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The efficacy of sorghum and millet grains in spawn production and carpophore formation of *Pleurotus ostreatus* (Jacq. Ex. Fr) Kummer

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Abstract: Sorghum and millet grains were assessed as single treatments and combined in various proportions to determine their suitability for production of spawns and carpophores of *Pleurotus ostreatus* (Jacq. Ex. Fr) Kummer. The mycelia growth rates on the grains, one of the parameters assessed, were measured from the third to the ninth day of incubation. In addition, for each replicate of the various treatments, the days from inoculation of the bottles till total colonization was recorded. The combination of sorghum and millet grains in a 3:1 (w/w) ratio showed fastest mycelial growth of 16 days followed by sorghum only recording a value of 18 days. These were however not significantly different (P>0.5). The best grain treatments were used as inocula on composted sawdust of *Triplochiton scleroxylon* to compare their yield characteristics. Parameters assessed during fruiting included the number and weight of carpophores obtained, flush number and biological efficiency (BE). No significant difference in BE was observed. Based on the results obtained, for large-scale *P. ostreatus* spawn production, a combination of sorghum and millet grains in a 3:1 ratio would be most appropriate for use as substrate.

Keywords: ***Pleurotus ostreatus*, oyster mushroom, millet, sorghum, spawn**

Introduction

Edible mushrooms are nutritionally endowed fungi (mostly Basidiomycetes) that grow naturally on the trunks, leaves and roots of trees as well as decaying woody materials (Chang and Miles, 1992; Stamets, 2000; Lindequist *et al*., 2005). These edible mushrooms include *Agaricus* spp. (button mushrooms), *Volvariella volvacea* (oil palm mushrooms), *Auricularia auricula* (wood ear mushroom), as well as *Pleurotus ostreatus* (oyster mushrooms).

P. ostreatus, an oyster mushroom, is primarily consumed for its nutritive value and is used industrially as a bioremediator (Solomko and Eliseeva, 1988, Fountoulakis *et al*., 2002, Tsioulpas *et al*., 2002). Nutritionally, the mushroom has been found to contain vitamins B1 (thiamin), B2 (riboflavin), B5 (niacin), B6 (pyridoxine) and B7 (biotin) (Solomko and Eliseeva, 1988). Medically, in Bobek *et al*. (1995; 1998) and Hossain *et al*. (2003), *P. ostreatus* has been reported to decrease cholesterol levels in experimental animals. The carpophore of the mushroom is also a potential source of lignin and phenol degrading enzymes (Fountoulakis *et al*., 2002).

Though these mushrooms grow in the wild, they have been domesticated in most parts of the world to ensure ready availability all year round and to avoid incidences of mushroom poisoning of inexperienced collectors of wild mushrooms. The major practical steps of mushroom cultivation are: (a) selection of an acceptable mushroom species; (b) secreting a good quality fruiting culture; (c) development of active spawn; (d) preparation of selective substrate/ compost; (e) care of mycelial (spawn) running; (f) management of fruiting/ mushroom development; and (g) harvesting mushrooms carefully (Chang and Chiu, 1992; Chang, 1998).

Oyster mushrooms are grown from hyphae (threadlike filaments) that become interwoven into mycelium and propagated on a base of steam-sterilized cereal grain usually sorghum, rye or millet (Royse, 2003). This mycelium-impregnated cereal grain is called spawn and is used to inoculate mushroom substrate (Royse, 2003). Failure to achieve a satisfactory harvest may often be traced to unsatisfactory spawn used (Chang, 2009).

A number of materials, mostly agricultural wastes, can be used to prepare mushroom spawn. The type of waste available varies from region to region. Some of these wastes are chopped rice straw, sawdust, water hyacinth leaves, used tea leaves, cotton wastes and lotus seed husks (Chang, 2009). In most laboratories, cereal grains such as wheat (Elhami *et al*., 2008; Chang, 2009; Stanley, 2010), rye (Chang, 2009), sorghum (Chang, 2009; Stanley, 2010), rice (Oei, 1996), millet (Oei, 1996; Elhami *et al*., 2008; Stanley, 2010) and white maize (Stanley, 2010) are used as mother spawn.

