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Suitability of Selected Supplemented Substrates for Cultivation of Kenyan Native Wood Ear Mushrooms (*Auricularia auricula*)

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ABSTRACT

Different organic substrates namely maize cobs, wheat straw, grass straw and sugarcane bagasse supplemented with either wheat or rice bran were evaluated for production of two Kenyan native strains of wood ear mushroom [*Auricularia auricula* (L. ex Hook.) Underw.]. The objective was to evaluate the suitability of these substrates for cultivation of Kenyan native wood ear mushroom. Plastic bag technology was used with treatments arranged in a completely randomized design replicated three times. Samples of black and brown strains of the wood ear mushroom collected from woody stems of dead and dying trees within Kakamega forest were used in this study. Data was collected on days to pinning, fruit body quality, fruit body yields (number and 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 two mushroom strains were not significantly ($p>0.05$) different in performance except for the number of fruit bodies where the black strain yielded significantly ($p<0.05$) higher than the brown one. The best performance was obtained from maize cobs and wheat straw substrates supplemented with wheat bran and these combinations were recommended to wood ear mushroom growers.

Key words: *Auricularia auricula*, pinning, fruit bodies, biological efficiency, Kenya

INTRODUCTION

Strong consumer demands and threats of depletion of mushrooms have stimulated increased worldwide production in the past few decades (Chang and Miles, 2004). The increased demand of mushrooms is due to their unique culinary and medicinal properties (Yan *et al.*, 2003). However, Africa contributes a paltry 1% of the annual worldwide production of mushrooms (Adejumo and Awosanya, 2005). In Kenya, commercial mushroom production is limited to two exotic species namely; oyster and the white button mushroom which are solely cultivated for the hotel industry (Gateri *et al.*, 2004). Indigenous mushrooms such as wood ear mushrooms [*Auricularia auricula* (L. ex Hook.) Underw.] found in Kakamega forest in western Kenya have not been studied to establish their cultivation potential.

The wood ears occur as saprophytes on stumps or at the bases of dead or dying woody trees. The jelly-like fruit bodies of wood ears contain various bio-compounds that have anti-tumor, antiviral, antibacterial and anti-parasitic effects making it a choice food (Chang and Miles, 2004). Wood ear mushrooms are a delicacy to communities residing around the Kakamega forest where they are

known as *matere* (Palapala *et al.*, 2006). The communities harvest the mushrooms throughout the year causing rapid diminishing of the few remaining species (Palapala *et al.*, 2006). Further, the forest habitats are rapidly destroyed alongside the germplasm of this fungus to create land for settlement and agriculture. These threats of depletion necessitate development of cultivation techniques that can be easily adopted by local farmers for conservation of this resource.

Extensive research has been carried out on the most efficient cultivation methods for specialty mushrooms other than the wood ears (Royse *et al.*, 1990; Stamets, 2000). One of the greatest challenges to mushroom cultivation is production of spawn used for bulk inoculation of substrates (Oei, 2005). Another limiting factor towards domestication of native mushrooms is identification of suitable lignocellulosic substrates for cultivation. Palapala *et al.* (2006) reported that Kenyan native wood ear mushrooms have the potential to be grown on locally available substrates such as wheat straws, sugar bagasse, sawdust, maize cobs and maize stalks. In order to achieve maximum yields, supplementation of substrates with other nutrient bases such as soybean meal, rice and wheat brans is necessary as they can reportedly increase mushroom yield two-fold (Royse *et al.*, 1991). Supplementation of substrates has therefore become one of the major aspects of mushroom cultivation (Ayodele and Akpaja, 2007).

This study utilized maize cobs, wheat straw, sugarcane bagasse and grass straw as the main ingredients to determine the best substrate for cultivation. Equal levels of rice and wheat brans were used as supplements to the substrates. The objective was to evaluate the suitability of these locally available organic substrates in production of Kenyan native wood ear mushroom. The study therefore outlines the cultivation protocols for native wood ears that can be adopted by rural farmers for nutritional and conservation purposes. Experiments for house management were designed and comparison of outputs done to recommend superior procedures for domestication.

