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Cultivation of three types of indigenous wild edible mushrooms: *Coprinus cinereus, Pleurotus flabellatus* and *Volvariella volvocea* on composted sisal decortications residue in Tanzania

Anthony Manoni Mshandete[1]* and Joyce Cuff[2]

[1]Department of Molecular Biology and Biotechnology, University of Dar es Salaam, P.O. Box 35179, Dar es Salaam, Tanzania.
[2]Biology Department, Thiel College, Greenville, PA, 16124, USA.

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The periods for spawn running, pinhead and fruit body formation, number of flushes, yield and biological efficiency of the three Tanzanian wild edible mushrooms, *Coprinus cinereus, Pleurotus flabellatus* and *Volvariella volvocea*, grown on composted sisal decortications residue were studied. Results revealed that the organic ingredients in sisal decortications residue composted well within 21 days of composting, resulting in the formation of suitable compost, to support the growth of mycelia of the three edible mushrooms. The time for the first appearance of mushrooms was shortest for *C. cinereus* (10-11 days), followed by *V. volvaceae* (12-14 days), while that for *P. flabellatus* was 16-18 days. All three mushrooms produced at least five flushes; flush 1 gave the highest yield while flush 5 the lowest yield. The biological efficiency (B.E.) for *C. cinereus, P. flabellatus* and *V. volvaceae* was 68, 64 and 28%, respectively. Significant differences (P<0.05) in mushroom size, yield and % B.E. of the three mushrooms species were recorded. The results also showed that the B.E. (74%) of *P. flabellatus* grown on non-composted sisal decortications residue was significantly higher (P<0.05) than that grown on composted sisal decortications residue. The implications of this study are that sisal decortications residue could be used to cultivate very protein rich mushrooms for food while at the same time promoting environmental sustainability.

Key words: Composting, *Coprinus cinereus*, cultivation, biological efficiency, *Pleurotus flabellatus, Volvariella volvaceae.*

INTRODUCTION

Saprophytic mushrooms are found growing on rotten logs of woody tree trunks, decaying or dead organic matter, and dump soil rich in organic substances. Mushroom cultivation is a world wide practice which utilizes almost all agricultural and agro-industrial residues as substrate (Chang, 1999). In Tanzania, it is estimated that the conventional wet sisal decortications process generates 25 tons of solid residue per ton of sisal fibres produced. With the projected production of 45,000 tons of sisal fibres for the year 2007, about 1,125,000 tons of sisal solid residues will be produced (Mshandete et al., 2008). Sisal residues currently are conceived as a negative factor in both the industrial and agricultural settings, since they generate adverse environmental and economic effects related to their disposal (Mshandete et al., 2008).

Cultivation of mushroom can be viewed as an effective means to extract bioresource left behind in agro-industrial solid residues and simultaneously as a sound environmental protection strategy. Furthermore, the use of these residues in bioprocesses may be one of the solutions to bioconversion of inedible biomass residues into nutritious protein rich food in the form of edible mushrooms (Chiu et

*Corresponding author. E-mail: mshandete@amu.udsm.ac.tz, anthonymshandete@yahoo.co.uk. Tel: +255 22 2410223. Fax: +255- 22-2410 078.

al., 2000; Obodai et al., 2003a; Mshandete and Cuff, 2007). However, mushroom cultivation is not easy; it involves many steps, from selecting a suitable technique and strain to spawn manufacturing, growing the crop, and marketing the final crop (Oei, 2003). To make mushroom cultivation an environmentally friendly and cost-effective industry, the basic biology of wild edible mushrooms coupled to indigenous substrate formulation and locally adapted cultivation biotechnology under ambient climatic conditions must be researched and developed (Obodai et al., 2003a; Colak, 2004; Guler et al., 2006). Nevertheless, there is no information on the use of composted sisal decortications residue (CSDR) as substrate for mushroom cultivation, which has been reported so far in the literature (Härkönen et al., 1995; 2003). Since fresh sisal decortications residue is highly acidic and mildly corrosive, its utilization as a substrate for the cultivation of indigenous tropical mushrooms would preferably be by drying and/or by composting. Although in many developing countries, the collection and sale of wild edible mushrooms has become an important source of income for many people in remote forested regions. The knowledge about edible basidiomycetes that could be cultivated in tropical regions is very meager indeed, since at present science has been studying tropical mushrooms comparatively little (Oei, 2003). Therefore strains suitable for tropical and subtropical countries are hard to obtain (Guler et al., 2006). Most of the strains available are suited for cultivation in temperate climates (Oei, 2003; Colak, 2004).

In Tanzania almost every ethnic group has a traditional knowledge of mushrooms growing in the wild and members of each group harvest, consume and sell them during rainy seasons (Härkönen et al., 2003). Mushrooms cultured on agro-industrial residues could ensure year-round availability of mushrooms. However, so far only two edible Tanzanian mushrooms of the genera *Oudemansiella* and *Pleurotus* have recently been domesticated and cultivated on some agro-industrial residues and water hyacinth (Kivaisi et al., 2003; Magingo et al., 2004). The number of unexplored species of edible mushrooms, which may be artificially cultivated, is definitely still enormous considering Tanzania's rich mushroom diversity (Buyck et al., 2000; Härkönen et al., 1995, 2003). At the same time Tanzania's forests are becoming depleted, which could lead to the extinction of many of its wild indigenous mushroom species (Härkönen et al., 2003). Therefore the need arises to develop indigenous mushroom biotechnology to utilize Tanzania's diverse mushroom potential and the abundant agro-industrial and agricultural residues.

Indigenous mushrooms species and strains currently are screened for domestication and cultivation under ambient climatic conditions. This screening is a part of a long-term genetic resources culture collection and conservation of Tanzanian wild edible mushrooms, which may contribute to our understanding of the genetic diversity of Tanzania's mushrooms (Magingo et al., 2004). Cultivation of Tanzanian edible mushroom strains will help to solve agro-industrial and agricultural residue disposal problems and increase domestic proteinaceous human food production (Salmones et al., 1996). Also mushrooms could be a new high–value cash crop; it could serve as an effective means for alleviating poverty, which is so rampant among African countries such as Tanzania. Various workers have studied the growth conditions and substrate utilization of various mushrooms in many countries (Poppe and Hofte, 1995; Salmones et al., 1996; Stamets, 2000; Oei, 2003; Poppe, 2000; Guler et al., 2006). This is the first study on the isolation of pure culture mycelia, spawn run period, time of first appearance, and biological efficiency of three wild edible mushroom strains from Tanzania using CSDR as a substrate under ambient growth conditions. *Pleurotus flabellatus* cultivation on non-composted sisal decortications residue (NCSDR) is also being reported for the first time.

