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SOME FACTORS INFLUENCING OYSTER MUSHROOM *(Pleurotus ostreatus)* DEVELOPMENT ON RICE STRAW

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ABSTRACT

Some factors influencing Oyster fungus, *Pleurotus ostreatus* (Jacq. Ex. fr) Kummer, development on rice straw were investigated. Generally grinding method yielded better results concerning Oyster yield, biological efficiency and substrate dry matter loss than chopping method. *P. ostreatus* mushroom also grew faster on the ground straw, than on the chopped straw, with their growth cycles being shorter than on the chopped straw. No significant difference was found between the two sizes, 2.5 and 5.0 cm, of the chopped straw. Further reduction of the particle size by grinding the straw to 0.5 cm, however, resulted in lower mushroom yield. With the tested spawn levels, 6% and 9% levels (on the basis of dry weight of organic matter) resulted in significantly lower mushroom yield than the other levels. Spawn level 12% (on the basis of dry weight of organic matter) enhanced mushroom yields. Inoculation with *Pseudomonas fluorescence* had beneficial impact for mushroom production and biological efficiency. *P. fluorescence* inoculum appreciably reduced total number of days for cultivation of about 2 days compared with uninoculated treatments. The crude protein content reached 6.14 g/100 g dry matter basis of spent rice straw. The fungal cultivation reduced ($p<0.05$) the cellulose content of the rice straw. Hemicellulose content in spent rice straw showed a similar trend. Organic matter content decreased from 80.15 to 64.20 g / 100 g dry matter basis.

Keywords*:* Degradation, *Pleurotus ostreatus* mushroom, lignocellulosic wastes, yield, rice straw, *Pseudomonas fluorescence*.

INTRODUCTION

Cultivation of *P. ostreatus* on agricultural residues, such as rice straw, is a value-added process to convert this substance, which is otherwise considered to be waste, into human food. Cultivation is one of most efficient biological ways by which this waste can be recycled (Madan *et al*., 1987, Zhang *et al*., 2002 and El-Sawah *et al*., 2008).

There have been various reports on other factors, than substrate used for mushroom production, that influence the development of Oyster mushrooms (Gregori *et al*., 2007). None of these factors has been studied in depth. Straw size reduction method and particle size had effect on mushroom production (Zhang *et al*., 2002). Spawn inoculation level also effect the economics of the productivity process (Zhang *et al*., 2002). Inoculation of pure *P. ostreatus* mycelium cultures with strains of fluorescent *Pseudomonas* spp., isolated from the mycelial plane of commercially produced mushrooms, promoted the formation of primordia and enhanced the development of the basidiomata. Thus, inoculation of the mycelium with specific bacteria may have beneficial applications for mushroom production (Cho *et al*., 2003).

Irradiation by red and green light stimulated vegetative growth of *P. ostreatus* mycelium and shortened the substrate colonization and fructification time. The increased fruiting body yield in irradiated cultures

reached 36–51% (Poyedinok *et al*., 2003). The cytolytic protein ostreolysin, isolated from *P. ostreatus* mushroom fruiting bodies, was specifically expressed during fruiting initiation, suggesting its involvement in fruiting body formation. When purified ostreolysin was used as a supplement on nutrient media plates inoculated with *P. ostreatus* mycelium, the protein stimulated primordial and fruiting body formation (Berne *et al*., 2005).

Two compounds from olive mill waste, 4-methylcatechol and catechol, were found to be effective against *P. tolaasii* mushroom and supplementation with up to 10% olive mill waste reduced bacterium-related symptoms (Soler-Rivas *et al*., 2006). The influence of heavy metals in substrates on *P. eryngii* primordial formation, fruiting body development and biological efficiency was demonstrated. Heavy metal (As, Hg and Cd) supplementation decreased average growth yields and biological efficiency of *P. eryngii*, whereas Pb supplementation improved both parameters (Qu *et al.*, 2006). The bacterial blotch disease in mushrooms caused by *Pseudomonas tolaasii* was more severe when substrates were amended with Cu in *P. eryngii* cultivation (Rodriguez Estrada and Royse, 2007).

