African Journal of Biotechnology Vol. 10(7), pp. 1172-1180, 14 February, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.1220
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

The effect of the interaction of varying chicken manure supplement levels with three different solid sisal wastes substrates on sporocarp cap lengths and diameters, stipe lengths and diameters and dry weights of *Coprinus cinereus* (Schaeff) S. Gray s.lat

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Accepted 25 November, 2010

***Coprinus cinereus* (Schaeff) S. Gray s.lat is an indigenous Tanzanian edible and medicinal mushroom, which grows in the wild on decomposed sisal wastes heaps. In the laboratory, it was cultivated on three types of sisal wastes substrates each supplemented with varying chicken manure concentrations 0, 5, 10, 15, 20 and 25% using solid-state fermentation bioreactors. The results showed that the interaction of sisal dust, sisal fibres and sisal decortications leaves wastes substrates with chicken manure at varying concentrations, produced a highly significant effect ($p < 0.05$) on cap lengths and diameters, stipe lengths and diameters and dry weights of *C. cinereus* (Schaeff) S. Gray s.lat. The interactions of sisal dust waste with 5% chicken manure concentration induced longest mean cap length and widest mean cap diameter. On the other hand, the interaction of sisal leaves decortications wastes with 25% chicken manure concentration produced heaviest sporocarps mean dry weight. These findings for the first time illustrate importance of chicken manure supplementation in *C. cinereus* (Schaeff) S. Gray s.lat cultivation on sisal decortications wastes substrates.**

Key words: *Coprinus cinereus*, chicken manure, solid sisal wastes, sporocarps, pileus, stipe.

INTRODUCTION

Mushrooms are fruiting bodies of macro fungi. They include edible, medicinal and poisonous species. They have been valued as edible and medical provisions for humankind since time immemorial. Mushroom production can convert the vast lignocelluloses waste materials into a wide diversity of fungal biomass products (edible or medicinal food, feed and fertilizers), protecting and minimizing environmental pollution (Fan et al., 2006; Nwanze et al., 2008). Moreover, the mushroom production contributes to products of commercial interest that can generate equitable economic growth that has already had an impact at national and regional levels (Nwanze et al., 2008). Mushrooms are delicacy, which can be taken regularly as part of human diet, or be treated as health foods or functional food. The extractible bioactive compounds from medicinal mushrooms for health enhancement and fitness are classified as dietary supplement or mushroom nutriceuticals (Chang and Buswell, 1996).

Mushroom cultivation is a worldwide practice, which utilizes almost all agricultural and agro-industrial residues as substrate, an efficient and relatively short biological process of food protein recovery from lignocellulosic materials (Chang, 1999). In Tanzania sisal wastes name-

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Abbreviations: SSFBs, Solid-state fermentation bioreactors; **TS,** total solids; **VS,** volatile solids; **TOM,** total organic matter.

ly: sisal fibres, sisal leaf decortications, sisal dust and sisal processing wastewater are currently conceived as a negative factor in both the industrial and agricultural sectors, since they generate adverse environmental and economic effects related to their disposal (Mshandete et al., 2008). Therefore cultivation of mushroom on sisal wastes could alleviate pollution as well as provide protein food and income (Mshandete and Cuff, 2007, 2008).

To increase production of fruiting bodies, genetic crossing may be used. Also environmental factors such as temperature, light, carbon dioxide concentration, humidity and pH have been shown to influence fruiting bodies production (Hibbett et al., 1994; Chang and Miles, 2004). On the other hand, supplements or additives usually change the decomposition rate and also the sequence of decomposition of substrates components (Zadrazil, 1993). To that effect supplementation of substrates with different levels of inorganic (chemical) carbonates and nitrogen-based additives has been shown to increase mushroom production (Royse et al., 1991; Fasidi and Kadiri, 1993; Zadrazil, 1993; Isikhuemhen et al., 1999; Royse, 1996). There are also various organic additives that are known to stimulate fruiting. They include rice bran, maize bran, cassava peels, carbohydrates (such as glycogen), natural extracts like yeast and malt extract as well as cell-free extracts (Fasidi and Kadiri, 1993; Oei, 2003). Highly proteineous materials such as ground pigeon pea and soybean also have been reported to stimulate high fruit yield. Cereals such as wheat, sorghum, rye and millet that are used in spawn making also influence fruiting (Nwanze et al., 2004a,b, 2008). Conversely, the effects of post-anaerobically digested effluents/biogas and fluid/slurry on mushroom growth are frequently being reported in recent years to modify substrate composition such as of rice straw, water hyacinth wastes and consequently improving yields of some edible mushroom of *Pleurotus* and *Agaricus* species (Banik and Nandi, 2004; Chen et al., 2010). Supplementation of mushroom substrates with animal protein rich biowastes like chicken manure, dung manure has a potential to increase mushroom yield and productivity (Morimoto et al., 1981;Royse et al., 2004). Nwanze et al. (2004a,b, 2008) earlier reported on the effect of interaction of factors such as spawn grain, culture media, oil types and rate on fruiting bodies weights, length of stipe and pileus diameter of *Psathyrella atroumbonata* and *Lentinus squarrosulus*.

