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Nutritional Analysis of Some Composted and Non-Composted Agricultural Substrates used for Production of Kenyan Native Wood Ear Mushrooms [*Auricularia auricula* (L. Ex Hook.) Underw.]

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ABSTRACT

Wood ear mushrooms [*Auricularia auricula* (L. ex Hook.) Underw.] native to Kenya were cultivated on composted and non composted agro-wastes. The wastes included maize cobs, sawdust, sugarcane baggase grass straw and wheat straw each supplemented with wheat or rice bran at a ratio of 80:20. Both the composted and non composted substrates and supplements were nutritionally analyzed to determine their lignin, ash, cellulose, crude protein and moisture content. The cultivation experiment was arranged in a Completely Randomized Design (CRD) and replicated three times. Data was collected on days to pinning, fruit body quality, fruit body yield (fresh weight) and biological efficiency. The data collected was subjected to analysis of variance using SAS version 9.1. Mean separation was done using LSD and effects declared significant at 5% level. The substrates and supplements were significantly ($p<0.05$) different in their nutritional content with maize cobs and wheat bran containing higher cellulose, crude protein and moisture content. Remarkably, the dark brown strain had significantly ($p<0.05$) higher productivity factors on composted maize cobs supplemented with wheat bran. Saw dust and rice bran gave significantly the lowest growth performance on all the strains.

Key words: Wood ear mushrooms, composted and non composted substrates, nutritional content

INTRODUCTION

Domestication of wild edible mushrooms in tropical countries is underdeveloped despite the high quantities of agricultural wastes which can be utilized as ingredients for cultivation. In Kenya the mushroom industry is at infancy despite the abundance of agro-wastes produced at the end of each cropping season which offer a high potential for improvement. Currently, some non-governmental organizations are involved in assisting community based organizations to collect *Pleurotus* sp. from Kakamega and Karura forests for cultivation (Khare *et al*., 2008). This advancement is due to the rapid destruction of Kenyan forests leading to destruction of the natural habitat for such wild mushrooms which can cause their depletion. [*Auricularia auricula* (L. ex Hook.) Underw.] commonly known as wood ear mushrooms are native to Kenya and occur in Kakamega forest in Western Kenya (Wambua, 2004). Three main strains (brown, dark brown and yellow brown) occurring in this forest were previously identified through characterization using morphological markers (Onyango *et al*., 2010). The jelly-like fruit bodies have been shown to contain various bio-compounds that have anti-tumor, antiviral, antibacterial and anti-parasitic effects making it

a choice food (Chang and Miles, 2004; Yan *et al*., 2003). In addition, *Auricularia* mushrooms have been known to have marked ability to assist the body in healing complex ailments such as cancer, AIDS, diabetes and heart disease (Sultana and Qureshi, 2007). Tambekar *et al*. (2006) indicated that mushrooms have been used extensively in traditional medicine for curing various types of bacterial infections. In other parts of Africa, the wood ear mushrooms have been reported in diverse places such as Nigeria where it is being conserved through cultivation on palm substrates (Osemwegie and Okhuoya, 2009). In Kenya, the wood ears have not been previously cultivated. The mushrooms are currently faced by threats of depletion due to destruction of its forest habitat to clear land for settlement and agriculture (Gateri *et al*., 2004). In addition, communities residing around the forest harvest it throughout the year causing rapid destruction of the few remaining species (Palapala *et al*., 2006). Therefore, there is need to develop cultivation protocols which will protect this important resource from extinction.

In order to conserve the germplasm of this native mushroom, protocols for its domestication on locally available agricultural wastes are necessary. In Kenya, agricultural wastes such as maize cobs, sugar cane baggase, wheat straw and grass straw are of particular interest since they are produced in large quantities and their post harvest treatment is mainly accomplished through burning, incorporation into the soil or as livestock fodder. Tripathy *et al*. (2009) observed that cereal based substrates are the most popular ingredients used in synthetic formulations for mushroom production. According to Philippoussis *et al*. (2001) most agricultural residues are rich in lignocellulosic compounds whose handling and disposal are often problematic due to their chemical structure and chemical properties. However, mushroom cultivation technology can be exploited as a means of biodegradation of the plant matter thereby converting them into human food of high nutritive and medicinal value (Philippoussis *et al*., 2001). Gardezi and Ayub (2003) have previously illustrated such potentials for characterization and cultivation of mushrooms from the wild using locally available substrates. According to Chang and Miles (2004) the ability of a mushroom species to colonize a given substrate depends on its ability to produce lignolytic enzymes that can break down a wide range of plant matter.

