Proceedings of the International Conference on Agro-Biotechnology, Biosafety and Seed Systems in Developing Countries

www.scifode-foundation.org

Bioconversion potential of common agricultural lignocellulosic wastes

I. Nakalembe[a]* and J. Wong[b]

[a]*Department of Physiological Sciences, Faculty of Veterinary Medicine, Makerere University, P.O. Box 7062 Kampala, Uganda.*
[b]*State Key Laboratory for Agrobiotechnology, China Agricultural University, Beijing 100293, China.*
[*] *Corresponding author: e-mail, immynakalembe@vetmed.mak.ac.ug*

ABSTRACT

With increasing interest in organic farming in the developing world, the potential use of common agricultural wastes in wild *Pleurotus* species cultivation in Mid-Western Uganda was investigated. Growth rate, yield performance and nutrient contents of the mushroom were investigated on different agricultural wastes with and without supplementation of rice bran or molasses. Sugarcane bagasse (24.7days) gave minimum time throughout the growth period, then rice hulls (34.3 days) and coffee husks (35 days). Maximum mushroom growth period was observed on rice straws (44.7 days) and saw dusts (47 days). Organic supplementation improved growth rate, yield performance and nutrient composition of the mushroom. Molasses was the best supplement for growth and rice bran in addition, enhanced yield performance. Organic supplementation of soybean straws and saw dusts exhibited drastic results on growth rate. The cultivated *Pleurotus* species had significant nutrient profile compared to the wild species. Crude protein was significantly high ($p<0.05$) for cultivated mushrooms with supplementation, relatively high mineral contents (K, P and Mg) and no significant change on crude lipid and fatty acids. Bioconversion of lignocellulosic biomass by mushroom cultivation may increase productivity of high quality food, a solution to malnutrition and food security in the country.

Keywords: *Growth rate and yields, mushroom, Pleurotus species, nutrition, supplementation.*

Received*: 6 December 2010,* ***Accepted****: 28 November 2011*

Introduction

Unlike green plants, mushrooms lack chlorophyll and are unable to produce their own food but derive all their energy and growth materials from their growth medium, through biochemical (decomposition) processes. Mushrooms serve as the most efficient and economically living organisms that can convert lignocellulosic materials into high quality food and feed rich in protein (Vijay *et al*,. 2007). They can grow well on all crop residues such as cereals and legumes, corncobs, tree leaves, sawdust, coffee wastes, banana leaves, bagasse, cotton wastes, brewer's wastes, papyrus reeds and elephant grass (Quimio, 1986). The spent wastes can be reused in agriculture and agroforestry for soil conditioning, as fertilisers or as animal fodder as well as reducing environmental pollution (Zadražil, 1993).

Uganda being an agricultural country has a big potential to produce mushrooms due to availability of large quantities of agro residues. Unfortunately, these agro wastes are generally low in nutrients required for mushroom production. Supplementation of these wastes with the required nutrients such as nitrogen or a combination of one or more substrates is a crucial factor for growth and yield of mushrooms (Royse, 2002). This being the case, however, it remains unclear why the world mushroom thrive without supplementation and hence the need for robust data to guide decisions on supplementation in the production of edible mushrooms. In this study, therefore, growth performance, yield and nutrient composition of wild *Pleurotus ostreatus* on supplemented and main substrates compared to the edible wild *Pleurotus* species were established.

Uganda has a rich diversity of edible and medicinal mushrooms like *Armillaria mellea*, *Lentinus prolifer*, *Termitomyces aurantiacus*, *Termitomyces eurrhizus*, *Pleurotus* species and *Termitomyces microcarpus* which grow in most parts of the country (Katende *et al.*, 1999, Opige *et al.*, 2006, Engola *et al.*, 2006). These mushrooms constitute a traditionally very important nutritious food besides having broad ritual and medicinal acceptance (Kamatenesi *et al.*, 2006, Opige *et al.*, 2006, Nakalembe *et al.*, 2009). Mushrooms contribute to the welfare of some households when they are sold to earn an additional income (Nakalembe *et al.*, 2009). *Pleurotus*

species are grown on small-scale basis, specifically to improve on the socio-economic status of farmers, and they can easily be cultivated on various agricultural wastes (Igbal *et al.*, 2005, Vijay *et al.*, 2007, Pushpa and Manonmani, 2008) with minimal input to give high yield of valued food protein for direct human consumption. In this study, six common main substrates namely: rice straws, rice hulls, saw dusts, coffee husks, sugarcane bagasse and soybean straws were selected to examine their bioconversion potential through determination of the growth rate and yield performance of Ugandan edible wild Pleurotus species as well as the nutrient composition of the fruit bodies.

