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INFORMATION PROCESSING IN AGRICULTURE 4 (2017) 78–82

journal homepage: www.elsevier.com/locate/inpa

# Applicable properties of the bio-fertilizer spent mushroom substrate in organic systems as a byproduct from the cultivation of *Pleurotus* spp

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ARTICLE INFO

*Article history:*
Received 14 October 2016
Received in revised form
27 December 2016
Accepted 10 January 2017
Available online 2 February 2017

*Keywords:*
C:N ratio
Nitrogen
Organic agriculture
pH
SMS

ABSTRACT

The spent mushroom substrate (SMS) is a byproduct of cultivation of oyster mushroom (*Pleurotus* spp.) and represents the composted substrate that remains after completion the harvested crop. This study mentioned the role of some effective date palm wastes in improving spent mushroom substrate properties which containing fibers of date palm *Phoenix dactylifera* L. (Fibrillum), mixed with white sawdust and wheat straw in three formulas. These mixtures of SMS namely, SMS1 (wheat straw), SMS2 (wheat straw 70%, sawdust 20% and date palm fiber 10%) and SMS3 (wheat straw 50%, sawdust 30% and date palm fiber 20%) were obtained from locally mushroom farm in western Iraq and sent to determine some properties such as moisture content, dry matter, EC, pH, ash, carbon, nitrogen, protein contents and C:N ratio. Generally, determinations of Hydrogen ion concentration (pH) for SMS extracts had acidic value at average 5.06. The higher EC was 3.30 ms/cm for SMS1-P. ostreatus (white), while the lower value reached to 1.13 ms/cm for SMS3 of same species. The higher nitrogen content was 9.98 g/kg for SMS3-*P. ostreatus* (white), SMS1 of *Pleurotus salmoneostramineus* and SMS2-*P. ostreatus* (white), while, SMS3-*P. salmoneostramineus* had lower nitrogen content (6.65 g/kg). The higher C:N ratio was reported with SMS3 of *P. salmoneostramineus* at value 35.36, while SMS2-*P. ostreatus* (grey) had ratio 22.03, significantly ($p < 0.05$). Overall, these SMS was suitable as a natural fertilizer and soil amender in agriculture and horticulture fields.



## 1. Introduction

The genus of oyster mushroom *Pleurotus* spp. belongs to Basidiomycota division [1]. Some oyster mushrooms species found in eco-system on dead trunks in gardens wildly [2]. Cultivation of oyster mushroom is a biotechnological process for lignocellulosic wastes recycling. This current process has two targets; the production of fresh food and the reduction of the environmental pollutants [3]. Although some physical and chemical factors are affecting on bioconversion efficiency of *Pleurotus* spp. such as carbon and nitrogen sources [4], but this

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Peer review under responsibility of China Agricultural University.
http://dx.doi.org/10.1016/j.inpa.2017.01.001
2214-3173 

fungus is an important to bio-convert cellulosic matter to a rich protein food simply [5].

In mature green plants, the cellulosic matter has 40–50% of cell wall composition, which is a useful source for macro-fungi, especially Basidiomycetes because of their ability to release a high amount of exoenzymes, then huge biomass is formed [6]. From another side, lignin has a second level of carbon sources after cellulose, and the soil microbes (fungi in the first level and bacteria in the second level) decompose and use it as an energy and carbon source, significantly [7].

Fungi are termed as suitable microorganisms for solid state fermentation (SSF) according to the theoretical concept of water activity, whereas bacteria have been considered unsuitable [8]. SSF was used to decompose cellulose and lignin of agro-wastes for mushroom cultivation, animal feeding and enzymes production [9]. In many countries, the spent mushroom substrates (SMS) remaining after mushrooms harvesting are often discarded as wastes [10]. However, the results indicate the viability of reusing SMS with coconut fibers (CF) pith as an ingredient of casing material for mushroom cultivation [11]. Siddhant and Ayodhya [12] used SMS of three oyster mushrooms as fertilizer for growing the plant *Spinacea oleracea*. Sendi et al. [13] used it as a media replacement for peat moss to produce Kai-lan/Kale (*Brassica oleracea* var. *alboglabra*) in Malaysia. While Ashrafi et al. [14] reused SMS for the cultivation of *Pleurotus ostreatus* and *Pleurotus florida*. Also, SMS was used to tomato *Lycopersicon esculentum* Mill. seedling production [15].

