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PROPERTIES OF FRUIT BODIES OF OYSTER MUSHROOM *(PLEUROTUS OSTREATUS)* CULTIVATED ON SOME LOCAL CELLULOSIC RESIDUES IN IRAQ

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Abstract

In this work, some available local cellulosic substrates namely; S1 (wheat straw) as a control, S2 (70% wheat straw, 20% sawdust and 10% date palm fiber), S3 (50% wheat straw, 30% sawdust and 20% date palm fiber), S4 (sawdust) and S5 (date palm fiber), were used for cultivation white oyster mushroom *Pleurotus ostreatus*. The shorter period for completion of mycelial growth in bags was 12 days on S2 and S3 media, significantly (P <0.05), whereas, the longer time was 15 days on S4 and S5 media compared with the control (S1) at average 13 days. Biological efficiency percent was 26.1% on S2 medium as a higher percent compared with 23.8% on control medium, while S4 medium was unsuitable for cultivation this strain. In significant (P <0.05), S5 medium showed higher diameter of cap (67 mm) compared with the control medium (S1) at value approx. 60 mm.

Key Words: Mycelial growth, date palm wastes, cultivation, agricultural wastes, basidiomycetic.

IRAK'TA İSTİRİDYE MANTARI *(PLEUROTUS OSTREATUS)* ÜRETİMİ İÇİN BAZI YEREL SELÜLOZİK ARTIKLARININ DEĞERLENDİRİLMESİ

Özet

Bu çalışmada, bazı yerel kullanılabilir selülozik ortamlar olarak buğday samanı (kontrol; S1), % 70 buğday samanı, %20 talaş ve %10 hurma lifi (S2), %50 buğday samanı, %30 talaş ve %20 hurma lifi (S3), talaş (S4) ve hurma lifi (S5) beyaz istiridye mantarı *Pleurotus ostreatus* yetiştirilmesi için kullanılmıştır. Misel gelişiminin tamamlanması için en kısa süre S2 ve S3 ortamlarında 12 gün olmuştur (P <0.05). Bu süre, S4 ve S5'te 15 gün iken kontrol olarak kullanılan S1'de 13 gün olmuştur. Biyolojik etkinlik değeri S2'de %26.1 iken kontrol ortamında %23.8 olarak bulunmuştur. S4 ortamı, bu suşun gelişimi için uygun bulunmamıştır. S5 ortamında çap 67 mm ile kontrol ortamındaki (S1) yaklaşık 60 mm'den daha büyüktür (P <0.05)

Anahtar kelimeler: Misel gelişesi, hurma atıkları, tarım, tarımsal atıklar, basidiomycetes.

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INTRODUCTION

Oyster mushroom, *Pleurotus* spp., is one of twenty four edible and medicinal mushrooms produced within special cultivation farms (1). The genus *Pleurotus* spp. belongs to division Basidiomycota in more than seventy species such as *Pleurotus ostreatus* (2). Cultivation of *P. ostreatus* is an easy biotechnological method through bio-recycling of cellulose, hemicellulose and lignin matters, its outcome fresh food highly protein with reducing of ecological pollution (3).

Pleurotus ostreatus is source for trace elements (4), vitamins, essential amino acids (5) and low caloric level (6). *P. ostreatus* has high medicinal activities such as antiviruses, anti-inflammatory (7), anticancer (8), antioxidant (9), anti-parasitic (10), antibacterial (11) and antifungal activities (12).

Pleurotus spp. can be cultivated on different substrates containing cellulose, hemicellulose and lignin such as soybean straw, paddy straw, coffee pulp, cotton wastes, corn cobs wastes (13), bean straw, crushed bagasse, molasses wastes (14), cardboard and paper wastes (15-17) and sawdust (18, 19). Presently, *P. ostreatus* is cultivating on date palm residues such as empty palm fruit bunch (20, 21), date palm leaves (22-24), stalk and base stalk of date palm (25) and date palm fibers (25-27) mixed with other cellulosic wastes. Recently, *Pleurotus florida* is cultivate on date palm residue in Saudi Arabia (28), while cultivation of *P. salmoneostramineus* and *P. cornucopiae* were achieved in Iraq (26).

Date palm wastes represent major quantities of biomass as lignocellulosic matters. These biomasses are mostly including cellulose, hemicelluloses and lignin (29). More quantities of date palm wastes come out within environment from the carelessness or management of date palm trees, which use as substrate instead of burning processes that lead toward pollution of air (30). The aim of this work is use date palm fibers mixed with wheat straw and sawdust, supplemented with phosphate rock as a nutrient to improve agricultural media value, for cultivation of *P. ostreatus*.