In Ghana, *Pleurotus ostreatus* (Jacq. Ex. Fr) Kummer, strain EM-1, is the most cultivated mushroom (Obodai and Johnson, 2002). The spawns

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of this mushroom has been prepared using sorghum grains as well as composted sawdust of *Triplochiton scleroxylon* (wawa) (Sawyerr and Obodai, 1995; Obodai *et al*., 2002). This report seeks to compare the efficiency of sorghum and millet grains as single substrates, and combinations of these cereal grains in various proportions for the production of spawns of *Pleurotus ostreatus* (Jacq. Ex. Fr) Kummer and to assay the yield and biological efficiency of the mushroom when the spawns are used as inocula on composted sawdust of *T. scleroxylon*.

Materials and Methods

Spawn preparation

The spawns were prepared using a modified form of the method of spawn preparation outlined by Stamets and Chilton (1983). The cereal grains used were sorghum and millet obtained from the Nima Market in Accra, Ghana. The grains were separately washed and steeped overnight in water. They were then thoroughly washed separately with tap water to ensure that dust and other particles had been removed, drained, tied in a wire mesh and steamed for 45 mins in an autoclave (Priorclave, Model PS/LAC/EH150, England) at 105°C to ensure that the steamed grains were cooked but intact. Broken grains are more prone to contamination. Thereafter, they were air-dried to cool on a wooden frame with a wire mesh. To each grain, 3 percent (w/w) of calcium carbonate ($CaCO_3$) was added and thoroughly mixed manually. One hundred and fifty grams (150 g) aliquots of the grains was then weighed into transparent 330 ml narrow mouthed glass bottles, plugged with cotton wool and covered with plain sheets. The sheets were held in place with rubber bands (Plate 1). The bottled grains were sterilised in an autoclave (Priorclave, Model PS/LAC/EH150, England) at 121°C for 1hr.

The treatments used were

- Sorghum (S) only (100% S) – control treatment
- Millet (M) only (100% M)
- Combination of sorghum and millet in a 3:1 (w/w) ratio (75% S+25% M)
- Combination of sorghum and millet in a 1:1 (w/w) ratio (50% S+50% M)
- Combination of sorghum and millet in a 1:3 (w/w) ratio (25% S+75% M)

For each of the combinations, the prepared grains were weighed and properly mixed together manually according to the specified ratios. There were five replicates for each treatment.

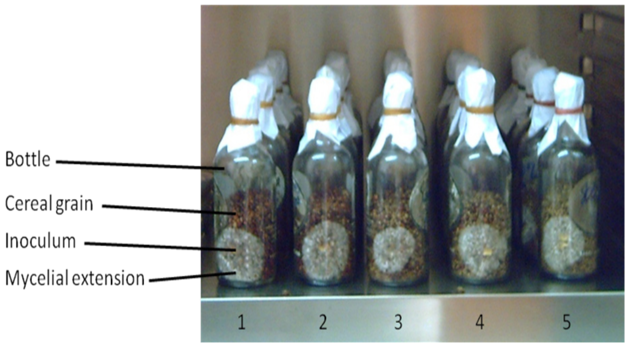

Plate 1. Mycelial growth on grains on the 7th day of incubation
Legend
1 – 100% S, 2 – 75% S+25% M, 3 – 50% S+50% M, 4 – 25% S+75% M and 5 – 100% M

Inoculation and incubation of grains

One-week-old pure tissue cultures of *Pleurotus ostreatus* (Jacq. Ex. Fr) Kummer, strain EM-1, were obtained from the National Mycelium Bank at the CSIR- Food Research Institute in Ghana. Each of the bottled sterilized grains was aseptically inoculated with one $1cm^2$ of the one-week-old tissue culture of the experimental strain grown on Malt Extract Agar (OXOID™ Ltd., Basingstoke Hampshire, England) using a flamed and cooled scalpel in a laminar flow hood. Thereafter, the spawns were incubated for 16-21 days without illumination in an incubator (Tuttlingten™ WTC Binder, Germany) set at 28°C (Plate 1).

