EFFECT OF FARM WASTE SUBSTRATES ON GROWTH, YIELD AND QUALITY OF OYSTER (*PLEUROTUS SPP.*) MUSHROOM.

BY

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DECLARATION

Declaration by the candidate

This thesis is my original work and has not been presented for a degree in any other University. No part of this thesis may be reproduced without the prior permission of the author and /or University of Eldoret.

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DEDICATION

To my wife Nelly Jerotich and my children; Cynthia, Moen and Abel who despite financial constraints continued to pray for me and also gave me moral support during the entire period of my studies. May God richly bless you, and meet you at the point of need for He is our God full of riches. To my parents, sisters, brothers and cousins, I sincerely thank you for your prayers and financial support.

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ABSTRACT

Mushrooms are grown under natural conditions on living trees as saprophytes, on dead woody branches or artificially on agricultural and agro-industrial wastes. Oyster is rich in protein (30.4%), fats (2.2%), carbohydrates (57.6%), fibre (8.7%), ash (9.8%), vitamins and minerals salts. Oyster farmers are not conversant with suitable substrates to use or species to grow. The objective of this study was to determine the effect of farm waste substrates on growth, yield and quality of oyster mushroom and also the suitability of cereal grains substrates for spawn production. Two experimental sites namely; Kaimosi Agricultural Training Centre and Kapsabet Divisional Headquaters in Nandi Central District were used. The nine substrates were wheat straw (*Trichum aestivum*), maize cobs *(Zea mays*), bean husks (*Phaseolus vulgaris*), sugarcane bagasse (*Saccharum officinarum*), papyrus (*Cyperus papyrus*) and a combination of bean husks with each of the mentioned substrates separately. These were tested to determine the best farm waste substrates for oyster production. The substrates were mechanically shredded and sterilized and pH adjusted to 4.2 -7.5. Upon cooling the substrates were packed in 2 kg heat resistant polythene bags (10'' x15'') and pasteurized for 90 minutes using an oil drum and allowed to cool before being spawned with *Pleurotus* spp at the rate of 30 g per 2kg substrate. To determine the best cereal grain substrates for spawn multiplication, four cereal grain types namely, sorghum, corn, wheat and millet were tested for their suitability. The experiment was laid out as Completely Randomized Design - 2 oyster spp. x 9 media combinations (substrates) x 3 replicates. The inoculated bags spawned with *Pleurotus* spp were incubated in an incubation room with relative humidity of 70-80% and temperature of 22-30^0 C for 3 weeks to allow rapid mycelium colonization, pin head formation and subsequent emergence of fruiting bodies. Parameters observed were both fresh and dry weights, the rate of mycelium colonization, time taken for pinning, days to maturity, height of stipe, stem diameter, cap diameter, and Biological Efficiency. Quality of oyster mushroom was determined based on biochemical and microelements content at Kenya Industrial Research and Development Institute Laboratories. Data analysis was subjected to analysis of variance using general linear model of statistical analysis (SAS 2000) package. Treatment means were separated by multiple comparisons using Tukey's Honest Difference. Results showed that the best substrates in terms of growth, yield, and biological efficiency were maize cobs followed by wheat straw and sugarcane bagasse respectively. A combination of maize cobs with bean husks also proved to be a superior substrate. Sorghum (*Sorghum bicolar*) grains were found to be the best cereal grains substrates for spawn multiplication. Maize cobs are recommended as the best substrate for oyster (*Pleurotus* spp.) cultivation while sorghum grains are recommended as the most suitable cereal grains for oyster spawn production. White oyster are recommended for cultivation due to its faster rate of colonization on cereal grains during spawn multiplication. Analysis of biochemical and micro-element (nutrient) content revealed that oyster contains the much needed proteins, vitamins and mineral salts and hence this study recommends cultivation and consumption of oyster which will address the current protein deficit in most rural areas.

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LIST OF AGRONYMS AND ABBREVIATIONS

AOAC……..Association of Official Analytic Chemists

APEMTC…….Asian Pacific Edible Mushroom Course.

BE……....Biological efficiency

DOS…….....Diameter of stem

DTP……..Days to pinning

DTM…….....Days to maturity

DOC…….Diameter of the cap

EPC……..Export Promotion Council

ERSE……...Economic Recovery on Wealth Creation and Employment

FAO…….Food and Agriculture Organization

HOS…….....Height of stipe

ILRI…….International Livestock Research Institute

KIRDI…….....Kenya Industrial Research and Development Institute

LOM……....Length of mycelium

MOA……....Ministry of Agriculture

SRA…….....Strategy for Revitalization of Agriculture

Spp…….....Species

CHAPTER ONE

1.0 Introduction

1.1 Overview

Oyster mushrooms (*Pleurotus* spp) are fleshy fungi and are the premier recyclers on the planet (Boa, 2004; Oei and Niuwenhuijzen, 2005). They do not contain chlorophyll like green plants and as a result cannot manufacture their own food. In respect, they resemble animals because they feed themselves by digesting other organic matter and hence they are essentially important in recycling organic wastes. They are also efficient in returning nutrients back into the ecosystem (Hayes, 1978).

Oyster (*Pleurotus* spp) is far the easiest and least expensive to grow. For small scale farmers with limited budgets, oyster mushrooms are the clear choice for gaining entry into the gourmet mushroom industry (Stametes, 1993). Few other mushrooms demonstrate such adaptability, aggressiveness and productivity as the genus *Pleurotus.* Prominent farm waste decomposers, *Pleurotus* spp. grow on wider array of forest and agricultural wastes than species from other groups. They thrive on some hardwoods, wood by-products, cereal straws, corn and corn cobs, coffee residues (coffee grounds, hulls, stack and leaves), cotton seed hulls, agave wastes, soy pulp and other materials (Sun Pei-ji and Jian-jun, 1989). Oyster mushroom can best serve to reduce hunger in developing countries and revitalize rural economies.

Edible mushrooms or wild fungi have been collected and consumed by people for thousands of years. The archeological records reveals that certain species are associated

with people living 1300 years ago in Chile (Mansure and Rojas, 1992) but it is in China where eating of fungi was first reliably documented (Aaronson *et al.*, 2000). Mushrooms are found growing in forests or hilly areas and have been successfully cultivated in temperate and tropical regions of the world (Ibekwe *et al*., 2008). Habitat and substrate requirement are important factors in distinguishing groups of mushrooms (Westhuizen and Eicker, 1994).

Oyster mushrooms are highly nutritive and very rich in protein, vitamins, and minerals and low in lipid and sugar content (Ayodele, 2006). Cultivation of oyster mushroom in Kenya will reduce incidences of malnutrition arising from protein deficiency which is responsible for high mortality and morbidity in rural areas. In Kenya, availability of abundant agricultural wastes offers opportunity for oyster mushroom production since only a tenth of crop residues and weeds left after crop harvest is eaten by livestock (ILRI, 1999).

Pleurotus ostreatus is not only important within the environment for disposing of agricultural waste but also for their effect on human health, yet their inherent biological power embodied within the mycelia network remains a vast untapped resource (Stametes, 1993; Diamantopoulou and Philippoussis, 2001). Some species of oyster mushroom and Reishi (*Ganoderma lucidum*) contains powerful stimulants of the immune system and numerous studies have demonstrated the anti-cancer and inferon stimulating properties of these mushrooms (Yamura and cohran, 1974).

The numbers of oyster mushroom species and suitable growing substrate for growing them are not fully exploited. Surveys carried out in Lake Victoria basin in Kenya shows

that a lot of research has been done on edible mushroom with little research on medicinal mushroom (Engola *et al.*, 2007; Munishi *et al.*, 2007; Olila *et al*., 2008). *Pleurotus* spp such as; *P. flabellatus, P. sojacaju, P. ergngii, P. sapidus, P. adjamor, P. citrinopileatus and P. cornucopiae* should be cultivated in Kenya. Agina and Joshua (2004) reported that rapid agricultural and urban development is destroying the natural habitats of edible mushrooms.

In China, mushrooms form the main ingredients in medicines and health products. In Kenya, however, oyster mushrooms are a recent introduction. Historically, they were considered a luxury food reserved for the rich. Production of oyster was a guarded preserve of a few large scale farmers who could afford the "state of the art" capital intensive outfits required for cultivation (Nita, 1987). Mushroom production by small scale growers in Kenya accounts for only 5%. Large scale farms in Kenya include Agridutt which accounts for 35% of the total annual production, along with Rift Valley mushroom producers (30%), Olive farm (20%) and Devan (10%). There has been a growing interest by small scale farmers to venture into oyster mushroom cultivation. However, cultivation has been hampered by several constraints which include lack of know-how on the best type of substrates to use and the necessary technical knowledge involved in spawn production and hygiene requirements.

Wambua (2004) reported that Kenya's ethnic communities were not completely alien to mushroom. Although Kenya is endowed with adequate agricultural wastes for oyster mushrooms, she has not fully exploited the existing production potential (Asian Pacific Edible Mushroom Course, 2007). World mushroom production is estimated at 12 million

tons annually and it is estimated that there are about 1.5 million different species of mushrooms and only 64,000 species have been described so far. Many species from the tropical rain forests and the remote areas may have disappeared due to human activities.

1.2 Problem statement.

The Kenyan population has over the years continued to increase against a declining acreage of arable land (Fermont *et al*., 2008). The ultimate impact has been a decline in agricultural productivity and increased poverty levels. Since 50% of Kenyan population is food insecure, farmers are faced with the task of oyster mushroom cultivation which requires only a small unit of land (KARI, 2007). Important challenges facing mushroom production is lack of knowledge on the type of substrates which support fast growth of high quality oyster, technical skills and proper control of environmental conditions (Tisdale *et al.,* 2006). Small scale farmers are not conversant with species of mushroom to grow, construction of local mushroom houses and maintenance of high level of hygiene. Most farmers who begin oyster production are not trained in production technologies and are therefore not able to sustain supplies. In addition, farmers willing to offer training, charge exorbitant prices which small scale farmers cannot afford. The government has very few trained extension staff that provide lean extension services to potential and practicing oyster farmers (Gateri *et al.,* 2007).

In Kenya there are no regulatory standards for the control of quality spawn production (Gateri *et al.*, 2007). This has led to proliferation of unscrupulous businessmen selling fake spawn to innocent farmers. Being an emerging crop, limited research has been undertaken to identify the best substrates and clean spawn for oyster farmers. Among the

problems affecting mushroom production in Kenya, include; the types of substrates to use, the strains of mushroom to grow and the kind of cereal grains substrates for spawn production which need to be investigated.

1.3 Justification of the Study

An adequate food intake is one of the fundamental human requirements, but there is no denying the facts that millions of people, especially in developing countries like Kenya are beset with danger of their very survival due to its non availability. Oyster mushroom requires no arable land for production and the abundant agricultural wastes found countrywide offers opportunity for production, which in turn provides a more environmentally disposal system (Stametes, 2000).

Low food production due to limited land resources coupled with poor quality protein is creating a protein gap of alarming proportion which has led to widespread malnutrition in several parts of Kenya, especially in infants, children, pregnant and lactating mothers. Increasing human population and diminishing farm sizes has resulted in reduced soil fertility and land degradation resulting in decreased land productivity and increasing poverty levels (Sanchez and Leaky 1997)

In Kenya, mushroom production stands at 500 tons annually. Kenya has to import 150 tons of canned mushrooms worth Ksh.10 million annually (EPC, 2004). Kenya can save on foreign exchange by promoting mushroom industry using the locally available agricultural and industrial wastes. Globally, 14% of the population is undernourished.

Some 10 million people die annually from hunger; about 27.4% are in Africa and 33% in sub Saharan Africa (FAO, 2003).

It is estimated that about 300 million tons of mushroom can be produced from just ¼ of the world's annual yield of straw (2557.5 million tons). Such an amount would provide 4,100 million people with 250g of fresh mushroom daily (Bahl, 1984). Considering the yearly agricultural wastes in Kenya, and other parts of the world estimated at 500 billion kg, and forest wastes (100 billion kg) we can easily grow 360 billion kg of mushroom on a total of 600 billion kg of dry waste. This could produce 60 kg of mushroom per head per year (Courvoisier, 1999). Currently there is a big protein deficit gap, and hence the need to provide protein rich food for Kenyans as per the millennium development goals (MDG's) using cheap, unutilized agricultural and agro-industrial wastes "Economic Recovery Strategy on Employment and Wealth Creation, 2003). Mushroom products of high standards should be promoted so as to favorably compete in the regional and international market. Tinned oyster imported from China and India indicates that there is an opportunity for local production that smallholder farmers can exploit to generate income and create job opportunities and boost food security (Family Concern, 2005).

In Nandi County, there is over 100 ha of naturally growing papyrus (at Kingwal-Kimondi swamp) which can ensure a constant supply of the substrate throughout the year. The most significant aspect of oyster cultivation is to create "zero-emissions" since more than 70% of agricultural and forests materials are non-productive and are wasted in processing (Poppe, 2000). Creating zero-emissions means a practical approach to improving the livelihoods of rural communities in Kenya (Chiang and Mshigeni, 1997).

According to Strategy for Revitalization of Agriculture (2005), Kenya has a high potential to produce and even export oyster mushroom, but disappointingly, the sector has largely remained unexploited. Favorable climatic conditions allows for production of a variety of cereal crops required by mushroom industry. According to Farm Concern (2005) there is huge underlying potential for oyster cultivation if prices were put right and farmers sensitized on mushroom nutritional and medicinal values.

1.4 Objective

1.41 Broad objective

To determine substrates for production of high yield and quality oyster mushroom (*Pleurotus* spp.)

1.42 Specific objectives

1. To determine the effect of various local grains on oyster mushroom (*Pleurotus* spp.) spawn production.

2. To determine the most suitable local farm waste substrates for optimal growth, yield and quality of oyster mushroom (*Pleurotus* spp.)

3. To determine biochemical and nutrient content of oyster mushroom.

1.43 Experimental Hypotheses

1. **H_o:** Different local cereal grain substrates have no effect on oyster spawn production.

2. **H_o:** Different local farm waste substrates have no significant differences on growth, yield and quality of oyster (*Pleurotus* spp.) mushroom

3. **H_o:** Oyster mushroom has no nutritive value.

CHAPTER TWO

2.0 Literature review

2.1 History of mushroom cultivation

Fungi have been at work since life began on earth. To quote Caryle "Nature alone is antique and oldest art a mushroom" (Nita, 1987) .The Romans referred to mushrooms as "food of the gods ''and the Greeks thought mushrooms provided strength for warriors in battle (Daba, 2007). The first record of the cultivation of mushrooms was during the reign of Lous (1638-1715). The earliest description and knowledge of growing mushroom was written by de Tournforte, a Frenchman, who grew mushrooms underground in quarries around Paris on horse manure (Bahl, 1995). A breakthrough in commercial production was achieved when spawn culture was made from mushroom tissue (Duggar, 1905). In Latin 'fungi' means to flourish. It was a term which was used to refer to mushroom and to excrescence from the ground or farm trees. The Greek term "mushroom" was derived from the word 'sponges" or "sphoggos" which meant "spong" and refer to the sponge –like structure of some species.