MATERIALS AND METHODS

Tanzanian wild edible mushroom species collection and identification

Taxonomic studies of some saprophytic edible mushrooms growing in the wild were carried out in the field and in the laboratory, with the objective of identifying those with the potential of being cultivated under natural ambient tropical conditions in Tanzania. Three out of the four wild edible mushrooms collected and identified namely, *Auricularia auricula* (Hook.) Underwood (Sny. *A.auricularia-judae* (St. Amans), *Coprinus cinereus* (Schaeff) S. Gray s.lat. and *Volvariella volvacea* (Bull.ex.Fr) Singer were found growing on sisal decortications residue dumps under natural environmental conditions at Alavi and Kingolwira sisal estates (Coast and Morogoro, Tanzania) while *P. flabellatus* (Berk and Br.) Sacc was found growing on the bark of a dead *Ficus benjamina* tree at the University of Dar es Salaam, Mlimani main campus (Dar es Salaam, Tanzania). Morphological features of the specimens collected were the main taxonomic criteria. The collected specimens were identified according to methods of classical herbarium taxonomy. The main taxonomic works on mushrooms of Ainsworth (1973), Peglar (1977), Buckzaki (1992), Phillips (1994) and Harkonen et al. (1995, 2003) were used to identify the species. A detailed account of taxonomy of the four edible wild mushroom species will be reported separately elsewhere.

Tissue culture

The first stage in any mushroom cultivation process is to obtain a pure mycelial culture of the specific mushroom strain. In this study three varieties of wild edible mushrooms were included: *C. cinereus* (Schaeff) S.Gray s.lat, *P. flabellatus* (Berk and Br.) Sacc and *V. volvacea* (Bull.ex.Fr) Singer. For simplicity, each will be referred to by the species name only for the remainder of the paper. The starting culture from living mushroom fruit bodies of the three varieties of wild edible mushrooms were obtained by tissue culture according to Stamets (2000) and Dhouib et al. (2005). Young and healthy fruit bodies were chosen to establish the mycelial culture of the wild edible mushrooms. Fruiting bodies were thoroughly pre-washed in water, and then wiped clean with a damp paper towel to remove any dirty and damaged external tissue. They were then swabbed with either 3% (v/v) hydrogen peroxide or 70% (v/v) ethanol to remove any contaminant on the surface. Tissue cultures were done

by breaking either mushroom cap or stem to expose the interior tissue, followed by inoculating small tissue fragments using a sterile scalpel in a 9 cm diameter Petri dish containing potato dextrose agar (PDA), (OXOID Ltd Basingstoke Hampshire, England). Two hundred and fifty (250) mg/l ampicillin (Sigma, USA) was added to the medium to inhibit the growth of bacteria. Prior to addition to the medium ampicillin was filter sterilized using 0.2 µm syringe filters (Microgon Inc, Califonia, USA). The inoculated Petri dishes were incubated upside down at 28°C in the dark, 4 days for *C. cinereus*, 5 days for *V. volvocea* and 6 days for *P. flabellatus*. Pure cultures obtained were used as stock cultures and were preserved on malt extract agar (MEA) (OXOID Ltd Basingstoke Hampshire, England) at 4°C by subculturing to fresh media every three months for *C. cinereus* and *P. flabellatus*. The cultures of *V. volvocea* are unique among edible mushrooms because they do not survive at chilling temperatures of 5 to 10°C. They cannot be refrigerated as other mushroom cultures (Quimio, 2002). Therefore *V. volvocea* culture was preserved at 30°C on MEA (Salmones et al., 1996).

Spawn preparation

Spawn of the three varieties of wild edible mushrooms were prepared with intact sorghum grains, which were bought from Kariakoo market in Dar es Salaam. The grains were first soaked in water overnight and thereafter parboiled for 10 min. After draining excess water, 1% (w/w) of calcium carbonate ($CaCO_3$) was added and properly mixed into the grains before spreading them out on a clean plastic sheath. After air-drying for about 20 min, 150 g of the grains were packed in 330 mL wide mouth bottles (Kioo Ltd, Dar es Salaam) and sterilized in an autoclave (Koninklijke AD Linden JR.BN-Zwijinderect, Holland) at 121°C and 1 atm for 1 h. Thereafter each cooled bottle of sterilized grains was aseptically inoculated with three 1 cm^2 pieces of mycelium MEA taken from 4-7-day-old cultures of one of the three different strains used. Each inoculated bottle, with its cap closed, was shaken thoroughly by hand to distribute the mycelia to the grains. Before use the bottles were incubated with their caps loosely in a ventilated incubator (Memmert GmbH KG, Schwabach FRG, Germany) set at 28°C, 10 days for *C. cinereus*, 12 days for *V. volvacea* and 13 days for *P. flabellatus*.

Substrates and their preparation

Fresh sisal decortications residues were collected from Alavi sisal decortications factory at Coast region, in Tanzania and were sun dried for 5 days. The fibrous sisal decortications residue was chopped into 3-4 cm lengths using a locally made manual chopper. The substrate was used before any degradation had occurred. The dried biomass was wetted over night to 68-72% moisture level and subjected to a composting process for 21 days. We used sisal decortications residue as the substrate since in nature it supports the growth of three wild edible mushrooms *A. auricula, C. cinereus* and *V. volvacea.* The outdoor composting method which manipulates the natural succession of microorganisms was used as previously described by Stamets and Chilton (1983). The following modifications were made in the present investigation:

(i) Sisal decortication residue was used instead of cereal straws.
(ii) Organic and inorganic manures were not used but the formulation used was 150 kg sisal decortication residue and 3.75 kg calcium sulphate (gypsum).
(iii) Dimensions of the three piles employed were 1.4 m high x 1.2 m wide x 1.0 m long (Oei, 2003). Wooden frames were not used as it is not cost effective for rural Tanzania.
(iv) Compost was turned every 3 days, starting on day 5 and ending on day 21 and on the same day the piles were dismantled.

Measuring of inner compost temperatures

Inner temperatures were measured as an indicator of microbial activity within the compost piles. The method of measuring points of inner compost temperature described by Colak (2004) was used. Temperature measurements were made at 24 h intervals. In this study the outdoor composting method was altered to suit the ambient tropical temperature and the fibrous nature of the sisal decortications residue which can be closely compacted together to maintain the inner high temperature in the pile. The qualitative characteristics of the finished compost which include colour, smell of ammonia expected due to deammonification, softness, and greasiness were observed at the end of the composting process as previously described by Stamets and Chilton (1983). The finished compost equivalent to 2 kg dry weight used as a substrate for mushroom cultivation was packed into transparent heat-resistant 0.1 µm polypropylene bags 40 cm x 80 cm (Simba Plastics, Dar es Salaam). The ends of each were tied loosely with a sisal rope before steaming at 71°C, for 2 h in a 200 L food grade drum (not used for chemicals) in order to deactivate any residual microorganisms. The wetted non-composted sisal decortications residue equivalent to 300 g of dry weight was packed into transparent, heat resistant, 0.1 µm polypropylene bags, 40 cm x 20 cm (Simba Plastics, Dar es Salaam) and pasteurized at 70°C, for 3 h in a 200 L food grade drum.