Materials and Methods

Wild Pleurotus species was obtained from Mid-Western Uganda, in the Albetine region. Identification of this species of mushroom was done locally by the indigenous people and then identified up to genus level by Dr. Ipulet Perpetua of the department of Botany, Makerere University. A fresh and healthy mushroom provided the required cultures through tissue culturing. The cultures were maintained on acidified potato dextrose agar (PDA). A small sterile tissue (3x3mm) was obtained from inside of the stalk near the veil of the mature, fresh and health mushroom with a sterile blade and inserted onto the sterile agar under the laminar flow hood.

The cultures were incubated at room temperature. Radial growth of mycelium of different portions was observed everyday until the Petri dishes were approximately 90% filled with mycelia. Subculturing was done three times on PDA until a pure culture was obtained. Spawn preparation was carried out on millet grain in a small-necked glass flask. Dried and clean grains (200 g) of millet were boiled for 10-15 min. The excess water was removed leaving the grains with approximately 60% of moisture content. Then the grains were spread on newspapers for cooling and mixed with 2% of lime (to improve and maintain pH) and gypsum (to prevent stickiness and absorb excess moisture) before sterilization in flasks at 15 psi at 121 ºC for 45 minutes. The sterilized grains were inoculated aseptically with small squares of 4x4mm mycelial culture from the full-grown pure agar culture and incubated at room temperature until mycelium fully covered the grains.

The dried substrates were chopped into small pieces of 2-3cm, with exception of sugarcane bagasse, weighed and soaked in water overnight before boiling them for one hour. The substrates were spread over a clean, inclined surface for cooling and draining off the excess water. Half a kilogram of each substrate (about 65-70% of moisture content) was packed in polythene bags before sterilization in an autoclave at 15 psi for 45 min. Some bags of each main substrate were supplemented with 10% of sterile molasses by weight (with exception of sugar baggasse) or 38% of rice bran alone by weight in dry form. Three replicates of each substrate were also prepared. The bags were inoculated with the pure grain spawn of Pleurotus species at the rate of 20g/kg of substrate dry weight. The inoculated bags were placed in a dark room with 65-70% humidity, temperature of (27±2)°C, and good ventilation for spawning run. Time of spawning run i.e. the time mycelia is seen in the substrate to full growth and that of pin-heads starting to appear were recorded daily for all treatments. The bags were mouth opened and slits made on their sides to facilitate development of fruit bodies. Watering of the mushrooms was done three times by hand spraying and discontinued a day prior harvest of the fruiting bodies. The time taken for the maturity of fruiting bodies was recorded while the mushrooms harvested in three flushes on each substrate with or without supplementation were weighed in g/kg substrate and the maximum average yield estimated from each substrate.

Chemical compositions of all fruit bodies with or without supplementation were determined. Proximate compositions were performed in accordance with the official Methods of Analysis of the Association of Official Analytical Chemists, AOAC (2002). Minerals were determined using an atomic absorption spectrophotometer and a flame photometer (AOAC, 2002) while fatty acids were determined by AOAC Official Method Ce-2-66 (Modified) Gas-liquid chromatography). The content of total carbohydrates was calculated using the following formula: Total carbohydrates (g/100g fresh weight) = 100 - moisture (g/100g fresh weight) - protein content (g/100g fresh weight) - crude fat (g/100g fresh weight) - ash (g/100g fresh weight). Data were subjected to a one-way ANOVA test and differences between means were detected using the t-test and least significant differences (LSD) at the 95% confidence level. All analyses were computed using SPSS 12.0 Windows program (SPSS Inc., 2003, Chicago, IL, USA).

