ORIGINAL RESEARCH



Fruiting bodies yield of oyster mushroom (*Pleurotus columbinus*) as affected by different portions of compost in the substrate

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Abstract

Purpose A study was conducted to assess production of *Pleurotus columbinus* mushroom fruiting bodies for different formulations of rice or corn straw substrates mixed at different percent portions with the corresponding composted straw.

Methods These formulations were: (1) raw straw (RS) mixed with 5 % composted straw (CS), (2) RS mixed with 10 % CS, (3) RS mixed with 15 % CS, (4) RS mixed with 25 % CS, (5) RS mixed with 50 % CS, and (6) 100 % RS. Composted straw (CS) was made of moistened chopped RS mixed with chicken manure and soil (4:1:1, v/v).

Results Data showed a magnificent impact of the substrate on oyster mushroom fruiting bodies yield and characteristics. There was a significant progressive upgrading in all parameters studied of mushroom growth and crop outcome with increasing the percentage of CS mixed with the RS substrate up to 15 %. Utilizing CS at 25 % significantly downgraded these parameters. No mushroom growth was observed at all when cultivated in medium contained 50 % CS. Instead, molds of different colors grew on that latter substrate mixture. The formulation containing 15 % CS distinctly gave the uppermost fruiting bodies yield, biological efficiency, earliness for pinheads formation, fruiting body cap diameter, thickness and weight and stem diameter, length and weight. Up to 80 % increase in fruiting bodies crop outcome relative to sole RS was detected.

Conclusions This study suggests that composted straw substrates hold a great promise for the development of *Pleurotus* mushroom production industry.

Keywords Fruiting bodies · Edible fungi · Lignocellulosic wastes · Macrofungi · *Pleurotus columbinus* · Primary decomposer

Introduction

The cultivated *Pleurotus* mushrooms include a number of different species: *Pleurotus ostreatus, Pleurotus sajor-caju, Pleurotus columbinus, Pleurotus cystidus, Pleurotus citrinopileatus,* and *Pleurotus flabellatus. Pleurotus* mushrooms are widespread in the temperate zones representing the third largest groups of the cultivated edible mushrooms in the world (Mendez et al. 2005; Sarangi et al. 2006; Sher et al. 2010). China is the major producer of oyster mushroom. The production of this mushroom species is estimated to be 25 % of the total world production of cultivated mushrooms. The *Pleurotus* mushrooms are nutritionally and gastronomically important (Sarangi et al. 2006; Valverde et al. 2015). In addition, mushroom spent substrate can be potentially utilized in crop organic production (Lopes et al. 2015).

Oyster mushroom production using various sources of agricultural wastes has received a renewed interest of researchers (Jeznabadi et al. 2016; Mohamed et al. 2012). Furthermore, the appropriate preparation of the substrate is crucial for the production of maximized yield of *Pleurotus* mushrooms (Choi et al. 2009; Obodai and Johnson 2002;



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Soliman 2011). *Pleurotus* mushroom species, unlike button mushroom (*Agaricus bisporus*), are primary decomposers (Mohamed et al. 2014). They can break down and absorb the components of substrate materials that have not been degraded. Therefore, cultivation of *Pleurotus* mushrooms is considered to be a simple, low cost, and environmentally friendly technology for the utilization of rural and agro-industrial residues in the developing countries (Kirbag and Akyüz 2008; Mohamed et al. 2011; Zhang, et al. 2014). However, it has been found that fermented substrate materials produce high yield and quality fruiting bodies (Choi 2004). As shown by Obodai and Johnson (2002), composted *Triplochiton scleroxylon* sawdust mixed with other substrates significantly increased the yield of *P. ostreatus*.

Substrate materials in nature have microorganisms attached to their surfaces. The initial microorganisms that exist come mainly from the soil. Activity of these microorganisms is suppressed on dry material. When such organic materials are moistened, microorganisms can propagate and the nutritive substances of the substrate are accumulated in form of protein and other useful compounds. Thus, fermentation during composting can be defined as the conversion of the nutrients of substrates by microorganisms into other important compounds (Choi 2004). By pasteurization of substrate, most of contaminating microorganisms are killed and protein can be utilized by growing mushroom. This study presents an assessment of P. columbinus mushroom productivity when cultivated on substrate formulations of composted rice (Oryza sativa L.) or corn (Zea mays L.) straws mixed at different portions with raw straw.