MATERIALS AND METHODS

Strains

White oyster mushroom species *Pleurotus ostreatus* is obtained from Mushroom Box Company, Monmouth, UK, in form spawn and sub cultured it on potato dextrose agar (PDA) medium at 25 C° for this experiment. Spawn was achieved on millet plant (*Pennisetum americanum*) seeds as mentioned by Owaid et al. (31).

Substrates

In this test, available local cellulosic wastes, in Iraq, were used namely, wheat straw, sawdust (from factories of wood) and chopped date palm (*Phoenix dactylifera* L.) fibers. Five combinations were used in this experiment; S1 (wheat straw) as a control, S2 (70% wheat straw, 20% sawdust and 10% date palm fiber), S3 (50% wheat straw, 30% sawdust and 20% date palm fiber), S4 (sawdust) and S5 (date palm fibers). All mixtures were supplemented with 5% rock phosphate based on dry matter is brought from State Company For Phosphate in Anbar province.

Substrates preparation and mushroom cultivation

After the soaking these mixtures, pasteurization process was applied using boiling in water for 2 h, cooled, put on clean place to drain out excess water and mixed with five percent of rock phosphate powder. Inoculation was achieved at percent 4% mushroom spawn (based on wet matter) within 1.5 kg substrate in layers method, which packed in polyethylene bags (their capacity 30x50 cm) then closed. The inoculated bags were transferred into incubation room, darkly incubated at 25 °C and 90% relative humidity for spawn running. During the fruiting stage, the bags were opened when the mycelia had covered all the substrates, shocked using 15 °C for 2 days, lighted 12 h/d using fluorescent light and fresh aeration twice a day. Spray watering was also given twice a day with 90% humidity (30).

Determinations are including mycelial completion time (MCT), growth intensity level (GIL), primordial formation time (PFT), flushes numbers, total yield, fruit bodies' number, diameter of pileus, thickness of pileus, length of stipe and diameter of stipe

(30). Biological efficiency also was calculated according to the following equation: Biological efficiency 9(%) = (weight of harvested fresh mushrooms / weight of dried substrate) x 100 (32).

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

In Table 1, status of white oyster mushroom *Pleurotus ostreatus* before fruits emergence was demonstrated, that including mycelial completion time (MCT), growth intensity level (GIL) and primordial formation time (PFT). The shorter time to complete mycelial growth in bags was approx. 12 days, as a best period for MCT, on S2 (70% wheat straw, 20% sawdust and 10% date palm fiber) and S3 (50% wheat straw, 30% sawdust and 20% date palm fiber) media, significantly (P <0.05). Whereas, the longer time was 15 days on S4 (sawdust) and S5 (date palm fiber) media compared with the control (wheat straw) medium at average 13 days. That agrees with results of Owaid et al. (30) who elucidated MCT after 13 days on wheat straw substrate in case blue oyster mushroom *P. ostreatus*, also it took time at average 17 days on sawdust substrate.

The shorter time to overgrow mycelia of *P. ostreatus* on the agro-substrate may be return to capability of this fungus for producing enzymes to decompose lingo-cellulosic substrates (25). Also, type of cellulosic waste affected on speed of mycelial growth and the covering time (MCT) appreciably (30, 33). All those agree with using *P. ostreatus* as a microbial factor to decompose mixture of date palm wastes and wheat straw, which led to increase digestion of dry matter by produced oyster mushroom's enzymes by their mycelia (34).

Growth intensity level (GIL) was assessed according to three levels; (1st level: Light, 2nd level: Moderate, 3rd level: Vigorous) (30). S4 and S5 media had 3rd level (vigorous level), significantly (P <0.05), while the lesser intensity was recorded a light growth (1st level) on S2 medium compared with the 2nd level (moderate intensity) on the control (S1) and S3 media. Table 1, also, showed primordial formation time (PFT) which reached to 41 days on S5 medium in significant (P <0.05) followed by S4 medium at average 24 days. S5 medium led to form fruit bodies; also other substrates, while S4 medium inhibited the fruits emergence (Table 2). The lesser primordial formation time was 6 days on S3 medium compared with 8 and 9 days on the control and S2 media respectively. Conversely, in spite of S2 substrate showed lower growth intensity (GIL) but this mixture had given preferable results for cultivation this fungus (Table 2) and took lesser period for MCT.

