EVALUATION OF PHENETIC CHARACTERS AND GROWTH PERFORMANCE OF NATIVE WOOD EAR MUSHROOMS (*Auricularia* species) ON PARTIALLY COMPOSTED AGRICULTURAL WASTES

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ABSTRACT

Wood ear mushrooms (*Auricularia* species) are a group of jelly fungi which occur as saprophytes on stumps or at the bases of dead or dying woody trees found in tropical regions. They have an ear shaped fruiting body from which they derive their common name. In other parts of the world, wood ear mushrooms have been domesticated and are valued for their nutritional and medicinal properties. Kenyan indigenous wood ear mushrooms which occur abundantly in Kakamega Forest have not been studied to evaluate their cultivation potential. Currently, the indigenous wood ear mushrooms face a threat of depletion due to over harvesting by the rural populace and rampant forest destruction. There is need to investigate their domestication potential in order to conserve their genetic resource. The objectives of this study were to evaluate the genetic diversity and to develop cultivation protocols for the indigenous wood ear mushrooms on malt extract agar, millet and sorghum grains and partially composted agricultural wastes. Mushroom germplasm collection was done at 3 different forest reserves within Kakamega Forest in Western Kenya. Mycelia growth was done through culturing of collected fruiting bodies on malt extract agar. External and internal mycelia and fruiting body features were evaluated and used to develop a descriptor for phenetic characterization of the wood ears. Data obtained from nine randomly selected accessions were used to calculate genetic similarity and to construct a dendogram. Some mycelia were used to raise spawns, which were used to inoculate the agricultural wastes. The agricultural wastes were treated to a short composting procedure, packed in heat resistant polypropylene bags and sterilized before inoculation. Inoculated substrates were kept in enclosed cabinets to allow mycelia propagation. They were then transferred to a cultivation room for mushroom growth. The cultivation experiment was set up as a two factorial Randomized Complete Block Design (RCBD) with variations in selected mushroom strains, different substrates and supplements. Three wood ear mushroom strains (Brown, Black, and White), four partially composted substrates (Wheat straws, Sugarcane bagasse, Grass straws and Corn cobs) and two supplements (Wheat bran and Rice bran) were used as treatments. Agronomic factors such as mycelia colonization rate, primordia initiation, yield and fruiting body quality were measured. Data collected were subjected to Analysis of Variance (ANOVA) to determine differences due to treatment effects. Mean separation was done using Least Significant Difference (LSD) at $p \leq 0.05$. Correlation analysis was done to compare some of the variables. The results demonstrated significant genetic and agronomic variations between the strains. A significant dichotomy separating three (all white strains) out of the nine accessions at dissimilarity index of 0.318 was observed. The other two groups (black and brown strains) separated at a narrower dissimilarity index indicating that they were more closely related. From the cultivation experiment, no significant differences occurred between the strains on their mycelia growth on malt extract agar. The treatment 40% millet, 40% sorghum and 20% wheat bran gave the shortest duration of 10 days for spawn production. Averagely, the black strains gave the best performance on corn cobs supplemented with wheat bran giving values of 3.7, 21, 318 g and 79% for fruiting body quality, number of fruiting bodies, fresh weight and biological efficiency respectively. The second best production was observed in wheat straw supplemented with wheat bran. Corn cobs and wheat straw supplemented with wheat bran were recommended to rural growers. Whereas morphological and productivity traits significantly separated the strains, further differentiation using molecular markers was recommended. Further investigation on their growth performance on other agricultural wastes and in different environmental conditions is necessary.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Mushrooms, the fruiting bodies of basidiomycetous fungi are important natural resources whose cultivation has markedly expanded all over the world in the past few decades (Liu *et al.*, 2005). Mushrooms play an important role in improving nutrition and health of communities that have domesticated them (Royse, 1996). This is because they contain substances of various kinds that are highly valued as food, medicines, flavourings and perfumes (Yan *et al.*, 2003). Since mushrooms have a high protein content, its production has tremendously increased worldwide making it a prominent source of alternative foods (Yan *et al.*, 2003). Mushroom cultivation mainly utilizes agricultural and industrial wastes as growth substrates. This confers ecological benefits through transformation and recycling of agricultural wastes. Cultivated mushrooms as such have potential to provide nutritional, medicinal and ecological benefits.

Mushrooms play an important role in world trade. In 1997, the total world population of edible and medicinal mushrooms was estimated to exceed six million metric tones with a value of about U.S. $ 26 – 30 billion (Sobal *et al.*, 2007). Between the years 1994 and 2007, the total world production of different species of mushrooms has tremendously increased as shown in Table 1 (APEMTEC Manual, 2007). The current world production of edible mushrooms is estimated at 12 million metric tonnes with China taking the lead and accounting for 86.6% of the total world production ((Beetz and Kaustudia, 2004). Apart from China, production and consumption of mushrooms has increased in other parts of the world. In America, consumption has overtaken production with the deficit being supplied through imports from Asian countries such as Chile and India (Chang and Lin, 1997). Africa trails the rest of the world in mushroom production with South Africa and Nigeria leading in commercial mushroom production (Adejumo and Awosanya, 2005). Kenya produces an insignificant quantity of mushrooms for export and local consumption compared to the rest of the world (Magingo *et al.*, 2004). Mushrooms have been considered as luxury food in Kenya reserved for the affluent, and their production, a guarded preserve of a few large scale farmers who could afford the 'state of the art' capital intensive outfits required for cultivation (Gateri *et al.*, 2004). Consequently, there is little awareness on mushroom production and utilization and the market prices are high and out of reach for most Kenyans. According to

Gateri *et al.*, (2004) Kenyan mushroom cultivation has concentrated on exotic species such as *Pleurotus ostreatus* (Oyster mushroom) and *Agaricus bisporus* (Button mushroom). In rural areas, locals collect wild mushrooms and prepare them traditionally with other foods Gateri *et al*, (2004). Some non-governmental organizations are currently involved in assisting community based organizations to collect Oyster mushrooms from Kakamega and Karura forests for cultivation (Kihumbu *et al.*, 2008). This advancement is due to the rapid destruction of Kenyan forests which decreases the natural habitat for such wild mushrooms and can cause their depletion.

Table 1: World production of cultivated edible mushrooms (1994 and 2007).

Species	**1994 (× 1000 tons)**	**2007 (× 1000 tons)**	**% increase**
Agaricus bisporus (Button)	1,846	4230	120
Lentinula edodes (Shiitake)	826	2250	115
Pleurotus spp. (Oyster)	797	1387	98
Auricularia spp. (Wood ear)	420	1100	160
Volvariella volvaceae (Straw)	299	877	320
Flamulina velutipes (Enokitake)	230	674	240
Tremella fuciformis (Jelly fungus)	156	532	380
Hysizygus marmoreas (Bonashimeji)	55	221	410
Pholiota mameko	27	114	420
Grifola frondosa (Maittake)	14	87	630
Others	237	872	320

(Adopted from APEMTEC Manual, 2007)

Kenyan indigenous such as *Auricularia* species commonly known as wood ear mushrooms are faced by threats of depletion due to over harvesting and forest destruction. These indigenous mushrooms have not only a high potential for domestication but can also provide a prominent source of biosynthetic products and genes for biotechnology. The fruiting bodies of wood ear mushrooms inhabit fallen logs of several tree types. Morphologically, they resemble ears in shape and are gelatinous, elastic, rubber to leathery in texture and brownish-purplish, dark-brown or white in color (Rogers, 2007). The rubbery, earlike structures may reach 15 centimeters in

diameter and are produced on dead stumps, logs and branches of hardwood trees (Anon, 1994). According to Palapala *et al.*, (2006) native wood ear mushroom production has the potential to provide employment opportunities, raise farm income and improve household food security without posing competition for land. Despite the high production potential of indigenous wood ear mushrooms, no reported work has been done on Kenyan native species which occur abundantly in Kakamega Forest in Western Kenya. Due to impacts of forest destruction and over-harvesting, availability of native wood ears has greatly diminished in recent times. To utilize and conserve the native wood ear mushroom resource, there is need to obtain information on its cultivation potential on locally available agricultural wastes.

Discovering the methods most successful for specific mushroom cultivation has been a long and arduous task, evolving from the experience of lifetimes of research (Stamets *et al.*, 1983). A great challenge has been witnessed in identification of appropriate substrates for mushroom growth and development (Royse, 1997). Most of the worldwide cultivation procedures have used logs of fallen trees, locally available agricultural substrates and synthetic media (Royse, 1997). Massfat (2004) demonstrated that precise conditions for mushroom growth may be difficult to obtain. Massfat (2004) therefore recommended testing of many different substrates together with different levels of supplements for development of appropriate cultivation protocols. This approach has the potential to aid in the development of reliable cultivation methods of indigenous mushrooms for rural utility.

Palapala *et al.*, (2006) reported that Kenyan native wood ear mushrooms have the potential to be grown on locally available agricultural wastes such as wheat straws, sugar baggase, saw dust, corn cobs and maize stalks. However, their agronomic performance such as comparative fruiting abilities, quality of basidiocarps and biological efficiency is yet to be ascertained to authenticate sustainable utilization before adoption. Before cultivating mushrooms on growth substrates, grain spawns are required for substrate inoculation. According to Stamets, (2000) grain spawn is made by sterilizing a cereal substrate, inoculating the substrate with a pure culture of mother spawn and incubating until it is fully colonized. Spawn is typically rye, millet or sorghum, supplemented with brans and chalk with a moisture content of 45 – 48% (Royse, 2007b). Development of appropriate grain combinations for production of native wood ear mushroom spawn is necessary. In order to achieve maximum yield of mushrooms, supplementation and composting of substrates

is necessary since it can increase mushroom yield two-fold (Royse, 1991). Supplementation refers to addition of nutrient bases such as soybean meal, rice and wheat brans (Stamets, 2000). Factories that process wheat and rice products in Kenya produce large quantities of brans which can be used to determine the growth of native mushrooms. Composting of substrates is an accelerated process of natural decomposition and humidification of plant residues accomplished by thermophillic microorganisms (Stamets *et al.*, 1983). Composted substrates have successfully been used to increase agronomic performance of mushroom (Royse, 1991). For this reason an experimental protocol for growth of native wood ear mushrooms on supplemented and composted substrates needs to be designed.

Investigating the genetic variability of native wood ears is necessary to delineate phylogenetic relationships among the local species. According to Weber and Webster (2006), studies on morphological characteristics to delimit strains of wood ears have previously provided insufficient information. Initial phenotypic studies by Saccardo (1885) considered external features such as basidiocarp size, color, shape, texture and length of abhymenial hairs to describe the species. Lowy, (1951) described and named several hyphal zones visible in transverse sections of basidiocarps. More recently, microscopic characters such as spore morphology, colony growth, dimension of hyphal zones and internal anatomies of the basidiocarp have also been used (Anderson *et al.*, 1998). The correlation of external, internal and microscopic features for phenotypic characterization of indigenous wood ear isolates is an important step towards a better understanding of their population biology.

In this study phenotypic analysis of wood ear mushroom samples obtained from Kakamega Forest was been done to provide a better understanding of genetic variations occurring amongst local strains. Before this study, little was known about such variations in Western Kenya and Kakamega Forest in particular. Therefore an investigation on appropriate cultivation protocols using different agricultural substrates was done to determine the best combination for their domestication. This project was formulated to answer the following research questions:

i) Are the indigenous wood ear mushrooms of Kakamega Forest morphologically diverse?

ii) Are there significant differences in the growth performance of wood ear mushroom phenotypes on partially composted agricultural substrates?

1.2 Problem statement

Food insecurity in Kenya and other African countries is directly related to insufficient food production and diets deficient of important food components such as proteins (Gateri *et al.*, 2004). This problem manifests in form of perennial hunger and malnutrition amongst the population. The rural Kenyan populace diet is restricted to food crops most of which require numerous cultural practices and are dependent on unreliable weather conditions. Unfortunately, rapid changes in weather patterns have led to reduction in food quantities and have not kept pace with the ever-increasing demand (Kihumbu *et al.*, 2008). There is a growing need to seek alternative sources of food that can subsidize the dwindling food crop production. Inadequate supply and high cost of animal proteins necessitate the search for locally available and cheap protein sources. One such source is mushrooms, which have a tremendous value both as food and medicine. In addition, increasing cases of illnesses in the local populations are attributed to diets deficient of important food nutrients. Meals deficient of essential proteins and vitamins have led to numerous deficiency diseases.

Kenya and other African countries with relics of tropical rainforests such as Kakamega Forest have a high quantity of indigenous wood ears. The wood ear mushrooms locally known as *matere* are under threats of depletion due to activities of people residing around the forest. Currently, wood ears are a special delicacy in Western Kenya and are over-harvested throughout the year from Kakamega Forest (Palapala *et al.*, 2006). Wanton forest destruction and over-harvesting has led to rapid diminishing of the few remaining wood ear species in recent times. These indigenous species have not been studied in order to isolate, characterize and select them for commercial production. It is therefore necessary to develop appropriate cultivation techniques that can be easily adopted by local farmers. Despite the high production potential of indigenous wood ear mushrooms, no documented work has been done on Kenyan native species (Palapala *et al.*, 2006). According to Palapala *et al.*, (2006) native wood ear mushroom production has the potential to provide employment opportunities, raise farm income and improve household food security without posing competition for land. To utilize and conserve the native wood ear mushroom resource, there is need to obtain information on its cultivation potential on locally available agricultural wastes. Cultivation methods will encourage propagation and therefore conserve this resource protecting it from extinction.

Whereas Palapala *et al.*, (2006) proposed use of locally available agricultural wastes for cultivation of wood ears, there is need to ascertain their growth characters such as period to maturity, fruiting abilities and quality of basidiocarps. Western Kenya, being an active agricultural zone has an abundance of agricultural wastes that can be utilized for mushroom cultivation. Large quantities of agro-wastes often cause environmental pollution due to problems with their safe disposal. This together with a growing need for production of cheap nutritious foods could lead to fast development of mushroom cultivation (Zervakis *et al.*, 2001). In fact after every harvest, most local farmers' burn crop remains. Factories that process agricultural products also have a problem of disposing remains such as sugar baggase, rice and wheat brans. These substances can be used to investigate the growth potentials of the native wood ears. Since mushroom mycelia grow on a wide range of plant matter (Stamets, 2006) there is a high potential for this region to develop a sustainable mushroom production industry.

Currently, no information is available on phenotypic differentiations on Kenyan native wood ear mushroom species (Palapala *et al.*, 2006). This information is required for varietal breeding of the species that can support extension work for rural cultivation and commercial purposes. Preliminary investigations in Kakamega Forest revealed phenotypically diverse wood ear mushrooms in terms of fruiting body color (Palapala, pers comm). These include strains that are black, brown or white/cream in color. There is therefore need to distinguish useful characters through genetic characterization of the different species that are indigenous to Kakamega Forest. A genetic evaluation of these species will provide vital information to characterize the western Kenya wood ear mushroom strains and offer information useful to cultivation. In addition, knowledge of the varietal differences can be used in breeding programs (Pei-Sheng and Chang, 2004) and in mass production of spawns, which will be used for local production of the wood ear mushrooms.

1.3 Objectives

1.3.1 General objectives

The ultimate goal of this study was to evaluate the genetic diversity of native wood ear mushrooms and to develop authentic cultivation protocols for improved livelihoods of small scale farmers in Western Kenya.

1.3.2 Specific objectives

1. To evaluate the genetic diversity among the native wood ear mushrooms of Kakamega Forest using phenetic characters.
2. To evaluate the growth of native wood ear mushroom mycelia on malt extract agar.
3. To determine the effect of millet and sorghum grains in production of wood ear mushroom spawn.
4. To compare agronomic performance of native wood ear mushrooms cultivated on some partially composted agricultural wastes.

1.4 Hypothesis

H1: There are significant character variations amongst phenotypes of native wood ear mushroom species of Kakamega Forest.

H2: There are significant differences between mycelia growth of native wood ear mushrooms on malt extract agar.

H3: Millet and sorghum grains can significantly affect the production of native wood ear mushroom spawn.

H4: There are significant differences in the growth performance of native wood ear mushrooms of Kakamega Forest on partially composted agricultural wastes.

1.5 Justification

Kenya's economy largely depends on agriculture with about 80% of the population depending on it for their livelihoods. Despite the fact that about 50% of Kenyans are food insecure, the potential for increased food production continues to remain unexploited. An ever-increasing human population and diminishing farm sizes have resulted in declining soil fertility and associated land degradation culminating in decreased land productivity and increasing poverty levels. Diversification of agriculture to high value crops and transformation of smallholder agriculture from subsistence to commercial business enterprises offer good promising options for revitalization of agriculture and wealth creation among the rural poor. Mushrooms such as wood ears are high value crops with a great potential for income generation and enterprise diversification and can benefit rural areas by alleviating poverty.

