African Journal of Biotechnology Vol. 9 (19), pp. 2756-2761, 10 May, 2010
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2010 Academic Journals

Full Length Research Paper

Evaluation of various substrates and supplements for biological efficiency of *Pleurotus sajor-caju* and *Pleurotus ostreatus*

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Accepted 23 October, 2009

An experiment was conducted to determine the effects of different substrates namely wheat straw (*Triticum aestivum*), maize stover (*Zea mays* L), thatch grass (*Hyparrhenia filipendula*) and oil/protein rich supplements (maize bran, cottonseed hull [*Gossypium hirsutum*]) on biological efficiency of two oyster mushroom species (*Pleurotus sajor-caju* and *P. ostreatus*). Wheat straw had superior performance over maize stover and thatch grass when cultivating *P. sajor-caju*. However, maize stover was more suitable for *P. ostreatus* than wheat straw. Supplementation with cottonseed hull improved yields when cultivating *P. ostreatus* using wheat straw. These findings suggest that at 25% inclusion rate, farmers should not supplement with maize bran, as this would reduce yields significantly. Further investigations are needed to test both lower and higher rates of inclusion of supplements.

Key words: *Triticum aestivum, Pleurotus sajor-caju, Pleurotus ostreatus.*

INTRODUCTION

Culture of oyster mushroom is becoming popular throughout the world because of their abilities to grow at a wide range of temperatures and to utilize various lignocelluloses (Baysal et al., 2003). *Pleurotus* species have extensive enzyme systems capable of utilizing complex organic compounds that occur as agricultural wastes and industrial by-products (Baysal et al., 2003). For this reason, it is not necessary to process substrates for cultivation of *Pleurotus* species (Khan and Chaudhary, 1987; Yalinkiliç et al., 1994). These mushrooms are also found to be one of the most efficient lignocelluloses solid state decomposing types of white rot fungi (Baysal et al., 2003). Thus, many agricultural and industrial wastes can be utilized as substrates for production of *Pleurotus* species (Zadrazil and Brunnert, 1981; Platt et al., 1983; Platt et al., 1984; Baysal et al., 2003). Zadrazil and Kamara (1997) reported a 300% increase in the yield of *P. sajor-caju* from the addition of either 30% soybean or 40% alfalfa (*Medicago sativa)* meal. Industrial wastes such as apple pomace and chicken manure have also been reported as cheap nitrogen sources for higher mushroom yield with high dry matter content for several *Pleurotus* and *Auricularia* species (Vijay and Upadhyay, 1989). Rinker (1989) found 37 and 42.6% more yield in *P. ostreatus* from supplementation of barley straw with brewer's grain and 17, 27, 65 and 118% more yield by addition of alfalfa hay at 5, 10, 20 and 40% (dry weight basis).

Zimbabwe is an agro-based country and as such, oyster mushroom is produced using plant residues and agricultural waste. Since oyster mushroom is a first decomposer, it assists in the recycling of agro-waste, which would potentially pollute the environment. Mushroom production is therefore a favourable income-generating project for developing countries such as Zimbabwe, from both an environmental and cost point of view. However, in this case, the major challenges in oyster mushroom production appear to be choice of substrates and supplements to achieve high yield as there seems to be a wide range of agricultural crop residues from which

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Table 1. Some chemical constituents of substrates and supplements used in the study.

Properties	Cotton seed hull	Thatch grass	Maize bran	Wheat straw	Maize stover
pH (CaCl)	6.0	5.8	5.0	7.0	6.4
Fat (Ether extract)	2.6	-	9.3	-	-
Organic carbon (%)	47.0	44.0	47.0	40.0	35.0
Nitrogen (%)	0.8	0.4	2.0	1.1	0.4
C:N ratio	58.8	55.0	23.5.	37.3	87.5

Zimbabwean farmers can choose. Media type, quantity and supplementation may affect some substrate qualities such as water holding capacity and degree of aeration; characteristics that subsequently have an effect on mushroom yield (Dietzler, 1997). If the substrate is too tight or too loose, the mycelium will have difficulties in colonizing it. In addition, species of mushroom within the same genus may vary significantly in their nutritional and climate requirements (Choi, 2003).

