

Impact Palm Date Fibers (Fibrillum) and Sawdust extract on Mycelial Growth

Rate of Four Species of *Pleurotus*

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Abstract.

Five extracts of plant substrates including palm date fiber (fibrillum), wheat straw, sawdust and their mixtures were investigated to grow four species of oyster mushrooms: *Pleurotus ostreatus* (grey strain), *Pleurotus ostreatus* (white strain), *Pleurotus cornucopiae* (bright yellow strain) and *Pleurotus salmoneostramineus* (pink stain). In significant difference ($P > 0.05$), the best mycelial growth rate was 14.73 mm day⁻¹ by *P. salmoneostramineus* on solid medium of F1 (Solid medium of wheat straw 100%) followed 14.13 mm day⁻¹ by *Pleurotus ostreatus* (grey) on solid medium of F5 (Solid medium of date palm fiber 100%) after four days from growth. Seven days were less time to cover whole plate by mycelia of *P. ostreatus* (grey strain) and *P. ostreatus* (white strain) on F5 medium.

تأثير مستخلص ألياف النخيل ونشارة الخشب على معدل نمو غزل أربع أنواع من الفطر المحاري *Pleurotus* spp.

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الخلاصة:

الكلمات الدالة:

أختبرت خمسة مستخلصات من المخلفات النباتية المتضمنة: ألياف النخيل وتبن الحنطة ونشارة خشب معامل النجارة وخلانطها (في الأطباق) في تنمية أربع أنواع من الفطر المحاري: السلالة الرمادية *Pleurotus ostreatus* والسلالة البيضاء *Pleurotus ostreatus* والسلالة الصفراء *Pleurotus cornucopiae* والسلالة الوردية *Pleurotus salmoneostramineus*. وتحقق أفضل معدل نمو معنوي ($P > 0.05$) للغزل الفطري بمعدل 14.73 ملم يوم-1 للفطر *P. Salmoneostramineus* على الوسط 1 (مستخلص تبن الحنطة 100%) تلتها السلالة الرمادية *P. ostreatus* بمعدل 14.13 ملم يوم-1 على وسط مستخلص ألياف النخيل لوحده بعد أربعة أيام من النمو. وامتألت الأطباق الحاوية على مستخلص ألياف النخيل بغزل الفطر ذي اللون الرمادي والأبيض *P. ostreatus* بعد سبعة أيام وهي أقل مدة لتغطية الأطباق بغزل الفطر بالكامل.

للمراسلة:

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Introduction

Pleurotus spp. belongs to Basidiomycota, order Agaricales, and the family Pleurotaceae. The oyster mushrooms are the second most important mushrooms in production in the world, accounting for 25% of a total world production of cultivated mushrooms (Chang and Miles, 2004). Oyster mushroom is an edible mushroom, can be cultivated on a wide variety of substrates containing lignin and cellulose. It has nutritional and medicinal properties (Gregoriet *al.*, 2007). *Pleurotus* spp. was cultivated on different agro-wastes such soybean straw, rice straw, paddy straw, coffee pulp, sawdust, cotton wastes, cotton seed hulls, corn cobs waste (Poppe, 2004), bean straw, crushed bagasse, molasses wastes (Ahmed *et al.*, 2009), wheat straw, palm date waste (Kabirifardet *al.*, 2012), handmade paper wastes, cardboard industrial wastes and some their combination (Kulshreshthaet *al.*, 2013) to determine the effect of these agro wastes on yield and other properties. In Iraq, the first research on cultivation of some *Pleurotus* species on various agriculture wastes available locally in Iraq was by Hassen (1996).

The number of date palm tree about 8 millions in Iraq according to Central Organization for Statistics (COS) (Ismail *et al.*, 2010). Fiber which comes from the bark surface called "Fibrillum" which contain 50.6% cellulose, 8.1% hemicelluloses and 31.9% lignin, around 91.2% dry weight and 6.2% protein (wt%)(Saadaoui *et al.*, 2013). Wheat stubble and date palm leaf used to cultivate *P. ostreatus* and *P. florida* in Iran (Kabirifardet *al.*, 2012). The substrates that prepared from date palm wastes have low nitrogen, therefore; added some nutrients such urea and K_2SO_4 to date palm wastes such fibers, stalk and base stalk (Hassan, 2011). Muhamadet *al.* (2008) and Tabi (2008) studied production *Pleurotus sajor-caju* and *Pleurotus ostreatus* on empty palm fruit bunch with other cellulosic wastes. Daneshvar and Heidari (2008) were used date palm leaves and alfalfa to produce oyster mushrooms. *Pleurotus* spp. produced many enzymes when decomposed lignocellulolytic substrates; oxidative enzymes such laccase, manganese peroxidase and lignin peroxidase; and hydrolytic enzymes such cellulases, xylanases and tanases (Luz *et al.*, 2012). The objective of this study is test

mycelial growth rate of four *Pleurotus* spp. by using extracts of date palm fiber, sawdust, wheat straw and their combinations, and then knows the best formula for using it in production this mushroom in farm.

