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Full Length Research Paper

High protein complementation with high fiber substrates for oyster mushroom cultures

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Agricultural residues have been world widely accepted for oyster mushroom culture. In this study, we used wheat straw, barley straw, maize stem residue, and lawn residue as substrates coupled with wheat bran, rice bran and soybean powder as complements for the growth of *Pleurotus florida* and *Pleurotus ostreatus* as edible mushrooms. Shorter growth period and higher yield and biological efficiency were obtained for *P. florida* than *P. ostreatus*. Wheat and barley straws which contained high fiber and carbon to nitrogen (C/N) ratio caused the best growth period, fruiting body weight, yield and biological efficiency. Assessment of substrate and complement combinations indicated that the longest growth period was obtained from barley straw enriched with rice bran (27.00 day). However, the highest fruiting body number (36.33), fruiting body weight (31.17 g), yield (939.33 g), and biological efficiency (187.87%) belonged to wheat straw complemented by soybean powder plus rice bran. In conclusion, the highest yield and biological efficiency was achieved by implementation of composts in which high fiber substrates and high protein complements were combined.

Key words: Oyster mushroom, wheat straw, barley straw, high fiber.

INTRODUCTION

Oyster mushroom production technology under controlled condition has been broadly developed specially via recycling of agricultural wastes. It has been widely accepted in rural systems mainly due to simple cheep procedure requirements (Soto–Cruz et al., 1999). Most *Pleurotus* species are able to grow on lingo-cellulose materials like rotten woods, wood chips, and agricultural postharvest residues because of having high saprophyte characters (Straatsma et al., 2000; Stamets, 2000). In tropical and subtropical regions, high amount of lingo-celluloses from agricultural wastes are inappropriately buried or burned in farms. Recycling of these unfavorable materials through mushroom culture can increase agricultural efficiency and enhance degradation process of lingo-cellulose sources (Obodai et al., 2003).

There are various numbers of parameters affecting on the growth and performance of oyster mushroom, including substrate source, substrate quality, spawn, strain, compost and complement (Royse et al., 2004; Jafarpour et al., 2010). Having high content of protein and nitrogen source was reported to be effective in shortening growth period and increasing both yield and biological efficiency (Peksen and Yakupoglu, 2009; Adebayo et al., 2009; Fanadzo et al., 2010; Jafarpour et al., 2010). In contrast, high nitrogen content of substrates was considered as an obstacle for mushroom culture as a result of raising the media temperature and subsequently postponing the mycelium run (Gurjar and Doshi, 1995; Royse and Schisler, 1986). However, substrates enriched by plant origin complements led to slow release of organic materials which could be absorbed by mycelium structures (Royse et al., 1991).

Our previous study indicated that enrichment of sugar beet pulp, palm fiber and boll complemented with wheat bran, rice bran, carrot pulp and soya cake powder improved growth characters of *Pleurotus ostreatus* (Jafarpour et al., 2010). This study was therefore aimed to assess substrate and complements having different fiber and nitrogen contents, on growth performance and the yield of edible mushrooms.

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MATERIALS AND METHODS

Agricultural residues including wheat straw, barley straw, maize

Table 1. Environmental condition based on *P. florida* and *P. ostreatus* requirements.

Parameter	Spawn run phase	Pin head	Fruiting body
Temperature (°C)	21 - 24	10 - 16	16 - 20
Relative humidity (%)	85 - 95	95 - 100	85 - 90
CO2 (ppm)	5000 - 20000	≤1000	≤1000
Air replacement	1	4 - 8	4 - 8
Light (Lux/day)	-	1000 - 2000	1000 - 2000

Table 2. Chemical composition of substrates and food complements (dry weight based).