Materials and methods

This study was conducted in the mushroom research and production laboratory, Department of Horticulture, Faculty of Agriculture, Assiut University. Production of Pleurotus columbinus mushroom fruiting bodies was assessed for different formulations of mixed composted straw with raw straw substrate (w/w). The compost was made of moistened chopped raw rice (Oryza sativa L.) straw or corn (Zea mays L.) straw mixed with chicken manure and soil (4:1:1, v/v). The soil was clay loam and was collected from the top 20 cm in the Vegetable Crop Research Farm, Faculty of Agriculture, Assiut University, Assiut city. Soil was added to enrich the materials with biodegrading mesophile microorganisms. Raw straw was used as a carbon source for the biodegrading mesophile microorganisms. Detailed procedure for compost preparation is described elsewhere here under general procedure. Spawn of Pleurotus columbinus mushroom used in this study was obtained



from Agricultural Research Center, Food Technology Research Institute, Giza.

Pilot assessment and particular experiment designation

In a preliminary assessment, five treatments were studied. These were: (1) 100 % raw straw (RS) (control), (2) 75 % RS mixed with 25 % compost (CS), (3) 50 % RS mixed with 50 % CS, (4) 25 % RS mixed with 75 % CS, and (5) 100 % CS. Based on this preliminary assessment, the treatments of the substrate formulations were modified to be: (1) RS mixed with 5 % CS, (2) RS mixed with 10 % CS, (3) RS mixed with 15 % CS, (4) RS mixed with 25 % CS, (5) RS mixed with 50 % CS, and (6) 100 % RS. Two experiments were executed and each of them was repeated twice (2 trials). Both experiments were conducted in randomized complete-blocks with four replicates. Each treatment was presented by five culture bags per replicate.

Experiment I: composted rice straw formulations

In this experiment, the raw rice straw (RRS) was used either sole or mixed with compost (CS) added at different percent portions (5, 10, 15, 25, and 50 %). Compost (CS) was made of moistened chopped RRS mixed with chicken manure and soil (4:1:1, v/v).

Experiment II: composted corn straw formulations

In this experiment, the raw corn straw (RCS) was used either sole or mixed with compost (CS) added at different percent portions (5, 10, 15, 25, and 50 %). Compost (CS) was made of moistened chopped RCS mixed with chicken manure and soil (4:1:1, v/v).

General procedure

Preparation of composted substrate

The chopped raw straw mixed with chicken manure and soil was piled up outdoors and moistened. The heap was kept for 2 weeks. Then, the pile was turned weekly to provide fresh air and prevent overheating. The composting continued under this process for 6 weeks. The mature compost was then used in preparation of the different substrate formulations.

Preparation of substrate formulations and spawning

The raw substrate was moistened thoroughly by soaking overnight in water. The raw and the composted substrates were pasteurized for 2 h in hot water at 80 $^{\circ}$ C (Bahukhandi

and Munjal 1989; Balasubramanya and Kathe 1996). The pasteurized substrate was left overnight (15–18 h) to cool down and to drain excess water. Subsequently, the raw substrate was thoroughly mixed with the corresponding composted substrate and checked to assure average moisture of about 70 %. The prepared substrate was manually packaged into 20×40 cm clear polyethylene bags of mean thickness 0.2 mm containing 1-kg moistened substrate. The spawn was inoculated at rate of 5 % based on wet mass of the substrate.

Culture conditions for spawn running and fruiting bodies formation

The inoculated substrate was incubated for spawn running at 24–28 °C in the darkness for 3–4 weeks. The mushroom cultures were subsequently transferred into fruiting room for basidiocarp formation. Polyethylene bags were removed, and the cultures were kept at 22 ± 1 °C under light provided by cool white fluorescent tubes for 12 h/day. Electric fans were used 4 h/day during incubation for basidiocarp formation to provide homogenous ventilation condition in the incubation room. The bags moisture was maintained by spraying with tap water two times a day during the whole cropping period. Mushroom fruiting bodies were harvested about a week after pinheads formation that was as soon as the gills were well formed, and while the edge of the caps is still curled under.

