PERFORMANCE OF *PLEUROTUS FLABELLATUS* ON WATER HYACINTH (*EICHHORNIA CRASSIPES*) SHOOTS AT TWO DIFFERENT TEMPERATURE AND RELATIVE HUMIDITY REGIMES

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ABSTRACT

Efforts are being made in Tanzania to promote mushroom cultivation, and identification of abundantly available plant biomass residues appropriate for growing mushrooms is part of the efforts. This study investigated the suitability of water hyacinth as a bulk substrate for growing a newly domesticated local oyster mushroom, Pleurotus flabellatus. The performance of the mushroom was investigated under ambient temperature and relative humidity (RH) regimes of 18-25/27-29 ^{O}C and 55-85/78-93%, respectively. The growth cycle of the mushroom was completed in 40 days with three and four flushes respectively. At the higher temperature and RH regime, the mushroom grew faster and the first flush was harvested at the 13th day after substrate inoculation with a Biological Efficiency (B.E.) of 84%, whereas the first harvest was done on the 19th day after inoculation at the lower temperature and RH regime with a B.E. of 53%. Substrate total fibre loss at the end of the growth cycle was in the range of 31-40%, and cellulose the most utilized fraction, decreased by 35-48%. The rates of fibre loss increased over time during the mushroom growth and were highest during the first and second flush during which about 80% of the total mushroom yield were obtained. Water hyacinth shoots proved to be a good substrate for growing the local oyster mushroom at ambient environmental conditions.

INTRODUCTION

More than 200 agro-forestry residues have been used for small or large scale cultivation of oyster mushrooms worldwide (Poppe 2000). In Tanzania, a wild oyster mushroom which was recently isolated from Dar es Salaam and domesticated, has been found to grow well on rice straw, sawdust, banana leaves, sugarcane bagasse, sisal fibre and hay (Mshandete 1998; Mtowa 1999). The mushroom is being cultivated at a small scale by individual farmers, and the extent of utilization of the materials tested by these farmers depends on their availability in abundance. The agro-industrial residues are easily obtainable by farmers in close proximity to the factories, and the agro-residues are abundantly available only in areas that grow the respective crops such as rice and bananas. In an attempt to promote mushroom cultivation in Tanzania, there has been therefore, a need to identify other abundantly available potential substrates for mushrooms.

A potentially suitable substrate for mushroom cultivation is water hyacinth (*Eichhornia crassipes*). This prolific aquatic weed has been shown to be a more viable substrate for mushroom cultivation than rice straw (Murugesan et al. 1995). The weed is a menace in several fresh water bodies in Tanzania and it is currently harvested and disposed of by burning. Its use for

mushroom cultivation could be a cost effective way of controlling it while at the same time producing protein rich food and fertilizer.

This study was therefore undertaken to determine the suitability of the weed for growing the domesticated strain (*Pleurotus flabellatus)* at two places with different temperature and relative humidity regimes. The two climatic regimes are typical of many regions in the country.

MATERIALS AND METHODS

Mushroom strain, mycelial culture and spawn

The oyster mushroom found growing on a dead *Ficus benjamina* tree on the main campus of the University of Dar es Salaam during the long rains was used in this study. The mushroom was identified by Dr. Bart Buyck of the Paris Museum of Natural History as *Pleurotus flabellatus*. The mushroom is kept at the Applied Microbiology Unit, University of Dar es Salaam.

Pure cultures were established from a young fruit body of the mushroom by tissue culture method as described by Oei (1991). Aseptically, pieces of the mushroom were cut from the inner tissue of a fruit torn length wise and inoculated in malt extract agar Petri dishes. The inoculated dishes were incubated at room temperature (25°C and 28°C), in darkness for 7 days.

The obtained mycelium was used for spawn preparation. Proof that the pure mycelial cultures belonged to the same mushroom species as the fruits from which pure cultures were prepared was provided by a Polymerase Chain Reaction – Restriction Fragment Length Polymorphism (PCR-RFLP) analysis (Muruke et al. 2002).