Bag preparation and mushroom cultivation

Compost bags were prepared as described by Obodai *et al*., (2002) using composted sawdust of T. scleroxylon as substrate and inoculated with the best grain combination (i.e. 75% S+25% M) and the control treatment (i.e. 100% S). Five each of the bags were inoculated with 5 g of spawns produced with a 3:1 (w/w) combination of sorghum and millet as substrate (75% S + 25% M) and spawns produced with sorghum only as substrate (100% S). The bags were labelled HC and OC respectively. The compost bags were placed horizontally on shelves in a cropping house for 2 months (December 2009 and January 2010) and fruited according to Obodai and Johnson (2002).

Assessment

Determination of moisture content and pH of the treatments

Dry weight of the sterilized treatments was determined by drying 5 g of each treatment at 103°C for 4 hrs in a hot oven (Gallenkamp oven, 300 plus series, England). The moisture contents of the treatments were calculated using the formula below:

Moisture content = [(Initial weight – Dry weight)/ Initial weight] x 100%

The acidity of the treatments was also measured by steeping 5 g of the prepared grains in 100 ml of distilled water for 1hr and using a pHM92 Lab pH meter (MeterLabTM, Radiometer Analytical A/S, Copenhagen, Denmark) to measure the pH.

Mycelial growth on grains

At 24-hour intervals, the mycelial radial growth was determined. This was carried out over a 7-day period from the third day of incubation. The length of mycelia (from the edge of the inoculum to the edge of the mycelia) was measured at right angles with a ruler and the average of the two readings per replicate, recorded. Parameters determined included the radial growth rate, the days till total colonization and the mycelial density.

Mushroom Cultivation

Data recorded included the spawn run period ie. the number of days from inoculation to complete colonization of the compost bag by the mycelia, mycelial density (taken by direct observation), number of days taken till appearance of pinheads and the number of flushes per treatment. The days from bag opening to first flush, weight and number of carpophores per flush, weight and number of carpophores per bag, interval between flushes (the average number of days that lapses between consecutive flushes) and the biological efficiency (BE) were also determined. BE values were calculated in accordance to Royse *et al*. (2004).

B.E. = [Weight of fresh mushrooms harvested / dry weight of substrate] x 100.

Dry weight of substrate = [(100 - % moisture content of substrate)/100] x Fresh weight of substrate.

A Digital Computing Scale (Hana Electronics Company Limited, Korea) was used to take all weight measurements and the unit for measurement was in grams (g).

Statistical analysis

Data analysis was conducted by the separation of means by Fischer's Least Significant Difference (LSD). Values reported are the means and standard errors of all analysis. There were five replicates for each analysis. Statistical significance was set at P≤0.05.

Results and Discussion

pH and moisture content of treatments

The pH of the combination of sorghum and millet grains in a 3:1 w/w ratio (75% S+25% M) and 1:1 w/w ratio (50% S+50% M) were 4.87 and 4.69 respectively (Table 1). These pH values were lower than the optimum pH range of 5.5-6.5 for culture media for *P. ostreatus* production stated by Stamets (2000). The pH of the other grain treatments at 25ºC which ranged from 5.12-5.89 (Table 1), were not significantly different from each other and were generally within the optimum range. While the pH of the fresh sorghum reduced from 5.25 to 5.12 after preparation, that of millet increased slightly from 5.87 to 5.89 (Table 1).

Table 1. Mean pH at 25ºC and moisture content of substrates and sterilized treatments

Treatment	pH at 25ºC	% Moisture Content
Fresh Sorghum	5.25	15
Fresh Millet	5.87	10
100% S	5.12	35
75% S+25% M	4.87	35
50% S+50% M	4.69	35
25% S+75% M	5.66	35
100% M	5.89	40
Composted Sawdust of *T. scleroxylon*	7.31	60