Substrate and complement	Component (%)					
	C/N	Nitrogen	Carbon	Carbohydrate**	Food fiber	Protein*
Wheat straw	21.1	1.45	30.6	41.29	36.95	9.06
Maize stem residue	10.19	2.05	20.9	50.04	17.75	12.81
Barley straw	28.33	0.99	28.05	43.42	37.9	6.18
Lawn cut	8.77	3.55	31.15	41.62	18.45	22.18
Wheat bran	10.07	2.1	21.15	66.23	11.8	13.12
Rice bran	21.4	1.25	26.75	38.19	33.35	7.81
Soybean powder	3.16	8.85	28	29.74	6	55.31

* N × 6.25; ** Lane and Eynon's method.

Table 3. Growth and yield statistics for *P. florida* and *P. ostreatus* oyster mushrooms.

Mushroom type	PH	PFB	CFB	Growth period	Yield	Body number	Body weight	Biological efficiency
P. ostreatus	33.72^{a}	2.55	4.98	41.25^{a}	582.20^{b}	23.75^{b}	24.95	116.43^{b}
P. florida	30.25^{b}	2.48	4.43	37.23^{b}	634.18^{a}	26.12^{a}	24.38	126.84^{a}

PH, Pin head formation; PFB, primary fruit body formation; CFB, completely fruit body formation.

stem residue, and lawn residue were used as substrates, while wheat bran, rice bran, soybean powder and a mixture of soybean powder and rice bran (1:1 w/w) were considered as complement groups. Substrates with no complement were designated as the control for complements groups. Chemical composition of all substrates and complements were analyzed prior to experiment conduction (Table 1).

Food complements were sterilized at 121°C and 15 psi pressure for 1 h. Pasteurization of the substrates was conducted through water absorption for 1.5 h following tissue softening at 100°C for 1.5 h (Jafarpour et al., 2010). Spawns were purchased from Karaj Spawn Production Institute. Fifty grams of organic complements and 80 g of spawns (based on 10 and 16% of substrate's dry weight, respectively) were added to 500 g of substrates in each experimental unit (Zhang et al., 2002; Jafarpour et al., 2010). All environmental conditions in the culture hall were managed according to growth requirements of Pleurotus as indicated in Table 2 (Stamets, 2000; Jafarpour et al., 2010).

Total growth period of *Pleurotus florida and P. ostreatus were* classified into three basic phases including spawn run to pin head stage, primary fruiting body, and complete fruiting body phases which were designated as PH, PFB, and CFB, respectively. Moreover, total growth period, fruiting body number and weight, yield and biological efficiency were measured.

Statistical analysis

Data was analyzed using general linear model (GLM) procedure in SAS package. Mean comparisons for substrates and complements coupled with their combinations were conducted using Tukey's post-hoc and considering P-value ($p < 0.05$) as significant level.

RESULTS

Growth period

Substrate effect on growth performance is presented in Table 4. Total growth period varied from 37.30 (wheat straw) to 42.40 days (maize stem residue). This total period was partitioned into PH, PFB, and CFB phases. Duration of PH ranged from 30.23 (wheat straw) to 33.73 days (maize stem residue). The PH phase was significantly shorter in barley straw and wheat straw rather than both maize stem residue and lawn residue ($p < 0.05$). The differences among substrates were not

Table 4. Substrate effect on *P. florida* and *P. ostreatus* growth characteristics.

Characteristic	Wheat straw	Maize stem residue	Barley straw	Lawn cut
Growth period (day)	37.30^{b}	42.40^{a}	37.43^{b}	39.83^{ab}
PH (day)	30.50^{b}	33.73^{a}	30.23^{b}	33.47^{a}
P.F.B. (day)	2.57	2.6	2.57	2.33
C.F.B. (day)	4.17^{b}	6.07^{a}	4.50^{b}	4.10^{b}
Fruiting body weight (g)	29.77^{a}	20.06^{c}	27.11^{b}	21.73^{c}
Fruiting body number	26.63^{b}	28.67^{a}	24.67^{b}	19.77^{c}
Yield (g)	776.60^{a}	575.67^{c}	658.70^{b}	421.80^{d}
Biological efficiency	155.32^{a}	115.13^{c}	131.73^{b}	84.36^{d}

Means with common letters in each row were not significantly different at $p \leq 0.05$.