Sorghum grains were washed in water and boiled for 20 minutes. The grains were then left in the water for about ten minutes to allow for absorption of enough water to bring the moisture content to 49-54% and then spread on a sieve to allow excess water to drain and to allow the surface to dry. Calcium sulphate (20 g) and Calcium carbonate (5 g) were added to the grains to adjust the pH to about 7.0-7.8 and to prevent grains from sticking together. After mixing thoroughly, about 100 g of the grains were packed into wide mouthed 250 ml bottles and then autoclaved at 121°C and 1 atmosphere for 2 hours. Upon cooling while shaking the bottles to keep the grains separate, each bottle was inoculated with five 1-cm2 pieces of mycelium-covered agar, and was shook to distribute the mycelia evenly onto the grains. Incubation of the inoculated bottles was done at 27°C for 14 days with their caps loosely closed.

Substrate preparation and inoculation

Water hyacinth was harvested from a roadside rainwater drainage trench at Regent Street, Mikocheni in Dar es Salaam. The roots were removed and the water hyacinth shoots (WHS) were dried in the sun for four days. The dry WHS were chopped to approximately 4-6 cm long segments and soaked in water for 5 minutes to allow for moisture absorption. About 1.0 kg of the shoots were then packed in 25 x 40 cm heat resistant polyethylene bags with both ends open, which were then loosely tied with sisal ropes. The packed bags were then steam pasteurized at 100 °C for 4 hours in a 200-litre steel drum. On cooling to room temperature, the bags were removed from the pasteurization vessel and placed onto a disinfected bench in a spawning room. Composition of the pasteurized WHS is shown in Table 1.

The sisal ropes were removed and replaced by PVC collar necks (Quimio et al. 1990) and spawning of the WHS was done at a rate of 1.5 % (w/w). The collar neck openings were closed with cotton plugs. The bags were perforated using a clean needle in order to facilitate gaseous exchange. The holes were of about 1 mm diameter and spaced about 10 cm from each other.

Table 1. Composition (mean ± sd[a]) of pasteurized water hyacinth shoots.

Component	Amount (% dry wt)
Neutral Detergent Fibre	77.0 ± 0.8
Acid Detergent Fibre	52.6 ± 0.4
Lignin	12.1 ± 0.5
Cellulose	40.0 ± 0.9
Hemicellulose	24.9 ± 0.2
Total carbon	37.1 ± 0.5
C : N ratio	21.6 : 1
Total solids	23.1 ± 0.1
Volatile solids	82.4 ± 0

[a] : sd=standard deviation, n=6

Cultivation and harvesting

The inoculated bags were placed onto disinfected shelves in a disinfected dark room with a cement floor for spawn running. The 10 x 8 x 2.8 m growing room had a door of 1.8 x 2 m and windows of 0.8 x 0.9 m. The window and doorframes were fitted with wire gauze with mesh size small enough to keep out flies, snails and rodents. The room was kept humid by pouring 10 litres of water per day on the floor. A data logger (HOBO from Onset Computer Cooperation, Pocasset, Massachusetts, USA) monitored humidity, temperature and illumination in the room. The spawn-running period ended when the substrate was sufficiently colonized by mycelia.

Table 2. Environmental conditions at experimental sites during spawn running and at fructification of the mushroom

Site	Spawn running		Fructification	
	Temperature (°C)	RH (%)	Temperature (°C)	RH (%)
Moshi	21-25	55-78	18-22.5	74-85.5
Dar es Salaam	27-29	78-83	25-27	86-93

The fructification conditions included increased light and ventilation by opening windows and the black curtains, and increased humidity by pouring water on the floor. Conditions during spawn running and fructification are shown in Table 2. The induction of fruiting conditions caused formation of primordia, which grew into mushroom fruits.