Cropping containers and spawning

Cropping containers depend upon a number of variables: mushroom species, the cultivator and the equipment/facility at hand (Stamets, 2000). Wooden trays were used for cultivation of *C. cinereus* and *V. volvacea.* These trays were 30 cm long x 20 cm wide x 10 cm deep having no top cover but with a bottom and sides made up of five 2.5 x 5 cm wooden slats placed 2 cm apart across the bottom base and sides. The tray bottom and the sides across were lined with clear plastic sheet pre-punched using a 6-inch nail. Each hole had a diameter of 6 mm and there were 50 holes on the entire plastic sheet. The holes facilitated drainage, aeration (free diffusion of gases and heat generated during spawn-running) and helped to maintain humidity. On the other hand transparent heat resistant polypropylene mushroom cultivation bags measuring 40 by 20 cm were used for *P. flabellatus*. The prepared sisal decortications residue compost was used for the growth of *C. cinereus, P. flabellatus* and *V. volvacea* when cooled to ambient temperature after steam pasteurization.

Similarly, non-composted sisal decortications residue substrate was used for cultivation of *P. flabellatus* when cooled to room temperature after steam pasteurization. Inoculation of the cooled substrate was done on the transparent plastic sheet disinfected with 3% domestic bleach and placed on the simple wooden platform in a clean room to simulate what is expected in the rural areas. The pasteurized substrate was inoculated by adding 20 g of previously prepared spawn (2% wet weight spawn/wet weight substrate) based on wet substrate weight (1000 g) equivalent to dry weight (300 g non-composted substrate) and 400 g composted substrate in each bag for *P. flabellatus* (Oei, 2003). Similarly the spawning rate for *V. volvacea* was 2% wet weight spawn/wet weight substrate (100 g spawn) based on 5 kg wet weight substrate equivalent to 2 kg dry weight in each wooden tray.

On the other hand the spawning rate for *C. cinereus* was 5% wet weight spawn/wet weight substrate (250 g spawn) based on wet substrate weight (5 kg wet weight) equivalent to 2 kg dry weight in each wooden tray (Kurtzman, 1978). Surface spawning was employed for *P. flabellatus*. Before spawning the sisal ropes were removed and replaced with collar necks. The collar necks made from poly-vinyl-chloride pipe (Simba Plastics, Dar es Salaam), were 2.5 cm height and 6 cm in diameter. After inoculation the bag was folded down over the outside of the collar and closed with new paper plugs tied with rubber bands. Forty bag replicates were prepared and evaluated; twenty bags for composted substrate and the other twenty bags for

non-composted substrate were prepared for *P. flabellatus*. On the other hand, the layer spawning method was employed for *C. cinereus* and *V. volvacea*; each 3 cm depth of the substrate was inoculated with the spawn until each wooden tray was filled up to 9 cm depth. A total of forty tray replicates, twenty per each *C. cinereus* and *V. volvacea* mushroom strains were prepared and evaluated. After spawning 1 cm layer of the substrate was applied on the top and the perforated plastic sheet was folded to cover the top. The controls in these experiments were sets of three bags/trays prepared as above but not inoculated (`unspawned').

Spawn- running: mycelia colonizing the substrate

In this study an environment similar to mushroom cultivation conditions present in rural areas was created to insure that the adoption of the cultivation technology of the studied mushroom is technically feasible at grass-roots level. After inoculation the bags and trays were placed on wooden shelves disinfected by 3% domestic bleach in a spawning running room (dimensions in m: 10 l x 8 b x 2.8 h) at the Molecular Biology and Biotechnology Department, University of Dar es Salaam. The room had a concrete floor pre-disinfected with 3% domestic bleach. The windows and the door frame were covered with wire gauze to bar insects and rodents; they were hung with black cotton curtains to create darkness and to limit fresh air circulation as recommended by Stamets and Chilton (1983). The spawning room was kept humid by pouring 15 litres tap water per day on the floor; however no artificial lighting was applied to enhance darkness. Vegetative development was followed by direct observation of the inoculated substrates until the substrates were completely invaded by mycelia of the three mushroom strains. On the other hand pests and contaminants were observed and noted, however not quantified. The number of spawn run days for mycelia of the three strains was recorded. During spawn running and fructification, a data logger (HOBO, Onset Computer Corporation, MA, USA) monitored humidity, temperature and light. The conditions during spawn-running in the room were 28±1°C, relatively air humidity 70±1% and light intensity only at 2.0±1 l m/sqf. The temperature recorded inside spawned substrate containers during spawn running ranged between 29±1°C for *P. flabellatus*, 31±1°C for *C. cinereus* and 32 ±1°C for *V. volvocea*.

Fructification: fruit body (mushroom) development

The fruiting body formation was triggered by shifting the environmental variables namely moisture, air exchange, temperature and light in the cropping room (Stamets, 2000). Once the mycelia of the three wild edible mushrooms strains had grown throughout the whole substrate the bags and the trays were removed and transferred to a fruiting room with same dimensions and shelves as that of the spawning room. Fruiting was stimulated by changing conditions which were suitable during spawning. In the fruiting room, to increase ventilation (air exchange) there were no curtains placed on the windows. Such a primordia initiation strategy introduced sufficient fresh air which at the same time lowered carbon dioxide concentration in the cropping room. To further lower carbondioxide newspaper plugs tied with rubber bands on the collar necks of bags were removed. Also for the same reason the perforated plastic sheet which was folded to cover the top of trays was opened to expose the fungal colonized substrate. In the fruiting room, the light intensity was increased from 2.0±1 to 16±3 lm/sqf by allowing indirect natural day light to diffuse in the room through windows which lacked curtains. Relative humidity and the temperature in the room were increased to 86±4% and temperature decreased to 26.5±0.05°C, respectively by pouring 25 litres of water per day on the floor and on the walls. When necessary, the moisture of the bags and trays was maintained with the use of mist sprayers.