The moisture content of the various treatments were also not significantly different and ranged between 35-40% (Table 1). Percentage increases of 133 and 300 in the moisture content between the fresh grains (sorghum and millet) and the prepared grains (100% S and 100% M respectively) were observed. Excess moisture in spawn substrate has been seen to inhibit mycelial growth within the substrate. It has been observed that where the excess water sets at the bottom of the substrate, the mycelia colonizes the substrate just to the level of the water. A higher contamination rate by bacteria has also been observed where there is excess moisture. Various reports (Golueke, 1992; Tiquia *et al*., 1996) state that while a moisture content below 30% decreases microbial activity causing microorganisms to become dormant, a moisture content above 65% causes oxygen depletion and nutrient loss through leaching. Hence, a moisture content ranging between 30-40% would be appropriate when cereal grains such as sorghum and millet are being used as substrate for EM-1 spawn production.

Spawn growth

The mycelial growth of the *P. ostreatus* on the grains showed a steady linear growth (Figure 1). The mycelial growth rates on sorghum only (100% S) and the combination of sorghum and millet in a 3:1 w/w ratio (75% S+25% M) were not significantly different (P>0.5) from each other but were significantly different from the mycelial growth rate seen on all the other treatments during the period of incubation (Figure 1). Treatment 3 (75% S+25% M), however, generally showed a higher growth rate compared to 100%S (Figure 1). The mycelial growth rates were also not significantly different (P>0.5) among the combination of sorghum and millet in a 1:1 and 1:3 w/w ratio (50% S+50% M and 25% S+75% M respectively) and the millet only (100% M) treatments (Figure 1).

Larger surface area and pore of substrates support faster mycelium growth rate (Tinoco *et al*., 2001). This could account for the significant difference between the mycelial growth rates recorded for the sorghum only and the millet only treatments. Sorghum grains have a larger surface area compared to millet grains. Since smaller particles are generally more compact than larger particles, 100% S would have larger air spaces than 100% M. This increased ventilation within the sorghum only treatment resulting in improved respiration by the mycelia. Respiration rate is directly related to O_2 concentration of substrate (Mehravaran, 1993). Hence, the significantly higher growth rate of mycelia in the 100% S treatment compared to the 100% M treatment.

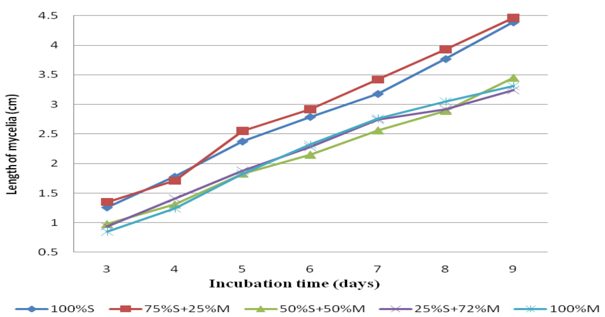

Figure 1. Mean radial mycelial growth per treatment per day

Total colonization of grains and mycelial density

The number of days from inoculation to the total colonization of a substrate is related to the mycelial growth rate on the substrate. A faster growth rate results in a corresponding reduction in the days required for complete colonization of the substrate by the mycelia (Figure 1 and Table 2). The days until total colonization of the grains by the mycelia varied for the various treatments (Table 2), these differences were not significantly different (P>0.5).

The period required for total colonization could be reduced significantly if the bottles are shaken after the fifth day of incubation, when there is considerable mycelia growth (mycelial diameter of about 4 cm). This would agitate and break the mycelia into fragments, redistribute the fragments of mycelia within the substrate, and hence serve as more inocula within the substrate. Eventually, there would be various points of growth of the mycelia within the substrate, resulting in full substrate colonization within a shorter period. Among the various treatments, there were no notable differences in the density of mycelia (Table 2). The mycelia were dense on all the treatment.