Coprinus cinereus (Schaeff) S. Gray s.lat is an indigenous Tanzanian wild edible and medicinal mushroom, which grows naturally in sisal waste dumps around sisal processing factories. Its domestication on composted sisal decortications waste has been recently reported (Mshandete, 2008). However, so far no studies have been done to investigate the effect of interaction of chicken manure with sisal fibres, sisal leaf decortications and sisal dust wastes as substrates on sporocarp cap lengths diameters, stipe lengths and stipe diameters and dry weights of the Tanzanian *C. cinereus* (Schaeff) S. Gray s.lat.

MATERIALS AND METHODS

Origin of materials

Coprinus cinereus (Schaeff) S. Gray s.lat and sisal decortications wastes and chicken manure

Although *C. cinereus* was classified as species of Agaricaceae, it is currently named as *Caprinosis cinerea* classified under Psathyrellaceae (Redhead et al., 2001; Srivilai and Loutchanwoot, 2009). The name of *C. cinereus* has changed to *C. cinerea* due to the recent phylogeny analysis with molecular makers (ITS sequence) by Redhead et al. (2001). However, some current publications still use the name *C. cinereus* (Arima et al., 2004; Riquelme et al., 2005; Heneghan et al., 2007; Mshandete and Cuff, 2008). In this paper the name *C. cinereus* (Schaeff) S. Gray s.lat of Tanzanian material established by traditional morphological taxonomy by Härkönen et al. (1995, 2003) will be used until molecular characterization is established. Starter fruiting bodies of *C. cinereus* were collected from sisal waste dumpsite at Alavi sisal estate privately owned by Mohamed Enterprises at Soga-Kongowe, Kibaha, Coast region, Tanzania, where they grow in nature. These mushrooms were brought to the laboratory the same day for tissue culture. Sisal wastes (sisal dust, sisal fibres and sisal decortications leaves) were obtained from sisal processing factory at Alavi sisal estate. Sisal wastes were packed separately and brought to the University of Dar es Salaam. Sisal fibres and sisal leaf decortications wastes were sun dried for seven days. Fresh free-range chicken (*Gallus gallus domesticus*) manure (chicken droppings) was obtained from local poultry keeper at Upanga, Dar es Salaam, Tanzania and was sun dried for five days and ground to fine powder using a laboratory blender (Snijders Scientific Tilburg, Holland, Waring Blender, Torrington, CT and USA).

The batches of sisal wastes and chicken manure used in this study were kept at -20°C in order to avoid changes of compositions (nutritional quality). The composition of sisal dust and both fresh and dry chicken manure are given in Table 1. The compositions of sisal leaf decortications wastes and sisal fibres wastes were as previously reported by Mshandete et al. (2005, 2006).

Mushroom cultivation

Tissue culture

Tissue culture is the beginning of mushroom cultivation. Establishments and maintenance of pure mycelium of the starter fruiting bodies was done according to Mshandete and Cuff (2008). A healthy fruiting body of *C. cinereus* was swabbed to sterilize with 70% alcohol. The fruiting body was split into halves to expose the inner tissues. A sterile scalpel was used to cut thin layer of the inner tissue, which was inoculated in the Petri dish containing the media malt extract agar (MEA). The latter was procured from (Oxoid, Unipath Ltd, Basingstoke, Hampshire, England). The media was prepared according to the manufacturer's instruction by dissolving 17.75 g of agar in a litre of distilled water. After the agar dissolved, the media was sterilized by autoclaving (Koninklijke AD Linden JR.BN-Zwijinderect, Holland) at 121°C for 20 min. The sterilized media was left to cool at room temperature and poured in pre-sterilized glass plates at 150°C for 3 h, each plate contained 20 ml. The plates were left to solidify at room temperature. The plates were then inverted and incubated at room temperature range of 27 to 30°C for 4 days to allow mycelia to grow. The sub culturing of the pure culture isolate was done on the same media employed during tissue culture.