The use of mushrooms to convert lignocellulosic wastes into food offers an alternative for developing unconventional source of proteins as food. A wide range of diverse lignocellulosic substrates have been used for cultivation of *Auricularia* mushrooms. Amongst cereal straws, rice and wheat straws were reported to be the best substrates for the cultivation of wood ear mushrooms (Bonatti *et al*., 2004). Chang and Miles (2004) reported the utility of sorghum straw and maize cobs for effective cultivation of wood ear mushrooms. Initial reports by these researchers indicate that Kenyan native wood ear mushrooms can successfully grow on substrates of maize cobs, sugarcane bagasse, wheat straw and sawdust (Onyango *et al*., 2010). Subsequent findings by Otieno (2010) indicated that composted substrates gave higher production values with wood ear mushrooms than non composted substrates. Whereas Shin *et al*. (2007) evaluated the nutritional properties of wild mushrooms cultivated on various substrates; the proximal composition of these substrates causing variations in mushroom productivity has not been established. The objective of this study was to evaluate the nutritional composition of composted and non composted agricultural wastes used for production of Kenyan native wood ear mushrooms.

MATERIALS AND METHODS

Source of germplasm: Three strains of native wood ear mushrooms were collected from Isecheno, Kisere and Malava forest reserves within Kakamega forest and phenetically characterized as previously described by Onyango *et al*. (2010). Identification of strains was mainly based on fruit body color with dark brown, brown and yellow brown strains selected for this study.

Substrate preparation: Five agricultural wastes, namely sugarcane baggase, maize cobs, wheat straw, grass straw and sawdust were used in this study. Baggase was obtained from West Kenya sugar factory, maize cobs, wheat and grass straws were obtained from farms within Kakamega, Eldoret and Kisumu counties, respectively.

Non Composted substrates (NC) were prepared by drying for 4 h under the sun and cut into small pieces (<4cm) using a sharp knife. They were soaked in water for 24 h and the surplus water drained off. The substrates were supplemented with either wheat or rice brans at a substrate supplement ratio of 80:20 and then mixed with $CaCO_3$ at a ratio of 3:100.

Compost substrates (C) were prepared using the short composting procedure derived from Sinden and Hauser (1980) in an enclosed room at the prevailing environmental conditions. Individual substrate combinations were laid in piles on the floor of the cultivation room and left exposed to free aeration. The substrate formulations were separately subjected to the short composting procedure for 9 days as shown in Table 1.

Nutritional analysis of the substrates: The following analysis were done for both the composted and non composted substrates; moisture, ash, crude protein, lignin and cellulose contents. All the samples were ground into a fine powder using before being analyzed for their nutritional content. The experiment was carried out at the microbiology laboratory of the Faculty of Agriculture, Egerton University. All the nutritional analysis procedures were replicated three times.

Determination of moisture content: Clean individually marked silica dishes of 3 cm in diameter were placed in an oven set at 105°C for at least one hour and cooled in a desiccator. They were weighed and recorded accurately. A small portion of the samples were placed in the dishes and the weighed on an electronic balance. The dishes with the sample were transferred to an oven set at 105°C and left for 8 h. After 8 h the dishes were transferred to a desiccators and left to cool. The samples were then removed from the desiccator one at a time and weighed again accurately. The loss of weight was considered as the moisture content of the samples in grams.

Determination of ash content: Clean silica dishes were appropriately marked and placed in an oven set at 105°C for 1. They were the removed, placed in desiccators to cool and immediately weighed. Four gram samples were placed in the dishes and weighed. The dishes with their samples were then placed in a furnace set at 550°C and the temperature left to rise gradually for 4 h. The furnace was then switched off and the sample left to cool to about 100°C before transferring into desiccators for cooling to room temperature. The samples were then weighed immediately in grams and net weight taken as the total ash content.

Table 1: Substrate composting procedure

Day	Procedure
1	Substrates were soaked in excess water and left for 3 days
3	Substrates were drained of excess water then arranged in a pile and compressed manually using wooden implement
5	First turn-30 g of $CaCO_3$ (for every 1 kg of mixture) was added as the pile was turned using a forked spade
7	Second turn-Little water was sprinkled while turning until the substrates were completely wet
9	Third turn-Little water was added as needed after performing a squeeze test. Where there was excess water, sun drying was done for a few hours. Compost was then ready for spawn running

Determination of crude protein content: 0.5 g of air dried samples were weighed and put in a micro-Kjeldahl tube. A tablet of selenium catalyst was added into each tube (making sure the entire sample was soaked). The flasks were placed in a digestion rack and the heat and exhaust fans switched on. The samples were heated until completely digested to colorless/blue solution followed by cooling in a desiccator to room temperature. Receivers were prepared by pipetting 15 mL of 0.1N HCL into Erlenmeyer flasks and 2-3 drops of the mixture indicator added. Kjeldahl tube was connected to the distilling unit. The volumes of acid, base and water was checked, keyed in program one. The door was closed and the distilled for 5 min. Two hundred and fifty millilitre of the distillate was collected and the Erlenmeyer flask was removed for titration. It was titrated against 0.1N sodium hydroxide to obtain a titer value. The amount of nitrogen (N) ×6.25 was taken as the amount of crude protein in the sample, given in micrograms.

