COMPARATIVE STUDY OF THE GROWTH AND YIELD OF THREE CULTIVATED *Pleurotus* SPECIES ON SELECTED TROPICAL TREES SAWDUSTS

BY

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ABSTRACT

The quest to boost dietary protein production from readily available and affordable sources is ever increasing in developing countries. The indigenous edible *Pleurotus* species (oyster mushrooms), which grow naturally on wood wastes, are potential protein supplements. However, little information is available on the usage of selected tropical trees for optimum mushroom cultivation. Therefore, this research was designed to investigate the growth and yield of oyster mushrooms on sawdust of selected tropical trees.

Mature stems of *Mangifera indica* L. (PTBG0000039360), *Senna siamea* Lam. (BISH0000032830) and *Azadirachta indica* A. Juss (BISH0000015188) were harvested and identity authenticated at the National Horticultural Research Institute (NIHORT), Ibadan. The samples were air-dried, separately milled into sawdust, composted and used as three substrates. The fourth substrate was the mixed bed derived from the mixtures of the three substrates in ratio 1:1:1 by weight. Three mushroom species (*Pleurotus ostreatus, P. pulmonarius* and *P. tuber-regium*) were collected from Mycology Unit, NIHORT. A total of 108 polyethylene substrate bags (27 for each substrate) were filled with 300 g of the sawdust, each tightly packed, sterilised and inoculated with 30 g each of mushroom spawn. Mycelial growth was determined using standard method. Fruiting body production was obtained for the three mushrooms on all the substrates and sclerotia weight recorded at different Weeks of Composting Intervals (WCI) of 4, 8 and 12. Biological and production efficiencies were determined using mathematical methods. The experiment was a 4 x 3 x 3 factorial arrangement laid out in a complete randomised design with three replicates each. The data were analysed using descriptive statistics and ANOVA at p =0.05.

The longest and the shortest mycelial extensions (13.3 and 4.6 cm) were observed in *P. pulmonarius* grown on *A. indica* at 4WCI and 8WCI respectively. At 12WCI, the highest Fruit Weight (FW) of 86.8±1.2 g for *P. pulmonarius* was observed on *S. siamea* which was not significantly different from *P. ostreatus* (84.9±1.2 g) on *M. indica*. The most significant Biological Efficiencies (BE) of 82.7% and 80.9% for *P. pulmonarius* and *P. ostreatus* at 12WCI respectively were observed on *S. siamea* and *M. indica*. At 12WCI, the Production Efficiency (PE) was highest (42.8%) for *P. ostreatus* on *M. indica* and 41.1% for *P. pulmonarius* on *S. siamea*. Also, the highest mean sclerotia weight of 42.1±0.9 g was obtained for *P. tuber-regium* on *M. indica* at 12WCI. However, at 4WCI, the least FW (13.7±0.1 g) was in *P. pulmonarius* cultivated on *S. siamea* while the least BE (13.0%) was observed for *P. pulmonarius* on *S. siamea*. Also, the least PE value of 4.9% for *P. pulmonarius* was observed on *S. siamea* while the least sclerotia weight of 9.0±0.6 g was obtained on mixed bed. The longer the decomposition period of the substrate, the more significant was the yield.

The best substrate for the production of fruiting body of *Pleurotus ostreatus* and sclerotia of *Pleurotus tuber-regium* was *Mangifera indica* while *Senna siamea* was most suitable for *Pleurotus pulmonarius.*

Keywords: Biological and production efficiencies, Composting intervals, Oyster mushrooms, Tropical trees sawdust

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DEDICATION

This work is dedicated to the Almighty God, the "I AM THAT I AM" (Exodus 3: 14) and the Fountain of all knowledge. Surely, your persistence in God's presence will perpetuate your prosperity.

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CERTIFICATION

I certify that this work was carried out by Mr. C.A. Otunla in the Department of Botany, University of Ibadan.

.....

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CHAPTER ONE

INTRODUCTION

1.1 Mushrooms

The word mushroom is believed to have originated from the french word "mousseron" derived from the word moss (Mau *et al.*, 1993). Mushrooms belong to the Kingdom Mycetae which are non-green, edible fungi and that they are large heterogeneous group with various shapes, sizes and appearance. They are macro fungi with distinctive fruit bodies which are visible to the naked eye (Alexopoulos and Mims, 1979). They are neither plants, due to lack of chlorophyll, nor animals because they store glycogen (Alexopoulos and Mims, 1979). Mushrooms are seasonal and highly perishable crop and contain about 90% moisture. They are non-traditional horticultural crops of high quality proteins, high fibre value, vitamins and minerals (Narayanasamy *et al*., 2009). Mushrooms gathered from farmlands, fields and meadows were valued as food (Quimio *et al*., 1990). They could be cultivated on composted substrates, chemically treated or untreated wood logs, or picked up in the field during the rainy season (Aletor, 1995; Kadiri, 1999; Kadiri, 2002). All edible fungi are saprophytes, and only the reproductive structure comes out of the substrate and forms a fruiting body which is visible, called mushroom. Although, some mushrooms are unpalatable and others even poisonous, the mushrooms of many species are not only edible but also delicious and nutritious (Chang and Miles, 1986; Chang and Miles, 1989).

1.2 Oyster Mushrooms

Oyster mushrooms are scientifically called *Pleurotus* and the latin name *Pleurotus ostreatus* means "sideways oyster", referring to the oyster-like shape of the mushroom (Dike *et al.*, 2011). *Pleurotus* species are found to be one of the most efficient lignocelluloses solid state decomposing types of white rot fungi (Baysal *et al.*, 2003). Thus, many agricultural and industrial wastes can be utilized as substrates for the production of *Pleurotus* species (Baysal *et al.*, 2003). They require shorter growth time when compared to other edible mushrooms, they demand few environmental controls, their fruiting bodies are not very often attacked by diseases and pests and they can be cultivated in a simple and cheap way (Jwanny *et al.*, 1995; Patrabansh and Madan, 1997). Sawdust and sugarcane bagasse were once reported as the best substrates for growing of Oyster Mushroom than other agro-based substrates (Ahmed, 1998).

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1.2.1 *Pleurotus ostreatus*

P. ostreatus is an excellent producer of the industrially important enzyme laccase and also used for the decolorization of anthraquinone dye (Ho u *et al*., 2004). Although, it contains lower concentration of major elements like phosphorus, potassium and calcium but trace mineral elements like magnesium, boron, cadmium, iron and manganese are at a higher concentration than that of *Agaricus bisporus* (Vetter, 1994). It has extensive use in biodegradation (Adamovic *et al*., 1998), immunomodulatory, anti-hypocholesterolemic, anti-mutagenic and anti-tumour activities (Wasser, 2002a; Hossain *et al*., 2003; Lakshmi *et al*., 2004; Maiti *et al*., 2011).

1.2.2 *P. pulmonarius*

It is commonly known as the Indian Oyster, Italian Oyster, Phoenix Mushroom, or the Lung Oyster, is a mushroom very similar to *Pleurotus ostreatus*, the pearl oyster, but with a few noticeable differences (Stamets, 2000). The caps of this mushroom are much paler and smaller than that of *P. ostreatus*. It also prefers warmer weather than *ostreatus* and will appear later in the summer. Otherwise, the taste and cultivation of the two species is generally described as largely the same (Stamets, 2000). It is widespread in temperate and subtropical forests throughout the world. In the eastern United States, this species is generally found on hardwoods, while in the west it is commonly found on conifers (Stamets, 2000).

A polysaccharide called β-D-Glucan from *P. pulmonarius* reduced sensitivity to pain in mice (Baggio *et al*., 2010), a basis for new analgesic medications (Baggio *et al*., 2011). While a glucan from it showed potent anti-inflammatory and analgesic properties, a methanol extract of it displayed anti-inflammatory and antitumor activity (Jose *et al*., 2002; Smiderle, 2008). The extracts of *P. pulmonarius* may slow the proliferation of cancer cells and be useful as an adjuvant to cancer therapies and can halt the progression of diabetes (Badole *et al*., 2008; Wasonga *et al*., 2008; Lavi *et al*., 2010). It may be effective in the treatment of hay fever, cause a significant reduction in sneezing and nasal rubbing by inhibiting the release of histamine, can be used in the treatment of colitis and also inhibit colon cancer formation associated with colitis in mice (Yatsuzuka *et al*., 2007; Lavi *et al*., 2010; Lavi *et al*., 2011). Extracts of *P. pulmonarius* have antimicrobial properties (Ramesh and Pattar, 2010).

1.2.3 *P. tuber-regium*

It is a popular edible mushroom and is considered a profound health promoting mushroom in traditional Chinese medicine (Isikhuemhen *et al*., 2000; Huang, 2002). It has nutritive values and some medicinal properties which include relief for stomach ailments, fever,

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asthma, smallpox, high blood pressure, and cancer (Wong *et al*., 2011). It is believed by many Nigerians to be capable of curing ailments such as headaches, stomach pain, small pox, fever and chest pain (Oso, 1977a; Isikhuemhen and Okhuoya, 1995; Okhuoya *et al*., 1996). It is used in the Asaba area of Nigeria in herbal preparation for pregnant women to aid the development of foetus.

In Ghana, the sclerotia are used mainly for fattening of malnourished babies and as one of the ingredients in the embalming of dead bodies (Okhuoya *et al*., 1998). The fruiting bodies of *P. tuber-regium* is highly nutritive and very rich in protein, while sclerotium is rich in fiber, especially non-starch polysaccharides (Kadiri and Fasidi, 1990), mainly composed of bioactive β-glucans responsible for pharmacological actions (Cheung and Lee, 2000; Tao *et al*., 2006). It has antihyperglycemic, antihyperlipidemic, and antioxidant properties (Huang *et al*., 2012). Analysis of its sclerotia has shown the presence of Calcium, Magnesium, Iron and Zinc (Okhuoya and Ajerio, 1988).

1.3 Substrates for mushroom cultivation

Mushrooms are grown on great variety of substrates. Mushroom substrate may be defined as lignocellulotic material which supports the growth, development and fruiting of mushroom and their choice depends on availability and cost (Chang and Miles, 1988a).

A lot of studies were reported on the various substrates for mushroom production namely straws of rice (*Oryza sativa*), wheat (*Triticum vulgare*), ragi (*Elucine coracana*), bazra (*Pennisetum typhoides*), sorghum (*Sorghum vulgare*), maize (*Zea mays*), wood of poplar (*Populus robusta*), oak (*Quercus luecothricopora*), horse chest nut (*Aesculus indica*), *Acasia* species, chopped banana pseudostem, cotton stalk, pea shells and poplar sawdust (Saidu *et al.*, 2011). Most of the commercial producers of mushroom in Malaysia are currently using sawdust and rice husk (Saidu *et al.*, 2011).

Substrates may also be obtained from various plant remnants without enrichments by expensive additives. Composted or uncomposted wheat and paddy straw, banana leaves, sugarcane bagasses and leaves, wheat bran, rice husk, sawdust etc can be used as substrate for growing mushroom (Gupta, 1986).

1.4 Choice of Oyster mushrooms

Oyster mushroom is the second most cultivated edible mushroom worldwide after *Agaricus bisporus* (Sánchez, 2010). Members of the mushroom genus *Pleurotus* form a heterogeneous group of edible species of high commercial importance (Georgios *et al*., 2004). It

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has economic and ecological values with medicinal properties (Gregori *et al*., 2007; Sánchez, 2010; Khan and Tania, 2012). It has abilities to grow over a wide range of temperatures utilizing various lignocelluloses (Sánchez, 2010).

Oyster mushroom cultivation also help in managing organic wastes whose disposal is very complicating and time consuming (Das and Mukherjee, 2007). It converts a high percentage of the substrate to fruiting bodies thus increasing profitability (Sanchez, 2010). Particularly, *P. ostreatus* requires a shorter growth time in comparison to other edible mushrooms, demands few environmental controls, their fruiting bodies are not often attacked by diseases and pests, and they can be cultivated in a simple and cheap way (Sánchez, 2010).

1.5 Choice of the selected tropical trees

The selected tropical trees are mango (*Mangifera indica*), cassia (*Senna siamea*) and neem (*Azadirachta indica*) trees. They are readily available in the tropics and consequently, our environment. They have lignin and cellulose which support growth of mushrooms and are easily converted to sawdust during milling for mushroom cultivation. They can be easily colonized and degraded by edible mushrooms (Sánchez, 2010). They have medicinal and seasoning or flavouring properties (Lose *et al.*, 2000; Kiepe, 2001; Subapriya and Nagini, 2005).

1.6 Statement of problem

Malnutrition cases occur in the most people of developing countries due to poverty level and their inaffordability in sourcing for animal proteins. Also, there is an increased demand for animal protein in these countries as a result of population explosion and proper diet.

1.6.1 Justification

Animal protein is beyond the reach of most people in developing countries because most of them live below poverty level (World Bank, 1992). Therefore, there is the need to find out an alternative source of protein due to malnutrition as a result of population explosion predicated upon by an increasing protein gap. More so, FAO recommended edible mushrooms as food that can contribute to protein nutrition of developing countries which depend largely on cereals (Islam *et. al.,* 2009). *Pleurotus* species are one of the choice edible mushrooms that can be cultivated in the tropics (Quimio *et al.*, 1990). They possess extensive enzyme systems that are able to degrade varieties of lignocellulotic materials (Baysal *et al.*, 2003), thereby utilizing them for growth.

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1.6.2 Aim

The aim of this study is to evaluate the growth and yield of *Pleurotus ostreatus, P. pulmonarius* and *P. tuber-regium* on the sawdust of some selected tropical trees.

1.6.3 Objectives

i. To compare the growth and yield of *Pleurotus ostreatus, P. pulmonarius* and *P. tuber-regium* as affected by weeks of compositing interval.

ii. To select a suitable substrate for the cultivation of *Pleurotus ostreatus, P. pulmonarius* and *P. tuber-regium*.

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CHAPTER TWO

LITERATURE REVIEW

2.1 Origin and Geographical Distribution of Mushrooms

Evidence from molecular systematic studies suggests that many mushroom species may be quite ancient (Vilgalys and Sun, 1994; Moncalvo *et al*., 2000). The Chinese and Japanese chronicles indicate that the shiitake mushrooms were collected in the wild and given to the Emperor as tribute. Throughout the middle ages and the preceeding century, the Greeks and the Romans considered mushrooms as special food and ate mushrooms on special occasions while some cultures considered all mushrooms as toadstools and poisonous gifts from the devil. At this time, mushrooms could only be obtained in autumn and spring (Quimio *et al.*, 1990).

Fungi are commonly associated with thunderstorms as it was believed in mythology that mushrooms were formed by lightening. According to Oso (1977a), a lot of myths surround the origin of Nigerian mushrooms. The myth surrounding the origin of *P. tuber-regium* says that "orunmila", the supreme deity who represent God on earth had several messengers, one of whom was "Ifa", the god of divination. "Ifa" had 16 disciples one of whom was "Ejiogbe" (the god of all goodness) who introduced *Pleurotus tuber-regium* on earth. *Termitomyces robustus* origin is associated with a poor man called "Ogogo", who after consulting "orunmila" had some rites performed for him. These rites resulted in the man producing *T. robustus* fruiting bodies which people liked and eventually made the man wealthy (Oso, 1977b). *Termitomyces microcarpus* origin is associated with a childless Yoruba woman called "Oran" who approached "Orunmila" about her childlessness."Orunmila" prescribed certain rites for her to perform which the woman disobediently failed to do. This made her to start having *T. microcarpus* fruiting bodies instead of having children. Human beings became fond of them due to their sweetness so this mushroom was named after the woman and is popularly called "Olu Oran" (Oso, 1977b). The popular acceptability of *T. globulus* is linked with a poor woman who after consulting a "Babalawo" was asked to procure a *T. globulus* fruiting body which was divined upon, making it to multiply many fold and the woman became extremely rich after selling the fruiting bodies (Oso, 1977b).

Mating compatibility studies have demonstrated the existence of discrete intersterility groups (biological species) in *Pleurotus*, many of which are broadly distributed over one or more continents (Andersen and Stasovaki, 1992). At least, fifteen intersterility groups have been identified, with each group associated with one or more morphological species (Vilgalys *et al*., 1996).

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2.1.1 Botany of Mushrooms

Mushrooms are familiar to adults and children alike from their most characteristic shape, a rounded cap known as pileus on a central stalk called stipe (Turner and Szczawinski, 1992). Mushrooms belong to a large, complex group of organisms called fungi, all of which lack chlorophyll, the green substance that enables green plants to manufacture their own food through photosynthesis (Turner and Szczawinski, 1992). The fruiting body lasts for a few years but the mycelium living on the organic materials in the soil may survive for years (Oyetayo and Oyetayo, 2008). The mycelium branches and produces enzymes that digest complex carbohydrates, lipids and proteins, which are then easily absorbed by the hyphae. The hyphae penetrate the substrate until it is time to form fruiting bodies or to start reproduction (Oei, 2003).

2.1.2 Taxonomy of Mushrooms

The modern classification has grouped mushrooms into the Kingdom Mycetae. The large fleshy mushrooms are found in two classes of fungi (Eumycophyta); the Basidiomycetes and the Ascomycetes. Most of the edible mushrooms belong to Ascomycotina and Basidiomycotina (Pathak *et al.,* 2003). Some mushrooms, such as truffles and morels are Ascomycetes, while the vast majority of the large fungi are Basidiomycetes (Chang, 1981). The size, texture, shape and colour of the fruiting body vary according to the types of genera and species of mushroom (Alexopoulos and Mims, 1979).

The fungal class *Basidiomycetes* is further divided into sub-class *Hymenomycetes*, whose fruiting bodies are exposed to the air and *Gasteromycetes*, which have unexposed fruiting layers. Members of the sub-class *Hymenomycetes* comprises of four divisions: gill fungi (fungi with gills), pore fungi (fungi with tubes or pores), teeth fungi (fungi that are club or coral-like in shape) and jelly fungi (fungi that form jelly-like masses) (Kadiri *et al.,* 2003). On the basis of spore print colour, the gill fungi are sub-divided into white spore-genera (example include; *Amanita* sp, *Lepiota* sp, *Pleurotus* sp, *Tricholoma* sp, *Lentinus* sp), pink-spore genera (which include *Volvariella* sp, *Enteloma* sp), brown-spore genera (which include; *Pholiota* sp, *Cortainarius* sp), purple-spore genera (which include; *Psalliota* sp, *Panaelous* sp) and black-spore genera (*Coprinus* sp). The pore fungi consist of the genera *Boletus* sp, *Boletinus* sp and *Polyporus* sp. The teeth and jelly-fungi have only one genus each and these are *Clavaria* and *Auricularia* respectively (Jonathan, 2002; Kadiri *et al.,* 2003). The *Gasteromycetes* consist of the genera *Gaester* (star-shaped), *Lycoperdon* (puff-balls) and *Calvatia* (giant puff-balls). In general,

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mushrooms are identified on the basis of their spore prints, morphological features and results produced by some chemical tests (Kadiri *et al.,* 2003).