The mushrooms were harvested when the cups were fully open by grasping the basidiocarp by the stalk followed by gentle twisting and pulling them off the substrate (Quimio et al. 1990). The harvested fruits were immediately put in thin polythene bags, and were weighed. The flushing patterns were noted throughout the cropping period. The biological efficiencies were calculated as the fresh weight of mushrooms produced divided by the dry weight of the original substrate, expressed as a percentage (Miles and Chang (1997).

Substrate utilization, analytical methods and statistical analysis

The extent of substrate utilization was determined by subtracting residual components of Volatile solids (VS), Total fibre (NDF), lignin, cellulose and hemicellulose from the initial amounts. The degradation of the lignocellulosic components was monitored at 10-day

intervals for 40 days.

The WHS were analysed for total solids (TS), VS, total Carbon, and total Nitrogen prior to substrate inoculation and at the end of the harvesting period. Total fibre, lignin, cellulose and hemicellulose were analysed prior to spawning and at regular intervals of 10 days during the spawn running period through the mushroom harvesting period to monitor the rate of lignocelluloses degradation at the two different temperature regimes. Unless otherwise stated, all parameters were done in six replicates.

TS, and VS were determined as described in Standard methods (APHA 1995) and total Carbon was determined according to the method of Allen (1989). Total Nitrogen and Nitrate-N were estimated in triplicate by Semi-micro Kjeldahl digestion followed by colorimetric method as described by Emteryd (1989) and Allen (1989). NDF, lignin, cellulose and hemicellulose were determined by the method of Goering and van Soest (1970).

The data was statistically analysed using the t-test and 95% error bars were attached to means in line graphs. Zar (1996).

RESULTS AND DISCUSSION

Effect of environmental conditions on rate and pattern of growth

At lower temperatures and humidity similar to conditions in Moshi, the mushroom took 10 days to colonize the substrate fully whereas at higher temperature range and humidity as in Dar es Salaam (DSM), colonization was complete on the 7th day after inoculation. In Moshi, pinheads started appearing in some bags after 15 days of incubation, but grew very slowly to maturity. They took 5 to 7 days to grow into grayish, highly clustered, fairly small fruits weighing between 3 g and 15 g with very short, almost absent stipes. The first harvest was done on the 19th day. The fruits were few in the first harvest but increased in the subsequent flushes and 87% of the total yield was obtained in the first two flushes with an overall B.E. of 55.3%. On the contrary, at higher temperatures and RH in Dar es Salaam, the mushroom grew much faster and the first mushroom harvest was done 13 days after substrate inoculation. Four flushes were observed with the second having the highest mushroom weight after which the weight decreased by about 50 % in the second flush and finally by 84 % in the fourth and last flush. About 76% of total production was obtained in the first two flushes and the overall B.E. was 84.4%. Results of the rate of growth of the mushroom at Moshi and Dar es Salaam are summarized in Table 3. Comparison done between the mushroom B.E. obtained in the two sites showed a significantly higher yield for Dar es Salaam ($P<0.0001$) than for Moshi using a student t-test.

Table 3. Rate of growth and yield of the mushroom in Moshi and Dar es Salaam

Place of growth	Time to full mycelia colonization (days)	Time to primordial appearance (days)	Mushroom yield (grams)				BE (%)
			First[a] flush	Second[b] flush	Third[b] flush	Fourth[b] flush	
Moshi	10	15	154(19)	402(3)	80(4)	none	55.3
Dar es Salaam	7	10	362(13)	378(3)	199(4)	31(6)	84.8

[a]: in brackets are days to first flush
[b]: in brackets are days in between the flushes
BE: Biological efficiency