Greek king Perseus, being thirsty, had a chance to squeeze out water from mushroom and drank it. He was so pleased that he gave his new kingdom the name Mycenae, Thus one of the greatest civilizations of history. (Alexopoulus, 1962). The Greek, Roman, Egyptian and Chinese civilization is sprinkled with reference of mushroom as delicate food. Saad bin Zaid, relates that mushroom were part of Manna-O-Salva, the biblical God-ordained food of pre-Jewish communities. The Buddha is believed to have eaten mushroom before

being transported to Nirvana. The Aryans used an intoxicating drink “soma” in religious rites. Soma in Riq Veda refers to *Amanita muscaria*, the flying mushroom (Wasson, 1969).

One of Wasson’s most provocative findings can be found in Soma: Divine mushroom of immortality, where he postulated that the mysterious Soma in Vedic literature, a red fruit leading to spontaneous enlightment for those who ingested it, was actually a mushroom. In Central America, the highlands of Guatemala mushroom-shaped stone carvings have been found. These are considered to belong to the Mayan period and point to the fact that they were being used in ceremonies (Bahl, 1987)

The ancient Indian, Greek and Roman myths agree that mushrooms sprang from a stroke of lightening. In Mexico, Indians believed that mushroom are sacred because they are born of sexual intercourse between a bolt of lightning and the earth (Bahl, 1987). Regarding fairy rings, it was once believed that fairies used to dance in the midnight in circles but actually the dark green circles in the grass around which mushrooms appear are caused by the radial growth of the fungus in the soil. Another delightful superstition is mentioned in “Alice in wonderland” when a bite of one side of a certain mushroom would make one grow and a bite of the other side would make one smaller, so by little judicious nibbling it was possible to adjust oneself to any dimension (Bahl, 1995)

2.2 Biology of Mushroom

Oyster mushroom belongs to the kingdom of fungi and very distinct from plants, animals and bacteria. Fungi lack the most important feature of plants; the ability to use energy

from the sun directly through chlorophyll. Fungi reproduce by means of microscopic reproductive units called spores (Stametes and Chilton, 1983a).The term mushroom is often used to describe the reproductive structure (fruiting body) of fungus. Some mushroom produce their spores on the exterior of the club-shaped cells called basidia (singular: *basidium*), hence called basidiomycetes. A smaller number produce spores inside microscopic sac-like mother cells called *asci*, hence they are called *Ascomycetes* (Chang and Miles 1992). A typical gilled mushroom is a straight forward structure consisting of cap, gills and a stalk. A protective covering called veil may also be present.

2.3 Mushroom classification

Khan *(*1982) reported that there are 100,000 known fleshy fungi out of which 50 are extremely delicious, 50 less tasty and 50 just edible. Oyster mushrooms, the best converters of straw, into food are quite delicious (Stametes, 2005). Chang and Miles (1997) classified mushroom as class: (*Basidiomycetes*), Sub class: (*Hymenomycetes*), Order: (*Agaricales*), Family: (*Agaricacea*e). Genus (*Pleurotus*)

2.4 Fungi Ecology

There are three modes of living which is comprised of saprophytes (degrading already dead materials), symbionts (living together with other organisms especially trees, in mutually beneficial relationships), and parasites (living at the expense of other organisms) (Pani and Naik, 1998). Oei (2003) reported that oyster mushroom degrades dead wood in nature and they can grow on a wide range of lignocellulosic materials.

2.5.0 Importance of mushroom

2.5.1 Importance of mushroom in food security and national income

Increase in human population is creating an alarming food deficit in the world. Exploiting non-traditional food resources can make a substantial breakthrough to meet the serious food deficit (SRA, 2005). Diversification of agriculture to high value crops and transformation of smallholder agriculture from subsistence to commercial enterprises offers good promising option for revitalization of agriculture and wealth creation among rural poor.

In Malawi the rural communities used to generate their income mainly from tobacco products but due to the world anti-smoking campaign, the farmers turned to mushroom production as their income sources (Afrol news, 2008). Apart from being used as human food, spent mushroom compost can be fed to livestock (Chantaraj, 2002). This reduces costs incurred in purchasing commercial feeds by small scale dairy farmers. In Tanzania, Magu district around Lake Victoria a low cost, small scale mushroom project is generating vast profits for women and children, hence helping them eradicate poverty (Nyawangah, 2008).

In Kenya an increasing human population coupled with diminishing farm sizes has resulted in decreased land productivity and increased poverty level among rural communities. In addressing food deficiencies and poverty issues, the government in its key economic policy documents “Economic Recovery Strategy on Employment and Wealth creation and the strategy for revitalization of agriculture” calls for incorporation

of crops such as mushroom that requires less land and can compete in national and international markets (ERSE and WC, 2003).

Benzinger (1996) reported that in order to help farmers make a transition, the government of Thailand and Taiwan supported the private sector agro- production and marketing program

(APMP). APMP staff acted as catalyst between firms and farmers producing crops such as mushrooms, tomatoes, watermelons and asparagus. In Taiwan the government supported the development of two industries through Taiwan Agricultural Research Institute (TARI) where local farmers association through the farmers techniques, inspection, grading and canning of mushroom. All canners acted as a cartel in the international markets citing a single price and hence Taiwan became the largest exporter of canned mushroom in the world. Most farmers in Africa appreciate the value of fertilizer but seldom use them because of high cost and lack of credit. Therefore, there is need for an enabling environment for small-holder sector (infrastructure, education, credit, inputs, markets and extension services), reversing soil fertility depletion and intensifying land use with high value crops such as oyster mushroom (Sanchez and Leakey, 1997)

2.5.2 Importance of mushroom in medicinal field

Extracts from Shiitake spores and isolation of "mushroom RNA" from them have proved effective against influenza. Several compounds from *Pleurotus* spp. and shiitake spores with immune-stimulatory activities on humorial and cell mediated immunity have been

isolated (Cheung and Lee, 2000). Antitumor, immunopotentiator and inferon stimulating polysaccharides have been found in several mushrooms such as *Boletus edulis, Calvata gigantean, Coriolus vericolor, Ganoderma applanatum, Ganoderma lucidum, Amillaria ponderosa* and *Phaliota nameko* and some *Pleurotus*

spp. (Yamura and Cohran, 1974). They further reported that *Panaeolus sub-balteatus*, a mushroom producing psilocybin and psilocin, provided significant protection from polio virus. Antioxidant compounds from mushroom have been known to prevent oxidative damage related to aging, and diseases such as atherosclerosis, diabetes, cancer and cirrhosis (Yang *et al.,* 2002; Jonathan *et al*., 2006). Oyster (*Pleurotus sojar-caju* and *Pleurotus* spp.) water extracts has been known for its capability to lower the effect of HIV. (Wang and Wang, 2007).

Human and fungi share common microbial antagonists for example *Escherichia coli*, *Staphylococuss aureus* and *Pseudomonas aeruginosa.* Extracts from several wood inhabiting oysters have been shown to inhibit growth of a wide variety of microorganisms which include bacteria and viruses (Suay *et al*., 2000).The Tinder polypore (*Formes formentarius*) have been shown to inhibit the growth of *P. aeruginosa* and *S. marcescens*, while birch fungus *Piptoporus betulinus* is effective against the two bacteria, and further exhibit inhibitory activity against S. *aureus, B. subtilis and M. smegmatis,* a cousin of pathogenic mycobacterium tuberculosis (Suay *et al*., 2000).

Lentinam, a water soluble polysaccharide (B-1, 3 glucan with B-1, 6 and B-1, 3 glucopyranoside branching) extracted from mushroom is proving to be anti-cancer drug (Chihara, 1978). Jose *et al.,* (2002) showed that methanol extracts of *P. pulmonarius*,

and *P. ostreatus* fruiting bodies decreased pawpaw oedima. They also ameliorate acute and chronic inflamatoris (Gude and Plemenitus, 2001). Some *Pleurotus* spp have been found to contain blood pressure lowering activity (Hagiwara *et al*., 2005).

2.5.3 Importance of oyster mushroom in nutrition

The protein value of mushrooms is twice that of asparagus and potatoes, four times that of tomatoes and carrots and six times that of oranges (Adjumo and Awosanya, 2005). The sclerotic are usually harvested from decaying logs and the dark brawn exterior is peeled off and the white mycelia tissue used for food or medicine (Isikhuemhen and Lebauer, 2004*).* The fat content in mushroom comprises of palmitic acid 14%, stearic acid 3%, oleic acid 18%, and linoleic acid 65% (Hadar and Arazi, 1986). These authors further reported that the fat content in different species of mushroom ranges from 1.1 -8.3% on dry weight basis. Fresh mushrooms contain 0.95% manitol, 0.28% reducing sugars, 0.5% glycogen and 0.91% hemicelluloses and the absence of starch in mushrooms makes it ideal for diabetic patients and for weight watchers (Aletor, 1995)

2.5.4 Importance of oyster mushroom in soil conditioning

Stewart *et al. (*1998) reported that the spent substrate compost left after harvesting is a good soil conditioner and bio-fertilizer that can be used in crop production to reduce the required inorganic fertilizer leading to sustainable farming system. They also stressed that application of spent mushroom substrate caused a rapid increase in soil inorganic nitrogen concentration, increased both soil pH and cation exchange capacity (CEC) whereas inorganic fertilizers decreased both. Spent substrate improved the environment

for root development by increasing soil density, reducing clod and surfaces crust formation and increasing infiltration level (Stewart *et al.,* 1998). Spent mushroom substrate is the choice of ingredient by companies making potting mixtures sold in supermarkets.

2.5.5 Importance of mushroom in toxic wastes management and distilleries

A number of fungi can, however, be used to detoxify contaminated environments, a process called "bioremediation" (Atlas and Bartha, 1992). *Pleurotus. tuberregium* (a white-rot fungus) has been reported to ameliorate crude oil polluted soil and the resultant soil sample supported germination of *Vigna unguiculata* (Isikhuemhen *et al.,* 2003). Apart from the use of white rot fungi (*Phanerochaete chrysosporium*) to detoxify the polluted environment, the brown rot fungi (*Gloephyllum spp.*) are also widely used. Most of these wood rotters produce lignin peroxidase and celluloses. These extracellular enzymes have evolved to break down fiber, primarily lignin-cellulose the primary component of woody plants into simple forms (Mira and Ragini, 1984). Fungi which detoxify the enviroment reduce recalcitrant hydrocarbons and other man-made toxins. Current and prospective future uses include the detoxification of Polychlorobiphenols (PCB), Pentachlorophenol (PCP), oil, pesticides residues and more so are being explored for ameliorating the impact of radioactive wastes (Adenipekun, 2008).

Distilleries are among the most polluting industries as their effluent, if discharged into water bodies defile the natural ecosystem. The waste water from distilleries using molasses as the main substrate for fermentation is characterized by high organic pollutant load. The biochemical and oxygen demand (BOD) and chemical oxygen demand (COD)

typically range from 35,000-50,000 and 10,000-150,000mg/l, respectively (Nandi and Mukherjee, 2002). Molasses spent wash (MSW) is a potential water pollutant since it can block out sunlight from rivers and streams thereby reducing oxygen of the water by the photosynthesis and hence become detrimental to aquatic life. Also the MSW has high pollutant load which would result in eutrophication of contaminated water courses. If disposed on land the spent wash functions as a soil pollutant with an ability to inhibit seed germination, reduce soil alkalinity, cause soil manganese deficiency and damage agricultural crops (Deepak *et al.,* 2006). Oyster cultivation protects the environment from entry of biodegradable pollutants (Krithiga *et al.,* 2005).

2.6. 0 Oyster mushroom production on different substrates

The great attraction of mushroom production is that it can be grown on a wide range of agricultural by-products such as cereal straws, sisal wastes, banana pseudo stems, and potato peels. These wastes are of little or no value in their original form (Oei, 1991; Murugasen *et al.,* 1995). These materials are mostly resistant to natural biodegradation because they contain mainly cellulose, hemicelluloses and lignin. Mushroom mycelia excrete enzyme complexes that can degrade these components. Integrating mushroom production into the farm system will convert these otherwise waste products into high quality protein. This will alleviate the protein deficit and improve incomes (FAO, 1983*).*

A sizeable proportion of farm wastes are burned up during land preparation and only a tenth of the crop residues and weeds left on the fields after harvest are fed on by livestock (Zanchez *et al,* 2002). Although commonly grown on pasteurized wheat straw or rice straw, oyster mushroom can be grown on a wide variety of lignocelluloses substrates,

enabling it to play an important role in managing organic wastes (Isikhuehmem *et. al.*, 2000). Holcker and Lenz (2004) reported that new technologies are being developed using different substrates as the number of environmental parameters also increase.

Imbarnon *et al.* (1977) studied the number of saprophytic wild edible fungi of France and reported that several species of fungi (*Pleurotus* spp.) could be grown successfully on non-composted substrate like tree bark (industrial wastes from deciduous and coniferous forest trees). According to Poppe *et al.* (1995a), cultivation of *Pleurotus ostreatus* showed a better yield performance when grown on saw dust of Lipi lipi (*Leucena leucocephala*) and kikwati (*Gliricidia sepium*) without any supplementation.

According to Obodai *et al.* (2003) rice straw appeared to be the best substrate for *Pleurotus* as opposed to banana leaves, maize stovers, corn husks and elephant grass. When cultivating *P. florida,* the incorporation of cotton seed powder into rice straw substrate enhanced mushroom growth, mycelial growth, net total protein, free amino acids and total lipid content. Baysal and Temiz (2003) reported that cultivation of *Pleurotus* on waste paper with addition of chicken manure, peat and rice husks accelerated spawn running, pinhead formation and fruiting body formation.

Balaz (1981) observed that *P. florida* can be cultivated on cereal straws and that the substrates could be enriched by some other agricultural wastes such as alfalfa flour, oat meal, rape straw and soya straw. Cho *et al.* (1981) found that a mixture of cotton seed hulls and saw dust was a good substrate for *Pleurotus* spp. Mycelia extension and supplementation of the substrate with wheat brand resulted in a significant increase in yield and quality. They further noted that strong light was inhibitory to mycelia growth at

initial stages and that exposure to it after pinhead formation was essential to prevent abnormalities in fruiting bodies.