Maize (*Zea mays*) stover and thatch grass (*Hyparrhenia filipendula*) which are readily available, may be a better and cheaper alternative for growing oyster mushrooms as opposed to the recommended wheat (*Triticum aestivum*) straw, which is generally scarce and expensive as smallholder farmers in Zimbabwe are non-wheat growers. It has been reported that often an addition of a limited amount of supplement to the lignocellulose-based substrates will increase yields (Quinio et al., 1990; Choi, 2003). For the smallholder grower and to the income of oyster mushrooms, the implications of using the ‘inferior’ substrates and supplementing them with protein and oil-rich supplements have not been systematically examined. An experiment was therefore conducted to determine the effects of different substrates and oil/protein rich supplements on biological efficiency of two oyster mushroom species.

MATERIALS AND METHODS

Substrates and preparation

Samples of the substrates were collected as agricultural wastes from smallholder farmers in Zimbabwe after the harvest of the 2006/7 cropping season. The three base substrates; wheat straw, maize stover and thatch grass were then chopped manually into 5 cm lengths before being milled and passed through a 5 mm sieve. The two supplements; maize bran and cotton seed hull were purchased from local millers. The chopped substrates were soaked in clean water overnight in a 200 L drum. Enough water was added to raise the substrate moisture to 75%. The soaked substrate was then boiled for three hours to increase temperature of the substrate to 95°C. The same pasteurization process was applied to the supplements. When pasteurization was completed, samples of the substrates and supplements were analyzed for percent organic carbon, pH, nitrogen and fat as indicators of substrate quality. Organic C and N were determined by dry combustion using a LECO TRUSPEC C/N auto-analyzer (LECO Corporation, 2003) and fat by ether extract. The composition and characteristics of the substrates and supplements is shown in Table 1.

Spawning

The substrates from the pasteurization drum were cooled to a temperature of 24°C under clean and sterile conditions inside the growing room and mixed with supplements before combining with 4% of spawn, based on wet weight. Spawn was purchased from local supply companies. The spawn was thoroughly mixed with the substrate, some being placed underneath the substrate surface and a small amount of spawn sprinkled uniformly on the surface. Each polythene bag contained 10 kg of moist substrate.

Experimental design and data analysis

The factors under study were substrate, supplement type and mushroom variety. The experiment was designed as a 3×3×2 factorial arrangement in a completely randomized design with three replicates per treatment. The three substrates were wheat straw, maize stover and thatch grass; the two supplements were maize bran and cottonseed hull and the two species were *P. ostreatus* and *P. sajor-caju*. All the data collected was subjected to an analysis of variance (ANOVA) using Genstat Release 7.22 DE (2005).

Experimental conditions

After spawning, the bags were moved to a production room where temperature was maintained at 23 - 26°C and relative humidity at 95 - 98%. The temperature and relative humidity ranges were maintained through ventilation and wetting. The first 15 days of spawn run were completed without artificial lighting. At the end of this period, holes were punched on the top of the polythene bags and sides (about 6 cm apart) and light was introduced into the growing room. The surface of substrate was briefly illuminated with a 100-lux lamp to facilitate pinhead formation. At the end of pinning, sufficient fresh air was introduced through ventilation in order to lower CO_2 concentration below 700 ppm. The floors, walls and asbestos roof of the growing room were covered by a black plastic sheet while peat sand was evenly spread on the floor to cover the plastic sheet. The purpose of the plastic sheet on the floor was to prevent the percolation of water applied on the surface to maintain relative humidity and to cool the room.