Harvesting and crop yield

Fruit primordia were allowed to grow to the recommended harvesting stage and were picked. Mushrooms were harvested from the substrate, the substrate clinging to the stipe or to the volva was taken away and the mushrooms in their entirety were weighed the same day. Fresh *C. cinereus* fruit bodies were harvested when young, firm and fleshy (immature/juvenile stage). When the mushroom caps turned into an inky mass they were considered over matured and hence not suitable as food (Härkönen et al., 2003). *P. flabellatus* mushrooms were harvested when the in–rolled margins of the basidiomes began to flatten (Tisdale et al., 2006). *V. volvacea* harvesting was done at the stage before the volva enclosing the cap broke or just after the volva ruptured (button/elongation stages of the fungi). Dates of each harvest were also recorded. Total number of flushes (flush number) produced per each tray and/or bag was noted. The distribution of the yield per flush was tabulated to observe changes in yield over the course of multiple flushes. Duration of time from inoculation to final harvest was calculated. Five aspects of crop yield were evaluated according to Morais et al. (2000) and Tisdale et al. (2006): (i) Mushroom size was determined as total weight of fresh mushroom harvested/total number of mushrooms harvested (ii) biological efficiency (B.E.), (iii) mushroom yield (MY) (iv) flush number (v) crop period (sum of incubation and fruiting periods). BE values were calculated according to Stamets (2000), Royse et al. (2004). B.E. = [Weight of fresh mushrooms harvested (g) /dry substrate weight (g)] x100. On the other hand MY values were calculated as previous reported by Morais et al. (2000). MY= [Weight of fresh mushrooms harvested (g) per fresh substrate weight].

Analytical procedures

Composted and non-composted sisal decortications residue substrate samples were dried to a constant weight in a GallenKamp (Sanyo OMT oven, UK) drying oven at 45°C. Dried samples were ground using a laboratory mill to pass through a 1 mm sieve (Model 4, Thomas Wiley; Arthur K Thomas, Philadephia, PA, USA). All ground samples were transferred to airtight plastic bottles with well-fitting caps, labeled and then sealed in polythene bags to prevent any water intake and were stored in a refrigerator at 4°C until required for analysis. The cold stored samples were allowed to attain room temperature and mixed thoroughly with a spatula before withdrawing samples for proximate constituent analysis. Analysis was done on ground samples in triplicate to yield results from which mean compositions were computed. All weight measurements were done using an Adventurer TM balance (Ohaus Corp. Pine Brook, NJ, USA). The total carbon and nitrogen of the substrate was determined, according to Allen (1989) and AOAC (2000), respectively. Lignin, cellulose and hemicelluose (fibre content) were analysed according to the method of Goering and Van (1970). Fibre content was determined by analyzing the Acid detergent fibres (ADF), which is the lignocellulosic fraction of the substrate, followed by analyzing the Neutral detergent fibres (NDF), which includes lignin, cellulose and hemicellulose fractions of the substrate. The hemicellulose content of the substrate then is determined by subtracting ADF from NDF. Dry substrate weights were determined by heating 100 g of freshly prepared and composted substrates at 105°C for 24 h (AOAC, 2000). Ash mineral Constituents (calcium, phosphorous, sodium, potassium, magnesium, iron, copper, manganese, zinc and cobalt) were determined by atomic absorption spectrophotometry (AOAC, 2000).

Statistical analysis

The data on mushroom size, mushroom yield, and biological efficiency of *C. cinereus*, *P. flabellatus* and *V. volvocea* cultivated on

Table 1. Composition of dried non-composted and composted sisal decortications residue substrates for cultivation of wild edible mushrooms.

Parameter	Non-composted	Composted
pH	7.6	7.4
Moisture (%) fresh sample	75	68
Lignin (% of TS)	8.11	3.82
Cellulose (%TS)	60.1	40.37
Hemicellulose (%TS)	15.05	9.63
Total carbon (%TS)	51.84	21.80
Total nitrogen (%TS)	1.75	1.00
C:N	29.97	21.7
Minerals (g/100g dry matter)		
Phosphorous (P)	1.65x10-2	nd
Potassium (K)	1.75x10-2	nd
Calcium (Ca)	2.35x10-2	nd
Sodium (Na)	7.30x10-3	nd
Magnesium (Mg)	9.20x10-3	nd
Iron (Fe)	5.40x10-3	nd
Copper (Cu)	6.40x10-3	nd
Zinc (Zn)	3.30x10-4	nd
Manganese (Mn)	7.30x10-4	nd

All values are the means of three replicates, except for the fibres and minerals contents which were obtained from duplicate measurements. nd = Not determined.

composted sisal decortication residue were subjected to analyses of variance (one-way ANOVA) at the 5% level using the Statistical Package for Social Sciences (SPSS) Program 15.0. Version (SPSS, 2006). The yield performance of *P. flabellatus* on compost and non-composted sisal decortications residue was tested using t - test (SPSS, 2006). Data for analysis of substrate compositions were reported as the mean ± SD for three replicates, while two determinations per sample were carried out for fibres contents. The results are given as mean ± SD.

RESULTS AND DISCUSSION

Chemical composition of sisal decortications residue mushroom substrate

The results regarding the major chemical components of dried non-composted and composted sisal decortications residue substrates for the cultivation of wild edible mushrooms are indicated in Table 1. The main chemical constituents of sisal decortications residue prior to composting in terms of total fibre (lignin, cellulose and hemicellulose), total nitrogen total carbon and C:N ratio were higher than that of composted sisal decortications residue. The composting process showed that the major chemical components of sisal decortications residue were biodegradable as illustrated by a decrease in C:N ratio. Similar observations have been reported on different compost formulations with various composting materials employed for preparation of substrates for mushrooms cultivation (Oei, 2003; Colak, 2004; Yigitbasi et al., 2007; Atkipo et al., 2008).

Inner temperature profiles of composts and physical characteristics of the finished compost

The mean inner compost temperature values of 27 measuring points determined is given in Figure 1a. Also, Figure 1b shows the average temperature at turning stages for the compost formula used. The highest temperatures were recorded at the second turning stage. Increased temperature at this stage (6^{th} to 11^{th} days of composting) is an indicator of rapid and exothermic microbial activity within compost layers that might constitute a critical stage for decomposition of carbohydrates necessary to produce a better selective substrate for mushroom growing. These results are consistent with findings reported earlier by other researchers (Colak, 2004; Yigitbasi et al., 2007). As it can be seen from Figure 1a, the inner temperature obtained from the compost piles steadily rose to a peak level at the 2^{nd} and 3^{rd} turning stages followed by a gradual decrease. The composting process was completed at 21 days, with the end temperature levels ranging from 36 to 45°C. Ammonia is a respiration inhibitor; its complete release from the compost is critical. Previous findings by Ross and Harris (1982) had found that ammonia disappears most rapidly in the compost temperature range of 40 to 45°C. Thus the end temperature results of this study falls within