Wood ear mushroom production has recently expanded worldwide and this is attributed to its high nutritional and medicinal value. Nutritionally, its fruiting bodies contain high protein levels (about 30% of dry weight) with all the essential amino acids. The wood ear fruiting body also contains other essential elements such as vitamins, minerals, proteins and polysaccharides. This makes it a choice food since it contains most of the basic food requirements. Due to their high protein content, wood ears can act as a dietary alternative to animal proteins whose quantities and qualities are constantly decreasing in the world. They are known to produce many different polysaccharides, which have medicinal uses. These polysaccharides have been found to stimulate the immune system in humans and in some cases cause production of interferon and interleukins that can stop proliferation of cancer cells. They have also been found to have antitumor, cardiovascular and hypocholesterolemia, antiviral, antibacterial and antiparasitic properties.

Several reasons can be advanced for development of the mushroom industry in Kenya. For instance, there is no requirement for arable land for production. The industry has a potential to increase incomes, employment, food and nutritional security (Palapala *et al.*, 2006). In addition, utilization of agricultural wastes in mushroom production confers environmental conservation benefits. The abundant agricultural wastes found countrywide offers opportunity for mushroom production, which in turn provides a more economical and environmentally friendly disposal system of the wastes (Palapala *et al.*, 2006). Therefore, the potential for development of a mushroom industry in Kenya is high. Regarding the role of mushrooms as food, demand outstrips

supply as Kenya imports 1.5 tones annually not to mention the feasible export market (Wambua, 2004). However, there are constraints to sustainable production of mushrooms as revealed by (Gateri *et al.*, 2004). These include high input costs, lack of quality spawn, incidences of diseases and pests, lack of proper skills in production and post harvest handling and absence of government extension service for the industry.

In order to make mushroom cultivation sustainable and highly productive, strains with improved characteristics are a prerequisite (Sonnenberg, 2007). Investigation of the genetic variability of native mushrooms such as wood ears in relation to their growth performance is necessary. Such studies based on external, internal and microscopic features of the wood ears may provide relevant information on growth performance of different strains on different substrates. No previous work on genetic variability of wood ear mushrooms of Kakamega Forest has been conducted (Palapala *et al.*, 2006). An understanding of the genetic variability of native wood ear mushrooms may aid in delineation of phylogenetic relationships that can be used to enhance breeding efficiency. Genetic selection and improvement of cultivated isolates for commercial mushroom production may also be facilitated by such a study.

CHAPTER TWO

LITERATURE REVIEW

2.1 Classification of the genus *Auricularia*

Auricularia fungi belong to the division Basidiomycota that includes about 30,000 described species (Kirk *et al.*, 2001). This division is further distributed across three classes: Ustilagonomycetes (true smuts and yeasts), Urediniomycetes (rusts, anther smuts and diverse yeasts) and Heterobasidiomycetes (mushrooms and molds). The Class Heterobasidiomycetes in which *Auricularia* belong are characterized by a fruiting body known as basidiocarp. The basidiocarp is strongly lobed and is divided by transverse or longitudinal septa (Kirk *et al.*, 2001). Basidiospore germination in this class is unusual in that it can proceed in different ways including repetitious germination to produce ballitospores. There also occurs formation of monokaryotic microconidia and emission of a germ tube to give rise to a homokaryotic primary mycelium (Kirk *et al.*, 2001). This class is further subdivided into the subclass Heterobasidiomycetidae in which the homokaryotic spores are microconidia in which the homokaryotic spores are microconidia and Holobasidiomycetidae which bear holobasidia.

Ingold (1982) identified three orders within the class Heterobasidiomycetes all of which are commonly known as jelly fungi. They include *Auriculariales* (ear jelly fungi), *Tremellales* (jelly fungi) and *Dacrymycetales* (tuning fork fungi). Members of *Tremellales* tend to be softer in texture and more easily dehydrated. They have cruciate-septate basidia (vertically septate parallel to their direction of growth). The *Dacrymycetales* are typically tougher and more orange in color and have a septate basidium in the shape of tuning forks. *Auriculariales* are characterized by their typically brown colors and are known for their tough rubbery consistency. However, when dried they become hard and do not closely resemble the fresh fruiting body. The *Auriculariales* have transversely septate basidia perpendicular to their direction of growth. Their fruiting bodies grow to about 2-15 cm across. The fruiting bodies are centrally or laterally cuplike, gelatinous when fresh and hard when dry. Phenotypically, the outer surface is tan brown, black or cream with minute grayish dowry hairs. The inner surface is grey-brown, smooth or often wrinkled and ear like. The basidia produce spores which are white, cream or yellowish; sausage shaped and is about 18 x 6.8μm in size (Rogers, 2007).

2.2 Origin and domestication of wood ear mushrooms

Historically mushrooms have been known from fossil records as far back as the Siluvian period (about 438 million years ago) in the Paleozoic era, (Chang and Miles, 2004). Pre-historic humans probably used mushrooms collected in the wild as food and possibly for medicinal purposes. With the widespread cultivation of plants for food in the ancient civilization such as Greece, Rome, Egypt and China, it was inevitable that this choice source of food would eventually be cultivated and not just sourced from the wild (Yang, 1996). Mushroom cultivation did not come into existence until 600 AD when *Auricularia auricula* (wood ear mushroom) was first cultivated in China on wood logs, (Yang, 1996). Other wood rotting fungi such as *Flammulina velutipes* (800 AD) and *Lentinula edodes* (1000AD) were grown in a similar manner (Chang and Miles, 2004). The biggest advance in mushroom cultivation came in France in about 1600 AD when *Agaricus bisporus* was cultivated upon a composed substrate in the Western world (Yan *et al.*, 1995). *A. bisporus* commonly known as button mushroom increased steadily in popularity from the beginning and is today the mushroom that is produced in the greatest quantity (Chang and Miles, 2004). The Shiitake mushroom (*L. edodes)* is the second most important mushroom ranking behind *A. bisporus* (Yan *et al.*, 1995). In 1999 the total world production of *L. edodes* was estimated to be very close to that of the button mushroom (Chang and Miles, 2004). The wood ear mushrooms are currently the fourth most important cultivated species in the world (Yan *et al.*, 1995).

2.3 Morphology of wood ear mushrooms

Wood ears are fruiting bodies of a fungus that invades and lives in wood of fallen logs of several tree types. They resemble ears in shape and are gelatinous, elastic, rubber to leathery in texture and brownish-purplish, dark-brown or cream in color. The brown, rubbery, earlike structures may reach 15 cm in diameter and are produced on dead stumps, logs and branches of hardwood trees (Anon, 1994). Morphologically, species of *Auricularia* has been traditionally difficult to delimit (Koboyasi, 1942). Saccardo (1885) emphasized size, color, shape of the fruiting body (basidiocarps) and length of abhymenial hairs for its characterization. Teixeira (1945) recognized the medulla, a central compact zone with parallel hyphae in transverse sections of basidiocarps. Lowy (1951) proposed that microscopic characters such as size, shape and color varied with age and environmental conditions and therefore were not of taxonomical importance. Instead, Lowy (1951) placed emphasis on internal anatomy of the basidiocarp. Lowy (1951) proceeded to divide

the genus into two groups on the basis of presence, absence or poor definition of a medulla. Lowy (1951) considered width of the medulla as an important feature and followed earlier workers in emphasizing length of abhymenial hairs. In addition to medulla, Lowy (1951) described and named several other hyphal zones visible in transverse sections of basidiocarps. These zones are grouped as medullated and non-medullated. Recent studies have revealed that dimensions of hyphal zones have more variations than indicated by Lowy (1952). For this reason, dimensions of hyphal zones are inappropriate for identification of the species. Rather, they can be characterized as follows:

i. *Auricularia polytricha*

Cross-sections of this species are composed of a single layer medulla bordered by two relatively wide *laxa* zones.

ii. *Auricularia cornea*

This species has a wide medulla with a loosely arranged band of hyphae that merges abruptly with a *Zona laxa.* The *zona laxa* is very narrow and sometimes absent.

iii. *Auricularia tenuis*

The cross-section reveals a medulla composed of two bands separate by a loosely interwoven zone.

2.4 Nutritional and medicinal values of wood ear mushrooms

2.4.1 Nutritional Values

The wood ear mushrooms have a chemical composition, which is attractive from a nutritional point of view. When fresh, the fruiting bodies contain 90% water and 10% dry matter and their protein content varies between 27% and 48% of the dry weight (Chang and Miles, 2004). Carbohydrates are less than 60% and lipids composition is 2 – 8 %, (Morais *et al.*, 2000). The total energy value of the wood ear mushrooms is between 1.05 – 1.50 J/ Kg of fresh weight (Laborde, 1995). According to Morais *et al.*, (2000) the wood ears fruiting bodies contain minerals such as phosphorus, potassium, iron and copper. Studies conducted by Hardec (2001) revealed that the wood ear fruiting bodies are also rich in the minor elements such as vitamins B1, B2, B12 and C. Hardec (2001) showed that they are rich in essential amino acids but are low in fibers and contain no starch. The fungal mycelia produce antibiotics used in medicine (Sur and Ghosh, 2004), fermentation products used mostly in food industry (Koizumi, 2001), enzymes

used industrially for biotechnology of wood (Mei and Militz 2004) and chemicals used ecologically for bioremediation of contaminated items (Stamets, 2005)

2.4.2 Medicinal Values

Over 200 species of mushrooms have been collected from the wild for their medicinal purposes. Such purposes include uses as anti-tumour, immuno-stimulating hypolipideamic and hypocholesteroleamic effect (Yang *et al.*, 2002). *Auricularia auricula-judae* and *A. polytricha* have been used as medicines for many centuries in China, particularly to cure hemorrhoids and strengthen the body, perhaps by stimulating the immune system (Chang and Miles, 2004). It was also used to treat such widely varying conditions as hemoptysis (spitting up blood), angina (cardiac pain), diarrhoea, and warding against gastrointestinal upset (Volk, 2004).

The wood ear mushrooms have been shown to block blood clotting by obstructing the platelets (Liu *et al.*, 2005). There is some evidence that regular ingestion of *Auricularia* in small doses can be therapeutic in preventing strokes and heart attacks. Other therapeutic uses of *Auricularia* from modern medicine include lowering blood cholesterol and triglycerides (Volk, 2004). According to Linderquist *et al.*, (2005) the hypercholesterolemic effect of this mushroom is attributed to its ability to treat obstructions of arteries and veins, vascular sclerosis and hypertension. It is hypothesized that the wood ear mushrooms can help reduce the blood glucose levels by reducing emptying time of the stomach (Wasser and Weis, 1999). There is even evidence that it can play a role in treating diabetes and cancer, while some studies claim it can reverse ageing by increasing activity for DNA repair (Wasser and Weis, 1999). However, due to possibility of anti-fertility effects, this fungus is not recommended for pregnant or lactating women, as well as those intending to conceive (Linderquist *et al.*, 2005).

2.5 Cultivation systems of mushrooms

2.5.1 Cultivation of specialty mushrooms

Different approaches have been undertaken to cultivate mushrooms for commercial purposes. According to Royse (1996) a great challenge has been witnessed in identification of appropriate substrates for mushroom growth and development. Most worldwide cultivation procedures have used logs of fallen trees, locally available agricultural substrates and synthetic media (Royse, 1996). Royse (1997) demonstrated that precise conditions for mushroom growth may be difficult

to obtain. Royse (1997) therefore recommended testing of many different substrates together with different levels of supplements for discovering appropriate cultivation protocols. This approach has the potential to aid in development of reliable cultivation methods of indigenous mushrooms for rural utility. Whereas most previous cultivation methods for wood ear mushrooms used saw dust from specific tree species Yan *et al.*, (2003), there is need to determine their performance on agricultural wastes (Royse, 1997). Most mushrooms being consumed today such as *Agaricus, Pleurotus Lentinula* and *Auricularia* are artificially cultivated. The three methods used for artificial mushroom cultivation include log, bag and bottle culture (Royse, 1997).

2.5.1.1 Log culture

Logs of hard wood trees (about 1 m in length) can be bored to obtain small holes, which can be stuffed with mushroom spawns (a mix of grain and mushroom mycelium) (Oei, 1996). Hard woods in the Oak family (Fabaceae) such as *Sesbania grandifolia* and *Ficus racemosa* are preferred for log culture though many soft woods such as Aspen have been used (Mei and Miltz, 2004). The inoculated logs are then kept in dark for about 6 months before being transferred to a cool, moist yard for mushroom development (Oei, 1996). However, this method is hardly used today because mushroom mycelia take a longer period to colonize wood compared to synthetic media. There is also a high probability of other fungal infection and long periods taken by the mushrooms to fruit (Mei and Militz, 2004). These shortcomings together with reduction in preferred tree species led to use of synthetic logs made from substrates such as saw dust, corn cobs and grain straws (Chang and Miles, 1989). Currently, such synthetic logs account for about 80 % of mushroom production.

2.5.1.2 Bag culture

Bag culture involves use of heat resistant polypropylene bags for carrying growth substrates (Yan *et al.*, 1995 ; Peng *et al.*, 2000). Bag culture has been widely used in cultivation of mushrooms of the genus *Agaricus, Pleurotus* and *Lentinula* (Sonnenberg, 2007). The substrates are blended with calcium carbonate (for buffering and achieving the appropriate pH) and nutritional supplements such as brans before being packaged into heat resistant polypropylene bags (Stamets, 2000). The bags are pasteurized or sterilized to remove contaminating microorganisms. Pasteurizing can be done using hot water (steam baths) or commercial steam sterilizers (Oei, 1996) at 121^0C for 1 hour. The sterilized substrates are cooled, inoculated with desired mushroom mycelia and placed

into spawn run rooms where temperature, humidity, light and other atmospheric factors are controlled (Stamets, 2000). After complete mycelia run, substrates are transferred to a designated cultivation room with appropriate environmental conditions. The bags are cut off to allow emergence of mushroom primordia from the bag tops and sides.

2.5.1.3 Bottle culture

In bottle culture, the procedure for curation of substrates is similar to that of bag culture. After blending substrates with calcium carbonate, they are moistened to about 60% (Oei, 1996). The prepared substrates are filled into heat resistant bottles and sterilized in a steam sterilizer at 121 ^{0}C for one hour (Stamets, 2000). After cooling, they are inoculated with a small portion of grain spawns. The spawn can be mixed into the substrate mechanically or by hand. In some cases, the spawn can be placed on substrate surfaces and allowed to grow downwards. Temperatures for spawn run are maintained at about 23 - 25^{0}C (Stamets, 2000). The bottles are usually kept in dark cabinets since light intensity of more than 500 lux during the spawn run may result in premature formation of primordia (Balakrishnan and Nair, 1993). After completion of spawn run, the bottles are transferred to a cultivation room where mushrooms will emerge from the top surface.

2.5.2 Cultivation systems of wood ear mushrooms

There are limited reference texts available for production of wood ear mushrooms. Presently, techniques used to grow wood ears are mostly adopted from other specialty mushroom cultivation with a few modifications (Sobal *et al.*, 2007). Log, bag and bottle culture have previously been successfully used in wood ear cultivation (Chang *et al.*, 2004). China which accounts for the worlds largest production of wood ear mushrooms mostly uses synthetic log culture though bag culture is increasingly becoming dominant (Yan *et al.*, 1995). Apart from cultivation systems, substrate type is important in determining the output in mushroom cultivation (Sanchez *et al.*, 2002).

2.5.2.1 Selection of substrates

One of the challenges in mushroom cultivation is identification of substrates that are readily available which are suitable for mushroom growth and development (Sanchez *et al.*, 2002; Royse, 1997). Fortunately, extensive research has been done on substrate selection for cultivation of mushrooms other than wood ears (Royse, 1997). The most common substrate used for wood ear

mushroom cultivation is supplemented saw dust (Stamets, 2000). This is probably due to their saprophytic tendencies on logs in their natural habitats. In China, cottonseed hulls have been used as a substitute for saw dust with acceptable yields (Chang and Miles, 2004). Other efforts on wood ear mushroom cultivation have utilized a wide range of organic wastes such as cereal straws, corn cobs, coffee bean residues, sugarcane baggase, forest saw dust and cotton crop residues (Magingo *et al.*, 2004). Palapala *et al.*, (2006) reported that Kenyan native wood ear mushrooms have the potential to be grown on locally available agricultural wastes such as wheat straws, sugar baggase, saw dust, corn cobs and maize stalks.

2.5.2.2 Supplementation of substrates

High yields of mushrooms can be achieved through supplementation of substrates with other nutrient bases such as soybean meal, rice and wheat brans. The most important quality to consider in choice of a supplement is its nutritional value and especially the level of proteins it contains (Peng *et al.*, 2000). Brans derived from cereal grains can be used as supplements to provide a nutritionally consistent substrate. Brans such as rice (Takama *et al.*, 1987) and wheat (Sanchez *et al.*, 2002) are widely used as supplements in wood ear mushroom production. However other sources of supplements such as chicken manure (Magingo *et al.*, 2004) have been used in wood ear mushroom cultivation. Previous studies on wood ear mushroom cultivation have shown increase in mushroom yield and quality if substrates are supplemented with cereal bran (Peng *et al.*, 2000). Supplementation can increase mushroom yield two-fold (Royse *et al.*, 1990). The addition of a carbon - nitrogen based supplement helps in getting higher rates of mycelia growth (Royse, 2007a). This results in faster and denser ramification of substrates that leads to higher yields and production of better quality mushrooms.