MATERIALS AND METHODS

The study was conducted at Masinde Muliro University of Science and Technology between September, 2007 and May, 2008. Four substrates namely grass straw, sugar bagasse, wheat straw and maize cobs were tested in this experiment. To each of the substrates, 20% of rice and wheat bran supplements were added. Plastic bag technology was used with treatments arranged in a completely randomized design replicated three times. Samples of black and brown strains (Fig. 1a,b) of wood ear mushrooms collected from woody stems of dead and dying trees within Kakamega forest were used in this study.

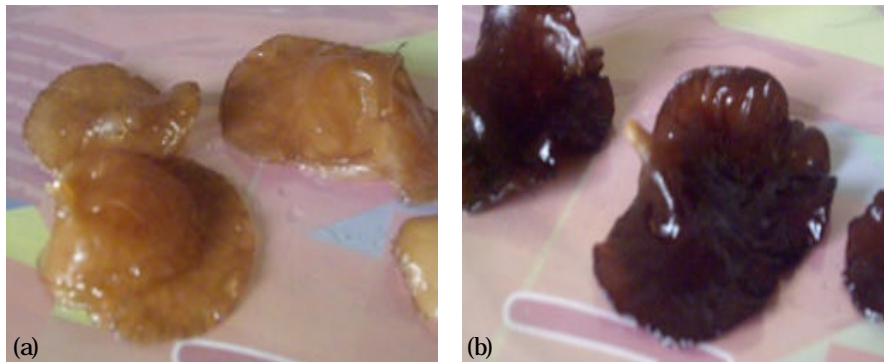


Fig. 1: (a) Brown and (b) black wood ears mushrooms from Kakamega forest in western Kenya

Table 1: 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

The substrates were cut into small pieces (<4 cm) using a sharp knife. They were then submerged in water for 12 h to soften them. The substrates were separately subjected to the short composting procedure using the methods of Sinden and Hauser (1980). The 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 ends of 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 the two mushroom strains. The inoculated substrates were labeled and kept in total darkness in enclosed cabinets for 14-25 days to allow complete colonization of the substrates. Upon completion of spawn run, two holes 10 mm diameter were made on each bag.

After the substrate bags were fully colonized by mycelia, they were slit using a sharp razor at the sides while the tops were opened. Substrate temperatures were lowered to the fruiting range of 18-23°C by submerging the sealed substrates in cold-water refrigerator for 10 min. Air temperatures were lowered using two electric fans during the day. At night, the windows were left open to lower the room temperature. Humidity was maintained at between 90-95% through constant flooding of the floor with sterile water and spraying each bag of substrate with 1 liter of water twice a day. Fresh air was circulated using two electric fans. The room was lighted on a 12 h on/off cycle using two fluorescent bulbs of 100 watts. The duration taken by each bag to produce primordial was recorded and averaged for each replicate.

Three to four days after primordial formation, mature mushrooms were harvested. Data was recorded on quality, number and fresh weight of fully mature fruit bodies. Fruit body quality was evaluated on a scale of 1-4 using the descriptors in Table 1. The second flush grew more rapidly and was harvested in the same manner after 6-10 days. Used substrates from each bag were wrapped in aluminum foil and dried in an oven set at 80°C for 36 h. Percentage Biological Efficiency (BE) was calculated using the following formula:

$$BE = \frac{\text{Fresh weight of mushroom fruit bodies per bag (g)}}{\text{Dry weight of spent substrates (g)}} \times 100$$

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.

RESULTS

Organic substrates and supplements tested were significantly different ($p < 0.05$) in suitability for wood ear mushroom cultivation. Generally, maizcob substrate consistently gave the best results followed by wheat straw, sugar bagasse and grass straw in that order. On the other hand, wheat bran supplement proved to be better than rice bran (Table 2). Analysis of variance indicated that the two mushroom strains were not significantly ($p > 0.05$) different in all the parameters that were

Table 2: Effects of separate substrates and supplements on growth, yield and quality