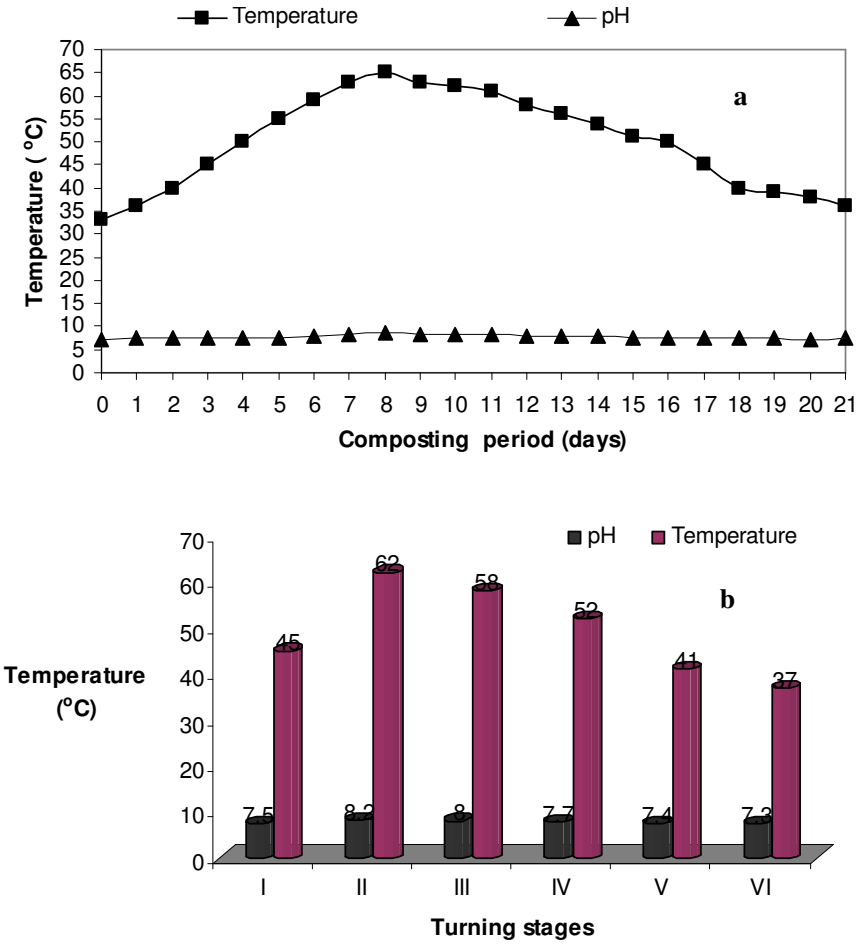

Figure 1. Inner compost temporary change in temperature and pH profiles during composting of sisal decortications residue.

the range of temperature necessary for ammonia removal from the compost.

In this study the highest inner compost temperature obtained was 65°C. The data are in agreement with the highest inner compost temperature in the range of 52 to 82°C, which has been reported by other investigators depending on the compost formulations (Colak, 2004; Yigitbasi et al., 2007; Atikpo et al., 2008). The rise of temperature during composting sisal decortications residue was obviously a result of indigenous microbial activity on the organic ingredients used in compost formulation and on the size of the pile. Similarly, Yigitbasi et al. (2007) reported that during phase 1 composting process, the raw ingredients are mixed, wetted and stacked to recommended dimensions to allow local microbial flora (fungi and bacteria) to break down the organic raw materials. Owing to microbial activity large quantities of heat are generated in distinct zones within the cross section of the compost stack, which is sufficient to kill pests and pathogens in the raw organic materials (Yigitbasi et al., 2007).

The results regarding physical characteristics of the finished sisal decortications residue revealed that all the organic ingredients used in sisal decortications compost composted well in three weeks of outdoor compositing, resulting in compost with dark brownish white spots, and a pleasant/sweet odor, not pungent or smelling of ammonia. Furthermore the compost was easily torn, soft and not greasy and by a squeeze test the compost could hold its form without water oozing between fingers. The volume of the compost was less by 50% compared to the initial biomass. The aforementioned physical attributes of the finished sisal decortications compost are similar to those reported by Stamets and Chilton (1983) on various composts formulations particularly for *Agaricus* spp cultivation.

Spawn running (mycelia development), pin head and fruit body formation of the three wild edible mushrooms

This is the first report on spawn running, pinhead formation and fruiting body formation periods of the three local wild edible mushrooms grown on sisal decortications residue

substrate in Tanzania and elsewhere. The three phases which are important in the cultivation of any mushroom differed for the three local edible mushrooms investigated as shown in Table 2. The duration of different growth stages of cultivated mushrooms depends on the type of substrate and substrate formulation (composted or non-composted or spent mushroom substrate and supplements) used, the type of species and/or the strain employed, spawn type and the level spawning rate applied, as well as on the prevailing mushroom growing conditions (Fasidi, 1996; Philippoussis et al., 2001; Obodai and Vowotor, 2002; Obodai et al., 2003a; Obodai et al., 2003b; Royse et al., 2004; Shah et al., 2004; Vetayasuporn, 2006, Mamiro and Royse, 2008; Olfati and Peyvast, 2008). It is evident from Table 2 that spawn running took one to two weeks after inoculation depending on the mushroom species. All the CSDR and NCSDR substrates after heat treatment were inoculated on the same day and were incubated in darkness. *C. cinereus* recorded the shortest colonization time of 7±1 day, followed by *V. volvacea* at 9±1 day. *P. flabellatus* recorded a spawn run of 12±1 day for CSDR and 13±1 day for NCSDR. These results agree with the findings of Atikpo et al. (2008) who reported that the genetic nature of the mushroom species/strains determine their mycelia colonization on different substrates. To this effect, a range of 7 to 20 days has been recorded for full mycelia colonization of *C. fimentarius* Fr (possibly could be *C. cinereus* (Schaeff. ex Fr.) S. F. Gray according to Kües, (2000) and *C. comatus* (O.F.Müll.) Gray on various substrates, incubation temperatures and incubation containers employed (Kurtzman, 1978; Stamets and Chilton, 1983; Stamets, 2000; Chaiyama et al., 2007). Mycelia of *Pleurotus* species are well known to colonize various lignocellulosic materials due to their extensive enzyme systems capable of utilizing complex organic compounds which occurs in organic matter residues (Tisdale et al ., 2006; Mane et al., 2007; Olfati and Peyvast, 2008; Atikpo et al., 2008). Thus a range of 12 to 41 days has been reported as time periods of spawn running for various *Pleurotus* species on composted or non-composted substrates (Stamets, 1993; Baysal et al., 2003; Obodai et al., 2003a; Royse et al., 2004; Shah et al., 2004; Tisdale et al., 2006; Vetayasuporn, 2006, 2007, Mane et al., 2007). The results from this study concur with the previous spawn running periods reported by others on *Pleurotus* species. The mycelia of the local *V. volvacea* isolate fully colonized the CSDR substrate in 8-10 days. This falls within spawn run periods previously reported by others for *Volvariella* species and strains on various substrates. Salmones et al. (1996) reported that it took 3-4 days for mycelia of Mexican *V. volvacea* (Bull:Fr.) Sing IE -158 strain to cover 13 different fermented agroindustrial wastes. Balewu and Balewu (2005) reported a spawn run time of 15 days on banana leaves for *V. volvacea* in Nigeria. Previously Kasidi (1996) reported spawn running of 11 days on agricultural wastes for a Nigerian edible mushroom *Volvariella esculenta* (Mass) Singer.