Table 1. Proximate chemical composition of sisal dust and chicken manure (mean ± SD, n = 3).

Parameter	Sisal dust waste	Fresh chicken manure	Dried chicken manure
Total solids (%fresh)	12 ± 0.53	18 ± 0.43	11 ± 0.64
Volatile solids (%TS)	79 ± 0.50	60 ± 1.50	59 ± 4.70
VS/TS ratio	6.7	3.3	5.4
Ash content (%TS)	20 ± 1	39 ± 1	40 ± 1
Organic Carbon (%TS)	42 ± 2.0	38.3 ± 1.0	32 ± 2.0
Total Organic Matter	73 ± 7.0	62 ± 1.30	56 ± 8.10
Total nitrogen (TN) (%TS)	1.06 ± 0.5	1.99 ± 0.8	2.92 ± 0.6
Crude protein (TN x 6.25)	7	12.50	18
Carbon Nitrogen ratio	40	19	11

Spawn (mushroom seed planting material) preparation

Spawn preparation of *C. cinereus* was done as described according to Mshandete and Cuff (2008) using intact sorghum grains, which were bought from Kariakoo market in Dar es Salaam, Tanzania. The grains were first soaked in water for 12 h and thereafter parboiled for 10 min. After draining excess water, for every 1 kg of parboiled grains, 1.35 g of calcium sulphate and 0.35 g of calcium carbonate were added and properly mixed into the grains before spreading them out on a clean plastic sheet. 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 for one hour at 121°C and 1.54 kg/cm^2 in a 17 l autoclave (International pbi Sp, Milano, Italy). After cooling to room temperature, the bottles were shaken vigorously to avoid clumping of the grains. Then each cooled bottle of sterilized grains was aseptically inoculated with three 1cm^2 pieces of mycelium MEA taken from 4 day old cultures of one *C. cinereus* and incubated in a ventilated incubator (Memmert GmbH KG, Schwabach FRG, Germany) set at 28 ± 2°C for 10 days. Each inoculated bottle, with its cap loosely closed, was shaken twice thoroughly by hand to distribute the mycelia to the grains.

Solid-state fermentation bioreactors and inoculation of the substrates

The substrates used were sisal wastes, dust, leaves and fibers. They were added to an amount of water enough for making it moist. Every substrate and chicken manure was pasteurized at 70°C for three hours (Koninklijke AD Linden JR.BN-Zwijinderect, Holland). After pasteurization, they were left to cool. The cooled substrates supplemented with chicken manure were introduced in solid-state fermentation bioreactors (SSFBs). These SSFBs each consisted of 3 L rectangular plastic containers measuring 230 x 140 x 90 mm (length, width and height, respectively) (Cello® Domestoware (Mkate), Dar es Salaam, Tanzania). A total of 136 aeration holes of 7 mm in diameter and 30 mm apart were made in all the sides of the bioreactor. Four hundred and fifty grams of the substrate was introduced in each bioreactor and the weight of the chicken manure introduced in the bioreactor depended on the percentage of supplementation. The supplementation of chicken manure was based on the dry weight of the substrate. Each substrate was supplemented with 5 to 25% concentrations of free range chicken manure (Table 2). There were control SSFBs in which no chicken manure was added and these were labelled as 0% supplementation, that is, contained the substrate only (non-supplemented). The spawn rate employed was 5% based on wet weight of the substrate (22.5 g per 450 g wet weight substrate). After inoculation, the SSFBs were placed on wooden shelves disinfected by 3% domestic bleach (JIK, Reckitt Benckiser East Africa Limited, Kenya) in a spawn running room at the Molecular Biology and Biotechnology Department, College of Natural and Applied Sciences, 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 L tap water per day on the floor.

