CULTIVATION OF EDIBLE OYSTER MUSHROOMS (*Pleurotus ostreatus* AND *Pleurotus sajor-caju)* USING SOME SELECTED AGRICULTURAL WASTE SUBSTRATES (COTTON SEED, KHAT LEFT OVER AND SPENT COFFEE GROUND)

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Cultivation of Edible Oyster Mushrooms (*Pleurotus ostreatus* and *Pleurotus sajor-caju)* Using Some Selected Agricultural Waste Substrates (Cotton Seed, Khat Left Over and Spent Coffee Ground)

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By

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APPROVAL SHEET

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As thesis research advisors, we here by certify that we have read and evaluated this thesis prepared, under our guidance, by Teklu Tadesse entitled: cultivation of edible oyster mushrooms (*Pleurotus ostreatus and Pleurotus sajor-caju)* using some selected agricultural waste substrates (cotton seed, khat left over and spent coffee ground).

We recommend that it be submitted as fulfilling the thesis requirement.

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As members of the Board of Examiners of the M.Sc. Thesis Open Defense Examination, we certify that we have read, evaluated the thesis prepared by **Teklu Tadesse** and examined the candidate. We recommend that the thesis be accepted as fulfilling the thesis requirement for the Degree of Master of Science in **Biotechnology.**

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DEDICATION

This thesis is dedicated to my mother W/ro Alemnesh Ayele, my sister W/ro Seblework Tadesse and her husband D/r Kidane Bekele for their financial support and encouragement as well as to my brothers and sisters for their continuous love, support and encouragement to complete this work.

STATEMENT OF THE AUTHOR

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The author was born on October 27/1990 G.C in Addis Ababa. He joined Qusquam Titu Primary School and attended up to grade 8. Then he continued his Secondary and preparatory school education at Yekatit 12 preparatory School.

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ACRONYMS AND ABBREVIATIONS

AC	Ash Content
AREU	Agricultural Research and Extension Unit
BE	Biological Efficiency
CC	Carbohydrate Content
CFC	Crude Fat Content
CG	Coffee Ground
CPC	Crude Protein Content
CRD	Completely Randomized Design
CS	Cotton Seed
NEFB	Number of Effective Fruiting Bodies
FAO	Food and Agricultural Organization
FC	Fiber Content
KL	Khat Leftover
MC	Moisture Content
NEFB	Number Effective Fruiting Bodies
PDA	Potato Dextrose Agar
SCG	Spent Coffee Ground
SSF	Solid State Fermentation

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ABSTRACT

Cultivation of edible mushrooms is one of the strategies of production of protein-rich food with the reduction of environmental pollution with full use of all materials in which nothing left as waste. In view of this, an experiment was conducted to evaluate substrates and substrate combination (Cotton seed (CS), khat left over (KL) and spent Coffee Ground (CG)) for oyster mushrooms (Pleurotus ostreatus and Pleurotus sajor-caju) cultivation at Mushroom Research, Production and Training Laboratory of Haramaya University. The results revealed that oyster mushroom showed varying growth performances on different substrate and substrate combinations. Highest total number of effective fruiting bodies (TEFB) (77.50 and 64.33) for Pleurotus ostreatus and Pleurotus sajor-caju respectively, were obtained from 100% CS which taken minimum total time (27.33 day) for both species. Lowest TEFB (21.00 and 15.00) for Pleurotus ostreatus and Pleurotus sajor-caju respectively, were recorded from 100% CG which taken maximum total time (39.04 and 39.37 day) for Pleurotus ostreatus and Pleurotus sajor-caju respectively. For Pleurotus ostreatus, highest (95.18%) and lowest (42.27%) biological efficiency (BE) were recorded on 100% CS and 100% CG respectively. For Pleurotus sajor-caju, highest (93.47%) and lowest (38.97%) BE were recorded on 100% CS and 100% CG respectively. The highest moisture content (MoC) (93.42%) obtained from 100% CS, crude fat (CFa) (1.85%) from 50% CS +50% CG, total ash(7.96%) from 50% CG +50% KL, crude fiber (CFi)(11.95%) from 100% CS, crude protein (CP)(34.61%) from 100% CS and Total carbohydrate (TC)(49.62%) from 100% CS was obtained from Pleurotus ostreatus. On the other hand for the same species the lowest MoC (75.08%) was obtained from 100% CG, CFa (1.76) from 100% CG, TA (6.77%) from 100% KL, CFi (8.68%) from 100% CG, CP(23.27%) from 100% CG and TC (22.80%) from 100% CG was obtained. For Pleurotus sajor-caju, highest MoC(92.84%) from 100% CS, CFa (2%) from 50% KL +50% CG, TA(8.1%) from 100% CS, CFi(11.4%) from 50% CS + 50% CG, CP(32.97%) from 100% CS and TC(46.93%) from 100% CS. The lowest MoC, CFa, TA, CFi, CP and TC were 73.77%, 1.76%, 6.63%, 8.43%, 23.71% and 23.93% respectively, all were obtained from 100% CG except fat (1.76%) obtained from 100% CS. This study revealed that the substrate (CS, KL and CG) and their combinations are more suitable for Pleurotus ostreatus than for Pleurotus sajor-caju in terms growth and nutritional value. From these growth substrates (100% CS) was the most suitable substrate than others. Even though KL and CG alone showed lowest results, their supplementation with CS resulted better result (effective fruiting bodies in terms of number and time), biological efficiency and nutritional value).

Key words: Cotton seed, khat left over, spent Coffee Ground, *Pleurotus ostreatus* and *Pleurotus sajor-caju*.

1. INTRODUCTION

Mushrooms are the reproductive structures of fleshy macro fungi (Nair, 1994). Nutritionally they are classified as saprophytes, that obtain nutrients from dead organic materials; pathogens, which depend on living plants and animal bodies; mycorrhiza, through a close physiological association with host plants and animals, thereby forming a special partnership where each partner enjoys some vital benefits from the other (Stamets and Chilton, 2005).

The Ethiopian population is on a continuous increase against a declining acreage of arable land (Diriba *et al.*, 2013) consequently, the available arable land is being subdivided into smaller parcels which are intensively cultivated (MoARD, 2010). In order to encounter this problem, cultivation of edible mushrooms is one of the strategies that do not require large tracts of land (Kakon *et al*., 2012; WHO, 2011).

Ethiopia has a favorable climate, labor and good water resources that create ample opportunities for horticultural production. However, the production and utilization of mushrooms in Ethiopia is neglected. As a result this country is not benefited from mushrooms as the rest of the world (Kiflemariam, 2008).