Determination of mushroom yield and biological efficiency

Mushrooms were harvested from the substrate the same time each day when the in-rolled margins of the basidiomes began to flatten. The substrate clinging to the stipe was cut away and the mush-

Table 2. Interaction among supplements and substrates on BE of *P. sajor-caju.*

Substrates	BE (%)		
	Maize bran supplement	Cotton seed hull supplement	Non- supplemented
Wheat straw	23.4^{de}	82.6^{a}	71.0^{ab}
Maize stover	11.4^{de}	76.4^{a}	40.0^{bcd}
Thatch grass	1.0^{e}	54.0^{abc}	35.4^{cd}
LSD (0.05)	29.0		

Means followed by the same letter are not significantly different.

Table 3. Effect of substrate type and supplementation on BE of *P. ostreatus.*

Substrates	BE (%)		
	Maize bran supplement	Cotton seed hull supplement	No supplement
Wheat straw	49.4^{bc}	70.4^{ab}	45.6^{bc}
Maize straw	23.0^{cd}	52.0^{bc}	97.0^{a}
Thatch grass	15.0^{d}	41.8b^{cd}	48.6^{bc}
LSD (0.05)	29.0		

Means followed by the same letter are not significantly different.

rooms were counted and weighed. At the end of the 40-day harvest period, biological efficiency (BE) was calculated. The BE percentage was calculated using the substrate dry weights as follows: ([weight of fresh mushrooms harvested/substrate dry matter content] × 100) (Royse, 1985).

RESULTS

There was a significant ($p < 0.01$) third order interaction among substrates, supplements and species. There was also a significant ($p < 0.01$) interaction between species and supplements. However, the interactions between supplements and substrates as well as substrates × species were not significant ($p > 0.05$). The main effects of substrates and supplements were significant ($p < 0.01$) whereas the differences between the two species were not significant ($p > 0.05$). Results are presented separately for *P. ostreatus* and *P. sajor-caju* to show interactions between substrates and supplements, in Tables 2 and 3.

When *P. sajor-caju* was cultured without addition of supplements, wheat straw achieved the highest BE while maize stover and thatch grass achieved similar, but lower BE. However, when the substrates were supplemented with cottonseed hull, similar and higher BEs to non-supplemented wheat straw were achieved regardless of substrate type (Table 2). On the other hand, supplementation with maize bran resulted in significantly lower and similar BEs compared to non-supplemented wheat straw (Table 2).

For *P. ostreatus* cultured without supplements, the highest BE was achieved with maize stover. Supplementation with cotton seed hull did not significantly modify the BEs of wheat straw and thatch grass but had a significant yield reducing effect on maize stover. Supplementation with maize bran also had a significant yield reducing effect on both maize stover and thatch grass (Table 3).

Analysis of the changes in BE with a switch from wheat straw (control) to the supposedly inferior substrates indicated that maize straw with no supplement was superior to wheat straw when cultivating *P. ostreatus*. Maize straw resulted in the highest BE increase of 112% (Figure 1). However, supplementation of the wheat straw with cottonseed hull resulted in a significant improvement in BE of 54% (Figure 1).

In the cultivation of *P. sajor-caju*, wheat straw remained the superior substrate, yielding 44 and 50% above maize stover and thatch grass, respectively (Figure 2). Cotton seed hull supplementation did not result in significant yield gains whilst supplementation with maize bran had a significant yield reducing effect in all cases.

DISCUSSION

The effects of physical and chemical properties of substrates on yield and BE have been investigated in *Agrocybe aegerita*, *Volvariella volvacea*, *Pleurotus* spp., *Lentinula edodes* and *Ganoderma lucidum* (Philippoussis et al., 2001; Obodai et al., 2003; Ozcelik and Peksen, 2007; Peksen and Yakupoglu, 2009). Variable ranges of BE have been reported when different lignocellulosic materials were used as substrates for cultivation of oyster

