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Cultivation of *Oudemansiella tanzanica* nom. prov. on agricultural solid wastes in Tanzania

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Abstract: The edible mushroom *Oudemansiella tanzanica* nom. prov., which is new to science, has been studied as a potential crop to reduce agricultural solid wastes and increase domestic mushroom production. The substrates sawdust, sisal waste and paddy straw supplemented with chicken manure resulted in the highest biological efficiencies of any mushroom cultivated in Tanzania so far. In addition, the mushroom has one of the shortest cultivation cycles at 24 d. Despite the fact that the mushroom extracts substantial amounts of nutrients, the spent substrate can be used as fodder, as a soil conditioner and fertilizer and in bioremediation.

Key words: Africa, Agaricales, mushroom, paddy straw, sawdust, sisal waste, Tricholomataceae

INTRODUCTION

Mushrooms of the genus *Oudemansiella* are consumed worldwide. The species include, *O. radicata* (Kasik 1994), *O. canarii* (Maziero et al 1995), *O. orientalis* and *O. hongoi* (Yang 2000). All *Oudemansiella* intended for human consumption thus far have been harvested in the wild. During the March–May rainy season in Tanzania, a species *Oudemansiella* frequently has been found to grow on the lower part of a stump of the tree *Pteleopsis myrtifolia* (Combretaceae) on the main campus of the University of Dar es Salaam. The mushroom was identified by Dr. Bart Buyck of the Paris Museum of Natural History (PC) to be a new species of *Oudemansiella*. The name *Oudemansiella tanzanica* is a nomen provisorum until the description is published. The holotype is kept at PC (Bart Buyck collection, No. 98086). The isotype is kept at Uppsala University (UPS F-015307) and color pictures of the species are at http://www.mykopat.slu.se/mycorrhiza/kantarellfiler/texter/tanzania.html. The entire fruiting body was found to be edible. We propose the Kiswahili name *Uyoga-Kunukia* (mushroom with a spicy odor) because the dried specimen has this appealing trait. Although *Oudemansiella* has been grown on chemically defined media (Semerdzieva and Cejp 1966, Musilek et al 1969, Volc et al 1995, Maziero et al 1995, Ypema and Gold 1999, Sede and Lopez 1999), cultivation with solid organic substrates has not been reported.

Tanzania produces large amounts of solid agricultural wastes. The annual amount of waste in metric tones includes: sisal pulp 100 000, robusta coffee pulp 58 860, sugarcane bagasse 5374, maize straw 5280, sorghum 1089, rice 600, millet 367 and wheat straw 39 (Kivaisi 1997).

Because edible mushrooms traditionally are consumed by tribes in Tanzania (Härkönen et al 1995, Buyck et al 2000), small-scale mushroom cultivation might help solve the waste problem. Indigenous species and strains currently are screened for cultivation. This screening is a part of a long-term inventory of Tanzanian macromycetes, an inventory that might contribute to our understanding of Tanzania's biodiversity, solve waste problems and increase domestic food production. Our aim in this study was to determine if *Oudemansiella tanzanica* nom. prov. is suitable for cultivation on solid-waste products.

MATERIALS AND METHODS

Establishing mycelial culture and spawn.—A young and healthy fruit body was chosen to establish the mycelial culture of the mushroom. Pieces of tissue were cut from the stipe, near the point where it joins the cap, and inoculated in a 9 cm diam Petri dish with malt-extract agar (Stamets and Chilton 1983). The inoculated Petri dishes were incubated upside down at 25 C in the dark for 7 d.

The mycelium obtained was identified as *Oudemansiella* using PCR/RFLP (Restriction Fragment Length Polymorphism in Polymerase Chain Reaction products) of rDNA ITS (internal transcribed spacer) (Muruke et al 2002). The mycelium was used for spawn production. Millet grains were washed in water and boiled 15 min. The boiled grains then were placed on a sieve to drain, after which they were

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spread on a clean plastic sheet to dry. The grains then were packed into wide-mouth, 1.0 L jars, until each jar was three-quarters full, and then autoclaved at 121 C and 1.0 atm for 1 h.

Each jar was inoculated aseptically with five 1 cm^2 pieces of mycelial agar. Each inoculated jar, with its cap closed, was shaken thoroughly by hand to distribute the mycelia to the grains. The jars then were incubated with their caps loosely closed, in a ventilated incubator set at 25 C for 21 d.