Nutritionally mushrooms have high contents of qualitatively good protein, crude fiber, minerals, vitamins, abundance of essential amino acids, mono and disaccharides, alcohols, glycogen and chitin but are poor sources of lipids (Park and Kwang, 2001).Medicinally also important for cholesterol reduction, immune enhancement, cancer fighting, anti-allergic activities, anti-microbial and cardiovascular treatment (Wasser, 2000), physical and emotional stress relief (Menoli *et al.*, 2004; Guterrez *et al*., 2004).

Mushroom cultivation is a potential biotechnological process where waste plant materials or crop residues can be converted into valuable food (Baysal and Peker, 2001). Mushroom cultivation drives towards full use of all materials in which nothing will be left as waste, without any adverse impacts on the environment through sustainable utilization of lignocellulosic wastes available in abundance everywhere, usually as by-products from agriculture, forestry and households (Chang, 2007). An alternative way of using agricultural

residues/wastes is in the use of the organic material in mushroom production (Chang and Miles, 2004; Khare *et al*., 2010).

Oyster mushrooms are one of the most popular edible mushrooms and belong to the genus *Pleurotus* and the family *Pleurotaceae*. Many of the *Pleurotus* mushrooms are primary decomposers of hardwood trees and are found worldwide (Singh *et al*., 2005). The oyster mushrooms can be cultivated successfully under semi-controlled conditions in a small space by using agricultural as well as industrial wastes and other refuse as substrate (Khare *et al*., 2010).

Cotton seed is one of the most efficient substrate materials for oyster mushroom cultivation (Rajarathnam, 1986). It contains higher concentration of nitrogen and high proportion of cellulose (Hang, 1984). Cotton seed can be stored for relatively long period of time. It is suitable to be sterilized or pasteurize, because cotton waste emits extra heat by itself. During fermentation, concentrated gas cannot escape to the outer surface of the cottonseed hull. The control of gas and water is essential for successful cultivation (Qian, 2005).

Spent coffee grounds are produced during processing of raw coffee powder to prepare instant coffee (Fan and Soccol, 2005). Since coffee ground is extracted in water, most of the hydrophobic compounds, including oils, lipids, triglycerides, fatty acids and carbohydrates like cellulose and various indigestible sugars remain in the brewing process (Fan, 2005). Spent coffee grounds have relatively low amount of caffeine therefore, it do not require caffeine removal process (Thieke, 1989).

Khat (*Catha edulis*) is one of the most important cash crops which fetch foreign currency for the economy of Ethiopia (CSA, 2008. The khat leftover consists of over-matured leaves, twigs, stems and shoots that are discarded as not chewable by humans. These tons of khat left-over remains to be a serious environmental hazard due to the tannins and other toxic materials it contains (Mekasha *et al*., 2007). The presence of tannins, especially the condensed ones, is toxic to soil microbial population and as a result decrease net soil nitrogen mineralization (Bradley *et al*., 2000.

Despite some research conducted on theses substrates to cultivate oyster mushroom (Anteneh *et al*., 2015; Leifa *et al*., 2001), they required further enhancement with nitrogen containing substrates to increase the yield of oyster mushroom while they are cheap and easily available together to convert them to protein rich food and to save our environment from pollution.

Thus, the overall objective of this study was to cultivate oyster mushrooms (*Pleurotus ostreatus and Pleurotus sajor-caju)* using some selected agricultural wastes (cotton seed, *khat* leftover and spent coffee ground) in Haramaya University.

The specific objectives of the study were

1. To evaluate substrates or substrate combinations (cotton seed, khat leftover and spent coffee ground) in terms of time required to harvest oyster mushrooms (*Pleurotus ostreatus* and *Pleurotus sajor-caju*).
2. To determine the yields (biological efficiency and number of effective fruiting bodies) of *Pleurotus ostreatus* and *Pleurotus sajor-caju* cultivated in different substrate and substrate mixtures of cotton seed, khat leftover and spent coffee ground.
3. To determine nutritional composition of the two mushroom species cultivated using different substrate mixtures of cotton seed, khat leftover and spent coffee ground.

2. LITERATURE REVIEW

2.1 Biology of Mushroom

Fungi are regarded as being the second largest group of organisms in the biosphere after the insects. There are over 1,500,000 species of fungi on earth (Halpern, 2007). Among this, the number of mushroom species is estimated at 140,000 (Wasser, 2002). Known fungal species constitute only about 5% of their species in the world. Thus, the large majority of fungi are still unknown. Out of about 70,000 described species of fungi, it has been suggested that around 14,000-15,000 species produce fruiting bodies of sufficient size and suitable structure to be considered as macro fungi (mushrooms). Of these, about 5,000 of the species are considered to possess varying degrees of edibility, and more than 2,000 species from 31 genera are regarded as prime edible mushrooms. But only 100 of them are experimentally grown, 50 economically cultivated, around 30 commercially cultivated, and only about 6 to have reached an industrial scale of production in many countries. Furthermore, about 1,800 are medicinal ones. The number of poisonous mushrooms is relatively small (approximately 10%), of these some 30 species are considered to be lethal (Miles and Chang, 1997). Out of 38,000 known mushroom varieties, the most popular are *Agaricus bisporus* (white button mushroom), *Lentinus edodes* (Shiitake or Japanese mushroom), *Pleurotus* species (oyster mushroom), *Volvarella volvacea* (paddy straw mushroom*), Flammulna velutipis* (winter mushroom) and *Auricularia polytricha* (Jew's ear mushroom) (Singh *et al*., 1999). Among these, oyster mushrooms (*Pleurotus spp*.) are in the third place after the white button and shiitake among the world mushroom production (Gyorfi and Hajdu, 2007).

Mushrooms belong to the kingdom of Fungi, a group very distinct from plants, animals and bacteria. Fungi lack the most important feature of plants: the ability to use energy from the sun directly through chlorophyll. Thus, fungi depend on other organisms for food, absorbing nutrients from the organic material in which they live. The living body of the fungus is mycelium made out of a tiny web of threads (or filaments) called hyphae. Under specific conditions, sexually compatible hyphae will fuse and start to form spores.

