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ASSESSMENT DATEPALM FIBERS EXTRACT AGAR WITH OTHER LIGNOCELLULOSE RESIDUES ON MYCELIAL GROWTH OF *PLEUROTUS ERYNGII*

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ABSTRACT

The influence of various culture media on the mycelial growth of *Pleurotus eryngii* (king oyster mushroom) was investigated on five agro-substrate agar media; wheat straw extract Agar (S1EA), 70% wheat straw, 20% white sawdust and 10% date palm fibers extract agar (S2EA), 50% wheat straw, 30% white sawdust and 20% date palm fibers extract agar (S3EA), white sawdust extract agar (S4EA) and date palm fibers extract agar (S5EA) media. The best medium for mycelial growth of *Pleurotus eryngii* was date palm fiber extract agar (S5EA) while less mycelial growth was on white sawdust extract agar (S4EA). In significant difference ($P < 0.05$), the periodic growth after 4 days was 9.4 mm day^{-1} on fibrillum extract agar medium (S5EA), while the lower growth was 6.7 mm day^{-1} on S4EA. The best cumulative growth of mycelia was

9.4 mm day⁻¹ on S5EA medium, while the less growth was 6.3 mm day⁻¹ verified on S4EA, which take more time for covering plate by mycelia.

Keywords: *Pleurotus eryngii*, date palm fibers, white sawdust, wheat straw, mycelial growth rate.

INTRODUCTION

The king oyster mushroom *Pleurotus eryngii* belongs to the genus *Pleurotus*, the family Pleurotaceae, the order Agaricales and the division Basidiomycota (Kang, 2004). And as a local name, it is called Eryngii (Reis *et al.*, 2012). The world distribution of king oyster mushroom placed in Europe, Asia and Africa (Kong, 2004); other varieties were grown naturally as a wild mushroom in Pakistan (Sheret *et al.*, 2010) and in Iraq (Owaid *et al.*, 2014b). *P. eryngii* is commercially cultivated on various raw plant materials, which due to its remarkable flavor, high nutritional value, and numerous medicinal features (Stajic *et al.*, 2009).

Nutritionally, *P. eryngii* used as food supplement that due to dietary fibers and glucans (Synytsya *et al.*, 2008). It has a highly nutritive value that due to its essential amino acids : valine, leucine, isoleucine, threonine, methionine, phenylalanine, lysine and tyrosine, low energy 276.33kcal/100g, chemical compositions: approx.19% proteins, 7.5% fat and 40% carbohydrates on based dried weight (Dundaret *et al.*, 2008), Fe, Zn, Mn and Cu as micro elements (Akyuz and Kirbag, 2010) and A, C and E vitamins (Akyuz *et al.*, 2011).

Medicinally, it has a potential prebiotic activity, because of its polysaccharides as glucans 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-bacteria, anti-yeast, anti-dermatophyte (Akyuz and Kirbag, 2009; Akyuz *et al.*, 2010), antitumor activities (Yang *et al.*, 2013) and to induce the host immune system (Choi *et*

al., 2013). Also, it has antioxidant activity (Oke and Aslim, 2011; Mishra *et al.*, 2013) that revert to its contents of selenium and ergothioneine (Estrada *et al.*, 2009; Estrada and Royse, 2011).

P. eryngiis is an edible mushroom, can be cultivated on a wide variety of substrates containing lignin and cellulose. It was cultivated on various agro-wastes to increase its bioconversion efficiency; such substrate of umbrella plant *Cyperus alternifolius* (Ohga and Royse, 2004), sawdust and rice straw (Moonmoon *et al.*, 2010), wheat straw mixed with corn stalk, millet straw, soybean straw, bean stalk and cotton stalk (Akyuz *et al.*, 2011), lentil straw with wheat and cotton straws (Kirbag and Akyuz, 2008), soy stalk with rice bran (Yildirim and Yildiz, 2010), sawdust, soybean straw, rice straw and sugar cane (Hassan *et al.*, 2010) and spent compost casing materials (Mishra *et al.*, 2013).