2.1.3 Morphology of Mushroom

Mushrooms are fleshy, spore-bearing structures which are easily visible. Some have a ring or annulus on the upper part of the stalks while others have none. They are variable in form, but they have a common function which is to bear the sexual spores. These spores are borne in or on specialized hyphal structures (asci or basidia) which make up a fertile layer called a hymenium. Most mushrooms bear their hymenia on gills, while there are also some with hymenium-lined canals, tubes, or cavities (Chang and Miles, 1989). Like all filamentous fungi, the mushroom hyphae can grow over, into, or through the substrate by the extension of hypha. This extension occurs at the tips of the hyphae. The older portions of the hyphae are not capable of growth, but they have an important role in supporting the growth of the tip and the development of fruiting bodies as new protoplasm is formed and the absorbed nutrients are transported to the active growing apices (Chang and Miles, 1989). The morphology of a typical mushroom is as shown in figure 2.1.

Mushrooms are only the reproductive part of the organism- the "fruit"; the main part is a seldom-seen mass of tiny thread-like growths, or hyphae, called the mycelium. Ever present but usually inconspicuous mycelia penetrate soil, bark, and wood (Turner and Szczawinski, 1992). Mushrooms make up only a small fraction- about 5,000 to 10,000 of the total number of fungi, which includes an estimated 200,000 or more species, the majority being inconspicuous or microscopic. Although many fungi are edible or useful to people in some way, many are potentially harmful (Turner and Szczawinski, 1992). The basic life cycle is from spore to mycelium to fruiting body, which later bears the spores again (Hayes and Hand, 1981). The fruiting stage is the formation of visible mushrooms. Hence, mushrooms are actually fruits of the fungus (Okhuoya and Okogbo, 1990). The life cycle of mushroom is as shown in figure 2.2.

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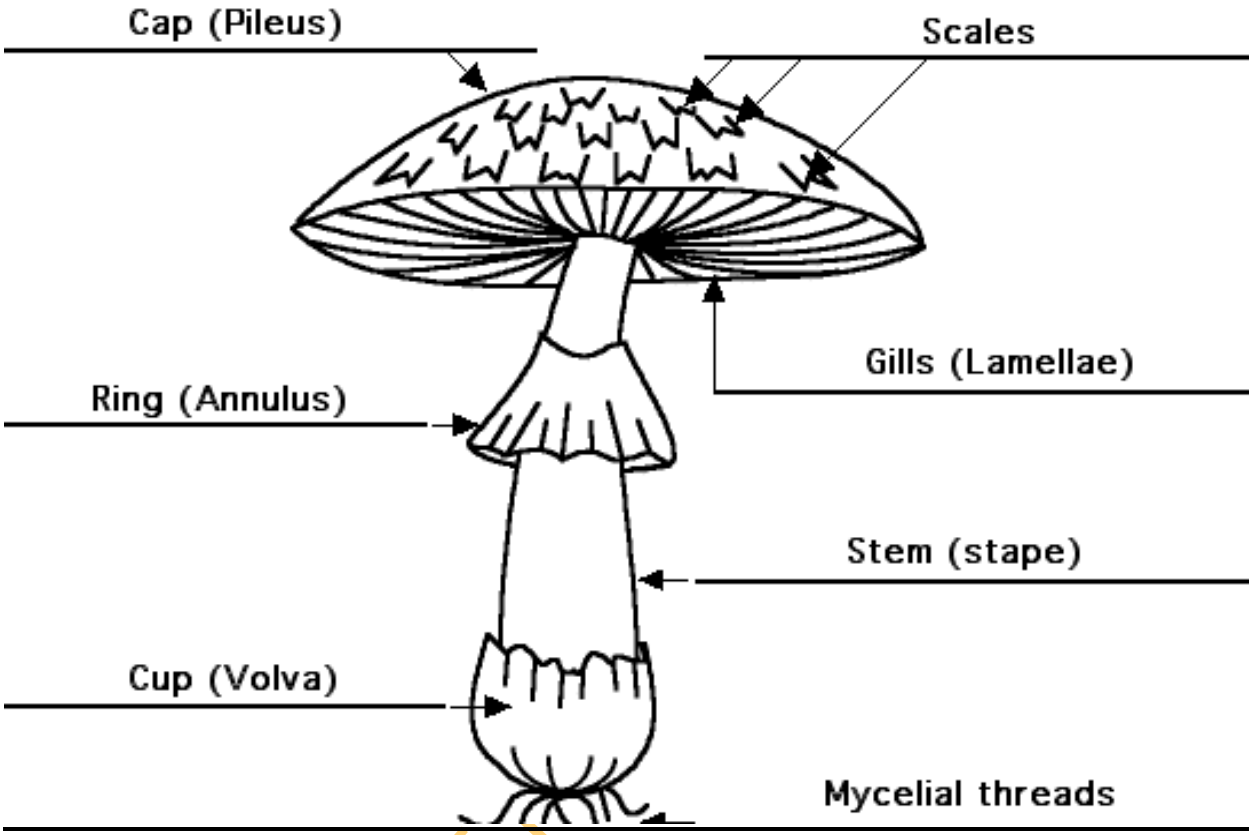

Fig. 2.1: Morphology of mushroom fruiting body

Source: Stamets (2000)

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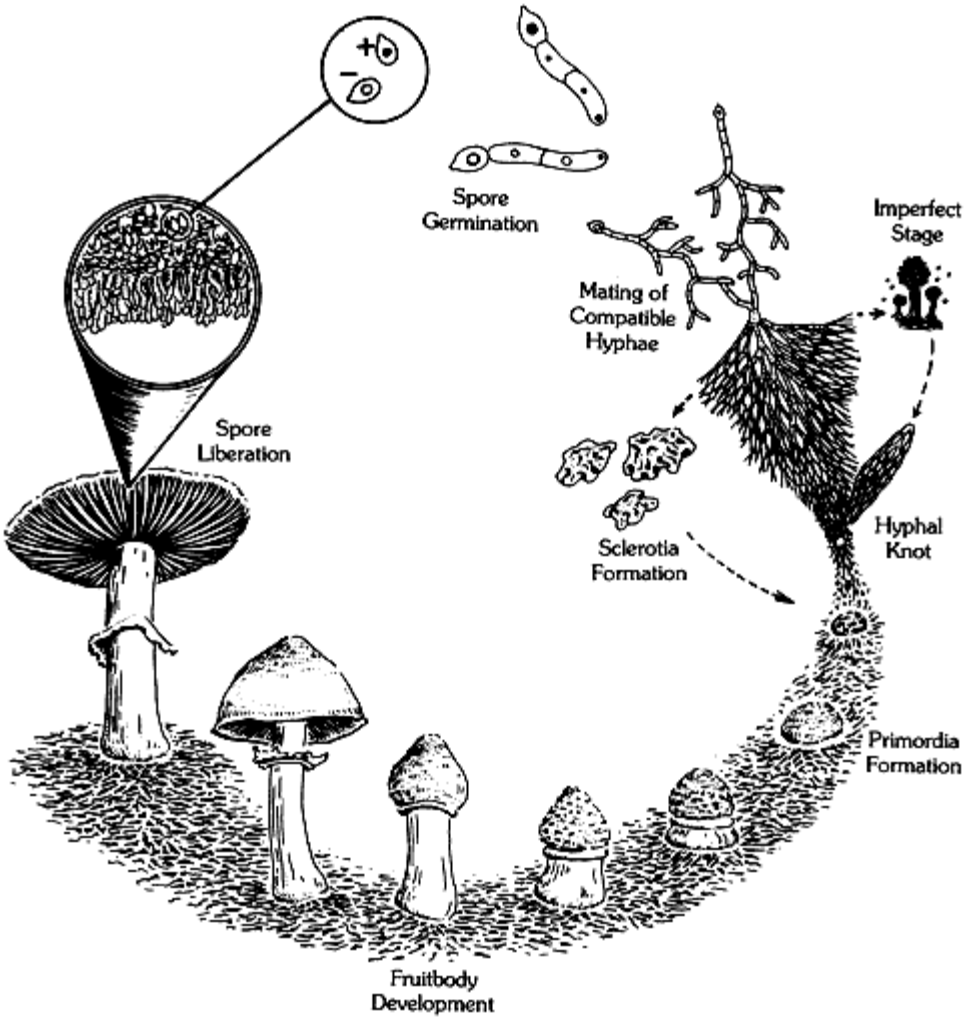

Fig. 2.2: Life cycle of mushroom

Source: Stamets (2000)

2.2 Mushroom cultivation

Cultivation of saprophytic edible mushrooms may be the only currently economical biotechnology for lignocellulose organic waste recycling that combines the production of protein rich food with the reduction of environmental pollution (Obodai *et al.*, 2003). The cultivation of edible mushrooms is a prime example of how low-value waste produced primarily through the activities of the agricultural, forest and food-processing industries can be converted to a higher value commodity useful to mankind. It is a profitable agribusiness, particularly edible oyster mushroom, with excellent flavour and taste (Shah *et al.*, 2004). It helps in the conversion of lignocellulosic waste material into high quality protein food.

The cultivation of mushrooms needs preparation of substrate and compost; preparation of spawn and seeding of the spawn on suitable substrate for mycelial growth and production of fruiting bodies (Meera, 2004). The materials that are most widely adopted for mushroom cultivation are `lignocellulosic' materials, the major components of which are cellulose, hemicellulose and lignin. These three polymeric substances form the bulk of most plant cell walls.

The white button mushroom, *Agaricus bisporus* (Lange) Imbach, is the most commonly grown mushroom in Turkey, accounting for up to 94.8% of the total mushroom production, and productivity of this species is 20-22% of compost fresh weight (Erkal and Aksu, 2000; Erkel, 2004). It is produced on a composted mixture of various cereal straws (wheat, rye, corn) hay, corncobs, distillers' grain, cottonseed meal, poultry manure and other raw materials (Van Griensven, 1988).

Mushrooms have been recognized as a high potential converter of cheap celluloses into valuable protein (Poppe, 2000). There are about 100 species of edible mushrooms all over the world. Unfortunately, it is realized that mushrooms did not receive universal acceptance over the years since a number of naturally growing mushrooms are poisonous. Now the situation has been changed because the cultivated edible mushrooms are totally safe for human consumption. Mushroom cultivation fits in very well with sustainable farming and has several advantages: it uses agricultural waste products, a high production per surface area can be obtained, after picking; the spent substrate is still a good soil conditioner (NPCS, 2011).

Pleurotus species can produce a broad spectrum of lignocellulolytic enzymes and have been grown on different kinds of sawdust, straw and many other agricultural and industrial wastes (Hadder *et al.*, 1993). Various works using different substrates like sawdust (Block *et al*.

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1958), paddy straw (Bano and Srivastava, 1962), banana pseudo stems (Jaindaik, 1974), newspaper (Hashimoto and Takahashi, 1974), wheat straw (Zadrazil, 1974), cotton and sugar cane wastes (Chang,1980), maize cobs (Sivaprakasam and Kandaswamy, 1981), hulled cocoa shells (Phettipher, 1987) have been done. Waste paper and used cotton have been reported (Shakil *et al*., 2014).

Presently three mushrooms namely *Pleurotus* species (Oyster Mushroom), *Volvariella volvaceae* (Straw Mushroom) and *Auricularia* spp (Ear Mushroom) are under commercial cultivation in Bangladesh.

Most mushrooms performed better during the rainy season (Aletor, 1995). *Lentinus subnudus* and *P. tuber-regium* had been successfully cultivated on various cellulolytic agricultural wastes (Fasidi and Ekuere, 1993; Fasidi and Kadiri, 1993). Similarly, utilization of agricultural waste as growing media for the production of mushroom plays a key role in reducing the waste and at the same time useful as a bio-fertilizer (Sher *et al*., 2011). For this reason, it is not necessary to process substrates for cultivation of *Pleurotus* species (Khan and Chaudhary, 1987; Yalinkiliç *et al.*, 1994).

Other basal ingredients that may be used include palm-bunches, straw and corn cobs or mixtures thereof. Idowu (2003) observed that oil palm bunch waste singly and in combination with other substrates was stimulatory to the growth of the mushroom mycelia which resulted in higher fruiting body yield and biological efficiency. Furthermore, it was observed that *P. pulmonarius* performed best on oil palm bunches in comparison with other substrates investigated (Idowu, 2003). Mushroom production is completely different from growing green plants because it does not contain chlorophyll, and therefore depends on other plant material (substrate) for its food. Therefore, the substrate is an important item for growing mushroom.

2.3 Nutritional Requirements for the growth of Mushrooms

Sawdust is the most popular basal ingredient in synthetic formulation of substrate used to produce shiitake mushrooms (Miller and Jong, 1987). In almost all cases, the efficiency of these waste constituting substrates is considerably enhanced when supplemented with protein-rich materials such as bran of rice and wheat. Regardless of the main ingredient used, starch-based supplements such as wheat bran, rice bran, millet, rye, corn, etc are added to the mixture in a 10 to 40% ratio (dry weight) to the main ingredient. Royse and Sánchez (2008) indicated that mushroom yields may also be stimulated by supplementation of first break mushroom compost with hydrolyzed protein, commercial supplements and crystalline amino acids. These

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supplements serve as nutrients to provide an optimum growing medium (Royse *et al.,* 1990). However, all kinds of lignocellulosic substances are likely to be used as substrate for *Pleurotus* species cultivation, the main and co-substrate differ among countries and even regions on the basis of availability and cost (Balazs, 1995; Croan, 1999; Labuschagne *et al.*, 2000; Oei, 2003).

Mushroom cultivation ensures their availability throughout the world irrespective of season since cultivated mushrooms can be grown under different climatic conditions on cheap, readily available agro wastes. The cultivation process guarantees edibility and serve as the most economically viable process for the bioconversion of low value wastes produced primarily from the activities of agricultural, forest and food processing industries to produce higher value fungal protein for human consumption (Wasser, 2002b). *Pleurotus* species are also found to be one of the most efficient lignocelluloses solid state decomposing types of white rot fungi (Baysal *et al.*, 2003). Thus, many agricultural and industrial wastes can be utilized as substrates for production of *Pleurotus* species (Zadrazil and Brunnert, 1981; Platt *et al.*, 1983; Platt *et al.,* 1984; Baysal *et al.,* 2003).

All edible fungi are saprophytes (Chang and Miles, 1989) and they need organic matter to decompose (Oei, 2003). Mushroom hyphae liberate large amounts of extracellular enzymes which bring about the degradation of the many types of macromolecules, such as cellulose, hemicelluloses, lignin, protein, etc., present in the substrate. The simple, soluble smaller molecules resulting from the activities of these extracellular enzymes are then absorbed by the fungal cells. Thus, mushroom hyphae can easily grow and colonize the substrate (Chang and Miles, 1989). Mushrooms require a temperature of 20-32^{o} Celsius and about 35-90% humidity. They also require adequate ventilation, diffused light and semi-darkness. Too much light makes mushrooms dark in colour.

2.4 Economic importance

For many reasons, the fungi of the *Pleurotus* genus have been intensively studied in many parts of the world; they have high gastronomic value. They are able to colonize and degrade a large variety of lignocellulosic residues, reduce wastes, control environmental pollution and the spent mushroom compost (SMC) can be utilised as bio-fertilizer (Sher *et al*., 2011).

Fungi have been extensively studied by Mycologists in educational research fields. Antibiotics, therapeutic agents have been produced for medicinal use from some fungi such as *Penicillium notatum*, *Aspergillus* species, *Pleurotus* species, *Lycoperdom* species, *Polyporus*

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species (Jonathan, 2002). They have less carbohydrate so they are believed to be suitable for diabetic patients. Fresh mushrooms have very limited life and hence they need to be consumed within few hours but processing and canning increases their shelf life to few months. Osmotic dehydration is one of the important methods of processing mushroom which involves drying technology of mushroom. Mushrooms are very popular in most of the developed countries and are becoming popular in many developing countries like India (NPCS, 2011).

Mushrooms are regarded as highly nutritive food delicacies and are important features of human diets worldwide (Oso, 1981). Edible mushrooms have been consumed as food and delicacies in many cultures (Fasidi and Kadiri, 1990; Jonathan *et al.*, 2008). They are good sources of non-starchy carbohydrates, dietary fibre, mineral and vitamins (Bano and Rajarathanum, 1988). Several species of edible mushroom have been reported to have protein as high as 50.8% dry matter (Eiker, 1993; Alofe *et al.*, 1996), thus surpassing most vegetable protein sources. Mushrooms have high contents of qualitatively good protein, crude fibre, minerals but are poor sources of lipids (Fasidi and Kadiri, 1990). They have high nutrient value of almost twice that of any other vegetable or fruits (Sivrikaya *et al.*, 2002).

Mushrooms are superior to many vegetables and beans in their nutritive value. They are very rich in protein, vitamins and minerals. Fresh mushrooms contain about 85% water and 3.2% protein. But dried mushrooms have low water content and protein level is as high as 34 to 44% while the fat content is less than 0.3% (NPCS, 2011). Mushrooms are rich sources of mineral elements and vitamins, with mineral elements already detected being potassium, phosphorus, calcium, sodium, magnesium, zinc, iron, manganese, nickel, copper, chromium, cobalt and vitamins (B, C, K) and niacin (Ogundana and Fagade, 1982, Zakhary *et al.*, 1983). They are rich in protein, minerals, and vitamins, and they contain an abundance of essential amino acids (Sadler, 2003). Therefore, mushrooms can be a good supplement to cereals (Chang and Buswell, 1996).

Mushrooms have been used as human food for centuries, being valued particularly for the variety of flavours and textures they can provide (Sadler, 2003). Furthermore, Fasidi and Akwakwa (1996) stated that edible mushrooms are eaten in Nigeria as alternatives to meat and also for medicinal purposes. Mushrooms can also be canned for consumption and exported to foreign countries (Jonathan and Fasidi, 2003a). Protein tends to be present in an easily digestible form and on a dry weight basis. The protein content of mushroom normally ranges between 20 and 40% which is better than many legume sources like soybeans and peanuts, and protein-

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yielding vegetable foods (Chang and Buswell, 1996; Chang and Mshigeni, 2001). Moreover, mushroom proteins contain all the essential amino acids needed in the human diet and are especially rich in lysine and leucine which are lacking in most staple cereal foods (Chang and Buswell, 1996; Sadler, 2003). Mushrooms are low in total fat content and have a high proportion of polyunsaturated fatty acids (72 to 85%) relative to total fat content, mainly due to linoleic acid. The high content of linoleic acids is one of the reasons why mushrooms are considered a health food (Chang and Mshigeni, 2001; Sadler, 2003). Furthermore, they contain significant amounts of carbohydrates and fibres (Chang and Buswell, 1996).