2.5.2.3 Composting of substrates

Composting refers to the process of microbial decomposition on preferred substrates (Stamets, 2000). Its role is to prepare a nutritious medium for mycelia growth in exclusion of competitor organisms. Microbes present in substrates may not readily decompose them. They require addition of supplements to supply energy for nitrogen utilization. Composted agricultural byproducts have been used as inexpensive substrates for economical production of mushrooms (Stamets, 2000). Various researchers have reported increased yield and better quality mushrooms when grown on composted substrates. Recent advances in commercial production of mushrooms

such as wood ears involve composting of substrates after combining them with appropriate supplements such as rice and wheat brans (Sanchez *et al.*, 2002). The level of moisture in substrates will determine the period of composting and Stamets (2000) recommends 50% of dry weight of substrates. The short composting period for prepared substrates ranges from 5-14 days depending on the substrates and supplements used (Sinden and Hauser, 1980). The short composting achieves partial breakdown of substrates and is suitable for mushrooms which are primary decomposers.

2.5.2.4 Spawn production

Grain spawn is made by sterilizing a cereal substrate, inoculating the substrate with a pure culture of mother spawn and incubating until it is fully colonized (Stamets, 2000). Spawn is typically rye, millet or sorghum, supplemented with chalk, and contains a moisture content of 45 – 48% (Royse, 2007b). The colonized grain may readily be mixed with various substrate formulations, thus providing many points of inoculation. Since spawn is normally sold on a weight basis, grains that have small seeds such as millet give a greater number of inoculation points per kg than large grains such as rye. However, large grains have a greater food reserve and can sustain mycelia for longer periods of time during stress (Royse, 2007b). Most spawn of wood ear mushrooms are prepared by commercial manufacturers (Oei, 2005).

2.5.2.5 Environmental conditions

Successful mushroom cultivation involves overcoming factors such as temperatures, light intensity, humidity, pest and diseases (Stamets, 2006). According to Royse (1996) and Stamets (2000) achieving appropriate conditions is crucial for growth of mushrooms. The time it takes for mushrooms to mature is influenced by temperature and moisture (Stamets, 2000). High temperatures shorten the crop period by promoting rapid intense flushes. Lower temperatures lengthen the harvesting period and allow for maximum quality control. The recommended spawn run temperatures is 21-25 ^{0}C and moisture levels of 55-58% of the fresh weight of the substrates (Stamets, 2005). Light intensity should be maintained below 500 flux since above this level may result in premature primordia formation (Balakrishnan and Nair, 1993). Pest and disease control for mushroom cultivation is ensured through maintenance of high standards of hygiene (Stamets, 2005).

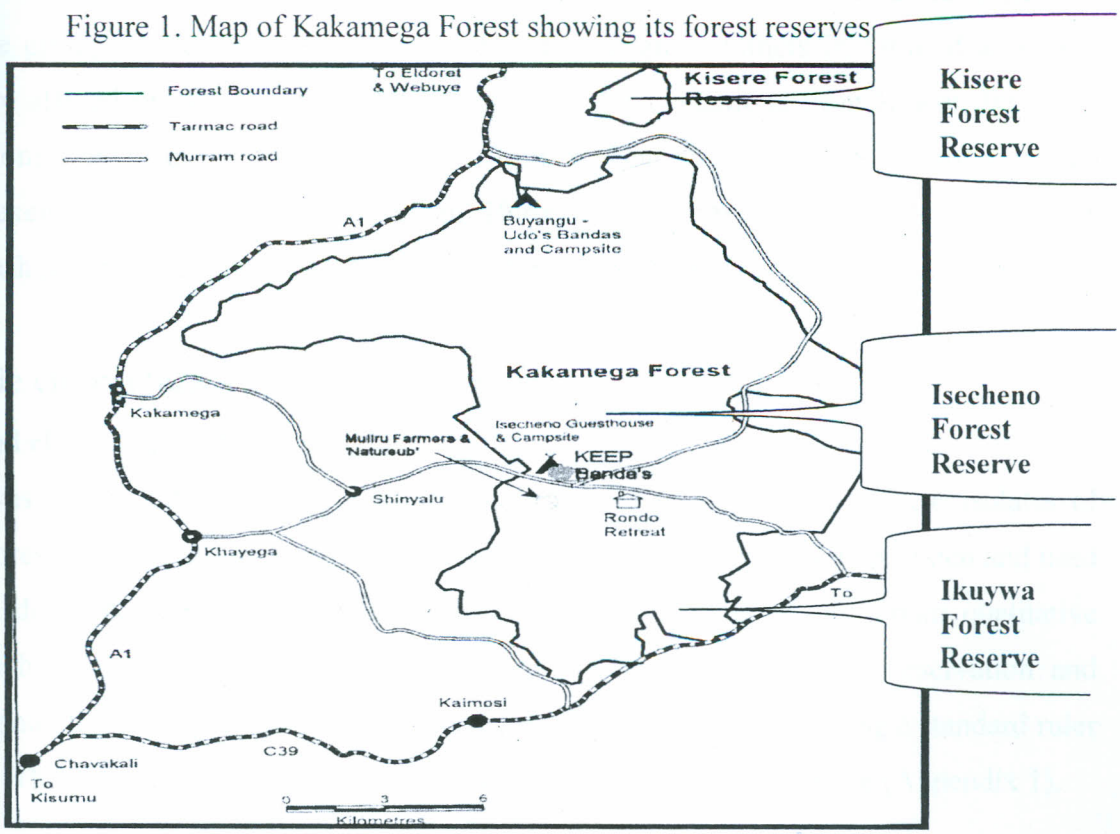
CHAPTER THREE

MATERIALS AND METHODS

3.1 Study sites

Collection of indigenous wood ear mushroom fruiting bodies was done at Kakamega Forest. Kakamega Forest covers an area of about 154.2 sq. km out of which 15.92 sq. km is plantation forest while the rest is under natural forest (Wambua, 2004). It is a tropical rain forest located between longitudes of $34^0 32'0''E$ and $34^0 57'0''W$ and latitudes of $0^0 07'30''N$, $0^0 10'15''S$ of the equator (Wambua, 2004). This study was conducted at Isecheno, Kisere and Ikuywa reserves of Kakamega Forest as shown in the map in figure 1. The topography of the forest reserves ranges between 1250 – 2000 m altitudes above sea level. The total annual rainfall averages between 1500 - 2000mm with long rains occurring between March to June while the short rains occur between July and September each year. Daily temperature variations are minimal with maximum ranges of 28 – 32 ^{0}C and minimum ranges of 11 – 13^0C. Studies of collected mushroom specimen were done at Masinde Muliro University laboratory in Kakamega District while the cultivation experiment was carried out at a farm in Milimani area within Kakamega town.

Figure 1. Map of Kakamega Forest showing its forest reserves



3.2 Collection of indigenous wood ear germplasm

Samples of wood ear mushrooms were collected from woody stems of live and dead trees at Isecheno, Ikuywa and Kisere forest reserves within Kakamega Forest. Line transect method was used along 10 forest trails in each reserve to identify tree species from which mushroom samples were collected. In each forest reserve, 100 m long transects were laid along the trails. Since the trails were about 50 m apart, the area for sample collection was about 0.5 square kilometers. Marks at intervals of 10 m were located along each transect to guide exact position of sample collection. Tree species on which specimens were collected were identified by a forest assistant working with Kakamega Environmental Education Programme as shown on Tables 7a, 7b and 7c. The fungus position on the logs, whether exposed or hidden was recorded. Those collected from varied places such as underside of fallen trees, tree trumps and in open or dark places were noted. During harvesting, fruiting bodies were detached from the logs using a sharp knife. Care was taken to ensure collected samples were at different stages of growth and development in order to ensure proper representations of species. Also, diseased parts were avoided to eliminate contamination. Collected specimens were grouped depending on external color of fruiting body and the forest reserve where they were collected. They were labeled, placed in plastic containers and sealed. Five specimens of black, brown and white fruiting bodies were collected from each forest reserve giving a total of 45 fruiting bodies. Identification epithets in form of accession numbers were affixed on the sample bags bearing Kis – for collections from Kisere, Iky – for collections from Ikuywa and Isc – for collections from Isecheno forest reserves. Samples of each type were taken to Kenya National Museum Herbarium for taxonomic identification. The remaining fresh samples were immediately taken to laboratory for characterization and culturing.

3.3 Phenetic characterization procedures

3.3.1 External characters of fruiting bodies

This study was conducted in order to determine phenotypic variation of wood ear isolates of Kakamega Forest. Nine accessions were selected randomly from the collected specimen and used to develop a descriptor (Appendix 1) for construction of a dendogram. Important qualitative characters such as color, shape, and stipe presence were evaluated by eye observation and recorded. Quantitative characters such as size were measured in millimeters using a standard ruler and recorded. The external variations observed were used to develop a descriptor (Appendix 1).

3.3.2 Internal morphology of the fruiting bodies

Sections obtained from fresh fruiting bodies were studied using microscopy to observe differences in their internal structures. Thin transverse sections were obtained using sharp scalpel blades and immersed in a diluted solution of methyl blue stain for 10 minutes. The thinnest sections were selected and placed on glass slides and covered with cover slips. Low power (×100) and high power (×400) objectives of a standard light microscope were used to observe internal sections of the fruiting bodies. The variations observed were used to develop a descriptor (Appendix 1) for construction of a dendogram.

3.4 Mushroom tissue culture on malt extract agar

The method used for tissue culture of was derived from Weber and Webster, (2006). A laminar flow hood was used to perform the procedure. The lamina flow hood was thoroughly cleansed using cotton swabs soaked in 80% ethanol after which the fan and UV light were set for 30 minutes to sterilize the working chamber. Petri - plates were prepared by washing thoroughly with ordinary detergents and then autoclaved at 115 °C for 1 hour. Sterilized plates were transferred to an oven for drying at 140 °C for 30 minutes. Preparation of culture media was done by weighing 25 grams of 2% Malt extract agar which was dissolved in 500 milliliters of distilled water (HI Media lab PVT Ltd.) and then sterilized by autoclaving at 115 °C for 1 hour. The media was poured in sterile Petri dishes and quickly covered using Petri dish lids and allowed to cool and solidify.

Freshly harvested fruiting bodies of accessions Isc006, Kis002, Iky007, Isc014, Kis008, Iky008, Isc001, Kis003 and Iky013 were re-hydrated by placing them on moist filter paper at room temperature (about 23–25 °C). They were washed thoroughly in sterile water and 5% sodium hypochlorite. A sharp scalpel was dipped in 80% ethanol and flamed until it was red-hot then allowed to cool for 10 seconds. Cleaned mushroom sections were broken lengthwise while avoiding handling the inner surface. Sterilized scalpels were used to remove fragments (about 2×2 mm^2) from inner surfaces of the basidiocarps. Cut fragments were placed in the middle surface of the media, covered with a Petri - dish lid and tightly sealed with a parafilm. Inoculated dishes were then labeled. Distinction of cultures was established depending on collection site using accession numbers previously listed. Cultured plates were incubated in dark sterile cabinets for 5-8 days at 25 °C to enable mycelia establishment.

Sub culturing was done on fresh media to obtain pure cultures. A scalpel was sterilized in a flame until it became red hot. Petri dishes containing established mycelia were uncovered within a sterile lamina flow hood. A small square piece of colonized agar (about 3×3 mm^2) was cut using sterilized scalpels and placed in the middle of a freshly prepared agar plate. For each accession three fresh agar plates were prepared and inoculated. Inoculated Petri dishes were held near a flame for a few seconds to get rid of aerial contaminants after which Petri plate covers were replaced and tightly sealed with a parafilm. Newly inoculated petri dishes were correctly labeled and transferred to dark sterile cabinets for 5 – 8 days.

3.4.1 Measurement of mycelia growth in malt extract agar plates

Mycelia growth data obtained using a standard ruler by measuring the diameter of each colony in millimeters as it grew on Petri dishes (Plate 1). Mycelia diameter along four planes at right angles across the media was measured daily for three plates per accession and recorded. The average of diameter readings was calculated and the rate of mycelia growth was obtained as shown below.

Mycelia Growth Rate = $(m_2 - m_1) + (m_4 - m_3) + \ldots /$Number of days

Where; m_1 – first measurement

m_2 - second measurement

m_3 – third measurement

m_4 – fourth measurement

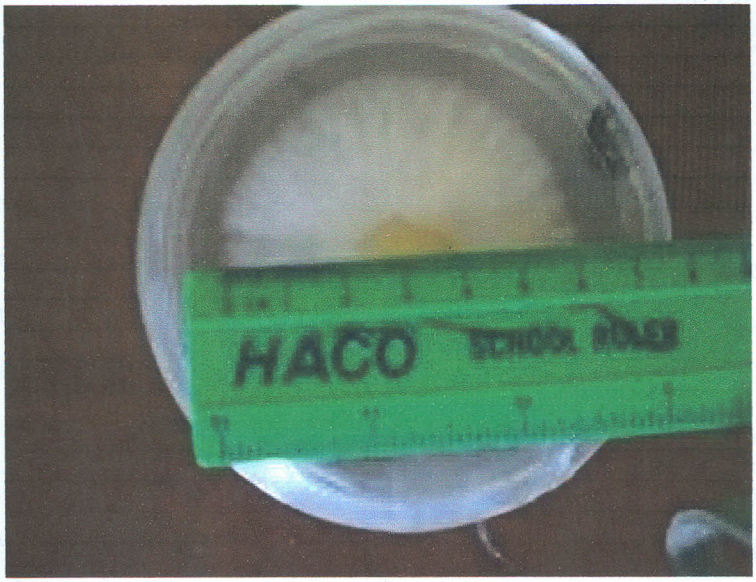


Plate 1: Measurement of radial mycelia growth on malt extract agar plate.

3.4.2 External structure of mycelia colony

When pure mycelia cultures had been obtained through a series of sub culturing, they were studied for variation in mycelia colony. Mycelia were exposed from petri-plates and qualitative features of colonies observed by eye included density, texture, presence of aerial hyphae, type of colony growth and presence of exudates. Mycelia sectoring as described by Stamets (2006) whether rhizomorphic (strandy), linear (longitudinally radial) or tormentose (cottony) was determined through eye observation. Results obtained from samples were used to develop a descriptor (Appendix 1) for construction of a dendogram.

3.4.3 Microscopic analysis of hyphae structure

Microscopy was done to determine differences between hyphal structures of wood ear mushroom strains. After 3 days of mycelia growth, a square piece of colonized agar (2×2 mm^2) was cut using a sterile scalpel and mounted in water on a slide. The specimen was stained with methyl blue solution and observed at medium ($\times100$) and high power ($\times400$). Photographic records of hyphal features were taken (Plate 6 and 7). Data collected included presence of conidiophores, type of septation and type of hyphae. Information obtained from samples was used to develop a descriptor (Appendix 1) that was used to construct a dendogram.

3.5 Spawn production procedures

Mycelia obtained from tissue culture were used to develop grain spawns. Two different cereal grains supplemented with brans at varying combinations were tested to determine their suitability for spawn production.

3.5.1 Selection of grains and formulations

Millet and sorghum grains obtained from farmers within Kakamega District were used with wheat and rice bran supplements. Rice and wheat brans were obtained from Ahero and Eldoret grain factories respectively and were used as nutrient additives. The grains were soaked in water for four hours to soften before being utilized for spawn production. A total of eight different treatments (300 g each) were used as adopted from Royse (1997) as shown in (Table 2). Grains of 100%, 80%, 50% and 40% were obtained by weighing 300 g, 240 g, 150 g and 120 g respectively. Similarly, 20% bran was obtained by weighing 60 g of either wheat or rice bran. The weighed grains and brans were then thoroughly mixed by hand.

Table 2: Grain and supplement formulations for spawning

Treatment	Grain and Supplement Formulations
1	40% millet + 40% sorghum + 20% wheat bran
2	80% millet + 20% wheat bran
3	80% sorghum + 20% wheat bran
4	40% millet + 40% sorghum + 20% rice bran
5	80% millet + 20% rice bran
6	80% sorghum + 20% rice bran
7	50% millet + 50% sorghum
8	100% millet

Formulations adapted from (Royse, 1997).