The larger spore producing structures (bigger than about 1 mm) are called mushrooms. In nature this is the most striking part of the organism, but in fact it is just the fruiting body and the major part of the living organism is found under the ground or inside the wood (Oei, 2005). Since mushrooms lack chlorophyll they cannot, like green plants, get their energy from the sun through photosynthesis. Instead, during their vegetative growth stage, mushroom mycelia secrete. Enzymes that break down compounds such as cellulose and lignin present in the substrate. The degraded compounds are then absorbed by the hyphae and the mycelium enlarges-usually ate rally, and in some cases growing several meters in diameter with the substrate. Partially understood environmental factors (temperature and light are known to be critical) stimulate the second or reproductive growth stage. Cells of one mycelia strain fuse with cells of the opposite type to form a mycelium that contains both types of nuclei. The new mycelium continues to grow and eventually develops into a mature fruiting body, the gills of which are lined with spore bearing cells called basidia. Various mechanisms trigger the dispersal of spores, which in turn lodge in a substrate, become hyphae and begin the cycle anew (James, 1995).

The structure that we call a mushroom is in reality only the fruiting body of the fungus. The vegetative part of the fungus, called the mycelium, comprises a system of branching threads and cord-like strands that branch out through soil, compost, wood log or other lignocellulosic material on which the fungus may be growing. After a period of growth and under favorable conditions, the established (matured) mycelium could produce the fruit structure which we call the mushroom (Chang and Buswell, 1996).

2.2. Life Cycle

The life cycle of most mushrooms is the same or very similar but macroscopic and microscopic features are different; such morphological variations enable us to identify individual mushroom species. The general life cycle of mushrooms start from a mature fruit body or basdiospore when the condition is suitable, the basdiospores germinate and grow as threads (hyphae) in the substrate. Hyphae from two different compatible spores fuse and form

cells containing two nuclei, one from each, and such hyphae are called dikaryotic. The dikaryotic hyphea grow extensively and later form diploid cells through fusion of the two nuclei. The genetic material undergoes division and the cell develops into a basidium, which forms the basdiospores at the same time as the fruit body matures. The life cycle continues through the fruit body disappear for most of the year (Dawit Abate, 1998). Four basidiospores form at the end of each basidum on the gill of a fruit body if a section of the gills is cut and examined under the microscope, spores will be observed on their surface. The spores will start to fall as the cap fully expands, indicating maturity of the mushroom. The spores are so minute that they float in the air and are carried by the wind. Eventually, they fall to the ground, usually with rain (Wasser, 2002a). If conditions are favorable (optimum temperature and moisture), the spores will germinate to form a mass of mycelium. This is the start of the vegetative phase of the mushroom. Given an unrestricted amount of nutrients and favorable growing conditions, it is capable of unlimited growth. The mycelium developing from the germinating spore is the so-called primary mycelium and is usually uni nucleate and haploid. This stage is short-lived because mycelia from different spores tend to ramify and fuse to form the secondary mycelium with two compatible nuclei, which continues to grow vegetatively and is able to form fruiting bodies (Kang, 2004)

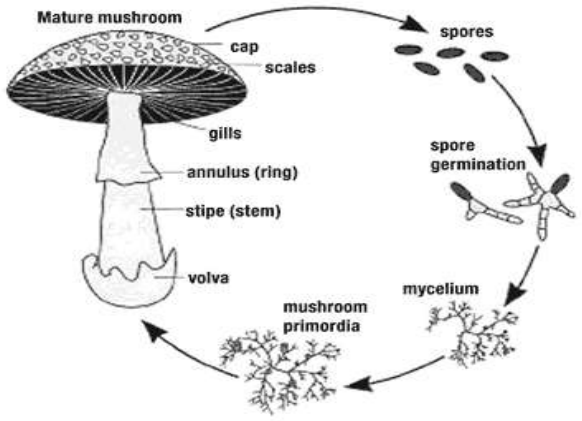

Figure 1. Life cycle of *Pleurotus ostreatus* (Source: Kang, 2004).

2.3. Mushroom Cultivation

The use of mushrooms as food is probably as old as civilization itself Chaube (1995) and mushrooms have been treated as a special kind of food (Tripathi, 2005). Mushroom cultivation is both a science and an art. The science is developing through research; the art is perfect through curiosity and practical experience (Chang, 2008). Cultivated mushroom are generally saprophytes, utilizing substrate as primary or secondary decomposers (Stott and Caroline, 2004).

Mushroom cultivation is a highly technical and skilled activity it involves investment depending on the size of the unit/production targets. In most developing countries like India, mushroom growing is a seasonal activity for marginal and small farmers around cities. These farmers prepare compost either by long method or purchasing from composting units and sale fresh mushroom in nearby market or on to the canners of the locality (Quimio, 1998). On the other hand in most countries, the commercial large scale, year round mushroom production units, are equipped with composting, growing, spawn production and processing units. These producers sale their products for domestic consumption and external markets mostly in canned and partially in freeze dehydrated form (Singh and Chaube, 1995). Only about 45% of mushrooms produced are consumed in the fresh form. The rest of the 55% is processed with 5% in the dehydrated form and 50% in the canned form. This is because their shelf life in the fresh form is very short. Hence mushrooms are traded in the world market mostly in the processed form (Lal, 2005)

The habitual use of mushrooms is well documented in several cultures and religions. They began to be used as food and medicine in 600 A.D. in Asia. At first, they were harvested in forests only, and some time later began to be cultivated by man. Cultivation of edible mushrooms combines both skill and scientific technology in which agricultural wastes are recycled to produce a protein rich but cheap human food (Chang, 2008). Mushroom production is a complicated business as it needs training and adaptation of new modern techniques for profitability. On top of poor post- and pre- harvest handling facilities, capacity

of smallholders and private sector is at its nascent stage in using pre- and post harvest handling technologies and know- how (Crisan and Sands, 1978). The art and science of mushroom growing are not known in this country; rather it is a new type of technology and production system. Lack of skilled labor on production, processing, etc that can meet the demand of emerging mushroom farms; and dearth of qualified personnel in the area of production, research, and extension. And apart from the different phases of mushroom production that occur in phases, good knowledge of mycology, microbiology, biochemistry (fermentation) and environmental engineering must be well known to commercial growers (Tripathi, 2005).