It can be naturally found in tropical and subtropical rainforests, and can be artificially cultivated (Maziero *et al.*, 1992). Mushroom is appreciated because of its delicious taste and that it has high quantities of proteins, carbohydrates, minerals (calcium, phosphorus, iron) and vitamins (thiamin, riboflavin and niacin) as well as low fat (Manzi *et al.*, 1999). Earlier in his investigation, Arora (1986) observed that over the centuries, mushrooms, especially the wild poisonous forms or toadstools, became objects of fear and distrust because of the stories of mushroom poisoning. Most poisoning cases were characterized by extreme pain and suffering before death which were recorded not only in America but also in Great Britain.

2.5 Major phases in mushroom cultivation

Mushroom farming is a complex business, which requires precision. The major practical steps/ segments of mushroom cultivation are: (a) selection of an acceptable mushroom species; (b) secreting a good quality fruiting culture; (c) development of robust spawn; (d) preparation of selective substrate/ compost; (e) care of mycelial (spawn running); (f) management of fruiting/ mushroom development; and (g) harvesting mushrooms carefully (Chang 1999). The major phases in mushroom cultivation are as shown in figure 2.3.

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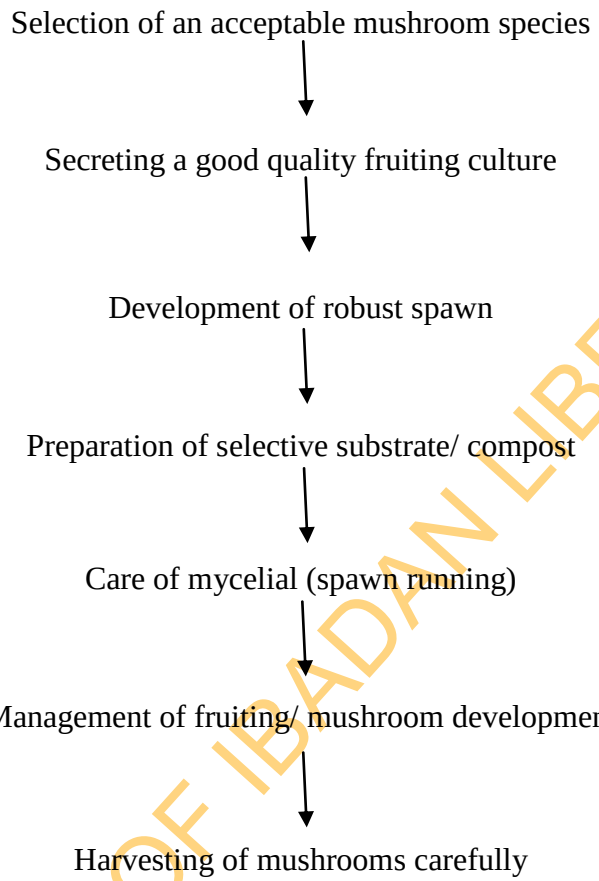

Fig. 2.3: Flow Chart of Mushroom Production

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(a) Selection of Acceptable Mushroom Species/ Strains

Before any decision to cultivate a particular mushroom is made, it is important to determine if that species possess organoleptic qualities acceptable to the indigenous population or to the 33 international markets, if suitable substrates for cultivation are plentiful, and if environmental requirements for growth and fruiting can be met without excessively costly systems of mechanical control (Chang, 2000).

(b) Secreting a Good Quality Fruiting Culture

A "fruiting culture" is defined as a culture with the genetic capacity to form fruiting bodies under suitable growth conditions. The stock culture which is selected should be acceptable in terms of yield, flavour, texture, fruiting time, etc. (Stamets, 2000)

(c) Development of Robust Spawn

A medium through which the mycelium of a fruiting culture has grown and which serves as the inoculum or "seed" for the substrate in mushroom cultivation is called the "mushroom spawn". Failure to achieve a satisfactory harvest may often be traced to unsatisfactory spawn used. Ragunathan *et al.* (1996) reported that consideration must also be given to the nature of the spawn substrate since this influences rapidity of growth in the spawn medium as well as the rate of mycelia growth and filling of the beds following inoculation.

(d) Preparation of Selective Substrate/ Compost

The process of substrate preparation is broadly termed “composting”. The final product of “composting” is called the “compost” or prepared substrate. While a sterile substrate free from all competitive micro-organisms is the ideal medium for cultivating edible mushrooms, systems involving such strict hygiene are generally too costly and impractical to operate on a large scale. Substrates for cultivating edible mushrooms normally require varying degrees of pre-treatment in order to promote growth of the mushroom mycelium to the practical exclusion of other micro-organisms. The substrate must be rich in essential nutrients in forms which are readily available to the mushroom, and be free of toxic substances which inhibit growth of the spawn. It was observed by Stamets (2000) that moisture contents, pH and good gaseous exchange between the substrate and the surrounding environment are important physical factors to consider. Mushroom substrate may be simply defined as

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a lignocellulosic material which supports the growth, development, and fruiting of mushroom mycelium (Chang and Miles, 1988a).

The process for the preparation of substrates has been the subject of much scientific and practical interest over the past two decades. The different types of mushrooms require different types or substrate/ compost. *Lentinula edodes* and *Pleurotus* species are fungi that can grow on wood. The composting conditions produce a favourable medium for the development of mushrooms due to the development of microbial population that paved the way for the subsequent growth and fructification of mushrooms. The large amount of substrate left after the mushrooms have been harvested is known as spent compost. It is certainly not desirable to leave it without utilization because it can be a possible source of pollution. The remains of spent compost consists considerable amount of lignocellulosic material in addition to the mushroom mycelia as well as other products formed by the metabolic activities of the mycelium. Thus, the spent compost is capable of supporting further biological activities. For example, the growth of another species of edible mushroom is used as fodder for livestock; as a soil conditioner in bio-fertilizer; and also in bioremediation.

(e) Care of Mycelia (Spawn Running)

Following composting, the substrate is placed in beds where it is generally pasteurized by steam to kill off potential competitive microorganisms. After the compost has cooled, the spawn may be broadcast over the bed surface and then pressed down firmly against the substrate to ensure good contact, or inserted 2 to 2.5 cm deep into the substrate. Spawn running is the phase during which mycelium grows from the spawn and permeates into the substrate. Good mycelial growth is essential for mushroom production and depends on proper maintenance of the beds/ innoculated substrate, and also of the mushroom house, in terms of temperature, moisture content, humidity and aeration (Quimio, 1988).

(f) Management of Fruiting/Mushroom Development

The management of fruiting under suitable environmental conditions may differ from those adopted for spawn running. Primordial formation occurs which is then followed by the production of fruiting bodies. The appearance of mushrooms normally occurs in rhythmic cycles called "flushes"(Jandaik and Goyal, 1995).

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(g) Harvesting Mushrooms Carefully

According to Jandaik and Goyal (1995), harvesting is carried out at different maturation stages depending upon the species, consumer preferences and market value.

2.6 The selected tropical plants as substrates

2.6.1 MANGO PLANT

This plant (*Mangifera indica*) belongs to the order Sapindales and the family Anacardiaceae. It is a group of tropical trees native to North India, Burma, and Malaya. The mango tree is believed to have evolved as a canopy layer or emergent species of the tropical rainforest of South and South-east Asia (Kaur *et al.*, 1980). It is found in the wild and have been introduced to other warm regions of the world. It is the largest fruit-tree in the world, capable of a height of one-hundred feet and an average circumference of 12 to 14 feet, sometimes reaching twenty 20. This tree can grow up to 90 feet and have a spread of 120 feet or more (Mukherjee and Litz, 2009). It is widely grown in the tropics for its delicious fruit. In the United States of America, it is grown outdoors in Southern Florida and the warmest parts of California. Young plants can be grown indoors as houseplants. Their foliage is dark green and shiny. When new leaves unfold, they have a rich brownish-red color (Mukherjee and Litz, 2009).

It is believed that the Portuguese transported the mango from their colonies in India to their African colonies, although Purseglove (1972) suggested that it might also have been introduced to Africa via Persia and Arabia in the 10th century by Arab traders. The Portuguese later introduced the mango into Brazil from their African colonies of Mozambique and Angola. On the basis of ancient accounts of travellers and the written historical record, it was believed for many years that mango must have originated in India and spread outwards from there to South-east Asia and thence to the New World and Africa (Mukherjee and Litz, 2009). The mango is cultivated commercially throughout the tropics and in many subtropical areas. The *Mangifera* species, like many other tropical fruit trees, are canopy and emergent trees of the tropical rainforest (Kaur *et. al.*, 1980). These trees are widely scattered in the tropical rainforest, flower erratically and reproduce from large seeds that deteriorate rapidly.

2.6.2 Local names:

Yoruba:	Mango
Hausa:	Mangwaro
Igbo:	Mangolo
Efik:	Mangoro

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2.6.3 Chemical components

From the young leaves (172 g/kg), bark (107 g/kg), and from old leaves (94 g/kg), mangiferin (a pharmacologically active flavonoid, a natural xanthone C-glycoside) is extracted from mango at high concentrations (Barreto *et al.*, 2008). Chlorophyll, carotenes, anthocyanins and xanthophylls are all present in the fruit. The skin is generally a mixture of green, red and yellow pigments, although fruit colour at maturity is genotype dependent. During ripening, the chloroplasts in the peel become chromoplasts, which contain yellow and red pigments (Krishnamurthy and Subramanyam, 1970; Akamine and Goo, 1973; Salunkhe and Desai, 1984; Mitra and Baldwin, 1997). The pulp carotenoids in ripe fruit also vary with respect to cultivar (Mitra and Baldwin, 1997).

2.6.4 Scientific Classification

Kingdom: Plantae

Division: Angiospermae

(Unranked): Rosids

Order: Sapindales

Family: Anacardiaceae

Genus: *Mangifera*

Species: *M. indica*

2.6.5 Mango stem as mushroom substrate

An enhanced growth of mushroom pileus has been observed on mango sawdust (Veena *et al.*, 1998) as Ashrafuzzaman *et al.* (2009) also observed that mango sawdust gave the largest diameter of mushroom pileus. Islam *et al.* (2009) evaluated the growth and yield of *Pleurotus flabellatus* on seven different type of substrates viz. Mango, Jackfruit, Coconut, Jam, Kadom, Mahogony, Shiris sawdust with wheat bran and $CaCO_3$ and obtained the maximum biological yield on mango sawdust. Islam *et al.* (2009) also stated that the cost benefit analysis revealed that mango and shiris sawdusts were promising substrates for the growing of Oyster Mushroom (*Pleurotus flabellatus*).

2.7 CASSIA PLANT

The genus *Cassia* is in the family *Leguminosae* in the major group *Angiosperms* (Flowering plants). *Cassia* is a genus of Fabaceae in the subfamily Caesalpinioideae. Commonly called cassias, "cassia" is also the English name of *Cinnamomum aromaticum* in the Lauraceae

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(from which the spice cassia bark is derived), and some other species of *Cinnamomum*. It is commonly called Thailand shower, minjiri, or kassod and has many regional names (F/FRED, 1994). It is native to South and Southeast Asia and is usually planted as a shade tree in cocoa, coffee, and tea plantations (National Academy of Sciences, 1984; Webb *et al.*, 1984).

Senna siamea, the species used for this study, is a medium sized evergreen tree attaining 5 m height (F/FRED, 1994). It is a medium size tree up to 15-20 cm tall, with a straight trunk up to 30 cm in diameter, bole short, crown usually dense and rounded at first, later becoming irregular and spreading with dropping branches, bank grey or light brown, smooth but becoming slightly fissured with age (Bernard, 2005). It is an evergreen tree commonly cultivated in fuel plantation (Smith, 2009).

It rarely exceeds 20 m height and 50 cm diameter at breast height (Jensen, 1995). It has a dense, evergreen, irregular, spreading crown, a crooked stem, and smooth, grayish bark that is slightly fissured longitudinally. Its young branches have fine hairs. The leaves are pinnately compound with an even leaf arrangement of 7-10 pairs of ovate-oblong leaflets 7-8 cm long and 1-2 cm wide. Its flowers are yellow, borne in large terminal panicles that are often 30 cm long. The flowering period is long, and flowers may often be found at various seasons (Troup, 1921). The fruit is a flat pod 15-25 cm long, thickened at both sutures, containing many seeds (Gutteridge, 1997).

It grows well in many environments, but it grows particularly well in lowland tropics having mean annual rainfall of 500-2800 mm (optimum about 1000 mm), mean minimum temperature of 20 ^{o}C, and mean maximum temperature of 31^{o}C. In semiarid environments with mean annual rainfall of 500-700 mm, it will grow only where its roots have access to groundwater and where the dry season does not exceed 4-6 months. Best growth occurs in deep, well drained, rich soils with pH 5.5-7.5. It tolerates well drained lateritic or limestone soils and moderately acid soils (pH 5.0). It is susceptible to cold and frost and generally does not grow well above 1300 in. It requires full sun (Gutteridge, 1997; Davidson, 1985).

It is native to South and Southeast Asia, from Thailand and Myanmar to Malaysia, India, Sri Lanka, and Bangladesh (Khan and Alam, 1996). It has been cultivated worldwide and is naturalized in many locations (Gutteridge, 1997). No significant pest or disease damage has been recorded, but minor damage can be caused by the wood rot *Ganoderma lucidum* (Khan and Alam, 1996). Insects that damage seed include *Caryedon lineaticollisi*, *Bruchidius maculatipes*,

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Aspergillus niger and *Curvularia pallescens* while *Phaeolus manihotis* occasionally causes damage to the root system (Gutteridge, 1997).

It is effective in managing constipation associated with a number of causes including surgery, childbirth and the use of narcotic pain relievers (Hill, 1992). It is used locally as anti-malaria drugs especially when decocted (the leaves and the bark) (Lose *et al.*, 2000). In traditional medicine, the fruit is used to charm away intestinal worms and to prevent convulsion in children. The young fruits and leaves are also eaten as vegetables in Thailand. The flowers and young fruits are used as curries (Kiepe, 2001).

2.7.1 Common names

Amharic: Yeferenji digita

Creole : Kasya

Filipino : Robles

English : Black-wood cassia, Bombay blackwood, cassia, iron wood, kassod tree, Siamese senna, thai copper pod, Thailand shower, yellow cassia.

It has been cultivated for so long that its exact origin is unknown. It is widely planted throughout the tropics and is locally naturalized. Plantations were established in the 1920s in Ghana, Nigeria and Sierra Leone, mainly for its quality fuel wood (National Academy of Sciences, 1984; Webb *et al.*, 1984).

2.7.2 Scientific classification

Kingdom:	Plantae
(Unranked):	Angiosperms
(Unranked):	Eudicots
(Unranked):	Rosids
Order:	Fabales
Family:	Fabaceae
Subfamily:	Caesalpinioideae
Tribe:	Cassieae
Subtribe:	Cassinae
Genus:	*Senna*
Species:	*S. siamea*

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2.7.3 Cassia stem as mushroom substrate

Das and Mukherjee (2007) observed a longer fruiting time when *Pleurotus ostreatus* was cultivated on dry weed plants; *Cassia sophera*, *Leonotis* sp, *Sida acuta*, *Parthenium argentatum*, *Ageratum conyzoides*, *Tephrosia purpurea* and *Lantana camara*. Furthermore, Das and Mukherjee (2007) stated that the protein contents of the fruit bodies obtained from *Cassia sophera*, *Parthenium argentatum* and *Leonotis* species were better than cultivating it purely on rice straw and as well as the rice straw supplemented with weeds.

2.8 NEEM PLANT

The neem tree (*Azadirachta indica*) originated in India and the word NEEM is derived from Sanskrit Nimba which means 'bestower of good health' (Chaturvedi *et al*., 2003). It is a medicinal plant belonging to the Meliaceae family and indigenous to Southern Asia (Akula *et al*., 2003). The tree (the seeds of which contain up to 45 per cent of a non-edible oil), grows quickly, drought resistant and ideal for re-forestation of semi-arid areas.

It has been used for centuries in Asia as insecticides, fungicides and anti-conceptionals in popular medicine. Almost every part of this tree: seeds, leaves, roots, bark, trunk and branches have multiple uses (Chaturvedi *et al*., 2003; Hashmat *et al*., 2012). It has also been known as Ravisambha – sun-ray like effects in providing health. It is recommended for planting in African and Asia by many international organizations and regarded as a source of fuel wood in other countries (Chaturvedi *et al*., 2003).

The neem tree has been venerated through the ages in the Indian countryside as it provided hope in any situation and the faith in the miraculous healing powers of this amazing tree led patients with incurable diseases to adopt neem as way of life. It has been extensively used in Ayurveda, Unani and Homoeopathic medicine and has become a cynosure of modern medicine. It is a valuable source of unique natural products for development of medicines against various diseases and also for the development of industrial products (Hashmat *et al*., 2012). It elaborates a vast array of biologically active compounds that are chemically diverse and structurally complex. More than 140 compounds have been isolated from different parts of neem.

All parts of the neem tree- leaves, flowers, seeds, fruits, roots and bark have been used traditionally for the treatment of inflammation, infections, fever, skin diseases and dental disorders. Neem leaf and its constituents have been demonstrated to exhibit immunomodulatory, anti-inflammatory, antihyperglycaemic, antiulcer, antimalarial, antifungal, antibacterial,

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antiviral, antioxidant, antimutagenic and anticarcinogenic properties (Biswas *et al.*, 2002; Subapriya and Nagini, 2005).

The tree protects itself from pests with a cocktail of pesticidal compounds called limonoids. The most common found in neem include azadirachtin, salannin, meliantriol and nimbin. The first is chemically similar to insect hormones that control the process of metamorphosis as insects pass from larva to pupa to adult, and appears to block the hormones that insects need to moult (Subapriya and Nagini, 2005).

