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Cultivation Performance of *Pleurotus salmoneostramineus* Mushroom on Wastes of Date-palm Trunk, *Phoenix dactylifera* L., and Woodworking Sawdust

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Abstract

The objective of this study is to produce *Pleurotus salmoneostramineus* pink oyster mushroom using organic farming from some surplus local organic wastes, viz., the WSF1 formula (70 % wheat straw, 20 % woodworking sawdust, and 10 % date-palm fiber), the WSF2 formula (50 % wheat straw, 30 % woodworking sawdust, and 20 % date-palm fiber), the S formula (woodworking sawdust), the F formula (fibers of date-palm (*Phoenix dactylifera* L.) trunk), and the W formula (wheat straw) as a control. The WSF1 formula gave the best yield and biological efficiency (163.2 g/bag and 45.5 %, respectively) with significant differences ($p < 0.05$), while the S formula test recorded 27.8 g/bags and 6.5 %, respectively. However, F formula showed the highest number, reaching 35 basidiocarps in comparison to the control (wheat straw, 30 basidiocarps), followed by 28 basidiocarps with the WSF1 formula, whereas the S formula showed the lowest number of basidiocarps (6 basidiocarps). However, the WSF1 formula showed the best rate of basidiocarp weight (5.7 g) and diameter of pileus (9.0 cm). Significantly ($p < 0.05$), the WSF1 formula showed a shorter length (11.9 mm), which was considered to be the best value in comparison with the control (19.5 mm). The S formula exhibited a low diameter of pileus (6.6 cm) and a longer stipe (18.6 mm), respectively. In conclusion, WSF1 is considered to be the best formula for the cultivation of this mushroom.

Keywords: Myco-degradation, higher basidiomycetes, pink oyster mushroom, biological efficiency, recycling.

Introduction

The species *Pleurotus salmoneostramineus*, pink oyster mushroom, belongs to the class Basidiomycetes, order Agaricales, and grows naturally in nature [1]. Industrially, *P. salmoneostramineus* can be grown on some agro-substrates containing lignin, cellulose, and hemicellulose, because of its ability to degrade complex organic materials like rice straw and wheat straw [2]. Generally, oyster mushroom is considered to be a natural health food and is used in various industrial processes for food [3]. Also, this mushroom is used to treat different diseases. *P. salmoneostramineus* reduces total cholesterol, low-density lipoprotein (LDL), triglyceride, and phospholipids, and has no adverse effects on total protein, glucose, total bilirubin, urea, Creatinine, uric acid, sodium, potassium, calcium, or enzyme profiles in rats [4]. Additionally, *P. salmoneostramineus* is capable of improving the digestibility of

sugarcane bagasse for ruminant feed [5] because of its enzymes, which biodegrade cellulosic materials [2]. *P. salmoneostramineus* has antioxidant components, such as phenols, flavonoids, and tannins [6]; thus, this species has antifungal activities (*in vitro*) [7], and anticancer and antioxidant characteristics in rats (*in vivo*) [8]. Recently, it has been considered to be a healthy good food [3] because of its compositions from micro and macro minerals, such as Ca, K, Mg, Fe, Zn, Cu, and Mn, and is a good source of fatty acids [9].

P. salmoneostramineus is important in biodegrading lignocellulosic wastes in solid state fermentation; thus, this species has been successfully cultivated on wheat straw [10], rice straw [2], sunflower (*Helianthus annuus*) husks [11], and some mixtures of agro-substrates. In general, only a few studies have been made on the cultivation of this species compared with others. On the other hand, some studies recently evaluated date-palm wastes as an agrosubstrate for cultivating some edible and medicinal mushrooms like *Agaricus bisporus* [12], *Calocybe indica*, *P. sajor caju* [13], *P. florida* [14,15], and *P. ostreatus* [16-21]. Finally, palm fibers mixed with others as substrates have been successfully used with plants in soilless [22].