Effects	Days to pinning		Fruit body quality (Scale 1-4)		No. of fruit bodies		Fresh weight of fruit bodies (g)		Biological efficiency	
	Brown	Black	Brown	Black	Brown	Black	Brown	Black	Brown	Black
Substrate										
Grass straw	35.0a	33.8a	1.1d	1.1c	4.4c	2.5d	125.6c	96.4 ^d	23.8c	18.2d
Bagasse	28.2b	30.1b	1.3c	1.7b	4.0c	5.4c	117.5c	136.6 ^c	25.3c	30.3c
Wheat straw	25.7c	24.4c	2.7b	2.7a	8.1b	11.1b	180.0b	223.8 ^b	40.8b	55.2b
Maize cobs	21.7d	20.6d	3.2a	2.9a	15.4a	15.5a	266.2a	262.4 ^a	67.0a	63.7a
LSD _(5%)	0.695	1.134	0.180	0.221	1.067	1.37	11.805	19.82	4.483	6.745
Supplement										
Rice bran	28.7a	28.35a	1.8b	1.8b	6.8b	7.9b	156.8b	169.1 ^b	35.4b	37.0b
Wheat bran	26.6b	26.0b	2.3a	2.5a	9.1a	9.4a	187.9a	190.5 ^a	43.1a	46.7a
LSD _(5%)	0.491	0.802	0.127	0.156	0.755	0.967	8.347	14.02	3.170	4.770

Table 3: Effects of substrate supplementation on growth, yield and quality parameters

Substrate+ supplement combination	Days to pinning		Fruit body quality (Scale 1-4)		No. of fruit bodies		Fresh weight of fruit bodies (g)		Biological efficiency	
	Brown	Black	Brown	Black	Brown	Black	Brown	Black	Brown	Black
GS+RB	35.1 ^a	33.9 ^a	1.0 ^b	1.0 ^f	4.1 ^{ef}	2.8 ^{ef}	121.7 ^d	96.6 ^d	18.7 ^d	18.0 ^d
GS+WB	34.8 ^a	33.6 ^a	1.2 ^{gh}	1.2 ^{ef}	4.7 ^{ef}	2.2 ^f	129.7 ^d	96.2 ^d	29.0 ^c	18.3 ^d
SB+RB	29.8 ^b	31.6 ^b	1.4 ^f	1.2 ^{ef}	2.8 ^f	4.5 ^{de}	100.2 ^e	123.4 ^d	19.3 ^d	21.3 ^d
SB+WB	26.6 ^c	28.5 ^c	1.9 ^e	1.4 ^e	5.3 ^{de}	6.3 ^d	134.8 ^d	149.9 ^c	31.3 ^c	39.3 ^c
WS+RB	26.5 ^c	25.6 ^d	2.3 ^d	2.1 ^d	6.2 ^d	10.6 ^c	146.9 ^c	221.1 ^b	38.3 ^b	51.7 ^b
WS+WB	24.8 ^d	23.2 ^e	3.2 ^b	3.3 ^b	9.9 ^c	11.5 ^c	213.1 ^b	226.5 ^b	43.3 ^b	58.7 ^b
MC+RB	23.3 ^e	22.3 ^e	2.8 ^c	2.2 ^d	14.2 ^b	13.6 ^b	258.2 ^a	235.4 ^b	65.3 ^a	57.0 ^b
MC+WB	20.1 ^f	18.8 ^f	3.6 ^a	3.7 ^a	16.6 ^a	17.5 ^a	274.1 ^a	289.4 ^a	68.7 ^a	70.3 ^a
LSD _(5%)	0.98	1.60	0.25	0.31	1.51	1.93	16.69	28.03	6.34	9.54
CV (%)	2.05	3.41	7.17	8.52	10.93	12.94	5.60	9.01	9.33	13.17

Means followed by the same letter along the same column are not significantly different, GS: Grass straw, SB: Sugarcane bagasse, WS: Wheat straw, MC: Maizecobs, RB: Rice bran, WB: Wheat bran

measured except number of fruit bodies. However, the black strain recorded numerically better results than the brown one in all aspects. Strain by substrate interactions were highly significant ($p < 0.0001$) indicating that different mushroom strains responded differently to different substrates.

Primordia formation was preceded by whitening of specific parts of the substrate surface and formation of sclerotia. Pinheads emerged as small rounded lumps that were grouped at particular parts of substrate surfaces. The substrates had variable effects on the duration to primordial formation ranging from 21 to 35 days for maize cobs and grass straw respectively. The supplements were also significantly variable with wheat bran taking a shorter time to pin averaging 26 days than rice bran which took an average of 29 days (Table 2). Consequently, the combination of maize cob and wheat bran resulted to the earliest primordial formation while grass straw and rice bran combination significantly delayed the pinning (Table 3). Analysis of variance indicated no significant ($p > 0.05$) difference in primordia formation between the two mushroom strains.