Oyster mushroom (*Pleurotus* spp.) can grow and utilize various kinds of substrate materials than any other mushrooms (Cohen *et al.*, 2002). Oyster mushroom (*Pleurotus* spp.) is commonly known as Dhingri in Pakistan and India because of its oyster like shape. The genus *Pleurotus* belongs to family Tricholomataceae and has about 40 well-recognized species, out of which 12 species are cultivated in different areas of Pakistan and India. *Pleurotus* is an efficient lignin- degrading mushroom and can grow and yield well on different types of lignocellulolosic materials. Cultivation of oyster mushroom is very simple and low cost production technology, which give a continuous growth with high biological efficiency. Different species of *Pleurotus* can grow well in variable temperature conditions; hence they are ideally suited for cultivation throughout the year in various tropical regions of the country (Ahmed *et al*., 2009). It can be cultivated in containers like jars, basins, trays, plastic bags and other similar substances by providing artificial controlled conditions (Quimio, 1998).

2.4. Benefits of Mushrooms

2.4.1. Nutritional value

Mushrooms can be taken in various forms. The most popular are the cooked mushrooms. However, it was proven that taking raw mushroom could give better effects nutritionally

(Stametes, 2005). It was reported that raw mushroom contains three times more nutrients than the cooked ones (Berch *et al*., 2007).

Mushrooms are highly nutritious and are important features of human diet worldwide. Edible mushroom are highly nutritious and can be compared with eggs, milk and meat. The content of essential amino acids in mushroom is high and close to the need of the human body (Belewu, 2005; Oei, 2003).

Edible mushrooms are recommended by the FAO as food, contributing to the protein nutrition of developing countries dependent largely on cereals with it became a new and alternative demand for poultry and animal protein in fresh mushrooms. In general mushrooms are highly nutritious, their taste and delightful aroma makes them one of the delicious preferred foods in restaurants throughout the world (Chang and Mshignei, 2000).

According to Carvalho *et al*. (2010) mushrooms are considered as food with delicious taste and high nutritional value because their contents (g/100g) of protein (23.22), carbohydrate (63.17), phosphorus (104.13) and fiber (34.0) are high, and the amount of lipids (4.71) is low. High protein content of as much as 50 to 84% dry matter has been detected in the fruit bodies and mycelia of *P. ostreatus, L. edodes, V. esculenta & T. clypetus*. Their mycelia also contain amino acids like glycine, valine, threonine, serine, leucine, proline, methionine, asparagine, glutamine, lysine, arginine, histidine, cysteine and alanine (Nwokye *et al*., 2010).

The chemical composition of the fresh fruiting bodies of oyster mushroom, *Pleurotus ostreatus* indicates a large quantity of moisture (90.8%), whereas fresh as well as dry oyster mushrooms are rich in proteins (30.4%), fat (2.2%), carbohydrates (57.6%), fiber (8.7%) and ash (9.8%) with 345 K (cal) energy value on 100 g dry weight basis; while vitamins such as thiamin (4.8 mg), riboflavin (4.7 mg) and niacin (108.7 mg), minerals like calcium (98 mg), phosphorus (476 mg), ferrous (8.5 mg) and sodium (61 mg) on 100 g dry weight basis (Bhatti *et al*., 2007)**.**

Among the various edible mushroom types, *Pleurotus* species have become more popular and widely cultivated throughout the world particularly in Asia and Europe as they have simple

and low cost production technology shows higher bio-efficiency. Oyster mushrooms (*Pleurotus* species) are rich source of vitamin C, B-complex (thiamin, riboflavin, folic acid and niacin), minerals (Ca, P, Fe,K and Na) and protein (Sturion *et al.*,1995).

Table 1.Nutritional property of culinary-medicinal mushrooms

Mushroom	**Dietary Fiber (g/100g)**	**Thiamine B1 (mg /100g)**	**Riboflavin B2 (mg /100g)**	**B2 Niacin B3 (mg /100g)**	**Pantothenic acid B5 (mg /100g)**	**Vitamin C (mg /100g)**	**Vitamin D (lU/100g)**
A.bisporus	20.90	0.27	4.13	69.20	12.70	0	235
A.bisporus Crimni	19.90	0.23	3.49	38.50	21.70	0	26
Lentinus edodes Shiitake	28.80	0.25	2.30	20.40	11.60	0	110
Pleurotus ostreatus Pearl oyster	34.10	0.16	2.40	54.30	12.30	0	116
Pleurotus pulmonau (*sajorcaj*	48.60	0.10	1.68	23.80	8.80	0	178

Source: Stamets and Paul (2005)

2.4.2. Medicinal value

Due to present day high pressured work demands resulting in great stress to the human body and causing a weakening of the human immune system, there are now many new diseases. These have developed as a consequence of lower natural body resistance (Beyer, 2005). There is some evidence that the beneficial treatment of these diseases can be obtained by consumption of mushrooms as a functional food, or through the use of extracted biologically active compounds as a dietary supplement, in order to enhance immune response of the human body, thereby increasing resistance to disease and, in some cases, causing regression of a diseased state (Oei, 1996). Differing from most pharmaceuticals, these biologically active compounds extracted from medicinal mushrooms have extraordinarily low toxicity, even at high doses. Long viewed as tonics, now it has been known that they can profoundly improve the quality of human health (Wasser *et al.*, 2002a).

Mushrooms are alprobiotic, help our body strengthen itself and fight off illness by maintaining physiological homeostasis, restoring our bodies balance and natural resistance to disease. They have a beneficiary effect on prebiotics in the gastrointestinal tract, helping promote healthy bacteria. They are also adaptogens, substances that help the body cope during times of stress.

The compounds they contain have been classified as host defense potentiators (HDP) which can have immune system enhancement properties. That is the reason why currently used as adjuncts to cancer treatments in Japan and China (Halpern, 2007). Mushrooms produce several biologically active compounds that are usually associated with the cell wall. Most notably, a group of polysaccharides comprising high molecular weight sugar polymers has been reported to contribute to their immune enhancing and tumor retarding effects. It has been reported that the anti-tumor and anti-cancer effects of the polysaccharides are based on the enhancement of the body's immune systems, including activated macrophages, natural killer cells, cytotoxic T cells, and their secretary products, such as the tumor necrosis factor, reactive nitrogen and

oxygen intermediates, and interleukins, rather than direct cytocidal effects (Mizuno *et al*., 1995; Liu *et al*., 1996).

Some mushrooms are used for the treatment of gastric ulcer, duodenal ulcer and chronic gastritis. A good example is *Hericium erinacius*. Some mushrooms such as *Tremella fulciformis* are used for curing leukaemia, coughing, phlegm and asthma of patients suffering from chronic bronchitis (Oei, 1996).