Recently, many scientific reports have shown that the main use of date-palm wastes is as an energy source in burning processes in rural districts for cooking, heating, and other activities. In date-palm-rich countries like Iraq, Saudi Arabia, Iran, and the United Arab Emirates, date-palm residues and wastes have been used by many researchers as agrosubstrates for the growing and cultivation of some famous edible mushrooms such as *A. bisporus*, *C. indica*, *P. ostreatus*, *P. sajor caju*, *P. eryngii*, *P. florida*, and *P. cornucopiae*. In Iraq, huge amounts of date-palm wastes are an outcome; thus, this study investigates the mycological recycling of this residue to evaluate mycelial growth, total yield, and biological efficiency (BE) of pink oyster mushroom (*P. salmoneostramineus*) on date-palm residue, woodworking sawdust, wheat straw, and their mixtures, supplemented with phosphate rock in a low-cost route. The goal of this work is to myco-recycle date-palm wastes and produce pink oyster mushroom.

Materials and methods

Mushroom spawn

Mycelium of pink oyster mushroom *P. salmoneostramineus* was obtained from the UK (MushroomBox Company). It was subcultured on potato dextrose agar (PDA) at 25±1 °C. Spawn was prepared on seeds of *Pennisetum americanum* millet, as mentioned by Chang and Miles [23].

Formulas of agricultural substrates

Some locally surplus agro-wastes were obtained from Heet district, viz., wheat straw (*Triticum aestivum*), woodworking sawdust, and date-palm fibers (*Phoenix dactylifera* L.); Phosphate rock supplement was obtained from State Company for Phosphate in Anbar province, Iraq. After chopping the substrates, 5 formulas were applied in this experiment. They were the W formula (wheat straw, control), the WSF1 formula (70 % wheat straw, 20 % woodworking sawdust, and 10 % date-palm fiber), the WSF2 formula (50 % wheat straw, 30 % woodworking sawdust, and 20 % date-palm fiber), the S formula (woodworking sawdust), and the F formula (date-palm fibers). All formulas were supplemented with phosphate rock (5 % based on dry matter).

Preparation of formulas and cultivation of pink oyster mushroom

All agricultural formulas were soaked, then boiled in water for 2 h, cooled, drained of excess water, and supplemented with 5 % rock phosphate powder. Inoculation with 4 % spawn of pink oyster mushroom (based on wet matter) used the Layers Method using polyethylene bags with a capacity of 30×50 cm, containing 1500 g of each formula separately [24]. The bags were incubated at 23±2 °C in a dark place for spawn running, as shown in the mushroom cultivation room (**Figure 1**). In the fruiting stage, after full colonization in each bag, it was opened at 16 - 18 °C temperature and approx. 90 % relative humidity (HR), given light 12 h/day using fluorescent light and fresh aeration (O_2), and was spray watered twice a day.

Determinations included the period of full colonization of mycelia, mycelial growth strength, primordial formation period, flushes number, weight rate of each flush, total yield, biological efficiency, number of basidiocarps, weight average of basidiocarp, diameter and thickness of pileus (cap), and length and diameter of stipe (stem) [25]. Biological efficiency and DP/LS ratio were calculated, as per the equations below;

Biological efficiency (BE) = (fresh weight of mushroom/dry weight of substrate/compost)×100 (1)

DP/LS ratio = diameter of pileus/length and stipe (2)

Figure 1 Room for mushroom cultivation.
Legend: W: wheat straw (control); S: woodworking sawdust; F: fibers of the meshed surface of date-palm trunk, called fibrillum. WSF1 formula: (70 % wheat straw, 20 % woodworking sawdust, and 10 % date-palm fiber), WSF2 formula: (50 % wheat straw, 30 % woodworking sawdust, and 20 % date-palm fiber).

Statistical analysis

The data (in triplicate) were analyzed by one-way analysis of variance using an ANOVA table from SAS program for Windows, version 9.0, SAS Institute Inc., USA. The significance of differences was calculated using Duncan's Multiple Range Test (DMRT). A probability value of less than 5 % was considered to be statistically significant.

Results and discussion

Characterization of *P. salmoneostramineus* mycelia in bags before fruiting stage

Some properties of mycelia of pink oyster mushroom *P. salmoneostramineus* were investigated in bags; see **Table 1**. This comprised a period of full colonization of mycelia, mycelial growth strength, and primordial formation, which was used to determine the preferability for selecting agrowastes formulas and the best application for the production of fruiting bodies of the tested mushroom.