Results and discussion

The growth rate of the cultivated Pleurotus species was determined using the spawn running time, pin-head-formation time and maturation time. The spawn running ranged from 16-40 days on the main substrates (Tables 1-3). The spawn running completed earlier on sugarcane bagasse at 16th day followed by rice hulls (24 days) and coffee husks (26 days). Maximum time was observed on saw dusts and rice straws at 40.3 days and 35 days, respectively. This could be attributed to the fact that the substrate needed to undergo fermentation due to tough lignin mainly in the saw dusts. Shah *et al.*, (2004) reported spawn running time of 17.33 days for Pleurotus species on fermented saw dusts while Iqbal *et al.*, (2005) reported spawn running time of 14 days on sugarcane bagasse.

Substrate supplementation significantly reduced the spawn running time in all treatments. The best supplementation was observed with molasses (15-29 days) compared to 17-32 days of rice bran supplementation. Supplementation of soybean straws and saw dusts tremendously decreased the days of spawn running with the minor decrease on sugarcane bagasse (Table 1).

Table 1 Time (days) taken for completion of spawn running on different agricultural wastes

Agricultural wastes	Supplementation		
	Main substrate	Rice bran	Molasses
2Rice grass	35.3	30.3	24
2Rice hulls	24.7	18.7	16.3
1Saw dusts	40.3	32.3	29
2Coffee husks	26	20.3	17.7
Sugarcane baggasse	16	14	ND
1Soybean straws	32.3	17	15

ND, not done, [1]tremendous decrease in time (days) on supplementation;

[2]moderate decrease in time (days) on supplementation

The same trend as in spawn running was observed in the case of pin-head formation for all treatments. Sugarcane bagasse was the best main substrate whereby 20 days were required for the first appearance of pin-heads after spawning of the substrate. This was followed by rice hulls (28.3 days). Longer time was taken for the appearance of pin-heads on rice grass and saw dusts with 39 and 43 days, respectively. Several studies report different timings of pin-head formation of *Pleurotus* species on various substrates. For instance, Iqbal *et al.*, (2005) reported time for pin-head formation ranging from 43-47 days and 16-23 days on wheat straw and sugarcane bagasse, respectively; while Kirbag and Akyuz, 2008 reported 26.2 days on wheat straw, 2-4days on composted sisal decortications residue (Mshandete and cuff, 2008) and 11.3days on paddy straws (Jansi, 2010).

The best main substrate to give drastic results for pin-head formation after supplementation was soybean straws (Table 2). *Pleurotus* species mushrooms attained maturity at an early time on sugarcane bagasse (24.7 days) and maximum on rice straws (44.7 days) and saw dusts (47 days). A minimum time of maturation of fruiting bodies (20.3, 22 and 37 days) for *Pleurotus* species was reported by Iqbal *et al.*, (2005) on sugarcane bagasse while the first fructification on coffee husks started on the 20 days. Supplementation of main substrates significantly reduced the maturation time in all substrates. Soybean straws supplemented with molasses exhibited drastic results on all stages of mushroom growth (Table 3). This shows that supplements modified the substrates for better conversion and utilization of the substrates. This observation concurs with Zadražil (1993) report that supplements usually change the decomposition rate and the sequence of decomposition of substrate components during mushroom growth. Zadražil, (1980) also observed that organic supplements such as soybean meal, alfalfa meal, and cotton seed powder increase not only yields but also proteins of mushrooms. Okeke *et al*., (1994) also observed that high levels of soluble protein provide greater biomass in mushroom cultivation. Therefore, the stimulating effects of rice bran may be due to the presence of high levels of carbohydrates, amino acids and minerals in the supplement (Fasidi and Kadiri, 1993) whereas the presence of the additional sugars in molasses facilitated degradation of lignocelluloses in the main substrates, and hence their utilization by the growing mushrooms. Molasses, in addition, provides relative levels of nitrogen, minerals, protein and non-nitrogenous acids (Paterrson-Beedle *et al.*, 2002) needed for the growth of the fungus.

