INTERNATIONAL JOURNAL OF ENVIRONMENT
Volume-5, Issue-3, June-Aug 2016 ISSN 2091-2854
Received:7 January *Revised:3 June* *Accepted:14 August*

MYCELIAL GROWTH OBSERVATION OF *Pleurotus eryngii* (Higher Basidiomycota) *In Vitro*

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Abstract

Five agro-substrates including date palm fibers (fibrillum), wheat straw, white sawdust and their combinations were investigated to grow *Pleurotus eryngii*. The longer mycelium complete time within bags was 20 days on sawdust (S4), in contrast, the shorter time for mycelium overgrew was completed after 15 days on date palm fiber (S5). In significant (p<0.05), S5 showed the higher growth intensity level (vigorous growth) than other substrates. Thus use of date palm wastes (S5 medium) may be useful for successfully cultivation king oyster mushroom in fa rm.

Keywords: Cellulosic Wastes, Date Palm Fiber, King Oyster Mushroom, Mycelial Completion Time.

Introduction

The king oyster mushroom (*Pleurotus eryngii*) belongs to the genus *Pleurotus*, the family Pleurotaceae in the division Basidiomycota (Kang, 2004). It is called Eryngii as a common name (Reis *et al*., 2012). *P. eryngii* distributes in Asia, Africa and Europe (Kong, 2004). Nutritionally, *P. eryngii* has a highly nutritive value that due to its essential amino acids, low energy, low fat, proteins, carbohydrates (Dundar *et al*., 2008), micro elements (Akyuz and Kirbag, 2010), A, C and E vitamins (Akyuz *et al*., 2011), selenium and ergothioneine (Estrada *et al*., 2009; Estrada and Royse, 2011). Medicinally, it has a potential prebiotic activity because of its glucan and dietary fibers (Synytsya *et al*., 2008), that important to stimulate the growth of colon microorganisms (Synytsya *et al*., 2009), anti-allergy potential (Han *et al*., 2011), antifungal (Wang and Ng, 2004), anti-bacterial, anti-yeast, anti-dermatophyte (Akyuz and Kirbag, 2009; Akyuz *et al*., 2010), antioxidant (Oke and Aslim, 2011; Mishra *et al*., 2013), antitumor activities (Yang *et al*., 2013) and to induce the host immune system (Choi *et al*., 2013).

P. eryngii is commercially cultivates on different plant residues and wastes such as residues of umbrella plant (*Cyperus alternifolius*) (Ohga and Royse, 2004), lentil straw, cotton straw (Kirbag and Akyuz, 2008), rice straw, sawdust (Moonmoon *et al*., 2010; Hassan *et al*., 2010), soybean straw, sugar cane (Hassan *et al*., 2010), soy stalk (Yildirim and Yildiz, 2010), wheat straw, corn stalk, millet straw, bean stalk and cotton stalk (Akyuz *et al*., 2011) and date palm fibers (Owaid *et al*., 2014b). *P. eryngii*is an edible mushroom, can be cultivated on a wide variety of substrates containing lignin and cellulose. The objective of this study is test mycelial growth observation of *P. eryngii* on some local agro-substrates such as date palm fibers, sawdust, wheat straw and their combinations (*in vitro*) to know the ability using these substrates for production of eryngii mushroom in farm.

Materials and Methods

1. Mushroom Strain

King oyster mushroom species *Pleurotus eryngii*is obtained from Mushroom Box Company, Monmouth, UK, in form spawn and sub cultured it on Potato Dextrose Agar

medium at 25 C° for this experiment. Spawn was achieved on millet *Pennisetum americanum* seeds.

2. Collection of cellulosic substrates

In this experiment, using locally agro-residual wastes, available in Hit city, Anbar Province, Iraq, were wheat straw (1-5) cm, sawdust from factories of wood and fibers of Iraqi date palm *Phoenix dactylifera* L., called (Fibrillum), which first chopped into small pieces (5×5) cm and mixed. Five combinations were used in this experiment as shown in table 1. All mixtures were supplemented with 5% rock phosphate based on dry matter.

Table 1. Contents of agricultural mixtures

Agricultural wastes	**Compositions**		
	Wheat straw	**Sawdust**	**Date palm fiber**
S1	100%	-	-
S2	70%	20%	10%
S3	50%	30%	20%
S4	-	100%	-
S5	-	-	100%

3. Mycelial growth observance

After soaking of substrates in tap water, all mixtures were pasteurized using boiling in water for 2 h, cooled, put on clean place to drain out excess water and mixed with five percent rock phosphate powder. Inoculation 4% mushroom spawn (based on wet weight) with1.5 kg substrate was achieved in layers method that packed in polyethylene bags which capacity 30×50 cm then closed. The inoculated bags were transferred into incubation room, darkly incubated at 25 °C and 90% relative humidity for spawn running. Determinations are included time of mycelial growth completion time and growth intensity level (Owaid *et al*., 2015).