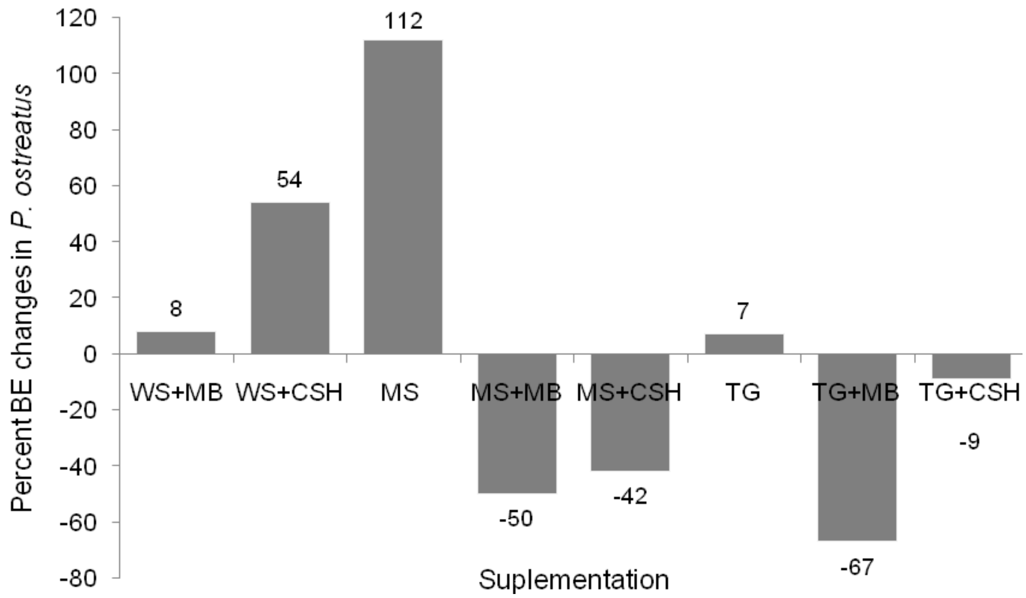

Figure 1. Percentage BE changes of *P ostreatus* in different substrate/supplement combinations compared to wheat straw (X-axis represents wheat straw BE as the basis of comparison). Key: WS = wheat straw; MB = maize bran; CSH = cotton seed hull; MS = Maize stover; TG = thatch grass.