Determination of lignin and cellulose content: 0.5 g of air dry sample was ground to pass 20-30 mesh/1 mm into refluxing beakers. 50 mL of neutral detergent solution and 1 mL of decahydronaphthalene was added. It was then placed in hot refluxing apparatus and condensers put in place. It was heated to boil for 5-10 min and adjusted to continue boiling gently. Refluxing was done for 60 min timed from the onset of boiling. The previously dried and tarred crucibles were placed on the filtering apparatus. The beakers were swirled to suspend the solids and fill the crucibles. The samples were rinsed into crucible with minimum hot water (90-100°C). The vacuum was removed, the mat broken up and crucible filled with hot water which was then filtered again. The samples were washed twice with acetone then suck dried. The crucible was dried at 105°C for 8 h. The desiccators were cooled and weighed. The crucible and their residue were then placed into the furnace set at 550°C and the temperature left to rise gradually for 4 h. The furnace was then switched off and the residue left to cool to about 100°C before transferring it into a desiccators for cooling down to room temperature. Evaluation of amount of cellulose as determined using the A. D. F method. To find the amount of lignin, 72% of H_2SO_4 was added to the samples and stirred with glass rod to smooth paste. The paste was left to stand for 4 hrs while stirring it hourly. The rod was then rinsed with hot water and the contents filtered into crucible half way with hot water and then filtered as completely as possible with vacuum to obtain the lignin. This was then weighed in micrograms.

Mushroom cultivation procedures: The mushroom cultivation procedure was conducted according to the methods of Oei (1996, 2005). Both the composted and non composted substrates were divided into lots of 1 kg each and packed into heat resistant polypropylene bags with a diameter of 12 cm and a length of 20 cm. The open ends of the bags were tightly tied using sterile cotton strings and autoclaved at 121°C for 1 h. The substrate bags were cooled to room temperature for 30 min and inoculated using grain spawns obtained from mycelia cultured from three strains of Kenyan native wood ear mushrooms-Yellow brown, Brown and Dark brown strains as previously characterized by the same author (Onyango *et al*., 2010). Several parameters were averaged from three replicates per treatment to test for the suitability of composted and non composted substrates for cultivation of wood ear mushrooms. The parameters included:

- **Duration to pinning:** Time in days that elapsed between the day of inoculation and the day of first pinhead formation
- **Yield:** Fresh weight measured in grams for all mature fruiting bodies collected from each bag
- **Fruit body quality:** Evaluated on a scale of 1-4 using the scale given in Table 2
- **Biological efficiency:** Percentage yield of fresh mushrooms over the dry weight of substrates.

Table 2: Descriptors for fruit body quality

Descriptor name	Shape	Fruit body diameter	Texture
Descriptor state	Cup-shaped/discoid	Very small (<10 mm)	Soft
	Lobed	Small (11-20 mm)	Rubbery
	Flattened/Appressed	Large (21-40 mm)	Leathery
	Ear shaped	Very large (>40 mm)	Gelatinous

Data analysis: Data collected on quantitative growth characters were subjected to Analysis of Variance (ANOVA) at 5% level of significance using the SAS version 9.1 (SAS, 2005). Duncan Multiple Range Test was used to separate the means where ANOVA was significant.

RESULTS

Nutritional content of the substrates: Fresh and composted substrates showed highly $p \leq 0.05$ significant differences in most of the nutrients tested with composted substrates leading in most of them. The ash content was significantly $p \leq 0.05$ higher in composted substrates than the non-composted ones. Composted sugarcane baggase gave the significantly the highest $p \leq 0.05$ ash content of 31.65 g while the least ash content was observed in non composted grass straw. Generally, composting increased the ash content in all the substrates. Positively significant $p \leq 0.05$ differences were also recorded in the lignin and cellulose content of the substrates. Composted sawdust gave the highest lignin and cellulose contents of 35.33% though it recorded much lower values for cellulose content of 24.80%. Comparatively, wheat straw and grass straw trailed all the substrates tested in their lignin and cellulose composition. The quantity of crude proteins was lowest in composted sawdust (3.20) while non composted maize cobs gave significantly $p \leq 0.05$ the highest amount of crude protein (8.20). The highest moisture content occurred in composted corn cobs with 52.12% while the lowest was non composted saw dust which gave 8.00% moisture content (Table 3).