Table 2 Time (days) taken for completion of pin-head formation on different agricultural wastes

Agricultural wastes	Supplementation		
	Main substrate	Rice bran	Molasses
2Rice grass	39	34	27
2Rice hulls	28.3	22	20
2Saw dusts	43	36	33
2Coffee husks	30.7	24	22
Sugarcane baggasse	20	19	ND
1Soybean straws	35.7	21.7	17

ND, not done, [1]tremendous decrease in time (days) on supplementation;

[2]moderate decrease in time (days) on supplementation

Table 3 Time (days) taken for the fruit bodies to attain maturity on different agricultural wastes

Agricultural wastes	Supplementation		
	Main substrate	Rice bran	Molasses
Rice grass	44.7	38.7	31
Rice hulls	34.3	27	23.7
Saw dusts	47	40.7	37.7
Coffee husks	35	27.7	25
1Sugarcane baggasse	24.7	22	ND
Soybean straws	39	27	26.7

ND, Not done, 1Presented early maturity of fruit bodies and late for rice grass and saw dusts

Table 4 Yield of Pleurotus species on different agricultural wastes with or without supplementation

Agricultural waste	Mean fresh weight (g/kg substrate)		
	Main substrate	[1]Rice bran	[2]Molasse
Rice grass	321.05±1.34[a]	489.55±6.85[b]	432.00±5.11[bc]
Rice hulls	406.56±4.51[ab]	**590.45±1.76[d]	434.74±1.92[bc]
Saw dusts	177±5.06[ac]	**392.92±2.13[ed]	240.55±7.44[e]
Coffee husks	[a]476.2±2.99[b]	601.85±8.21[d]	484.86±3.35[b]
Sugarcane bagasse	[a]430.6±3.06[c]	522.32±1.25[ad]	ND
Soybean straws	[a]500.62±1.50[c]	525.22±6.42[ad]	518.62±7.71[ad]

*Figures having different letters are significant different at 5% level of probability,

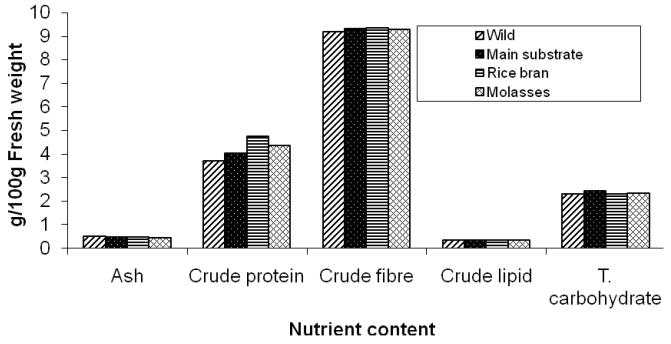
ND= not done [1]Moderate increase in fresh weight, [2]Minimal increase in fresh weight, [a]High mean fresh weight on main substrate

**Drastic increase in mean fresh weight on Rice bran supplementation.

There was a significant difference between the mean fresh weights of fruit bodies produced on all the substrates. However, molasses supplementation had minimal increase in mean fresh weights of fruit bodies compared to rice bran. Considering the main substrates, mushrooms cultivated on sugarcane bagasse, soybean straws and coffee husks weighed higher than those of rice straws, rice hulls and saw dusts (Table 4). Supplementation of the main substrates produced more mean fresh weight of fruit bodies. This could be attributed to the improved nutrient content of the main substrates. Saw dusts and sugarcane bagasse showed a drastic increase in mean fresh weight of fruit bodies in three flushes on rice bran supplementation with the lowest being observed on soybean straws.

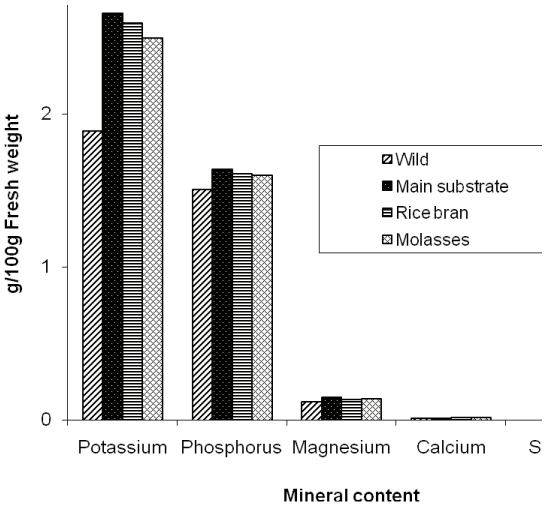
There were also significant differences of some nutrient composition among the fruit bodies of wild Pleurotus species and the cultivated ones (Figs. 1-3). Crude protein was significantly ($p<0.05$) high for cultivated mushrooms as compared to the wild Pleurotus species, ranging from 4.42-5.91±(0.02-0.05) g/100g fresh weight. This was attributed to the presence of high protein and the availability of the degraded components in the substrates. There were no significant differences in crude fat and their fatty acids among the respective treatments (Fig. 3). Potassium (K), phosphorus (P) and magnesium (Mg) were relatively higher in cultivated mushrooms. This shows that the mushrooms could be able to assimilate additional minerals from the media tested.

