African Journal of Agricultural Research Vol. 5(23), pp. 3291-3296, 4 December, 2010
Available online at http://www.academicjournals.org/AJAR
ISSN 1991-637X ©2010 Academic Journals

Full Length Research Paper

Evaluation of agricultural wastes and food supplements usage on growth characteristics of *Pleurotus ostreatus*

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Accepted 9 November, 2010

As agricultural wastes are easily accessible and quite free sources, re-use of them could be considered as a break through in production management, especially if it sustains the production efficiency as standard materials do. In this study, we evaluated combination usage of substrates including wood chips, boll, Sugar Beet Pellet Pulp (SBPP) and palm fiber along with wheat bran, rice bran, soya cake powder, Soya Cake Powder + Rice Bran (SCPRB) and carrot pulp as supplements. Substrates with no supplement were regarded as control groups. Results revealed that boll, SBPP and palm fiber, which could be abundantly in Iran, had better growth period, fruiting body weight, yield and biological efficiency than wood chips. Moreover, SCRBP had the highest yield, biological efficiency and fruiting body weight among all substrates. We also assessed combination effects of substrates and complements. The least growth period (30.3 d) belonged to sugar beet pulp enriched with soya cake powder. The lowest fruiting body weight (12.4 g) was produced in boll substrate with no supplement, though maximum fruiting body number (34.3) belonged to this group. The highest fruiting body weight (41.5 g) was achieved when the enriched palm fiber by rice bran supplement was used. In addition, the highest yield and biological efficiency was found on boll substrate enriched with a mixture of soya cake powder and rice bran supplements (794.3 and 158.9 g, respectively).

Key words: Oyster mushroom, yield, biological efficiency, substrate, protein.

INTRODUCTION

Pleurotus spp. grows on most lignocellulosic materials such as rotten or rotting wood, wood residues and most of agricultural wastes (Stamets, 2000; Straatsma et al., 2000). Accordingly, oyster mushrooms are among active decomposers of wood and other substrates. This is because of the large capacity of oyster mushrooms that secrete vast numbers of enzymes which enable them to live on various substrates, decompose lignin, protein, carbohydrate, cellulose and starch containing materials (Straatsma et al., 2000). Hence, agricultural wastes can be used as substrates for the growth of oyster mushrooms. Usage of substrate source in different parts of the world depends on the cultivation of different agricultural products and their attributed residues for oyster mushrooms growth (Obodai et al., 2003). Having low cost is the main advantage of these particular substrates which increased high interests in producers (Royse, 2003). In Asia, rice straw is widely accepted as the main substrate to cultivate oyster mushrooms. In Europe, wheat straw is the best substrate for oyster mushrooms cultivation, due to high content of yield and protein (Mandeel et al., 2005).

Obodai et al. (2003) reported that a mixture of lignocelluloses basis such as saw dust, rice bran, corn bran, banana leaves, field corn, grass and rice straw substrate positively affected the yield, mycelium run and the growth period of fruiting body of oyster mushrooms. Moreover, other studies on nutritional quality, yield, biological efficiency, mycelium growth, quantity and quality of fruiting bodies of oyster mushroom indicated

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Table 1. Chemical composition of substrates and food supplements (Dry weight based).

Substrate and supplement	Components (%)							
	C/N	Nitrogen	Carbon	Carbohydrate**	Food fiber	Fat	Ash	Protein*
Wood chips	67.5	0.4	28.4	28.9	65.3	1.2	1.9	2.7
Boll	19.1	1.4	27.2	49.4	28.8	2.1	10.9	8.9
Sugar beet pellet pulp	15.6	1.5	24.3	67.7	15.9	1.6	5.2	9.7
Palm fiber	9.3	3.0	27.8	20.6	56.6	1.1	3.0	18.8
Wheat bran	10.0	2.1	21.2	66.2	11.8	4.1	4.8	13.1
Rice bran	21.4	1.3	26.8	38.2	33.4	4.5	16.2	7.8
Soya cake powder	3.7	8.9	28.0	29.7	6.0	2.7	6.3	55.3
Carrot pulp	20.5	1.5	30.1	67.5	13.4	2.0	8.0	9.1