Similar variations were observed in the wheat and rice bran supplements used in this study. Composted wheat bran gave significantly higher ash, lignin and moisture content compared to the non composted wheat bran. However, higher crude protein and cellulose contents were observed in non composted rice bran in comparison to the composted rice bran.

Effect of composted and non-composted substrates on duration to pinning: The type of substrate used had a pronounced effect on duration to pinning of the three wood ear mushroom strains tested. At the same time, the effect of composting and non-composting produced varying results with composted substrates mostly showing faster earliness. Composted maize cobs supplemented with wheat bran was found to be significantly the best substrate for all the wood ear strains examined. At the same time, the dark brown mushroom strain took numerically a shorter duration to produce pin heads followed by the brown and yellow brown strains respectively. The dark brown strain took significantly $p \leq 0.05$ the shortest duration of 15 days to produce pinheads when cultivated on composted maize cob and wheat bran. This was unlike the 19 days taken by the same strain to pin on non composted maize cob and wheat bran. Longer days to pinning were progressively recorded on wheat straw, sugar cane baggase and grass straw substrates. The longest duration to pinning of 37 days was observed on the dark brown strain when cultivated on non-composted saw dust. The brown strain lasted 16 days on composted maize cob and wheat bran combination to produce pinheads while it took 22 days on the same substrate but non-composted.

Table 3: Analysis of nutritional content of different substrates and supplements

	Ash (g)		Lignin (mg)		Cellulose (mg)		Crude protein (mg)		Moisture (%)	
Analysis	C	NC	C	NC	C	NC	C	NC	C	NC
Substrate										
Grass straw	12.93^{d}	8.36^{d}	7.38^{c}	8.83^{b}	25.46^{d}	27.63^{d}	6.88^{b}	7.36^{b}	42.60^{d}	35.75^{c}
Bagasse	21.65^{a}	10.90^{b}	12.00^{b}	14.16^{a}	28.51^{c}	29.11^{c}	5.43^{c}	6.40^{bc}	47.36^{b}	38.40^{a}
Wheat straw	12.90^{d}	9.33b^{c}	6.10^{d}	7.40^{c}	31.18^{b}	32.06^{b}	7.03^{a}	7.66^{b}	43.35^{c}	31.70^{d}
Maize cobs	18.50^{c}	9.28^{bc}	7.90^{c}	8.10^{b}	37.50^{a}	37.83^{a}	7.20^{a}	8.20^{a}	52.12^{a}	36.00^{b}
Saw dust	20.50^{b}	15.00^{a}	13.33^{a}	13.88^{a}	24.80^{e}	25.55^{e}	3.20^{d}	3.68^{d}	11.85^{e}	8.00^{e}
$LSD_{(5\%)}$	1.67	2.01	0.93	1.24	0.73	1.05	0.44	0.95	1.26	1.01
CV (%)	2.71	2.88	4.65	5.26	7.11	7.63	0.94	1.07	4.31	7.19
Supplement										
Rice bran	19.94^{b}	7.54^{a}	14.49^{b}	13.75^{b}	16.31^{b}	20.71^{a}	6.65^{a}	7.01^{b}	17.60^{a}	9.60^{a}
Wheat bran	21.89^{a}	8.01^{a}	16.59^{a}	14.82^{a}	21.07^{a}	20.17^{b}	6.97^{a}	7.87^{a}	17.30^{b}	9.14^{b}
$LSD_{(5\%)}$	1.05	2.14	0.95	1.06	0.88	0.51	0.77	0.05	0.13	0.16
CV (%)	5.61	4.93	9.24	5.66	4.45	6.72	0.27	0.83	088	0.95

Means followed by the same letter in the same column are not significantly different ($p \leq 0.05$). C: Composted, NC: Non-composted

On composted wheat straw, sugarcane baggase, grass straw and saw dust all supplemented with wheat bran, the brown strain took 20, 23, 29 and 33 days respectively while it lasted 22, 26, 32 and 33 days when the respective substrates were supplemented with rice bran. The yellow brown strain recorded the slowest mycelia progression taking up to 41 on non composted saw dust supplemented with rice bran. It however took 35 days on the same substrate supplemented with rice bran. On composted grass straw, sugar cane baggase, wheat straw and maize all supplemented with wheat bran, the yellow brown strain took 27, 25, 20 and 18 days respectively to pin. These values were numerically better than those observed in the same substrates supplemented with rice bran.