The average of the period of full colonization of mycelia in bags reached 17.7±0.27 days; generally. The WSF1 formula (70 % wheat straw, 20 % woodworking sawdust, and 10 % date-palm fiber) had the

best result significantly ($p < 0.05$). The WSF1 test recorded a lower period (14 days) than the W formula (control, wheat straw) test at 15 days. The F (date-palm fiber) and S (woodworking sawdust) formulas had additional time to complete full colonization, reaching 20.6 and 23.0 days respectively, whereas the WSF2 formula (50 % wheat straw, 30 % woodworking sawdust, and 20 % date-palm fiber) did not show any change in the period.

Mycelial growth strength is an important parameter for nutrient availability in the medium. It is estimated by 3 levels: first (poor), second (moderate), and third (vigorous). The WSF1 and S formulas had significant differences ($p < 0.05$) and had the best level recorded (first level), while the control (W), WSF2, and F formulas were estimated at the second.

Significantly, ($p < 0.05$), the primordial formation period took 41 days for the S formula compared with the control (6 days), followed by the F formula (24.6 days), while the WSF1 and WSF2 formulas showed 5.6 and 5 days, respectively.

The differences in sources of carbon led to the speed of complete growth of mycelia, which took a short time in this test. In general, fungi have certain requirements in order to utilize and decay date-palm wastes which contain complex carbohydrates [26]. Using different agricultural substrates in the cultivation of oyster mushroom has an important effect on its growth, quality and quantity properties [25].

Table 1 Characteristics of *Pleurotus salmoneostramineus* mycelia in cultivation bags.

Formula	Period of full colonization of mycelia (days)	Mycelial growth strength	Primordial formation period (days)
WSF1	14.0^{d}	1^{b}	5.6^{c}
WSF2	15.0^{c}	2^{a}	5.0^{c}
S	23.0^{a}	1^{b}	41.0^{a}
F	20.6^{b}	2^{a}	24.6^{b}
W (control)	15.0^{c}	2^{a}	6.0^{c}
Mean ± SE	17.7 ± 0.27	1.5 ± 0.0	17.2 ± 1.01

Legend: Mycelial growth strength: 1st: Light, 2nd: Moderate, 3rd: Vigorous. WSF1 formula: (70 % wheat straw, 20 % woodworking sawdust, and 10 % date-palm fiber), WSF2 formula: (50 % wheat straw, 30 % woodworking sawdust, and 20 % date-palm fiber), S formula: woodworking sawdust, F formula: date-palm fiber, W: wheat straw formula (control). SE: standard error. Means within a column followed by superscript letter(s) are not significantly different ($p < 0.05$) using Duncan's multiple range test (DMRT).

Characteristics of the yield of pink oyster mushroom

Quantity characteristics of *P. salmoneostramineus* cultivated on various substrates are reported in **Table 2** and **Figure 2**. The formula which was composed of only woodworking sawdust had only one flush, while the others gave 3 flushes in the producing cycle. The rate of basidiocarp weight of each flush was recorded at 42.9 ± 2.27 g in this experiment. The WSF1 formula gave us best total yield (163.2 g/bag) significantly ($p < 0.05$), followed by the WSF2 formula (146.3 g/bag), while the F and S formulas were recorded at 90.8 and 27.8 g/bags respectively, compared with the control (W formula), which showed 176.8 g/bag.

The use of these different carbon sources influenced the composition of each formula [27]. Owaid *et al*. [25] reported that date-palm fibers have a positive correlation toward total yield, whereas woodworking sawdust has a negative correlation.

The results of the biological efficiency of *P. salmoneostramineus* corresponded to their total yield results, as shown in **Table 2**. The WSF1 formula was recorded at 45.5 %, while the S formula exhibited

the lowest biological efficiency, reaching 6.5 %, in comparison with the control percentage (51.3 %). The results of the formulas agree with the biological efficiency of a mixture of barley and rice straws (20 %) [28]; also, it is compatible with the results of the biological efficiency of *Pleurotus* spp. (20 - 30 %) on date-palm fibers [19].

The number of *P. salmoneostramineus* basidiocarps has a significant difference ($p < 0.05$). The F formula, which was composed of only date-palm fibers, showed the highest number, reaching 35 basidiocarps, in comparison to the control (W formula, 30 basidiocarps), followed by the WSF1 formula, at a rate of 28 basidiocarps. The lowest number of basidiocarps was 6, recorded in the test with the S formula, which was composed only woodworking sawdust substrate only.