Preparation of the substrates and their spawning.—The substrates were paddy straw from a small-hold farmer near the university campus, hardwood sawdust from a university carpentry workshop and sisal waste from Ubena Zomozi sisal estate in Morogoro, Tanzania. We used sawdust because it is another form of its natural substrate; the other two substrates were used because they are agricultural byproducts. All fresh substrates had been dried before any degradation had occurred. Paddy straw and sisal waste were chopped into 5 cm long pieces. Each substrate then was soaked separately in water 24 h, for moisture absorption. The substrates were placed on wire sieves to drain.

Substrates then were combined with nitrogen supplements—rice bran or dried chicken manure at 1, 2 and 5% (w/w) of the wet weight of the substrates (FIGS. 1a–c). Calcium carbonate was added and thoroughly mixed with each substrate at 2% (w/w) of the wet weight of the substrate. After supplementation, the substrates were divided into 500 g lots. Each lot was packed into separate transparent polypropylene bags (Simba Plastics, Dar es Salaam). Each bag was kept open at both ends. The ends of each were tied loosely with a sisal rope before steaming at 100 C for 6 h in a 200 L steel vessel. Thirty bags of each substrate were prepared, five for each supplementation level. In total, 90 bags were prepared for the experiment.

After cooling to room temperature, the polypropylene bags were removed from the pasteurization vessel and placed on a disinfected laminar-flow hood. The ropes were removed and replaced with disinfected collar necks (Quimio et al 1990), attached at each end of each bag with disinfected rubber bands. The collar necks, made from poly-vinyl-chloride pipe, were 2 cm wide and 2.5 cm in circumference.

The spawn was added at 2% (w/w) of the substrate in each bag, and the collar neck openings were closed with cotton-wool plugs.

Spawn running and fructification.—We created an environment similar to mushroom cultivation conditions in rural Tanzania. The bags containing spawned substrates were placed on disinfected shelves in a disinfected spawn-running/fructification room. The 10 × 8 × 2.8 (l × b × h) m spawn-running/fructification room had a concrete floor. Windows and the doorframe were covered with wire gauze to bar insects and rodents; they were hung with black cotton curtains to create darkness. The spawn-running room was kept humid by pouring 10 L of water per day on the floor. During spawn running, a data logger (HOBO from Onset Computer Corp., in Pocasset, Massachusetts) monitored humidity, temperature and light.

Substrates were subjected to fructification conditions

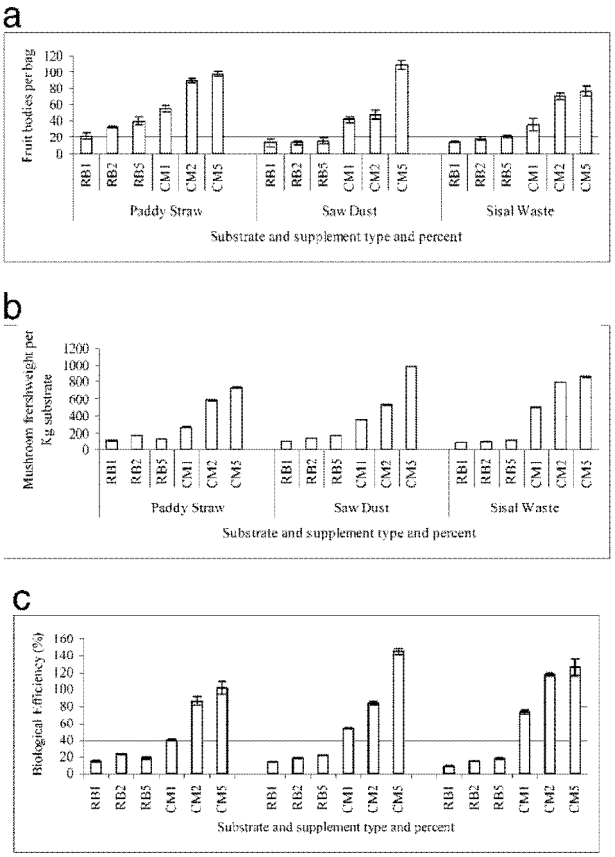


FIG. 1. Mean number of fruit bodies per bag (FIG. 1a), total mushroom fresh weight (g) per Kg (wet weight) substrate (FIG. 1b) and biological efficiency (B.E.) (FIG. 1c) (B.E. = mushroom freshweight/substrate dry weight × 100) of *Oudemansiella tanzanica* nom. prov. on the substrates. (RB = rice bran, CM = chicken manure; 1, 2 and 5 = percent supplementation.) Bars represent confidence intervals at $P = 0.05$. The substrate dry weights needed to make 1.0 Kg wet weight substrates were 730 g, 700 g and 690 g for paddy, sawdust and sisal waste respectively.