2.8.1 Local names:

Yoruba:	Eke-oyinbo
Hausa:	Daldejiya/ Dogonyaro
Igbo:	Ogwu iba

2.8.2 Scientific classification of neem

Kingdom:	Plantae
Order:	Rutales
Family:	Meliaceae
Tribe:	Melieae
Genus:	*Azadirachta*
Species:	*A. Indica*

Source: Girish and Shankara, 2008

2.8.3 Neem as mushroom cultivation material

It has been reported that neem extracts can be used as botanicals to improve and enhance mushroom growth and yield. Neem extracts played an important role on the yield and productivity of *Agaricus bisporus* (Polat *et al.*, 2000) and regarded as potential alternatives to conventional pesticides for the control of mushroom phorid fly (Erler *et al.*, 2009). Inam-ul-haq *et al.* (2010) and Shah *et al.* (2011) observed that the extract also reduced the incidence of microbes in Oyster mushroom production and is more preferable than chemicals due to their lethal effects during human mushroom consumption.

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CHAPTER THREE

MATERIALS AND METHODS

3.1 Source of spawn (Mushroom seed)

The spawns of the selected mushrooms; *Pleurotus ostreatus, P. pulmonarius* and *P. tuber-regium*, were obtained from the mushroom unit, NIHORT, Ibadan. The inocula used were generated from the mushroom sporophore obtained at Songhai farm in Porto Novo in Benin Republic and maintained on Potato Dextrose Agar (PDA) for regular sub-culturing as described by Quimio *et al.* (1990).

3.1.1 Preparation of mother spawn

Extraneous debris was picked from Sorghum seeds (*Sorghum bicolo*r), soaked in water for 2 hrs, drained and boiled for 10 minutes. The seeds were then drained again. About 1% calcium carbonate ($CaCO_3$) was added to the seeds to adjust the pH to 7.5. The aliquot portions of the seeds were poured into 200 ml bottles and sterilized at 121 ^oC for 15 min and allowed to cool down to room temperature, 30 ± 2 ^oC. The sterilized substrates (sorghum seeds) in the bottles were separately inoculated with actively growing mycelia of all the selected cultivated mushrooms. These inoculated substrate bottles were then incubated in the dark room at 30 ± 2 ^oC for 15 days.

3.1.2 Preparation of the planting spawn

Sorghum seeds were prepared by removing unwanted particles and soaked overnight. They were drained in a strainer the following morning to remove excess water (to about 65% moisture content), calcium carbonate ($CaCO_3$) of about 1% was added to the seeds to adjust the pH to 7.5 and poured into 200 ml bottles. The mouth of these bottles were plugged with cotton wool, covered with aluminium foil and sterilized in an autoclave at 121 ^oC for 15 min. They were allowed to cool to room temperature of 30 ± 2 ^oC and inoculated asceptically (in an inoculating chamber) with freshly prepared mother spawn of all the selected cultivated mushrooms separately according to the method described by Quimio *et al.* (1990). These were then incubated under the conditions of 70% relative humidity and at the temperature of 30 ± 2 ^oC in a dark room for two weeks. They were kept in the refrigerator until when needed.

3.2 Collection of substrates

The mango (*Mangifera indica*), Cassia (*Senna siamea*) and neem (*Azadirachta indica*) trees used for this research work were obtained within the premises of the National Horticultural Research Institute (NIHORT), Ibadan located in the forest Savannah zone of South-West Nigeria

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(Latitude 7^{o} 22`N, Longitude $3^{o}$ 50`E). Their identities were authenticated at the Floriculture Unit of NIHORT and assigned voucher numbers as follow: *Mangifera indica* L. (PTBG0000039360), *Senna siamea* Lam. (BISH0000032830) and *Azadirachta indica* A. Juss (BISH0000015188). The research work was conducted at the Mycology Laboratory in NIHORT.

3.2.1 Substrate preparation

The selected tropical trees (substrates) were taken to Sanngo sawmill within Ibadan metropolis for milling into sawdust to improve their water retention capacity. The sawdust of these tropical trees was sundried for one week and stored to reduce the presence of inhibitory substances. Drying and further storage will ensure further decomposition of the sawdust, and permit faster growth of the fungi (Chang and Miles, 1988b).

3.2.2 Substrate composting

The sawdusts (substrates) were separately soaked overnight and pressed the following morning to remove excess water until the moisture content was about 65% and about 1% calcium carbonate ($CaCO_3$) was added to adjust the pH to 7.5. Polyethylene bags of size 25 x 15 cm were filled with the substrates with each bag weighing 300 g and packed tightly. The neck of the bag was made using heat resistant PVC (Poly Vinyl Chloride) tube. The opening was covered with a cotton plug through which the spawn was inoculated later.

3.3 Research Design

The three levels of composting interval used in this research were 4, 8 and 12 weeks. The experiment was a 4 x 3 x 3 factorial arrangement laid out in a complete randomised design with three replicates each. For all the experiments, each treatment was replicated three times at each of the three levels of composting interval. For the evaluation of mycelia growth and fruiting body production at each level, incubation was carried out at the room temperature of $30 \pm 2\,^{o}$C.

A total of 36 substrate bags were produced for each variety of the mushroom. Therefore, a sum total of 108 substrate bags were produced for the three varieties of the mushrooms.

SUBSTRATES	WEEKS OF COMPOSTING INTERVAL (Weeks)		
	4	8	12
Mango	3 replicates	3 replicates	3 replicates
Cassia	3 replicates	3 replicates	3 replicates
Neem	3 replicates	3 replicates	3 replicates
Mixed Bed	3 replicates	3 replicates	3 replicates

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3.4 Mycelial ramification

3.4.1 Growth of the mycelia of *Pleurotus ostreatus, P. pulmonarius* and *P. tuber-regium.*

The sawdusts of mango, cassia, neem and their mixed bed (fourth substrate) were evaluated. The fourth substrate was derived from the mixtures of the three substrates in ratio 1:1:1 by weight. They were separately moistened with water and left overnight. Portions of each substrate were packed in a boiling tube of size 16 x 150 mm, plugged with cotton wool, covered with aluminum foil before sterilization in an autoclave at 121 ^{o}C for 15 min. These boiling tubes were inoculated asceptically with actively growing mycelia of the test mushrooms (*P. ostreatus, P. pulmonarius* and *P. tuber-regium*) after cooling. Vertical mycelia extension was taken every other day from the first day of inoculation for two weeks.

3.5 Fruiting body production

3.5.1 Cultivation of the fruiting bodies of *P. ostreatus, P. pulmonarius*.

The substrates were separately moistened until the moisture content was about 65% and left overnight. The moisture content was considered appropriate if there was no escape of water when a handful of the mixture was pressed. Calcium carbonate (1%) was added to adjust the pH. Polyethylene bags of size 25 x 15 cm were filled with each substrate, each bag weighing 300 g, and tightly packed together. The neck of the bag was prepared by using heat resistant PVC (Poly Vinyl Chloride) tube. The opening was covered with a cotton plug and wrapped with aluminum foil. The neck served as the opening through which the spawn was introduced. The bags were sterilized in an autoclave at 121^{o}C for 15 min, and allowed to cool to room temperature.

After cooling to room temperature of 30 ± 2 ^{o}C, each of the bags was inoculated separately with 30 g of the inoculum (10% by weight of the substrate in each bag) for two of the selected cultivated mushrooms (*P. ostreatus* and *P. pulmonarius*) through the neck with three replicates per treatment. They were incubated in the dark room for 30 days from the day of spawning to allow the mycelia to ramify in the substrates. The fully colonized bags were brought out for weighing with an electronic weighning balance and then transferred into the cropping house where fruiting bodies emerge and then harvested manually by gently twisting the base of each fruiting body without leaving any remnant on the bags to avoid rotting. These procedures were carried out on each of the four substrates at the three levels of weeks of composting intervals (WCI) of 4, 8 and 12.

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3.6 Cultivation of the sclerotia of *P. tuber-regium.*

The sawdusts (substrates) of mango, cassia, neem and their mixed bed were prepared. About 300 g each of the various sawdust types were packed in polyethylene bags. The neck was made with heat resistant polyvinyl chloride (PVC) pipe plugged with cotton wool and covered with aluminum foil. These bags were also sterilized in an autoclaved at 121 ^{o}C for 15 min, allowed to cool down to ambient temperature of 30 ± 2 ^{o}C and thereafter inoculated with the freshly prepared spawn of *P. tuber-regium*. The spawned substrate bags were incubated in the dark at 30±2 ^{o}C. Each treatment was replicated three times.

About 3½ months after spawning, harvesting of the sclerotia was done. Sclerotia were harvested from the substrates when the substrate moisture and the mycelium were dried up. Substrates clinging to the sclerotia were removed and the sclerotia were weighed.

3.7 Evaluation of growth and yield characters in *Pleurotus ostreatus* and *P. pulmonarius*

The following data were collected on the mushroom: number of fruiting bodies (NF), fruit weight (FW), average fruit weight, width of pileus, length of stipe, primordial initiation and days to full colonization, biological and production efficiencies.

i. **Number of Fruiting Bodies:**

Fully opened fruiting bodies of *P. ostreatus* and *P. pulmonarius* were harvested per flush by gently twisting their base without leaving remnants on the bags and recorded.

ii. **Fruit weight**

The weight of the harvested fruiting bodies of *P. ostreatus, P. pulmonarius* and the sclerotia of *P. tuber-regium* were taken with a sensitive weighing balance and recorded.

iii. **Average fruit weight**

This was calculated as the ratio of total fruit weight to the total number of fruits.

$$\text{Average fruit weight} = \frac{\text{Total fruit weight}}{\text{Total number of fruits harvested}}$$

iv. **Width of pileus**

With the aid of a slide calliper, the width or diameter of the pileus of *P. ostreatus* and *P. pulmonarius* were measured and recorded.

v. **Length of stipe**

The length of stipe of *P. ostreatus* and *P. pulmonarius* were taken using a ruler.

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vi. **Primordial initiation**

This was carried out by counting the number of days to first appearance of mushroom primordia (pin-head formation).

vii. **Biological efficiency**

This was calculated by using the formula:

$$\text{Biological efficiency (\%)} = \frac{\text{Total biological yield (g)}}{\text{Total substrates used (g)}} \times 100$$

viii. **Production efficiency**

This was calculated as follow:

$$\text{Production efficiency} = \frac{\text{Mushroom life weight (g)}}{\text{Substrate weight before cropping (g)}} \times 100$$

where; **mushroom life weight** is the total weight of mushrooms harvested till the nutrients in the substrate were expended, while the **substrate weight before cropping** was the weight of the substrate taken just before it was transferred to the cropping house.

3.7.1 Data analysis

The data were analysed using descriptive statistics and ANOVA. Significant means were separated using Duncan's multiple range test (DMRT) at 5% level of probability (Gomez and Gomez, 1984).

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CHAPTER FOUR

RESULTS

4.1 Preparation of spawn

The various stages of spawn preparation and growth are shown in plates 4.1- 4.3.

4.2 Effects of different substrate types on the growth of mushroom mycelium and fruit body production.

4.2.1 Effects of different substrate types on the growth of the mycelia of *P. ostreatus, P. pulmonarius* and *P. tuber-regium.*

It was observed that all the substrates used for this study supported the growth of the mycelia of the three tested cultivated mushrooms. Their mycelia grew well on all the tested substrates (Plate 4.4).

4.2.2 Effects of different substrate types on the fruiting bodies production of *P. ostreatus* and *P. pulmonarius.*

There were fruiting bodies yield for the two tested mushrooms on the substrates during cultivation (Plate 4.5). The mean square result of analysis of variance (ANOVA) of two mushroom varieties of the mushrooms above grown on four substrates is presented in Table 4.1. The effect recorded high significant mean squares for all the parameters except the width of pileus which was not significant. Likewise, the mean square value for the interaction of substrates x varieties was found not to be significant in the number of days for full mycelia colonization and days to primordia initiation. Significant means were observed for the three-way interaction effect of substrates x varieties x weeks of composting intervals (WCI) except days for full mycelia colonization that was significant at $p < 0.05$ (Table 4.1).

Figure 4.1 represents the effect of weeks of composting intervals (WCI) on the yield parameters of *Pleurotus ostreatus* and *P. pulmonarius*. As WCI increases, the yield parameters also increased.

Figure 4.2 represents the effect of weeks of composting intervals (WCI) on the growth parameters of *Pleurotus ostreatus* and *P. pulmonarius* on the biological efficiency (BE), production efficiency (PE), mycelia extension, days to full mycelia colonization and extension per day. While there was a progressive increase in PE and BE, the number of days for full mycelia colonization revealed a progressive decrease in values with the highest as 24.83 days at 4WCI and the least as 20.13 days at 12WCI. Similar trend was observed in the number of days to primordial initiation.

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Plate 4.1: Spawn preparation (Sorghum seeds in bottles ready for sterilization)

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Plate 4.2: Growth of spawn in bottles one week after inoculation

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Plate 4.3: Mushroom spawn fully grown in bottles and ready for use

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Plate 4.4: Growth of mycelia on the different substrate types

A: Mycelial growth on mango (*Mangifera indica*) sawdust

B: Mycelia growth on cassia (*Senna siamea*) sawdust

C: Mycelial growth on neem (*Azadirachta indica*) sawdust

D: Mycelial growth on the mixed bed

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Plate 4.5: The fruiting bodies of *Pleurotus ostreatus* on substrates

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Table 4.1**:** Interactions of weeks of composting intervals, substrates, varieties and their combinations on growth and yield of *Pleurotus ostreatus* and *P. pulmonarius*.

Source of variation	DF	Number of fruits	Fruit weight	Average fruit weight	Width of pileus	Length of stipe	Biological Efficiency	Production Efficiency	Mycelial extension	Full mycelial colonization	Primordial initiation	Extension per day
	2	662.35**	15399.59**	11.14**	8.68**	5.38**	13755.44**	3975.65**	220.08**	138.29**	307.04**	0.15**
Substrate (S)	3	7.71**	577.39**	9.95**	0.03	0.60**	576.44**	127.51**	1.92**	10.87**	12.76**	0.05**
Varieties (V)	1	435.31**	404.29**	79.91**	16.72**	24.04**	323.30**	120.67**	14.37**	37.56**	25.68**	0.02**
S x WCI	6	6.27**	95.12**	2.51**	1.56**	0.41**	91.32**	25.07**	10.45**	2.44**	5.23**	0.00**
V x WCI	2	115.79**	43.76**	12.46**	2.16**	2.50**	45.83**	13.70**	25.68**	4.26**	4.68**	0.03**
S x V	3	5.46**	385.41**	7.66**	1.03**	0.77**	367.07**	107.03**	5.11**	0.74	1.16	0.02**
S x V x WCI	6	9.79**	194.09**	3.42**	1.58**	1.54**	162.63**	39.44**	2.02**	1.89*	3.94**	0.00**
Error	48	1.18	4.36	0.33	0.04	0.04	2.03	1.33	0.32	0.71	0.75	0.00
Total	71											

** Significant at 1% probability

* Significant at 5% probability

WCI: Weeks of Composting Intervals

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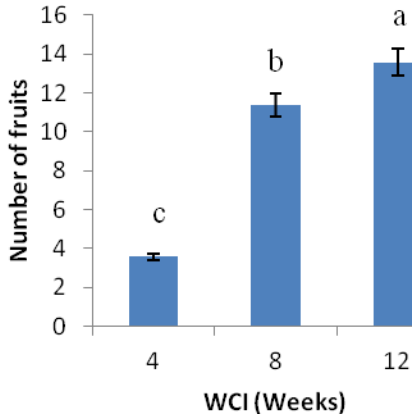
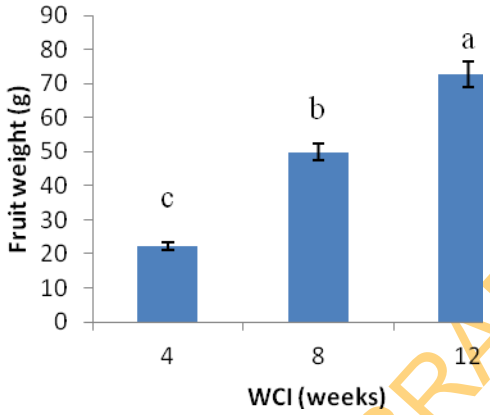
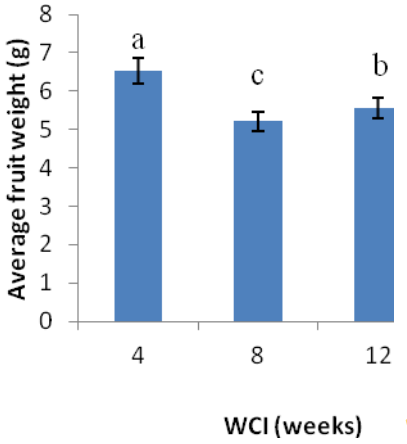
(a) Number of fruits



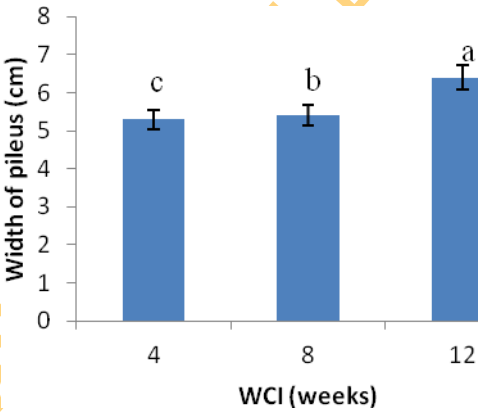
(b) Fruit weight (g)



© Average fruit weight (g)



(d) Width of pileus (cm)



(e) Length of stipe (cm)

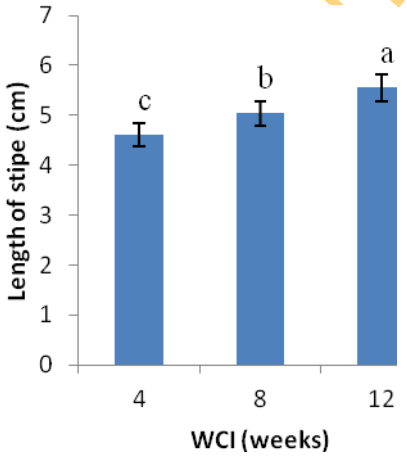


Fig. 4.1: Combined effect of weeks of composting intervals (WCI) on the yield parameters of *Pleurotus ostreatus* and *P. pulmonarius*.