*N × 6.25,** Lane and Eynon method.

that there were significant difference among each type of substrates such as cotton, coconut fiber and field corn (Ragunthan and Swamin, 2003; Mendeel et al., 2005). Application of grass, boll and wheat straw on *Pleurotus ostreatus* cultivation showed that the type of substrate enhanced the nutrient release of oyster mushrooms and subsequently their yield and growth period compared to control group (Royse et al., 2004). Another research results showed that rice straw as substrate increased biological efficiency of *Pleurotus sajor–caju* about 182.3 (Banik and Nandi, 2004). Estrada et al. (2008) investigated and applied maize wastewater to produce different types of *P. ostreatus* and verified the useful effects of them.

The production of *Pleurotus* spp. requires good quality of substrate, sufficient amount of spawn, favorable environmental growth condition, mushroom strain, culture bed preparation and supplement usage (Royse, 2003; Royse et al., 2004; Banik and Nandi, 2004). Since 1980, enrichment of cultivation environment has been of great concern to increase oyster mushroom yield. Basically, food supplements increase mushroom yield and quality, while there decrease in its growth period due to their nutritional values (Royse, 2003). Studies indicated that adding 6% food supplement to substrate can increase the *Pleurotus cornucopiae* output up to ninety percent (Royse, 2003). Poultry manure, rice bran, wheat bran and peat moss were successfully used as food supplements to improved yield, biological efficiency and growth period via providing sufficient nitrogen and slow nutrient releasing (Baysal et al., 2003; Tisdale et al., 2006; Loss et al., 2009).

In this study, we aimed to assess (i) agricultural waste usage and (ii) their combination with food supplements on yield and quality of *P. ostreatus*.

MATERIALS AND METHODS

The substrates used in this research basically included agricultural industry residues such as wood chips, boll, and Sugar Beet Pellet Pulp (SBPP) and palm fiber. Moreover, plant supplements like wheat bran, rice bran, soya cake powder and carrot pulp were being much cheaper and more easily available compared to other supplements were employed for compensation for probable nutrients deficiency of substrates in the culture medium of oyster mushroom. Chemical composition of all substrates and supplements were analyzed prior to conduction of experiments (Table 1).

Food supplements were sterilized via autoclave at 121°C and a pressure of 15 psi for 1 h. Pasteurization of the substrates was conducted through water absorption for 1.5 h following tissues softening at 100°C for 1.5 h. Sterilization and pasteurization processes eradicated bactericides and fungicides along with removal of all probable types of dormant spores of competitive fungi or mold producers which naturally reside on post-harvest agricultural residues. After pasteurization, substrates were placed separately on double storied reticulate tables for the process of ventilation to reduce their moisture and temperature to 70% and 20°C, respectively. Finally, 50 g organic supplement and 80 g spawns (10 and 16% of substrate's dry weight, respectively) were added to 500 g substrate in each experimental unit (Zhang et al., 2002). After exact weighting via electronic scale for each three replicates, the spawn was added and uniformly blended. All environmental conditions in the culture hall were provided according to growth requirements of *P. ostreatus* as indicated in Tables 2 and 3 (Stamets, 2000).

To measure the growth period of *P. ostreatus*, three basic phases are mostly considered:

First phase: Spawn run phase or mycelium run.
Second phase: Pin head stage.
Third phase: Fruiting body formation phase. Usually this phase can be divided into two separate growth phases; primary fruiting body formation and complete fruiting body formation. Concerning the growth of *P. ostreatus*, it must be mentioned that, in all cases, mycelia colonize the whole surface of substrate, they start to form pin head right away. The growth of mycelium is heterogeneous and can not be seen with naked eyes. So, in most cases, the end phase of mycelium growth can not be determined. Neither when the pin head formation started, protuberances emerge ending to formation of pin heads, unless mycelium comes up. Hence, to minimize measurement error, the measurement of pin head period was omitted. The mycelia and pin head phase were measured and recorded together. After the maturation of *P. ostreatus*, and galea attained the diameters of 10 cm the harvesting phase started. Harvesting took four weeks and each time the amount of *P. ostreatus* was electronically weighed and recorded in special forms. Finally, the total weights of recording for each experimental unit were added up to obtain the total weight or the yield of *P. ostreatus*. It should be noted that since mushrooms in majority of cases

Table 2. Environmental conditions based on *P.ostreatus* requirements.