*Pleurotus* spp can be cultivated on a wide variety of cellulosic substrates such as cotton wastes, corn cobs wastes [16], bean straw, crushed bagasse, molasses wastes [17], coffee husks [18], paper wastes, industrial cardboard wastes [19,20], trees sawdust [21] and rice straw [22]. But *Pleurotus* spp. had no any SMS quality assessing when grown on some date palm wastes such empty palm fruit bunch [23,24], date palm leaves [25,26] and other date palm residues [27] such as fibers, stalk and base stalk [28–30] with other cellulosic wastes.

This experiment must be put in the field of sustainable development on based wastes hierarchy. The importance of the current study is due to determine properties of spent mushroom substrate containing date palm fibers mixed with other cellulosic matters. Thus, the present study seeks to access the optimal condition of SMS of *Pleurotus* spp. namely, *P. ostreatus* (grey oyster), *P. ostreatus* (white oyster), *Pleurotus cornucopiae* var. *citrinopileatus* (bright yellow oyster) and *Pleurotus salmoneostramineus* (pink oyster) for vegetation or cultivation another mushroom such *Agaricus bisporus*.

## 2. Material and methods

### 2.1. Agro-substrates

In this experiment, using locally agro-residual wastes, available in University of Anbar from Hit city in Iraq, wheat straw, hardwood sawdust (from factories of wood) and fibers of date palm *Phoenix dactylifera* L. Three combinations were used in this experiment; S1 (wheat straw), as a control, S2 (70% wheat straw, 20% white sawdust and 10% date palm fiber) and S3 (50% wheat straw, 30% white sawdust and 20% date palm fiber). All mixtures were supplemented with 5% phosphate rock, based on dry matter, which brought from State Company For Phosphate in Anbar.

### 2.2. Oyster mushroom strains

Twelve SMS of Oyster mushroom (*P. ostreatus* (grey), *P. ostreatus* (white), *P. cornucopiae* var. *citrinopileatus* (bright yellow) and *P. salmoneostramineus* (pink)) were obtained from University of Anbar.

### 2.3. Determination of SMS properties

After 30 days from mushroom harvesting, moisture content, ash and carbon content were determined by the method of Page [31] nitrogen content and proteins according to this equation: proteins% = Nitrogen% $\times$ 6.25 [32]. Compacting factor was calculated according to equation (density of wet SMS - the density of dry SMS) [33]. EC (electrical conductivity) and pH of SMS extracts were determined in distilled water at a ratio (1:10) according to Liu and Price [34].

### 2.4. Statistical analysis

Experimental values are given as means. Statistical significance was determined by two variances (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 were applied at three replicates.

## 3. Results and discussion

### 3.1. Physical properties of agro-substrates

Results of physical conditions of agro-substrates before oyster mushroom cultivation were appeared in Table 1. Wheat straw substrate (S1) showed higher moisture content reached to 77.04%, followed S2 substrate (70% wheat straw, 20% white sawdust and 10% date palm fiber) at percentage 76.08% then declined significantly ($p < 0.05$) to 73.70% with S3 substrate (50% wheat straw, 30% white sawdust and 20% date palm fiber). The last medium exhibited higher significant ($p < 0.05$) dry weight 26.30%, followed 23.92% and 22.96% by S2 and S1 substrates, respectively. From another side, pH values of the substrate have lightly acidity (6.96–6.40). Total salt in substrates was determinate using EC criterion/test, which recorded 2.33 ms/cm for S1 and S2 substrates then touch bottom to 1.30 ms/cm for S3 substrate.

### 3.2. Chemical properties of agro-substrates

The higher carbon content was achieved on the S3 substrate at value 244.33 g/kg significantly ($p < 0.05$), while S1 and S2 substrates decreased to 243.33 g/kg and 239.00 g/kg, respectively. Nitrogen content was 7.71 g/kg for S2 substrate in significant ($p < 0.05$), but that declined to 6.65 g/kg with the others. C:N ratio decreased to 34.06 with S2 substrate then

**Table 1 – Physical and chemical properties of agro-substrates before oyster mushroom cultivation.**

| Futures | S1 | S2 | S3 | LSD ($p < 0.05$) |
|---|---|---|---|---|
| Moisture content % | 77.04 | 76.08 | 73.70 | 1.669 |
| Dry matter % | 22.96 | 23.92 | 26.30 | 1.669 |
| pH | 6.96 | 6.63 | 6.40 | 0.148 |
| EC (ms/cm) | 2.33 | 2.33 | 1.30 | 0.358 |
| Carbon g/kg | 239.00 | 243.33 | 244.33 | 1.489 |
| Nitrogen g/kg | 6.65 | 7.71 | 6.65 | 0.541 |
| C:N Ratio | 38.50 | 34.06 | 40.20 | 0.176 |
| Ash % | 12.27 | 10.63 | 10.23 | 0.621 |