Data records and statistical analysis

Data were recorded for days lapsed to visible pinhead (primordia) formation, total fruiting bodies yield (overall flushes) (g) per kg moistened substrate, weight (g) of mushroom spent substrate, biological efficiency (%), average fruiting body weight (g), diameter (mm) and thickness (mm) and average stem weight (g), diameter (mm), and length (cm). Biological efficiency (BE) was calculated as follows: BE (%) = (weight of fresh mushroom fruiting bodies/weight of dry substrate) \times 100 (Ahmed 1995; Kirbag and Akyüz 2008). All data were subjected to analysis of variance (Gomez and Gomez 1984), and the means were compared using "the least significant difference" (LSD) test at 0.05 probability level.

Results

Both rice and corn substrate formulations containing 50, 75, and 100 % composted straw (CS) showed no mushroom colonization and, subsequently, no fruiting bodies formation in the pilot assessment. Meanwhile, mushroom fungus spawn running and fructification were observed on the sole raw rice straw (RRS) and sole raw corn straw (RCS) substrates in this assessment.

Experiment I: composted rice straw formulations

Fruit bodies yield of Pleurotus columbinus mushroom grown on raw rice straw (RRS) mixed with 5 or 10 % composted straw (CS), in contrast to sole RRS substrate, exhibited significant increase in the first trial but not in the second one (Table 1a). When grown on substrate formulation containing 25 % CS, mushroom showed tendency to produce lower yield in the first trial, while significant reduction was detected in fruiting bodies yield in the second trial. Consistently, mushroom grown on substrate formulation containing 15 % CS produced fruiting bodies yield significantly surpassing all the other treatments in the first trial, but except substrate formulation containing 10 % CS in the second trial. Again in both trials, there were no fruiting bodies produced when the fungus was grown on rice substrate formulation containing 50 % CS (negative control treatment). Data obtained for spent weight substantiated those of the fruiting bodies yield (Table 1a). Spent weight was the least for mushroom grown on the substrate formulation composed of 85 % RRS and 15 % CS. Thus, the fungus consumed larger amount of components of this substrate formulation. Mushroom grown on formulation containing 5 % CS utilized greater amount of the substrate than that one grown on RRS (positive control treatment). Meanwhile, greater amount of the substrate formulation containing 10 % CS was utilized by the fungus than that one containing 5 % CS. However, the utilized quantity of the substrate formulation containing 25 % did not differ from that of the RRS (positive control). Apparently, the substrate formulation containing 50 % CS had the greatest spent weight.

Due to the highest fruiting bodies yield produced on the substrate formulation containing 15 % CS, the fungus exhibited the highest biological efficiency for this formulation (Table 2a). The other substrate treatments, except 50 % CS, showed inconsistence with regard to the significance of the difference in biological efficiency from the RRS substrate. Apparently, the substrate formulation containing 50 % CS was inferior to the RRS substrate regarding the biological efficiency. As a result of the superior capability for biodegradation of substrate having 15 % CS, the fungus formed its pinheads exceptionally earlier than the others (Table 2a). Differently, the days lapsed to visible pinhead formation were similar for the mushroom grown on 0, 5, 10, and 25 %. Obviously, the availability of nutrient components from the substrate for the mushroom grown on the formulation containing 15 % CM enabled developing fruit bodies with caps of large diameter and great thickness and weight (Tables 3a, 4a).