Effect of composted and non-composted substrates on fruit body quality: Evaluated on a scale of 1-4, fruit body quality of wood mushrooms harvested from different substrates showed significant differences. The dark brown strain produced significantly $p \leq 0.05$ the highest quality mushrooms on composted wheat straw and maize cobs ranging from 2.9 to 4.0 compared to a range of 2.8-3.9 for the same strain on non composted substrates. The lowest quality mushrooms of the dark brown strains were observed on saw dust and grass straw irrespective of the supplement used since they ranged from 2.0 to 1.0. This indicated that saw dust and grass straw were the worst substrates in terms of production of good quality mushrooms. On the other hand, maize cob, wheat straw and sugar cane baggase produced superior quality mushrooms. This was evident in the brown mushroom strains collected from composted maize cob, wheat straw and sugar cane baggase which gave values ranging from 2.1 to 3.7. A similar trend was observed in yellow brown fruit bodies with significantly $p \leq 0.05$ the highest qualities (3.6-2.3) observed in composted maize cobs and wheat straws supplemented with wheat bran. Saw dust, grass straw and sugarcane baggase all gave low quality yellow brown mushrooms. At the same time, it emerged that supplementation of substrates with wheat bran resulted in production of better quality mushrooms. This was noted in all the strains growing on either composted or non composted substrates (Table 4).

Effect of composted and non-composted substrates on fresh weight: Results for fruit body fresh weight per bag varied in composted and non composted substrates. Composted substrates supplemented with wheat bran supported higher fresh weight in all the strains. The most

Table 4: Effect of substrates and supplements on duration to pinning and fruit body quality

	Days to pinning						Fruit body quality (Scale 1-4)					
Substrate+ supplement	Yellow brown strain		Brown strain		Dark brown strain		Yellow brown strain		Brown strain		Dark brown strain	
combination	C	NC	C	NC	C	NC	C	NC	C	NC	C	NC
SD+RB	35.1^{a}	41.2^{a}	33.9^{a}	40.5^{a}	32.1^{a}	37.1^{a}	1.0^{h}	1.0^{f}	1.0^{f}	1.0^{e}	1.2^{j}	1.0^{gf}
SD+WB	34.8^{a}	39.4^{ab}	33.6^{a}	38.7^{a}	30.8^{a}	37.3^{a}	1.0^{h}	1.0^{f}	1.0^{f}	1.0^{e}	1.4^{i}	1.1^{gf}
GS+RB	29.8^{b}	38.8^{ab}	31.6^{b}	36.4^{b}	29.1^{b}	34.8^{b}	1.0^{h}	1.0^{f}	1.0^{f}	1.0^{e}	1.8^{h}	1.1^{gf}
GS+WB	26.6^{c}	37.3^{abc}	28.5^{c}	34.3^{c}	27.7^{c}	33.6^{c}	1.2^{gh}	1.0^{f}	1.2^{f}	1.1^{e}	2.0^{g}	1.3^{f}
SB+RB	26.5^{c}	35.7^{cd}	25.6^{d}	32.8^{cd}	24.3^{d}	31.1^{d}	1.4^{f}	1.1^{e}	1.2^{f}	1.1^{e}	2.1^{f}	1.6^{f}
SB+WB	24.8^{d}	34.3^{d}	23.2^{e}	30.9^{d}	22.8^{e}	28.4^{e}	1.9^{e}	1.3^{d}	1.8^{e}	1.6^{d}	2.5^{e}	2.1^{e}
WS+RB	23.3^{e}	32.4^{de}	22.3^{e}	28.3^{e}	21.3^{e}	27.2^{f}	2.3^{d}	1.3^{d}	2.1^{d}	1.9^{c}	2.9^{d}	2.8^{d}
WS+WB	20.1^{f}	29.5^{f}	20.1^{f}	27.3^{e}	18.8^{f}	24.9^{g}	3.2^{b}	1.6^{c}	3.3^{b}	2.1^{c}	3.1^{c}	3.5^{b}
MC+RB	18.3^{g}	28.1^{f}	16.2^{g}	25.4^{f}	17.4^{g}	21.3^{h}	2.8^{c}	2.0^{b}	3.1^{d}	2.8^{b}	4.5^{b}	3.2^{d}
MC+WB	17.6^{g}	27.1^{f}	16.0^{g}	22.3^{g}	14.8^{g}	18.7^{i}	3.6^{a}	2.4^{a}	3.7^{a}	3.1^{a}	4.0^{a}	3.9^{a}
$LSD_{(5\%)}$	0.98	2.53	1.60	1.93	1.22	0.97	0.25	0.18	0.31	0.24	0.16	0.35
CV%	2.05	1.18	3.41	2.17	4.82	4.68	7.17	5.28	8.52	6.93	11.20	8.52

Means followed by the same letter in the same column are not significantly different ($p \leq 0.05$). GS: Grass straw, SB: Sugarcane baggase, WS: Wheat straw, MC: Maize cobs, SD: Saw dust, RB: Rice bran and WB: Wheat bran, C: Composted, NC: Non-composted

pronounced effect was observed on combination was on the dark brown strain cultivated on composted maize cobs and wheat bran recording significantly $p \leq 0.05$ the highest fresh weight of 282 g. This was followed by the brown and yellow brown strains giving 274 g and 259 g respectively on the same substrate combination. In many cases, rice bran supplementation of maize cob lowered fresh weight with 266 g, 258 g and 235 g observed for the dark brown, brown and yellow brown strains respectively. Fresh weight reduced in non-composted substrates with 230 g being the highest recorded on maize cob and wheat bran. Collectively maize cobs was progressively followed by wheat straw, sugar cane baggase, grass straw and saw dust in terms of suitability for high fresh weight production. Significantly the least fresh weight was observed in the yellow brown strain cultivated in non composted sawdust and rice bran with a value of 78 g (Table 5).