S1: a substrate formed from wheat straw, S2: a substrate formed from 70% wheat straw, 20% sawdust and 10% date palm fibers, S3: a substrate formed from 50% wheat straw, 30% sawdust and 20% date palm fibers.

increased significantly ($p < 0.05$) to 38.50 and 40.20 for S1 and S3 substrates respectively. Also, table 1 showed ash percentage around 12.27% for S1, but S2 and S3 substrates had ash content at percent 10.63% and 10.23% respectively. These results were attributed to use a mixture from more than one substrate instead of which composed from one [28].

### 3.3. *Physical properties of SMS*

According to Table 2, type of carbon source had been affected on the loss of mixture weight significantly ($p < 0.05$). The high miss in weight was 38.52% for SMS2-G, followed by SMS2-W at 32.36%, while lower lose 5.78% exhibited on SMS1-P then 7.24% and 8.59% by SMS3-Y and SMS2-Y respectively.

Table 2 showed results of a compacting factor of agro-substrates after oyster mushrooms cultivation which reached to 0.17 and 0.16 on SMS2-Y and SMS3-Y but declined to factor 0.13 for SMS3-P significantly ($p < 0.05$). While SMS2-G had lesser compacting factor (0.05), followed 0.9 by SMS2-W and SMS2-P. The compacting factor raised when the density of SMS raised too compared with agro-substrates before mushroom cultivation, which leads to bad growth condition reflected on mushroom mycelium growth. This factor depended on the type of substrates and variety of fungi basically.

Determination of Hydrogen ion concentration (pH) for extracts of SMS was acidic at average 5.06. The higher pH was reported 5.73 and 5.70 for extracts of SMS2-Y and SMS3-Y. SMS2-G had lesser pH (4.70), followed 4.77 for SMS1-Y.

From comparative results of pH before (agro-substrates) and after oyster mushrooms cultivation (SMS), that were similar to findings of Ahmed [35] and Danai et al. [36], they reported producing *P. ostreatus* on various wheat straws at a pH value between 5.80 and 5.90 then declined in SMS to 4.62–4.82. That due to increasing organic acids from phosphate rock which reduced the pH value by taking place solubility phosphate compounds [37] or byproducts/metabolites of mushroom [19]. Also, *In vitro*, Sobal et al. [38] referred to the pH value of PDA between (6.8 and 5.8) after growth of *P. ostreatus*. Rawte and Diwan [39] reported that PDB (pH = 7) had final pH value around 5.27–4.59 after nine days from oyster mushroom inoculation.

SMS1-W showed the higher EC 3.30 ms/cm, while the lower value reached to 1.13 ms/cm for SMS3-W; these observed values were in the range considered optimal for edible mushroom and plants production [40]. The low EC made

**Table 2 – The evaluation of spent mushroom substrates.**

| Type of SMS | Losing weight (%) | Compacting factor | pH | EC (ms/cm) | Carbon content (g/kg) | Protein (%) | Nitrogen (g/kg) | C:N ratio | Ash (%) |
|---|---|---|---|---|---|---|---|---|---|
| SMS1-G | 17.47 | 0.10 | 4.93 | 2.46 | 216.33 | 5.41 | 8.65 | 25.03 | 20.50 |
| SMS2-G | 38.52 | 0.05 | 4.70 | 2.10 | 220.33 | 6.24 | 9.98 | 22.03 | 19.00 |
| SMS3-G | 20.42 | 0.11 | 4.80 | 1.93 | 224.33 | 5.82 | 9.31 | 24.13 | 17.50 |
| SMS1-W | 19.49 | 0.10 | 5.23 | 3.30 | 227.33 | 6.24 | 9.98 | 22.73 | 16.50 |
| SMS2-W | 32.36 | 0.09 | 4.94 | 1.93 | 234.00 | 5.41 | 8.65 | 27.10 | 14.00 |
| SMS3-W | 15.75 | 0.12 | 4.83 | 1.13 | 238.00 | 6.24 | 9.98 | 23.80 | 12.50 |
| SMS1-Y | 11.38 | 0.12 | 4.77 | 3.07 | 221.67 | 4.58 | 7.32 | 30.30 | 18.50 |
| SMS2-Y | 8.59 | 0.17 | 5.73 | 2.43 | 214.67 | 4.99 | 7.98 | 26.90 | 21.00 |
| SMS3-Y | 7.24 | 0.16 | 5.70 | 1.73 | 234.00 | 5.19 | 8.31 | 28.16 | 14.00 |
| SMS1-P | 5.78 | 0.11 | 5.03 | 2.26 | 228.67 | 6.24 | 9.98 | 22.86 | 16.00 |
| SMS2-P | 16.60 | 0.09 | 5.03 | 1.87 | 237.00 | 4.58 | 7.32 | 32.40 | 13.00 |
| SMS3-P | 14.52 | 0.13 | 5.03 | 1.87 | 235.33 | 4.16 | 6.65 | 35.36 | 13.50 |
| LSD $p < 0.05$ | 0.990 | 0.027 | 0.084 | 0.584 | 1.895 | 0.420 | 0.420 | 0.462 | 1.418 |