Pinhead formation is the second stage of mycelial growth during cultivation of mushrooms. Small pinhead-like structures was observed. As shown in Table 2 the time taken for these pinheads to be formed after the spawn running differed for each of the three mushrooms. *C. cinereus* recorded the earliest pinhead formation, followed by *V. volvacea* and lastly *P. flabellatus.* A similar trend was observed for the third and final stage of fruiting body formation during the cultivation of the three wild edible mushrooms. For *C. cinereus* the time taken for the small pinhead (primordia) to be formed was 8 -10 days starting from the first day of spawning. These pinheads grew into mushrooms which were harvested 1- 2 days later when young, firm and fleshy (immature/juvenile stage). The length of the fruiting period was between 11-12 days. The entire crop cycle took about three weeks. These observations indicated that *C. cinereus* has a short fruiting time, that is, in less than two weeks from the first day of spawning, mushroom biomass can be obtained. Furthermore in less than a month a *C. cinereus* crop can be realised. These findings are in agreement with Kurtzman (1978) who stated that pinheads of *C. fimentarius* Fr (possibly could be *C. cinereus* (Schaeff. ex Fr.) S. F. Gray according to Kües (2000) cultivated on straw supplemented with calcium nitrate as a fertilizer could be harvested 2 days after their appearance and the entire crop cycle lasts about 20 days. However, the present findings and that of Kurtzman (1978) differed from those reported by Stamets and Chilton (1983) for *C. comatus* (O.F.Müll.) Gray cultivated on wheat straw/horse or chicken manure compost. In their study pinheads were formed three days after 12 day casing layer run (which followed 12 days of normal spawn running). Then the first mushrooms were harvested 10 days afterwards, and the crop cycle took 97 days. Recently, Chaiyama et al. (2007) reported three weeks for the first mushroom to be harvested after the casing layer running period for *C. comatus* (O.F.Müll.) Gray grown on a pararubber sawdust: kapok waste: boiled sorghum (3:3:1) substrate mixture. The pinheads for *P. flabellatus* were formed 2-4 days after the spawn running. The fruiting bodies appeared 1-6 days after pinhead formation and the crop period was noted as 28-34 days. Quimio et al. (1990); Philippoussis et al. (2001); Baysal et al. (2003); Obodai et al. (2003a); Shah et al. (2004) and Royse et al. (2004) reported that *Pleurotus* spp on different substrates took 2-4 weeks for fruiting bodies to be formed after inoculation of spawn. Additionally, Philippoussis et al. (2001) reported a cropping cycle in the range of 20-52 days for 2-3 numbers of flushes on different composted and non- composted substrates for *Pleurotus* species. Our results were supportive of these earlier findings.

The results in Table 2 also showed that for *V. volvocea* it took 1-2 days after substrate lumps were exposed to fructification conditions to form small pinheads on the surface which, within 2 - 4 days, developed into the closed egg shaped mushrooms which were ready for harvesting. It took 12-14 days after spawning for the first mushrooms to

Table 2. Days for completion of spawn running, pinhead and fruiting bodies formation and crop cycles of three wild edible mushrooms grown sisal decortications residues.

Species	Substrate	Spawn Running (days)	Primordia/pinhead formation (days)	First harvest from inoculation (days)	Flush Interval (days)	Total fruiting period (days)	Entire crop cycle (days)
Coprinus cinereus	CSDR	7±1	9±1	10-11	1-2	11-12	21-23
Pleurotus flabellatus	CSDR	12±1	14±1	16-18	6	12	28-30
Pleurotus flabellatus	NCSDR	13±1	15±1	18-20	6-7	14	32-34
Volvariella volvocea	CSDR	9±1	11±1	12-14	4-5	13-14	25-28

Table 3. Fresh weight, number of fruiting bodies, average weight, mushroom size, yield and biological efficiency of three wild edible mushrooms. nd= not determined.

Species	Substrate	Total weight of fresh mushroom harvested (g)/bag or tray	Total number of fruiting bodies	Average weight of fruiting body (g)	Mushroom size	Mushroom Yield (g/kg wet substrate)	% BE
Coprinus cinereus	CSDR	1368	465	3.26±0.55	3	238±2.1	68± 0.72
Pleurotus flabellatus	CSDR	326	144	3.96±0.86	6.03	290±1.23	65± 1.37
Pleurotus flabellatus	NCSDR	371	nd	nd	nd	371±0.91	74± 2.03.
Volvariella volvocea	CSDR	569	54	6.22±2.15	3.95	114±08	28±1.52

be taken. Thus a crop of *V. volvacea* local isolate grown on CSDR substrate can be realised within two weeks. These results are in conformity with Quimio (2002) and Oei (2003) who reported that small white fruiting bodies of *V. volvacea* develop into the button stage in 2-3 days and it takes 10-14 days after spawning for the first crop to be harvested, preferably when the volva still encloses the pileus and/or immediately prior to rupture for extended shelf life.

Mushroom size, mushroom yield and biological efficiency

The type of mushroom species grown on CSDR significantly influenced the mushroom size (Table 3). Mushroom size was significantly larger on *P. flabellatus* but significantly smaller for *C. cinereus* and *V. volvocea* ($p < 0.05$). As expected, mushroom size of *P. flabellatus* was significantly larger than that of *C. cinereus* and *V. volvocea*. This was due to primarily to the stage of growth at harvest. *P. flabellatus* was harvested when the mushroom caps are open like a flower and the lamellae are exposed (Tisdale et al., 2006) as compared to *C. cinereus* which are harvested when immature with closed lamellae before the mushroom cap undergoes deliquescence (Härkönen et al., 2003) while *V. volvocea* are harvested when the volva encloses the pileus and/or immediately prior to rupture (Quimio, 2002). The results obtained on mushroom size variations were in line with earlier research which reported that there are several sources of variations for mushroom size which include the type substrate, spawn rate, type and level of supplements, and type of mushroom species as well as their strains (Royse et al., 2004; Mamiro and Royse, 2008).