4. Statistical Analysis

The data, collected in triplicates, has been expressed by its mean value and standard deviation (SD). The results were subjected to one way analysis of variance (ANOVA) using SAS statistical program for windows (version 9.0, SAS Institute Inc., Cary, NC, USA). The significance of difference was determined according to Duncan's Multiple Range Test (DMRT). P values < 0.05 were considered to be statistically significant.

Results and Discussion

Significantly (p<0.05), type of agricultural substrate effected on mycelium completion time (MCT) and growth intensity level (GIL) (Table 2).The longer MCT within growth bags was 20±0.57 days on sawdust substrate (S4), as a longest time for mycelium growth completion compared with the control (wheat straw alone, S1) which had 18±0.57 days. While the growth completion completed after16±0.57 days and 16±0.00 days on S3 (50% wheat straw, 30% sawdust and 20% date palm fiber) and S2 (70% wheat straw, 20% sawdust and 10% date palm fiber), respectively. In contrast, the short time for mycelium overgrew completed after 15.3±0.33 day on S5(date palm fiber) as a best result, significantly (p<0.05).

From other side, growth intensity level (GIL) was assessed according to three levels (1: Light, 2: Moderate, 3: Vigorous) as mentioned by Owaid et al. (2015). In significant (p<0.05), S1 and S5showed the higher GIL than other substrates at 3rd level (vigorous mycelial growth). S2, S3 and S4observed showed 2nd level (moderate) as a less GIL, Table 2. Generally, these results reflect on lessening time of the production cycle.

Table 2. Status of king oyster mushroom's mycelium on agricultural wastes in polyethylene bags

Agro-wastes	**Mycelium Completion Time MCT (days)**	**Growth Intensity Level (GIL)**
S1	18±0.57^{b}	3^{a}
S2	16±0.00^{c}	2^{b}
S3	16±0.57^{c}	2^{b}

S4	20 ± 0.57^{a}	2^{b}
S5	15.3 ± 0.33^{c}	3^{a}
Mean ± MSD	17.07 ± 0.81	2.4 ± 0.00

Legend: Growth Intensity Level (GIL): 1: Light, 2: Moderate, 3: Vigorous. S1: wheat straw substrate, S2 (70% wheat straw, 20% sawdust and 10% date palm fiber), S3: (50% wheat straw, 30% sawdust and 20% date palm fiber), S4: sawdust, S5: date palm fiber. Values followed by the same superscript letter(s) along each column are not significantly different by Duncan's multiple range test (DMRT) ($p<0.05$). Values in the same column followed by a common superscript letter do not differ significantly ($p>0.05$). MSD: Mean of standard deviation.

Pleurotus eryngii was efficacy in using nutrients from various lignocellulosic wastes in this experiment is based on possession of a potent ligninolytic enzyme system, constituted of lignin peroxidase, manganese peroxidase (Camarero *et al*., 2000), laccase and aryl-alcohol oxidase (Stajic *et al*., 2009), which successfully degrade different agro-substrates as soy stalk using bio-treatment by its mycelia, and using that as a feed for ruminants (Yildirim and Yildiz, 2010). These results are in line with the finding of Kabirifard *et al*. (2012) they reported, this genus grew on date palm leaves faster than wheat stubble. The lower time to overgrow mycelia of *P. Eryngii* on S5 may be return to capability of this fungus for producing enzymes to decompose various aromatic compounds (Hassan, 2011). Also, Hassan *et al*. (2008) induced for using *P. ostreatus* as microbial method for decomposing mixture of date palm leaves with wheat straw, which lead to decrease lignin and phenolic content and increase digestion of dry matter. From other side, kind of substrate affected on speed of mycelial growth and the covered time (Kashangura, 2008).

Intensity of mycelia may be good to raise the productivity and number of fruits (Owaid *et al.,* 2015).The extended time of MCT on S4 and poor mycelial growth attributed to this medium which composed from sawdust alone that unsuitable for oyster mushroom cultivation thereby it must be mixed with other agro-residues (Alheeti, 2013; Alheeti *et al*., 2013; Owaid *et al*., 2014a). Also, Davis and Aegerter (2000) emphasized that sawdust must

be not used alone but in mixtures with various agro-residues because sometimes the sawdust may be reduced oyster mushroom production relatively (Onuoha, 2007). The reason may be that sawdust which produced in wood factories was pretreated with fungicides (Kalpana *et al*., 2011).

Conclusion

Five agro-substrates including: S1 (wheat straw), S4 (sawdust), S5 (date palm fiber) and their combinations S2 (70% wheat straw, 20% sawdust, 10% date palm fiber) and S3 (50% wheat straw, 30% sawdust, 20% date palm fiber) were investigated to grow mycelia of *Pleurotus eryngii*. S5 medium (date palm fiber) is best medium *in vitro* in this work, which showed shorter mycelium complete time 15 days and vigorous mycelial growth compared with other substrates at level 3. That is important to apply cultivation of of *P. eryngii* in farm.

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