The number of date palm trees about 8 millions in Iraq according to Iraqi Central Organization for Statistics (Ismail *et al.*, 2010). In Iraq, the first research on some *Pleurotus* species cultivation was achieved on various agriculture wastes available locally by Hassen (1996); recently, date palm wastes such fibers, stalk and base stalk (Hassan, 2011) and date palm fibers mixed with the other agro wastes were used to determine their effects on yield (in farm) (Alheeti, 2013) and mycelium growth properties (in Petri dish) of *P. ostreatus*, *P. cornucopiae* and *P. salmoneo stramineus* (Owaid *et al.*, 2014a). In other countries, like Iran, date palm leaves (leaflets and rachis) were used for cultivation of *P. ostreatus* (Daneshvar and Heidari, 2008) and *P. Florida* (Kabirifard *et al.*, 2012), in Saudi Arabia, *P. ostreatus* (Alananbeh *et al.*, 2014), in Malaysia, *P. ostreatus*, on oil palm pressed fibers (Tabi, 2008) and *P. sajor-caju* empty on oil palm fruit bunch with other cellulosic wastes (Mohamad *et al.* 2008).

Generally, oyster mushrooms can be cultivated on various agro-wastes such rice straw basal substrate, wheat straw basal substrate, cotton seed hull basal substrate, and wheat straw or rice straw supplemented with different proportions (Yang *et al.*, 2013)

Date palm Fiber which come from the bark surface is called “Fibrillum” which contain 50.6% cellulose, 8.1% hemicelluloses, 31.9% lignin and 6.2% protein (wet weight) (Saadaoui *et al.*, 2013). *P. eryngii* is efficacy in using nutrients from lignocellulose residues 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).

In Iraq, wheat straw is widely used as the main substrate; firstly, for cultivation of white button mushroom *Agaricus bisporus* and secondly for *Pleurotus ostreatus* in limited use. But still no any work has been done to find out the suitability of locally available ligno-cellulosic wastes as date palm wastes for *P. eryngii* cultivation. The objective of this study is test mycelial growth rate of *P. eryngii* using extracts of date palm fibers, white sawdust, wheat straw and their combinations (*In Vitro*) to know the ability using their for production of *eryngii* mushroom in farm especially in winter season, under outdoor conditions, which important achievement.

MATERIALS AND METHODS

1. Strains

King oyster mushroom *Pleurotus eryngii* obtained from Mushroom Box Company, Monmouth, UK, in form spawn and sub cultured it on Potato Dextrose Agar medium (PDA) at 25 °C for this experiment.

2. Agro-wastes

In this experiment, the used locally agro-residual wastes, available in Hit, Iraq, were wheat straw, white sawdust from industry of woods factories and fibers of date palm *Phoenix dactylifera* L., called (fibrillum) to cultivate *P. eryngii*.

3. Preparation of extracts solid media

Five substrates extract agar used in Table I as tested by Owaid *et al.*, (2014a). Firstly, each formula (Table I) was chopped into small pieces and grinded to nearest powder using blender. Seven grams of each powder was put in flask 500 ml, added 250 ml of distilled water, boiled for 20 minutes, filtrated by gauze and completed the volume to 350 ml by distilled water without glucose adding, added agar (1.5%), sterilized using Autoclave at 121 °C and 1.5 psi for 25 minutes and poured into Petri dishes 85 mm. PDA used as control.

4. Determination of mycelial growth rate (MGR)

Five mm disk of 10 days old culture was put in center of plate and incubated at 25 ± 1 °C. The diameter of colonies, the periodic growth of mycelia (mycelial growth rate after 2 and 4 days), the cumulative growth of mycelia and time of overly covering Petri dishes were calculated.

Table I: Contents of agro-substrates extract agar media

Solid media	compositions		
	Wheat straw	White sawdust	Date palm fibers
S1 Extract Agar medium (S1EA)	100%	-	-
S2 Extract Agar medium (S2EA)	70%	20%	10%
S3 Extract Agar medium (S3EA)	50%	30%	20%
S4 Extract Agar medium (S4EA)	-	100%	-
S5 Extract Agar medium (S5EA)	-	-	100%

5. Statistical Analysis

Experimental values are given as means. Statistical significance was determined by One Way ANOVA (no blocking) with three replications. Data were analyzed and graph

was constructed by statistical program, GenStat Discovery Edition computer program version 7 DE3 and Microsoft Excel version 2010. Differences at $P < 0.05$ were considered to be significant.