The present study aimed at investigation some factors other than substrate influencing *P. ostreatus* oyster development on rice straw. The chemical composition of spent rice straw was determined.

MATERIALS AND METHODS

The present experiments were conducted in the Mushroom Unit, Agric. Res. and Experimental Center (AREC), Fac. of Agric., Mansoura Univ., Mansoura, Egypt, in the period between October 2005 and March 2008. All treatments were replicated four times and arranged in completely randomized block design.

Organisms

Pleurotus ostreatus (Jacq. ex. fr) Kummer strain PO3 was used in this study (El-Sawah *et al*., 2008). The strain was maintained on malt extract agar slants (Atlas, 1995). Spawn was prepared on sorghum grains (Zadrazil, 1978). *Pseudomonas fluorescence* isolated from grown mushroom plane was maintained on nutrient agar (Atlas, 1995). All cultures were provided kindly by Mushrooms Unit, Agric. Res. and Experimental Center (AREC), Fac. of Agric., Mansoura Univ., Mansoura, Egypt.

Spawn preparation

The mycelium was transferred from fungal strain grown on malt agar medium to bottles with sterilized sorghum grains and incubated again at 28ºC until colonization of the substrate was observed (approximately one week). These fermented grains were used as inocula for the growth tests in bags at 28ºC.

Oyster growing

Rice straw (*Oryza sativa*) was chopped or grinded. The substrates were also thoroughly mixed with 5% wheat bran and 5% of calcium sulfate and pasteurized. Excess water was drained and the substrates dried at 25°C±3°C for 12 h. Plastic bag technology was used in production experiments. Substrates were spawned at a rate 4% of sorghum spawn

(w/w). Each bag was closed with a plastic neck. The spawned bags were subjected to these different treatments to ensure maximum yields. The spawned bags were then incubated at 25°C and 60–65% relative humidity for ~ 3 weeks in a well-ventilated, semi-dark room until spawn run was completed (El-Sawah, 2000).

Oyster cropping

Upon completion of the spawn run, the bags were transferred onto horizontal racks in a cropping room–a wooden-frame structure covered with woven mats. The bags were then opened and fortification conditions for providing more light and ventilation were created. The mats were watered twice a day to increase the humidity and induce fruit body formation. The interior of the house reached 25°C and 90–95% relative humidity. Time was recorded in days for the completion of growth of mycelium on substrates, appearance of pin heads, and maturation of fruiting bodies in different treatments. The data were also recorded for the yield number of fruiting bodies and biological efficiency of substrates. The total biological efficiency was worked out against the dry weight of each substrate.

The loss of organic mass

At the end of the cultivation, the organic mass was dried at 105°C until the constant weight, weighed and the loss of organic mass was determined. The dry mass of rice straw cultivated under the same conditions without fungus was used as a control. Three replicates were used for each strain and treatment.

Biological efficiency

The total biological efficiency was worked out against the dry weight of each substrate using the formula outlined by Shah *et al*. (2004).

Rice straw size reduction and particle size

The effect of size reduction method and particle size on *P. ostreatus* mushroom growth and substrate degradation was investigated.

Spawn inoculation level

Spawning levels (6-18% on dry weight basis of rice straw) on the yield of the Oyster mushroom was investigated.

Inoculation with *Pseudomonas fluorescence*

Inoculation of agrowaste medium spawned with pure *P. ostreatus* mycelium cultures with the fluorescent *Pseudomonas* was investigated. *Pseudomonas* was added with the rate of 7 x 10^3 cell/g Oyster mushroom medium.

Chemical analysis

Analyses were carried out on the wastes on which the mycelium grew. The wastes were powdered and analysed for various constituents: cellulose, hemicellulose, lignin, fibre and, crude protein and ash (AOAC, 1990). Moisture content was determined by drying 5 g of each substrate at 105°C overnight. pH was measured using an Alpha 500 model laboratory pH/mV meter.