Intensity of mycelia may be signaled to reduce the total yield and number of fruits as showed in Tables 2 & 3. The extended time of MCT of S4

Table 1. Properties of oyster mushroom's mycelia before fruiting bodies formation

Type of agro-substrate	Mycelium Completion Time (MCT) days	Growth Intensity Level (GIL)	Primordial Formation Time (PFT) days
S1	12.7^{b}	2^{b}	7.7*dc*
S2	11.7^{c}	1^{c}	9.3^{c}
S3	12.0^{cb}	2^{b}	6.3^{d}
S4	15.0^{a}	3^{a}	24.0^{b}
S5	15.3^{a}	3^{a}	41.3^{a}
Mean ± MSD	13.3 ± 0.44	2.2 ± 0.0	17.7 ± 1.57

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). MSD: Mean of standard deviation.

medium was attributive to the substrate type which composed from sawdust alone that unsuitable for *P. ostreatus* cultivation, thereby it must be mixed with other cellulosic matters. Thus, the shorter time for primordial formation of *P. ostreatus* was on the mixture substrates (30).These results are in agreement with the finding of Owaid et al. (27), they reported that the sawdust extract exhibits poor mycelial growth of *P. ostreatus in vitro*. Davis and Aegerter (35), emphasized that sawdust must be not used alone but in mixtures with various agro-residues. Also, Onuoha (36) mentioned that the sawdust may be reduced *P. ostreatus* production relatively. The reason of this case may be belongs to pretreat sawdust, which made in factories of wood, by fungicides and antibacterial chemicals (37). When MCT increased, GIL increased too; the reason returns to hat MCT has been associated (r= 0.86) with GIL at a positive correlation at probability level <0.01.

Table 2 showed some quantity characteristics for *Pleurotus ostreatus* (white strain) which cultivated on various agricultural wastes supplemented with phosphate rock which has high trace minerals value (38). Number of flushes was 2 flushes on all substrates except S5 medium which given only one flush. In contrast, S4 medium was unsuitable for formation of fruit bodies in spite of highly intensity of mycelial growth (Table 1). In significant (P <0.05), the higher total yield of oyster mushroom was recorded on S2 and S1 media (93.81 and 82.28 g/bag) respectively. S3 medium given crop outreach 50 g/bag while S5 medium recorded lower crop come to 31.84 g/bag. Biological efficiency percent was 26.1% on S2 medium as a higher percent compared with 23.8% on the control, followed by S3 medium at percent 12.9%, while S5 medium showed lower biological efficiency (only 6.4%). In contrast, S4 medium did not show any biological efficiency because no yield achieved on it due to consider this medium was unsuitable for cultivation *P. ostreatus*. S3 medium showed higher fruit bodies weight (11.1g) and lower fruit bodies number (approx. 4 fruits). The higher fruit bodies' number was approx. 12 fruits on S2 medium compared with 11 fruits on S1 medium (control), while, S5 medium showed 5 fruits. Average of fruits weight was changeable from 8 g on S2 and S5 media to 7 g on control medium (S1).

Table 2. Quantity properties of cultivated white oyster mushroom on various cellulosic matters

Type of agro-substrate	No. of Flushes	Flushes weight (yield) (g/bag)	Biological efficiency (%)	Fruits number	Fruit weight (g)
S1	2^{a}	82.28^{ab}	23.89^{a}	11.3^{a}	7.26^{a}
S2	2^{a}	93.81^{a}	26.15^{a}	11.7^{a}	8.03^{a}
S3	2^{a}	50.92^{bc}	12.91^{b}	4.3^{b}	11.13^{a}
S4	0^{b}	0.00^{d}	0.00^{c}	0.0^{c}	ND
S5	1^{a}	31.84^{dc}	6.44b^{c}	5.0^{b}	8.43^{a}
Mean ± MSD	1.4 ± 0.0	51.77 ± 18.04	13.87 ± 4.58	6.4 ± 1.48	8.71 ± 4.01

Legend: 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). MSD: Mean of standard deviation.