Effect of composted and non-composted substrates on biological efficiency: Percentage biological efficiency was evaluated to determine the rate at which the lignocellulosic compounds in the substrates were degraded by the mushroom strains. Results obtained revealed significantly different $p \leq 0.05$ biological efficiencies for the various combinations as shown on Fig. 1. Maize cob supplemented with wheat bran gave significantly the highest B.E values for all the three strains tested. For composted maize cob supplemented with wheat bran, all the strains recorded B.E values above 60 whereas when the same substrate was not composted, B. E values of below 60 were only recorded in the yellow brown and brown strains. It clearly emerged that supplementation with rice bran lowered the B. E for composted and non-composted substrates. For instance, both composted and non composted sawdust all produced B.E values below 20 except when this substrate was supplemented with wheat bran. It was therefore evident that wheat bran supplementation greatly improved biological efficiency. On the other hand, B.E values progressively increased from maize cobs to wheat straw to grass straw and finally sawdust producing a linear increase. However, a departure from this trend was noted on the yellow brown strain cultivated on composted and non composted sugar cane baggase supplemented with rice bran.

Table 5: Effect of substrates and supplements on yield (fresh weight/bag)

	Fresh weight of fruit bodies (g)					
	Yellow brown strain		Brown strain		Dark brown strain	
Substrate+supplement combination	C	NC	C	NC	C	NC
SD+RB	81.7^{e}	78.4	106.7^{f}	98.4^{f}	110.3^{g}	101.8^{g}
SD+WB	92.4^{d}	83.9	114.1^{e}	102.7^{e}	117.7^{fg}	111.4^{fg}
GS+RB	96.6^{d}	88.7	121.7^{d}	108.3^{e}	126.9^{f}	116.9^{fg}
GS+WB	96.2d	92.2	129.7d	116.9de	141.8e	128.4^{ef}
SB+RB	123.4^{cd}	106.5	100.2^{e}	122.4^{de}	144.7^{e}	133.2^{e}
SB+WB	149.9^{c}	118.9	134.8^{cd}	125.0^{d}	153.5^{de}	143.7^{de}
WS+RB	221.1^{b}	134.4	146.9^{c}	136.6^{d}	166.3^{d}	154.1^{cd}
WS+WB	226.5^{b}	156.5	213.1^{b}	168.1^{c}	223.7^{c}	168.2^{c}
MC+RB	235.4^{b}	177.8	258.2^{a}	192.7^{b}	266.0^{b}	198.5^{b}
MC+WB	259.4^{a}	198.2	274.1^{a}	225.8^{a}	281.8^{a}	230.0^{a}
LSD (5%)	28.03	31.37	16.69	21.48	15.16	23.53
CV%	9.01	7.24	5.6	8.46	11.25	10.81

Means followed by the same letter in the same column are not significantly different ($p \leq 0.05$). GS: Grass straw, SB: Sugarcane baggase, WS: Wheat straw, MC: Maize cobs, SD: Saw dust, RB: Rice bran and WB: Wheat bran, C: Composted, NC: Non-composted

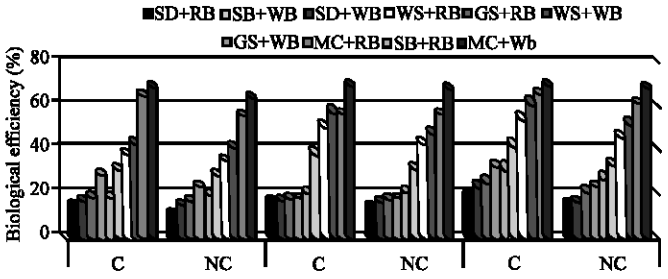

Fig. 1: Bar graphs showing effect of composted and non composted substrates on percentage biological efficiency. GS: Grass straw, SB: Sugarcane baggase, WS: Wheat straw, MC: Maize cobs, SD: Saw dust, RB: Rice bran and WB: Wheat bran, C: Composted, NC: Non-composted

DISCUSSION

Composted and non composted maize cobs, wheat straw, sugarcane baggase, grass straw and sawdust wastes evaluated for production of three strains of Kenyan native wood ear mushroom all revealed high utilization potentials for domestication. All the substrates were successfully colonized and reported emergence of fruiting bodies though at different rates. The influence of these substrates on mycelia growth of this mushroom was previously reported. This study investigated the nutritional role of composting the substrates in mushroom productivity factors such as pinhead formation, mushroom quality, yield and biological efficiency. Important nutrients such as moisture, lignin, cellulose and crude protein contents significantly affected the outcome of all the investigated parameters. At the same time, the role of added supplements was found to be high and it emerged that wheat bran supplement generally gave higher productivity values than rice bran.