RESULTS AND DISCUSSION

1. Mycelium growth rate of *Pleurotus eryngii*

The periodic growths after 2 and 4 days and cumulative growth of king oyster mushroom were achieved on six types of culture media, Table II. The best Mycelial Growth Rate (MGR) of *P. eryngii* after 2 days was 9.6 mm day^{-1} on date palm fibers extract agar medium (S5EA), followed by 9.1 mm day^{-1} on medium S3EA which contain 10% date palm fibers. While the lower growth 7.6 mm day^{-1} on white sawdust medium (S4EA) and PDA as control after 2 days. In spite of increasing MGR on S5EA medium but the density of mycelia was low compared with mycelia of PDA because the date palm fiber medium do not contain glucose compared with PDA.

The results of MGR after 4 days were similar to same growth levels after 2 days as shown in Table II. The best significant ($P < 0.05$) periodic growth after 4 days was 9.4 mm day^{-1} on fibrillum extract agar medium (S5EA), followed 8.2 mm day^{-1} by the medium that contain 10% fibrillum (S2EA), whereas, the lower growth was achieved at rate 6.7 mm day^{-1} on S4EA.

The cumulative growth after spreading of mycelia was completed on plate reached to best growth 9.4 mm day^{-1} on S5EA medium, followed by S1EA, S2EA and S3EA media at rate 7 mm day^{-1} . The less growth was 6.3 mm day^{-1} verified on S4EA medium too, that give no differences between periodic and cumulative growths in all media (Table II). But Fig. 1 showed the growth like as stationary phase in bacteria with all media except S4EA medium (declined) and S5EA medium (increased) after 8th day. The last medium was suitability choice for cultivation *P. eryngii* because of the high

nitrogen content (6.2% protein by wet weight) in this lignocellulosic residue (Saadaoui *et al.*, 2013), which make fibrillum uses for growth oyster mushroom strains. These results agree with Owaïd *et al.* (2013) who use this substrate to estimate mycelia growth in same mixtures (Table I) with *P. ostreatus* (grey and white), *P. cornucopia* and *P. salmoneostramineus*, and has similar results for this investigation test.

Table II: Mycelial growth rate of *Pleurotuseryngii* on solid media of substrates extract (mm day⁻¹)

Solid Media	Periodic growth		Cumulative growth
	MGR after 2 days	MGR after 4 days	
PDA	7.6	6.8	5.6
S1EA	8.8	7.9	7.0
S2EA	9.1	8.2	7.0
S3EA	8.3	7.6	7.0
S4EA	7.6	6.7	6.3
S5EA	9.6	9.4	8.5
LSD P< 0.05	1.027	0.49	0.42

MGR: Mycelial Growth Rate. PDA: Potato Dextrose Agar, S1EA: 100% wheat straw extract agar, S2EA: 70% wheat straw, 20% white sawdust and 10% date palm fibers extract agar, S3EA: 50% wheat straw, 30% white sawdust and 20% date palm fibers extract agar, S4EA: 100% white sawdust extract agar, S5EA: 100% date palm fibers extract agar.

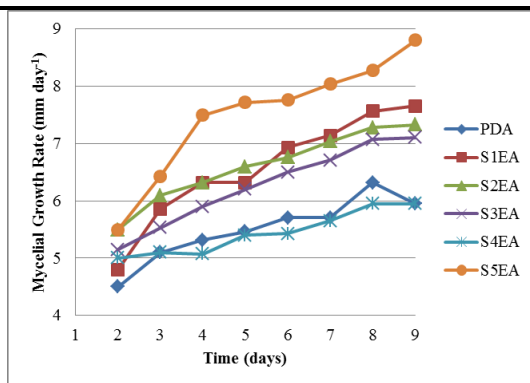


Fig 1:Periodic growth of *P.eryngii* mycelia

PDA: Potato Dextrose Agar, S1EA: 100%wheat straw extract agar, S2EA: 70% wheat straw, 20% white sawdust and 10%date palm fibers extract agar, S3EA: 50%wheat straw, 30% white sawdust and 20%date palm fibers extract agar, S4EA: 100% white sawdust extract agar, S5EA: 100%date palm fibers extract agar.