3.5.2 Grains preparation and inoculation

Each grain formulation (300 g) was mixed with 10 g of $CaCO_3$ powder to regulate their pH. To produce grain spawn of 50% moisture level, water was added at a rate of 225 milliliters per 300 g of formulation. Grain combinations were put in 500 ml heat resistant glass bottles and autoclaved for 1 hour at 121°C. Bottles were allowed to cool in a sterile lamina flow hood after shaking them to loosen and evenly distribute wet and dry grains. Flame sterilized scalpels were used to cut out (1×1 cm) square pieces of agar covered mycelium. The agar pieces were carefully transferred to upper surfaces of prepared grains. For each grain combination, three wood ear mushroom strains were inoculated and were replicated three times. Inoculated grain bottles were tightly secured using moist cotton wool and covered with sterile aluminum foil and bottle lids. They were kept in dark sterile cabinets at ambient room temperatures for 7- 20 days until they were fully colonized.

3.5.2.1 Duration to complete mycelia colonization of grains

The number of days it took to completely colonize the grains by each mushroom strain was recorded. The average duration was calculated by dividing sum of three recordings (from replicates) by three.

3.6 Selection of agricultural substrates and composting procedure

Four agricultural wastes, namely sugar cane baggase, corn cobs, wheat straw and grass straw were used as the main ingredients in this study. Sugar cane baggase was obtained from West Kenya sugar factory. Corn cobs, wheat and rice straws were obtained from farms within Kakamega, Uasin Gishu and Kisumu districts respectively. All substrates used were completely dry and devoid of moisture that could encourage microbial growth. Before being used, each of the substrates were cut into small pieces (< 4 cm) using a sharp knife. They were then submerged in water for 12 hours to soften them. To each substrate rice and wheat bran supplements were added at a substrate: supplement ratio of 80:20 as shown in Table 3. To achieve this ratio, for every 800 g of substrate used, 200 g of either wheat or rice supplement was added. 1kg of each treatment was prepared and each was replicated three times giving a total of 24 kg. The combinations were thoroughly mixed in readiness for composting.

Table 3: Substrate and supplement formulations

Treatment	Substrate (80%)	Supplement (20%)
I	Sugar cane baggase	Wheat bran
II	Sugar cane baggase	Rice bran
III	Corn cobs	Wheat bran
IV	Corn cobs	Rice bran
V	Wheat straw	Wheat bran
VI	Wheat straw	Rice bran
VII	Grass straw	Wheat bran
VIII	Grass straw	Rice bran

Composting was done in an enclosed room at prevailing environmental temperatures. Individual substrate combinations were laid in piles on the floor of a cultivation room and left exposed to free aeration. The door of the room was tightly secured to prevent entry of rodents. Eight substrate formulations were separately subjected to short composting procedure using the methods of Sinden and Hauser (1980) as quoted by Stamets (2000) (Table 4).

Table 4: Substrate composting procedure used in the study

Day	Procedure
1	Substrates were soaked in excess water for 12 hrs to soften them
3	200 g of cereal supplements (wheat and rice brans) were added for every 800 g of substrate and mixed as 500 ml of water was added. The mixture was arranged in a pile and compressed manually using wooden implement.
5	1st turn – 30 g of $CaCO_3$ (for every 1 kg of mixture) was added as the pile was turned using a rake.
7	2nd turn – Excess water was added while turning until the substrates were completely wet.
9	3rd 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.

Adapted from (Sinden and Hauser, 1980)

At the end of composting period, a squeeze test was done on substrates according to the methods of Palapala (Pers. comm.) to determine their moisture content. The test was done by squeezing the substrates between two hands. Substrates that produced only a few drops of water were considered to have adequate moisture content. Those that had more water were aired for a few hours while those that had less water were sprinkled with little water. The substrates were then divided into lots of 0.5 kg each and packed into heat resistant polypropylene bags. The bags used had a diameter of 12 cm and were 20 cm long. The ends of bags were tightly tied using sterile cotton strings and autoclaved at 121^0C for 1 hour. They were then cooled to room temperature for 30 minutes while subjecting them to ultra-violet light in a lamina flow hood to kill any microorganisms on bag surfaces. The cool sterile substrates were ready for inoculation with mushroom mycelia.

3.7 Cultivation of mushrooms on agricultural substrates

3.7.1 Preparation of cultivation room.

A designated room for mushroom cultivation with specifications derived from Oei (2005) was prepared. The room had a slightly inclined cemented floor to provide a smooth surface that could be easily cleaned and allowed excess water to drain. It had well fitted wooden doors and windows that could close properly preventing insects from entering. The windows were covered with a cloth barrier to keep out insects and rodents. Care was taken to ensure surrounding of the

cultivation room was free from possible contamination by disease causing microorganisms and foreign spores by regular cleaning and spraying. Wooden shelves for placing cultivation bags were constructed against the wall with each wall having six rows. The surfaces of the shelves were covered with formica to ease in cleaning and to reduce possibilities of contamination.

3.7.2 Spawning and spawn running

Inoculation of substrates with grains fully covered with mushroom mycelia was done in a lamina flow bench. Fully colonized grains were removed from cabinets and used as spawn seed. Small sized pieces (about 10 g) of spawns were planted in holes poked on substrate bags at the top. Bags were rapidly passed near a flame and covered using sterile cotton wool. A 2 cm long perforated plastic pipe was introduced on top of the bags then they were covered with sterile cotton wool and tightly sealed with a rubber band. A total of 72 bags were inoculated with grain spawns obtained from three mushroom strains. Inoculated substrates were labeled and kept in total darkness in enclosed cabinets for 14 - 25 days to allow complete colonization of the substrates (Plate 2). The duration to complete colonization was recorded in days.

Plate 2: Completely colonized substrate bag.

3.7.3 Primordia initiation

In order to convert mature mushroom mycelia into primary fruiting bodies called primordia, it was necessary to alter environmental conditions of the substrates. The method described below that was used to initiate pinheads was derived from Stamets, (2006). The bags were cut from sides while their tops were opened up to give room for fruiting body development. The following steps were also undertaken to initiate primordia development after complete colonization of the substrates.

a) Substrates and air temperatures were lowered to fruiting range of 18 - 23 ^{0}C. This was done by submerging sealed substrates in a cold-water refrigerator at about 10 ^{0}C for 10 minutes in a process referred to as temperature shock. Air temperatures were lowered using two electric fans during the day. At night, windows were left open to lower the room temperature.

b) Humidity was maintained at between 90 - 95%. High humidity was ensured through constant flooding of the room with sterile water and spraying each bag of substrate with 1 liter of water every morning and evening.

c) Fresh air was circulated using two electric fans to reduce CO_2 concentration. Though measurement of CO_2 concentration was not done, it was assumed to be low enough to allow fruiting.

d) The room was lighted on a 12 hour on/off cycle using two 100 watts fluorescent bulbs.

Between 8–15 days after colonized substrates were exposed to different environmental conditions, young fruiting bodies known as pinheads began to form. Average number of days to primordia initiation for each strain was obtained from three replicates per substrate by dividing the sum of means obtained by three.

3.7.4 Sustaining the mushroom crop

Development of fruiting bodies of wood ear mushrooms required controlled environmental conditions. The procedure for this maintenance was derived from previous work done by Stamets, (2006). Temperature was maintained at between 23 – 25 ^{0}C by spraying growth substrates with 1 liter of sterile water of about 20 ^{0}C each morning and evening. Aeration regimes were regulated through periodic fanning of the room using two fans installed in the cultivation room. Similarly, moisture levels of 90-95% were maintained by flooding the floor with water every evening and morning. Clean cotton clothes were spread on the floor to increase water retention capacity. A 12-hour periodic on/off cycle of lighting using a 100 watts fluorescent bulb was introduced.

3.7.5 Fruiting body growth measurements

Measurements taken during mushroom growth included days to primordia initiation, days to fruiting body maturation and number of fruiting bodies per bag.

3.7.5.1 Days to fruiting body maturation

When fruiting bodies had increased in size and achieved adequate pigmentation they were assumed to be mature and ready for harvesting. Average number of days to fruiting body maturation for each strain was obtained from three replicates per substrate by dividing the sum of means obtained for each strain by three.

3.7.5.2 Number of fruiting bodies per bag

Only fruiting bodies that remained firmly attached to substrates and grew to maturation were counted in each bag. Average number of fruiting bodies per bag for each strain was determined by dividing the sum of means by three.

3.7.6 Harvesting

Duration taken for maturation of fruiting bodies was recorded in days. Mature fruiting bodies were harvested by twisting from the base using hands. The whole fruiting body was removed without leaving any stumps. Surfaces of substrates were gently rubbed to scrap off old, infected or dead mycelia to allow fresh cropping. Induction of substrates was also done through placing of small sized ice cubes on their surfaces to lower their temperatures. The second flush grew more rapidly and was harvested in a similar manner after 15 – 20 days.

3.7.7 Determination of mushroom productivity.

Fully mature fruiting bodies were analyzed for quality, fresh weight and biological efficiency to determine strain productivity. The procedures used to evaluate mushroom output from each bag were as illustrated below.

3.7.7.1 Mushroom quality

Large fruiting bodies harvested from each bag were selected and placed in clean transparent polythene bags and transferred to the laboratory for fruiting body quality analysis. Mushroom quality was evaluated by shape, diameter size and texture of fruiting bodies rated 1-4 as shown in Table 5 below. Average values for the three qualitative characteristics were obtained for three replicates per strain and recorded.

Table 5: Rating scale for fruiting body quality.

Fruiting body structure	Quality Rating	Fruiting body characteristics
Shape	1. 2. 3. 4.	Cup-Shaped/Discoid Lobed/Curved/Auriform Flattened/Appressed Ear shaped
Fruiting body diameter	1. 2. 3. 4.	Very small (<10 mm) Small (11-20 mm) Large (21-40 mm) Very large (>40mm)
Texture	1. 2. 3. 4.	Soft Rubbery Leathery Gelatinous

Adapted from (Rodgers, 2007)

3.7.7.2 Fresh weight

Fruiting bodies harvested from each bag were used to determine each strain yield. They were packed in clean polythene bags and weighed on a weighing scale in grams. Fresh weight was recorded as yield for every bag. Similar data was obtained after harvesting the second flush and average results for two crops calculated.

3.7.7.3 Biological efficiency

Investigation of percentage biological efficiency (B.E) was necessary to determine which mushroom strains utilized nutrients present in substrates most efficiently. The procedure used to determine B.E was obtained from Royse (1996). The fresh weight for fruiting bodies obtained from each bag was measured in grams using a weighing balance and recorded. The spent substrates from each bag were also wrapped in aluminum foil and dried in an oven set at 80°C for 36 hours. The output of mushrooms obtained from the second flush was determined in a similar manner and the results averaged.

Percentage Biological Efficiency was calculated as:

$$\frac{\text{Fresh Weight of Mushroom Fruiting bodies per bag (g)}}{\text{Dry Weight of Spent Substrates (g)}} \times 100$$

3.8 Experimental Design

Experiments on qualitative phenetic characters were done through random selection of nine accessions, which were assigned as treatments. Qualitative data from external and internal features were recorded and assigned numerical values using a 24-step descriptor (Appendix 1) for data analysis. All mushroom cultivation experiments were laid out in a two factorial Randomized Complete Block Design (RCBD). The treatments included four substrates (Grass straw-B1, Baggase-B2, Wheat straw-B3, Corn cobs-B4) each with two supplements (Rice Bran-C1, Wheat Bran-C2) at a substrate to supplement ratio of 80:20. The mushroom strains were three (Black-A2, Brown-A3 and White- A1). There were a total of 24 treatments with three replicates per treatment making a total of 72 experimental units which were arranged in three blocks as shown in Table 6.

3.9 Experimental layout

The following layout was used to set up the cultivation experiment.

Table 6: Layout of the mushroom cultivation experiment

A3B4C1	A2B3C1	A2B2C2
A1B3C2	A3B1C2	A3B4C2
A2B1C2	A3B3C2	A3B3C1
A1B4C1	A3B4C1	A2B1C1
A2B4C2	A2B1C1	A2B3C2
A3B1C1	A2B2C2	A2B2C1
A1B3C1	A3B4C2	A2B1C2
A3B3C2	A1B1C2	A1B4C2
A1B2C1	A2B2C1	A3B4C1
A2B2C2	A1B3C2	A1B1C2
A3B4C2	A2B4C1	A3B3C2
A3B2C2	A1B1C1	A1B1C1
A1B1C1	A2B3C2	A1B3C1
A2B4C1	A3B2C2	A3B3C2
A3B1C2	A1B3C1	A1B2C2
A1B1C2	A3B1C1	A2B4C1
A2B1C1	A1B4C2	A3B1C2
A1B4C2	A1B2C2	A3B1C1
A2B3C1	A3B2C1	A1B3C2
A3B3C1	A2B1C2	A2B3C1
A2B2C1	A1B4C1	A2B4C2
A3B2C1	A3B3C1	A3B2C1
A1B2C2	A2B4C2	A1B4C1
A2B3C2	A1B2C1	A1B2C1

3.10 Data Analysis

Numerical values obtained from a descriptor were organized into a matrix and subjected to cluster analysis using R statistical software (R Core Team, 2006). Variables were segregated into numericals and clustered using DAISY (dissimilarity calculation) function (Venables *et al.*, 2006). The clustering algorithm was done by Un-weighted Pair Group Method with Arithmetic mean (UPGMA). The optimum number of cluster groups representing the dendogram was calculated by the Kelly-Gardner-Sutcliff (KGF) penalty function (Venables *et al.*, 2006). 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 Institute, 2005). Separation of treatment means was done only for those parameters where the ANOVA was significant, using Least Significant Difference at 5% level [$LSD_{5\%}$].

CHAPTER FOUR

RESULTS

4.1 Phenetic characterization of wood ear mushrooms

Wood ear mushrooms collected from Kakamega Forest revealed similarities and differences in their external and internal phenotypic features (Tables 7a, 7b and 7c).

4.1.1 External structure of fruiting bodies

Three main fruiting body colors (black, brown and white) were observed (Plate 3, 4 and 5). However, a few sheds of these colors were encountered and were grouped within the colors depending on which were dominant. Measurement of fruiting body sizes showed differences amongst the strains (Table 7a, 7b and 7c). The black strain had the longest diameter which ranged from 4.7 to 5.9 cm, the brown strain ranked second (3.8 – 5.1 cm) while the white strain had the smallest fruiting bodies which ranged from 2.4 to 3.8 cm (Table 7a, 7b and 7c). No major variations occurred between collections obtained from different forest reserves since they all existed within the range. However, fruiting bodies obtained from Ikuywa were relatively smaller in all strains. With regard to fruiting body shapes, there occurred significant variations amongst studied mushrooms (Table 7a, 7b and 7c) ranging from auriform (ear shaped), discoid (cup-shaped), appressed (flattened) and campanulate (bell shaped). Most of black strains were ear shaped, majority of brown strain were discoid and campanulate while a good number of white strains were flattened. However, there were still many cases of disparities from this observation. There also occurred variations in the texture of the fruiting bodies. Black fruiting bodies were softest exhibiting gelatinous feels. Brown strains were rubbery while the white had tougher feels making them leathery. These variations were consistent for collections from the different forest reserves.