Compost portion (%)	Total fresh fruiting bodies yield (g/1 kg moistened substrate)		Spent weight (g)	
	1st Trial	2nd Trial	1st Trial	2nd Trial
(A) Rice straw				
0	148.357	170.724	668.5	691.7
5	175.661	171.136	466.7	504.7
10	206.007	186.249	401.1	400.4
15	248.169	201.923	267.1	246.1
25	133.251	136.036	685.5	689.9
50	$0.000^{\rm a}$	0.000	776.5	817.6
LSD ^b _{0.05}	19.732	18.065	33.7	49.6
(B) Corn straw				
0	126.039	115.081	766.5	831.5
5	152.368	158.001	687.3	713.8
10	158.128	168.289	506.3	587.7
15	207.965	206.525	316.8	331.3
25	119.325	108.250	770.2	735.3
50	0.000^{a}	0.000	824.1	786.7
$LSD_{0.05}^{b}$	29.014	29.802	54.8	24.0

 Table 1
 Means of total fruiting bodies yield and spent weight for *Pleurotus columbinus* mushroom grown on raw rice straw (A) or raw corn straw (B) mixed with different percent portions of the corresponding composted straw

Rice straw and corn straw were tested in separate experiments, and each experiment was repeated twice (two trials)

^a No mushroom growth occurred

^b The least significant difference at 0.05 level of probability to separate means of different compost portion treatments of the same experiment trial

Stems of the fruiting bodies for this treatment had increased weight, diameter, and length (Tables 4a, 5a).

Experiment II: composted corn straw formulations

Mushroom grown on raw corn straw (RCS) mixed with 5 % composted straw (CS), in contrast to sole RCS substrate (positive control treatment), exhibited significantly higher fruiting bodies yield only in the second trial (Table 1b). The substrate formulation containing 10 % CS significantly elevated fruiting bodies yield in both trials. Fruiting bodies yield was similar whether the mushroom was grown on substrate formulation containing 25 % CS or sole raw corn straw (RCS) substrate. Consistently, mushroom grown on substrate formulation containing 15 % CS produced fruiting bodies yield significantly surpassing all the other treatments. There were no fruiting bodies produced in both trials when the fungus was grown on corn substrate formulation containing 50 % CS (negative control treatment). The least spent weight was for mushroom grown on the substrate formulation contained 15 % CS (Table 1b). The fungus, therefore, degraded and consumed larger amount of components from this substrate formulation than any other substrates. Mushroom grown on formulation containing 5 % CS utilized greater amount of the substrate components than the one grown on RCS (positive control treatment). Meanwhile, greater components amount of the substrate formulation containing 10 % CS was utilized by the fungus than that one containing 5 % CS. The utilized quantity of the substrate formulation containing 25 % was significantly less than RCS (positive control) only in the second trial.

Due to the highest biodegradation occurred for the substrate formulation containing 15 % CS, the mushroom exhibited the highest biological efficiency (Table 2b). The other substrate formulations, except those containing 25 and 50 % CS, showed significantly higher biological efficiency than RCS substrate. In both trials, the substrate formulation containing 25 or 50 % CS did not differ from RCS substrate regarding the biological efficiency. As a result of the superior capability for biodegradation of substrate having 15 % CS, the fungus formed its pinheads exceptionally earlier than the others (Table 2b). Contrary to this result, the days lapsed to visible pinhead formation increased for the mushroom grown on RCS mixed with 25 % CS. On the other hand, significantly earlier visible pinhead formation was detected for cultures on RCS containing 5 and 10 % CS than sole RCS. Noticeably, the availability of nutrient components from the substrate for the mushroom grown on the formulation containing 15 %

apseu to visible plineaus (plinorula) formation
al 2nd Trial
34.5
34.3
35.2
29.8
35.4
0.0
1.2
39.7
37.5
38.3
31.1
41.6
0.0
1.2
a

 Table 2 Means of biological efficiency and days lapsed to visible pinheads (primordia) formation for *Pleurotus columbinus* mushroom grown on raw rice straw (A) or raw corn straw (B) mixed with different percent portions of the corresponding composted straw

Rice straw and corn straw were tested in separate experiments, and each experiment was repeated twice (two trials)

^a No mushroom growth occurred

^b The least significant difference at 0.05 level of probability to separate means of different compost portion treatments of the same experiment trial

CM enabled developing fruit bodies with caps of largest diameter and greatest thickness and weight (Tables 3b, 4b). Greatest weight, diameter, and length (Tables 4b, 5b) were found for stems of the mushroom fruiting bodies produced by the substrate formulation containing 15 % CS.