Spawn running (mycelia development), pin-head initiation, fruit body formation and harvesting of *C. cinereus*

Vegetative development was followed by direct observation of the inoculated substrates until the substrates were completely invaded by mycelia. Contaminants were also observed and noted but not quantified. The number of spawn run days for mycelia to colonize the substrate was recorded. During spawn running, fructification humidity and temperature was recorded using weather forecast clock (which simultaneously measures temperature and humidity) (Bright Weather care, Scholer Quartz, Swiss). The conditions during spawn-running in the room were 28 ± 2°C and relatively air humidity 78 ± 2%. 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 *C. cinereus* strain had grown throughout the whole substrate, the SSFBs were removed and transferred to a fruiting room with same dimensions and shelves as that of the spawning room. Changing conditions which were suitable during spawning, stimulated fruiting. In the fruiting room, in order to increase ventilation (air exchange), there were no curtains placed on the windows. Such a pin-head (primordial) initiation strategy introduced sufficient fresh air, which at the same time lowered carbon dioxide concentration in the cropping room. To further lower carbon dioxide, the lids of the SSFBs was removed to expose the fungal colonized substrate. In the fruiting room, the light intensity was increased by allowing indirect natural day light to diffuse in the room through windows which lacked curtains. In the room, relative humidity increased by 9% and temperature decreased by 5%, respectively (compared to that observed during spawn running) by pouring 25 to 30 L of water per day on the floor and on the walls. When necessary, the moisture of the SSFBs was maintained with the use of mist sprayers. Fresh *C. cinereus* fruiting bodies were harvested when young, firm and fleshy (immature/juvenile stage). *C. cinereus* fruiting bodies from different flushes in the different experiments were collected and the pileus and stipe diameters as well as the

pileus and stipe lengths were measured (Nwanze et al., 2008). Additionally, fresh weights of *C.cinereus* fruiting bodies were also taken (Mshandete and Cuff, 2008) and were dried in drying oven at 45°C to a constant weight (Mshandete and Cuff, 2007).

Analytical methods

Total solids (TS), volatile solids (VS) and ash content were determined according to standard methods (American Public Health Association, APHA, 1998). Total nitrogen contents were determined by indophenol-blue method using NH_4^+-N as standard according to Allen (1989). The UV absorbance of the solution was measured using spectrophotometer (Thermo Spectronic Helios β, England) at 660 nm. The organic carbon was determined by dry combustion method described by Allen (1989). The total organic matter (TOM) content of the substrate and chicken manure was calculated as differences in weight between dry weight (80°C until a constant weight) and ash weight (550°C for 4 h) according to Lyimo et al. (2002). All samples were determined in triplicate. Protein content was calculated by multiplying the total nitrogen content by the universal factor of 6.25. The composition and characteristics of sisal dust wastes substrate and chicken manure supplements is shown in Table 1.

Experimental design and statistical analysis

The experiment was carried out in SSBRs and was designed as split-split plot design with three replicates comprised of sisal waste substrates as the main plot, varying chicken manure supplementation concentrations as the sub-plot treatments. The data was collected and their means results are presented. To test the interactive effects of varying concentrations of chicken manure supplement and three solid sisal wastes substrates (sisal dust, sisal fibres and sisal leaves), cap diameter, stipe diameter, cap length, stipe length and dry weights of the fruiting bodies were recorded and the data subjected to one way analysis of variance (ANOVA). When significant differences were determined, post-test were made using Tukey-Kramer Multiple Comparisons Tests at the 5% probability level.

RESULTS

Chicken manure both from free range and poultry chicken is among the organic additives available in large quantities in Tanzania. The relative available even distributed and abudant bioresource chicken manure can be used to stimulate mushroom fruiting and productivity. In this study, an attempt was made in which *C. cinereus* an indigenous Tanzanian mushroom species was cultured on three different sisal wastes substrates that were inoculated with same sorghum spawn and supplemented with five varying concentrations of chicken manure. Generally, the results revealed that there was interaction of the three sisal wastes substrates with various chicken manure concentrations on the stipe and pileus, lengths and diameters and on dry weights of *C. cinereus* mushrooms. Various concentrations of chicken manure had effects on mushrooms dimensions, cap length (mm), cap diameter (mm), stipe length (mm), stipe diameter (mm) and dry weights (mg) regardless of the substrates used, ($p < 0.05$). Among the three substrates, sisal dust gave relatively overall best results compared to sisal fibres and sisal leaves decortications wastes, which was significant at ($p < 0.05$). It should be noted that, interaction of the three sisal wastes substrates with various chicken manure concentrations on the *C. cinereus* parameters measured in this study was very complex and in some situations the expected trend was not followed. For example, some interactions of chicken manure at lowest or highest concentrations with the three sisal wastes substrates induced negative effects on the parameters measured. Also, in some cases, chicken manure supplemented sisal waste substrates had smaller dimensions than that recorded from non-supplemented sisal wastes substrates. In particular, stipe diameters increase, were all negative for all five concentrations of chicken manure supplemented on sisal decortications waste. Such complex observations could possibly be attributed to the blending of sisal wastes substrates with chicken manure additive which may have affected the composition and qualities such as water holding capacity, degree of aeration, microclimate characteristics, nutrients resource distributions/allocations of the mixture that consequently induced varied effects on *C.cinereus* mushroom size/dimensions and dry weight. However, such detailed compositions analysis of sisal wastes and chicken manure mixtures used in this study (Table 2) were beyond the scope of this study and were not analysed.