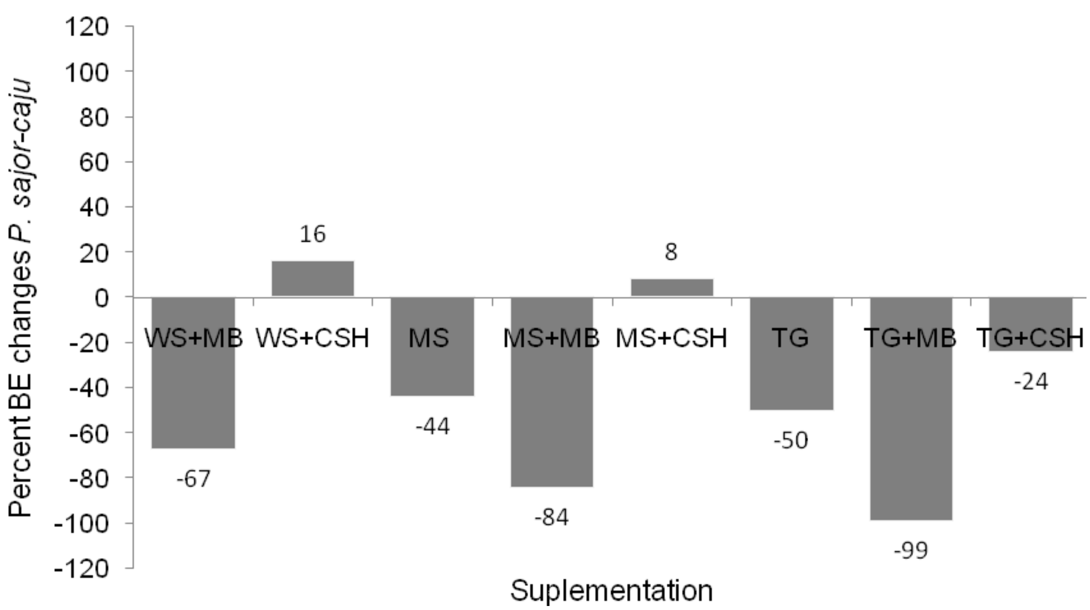

Figure 2. Percentage BE changes of *P. sajor-caju* in different substrate supplement combinations compared to wheat straw (X-axis represents wheat straw BE as the basis of comparison). Key: WS = wheat straw; MB = maize bran; CSH = cotton seed hull; MS = Maize stover; TG = thatch grass.

mushrooms (Liang et al., 2009). This study showed that the composition of substrate has a great influence on yield and BE. These results are consistent with findings by Peksen and Yakupoglu (2009) who reported a positive correlation among yield, N content of substrate and BE. The addition of protein-rich supplements is also a common practice for nitrogen-deficient composts in the cultivation of mushrooms. Various researchers have used supplements from animal and plant origins, including protein-, carbohydrate- or oil-rich substances, for

Agaricus bisporus (Gerits, 1983) and *Pleurotus* species (Gurjar and Doshi, 1995).

Substrate composition analysis showed that the proposed supplements (maize bran and cottonseed hull) were superior to the base substrates over both percentage nitrogen and fat. Since cotton seed hull has a fast decomposition rate, it is generally accepted as a superior substrate over lignocelluloses (Quinio et al., 1990). Cotton seed waste was also found to be a superior substrate in Kenya (Nout and Keya, 1983) and Australia (Choi et al., 1981). These results are consistent with our findings where cottonseed hull supplementation to wheat straw significantly improved yield of *P. ostreatus.* The same yield response was not achieved with *P. sajor-caju*. Such differences in response of the two mushroom species to supplementation is in line with findings by Upadhyay and Vijay (1991) who observed cottonseed meal as a better supplement for *P. fossulatus* and rice bran for *P. ostreatus*. It was also reported by Royse and Schisler (1986) that supplementation is necessary for getting fructification in some strains of *P. eryngii*. Choi (2003) also stated that even different species of oyster mushrooms from the same genus could have different requirements for nutrients.

Following supplementation of supposedly inferior base substrates, there were unexpected BE reductions in some cases. Whilst these results may appear to be confounding, they demonstrate that other factors beyond the scope of this study should come into consideration. These factors include availability of nutrients in these supplements, rather than the total content. Carbon is readily available from cellulose, hemicellulose and lignin from straw, but nitrogen occurs mainly in a bound form and is not available until it is enzymatically released. Some rich nitrogen sources may not yield as expected, such as happened with maize bran. Various researchers have also reported that *Pleurotus* species have the capability to fix atmospheric nitrogen (Bisaira et al., 1987), such that nitrogen-associated yield discrepancies cannot be credited to substrate composition. To further substantiate the above argument, an attempt to correlate nutritional composition (fat, N and C) of substrate/ supplement against BE showed insignificant correlations ($p > 0.05$) among these factors.

The greatest yield reduction effect was experienced when using maize bran as a supplement. Oei (2003) suggests that a supplement can be too rich and increase the risk of contamination, anaerobiosis, antibiosis and subsequently lower yields, in contrast to what would be expected. Dhandha et al. (1995) also observed no change in total mushroom yield in paddy straw mushrooms from the addition of oils of mustard, sunflower, groundnuts and cottonseed at 0.1 to 0.5. They concluded that *Pleurotus* species prefer non-supplemented and unfermented straw. Because of its relatively high starch and oil content, wet maize bran as used in this study is readily fermentable and this might explain the yield reductions.