Oyster mushrooms are also known to have multiple medicinal properties. Two of the more prominent medical attributes are cardiovascular and cholesterol-controlling benefits. Oyster mushrooms naturally produce mevinolin (lovastatin) in portions of the fruiting bodies (Gunde-Cimerman, 1999). Mevinolin inhibits the key enzyme in cholesterol biosynthesis in the liver and reduces cholesterol absorption (Bobek *et al*., 1998). *Pleurotus* species also content high potassium: sodium ratio, which makes mushrooms an ideal food for patients suffering from hyper tension and heart diseases (Bano *et al.,* 1993).

2.4.3. Waste Recycling and environmental bioremediation

Organic solid wastes are a kind of biomass, which are generated annually through the activities of the agricultural, forest and food processing industries. They consist mainly of three components: cellulose, hemicelluloses and lignin. The general term for these organic wastes is lignocelluloses (Oei, 2003). They can be treated by various chemical methods, e.g. with dilute hydrochloric acid and calcium chloride to increase the digestibility and nutritional qualities, and even to form sugars to serve as carbon sources(Chang, 2007). However, these chemical methods are tedious and costly. Furthermore, treatments to eliminate adverse side effects of the chemicals are also very complex (Miles and Chang, 1986). All lignocellulosic waste residues can be used as substrate for growing mushrooms; otherwise, they would cause health hazards. Mushroom enzymes can break down lignin, cellulose and hemicellulose present in these organic materials into simpler molecules, which the mushrooms then use for their growth and metabolism (Wasser, 2002b).

The oyster mushrooms are cultivated in many countries both in sub-tropical and temperate regions which can be grown on various agricultural waste products without the addition of enrichment materials (Rajapakse *et al*., 2007). An attractive feature of these group of mushroom is due to the capacity of secreting spectrum of enzyme and they can utilize a large variety of agricultural waste products containing (lignin, cellulose, starch, sugars and fermented proteins) and transform the lignocellulosic biomass into food of high quality, flavour and nutritive value (Baysal and Peker, 2001).

Environmental contamination can be ameliorated by the application of mushroom mycelial technologies. For example, (1) the use of bioconversion processes to transform the polluting substances into valuable foodstuffs, e.g., the proper treatment and reutilization of spent substrates/composts in order to eliminate pollution problems (Beyer, 2005; Noble, 2005). One of the most intriguing opportunities offered by mushroom mycelia in the area of bioconversion is the exploitation of their ability to degrade pollutants, many of which are highly carcinogenic, released into the environment as a consequence of human activity. And (2) the use of fungi/mushroom mycelia as tools for healing soil, what called “myco-restoration”, which is the use of fungi/mushrooms to repair or restore the weakened or damaged bio-systems of environment. The processes of myco-restoration include the selective use of mushrooms for myco-filtration, to filter water; myco-forestry, to enact eco-forestry policy; myco-remediation, to denature toxic wastes; and myco-pesticides, to control insect pests. Myco-resoration recognizes the primary role fungi/mushrooms can play in determining the balance of biological populations (Stamets, 2005).

It is therefore suggested that an integrated approach in the production of mushroom, biofertilizer and biogas should be considered as a feasible approach for the rural and urban lignocellulosic waste utilization and disposal. This is the “Zero Emission or Total Productivity” concept (Pauli, 1996; Chang, 2007).

2.4.4. Economic and social values

Mushroom cultivation is a space-confined technology and requires relatively small capital with declining land productivity and increasing interest in organic farming; the cultivation of edible saprophytic mushrooms offers prospects for using agricultural residues (Shasho, 2004). In most developing countries like India, mushroom growing is a seasonal activity for marginal and small farmers around cities. These farmers prepare compost either by long method or purchasing from composting units and sale fresh mushroom in nearby market or on to the canners of the locality. On the other hand in most countries, the commercial large scale, year round mushroom production units are equipped with composting, growing, spawn production and processing units. These producers sale their products for domestic consumption and external markets mostly in canned and partially in freeze dehydrated form (Singh and Chaube, 1995).

Since mushroom cultivation can be a labor-intensive agro-industrial activity, it could have great economic and social impact by generating income and employment for both women and youth, particularly in rural areas in developing countries (Reid, 1989). In addition to the nutritional and medical values, mushrooms cultivation practices have paramount importance in food self-sufficiency attempts Beteez and Kustudia (2004), especially for low-income countries like Ethiopia. Mushrooms can generate additional employment and income through local, regional and national trade offering opportunities through processing enterprises (FAO, 2009).

2.4.5. Enzyme production

Most enzyme manufacturers produce enzymes by submerged fermentation (SMF) techniques. However, in the last decades there has been an increasing trend towards the use of the solid-state fermentation (SSF) technique to produce several enzymes **(**Herrera *et al*., 2007). The food, agricultural and forestry industries produce large volumes of wastes annually which cause a serious disposal problem worldwide. Most of such wastes are rich in soluble

carbohydrates and also contain inducers of laccase synthesis, ensuring an efficient production of laccase enzyme (Elisashvili *et al*., 1976).

The white-rot fungi like *Pholiota nameko, Pleurotus ostreatus, Pleurotus cystidiosus and Pleurotus pulmonarius* are the organisms able to degrade the whole wood components due to the secretion of an extracellular ligninolytic complex during their secondary metabolism. The main components of this ligninolytic complex consist of a family of peroxidases named lignin peroxidases (LiPs) and manganese-dependent peroxidases (MnPs) and a family of multicopper oxidases named laccases (Kirk and Fenn, 1982). Laccases (*p*-diphenol: dioxygen oxidoreductases) catalyses the oxidation of both phenolic and non-phenolic compounds and are able to mineralize a wide range of synthetic dyes (Russell and Paterson, 2006). Laccase catalyzed reaction where diphenol is oxidized to form a free radical, which can further undergo a second enzymatic catalysis to form a quinone (Tavares, 2006). Most laccases are extracellular enzymes, making the purification procedures very easy and they generally exhibit a considerable level of stability in the extracellular environment. Such characteristics make laccases very suitable for their application to several bioprocesses such as bio pulping, bio bleaching and the treatment of industrial waste water (Kapich *et al.*, 2004).

2.4.6. Substrate for animal feed and soil conditioner

Spent after mushroom cultivation on different substrates such as cottonseed waste, cereal straw and bagasse serve as animal feed supplements (Dawit Abate, 1998). Spent mushroom substrate has been as animal feed, since its degradation by the mushroom can improve its nutritional quality and digestibility (Suzuki *et al*., 1994).