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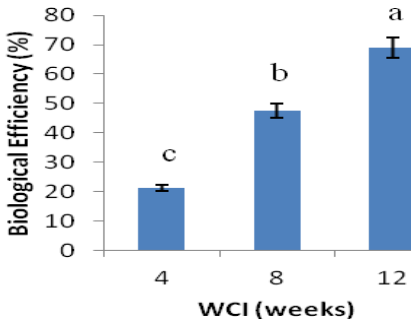
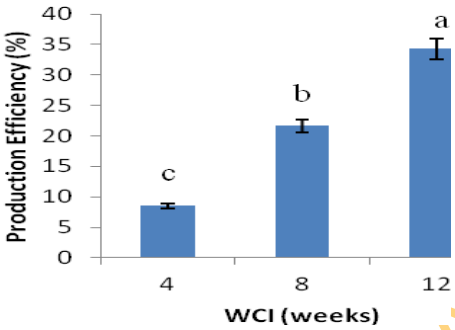
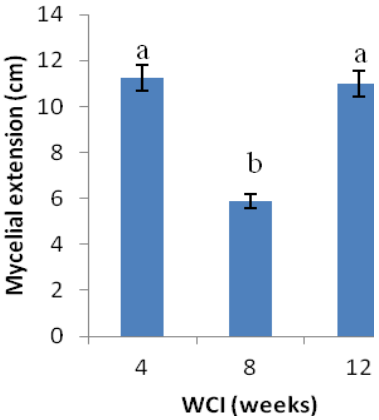
(a) Biological Efficiency (%)



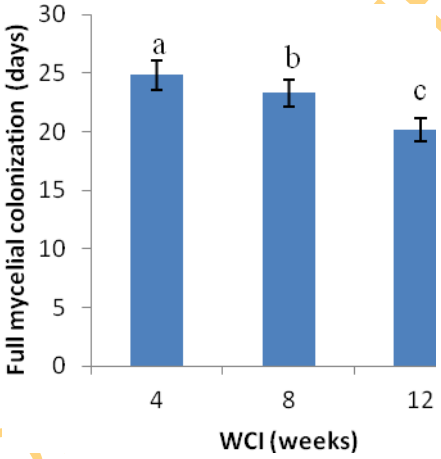
(b) Production Efficiency (%)



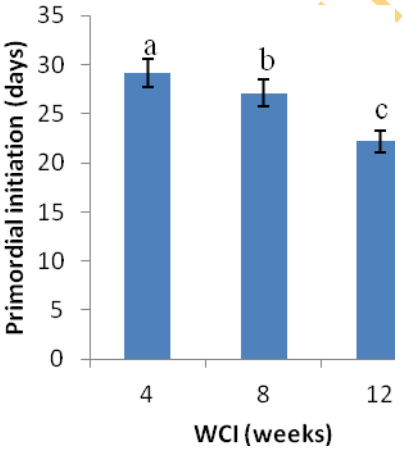
© Mycelial extension (cm)



(d) Full mycelia colonization (days)



(e) Primordial initiation (days)



(f) Extension per day (days)

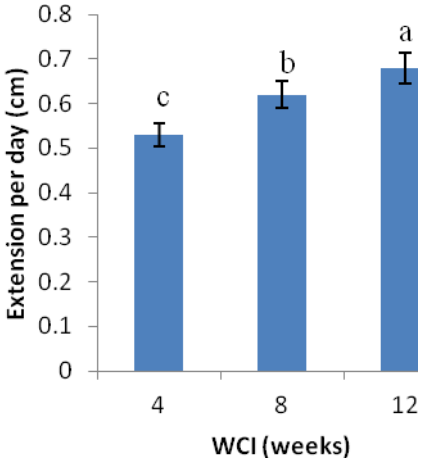


Fig. 4.2: Combined effect of weeks of composting intervals (WCI) on the growth parameters of *Pleurotus ostreatus* and *P. pulmonarius*.

The effect of substrate types on the yield of *Pleurotus ostreatus* and *P. pulmonarius* is represented in Table 4.2. Mango, as a substrate, produced the highest number of fruits (10.17) followed by mixed bed and neem which were not significantly different from each other (i.e. 9.72 and 9.56 respectively). Cassia produced the least (8.61). The heaviest fruit weight was observed on mixed bed (52.50 g) followed by cassia (50.45 g) and mango (50.23g) which were not significantly different from each other, while the lowest was produced by neem (39.92 g). Cassia recorded the highest average fruit weight (6.55 g) but was not significantly different from mixed bed, while mango produced the least (5.08 g) which was also comparable to what was produced by neem (5.18 g). The longest width of pileus was observed on neem (5.73 cm). However, this was not significantly different from all other substrates.

The longest length of stipe was obtained from the mixed bed (5.21 cm) which was comparable to what was obtained from cassia and mango but the least was from neem (4.79 cm). Similar trend was observed in both biological and production efficiencies. The fastest mycelia extension was observed in neem (9.73 cm) which was not significantly different from that of the mixed bed (9.59 cm). The least was observed on cassia (9.04 cm) but was not significantly different from mango (9.18 cm). Cassia recorded the longest number of days for full mycelia colonization (23.50 days) followed by the mixed bed (22.61 days), as a substrate, while the significantly lowest number of days was observed in mango (21.72 days). The longest number of days for mushroom primordial initiation was observed in cassia (27.28 days) followed by neem (26.22 days). The least was observed on the mixed bed (25.44 days) which was not significantly different from mango (25.56 days). The fastest mycelia extension per day was observed on mango (0.66 cm) followed by cassia (0.64 cm) and the mixed bed (0.57 cm) while the least was on neem (0.55 cm).

Figure 4.3 showed the effects of varieties on the yield parameters of *P. ostreatus and P. pulmonarius*. In terms of number of fruits, *P. ostreatus* was more (11.97) than *P. pulmonarius* (7.06). The same trend was observed in the fruit weight. In contrast, the average fruit weight of *P. pulmonarius* (6.82 g) was more than that of *P. ostreatus* (4.71 g). This trend was also observed in the width of pileus and length of stipe.

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Table 4.2: The effect of substrate types on the yield and growth parameters of *Pleurotus ostreatus* and *P. pulmonarius*.

Substrate	Number of fruits	Fruit weight (g)	Average fruit weight (g)	Width of pileus (cm)	Length of stipe (cm)	Biological Efficiency (%)	Production Efficiency (%)	Mycelial extension (cm)	Full mycelial colonization (days)	Primordial initiation (days)	Extension per day (cm)
Mango	10.17a	50.23b	5.08b	5.72a	5.12a	47.84b	23.05a	9.18b	21.72c	25.56c	0.66a
Cassia	8.61b	50.45b	6.55a	5.64a	5.13a	48.05b	22.66a	9.04b	23.50a	27.28a	0.64b
Neem	9.56a	39.92c	5.18b	5.73a	4.79b	37.50c	17.50b	9.73a	23.17ab	26.22b	0.55d
Mixed Bed	9.72a	52.50a	6.25a	5.71a	5.21a	50.03a	22.71a	9.59a	22.61b	25.44c	0.57c

Means with the same letter along the column are not significantly different from one another at $p \leq 0.05$

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Figure 4.4 represents the effects of varieties on the growth parameters of *P. ostreatus and P. pulmonarius*. Higher biological and production efficiencies were observed in *P. ostreatus* than in *P. pulmonarius*. Number of days for full mycelia colonization observed in *P. ostreatus* was more than that of *P. pulmonarius*. However, total mycelial extension, number of days for primordial initiation and average extension per day were more in *P. pulmonarius* than in *P. ostreatus.*

Tables 4.3- 4.5 showed the performance of the substrates as affected by the number of weeks of composting interval (WCI). It was generally observed that all the parameters were significantly highest at 12WCI except mycelia extension, days to full mycelia colonization and primordial initiation.

Table 4.3 showed the performance of the substrates as affected by the number of weeks of composting interval (WCI) on the number of fruits, fruit weight, average fruit weight, width of pileus and length of stipe of *Pleurotus ostreatus* and *P pulmonarius*. At 4WCI, the highest number of fruits (NF) were observed in mango (5.00) followed by mixed bed and cassia which were not significantly different from each other (3.83 and 2.83) while the least number of fruits was observed in neem (2.67). At 8WCI, the mixed bed produced the highest NF (12.17) followed by mango and cassia substrates (11.17 each) with the least from neem (11.00). Neem and mango produced the highest NF (15.00 and 14.33 respectively) at 12WCI followed by the mixed bed (13.17) while the least was observed in cassia (11.83). The heaviest fruiting body weight (FW) was observed in the mixed bed (27.75 g) at 4WCI which was not significantly different from what was obtained in mango (25.50 g). This was followed by comparable results in cassia and neem (18.68 g and 16.75 g respectively). At 8WCI, mixed bed (56.47 g) produced the heaviest FW followed by cassia (52.79 g) which was not significantly different from what was produced by mango (50.49 g). The smallest FW was obtained in neem (39.88 g) at 8WCI. Cassia substrate (79.90 g) produced the heaviest FW at 12WCI followed by comparable results from mango and mixed bed (74.70 g and 73.28 g respectively). The least was produced by neem (63.12 g).

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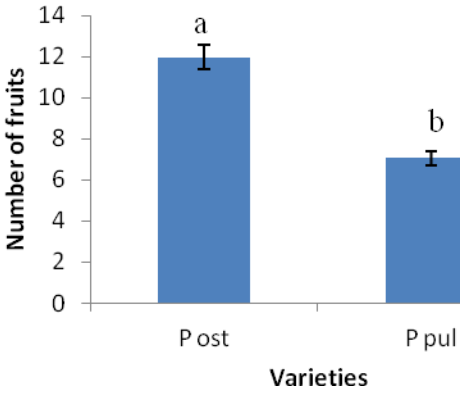
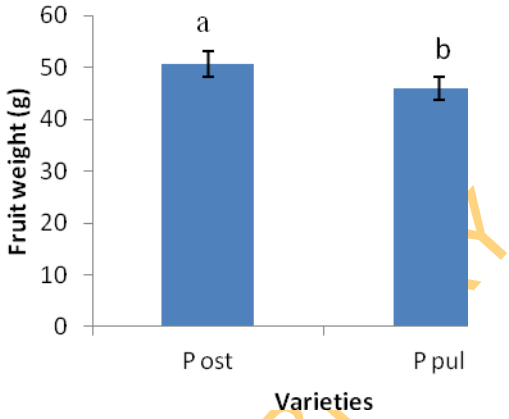
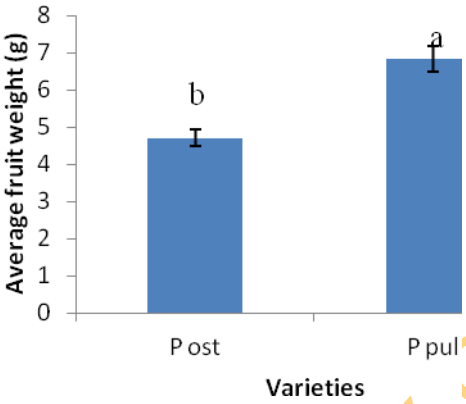
(a) Number of fruits



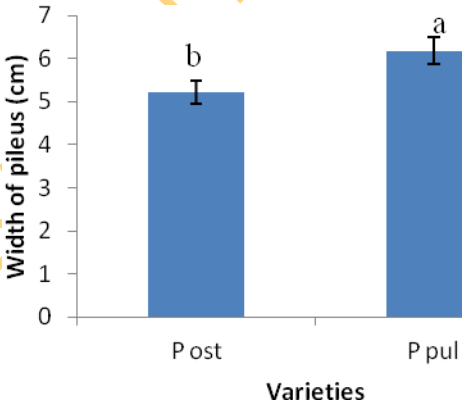
(b) Fruit weight (g)



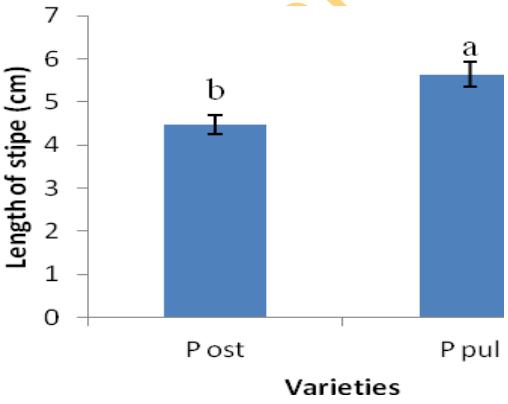
© Average fruit weight (g)



(d) Width of pileus (cm)



(e) Length of stipe (cm)



P ost: *Pleurotus ostreatus* P pul: *Pleurotus pulmonarius*

Fig. 4.3: The effects of varieties on the yield parameters of *Pleurotus ostreatus and P. pulmonarius*

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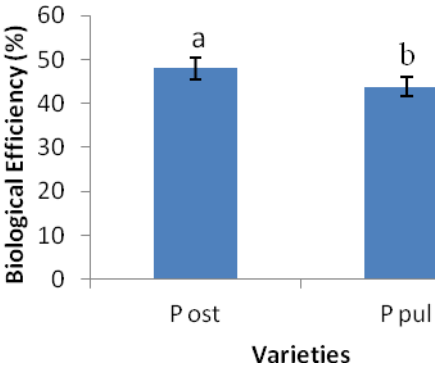
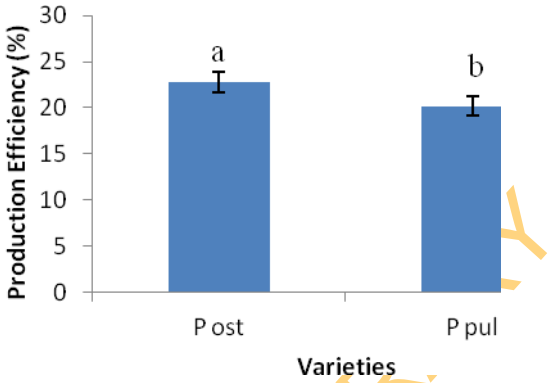
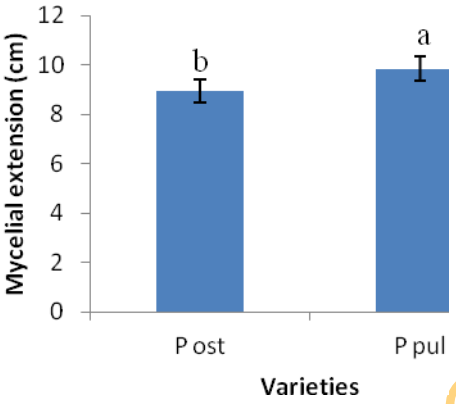
(a) Biological Efficiency (%)



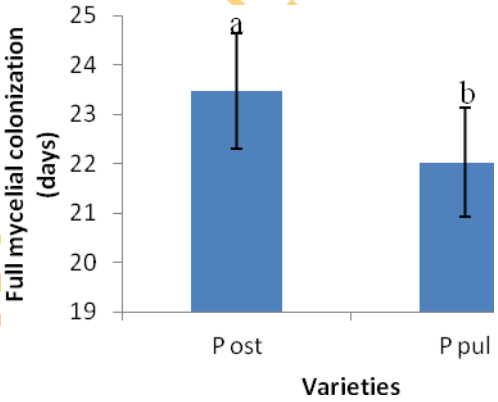
(b) Production Efficiency (%)



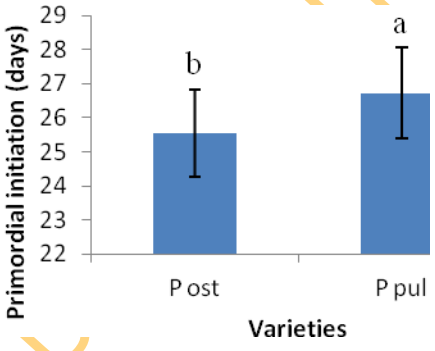
© Mycelial extension (cm)



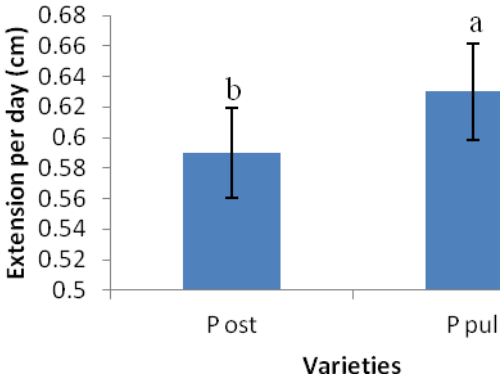
(d) Full mycelia colonization (days)



(e) Primordial initiation (days)



(f) Extension per day (days)



P ost: *Pleurotus ostreatus* P pul: *Pleurotus pulmonarius*

Fig. 4.4: The effects of varieties on the growth parameters of *Pleurotus ostreatus and P. pulmonarius*

The highest average fruit weight, at 4WCI, was obtained in mixed bed (7.31g) which was comparable with what was obtained in neem (6.81g). This was followed by cassia (6.68 g) while mango substrate produced the lowest (5.31 g). The largest width of pileus obtained at 4WCI in cassia and mango were not significantly different from each other (5.47 cm and 5.40 cm respectively). This was also followed by comparable results from neem and mixed bed (5.17 cm and 5.13cm respectively). At 8WCI, the widest pileus was produced by cassia (5.87 cm) followed by neem (5.65 cm) which was significantly different from mixed bed as substrate (5.23 cm). The shortest was obtained in mango (4.93 cm). Mango (6.83 cm) produced the largest width of pileus which was not significantly different from what was obtained in mixed bed (6.77 cm). This was followed by neem (6.37 cm) while least was observed in cassia (5.60 cm). In terms of length of stipe, the longest was produced on the mixed bed (4.83 cm) at 4WCI but this was not significantly different from what was obtained in mango (4.68 cm) and cassia (4.65 cm). The least was observed in neem (4.23 cm). At 8WCI, the longest was obtained on cassia (5.40 cm) comparable to what was produced by the mixed bed (5.22 cm) as substrates. This was followed by similar results from mango and neem (4.77 cm each). At 12WCI, the longest length of stipe was produced by mango (5.90 cm) followed by the mixed bed (5.57 cm) comparable with what was obtained in neem (5.38 cm). Cassia sawdust produced the least (5.33 cm).

Table 4.4 showed the performance of the substrate as affected by weeks of composting intervals (WCI) on the biological and production efficiencies of *Pleurotus ostreatus* and *P pulmonarius*. The biological efficiency (BE) was highest in mixed bed (26.51%) at 4WCI followed by mango and cassia (24.28% and 17.79% respectively). The least BE was observed in neem (15.95%). Mixed bed (53.78%) was the most biologically efficient substrate at 8WCI. This was followed by cassia and mango which were not significantly different from each other. Neem possessed the least BE of 37.84% at 8WCI. In contrast, cassia (76.10%) was the most biologically efficient substrate at 12WCI followed by mango (71.14%) comparable to the mixed bed (69.79%). The least was also observed in neem (58.69%) similar to what was observed in other WCI. The production efficiency (PE), as observed, in the mixed bed (10.77%) was the greatest at 4WCI followed by mango (9.66%). This was followed by comparable results from cassia and neem (7.14% and 6.54% respectively). The same trend was observed at 8WCI.