Table 7a. Collections from Isecheno forest reserve (Acc No-Accession number, Isc-Isecheno)

Acc No.	Collection site features		Phenetic characters			
	Host tree	Position on log	External fruiting body structure			
			Color	Size (cm)	Shape	Texture
Isc001	*Craibia brownii*	Dark underside	Black	5.5	Auriform	Gelatinous
Isc002	*Croton macrostachyus*	Light underside	Brown	4.3	Discoid	Rubbery
Isc003	*Albizia gummifera*	Light surface	White	3.2	Flattened	Leathery
Isc004	*Markhamia lutea*	Light underside	Brown	4.8	Discoid	Rubbery
Isc005	*Ficus elastica*	Dark underside	Black	5.7	Auriform	Gelatinous
Isc006	*Prunus africana*	Light surface	White	3.6	Fattened	Leathery
Isc007	*Markhamia lutea*	Light surface	White	3.3	Fattened	Leathery
Isc008	*Craibia megalocarpus*	Light underside	Brown	4.2	Discoid	Rubbery
Isc009	*Croton macrostachyus*	Light surface	White	3.7	Fattened	Leathery
Isc010	*Funtumia africana*	Dark underside	Black	5.3	Auriform	Gelatinous
Isc011	*Funtumia africana*	Light underside	Brown	4.9	Discoid	Rubbery
Isc012	*Prunus africana*	Light surface	White	3.1	Fattened	Leathery
Isc013	*Croton macrostachyus*	Dark underside	Black	5.8	Auriform	Gelatinous
Isc014	*Markhamia lutea*	Light underside	Brown	4.5	Discoid	Rubbery
Isc015	*Albizia gummifera*	Dark underside	Black	5.1	Auriform	Gelatinous

Table 7b. Collections from Kisere forest reserve (Acc No-Accession number, Kis-Kisere)

Acc No.	Collection site features		Phenetic characters			
	Host tree	Position on log	External fruiting body structure			
			Color	Size (cm)	Shape	Texture
Kis001	*Albizia gummifera*	Light underside	Brown	4.4	Discoid	Rubbery
Kis002	*Albizia gummifera*	Light surface	White	3.8	Fattened	Leathery
Kis003	*Antiaris toxicarius*	Dark underside	Black	5.6	Auriform	Gelatinous
Kis004	*Croton macrostachyus*	Light underside	Brown	4.2	Discoid	Rubbery
Kis005	*Funtumia africana*	Dark underside	Black	5.3	Auriform	Gelatinous
Kis006	*Prunus africana*	Light surface	White	3.3	Fattened	Leathery
Kis007	*Ficus elastica*	Light surface	White	3.9	Fattened	Leathery
Kis008	*Ficus elastica*	Light underside	Brown	4.7	Discoid	Rubbery
Kis009	*Croton macrostachyus*	Light surface	White	3.1	Fattened	Leathery
Kis010	*Funtumia africana*	Dark underside	Black	5.5	Auriform	Gelatinous
Kis011	*Funtumia africana*	Light underside	Brown	4.6	Discoid	Rubbery
Kis012	*Prunus africana*	Dark underside	Black	5.4	Auriform	Gelatinous
Kis013	*Antiaris toxicaria*	Dark underside	Black	5.1	Auriform	Gelatinous
Kis014	*Crappia brownii*	Light surface	White	3.7	Fattened	Leathery
Kis015	*Croton macrostachyus*	Light underside	Brown	4.6	Discoid	Rubbery

Table 7c. Collections from Ikuywa forest reserve. (Acc No-Accession number, Iky-Ikuywa)

Acc No.	Collection site features		Phenetic characters			
	Host tree	Position on log	External fruiting body structure			
			Color	Size (cm)	Shape	Texture
Iky001	*Kigelia pinnata*	Light surface	White	2.4	Fattened	Leathery
Iky002	*Ficus elasticas*	Light surface	White	2.8	Fattened	Leathery
Iky003	*Kigelia pinnata*	Light surface	White	3.0	Fattened	Leathery
Iky004	*Markhamia lutea*	Dark underside	Black	4.7	Auriform	Gelatinous
Iky005	*Prunus africana*	Light surface	White	2.7	Fattened	Leathery
Iky006	*Antiaris toxicaria*	Light underside	Brown	4.3	Discoid	Rubbery
Iky007	*Markhamia lutea*	Light surface	White	3.1	Fattened	Leathery
Iky008	*Pinus sylvestiris*	Light underside	Brown	3.8	Discoid	Rubbery
Iky009	*Ficus elasticas*	Light surface	Black	5.1	Auriform	Gelatinous
Iky010	*Funtumia africana*	Light underside	Brown	4.2	Discoid	Rubbery
Iky011	*Funtumia africana*	Light underside	Brown	3.3	Discoid	Rubbery
Iky012	*Prunus africana*	Dark underside	Black	4.9	Auriform	Gelatinous
Iky013	*Croton macrostachyus*	Dark underside	Black	5.3	Auriform	Gelatinous
Iky014	*Kigelia pinnata*	Dark underside	Black	5.7	Auriform	Gelatinous
Iky015	*Markhamia lutea*	Light underside	Brown	4.2	Discoid	Rubbery

Table 8. Morphological features of selected mushroom specimen (Isc-Isecheno, Kis-Kisere, Iky-Ikuywa)

Identity	Internal fruiting body structure			External features of mycelia				Structure of hyphae		
	Medulla presence	Zona laxa	Abhym. hairs	Color	Density	Mycelia appearence	Aerial hyphae	Septation	Type of hyphae	Conid. presence
Isc001	Present	Large	Absent	White	High	Cottony	Abundant	Longt. septate	Hyaline	Absent
Isc006	Present	Small	Present	Pale brown	Low	Velvety	Scarce	Longt. sptate	Hyaline	Present
Isc014	Present	Large	Present	Offwhite	Medium	Floccose	Regular	Longt. septate	Hyaline	Absent
Kis003	Present	Large	Absent	White	High	Cottony	Abundant	Longt. septate	Hyaline	Absent
Kis002	Present	Small	Present	Pale brown	Low	Velvety	Scarce	Longt. septate	Hyaline	Present
Kis008	Present	Large	Present	Offwhite	Medium	Floccose	Regular	Longt. septate	Hyaline	Absent
Iky007	Present	Large	Absent	White	High	Cottony	Abundant	Longt. septate	Hyaline	Absent
Iky013	Present	Small	Present	Pale brown	Low	Velvety	Scarce	Longt. septate	Hyaline	Present
Iky008	Present	Large	Present	Offwhite	Medium	Floccose	Regular	Longt. septate	Hyaline	Absent

Plate 3. Brown wood ear mushroom

Plate 4. Black wood ear mushroom

Note: Plates 3, 4 and 5 represent external morphology of wood ear mushroom obtained from three different forest reserves of Kakamega Forest.

Plate 5. White wood ear mushroom

4.1.2 Internal structure of fruiting bodies

Microscopic analysis of fruiting body sections did not reveal major differences between the three strains (Table 8). Centrally placed was a single region of compact, parallel hyphae (the medulla) in all accessions. The hyphae of this region appeared tightly arranged forming a clearly distinct band from other parts. Occurring above and below the medulla were two layers known as zona laxa. However these regions were distinctly smaller in accessions Isc006, Kis002 and Iky007. The lower side bore abhymenial hairs making the surface to appear rough. This characteristic was consistent to all accessions. However, a good number of accessions Isc001, Kis003 and Iky013 lacked the hairs and had completely smooth undersides. The other two groups had clearly distinguishable hairs in all studied samples (Table 8).

4.2 Characterization of mycelia colonies

4.2.1 External structure of mycelia colonies

Differences in mycelia colonies are listed on Table 8. Mycelia obtained from accessions Isc001, Kis003 and Iky013 were mainly white in color and showed a slight range in mycelia density of between regular and high. Their mycelia surface textures mainly appeared cottony and hairy and had the greatest amount of aerial hyphae. A distinction was noted with accessions Isc014, Kis008 and Iky008 since their colonies appeared off white making them look like a straw hat with a little gibbose at the centre. Their textures were distinctly floccose with a highly regular density. These accessions presented aerial hyphae after 5 days of culture that appeared regular though not as thick as the one observed in the first group. Mycelia from accessions Isc006, Kis002 and Iky007 had the least resemblance to the other two since they were pale brown in color. They had low densities and their texture appeared ropy and velvety. They also showed the least amount of aerial hyphae when compared to the other two groups (Table 8).

4.2.2 Structure of individual hyphae.

When individual hyphae from the nine accessions were observed under low and high power microscopy, no major differences were noted with regard to their structures. They were all longitudinally septate, hyaline and ramose (Plate 6). A high power observation of the hyphae at later stages of growth showed variations in presence of conidiophores. Accessions Isc006, Kis002 and Iky007 had conidiophores that appeared single and erect, but this was absent in the other two.

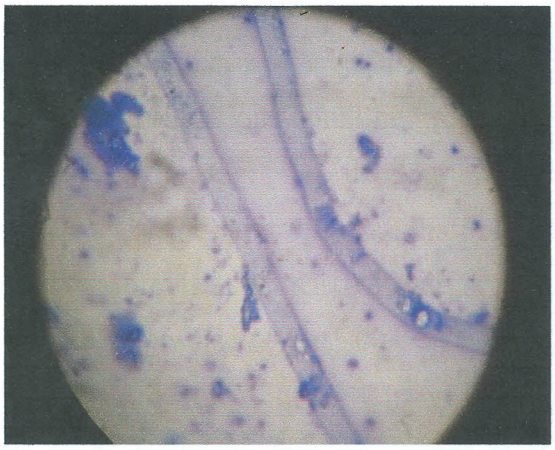


Plate 6. Hyaline hyphae in wood ear mushrooms.

4.3 Cluster analysis of phenetic data

All the data obtained from phenetic variations (Table 7-8) between collected and cultured mushrooms were organized into a matrix and subjected to a cluster analysis to obtain a dendogram shown in figure 2 below.

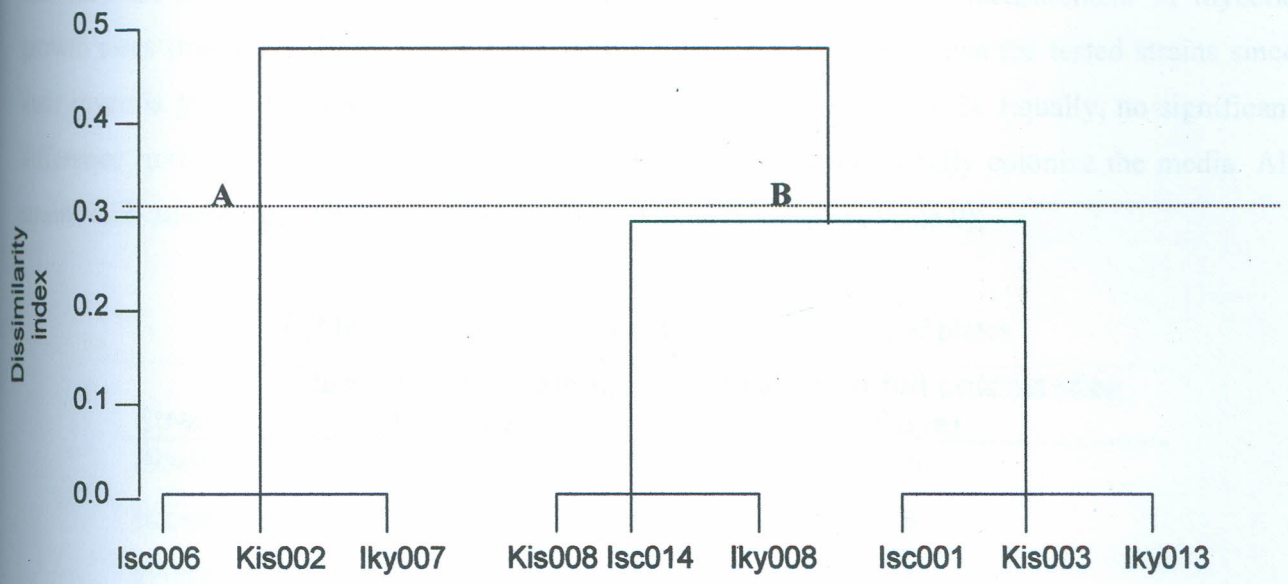


Fig 2. Cluster dendogram illustrating morphological diversity between nine wood ear mushroom accessions. The scale shows dissimilarity distance between the accessions. The faint line shows confidence limits of 95% giving a dissimilarity index of 0.318 (Appendix 5).

NB. Kis – Kisere, Isc – Isecheno, Iky – Ikuywa.

Analysis of the cluster dendogram showed that averaged dissimilarity coefficient of all accessions was significantly different (p=0.05). All accessions could be grouped together at 0.50 dissimilarity coefficient (Fig 2). Further analysis reduced the dendogram into two distinctly separate morphological clusters (shown by A and B on faint line) at a dissimilarity distance of 0.318 (Fig 2). Accessions separated into group A were Isc006, Kis002 and Iky007 (all white) while the rest clustered into group B (Fig 2). Accessions Isc001, Kis003 and Iky013 (all black) together with accessions Isc014, Kis008 and Iky008 (all brown) clustered together at B indicating that they were more closely related than those at A. However, the two groups at B later separated at a lower dissimilarity index of about 0.290.

4.3 Culture of mushrooms on malt extract agar

4.3.1 Mycelia growth on malt extract agar plates.

Evaluation of phenetically distinguished mushrooms through culturing on malt extract agar revealed that they all successfully colonized the media. Results obtained from measurement of mycelia growth rates (mm/day) showed no significant differences (p>0.05) between the tested strains since their mycelia grew at equal rates of 1.3 mm/day (Table 9 and appendix 2). Equally, no significant difference (p>0.05) occurred between them regarding the duration to fully colonize the media. All strains took an average of 6 days to completely colonize the media (Table 9).

Table 9. Mycelia colonization of malt extract agar plates

Strain	Rate of Colonization (mm/day)	Duration to full colonization (Days)
Black	1.3	6
Brown	1.3	6
White	1.3	6
$LSD_{0.05}$	NS	NS
Mean	1.3	6
CV	0.016	0.024

NS – Not significant

4.4 Growth of mycelia on millet and sorghum grains

4.4.1. Duration to complete colonization of grains

After bottles containing millet and sorghum grains were inoculated, it took between 3-4 days for mycelia to be established. Mycelia growth occurred rapidly downwards as shown in (Plate 7). Significant differences ($p \leq 0.05$) occurred on days taken to completely ramify the grains (Table 10 and appendix 3). The fastest mycelia growth was observed on 40% millet + 40% sorghum when supplemented with 20% wheat bran lasting 10 days after inoculation while treatment 80% millet + 20% wheat bran took one more day. Wheat and rice bran supplements had a positive effect with 20% wheat bran shortening the time compared to 20% rice bran. Much longer days were observed when grains were used without supplementation. For instance equal combination of millet and sorghum lasted 15 days similar non supplemented millet. Mycelia growth of black, brown and white mushroom strains showed significant difference ($p \leq 0.05$) on all treatments as shown on Table 10. All three strains grew optimally in different grain formulation extending their hyphae deep into the grains at different rates. The black strain was fastest in colonizing all grain combinations tested taking 10 days to completely ramify them. This was followed by the brown strain with 13 days. The slowest colonization was in the white strain which took 15 days.

Table 10. Duration to complete colonization of different grains by mushroom strains (days)

Grain Formulation	Strains			Mean formulation
	Black	Brown	White	
40% millet + 40% sorghum + 20% wheat bran	10.0	12.0	13.0	11.6
80% millet + 20% wheat bran	10.6	12.6	13.6	12.3
80% sorghum + 20% wheat bran	11.0	12.6	13.3	12.3
80% millet + 20% rice bran	11.6	13.7	15.6	13.6
80% sorghum + 20% rice bran	12.0	13.3	15.3	13.5
40% Millet + 40% sorghum + 20% rice bran	12.6	14.0	15.3	14.0
50% millet + 50% sorghum	13.6	14.3	16.0	14.6
100% millet	13.6	14.6	16.3	14.8
Mean strain	11.9	13.4	14.8	
CV (%)		10.1		
$LSD_{0.05}$		1.7		1.47

Plate 7. Mycelia colonizing millet and sorghum grains

4.5 Cultivation of mushrooms on agricultural substrates

4.5.1 Duration to complete mycelia colonization of substrates

There were significant differences ($p \leq 0.05$) between the strains in duration taken by their mycelia to fully colonize the substrates (Table 11 and appendix 4a). Black strains took significantly ($p \leq 0.05$) the least duration of 14 days when cultivated on corn cobs and wheat bran. Brown and white strains averaged 17 days on corn cobs and wheat bran. Wheat straw supplemented with wheat bran was second best in rate of mycelia ramification of substrates lasting 15 days in black strains followed by brown and white strains, which took 17 and 18 days respectively. It was evident that corn cobs and wheat straw were the best substrates in terms of reduction of duration for mycelia growth. White strains took significantly ($p \leq 0.05$) the longest duration of 24 days to fully colonize grass straw and rice bran. Wheat straw and sugar cane bagasse gave average days of between 18 and 21 when supplemented with either wheat or rice brans. Supplementation with rice bran showed low performance giving longer days to complete colonization of all substrates tested. Significant strains-substrate interactions ($p \leq 0.05$) were observed for duration to complete mycelia growth.

Table11. Duration to complete mycelia colonization by mushroom strains in different substrates (days)

Substrate and supplement combination	Strains			Mean combination
	White	Brown	Black	
80% grass straw +20% rice bran	24.3	22.6	20.9	22.6
80% grass straw +20% wheat bran	22.4	21.9	19.9	21.4
80% baggase +20% rice bran	20.9	20.2	19.2	20.1
80% baggase +20% wheat bran	20.3	20.0	18.5	19.6
80% wheat straw +20% rice bran	21.8	19.1	17.6	19.5
80% corncobs +20% rice bran	19.3	18.8	16.8	18.3
80% wheat straw +20% wheat bran	18.3	17.7	15.3	17.2
80% corncobs +20% wheat bran	17.0	16.8	14.0	15.9
Mean strain	20.5	19.6	17.7	
CV (%)		4.43		
$LSD_{0.05}$		0.43		1.41

4.5.2 Duration to primordia initiation

Significant variations ($p \leq 0.05$) were found between strains in terms of duration to pinheads production (Table 12 and appendix 4b). Black strains took the shortest period of about 23 days when grown on corn cobs and wheat bran while it took 25 days on wheat straw and wheat bran. When corn cobs were supplemented with rice bran, white strains lasted 24 days to produce primordia while the brown and black strains took 25 and 26 days respectively. White strains took the longest period to form pinheads taking 29 days in grass straw and rice bran. Even though no strains-substrate interactions ($p \leq 0.05$) were observed, it clearly emerged that wheat bran was a better supplement for early pinhead formation (Table 12 and appendix 3b). Black strains cultivated in substrates supplemented with wheat bran produced the earliest primordia. This was 24 days after inoculation and was significantly faster ($p \leq 0.05$) than the brown strain, which took 26 days on the same supplement. The longest duration to primordia formation was observed in white strains growing on substrate supplemented with rice bran.