Figure 1 Mean proximate chemical composition of a wild and cultivated mushrooms (Mean±SD, n=2).



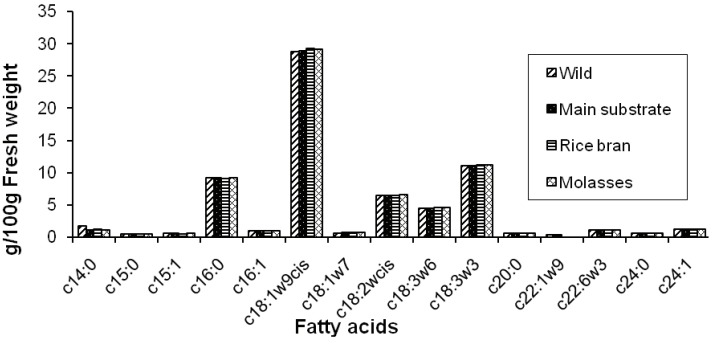
*An arrow indicates significant difference between crude proteins at 5% level of probability

Figure 2 Mineral contents of wild and cultivated mushrooms (Mean± SD, n=2).



*Relatively high Potassium, Phosphorus and Magnesium contents, low sodium content

Figure 3 Mean fatty acid contents of wild and cultivated mushrooms. (Mean±SD, n=2)



Legends: c14:0 (Myristic acid), c15:0 (Pentadecanoic acid), c15:1 (cis-10-pentadecanoic acid), c16:0, (Palmitic acid), c16:1 (Palmitoleic acid), c18:1w9cis (Oleic acid), c18:1w7 (Vaccenic acid), c18:2:cis (Linoleic acid), c18:3:w6, gamma-linolenic acid, c18:3:w3, Alpha-linolenic acid, c20:0 (Arachidic acid), c22:1w9 (Erucic acid), c24:0 (Lignoceric acid), c22:6w3 (Docosahexaenoic acid (DHA), c24:1 (Nervonic acid)

Conclusion

Wild Pleurotus species has a potential for domestication using Uganda's available agro wastes namely: sugar bagasse, rice hull, saw dusts, rice straws, soybean straws and coffee husks. The best main substrates were sugar bagasse, rice hulls and coffee husks. Supplementation of the main substrates enhanced the growth rate, the yield and the nutritive value of the cultivated mushrooms. Molasses and rice bran gave promising results as supplements in cultivation of wild Pleurotus species. Molasses being the best supplement during mushroom growth but it does not significantly affect the yields. Rice bran would be another alternative supplement as it increases growth rate of mushrooms and their yields. Therefore, more sensitization about use of these available agro wastes in the domestication of wild Pleurotus species should be done. This will help to reduce on the environmental pollution, and enhance better utilization of the agro-wastes in agriculture.

Acknowledgement

We are grateful to Carnegie Corporation of New York and DAAD for financial support towards this research work. Our sincere thanks also go to the people of Mid-Western Uganda (Hoima and Kibaale districts) for their efforts in identification of the edible mushroom.

REFERENCES

AOAC, 2002. Official Methods of Analysis - 17th ed. Association of Official Analytical Chemist, Maryland.

Chang, S.T., Miles, P.G., 1984. A new look at cultivated mushrooms. Bioscience. 34, 358-362.

Engola, A.P.O., Eilu, G., Kabasa, J.D., Kisovi, L., Munishi, L.P.K., Olila, D., 2006. Ethnomycology and nutraceutical potential of indigenous edible mushrooms of Rakai district, Uganda. African Journal of Animal Biomedical Sciences. 1: 1, 71-79.