Interaction of sisal waste with chicken manure on *C. cinereus* pileus and stipe lengths

Results in Tables 3 and 4 demonstrated the interactive effects of the three sisal wastes substrates and chicken manure on the *C. cinereus* pileus and stipe lengths. The interaction of the three substrates and 5% chicken manure concentration had significant effect ($p < 0.05$) on pileus length. The longest pileus average length of 18 mm was produced by sisal dust wastes interaction with 5% chicken manure concentration and sisal dust wastes interaction with 15% chicken manure concentration than comparable to sisal fibres wastes and sisal decortications leaves wastes substrates interactions with respective chicken manure concentrations. On the other hand, the sisal decortications leaves wastes interaction with 25% chicken manure concentration produced the shortest average pileus length of 6 mm. The observed pattern was supported by percentage increase of mean pileus length of *C.cinereus* in three sisal wastes substrates and five varying chicken manure concentrations compared to non-supplemented substrates. The interaction of sisal decortications wastes with all five chicken manure concentrations compared to non-supplemented sisal decortication wastes demonstrated highest percentages pileus length increase which ranged between 74 and 114%. The interaction of the three sisal wastes substrates with five

Table 2. Mixtures of substrates (wet weight) and chicken manure (dry weight) loaded in SSFBs.

Substrates/chickenmanure varying concentrations	Sisal dust	Sisal fibres	Sisal leaves
Control (0%)	450 g sisal dust	450 g sisal fibres	450 g sisal leaves
5%	4.9 g chicken manure, 427.5 g sisal dust	6.5 g chicken manure, 427.5 g sisal fibres	7.63 g chicken manure, 427.5 g sisal leaves
10%	9.88 g chicken manure, 405g sisal dust	13.1 g chicken manure, 405 g sisal fibres	15.26 g chicken manure, 405 g sisal leaves
15%	14.82 g chicken manure, 382.5 g sisal dust	19.6 g chicken manure, 382.5 g sisal fibres	28.88 g chicken manure, 382.5 g sisal leaves
20%	19.76 g chicken manure, 360 g sisal dust	26.2 g chicken manure, 360 g sisal fibres	30.51 g chicken manure, 360 g sisal leaves
25%	24.71 g chicken manure, 337.5 g sisal dust	32.72 g chicken manure, 337.5 g sisal fibres	38.14 g chicken manure, 337.5 g sisal leaves

Table 3. Pileus length (mm) of *C. cinereus* as affected by the interaction of sisal wastes and varying chicken manure concentrations (mean ± SD, n = 9).

Substrate	Non supplemented	5% chicken manure	10% chicken manure	15% chicken manure	20% chicken manure	25% chicken manure
Sisal dust	8.4a ± 6.3	18.0 ± 3.8 (114%)	14.6 ± 5.9 (74%)	18.0 ± 7.9 (114%)	15.6 ± 3.5 (86%)	16.2 ± 4.6 (93%)
Sisal fibres	7.2a ± 3.4	10.2 ± 4.9 (42%)	9.7 ± 1.5 (35%)	12.1 ± 6 (68%)	11.5 ± 2.3 (60%)	9.2 ± 3.8 (28%)
Sisal leaves	7.8a ± 3.3	12.5 ± 5.8 (60%)	9.3 ± 2.9 (19%)	11.7 ± 5.2 (50%)	7.6 ± 2.7 (-3%)	6.0 ± 2.8 (-23%)

Means followed by letter 'a' within the same column in a treatment group are not significantly different statistically at 5% probability using Tukey-Kramer Multiple Comparisons Test.Value in patenthesis percentage; increase of mean pileus length of *C.cinereus* in three sisal wastes substrate and five varying chicken manure concentrations compared to non-supplemented sisal wastes substrates.

Table 4. Stipe length (mm) of *C. cinereus* as affected by the interaction of sisal wastes and varying chicken manure concentrations (mean ± SD, n = 9).