Statistical analysis

For each analysis, there were four replicates. Data were submitted to a one-way analysis of variance. The total yield of mushroom per waste was

separated by Duncan's multiple range tests at a=0.05. Correlation analyses were carried out in order to determine the relation of each chemical constituent with the total yield of mushroom pooled from all the substrates. All statistical analyses were performed using SPSS 10 for Windows (SPSS, 1999).

RESULTS AND DISCUSSION

Effect of rice straw size reduction and particle size

This experiment was designed to investigate the effect of size reduction method and particle size of rice straw on Oyster mushroom *P. ostreatus* strain PO 3 growth and substrate degradation. The mushroom yield, biological efficiency and rice straw dry matter loss % after the fungal growth on different straw types, namely ground or chopped straws, are presented in Table 1. The obtained results revealed that generally grinding method yielded better results than chopping method. This may be due to grinding as mechanical method ruptured the cell walls of the rice straw to a greater degree, potentially making the nutrients in the rice straw more accessible for *P. ostreatus* strain PO 3 growth.

Table 1: Effect of rice straw size reduction and particle size on Oyster mushroom *P. osteratus* strain PO 3 yield.

Type of substrate	Size (cm)	Growth cycle	Mushroom yield	Biological efficiency %	Substrate dry matter loss (%)
Ground rice straw	0.5	-1	650.06	65.01±3.26	36.04±0.14
	2.5	-3	686.12	68.61±1.02	36.02±0.09
Chopped rice straw	2.5	-2	656.07	65.61±0.74	30.08±0.49
	5.0	-2	651.08	65.11±0.19	29.07±0.12
	7.5	-1	631.10	63.11±0.58	28.48±0.13
	10.0	-1	598.09	59.81±0.13	27.92±0.12
Control	-	-	590.04	59.00±0.70	26.97±0.24
LSD 5%			16.04	1.60	0.27

The Oyster mushroom *P. ostreatus* strain PO 3 also grew faster on the ground rice straw, than on the chopped rice straw, with their growth cycles being three days shorter than on the chopped straw.

Among the types of rice straw used in Oyster mushroom *P. ostreatus* strain PO 3 cultivation, the ground 2.5 cm rice straw showed the best results in terms of *P. ostreatus* mushroom yield, biological efficiency, and rice straw dry matter loss (%). No significant difference was found between the two sizes, 2.5 and 5.0 cm, of the chopped straw. With the same particle size (2.5 cm), the ground substrate resulted in 15% higher mushroom yield than the chopped rice straw, which was significant. Further reduction of the particle size by grinding the straw to 0.5 cm, however, resulted in lower mushroom yield. This could be because the particles that were to small resulted in the over-compaction of rice straw in the cultivation bags, which may have led to hindered air exchange between the void spaces in the substrate and headspace, especially when the bags were closed. These results are in

agreement with those reported by Zhang *et al.* (2002). They revealed that different types of straw are commonly used for *Pleurotus* spp. cultivation. Straw can be composted or pasteurized and extra additives can be used to increase the biological efficiency. When using rice and wheat straw for *P. sajor-caju* cultivation, higher yields were obtained on ground than on chopped straw, and yields were 10 % higher on rice than on wheat straw. The dry matter loss of the substrate after mushroom growth varied from 30.1% to 44.3%.

Effect of spawn inoculation level

Spawn inoculation level is an important factor that relates to the production costs of the Oyster mushroom cultivation. Compared to rice straw, spawn is a relatively expensive item. Using the lowest inoculation level possible without sacrificing the mushroom yield would give the best economics (Royse, 2002 and Zhang *et al.*, 2002). In this experiment, the effect of spawning levels on Oyster mushroom development was investigated. The obtained results are given in Table 2. It is seen that with the five spawn levels, 6, 9, 12, 15 and 18%, the 6% and 9% levels resulted in significantly lower mushroom yield than the other three levels. Higher spawn levels namely 12%, 15% and 18% enhanced mushroom yields. However, the level 12% represented the best economic level taking the costs in consideration. Several authors confirmed these results. Royse (2002) found that maximum yield of Oyster mushroom (weight of fresh mushrooms harvested at maturity) was obtained at 3.75–5.00% spawn level. Zhang *et al.* (2002) found that with three spawn levels tested, 12%, 16% and 18%, the 12% level resulted in significantly lower mushroom yield than the other two levels. Higher spawn levels namely 16% and 18% enhanced mushroom yields.