Khan *et al.* (1981) studied the yield performance of four strains of oyster mushroom viz. *P. ostreatus* (strain 467), *P. florida* (strain 3526), *P. sajor-caju* and *P. ostreatus* on paddy straw in winter where the temperatures were varied between 16-24^{0}C and reported that *P.ostreatus* (strain 467) was the most productive followed by *P. florida* (strain 3526) and *P.sajor-caju*. Tawiah and Martin (1986), cultivated *P. ostreatus* in *peat* moss based substrate and found that the highest yield gave 10% conversion of substrate into mushroom biomass after 45 days. The mushroom had 36% crude protein and all the essential amino acids were present. Cho *et al. (*2003) reported that inoculation of pure *P. ostreatus* mycelium cultures with strain of *Pseudomonas* spp isolated from the mycelia plane of commercially produced mushrooms promoted the formation of premordia and enhanced the development of the basidiomata.

The ability of different mushrooms species to utilize various substrates depend on both the mushroom and the substrate associated factors. Therefore the growth, quality and yield of an individual mushroom species on particular substrate will depend largely upon the ability of the mushroom enzymes to digest the components of the substrates. It also indicates that mushroom produce hydrolyzing and oxidizing enzymes which can hydrolyze the wastes (Okhuoya, 1997).

Apart from growing mushroom in underground quarries, it can be successfully grown above ground or on farm waste substrate (Stevenson and Lentz, 2007). Utilization of agro-industrial wastes for mushroom production in Kenya will be in line with reports of

Okhuoya and Okugbo *(*1991), Okhuoya and Etugo (1993) where they emphasized on the exploitation of agro-industrial wastes in mushroom cultivation. Okhuoya *et al.* (1998), and Kufonji *et al.,* (2003), reported that *P. tubberegium, P. ostreatus, and Lentinus spp,* grew successfully on diverse farm wastes, and saw dust of some forest trees.

Fasidi and Ekuere (1993) successfully cultivated oyster (*Pleurotus pulmonarius*) on grass straw, corn cobs, cassava leaves, rice straw, saw dust and banana leaves and reported that the best stimulatory wastes were saw dust, rice straw and banana leaves, respectively.

Bhavani and Nair (1989) reported that dried banana leaves with 1.45 N proved a good substrate for oyster while Poppe and Hofte (1995 b) found that both bean and bean pod straws produced a good yield of oyster when used as substrate during cultivation. Khan and Chandary (1989) found that post shelling and broken bits of maize cobs (*Zea mays*) were useful in production of quality oyster since mushroom enzymes was able to efficiently hydrolyze the wastes. Water hyacinth (*Eichhornia crassipes*) an aquatic plant found in Africa and Philippines has gained prominence as a substrate that produce high yield of oyster crop (Gujral *et al.,* 1989).

According to Jiskani *et al.* (1999) barley straw, wheat straw, rye, oat, and rye grass can be used for cultivation of mushroom (*Pleurotus* spp*).* Other materials include paddy straw, banana leaves, sugarcane bagasse, millet heads, cotton wastes, saw dust and waste paper. Wheat straw substrates contain 36% cellulose, 25% pentoson and 16% lignin. Cellulose and pentason are carbohydrates which upon breakdown by oyster enzymes yield simple sugars. These sugars supply the energy for microbial growth. Lignin a

highly resistant material is changed during composting to a "nitrogen –rich-lignin complex" a source of protein.

2.6.1. Purpose of composting

The purpose of composting is to create a physically and chemically homogenous substrate where mushroom mycelium thrives better than competitor microorganisms (Schisler, 1980). Composting helps in concentrating nutrients for use by oyster, exhaust nutrients which favor oyster competitors and also help remove the heat generating capacities of the substrate (Stamets and Chilton, 1983b)

2.6.1.1 Importance of ammonia in oyster mushroom compost

Production of ammonia during compost preparation is essential for microbes and just as carbohydrates it must be in form that microbes can utilize. Ammonia supplies nitrogen for microbial use and is produced by microbes acting upon the protein contained in supplements (Rasmusen, 1981). Ammonia content of 0.3% reduces the yield of mushroom (Curvetto *et al.,* 2002.) Energy supplied by readily available carbohydrates, the microbes has been known to use ammonia in forming their body tissues. A microbial succession of generations is established, with each new generation decomposing the remains of the previous one. Microbial action fixes a certain amount of ammonia, forming the "nitrogen-rich-lignin-humus complex" (Cotnair, 1978).

2.6.1.2 Importance of Carbon: Nitrogen ratio in compost substrate.

The importance of C: N balance cannot be underestimated. Well balanced compost

holds an optimum nutritional level of microbial growth. Because organic matter is reduced during composting, the C: N ratio gradually decreases (30:1 at makeup, 20:1 at filling and 17:1 at spawning). Increase of nitrogen should be up to 3% level of the finished compost at the time of spawning (Schisler and Sinden, 1962) while over supplementation with nitrogen results in residual carbon compounds (Schisler and Sinden, 1962). Readily available carbohydrates which are not consumed by the microbes during composting can become food for the competitors. It is therefore important that these compounds are no longer present when composting is finished.

2.6.1.3 Importance of water and air in compost substrate

Water governs the level of microbial activity in the substrate (Wuest and Schisler 1979). In turn, this activity determines the amount of heat generated within the compost pile because the microbes can only take up nutrients in solution. Microbes in the substrate also require oxygen for their activities. An inverse relationship exists between the amount water and the amount of oxygen in compost. Over wetting of compost causes the air spaces to be filled with water, hence limiting oxygen penetration resulting in an aerobic condition. Insufficient water results in compost that is airy while high temperatures are never reached because the heat generated is quickly converted away.

2.6.1.4 Importance of pre-wetting of compost substrate

The first step in composting process is the initial watering of the starting materials. The purpose of pre-composting or pre-wetting is to activate the microbes. Once activated, the microbes begin to attack the straw and decompose the waxy film which encases the straw

fibres. Until the film is degraded, water will not penetrate the straw and its nutrient will remain unavailable. Pre-wetting may be done by dipping or dunking the materials in a tank of water, spraying using a horse pipe or spreading it out in a flat pile 2-3 ft high and running a sprinkler over it.

2.6.1.5 Importance of appropriate temperature at composting of substrates

Given the proper balance of raw materials, air and water, a continuous microbial population has been known to produce temperatures up to 82.2^{o} C. The mesophiles (microbes) are active under 32.2^{o} C and thermophiles are active from 32.2-71.1^{o} C (Stametes and Chilton, 1983b). Bacteria and fungi that utilize available carbohydrates, attack the nitrogenous compounds, thereby releasing ammonia. The ammonia is then utilized by successive microbial populations and the temperatures rise From 73.8-82.2^{o}C decomposition is mainly due to the chemical reactions of humification and caramelization, the later taking place under conditions of high temperatures, high pH 8.5 and in the presence of ammonia and oxygen. Several scientific studies have revealed that compost taken from a pile having temperature of 46.1-60^{o}C (fire fang area) produces the highest yield of mushroom.

2.6.2 Pasteurization of substrates: The air and compost temperature are held at 57.2 - 60^{o} C for 2- 6 hours. The purpose of pasteurization is to kill or neutralize all harmful organisms in the compost, compost container, and the room. These are mainly nematodes, eggs and larva of flies, mites, harmful fungi and their spores (Hussey, 1972). The length of time needed depends on the "depth of fill". In general two hours at 60^{o}C has been reported to be sufficient. Compost temperature above 60^{o} C must be avoided

because they inactivate fungi and actinomycetes while at the same time stimulating the ammonifying bacteria. If temperatures do go above 60^{o} C, be sure there is a generous supply of fresh air.

Experimental data from tunnels during phase II composting revealed that pasteurization temperature between 55-63^{o}C, conditioning temperature between 40-48^{o}C, air flow between 120-200 $m^{3}h^{-1}tonne^{-1}$, oxygen concentration between 15 and 20% (v/v) and moisture content of 75% produced the highest yield of mushroom (Gerrits, 1988).

Hogan *et al.* (1989) reported that when composting is done in a tunnel, 70% of the total heat produced is discharged as latent heat, 12% is used for heating the compost and process air, 14% is lost through the walls by conduction and another 4% is lost through condensation in the air ducts and return channels. Importance of composting in tunnels will increase since both phase I and phase II have to be carried out in tunnels in the near future, to avoid emission of stench and ammonia.

2.6.3. Conditioning of substrates: Many mushroom researchers have found that high mushroom yields are obtained from substrates when compost temperature is held at 47.7-54.4^{o}C during conditioning (Wuest, 1978). Once pasteurization is completed, the compost temperature should be lowered gradually (conditioning) over 24 hours. At depths up to 8 inches (depth of fill), 50^{o}C is more frequently used. By adjusting the amount of fresh air, the compost is held at 47.7-50^{o} C until all ammonia is gone (Tunney, 1971) Once the ammonia is below 10 parts per million, full fresh air is given to reduce compost temperature to 26.6^{o}C, then cooling down should proceed as rapidly as possible.

2.7.0 Substrate sterilization methods

2.7.1 Hydrated bath method: Hydrated lime (calcium hydroxide) is extremely alkaline and water soluble. By immersing straw into baths high in hydrate lime, competitor fungi and bacteria are largely rendered inactive from drastic change in pH. In hydrated bath method 0.9-1.8 Kg of lime is added for every 189 litres of water. The pH of water increases to 9.5 or higher. Once dissolved, chopped straw is immersed into this alkaline bath, pH sensitive micro-organisms soon die.

2.7.2 Bleach bath method: Five percent sodium hypochlorite is used as disinfectant; 5-6 cups of household bleach to 189 litres of water. Straw is kept submerged for a minimum of 4 hours and no more than 12 hours. The bleach leachate is drained off and straw immediately inoculated (source)

2.7.3 The detergent bath method: The method simply utilizes biodegradable detergents containing fatty oils to treat bulk substrates. Surfactants kill majority of the contaminants competitive to mushroom mycelium.

2.8.0 Spawning methods

The basic principle of spawn running is the same regardless of the type of mushroom or substrate. Colonization must proceed as rapidly as possible to prevent other organisms from becoming established (Cooke, 1962)**.** Flegg *et al.* (1966) observed that highest mushroom yields was obtained from super spawning as opposed to shake up spawning and top spawning " vis a vis the Hunkle-Till process" (A small amount of substrate is inoculated and the fully run substrate is then used as inoculums to spawn higher amounts

of a similar substrate) . Shandilya *et al.* (1974) tried spot spawning, surface spawning, through spawning, shake up and double layer spawning and reported that through spawning method gave the highest yield followed by double layer spawning method**.** In double layer spawning**,** the spawn is scattered on tray beds or polythene bags. The spawn is gently pressed into the substrate. **.** In top spawning, the spawn is planted on the surface of the prepared substrate and then a thin layer of substrate is spread on top of the spawn to prevent it from drying.

2.9.0 Moisture content of substrate

Oyster mushroom mycelium does not grow in a substrate that is either too dry or too wet. A dry substrate produces a fine wispy mycelia growth and poor mushroom formation because the water essential for the transport and assimilation of nutrients is lacking while an over wet substrate has been known to inhibit mycelia growth and also results in an overly sticky mycelia. Controlled experiments with *Avarices brunnescens* and *Pleurotus* spp grown on horse manure composts have shown yield depressions when the moisture content deviates more than 2% from optimum. They further reported that deviations of greater than 5% often results in a spawn run that does not support fruit body production. The growth of oyster mushroom requires humidity of about 80-90% and temperature 25-30^{o}C for the vegetative growth called spawn running and lower temperature (18-25^{o}C) for fruit body formation (Viziteu, 2000).

2.9.1 Temperature of the substrate

Bano and Patwardan (1979) reported that *P. flabellatus* require a temperature of 20-28^{o}C

for rapid growth on the substrate while Ayodele and Okhuoya (2007a) found that *P. ostreatus and P. astroumbonata mycelia* spread was favored by 20-40^{o}C. It has been found that there is need to place a thermometer at the centre of the substrate randomly and in the spawning room's atmosphere. If the hottest point in the substrate is 26.2^{o}, and the air is 21.1^{o}C, then the temperature of the total mass must lie within this range.

During spawn running the metabolism of the growing mycelium generates tremendous quantities of heat. Substrate temperature normally reaches a peak on the 7th and 9th days after spawning and can easily reach 32^{o}C. At this temperature the thermophilic micro-organisms become active. Temperatures between 35-43.3^{o}C can kill the mushroom mycelia. Mushrooms currently cultivated at temperatures around or just below 30^{o}C includes, *Pleurotus cystidiosus, P. abalones, P. ostreatus*(var. florida), *Volvariella volvaceae, Agaricus bitorquis, Stropharia rugusa-anulata* and *Auricularia politrichia* (Tschierpe, 1972)

2.9.2 Light requirements for oyster production

Eger *et al.,* (1974) determined that *P. ostreatus* forms the most primordial in response to light intensity of 2000 lux or about 185 foot candles. They reported that light intensities exceeding 2000lux/hour caused precipitous drop in number of primordial forming. At 10,000 lux/hr (>925 foot candles) primordial failed to form hence lowering growth and quality of oyster. They further observed that the total yield was maximized at 300-430 lux at 12 hours per day when crop wastes was used as substrate. For rapid growth, high yields, and quality of oyster crop parameters such as water, carbon dioxide and relative humidity are very essential.

CHAPTER THREE

3.0 Materials and methods

3.1 Study site

The study sites were Kaimosi Agricultural Training Centre (KATC) and Kapsabet Divisional Headquaters in Nandi Central District, Nandi County. The district has an altitude range of between 1300-2500 m above sea level and is underlain by outcrops of basement rock system which are distinct to the north giving way to thick layers of red soils. The soils are generally sandy clay loam. The area receives an annual rainfall range of between 1200-2000 mm per annum. The long rains starts in March and continue up to the end of June while the short rains usually fall from mid- September to end of November. The mean temperature of the study site is between 18^{o}C - 22^{o}C during the rainy season while the highest temperatures averaging 23^{o}C was recorded during the drier months of December and January. The coolest temperatures as low as 12^{o}C is experienced during the cold spell of July and August (G.O.K, 2002).

3.2 Mushroom growing room

The mushroom house was constructed using locally available materials such as grass, poles and timber. Availability of clean water and access to essential facilities for spawn multiplication was important factors for site selection. Mushroom house used in first trial was grass thatched with mud smeared walls. The size of the house was 4 m x 6 m x 2.5 m with six slatted shelves. Doors, windows and other openings were frequently closed to prevent entry of insect pests. Foot paths were disinfected with 2% formalin solution,

chloropicirin, and oxysan to prevent introduction of insect pests and diseases into the growing room. Second trial was done in a room fitted with wooden shelves. Humidity was regulated by periodic misting and hanging of moistened sisal bags along the walls. Light entry was controlled by small openings along the beds. Temperature was regulated by frequent cold water misting and good hygiene was maintained in the growing room. To control notorious pests such as mushroom flies, fly catcher traps were placed along the oyster shelves (Appendix 11, plate 13)

3.3 Determination of water pH, dissolved oxygen, water conductivity and temperature.

In order to determine dissolved oxygen (DO), water conductivity (WC), and water temperature (WT), Vernier Lab Quest was used which utilized oxygen, water conductivity, and Temperature. Dissolved oxygen was between 1.5 - 2.5 mg / l, water conductivity 220-230 us per cm, water temperature, 16.6 ^{0}C -18^{o}C and water pH range of 6.5-7.5. Oyster mycelium has been known to thrive well at a pH range of 4.2-7.5.