Parameters	Spawn run phase	Pin head stage	Fruiting body stage
Temperature (℃)	21 - 24	10 -16	16 - 20
Relative humidity (%)	85 - 95	95 -100	85 - 90
CO_2 (ppm)	5000 - 20000	≤1000	≤1000
Air replacement	1	4 - 8	4 - 8
Light (Lux/day)	-	1000 - 2000	1000 - 2000

Table 3. Substrate effect on *P. ostreatus* growth characters.

Characteristics	Wood chips	boll	sugar beet pellet pulp	Palm fiber
Growth period	44.9 ^{a*}	41.1 ^{b}	37.3 ^{c}	38.2 ^{bc}
M.R./P.H. stage	37.1 ^{a}	33.3 ^{b}	30.8 ^{bc}	28.8 ^{c}
P.F.B. Stage	2.6 ^{a}	2.6 ^{a}	2.7 ^{a}	2.8 ^{a}
C.F.B. stage	5.3 ^{b}	5.3 ^{b}	3.8 ^{c}	6.6 ^{a}
Fruiting body Weight	19.2 ^{c}	21.9 ^{b}	28.9 ^{a}	27.7 ^{a}
Fruiting body number	21.0 ^{b}	28.9 ^{a}	19.8 ^{bc}	17.6 ^{c}
Yield	404.2 ^{d}	604.8 ^{a}	558.9 ^{b}	486.1 ^{c}
Biological efficiency	80.8 ^{d}	120.7 ^{a}	111.8 ^{b}	97.2 ^{c}

* M.R. mycelium run, P.H. pin head, P.F.B. primary fruiting body formation and C.F.B. complete fruiting body formation. Means with common letters in each row were not significantly different at $P \leq 0.05$.

Table4. Food supplements effect on *P. ostreatus* characters.

Characteristics	Wheat bran	Rice bran	Soya cake powder	Soya cake powder and rice bran	Carrot pulp	Control
Growth period	42.4 ^{ab*}	35.8 ^{c}	39.0 ^{bc}	44.2 ^{a}	40.9 ^{ab}	40.0 ^{b}
M.R./P.H. stage	35.2 ^{ab}	27.8 ^{d}	31.8 ^{bc}	36.3 ^{a}	33.4 ^{abc}	30.5 ^{cd}
P.F.B. stage	2.6 ^{a}	2.7 ^{a}	2.8 ^{a}	2.5 ^{a}	2.7 ^{a}	2.8 ^{a}
C.F.B. stage	4.7 ^{b}	5.3 ^{b}	4.4 ^{b}	5.4 ^{b}	4.8 ^{b}	6.8 ^{a}
Fruiting body weight	27.8 ^{a}	23.5 ^{b}	22.9 ^{b}	29.8 ^{a}	34.8 ^{b}	18.5 ^{c}
Fruiting body number	21.6 ^{ab}	24.2 ^{a}	23.9 ^{a}	21.7 ^{ab}	18.9 ^{b}	20.8 ^{b}
Yield	560.7 ^{b}	559.1 ^{b}	546.5 ^{b}	638.8 ^{a}	421.9 ^{c}	354.0 ^{d}
Biological efficiency	112.1 ^{b}	111.5 ^{b}	109.3 ^{b}	127.8 ^{a}	84.4 ^{c}	70.8 ^{c}

* M.R. mycelium run, P.H. pin head, P.F.B. primary fruiting body formation and C.F.B. complete fruiting body formation. Means with common letters in each row are not significantly different at $P \leq 0.05$.

grow in clusters, they might not mature concomitantly therefore, during harvesting time only the *P.ostreatus* whose galea had reached 10 cm were harvested carefully and others were allowed to reach maturation. Biological efficiency is one of the important characteristics of production of *P. ostreatus* indicating conversion of substrate mass to mushroom fruiting bodies. To measure this index, oyster mushroom fresh weight g/100 g substrate dry weight was calculated. In this respect, after finishing harvesting all experimental units, the biological efficiency of each individual block was calculated. To measure mean fruiting body weight, it is enough to divide the total weight of *P. ostreatus* from each experimental unit to the number of harvested fruiting bodies. To do so, after each harvesting from the experimental units, the fruiting body number and their weights were calculated as well and finally, when harvesting was terminated, in all experimental units mean fruiting body weight was separately computed.