when, after 18 d on paddy straw and sawdust substrates and 19 d on sisal waste, the mycelium had sufficiently colonized them. Spawn running longer than 20 d resulted in mycelial degeneration (i.e., hyphae collapsed and patches of substrate became visible again. Fructification conditions included opening the curtains to provide more light and ventilation). Under these conditions, 2 d were allowed for pinhead formation.

Conditions in the spawn-running room were 23–25 C, relative humidity of 73–78% and light at only 1.0 lm/sqf. During the fructification period, the conditions included an increase in temperature to 24.5–26 C, a reduction in humidity to 50–53% and an increased light intensity of 13.5–19 lm/sqf.

Harvesting of fruit bodies and comparison of biological efficiencies on different substrates.—Fruit bodies were harvested when the caps were open. All fruit bodies were collected in 3 d. The substrates were incubated another 7 d after har-

TABLE I. Cultivation cycle of *O. tanzanica* nom. prov. in comparison with other mushrooms (N/A = not applicable)

Mushroom	Substrate	Duration in days for the activities: Composting	Spawn running	Casing layer run	Primordia/ pinhead formation	To first fruitbodies	Total days
Oudemansiella tanzanica nom. prov. (This work)	Sawdust, sisal waste, paddy straw	N/A	19	N/A	2	3	24
Volvariella volvacea (C, S&T)	Paddy (rice) straw	N/A	6	N/A	4	14	24
Flammilina velutipes (T)	Sawdust/bran	N/A	25	N/A	3	7	35
Pleurotus florida (Z)	Cereal straws	N/A	20	N/A	10	10	40
Pleurotus ostreatus (Z)	Cereal straws	N/A	20	N/A	10	10	40
Auricularia spp. (S&T)	Wood logs	N/A	40	N/A	7	10	57
Coprinus comatus (S&C)	Wheat straw/horse or chicken manure	60	12	12	3	10	97
Agaricus bisporus (S&C)	Wheat straw/ horse manure	60	14	12	3	10	99
Agaricus bitorquis (S&C)	Wheat straw/ horse manure	60	14	12	3	10	99
Lentinula edodes (S&C)	Saw dust	60	60	N/A	6	15	141
Lentinula edodes (S&C)	Tree logs	N/A	360	N/A	14	15	389

References: C = Chang (1978); S&C = Staments and Chilton (1983); S&T = Cheng and Tu (1978); T = Tonomura (1978); Z = Zadrazil (1978).

vesting. During that extra time, the pinheads that previously had formed never matured. Instead, they continued to degenerate like the mycelium. Mushrooms from different substrates and treatments were kept separately for fresh-weight measurements, and biological efficiencies were calculated (Oei 1991, Miles and Chang 1997). The biological efficiency (B.E.) is computed as the fresh weight of mushrooms produced divided by the dry weight of the original substrate, expressed as a percentage.

Measurements of substrate utilization.—Analyses of the substrate before spawning and after harvesting included: mois-

TABLE II. A comparison of Biological Efficiencies of *Oudemansiella tanzanica* nom. prov. and other mushrooms cultivated on various substrates supplemented with 5% chicken manure