3.4 Sources of materials

3.4.1 Sources of substrates

Agricultural wastes mainly maize cobs (*Zea mays*) and bean husks (*Phaseolus vulgaris*) were obtained from farms adjacent to Kaimosi Agricultural Training Centre (KATC) while wheat (*Ttritichum aestivum*) straw was sourced from Uasin Gishu County where large farms are under wheat cultivation. Agro-industrial wastes notably sugarcane bagasse was obtained from Chemelil Sugar Factory. Papyrus (*Cyperus papyrus*) an

aquatic weed was obtained from Chepkoilel River in Wareng County and Nandi Central District (Kimondi-Kigwal swamp) which covers over 100 ha and hence offers a reliable and cheap source of biomass throughout the year.

Cereal grain substrates (sorghum, millet and corn) for spawn production were obtained locally from Nandi central district while wheat grain was sourced locally from Uasin Gishu County.

3.4.2 Sources of spawn

Oyster spawn was obtained from Jomo Kenyatta University of Agriculture (JKUAT**)-** Department of Botany and University of Nairobi - Microbiology laboratory**.**

3.4.3 Spawn multiplication

Spawn preparation method was done according to the method described by Nwanze *et al.,* (2005a). One kilogram each of the cereal grains sorghum, wheat, millet and corn was boiled separately in 1.5 litres of water for 15 minutes. Upon cooling, the water was drained off and 1000 g of each of the boiled grains was mixed with 12 g gypsum ($CaSo_4.2H_2O$) and 3.0 g calcium carbonate before being sterilized. The inoculated cultures were packed in autoclavable polypropylene bags and incubated at 25-28^{0}C.

3.5. Substrate preparation methods

3.5.1 Shredding and pasteurization of substrates

Shredding of agricultural wastes into small pieces of 1-3cm was carried out to ease bagging and water absorption (Appendix 11, plate 2). The chopped substrate wheat straw,

bean husks, maize cob, sugarcane bagasse, and papyrus were immersed in cold water for 24 hours in order to attain an approximate moisture content of 70% (Zandrazil, 1978; Quimio *et al*., 1990). Thereafter, the soaked substrates were sterilized by submerging in hot water in order to kill harmful microorganism leaving friendly thermophiles. The air tight drum (Plate 3) containing substrate was heated for 1-2 hours at temperatures of 71.1^{0}C -76.6^{o}C. To maintain a desirable level of steam, a lid was tightly fastened on top of the drum with only a small hole for escape of excess steam.

Sterilization of substrate bags was to kill any remaining harmful micro-organisms and also sterilize the packaging material containing the substrates. Two kg of each different substrate (wheat straw, sugarcane bagasse, corn cobs, bean husks and papyrus and their combinations with bean husks in a ratio of1:1) were staked in clear heat resistant autoclavable polypropylene transparent bags of size 25.4 cm x 38.1 cm. The mouth of the bags were plugged with cotton wool and covered with paper foil tightened with rubber band. A wooden rack with a height of 20 cm was put at the bottom of the drum. Having filled the water up to the height of the rack (20 cm), the drum was heated from below using firewood as a source of energy. Substrate filled bags were packed inside the drum on top of the rack to prevent direct contact of the bags with water. The packed substrates were sterilized for 90 minutes at 121^{o} C and allowed to cool ready for spawning. Calcium carbonate was added to the substrate in order to achieve a desirable pH range of 4.5 - 7.5 and also improve aeration.

3.5.2 Spawning

After pasteurization of substrates, the substrates were rapidly cooled to a temperature of 30^{o}C by exposing it to natural air flow and then spawned at a rate of 30 g per 2 kg of substrate using "spot spawning method" according to procedure developed by Bano and Srivastava (1974). Polyvinyl pipes measuring 2-4 cm long were inserted into each of the 2 kg substrate bag plugged with cotton wool. The polyvinyl pipes were fastened to the neck of the polythene bags using a rubber band to serve as a neck bottle. The pipes allowed free air exchange to and from the inoculated substrate. The spawned bags were then placed in an incubation room at 22^{0}C and relative humidity 60 % to allow rapid colonization of substrates by oyster mycelia.

3.5.3 Incubation of spawned bags

The inoculated bags (Appendix 11, plate 4) were placed in a dark incubation room with relative humidity of 70- 80% and a temperature range of 22-27^{0} C for 3 weeks to allow rapid mycelia colonization. Periodic misting with spray of water thrice a day was carried out to keep the growing room moist to prevent drying up of colonized substrate. Thickening of mycelia in the substrates (colonization) was an indication of complete mycelia run on the substrate which called for bag opening to allow pinhead formation and development of fruiting bodies.

3.6 Parameters for observation

A metre rule was used to measure oyster mycelial extension (LOM) from the centre of the bags to the periphery on different cereal grains. Rate of mycelia colonization (RMC) on different substrates

(Time taken for completion of mycelial growth on different substrates) was recorded according to procedures developed by Kadiri and Fasidi (1974). Rate of mycelial colonization (Spawn running) on each substrate was estimated on the basis of the ratio between the distance covered by the mycelium and the time needed for growth.

$$RMC = \frac{\text{Length mycelia (cm)}}{\text{Time (days)}}$$

NB. In this study dry weight of substrate used was 2 kg.

Days to pinning (DTP) for oyster to form pinheads was observed for all the substrates after inoculation with oyster spawn. Time for oyster fruiting bodies from different substrates to mature (Days to maturity-DTM) after inoculation was recorded. Height of the stipe (Hos) was measured in centimeters using a meter rule from the base of the stem to the pileus (Plate1).

Vanier caliper was used to determine stem diameter (Dos) in cm of the fruiting bodies of both species grown on different substrates at the time of maturity (Plate 1). Cap diameter (Doc) of oyster mushroom (both white and grey oyster) was measured from one edge of the pileus (cap) to the other at maturity in centimeters. Measurements were taken from

the shortest and the longest edges of the pileus and the average determined because the caps were not uniformly round in shape (Plate 1).

3.6.1 Determination of oyster fresh weight, dry weights and total yield

Freshly harvested oyster mushrooms were weighed using highly sensitive analytical balance (Model SHIMADZU-BL320H, Tokyo, Japan) (Appendix 11, Plate 17). They were then placed in a solar drier and dried for 8 hours before determining dry weight (Dw). Total yield of mushroom was determined by taking the weights in grams of mushrooms obtained after 1st, 2nd and 3rd flush.

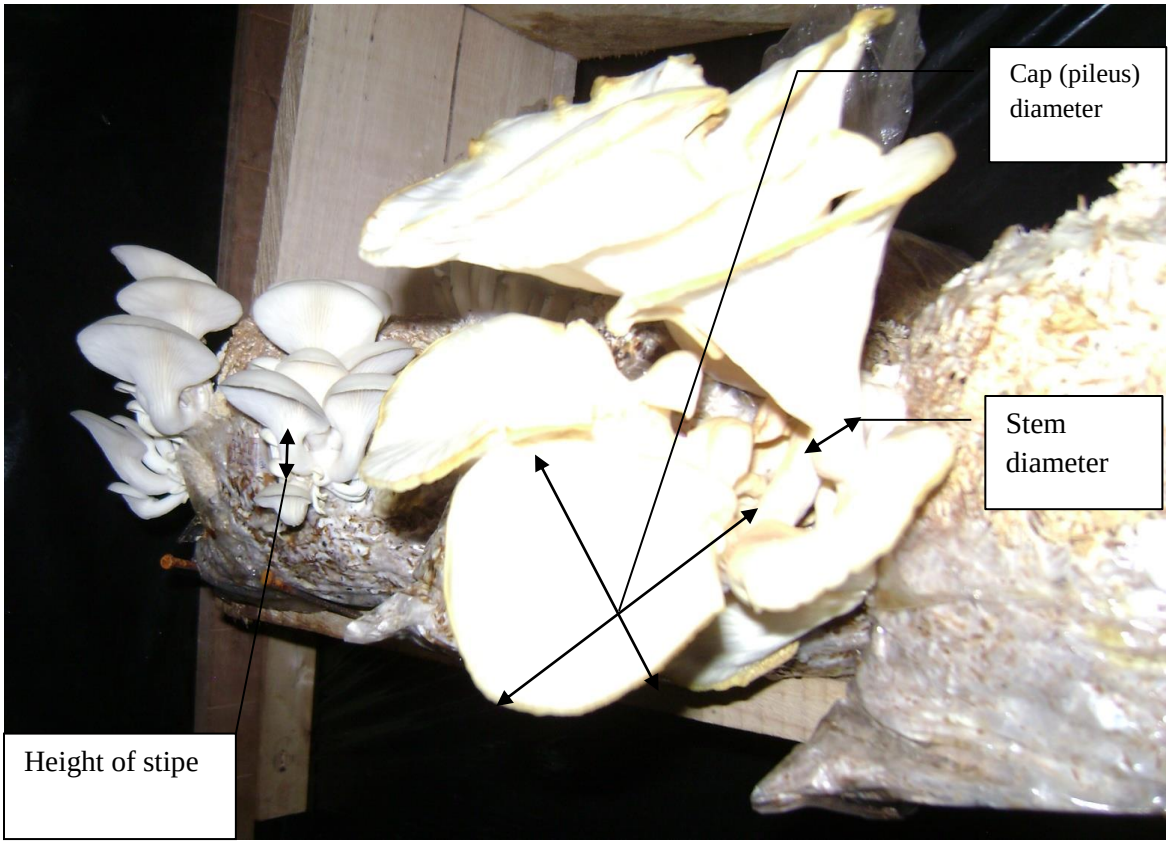

Plate 1: Determination of oyster height, stem and cap diameter. (Source, Author 2010)

3.6.2 Determination of biological efficiency

Biological efficiency (BE %) was determined using the formula:

$$BE\% = \frac{\text{Fresh weight (FW) or dry weight (DW) of mushroom (g)}}{\text{Dry weight of substrate used (g)}} \times 100$$

NB: In this study the dry weight of substrate used was 2 kg.

3.7. Experimental design and data analysis.

The experiment was laid out as Completely Randomized Design (CRD). Two oyster species x 9 substrate combination (Treatments) x 3 replications randomized within the experimental unit in a factorial manner (Appendix 1, Table 2).

Data was subjected to analysis of variance (ANOVA) using Generalized Linear Model (GLM) procedure of Statistical Analysis System (SAS 2000 program package) to examine significant treatment effects. Multiple comparisons among treatment means were done using Tukey's Honest Significant Difference (HSD) at P=0.05.

3.7.1 Treatments.

Two oyster species were evaluated on 9 substrates which were replicated three times. Mushroom strains (Species**)**: Sp_1- White oyster, Sp_2- Grey oyster **(**Appendix 1, Table 2)

Substrates:

The substrates used were; Sb_1- maize cobs (mc), Sb_2 –wheat straw (ws), Sb_3- sugarcane bagasse (sb), Sb_4-bean husks (bh),
Sb_5-papyrus (py), Sb_6- bean husks + maize cobs1:1 (bh, mc), Sb_7- bean husks + wheat straw 1:1 (bh, ws), Sb_8-bean husks + sugarcane bagasse 1:1 (bh, sb), Sb_9- bean husks + papyrus 1:1(bh, py)

3.7.2 Generalized mathematical model

$$X_{jkl} = \mu+\alpha_j+\alpha_k + \varepsilon_{jk}$$

Where jkl = plot observation α_j = species

μ = mean of plot observation Ejk = experimental error effect

α_k = substrate

3.8 Biochemical and nutrient analysis

Nutrient analysis was done at Kenya Industrial Research and Development Institute (KIRDI) to determine proteins, fats, vitamins, ash, fibre, calcium, iron, zinc, potassium, manganese and sodium content. Nutrient analysis was important in determining the quality of oyster.

Proteins

Protein content was analyzed using Khjeldals method (*AOAC*, 1990). Oven dried samples of oyster *(Pleurotus* spp.) weighing 0.5 g was put in 30 mls khjeldals flask and 15 ml conc. H_2SO_4 added. The mixture was cautiously heated in a fume hood until a greenish clear solution appeared. The digest was allowed to cool for 30 minutes and 10 ml distilled water added to prevent caking. The sample was then distilled and 35 ml of distillate collected in a receiver flask. This was titrated with 0.01M HCL until a pink colour emerged. The percentage protein was calculated as percentage (%N) x 625.

Vitamins and microelements content

Total sugars were determined according to the method developed by Dubois *et al. (*1956). Vitamin content was estimated according to the method described by Roe and Keuther (1953), Bayfield and Cole (1980) where mushroom samples weighing 0.5g was mixed slowly with 0.1M sulphuric acid (H_2SO_4) and incubated at room temperatures overnight. The reaction mixture was filtered through Whatman no.1 filter paper and final substrate used for determination of vitamin. The micronutrients of oyster mushroom samples such as potassium, calcium, iron, zinc, manganese were estimated according to procedure of AOAC (1990). Fibre Tech method was used to determine fibre content while Soxhlet method was employed in determining fat content. Muffle furnace method was used in determining ash content.

CHAPTER FOUR

4.0 Results

4.1 Effect of spawned cereal grains on the rate of oyster (*Pleurotus* spp.) mycelial extension

Cultivation of *Pleurotus* spp. spawn on sorghum grains resulted in significantly (p= 0.05) faster mycelial extension throughout the time of the experiment (Table 1). Sorghum grain was followed by wheat grains and corn in the order of effectiveness in mycelial extension (Table 1) Millet grains (*Eleusine corocana*) surprisingly resulted in significantly lower rate of mycelial extension in the entire experimental period (Table1). In all the cereal grains, White oyster had significantly faster rate of mycelia extension than Grey oyster in the entire period of study (Table1).

4.2 Effect of crop waste substrates on the rate of mycelial colonization

Pure maize cobs had the fastest rate of mycelial colonization (spawn running) throughout the study period (Table 2). Maize cobs (100 %) substrate was closely followed by a combination of maize cobs 50% and 50% bean husks (Table 2). A combination of 50% papyrus and 50 % bean husks or 100% papyrus resulted in the slowest rate of spawn running in the entire period of study (Table 2). The rest of substrates and /or combinations had intermediate rate of mycelia colonization (Table 2). There was significant difference (p= 0.05) among the substrates.

