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Effect of Substrate Type, Different Levels of Nitrogen and Manganese on Growth and Development of Oyster Mushroom (*Pleurotus florida*)

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ABSTRACT

An experiment was conducted to increase oyster mushroom production and to evaluate the effect of different treatments on the quantitative and qualitative growth of *Pleurotus florida*. Two substrates (sugarcane bagasse and wheat straw) were tested, supplemented with different levels of N (0, 500, 750 µg/g), and Mn (0, 100, 200 µg/g). Different levels of nitrogen and manganese significantly affected mycelia lineal growth rate, spawn running, pinhead and fruit body formation, protein content, yield and biological efficiency but there was no significant effect on ash, dry matter and manganese content of fruit bodies. Substrate type had a significantly effect on all characters with the exception of manganese content of the fruit body. The most positive effect of N and Mn on measured characters was observed at 750 and 100 µg/g, respectively. Wheat straw substrate caused an increase in yield and reduced fruiting time compared to sugarcane bagasse. Sugarcane bagasse, on the other hand, had a higher feed value (content of protein, ash, and dry matter) than wheat straw when used as a substrate.

Keywords: bagasse, pinhead, protein, spawn running, wheat straw

INTRODUCTION

Mushroom production not only reduces environmental pollution of agricultural and industrial residues when used as substrates, but also provides an economically acceptable alternative for the production of food of superior taste and quality, as well as high value-added secondary metabolites such as enzymes or polysaccharides (Adholeya *et al.* 2006; Philippoussis *et al.* 2007). Among the *Pleurotus* mushrooms, the species *florida* can be grown on a wide range of agricultural and industrial wastes such as rice straw, wheat straw, sawdust, corncobs, and sugarcane bagasse (Baysal *et al.* 2003; Adholeya *et al.* 2006). Among these, wheat straw is the most commonly used substrate (Philippoussis *et al.* 2001). According to reports, 234 million tons of sugarcane bagasse is produced every year in the world, which are normally wasted, and represent one of the largest potential for mushroom cultivation (Shin *et al.* 1998; Moda *et al.* 2005). Nevertheless, little is known about the performance of the fungus on different waste substrates, especially regarding mushroom yield and quality (Philippoussis *et al.* 2007). Generally, growth and nutritional values of *Pleurotus* mushrooms mainly depend on the chemical profile of substrates (Curvetto *et al.* 2002) since these, when used for cultivation, include large quantities of lignin, cellulose and hemi cellulose. Therefore, degradation of these compounds needs the action of lignin peroxidase, manganese peroxidase, laccase, endoglucanase, cellobiohydrolase and xylanase (Papinutti and Lechner 2006). The macro- and microelements play the greatest role in optimizing yield potentials by the action of ligninolytic enzymes and lignin degradation (Curvetto *et al.* 2002). Nwanze *et al.* (2005) and Mao and Zhong (2006) reported increased mycelial growth, yield and biological efficiency (BE) on substrates supplemented with different nitrogen sources. Queiroz *et al.* (2004), who examined the effect of three levels (0, 0.05, 0.5%) of ammonium sulfate on the culture of *Lentinula edodes*, showed that ammonium sulfate (0.5 %) increase mycelia growth rate, BE and protein content of fruit body, but did not have a significant effect on the ash and dry matter content of fruit bodies.

The importance of microelements in the lifecycle (e.g. in the cultivation of *Pleurotus* spp.) has often been emphasized in the literature. Among these, Mn is an important element in the selective delignification of substrates by fungi. It increases growth and yield of mushrooms (Racz and Tasnadi 1998). Royse *et al.* (2007) examined the effect of supplementation of cotton seed hull substrate with $MnSO_4$ (50, 150, 250 µg/g) in the cultivation of *Pleurotus eryngii*. Yield, BE and mushroom size were significantly higher on substrates containing $MnSO_4$ at 50 µg/g while the addition of $MnSO_4$ at 250 µg/g to the substrate increased protein, P, K, Bo and Zn contents of the fruit body but Mg, Mn, Al and Na were not influenced by any of the $MnSO_4$ levels. Previously, Wuyep *et al.* (2003) reported that growth and extension of fungal mycelia increased when Mn was added to the substrate used for the cultivation of *Lentinus squarosulus* and *Psathyrella atroumbonata.*

The objective of this research was to evaluate quali- and quantitative characters of *Pleurotus florida* grown on two substrate types (wheat straw and sugarcane bagasse) supplemented with various levels of Mn and N.