Supplementation has been reported to cause a rise in substrate temperature, possibly due to faster metabolic activities triggered by extra nitrogen. Royse and Schisler (1986) observed overheating (from 30 to 47°C) in bags where a nitrogen-rich supplement was applied without benomyl (fungicide) treatment and proposed that it could be due to the growth of competitor moulds. Gurjar and Doshi (1995) did not find any effect on yield of *P. cornucopiae* with 5 and 7.5% addition of soybean meal in wheat straw and assumed this could be due to a rise in temperature, the reason why gains in yield were not realized. This suggests that supplements should be cautiously used because excessive bed temperature (more than 35°C) may kill the mycelium. In the current study, the temperatures of the various substrates were monitored and none rose above 30°C. The growing conditions were also maintained at optimal temperatures of 23 - 25°C through ventilation and humidification. Overstijns (1995) observed an increase of 19% in mushrooms with the addition of only 0.5% corn steep liquor and recorded a rise in temperature from 0.3, 1.4 and 2.3°C with the addition of only 0.5, 1 and 2% corn steep liquor. Higher supplement doses gave even higher temperatures, which were harmful and attracted growth of *Coprinus* spp. (Gunasegaran and Graham, 1987).

Conclusions

Wheat straw is a superior substrate over maize stover and common thatch grass when cultivating *P. sajor-caju*. However, maize stover is more suitable for *P. ostreatus*. Supplementation with cotton seed hull at an inclusion rate of 25% can be used to improve yields when cultivating *P. ostreatus* using wheat straw. Further investigations are needed to test both lower and higher rates of inclusion of supplements. However, at 25% inclusion rate, farmers should not supplement with maize bran, as this will reduce yields significantly. It appears also that oyster mushroom species should be an important consideration when selecting the appropriate substrate and/or supplement.

REFERENCES

Baysal E, Peker H, Kemal M, Temiz A (2003). Cultivation of oyster mushroom on waster paper with some added supplementary materials. Bioresour. Technol. 89: 95-97.

Bisaira R, Bisaria VS, Hobson PN (1987). An integrated approach to utilization of agro-residues through *Pleurotus* cultivation. Crit. Rev. Biotechnol. 7 (1): 17-41.

Choi, KY, Nair NG, Bruniges PA (1981). The use of cotton seed hulls for cultivation of *P. sajor-caju* in Australia. Mush Sci. XI: 679-690.

Choi KW (2003). Oyster cultivation: Shelf or bag? Available online at http://www.mushworld.com/tech/view.asp?cata_id=1110&vid=5553. Accessed 14 February 2009.

Dhandha S, Garcha HS, Kakkar VK, Makkar GG (1995). Effect of supplementation of Pleurotus related paddy straw on its nutritive value and cultivative mushroom yield. Mush. Res. 4: 15-22.

Dietzler G (1997). About oyster mushrooms. 2004 greenmuseum. Org. Genstat release 4.24 DE (2005). Lawes Agricultural Trust (Rothamsted Experimental Station), UK.

Genstat release 4.24 DE (2005). Lawes Agricultural Trust (Rothamsted Experimental Station), Hertfordshire, England, UK.

Gerits JPG (1983). New Products for compost supplementation. Mush. J. 126: 207-213.

Gunasegaran K, Graham KM (1987). Effect of organic additives on yield of the Phoenix mushroom grown on cellulose waste. Mush. J. Tropics, 7: 101-106.

Gurjar KL, Doshi A (1995). Effect of substrate supplements on fruit bodies production of Pleurotus cornucopiae (Paul ex Pers.) Rolland. Mushroom Information, 10-12: 12-23.

Khan SM, Chaudhary IA (1987). Some studies on oyster mushroom (*Pleurotus* spp.) on the waste material of corn industry in Pakistan. In: Mushroom Science XII (Part II) Proceedings of the Twelfth International Congress on the Science and Cultivation of Edible Fungi, Braunschweig, Germany.