The rate of basidiocarp weight was 5.0±0.43 g generally. The best rate of basidiocarp weight was 5.7 g in the WSF1 formula, followed by 5.5 g in the WSF2 formula, then decreased significantly ($p < 0.05$) to 4.7 g with basidiocarps in the S formula. The F formula test recorded 2.6 g, the lowest rate in comparison with the control (5.9 g), as per **Table 2**.

These results agree with some of the results of works which mention that woodworking sawdust is improper for the cultivation of oyster mushroom [21]; these works also reported that the extract of this sawdust shows poor growth of mycelia of oyster mushroom. Moreover, Onuoha [29] reported that this sawdust might relatively decrease the production of oyster mushroom. The main reason for this issue is related to pretreatment by fungicides in factories of wood production [30]; therefore, this sawdust would not be used alone in the cultivation of oyster mushrooms, but mixed with other agricultural wastes and residues [31].

Oyster mushroom is able to mycodegrade the complex carbohydrates of date-palm fibers [21]. The formula WSF1 (containing 10 % of date-palm fibers) is considered to be the best formula, with the test recording the best results in comparison with the formula WSF2 (containing 20 % date-palm fibers) and other formulas which were composed of one carbon source. The results of this study are compatible with the results of *P. ostreatus*, which was carried out by Alananbeh *et al*. [20]. The total yield of oyster mushroom depended on the kind of straw which was used as agrosubstrates [32]. In solid-state fermentation, oyster mushroom is able to mycodegrade lignocellulosic compounds into protein-rich food [24]. *P. salmoneostramineus* is able to secrete lignocellulolytic (phenol oxidase) enzymes like laccase and manganese peroxidase, which are used in the biodegradation of phenolic and polycyclic aromatic compounds [33].

Table 2 Quantity characteristics of *P. salmoneostramineus* yield on different agricultural formulas.

Formula	Flushes number	Weight of each flush (g)	Total yield (g/bag)	Biological efficiency (%)	Number of basidiocarps	Rate of basidiocarp weight (g)
WSF1	3^{a}	54.3^{b}	163.2^{b}	45.5^{b}	28^{b}	5.7^{a}
WSF2	3^{a}	48.8^{c}	146.3^{c}	37.1^{c}	27^{b}	5.5^{ab}
S	1^{b}	27.8^{d}	27.8^{e}	6.5^{e}	6^{c}	4.7^{b}
F	3^{a}	30.3^{d}	90.8^{d}	18.4^{d}	35^{a}	2.6^{c}
W (control)	3^{a}	58.9^{a}	176.8^{a}	51.3^{a}	30^{b}	5.9^{a}
Mean±SE	2.5±0.00	42.9 ±2. 27	117.0 ±6.66	30.3±1.92	24 ±1.57	5.0 ±0.43

Legend: WSF1 formula: (70 % wheat straw, 20 % woodworking sawdust, and 10 % date-palm fiber), WSF2 formula: (50 % wheat straw, 30 % woodworking sawdust, and 20 % date-palm fiber), S formula: woodworking sawdust, F formula: date-palm fiber, W: wheat straw formula (control). SE: standard error. Means within a column followed by superscript letter(s) are not significantly different ($p < 0.05$) using Duncan's multiple range test (DMRT).

Figure 2 Formation of *P. salmoneostramineus* basidiocarps on agrosubstrates.
Legend: F formula: date-palm fibers, W formula: wheat straw (control), and WSF1 formula (70 % wheat straw, 20 % woodworking sawdust, and 10 % date-palm fiber).

Characteristics of basidiocarps of pink oyster mushroom

Quality characteristics of *Pleurotus* spp. have been related to fruiting bodies size, such as thickness and diameter of pileus, length and diameter of stipe, and the DP/LS ratio (ratio of the diameter of pileus to the length of stipe) [25]. The size of the fruiting body is an important parameter to determine the quality of *P. salmoneostramineus* pink oyster mushroom, as shown in **Table 3**. The diameter of pilei changed and the recorded rate was 7.6±2.44 cm, as per **Table 3**. The WSF1 formula exhibited (9.0 cm), the best diameter of pileus, followed by 8.6 cm in the WSF2 formula compared with the control (W formula) (8.8 cm). The S and F formulas had lower diameters, reaching 6.6 and 5.5 cm, respectively. The thickness of pileus ranged from 1 to 3 mm. The WSF2 formula had the best value, 3.5 mm, compared with the control, 3.4 mm. The WSF1, S, and F formulas recorded thickness of pileus between 2.8 - 3.0 mm.