Mushroom	Substrate	Biological efficiency	Reference
Pleurotus ostreatoroseus	Sugar cane bagasse	4.9	Bononi et al 1991
Ganoderma lucidum	Saw dust	17.3	Triratana et al 1991
Volvariella volvacea	Sugar cane bagasse	19.4	Khan et al 1991
Volvariella volvacea	Sisal waste compost	28.5	Mtowa 1999
Pleurotus ostreatus	Wheat straw	40.0	Upadhyay and Vijay 1991
Pleurotus flabellatus	Corn cobs	50.2	Mshandete 1998
Pleurotus citrinopileatus	Sugar cane bagasse	64.0	Mtowa and Magingo 1996
Pleurotus cornucopiae	Wheat straw	65.0	Upadhyay and Vijay 1991
Pleurotus flabellatus	Sisal waste compost	65.1	Mshandete 1998
Coprinus cinereus	Sisal waste compost	68.4	Mshandete 1998
Pleurotus flabellatus	Banana leaves	72.9	Mshandete 1998
	Sugar cane bagasse	74.1	Mshandete 1998
	Pasteurized sisal waste	74.2	Mshandete 1998
Coprinus cinereus	Market waste component	80.0	Chuwa et al 1997
Pleurotus pulmonarius	Saw dust	83.0	Magingo 1998
Oudemansiella tanzanica nom. prov.	Paddy straw	101.9	This work
Pleurotus sajor-caju	Wheat straw	112	Rajarathnam and Bano 1987
Oudemansiella tanzanica nom. prov.	Sisal waste	126.1	This work
	Saw dust	145.4	This work

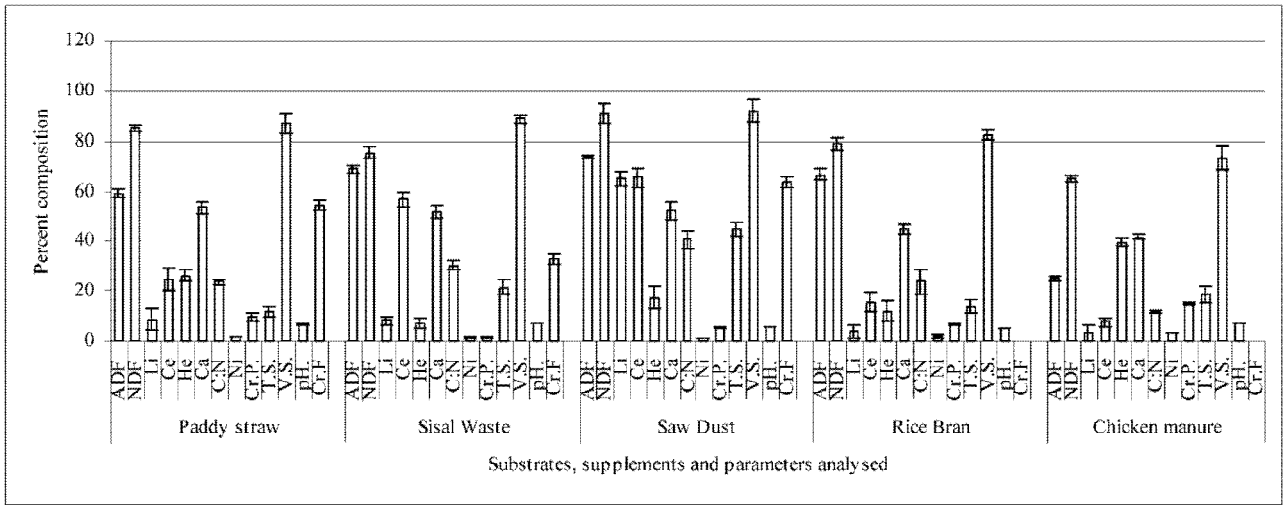


FIG. 2. Composition of the substrates and supplements in terms of Acid Detergent Fiber (ADF), Neutral Detergent Fiber (NDF), Lignin (li), Cellulose (Ce), Hemicellulose (He), Carbon (Ca), C:N ratio (C:N), Nitrogen (Ni), Crude Protein (Cr. P.), Total Solids (T.S.), Volatile Solids (V.S.), pH and Crude Fiber (Cr. F.). Bars represent confidence intervals at $P = 0.05$.

ture, fiber, lignin, cellulose and hemicellulose content, total and volatile solids, crude fiber of the substrate, total carbon content and nitrogen content.

Substrate samples were dried 24 h in an oven at 105 C. They then were ground and sieved through a 5 mm mesh. Each sample was stored separately in bottles with airtight lids in a refrigerator until analyzed.