MATERIALS AND METHODS

The experiment was carried out in the edible fungi laboratory, Horticulture Department, Agriculture College, Shahid Chamran University, Ahwaz, Iran in 2006. The experiment was conducted in a factorial experiment design, completely randomized with three replications. In this study, two substrate types [wheat straw (S1) and sugarcane bagasse (S2)] supplemented with varying levels of urea (N_1:0, N_2:500, N_3:750 µg/g) and $MnSO_4$ (M_1:0, M_2:100, M_3:200 µg/g) were used.

The wheat grain spawns were prepared as previously described by Elhami and Alemzadeh Ansari (2008). The substrates

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Table 1 Mean simple effect of different substrate types, nitrogen, and manganese levels on some of morphological and physiological characters of oyster mushroom.

Treatment	Mycelia linear growth rate (mm)	Spawn running (days)	Pinhead formation (days)	Fruit body formation (days)	Yield (g)	Biological efficiency (%)	Protein (%)	Dry matter (%)	Ash (%)	Manganese (µg/g)
Substrate types										
Sugarcane bagasse	89.6 b*	19.5 a	24.8 a	29.5 a	1001.8 b	79.5 b	17.5 a	8.2 a	7.0 a	7.0 a
Wheat straw	93.1 a	14.8 b	19.4 b	22.5 b	1291.7 a	107.6 a	15.6 b	6.4 b	6.0 b	6.5 a
Nitrogen doses										
0	86.1 b	18.5 a	23.3 a	27.3 a	960.0 c	78.4 c	14.7 b	7.4 a	6.6 a	6.8 a
500	90.2 b	16.8 b	21.9 b	25.7 b	1199.2 b	97.4 b	17.1 a	7.3 a	6.5 a	6.8 a
750	97.7 a	15.9 c	20.8 c	24.7 c	1281.1 a	104.4 a	17.8 a	7.2 a	6.4 a	6.7 a
Manganese doses										
0	92.6 b	16.9 b	21.7 b	25.7 b	1190.9 b	97.1 b	15.1 c	7.2 a	6.4 a	6.3 a
100	100.4 a	14.9 c	19.9 c	23.7 c	1341.9 a	109.4 a	16.5 b	7.2 a	6.4 a	6.8 a
200	81.1 c	19.7 a	24.7 a	28.6 a	907.5 c	74.2 c	18.1 a	7.4 a	6.7 a	7.2 a

*Means followed by the same letter did not differ significantly at P=0.05.

were pasteurized by boiling vapour and supplemented with different levels of manganese ($MnSO_4$ monohydrate was purchased from Merck Company and used without further purification and nitrogen (urea) on a dry weight basis. Substrates were packed in 200 mm long and 16 mm diameter glass tubes after first adding 6-7 wheat spawn grains of mushroom inoculum to the bottom of each tube. All the tubes were plugged with cotton and incubated at 25°C± 1°C in the dark. The rate of mycelia lineal growth was determined after 5 days of spawn running (Curvetto *et al.* 2002).

Also, the pasteurized substrates (60-70% RH) were manually packaged into polypropylene bags (3 Kg wet substrate) after being supplemented with different levels of Mn and N, then the bags were inoculated with wheat grain spawns at 5% (w/w) of substrate fresh weight (Moda *et al.* 2005; Papinutti and Lechner 2006). Colonization of the substrates took place in a growth room in the dark, at 25 ± 1°C. After complete colonization of the substrate, polypropylene bags were removed and environmental conditions were controlled at 20 ± 1°C, 85-90% RH, 8 h light/16 h dark (Adholeya *et al.* 2006).

Several parameters were evaluated to select the suitability of substrate, levels of N and Mn for the cultivation of *P. florida*: the length of each phase of the fungus production cycle such as spawn running (days), pinhead formation (days), fruit body formation (days), yield (g/bag), biological efficiency [(BE), the ratio of fresh mushroom weight to the dry weight of the substrate, by percent], protein contents by the Kjehldal method (N×4.38), dry matter (%), Mn (by atomic absorption spectrophotometer) and ash (by Rajarthnam *et al.* 2002) contents of fruit body. The data were analyzed using GLM producer SAS 9.1 version software package and the means were separated by Duncan's multiple range tests (Jamalzadeh and Shareghi 2004).