The lignocellulosic substrate used for mushroom production and which is left after harvesting of the mushrooms can be used as compost for soil conditioning. It should be noted that this compost besides being rich in nitrogenous material contains partly degraded lignocelluloses components, when combined with animal dung or human excreta in a biogas digest would yield not only biogas but also a good quality organic nitrogenous fertilizer in the form of sludge (Singh and Chaube, 1995). The sludge from the biogas plant as a nitrogenous fertilizer

is far more beneficial than the compost from which it has been derived. Part of the biogas that is produced in the vicinity of the mushroom house can also be conveniently used for pasteurization of the mushroom bed material (Chang, 2004).

2.5. Growing Oyster Mushrooms

2.5.1. Substrates

Mushroom substrates defined as a kind of lignocellulosic material which supports the growth, development, and fruiting of mushroom mycelium. The process of preparation of substrate is broadly termed “composting”. The final product of “composting” is called the “compost” or prepared substrate. The process for preparation of substrates has been the subject of much scientific and practical interest over the past two decades. It should be noted that different types of mushrooms require different types or substrate/compost (Chang, 2007).

Oyster mushrooms are a good choice for beginning mushroom cultivators because they are easier to grow than many of the other species and they can be grown on a small scale with a moderate initial investment. Another advantage of growing oyster mushrooms is that a high percentage of the substrate converts to fruiting bodies, increasing the potential profitability (Beetz and Kustudia, 2004**).**

The properties of a substrate determine which mushrooms and microbes can grow in it. The more selective it is, the better the substrate meets the demands of a specific mushroom and the less suitable it is for others. Oyster mushrooms can become an integral part of a sustainable agriculture system. Many types of organic wastes from crop production or the food processing industry can be used to support oyster mushroom production (Oei, 2005).

Table 2.Mushroom Cultivation substrates

Mushroom Cultivation substrates

Growing Medium	**Mushroom Species**
Rice straw	Straw (*Volvariell*a), *Oyster (Pleurotus*),Common (*Agaricus*)
Wheat straw	*Oyster (Pleurotus*),Common (*Agaricus*), Stropharia
	Straw (*Volvariella*)
Coffee pulp	Oyster (*Pleurotus*), Shiitake (*Lentinus*)
Sawdust	Shiitake (*Lentinus*), *Oyster (Pleurotus*), Hericium
	Ear (Auricularis), Ganoderma (Reishi)
	Winter (Flammulina)
Cotton waste from textile industry	Oyster (*Pleurotus*) , Straw (*Volvariella*)
Bean and cotton straw	*Oyster (Pleurotus*)
Cotton seed hulls	*Oyster (Pleurotus*), Shiitake (*Lentinus*)
Logs	Nameko (Pholiota), Shiitake (*Lentinus*)
	Whitejelly (Tremella)
Sawdust-rice bran	Nameko (Pholiota), Ear (Auricularis)
	Coprinus,Winter (Flammulina), Shiitake (*Lentinus*)
Crushed bagasse and molasses wastes	*Oyster (Pleurotus*), Shiitake *(Lentinus*)
from sugar industry	
Banana leaves	Straw (*Volvariella*), Common (*Agaricus*)
Horse manure	

Source: Rafats and Jerry (1996)

2.5.2. Environmental conditions

Mushroom cultivation requires firstly the manufacture of composts and secondly management of growing environments. The cultivation of the fungus in compost and the way in which nutrition and growing environments are manipulated to force mushrooms to emerge for harvesting is the key to success (Robin, 1997). Each *Pleurotus* species needs different environmental conditions for fruit body development (Chang and Miles, 1989). Substrate formulation, strain, temperature, light, air composition and cultivation technique have been identified as important factors affecting cultivation (Pettipher, 1988).

2.5.2.1. Temperature

The optimal temperatures for the development of fruiting bodies can vary among the species. Some previous studies subjected to the effects of temperature on the spawn run for *Pleurotus* species were also carried out (Bano and Rajarathram, 1982; Koçyi"it and Günay, 1984; Zervakis *et al.*, 2001). The ambient temperature has to fit the chosen mushroom strain. If the temperature in the mushroom house is too high for the chosen strain, it will be necessary to frequently mist the house. Opening the doors and windows at night will also help keep the temperature down (Oei, 2005)

Oyster mushrooms are able to grow and thrive in a wide range of temperature environments. Stamets (2000) recommends temperatures between 10°C and 21°C for development of oyster mushrooms. *Pleurotus sajor-caju* (grey oyster mushroom) is comparable to the high temperature species in the group of *Pleurotus* (*oyster*) mushrooms, with high temperatures required for fructification (Chang and Miles, 2004; Kaul and Dhar, 2007). The temperature for growth of mycelium is 10-35^{o}C. The optimum growing temperature of the mycelium is 23-28^{o}C. The optimum developmental temperature of the fruiting body is 18-24^{o}C. (Pettipher, 1987)

Table 3.Temperature ranges for mycelial growth, optimal growth and fruiting for specific mushroom species.

Mushroom species	**Tmg**	**T optimal mg**
Shiitake (*Lentinus edodes*)	5-35	20-30
Abalone oyster mushroom (*Pleorotus abalone*)	15-35	20-30
Oyster mushroom (*Pleorotus cytidiosus*)	5-35	20-25
Winter mushroom(*Pleorotus ostreotus*)	5-35	20-25
Oyster mushroom(*Pleorotus pulmonarius*)	5-35	20-25
Yellow oyster mushroom(*Pleorotus cornucopiae*)	15-35	20-28
Pink oyster mushroom *(Pleorotus djamr*)	15-35	24-30

Source: Oei (2005)

Tmg: The range at which the mycelium stays viable; the growth speed declines at both high and low ends of this range.

T optimal mg: The optimal temperature range required for fruiting; the most important temperature.

2.5.2.2. Light

The requirements for light are different for the various stages of growth. Many study results showed that light prevented spawn run, enhanced primordia formation and it was necessary for the mushroom formation in *Pleurotus* species. However, *Pleurotus* species can grow to benefit from natural light in greenhouses and high plastic tunnels throughout a year (Okwujako, 2001)

Although the mycelium of the oyster mushroom does not require light, proper fruit body formation requires moderate light. Too little or too much light can lead to discolored, malformed fruit bodies or the inability to fruit. In the complete absence of light, oyster mushrooms will form no cap but stipes (mushroom stalks) forming a coral-like structure. When the small mushrooms emerge, their form will reveal whether they get sufficient light and aeration. If the stems are long and the caps small, the aeration and light requirements were not met (Chang and Miles, 1989).