Table 2. Total colonization of grains and mycelia density

Parameters	Total colonization(days)	Mycelial density*
100% S	18[a]	++
75% S+25% M	16[a]	++
50% S+50% M	20[a]	++
25% S+75% M	20[a]	++
100% M	21[a]	++

Values in the same column followed by a common letter do not differ significantly (P>0.05)
*: Degree of mycelial density when mycelia fully colonize the substrate
++: Mycelium totally grows through the bottle and is uniformly white

Mushroom yield

The best grain combination for the *P. ostreatus* spawn production (3:1 ratio: (75% S+25% M) was compared with the control grain treatment (100% S) to determine differences, if any, in their yield characteristics when fruited on composted *T. scleroxylon* sawdust. The compost bags inoculated with spawns produced with the 3:1 (w/w) combination of sorghum and millet (HC) were fully colonized within an average of 32 days whereas those inoculated with spawns produced with sorghum only (OC) were fully colonized within an average of 36 days (Table 3). A range of 12 to 41 days has been reported as the spawn running period for various *Pleurotus* species on composted or non-composted substrates (Baysal *et al*., 2003; Obodai *et al*., 2003; Royse *et al*., 2004; Shah *et al*., 2004; Tisdale *et al*., 2006; Mane *et al*., 2007).

Both the HC and OC compost bags had 6 days from bag opening to first flush and 14 days as the interval between flushes (Table 3). An interval between flushes of 10 days has been recorded for *Pleurotus* spp. cultivated on sawdust (Mandeel *et al.*, 2005). The specific wood from which the sawdust is obtained and the pre-treatment given to it can result in varying growth characteristics of mushrooms when the sawdust is used as substrate for mushroom cultivation. Four flushes were obtained from the bags from both treatments within the period (Table 3). Shahid *et al*. (2006) have recorded four to six flushes for *P. sajor-caju* cultivated on wheat straw given various pre-treatments. The mean number of carpophores per bag obtained within the cropping period and mean weight of each carpophore obtained from the HC compost bags were 34 carpophores and 4.85 g respectively whereas those for the OC compost bags were 37 carpophores and 5.12 g respectively (Table 3).

Though biological efficiencies (BEs) obtained in this study (50.86% and 59.84% for HC and OC respectively) were comparable to the BE of 59.6% for *P. ostreatus* cultivated on white sawdust (Mandeel *et al.*, 2005), none of the results for the parameters observed for the two treatments varied significantly (Table 3). This implied that the two spawn types cultivated on the composted *T. sceroxylon* sawdust are comparable in their yield characteristics. However, significantly different yields have been reported when spawns produced with various grains (corn, millet, rice, sorghum and wheat) were used to cultivate *Lentinus subnudus* and *Lentinula sqarrosulus* (Fasidi and Kadiri, 1993; Nwanze *et al*., 2005). Also, Pathmashini *et al*. (2008) recorded significant differences in BEs for *P. ostreatus* spawns produced with sorghum (*Sorghum bicolar*), kurakkan (*Eleusine coracana*), maize (*Zea mays*) and paddy (*Oryza sativa*) and separately fruited on sawdust of mango supplemented with rice bran, chalk and epson. BE values obtained were 25.38%, 30.76%, 16.57% and 11.99% respectively. These results indicate that the grain used for spawn production has a significant effect on the carpophore yield of the mushrooms. Subsequent work will be carried out to ascertain this observation using other cereal grains as substrate for *P. ostreatus* strain EM-1 spawn preparation.

Table 3. Effect of spawn inoculum on the growth characteristics and yield of *P. ostreatus* strain EM-1

Parameters	Treatments	
	HC[1]	OC[2]
Spawn run period (days)	32±1.500	36±1.562
Days taken till appearance of pinheads (days)	3±0.200	4±0.000
Days from bag opening to first flush (days)	6±0.490	6±0.490
Weight of carpophores per flush (g)	40.7±0.632	47.8±0.274
Weight of carpophores per bag (g)	162.8±19.891	188.5±10.384
Weight of single carpophore per treatment	4.84±0.632	5.12±0.274
Number of carpophores per flush	6±0.294	7±0.647
Number of carpophores per bag	34±1.806	37±3.308
Flush number	4±0.000	4±0.000
Interval between flushes (days)	14±1.208	14±0.600
Biological Efficiency (%)	50.86%±6.216	59.84±3.296

Values recorded are mean ± standard error
[1]HC: Compost bags inoculated with spawns produced with combination of sorghum and millet in a 3:1 (w/w) ratio
[2]OC: Compost bags inoculated with spawns produced with sorghum only

Conclusion

The combination of sorghum and millet in a 3:1 ratio (75% S+25% M) is the best combination of sorghum and millet grains for the production of *P. ostreatus* strain EM-1 spawns. As single substrates, sorghum is more suitable for the production of EM-1 spawns compared to millet. No significant differences (P>0.05) were observed in the spawn run period, days till total colonization and yield of carpophores formed using these different spawn types.