Discussion

The main components of the lignocellulosic substrates are cellulose, hemi-cellulose, and lignin. Pleurotus mushrooms can biodegrade cellulose and lignin of lignocellulosic materials to get their carbon requirements. However, Pleurotus mushrooms also need nitrogen and inorganic compounds. The substrate material utilized alone for cultivation of Pleurotus mushrooms sometimes cannot provide enough nitrogen and/or other nutrients required for optimal growth (Siqueira et al. 2012; Soliman 2011; Soliman et al. 2011). Therefore, supplements, such as rice and wheat bran, are added as a nitrogen source (Jafarpour and Eghbalsaeed 2012; Soliman 2011). In this regard, composting converts the components of raw lignocellulosic materials into a superior nutritional source especially protein for mushroom (Rajarathnam and Bano 1989) through the actions of a succession of microorganisms. However, total nitrogen should not exceed the amount from which the ammonia nitrogen created during fermentation can restrain the mycelial growth of the mushroom (Choi 2004). For the cultivation of *Pleurotus* spp., the compost containing 0.6–0.9 % N of its dry weight, depending on the species, is recommended (Imbernoon et al. 1983; Laborde 1987).

Noticeably, there was a significant progressive upgrading in this study for growth and crop outcome with increasing the percentage of composted straw materials mixed with the raw substrate up to 15 %. Utilizing the composted materials at 25 % apparently downgraded these parameters. No mushroom growth was observed at all when cultivated in medium contained 50 % or more of composted straw materials. Instead, molds of different colors grown on this latter substrate mixture. Unlike the end up result of our study, composted straw materials can be utilized entirely as substrate for cultivation of oyster mushroom, and it has been found to definitely improve vield and quality of oyster mushroom crop outcome (Choi 2004). In the method used by Choi (2004), raw substrate without supplements was moistened and left for composting by microorganisms naturally subsist on the surface of the dry raw substrate.

Here, we prepared the composted materials from rice or corn straw mixed with chicken droppings. Diminutive



Compost portion (%)	Average diameter of fruiting body cap (mm)		Average thickness of fruiting body cap (mm)		
	1st Trial	2nd Trial	1st Trial	2nd Trial	
(A) Rice straw					
0	86.8	91.5	7.3	8.3	
5	102.8	99.3	8.8	9.0	
10	115.3	116.5	8.9	9.8	
15	136.8	130.8	14.7	13.7	
25	92.5	85.0	7.6	8.1	
50	0.0^{a}	0.0	0.0	0.0	
LSD _{0.05}	5.1	8.9	2.3	1.7	
(B) Corn straw					
0	104.1	107.2	8.1	7.9	
5	108.9	112.2	8.5	7.9	
10	118.3	121.8	10.1	9.1	
15	145.2	146.7	12.5	11.5	
25	96.0	98.9	5.4	5.0	
50	0.0^{a}	0.0	0.0	0.0	
LSD ^b _{0.05}	6.2	5.2	2.4	2.3	

Table 3 Average diameter and thickness of fruiting body cap for *Pleurotus columbinus* mushroom grown on raw rice straw (A) or raw corn straw (B) mixed with different percent portions of the corresponding composted straw

Rice straw and corn straw were tested in separate experiments, and each experiment was repeated twice (two trials)

^a No mushroom growth occurred

^b The least significant difference at 0.05 level of probability to separate means of different compost portion treatments of the same experiment trial

Compost portion (%)	Average weight of fruiting body cap (g)		Average wei	Average weight of stem (g)	
	1st Trial	2nd Trial	1st Trial	2nd Trial	
(A) Rice straw					
0	7.318	7.603	2.905	3.025	
5	7.728	8.504	3.255	3.227	
10	8.761	9.061	3.387	3.622	
15	9.853	9.625	3.975	3.932	
25	7.172	7.352	2.988	3.037	
50	0.0^{a}	0.0	0.0	0.0	
LSD _{0.05}	1.340	1.455	0.141	0.132	
(B) Corn straw					
0	7.303	7.174	4.042	3.188	
5	9.555	9.266	4.263	3.417	
10	9.890	9.537	5.033	4.321	
15	12.328	11.968	6.667	5.517	
25	7.353	6.838	4.554	3.808	
50	$0.000^{\rm a}$	0.000	0.000	0.000	
LSD ^b _{0.05}	0.447	0.434	0.582	0.544	