White oyster had significantly (p = 0.05) faster rate of spawn running than Grey oyster from day 5 to day 15 (Table 2). However, this difference in the rate of spawn running was not apparent at day 20 (Table 2).

Table 1: Effect of Spawned Cereal Grains Substrates on Mycelial Extension in Oyster Mushroom (*Pleurotus* spp.)

Cereal grains	**Mycelial Extension (cm).**				
Substrate	**Day5**	**Day10**	**day15**	**Day20**	**Day25**
Sorghum grains	4.55a	10.15a	16.03a	17.67a	19.78a
Wheat grains	4.15b	9.75b	12.06b	14.77b	18.80b
Millet grains	2.30d	4.5d	6.17d	6.51d	10.76c
Corn	3.05c	5.10c	7.75c	9.87c	11.02d
Significance	*	*	*	*	*
L.S.D $_{0.05}$	**0.310**	**0.37**	**0.27**	**0.39**	**0.29**
Species:					
White Oyster	3.72a	7.76a	10.55a	12.38a	15.58a
Grey Oyster	3.30b	6.98b	10.45b	12.03b	14.60b
Significance	*	*	*	*	*
L.S.D $_{0.05}$	**0.16**	**0.19**	**0.14**	**0.201**	**0.15**

Means followed by the same letter (s) within the columns are not significantly different according to Tukey's HSD ($P \leq 0.05$) * = Significant.

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Table 2: Effect of Crop Waste Substrates on Mycelia Colonization

Substrate	**Day5**	**Day10**	**Day 15**	**Day20**
Maize cobs	5.40a	12.03a	17.85a	24.70a
Wheat straw	5.06ab	10.15dc	16.92cd	23.07c
Sugar cane bagasse	5.13ab	10.28c	16.95cd	22.17c
Bean husks	4.88b	9.70e	16.83cd	22.37cd
Papyrus	4.85b	9.86de	16.80d	20.91f
Maize cobs 50% + Bean husks (50%)	5.15ab	10.30 c	17.51ba	23.58b
Wheat straw (50%) + Bean husks (50%)	5.03ab	10.88b	17.25bcd	22.82cd
Sugarcane bagasse (50%) + Bean husks (50%)	4.88b	9.95dce	17.30bc	23.63b
Papyrus (50%) + Bean husks (50%)	4.92b	9.21f	16.22e	18.58g
Significance	*	*	*	*
LSD $_{0.05}$	**0.52**	**0.41**	**0.47**	**0.48**
Species:				
White oyster	5.14a	10.57a	17.14a	22.40a
Grey Oyster	4.92b	9.95b	17.00b	22.45a
Significance	*	*	*	NS
LSD $_{0.05}$	**0.11**	**0.12**	**0.14**	**0.14**

Means with the same letters (s) within columns are not significantly different according to Tukey's HSD ($P \leq 0.05$) *****=Significant NS = Not significant

4.3 Effect of substrates and species on days to pinhead formation and maturity of fruiting bodies

4.3.1 Days to pinning

A combination of maize cobs with bean husks in the ratio of 1:1 resulted in the least number of days for oyster (*Pleurotus* spp.) to form pinheads (Table 3). Oyster grown on pure maize cobs substrate came second in terms of days to pinhead formation (25 days). Wheat straw and its combination with bean husks in the ratio of 1:1 were not significantly different (Table 3). The same effect was observed for sugarcane bagasse alone and its combination with bean husks (1:1). A combination of papyrus and bean husks (1:1) or papyrus alone resulted in the longest time (34 and 33 days, respectively) for pinheads to form (Table 3). Substrates were significantly different (Table 3) White oyster took significantly shorter time to form pinhead than Grey oyster (Table 3).

4.3.2 Days to maturity

Wheat straw alone or its combination with bean husks (1:1) resulted in the shortest time for oyster fruiting bodies to reach maturity (Table 3). This was followed by maize cobs alone and a combination of sugarcane bagasse and bean husks in the ratio of 1:1 (Table 3). In contrast, papyrus alone and its mixture with bean husks (1:1) resulted in the longest time for oyster fruiting bodies to reach maturity (Table 3). Sugarcane bagasse alone or a combination of maize cobs and bean husks at the ratio of 1:1 were intermediate in the number of days for fruiting bodies to reach maturity (Table 3).There was significant difference among the substrates (Table 3)

The two oyster species were not significantly different in respect to the number of days for the fruiting bodies to reach maturity (Table 3).

Table 3: Effects of Substrates and Species on Days to Pinhead Formation (Pinning) and Maturity

Substrate	Days to Pinning (DTP)	Days to maturity (DTM)
Maize cobs	25.25f	30.53e
Wheat straw	26.85e	29.98fe
Sugar cane Bagasse	31.26c	33.95b
Bean husks	28.56d	31.88dc
Papyrus	32.65b	37.05a
Maize cobs (50%) + Bean husks (50%)	24.31g	32.66c
Wheat straw (50%) + Bean husks (50%)	26.68e	29.45f
Sugar cane Bagasse (50%) + Bean husks (50%)	31.78c	33.91de
Papyrus 50% + Bean tusks 50%	33.63a	37.38a
Significance	*	*
L.S.D $_{0.05}$	**0.71**	**1.03**
Species:		
White Oyster	28.26b	32.41a
Grey Oyster	29.73a	32.87a
Significance	*	NS
L.S.D $_{0.05}$	**0.21**	**0.29**

Means with the same letters (s) within columns are not significantly different according to Tukey's HSD ($P \leq 0.05$) NS = Not Significant * = Significant.

4.4 Effect of different substrates and species on oyster mushroom mycelia length, height of stipe, stem and cap diameter

4.4.1 Length of mycelia

Maize alone or its combination with bean husks (1:1) significantly produced the longest oyster mycelia (Table 4). Wheat straw alone and its combination with bean husks (1:1) were the second best substrates and they were not significantly different from each other (Table 4). Sugarcane bagasse alone and pure bean husks produced mycelia of intermediate length. Combination of papyrus and bean husks in the ratio of 1:1 or papyrus alone produced the shortest oyster mycelia (Table 4).There was significant difference among the substrates.

There was no significant difference between White oyster and Grey oyster with respect to the length of mycelia (Table 4).

4.4.2 Height of stipe

Maize cobs alone or its combination with bean husks in the ratio of 1:1 significantly (P=0.05) increased stipe height in oyster mushroom (Table 4). Pure wheat straw and sugarcane bagasse were second best in enhancing stipe height. A combination of papyrus and bean husks (1:1) and papyrus alone had the shortest height of stipe and were not significantly (p=0.05) different (Table 4). There was no significant difference between White and Grey oyster regarding the height of the stipe (Table 4).

4.4.3 Stem diameter

Oyster mushroom grown on maize cobs alone or in maize cobs combined with bean husks (1:1) had the largest stem diameter followed by those grown on sugarcane bagasse alone. Oyster grown on wheat straws alone or in combination with bean husks were not significantly different from each other (Table 4). Surprisingly, bean husks alone, papyrus alone or in combination with bean

husks (1:1) and sugarcane bagasse combined with bean husks (1:1) had the smallest stem diameter. Substrates were significantly different (Table 4). There was no significantly ($p=0.05$) difference between White oysters Grey oyster with respect to stem diameter (Table 4).

4.4.4 Cap diameter

All substrates tested, except combination of papyrus with bean husks (1:1) and sugarcane bagasse with bean husks (1:1) resulted in mushrooms with large cap diameter (Table 4). Oyster caps were largest in pure maize cobs. There was no significant difference between White oyster and Grey oyster regarding diameter of mushrooms (Table 4).There was significant difference among substrates (Table 5)

Table 4: Effects of Different Substrates and Species on Oyster (*Pleurotus* spp.) Mycelial Length, Height of stipe, Stem and Cap Diameter

Substrate	Mycelial Length (LOM) (cm)	Height of Stipe (HOS) (cm)	Stem Diameter (DOS) (cm)	Cap Diameter (DOC) (cm)
Maize cobs	24.23**a**	8.75a	2.20a	9.86a
Wheat straw	22.90bc	7.87b	1.92c	9.41ab
Sugarcane Bagasse	21.93d	7.67b	1.95bc	9.45ab
Bean husks	22.23cd	7.03cd	1.5bd	9.51ab
Papyrus	20.90e	6.98cd	1.57d	8.98abc
Maize cobs 50 + Bean husks50%	23.58ab	8.40a	2.11ab	9.48ab
Wheat straw 50% + Bean husks 50%	22.98bc	7.70c	1.90c	9.18abc
Sugarcane bagasse 50 +Bean husks 50%	23.60ab	7.46bc	1.56d	8.50bc
Papyrus 50% + Bean husks 50%	18.71f	6.88d	1.55d	8.15c
Significance	*	*	*	*
L.S.D $_{0.05}$	**0.52**	**0.79**	**0.19**	**1.11**
Species:				
White oyster	22.31a	7.30a	1.81a	9.21a
Grey oyster	22.37a	7.28a	1.81a	9.13a
Significance	**NS**	**NS**	**NS**	**NS**
L.S.D	**0.23**	**0.51**	**0.56**	**0.32**

Means with the same letters (s) within columns are not significantly different according to Tukey's HSD ($P \leq 0.05$). NS = Not Significant * = Significant.

4.5 Effect of substrate and species on fresh, dry weight and biological efficiency

4.5.1 Fresh weight

The highest mushroom fresh weight was recorded when it was grown in pure maize cobs, pure wheat straw, pure sugarcane bagasse and maize cobs combined with bean husks in the ratio of 1:1 (Table 5). In contrast, papyrus alone or in combination with bean husks (1:1), bean husks alone and sugarcane bagasse combined with bean husks in the ratio of 1:1 significantly decreased fresh weight of *Pleurotus* spp (Table 5). Both species of oyster were not significantly different in regard to accumulation of fresh weight (Table 5).

4.5.2 Dry weight

All the substrates tested were not significantly different from one another with respect to dry weight of oyster (*Pleurotus spp*) (Table 5), Likewise White oyster was not different from Grey oyster in their accumulation of dry weight (Table 5).

4.5.3 Biological efficiency

The highest biological efficiency was recorded on pure maize cob (Table 5). In addition pure maize cobs were not different from its combination with bean husks in the ratio of 1:1 (Table 5) Wheat straw alone or sugarcane bagasse alone was the second best substrates regarding biological efficiency. Sugarcane bagasse combined with bean husks (1:1) produced intermediate dry weight

(Table 5). The lowest biological efficiency was found in pure bean husks or papyrus and bean husks combined with either sugarcane bagasse or papyrus (1:1) (Table 5).There was significant difference among substrates (Table 5)

Table 5: Effects of Substrate and Species on Oyster Fresh and Dry Weights and Biological Efficiency

Substrate	Fresh weight (g)	Dry weight (g)	Biological Efficiency
Maize cobs	1925.50a	0.36a	95.00a
Wheat straw	1830.00ab	0.36a	92.17bc
Sugar cane bagasse	1744.17abc	0.18a	85.48bcd
Bean husks	1563.33cde	0.16a	75.68ef
Papyrus	1461.33e	0.37a	73.25f
Maize cobs (50%) +Bean husks (50%)	1765.00ab	0.17a	87.58abc
Wheat straw (50%) +Bean husks (50%)	1659.83bcd	0.16a	83.00cde
Sugar cane bagasse (50%) + Bean husks (50%)	1574.83cde	0.15a	78.10def
Papyrus (50%) + Bean husks (50%)	1529.67de	0.13a	76.00ef
Significance	*	NS	*
L.S.D $_{0.05}$	**182.23**	**0.35**	**8.53**
Species:			
White oyster	1686.74a	0.25a	84.15a
Grey oyster	1658.52a	0.21a	81.68a
Significance	**NS**	**NS**	**NS**
L.S.D $_{0.05}$	**52.84**	**0.10**	**2.48**

Means with the same letters (s) within columns are not significantly different according to Tukey's HSD ($P \leq 0.05$) NS = Not significant ***** = Significant.

4.6 Biochemical and nutrient analysis of oyster mushrooms (*Pleurotus* spp.)

Laboratory analysis of biochemical and nutrient (micro- elements) content of oyster sample (*Pleurotus* spp.) at Kenya Industrial Research and Development Institute (KIRDI) revealed that oyster mushroom had on weight by weight basis (w/w) : protein 17.66%, Fats 2.0%, Free fatty acid 1.39%, Fibre 20.64% Vitamin 'C' (mg /100 g) 0.192 (Table 6).

The micro elements content in the sample (white oyster harvested from maize cob substrate) maize was Ash 10.59%, magnesium 0.16%, iron 0.01%, zinc 0.014%, calcium 0.001%, and potassium 2.4%. Moisture content of dry sample was 11.1% while fresh sample had a moisture content of 90.2 % (Appendix 3).

CHAPTER FIVE

5.0 Discussion

5.1 Effect of spawned cereal grains on the rate of oyster (*Pleurotus* spp.) mycelial extension

Production of clean spawn from mother spawn using cereal grains as substrate during this study is in line with the findings of Elhami and Answari (2008) who found that cereal grains are good substrates for oyster (*Pleurotus* spp.) spawn production as a carrier material due to their ability to colonize these substrates. Spawn quality is counted the most important part in oyster mushroom production (Mohammadi and Purjam, 2003). Spawn production is a very technical process and requires a lot of expertise and specialized knowledge and care on the part of people producing it (Chinda and Chinda, 2007). They further reported that for a faster mycelial growth, clean and uncontaminated spawn is essential.

Sorghum grain resulted in faster rate of mycelial colonization by oyster (*Pleurotus* spp). Similar results were reported by Kumar *et al.* (1975). In contrast, Nwanze *et al.* (2005a) reported that corn unlike sorghum induced the highest rate of mycelial colonization and dry weight of fruiting bodies compared to wheat and millet. Motthagi (2004) reported that large grains of corn contain high amounts of nutrients and hence better substrates for faster mycelial colonization. Similar results were reported by Nwanze *et al*. (2005b) who reported that mycelial extension is also affected by the type of substrates used and environmental factors.

The ability of White oyster to effectively hydrolyze sorghum grains (substrate) than Grey oyster may be attributed to faster mycelial extension observed.

5.2 Effect of crop waste substrates on the rate of mycelia colonization

From this study it was evident that oyster (*Pleurotus* spp.) had the ability to colonize and grow on a variety of crop wastes and spawn running depend on the kind of substrates used. Similar results were reported by Ayodele and Okhuoya, (2007b), Olfati and Peyvast (2008), and Silveira *et al*. (2001). The rate of spawn running was faster in maize cobs which may be attributed to the ability of oyster to secrete a wide range of enzymes which breakdown lignocelluloses in maize cobs. Similar results were obtained by Yolisa (1997) and Bhatti *et al.* (1987) who reported that variation in the rate of oyster mycelial colonization (RMC) on different substrates may be due to variation in chemical composition and C: N ratio of the substrates.