Table 12. Duration to primordia initiation by mushroom strains in different substrates (days)

Substrate and supplement combination	Strains			Mean combination
	White	Brown	Black	
80% grass straw +20% rice bran	29.3	27.6	26.9	27.6
80% grass straw +20% wheat bran	26.4	26.9	24.9	26.0
80% baggase +20% rice bran	27.3	27.0	23.9	26.0
80% baggase +20% wheat bran	25.9	26.9	24.3	25.7
80% wheat straw +20% rice bran	26.8	26.1	22.6	24.9
80% corncobs +20% rice bran	24.3	24.8	25.8	24.8
80% wheat straw +20% wheat bran	24.3	23.7	25.3	24.4
80% corncobs +20% wheat bran	24.0	25.8	23.3	24.3
Mean strain	26.0	25.8	24.3	
CV (%)		3.10		
$LSD_{0.05}$		0.66		1.078

4.5.3 Duration to fruiting body maturation

Mushroom fruiting bodies of black strains cultivated on corn cobs and wheat bran grew fast and much more luxuriantly maturing significantly faster ($p \leq 0.05$) than white and brown strains. The shortest duration to fruiting body maturation was on corn cobs and wheat bran in which black strains took 7 days, brown 9 days while white took 8 days (Table 13 and appendix 4c). The longest time to mature of 22 days was observed on white strains cultivated on grass straw supplemented with rice bran. Significant interactions ($p \leq 0.05$) were observed between substrates and strains. In all substrates, supplementation with wheat bran gave better results compared to rice bran in terms of reduction of fruiting period (Table 13 and appendix 3c).

Table 13. Duration to fruiting body maturation of mushroom strains in different substrates (days)

Substrate and supplement combination	Strains			Mean
	White	Brown	Black	combination
80% grass straw +20% rice bran	22.4	21.0	17.8	20.3
80% grass straw +20% wheat bran	21.4	19.4	18.2	19.7
80% baggase +20% rice bran	19.6	18.1	15.2	17.8
80% baggase +20% wheat bran	17.3	16.6	14.8	16.1
80% wheat straw +20% rice bran	13.6	11.9	10.6	12.2
80% wheat straw +20% wheat bran	13.6	11.2	8.3	10.8
80% corncobs +20% rice bran	9.5	10.7	8.4	9.9
80% corncobs +20% wheat bran	8.6	7.7	7.0	7.7
Mean strain	15.6	14.5	12.6	
CV (%)		4.02		
$LSD_{0.05}$		0.33		0.543

4.5.4 Fruiting body quality

Fruiting body quality evaluated on a scale of 1-4 showed significant variations ($p \leq 0.05$) between all strains (Table 14 and appendix 3d). The lowest quality fruiting bodies were obtained from grass straw with a quality rating of 1.0 for all strains. Being large, gelatinous and auriform in shape, fruiting bodies from corn cobs supplemented with wheat bran were highest in quality giving values of 3-4 for all strains (Plate 8). Wheat straw and bagasse gave average quality values of 3 and 1 respectively since their characters were intermediary in terms of shape, size and texture. Significant interactions ($p \leq 0.05$) were observed between substrates and strains. High quality fruiting bodies of 3.6 on a scale of 1-4 occurred in all mushroom strains growing on corn cobs supplemented with wheat bran (Table 14 and appendix 4d). These were significantly of better quality ($p \leq 0.05$) than those cultivated on wheat straw (3.0) and sugar cane bagasse (1.8) on the same supplement. Rice bran caused production of poorer quality fruiting bodies of 2.8, 2.3, 1.5 and 1.0 as observed on corn cobs, wheat straw, sugar cane bagasse and grass straw respectively. Significant interactions ($p \leq 0.05$) between the substrates and the strains were also observed with respect to fruiting body quality.

Table 14. Fruiting body quality of mushroom strains in different substrates (Scale 1-4)

Substrate and supplement combination	Strains			Mean combination
	White	Brown	Black	
80% corncobs +20% wheat bran	3.4	3.6	3.7	3.6
80% wheat straw +20% wheat bran	2.7	2.9	3.4	3.0
80% corncobs +20% rice bran	2.2	2.8	3.3	2.8
80% wheat straw +20% rice bran	2.1	2.3	2.4	2.3
80% baggase +20% wheat bran	1.7	1.9	1.9	1.8
80% baggase +20% rice bran	1.3	1.4	1.8	1.5
80% grass straw +20% wheat bran	1.2	1.2	1.3	1.2
80% grass straw +20% rice bran	1.0	1.0	1.1	1.0
Mean strain	2.0	2.1	2.4	
CV (%)		3.10		
$LSD_{0.05}$		0.12		0.174

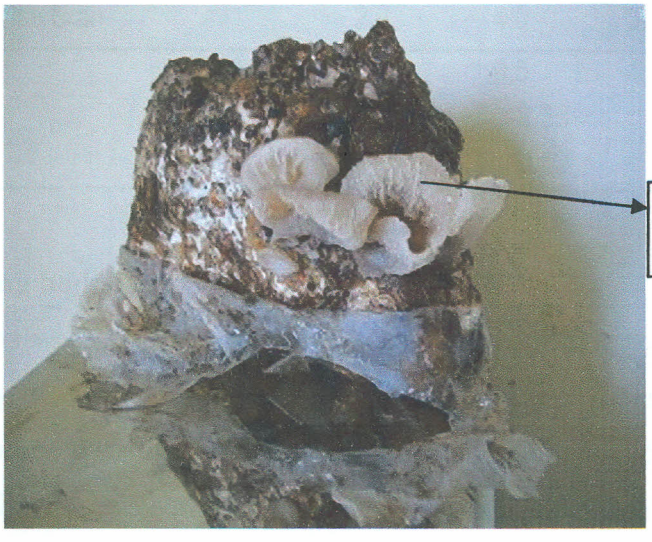


Plate 8. Brown wood ears fruiting on corn cobs

4.5.5 Number of fruiting bodies

The total number of mushrooms per bag was quite low in all substrates from which they were harvested (Table 15 and appendix 4e). Corn cobs and wheat bran produced significantly ($p \leq 0.05$) the highest number of 21 for black strains while the lowest number of 2 was seen in white strains cultivated in grass straw. Overally black, brown and white strains produced 13, 10 and 7 fruiting bodies respectively. Average numbers of 15, 10, 7 and 4 were obtained from corn cobs, wheat straw, sugar cane bagasse and grass straw respectively when each was supplemented with rice bran. This

was lower than 18, 11, 8 and 4 respectively when each of the substrates was supplemented with wheat bran. Supplementation with wheat bran therefore caused production of significantly higher ($p \leq 0.05$) number of fruiting bodies compared to rice bran.

Table 15. Number of mushroom fruiting bodies obtained from different substrates.

Substrate and supplement combination	Strains			Mean combination
	White	Brown	Black	
80% corncobs +20% wheat bran	15.4	18.6	20.8	18.8
80% corncobs +20% rice bran	13.6	14.2	19.2	15.1
80% wheat straw +20% wheat bran	8.6	11.2	15.3	11.7
80% wheat straw +20% rice bran	7.4	10.9	14.6	10.4
80% baggase +20% wheat bran	5.8	6.4	10.3	7.5
80% baggase +20% rice bran	3.3	6.2	10.3	6.6
80% grass straw +20% wheat bran	2.2	4.7	5.8	4.3
80% grass straw +20% rice bran	2.0	4.0	5.1	3.6
Mean strain	7.3	9.5	12.7	
CV (%)		3.39		
$LSD_{0.05}$		0.19		0.317

4.5.6 Fresh weight of fruiting bodies

Since most black mushrooms were larger at maturity, they gave significantly the highest ($p \leq 0.05$) fresh weight of 318 g per bag on corn cobs and wheat bran (Table 16 and appendix 4f). On the same substrate, brown fruiting bodies were of average size and weighed about 295 g. The least fresh weight was obtained from white strains cultivated on grass straw and rice bran giving 116 g per bag. The fresh weight of fruiting bodies harvested from substrates supplemented with wheat bran was significantly higher ($p \leq 0.05$) than those obtained from rice bran. There occurred significant interactions ($p \leq 0.05$) between supplements and strains.

Table 16. Fresh weight of fruiting bodies of mushroom strains cultivated on different substrates (g/ bag)

Substrate and supplement combination	Strains			Mean
	White	Brown	Black	combination
80% corncobs +20% wheat bran	267.6	295.9	318.1	298.6
80% wheat straw +20% wheat bran	191.8	224.0	263.4	226.3
80% corncobs +20% rice bran	235.3	258.2	303.4	265.6
80% wheat straw +20% rice bran	168.2	223.6	248.1	213.3
80% baggase +20% wheat bran	140.4	156.3	214.9	170.8
80% baggase +20% rice bran	106.9	147.7	213.2	155.6
80% grass straw +20% wheat bran	96.2	129.6	148.0	124.0
80% grass straw +20% rice bran	87.3	121.6	141.2	116.3
Mean strain	161.7	194.6	231.3	
CV (%)		5.01		
$LSD_{0.05}$		5.69		9.297

4.5.7 Biological efficiency

There were significant differences ($p \leq 0.05$) between all strains when percentage biological efficiency (B.E) was calculated (Table 17 and appendix 4g). With a B.E of 79%, black strains cultivated on corn cobs and wheat bran was significantly ($p \leq 0.05$) the highest. The second best B.E of 74% was seen on brown strains cultivated on corn cobs and wheat bran. Wheat straw, sugar cane bagasse and grass straw gave B.E values of between 63% and 20% for all strains. The lowest B.E of 15% was observed in white strains cultivated on grass straw supplemented with rice bran. Interactions between substrates and strains were significant at ($p \leq 0.05$).

Table 17. Biological efficiency of mushroom strains cultivated on different substrates (%/ bag)

Substrate and supplement combination	Strains			Mean
	White	Brown	Black	combination
80% corncobs +20% wheat bran	70.0	74.0	79.3	74.1
80% wheat straw +20% wheat bran	41.6	56.3	62.0	53.3
80% corncobs +20% rice bran	57.0	65.3	73.3	63.8
80% wheat straw +20% rice bran	40.0	54.0	56.3	49.7
80% baggase +20% wheat bran	24.3	46.6	48.0	40.6
80% baggase +20% rice bran	21.6	37.3	46.3	35.1
80% grass straw +20% wheat bran	18.3	29.0	30.6	25.8
80% grass straw +20% rice bran	15.0	18.6	26.0	19.8
Mean strain	35.7	47.2	53.0	
CV (%)		10.10		
$LSD_{0.05}$		2.09		3.408

4.6 Correlation analysis

4.6.1 Correlation analysis between some growth parameters.

There were strong and significant positive and negative correlation between the various growth parameters measured as shown in Table 18. The highest positive correlation ($R^2 = 0.99$) was found between fresh weight of fruiting bodies and biological efficiency. The second highest positive correlation ($R^2 = 0.95$) was found between duration to complete mycelia colonization of substrates and the quality of the fruiting bodies while the lowest positive correlation ($R^2 = 0.05$) was between duration to complete mycelia colonization and duration to primordia formation. Strong negative correlations also occurred between growth parameters measured. With negative correlation values of ($R^2 = -0.97$), biological efficiency showed the lowest correlation in duration to mycelia growth and duration to fruiting body maturation.

Table 18. Correlation analysis between some growth parameters measured.

Growth Parameters	DMC	DPF	DFM	FQ	NF	FW	BE
DMC		+0.05	+0.91	+0.95	-0.95	-0.84	-0.97
DPF			+0.84	-0.01	-0.78	-0.82	-0.86
DFM				-0.95	-0.94	-0.97	-0.97
FQ					+0.98	+0.90	+0.89
NF						+0.88	+0.88
FW							+0.99
BE							

(Note: All values are R^2 with (+) and (–) showing positive and negative correlations respectively.)

KEY.

DMC= Duration to complete mycelia colonization of substrates

DPF= Duration to primordial formation

DFM= Duration to fruiting body maturation

FQ= Fruiting body quality

NF= Number of fruiting bodies

FW= Fresh weight

BE= Biological efficiency

4.6.2 Linear correlation between fruiting body quality and biological efficiency

Positive linear correlations with an R^2 value of 0.92, 0.88 and 0.74 (Fig 3, 4 and 5) were observed for the black, brown and white strains respectively indicating that the strains differed in terms of the manner in which they utilized the nutrients in the substrates.

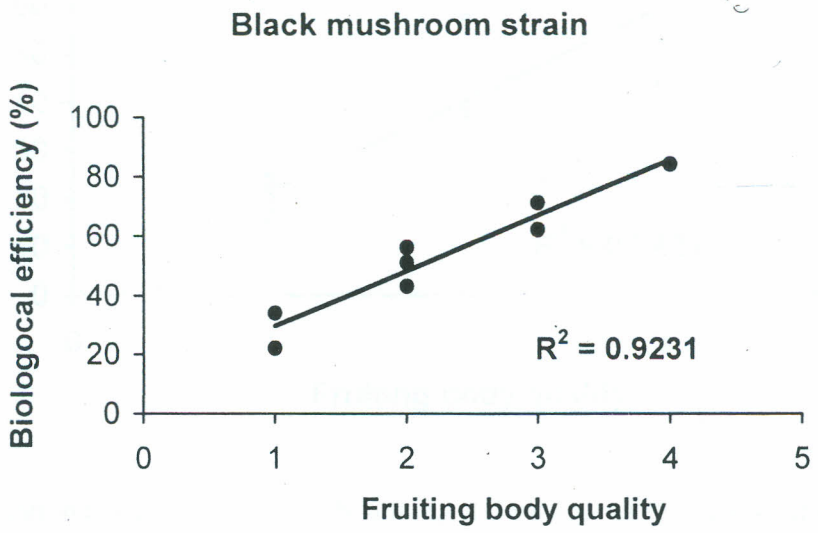


Fig 3: Linear correlation between fruiting body quality and biological efficiency for the black strain

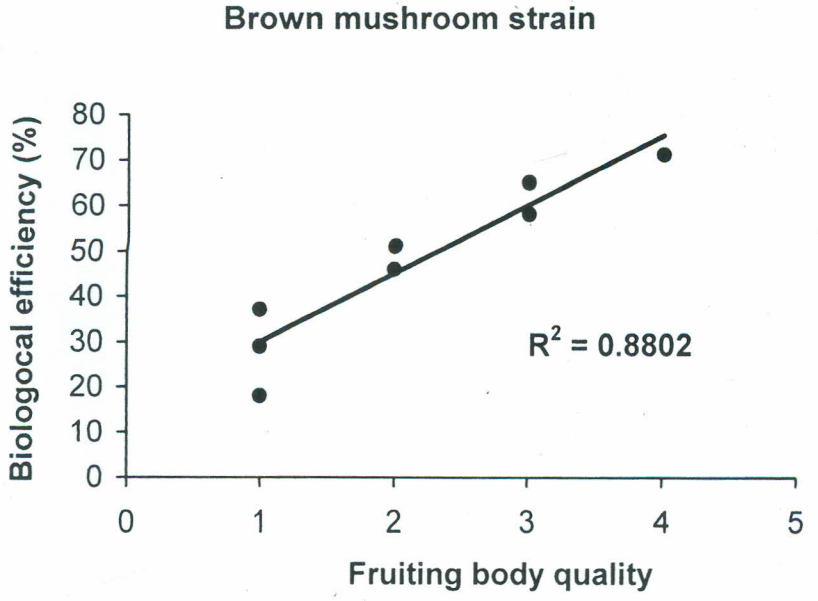


Fig 4: Linear correlation between fruiting body quality and biological efficiency for the brown strain

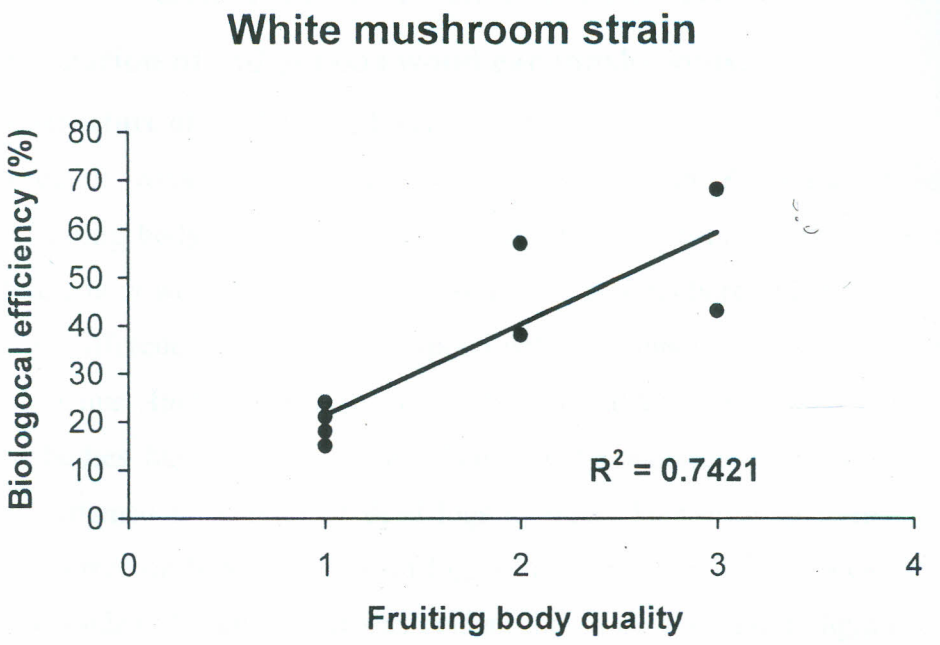


Fig 5: Linear correlation between fruiting body quality and biological efficiency for the white strain

CHAPTER FIVE: DISCUSSIONS

5.1 Characterization of indigenous wood ear mushrooms.

5.1.1 External structure of the fruiting body

External features of wood ear fruiting bodies obtained from Kakamega Forest revealed differences in fruiting body colors, shapes, sizes and texture (Table 8). Predominantly black, brown and white colors were observed though some mushrooms bore lighter or darker sheds of these colors. The differences observed correspond with previous work reported by Sobal *et al.*, (2007) that the genus *Auricularia* have mainly brown and dark fruiting bodies even though lighter fruiting bodies have been observed. Interestingly majority of black wood ears were collected from darkened undersides of dead logs of trees. This differed from the white ones which mainly occurred on lighted regions of logs while most brown mushrooms were collected from lighted undersides of such logs. It was evident that more exposure to light caused brighter fruiting body color while hidden mushrooms had greater pigmentation. This observation agrees with Wong (1993) that phenotypic differences observed on wood ear mushroom fruiting bodies could be due to factors such as age and level of exposure to light.