Substrate fiber content was analyzed according to Goering and van Soest (1970). Fiber content was determined by analyzing the acid-detergent fraction (ADF), which is the lignocellulosic fraction of the substrate, followed by analyzing the neutral detergent fraction (NDF), which includes lignin, cellulose and hemicellulose fractions of the substrate. The hemicellulose content of the substrate then is obtained by subtracting ADF from NDF. Lignin and cellulose content of the substrate were determined according the methods of Iiyama and Wallis (1988) and Yokoyama et al (2002).

The total solids of a substrate comprise the organic matter in it, excluding water, while the substrate volatile-solids fraction comprises the percentage of organic matter that can be used directly by the mycelia. Total solids and volatile solids were determined according to the methods in Browning (1967).

The total carbon and nitrogen substrate content was determined respectively according to Allen (1989) and Browning (1967).

RESULTS

Harvests.—Of the three substrates used, the highest mushroom fresh weights were produced on sawdust supplemented with 5% chicken manure (FIG. 1b). Similarly, the highest number of fruit bodies per bag was recorded on this treatment (FIG. 1a). An average of 976 g fresh-weight mushrooms were harvested per kg of wet-weight substrate. The number of fruit bodies was 109 per bag.

Sisal waste supplemented with 5% chicken manure had the second-best mushroom harvest (FIG. 1b, c). An average of 846 g fresh-weight mushrooms were harvested per kg wet-weight substrate in this treatment. The third best harvests were obtained on sisal waste with 2% chicken manure as the nitrogen supplement.

On paddy straw, the best yield of 728 g fresh-weight mushrooms was obtained when supplemented with 5% chicken manure. Cultivating *Oudemansiella tanzanica* nom. prov. took only 24 d (TABLE I). When compared to B.E. of other mushrooms, *O. tanzanica* nom. prov. has the best biological efficiency recorded so far (TABLE II).

Characteristics of fruit bodies harvested.—Pinheads were brown and had a gelatinous layer on the cap. The gelatinous substance tended to dry as the mushroom matured and the cap flattened out. The fresh fruit body had an earthy-woody smell, which turned spicy upon drying.

Mushrooms differed morphologically according to substrate. Those grown on paddy straw and sawdust had long, thin stems, while those grown on sisal waste had short, thick stems resembling the morphology found in nature. The mushrooms grew both solitarily and in tufts, regardless of substrate or supplementation.

Mycelial culture and spawn.—After 7 d of incubation at 25 C, mushroom tissues had developed mycelium that covered three-quarters of the malt-extract agar

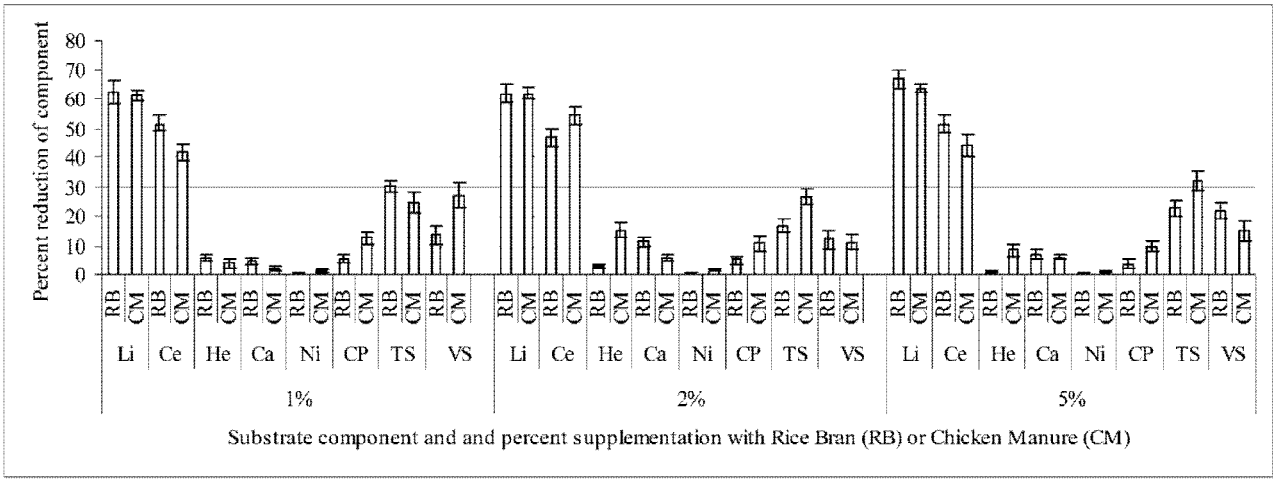


FIG. 3. Reduction in the components Lignin (Li), Cellulose (Ce), Hemicellulose (He), Carbon (Ca), Nitrogen (Ni), Crude Protein (CP), Total Solids (TS) and Volatile Solids (VS) on sawdust supplemented with rice bran (RB) or chicken manure (CM) after growth of *Oudemansiella tanzanica* nom. prov. (n = 5). Bars represent confidence intervals at $P = 0.05$.

medium. The mycelium was white and the texture cottony with some strands.