Papyrus (100%) had the least mycelial colonization which may be due to low levels of carbohydrates and the thin film covering the chopped substrates which could not allow easy attachment of oyster spawn and subsequent colonization. It was in this study found that spawn running (mycelial colonization) on various substrates took approximately three weeks which is in agreement with the findings of Tan (1981), who recorded completion of spawn running in approximately three weeks. Patra and Pani (1995) recorded completion of spawn running in 20 – 26 days on wheat straw which is close to that of wheat realized in this study. Ahmad (1986) also observed that oyster completed spawn running (mycelial colonization) in 17- 20 days on different cereal straws. Faster rate of mycelial colonization (spawn running) on maize cobs, wheat straw, and sugarcane bagasse respectively may be the answer to earlier reports by Stametes (2005) who

reported that mycelial running can save the world from food scarcity. This means the faster the spawn running, the better the colonization of oyster mycelium which translates to high oyster yield. Permana *et al.* (2004) reported that the capacity of mushroom to grow on ligno-cellulosic substrates is related to the vigor of its mycelium.

There was no significant difference in spawn running at the end of the study (day 20) between the two oyster species.

5.3 Effect of substrates and species on days to pinhead formation and maturity

5.3.1 Days to pinning

Days to pinning for maize cobs and wheat straw in this study are in agreement with the findings of Rangaswami *et al.* (1975) who reported pinheads of *Pleurotus-sojacaju* and *Pleurotus ostreatus* in 20-25 days after inoculation of maize cobs, wheat straw, and barley straw. Khan *et al.* (1981) got pinheads of *Pleurotus ostreatus* (strain 467) in 31.6 days which is close to that of papyrus. Ramazan (1982) obtained pin-head of five strains of *Pleurotus ostreatus* on wheat, maize cobs, and rice straw between 20-30 days. Vetayasuporn (2007) found that spawn running completed in 18-22 days on different crop wastes (maize cobs, wheat straw, and rice straw) and the time for pinhead formation (after inoculation) was between 6 to 7 days which fully supports the findings of this research. Fan *et al.* (2000) observed that first fructification occurred between 20-26 days of inoculation while Bhatti (1984) found that days to pinning after inoculation of substrate with *Pleurotus* spp was between 24-30 days on various crop waste substrates and the number of flushes ranged from 4-6.

The ability of *Pleurotus* spp to effectively biodegrade the lignin content in maize cobs may be attributed to reduced number of days to pinhead formation as compared to other substrates. Oyster (*Pleurotus* spp.) has also shown that it contains powerful enzymes which can effectively biodegrade lignin content in cereal straws (Yieldiz *et al.,* 2002; Ragunathan and Swaminathan 2003). They also reported that high percentage of lignin in wheat straw may have increased the number of days to pinning and spawn running since mushroom enzymes could not efficiently biodegrade it as compared to maize cobs. This was further enhanced by the fact that lignin is a complex aromatic polymer of phenylpropanoid units interconnected by a wide variety of non- hydrolysable carbon and ether bond which oyster enzymes took a longer time to biodegrade.

5.3.2 Days to maturity

Oyster (*Pleurotus* spp.) fruiting bodies matured in 4-6 days after pinning. These results are in agreement with observation made by Quimio (1987) who reported that fruiting bodies took less than 6 days to mature after pinning (Pinhead formation). Similar results were reported by Bhughio (2001) who reported maturity of oyster (*Pleurotus* spp.) in 5 -6 days after pinhead formation. Substrates which showed early pinhead formation also showed early maturity. High amounts of nutrients in the substrates and the ability of oyster mushroom to efficiently biodegrade these substrates may be attributed to early pinning and maturity respectively.

5.4 Effect of different crop waste substrates on mycelial length, height of stipe, stem and cap diameter

5.4.1 Mycelial length.

Maize cobs (100%) and its combination proved superior in all the treatments. Mycelial length of 24.23 cm was compared to that of Atikpo *et al.* (2008) who cultivated mushroom on fish wastes and obtained a mycelia length of 23.96 cm in 3 weeks. Papyrus recorded the least mycelia length which may be attributed to low nutrient content and poor spawn attachment to this substrate.

5.4.2 Height of stipe

Maize cobs also proved superior in terms of oyster height. Nutrient content of maize cobs may be the probable reason for fast growth in oyster stipe height, stem and cap diameter. Heltay and Zavoli (1960) reported that 40% corn cobs containing 40% cellulose, 15% lignin, 0.4% total N, 0.1% $P_2O_{5,}$ 0.25% $K_2O_{2,}$ 0.5% $Si0_2$, pH 7, and C/N 129 gave a satisfactory yield of oyster as a result of an increase in height, stem and cap diameter.

5.4.3 Stem diameter

Corn cobs (100%) and its combination with bean husks showed a considerable increase in stem diameter. Pure papyrus and its combination with bean husks and sugarcane bagasse combined with bean husks recorded short stem diameter. Veena *et al.* (1998) reported that substrates with a higher C: N ratio resulted in increased stem diameter and pileus thickness.

5.4.4 Cap diameter.

The number of fruiting bodies per cluster from maize cobs was comparatively low compared to other substrates and therefore development of larger caps. This agreed with the earlier findings of Kivaisi *et al*. (2003) who reported that the fewer the caps per cluster the greater the cap diameter. They also reported that cap diameter may be affected by aeration, light and nutrient content of substrates. Enhanced growth in diameter of oyster caps grown on wheat straw and maize cobs may be attributed to a higher C: N ratio compared to papyrus which has a lower C: N ratio. Veena *et al*. (1998) found that substrates with high C: N ratio enhanced growth in oyster cap (pileus) diameter, stem diameter and stipe height.

5.5 Effect of substrates on fresh weight, dry weight and biological efficiency

5.5.1 Fresh weight

Low yield from weed plants such as papyrus substrate in this study is in line with the findings of Das and Mukherjee (2007) that associated the low yield to low levels of nutrients and therefore supplemented the substrates with rice straw. Sivaprakasam and Kandasamy (1981) reported high yield from corn cobs may be attributed to high amounts of cellulosic materials. They further reported that corn produced better yield (fresh weight) compared to wheat straw and sugarcane bagasse Variation in fresh weight may be due to the differences in nutrient content of substrates as reported by Buswel and Chang (1993) who reported that oyster growth, fruiting and yield on a particular substrate depend largely upon the ability of mushroom to utilize the major components of the

substrate as a nutritional source. Similar findings were reported by Chang and Miles (2004) who showed that a substrate is a source of lignocellulosic material which supports growth, development and fruiting of oyster mushroom.

High yields from maize cobs, wheat straw and sugarcane bagasse may be attributed to nutrient content and the ability of mushroom to efficiently produce hydrolyzing and oxidizing enzymes which readily hydrolyze these substrates (Wuyep *et al.,* 2003). Ramasamy *et al.* (1985) reported that cellulosic materials are degraded very easily by growing oyster mushroom resulting in high quality crop yield while non-cellulolosic substrates are not easily degraded by oyster mushroom (*Pleurotus* spp.).

Maria *et al.* (2008) reported that corn cobs have a nitrogen content of 0.5% which enhance high crop yield. This is also supported by the findings of Onuoha (2007) who found that mushroom grown on corn cobs resulted in an increase in fresh weight than that grown on sugarcane bagasse. They reported that corn has 90% dry matter, 2.3% protein, 32.5% fiber, 545 free extracts and 16% total minerals and 0.37% sugar while bagasse has 95.5% dry matter, 1.1% protein, 40.% fiber, very low levels of minerals and only a trace of nitrogen. They further reported that high yield of mushroom is attributed to increasing levels of nitrogen. This may be the probable reason for better yields in corn than sugarcane bagasse.

Bassous *et al.* (1989) observed that corn stovers containing 57% polysaccharides + 30% lignin produce a substantial increase in oyster growth and subsequent yield. Low yield from weed plants such as papyrus substrate in this study is in line with the findings of

Das and Mukherjee (2007) that associated the low yield to low levels of nutrients and therefore supplemented the substrates with rice straw.

5.5.2 Dry weight

Dry weight of both White and Grey oyster was not significantly different. This may be due to the fact that on harvesting, mushrooms loose quite a substantial amount of water regardless of species (Stametes, 1993). Variation in environmental conditions such as temperature and humidity may have had an effect on oyster dry weight. High temperatures resulted in very dry mushroom with reduction in weight while high humidity extended drying period. Both species did not differ significantly in terms of fresh weight, dry weights and biological efficiency.

5.5.3 Biological efficiency

Biological efficiency was worked out against 2 kg of the dry weight of each substrate used (Table 5). Maize cobs showed the highest biological efficiency (BE %) for both *Pleurotus* spp. Maize cobs had a BE of 95%, wheat straw 92%, sugarcane bagasse 85.48%, bean husks 75.68%, and papyrus 73.25%. High biological efficiency from maize cobs may be attributed to greater amounts of nutrients especially cellulose, hemicelluloses and nitrogen levels. The lowest biological and economic yields were recorded from papyrus. Nutrient content of different substrates and environmental factors such as light, humidity, and temperature may have contributed to the difference in observations made by Hami (1990) who reported that crop wastes have a biological efficiency of 100%. Mushroom enzymes do not convert all the nutrients into dry matter,

hence the probable reason for biological efficiency being less than 100% in all the tested substrates during the entire study period.

Ruan *et al.* (2006) reported that different types of straw can be used in cultivation of oyster (*Pleurotus* spp.) after being composted, pasteurized, and additives added to increase BE %. Shah *et al.* (2004) showed that biological efficiency of substrate is linked to oyster yield which is in agreement with the findings of this study where an increase in biological efficiency resulted in increased oyster yield (Fresh weight).

5.6.0 Biochemical and nutrient analysis of oyster (*Pleurotus* spp.) mushroom

Oyster had 17.66% proteins, 0.01% iron, 0.014% zinc, 0.001% calcium, 0.06% sodium, and 0.192 mg/100 grams vitamin C. This confirms earlier reports by Selvi *et al.* (2007) who reported that oyster are rich in proteins, minerals, and vitamins (B2, C, and D, E). Protein content of 17.66% was compared to early reports by Shukla *et al.* (2005) who reported that oyster contains between 18 -42% protein. Ogundans and Florida (1982) reported that oyster mushroom contains 1620mg P, 2580mg K, 40 mg Ca, 10.0mg Fe and 2.3 mg Zn. Low amounts of zinc iron and calcium may be attributed to nutrient content of the substrate (maize cobs). Souci *et al. (*1989) reported that 30% of the world population of 6 billion is protein deficient yet only 200 g of oyster can replace 100 g of meat as a source of proteins. Moisture content of 90.25% was compared with early reports by Pandey and Ghosh (1996) who reported a moisture content of 90.8%. They further reported that oyster contains 2.2% fats, and 9.8% ash which are closer to the findings of this study (2.04 fats, 8.85 ashes respectively).

CHAPTER SIX

6.0 Conclusions and recommendation

6.1 Conclusions

Sorghum (*Sorghum bicolar*) grains was found to be the best cereal grain substrate for spawn multiplication as a carrier material since by the 25th day oyster mycelia had colonized most of the grains. This was followed by wheat grains (*Tritichum aestivum*) which also showed a faster rate of mycelial running (colonization). Millet (*Eleusine corocana*) and corn grain (*Zea mays*) recorded the least mycelial extension (colonization). This study concludes that sorghum grain is the most suitable substrate for oyster (*Pleurotus* spp.) spawn production. White oyster and Grey oyster were significantly different on the rate of mycelial colonization on the cereal grains since white oyster was found to induce a faster rate of mycelial colonization when inoculated on sorghum compared with wheat, millet and corn grains.

Maize cobs alone and its combination with bean husks (1:1) supported a faster rate of spawn running (mycelial colonization) compared to other substrates while pure papyrus (*Cyperus papyrus*) an aquatic plant and its combination with bean husks recorded the least mycelial running. Maize cob takes the shortest time to pin followed by wheat straw, bean husks, and sugarcane bagasse respectively. Papyrus recorded the longest period (days) for pinheads to emerge. White oyster took the shortest time to form pinheads compared to grey.

Performance of oyster (*Pleurotus)* on various substrates during the study period proved that maize cob (*Zea mays*) is the best farm waste substrate for oyster cultivation. This was followed by wheat straw (*Tritichum aestivum),* and sugarcane bagasse *(Saccharum officinarum*) respectively. These substrates performed better in terms of mycelial length (Lom), height of stipe (Hos), diameter of stem (Dos), cap diameter (Doc), yield (fresh weight) and biological efficiency (95%, 92.1%, and 85.4 respectively).

Maize cobs (substrate) other than having the highest yield and biological efficiency (BE %) had the fastest spawn running (mycelial colonization), early pinhead formation, early maturity and high yield compared to the other substrates. Substrates with the fastest rate of mycelial colonization (spawn running), also recorded increased yield and biological efficiency (BE %). This study confirmed that the higher the biological efficiency of a substrate the higher the yield.

6.2 Recommendations

1. This study recommends the use of sorghum grain (*Sorghum bicolar*) as substrate for spawn production for it was found to be the most suitable substrate. This will go a long way in reducing the high cost of imported spawn which currently is not within the reach of most rural farmers in Kenya.

2. This study recommends the use of white oyster during spawn multiplication due to its faster rate of colonization on sorghum grains. Farmers in rural areas should also be trained by the Ministry of agriculture on spawn production using cheap and readily

available local sorghum and wheat grains which according to this research finding have proved to be the most suitable grains for oyster mycelial development.

3. In terms of days to pinning (DTP) and the rate of mycelial colonization (RMC) the two species differed significantly. White oyster pinned early and had a faster rate of mycelial extension (mycelial running). It is therefore recommended as the best species for cultivation in Kenya.

4. It is recommended that large scale farmers especially in Rift valley engaged in production of maize (*Zea mays)* and wheat (*Tritichum aestivum*) whose residues are often burned after crop harvest during land preparation should be put in a more economic use such as oyster (*Pleurotus* spp.) cultivation.

5. Farmers especially those in sugarcane (*Saccharum officinarum*) growing zones in Keya should exploit the presence of large amounts of agro- industrial waste (sugarcane bagasse and mollases) from sugar factories such as Chemelil, Muhoroni, Mumias, and Nzoia in oyster (*Pleurotus* spp.) production.