2.5.2.3. Humidity

Good control of the humidity during cropping is very important for all types of mushroom. Keep the humidity high (80 - 90%) by spraying water several times per day. However, no water should be sprayed directly onto mushrooms that are ready for picking. Their shelf life will decrease drastically if they become too wet. Excessive moisture can cause lack of oxygen in the substrate, as well as encourage certain contaminates. Insufficient moisture can prevent primordia formation and stunt fruit body growth (Oei, 2005). Extremely high humidity (90 to100%) is recommended for optimal primordial formation. Once primordia have formed, humidity should be lowered to 85 to 90%. Ideally, humidity levels should be managed so that mushrooms are regularly receiving moisture but excess moisture can evaporate from fruit body surfaces (Stamets, 2000).

2.5.2.4. Oxygen and carbon dioxide

Fungal mycelium is extremely tolerant of carbon dioxide, thriving at 20% CO_2 levels. The high CO_2 concentration in the substrate serves as a shield for the *Pleurotus* against other microorganisms, which either grow or die off at higher concentration (Zadrazil, 1975). Four and six days after inoculation, carbon dioxide concentration had reached maximum then decreased, finally showing no material alteration from the 16^{th} to the 31^{st} day. Carbon dioxide production is nevertheless dependant on the ingredients in the substrates (Khan, *et al*., 2011).

Oxygen is required for formation of fruit bodies. A significant decrease in ambient CO_2 level and increase in oxygen is critical for the initiation and development of primordia. Thus sufficient air circulation within a mushroom fruiting site is vital. Excessive influx of outside air, however, greatly affects both temperature and humidity of the environment (Stamets, 2000).

2.5.2.4. pH

pH is an important factor for good production of Oyster mushroom. Most of the mushrooms grow and perform well at pH near to neutral or light basic. Lime (CaCO3) is an important constituent in mushroom cultivation. Commercial cultivation of mushroom depends upon proper adjustment of pH of substrate. The pH value of the nutrient (solid and liquid) is changed by mycelial growth. In very acid nutrient medium, the pH value change very slowly since the rate of growth is very slow. With rising mycelial growth, the pH value changes more rapidly (Khan, *et al*., 2011).

2.5.3. Type, availability and chemical composition of targeted substrates

2.5.3.1. Spent Coffee ground

Coffee is one of the most important beverages of the world. Green coffee beans are deemed as a commodity ranking second only to petroleum in terms of currency traded worldwide. The crop is cultivated in coffee growing countries of Latin America, Asia and Africa (Stanculescu, 2011). Coffee is a tropical plant which grows between the latitudes of 25 degree North and 25 degree south. Ideal average temperatures range between 15 – 24 ^{0}C for Arabica coffee and 24 - 30 ^{0}C for Robusta (Mutua, 2000). In general, coffee needs an annual rainfall of 1500 to 3000 mm, Arabica coffee need less than other species (Mutua, 2000). Coffee is produced in more than 60 countries; three of them account for more than half (52 per cent) of the world's production: Brazil, Vietnam and Columbia. Brazil is the largest coffee producer and exporter in the world and the second largest consumer (Gouvea *et al*., 2009).

There is actually a lot of research out there on reusing coffee wastes but much of it relates to what is generated through coffee production and coffee bean harvesting. These wastes include coffee pulp and peel (from the coffee fruit or “cherry”), coffee hulls and husks (covering the coffee seed or bean), and coffee effluent (the waste water used in several of the stages of coffee manufacture) (Chalkier - Scott, 2009).Spent coffee grounds are the waste product from brewing coffee. Coffee brews are usually prepared with an Arabica coffee or Arabica/Robusta blends, from single or different geographical origins, being available to consumers as roasted beans, whole or ground, or even as instant/soluble coffee. Thus, under the “spent coffee grounds” term, one can include those obtained from the soluble coffee industry as well as those produced after brewing at cafeterias or at home. About 0.91 g of the spent coffee ground is produced per 1 g of ground coffee (Dugmore, 2014).

Chemical composition of coffee brews is dependent on the extractive efficiency, which relies on diverse factors, including the coffee species, roasting degree, grinding grade, coffee/water ratio, water quality, temperature, pressure and percolation time. Therefore, different extraction processes will lead to sensorial and chemically distinct brews and, thus, distinct spent coffee grounds (Cruz, 2014).

SCG are composed of 12.4% cellulose, 39.1% hemicellulose (3.6% arabinose, 19.07% mannnose, 16.43% galactose, 23.9% lignin, 2.29% fat, 17.44% protein and 60.46% of total dietary fibers. It makes them interesting sources of raw materials for different applications (Ballesteros, 2014). One distinct advantage of using coffee grounds is that theoretically, they need not be pasteurized because the hot water of the coffee machine has already produced a similar effect. It involves storing the used grounds in a clean container on site, collecting them as soon as possible and then storing them in a freezer until use (Stamets, 2000 and Arora *et al*. 2012.

Coffee wastes are rich in anti-nutritional factors such as tannins. These substances have high capacity to bind proteins, making them unavailable to the organism and also act as enzyme inhibitors (Bressani, 1979; Mazzafera, 2002). Due to the presence of these anti-

physiological/anti-nutritional factors, coffee waste is not considered as an adequate feed supplement for cattle and other livestock consequently, most of them remain unutilized, or poorly utilized. These lead to the problem of environmental pollution (Pandey *et al*., 2000).

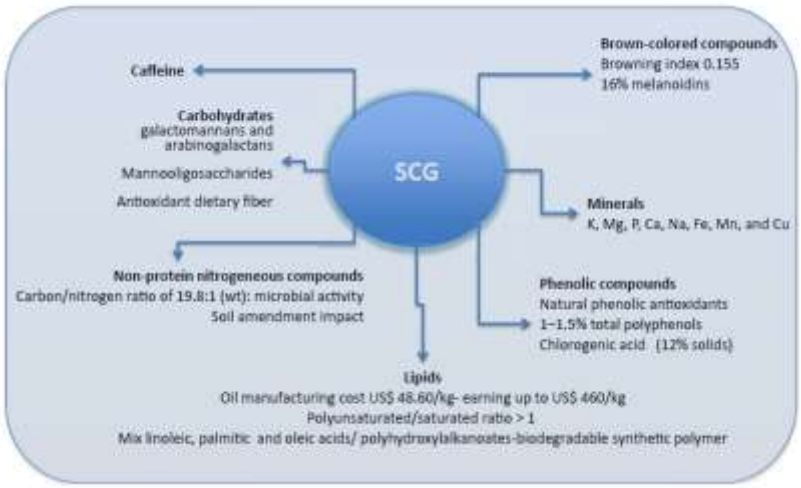

Figure 2. Composition of spent coffee ground.