Data analysis was performed using MSTAT C software. Treatment means for all mentioned traits were compared with Duncan multiple range test at 0.05 significance level.

RESULTS AND DISCUSSION

Growth period and other stage of growth

Mean comparison using Duncan method for substrates, food supplements, and their combinations are presented in Tables 3, 4, and 5, respectively. Results showed that sugar beet pulp, palm fiber had the least growth period among all substrate (37.3 days and 38.3 days respectively). Our result was in accordance with other reports (Mandeel et al., 2005; Obadai et al., 2003) which indicated that wood chips led to the longest growth period

Table 5. Combination effect of substrate and food supplement on *P.ostreatus* growth variables.

Substrate	Supplement	M.R./P.H. Stage	P.F.B. Stage	C.F.B. Stage	Growth period
Wood chips	Wheat bran	41.7 ^{ab*}	2.7 ^{ab}	4.7 ^{efgh}	49.0 ^{a}
	Rice bran	37.0 ^{abc}	2.3 ^{ab}	6.7 ^{abcde}	46.0 ^{ab}
	Soya cake powder	41.0 ^{ab}	2.7 ^{ab}	5.7 ^{bcdef}	49.3 ^{a}
	Soya cake powder and rice bran	35.3 ^{bcd}	2.7 ^{ab}	5.3 ^{cdef}	43.3 ^{abc}
	Carrot pulp	34.3 ^{bcd}	2.7 ^{ab}	2.7 ^{h}	39.7 ^{bcde}
	Control	33.3 ^{bcd}	2.3 ^{ab}	6.7 ^{abcde}	42.3 ^{abc}
Boll	Wheat bran	32.0 ^{cde}	3.3 ^{ab}	7.3 ^{abc}	42.7 ^{abc}
	Rice bran	27.3 ^{defg}	2.3 ^{ab}	3.0 ^{gh}	32.7 ^{def}
	Soya cake powder	38.0 ^{abc}	2.3 ^{ab}	3.0 ^{gh}	43.3 ^{abc}
	Soya cake powder and rice bran	30.0 ^{cdefg}	2.7 ^{ab}	5.7 ^{bcdef}	38.3 ^{bcdef}
	Carrot pulp	37.3 ^{abc}	2.3 ^{ab}	5.0 ^{dfg}	44.7 ^{ab}
	Control	35.3 ^{bcd}	2.3 ^{ab}	7.7 ^{ab}	45.3 ^{ab}
Sugar beet pellet pulp	Wheat bran	29.7 ^{cdefg}	2.3 ^{ab}	3.0 ^{gh}	35.0 ^{cdef}
	Rice bran	24.0 ^{efg}	2.7 ^{ab}	4.7 ^{efgh}	31.3 ^{ef}
	Soya cake powder	24.0 ^{efg}	3.7 ^{a}	2.7 ^{h}	30.3 ^{f}
	Soya cake powder and rice bran	44.3 ^{a}	2.3 ^{ab}	2.7 ^{h}	49.3 ^{a}
	Carrot pulp	31.3 ^{cdef}	2.7 ^{ab}	3.7 ^{fgh}	37.7 ^{bcdef}
	Control	31.3 ^{cdef}	2.7 ^{ab}	6.3 ^{abcde}	40.3 ^{bcd}
Palm Fiber	Wheat bran	37.3 ^{abc}	2.0 ^{b}	3.7 ^{fgh}	43.0 ^{abc}
	Rice bran	23.0 ^{fg}	3.3 ^{ab}	7.0 ^{abcd}	33.3 ^{def}
	Soya cake powder	24.3 ^{efg}	2.7 ^{ab}	6.3 ^{abcde}	33.3 ^{def}
	Soya cake powder and rice bran	35.7 ^{bcd}	2.3 ^{ab}	8.0 ^{a}	46.0 ^{ab}
	Carrot pulp	30.7 ^{cdef}	3.0 ^{ab}	8.0 ^{a}	41.7 ^{abc}
	Control	22.0 ^{g}	3.7 ^{a}	6.7 ^{abcde}	32.3 ^{def}