SMS1: spent mushroom substrate composed from wheat straw. SMS2: SMS composed from 70% wheat straw, 20% sawdust and 10% date palm fibers. SMS3: SMS composed from 50% wheat straw, 30% sawdust and 20% date palm fibers. SMS1-G: SMS1 of *P. ostreatus* (grey). SMS2-G: SMS of *P. ostreatus* (grey). SMS3-G: SMS of *P. ostreatus* (grey). SMS1-W: SMS1 of *P. ostreatus* (white). SMS2-W: SMS2 of *P. ostreatus* (white). SMS3-W: SMS3 of *P. ostreatus* (white).

excellent agronomic behavior [11]. This researcher mentioned to use the washing to reduce the EC of SMS, there is the possibility of mixing it with other substrates with a low EC to increase its re-use potential.

### 3.4. *Chemical properties of SMS*

Results of carbon content of SMS showed in Table 2, which reported the influence of the cellulosic composition of each SMS type on carbon level. The higher content was 238 g/kg for SMS3-W; conversely, lower content reached near 215 g/kg for SMS2-Y. All these results of SMS lesser than results of substrates before oyster mushrooms cultivation related with releasing $CO_2$ during the process of production by fungal exo-enzymes [41].

C:N ratio is very important in consideration of their role in mushroom growth. The treatments of this experiment recorded higher nitrogen content of SMS at average 9.98 g/kg for SMS3-W, SMS1-P, and SMS2-W. SMS3-P had lower content 6.65 g/kg. Treatments of this experiment reported higher Percentage of the protein content of SMS at average 6.24% in SMS3-W, SMS1-P, and SMS2-W respectively. That is similar to which reported (7.88%) by Foluke et al. [42]. While SMS3-P had lower content 4.16%; the higher C:N ratio was achieved on SMS3-P at value 35.36, while SMS2-G had ratio 22.03, significantly ($p < 0.05$). These results are in agreement with the findings of Ashrafi et al. [14] who referred to that the optimum C/N ratio for oyster mushroom *P. ostreatus* was 35.2. A high C/N ratio could enhance the digestibility of the lignocellulose content, followed by high availability of cellulose materials as mushroom nutrients [4].

These results agreed with numerous studies. The reason of increasing nitrogen and protein in SMS compared with agro-substrates before the cultivation, related with mushroom mycelium growth that led to decompose of the cellulosic matter then released $CO_2$ by fungal enzymes, that contributed to raising nitrogen content opposite the low carbon content [41,35]. Finally, in some cases, depletion of nutrients occurred in the substrate due to the subsequent utilization of nutrients by mushroom mycelium [14].

Ash content of SMS reached to percentage 21% as a lower level in case SMS2-Y, while 12.5% was reported for SMS3-W, that nearest to results of Foluke et al. [42], who reported ash percentage 9.92%. Results of ash content agreed with findings of Danai et al. [36], who referred to produce *P. ostreatus* on various types of wheat straws, with ash content approx. 11.68–19.61% then increased to 18.80–21.25% with SMS after the production cycle. In this experiment, firstly, the ash of media was calculated without any amendment. But, adding of rock phosphate to substrate during preparing substrates processes led to raising ash content after the production cycle because of high chemical value in this fertilizer [43], which reflected on the high value of ash in SMS.

## 4. Conclusion

Oyster mushroom *Pleurotus* spp. has been the ability of agro-wastes bio conversation and declining lignin content. The experiment was carried out to study the suitability of spent mushroom substrate (SMS) for the production of edible plants or edible mushrooms. In a few words, the material (SMS) can be integrated using new formulations and methodologies with the added advantages of lowering production cost and decreasing the environmental impact of its ever-growing accumulation. For bio-organic applications, SMS used as a type of bio-fertilization, due to the formula of carbon sources and rock phosphate used.

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