The crop of three mushroom species was harvested for five flushes and their mushroom yields are given in Table 3. Analysis of mushroom yields revealed significant differences ($p < 0.05$) among the three mushroom species. *P. flabellatus* grown on both CSDR and NCSDR was superior followed by *C. cinereus* and *V. volvocea*. These results demonstrated that mushroom yields are directly related to the mushroom species used. This indicates that the mycelia of different mushroom species or strains have different colonizing potentials for the substrates in which they are grown, and this corresponds to the yield obtained. With this observation in mind different researchers had reported different mushroom yields for different mushroom species and substrates. The mushroom yield of 238 g/kg wet substrate for *C. cinereus* falls within mushroom yield ranges reported by other researchers on similar species and strains. Chaiyama et al. (2007) reported a mushroom yield* range of 102-331 g/kg wet substrate from *C. coma-*

tus (O.F.Müll.) Gray cultivated on three different combinations of agricultural products. On the other hand, Chen et al. (2007) reported mushroom yield* of 141 and 190 g/kg wet substrate for *C. comatus* CC1 and CC2 strains, respectively from rice straw compost. In addition, they also reported a mushroom yield* of 243 g/kg wet substrate for *C. comatus* CC1 strain and 216 g/kg wet substrate for *C. comatus* CC2 strain cultivated on sawdust substrates. (*Mushroom yield calculated as g/kg wet substrate from data reported). The crop of *P. flabellatus* harvested from NCSDR had a mushroom yield 371 g/kg wet substrate. These results fall within the mushroom yield range of 61-796 g/kg wet substrate reported by others on *Pleurotus* species cultivated on various non-composted substrates, with and without supplements (Phillipoussis et al., 2001; Baysal et al., 2003; Shah et al., 2004; Tisdale et al., 2006; Mane et al., 2007; Vetayasuporn , 2006, 2007). On the other hand, mushroom yield in the range of 85-183 g/kg wet substrate has been reported from *Pleurotus* species and strains cultivated on composted sawdust in Ghana (Obodai and Vowotor, 2002; Obodai et al., 2003a). The results on mushroom yield of 290 g/kg wet substrate obtained for *P. flabellatus* cultivated on CSDR do not agree with those of the previous studies and was higher by 37-71%. The genetic background of the particular *Pleurotus s*pecies and strains, along with differences in composition and preparation of the substrates, as well as in cultivation methodologies and environmental conditions could provide the explanation for those mushroom yield variations on composted sisal decortications residue and composted sawdust. Nevertheless, from the results in Table 3, it is interesting to note that the mushroom yield of *P. flabellatus* from NCSDR was higher than that obtained from CSDR by 22%. Also analysis of mushrooms harvested from composted and non-composted sisal decortications residue were statistically different using t test $P < 0.05$. This demonstrated that substantial mushroom yields of *P. flabellatus* can be obtained from sisal decortications residue without composting which can simplify the cultivation technique in a low technology country like Tanzania. These observations agree with the earlier claims that *Pleurotus* spp are saprophytic fungi which secrete a wide range of enzymes, including peroxidases, laccases, cellulases, hemicellulases and xylanases, which enable them to grow on a greater variety of lignocellulosic substrates than any type of cultivated mushroom without the need for a composting or casing layer (Cohen et al., 2002).

Furthermore, the results in Table 3 showed that a mushroom yield of 114 g/kg wet substrate for *V. volvocea* could be raised from CSDR within two weeks. This does not compare favorably with the results of Phillipoussis et al. (2001) who recorded low mushroom yield* in the range of 8.5-51 g/kg wet substrate from composted substrates. However, the mushroom yield data of 114 g/kg wet substrate was in the range of 98-164* g/kg wet substrate reported for two strains of *V. volvocea* (VVO and V99) cultivated on non-composted agricultural residues in Ghana (Obodai et al., 2003b) (*Mushroom yield of mushrooms calculated as g/kg wet substrate from the data reported). This implied that experimentation with NCSDR as a substrate and addition of supplements could improve significantly *V. volvocea* yield.

The biological efficiency was worked out against the dry weight of CSDR and NCSDR and the fresh weight of mushrooms. It is clear from the Table 3 that the percentages of biological efficiency varied with mushroom species. The three local edible mushrooms harvested from CSDR in terms of fresh weight were analysed statistically using the F test. The fresh weight of mushrooms compared to the dry CSDR on which they grew differed significantly between species ($p < 0.05$). *C. cinereus* gave the highest biological efficiency (B.E.) of 68%, followed by 64% for *P. flabellatus. V. volvacea* gave a very low, B.E. of 28%. *P. flabellatus* harvested from NCSDR on a fresh weight basis gave the highest B.E. of 74%. The fresh weight of *P. flabellatus* harvested from CSDR and NCSDR were statistically different using t test, $P < 0.05$. This is not unexpected as other researchers have observed significant differences in percentage biological efficiency for different mushroom species or strains grown on the same substrate (Phillipoussis et al., 2001; Obodai and Vowotor, 2002). From the results, the B.E. of 68% for *C. cinereus* achieved in this study using CSDR was higher. Chen et al. (2007) reported B.E. of 41.1 and 55.5% for *C. cinereus* CC1 and CC2 strains, respectively grown on composted rice straw in Taiwan. However, the same strains when cultivated on non-composted sawdust had B.E. of 69.3% for CC1 and 61.6% for CC2 which were comparable to those reported in this study. Similarly, Kurtzman (1978) obtained a B.E. value of 60% growing *C. fimentarius* Fr. on paddy straw supplemented with calcium nitrate as a fertilizer. Conversely, very low B.E. ranging from 21.2 to 50.8% was obtained for *C. comatus* (O.F.Müll.) Gray cultivated on non- composted supplements of different combinations of agricultural products in Thailand (Chaiyama et al., 2007). This equally suggests that CSDR is a good substrate for mass production of *C. cinereus* local isolates, without the necessity of supplements which could add cost to growers. It follows that the artificial culture of *C. cinereus* local isolates on CSDR could lead to increased yield bearing in mind that they can grow very quickly, and thus a high output of fresh mushrooms can be realised in a short time. However, the possible utilization NCSDR and casing materials needs research to be conducted to establish their overall effect on productivity of *C. cinereus* local isolate. As far as the B.E.% of *P. flabellatus* is concerned, NCSDR substrate presented 74% which was higher than 65% obtained from CSDR substrate. Patra and Pani (1995) reported that substrates for the cultivation of oyster mushrooms should have B.E. values of at least 50%. In this context, CSDR and NCSDR substrates supported satisfactory biological efficiency. The differences in the B.E.% is an indication that the nature of the substrate as presented in Table 1 influenced the biological efficiency pattern as well. The overall B.E.% for CSDR was superior