The quality of fruit bodies evaluated on a scale of 1-4 varied significantly ($p = 0.05$) among substrates and supplements tested. Maize cobs and wheat bran combination produced large, erect and gelatinous fruit bodies that were auriform in shape (Fig. 2) and were rated of the highest



Fig. 2: High quality wood ears fruiting on maizecobs supplemented with wheat bran

quality ranging from a score of 2-4 (Table 3). The appearance of the mushrooms was excellent, with well-formed large caps and conspicuous stems (Fig. 2). Grass straw gave the lowest quality mushrooms followed by sugar baggasse. Wheat straw and wheat bran combination also gave good quality fruit bodies that ranged from 2-3 (Table 3). Supplemented wheat straw and sugarcane bagasse produced fruit bodies of average quality since their characters were intermediary in terms of shape, size and texture. The lowest quality fruit bodies were obtained from grass straw especially when supplemented with rice bran (Table 3).

The number of marketable fruit bodies varied significantly ($p < 0.05$) among substrates and supplements. Maizecobs and wheat bran combination produced the highest number of fruit bodies (17) followed by wheat straw and wheat bran combination (11). Grass straw produced the least number of fruit bodies ranging from 2-5 (Table 3). The fresh weight of fruit bodies also varied significantly ($p < 0.05$) among the substrates with maize cob recording the highest fresh weight of 266.2 and 262.4 g of fruit bodies of brown and black strains respectively. Wheat straw produced 180 and 223.8 g of fruit bodies of brown and black strains respectively. Grass straw and sugar bagasse produced the least number of fruit bodies of the black (96) and brown (118) strains respectively (Table 2).

Biological Efficiency (BE) was calculated to determine how the mushrooms utilized nutrients present in the substrates efficiently. Average biological efficiency was significantly ($p < 0.05$) different among different substrates and supplements tested. Maize cobs had the highest BE of 67% and 63.7% for the brown and black strains respectively. Wheat straw, sugar cane bagasse and grass straw had BE values of 40.8, 25.3 and 23.8%, respectively for brown strain and 55.2, 30.3 and 18.2%, respectively for black strain (Table 2). Rice bran was found to lower the BE of all substrates when used as a supplement unlike wheat bran which boosted the BE of all substrates (Table 3).

DISCUSSION

This study demonstrated that wheat straw and maize cobs are potentially suitable for use in wood ear mushroom production. Wheat bran also proved to be a suitable material for supplementing the two organic substrates. Ayodele and Akpaja (2007) reported that supplementation of sawdust with 20% oil palm fibers enhanced the mycelia growth and sporophore yield of *Lentinus squarosulus*. Kimenju *et al.* (2009) also demonstrated that mushrooms can be grown on several locally available organic substrates. Nutritional composition of substrates is a

crucial factor in determining how mycelia growth and primordial initiation occurs (Royse, 1997; Stamets, 2005). Narain *et al.* (2008) reported that mycelia growth and primordial development is dependent on the lignocellulosic materials especially the carbon: nitrogen ratio. Mushrooms are known to produce a wide range of hydrolytic and oxidative enzymes that enable them to colonize, degrade and bio convert many lignocellulosic substrates (Bano and Rajarathran, 1988).

Kimenju *et al.* (2009) indicated that the time taken by the mycelia to start pinning after ramification depends on the substrates used. Maize cobs and wheat straw took shorter time to primordial formation than sugar bagasse while grass straw took the longest time. This concurred with the findings of Kimenju *et al.* (2009) who obtained the shortest time to pinning of Oyster mushroom (*Pleurotus ostreatus*) on maize cobs. Ramzan (1982) obtained pin-heads of five strains of *P. ostreatus* on wheat straw between 20-40 days. 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. Materials with high quality lignin and cellulose contents reportedly take longer time to start pinning compared to the substrates with low contents of lignin and cellulose. This is because high nutrition materials make the mycelia to remain vegetative for a longer period resulting in vigorous growth and late pinning (Kimenju *et al.*, 2009). It can therefore be assumed that maizecobs and wheat straw had poor nutritional value compared to grass straw. 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. Maize cobs and wheat straw produced high quality mushrooms especially when supplemented with wheat bran. This contradicts with Shen (2001) who obtained poor quality mushrooms at high levels of wheat bran but conforms to Shen and Royse (2001) who reported that mushroom quality might be improved by lowering the quantity of the wheat bran supplement in the substrates. Shen (2001) also observed that increasing wheat bran levels in sawdust substrates containing millet and rye or both improved mushroom quality. 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.