Table 5. Food complements effect on *P. florida* and *P. ostreatus* growth traits.

Characteristic	Wheat bran	Rice bran	Soybean powder	Soybean powder and rice bran	Control
Growth period (day)	39.42^{b}	39.17^{b}	37.29^{b}	42.63^{a}	37.71^{b}
MR/PH (day)	32.29^{ab}	32.04^{ab}	30.25^{b}	34.58^{a}	30.75^{b}
P.F.B (day)	2.38	2.58	2.42	2.63	2.58
C.F.B. (day)	4.71^{ab}	4.54^{ab}	4.54^{ab}	5.42^{a}	4.33^{b}
Fruiting body weight (g)	24.43	24.95	24.46	24.81	24.72
Fruiting body number	26.17^{ab}	24.21^{b}	26.83^{a}	27.96^{a}	19.50^{c}
Yield (g)	622.54^{b}	607.00^{b}	654.92^{ab}	697.46^{a}	459.04^{c}
Biological efficiency	124.49^{b}	121.40^{b}	130.98^{ab}	139.49^{a}	91.81^{c}

Means with common letters in each row are not significantly different at $p \leq 0.05$.

significant at PFB step ($p < 0.05$). However, there were significant variations among different substrates at CFB step. The longest and shortest CFB period obtained in maize stem residue (6.07 d) and lawn cut (4.10 d), respectively.

Furthermore, the complement means comparison (Table 5) showed that total growth period was in the range of 37.29 (soybean powder) and 42.63 days (soybean powder plus rice bran mix). The longest PH and CFB period pertained to the mixture of soybean powder and rice bran (34.58 and 5.42 days, respectively), while the shortest mentioned stages belonged to soybean powder and control group, respectively (30.25 and 4.33 days). Interestingly, no significant difference was observed between wheat and rice bran for all growth period steps.

Combination effects of substrates and complements are shown in Table 6. The shortest and longest total growth period were attributed to lawn cut enriched by soybean powder (31.67 days) and maize stem residue enriched by soybean powder and rice bran (48.00 days), respectively. Complementation of rice bran with barley straw led to the shortest PH (27.00 days) while its complementation with lawn cut maximized the PH period (40.17 days). PFB period varied from 2.00 to 3.00 for lawn cut supplemented with soybean powder and barley straw supplemented with rice bran, respectively.

Fruiting body number and weight

Mean of fruiting body number and body weight for substrates, complements, and their combinations are given in Tables 4, 5 and 7, respectively. Mean of body number for different substrate groups changed from 19.77 (lawn residue) to 28.67 (maize stem residue). Tracking of average body weight showed that lawn residue caused to the least body weight (20.06) while wheat straw led to the highest body weight (29.77). For complements scenario, average fruiting body number was in the range of 19.5 (control) and 27.96 (soybean and rice bran). Average fruiting body weight varied from 24.43 (wheat bran) to 24.95 (rice bran) without significant difference.

The comparison of substrate and complement combinations indicated that the highest and lowest fruiting body number was attributed to wheat straw and rice bran mixture (33.00) and lawn residue with no complement (14.33), respectively. However, the maximum (34.30) and minimum (16.20) fruiting body

Table 6. Substrate and food complement combination effect on *P. florida* and *P. ostreatus* growth variables.