Table 2: Effect of spawning level on yield of Oyster mushroom *P. osteratus* strain PO 3.

Spawning level (%)	Mushroom yield	Biological efficiency %	Substrate dry matter loss (%)
6	565.27±13.25	56.06± 0.54	31.32±0.38
9	640.12±14.00	59.51±11.59	33.97±1.14
12	686.08± 4.04	68.61± 0.40	36.12±1.51
15	691.44± 5.43	69.14± 0.54	39.66±0.99
18	695.12± 3.13	69.51± 0.31	36.71±3.98
LSD 5%	8.90	0.89	2.41

Inoculation with fluorescent *Pseudomonas*

In this experiment, the effect of mixed inoculum of Oyster mushroom *P. osteratus* strain PO 3 and *Pseudomonas fluorescence* on growth cycle, mushroom yield, biological efficiency (%) and substrate dry matter loss (%) was investigated. The experiment was carried out in a congruency with the findings obtained in the preceding experiments. The results on the effect of inoculation of pure *P. osteratus* strain PO 3 with fluorescent *Pseudomonas* are recorded in Table 3. Inoculation of pure *P. ostreatus* mycelium culture with strain of fluorescent *Pseudomonas* revealed significant increase in

mushroom yield. Also, a significant positive effect was recorded with biological efficiency. Little positive effect was recorded in case of substrate dry matter loss (%). However, a positive effect on mycelial growth of *P. ostreatus* induced by the presence of *Ps. fluorescence* recently has been reported (Cho *et al*, 2003). *P. fluorescence*, a diazotroph has been isolated from the surface of *P. ostreatus*, and has been observed to promote pinning and to enhance the development of basidiome. Kim *et al*. (2008) found that the addition of *Pseudomonas* sp strain P7014 and its supernatant to the mushroom growing media resulted in mushroom mycelia run faster. Mycelial growth rate of *P. eryngii* was increased up to 1.6 fold and primordial formation was induced one day earlier. Moreover, it was supposed that inoculation with bacteria had beneficial applications for commercial mushroom production, which appreciably reduced total number of days for cultivation of about 5±2 days compared with uninoculated, which took 55±2 days.

Table 3: Effect of inoculation of pure *P. ostreatus* strain PO3 mycelium cultures with strain of fluorescent *Pseudomonas*.

Type of culture	Growth cycle	Mushroom yield	Biological efficiency (%)	Substrate dry matter loss (%)
Uninoculated control	-	685.90 ± 0.71	68.59 ± 7.14	35.37 ± 0.74
Inoculated	-2	940.76 ± 38.81	94.07 ± 3.88	35.78 ± 0.65
LSD 5%		35.89	3.59	0.89

Chemical composition and cell wall degradation

This experiment was designed to investigate the effect of Oyster mushroom *P. osteratus* strain PO 3 cultivation on the chemical composition of spent rice straws in comparison with untreated one. It was carried out in a congruency with the findings recorded in the preceding experiments. Data presented in Table 4 show that the crude protein content significantly increased in the spent rice straw. Crude protein content reached to 6.14 g/100 g dry matter basis of spent rice straw. Cell wall constituents of rice straw before and after *P. ostreatus* strain PO3 cultivation are shown in Table 4. The fungal cultivation reduced ($p<0.05$) the cellulose content of the rice straw. Hemicellulose content in untreated and spent rice straw showed a similar variation. Organic matter content decreased from 80.15 to 64.20 g/100 g dry matter basis.