Rice straw and corn straw were tested in separate experiments, and each experiment was repeated twice (two trials)

^a No mushroom growth occurred

 $^{\rm b}$ The least significant difference at 0.05 level of probability to separate means of different compost portion treatments of the same experiment trial

Table 4 Average weight of
fruiting body cap and stem for
Pleurotus columbinus
mushroom grown on raw rice
straw (A) or raw corn straw
(B) mixed with different percent
portions of the corresponding
composted straw



Table 5Average stem lengthand diameter for *Pleurotus*columbinusmushroom grownon raw rice straw (A) or rawcorn straw (B) mixed withdifferent percent portions of thecorresponding composted straw

Compost portion (%)	Average length of stem (cm)		Average stem diameter (mm)	
	1st Trial	2nd Trial	1st Trial	2nd Trial
(A) Rice straw				
0	2.5	2.5	7.3	9.9
5	2.8	2.8	8.6	12.1
10	3.1	3.3	9.9	14.2
15	4.1	4.2	14.0	15.7
25	2.5	2.5	9.9	10.5
50	0.0^{a}	0.0	0.0	0.0
LSD ^b _{0.05}	0.2	0.2	4.7	2.4
(B) Corn straw				
0	3.2	3.0	8.5	8.3
5	3.5	3.4	10.9	11.3
10	3.8	3.7	12.5	11.9
15	6.0	5.4	23.5	25.2
25	3.3	3.0	10.0	10.1
50	0.0^{a}	0.0	0.0	0.0
LSD ^b _{0.05}	0.4	0.2	1.2	1.3

Rice straw and corn straw were tested in separate experiments, and each experiment was repeated twice (two trials)

^a No mushroom growth occurred

^b The least significant difference at 0.05 level of probability to separate means of different compost portion treatments of the same experiment trial

information has been reported on use of chicken manure supplement to substrates in production of mushroom. As reported by Baysal et al. (2003), increased supplement of chicken manure to waste paper substrate had a negative effect on growing mushroom. Oyster mushroom yield decreases when the ammonia resulting from conversion of nitrogen during fermentation process is higher than 68 ppm as well as when total nitrogen is smaller than the optimal amount (Choi 2004). The chicken manure is rich in nitrogen in addition to phosphorus, potassium and calcium. It is suggested that the used compost here may have high total N. Mixing the composted straw materials at certain portions with raw substrate could have adjusted the total N to a suitable level for mushroom growth. In this study, the formulation composed of raw rice straw or raw corn straw mixed with 15 % composted straw materials showed superiority.

The microorganism species that may compete with *Pleurotus* spp. after pasteurization with hot water (80 °C for 2 h) includes the fungi *Penicillium* spp. and *Tricho-derma* spp. (green mold) (Balasubramanya and Kathe 1996). Pasteurization at high temperatures could make cellulose more available (Sturion and Oetterer 1995), due to the partial destruction of the lignin-cellulose bonds. Partial breakdown of cellulose and hemi-cellulose make them available to competitor microorganisms. We

conducted pasteurization at 80 °C, and this in conjunction with the lack of competition due to restrained mushroom growth on medium formulation contained 50 % or more composted straw materials can be accounted for the molds contamination observed.

This study, therefore, supports the notion of magnificent impact of substrate preparation on *Pleurotus* mushroom productivity. The biological efficiency enhanced relative to the sole raw substrate. The fruiting bodies crop outcome exhibited up to 80 % increase, relative to sole raw straws, when the mushroom was grown on substrate formulation containing 15 % composted straw material. The improved fruiting bodies yield was accompanied with magnificently enhanced weight and size for the harvested fruit caps. This substrate formulation produced mushroom crop earlier by 5-9 days, on average, than the cultivation on sole raw straws.

Conclusion

Composting may hold a promise for developing *Pleurotus* mushroom production industry. Under the conditions of this study, a formulation of mixed composted straw with raw rice straw or raw corn straw at rate of 15 % is proposed.



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