LECO Corporation (2003). TRUSPEC C/N Determinator. Instruction Manual. LECO Corporation, 3000 Lakeview Avenue. St Joseph, MI49085, USA.

Liang Z, Wu C, Shieh Z, Cheng S (2009). Utilisation of grass plants for cultivation of *Pleurotus citrinopeleatus*. Int. Biodeterior. Biodegrad. 63: 509-514.

Nout MJR, Keya SO (1983). Cultivation of *Pleurotus sajor-caju* in Kenya. Mush. Newslett. Trop. 4(2): 12-15.

Obodai M, Cleland-Okine J, Vowotor KA (2003) Comparative study different lignocellulosic by-products. J. Ind. Microbiol. Biotechnol. 30: 146-149.

Oei P (2003). Mushroom Cultivation-Appropriate Technology for Mushroom Growers. (Third Edition). Backhuys Publishers, Leiden. The Netherlands.

Overstijns A (1995). Influence of corn steep liquor in *Pleurotus* substrate. Mushroom information, pp. 6-11.

Ozcelik E, Peksen A (2007) Hazelnut husk as a substrate for the cultivation of shiitake mushroom (*Lentinula edodes*). Bioresour. Technol. 98: 2652-2658.

Peksen A, Yakupoglu G (2009). Tea waste as a supplement for the cultivation of *Ganoderma lucidum*. World J. Microbiol. Biotechnol. 25(4): 611-618.

Philippoussis A, Zervakis G, Diamantopoulou P (2001) Bioconversion of lignocellulosic wastes through the cultivation of the edible mushrooms *Agrocybe aegerita, Volvariella volvacea* and *Pleurotus* spp. World. J. Microbiol. Biotechnol. 17: 191-200.

Platt MW, Hadar Y, Chet I (1984). Fungal activities. Microbiol. Biotechnol. 20: 150-154.

Platt MW, Hadar Y, Henis Y, Chet I (1983). Increased degradation of lignocellulose by *Pleurotus* in Florida. Eur. J. Appl. Microbiol. Biotechnol. 17: 140-142.

Quinio TH, Chang ST, Royce DJ (1990). Technical guidelines for mushroom growing in the tropics. FAO plant production and protection paper, Rome, p. 65

Rinker DL (1989). Response of the oyster mushroom to supplementation prior to pasteurization. Mush. Sci. 13 (II): 189-198.

Royse DJ (1985). Effect of spawn run time and substrate nutrition on yield and size of the shiitake mushroom. Mycologia, 77: 756-762.

Royse DJ, Schisler LC (1986). Effect of benomyl application and spawn rate supplementation on yield and size of selected genotypes of *Pleurotus* spp. In: Proc. Int. Scientific and Technical aspects of cultivating edible fungi. Penn State University, Elsevier Science Publishers, Amsterdam, pp. 109-115.

Upadhyay RC, Vijay B (1991). Cultivation of *Pleurotus* species during winter in India. In: Maher MJ, ed. Science and cultivation of edible fungi. Mushroom Science XII. Vol. 2. Rotterdam, Netherlands: Balkema. pp. 533-536.

Vijay B, Upadhyay RC (1989). Chicken manure as a new nitrogen supplement in oyster mushroom cultivation. Ind. J. Mycol. Plant Pathol. 19(3): 297-298.

Yalinkiliç MK, Altun L, Baysal E, Demirci Z (1994). Development of mushroom cultivation techniques in Eastern Black Sea Region of Turkey. Project of the Scientific and Technical Research Council of Turkey (TUBITAK), No TOAG-875, p. 287.

Zadrazil F, Brunnert F (1981). Investigation of physical parameters important for the solid state fermentation of straw by white rot fungi. Eur. J. Appl. Microbiol. Biotechnol. 11: 183-188.

Zadrazil F, Kamara DN (1997). Edible mushroom. In: Anke T (ed). Fungal Biotechnology, Chapman and Hall. pp. 14-25.