. In: Advances in Biochemical Engineering Biotechnology. Germany: Springer-Verlag; 1997. p. 46-125.
- Muslat MM, Al-Assaffii IA, Alheeti MN. Efficient use of waste reed cane *Phragmites australis* supported by *Streptomyces* O3 in the preparation the compost for production mushroom *Agaricus bisporus* and affect spraying extract of liquorices *Glycyrrhiza* sp. Res J Aleppo Univ Agric Sci Ser 2011;91:xx (Page numbers in Roman).
- Owaid MN. Testing efficiency of different agriculture media in growth and production of four species of oyster mushroom *Pleurotus* and evaluation the bioactivity of tested species. Ph.D. Dissertation. College of Science, University of Anbar, Iraq; 2013.

- Owaid MN. Mineral elements content in two sources of *Agaricus bisporus* in Iraqi market. J Adv Appl Sci 2015;3:46-50.
- Owaid MN, Abed IA. Mineral analysis of phosphate rock as Iraqi raw fertilizer. Int J Environ 2015;4:413-5.
- Owaid MN, Abed AM, Nassar BM. Recycling cardboard wastes to produce blue oyster mushroom *Pleurotus ostreatus* in Iraq. Emirates J Food Agric 2015b;27(7):537-41.
- Owaid MN, Abed IA, Al-Saeedi SS. Using of date palm fiber mixed with other lignocelluloses toward *Pleurotus ostreatus* (Higher Basidiomycetes) cultivation. Emirates J Food Agric 2015c;27(7):556-61.
- Owaid MN, Al-Assaffi IA, Al-Saeedi SS. Assessment date palm fibers extract agar with other lignocellulose residues on mycelial growth of *Pleurotus eryngii*. Rev Microbiol Ind San et Environn 2014;8:172-86.
- Owaid MN, Al-Assaffi IA, Al-Saeedi SS. Antimicrobial activity of mycelia of oyster mushroom species (*Pleurotus* spp.) and their liquid filtrates (*in vitro*). J Med Bioeng 2015a;4:376-80.
- Owaid MN, Raman J, Lakshmanan H, Al-Saeedi SS, Sabaratnam V, Al-Assaffi IA. Mycosynthesis of silver nanoparticles from *Pleurotuscornucopiae* var. *citrinopileatus* and its inhibitory effects against *Candida* sp. Mater Lett 2015d;153:186-90.
- Owaid MN, Al-Saeedi SS, Sabaratnam V, Al-Assaffi IA, Raman J. Growth performance and cultivation of four oyster mushroom species on sawdust and rice bran substrates. J Adv Biotech 2015e;4:424-9.
- Royse DJ. Influence of spawn rate and commercial delayed release nutrient levels on *Pleurotuscornucopiae* (Oyster Mushroom) yield, size, and time to production. Appl Microbiol Biotechnol 2002;58:527-31.
- Sanchez C. Cultivation of *Pleurotus ostreatus* and other edible mushrooms. Mini-review. Appl Microbiol Biotechnol 2009;85:1321-37.
- Singh MP, Singh VK. Yield performance and nutritional analysis of *pleurotuscitrinopileatus* on different agrowastes and vegetable wastes. Proceedings of the 7th International Conference on Mushroom Biology and Mushroom Products, Section: Waste Conversion, Substrates and Casing; 2011. p. 355-392.
- Stamets P, Chilton JS. Mushroom Cultivator: A Practical Guide to Growing Mushrooms at Home. Washington: Agarikon Press; 1983. p. 415.
- Thomas MG, Schumann DR. Income Opportunities in Special Forest Products-Self-Help Suggestions for Rural Entrepreneurs. Washington: Agriculture Information Bulletin AIB, U.S. Department of Agriculture; 1993. p. 139-49.
- UNSD, United Nation Statistics Division. Environmental Indicators Waste, Municipal Waste Treatment. United Nation; 2011.
- Yabraq MM, Elias I, Mando H. Cultivation of Oyster Mushroom *Pleurotus* spp. Aleppo, Syria: General Commission for Scientific Agricultural Research (GCSAR); 2009.2010.p. 22.