The millet had an initial moisture content of 19% and a pH of 5.7. However, upon autoclaving the grains had 50% moisture content and a pH of 6.1. After inoculation, it took 25 d for the mycelium to colonize the grains in the mother-spawn culture. When the succeeding batches of millet were inoculated with the mother spawn, the mycelium took only 11 d to fully colonize that substrate.

Substrate utilization.—Three substrates—paddy straw, sawdust and sisal waste—had substantially similar amounts of carbon and volatile solids (FIG. 2). However, in terms of lignin, cellulose, C:N ratio and total solids, sawdust had the highest amounts, followed by sisal waste. Paddy straw had fewer of these constituents. Sawdust had the lowest values for nitrogen content and pH, while, in terms of hemicellulose, nitrogen and crude protein, paddy straw had the highest values.

While the supplements—rice bran and chicken manure—had statistically similar amounts of lignin, chicken manure had higher values of hemicellulose, nitrogen, crude protein, total solids and pH. On the other hand, rice bran was substantially higher than chicken manure in terms of cellulose, carbon and volatile solids.

Lignin and cellulose were the highly utilized com-

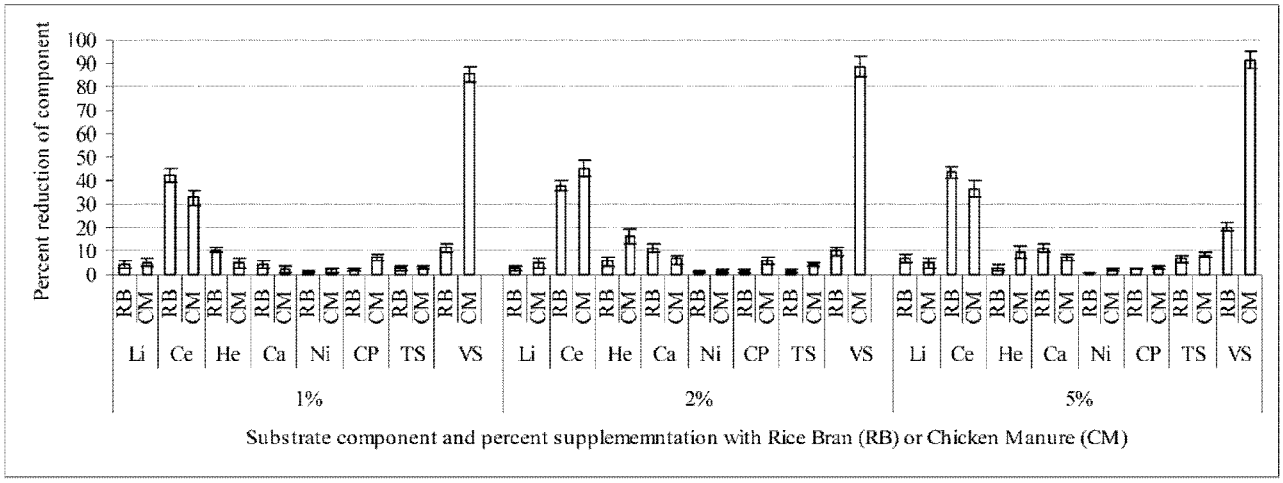


FIG. 4. Reduction in the components Lignin (Li), Cellulose (Ce), Hemicellulose (He), Carbon (Ca), Nitrogen (Ni), Crude Protein (CP), Total Solids (TS) and Volatile Solids (VS) on sisal waste supplemented with rice bran (RB) or chicken manure (CM) after growth of *Oudemansiella tanzanica* nom. prov. (n = 5). Bars represent confidence intervals at $P = 0.05$.