6. Biochemical and micro-element (nutrient) content of oyster at Kenya Institute of Research and Development Institute (KIRDI) showed that it is of high quality since it contains the much needed protein which is inadequate and expensive in most rural diets. This study recommends that oyster cultivation be up-scaled using local farm wastes in order to bridge the current protein deficit gap.

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APPENDICES

APPENDIX 1: EXPERIMENTAL DESIGN AND LAYOUT

Table 1: Randomization of treatments into plots laid out in RCD on effect of cereal grain substrates on the rate of (oyster) *Pleurotus* mycelial extension

Rep	**Treatments**			
I	Sp_1sb_a Sp_1sb_b	Sp_2sb_b Sp_2sb_a	Sp_1sb_c Sp_2sb_c	Sp_1sb_d Sp_2sb_d
II	Sp_2sb_b Sp_1sb_d	Sp_1sb_a Sp_2sb_c	Sp_2sb_d Sp_1sb_c	Sp_1sb_b Sp_2sb_a
III	Sp_1sb_c Sp_1sb_b	Sp_1sb_a Sp_2sb_a	Sp_2sb_c Sp_2sb_d	Sp_1sb_d Sp_2sb_b

Mushroom strains (oyster) Sp_1- White oyster, Sp_2 – Grey oyster.

Grain substrates Sb_a – wheat grains Sb_b – sorghum grains, Sb_c- millet grains, Sb_d corn grains.

Table 2: Randomization of treatments into plots laid out in RCD on the effect of farm waste substrates on growth and yield of oyster mushroom (*Pleurotus* spp)

Rep	**Treatments**								
I	Sp_1sb_3	Sp_2sb_2	Sp_2sb_4	Sp_1sb_7	Sp_1sb_5	Sp_2sb_5	Sp_1sb_8	Sp_2sb_3	Sp_1sb_2
	Sp_2sb_8	Sp_1sb_6	Sp_2sb_1	Sp_1sb_9	Sp_2sb_6	Sp_1sb_4	Sp_2sb_7	Sp_1sb_1	Sp_2sb_9
II	Sp_2sb_8	Sp_2sb_2	Sp_1sb_8	Sp_2sb_6	Sp_2sb_1	Sp_2sb_4	Sp_1sb_1	Sp_2sb_9	Sp_1sb_9
	Sp_1sb_6	Sp_1sb_5	Sp_2sb_5	Sp_1sb_4	Sp_2sb_3	Sp_1sb_2	Sp_2sb_7	Sp_1sb_7	Sp_1sb_3
III	Sp_1sb_4	Sp_1sb_1	Sp_2sb_3	Sp_1sb_6	Sp_2sb_6	Sp_1sb_3	Sp_2sb_1	Sp_1sb_9	Sp_2sb_5
	Sp_2sb_8	Sp_1sb_2	Sp_2sb_9	Sp_2sb_2	Sp_1sb_7	Sp_2sb_7	Sp_1sb_5	Sp_1sb_8	Sp_2sb_4

Mushroom strains (*Pleurotus* spp.) Sp_1- white oyster, Sp_2- grey oyster

Substrates

Sb_1- maize cobs (mc), Sb_2 –wheat straw (ws), Sb_3- sugarcane bagasse (sb), Sb_4-bean husks (bh),

Sb_5-papyrus (py), Sb_6- bean husks + maize cobs1:1 (bh, mc), Sb_7- bean husks + wheat straw 1:1 (bh, ws), Sb_8-bean husks + sugarcane bagasse 1:1 (bh, sb), Sb_9- bean husks + papyrus 1:1(bh, py)

APPENDIX (II) RESEARCH PROGRAM PHOTOGRAPHS

Plate 2: Shredding of cereal straws.

Plate 3: Pasteurization of substrates using oil drum.

Plate 4: Inoculation of pasteurized substrate with oyster (*Pleurotus* spp).

Plate 5: Oyster mycelia colonization/ fruiting on wheat straw.

Plate 6: Grey oyster on maize cobs substrate

Plate 7: White oyster on sugarcane bagasse

Plate 8: White oyster growing on papyrus

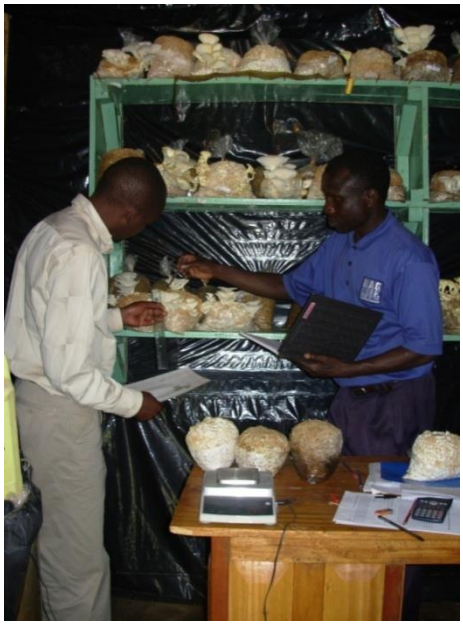


Plate 9: Determination of oyster growth

Plate 10: Oyster fruiting on maize cob/bean husk combination

Plate 11: Mature oyster pileus

Plate 12: Grey and white oyster on different substrates

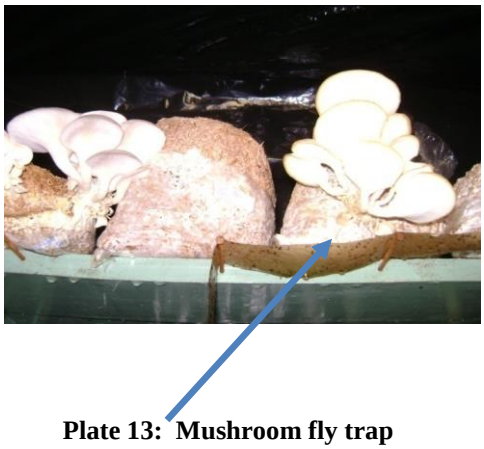


Plate 13: Mushroom fly trap

Plate14: Taking records on growth parameters.

Plate15: well colonized papyrus substrate with Oyster *(Pleurotus* spp.*)*

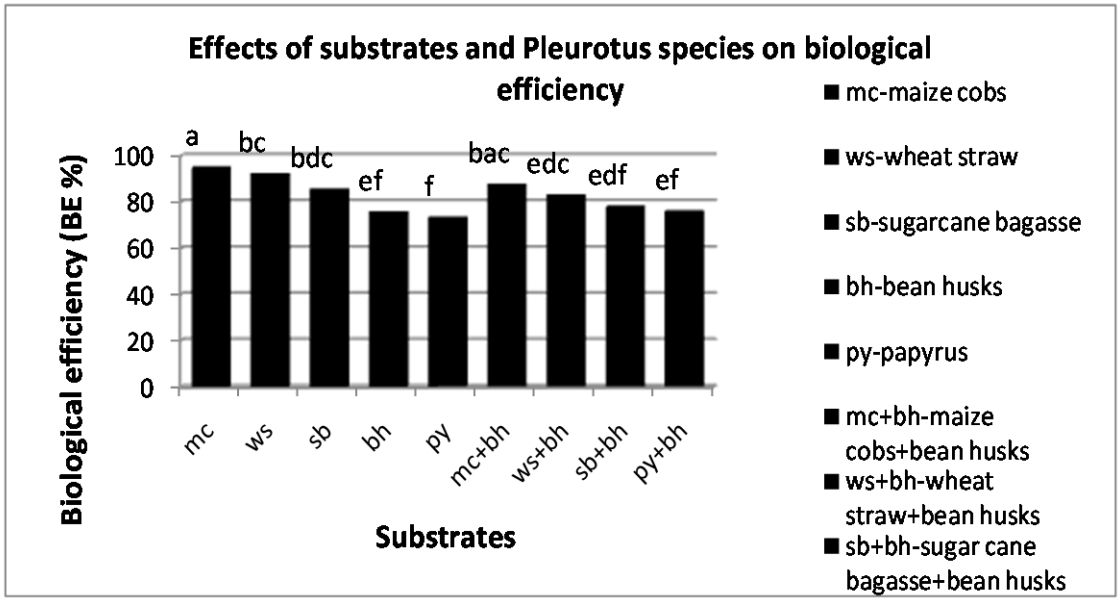
Plate 16: Spawn multiplication using sorghum grains.

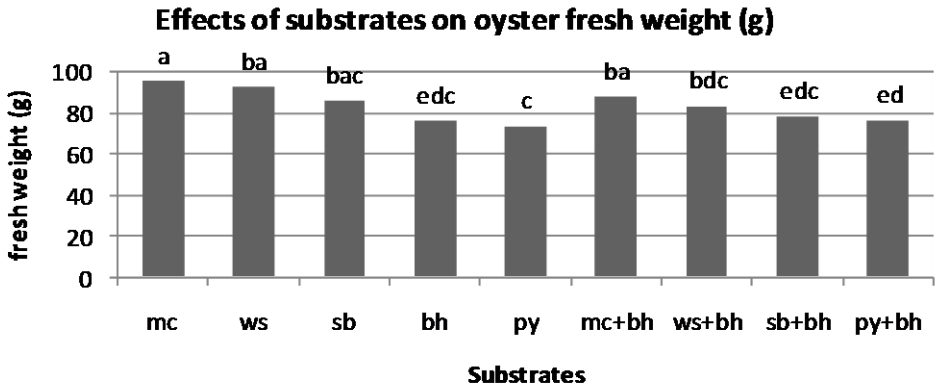
Plate 17: Electronic weighing of oyster.

(Source of research photographs- Author, 2010)

APPENDIX (I11)

GRAPHICAL PRESENTATION ON EFFECTS OF SUBSTRATES ON FRESH WEIGHT AND BIOLOGICAL EFFICIENCY (BE %)





Key

mc- maize cobs 100%

ws- wheat straw 100%

sb- sugarcane bagasse 100%

bh- bean husks 100%

py- papyrus 100%

mc+bh- maize cobs 50% +bean husks 50%

ws+bh- wheat straw 50% + bean husks 50%

sb+bh- sugarcane bagasse 50% + bean husks 50%

py+bh- papyrus 50% + bean husks 50%

APPENDIX (1V)

Biochemical and nutrient analysis of oyster (*Pleurotus* spp.) mushrooms

Parameter	Content (%)	Method
Proteins	17.66	Khjeldal method
Fats	2.04	Soxhlet method
Free fatty acids (Ascorbic acid)	1.39	Soxhlet method
Fiber	20.64	Fiber Tech. method
Vitamin "C "(mg/100g)	0.192	Titration method
Ash content	10.59	Muffle furnace methods
Magnesium	0.16	Atomic Absorption Spectrophotometer method
Iron	0.01	Atomic Absorption Spectrophotometer Method (AAS).
Zinc	0.014	Atomic Absorption Spectrophotometer method.
Calcium	0.001	Atomic Absorption Spectrophotometers method
Potassium	2.4	Atomic Absorption Spectrophotometer method
Sodium	0.06	Atomic Absorption Spectrophotometers method.
Dry weight moisture	11.10	Oven dry methods.
Fresh weight moisture	90.2	Weighing on digital scale

APPENDIX (V)

COMMON MUSHROOM CONTAMINANTS

(A)	(B)	(C)	(D)	(E)
Alternaria	*Cladosporium*	*Monillia*	*Papulospora*	*Sepedonium*
Aspergillus	*Corpinus*	*Mucor*	*Penicillium*	*Trichodema*
Bacillus	*Dactylum*	*Mycelia Sterilia*	*Pseudomonas*	*Trichothecium*
Botrytis	*Epicoccum*	*Mycogone*	*Rhizopus*	*Verticillium*
Chaetomium	*Fusarium*	*Neurospora*	*Scopoulariopsis*	*Yeast*
Chysosporium	*Gestrichum*			

Key to the Common Contaminants

This key is easy to use. Simply one has to follow the key that best describes the contaminant at hand.

- Contaminant parasitizing the mushroom fruit body (a pathogen)
- Contaminant not parasitizing the mushroom (an indicator)
- Droplets forming across the cap and stem but lacking sunken lesion, mushroom eventually reduced to a whitish foaming like mass…. Casual organism not known
- Cap not as above but first having brownish sports that enlarge deepen and in which brown slime forms. Mushroom eventually disintegrate into a dark slimy oozing mass

  ……. *Pseudomonas tosalisii*

  "Bacteria pit"

  "Bacteria Bloch"

- Contaminant eventually sporulating as a green mould on the mushroom .usually preceded by outbreak of green mould on the casing layer....*Trichoderma viride*

  Trichoderma Kiniigi

  Trichoderma blotch

- Contaminant in the form of another mushroom whose deliquesces (melts) into a blackish liquid with age. ……*Coprinus spp*

  "Inky cap"

- Contaminant turning young mushroom into a rotting amorphous ball-like mass from which an amber fluid oozes upon cutting stem typically not splitting or peeling (spores one two celled, the latter being darkly pigmented and a corn shaped. ……..……..…… …….*Mycogone peniciosa*

  "Wet bubble"

- Mycelium fast growing, aerial and never having a frosty texture, pinkish with spores maturity (spores unicellular with nerve – like ridges longitudinally arranged and ellipsoid…….…… *Neurospora spp*

  "Pink mold"

- More frequently seen in grain culture (spores produced on a short conidophore sickle cell shaped and multi-celled…… ……..……..***Fasarium***

  "Yellow rain mould

- Non – multi (not moving spontaneously) spores relatively large 4-2- microns in diameter not affected by bacterial antibiotics such as gentamycin ….. "*Yeast*"

(*Cytocoaus and Rhodotorula*)

- Cells variable in shape cram negative (not retaining a violet dye when fixed with crystal violet and an iodine solution ……..*Pseudonomas*

"Bacteria bloch"

- Spores elongated and ornamented with ridges generally exceeding 20 microns in length and 3 microns in diameter …….....*Alternaria*

"Black mould"

"Black pin mould"

- Conidiophores appearing swelled at apex, particularly covered by a sprouting membrane……. ……*Rhizopus*

"Black bread mould"

"Black pin mould"

- Mould developing small bread-like masses of cells easily visible with a magnifying lens. Never producing cup-like fruit bodies (dark pigmented cells clustered on a mycelial mat spores lacking) ……..*Papulospora byssina*

"Brownplaster mold"

- Mold forming a corky layer between a casing layer and the compost and mat like (spores borne on short vase shaped pegs…….....*Chrysosporium luteum*

"Yellow mat disease"

- Not occurring on compost (conidiophores short arising from cushions shaped cells) Spores appear to be compost of several tightly compacted cells. *Epicoccum* "Yellow mould"

- Appearing as a dense plaster-like stroma-like mycelium. Conidiophores brush shaped peniciliate ……..……..……. …….*Scopulariopsis* "White plaster mould"

- Spores forming from hyphae in chains ……..…*Monilla* *(*White flour mould)