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Table 4.3: The performance of the substrate as affected by weeks of composting intervals (WCI) on the yield parameters of *Pleurotus ostreatus* and *P. pulmonarius*.

Substrate x WCI	Number of fruits			Fruit weight (g)			Average fruit weight (g)			Width of pileus (cm)			Length of stipe (cm)		
	Weeks			Weeks			Weeks			Weeks			Weeks		
	4	8	12	4	8	12	4	8	12	4	8	12	4	8	12
Mango	5.00a	11.17ab	14.33a	25.50a	50.49bc	74.70b	5.31c	4.72b	5.21bc	5.40a	4.93d	6.83a	4.68a	4.77b	5.90a
Cassia	2.83b	11.17ab	11.83c	18.68b	52.79b	79.90a	6.68b	6.08a	6.88a	5.47a	5.87a	5.60c	4.65ab	5.40a	5.33c
Neem	2.67bc	11.00b	15.00a	16.75b	39.88c	63.12c	6.81ab	4.24b	4.51c	5.17b	5.65b	6.37b	4.23b	4.77b	5.38bc
Mixed bed	3.83b	12.17a	13.17b	27.75a	56.47a	73.28bc	7.31a	5.82ab	5.63b	5.13b	5.23c	6.77a	4.83a	5.22ab	5.57b

Means with the same letter along the column are not significantly different from one another at $p \leq 0.05$.

WCI: Weeks of composting intervals

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However, at 12WCI, PE was greatest in cassia (37.65%) which was not significantly different from what was obtained in mango (36.91%). Also, the least was observed in neem (28.93%).

Table 4.5 showed the performance of the substrate as affected by weeks of storage compositing interval (WCI) on the mycelia extension, days to full mycelia colonization, days to mushroom primordial initiation and extension per day of *Pleurotus ostreatus* and *P. pulmonarius*. The longest mycelia extension was observed in neem (13.07 cm) at 4WCI followed by followed by comparable results from mixed bed and mango (11.38 cm and 11.36 cm respectively). The least was obtained in cassia (9.32 cm). Neem (6.44 cm) also produced the longest mycelia extension at 8WCI which, however, was not significantly different from what was obtained in mango (5.82 cm). A comparable result was obtained in cassia (5.69 cm) while the least was produced by the mixed bed (5.63cm). At 12WCI, cassia (12.12 cm) produced the longest mycelia extension which was not significantly different from what was obtained in the mixed bed (11.75 cm). However, comparable results were observed in mango and neem (10.38 cm and 9.69 cm respectively) (Table 4.5).

In terms of full mycelia colonization, at 4WCI, the longest number of days was observed in neem (25.67 days) followed by comparable results from other substrates in the order cassia> mixed bed> mango (25.17 days, 24.50 days and 24.00 days respectively). At 12WCI, the longest was observed on cassia (21.67 days) which was not significantly different from what was observed in neem (20.67 days). This was followed by comparable results from mixed bed and mango (19.50 days and 18.67 days respectively).

The primordial was initiated at the shortest number of days, at 4WCI, in mango (28.17 days) which was not significantly different from what was observed in the mixed bed (28.67 days) and comparable to cassia (29.67 days). However, the longest was obtained in neem (30.00 days). All the substrates produced comparable results at 8WCI (Table 5) while at 12WCI, the shortest number of days for the mushroom primordial was observed in the mixed bed (20.67 days). This was followed by comparable results from mango and neem (21.83 days and 21.50 days respectively). The longest was observed in cassia (24.67 days).

The longest average extension per day was observed in mango (0.58 cm) at 4WCI followed by neem (0.55 cm) which was also significantly different from the mixed bed (0.55 cm)

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Table 4.4: The performance of the substrate as affected by weeks of composting intervals (WCI) on the biological and production efficiencies of *Pleurotus ostreatus* and *P. pulmonarius*.

Substrate x WCI	Biological Efficiency (%)			Production Efficiency (%)		
	Weeks			Weeks		
	4	8	12	4	8	12
Mango	24.28b	40.08c	71.14b	9.66b	22.58b	36.91a
Cassia	17.79c	50.28b	76.10a	7.14c	23.17ab	37.65a
Neem	15.95d	37.84d	58.69c	6.54c	17.01c	28.93c
Mixed Bed	26.51a	53.78a	69.79bc	10.77a	23.79a	33.59b

Means with the same letter along the column are not significantly different from one another at $p \leq 0.05$.

WCI: Weeks of composting intervals

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while the least was obtained in cassia (0.48 cm). At 8WCI, the same trend was observed (Table 5). Furthermore, mango substrate (0.73 cm) produced the longest but not stastistically different from what was obtained in neem (0.73 cm) at 12WCI. This was followed by the mixed bed (0.65 cm) which was significantly different from what was obtained in cassia (0.63 cm), as the least average extension per day.

Table 4.6 showed the mean performance of two mushroom varieties as affected by the number of weeks of composting intervals (WCI) on the number of fruits, fruit weight, average fruit weight, width of pileus and length of stipe. Of the two mushroom varieties, *Pleurotus ostreatus* produced more number of fruits and fruit weights than *P. pulmonarius* at the three levels of the WCI while in terms of average fruit weight, width of pileus and length of stipe, *P. pulmonarius* was more than *P. ostreatus*.

At 4WCI, the number of fruits produced by *P. osreatus* was 4.17, which is statistically higher than what was obtained in *P. pulmonarius* (3.00). *P. ostreatus* produced 16.25 fruits while *P. pulmonarius* produced 6.50 at 8WCI. At 12WCI, while *P. ostreatus* produced 15.50 fruits, *P. pulmonarius* produced 11.67. At 4WCI, the total fruit weight (FW) obtained in *P. osreatus* was 23.73 g while that of *P. pulmonarius* was 20.16 g. The total FW observed in *P. osreatus* at 8WCI was 53.83 g while 45.98 g was observed in *P. pulmonarius.* Furthermore, the FW observed in *P. osreatus* and *P. pulmonarius* were significantly different from each other (74.37 g and 71.13 g respectively).

The average fruit weight observed at 4WCI, in *P. pulmonarius* was more than what was obtained in *P. ostreatus* 7.16 g and 5.90 g respectively). This trend was also observed at 4 and 8WCI (Table 6). At 8WCI, *P. pulmonarius* produced an average fruit weight of 7.10g which was significantly different from what was obtained in *P. ostreatus* (3.33 g). At 12WCI, 6.02 g of *P. pulmonarius* was observed which was significantly different from what was observed in *P. ostreatus* (4.19 g). The width of pileus, as observed in *P. pulmonarius* at 4WCI, was 6.03 cm which was significantly different from what was observed in *P. ostreatus* (4.56 cm). At 8WCI also, the width of pileus observed in *P. pulmonarius* (5.98 cm) was significantly different from what was observed in *P. ostreatus* (4.86 cm) and at 12WCI, the larger was also observed in *P. pulmonarius* (6.54 cm) followed by *P. ostreatus* (6.24 cm). The longer length of stipe was recorded in *P. pulmonarius* (4.94 cm) followed by what was obtained in *P. ostreatus* (4.26 cm) at 4WCI. At 8WCI, it was also longer in *P. pulmonarius* than in *P. ostreatus* (5.48 cm and 4.59 cm

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Table 4.5: The performance of the substrates as affected by weeks of composting intervals (WCI) on the growth parameters of *Pleurotus ostreatus* and *P. pulmonarius*.

Substrate x WCI	Mycelia Extension (cm)			Full Mycelia Colonization (days)			Primordial Initiation (days)			Extension per Day (cm)		
	Weeks			Weeks			Weeks			Weeks		
	4	8	12	4	8	12	4	8	12	4	8	12
Mango	11.36b	5.82a	10.38b	24.00b	22.50b	18.67b	28.17b	26.67a	21.83b	0.58a	0.68a	0.73a
Cassia	9.32c	5.69ab	12.12a	25.17ab	23.67a	21.67a	29.67ab	27.50a	24.67a	0.48d	0.56d	0.63c
Neem	13.07a	6.44a	9.69b	25.67a	23.17ab	20.67a	30.00a	27.17a	21.50bc	0.55b	0.65b	0.73a
Mixed Bed	11.38b	5.63b	11.75a	24.50b	23.83a	19.50b	28.67b	27.00a	20.67c	0.50c	0.58c	0.65b

Means with the same letter along the column are not significantly different from one another at $p \leq 0.05$.

WCI: Weeks of composting intervals

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respectively). At 12WCI while 6.49cm was observed for *P. pulmonarius*, what was obtained as the length of stipe for *P. ostreatus* was 4.60 cm.

Fig. 4.5 represents the mean performance of two mushroom varieties as affected by the number of weeks of composting intervals (WCI) on the biological and production efficiencies of *Pleurotus ostreatus* and *P. pulmonarius*. Both Biological Efficiency (BE) and Production Efficiency (PE) followed the same trend. At 12WCI, *P. ostreatus* had the best BE (70.11%) followed by *P. pulmonarius* also at the same WCI (67.74%). However, the least was observed in *P. pulmonarius* at 4WCI (19.67%). Furthermore, the greatest PE was recorded in *P. ostreatus* at 12WCI (35.23%). This was followed by *P. pulmonarius* at the same WCI (33.31%). The least was also recorded in *P. pulmonarius* at 4WCI (7.76%).

Table 4.7 represents the mean performance of two mushroom varieties as affected by weeks of composting intervals period (WCI) on the mycelia extension, days to full mycelia colonization, days to mushroom primordial initiation and extension per day of *Pleurotus ostreatus* and *P. pulmonarius*. The longer mycelia extension (12.65cm) was obtained at 4WCI in *P. pulmonarius* which was significantly different from what was observed in *P. ostreatus* (9.91cm). In contrast, *P. ostreatus* (6.65cm) produced the longer mycelia extension which was significantly different from what was obtained in *P. ostreatus* at 8WCI while at 12WCI, *P. pulmonarius* produced a longer extension than what was observed in *P. ostreatus* (11.63cm and 10.34cm respectively).

The shortest number of days observed for the full mycelia colonization was in *P. ostreatus* (18.92 days) at 12WCI while 21.33 days was observed for *P. pulmonarius*. At 8WCI, a shorter number of days (22.83 days) were also observed in *P. ostreatus* as 23.75 days was observed for *P. pumonarius.* Shorter number of days (24.33 days) was observed for *P. ostreatus* as 25.33 days was recorded for *P. pulmonarius* at 4WCI (Table 4.7). At 12WCI, the number of days for primordial initiation was shorter in *P. ostreatus* than in *P. pulmonarius* (21.50 days and 22.83 days respectively). The same trend was observed at 8WCI while there was no significant difference between the two mushroom varieties at 4WCI; 29.00 days for *P. ostreatus* and 29.50 days for *P. pulmonarius*. In terms of average extension per day, it was longer in *P. pulmonarius* than in *P. ostreatus* (0.58 cm and 0.47 cm respectively). Similar trend was observed at 8WCI, 0.62cm was recorded for *P. pulmonarius* while 0.61 cm was recorded for *P. ostreatus*. However,

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Table 4.6: The mean performance of two mushroom varieties (*Pleurotus ostreatus* and *P. pulmonarius*) as affected by weeks of composting intervals (WCI) on the yield parameters.

Varieties x WCI	Number of fruits			Fruit weight (g)			Average fruit weight (g)			Width of pileus (cm)			Length of stipe (cm)		
Weeks	4	8	12	4	8	12	4	8	12	4	8	12	4	8	12
P ost	4.17a	16.25a	15.50a	23.73a	53.83a	74.37a	5.90b	3.33b	4.91b	4.56b	4.86b	6.24b	4.26b	4.59b	4.60b
P pul	3.00b	6.50b	11.67b	20.61b	45.98b	71.13b	7.16a	7.10a	6.20a	6.03a	5.98a	6.54a	4.94a	5.48a	6.49a

Means with the same letter along the column are not significantly different from one another at $p \leq 0.05$.

P ost: *Pleurotus ostreatus*

P pul: *Pleurotus pulmonarius*

WCI: Weeks of composting intervals

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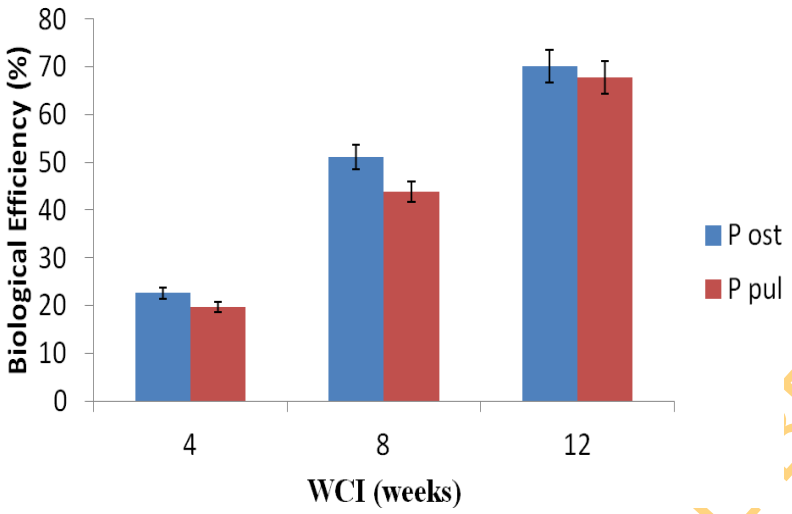
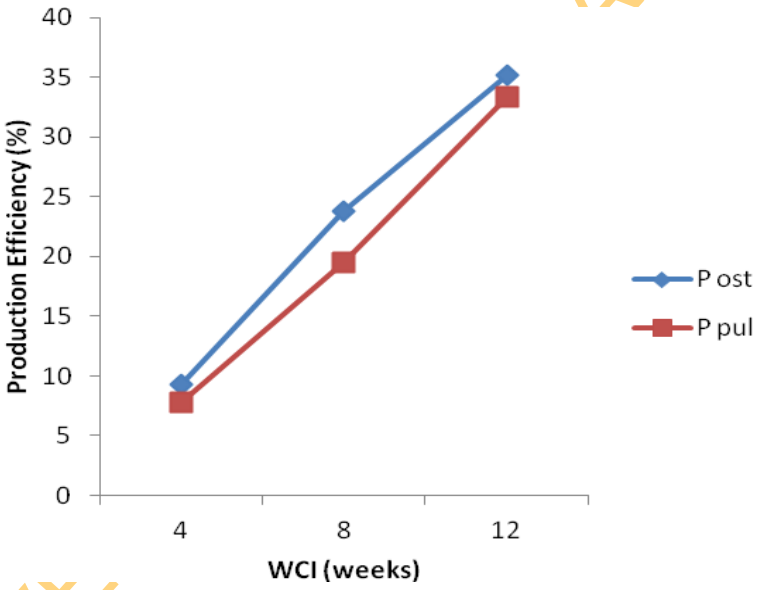
(a) Biological Efficiency (%)



(b) Production Efficiency (%)



P ost: *Pleurotus ostreatus* P pul: *Pleurotus pulmonarius*

Fig. 4.5: Performance of the substrate as affected by weeks of composting interval (WCI) on the biological and production efficiencies of *Pleurotus ostreatus* and *P. pulmonarius*.

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However, at 12WCI it was longer in *P. ostreatus* (0.70 cm) than in *P. pulmonarius* (0.67 cm).

Table 4.8 represents the effects of substrates x varieties interaction on the number of fruits, fruit weight, average fruit weight, width of pileus and length of stipe of *Pleurotus ostreatus* and *P pulmonarius*. The highest number of fruits (NF) observed during this study was in *P. ostreatus* as produced by neem (12.78) though not significantly different from what was obtained in mango (12.22) and also comparable to what was obtained in the mixed bed (11.78). The least was observed in by cassia (11.11). Comparable results were obtained on *P. pulmonarius* grown on mango and mixed bed (8.11 and 7.67 respectively) followed by 6.33 and 6.11 NF from neem and cassia respectively which were not significantly different from each other. *P. ostreatus* grown on mango produced the most significant fruit weight (59.52 g) followed by the mixed (52.37 g) which was comparable to what was obtained in cassia (51.04 g). The least was recorded in neem (39.66 g).

The highest weight in *P. pulmonarius* was recorded on the mixed bed (52.63 g) which was significantly different from what was observed in cassia (49.87 g). This was followed by comparable results from mango and neem (40.94 g and 40.18 g respectively). The average fruit weight observed in mixed bed (5.30 g) with respect to *P. ostreatus* production was highest but this was comparable to what was obtained in cassia (5.19 g). The least was also recorded in neem (3.48 g). The most significant average fruit weight was recorded in *P. pulmonarius* grown on cassia (7.91 g). This was followed by comparable results from mixed bed and neem (7.12 g and 6.89 g respectively). The least was obtained from *P. pulmonarius* cultivated on mango (5.27 g).

As observed, the largest width of pileus was recorded in *P. ostreatus* grown on mixed bed (5.47 cm) comparable to what was obtained in mango (5.34 cm). This was followed by the one grown on cassia (5.14 cm) which was significantly different from what was observed on neem (4.92 cm), as the least. In contrast, neem (6.53 cm) produced the largest width of pileus in terms of *P. pulmonarius* production followed by comparable results from cassia, mango and mixed bed (6.14 cm, 6.10 cm and 5.96 cm respectively). The longest length of stipe, in *P. ostreatus*, was produced by the mixed bed (4.87 cm) followed by mango (4.46 cm). Cassia and neem produced comparable results (4.31 cm and 4.30 cm respectively). The length of stipe observed in all the substrates with respect to *P. pulmonarius* production was significantly different from each other. It was in the order cassia> mango> mixed bed> neem (Table 4.8).

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Table 4.7: The mean performance of two mushroom varieties (*Pleurotus ostreatus* and *P. pulmonarius*) as affected by weeks of composting intervals (WCI) on the growth parameters.

Varieties x WCI	Mycelia Extension (cm)			Full Mycelia Colonization (days)			Primordial Initiation (days)			Extension per Day (cm)		
Weeks	4	8	12	4	8	12	4	8	12	4	8	12
P. ost	9.91b	6.65a	10.34b	24.33b	22.83b	18.92b	29.00a	26.08b	21.50b	0.47b	0.61b	0.70a
P. pul	12.65a	5.22b	11.63a	25.33a	23.75a	21.33a	29.25a	28.08a	22.83a	0.58a	0.62a	0.67b

Means with the same letter along the column are not significantly different from one another at $p \leq 0.05$.