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References

Baysal, E., Peker, H., Yilinkilic, M. K. and Temiz, A. 2003. Cultivation of oyster mushrooms on waste paper with some added supplementary materials. Bioresource Technology 89: 95-97.

Bobek, P., Ozdin, O. and Mikus, M. 1995. Dietary oyster mushroom (*Pleurotus ostreatus*) accelerates plasma cholesterol turnover in hypercholesterolaemic rats. Physiological Research 44: 287–291.

Bobek, P., Ozdin, L. and Galbavy, S. 1998. Dose- and time-dependent hypercholesterolaemic effect of oyster mushroom (*Pleurotus ostreatus*) in rats. Nutrition 14: 282–286.

Chang, S. T. 1998. Development of novel agroscience industries based on bioconversion technology. In Chou, C. H. and Shao, K. T. (Eds). Frontiers in Biology: The Challenges of Biodiversity, p. 217-222. Taipei: Academia Sinica,.

Chang, S. T. 2009. Training Manual on Mushroom Cultivation Technology, United Nations -Asian And Pacific Centre For Agricultural Engineering And Machinery (UN-APCAEM), Beijing, China.

Chang, S. T. and Chiu, S. W. 1992. Mushroom production—an economic measure in maintenance of food security. In DaSilva, E. J., Ratledge, C. and Sasson, A. (Eds). Biotechnology : Economic and social Aspects, p 110-141. USA: Cambridge University Press.

Chang, S. T. and Miles, P. G. 1992. Mushrooms biology—a new discipline. Mycologist 6: 64–65.

Elhami, B. and Ansari, N. A. 2008. Effect of substrate of spawn production on mycelium growth of oyster mushroom species. Journal of Biological Sciences 8(2): 474-477.

Fasidi, I. O. and Kadiri, M. 1993. Use of agricultural wastes for the cultivation of *Lentinus subnudus* (Polyporales: Polyporaceae) in Nigeria. Revista de Biologia Tropical 41(3): 411-415.

Fountoulakis, M. S., Dokianakis, S. N., Kornaros, M. E., Aggelis, G. G. and Lyberatos, G. 2002. Removal of phenolics in olive mill wastewaters using the white-rot fungus *Pleurotus ostreatus*. Water Research 36: 4735-4744.

Golueke, C. G. 1992. Bacteriology of composting. Biocycle 33: 55-57.

Hossain, S., Hashimoto, M., Choudhury, E., Alam, N., Hussain, S., Hasan, M., Choudhury, S. and Mahmud,

I. 2003. Dietary mushroom (*Pleurotus ostreatus*) ameliorates atherogenic lipid in hypercholesterolaemic rats. Clinical and Experimental Pharmacology and Physiology 30: 470.

Lindequist, U., Niedermeyer, T. H. J. and Julich, W. 2005. The pharmacological potentials of mushrooms. eCAM 2: 285–299.

Mandeel, Q. A., Al-Laith, A. A. and Mohamed, S. A. 2005. Cultivation of oyster mushrooms (*Pleurotus* spp.) on various lignocellulosic wastes. World Journal of Microbiology and Biotechnology 21: 601-607.

Mane, V. J., Patil, S. S., Syed, A. A. and Baig, M. M. V. 2007. Bioconversion of low quality lignocellulosic agricultural wastes into edible protein *Pleurotus sajor-caju* (Fr.) Singer J. Zhejiang. Universal Science Biology 8: 745-751.

Mehravaran, H. 1993. Principles of fungi. Urmiah University Publication. p 533.

Nwanze, P. I., Khan, A. U., Ameh, J. B. and Umoh, V. J. 2005. The effect of spawn grains, culture media, oil types and rates on carpophore production of *Lentinus squarrosulus* (Mont.) Singer. African Journal of Biotechnology 4(6): 472-477.