Substrate	Complement	P. H. (day)	P. F. B. (day)	C. F. B. (day)	Growth period (day)
Wheat straw	Wheat bran	31.33[bcde]	2.33[abc]	4.17[cdefg]	37.83[cde]
	Rice bran	27.67[de]	2.67[abc]	4.67[cdef]	35.00[de]
	Soybean powder	30.67[cde]	2.33[abc]	3.00[gf]	36.33[cde]
	Soybean powder and rice bran	34.50[abcd]	2.83[ab]	5.17[bcd]	42.50[abc]
	Control	28.33[de]	2.67[abc]	3.83[cdefg]	34.83[de]
Maize stem residue	Wheat bran	33.67[bcde]	2.67[abc]	4.83[cde]	41.17[abcd]
	Rice bran	33.33[bcde]	2.33[abc]	5.50[bc]	41.17[abcd]
	Soybean powder	30.33[cde]	2.50[abc]	4.00[a]	41.33[abcd]
	Soybean powder and rice bran	38.00[ab]	2.67[abc]	4.33[b]	47.33[a]
	Control	33.33[bcde]	2.83[ab]	4.83[cde]	41.00[abcd]
Barley straw	Wheat bran	28.33[de]	2.17[bc]	4.33[cdefg]	35.33[de]
	Rice bran	27.00[e]	3.00[a]	4.83[cde]	34.83[de]
	Soybean powder	33.00[bcde]	2.83[ab]	4.00[cdefg]	39.83[bcd]
	Soybean powder and rice bran	33.17[bcde]	2.33[abc]	4.33[cdefg]	39.83[bcd]
	Control	29.67[cde]	2.50[abc]	5.00[bcd]	37.33[cde]
Lawn cut	Wheat bran	35.83[abc]	2.33[abc]	5.50[bc]	43.33[abc]
	Rice bran	40.17[a]	2.33[abc]	3.17[efg]	45.67[ab]
	Soybean powder	27.00[e]	2.00[c]	2.67[g]	31.67[e]
	Soybean powder and rice bran	32.67[bcde]	2.67[abc]	5.50[bc]	40.83[abcd]
	Control	31.67[bcde]	2.33[abc]	3.67[defg]	37.67[cde]

Means with common letters in each column are not significantly different at $p \leq 0.05$.

weight were produced in wheat straw without complement and lawn cut residue without complement, respectively.

Yield and biological efficiency

Considering substrates as main effects showed that the highest and lowest rate of both yield and biological efficiency were observed in wheat straw (776.69 g and 155.32) and lawn residue (575.67 g and 115.13), respectively. There were no significant difference among wheat bran, rice bran, and soybean powder though all complements had significantly higher yield and biological efficiency than control group. Furthermore, the assessment of substrate and complement combinations showed that the mixture of wheat straw and soybean powder plus rice bran led to significantly higher yield and biological efficiency (939.93 and 187.87, respectively) than other combinations, except for wheat and rice bran combination ($p < 0.05$). On the other hand, lawn residue without complement had notably lower yield and biological efficiency (318.50 and 63.70, respectively) related to other combinations ($p < 0.05$).

DISCUSSION

In this study, we estimated the growth period at three stages including the spawning to PH formation, PFB formation and CFB formation along with total growth period (as a whole index) in two mushroom types (Table3). Longer total growth period was observed in *P. ostreatus* than in *P. florida*. Similar trend were presented in its compartments, which is the PH, PFB, and CFB stages. Even though *P. florida* had shorter growth period, it was associated with higher yield, body number and biological efficiency than *P. ostreatus*. Wheat and barley straws reduced total growth period to 5.4 weeks. In contrast, maize stem residue and lawn residue induced the growth period to 6 weeks. Variation in total growth period was attributable to changes in PH and CFB stages in which barley and wheat straws had significantly shorter period than both maize stem and lawn residue.

The highest fruiting body weight was also reached when wheat straw was used as substrate though its fruiting body number was not the highest among all substrates. Interestingly, wheat straw also maximized the yield and biological efficiency. This trend was observed in growth period, fruiting body number, yield and biological

Table 7. Substrate and food complement combination effect on *P. florida* and *P. ostreatus* production traits.