Substrate	Non-supplemented	5% chicken manure	10% chicken manure	15% chicken manure	20% chicken manure	25% chicken manure
Sisal dust	20.7a ± 11.7	16.2a ± 4.4 (-22%)	19.5 ± 8.6 (-6%)	19.2a ± 9.3 (7%)	17.6a ± 7.5 (15%)	22.7 ±10.3 (10%)
Sisal fibres	15.2a ± 4.6	16.3a ± 9.4 (7%)	9.7 ± 3.7 (-36%)	15.4a ± 12 (1%)	21.5a ±11.4 (41%)	11 ± 3.3 (-28%)
Sisal leaves	10.6a ± 4	24.8a ± 11.7 (134%)	14.9 ± 8.3 (41%)	14.7a± 5.7 (39%)	16a ± 3.1 (51%)	10.8 ± 5.3 (2%)

Means followed by letter 'a' within the same column in a treatment group are not significantly different statistically at 5% probability using Tukey-Kramer Multiple Comparisons Test. Values in parentheses are percentage increase of mean stipe length of *C. coprinus* in three sisal wastes substrate and five varying chicken manure concentrations compared to non-supplemented sisal wastes substrates.

varying chicken manure concentrations on stipe lengths was also significant. The stipe length produced by the interaction of sisal decortications leaves wastes with 5%chicken manure concentration was statistically longer than the comparable stipe lengths induced by sisal fibres and sisal dust wastes interactions with respective varying chicken manure concentrations. In fact, shortest mean stipe lengths were produced by the interaction of sisal fibres wastes with 10% chicken manure concentration. A similar trend was observed in terms of percentage stipe lengths

Table 5. Pileus diameter (mm) of *C. cinereus* as affected by the interaction of sisal wastes and varying chicken manure concentrations (mean ± SD, n = 9).

Substrates	Non-supplemented	5% chicken manure	10% chicken manure	15% chicken manure	20% chicken manure	25% chicken manure
Sisal dust	29.7a ± 11.8	44 ± 7.1 (48%)	35.8 ±11.8 (21%)	24.2a ± 12.4 (-19%)	35.4 ± 4.6 (19%)	37 ± 7.6 (25%)
Sisal fibres	21.7a ± 4.8	21.8 ± 8.7 (0.5%)	24.7± 3.8 (14%)	28.7a ± 11.8 (32%)	29.2 ± 5 (35%)	27 ± 9.4 (24%)
Sisal leaves	24a ± 6.10	31.6 ± 13.5 (32%)	21.7 ± 5.1 (-10%)	27.8a ± 6.5 (16%)	18.8 ± 3.7 (-28%)	24 ± 1.3 (0%)

Means followed by letter 'a' within the same column in a treatment group are not significantly different statistically at 5% probability using Tukey-Kramer Multiple Comparisons Test. Values in parenthesis are percentage increase of mean pileus diameter of *C.cinereus* in three sisal wastes substrates and five varying chicken manure concentrations compared to non-supplemented sisal wastes substrates.

Table 6. Stipe diameter (mm) of *C .cinereus* as affected by the interaction of sisal wastes and varying chicken manure concentrations (mean ± SD, n = 9).

Substrates	Non-supplemented	5% chicken manure	10% chicken manure	15% chicken manure	20% chicken manure	25% chicken manure
Sisal dust	19.6 ± 5.1	20.7a ± 4.6 (6%)	22.4 ± 4.1 (14%)	18.4a ± 4.0 (-6%)	19.3 ± 3.7 (-2%)	17.9a ± 3.9 (-9%)
Sisal fibres	16 ± 1.4	14.3a ± 5.4 (-11%)	16.7 ± 3.5 (4%)	17.8a ± 5.2 (11%)	18.8 ± 3.3 (18%)	18a ± 5.5 (18%)
Sisal leaves	23.2 ± 6.9	23.1a ± 11.7 (0.4%)	15.8 ± 4.1 (32%)	19.5a ± 7.3 (16%)	12.8 ± 1.8 (-45%)	14a ± 1 (-40%)

Means followed by letter 'a' within the same column in a treatment group are not significantly different statistically at 5% probability using Tukey-Kramer Multiple Comparisons Test.Values in parenthesis are percentage increase of mean pileus diameter of *C.cinereus* in three sisal wastes substrates and five varying chicken manure concentrations compared to non-supplemented.

increase. Compared to non-supplemented sisal wastes substrates, the interaction of sisal decortications leaves wastes with 5% chicken manure concentration recorded the highest percentage stipe lengths increase of 134% while the interaction of sisal fibres wastes with 10% chicken manure concentration recorded a negative increase effect on stipe length of -36%.