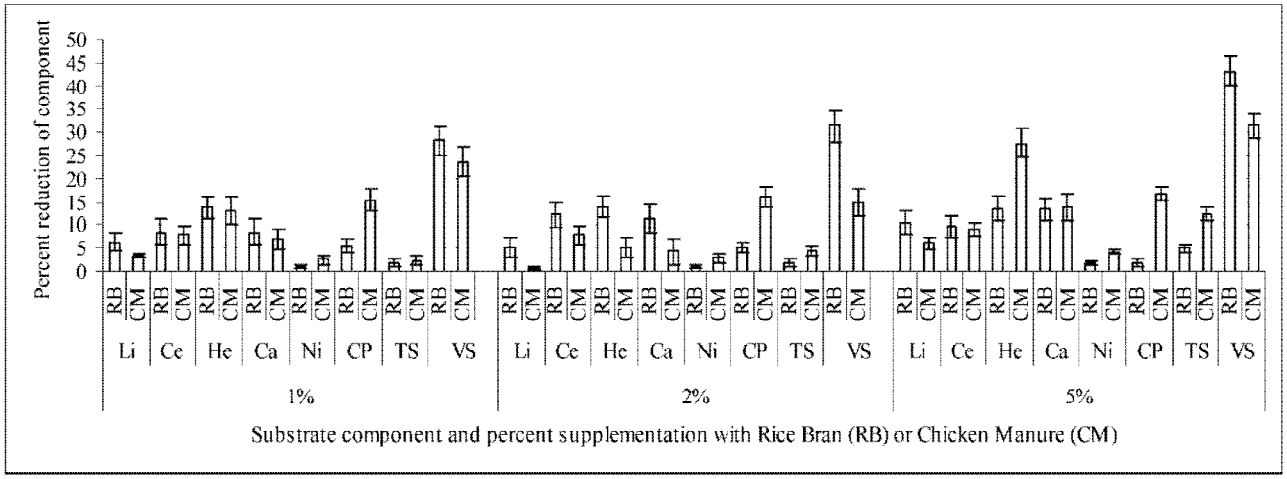


FIG. 5. Reduction in the components Lignin (Li), Cellulose (Ce), Hemicellulose (He), Carbon (Ca), Nitrogen (Ni), Crude Protein (CP), Total Solids (TS) and Volatile Solids (VS) on paddy straw supplemented with rice bran (RB) or chicken manure (CM) after growth *of Oudemansiella tanzanica* nom. prov. (n = 5). Bars represent confidence intervals at $P = 0.05$.

ponents in the sawdust treatment with the best harvests (FIG. 3), wherein 63.7% lignin and 43.97% of cellulose in the substrate were used by the growing mushroom. On sisal waste with 2 and 5% chicken manure supplement, the highly utilized components were volatile solids and cellulose (FIG. 4). On paddy straw with the best harvests, the highly utilized components were volatile solids and hemicellulose (FIG. 5).

DISCUSSION

Oudemansiella tanzanica nom. prov. has been successfully cultivated on sawdust, sisal waste and paddy straw. In general, *O. tanzanica* had high biological efficiencies compared to many other cultivated mushrooms (TABLE II). The most suitable substrate was sawdust supplemented with 5% chicken manure (FIG. 1). This preference corresponds well with its natural substrate which is wood, and our study indicates that *O. tanzanica* is a lignin degrader (causing white rot). Sawdust provided the richest source of lignin and cellulose, and chicken bran was the richest source of nitrogen. It could be speculated that, unlike *Pleurotus, O. tanzanica* needs nitrogen supplementation for optimum growth.

Spent substrates (FIGS. 3–5) had similar properties as those reported by Male (1981), Chong et al (1991) and Maher (1991). They concluded that the substrates could be used as fodder for livestock, as a soil conditioner and fertilizer and in bioremediation.

O. tanzanica is a promising mushroom for cultivation, in terms of its high productivity, simple means of cultivation on cheap organic substrates and a short production cycle compared to many other cultivated mushrooms (TABLE I).

Further studies could investigate the use of other organic substrates that are of environmental importance, such as the notorious water hyacinth (*Eichhomia crassipes*). Further research could investigate the mushroom's nutritional value and the light requirements for fructification. Because *Oudemansiella* has antimicrobial or antibiotic substances (Ypema and Gold 1999), the new mushroom also should be investigated on that score.

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