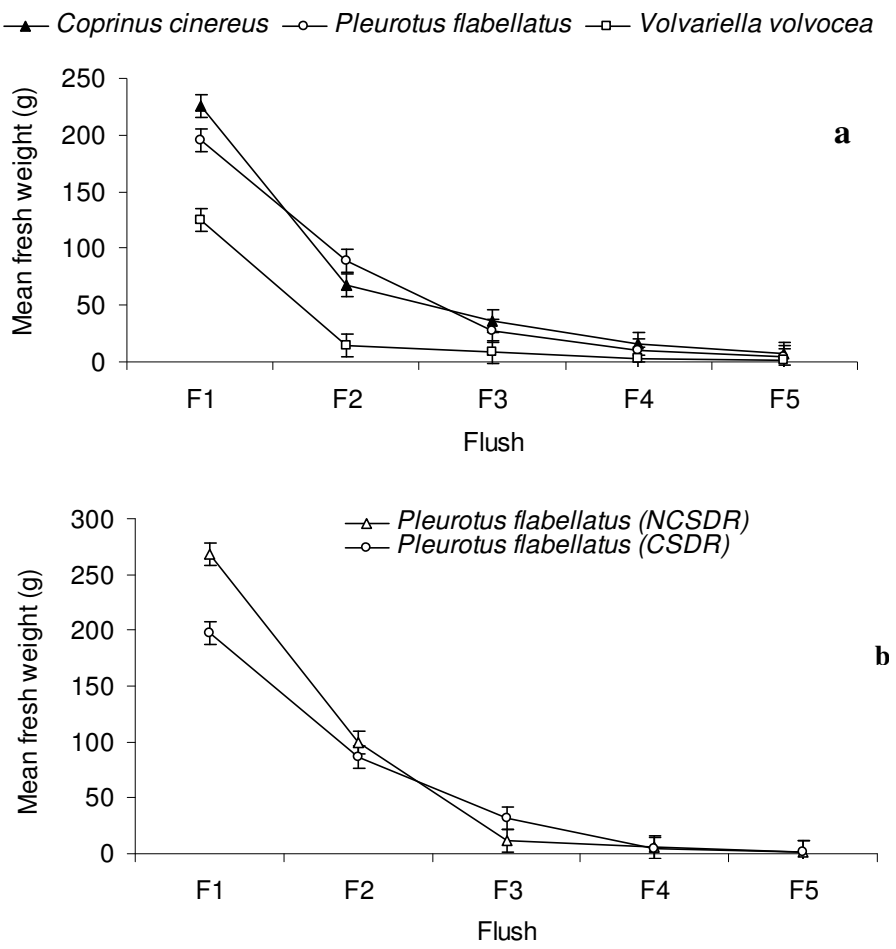

Figure 2. Change in mean yield throughout five flushes of (a) *Pleurotus flabellatus*, *Coprinus cinereus* and *Volvariella volvocea* harvested on CSDR and (b) *Pleurotus flabellatus* harvested on NCSDR and CSDR. Standard errors of mean are shown by error bars. (Bar: standard deviation).

to those recorded for eight (8) *Pleurotus* species grown on composted sawdust which ranged from 6.3 to 57.74% (Obodai and Vowotor, 2002). However, comparable B.E. of 61% was obtained from composted sawdust using *P. ostreatus* strain EM-1 (Obodai et al., 2003a). The B.E. 74% recorded for *P. flabellatus* from NCSDR is an indicative factor that non-composted substrates are more productive substrates in terms of bioefficiency as far as *Pleurotus* species are concerned. A range of B.E. between 53 to 84% has been reported for a local *P. flabellatus* from water hyacinth shoots at two different temperature and relative humidity regimes (Kivaisi et al., 2003). The B.E.% obtained in this study for NCSDR falls within the range of B.E. values reported for *P. flabellatus* using non-composted substrate. *V. volvacea* local isolate gave a B.E. of 28% when grown on CSDR. This result compares favourably with the range of 28.3 to 33.8% reported for *V. volvacea* cultivated on composted paddy straw (Chang, 1978; Salmoness et al. 1996). Moreover, composted cotton waste has been reported to give a higher and more stable B.E. of 30 to 45% than that obtained using straw as a substratum. This has lead to semi-industrialization cultivation of paddy straw on cotton wastes (Chang, 1974). However, in other cultivation studies on fermented substrates, B.E. values were inferior to that obtained in this study and ranged between 3.4 and 20.17% (Philippoussis et al., 2001), between 5.6 and 19% (Khan et al., 1991) or between 2.5 and 13.5% (Pani and Das, 1998). The marked B.E. % differences reported in the literature for *V. volvacea* showed that their yield is unpredictable on the same or different substrates (Oei, 2003). This was due to the ambient conditions for fructification (controlled or uncontrolled temperature and relative humidity), genetic nature of the species or strains, stage of harvesting (young stages or mature fungi) and the number of flushes harvested. Nevertheless, to confirm the suitability of CSDR for the cultivation of *V. volvacea* local isolate, additional experi-ments have to be conducted using other composted residues as cultivation substrates. Furthermore, utilization of non-composted residues as a substrate for *V. volvacea* could also be attemp-

ted since it has been reported recently to give higher B.E. in the range of 43 to 72% on agricultural wastes (Obodai et al., 2003b).

Flushing patterns of three wild edible mushrooms grown on sisal decortications residue

There was an expected progressive decline in yield over the course of five flushes which were harvested for the three wild edible mushrooms cultivated on CSDR (Figure 2a). The pattern was the same for the *P. flabellatus* grown on CSDR and NCSDR (Figure 2b). From the results it seemed that substrates did not affect this pattern. Approximately 93 to 99% of the total fresh weight was obtained in the first three flushes, with the fourth and fifth flushes producing 1 to 7%. These observations agree with those of Obodai and Vowotor (2002), Tisdale et al. (2006) who demonstrated that regardless of the mushroom species/strains and of the substrate (composted or non-composted) used to grow mushrooms, the pattern of gradually lessening mean yield per flush remains the same for any cultivated edible mushroom. This has been attributed to the finding that the quantity of mushrooms harvested in each flush is directly proportional to the nutrients disappearing from the substrate. The assimilable nutrient sources (carbon and nitrogen) in the organic waste substrate were absorbed by mycelia translocated and mobilized to supply the fruit bodies (Stamets and Chilton, 1983). It follows that in this study since the major part of mushroom production in three wild edible mushrooms investigated was obtained in the first three flushes, the economic flushes could be limited to three flushes; the fourth and fifth flushes can be ignored. Shortening cropping period by promoting rapid intensive early flushes could be of advantage in order to obtain maximum yield in a short time, which could ultimately lower the cost of production.

Conclusions

The results revealed that the organic ingredients in sisal decortications residue composted well within 21 days of composting, resulting in the formation of a suitable substrate for successful cultivation of *C. cinereus, P. flabellatus* and *V. volvacea* local edible mushrooms, using a locally adapted cultivation technique, under ambient tropical conditions. Also the NCSDR as substrate for *P. flabellatus* proved to be a better substrate than CSDR in terms of mushroom productivity. Therefore, the sisal organic waste could be recommended as a new substrate for cultivation of edible of mushrooms in Tanzania, which is being reported for the first time. In conclusion, the sisal industry in Tanzania has a great potential for mushroom production, because the substrates are abundant in the vicinity of sisal factories, which could make a commercial mushroom venture at the sisal factory level cost-effective, concerning handling and transport of substrates. However, the effects of additives, supplements, non-composted sisal decortications residue, and spawn rate aimed at more mushroom fruit body yield in as short a time as possible remains to be investigated.

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