Material and Methods

1. Strains

Four Oyster Mushrooms species were investigated. *Pleurotus ostreatus* (grey oyster), *Pleurotus ostreatus* (white oyster), *Pleurotus cornucopiae* var. *citrinopileatus* (bright yellow oyster) and *Pleurotus salmoneostramineus* (pink oyster) are obtained from MushroomBox Company, Monmouth, UK, in form spawn and sub cultured it on Potato Dextrose Agar (PDA) medium at 25 C° for this experiment.

2. Substrates

In this experiment, by using locally agro-residual wastes available in Heet city, Al-Anbar Province, Iraq were wheat straw (1-5) cm, sawdust from factories of wood and fibers of date palm *Phoenix dactylifera* L., called (Fibrillum), which first chopped into small pieces (5*5) cm and grinded to nearest powder by blender then mixtures prepared as Table 1 with using Potato Dextrose Agar (PDA) as control.

3. Preparation solid media of extracts of substrates

Seven grams of each powder formula of Table 1 was put in flask 500 ml, added 250 ml of distilled water, boiled for 20 minutes, filtrated and completed the volume to 350 ml by distilled water without glucose adding (Hassan, 2011), added agar (1.5%), sterilized by Autoclave at 121 C° for 25 minutes and 1.5 psi and poured into Petri dished 85 mm., PDA used as control (Stamets and Chilton, 1983).

4. Determination mycelial growth rate on solid medium of extract of substrates

Five mm disk of 10 days old culture was put in center of plate and incubated at 25±1 C°. The diameter of colonies were calculated daily (periodic growth of mycelium), time of covering whole plate and mycelium growth rate after 4 days.

5. Statistical Analysis

Experimental values are given as means. Statistical significance was determined by Completely Randomized

Design (CRD) in two variance (Two Ways) analysis (ANOVA) by using GenStat Discovery Edition computer program version 7 DE3 (VSN International Ltd., UK).

Differences at $P > 0.05$ were considered to be significant. The experiments used three replicates.

Table 1 Formula of substrates contents

| Formula | Substrates | | |
|-----------|-------------|---------|------------------------------|
| | Wheat Straw | Sawdust | Date Palm Fibers (Fibrillum) |
| Formula 1 | 100% | - | - |
| Formula 2 | 70% | 20% | 10% |
| Formula 3 | 50% | 30% | 20% |
| Formula 4 | - | 100% | - |
| Formula 5 | - | - | 100% |

Results

Effect of extract of substrates on *Pleurotus* spp. mycelium growth rate on solid medium

The best Mycelial Growth Rate (MGR) was 14.73 mm day⁻¹ for *P. salmoneostramineus* on solid medium of F1 after 4 days from growth followed 14.13 mm day⁻¹ by

Pleurotus ostreatus (grey) on solid medium of F5 with significant difference ($P > 0.05$) (Table 2). The less MGR was 5.63 mm day⁻¹ for *P. cornucopiae* when grew on F4 medium which gave less density of mycelia. In spite of increased MGR on F5 medium but the density of mycelia was low compared with mycelia of F1, F2 and F3 media.

Table 2 Mycelial growth rate of *Pleurotus* spp. on solid media of substrates extract after 4 days from growth (mm day⁻¹)

| Culture Media (F) | <i>Pleurotus</i> spp. (Oyster Mushroom) | | | | Culture Media Average |
|-------------------|---|-----------------------------|-----------------------|------------------------------|-----------------------|
| | <i>P. ostreatus</i> (grey) | <i>P. ostreatus</i> (white) | <i>P. cornucopiae</i> | <i>P. salmoneostramineus</i> | |
| PDA | 9.86 | 11.40 | 8.46 | 8.73 | 9.62 |
| Formula 1 | 12.90 | 12.50 | 9.73 | 14.73 | 12.47 |
| Formula 2 | 11.80 | 11.73 | 8.50 | 10.40 | 10.61 |
| Formula 3 | 11.90 | 11.63 | 8.40 | 9.13 | 10.27 |
| Formula 4 | 10.63 | 8.63 | 5.63 | 8.73 | 8.41 |
| Formula 5 | 14.13 | 13.63 | 10.70 | 10.80 | 12.32 |
| Mushroom Average | 11.87 | 11.58 | 8.57 | 10.42 | - |
| LSD $P > 0.05$ | F= 0.367, | M= 0.300, | F * M= 0.735 | | |