Table 3. Quality properties of white oyster mushroom's fruit bodies on various cellulosic matters

Type of agro-substrate	Diameter of pileus mm	Thickness of pileus mm	Length of stipe mm	Diameter of stipe Mm	D/L ratio
S1	60.3b	3.6bc	37.3ab	12.8b	1.6b
S2	44.7c	5.1a	31.3b	11.1b	1.4b
S3	60.6b	4.0b	41.0a	17.1a	1.5b
S4	ND	ND	ND	ND	ND
S5	67.0a	2.9c	33.5ab	9.8b	2.1a
Mean ± MSD	58.1 ± 3.40	3.9 ± 0.44	35.7 ± 4.21	12.7 ± 1.11	1.6 ± 0.21

Legend: 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. D/L ratio: diameter of pileus/length of stipe ratio. 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). MSD: Mean of standard deviation. ND: Not Detected.

Generally, hardwood sawdust substrate alone was unsuitable for cultivation white oyster mushroom *Pleurotus ostreatus*, that due either to phenol content of hardwood sawdust (39) or this wood was pretreated by fungicides in wood processing to protect it from microbial decomposition, which led to prevent fruiting bodies formation in this study (37). Also, Hami (40) reported that softwood sawdust is more suitable than hardwood sawdust for cultivation of *P. ostreatus*. This agrees with some researchers, they referred to use hardwood sawdust of factories in mixtures but no alone (27, 30, 35). That is agreement with the mentioned results by Alheeti et al. (12) and Owaid et al. (30) when they used same cellulosic mixtures for oyster mushroom cultivation. These results indicated to use mixtures from more than one substrate instead of that composed from one substrate (25, 27, 41). The reason of high biological efficiency in case S2 medium return to these combinations which have been formed from more one substrate or to use date palm wastes in these mixtures to improve its quality, which agreed with results of some studies (25, 26).

Quality characteristics of produced white oyster mushroom *Pleurotus ostreatus* did not detect on S4 medium because it was unsuitable to cultivate this mushroom strain, but it was suitable when mixed with other cellulosic wastes such as wheat straw and date palm wastes to improve its properties. Sawdust of wood factories was treated with antifungal and antibacterial chemicals to prevent decomposition of house's doors and furniture that may be lead to inhibit fruit bodies formation afterwards (26).

Quality properties of mushroom had been related with the fruit body size such as determination of pileus (cap) and stipe (stem in plant) (30). Generally, diameter of pileus recorded average 58.1±3.40 mm. S5 medium showed higher diameter 67 mm significantly (P <0.05), whereas S1 and S3 media followed that at value approx. 60 mm. The produced fruits on S2 medium exhibited lower diameter around 45 mm, but it exhibited higher thickness of pileus (5.1 mm), whereas S5 medium had lower thickness (2.9 mm). Diameter of stipe was approx. 10, 11, 13 and 17 mm for fruits which produced on S5, S2, S1 and S3 media respectively. In general, length of stipe was at average 35.7±4.21 whereas the diameter of pileus/length of stipe ratio (D/L ratio) recorded value at average 1.6±0.21, which was important to define the good quality for fruit bodies (Table 3).

Some of the studied properties link together in negative or positive correlations that is demonstrate the reasons of decreasing or increasing some results (30). According to the correlation, total yield has positive correlation (r= 0.60) at probability level <0.01 with average of fruit weight. Also, the last one has positive correlations (r= 0.80), (r= 0.74), (r= 0.70) and (r= 0.69) at probability level <0.01 with diameter of stipe, length of stipe, diameter of pileus and thickness of pileus respectively. All that lead to increase the biological efficiency that links together with total yield in positive correlation (r= 0.99) at P <0.01. That is illustrate increase of fruit size or its weight with line of diameter of pileus/stipe desirable in this field but this increasing in length of fruit stipe is undesirable; all that verified in this work.

CONCLUSION

Five substrates namely; S1 (wheat straw), S2 (70% wheat straw, 20% sawdust and 10% date palm fiber), S3 (50% wheat straw, 30% sawdust and 20% date palm fiber), S4 (sawdust) and S5 (date palm fibers), were used to cultivate white oyster mushroom *Pleurotus ostreatus*. In conclusion, the shorter period for completion of mycelia in bags was 12 days, as a best time on S2 and S3 media, significantly (P <0.05). Biological efficiency percent was 26.1% on S2 medium as a higher percent while S4 medium was unsuitable medium for this mushroom. Diameter of pileus was recorded average 58.1±3.40 mm.

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adreslerinden erişilebilir. Yazarlar, makale göndermeden önce yazım kurallarını tam olarak okumalı ve makalelerini burada verilen kurallara göre hazırlamalıdırlar.