P ost: *Pleurotus ostreatus*

P pul: *Pleurotus pulmonarius*

WCI: Weeks of composting intervals

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Table 4.8: Effect of substrates x varieties interaction on the yield parameters of *Pleurotus ostreatus* and *P. pulmonarius*

Substrate x Varieties	Number of fruits		Fruit weight (g)		Average fruit weight (g)		Width of pileus (cm)		Length of stipe (cm)	
	P. ost	P. pul	P. ost	P. pul	P. ost	P. pul	P. ost	P. pul	P. ost	P. pul
Mango	12.22a	8.11a	59.52a	40.94c	4.89ab	5.27c	5.34ab	6.10b	4.46b	5.78b
Cassia	11.11b	6.11b	51.04b	49.87b	5.19a	7.91a	5.14b	6.14b	4.31c	5.94a
Neem	12.78a	6.33b	39.66c	40.18c	3.48b	6.89bc	4.92c	6.53a	4.30c	5.29d
Mixed Bed	11.78ab	7.67a	52.37b	52.63a	5.30a	7.21b	5.47a	5.96b	4.87a	5.54c

Means with the same letter along the column are not significantly different from one another at $p \leq 0.05$.

P. ost: *Pleurotus ostreatus*

P. pul: *Pleurotus pulmonarius*

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Table 4.9 showed the effects of substrates x varieties interaction on the biological efficiency (BE) and production efficiency (PE) of *Pleurotus ostreatus* and *P. pulmonarius*. In *P. ostreatus*, the greatest BE was observed on mango (56.68%) followed by mixed bed (49.87%) comparable to what was obtained on cassia (48.61%). The least was recorded on neem (36.72%). However, in *P. pulmonarius*, the greatest BE was recorded in the mixed bed (50.18%) followed by cassia (47.50%). Both mango and neem produced comparable results (38.99% and 38.26% respectively). The greatest PE recorded for *P. ostreatus* was on mango (27.99%) followed by comparable results from cassia and mixed bed (22.94% and 22.86% respectively). The least was observed in neem also (17.30%). Mixed bed (22.57%) recorded the highest PE for *P. pulmonarius* which, however, was not significantly different from what was observed on cassia (22.37%). This was followed by comparable results from mango and neem (18.11% and 17.69% respectively).

Table 4.10 revealed the effect of substrates x varieties interaction on the mycelia extension, days to full mycelia colonization, days to mushroom primordial initiation and extension per day of *Pleurotus ostreatus* and *P. pulmonarius*. The longest mycelia extension in *P. ostreatus* was observed in neem (10.04 cm) followed by mixed bed (8.96 cm) which was comparable to that of cassia (8.50 cm). The least was recorded in mango (8.25 cm). In *P. pulmonarius*, the longest was obtained on mixed bed (10.21 cm) though not significantly different from the one grown on mango (10.11 cm) and comparable to what was recorded in cassia (9.58 cm). The least was found in neem (9.42 cm). *P. ostreatus* recorded the shortest number of days to full mycelia colonization in mango (20.89 days) followed by comparable results from mixed bed, neem and cassia (22.11 days, 22.22 days and 22.89 days respectively). The shortest number of days for full mycelia colonization in *P. pulmonarius* was observed in mango but comparable to that of mixed bed (22.56 days and 23.11 days respectively). This was followed by comparable results from cassia and neem (24.11 days each).

For primordial initiation, the shortest number of days observed for *P. ostreatus* was in mango (24.78 days) but not significantly different from what was obtained in the mixed bed (25.22 days). This was followed by neem (25.55 days) but comparable to what was recorded in cassia (26.56 days). The mixed bed (25.67 days) however, recorded the shortest number of days for primordial initiation in *P. pulmonarius* followed by mango (26.33 days) which was not

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Table 4.9: Effect of substrates x varieties interaction on the biological and production efficiencies of *Pleurotus ostreatus* and *P. pulmonarius*.

Substrates x Varieties	Biological Efficiency (%)		Production Efficiency (%)	
	P. ost	P. pul	P. ost	P. pul
Mango	56.68a	38.99c	27.99a	18.11b
Cassia	48.61bc	47.50b	22.94b	22.37a
Neem	36.72c	38.26c	17.30c	17.69b
Mixed Bed	49.87b	50.18a	22.86b	22.57a

Means with the same letter along the column are not significantly different from one another ($p \leq 0.05$).

P. ost: *Pleurotus ostreatus*

P. pul: *Pleurotus pulmonarius*

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significantly different from what was recorded for neem (26.89 days). Of all the substrates, cassia recorded the longest (28.00 days). The longest average extension per day was observed for *P. ostreatus* grown on mango (0.68 cm) followed by neem (0.60 cm). This was also significantly different from what was observed in cassia (0.56 cm) while the shortest was observed in mixed bed (0.54 cm). In *P. pulmonarius*, the longest average extension per day was recorded in neem (0.69 cm) followed by mango (0.64 cm). This was also significantly different from what was observed in mixed bed (0.61 cm) while the shortest was recorded in cassia (0.55 cm).

Table 4.11 represents the effects of substrates x varieties x week of composting interval on the number of fruits, fruit weight, average fruit weight and width of pileus of *Pleurotus ostreatus* and *P. pulmonarius*. At 4 weeks of composting interval (WCI), the most significant number of fruits (NF) was observed in *P. ostreatus* grown on mango (6.00) followed by *P. pulmonarius* grown on mango and mixed bed (4.00 each) though not significantly different from *P. ostreatus* grown on cassia, mixed bed and neem (3.67,3.67 and 3.33 respectively). The least NF was however obtained from *P. pulmonarius* grown on cassia and neem at 4WCI (2.00 each). Mixed bed (17.67) produced the highest NF of *P. ostreatus* though not significantly different from what was obtained on cassia (16.67) and comparable to that of mango and neem (15.33 each) at 8WCI. This was followed by results obtained from *P. pulmonarius* grown on mango, neem and mixed bed (7.00, 6.67 and 6.67 respectively) while the least was observed in *P. pulmonarius* cultivated on cassia (5.56). At 12WCI, *P. ostreatus* grown on neem (19.67) produced the highest NF followed by the result on mango (15.33). The least was also observed in *P. pulmonarius* grown on neem (10.33).

As observed, *P. pulmonarius* produced by the mixed bed (28.76±1.55 g) was the heaviest at 4WCI though not significantly different from what was obtained in *P. ostreatus* cultivated on mango (28.59±1.32 g). Furthermore, this was comparable to what was observed in *P. ostreatus* grown on mixed bed (26.74±1.44 g). *P. pulmonarius* was least produced on cassia (13.70±0.14 g). At 8WCI, the highest fruit weight (FW) was recorded on *P. ostreatus* grown on mango (65.02±1.83 g) followed by *P. ostreatus* cultivated on mixed bed (58.82±0.58 g) comparable to what was observed in *P. ostreatus* grown on cassia (56.44±1.65 g). *P. pulmonarius* produced by mango and *P. ostreatus* observed on neem (35.96±2.26 g and 35.05±0.76 g respectively) were the least at 8WCI. At 12WCI, *P. pulmonarius* produced on cassia (86.79±1.22 g) was the highest fruit weight (FW) comparable to the result of *P. ostreatus* grown on mango (84.94±1.23 g). This

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Table 4.10: Effect of substrates x varieties interaction on the growth parameters of *Pleurotus ostreatus* and *P. pulmonarius*

Substrates x Varieties	Mycelia Extension (cm)		Full Mycelia Colonization (days)		Primordial Initiation (days)		Extension per Day (cm)	
	P. ost	P. pul	P. ost	P. pul	P. ost	P. pul	P. ost	P. pul
Mango	8.25c	10.11a	20.89b	22.56b	24.78b	26.33b	0.68a	0.64b
Cassia	8.50bc	9.58ab	22.89a	24.11a	26.56a	28.00a	0.56c	0.55d
Neem	10.04a	9.42c	22.22a	24.11a	25.55a	26.89b	0.60b	0.69a
Mixed Bed	8.96b	10.21a	22.11ab	23.11b	25.22b	25.67c	0.54d	0.61c

Means with the same letter along the column are not significantly different from one another ($p \leq 0.05$).

P. ost: *Pleurotus ostreatus*

P. pul: *Pleurotus pulmonarius*

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was followed by *P. pulmonarius* grown on mixed bed (75.02±1.46 g). The least was recorded *P. pulmonarius* cultivated on neem (58.24±2.11 g).

The most significant average fruit weight (FW), at 4WCI, was observed in *P. pulmonarius* grown on neem (8.79±0.14 g). This was followed by *P. ostreatus* produced on mixed bed (7.39±0.87 g) with comparable results from *P. pulmonarius* grown on mixed bed and cassia (7.23±0.33 g and 6.85±0.07 g respectively). *P. ostreatus* produced on mango and neem were the least (4.86±0.84 g and 4.82±0.40 g respectively). At 8WCI, *P. pulmonarius* grown on cassia produced the heaviest average FW (8.74±0.96 g) though not significantly different from what was observed in *P. pulmonarius* cultivated on mixed bed (8.30±1.11 g). This was followed by *P. pulmonarius* grown on neem (6.18±0.82 g) while the least was observed in *P. ostreatus* cultivated on neem (2.30±0.22 g). At 12WCI, the heaviest average FW was recorded in *P. pulmonarius* grown on cassia (8.14±0.35 g) followed by *P. pulmonarius* grown on mixed bed and neem with *P. ostreatus* grown on caasia (6.09±0.39 g, 5.70±0.61 g and 5.63±0.47 g respectively). The least was observed in *P. ostreatus* cultivated on neem (3.31±0.08 g).

The largest widths of pileus, at 4WCI, were recorded in *P. pulmonarius* grown on cassia and neem (6.40 cm and 6.30 cm respectively). This was followed by *P. pulmonarius* grown on mixed bed (5.97 cm) while the least was observed in *P. ostreatus* cultivated on neem (4.03 cm). At 8WCI, the largest width of pileus was recorded in *P. pulmonarius* grown on neem (6.87 cm) but comparable to what was observed in the same mushroom cultivated on cassia (6.60 cm). This was followed by *P. pulmonarius* grown on mango and *P. ostreatus* cultivated on mixed bed (5.27 cm and 5.27 cm respectively). The least was observed in *P. ostreatus* grown on neem (4.43 cm). *P. pulmonarius* grown on mango (7.60 cm) recorded the largest width of pileus at 12WCI followed by *P. ostreatus* grown on mixed bed (6.83 cm) which was not significantly from *P. pulmonarius* produced by the same mixed bed (6.70 cm). However, the least was observed in *P. pulmonarius* cultivated on cassia (5.43 cm).

Table 4.12 showed the effects of substrates x varieties x weeks of composting intervals (WCI) interaction on the length of stipe, biological efficiency, production efficiency and mycelia extension of *Pleurotus ostreatus* and *P. pulmonarius*. As observed, the longest length of stipe, at 4WCI, was produced by *P. pulmonarius* grown on cassia (5.17 cm). However, this was not statistically different from what was recorded by the same mushroom cultivated on mixed bed and mango (5.17 cm and 5.03 cm respectively). This was followed by *P. ostreatus* grown on

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Table 4.11: Effects of substrates x varieties x weeks of composting intervals (WCI) interaction on the yield parameters of *Pleurotus ostreatus* and *P. pulmonarius*.

	Substrate	Number of fruits			Fruit weight (g)			Average fruit weight (g)			Width of pileus (cm)		
	WCI (Weeks)	4	8	12	4	8	12	4	8	12	4	8	12
	Mango	6.00a	15.33ab	15.33b	28.59a	65.02a	84.94ab	4.86d	4.26c	5.54bc	5.37c	4.60cd	6.07d
P. ost	Cassia	3.67b	16.67a	13.00c	23.6lb	56.44bc	73.01bcd	6.51bc	3.42cd	5.63b	4.53d	5.13bc	5.77de
	Neem	3.33b	15.33ab	19.67a	15.92cd	35.05f	68.02e	4.82d	2.30e	3.31d	4.03e	4.43cde	6.30cd
	Mixed Bed	3.67b	17.67a	14.00bc	26.74ab	58.82b	71.53d	7.39b	3.33cd	5.17bc	4.30de	5.27b	6.83b
	Mango	4.00b	7.00c	13.33c	22.40b	35.96f	64.46f	5.76cd	5.18c	4.87c	5.43c	5.27b	7.60a
P. pul	Cassia	2.00c	5.56d	10.67d	13.70d	49.13d	86.79a	6.85b	8.74a	8.14a	6.40a	6.60ab	5.43ef
	Neem	2.00c	6.67c	10.33de	17.58c	44.71e	58.24g	8.79a	6.18b	5.70b	6.30a	6.87a	6.43c
	Mixed Bed	4.00b	6.67c	12.33cd	28.76a	54.11c	75.02bc	7.23b	8.30a	6.09b	5.97b	5.20bc	6.70bc

Means with the same letter along the column are not significantly different from one another at $p \leq 0.05$.

P ost: *Pleurotus ostreatus*

P pul: *Pleurotus pulmonarius*

WCI: Weeks of composting interval

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mixed bed (4.50 cm) and *P. pulmonarius* cultivated on neem (4.40 cm). The least was recorded in *P. ostreatus* grown on cassia and neem (4.13 cm and 4.07 cm respectively). At 8WCI, *P. pulmonarius* grown on cassia (6.13 cm) also recorded the longest length of stipe but comparable with *P. pulmonarius* produced on neem (5.83 cm). This was followed by *P. ostreatus* cultivated on mixed bed (4.80 cm) while the least was recorded in *P. ostreatus* grown on neem (3.70 cm). *P. pulmonarius* grown on mango (7.47 cm) was observed to have the longest length of stipe at 12WCI followed by the same variety cultivated on cassia (6.53 cm). The least was *P. ostreatus* produced on cassia (4.13 cm).

The highest Biuological Efficiency (BE) observed at 4WCI was found in *P. pulmonarius* cultivated on mixed bed (27.55%) which, however, was comparable to what was recorded in *P. ostreatus* grown on mango and mixed bed (27.23% and 25.47% respectively). They were followed by comparable results of *P. ostreatus* grown on cassia and *P. pulmonarius* grown on mango (22.53% and 21.33% respectively). The least was observed in *P. pulmonarius* produced on cassia (13.04%). *P. ostreatus* grown on mango (61.92%) was the highest BE at 8WCI. This was followed by the same mushroom grown on mixed bed (56.02%). This was comparable to what was recorded on the same variety cultivated on cassia (53.77%) while the least was observed in *P. ostreatus* grown on neem (33.09%). At 12WCI, BE was highest in *P. pulmonarius* produced by cassia (82.66%) but comparable to *P. ostreatus* grown on mango (80.89%). This was followed by *P. pulmonarius* cultivated on mixed bed which was also comparable to what was recorded on *P. ostreatus* grown on cassia (71.45% and 69.53% respectively). The least was, however, observed in *P. pulmonarius* cultivated on neem (55.47%).

At 4WCI, *P. ostreatus* grown on mango (11.36%) was the recorded highest Production Efficiency (PE). However, this was comparable to the results of *P. pulmonarius* cultivated on mixed bed and *P. ostreatus* produced by the mixed bed also (11.13 and 10.42% respectively). This was followed by *P. ostreatus* observed on cassia (9.40%) while the least was observed in *P. pulmonarius* grown on cassia (4.89%). Mango (29.77%), as substrate, produced the highest PE at 8WCI with *P. ostreatus*. This was followed by the same variety producing comparable results on cassia and mixed bed (25.20% and 24.87% respectively). The least comparable PE values were obtained in *P. pulmonarius* grown on mango and *P. ostreatus* cultivated on neem (15.40% and 15.36% respectively). At 12WCI, *P. ostreatus* cultivated on mango (42.84%) also produced the highest PE. This was followed by comparable results of *P. ostreatus* grown on cassia with both

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P. pulmonarius and *P. ostreatus* cultivated on mixed bed (34.23%, 33.87% and 33.29% respectively).

At 4WCI, comparable values of the longest mycelial extension was recorded in *P. pulmonarius* grown on neem, mixed bed and mango with *P. ostresatus* produced by neem (13.25 cm, 13.13 cm, 13.08 cm and 12.88 cm respectively). This was followed by *P. pulmonarius* cultivated on cassia (11.13 cm) with the least produced by *P. ostreatus* also grown on cassia (7.50 cm). *P. ostreatus*, at 8WCI, recorded the longest mycelia extension on neem (8.25 cm) while all others showed no significant difference. At 12WCI, *P. pulmonarius* grown on mixed bed (12.25 cm) produced the longest mycelial extension but comparable to the same mushroom cultivated on cassia, *P. ostreatus* grown on cassia also with *P. pulmonarius* produced by mango (12.13 cm, 12.11 cm and 11.75 cm respectively). This was followed by comparable results of *P. ostreatus* grown on mixed bed (11.25 cm) and *P. pulmonarius* on neem (10.38 cm). The least comparable results were recorded in *P. ostreatus* cultivated on mango and neem (9.00 cm each).

Table 4.13 showed the effects of substrates x varieties x weeks of composting intervals (WCI) interaction on the number of days for full mycelia colonization, days to mushroom primordial initiation and extension per day of *Pleurotus ostreatus* and *P. pulmonarius*. At 4WCI, the longest number of days for full mycelial colonization was recorded in *P. pulmonarius* grown on neem (26.00days). However, this was not significantly different from the results obtained in *P. pulmonarius* grown on cassia and mixed bed and *P. ostreatus* grown on neem and cassia also (25.67 days, 25.33 days, 25.33 days, 24.67 days and 24.67 days respectively). This was followed by comparable results from *P. ostreatus* grown on mango (23.67 days) but not significantly different from *P. pulmonarius* grown on mango and *P. ostreatus* cultivated on mixed bed (24.33 days and 23.67 days respectively).

Also, at 8WCI, the longest was recorded in *P. pulmonarius* grown on neem (24.67 days). This was not significantly different from *P. ostreatus* cultivated on both mixed bed and cassia (24.00 days, 23.67 days, 23.67 days and 23.67 days respectively). This was followed by comparable result from *P. pulmonarius* cultivated on mango (23.00 days) but the least number of days for full mycelial colonization was observed in *P. ostreatus* grown on neem and mango (21.67 days and 22.00 days respectively). The longest number of days for full mycelial colonization at 12WCI was observed in *P. pulmonarius* grown on cassia (23.00 days) which

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Table 4.12. Effects of substrates x varieties x weeks of composting intervals (WCI) interaction on the growth parameters of *Pleurotus ostreatus* and *P. pulmonarius*.