ANNOVA

The SAS System

The GLM Procedure

Class Level Information

Class	Levels	Values
Species	2	SP1 SP2
Substrate	4	SB1 SB2 SB3 SB4
Number of observations	24	

The SAS System

The GLM Procedure

Dependent Variable: Diameter of Mycelial Extension day 5

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Source	DF	Type I SS	Mean Square	F Value	Pr > F
Species	1	1.08375000	1.08375000	30.60	<.0001
Substrate	3	19.00125000	6.33375000	178.84	<.0001
Species*Substrate	3	1.11458333	0.37152778	10.49	0.0005
Error	16	0.56666667	0.03541667		
Corrected Total	23	21.76625000			

R-Square	Coeff Var	Root MSE	DOMEday5 Mean
0.973966	5.357812	0.188193	3.512500

Source	DF	Type III SS	Mean Square	F Value	Pr >F
Species	1	1.08375000	1.08375000	30.60	<.0001
Substrate	3	19.00125000	6.33375000	178.84	<.0001
Species*Substrate	3	1.11458333	0.37152778	10.49	0.0005

The SAS System

The GLM Procedure

Dependent Variable: Diameter of Mycelial Extension day 10

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Species	1	3.6816667	3.6816667	73.63	<.0001
Substrate	3	160.6950000	53.5650000	1071.30	<.0001
Species*Substrate	3	2.8483333	0.9494444	18.99	<.0001
Error	16	0.8000000	0.0500000		
Corrected Total	23	168.0250000			

R-Square	Coeff Var	Root MSE	DOMEday10 Mean
0.995239	3.031957	0.223607	7.375000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Species	1	3.6816667	3.6816667	73.63	<.0001
Substrate	3	160.6950000	53.5650000	1071.30	<.0001
Species*Substrate	3	2.8483333	0.9494444	18.99	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Species	1	3.6816667	3.6816667	73.63	<.0001
Substrate	3	160.6950000	53.5650000	1071.30	<.0001
Species*Substrate	3	2.8483333	0.9494444	18.99	<.0001

The SAS System

The GLM Procedure

Dependent Variable: Diameter of Mycelial Extension day 15

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Source	DF	Type I SS	Mean Square	F Value	Pr > F
Species	1	0.0704167	0.0704167	2.60	0.1264
Substrate	3	356.4745833	118.8248611	4387.38	<.0001
Species*Substrate	3	0.4512500	0.1504167	5.55	0.0083
Error	16	0.4333333	0.0270833		
Corrected Total	23	357.4295833			

R-Square	Coeff Var	Root MSE	DOMEday15 Mean
0.998788	1.566713	0.164570	10.50417

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Species	1	0.0704167	0.0704167	2.60	0.1264
Substrate	3	356.4745833	118.8248611	4387.38	<.0001
Species*Substrate	3	0.4512500	0.1504167	5.55	0.0083

The SAS System

The GLM Procedure

Dependent Variable Diameter of Mycelial Extension day 20

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Source	DF	Type I SS	Mean Square	F Value	Pr > F
Species	1	0.7704167	0.7704167	13.50	0.0021
Substrate	3	445.3012500	148.4337500	2600.30	<.0001
Species*Substrate	3	0.3845833	0.1281944	2.25	0.1224
Error	16	0.9133333	0.0570833		
Corrected Total	23	447.3695833			

R-Square	Coeff Var	Root MSE	DOMEday20 Mean
0.997958	1.957702	0.238921	12.20417

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Species	1	0.7704167	0.7704167	13.50	0.0021
Substrate	3	445.3012500	148.4337500	2600.30	<.0001
Species*Substrate	3	0.3845833	0.1281944	2.25	0.1224

The SAS System

The GLM Procedure

Dependent Variable: Diameter of Mycelial Extension day 25

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Source	DF	Type I SS	Mean Square	F Value	Pr > F
Species	1	5.8016667	5.8016667	193.39	<.0001
Substrate	3	426.4483333	142.1494444	4738.31	<.0001
Species*Substrate	3	2.7483333	0.9161111	30.54	<.0001
Error	16	0.4800000	0.0300000		
Corrected Total	23	435.4783333			

R-Square	Coeff Var	Root MSE	DOMEday25 Mean
0.998898	1.147687	0.173205	15.09167

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Species	1	5.8016667	5.8016667	193.39	<.0001
Substrate	3	426.4483333	142.1494444	4738.31	<.0001
Species*Substrate	3	2.7483333	0.9161111	30.54	<.0001

ANOVA RESULTS

The GLM Procedure

Class Level Information

Class	Levels	Values
Species	2	SP1 SP2
Substrate	9	SB1 SB2 SB3 SB4 SB5 SB6 SB7 SB8 SB9

Number of observations 54

ANOVA RESULTS

The GLM Procedure

Dependent Variable: Length of Mycelia

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Source	DF	Type I SS	Mean Square	F Value	Pr > F
Species	1	0.0668519	0.0668519	0.39	0.5363
Substrate	8	136.9470370	17.1183796	99.83	<.0001
Species*Substrate	8	21.7448148	2.7181019	15.85	<.0001
Error	36	6.1733333	0.1714815		
Corrected Total	53	164.9320370			

R-Square	Coeff Var	Root MSE	LOM Mean
0.962570	1.853425	0.414103	22.34259

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Species	1	0.0668519	0.0668519	0.39	0.5363
Substrate	8	136.9470370	17.1183796	99.83	<.0001
Species*Substrate	8	21.7448148	2.7181019	15.85	<.0001

ANOVA RESULTS

The GLM Procedure

Dependent Variable: Mycelial Colonization DAY 5

Source	DF	Sum of Squares	Mean Square	F Value	Pr >F
Source	DF	Type I SS	Mean Square	F Value	Pr > F
Species	1	0.60166667	0.60166667	14.06	0.0006
Substrate	8	1.50814815	0.18851852	4.41	0.0009
Species*Substrate	8	3.85333333	0.48166667	11.26	<.0001
Error	36	1.54000000	0.04277778		
Corrected Total	53	7.50314815			

R-Square	Coeff Var	Root MSE	MCDAY5 Mean
0.794753	4.107652	0.206828	5.035185

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Species	1	0.60166667	0.60166667	14.06	0.0006
Substrate	8	1.50814815	0.18851852	4.41	0.0009
Species*Substrate	8	3.85333333	0.48166667	11.26	<.0001

ANOVA RESULTS

The GLM Procedure

Dependent Variable: Mycelial Colonization DAY 10

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Source	DF	Type I SS	Mean Square	F Value	Pr > F
Species	1	5.28907407	5.28907407	111.57	<.0001
Substrate	8	31.20148148	3.90018519	82.27	<.0001
Species*Substrate	8	10.16592593	1.27074074	26.80	<.0001
Error	36	1.70666667	0.04740741		
Corrected Total	53	48.36314815			

R-Square	Coeff Var	Root MSE	MCDAY10 Mean
0.964711	2.121153	0.217732	10.26481

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Species	1	5.28907407	5.28907407	111.57	<.0001
Substrate	8	31.20148148	3.90018519	82.27	<.0001
Species*Substrate	8	10.16592593	1.27074074	26.80	<.0001

ANOVA RESULTS

The GLM Procedure

Dependent Variable: Mycelial Colonization DAY 15

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Source	DF	Type I SS	Mean Square	F Value	Pr > F
Species	1	0.26740741	0.26740741	4.43	0.0424
Substrate	8	10.72925926	1.34115741	22.22	<.0001
Species*Substrate	8	4.20259259	0.52532407	8.70	<.0001
Error	36	2.17333333	0.06037037		
Corrected Total	53	17.37259259			

R-Square	Coeff Var	Root MSE	MCDAY15 Mean
0.874899	1.439358	0.245704	17.07037

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Species	1	0.26740741	0.26740741	4.43	0.0424
Substrate	8	10.72925926	1.34115741	22.22	<.0001
Species*Substrate	8	4.20259259	0.52532407	8.70	<.0001

ANOVA RESULTS

The GLM Procedure

Dependent Variable: Mycelial Colonization DAY 20

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Source	DF	Type I SS	Mean Square	F Value	Pr > F
Species	1	0.0362963	0.0362963	0.58	0.4513
Substrate	8	153.8770370	19.2346296	307.30	<.0001
Species*Substrate	8	25.7770370	3.2221296	51.48	<.0001
Error	36	2.2533333	0.0625926		
Corrected Total	53	181.9437037			

R-Square	Coeff Var	Root MSE	MCDAY20 Mean
0.987615	1.115607	0.250185	22.42593

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Species	1	0.0362963	0.0362963	0.58	0.4513
Substrate	8	153.8770370	19.2346296	307.30	<.0001
Species*Substrate	8	25.7770370	3.2221296	51.48	<.0001

ANOVA RESULTS

The GLM Procedure

Dependent Variable: Days to Pinning

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Source	DF	Type I SS	Mean Square	F Value	Pr > F
Species	1	29.3340741	29.3340741	209.53	<.0001
Substrate	8	563.0900000	70.3862500	502.76	<.0001
Species*Substrate	8	17.6359259	2.2044907	15.75	<.0001
Error	36	5.0400000	0.1400000		
Corrected Total	53	615.1000000			

R-Square	Coeff Var	Root MSE	DTP Mean
0.991806	1.290227	0.374166	29.00000

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Species	1	29.3340741	29.3340741	209.53	<.0001
Substrate	8	563.0900000	70.3862500	502.76	<.0001
Species*Substrate	8	17.6359259	2.2044907	15.75	<.0001

ANOVA RESULTS

The GLM Procedure

Dependent Variable: Days to Maturity

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Source	DF	Type I SS	Mean Square	F Value	Pr > F
Species	1	2.8016667	2.8016667	9.65	0.0037
Substrate	8	413.2692593	51.6586574	178.02	<.0001
Species*Substrate	8	47.9166667	5.9895833	20.64	<.0001
Error	36	10.4466667	0.2901852		
Corrected Total	53	474.4342593			

R-Square	Coeff Var	Root MSE	DTM Mean
0.977981	1.650075	0.538688	32.64630

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Species	1	2.8016667	2.8016667	9.65	0.0037
Substrate	8	413.2692593	51.6586574	178.02	<.0001
Species*Substrate	8	47.9166667	5.9895833	20.64	<.0001

ANOVA RESULTS

The GLM Procedure

Dependent Variable: Height of Stipe

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Species	1	0.00166667	0.00166667	0.02	0.8823
Substrate	8	68.58333333	8.57291667	114.31	<.0001
Species*Substrate	8	54.42333333	6.80291667	90.71	<.0001
Error	36	2.7000000	0.0750000		
Corrected Total	53	125.7083333			

R-Square	Coeff Var	Root MSE	HOS Mean
0.978522	3.754382	0.273861	7.294444

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Species	1	0.00166667	0.00166667	0.02	0.8823
Substrate	8	68.58333333	8.57291667	114.31	<.0001
Species*Substrate	8	54.42333333	6.80291667	90.71	<.0001

ANOVA RESULTS

The GLM Procedure

Dependent Variable: Diameter of Cap

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Source	DF	Type I SS	Mean Square	F Value	Pr > F
Species	1	0.06685185	0.06685185	0.20	0.6597
Substrate	8	14.20333333	1.77541667	5.24	0.0002
Species*Substrate	8	1.47148148	0.18393519	0.54	0.8166
Error	36	12.20666667	0.33907407		
Corrected Total	53	27.94833333			

R-Square	Coeff Var	Root MSE	DOC Mean
0.563242	6.348523	0.582301	9.172222

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Species	1	0.06685185	0.06685185	0.20	0.6597
Substrate	8	14.20333333	1.77541667	5.24	0.0002
Species*Substrate	8	1.47148148	0.18393519	0.54	0.8166

ANOVA RESULTS

The GLM Procedure

Dependent Variable: Diameter of Stem

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Source	DF	Type I SS	Mean Square	F Value	Pr > F
Species	1	0.00018519	0.00018519	0.02	0.8963
Substrate	8	3.13333333	0.39166667	36.47	<.0001
Species*Substrate	8	0.09481481	0.01185185	1.10	0.3838
Error	36	0.38666667	0.01074074		
Corrected Total	53	3.61500000			

R-Square	Coeff Var	Root MSE	DOS Mean
0.893038	5.704819	0.103638	1.816667

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Species	1	0.00018519	0.00018519	0.02	0.8963
Substrate	8	3.13333333	0.39166667	36.47	<.0001
Species*Substrate	8	0.09481481	0.01185185	1.10	0.3838

ANOVA RESULTS

The GLM Procedure

Dependent Variable: Fresh Weight

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Source	DF	Type I SS	Mean Square	F Value	Pr > F
Species	1	10752.667	10752.667	1.17	0.2859
Substrate	8	1134700.593	141837.574	15.48	<.0001
Species*Substrate	8	8852.667	1106.583	0.12	0.9980
Error	36	329918.667	9164.407		
Corrected Total	53	1484224.593			

R-Square	Coeff Var	Root MSE	FWT Mean
0.777716	5.723378	95.73091	1672.630

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Species	1	10752.667	10752.667	1.17	0.2859
Substrate	8	1134700.593	141837.574	15.48	<.0001
Species*Substrate	8	8852.667	1106.583	0.12	0.9980

ANOVA RESULTS

The GLM Procedure

Dependent Variable: Dry Weight

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Source	DF	Type I SS	Mean Square	F Value	Pr > F
Species	1	0.02406667	0.02406667	0.71	0.4064
Substrate	8	0.49603333	0.06200417	1.82	0.1056
Species*Substrate	8	0.66236667	0.08279583	2.43	0.0328
Error	36	1.22746667	0.03409630		
Corrected Total	53	2.40993333			

R-Square	Coeff Var	Root MSE	DWT Mean
0.490664	80.67313	0.184652	0.228889

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Species	1	0.02406667	0.02406667	0.71	0.4064
Substrate	8	0.49603333	0.06200417	1.82	0.1056
Species*Substrate	8	0.66236667	0.08279583	2.43	0.0328

ANOVA RESULTS

The GLM Procedure

Dependent Variable: Biological Efficiency

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Source	DF	Type I SS	Mean Square	F Value	Pr > F
Species	1	82.634074	82.634074	4.11	0.0502
Substrate	8	2860.488148	357.561019	17.77	<.0001
Species*Substrate	8	82.959259	10.369907	0.52	0.8368
Error	36	724.340000	20.120556		
Corrected Total	53	3750.421481			

R-Square	Coeff Var	Root MSE	BE Mean
0.806864	5.409641	4.485594	82.91852

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Species	1	82.634074	82.634074	4.11	0.0502
Substrate	8	2860.488148	357.561019	17.77	<.0001
Species*Substrate	8	82.959259	10.369907	0.52	0.8368