The better performance of the mushroom at the DSM site than at the Moshi site may be attributed to the fact that this particular mushroom strain was originally isolated from Dar es Salaam, and hence the ambient growth conditions *in vitro* were close to those of its natural growth conditions. Mshandete (1998) reported B.E.s in the range of 65-74 % for the same strain grown under ambient conditions in DSM on sisal waste compost, banana leaves, sugar cane bagasse and pasteurized sisal waste. The yield obtained in this study at DSM site was higher by 10-19%. On comparing the rates of growth of other oyster mushroom species on similar substrates, a marked higher rate of growth for *P. flabellatus* used in this study is noted. Ragunathan et al. (1996) reported 22, 22, 27 days to first primordia initiation and fructification for *P. sajor-caju*, *P. platypus*, and *P. citrinopilleatus*, respectively. Wang et al. (2001) reported 35-42 days for *P. ostreatus* on spent beer grains and Curvetto et al. (2002) reported 24-28 days for the same species on sunflower hulls. Unlike this study which applied ambient environmental conditions for mushroom growth, the above previous studies were carried out under controlled conditions of 24-25°C with a RH of 85-90% for spawn running, 21-24°C with a RH of 85-90% for fructification.

Substrate degradation

Residual fibre content that indicate the extent of substrate degradation are given in Table 4. While the decline trends in the various fibre fraction components of the substrates at Moshi and Dar es Salaam sites during mushroom cultivation are shown in Figure 1. The mushroom at Moshi utilized 40 % of the total fibre (NDF) (Table 4). Using student t-test, this was significantly higher ($P<0.0001$) than 31 % total fibre (NDF) utilization at Dar es Salaam. Initial total fibre contents of the substrates used for mushroom cultivation at the two sites were similar. The two extents of NDF degradation were far greater than that reported for *P. sajor-caju* (17%) on rice straw (Zhang et al. 2002) despite the fact that the latter had a lower content (63-67%) of total fibre than that of the WHS (77%) used in this study.

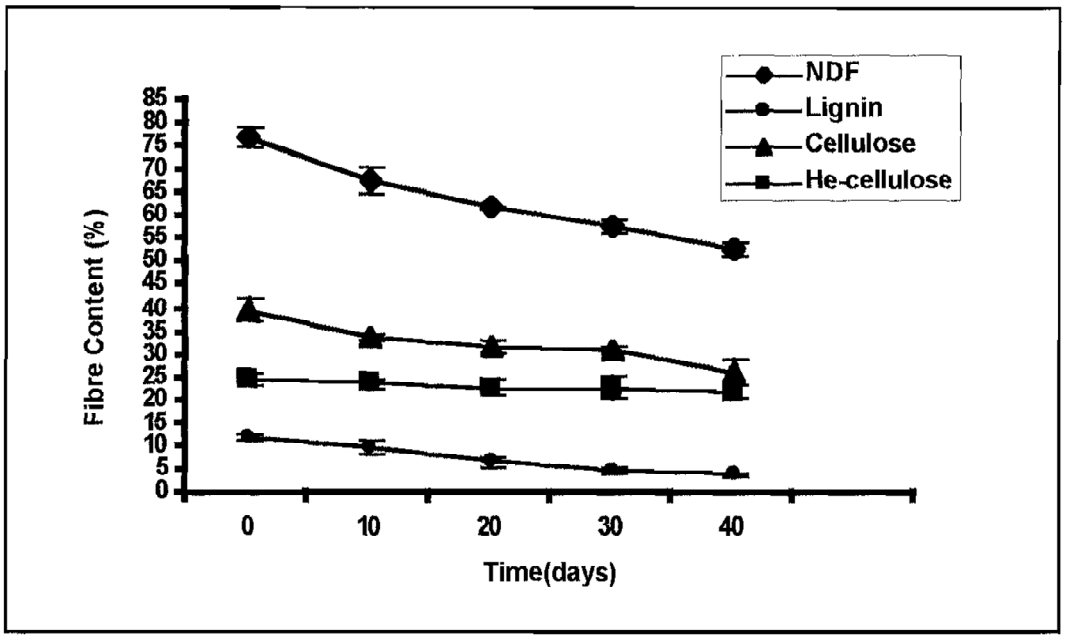


Fig. 1.A. Decline in fibre content of substrate during mushroom cultivation at the Dar es Salaam site