PDA: Potato Dextrose Agar, Formula 1: Solid medium of wheat straw 100%, Formula 2: Solid medium of wheat straw 70%, sawdust 20% and date palm fiber 10%, Formula 3: Solid medium of wheat straw 50%, sawdust 30% and date palm fiber 20%, Formula 4: Solid medium of sawdust 100%, Formula 5: Solid medium of date palm fiber 100%. F: Formula, M: Mushroom.

Time of covering plate by mycelia

Table 4 showed changeable in time of covering the whole plate according to type of mixture. The less time of covering the whole plate by mycelia was 7 days by *P. ostreatus* (grey and white) on F5 medium, followed by *P. salmoneostramineus* on F1 medium, *P. cornucopiae* on

same medium and *P. ostreatus* (white) on PDA in 8 days. The longer time was 15.66 days by mycelia of *P. cornucopiae* on F4 medium to cover Petri dish.

F5 medium take average 8 days with oyster mushrooms, then F1 medium 9 days, while others (PDA, F2, F3 and F4 media) take longer time 10 days. Mycelial growth rate was higher in F5 medium with *P. ostreatus*

(white and grey strains) and *P. cornucopiae*, whereas by *P. salmoneostramineus* showed higher MGR with F1 medium followed F5 medium. F4 medium was less MGR with all species especially with *P. ostreatus* (grey) and *P. cornucopiae*. Table 3 showed negative correlation between time of covering dishes and other properties.

Table 3 Correlation among number of characteristics of mycelium growth

| Correlation | MGR after 4 days | Time of covering plate |
|------------------------|------------------|------------------------|
| MGR after 4 days | 1.000 | |
| Time of covering plate | -0.899 | 1.000 |

Table 4 Time of covering Petri dishes by *Pleurotus* spp. mycelia on solid medium (days)

| Culture Media (F) | <i>Pleurotus</i> spp. (Oyster Mushroom) | | | | Culture Media Average |
|-------------------|---|-----------------------------|-----------------------|------------------------------|-----------------------|
| | <i>P. ostreatus</i> (grey) | <i>P. ostreatus</i> (white) | <i>P. cornucopiae</i> | <i>P. salmoneostramineus</i> | |
| PDA | 9.00 | 8.00 | 12.00 | 9.66 | 9.66 |
| Formula 1 | 8.00 | 8.66 | 10.33 | 8.00 | 8.75 |
| Formula 2 | 9.00 | 9.00 | 12.00 | 10.00 | 10.00 |
| Formula 3 | 9.00 | 9.00 | 12.00 | 11.00 | 10.25 |
| Formula 4 | 9.00 | 11.00 | 15.66 | 12.00 | 11.91 |
| Formula 5 | 7.00 | 7.00 | 9.00 | 9.00 | 8.00 |
| Mushroom | 8.50 | 8.77 | 11.83 | 9.94 | - |
| Average | | | | | |
| LSD P> 0.05 | F= 0.367, | M= 0.300, | F * M= 0.735 | | |

PDA: Potato Dextrose Agar, Formula 1: Solid medium of wheat straw 100%, Formula 2: Solid medium of wheat straw 70%, sawdust 20% and date palm fiber 10%, Formula 3: Solid medium of wheat straw 50%, sawdust

30% and date palm fiber 20%, Formula 4: Solid medium of sawdust 100%, Formula 5: Solid medium of date palm fiber 100%. F: Formula, M: Mushroom.