From another side, the characteristics of mushroom stipe (stem) include the length of stipe and diameter of stipe. The S formula test recorded a longer stipe (18.6 mm), followed by the WSF2 and F formulas (16.3 and 14.3 mm) respectively. Significantly ($p < 0.05$), the WSF1 formula test recorded a shorter length (11.9 mm), which was considered the best value in comparison with the control (19.5 mm). The biggest diameter of stipe was 8.3 mm in fruits using the WSF1 formula, followed by 7.4, 7.1, and 5.8 mm by the WSF2, W, and S formulas, respectively, but the F formula gave the lowest value (4.3 mm). The DP/LS ratio of the WSF1 formula reached 7.9, compared with 4.5 in control, as per **Table 3**.

The average of the DP/LS ratio is similar to the results of Owaid *et al.* [21], whereas the woodworking sawdust formula (S) exhibited a poor quality of oyster mushroom when used alone in the cultivation. This is compatible with the results of Davis and Aegerter [31], who recorded the use of woodworking sawdust with other raw agricultural wastes and residues in case cultivation performance of edible mushroom. The results of this study agree with many other studies which used date-palm wastes with other agrowastes to cultivate oyster mushroom [14-21,34]. The results of Khan *et al*. [34] showed that treatment of 25 % date palm leaves plus 75 % wheat straw and cotton waste recorded minimum days for spawn running, pinhead formation, and maximum number of fruiting bodies. Conversely, treatment of 100 % date palm leaves exhibited maximum days for the previous determinations, and minimum numbers of fruiting bodies [34].

Table 3 Quality characteristics of *P. salmoneostramineus* basidiocarps in various agricultural formulas.

Formula	Diameter of pileus (cm)	Thickness of pileus mm	Length of stipe (mm)	Diameter of stipe (mm)	DP/LS ratio
WSF1	9.0^{a}	3.0^{b}	11.4^{c}	8.3^{a}	7.9^{a}
WSF2	8.6^{a}	3.5^{a}	16.3^{b}	7.4^{ab}	5.3^{b}
S	6.6^{b}	2.8^{b}	18.6^{a}	5.8^{bc}	3.6^{d}
F	5.5^{c}	2.9^{b}	14.3^{b}	4.3^{c}	3.8^{d}
W (control)	8.8^{a}	3.4^{a}	19.5^{a}	7.1^{ab}	4.5^{c}
Mean ± SE	7.6 ±2.44	3.1 ± 0.19	15.8 ± 1.22	6.5 ± 0.82	5.0 ± 0.34

Legend: DP/LS ratio: ratio of diameter of pileus to length of stipe. WSF1 formula: (70 % wheat straw, 20 % woodworking sawdust, and 10 % date-palm fiber), WSF2 formula: (50 % wheat straw, 30 % woodworking sawdust, and 20 % date-palm fiber), S formula: woodworking sawdust, F formula: date-palm fiber, W: wheat straw formula (control). SE: standard error. Means within a column followed by superscript letter(s) are not significantly different ($p < 0.05$) using Duncan's multiple range test (DMRT).

Conclusions

The study investigates mycological recycling of date-palm residue by *P. salmoneostramineus* cultivation. In this study, mycelial growth, total yield, and BE of *P. salmoneostramineus* grown on date-palm residue, woodworking sawdust, wheat straw, and their mixtures, supplemented with phosphate, were evaluated. Five surplus organic substrates, consisting of wheat straw, woodworking sawdust, and date-palm fibers, were used to cultivate *P. salmoneostramineus* pink oyster mushroom. Significantly ($p < 0.05$), the WSF1 formula test recorded a higher total yield and biological efficiency, reaching 163.2 g/bag and 45.5 %, respectively. The F formula showed the highest number, reaching 35 basidiocarps, while the best rate of basidiocarp weight was 5.7 g in the WSF1 formula. The WSF1 formula exhibited the best diameter of pileus and length of stipe (9.0 cm and 11.9 mm) respectively. Finally, date-palm fiber is an important source in the cultivation of pink oyster mushroom (*P. salmoneostramineus*), but woodworking sawdust with other raw agricultural wastes and residues should be used in the case of cultivation performance of pink oyster mushroom. In conclusion, WSF1 is considered to be the best formula for the cultivation of this mushroom.

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