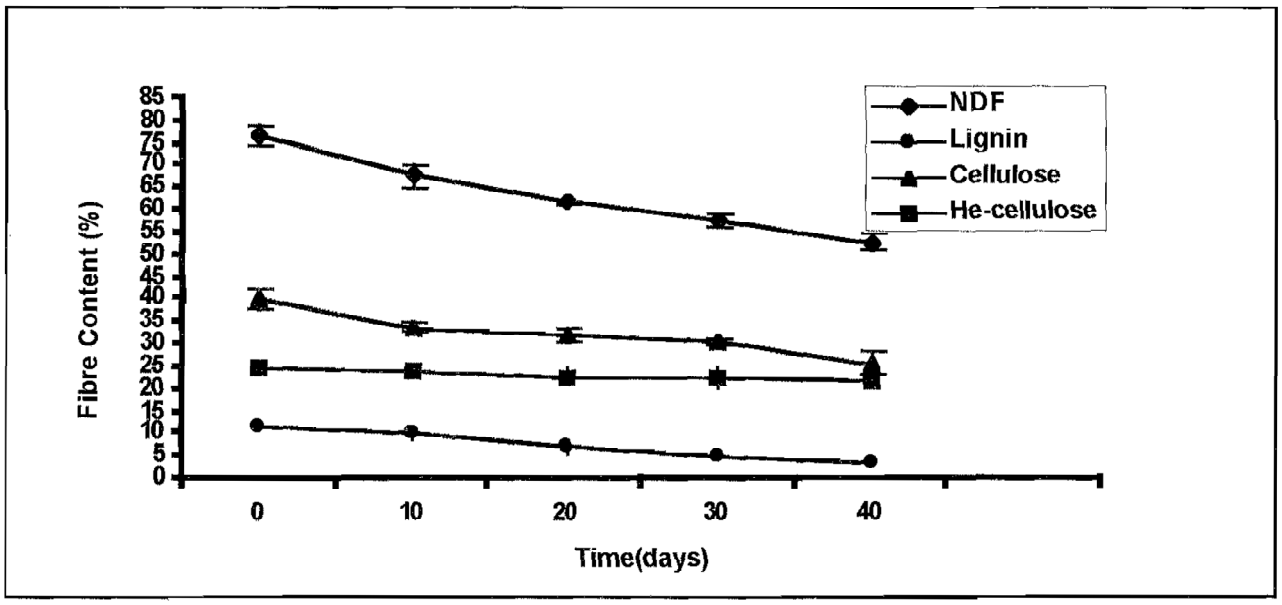


Fig. 1.B. Decline in fibre content of substrate during mushroom cultivation at the Moshi site

Table 4. Average substrate fibre utilization by the mushroom

Component	Moshi	Dar es Salaam
Total fibre	40	31
Lignin	42	66
Cellulose	48	35
Hemicellulose	24	12

The extents of utilization of the various fibre components at the two sites was significantly different under the student t-test ($P<0.0001$). Except for lignin of which the degradation extent was 24% higher at DSM than at Moshi, the rest of the components were utilized to a greater extent at Moshi than at DSM (Table 4). The cellulose fractions were the most utilized and hemicellulose fractions were the least utilized. On the contrary, Zhang et al. (2002) achieved the highest utilization of 40% for hemicellulose followed by 17 % for cellulose and lignin was hardly degraded in rice straw. The differences between the results of the two studies are most likely due to differences in the enzyme profiles of the two mushroom species and their activities. Although the enzymatic activities of the mushroom strains used were not studied, activities of lignolytic enzymes during growth of *P. flabellatus* on WHS in this study might have been higher than those of *P. sajor-caju* in the rice straw substrate used by Zhang et al. (2002).