2. Time of mycelia to overly cover plate

Significantly ($P < 0.05$), the mycelia of *eryngii* mushroom grown overly on S5EA medium after 10 days, Fig. 2, followed by S1EA, S2EA and S3EA media on 12th day. The white sawdust extract agar medium (S4EA) takes more time after 14 days for complete growth over plate. While PDA take 2 weeks for complete in same conditions. Fig.2 showed changeable in time of covering the whole plate according to type of mixture (Owaid *et al.*, 2014a). The longer time was carried out on S4EA medium to cover Petri dish, that agree with results Owaid *et al.* (2014a) on S4EA medium which was less growth with all species of oyster mushrooms.

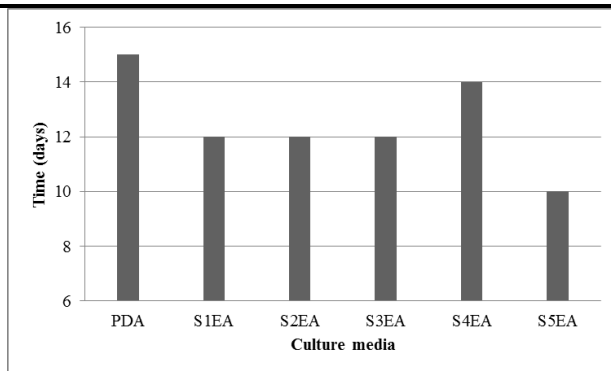


Fig 2: Time of covering Petri dishes by *P.eryngii* mycelia on culture media (days)

(LSD $P < 0.05 = 0.00$), PDA: Potato Dextrose Agar, S1EA: 100% wheat straw extract agar, S2EA: 70% wheat straw, 20% white sawdust and 10% date palm fibers extract agar, S3EA: 50% wheat straw, 30% white sawdust and 20% date palm fibers extract agar, S4EA: 100% white sawdust extract agar, S5EA: 100% date palm fibers extract agar.

Table III : Correlation among number of characteristics of mycelial growth

Correlation	Periodic growth		Cumulative growth	Time of covering plate
	MGR after 2 days	MGR after 4 days		
MGR after 2 days	1.000			
MGR after 4 days	0.735	1.000		
Cumulative growth	0.894	0.793	1.000	
Time of covering plate	-0.954	-0.785	-0.922	1.000

The species of oyster mushroom were differenced in mycelium growth rate, the substrates also affected on speed of mycelial growth and the covering time of whole plate by mycelia (Kashangura, 2008). The mycelium growth rate on date palm fiber extract agar was best medium may be due to cellulose ratio 48.93% (Al-Jabray, 2005).

Hassan *et al.* (2008) induced for using oyster mushroom as microbial method for decomposing mixture of date palm leaves and wheat straw, which lead to decrease lignin and phenolic content and increase digestion the dry matter by its enzymes as bio-treatment.

The solid medium of sawdust extract was decreased the mycelial growth, because of phenolic compounds which inhibit the mycelial growth after treated sawdust by heat (Chang and Quimio, 1982) that lead to decrease the mushroom production in farm (Onuoha, 2007), or revert to treat the wood of factories by fungicides to protect it from decomposed (Kalpana *et al.*, 2011; Ranjini and Padmavathi 2012). As per above, white sawdust medium (S4EA) will lead to decrease mycelial growth.

The periodic and cumulative growth have a positive correlation ($r=0.89$ and 0.79 after 2 and 4 days) as shown in Table III. Whereas a negative correlation between the time overly covering plate in one side and periodic ($r= -0.95$ and -0.78 after 2 and 4 days) and cumulative ($r= -0.92$) growths in other side, that is normal when increased speed of growth lead to take less time to covering plate by mycelia (Owaïd *et al.*, 2014).

CONCLUSION

Five extracts of agro-substrates including date palm fibers (fibrillum), wheat straw, white sawdust and their combinations were investigated to grow *Pleurotus eryngii*. The S5EA medium supported an excellent mycelial growth rate of *P. eryngii* while S4EA medium was observed to support less mycelial growth. That is important to use date palm wastes for successfully cultivation king oyster mushroom in field.

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