Guerrero *et al.*, (1978) investigated differences in the wood ears in terms of size and texture and concluded that such differences are due to a combination of inherent traits and the level of decomposition of the host tree. Also, the prevailing environmental conditions can influence the maximum size of a fruiting body (Martinez *et al.*, 2002). In this study, differences observed in fruiting body shapes included auriform, discoid and flattened types. It clearly emerged that black strains were mostly auriform, the brown were discoid while the white appeared flattened. According to Wong and Wells (1987) wood ear mushrooms are mainly ear-shaped but bell shaped and flattened types have been encountered. Minimal distinctions also occurred in size and texture of the native wood ear mushrooms. The black strains were generally larger and softer making them a better choice for cultivation experiments. The white strains were the smaller and tougher and could not provide a good source of cultivation material.

Traditionally, *Auricularia* species has been delimited according to fruiting body characteristics (Lowy, 1951). However recent work on this genus indicates that the morphological plasticity of the fruiting body has been inconclusive in establishing a stable characterization (Martinez *et al.*,

2002). Oberwinker and Weis (1997) proposed that delimiting the species of *Auricularia* using external features of the fruiting body alone is insufficient. Sobal *et al.*, (2007) redefined the characterization of this genus by proposing combination of the external and internal morphology together with growth features as important aspects of characterizing this species. According to Kirk *et al.*, (2001) traditional characteristics such as fruiting body color, shape, size and presence of abhymenial hairs may not be sufficient for dealinating the species because these characters tend to vary with age and the environmental prevailing conditions.

5.1.2 Internal structure of fruiting bodies

Phenetic evaluation of internal structure of fruiting body in collected native wood ears of Kakamega Forest could not distinguish between them since the medulla section was observed to exist in all the three strains (Table 8). Previous studies studies of the genus *Auricularia* have concentrated on the internal anatomy of the fruiting body as a principle distinguishing trait (Wong and Wells, 1987). Raitvir (1971) did indicate that the presence or absence of the medulla in the internal anatomy of the fruiting body was of value in delimiting the species. Wong and Wells (1987) gave evidence of variability of the medulla zone in different species of *Auricularia*. It is possible that no variations occurred in the fruiting bodies because of similar phylogeny of the local species.

5.1.3 External structure of mycelia colonies

The findings of this study showed that mycelia colonies of black and brown mushroom strains were white, cottony, and regular with abundant aerial hyphae while white mushroom strains produced pale brown, velvety and low-density mycelia with scarce aerial hyphae (Table 8). These varied results in colony morphology were unexpected especially after the fruiting body differences were established to be minimal. Given the high rate of uniformity in fruiting body characters of the genus *Auricularia*, further evaluation was necessary to establish differences between the three wood ear strains. As outlined by Sobal *et al.*, (2007) mycelia colony morphology traits such as color, density, texture and aerial hyphae are crucial in distinguishing between *Auricularia* species. However, Buswell and Chang, (1993) reported that fungal groups with genetically uniform populations can exhibit striking phenotypic variability. It may be possible that the three strains evaluated were genetically similar even though they showed marked phenotypic variability in the mycelia colony traits as they grew on malt extract agar.

Such phenotypic heterogeneity has been proposed to promote the fitness of fungal populations to enable them to adapt to different ecological conditions (Oei, 1996). Apart from readily scorable outputs of heterogeneity such as mycelia density, texture and color, the principal control processes are inherent and can only be differentiated through molecular studies (Philippoussis *et al.*, 2001). In this study, the use of environmental variations to explain the differences observed between the white, brown and black strains mycelia may not stand since the nutritional and ecological conditions of the media were similar for all strains.

5.1.4 Features of individual hyphae

Results obtained from microscopic observation of hyphal features of the three strains did not reveal any differences between them (Table 8). Hyphal features such as septation and hyphal type were identical for the three strains. The only difference that could not withstand statistical test was the pale brown color of the white strain, which is usually attributed to formation of conidiophores (Philippoussis *et al.*, 2001). Whereas it is obvious that utilization of microscopic data is a decisive step towards studying differences in the hyphae of basidiomycetes (Sobal *et al.*, 2007), this study did not establish differences between the hyphae of the three wood ear mushroom strains observed. Conidiophore formation observed may have been an adaptive strategy to counter inappropriate establishment. It is possible that when the vegetative stage of fungal growth is not very prolific, the hyphae resort to early formation of conidiophores, as was the case with the white mushroom strain. Evidently, at later stages of development, all the three mushroom strains lost their vibrant white color and became pale brown signifying the formation of conidiophores as the hyphae died off due to exhaustion of nutrients in the media.

5.1.5 Mycelia growth on malt extract agar

Mycelia for the tested wood ear mushroom strains grew at a rate of 1.3 mm per hour in 6 days on malt extract agar plates (Table 9). This may be attributed to uniformity in nutrient content of the media, which produced no variations in rate of mycelia extension. Kirk *et al.*, (1994) showed that studies on filamentous fungi have provided inconclusive results due to complexity of the microscopic scale of mycelia growth. Royse (2007b) reported equal mycelia extension in different strains of *Pleurotus* mushrooms and attributed it to close phylogeny between the species. Such similarities may be due to genetic relatedness between the strains used in the study (Sobal *et al.*, 2007).

5.1.6 Genetic diversity of indigenous wood ear mushrooms

A significant genetic diversity was found to exist between the nine accessions studied revealing two distinct groups. A dissimilarity coefficient analysis showed two morphological clusters with an index of 0.318 at p=0.05. Three (white strains) out of nine accessions were grouped together indicating that they were statistically indistinguishable using the morphological data analyzed. The other six accessions (brown and black strains) separated at a narrower genetic diversity indicating that they were more closely related. These results suggest that the genetic diversity was greater between the white strain and the two other strains and concur with the findings of Chang and Lin (1997) that some wood ear mushroom strains have a narrow genetic background. Sobal *et al.*, (2007) attributed such narrow variations in the morphology of wood ear mushrooms to the environmental conditions in which they grew. Since majority of the black and brown strains were collected from the underside of fallen logs, it is possible that lack of exposure to light led to the heavier pigmentation. The fruiting bodies of the white strain showed a consistent separation from the rest even under artificial cultivation conditions where they were exposed to moderate light. Therefore its classification and identification remain unclear according to Lowy's morphological characters (Lowy, 1951). Another important finding was that the observed phenetic relatedness united with different ecological locations from which the accessions were obtained. The white, brown and black strains clustered together irrespective of the forest reserve from which they were collected. It is also possible that the criteria of geographic origin and morphological characteristics used in evaluation of genetic diversity were not accurate because they might not reflect the genotypes.

The most uniting factor for the six accessions (black and brown strains) which clustered together at p=0.05 was their micro-morphology where they were distinctly identical. Oberwinkler and Weis (1997) and Wong (1993) indicated that it was not possible to distinguish between members of the genus *Auricularia* at the mycelia stage. According to Oberwinkler and Weis (1997), the wood ear group of mushrooms unites those with different fruiting body types but similar micro-morphology especially the longitudinally septate hyphae. However, a departure from this result was noted in the first three accessions (all white strains) in which mycelia sectoring and color appeared quite different. As reported elsewhere, qualitative features such as fruiting body color and mycelia sectoring were the major segregating features of the cluster analysis. It is however possible that some genetic differences might have gone unnoticed because morphological

markers are usually limited in determining genetic diversity. In addition, the samples used in this study were quite limited.

Remarkable about this study is that morphological variations sufficiently produced a dichotomy between the nine accessions revealing three distinct groups at low dissimilarity indices. Though it is mostly appropriate to distinguish between mushroom strains using molecular data Carrera (2002), it was clear that the morphological features studied produced variations within otherwise similar individuals. It is probable that the three groups that emerged belonged to different strains within the same species. Therefore, a conceivable evolutionary root of the wood ears native toKakamega Forest may be revealed by the cluster analysis. In addition, it was clear that ecological separation of the three strains has not affected their morphological similarities. Whereas the sites of collection were evidently different, the similar types grouped together since those collected from Isecheno, Ikuywa and Kisere forest reserves were phenetically identical.

5.2 Colonization of grains by mushroom mycelia

Mycelia growth was observed to vary in the different cereal grains used (Table 10). An equal combination of millet and sorghum grains supplemented with wheat bran gave the fastest rate of colonization of 500ml bottles of 10 days. Millet and sorghum grains have previously been successfully used to raise spawns for mushroom production. Shen and Royse (2001), Uhart *et al.*, (2008) and Narain *et al.*, (2008) have all reported differences in the growth of mushroom mycelia in millet and sorghum grains. Royse (1996) demonstrated that wheat bran supplementation of either millet or sorghum grains positively affected mycelia growth. Corresponding results were obtained in this study since cereal grains supplemented with wheat bran took shorter time to fully colonize compared to those combined with rice bran. According to Narain *et al.* (2008) high rates of colonization may be attributed to the mycelia getting the most suitable ratio of mixture with a high reservoir of energy and all the nutritional ingredients such as carbon, nitrogen, lipids and minerals. In addition, Royse (1997) reported that wheat bran was a good supplement rich in proteins and can increase rate of mushroom mycelia colonization. Shashireka *et al.* (2005) attributed enhanced performance of mycelia on availability of several amino acids and protease as well as transaminase enzyme activities on wheat bran.

Although nutritional evaluation of the brans used was not done, it is highly probable that the wheat bran used in this study had a much higher protein content increasing the nitrogen level in comparison to the rice bran used. Reportedly, wheat bran has protein components which increases the number of inoculation points making it a better supplement (Uhart *et al.*, 2008). It was not surprising that the spawn mixture of 40% millet + 40% sorghum with 20% wheat bran gave the best results since the small grains of millet contributed a greater food reservoir, whereas the larger sorghum grains provided a greater number of inoculation points. The influence of both wheat and rice brans on the spawn growth rate was further confirmed by very slow growth witnessed in the formulations that were non-supplemented.

The mushroom strains responded differently to the various grains used regarding the duration it took for their mycelia completely fill the grains. The fastest rate of colonization occurred in the black strain (10 days) followed by the brown strain (12 days) then the white (13 days). These values were higher than those reported by Narain *et al.* (2008) who obtained an average growth rate of 8 days with *Pleurotus florida* at similar growth conditions. Peng *et al.* (2000) reported that such variations are common during evaluation of spawn production in various strains of *Pleurotus* mushrooms. Factors that may have contributed to the difference may include quality of the inoculants used and aeration of the grains. Stamets (2000) recommended dense and thick mycelia to be used for inoculation of grains for spawn formation. Since the portion of inoculants obtained from the black strain used to colonize the grain spawns was dense, it progressed with vitality colonizing the grains at a faster rate. Slow colonization of sorghum may be attributed to compactness or poor aeration of the grains, which results in inefficient utilization of the nutrients. Vigorous substrate colonization by the mycelium during spawn run is desirable because it reduces mushroom cropping time and may allow mycelium to outgrow competitors in the substrate (Royse, 2007b).

5.3 Growth of mushroom strains on substrates

5.3.1 Colonization of substrates by mushroom mycelia

The substrates used in cultivation of the native wood ears could be colonized by the mycelia though at different rates (Table 11). Corn cobs and wheat straw supported the best mycelia growth in comparison to sugarcane baggase and grass straws. This was evidenced by complete

and heavy colonization of the corn cobs substrates forming a compact mass of white mycelium within 2-3 weeks of inoculation. This differed with hyphal growth in grass straw, which was quite slow and least profuse compared to the other substrates. In ecological terms, fast mycelia extension is often interpreted as an indication of hyphal progression on a nutritionally poor medium (Lee, 2000). A slower and denser growth can be attributed to favorable conditions and exploitation of nutrient resources by the fungus (Lee, 2000). The results obtained from this study partly differed from this principle since the fastest rate of colonization also proved to be denser in terms of ramification as was observed in the black strain.

Of all the substrates used, mycelia on corn cobs and wheat straw maintained a constant linear growth which was highly dense indicating that it was the most suitable substrate for wood ear mushroom cultivation. Previous work done on substrate evaluation has recommended the use of saw dust, corn cobs or wheat straw for wood ear mushroom cultivation. Oei (1996) highly regarded saw dust and corn cobs as appropriate substrates for the growth of the black wood ear mushroom in tropical regions. Mendeel *et al.* (2005) reported the wide use of wheat straw in Europe for mushroom cultivation. Wheat straw, rice straw and corn cobs are considered the best substrates in terms of yield and protein content in Asia (Thomas *et al.*, 1998). At the same time, Oei (2005) recommended supplementation of these substrates with very fine rice bran for good rates of mycelia growth. However rice bran had a lower effect in mycelia growth rate in comparison to wheat bran in this study.

In nature, *Auricularia* mushrooms grow on dead parts of plants which are generally poor in nutrients and vitamins (Narain *et al.*, 2008). It has been established that mycelia growth and fruiting body development is dependent on lignocellulosic materials especially the carbon: nitrogen ratio. Nutritional composition of substrates is a crucial factor in determining how mycelia growth occurs (Royse, 2007b ; Stamets, 2006). Even though this research did not involve nutritional analysis of substrates used, this may be the main reason for differences observed in rate of mycelia growth. According to Narain *et al.*, (2008) nutritional composition of a substrate is one of the limiting factors to saprobiotic colonization in cultivated mushrooms and particularly the fruit forming types. Bano and Rajarathran (1988) indicated that 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. A nutritional analysis done by

Silveira *et al.*, (2008) on banana fibre revealed the presence of cellulose (53.45%), hemicellulose (28.56%), lignin (15.42%), organic matter (95%), nitrogen (6.0%) and fibre (28.54%). All these nutrients were highly degraded through utilization by the mushrooms after colonization and fruiting. The ability of particular strains to utilize the nutrients and the physicochemical environment in the medium is crucial to its establishment and growth (Mukhopadhyay *et al.*, 2002). Philippoussis *et al.*, (2001) observed that the period between inoculation of substrates and the time of complete colonization up to fruiting body formation is favored in specific substrates ensuring linear extension of the mycelia. This probably explains the superior rate of growth of the black strain in comparison to the brown and white strains. Mycelia ramification was more condensed and vigorous in all the substrates colonized by the black strain. A slightly lower rate of colonization was observed in the substrates when colonized by the brown and white strains.

Results obtained on colonization of the substrates clearly revealed that supplementation of corn cobs and wheat straw with wheat and rice brans did not give significant differences ($p \leq 0.05$) between them. Whereas it was expected that either of the supplements would provide better mycelia growth within the substrates, this was not the case. These findings were in total contrast to previous work done with the same supplements. Various researchers have shown that supplementation of substrates with nutrients such as soybean, wheat and rice brans can increase mushroom colonization rate depending on the type and amount of nutrient used (Royse *et al.*, 2007). At the same time, contrary to the findings of Bechara *et al.*, (2005) that rice and wheat brans affect mycelia growth differently, the two did not show any significant difference. The type, quantity and quality of nutrient supplement should not only influence the rate at which various substrates are colonized but also the quantity and quality of the yield obtained (Royse *et al.*, 2007). Azizi *et al,* (1990) confirmed that addition of nitrogen based supplements may lead to higher rates of colonization.