* M.R. mycelium run, P.H. pin head, P.F.B. primary fruiting body formation and C.F.B. complete fruiting body formation. Means with common letters in each column are not significantly different at $P \leq 0.05$.

for oyster mushroom and subsequently was inappropriate for this purpose. This effect was 35.83 days for wheat bran supplement and soya cake powder respectively which were in good agreement with previous finding (Shashirekha et al., 2002; Baysal et al., 2003). The least growth period 30.33 days was observed when combining of substrate and SBPP with soya cake powder were applied SBPP with rice bran was in the next rank (31.3 days). Moreover, the source of substrate and food supplement was significantly effective on the growth period of oyster mushroom. This effect could be mainly due to growth and development state of mycelium on substrate and the development state of oyster's fruiting bodies. These result indicated that food supplement administrate had it's maximum effect at mycelium run state.

Fruiting body number

The minimum and maximum fruiting body number was pertained to palm fiber (17.6) and boll substrate (28.9), respectively (Table 6). Rice bran supplement and soya cake powder caused to 24.17 and 23.42 fruiting bodies, respectively. The best performance for fruiting bodies was achieved when both substrate and food supplement were applied together. (Combination boll substrate with supplement 34.3 and combination of boll substrate with wheat bran supplement (30)). However, addition of food supplements on boll substrate did not increased fruiting body number which was the only exception for combination usage of substrate and food supplements. Yildiz et al. (2003) also suggested applying combination of substrate and food supplements. Positive effect of food supplementation to substrate could be due to the compensation of nutrient deficiency through food supplements.

Fruiting body weight

The highest average to weight of fruiting bodies was achieved for application of SBPP substrate (28.4 g) and palm fiber (27.7 g). Use of all supplements significantly increased the average weight fruiting bodies related to

Table 6. Combination effect of substrate and food supplement on *P. ostreatus* production factors.

Substrate	Supplement	Fruiting body number	Fruiting body weight	Yield*	Biological efficiency
	Wheat bran	21.3 ^{defg**}	17.9 ^{i}	379.7 ^{ij}	75.9 ^{ij}
	Rice bran	24.7 ^{bcde}	20.3 ^{gh}	501.7 ^{fgh}	100.3 ^{fgh}
	Soya cake powder	18.7 ^{efghij}	19.9 ^{gh}	371.7 ^{ij}	74.3 ^{ij}
Wood chips	Soya cake powder and rice bran	22.7 ^{cdef}	21.7 ^{fgh}	490.0 ^{gh}	98.0 ^{gh}
	Carrot pulp	20.0 ^{efghi}	17.4 ^{hi}	348.0 ^{jk}	69.6 ^{ijk}
	Control	18.^{7efghij}	18.0 ^{hi}	334.0 ^{jk}	66.8 ^{jk}
	Wheat bran	30.0 ^{ab}	21.5 ^{fgh}	643.7 ^{bc}	128.7 ^{bc}
	Rice bran	28.3 ^{abc}	22.9 ^{efgh}	656.3 ^{b}	129.9 ^{bc}
	Soya cake powder	29.0 ^{abc}	20.4 ^{gh}	584.3 ^{bcdefg}	116.9 ^{bcdefg}
Boll	Soya cake powder and rice bran	24.0 ^{bcde}	33.2 ^{bc}	794.3 ^{a}	158.9 ^{a}
	Carrot pulp	27.7 ^{bcd}	20.7 ^{gh}	508.3 ^{fgh}	101.7 ^{fgh}
	Control	34.3 ^{a}	12.9 ^{j}	441.7 ^{hi}	88.3 ^{hi}
	Wheat bran	20.3 ^{efghi}	30.2 ^{bcd}	616.7 ^{bcde}	123.3 ^{bcde}
	Rice bran	25.0 ^{bcde}	22.0 ^{efgh}	550.3 ^{cdefg}	110.1 ^{cdefg}
	Soya cake powder	23.3 ^{bcdef}	25.8 ^{defg}	601.7 ^{bcdef}	120.1 ^{bndef}
Sugar beet pellet pulp	Soya cake powder and rice bran	19.0 ^{efghij}	36.2 ^{ab}	682.0 ^{b}	136.4 ^{efgh}
	Carrot pulp	14.3 ^{hij}	36.1 ^{ab}	518.0 ^{efgh}	103.6 ^{efgh}
	Control	16.7 ^{fghij}	23.1 ^{efgh}	385.0 ^{ij}	77.0 ^{ij}
	Wheat bran	14.7 ^{ghij}	41.5 ^{a}	602.7 ^{bcdef}	120.5 ^{bcdef}
	Rice bran	18.7 ^{efghij}	28.5 ^{cde}	528.0 ^{defgh}	105.6 ^{defgh}
	Soya cake powder	24.7 ^{bcde}	25.5 ^{cdef}	628.3 ^{bcd}	125.7 ^{bcd}
Palm fiber	Soya cake powder and rice bran	21.0 ^{defgh}	28.0 ^{cdef}	588.7 ^{bcdefg}	117.7 ^{bcdefg}
	Carrot pulp	13.7 ^{ij}	23.0 ^{efgh}	313.3 ^{jk}	62.7 ^{jk}
	Control	13.0 ^{j}	19.8 ^{gh}	255.3 ^{k}	51.1 ^{k}