Fasidi I.O., Kadiri, M., 1993. Use of agricultural wastes for the cultivation of Lentinus subsudus (Polyporale: Polyporaceae) in Nigeria. Review of Biolology in Tropics. 41, 411-415

Iqbal, M.S., Abdulrauf, C., Iqbal, M. S., 2005. Yield Performance of oyster mushroom on different substrates. International Journal of Agricultural Biology. 6: 7, 900-903.

Jansi, J.J., (2010). Studies on the effect of glutamic acid and gibberellic acid on the yield and protein content of oyster mushroom Pleurotus florida (mont.) Signer using paddy straw as a substratum. Journal of Basic and Applied Biology. 4:3, 217-220.

Kamatenesi, M.M., Origa, O.H., Odyek, O., Makawiki, D.W., 2006. The use of mushrooms in primary and feminine health care in Western, Uganda. Paper presented at First Africa conference in edible and medicinal mushrooms, 25th-29th, October 2006, Makerere University, Uganda.

Katende, A.B., Ssegawa, P., Birnie, A., 1999. Wild Food Plants and Mushrooms of Uganda. RELMA. Nairobi, Kenya.

Kirbag, S., Akyuz, M., 2008. Effect of various agro-residues on growing periods, yield and biological efficiency of Pleurotus eryngii. Food, Agriculture & Environment. 3:6, 402-405.

Mshandete, A.M., Cuff, J., 2008. Cultivation of three types of indigenous wild edible mushrooms: Coprinus cinereus, Pleurotus flabellatus and Volvariella volvocea on composted sisal decortications residue in Tanzania. African Journal of Biotechnology. 7: 24, 4551-4562.

Nakalembe, I., Kabasa, J.D., Olila, D., 2009. Indigenous knowledge and usage of wild mushrooms in Mid-Western, Uganda. Africa Journal of Animal Biomedical Science. 4: 1, 63-73.

Okeke, B.C, Paterson A., Smith, J.E., Whatson-Craik, I.A., 1994. The relationship between phenol oxidase activity, soluble protein and ergosterol with growth of Lentinus species in oak saw dust logs. Applied Microbiology and Biotechnology. 41, 28-31.

Opige, M., Kateyo, E., Kabasa, J.D., Olila, D. 2006. Indigenous knowledge and usage of indigenous edible and medicinal mushrooms among the Teso people of Eastern Uganda. Journal of Food Technology. 4: 4, 325-330.

Paterrson-Beedle, M., Kennedy, J., Melo, F.A.D., Lloyd, L.L., Medeiros, V. (2002). A cellulosic expolysaccharide produced from sugarcane molasses by a Zoogloea sp. Carbohydrate Polymers. 42, 375-383.

Pushpa, S.M., Manonmani, H.K., 2008. Bioconversion of coffee industry wastes with white rot fungus Pleurotus florida. Research Journal of Environmental Science. 2: 2, 145-150.

Quimio, T.H., (1986). Guide to low cost mushroom cultivation in the tropics. Department of Plant Pathology, UPLB. The Philippines. pg.73.

Royse, D.J., 2002. Influence of spawn rate and commercial delayed release nutrient level on Pleurotus cornucopiae yield, size and time to production. Applied Microbiology and Biotechnology. 58,527-531.

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

Vijay, P.M., Shyam, S.P., Abrar, A.S., Mirza, V.V.B., 2007. Bioconversion of low quality lignocellulosic agricultural waste into edible protein by Pleurotus sajor-caju (Fr.) Singer. Journal of Zhejiang University Science. 8:10, 745-751.

Zadrazil, F., 1980. Influence of ammonium nitrate and organic supplements on the yield of Pleurotus sajor-caju (Fr.) Singer. European Journal of Applied Microbiology and Biotechnology. 9, 31-35.

Zadrazil, I.F., 1993. “Conversion of lignocellulosic into animal feeds with white rot fungi”. In: Chang ST, Bruswell JA Siu-wai C (eds.) Mushroom Biology and Mushroom products. Chinese University Press, Hong Kong.