	Substrate	Length of stipe (cm)			Biological Efficiency (%)			Production Efficiency (%)			Mycelia Extension (cm)		
	WCI(Weeks)	4	8	12	4	8	12	4	8	12	4	8	12
	Mango	4.33bc	4.70de	4.33fg	27.23a	61.92a	80.89ab	11.36a	29.77a	42.84a	9.63c	6.13b	9.00c
P ost	Cassia	4.13c	4.67de	4.13fgh	22.53bc	53.77bc	69.53cd	9.40ab	25.20b	34.23b	7.50d	5.88b	12.11a
	Neem	4.07c	3.70f	5.13de	15.16ef	33.09fg	61.92f	6.01cd	15.36e	30.55c	12.88a	8.25a	9.00c
	Mixed Bed	4.50b	5.30bc	4.80ef	25.47ab	56.02b	68.12de	10.42a	24.87b	33.29b	9.63c	6.00b	11.25b
	Mango	5.03a	4.83d	7.47a	21.33bcd	34.25f	61.39f	7.95bc	15.40e	30.98c	13.08a	5.50b	11.75a
P pul	Cassia	5.17a	6.13a	6.53b	13.04fg	46.79d	82.66a	4.89d	21.14c	41.08a	11.13b	5.50b	12.13a
	Neem	4.40b	5.83ab	5.63cd	16.74e	42.58e	55.47g	7.08c	18.66d	27.32d	13.25a	4.63b	10.38b
	Mixed Bed	5.17a	5.13bcd	6.33bc	27.55a	51.53c	71.45c	11.13a	22.71c	33.87b	13.13a	5.25b	12.25a

Means with the same letter along the column are not significantly different from one another at $p \leq 0.05$.

P ost: *Pleurotus ostreatus*

P pul: *Pleurotus pulmonarius*

WCI: Weeks of composting interval

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however, was not significantly different from the same variety cultivated on neem (21.67 days). But this was comparable to the results from *P. ostreatus* grown on cassia with *P. pulmonarius* cultivated on mango and mixed bed (20.33 days each). This was followed by *P. ostreatus* grown on neem (19.67 days) while the least number of days was recorded in *P. ostreatus* cultivated on mixed bed (18.67 days).

The longest number of days for primordial initiation was observed in *P. ostreatus* grown on neem (30.33 days) at 4WCI but comparable with what was obtained in *P. pulmonarius* grown on cassia, neem and mixed bed with *P. ostreatus* grown on cassia (30.00 days, 29.67 days, 29.00 days and 29.33 days respectively). The least was however recorded in *P. ostreatus* grown on mixed bed (23.33 days). At 8WCI, the longest was observed in *P. pulmonarius* cultivated on neem but not significantly different from that of cassia (29.33 days and 28.00 days respectively). Comparable results were recorded in *P. pulmonarius* grown on mixed bed and mango with *P. ostreatus* cultivated on cassia (27.67 days, 27.33 days and 27.00 days respectively). The least number of days was, however, observed in *P. ostreatus* grown on neem (25.00 days) but not significantly different from the results of *P. ostreatus* cultivated on mixed bed and mango (26.33 days and 26.00 days respectively).

The least number of days were generally observed at 12WCI when compared with the other weeks of composting intervals. At 12WCI, *P. pulmonarius* grown on cassia (26.00 days) produced its primordial at the longest number of days. This was followed by comparable results from *P. ostreatus* cultivated on cassia and *P. pulmonarius* grown on mango (23.33 days each). The least was observed in *P. ostreatus* cultivated on mango (20.33 days) but not significantly different from *P. ostreatus* grown on neem and mixed bed with *P. pulmonarius* also cultivated on neem and mixed bed (21.33 days, 21.00 days, 21.67 days and 20.33 days respectively).

The longest mycelia extension per day at 4WCI was recorded in *P. pulmonarius* grown on neem (0.63 cm). This was followed by the same variety cultivated on mango (0.60 cm). The least was observed in *P. ostreatus* grown on mixed bed which was comparable to the same variety cultivated on cassia (0.42 cm and 0.44 cm respectively). At 8WCI, *P. ostreatus* grown on mango (0.72 cm) produced the longest extension per day. This was followed by comparable results from *P. pulmonarius* cultivated on neem and *P. ostreatus* grown on neem (0.69 cm and 0.68 cm respectively). The least results were observed in *P. ostreatus* grown on mixed bed and cassia with *P. pulmonarius* cultivated on cassia (0.55 cm, 0.56 cm and 0.56 cm respectively). At

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12WCI, the longest mycelial extension per day was recorded in *P. ostreatus* cultivated on mango (0.76cm) but not significantly different from *P. pulmonarius* grown on neem (0.75 cm). This was followed by *P. pulmonarius* grown on mango (0.69 cm) which also was not significantly from the results of *P. ostreatus* cultivated on both neem and cassia (0.71 cm and 0.68 cm respectively). The least was however, observed in *P. pulmonarius* cultivated on cassia (0.59 cm).

4.3 Effects of different substrate types on the yield of the sclerotia of *Pleurotus tuber-regium*.

At the different stages of this study, all the sawdusts supported the production of sclerotia of *Pleurotus tuber-regium* (Plates 4.6 and 4.7). Generally, the highest sclerotia weights were recorded on the sawdusts with the longest weeks of composting interval (WCI) (Table 4.14).

At 4WCI, the highest weight was recorded in *Pleurotus tuber-regium* sclerotia obtained from the sawdust of mango (23.24±1.81 g) followed by comparable results from cassia and neem sawdusts (17.30±1.59 g and 15.51±1.55 g respectively) while the least was on the mixed bed (9.00±0.60 g). At 8WCI, the highest weight was from *P. tuber-regium* sclerotia harvested on cassia sawdust but was not significantly different from the one grown on mango (35.34± 1.46 g and 32.42±1.48 g respectively).

The least was recorded in mixed bed (16.94±1.40 g) which however was not significantly different from what was obtained on the sawdust of neem (18.98±2.50 g). Furthermore, mango sawdust produced the heaviest sclerotia at 12WCI (42.13±0.85 g) followed by the mixed bed while neem sawdust produced the least (37.02±1.71 g and 26.77±1.79 g respectively).

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Table 4.13. Effects of substrates x varieties x weeks of composting interval (WCI) interaction on the growth parameters of *Pleurotus ostreatus* and *P. pulmonarius*.

	Substrate	Full Mycelia Colonization (days)			Primordial Initiation (days)			Extension per Day (cm)		
	WCI (Weeks)	4	8	12	4	8	12	4	8	12
P ost	Mango	23.67ab	22.00b	17.00d	28.00ab	26.00b	20.33c	0.55c	0.72a	0.76a
	Cassia	24.67a	23.67a	20.33ab	29.33a	27.00ab	23.33b	0.44f	0.56e	0.68b
	Neem	25.33a	21.67b	19.67b	30.33a	25.00b	21.33c	0.47e	0.68b	0.71b
	Mixed Bed	23.67ab	24.00a	18.67c	23.33c	26.33b	21.00c	0.42f	0.55e	0.64c
P pul	Mango	24.33ab	23.00ab	20.33ab	28.33ab	27.33ab	23.33b	0.60b	0.64c	0.69b
	Cassia	25.67a	23.67a	23.00a	30.00a	28.00a	26.00a	0.52d	0.56e	0.59d
	Neem	26.00a	24.67a	21.67a	29.67a	29.33a	21.67c	0.63a	0.69b	0.75a
	Mixed Bed	25.33a	23.67a	20.33ab	29.00a	27.67ab	20.33c	0.57c	0.61d	0.65c

Means with the same letter along the column are not significantly different from one another at $p \leq 0.05$.

P ost: *Pleurotus ostreatus*

P pul: *Pleurotus pulmonarius*

WCI: Weeks of composting intervals

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Plate 4.6: Sclerotia of *P. tuber-regium* on the substrates

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Plate 4.7: Harvested sclerotia of *P. tuber-regium*

A: At 4 weeks of composting interval

B: At 8 weeks of composting interval

C: At 12 weeks of composting interval

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Table 4.14. The interaction effects of substrate types and weeks of composting intervals (WCI) on the yield (g) of the sclerotia of *Pleurotus tuber-regium*

Substrates	Weeks of Composting Intervals		
	4	8	12
Mango	23.24a	32.42a	42.13a
Cassia	17.30b	35.34a	32.83c
Neem	15.51b	18.98b	26.77d
Mixed Bed	9.00c	16.94b	37.02b

Means with the same letter along each column are not significantly different from one another ($p \leq 0.05$).

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CHAPTER FIVE
DISCUSSION

The rapid growth and the ability to utilize various lignocellulosic substances make *Pleurotus* species cultivation possible in different parts of the world. *Pleurotus* species have been grown on different kinds of sawdust, straw and many other agricultural and industrial wastes (Hadder *et al*., 1993). Some of these otherwise valueless lignocellulosic wastes: cotton wastes, sawdust, cereal stover, corncob, wheat, paddy straw and sugarcane bagasse, have been used either mixed or singly as substrates for the cultivation of various species of edible mushrooms by various researchers (Fasidi and Kadiri 1993; Manzil *et al.*, 1999; Ragunathan and Swaminathan 2003). They can colonize and produce mushrooms on pretreated conifer (*Pinus* spp.) wood chips but they do not always readily colonize non-pretreated conifer wood, due to the presence of inhibitory components (Croan, 2004). Some strains can, however, be adapted for cultivation on conifer sawdust-based substrates (Ruan *et al.*, 2006). *Pleurotus* spp. can also be cultivated on wood waste or unused wood residues associated with harvesting or thinning operations, which can enhance economic returns needed to support ecosystem management (Croan, 2000). They have extensive enzyme systems capable of utilizing complex organic compounds that occur as agricultural wastes and industrial by-products (Baysal *et al.*, 2003).

Different substrates for cultivation have significant effects on the mushroom yield. Different yield amounts were obtained from varied substrate media. This result is in consonance with previous research findings of other researchers who reported various values for the yield (Ohga, 2000; Ragunathan and Swaminathan, 2003; Yildiz, 2003). Using varied substrate media for the cultivation of mushroom causes different yield amount because of the biological and chemical differences between the substrates medium and genotype of the cultured mushroom as previously observed (Imbernon, 1990; Olivier 1990).

Number of fruits, fruit weight, the width of pileus, biological and production efficiencies increased as weeks of composting interval (WCI) increases in all the treatments. The release of nutrients was constant in mango and mixed bed substrates, increased in neem but decreased in cassia. This could probably be due to the fact that the longer the substrates are allowed to decompose, the more the nutrients that will be released and this might have enhanced the

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increase of these parameters. However, the release of inhibitory substances might be atributable to the decrease in the yield parameters of neem.

The highest number of fruits and fruit weights, largest width of pileus, longest length of stipe, greatest biological efficiency (BE) and production efficiency (PE), shortest number of days for full mycelia colonization and primordial initiation with the fastest mycelia extension per day were recorded at 12WCI in the varieties. This could be attributed to prolonged decomposition of the components of the various sawdust used thus, making them available for the mushroom mycelia. The average fruit weight was however highest at 4WCI. It was observed that few numbers of fruits were produced at 4WCI resulting in the very high average fruit weight as against the emergence of more fruits as WCI increases. Various mushroom fruit weight values were recorded for the sawdust from the different wood types. This was similar to the report by Abott *et al.* (2009) where it was observed that that the yield of *Lentinus squarrosulus* was influenced by the type of sawdust used for cultivation. This means that saw dust type influences the yield of mushrooms.

Sawdust (wood) has been the traditional substrate for the growth of mushroom (Onuoha, 2007). Sawdust has been consistently reported to be the best substrate supporting mycelia growth and fruitification (Kadiri and Fasidi, 1990). It was observed in this study that mango tree sawdust produced the greatest number of fruits though not significantly different from that of mixed bed and neem while the least was obtained from cassia. It was also noted that the fruit weight recorded for mango significantly followed that of mixed bed and cassia however, it produced a significant width of pileus, length of stipe, production efficiency with the fastest mycelia extension per day.

Very high mycelium density was observed in mango sawdust, being a soft wood, resulting in very high yield. This could be due to the physical nature, high level of aeration and porosity within the substrate. Findings of Thomas *et al.* (1998) revealed that the yield of mushroom is directly related to the spread of mycelium into the substrate. Furthermore, values of BE observed varied as a result of the biological structure of the raw materials or substrates used in this study as previously reported (Akyüz and Yildiz, 2007, 2008). The variations may also stem from the difference in the nutrient content of the materials.

Of the three mushroom varieties under investigation, greater effects were observed in *P. ostreatus* in terms of number of fruits, fruit weights, biological efficiency, production efficiency,

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shorter days to full mycelia colonization and primordial initiation. This is attributable to the soft nature of mango tree. This agreed with the work of Hami (1990) that observed that *P. ostreatus* gave maximum biological efficiency on the sawdust of soft wood like mango.

Generally, it was observed that all the parameters were significantly highest at 12WCI except mycelia extension, days to full mycelia colonization and primordial initiation. The increased mushroom yield must have been due to the prolonged substrate decomposition period, causing increased decomposition and making more nutrients available in the substrate for the mushroom growth. This was in agreement with the research findings of Rohrer and Heimlich (1989) that compared the decomposition rates of shredded newsprints, straw and saw dust. The authors observed that as the period of decomposition increases, the materials under investigation became more decomposed. After five weeks, the sawdust and shavings bedding material tended to blend with the dry surface of the soil, sawdust that was in a moist condition crumbled readily and nearly disintegrated when rubbed between the thumb and forefinger. The fine shavings and sawdust material decomposed most readily. As these materials were kept moist, they completely decomposed at the end of the 16 weeks test period. The authors observed that newsprint and straw were more closely paired in decomposition rates. The newsprint did decompose or at least disintegrate more quickly than straw when kept in similar moist conditions on the soil. The straw was most persistent in retaining its color and strength because of the long fibers. At the end of the 16 weeks study period, the straw was still yellowish in color and tore very easily. Most of the newsprint and straw were not decomposed. However, my investigation spanned only 12 weeks of composting interval.

At 4WCI, the fastest mycelia extension was observed in neem. This could be as a result of minimal decomposition causing a sporadic spreading of mycelia because of the little available nutrients. The same reason is attributable to full mycelia colonization resulting in the shortest period for primordial initiation at 4WCI.

As observed, all the growth parameters revealed that *P. ostreatus* performed better than *P. pulmonarius* as WCI increases. This was indicated by the number of fruits, fruit weight, biological and production efficiencies and average extension per day, all at 12WCI. Also, as observed earlier, the yield of *P. ostreatus* was more than that of *P. pulmonarius*. This could stem from the resultant total yield harvested on mango sawdust, a soft wood. This was also reported by Hami (1990).

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Although neem sawdust produced the greatest number of fruits but it was not significantly different from what was obtained from mango. Of all the substrates under investigation in this study, mango sawdust produced the greatest yield with *P. ostreatus* performing better than *P. pulmonarius*. Furthermore, the biological and production efficiencies of mango were highest with *P. ostreatus*. This could have resulted from heavy mycelia ramification on mango sawdust because of its porosity and aeration as a soft wood. Mehravaran (1993) observed that active mycelia growth is directly proportional to maximum of respiration as oxygen (O_2) is one of the most important environmental factors. It plays a major role in the metabolism and respiration of mushrooms giving rise to a better yield.

However, both the mycelia extension per day and total mycelia extension of *P. ostreatus* were least in mixed bed and mango sawdusts respectively. Similar observation was made by Elhami and Ansari (2008) while conducting a research on the effects of substrates on spawn production with respect to mycelia growth of oyster mushroom species. They observed that mycelia growth was significantly affected by species (mushroom varieties). They stated that the best mycelia growth was obtained from *P. florida* followed by *P. citrinopileatus* while the least was from *P. ostreatus*. Nandi and Mukherjee (2004) also reported that *P. florida* was more effective than *P. citrinopileatus* in delignification while *P. ostreatus* also had the least growth rate. Furthermore, Smith and Margarel (1995) obtained similar report for *Agaricus* strain (W4II) while Jonathan and Fasidi (2003b) equally reported for *Psathyrella atroumbonata*.

It was generally observed that as WCI increases the yield also increases. At 12WCI, the number of fruits of *P. ostreatus* harvested was greater than that of *P. pulmonarius*. The fruit weights from both mango and cassia sawdusts were highest but not significantly different from each other. However, mango, as a substrate, performed best in terms of biological and production efficiencies and with the longest average extension per day. This could have been as a result of the temperature of the fruiting house, moisture level and compost preparation. The temperature of the fruiting house was kept low as the mushrooms were grown under controlled conditions. Wetting was applied when necessary to keep the relative humidity of the fruiting house very high. When the temperature and moisture level are at the best, maximum number of pinheads and mushrooms are formed. Compost preparation includes picking unwanted materials from the substrate (sawdusts), moistening with water and the duration of composting thereby releasing the nutrients for the maximum growth of the mushrooms. Rohrer and Heimlich (1989) stated that as

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the period of decomposition increases, the materials (substrates) under investigation become more decomposed.

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CHAPTER SIX
CONCLUSIONS

From the result, it may be concluded that mango sawdust is the most suitable for the production of the fruiting bodies *P. ostreatus* when compared with the other substrates. The use of this substrate gave the highest yields in terms of fruit number and weight, the width of pileus, length of stipe and mycelia extension. Thus, mango is the best of all the substrates investigated and may be useful for large scale production of *P. ostreatus*. Furthermore, mango sawdust also gave the best in terms of the growth and yield of the sclerotia of *P. tuber-regium*. In comparison with other substrates under this investigation, cassia sawdust was most suitable and efficient for the production of *P. pulmonarius.* Full mycelia colonization took the longest number of days to maximize the cellulose and lignin contents availability resulting in the highest yields in terms of fruit weight, average fruit weight, production and biological efficiencies.

The components of applied mushroom biology are closely associated with three aspects of wellbeing: food shortage, human health and environmental pollution. One of the most significant benefits of mushroom cultivation is their ability to create a pollution free and friendly environment. Mushroom is a short duration crop, its cultivation is land saving and can be welcomed by the poor farmers. The tropical trees usually constitute a nuisance in the environment, as they are allowed to rot naturally or set ablazed when cut down. This study has revealed that the sawdusts of mango, cassia and neem trees could be used to cultivate mushrooms. Thus, production of mushroom from tropical trees could compliment protein availability and a reduction in malnutrition.

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