* g per 500 g substrate dry weight basis, ** M.R. mycelium run, P.H. pin head, P.F.B. primary fruiting body formation and C.F.B. complete fruiting body formation. Means with common letters in each column are not significantly different at $P \leq 0.05$.

control group (no supplement). Accordance with the results for growth period and fruiting body number, mixture of food supplements and substrates enhanced the average of fruiting bodies. The combination of palm fiber substrate and wheat bran supplement caused to the maximum weight (41.5 g) among all groups. Mix application at soya cake powder with rice bran also caused high weight of fruiting bodies (24.8 g).

Yield and biological efficiency

Wood chips and boll had the minimum (80.8) and maximum (120.7) biological efficiency among all substrate. SBPP and palm fiber had significantly greater biological efficiency than wood chips group (P≤0.05). All food supplement groups and greater biological efficiency compared to control group. The best biological efficiency among all food supplements belonged to soya cake powder with rice bran (127.8). Yield of wheat bran (112), rice bran (111.5) and soya cake powder (104.3) were moderate with no significant difference between them.

Addition of soya cake powder with rice bran, wheat bran and soya cake powder as supplements to all substrates led to higher biological efficiency related to carrot pulp and no supplements. However, the maximum biological efficiency was obtained for combination of soya cake powder and rice bran with boll substrate (158.4) which was significantly greater than all other combinations.

Royse et al. (2004); Bysal et al. (2003); Royse (2002) reported high biological efficiency for supplement at wheat bran and rice bran to wood chips. Our results not only support that soya cake powder and rice bran supplements to boll, sugar beet pulp and palm fiber substrate could enhance the biological efficiency of *P. ostratus*.

Conclusion

Our findings revealed that the least growth period belonged to the use of SBPP enriched with soybean cake powder. The highest fruiting body number was observed on enriched boll substrate. The highest mean fruiting body weight was obtained on enriched palm fiber supplemented with rice bran. Also, the highest yield and biological efficiency of *P. ostreatus* was seen on the boll substrate enriched with a mixture of soybean cake powder and used as supplement bran. Combination effect of substrate and supplements showed different values from the summation of substrate and supplement main effect, that is, interaction effects between them existed. So, further studies are needed to determine the most appropriate combination for different sources of substrate and supplements.

ACKNOWLEDGMENT

The authors would like to thank Islamic Azad University, Khorasgan Branch, for supporting this project.

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