5.3.2 Duration to primordia formation.

Pinhead 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 the substrate surface. There occurred differences between the strains in duration to primordial emergence (Table 12). Only 20 days after the inoculation, the black strain produced primordia on corn cobs. This significantly differed ($p<0.05$) from the 21 days observed in the

white strains. There was however no significant difference between the brown strain and the two other strains. The differences observed in the period to primordia formation may be attributed to varied responses of the mycelia to the temperature shock that the substrates were subjected to. Stamets (2000) recommended a sudden temperature variation on the fully colonized substrate bags to initiate pinhead formation, which was used in this study. Also crucial in pinhead formation is water content, lighting, humidity and CO_2 removal from the fully colonized bags (Oei, 1996). These conditions were regulated though fluctuations were highly possible especially at night. Thomas *et al.*, (1998) attributed fast primordia formation to the moisture content of the substrates. Any substrate with high lignin and which is exposed for long periods before being used will have a low water retention capacity (Thomas *et al.*, 1998). Whereas the substrates were watered regularly, they may have differed in terms of their water retention abilities. It is plausible to state that corn cobs had the greatest ability to retain its water in comparison to wheat straw, sugar baggase and grass straw. The sugar cane baggase used in this study were very dry and had to be soaked for 12 hours before attaining adequate moisture. A similar state prevailed with the grass straw. Having been cut several months before the onset of the experiment, the grass may have lost most of its water making it absorb much water during composting before releasing it to the mushrooms.

5.3.3 Duration to fruiting body maturation

Corncobs, wheat straw, sugarcane bagasse and grass straw each took averagely 8, 11, 16 and 20 days respectively to produce mature fruiting bodies when supplemented with wheat bran (Table 15). Corn cobs therefore, were the best substrate in terms of fastening maturation of fruiting bodies. One factor that could cause fast maturation of fruiting bodies is the nutritional level of the substrates. It has been reported that nutritional content of the substrates is important in reducing the cropping cycle in mushrooms (Uhart *et al.*, 2008). In all cases, substrates supplemented with wheat bran gave lesser days to fruiting body maturation. According to Lelley and JanBen (1993) the nutritional content of any substrate can be improved by nitrogen supplementation. Since the protein level in wheat bran is relatively higher than in rice bran (Uhart *et al.*, 2008), there was faster mushroom growth in all the substrates combined with wheat bran compared to those supplemented with rice bran. This explains why fruiting bodies harvested from corn cobs and wheat bran matured significantly faster than all the other substrates. It is also possible that corn cobs provided a better surface for anchoring of the fruiting bodies as they grew

making it mature faster. As reported by Bechara *et al*., (2005) a firm substrate surface provides anchorage to the mushroom stipe as it grows upwards. This was converse in grass straw, wheat straw and sugar cane baggase which appeared loose even after being completely colonized by mycelia. It is therefore plausible to argue that longer days to fruiting body maturation in these substrates may have resulted from fruiting bodies spending most of their obtained nutrients in achieving better hold of the substrate. The black strain proved more vibrant being the first to form mature fruiting bodies followed by brown and white respectively.

5.3.4 Number of fruiting bodies per bag

The average number of fruiting bodies obtained in each bag was quite low when compared to work done on other specialty mushrooms (Table 15). From the harvest, only 20, 15, 10 and 4 black fruiting bodies were obtained from corn cobs, wheat straw, bagasse and grass straw respectively. This is in converse to an average of 21 fruiting bodies obtained by Wong and Wells (1985) when they cultivated the same genus on oats straw and sugar cane bagasse under similar conditions. A number of factors may have contributed to such a low number of fruiting bodies in this study. Spawn running to cause complete ramification of substrates by mycelia is a highly sensitive stage (Royse, 2007b). During spawn running, the bags were kept in dark cabinets to allow mycelia growth. It was quite difficult to control environmental conditions of the cabinets apart from lighting. It is probable that there occurred accumulation of CO_2, which led to decrease in points at which fruiting bodies emerged from the substrate bags. Oei (1996) indicated that successful spawn running is important in regulating the number of fruiting bodies that are eventually obtained. Apart from CO_2, temperature fluctuations may also have led to destruction of mycelia. It is probable that maintenance of required conditions was hindered especially at night where atmospheric temperature could be quite low. Stamets (2005) argued that wood ear mushroom mycelia are highly sensitive to temperature changes. Temperature fluctuations cause death of surface mycelia, which may reduce development of fruiting bodies (Oei, 2005). It was observed that some sections of colonized substrates became completely black indicating death of surface mycelia. Even though scrapping of substrate surface was done as recommended by Stamets (2005), only a few fruiting bodies emerged from such surfaces.

Even though very high standards of hygiene were maintained during the cultivation, incidences of contamination of substrates by other fungi were common. The presence of contaminating

fungi is one of the factors that may lead to low number of fruiting bodies (Oei, 1996). During the process of mushroom growth, competition occurs for space, nutrients as well as chemical alteration of the substrate, which will hinder mushroom development (Chang and Miles, 1989). The olive green mould (*Chaetomium olivacearum*) has an exclusive effect to *Auricularia* mushrooms resulting in inadequate compost formulation and deficiency of fresh air intake and circulation. Therefore, the areas of the substrate occupied by this fungus were unfavorable for mycelia growth. Only the areas devoid of infection were fully colonized and resulted in fruiting body production. The greatest effect of such fungi was observed in grass straw which produced the least number of fruiting bodies. In addition, the infection led to falling off of several young fruiting bodies during watering due to their fragile nature. This effect was greatest in wheat straw, sugarcane bagasse and grass straw where the pinheads regularly fell off.

5.3.5 Mushroom quality

Significant differences ($p \leq 0.05$) were observed in the quality of mushroom fruiting bodies obtained from various substrates (Table 14). Better quality mushrooms were obtained from corn cobs and wheat bran combination. The appearance of these mushrooms was excellent, with well-formed large caps and observable stems. This was in contrast to mushrooms obtained from grass straw and baggase that were much smaller with a minute stipe. Sonnenberg (2007) proposed that substrates with high nutrient bases produce higher quality mushrooms. This suggests that corn cobs and wheat straw combined with wheat bran had the highest quality and quantity of nutrients suitable for development of wood ear fruiting bodies. Black fruiting bodies produced on substrates supplemented with wheat bran were of good quality with large caps and identifiable short stipes. On the other hand, fruiting bodies obtained from substrates supplemented with rice bran had smaller caps with absent stipes. As expected, black mushroom strains were larger than both brown and white strains. These findings confirm work done by Uhart *et al.*, (2008) when they reported that wheat bran supplementation greatly improved the quality of mushroom basidiocarps. Even though large sized fruiting 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 fruiting bodies tend to break during packaging thereby reducing their quality. This might be improved by lowering the quantity of wheat bran supplement, which would slightly reduce fruiting body size while improving their durability (Shen and Royse, 2001).

Another way of obtaining high quality wood ears with average sizes would involve reducing light intensity of the cropping room since low light intensities promote development of hard and longer lasting caps (Lee, 2000). Oei (1996) proposed a proper control of environmental conditions to produce high quality mushrooms. For instance, Oei (1996) attributes poorly formed fruiting bodies to a high concentration of CO_2 during incubation. According to Shen and Royse (2001) stipe elongation is influenced by concentration of carbon dioxide in the room. It is possible that fanning of the room was interrupted during black outs and this may have led to accumulation of CO_2 within substrate bags causing production of poor quality mushrooms.

5.3.6 Mushroom fresh weight

Major differences occurred in yield (fresh weight of fruiting bodies per bag) of the mushrooms in different substrate and supplement combinations (Table 16). Significant interactions ($p \leq 0.05$) were also found between strains and substrates used for its cultivation. Corn cobs and wheat bran combination produced the highest fresh weight per bag for all strains. This result suggests that corn cobs and wheat bran provided the best nutritional base for wood ears and is most suitable for its cultivation. Numerous explanations can be given for differences observed in yield. Previous studies on mushroom cultivation suggest that cellulose content of substrates and enzyme production of mushrooms is important in determining yield of a mushroom crop. Schiler (1982) speculated that reduction in fresh weight of mushrooms might be associated with absence of certain specific nutrients especially the cellulose-based substrates. Thomas *et al.* (1998) showed that high lignocellulotic content of substrates is important in fruiting body production. Phillipoussis *et al.* (2001) compared various lignocellulotic contents of substrates such as wheat straw, sugar baggase and wood chips for various specialty mushrooms cultivation and concluded that varying results could be obtained. Martinez (2002) reported the capacity of wood ear mushrooms to grow on agricultural wastes such as corn cobs due to their lignolytic enzymes that are necessary for degradation of such substrates.

Variations observed in yield might also be due to complexity of substrates in terms of their cellulose content resulting in differences in rate of degradation by mushroom enzymes. For instance, 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 abundant free sugars that were present in the baggase and therefore

made limited use of the cellulose fraction (Phillipoussis *et al.*, 2001). As reported earlier, grass straw substrate used in this study had been stored for long accumulating phenolic acid. 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. A rapid reduction of the level of phenolics would accelerate colonization rate and reduce the risk of mould contaminants such as *Trichodema* and *Chaetomium*. This would eventually lead to higher yield.

5.3.7 Biological Efficiency

Determination of Biological Efficiency (B.E) involved relating mushroom yield per bag to the dry weight of spent substrates. This investigation revealed mushroom strains which utilized nutrients present in the substrates most efficiently. The biological efficiency of each strain responded more to the type of substrate and supplement used for its cultivation (Table 17). The highest ($p \leq 0.05$) biological efficiency (79%) was observed in the black strain cultivated on corn cobs supplemented with wheat bran. Since B.E values indicate how mushrooms assimilate available nutrients, high values depict better utilization of resources. Being mainly jelly like in form, wood ears require a medium with a high proportion of lipids. Therefore, lipids present in corn cobs must have been efficiently utilized resulting in the high B.E values. According to Mendeel *et al.*, (2005) growth and development of mushroom sporophores requires a substrate with a lipid component because lipids are stimulatory to mushroom growth. According to Narain *et al.*, (2008) corn cobs have a considerably higher lipid component in comparison to wheat straw, sugarcane baggase and grass straw. This may explain better performance of wood ears in corn cobs compared to other substrates. In addition, developing mushroom sporophores require an equal supply of lipids and proteins (ratio of 50/50) that are needed for enlarging cell membranes during growth (Schiler, 1982). Wheat bran supplemented on corn cobs may have provided the required protein component resulting in higher biological efficiencies.

Available reports show that spawn quality has a significant effect on Biological Efficiency. Bechara *et al.*, (2005) observed that biological efficiency is highly affected by the quality of spawn used to inoculate substrates. Rojas and Mansur (1995) reported high B.E values when high quality spawns were used compared to low quality spawns. In this study, the black strain whose mycelia were thick and condensed produced highest B.E values. Spawns obtained from the white strain were the least vibrant and therefore recorded low B.E values. The thick mycelia

of black strains rapidly colonized the substrate and caused conversion of its parts into fungal protoplasm during fruiting body development. Apart from spawn quality, B.E can be positively affected by supplementation. Mendeel *et al.*, (2005) reported that the effect of supplements on B.E is dependent on the cultivated mushroom strains. In a study conducted on *Pleurotus* mushrooms, Betterley (1988) suggested that with supplementation, a B.E of up to 90% could be obtained within 35 days of cropping. Similar observations have previously been made by other researchers (Balakrshnan and Nair, 1993; Mayuzumi and Mizuno, 1997). High B.E values may also be attributed to several other factors. First, increased level of nutrients in the substrate would provide more energy for mycelia growth and development. Second, more inoculums points would provide faster substrate colonization and thus more rapid completion of the production cycle. Finally, more rapid spawn run would reduce the time the non-colonized substrate is exposed to competitors such as moulds.

5.4 Comparison between some growth parameters

Correlation analysis between various growth parameters revealed strongly positive and negative correlations (Table 18). The highest positive correlation of (R^2 = 0.99) occurred between biological efficiency and the fresh weight of fruiting bodies for each bag. This result was expected since the biological efficiency values were derived from fresh weight of fruiting bodies in relation to dry weight of spent substrates. A high positive correlation (R^2 = 0.95) was found between duration to complete mycelia colonization of substrates and the quality of the fruiting bodies. This indicates that the best qualities of fruiting bodies were obtained from bags that had been colonized at faster rates. During the study it was observed that rapid and thick mycelia growth occurred in corn cobs and wheat straw substrates. These substrates eventually produced high quality fruiting bodies. Narain *et al.*, (2008) compared fruiting body qualities of *Pleurotus florida* mushrooms and the rate at which their mycelia had ramified substrates and found high correlations. Negative correlations observed in this study were all in agreement with standard mushroom agronomic principles as explained by Oei, (2005). For instance, substrates that produced more fruiting bodies, higher fresh weight and better biological efficiencies had been colonized faster reducing the days to complete colonization and duration to primordia initiation. Royse *et al.*, (2007) indicated that mushrooms with high fruiting body qualities produce high biological efficiency values than those with low qualities. This was confirmed by strong

correlations between quality and biological efficiencies for all strains. The black strain had the strongest $R^2 = 0.92$ (Fig 3) followed by the brown strain $R^2 = 0.88$ (Fig 4) and lastly the white strain $R^2 = 0.74$ (Fig 5).

CHAPTER SIX

CONCLUSIONS, RECOMMENDATIONS AND SUGGESTIONS FOR FURTHER RESEARCH

6.1 CONCLUSIONS

The following are the major conclusions that were drawn from this study:

- Genetic diversity among native wood ear mushrooms of Kakamega Forest was found to exist but relatively narrow. Evaluation of their phenetic characters revealed variations in fruiting body colors, texture, size, shape and presence of abhymenial hairs. However, comparison of internal of sections of fruiting bodies did not reveal any significant differences. Slight variations occurred in external features of mycelia when cultured in malt extract agar. Microscopic studies of individual hyphae did not reveal much variation between the strains. A dendogram analysis of phenetic data showed a clear distinction between white and the two other strains at a dissimilarity index of 0.318 at p=0.05 level of confidence. Black and brown strains were more closely related even though a dichotomy occurred separating them at a lower confidence level.

- Results from spawn production clearly showed that millet and sorghum grains can be successfully colonized by wood ear mushroom mycelia at different rates. An equal composition of these two grains supplemented with wheat bran produced the best spawn. It was also clear that un-supplemented grains were colonized but at very low rates indicating that rice and wheat brans greatly aided the growth of mushroom mycelia.

- Corn cobs combined with wheat bran provided the fastest rate of growth of mushroom mycelia. This combination also gave shortest period to primordia initiation and fruiting body maturation. It was evident that corn cobs and wheat bran was the best combination for rural cultivation of wood ear mushrooms. Wheat straw, bagasse and grass straw followed in that order.

6.2 RECOMMENDATIONS

The following are recommendations that arise from this study.

- Native wood ear mushrooms of Kakamega Forest have the potential for cultivation and expansion together with other non native species that are already being cultivated. Investigation on their growth performance on other agricultural wastes is necessary. As forests such as Kakamega Forest reduce in cover and land usage changes, it is important to secure the genetic stock necessary for cultivation of native mushrooms. This can only be achieved through a concerted effort to domestication of such native species.

- Morphological characterization considered in this study does not provide sufficient information for delimiting the strains of native wood ear mushrooms. There is need to employ genetic molecular analysis in order to obtain proper differences between them.

- Research and extension on diversity, abundance and distribution of native wood ears and other indigenous mushrooms is highly needed. In order to improve the management of native wood ear mushrooms, there is need to know more about their ecology, phenology, response to environmental disturbance and apply this knowledge in their protection.

- Commercialized mushroom production is highly underdeveloped in Kenya. This is attributed to low technological advancement and very low levels of consumption of mushrooms. There is need for more investment on equipment that would improve the quality of spawns and the general growing conditions. Also, better culinary research could explore the use of mushrooms as food additives and medicines thereby expanding the potential market.

6.3 SUGGESTIONS FOR FURTHER RESEARCH

The following are suggestions for further research.

- Evaluation of genetic diversity of the wood ear mushrooms was done using morphological characters. There is need to conduct molecular studies using genetic markers to determine possible genotypic differences between the native wood ear mushrooms.

- Cultivation of the wood ears utilized only four substrates and two supplements. There are numerous agricultural wastes in Kenya such as maize stalk, banana straw, coffee husks and bean pods. These should be evaluated to determine their potential as growth substrates for native wood ears.

- Biochemical analysis of nutrients in each of the substrates should be done to find out specific compounds responsible for variations in growth of the wood ear mushroom strains.

- Mushroom cultivation is extremely sensitive to variations in environmental conditions. Further experiments should be conducted with variations in temperature, light, humidity, and aeration to determine appropriate conditions for the wood ear mushrooms.

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