Interaction of sisal wastes substrates with chicken manure on *C. cinereus* pileus and stipe diameters

The mean of pileus and stipe diameters of *C.cinereus* as affected by the interaction of sisal waste substrates and chicken manure at varying concentrations are presented in Tables 5 and 6. The mean pileus diameters induced by sisal dust wastes interaction with 5% chicken manure concentration were significantly wider than that induced by sisal decortications leaves wastes and sisal fibres wastes interactions with their respective varying chicken manure concentrations. The percentage increase of pileus diameter induced by interactions of three sisal wastes substrates and five varying chicken manures concentrations compared to non-supplemented sisal wastes substrates showed that, the pileus diameter increase ranged between 5 and 48%. The highest mean pileus diameter increase was recorded from sisal dust waste interaction with 5% chicken manure concentration while the lowest (negative pileus diameter increase) of -28% was recorded from sisal leaves decortications wastes interaction with 20% chicken manure concentration followed by -19% from sisal dust wastes interaction with 15% chicken manure concentration and -10% from sisal leaves decortications wastes interaction with 10% chicken manure concentration. On the other hand, it was remarkable to note that, the interaction of sisal decortications wastes with five varying concentrations of chicken manure induced poorest results (all negative effect on stipe diameter increase) when compared with non-supplemeted sisal decortications leaves waste substrate. In contrast, the widest stipe diameter was induced in non-supplemented sisal decortications leaves waste, which was statistically wider than the comparable diameters induced by other two non-supplemented sisal wastes substrates. Thus implying

Table 7. Dry weight (mg) of *C. cinereus* sporocarp as affected by the interaction of sisal wastes and varying chicken manure concentrations (mean ± SD, n = 9).

Substrates	Non-supplemented	5% chicken manure	10% chicken manure	15% chicken manure	20% chicken manure	25% chicken manure
Sisal dust	120a ± 10	160a ± 130 (33%)	130a ± 21 (8%)	570a ± 70 (375%)	150a ± 39 (25%)	120 ± 23 (0%)
Sisal fibres	70a ± 30	50a ± 30 (-29%)	170a ± 37 (143%)	119a ± 47 (70%)	160 ± 30 (129%)	50 ± 6 (-29%)
Sisal leaves	340a ±129	710a ± 262 (109%)	420a ± 146 (24%)	435a ± 12 (28%)	490a ± 89 (44%)	2000 ± 39 (488%)

Means followed by letter a within the same column in a treatment group are not significantly different statistically at 5% probability using Tukey-Kramer Multiple Comparisons Test. Values in parenthesis are percentage increase of mean dry weight of *C.cinereus* fruiting bodies in three sisal wastes substrate and five varying chicken manure concentrations compared to non-supplemented sisal wastes substrate.

that, interaction of sisal decortications wastes with varying chicken manure concentrations induced insignificant effects as far as *C.cinereus* stipe diameter is concerned.

Interaction of sisal wastes substrates with chicken manure on *C. cinereus* dry weight

The interaction of sisal wastes substrate and chicken manure induced mean dry weights of *C. cinereus* fruiting bodies that were statistically significant ($p < 0.05$) (Table 7). The interaction of sisal leaves decortications wastes with 25% chicken manure concentration induced a mean dry weight of fruiting bodies (sporocarps) that was significantly heavier than that induced by varying chicken manure concentrations interactions on sisal fibres waste and sisal dust waste substrates. Conversely, the comparable mean dry weights sporocarps induced in sisal fibres wastes interaction with 5 and 25% chicken manure concentration revealed such interactions produced the poorest results. This was further supported by mean dry weight percentage increase data of chicken manure supplemented sisal wastes substrates when compared with non-chicken supplemented sisal wastes. The interaction of sisal leaves decortications waste with 25% chicken manure concentration produced the highest mean dry weight increase of 488% while sisal fibres wastes interactions with 5 and 25% chicken manure concentrations produced negative mean dry weight effect of -29%.