RESULTS AND DISCUSSION

Effect of substrate

The results of ANOVA showed that all characters with the exception of Mn content of fruit bodies were significantly affected by the choice of substrate. The maximum mycelia lineal growth rate, the shortest pre-harvest (spawn running, pinhead and fruit body formation), the highest yield and BE were obtained on wheat straw substrate (**Table 1**). This response is attributed to the higher N content, high levels of water-soluble sugars, particularly hemicelluloses, the low lignin content and the low C/N ratios of wheat straw as compared to sugarcane bagasse substrate (Ozcelik and Peksen 2007; Philippoussis *et al.* 2007).

However, the highest protein, ash and dry matter contents of fruit bodies were obtained on sugarcane bagasse (**Table 1**). For that reason, the growth and development period of mushrooms on sugarcane bagasse was longer than on wheat straw, therefore mycelia and fruit bodies had enough time to absorb inorganic matter and nutrients (Philippoussis *et al.* 2007). These results are in agreement with the findings of Ruegger *et al.* (2001) and Silva *et al.* (2002). Also, dry matter content of fruit bodies harvested on sugarcane bagasse was higher than on wheat straw because the water-holding capacity of sugarcane bagasse is less than that of wheat straw; consequently the fruit bodies produced on sugarcane bagasse will be a little more moist, and with a higher dry matter (Adholeya *et al.* 2006).

Effect of nitrogen

The mycelia growth rate, spawn running, pinhead and fruit body formation, yield, BE and protein content of fruit bodies were significantly affected by varying levels of nitrogen, although this had no significant effect on dry matter, ash and Mn content of fruit bodies. Comparison of means showed that the mycelia lineal growth rate, yield and BE increased with increasing N concentration in substrates, while this increase accelerated spawn running, and the period of pinhead and fruit body formation (**Table 1**). Nitrogen supplementation enhances the secretion of cellulase, hemicelluloses and laccase activities, cellular enzymes in the spawn running period, and responsible for the degradation of cellulose, hemicelluloses and lignin, respectively (Rajarathnam *et al.* 2002). Ultimately, the degraded carbohydrates are known to serve as energy sources for mycelia growth, the construction of fruiting bodies and also to serve as structural components of the fruit bodies (Curvetto *et al.* 2002; Nwanze *et al.* 2005). Also, the protein content improved when substrates were supplemented with different levels of N (**Table 1**), this result being similar to the observation of Queiroz *et al.* (2004), who reported that the protein content of fruit bodies for *Shiitake* mushrooms increased as the level of ammonium sulfate in substrates (eucalyptus logs) increased.

Effect of manganese

Varying levels of Mn supplementation significantly influenced mycelia lineal growth rate, spawn running, pinhead and fruit body formation, yield, BE and protein content of fruit bodies but did not have a significant affect on dry matter, ash and Mn contents of fruit bodies (**Table 1**). The highest mycelia lineal growth rate, yield and BE were produced from substrates supplemented with 100 µg/g Mn, while an increase in Mn (100 µg/g) accelerated spawn running, pinhead and fruit body formation periods.

Generally, Mn has a stimulating effect on mycelial growth (Racz and Tasnadi 1998). Mn is known to differentially affect transcription and activity levels of peroxidases that are responsible for lignin degradation (Papinutti and Lechner 2006; Royse and Estrada 2007) and the addition of Mn may facilitate enzymatic reactions in substrate for fungi, although higher Mn concentrations (200 µg/g) had a negative effect on enzyme activity and BE (Curvetto *et al.* 2002; Beelman *et al.* 2006). Curvetto *et al.* (2002) studied *Pleurotus ostreatus* and sunflower seed hulls substrate in which

Table 2 Mean interaction effect of different nitrogen and manganese levels on some of morphological and physiological characters of oyster mushroom.

Treatment	Mycelia lineal growth rate (mm)	Spawn running (days)	Pinhead formation (days)	Fruit body formation (days)	Yield (g)	Biological efficiency (%)
N1M1	85.9 b*	18.26 b	22.88 c	27.09 b	938.27 e	76.62 e
N1M2	94.9 b	16.31 d	21.27 d	25.14 d	1118.12 d	91.29 d
N1M3	77.6 c	21.13 a	26.01 a	29.87 a	823.76 f	67.27 f
N2M1	92.9 b	17.24 c	22.20 c	26.07 c	1266.63 c	103.35 c
N2M2	99.2 b	14.26 f	19.22 f	23.10 f	1384.96 b	112.91 b
N2M3	78.6 c	19.15 b	24.28 b	28.15 b	946.26 e	77.39 e
N3M1	98.9 b	15.33 a	20.29 e	24.17 e	1367.87 b	111.45 b
N3M2	107.0 a	14.26 f	19.22 f	23.10 f	1522.88 a	124.09 a
N3M3	87.2 b	18.91 b	23.86 b	27.73 b	952.66 e	77.90 e

*Means followed by the same letter did not differ significantly at P=0.05.