Obodai, M. and Johnson, P-N. T. 2002. The effect of nutrient supplements on the yield of *Pleurotus ostreatus* mushroom grown on composted sawdust of *Triplochiton scleroxylon*. Tropical Science 42: 78-82.

Obodai, M., Cleland-Okine, J., Awotwe, B., Takli, R. and Dzomeku, M. 2002. Training manual on mushroom cultivation in Ghana. pp 16-19. Technical manual of the CSIR-Food Research Institute

Obodai M., Cleland-Okine, J. and Vowotor, K. A. 2003. Comparative study on the growth and yield of *Pleurotus ostreatus* mushroom on different lignocellulosic by-products. Journal of Industrial Microbiology and Biotechnology 30: 146-149.

Oei, P. (1996). Mushroom cultivation with special emphasis on appropriate techniques for developing countries. CTA, The Netherlands.

Pathmashini, L., Arulnandhy, V. and Wilson-Wijeratnam R. S. 2008. Cultivation of oyster mushroom (*Pleurotus ostreatus*) on sawdust. Ceylon Journal of Science (Biological Sciences) 37 (2): 177-182.

Royse, D. J. 2003. Cultivation of Oyster Mushrooms. Pennsylvania State University Press, Pennsylvania.

Royse, D. J., Rhodes, T. W., Ohga, S. and Sanchez, J. E. 2004. Yield, mushroom size and time to production of *Pleurotus cornucopiae* (oyster mushroom) grown on switch grass substrate spawned and supplemented at various rates. Bioresource Technology 91: 85-91.

Sawyerr, L. C. and Obodai, M 1995. Physical characteristics of *Pleurotus* species of the National Mycelium Bank, Food Research Institute. Food Research Institute Technical Report.

Shah, Z. A., Ashraf, M. and Ishtiaq, Ch. M. 2004. Comparative study on cultivation and yield performance of oyster mushrooms (*Pleurotus ostreatus*) on different substrates (wheat straw, leaves and saw dust). Pakistan Journal of Nutrition 3: 158-160.

Shahid, M. N., Abbasi, N. A. and Saleem, N. 2006. Effect of different methods of compost preparation and lime concentration on the yield of *Pleurotus sajor-caju*. International Journal of Agriculture and Biology 8(1): 129-131.

Solomko, E. F. and Eliseeva, G. S. 1988. Biosynthesis of vitamins B by the fungus *Pleurotus ostreatus* in a submerged culture. Prikladnaia Biokhimiia i Mikrobiologiia 24(2): 164-169.

Stamets, P. 2000. Growing Gourmet and Medicinal Mushrooms. 3rd edn. California, Berkley: Ten Speed Press.

Stamets, P. and Chilton, J. S. 1983. The Mushroom Cultivator: A practical guide to growing mushrooms at home. Olympia, Washington: Agarikon Press.

Stanley, H. O. 2010. Effect of substrates of spawn production on mycelial growth of oyster mushroom species. Agriculture and Biology Journal of North America 1(5): 817-820.

Tinoco, R., Pick, M. A. and Duhalt, R. V. 2001. Kinetic differences of purified laccases from six *Pleurotus ostreatus* strains. Letters of Applied Microbiology 32: 331-335.

Tisdale, T. E., Miyasaka, S. C. and Hemmes, D. E. 2006. Cultivation of the oyster mushroom (*Pleurotus ostreatus*) on wood substrates in Hawaii. World Journal of Microbiology and Biotechnology 22: 201-206.

Tiquia S. M., Tam, N. F. Y. and Hodgkiss, I. J. 1996. Microbial activities during composting of spent pigmanure sawdust litter at different moisture contents. Bioresource Technology 55: 201-206.

Tsioulpas, A., Dimou, D., Iconomou, D. and Aggelis, G. 2002. Phenolic removal in olive oil mill wastewater by strains of *Pleurotus* spp. in respect to their phenol oxidase (laccase) activity. Bioresource Technology 84: 251-257.