Substrate	Complement	Fruiting body number	Fruiting body weight (g)	Yield (g)*	Biological efficiency
Wheat straw	Wheat bran	23.67^fghi	29.60^b	694.83^cdef	138.97^cdef
	Rice bran	33.00^a	26.17^bcd	870.67^ab	174.13^ab
	Soybean powder	27.00^def	29.20^b	786.00^bc	157.20^bc
	Soybean powder and rice bran	31.83^a	29.58^b	939.33^a	187.87^a
	Control	17.67^kl	34.30^a	592.17^ghijk	118.43^ghijk
Maize stem residue	Wheat bran	30.67^abcd	19.83^gh	606.50^fghij	121.30^fghij
	Rice bran	24.67^efgh	22.42^defg	552.50^ijk	110.50^ijk
	Soybean powder	31.33^abc	20.70^g	648.83^efghi	129.77^efghi
	Soybean powder and rice bran	31.67^ab	21.13^fg	668.00^defgh	133.60^defgh
	Control	25.00^efgh	16.20^h	402.50^mn	80.50^mn
Barley straw	Wheat bran	27.67^cdef	25.20^bcdef	688.17^cdefg	137.57^cdefg
	Rice bran	20.50^ijk	28.33^b	578.33^hijk	11.67^hijk
	Soybean powder	26.33^efg	29.08^b	761.17^cd	152.23^cd
	Soybean powder and rice bran	27.83^bcde	27.05^b	742.83^cde	148.57^cde
	Control	21.00^hijk	25.87^bcde	523.00^jkl	104.60^jkl
Lawn cut	Wheat bran	22.67^hijk	23.07^cdefg	500.67^klm	100.13^hi
	Rice bran	18.67^jk	22.87^cdefg	426.50^m	85.30^hi
	Soybean powder	22.67^ghij	18.72^gh	423.67^m	84.73^ij
	Soybean powder and rice bran	20.50^ijk	21.48^efg	439.67^lm	87.93^hi
	Control	14.33^l	22.52^defg	318.50^n	63.70^j

*Gram per 500 g substrate dry weight basis; ** means with common letters in each column are not significantly different at $p \leq 0.05$

efficiency for barley straw as well. Both wheat and barley straws have the highest C/N ratio among substrates. However, application of soybean powder plus rice bran and wheat bran with high protein and low C/N ratio caused long growth period.

Meanwhile, the application of nitrogen sources has been quite controversial for increase in yield and biological efficiency in some studies (Gurjar and Doshi, 1995; Royse and Schisler, 1986; Paksen and Yakupoglu, 2009; Adebayo et al., 2009; Fanadzo et al., 2010; Jafarpour et al., 2010). Temperature rise and species differences were considered as the main reasons for this discrepancy in different reports (Gurjar and Doshi, 1995). In our study, increase in nitrogen content of substrates was associated with longer PH stage. Having high fiber content and C/N ratio could enhance the digestibility of lingo-cellulose content followed by high availability of cellulose materials as mushrooms nutrients. In this issue, nitrogen might be in the bound form which needs more duration time to be delivered to the mushroom's mycelia (Fanadzo et al., 2010). In addition, it has been hypothesized that mushrooms have this ability to absorb and fix atmospheric nitrogen so that their nitrogen need discrepancy would be compensated (Bisaira et al., 1987).

The mixture of wheat straw and soybean powder led to the highest yield and biological efficiency among all combinations. Moreover, combination of soybean powder and soybean powder plus rice bran with all substrates maximized both yield and biological efficiency. Interestingly, combination of barley straw and soybean powder also led to the highest yield and biological efficiency. Our results on *P. florida* and *P. ostreatus* indicated that higher fiber substrates decreased total growth period, PH, and PFB stages, but increased the yield and biological efficiency. On the other hand, higher nitrogen complements induced the yield and biological efficiency in combination with almost all of substrates, except for lawn cut.

Conclusion

In this study, we assessed different substrates and complements implication on growth period, fruiting body weight, yield, and biological efficiency on *P. florida* and *P. ostreatus* edible mushrooms. The shortest growth period, mycelium run, and PH stages were obtained from media including high fiber and C/N ratio, whilst mushroom production on high protein content material extended total growth period and its compartments. Moreover, the highest fruiting body weight, yield, and biological efficiency was achieved by implementation of substrates containing high fiber and C/N ratio coupled with complements with higher protein and nitrogen contents.

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