Pinhead formation was found to vary between 15 days in the dark brown strain growing on maize cobs and wheat bran and 41 days for the yellow brown strain cultivated on sawdust supplemented with rice bran. A similar range of values for duration to pinning was observed by Iqbal *et al.* (2005) who obtained pin-heads of five strains of *P. ostreatus* on wheat straw between 20-40 days. At the same time, the variation in pinhead formation observed in this study agree with the findings of Kimenju *et al.* (2009) reporting time taken by mycelia to start pinning depends on the type of substrate used. Faster pinning occurred in composted substrates than the non composted ones. According to Philippoussis *et al.* (2001), duration to pinning of mushrooms is highly dependent on free circulation of moisture and air in the substrate. Analysis of the substrates showed that all composted substrates had higher moisture than the non composted ones. Therefore it is possible to argue that fast mycelia growth in composted substrates was due to the high moisture content which may have been conducive for the mycelia. However, composted maize cobs may have produced better results due to their ability to retain moisture as the substrates grew on them. Low rates of pinning on sawdust could be attributed to it inability to retain its moisture as noted in its moisture content. However, the findings of this study differed significantly with the findings of Ponmurugan *et al.* (2007) who reported a moisture content of 93.26 mg g^{-1} on sawdust. In addition to the substrate effect, supplementation with wheat bran further shortened the time to pinning unlike in a previous study where wheat bran reportedly caused contamination of the substrates (Nshemereirwe, 2004). These differences were attributed to nutritional variations among the substrates as observed in this study. All the substrates with high lignin and low cellulose contents took a longer time to start pinning compared to the substrates with low contents of lignin. The high lignin and low cellulose contents proved less efficient for pinning as it lacks ready carbon for rapid breakdown during mycelia growth. Therefore, the longer duration taken by the sawdust could be justified by the fact that sawdust has got very high amounts of lignin which take a long time to be decomposed hence unavailable to the mushroom. Conversely, substrates with high carbon and cellulose contents showed faster and denser mycelia growth and eventual pinning. The composting process may have initiated microbial breakdown of the cellulosic matter in the substrates releasing them faster for mycelia utilization (Kimenju *et al.*, 2009). High nutrition materials make mycelia to become vegetative rapidly resulting in vigorous growth and early pinning. Composted maize cobs and wheat straw had higher nutritional value compared to grass straw and sawdust. However, other factors such as high moisture content in a substrate have been reported to cause delayed pinning (Kimenju *et al.*, 2009).

The fruit body quality was significantly affected by the substrates and supplements. The dark brown strain produced superior quality mushrooms followed by the brown and yellow brown strains respectively. Composted maize cobs and wheat straw produced high quality dark brown mushrooms ranging from 2.9 to 4.0 compared to a range of 2.8-3.9 for the same strain on non composted substrates especially when supplemented with wheat bran. Nutritional evaluation of maize cobs and wheat straw substrates revealed high cellulose, crude protein and moisture content compared to saw dust, grass straw and sugar cane baggase. Sonnenberg (2007) proposed that substrates with high nutrient bases produce higher quality mushrooms. Wheat bran consistently gave high values of crude protein indicating high levels of nitrogen compounds. It was clear that all that the high nitrogen to carbon ratio in some of the substrates caused firm and large basidiocarps rated 3-4. In addition, Philippoussis *et al.* (2001) reported that high cellulase activity in mushrooms is increased in composted substrates and this shows better utilization of carbohydrates leading to production of high quality mushrooms. Non composted substrates may have produced mushrooms of less quality

due to reduction in cellulose activity. Even though large sized fruit bodies were considered to be of good quality and were rated highly, Shen and Royse (2001) commented that this is an inferior quality since such fruit bodies tend to break during packaging thereby reducing their quality. This might be improved by lowering the quantity of the wheat bran supplement in the substrates which would slightly reduce the size of the fruit bodies while improving their durability (Shen and Royse, 2001).