Table 3 Mean interaction effect of different substrate and manganese levels on some of morphological and physiological characters of oyster mushroom.

Treatment	Mycelia lineal growth rate (mm)	Spawn running (days)	Pinhead formation (days)	Fruit body formation (days)	Yield (g)	Biological efficiency (%)
N1S1	82.2 a*	21.1 a	26.1 a	31.0 a	808.5 e	64.1 d
N1S2	89.1 a	15.9 d	20.6 d	23.7 d	1111.6 c	92.6 b
N2S1	86.0 a	19.7 b	25.1 b	29.8 e	1032.6 d	81.9 c
N2S2	94.4 a	14.0 e	18.6 e	21.7 c	1366.0 a	113.8 a
N3S1	99.7 a	17.8 c	23.1 c	27.8 c	1164.6 b	92.4 b
N3S2	95.6 a	14.5 e	19.1 e	22.1 e	1397.6 a	116.5 a

*Means followed by the same letter did not differ significantly at P=0.05.

Mn (200 ppm) had a negative effect. Beelman *et al.* (2006) studied *Agaricus bisporus* and horse manure compost in which Mn (250 mg/ kg) had a negative effect.

Also, supplementing the substrate with Mn (200 µg/g) dramatically increased the protein content of fruit bodies, as was also observed by Royse and Estrada (2007) for *Pleurotus eryngii*; these authors also reported that protein, P, K, B, and Zn contents of fruit bodies increased when the level of Mn was increased in the substrate, cottonseed hull, but Mn, Ca, Al and Na did not increase these parameters.

Interaction effect of nitrogen and manganese levels

Mycelia lineal growth rate, spawn running, pinhead and fruit body formation, yield and BE were significantly influenced by the Mn × N interaction (**Table 2**), but protein, dry matter, ash and Mn contents of fruit bodies were not. Means comparison indicated that the highest mycelia lineal growth rate, yield and BE obtained by Mn (100 µg/g) × N (750 µg/g) interaction also accelerated spawn running, and pinhead and fruit body formation periods. These results findings support those of Curvetto *et al.* (2002) in which the interaction of Mn (100 ppm) and N (750 ppm) increased these factors hen sunflower hulls were used as a substrate for the growth of *Pleurotus ostreatus*.

Interaction effect of nitrogen and substrate levels

Spawn running, pinhead and fruit body formation, yield and BE were significantly affected by the N × substrate interaction (**Table 3**), but did not significantly affect mycelia growth rate, protein, dry matter, ash and Mn contents of fruit bodies. The faster spawn running, pinhead and fruit body formation periods, as well as the highest yield and BE were obtained from wheat straw substrate containing N (750 µg/g), with levels of N between 500 µg/g and 750 µg/g not having a significant affect on these parameters in wheat straw substrate. But in sugarcane bagasse substrate levels of N between 500 and 750 µg/g had a significant affect on these parameters, although N increases mycelial growth rate and yield, when above a certain level. Baysal *et al.* (2003) examined the effect of supplementation of waste paper with chicken manure as a source of organic nitrogen in the cultivation of *P. ostreatus.* The results showed that above 20% chicken manure mycelial growth and fruiting was inhibited. It is possible that, since wheat straw has more N (0.8% d.w.) than sugarcane bagasse (0.3% d.w.), that the addition of further N (750 µg/g) to wheat straw decreased mycelial growth.

Other interaction effects between factors were not significant.

CONCLUSIONS

1. Wheat straw substrate supported the shortest pre-harvest period (spawn running, pinhead and fruit body formation) and the highest yield and BE.
2. The highest protein, dry matter and ash contents of fruit bodies were produced on sugarcane bagasse substrate.
3. Supplementation of the substrate wheat straw and sugarcane bagasse with Mn (100 µg/g) shortened the pre-harvest period and also increased yield and BE; the highest protein content of fruit bodies was obtained on substrate wheat straw and sugarcane bagasse supplemented with Mn (200 µg/g).
4. Supplementation of the substrate wheat straw and sugarcane bagasse with N (750 µg/g) shortened the pre-harvest period and increased yield, BE and protein content of fruit bodies.
5. The interaction effect of Mn (100 µg/g) and N (750 µg/g) shortened the pre-harvest period and increased yield and BE.

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