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 variations observed in yield may 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. Maize cobs and wheat bran combination produced the highest fruit body yield (number and fresh weight) indicating that it was the most suitable for native wood ear mushroom growth. Iqbal *et al.* (2005) realized the best yield of *P. ostreatus* and *Pleurotus sajarcaju* from wheat straw. Ayodele and Akhuoya (2007) obtained the highest yield of *Psathyrella atroumbonata* on sawdust supplemented with wheat bran at 5%. 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. Zervakis *et al.* (2001) compared various lignocellulosic contents of substrates such as wheat straw, sugar bagasse and wood chips for various specialty mushrooms cultivation and concluded that varying results could be obtained.

Very low fresh weights were recorded for fruit bodies collected from sugarcane bagasse and grass straw. Schiler (1982) speculated that the reduction in fresh weight of mushrooms might be associated with the absence of certain specific nutrients especially the cellulose-based substrates. Thomas *et al.* (1998) reported that the very complex nature of sugar bagasse 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 bagasse and therefore made limited use of the cellulose fraction (Zervakis *et al.*, 2001). The grass straw used in this study had been stored for long accumulating phenolic acid, which reduced its fruit body forming ability. A study done by Royse (1996) showed that there is a significant relationship between phenolic acid concentration in a given substrate and the enzymatic activity of developing mushrooms that translates to the maximum size of the fruit bodies.

In this study, the average number of fruit bodies obtained in each bag was quite low in comparison to previous work done on wood ear mushrooms (Wong and Wells, 1987). This was attributed to accumulation of carbon dioxide and temperature fluctuations in the cabinets during spawn running since environmental conditions of the cabinets were not efficiently controlled. Shen and Royse (2001) reported that accumulation of CO₂ during spawn running may cause decrease in initiation points thus reducing mushroom productivity. Temperature fluctuations can also cause death of surface mycelia and sclerotia, which may reduce the number of fruit bodies formed (Oei, 2005). Contamination of the substrates by green mould (*Chaetomium olivacearum*) could also have contributed to low number of fruit bodies (Oei, 1996). The green mould competes with the mushroom for space, nutrients as well as causing chemical alteration of the substrate, which hinders mushroom development (Chang and Miles, 1989). The parts of the substrate occupied by this fungus were unfavorable for mycelia growth and only the parts devoid of infection were fully colonized and produced fruit bodies. In addition, the infection led to falling off of several young fruit bodies during watering due to their fragile nature. The contamination effect was greatest in sugarcane bagasse and grass straw. The effect of wheat bran in reducing contamination in sugar bagasse was significant.

The suitability of different substrates for mushroom cultivation was also confirmed by the average biological efficiency which was variable among the substrates. This concurred to the study done by Kimenju *et al.* (2009). Being mainly jelly like in form, the wood ears require a medium with a high proportion of lipids. According to Narain *et al.* (2008) maizecobs have a considerably higher lipid component than the rest of the substrates used in this study. Therefore, the lipids present in the maize cobs must have been efficiently utilized by the wood ears resulting in the high BE value. It was also observed that spawns of the black strains were of superior quality and yielded more especially on wheat bran supplemented substrates. The black strain also produced thick mycelia that were well condensed in the grains thus recording higher BE values than the brown strain whose mycelia were less vibrant. This confirms the previous reports that BE is highly affected by the quality of the spawn the cultivated strain (Mandeel *et al.*, 2005; Bechara *et al.*, 2005).

CONCLUSION

It is evident that many locally available organic substrates have high potential for utilization as substrates and/or supplements for mushroom production. In this study, 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.

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