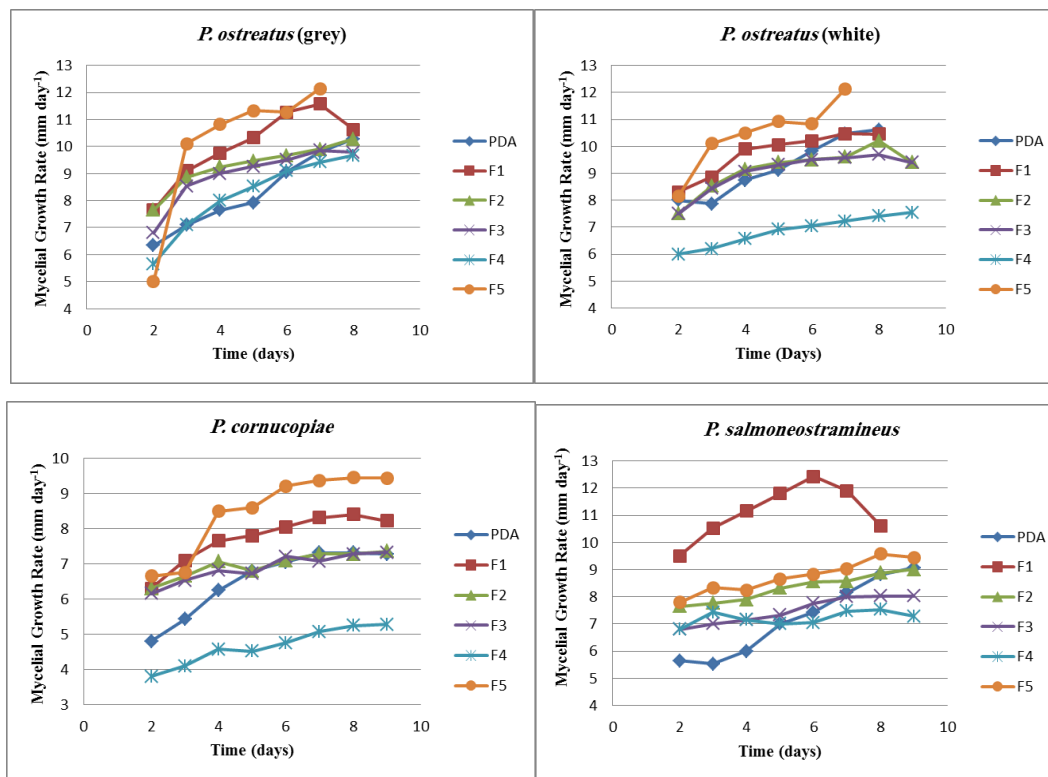


Fig.1 Periodic growth of mycelium

PDA: Potato Dextrose Agar, F1: Solid medium of wheat straw 100%, F2: Solid medium of wheat straw 70%, sawdust 20% and date palm fiber 10%, F3: Solid medium of wheat straw 50%, sawdust 30% and date palm fiber 20%, F4: Solid medium of sawdust 100%, F5: Solid medium of date palm fiber 100%.

Discussion

The species of oyster mushroom were differentiated in mycelium growth rate, the substrates also affected on speed of mycelial growth and time covered of the whole plate by mycelia (Kashangura, 2008). The results agree with Kabirifard *et al.* (2012), that mycelium growth rate of *P. ostreatus* differed according to type of medium to reach 8.1 cm² day⁻¹ on medium of leaves of date palm extract and 9.5 cm² day⁻¹ when grew on wheat straw extract and PDA medium. Mycelial growth rate agrees with Kashangura (2008) who obtained MGR 5 mm day⁻¹ for *P. ostreatus*.

The best mycelium growth rate with date palm fiber extract medium due to cellulose ratio in it around 48.93% low lignin 40.52% (Al-Jabray, 2005). This study agrees

with Hassan (2011) who registers lower time to grow mycelia of *P. ostreatus* in wheat straw substrate, which may be due to enable of *P. ostreatus* to produce enzymes for decomposed the aromatic compounds. Hassan *et al.* (2008) induced for using *P. ostreatus* as microbial method for decomposing mixture of leave of date palm and wheat straw, which lead to decrease lignin and phenolic content and increase digestion the dry matter compared with the digestion by chemical method due to produce many enzymes.

The solid media of 2 and 3 formula gave best mycelia growth compared as others (4 and 5 formula) that composed from one substrate, due to difference content of nutritional value with high density of mycelia, which agree with Hassan (2011). The solid medium of sawdust was decreased the growth that may be decreased the mushroom production in farm (Onuoha, 2007). Whereas Davis and Aegerter (2000) indicated for using sawdust with other substrates as mixture but not alone, because of phenolic compounds which inhibit the mycelia growth after treated sawdust by heat (Chang and Quimio, 1982). Kalpana *et al.*

(2011) and Ranjini and Padmavathi (2012) indicated phenol of wood of a factory or treated the woods by fungicides to protect wood from decomposed that lead to decrease mycelial growth. The density of *Pleurotus* spp. mycelia on PDA bigger compared with solid media of substrates (Fig 4) due to use three extracts without glucose, which agree with Stanley and Nyenke (2011) who referred to high density of mycelia on synthetic media compared with solid media of organic wastes.

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