DISCUSSION

It is obvious that there is a need to make more efforts to investigate various supplements (additives) both inorganic and organic, which are involved in mushroom substrate supplementation that affect mushroom productivity both quantitatively and qualitatively (Fanadzo et al., 2010). On the other hand, mushroom basal growth substrates have been reported to have significant effect on mushroom production whether it is at mycelia or fruiting bodies level due to the fact that mycelia growth and mushroom yield have different requirements (Oei, 2003, Nwanze et al., 2004a, 2005a, b). In this study, chicken manure from free-range chicken was evaluated as a possible additive to non-composted solid sisal wastes to ascertain its contribution to the pileus and stipe size and dry weight of *C. cinereus* fruiting body. The results in Tables 3 to 7 showed that interactions of three types of sisal wastes as substrates and varying chicken manure concentrations have a significant effect on *C. cinereus* sporocarps size and dry weight.

The mushroom size is essential for market purpose. The long and wide pileus as well as long stipe in particular could be of interest in the promotion of *C. cinereus* cultivation. It was observed that the interaction of sisal dust wastes with 5% chicken manure concentration and sisal dust wastes with 25% chicken manure concentration produced longest pileus and stipe lengths, respectively. Furthermore, the interaction of sisal dust wastes with 5% chicken manure concentration produced widest pileus diameters. However, in the case of non-supplemented solid sisal wastes the widest stipe diameters were observed in sisal leaves decortications wastes.

Weight of mushrooms whether wet or dry is an impor-tant agronomic parameter for evaluation of the fungi potent as biological agents in conversion of inedible organic wastes directly palatable mushroom human food. Therefore, the accumulation of more solids (nutrients) in the fruiting body measured as dry weight is important in nutritional consideration. The interaction of sisal leaves decortications wastes with 25% chicken manure concentration induced the heaviest sporocarps

dry weight. However, the present findings in this study cannot be rigorously compared with other studies since a review of the available literature of the information on the interaction of varying chic-ken manure supplement levels with three different solid sisal wastes substrates on sporocarp cap lengths and diameters, stipe lengths and diameters and dry weights of *C. cinereus* is lacking. Nevertheless, similar observations which can be indicative of the expected trends has been reported, although not on the same or similar mushroom species/strain, growth sub-strates and/or additives. The interaction of various spawn grains (corn, millet, wheat), oil types and rates, with different culture media (sawdust, grasses, wheat straw animal beddings, brown rice) have been reported to induce significant positive effects on carpophore dry weight, stipe length and stipe and pileus diameters of *Pleurotus sajor-caju*, *L. squarrosulus* (Mont.), Singer and *P. atroumbonata* (Bhandari et al., 1991, Nwanze et al., 2004a, 2005 a, b, 2008). The effectiveness of the chicken manure on *C. cinereus* fruiting bodies weight and dimensions (size) is most likely due to high protein in present chicken manure (Table 1). This observation sup-ports the previous findings that, in addition to increasing the surface area for fungal colonization, high protein additives are known to stimulate fruiting, increase mush-room weight and induce large size of fruiting bodies of course up to certain optimal concentrations of protein additives (Nwanze et al., 2005a).

Conclusions

Research on the use of lignocellulosic raw materials and additives to improve cultivation of *C. cinereus* has been an area of active continuous research in Tanzania. The experimental results showed that, fruiting bodies of *C. cinereus* can be cultured using solid sisal wastes based lignocellulosic waste materials. Sisal dust waste inte-raction with 5% chicken manure concentration induced longest pileus length and widest pileus diameter. Also, sisal dust waste interaction with 25% chicken manure concentration produced longest stipe lengths. Furthermore, chicken manure supplementation at 25% concentration on sisal decortications leaves wastes sub-strate have a profound effect on dry weight of *C. cinereus.* Therefore, cultivation of this mushroom can be recom-mended for economic exploitation particularly using sisal dust wastes and sisal leaves decortications wastes as a basal substrate in conjunction with readily available chicken manure as a protein additive.

ACKNOWLEDGEMENTS

We are highly indebted to the Management Alavi sisal estate for generously providing us with the substrates and granting access to sisal wastes dumps to collect *C. cinereus* fruiting bodies. The authors are grateful to the Department of Molecular Biology and Biotechnology, College of Natural and Applied Sciences (CoNAS) Uni-versity of Dar es Salaam (UDSM), Tanzania, for labora-tory facility support. We would like to thank Mr. Charles Kweyunga of Department of Botany, CoNAS, UDSM, for his assistance with total nitrogen analysis. We are also indebted to The Directorate of Research at the UDSM for financial support to publish this work.

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