The rates of fibre degradation at both sites increased over time and were highest between the 20th and 30th days after inoculation which corresponded with the periods of first and second flushes when about 80% of the total mushroom yields were obtained. These results imply utilization of the fibre fractions by the mushroom, particularly cellulose and lignin for biomass production. The faster rates for NDF and cellulose degradation at Moshi site than at DSM site appeared to have no obvious positive impact on the overall mushroom yield obtained.

In view of substrate upgrading for ruminant

feed, the spent WHS at the DSM site was of better quality than that generated at the Moshi site. In this case, the quality is based on lignin content that makes most feeds poorly or easily degradable by the animals. The residual lignin content of the DSM spent WHS was significantly lower than that of the Moshi spent WHS. On the basis of degradable fibre, the higher residual cellulose in the DSM spent substrate makes it a better potential ruminant feed than that of the Moshi substrate.

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REFERENCES

Allen SE 1989. *Chemical analysis of ecological materials.* Second Edition. Blackwell Scientific Publication. Oxford.

APHA 1995. *Standard methods for the examination of waste and wastewater.* Clesceri, S. S.E. Greenberg and R.R. Trussel (eds.). 19th Edition. American Public Health Association. Washington D.C.

Curvetto NR, Figlas D, Devalis R, and Delmastro S 2002. Growth and productivity of different *Pleurotus ostreatus* strains on sunflower seed hulls supplemented with $N\text{-}NH^{+}_{4}$ and/or Mn(II). *Bioresource Technology.* **84:**171-176.

Emteryd O 1989. Chemical and physical analysis of inorganic nutrients in plants, soil, water and air. Swedish University of Agricultural Sciences, Department of forest site research. UMEÅ, No. 10.

Goering, HK and van Soest PJ 1970. *Forage fibre analysis.* Agricultural handbook no. 379. Agricultural Research Services, United States Department of Agriculture.

Miles PG and Chang STJ 1997. *Mushroom Biology. Concise basics and current developments.* World Scientific Publishing Co. Pte, Ltd. P 40-46. 63-64, 105-106, 129.

Mshandete AM. 1998. Studies on morphology, taxonomy, cultivation and nutritive value of some local saprophytic edible mushrooms. [M.Sc. thesis]. Dar es salaam, Tanzania: University of Dar es Salaam.

Mtowa AC. 1999. Growth characteristics of some wild edible mushrooms from Tanzania. [M.Sc. thesis]. Dar es Salaam, Tanzania: University of Dar es Salaam.

Murugesan AG, Vijayalakshmi GS, Sukumaran N and Mariappan C 1995. Short communication. Utilization of water hyacitnth for oyster mushroom cultivation. *Bioresource Technology.* **51**: 97-98.

Muruke MHS, Kivaisi AK, Magingo FSS, Danell E. 2002. Identification of mushroom mycelia using DNA techniques. *Tanz. J. of Sci.* **28**: 115-128.

Oei P 1991. *Manual on mushroom cultivation.* First edition. Tool Foundation. **1:**249.

Poppe, J 2000. Use of agricultural waste materials in the cultivation of mushrooms. In: Van Griensven (ed.). *Science and cultivation of edible fungi.* Pp. 3-23.

Quimio TH Chang ST, Royse DJ 1990. *Technical guidelines for mushroom growing in the tropics.* FAO of UN Plant Production and Protection Paper. Rome Italy.

Ragunathan R, Gurusamy R, Palanisway M and Swaminathan K 1996. Cultivation of *Pleurotus* spp. On various agro-residues. *Food Chemistry.* **55:**139-144.

Wang D, Sakoda A and Suzuki M 2001. Biology efficiency and nutritional value of *Pleurotus ostreatus*

cultivated on spent beer grains. *Bioresource Technology*. **78:** 293-300.

Zar JH 1996. Biostatistical analysis. Third Edition. Pp 93-404. Prentice Hall International, Inc.

Zhang R, Li X and Fadel JG 2002. Oyster mushroom cultivation with rice and wheat straw. *Bioresource Technology*. **82:** 277-284.