Previous studies on wood ear cultivation suggest that the cellulose content of the substrate and enzyme production of the mushrooms is important in determining the yield of a mushroom crop. The consistent better performance of the dark brown mushroom strain could be attributed to faster and more vibrant primary growth at the mycelia stage (Onyango *et al*., 2010). This may have been translated to better uptake of nutrients for sporophore formation leading to higher fresh weights. The variations observed in yield may also be attributed to the complexity of substrates in terms of their cellulose content resulting in a difference in the rate of degradation by the mushroom enzymes. Martinez-Carrera *et al*. (2002) reported that the capacity of wood ear mushrooms to grow on agricultural wastes such as maize cobs is due to their lignolytic enzymes that are necessary for degradation of such substrates. Thomas *et al*. (1998) showed that high lignocellulosic content of the substrate is important in fruit body production. Philippoussis *et al*. (2001) compared various lignocellulosic contents of substrates such as wheat straw, sugar cane bagasse and wood chips for various specialty mushrooms cultivation and concluded that varying results could be obtained. Composted maize cobs and wheat bran combination produced the highest fruit body yield (fresh weight) indicating that it was the most suitable for native wood ear mushroom growth.

Iqbal *et al*. (2005) realized the best yield of *Pleurotus ostreatus* and *Pleurotus sajarcaju* from wheat straw and attributed this to the supplement base used. A similar conclusion can be derived from this study since the carbon:nitrogen ratio as evidenced by crude protein and cellulose content was found to be higher in all the substrates and supplements which produced high yielding mushrooms. However, wheat bran was found to have a greater effect on growth than rice bran. This may be attributed enhanced performance of mycelia due to availability of several amino acids, protease as well as transaminase enzyme activities on wheat bran (Shashireka *et al*., 2005). Ayodele and Okhuoya (2007) obtained the highest yield of *Psathyrella atroumbonata* on sawdust supplemented with wheat bran at 5%. At the same time Ayodele and Akpaja (2007) realised high fresh weight values on *Lentinus squarosulus*. However, these results differed markedly with the findings of this study since sawdust consistently gave low fresh weight values. Ponmurugan *et al*. (2007) protein contents of 20.88 mg g^{-1} in saw dust which was much lower than all the substrates studied. However, this value was higher than that recorded in this study. Schiler (1982) speculated that the reduction in fresh weight of mushrooms on sawdust might be associated with the absence of certain specific nutrients especially the cellulose-based substrates. Probably the greatest impediment to high productivity on saw dust was its small grains which limited adequate aeration and reduced its water retention capacity. Philippoussis *et al*. (2001) reported that small grained substrates achieve quick compactness which limits aeration. This may also apply to the sugarcane baggase used which was obtained from a milling factory that reduced them to powder form. Thomas *et al*. (1998) reported that the very complex nature of sugar baggase impedes its efficient conversion to fungal mycelium. In addition, it's possible that the mushroom received nutrition and energy from the abundant free sugars that were present in the baggase and therefore made limited use of the cellulose fraction (Philippoussis *et al*., 2001). The very low fresh weights recorded for fruit bodies collected from grass straw was surprising. However, the grass

straw used in this study had been stored for long accumulating phenolic acid which reduced its fruit body forming ability. Studies done by Bano and Rajarathran (1988) showed relationships between phenolic acid concentration in a given substrate and the enzymatic activity of developing mushrooms. This translates to maximum sized of the fruit bodies (Royse, 1996).

The suitability of different substrates for mushroom cultivation was also confirmed by the average biological efficiency which was variable among the substrates. Composted and non composted maize cob combinations produced the highest B.E values for all the strains. According to Narain *et al.* (2008) maize cobs have a considerably higher lipoprotein component than the rest of the substrates used in this study. This was evidenced by the highest value of crude proteins obtained in maize cobs. The wood ear mushrooms are known to be capable of breaking down such proteins and incorporating them rapidly into their protoplasm (Mandeel *et al.*, 2005). Therefore, the lipoproteins present in the maize cobs must have been efficiently utilized by the wood ears resulting in the high B.E value (Adejumo and Awosanya, 2005). These findings corresponds with the work of Kimenju *et al.* (2009) that stipe length in maize cob was longer than that in sawdust, sugarcane baggase and wheat straw. The present study also recorded pinhead abortion in some sawdust substrates and as a result sawdust recorded the least B.E (Biological Efficiency). However, contrary to these findings, sawdust from mango wood shavings was found to be best substrate for mushroom growth and fruiting body formation in a study conducted by Islam *et al.* (2009).

CONCLUSION

From the findings of this study, it is evident that many locally available organic substrates have high potential for utilization as substrates and or supplements for mushroom production. Composted maize cobs and wheat straw supplemented with wheat bran produced the best results and were recommended for wood ear mushroom production. Apparently, these locally available organic materials are rich in lignin and cellulose which are utilized by the mushroom mycelium as a source of nutrition. These can be used by the rural populace to increase production of the much needed proteins in the diet.

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