Oyster mushroom cultivation effect on the chemical composition of rice straw is in agreement with the studies of Gutpata *et al*. (1988), Tripathi and Yadav (1992), Jalk *et al*. (1996, 1998), Magnigo *et al*. (2004) and Mirzaei *et al.* (2007). They reported that cultivation of *P. osteratus* fungus on agrowastes led to decrease in cellulose, hemicellulose and lignin content of wheat straw. The reasons of these changes may be because white-rot fungi belonging to the basidomycetes produce enzymes such as lignin peroxidase, manganese peroxidase, H_2O_2 producer enzymes, arylalchol oxidase and laccase. These fungi are unable to supply all their carbon and energy requirements from lignin, and therefore they require substrate such as cellulose or other carbon sources for their growth and delignification, therefore they have greatest potential for degradation (Akin *et al*., 1995,

Martins, 2002, Ruggeri and Sassi, 2003). As result, they consume cellulose and hemicellulose and thus organic matter decreases and ash content increases. As well, delignification probably increases hemicellulose solubility, but cellulose remains insoluble and its content changes less than hemicellulose.

Table 4: Chemical composition and cell wall characteristics of untreated and spent rice straw after *P. ostreatus* strain PO3 cultivation.

Component*	Crude rice straw	Spent rice straw	LSD 5%
Crude protein	5.31 ± 0.07	6.14±0.08	0.16
Cellulose	41.20 ± 0.10	38.91±0.82	1.33
Hemicellulose	25.14 ± 0.15	16.05±0.94	1.53
Organic matter	80.15 ± 0.05	64.20±0.10	4.12

*** g / 100 g dry weight rice straw.**

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بعض العوامل المؤثرة على تطور فطر الأويستر (بليروتس أوستراتس) النامى على قش الأرز

محمـــود محمـــد عـــوض الله الســـواح، محمـــد منصـــور قاســـم علـــى و رشا يوسف يوسف النفاض
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اســتهدف البحــث دراســة بعــض العوامــل المــؤثرة علــى تطــور فطــر الأويســتر *P. ostreatus* PO 3 النامى على قش الأرز ، وقد أدى فرم قش الأرز إلى الحصول على نتائج أفضل فيما يتعلق بمحصول الأويستر والكفاءة البيولوجية ونسبة الفقد فى قش الأرز عن تقطيع القش . لوحظ سرعة فى نمو فطر الأويستر على قش الأرز المفروم ومن ثم قصر دورة نمو الأويستر على القش المفروم عن مثيله الذى تم تقطيعه . لم يلاحظ وجود فرق بين مخلف قش الأرز الذى تم تقطيعه بحجم ٢,٥ سم عن مثيله الذى تم تقطيعه بحجم ٥ سم . أدى فرم قش الأرز بحجم ٠,٥ سم إلى محصول أقل من الأويستر .

ومن بين مستويات الأسبون المختبرة ، أظهرت مستويات التلقيح ٦% و ٩% (على أساس الوزن الجاف للمادة العضوية) انخفاض معنوى فى محصول الفطر عن المستويات الأخرى ، وقد أعطى المستوى ١٢% (على أساس الوزن الجاف للمادة العضوية) أعلى محصول اقتصادى من الفطر المزروع .

أدى التلقيح ببكتيريا السيدوموناس الفلوروسنتية إلى تأثير إيجابى على النمو حيث نجم عنه زيادة معنوية فى كل من محصول الفطر والكفاءة البيولوجية ، ولوحظ قصر دورة إنتاج المحصول يومين فى حالة التلقيح ببكتيريا السيدوموناس الفلوروسنتية مقارنة بالمزارع غير الملقحة.

بلغ المحتوى البروتينى الخام ٦,١٤ جم / ١٠٠ جم مادة جافة من قش الأرز المتخلف عن زراعة فطر الأويستر ، ولوحظ وجود اختزال معنوى فى كمية السليلوز بقش الأرز المستخدم فى الزراعة ، وأظهر الهيميسليلوز بقش الأرز المتخلف عن الزراعة نفس اتجاه السليلوز ، كما تناقصت كمية المادة العضوية من ٨٠,١٥ جرام إلى ٦٤,٢٠ جم لكل ١٠٠ جرام قش أرز على أساس الوزن الجاف .

قام بتحكيم البحث

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