Source: R. Campos-Vega *et al*. / Trends in Food Science & Technology 45 (2015) 24e36

2.5.3.2 Cotton seed

Cotton seed is one of the most efficient substrate materials for oyster mushroom cultivation (Hang, 1984). If the substrate is to be sterilized or pasteurized, cotton seed is suitable because it emits extra heat by itself (Jawed, 2003). During fermentation, concentrated gas cannot escape to the outer surface of the cotton seed. The control of gas and water is essential for successful cultivation using cotton seed (Qian, 2004).

It contains 9.1% water and 90.9% organic matter that consist of 4% crude protein, 1.4% crude fat, 40.9% crude fiber, 34.9% soluble carbohydrate, and 2.6% ash. Its C: N ratio is about 59: 1 (Oei, 2005). This chemical composition shows how nutritious a raw material cottonseed hull

can be when cultivating oyster mushrooms. Thanks to its soft texture, high water-holding capacity, and good physical structure, cottonseed hull is used worldwide as a good substrate for cultivated oyster mushrooms (Chang, 1984).Being fresh and contamination-free are the basic requirements for cotton seed and should soak overnight to be used as substrate material (Obodai and Vowotor, 2003).

2.5.3.3. Khat (Catha eduis) leftover

khat is an evergreen perennial shrub plant that belongs to the *Celastraceae* family. The plant is known with different vernacular names: Khat in English and in Arabic, Jimaa in Oromiffaa and chat in Amharic. khat usually grows up to 7 meters but occasionally reaches as high as 15 to 25 meters. The buds and leaves contain an alkaloid and are chewed in a fresh or dried condition as a stimulant (Dechassa, 2001)

Some oral traditions claim that khat originated from Yemen; however the literature indicates that khat originated from Ethiopia, specifically in Hararghe with a gradual expansion to different parts of Ethiopia, Yemen and other parts of the world. Outside Africa it is planted in the Arabian Peninsula, Yemen, Afghanistan, India and Sri Lanka for consumption and in the USA, UK and France for experimental purposes (Peter, 1952).

Presently Ethiopia is the main producer and exporter of khat (Wabel, 2011). Khat is replacing coffee (Dube *et al*. 2014) and cereals (Taye and Jens 2003) in different parts of Ethiopia due to its economic benefits. The transport facilities have expanded the khat utilization across the country (Gesese, 2013).

The culture of chewing khat has increased the demand for leaves but results in consequences of health and socio economic conditions (Kandari *et al*. 2014). The Harar town of Ethiopia also produces large quantity of municipal solid waste. Major portion of it is khat waste discarded by users and exporters. The waste production is also enormous that needs proper disposal mechanism (Gessesse, 2013).

Table 4.Contents of Chat

Item	**Amount/mg**
Ash	1.6
Protein	5.2
Fiber	2.7
Ascorbic acid	160
Thiamin	20.05
Calcium	290
Iron	18.5

Source: UN-Emergencies Unit for Ethiopia Addis Ababa, June 2001

3. MATERIALS AND METHODS

3.1. Description of the Study Area

The study was conducted at Haramaya University, Mushroom Research, Production and Training Laboratory of Haramaya University within the campus, which is located at 42°3^{l}E longitude, 9°26^{l}N latitude and at altitude of 1980 m.a.s.l. (AUA, 1996). The experimental room average minimum and maximum temperatures were 12.96^{o}C and 25.72^{o}C, respectively. Relative humidity was in the range of 57.67-91.93% which has been managed by an instrument known as Hygrometer.

3.1. Experimental Design

The experiment was designed in a Completely Randomized Design (CRD) involving two edible oyster mushroom species (*Pleurotus ostreatus and Pleurotus sajor-caju),* six preparations of growth substrates with three replications (2 x 6 x 3).

3.3. Spawn Source and Preparation

Pure cultures of both *Pleurotus ostreatus and Pleurotus sajorcaju* were obtained from Mushroom Research, Production and Training Laboratory of Haramaya University. The pure cultures were initially multiplied on sterilized potato dextrose agar (PDA) medium to get enough stock culture of the pure species needed to prepare the required quantity of spawn (Sara, 2007). Mother spawn was prepared according to the procedure described by Singh and Chaube (1995). Nine kg of the maize seeds was boiled in 15 liters of water for 15 minutes and then allowed to remain soaked in the hot water for another 25 minutes. The water was drained off and the grain was put in a sieve to dry over night. Next day, 120 g calcium sulfate and 30 g calcium carbonate were mixed with each 9 kg of the boiled grains. The calcium sulfate and calcium carbonate were used to maintain the pH close to neutrality (5.5-6.5) and reduce grain adhesion (Smith and Love, 1995). The supplemented grains were filled in half liter sterilized bottles (225 g/ bottle). Bottles were plugged with cotton and covered with aluminum foil then

sterilized in an autoclave at 121ºC for half an hour. After cooled down, the bottles were inoculated with mother spawn and the bottles were shaken to mix thoroughly then incubated at 25ºC for 10 days.

3.4. Source of Substrates for Spawning

Cotton seeds were obtained from Shoa Cotton Ginning Plc, spent coffee ground was collected from coffee vending places at Haramaya University and khat leftover was collected from Bate town around Haramaya University.

3.5. Preparation and Formulation of Substrate for Spawning

The khat leftover, spent coffee ground and cotton seed were used as organic substrates for growing selected oyster mushrooms. The khat leftover was chopped into at least 2 cm then the three substrates (spent coffee ground, cotton seed and khat leftover) were spread separately on polyethylene sheet and allowed to dry for about one week at a regular turning interval of 3 days and then sterilized for 2 hours in a dry fire-heated drum at a temperature of 121°C to avoid contamination and to kill the seeds of cotton. The sterilized substrates were kept in a clean room and allowed to cool down overnight (for 12 hours) (Atikpo *et al*., 2008). Then after the three substrates (khat leftover, spent coffee ground and cotton seed) were kept in gunny bags and soaked in 80 liters of water and 110ml of 2% formalin for about 24 hours for further sterilization. After 24 hours of soaking in water, the excess water was removed from the moist substrate by decanting and manually squeezing by hand until the water stopped dripping (Sara, 2007).

The six different types of substrates and combinations of substrate (50% cotton seed +50% coffee ground, 50% cotton seed + 50% khat left over, 50% coffee ground +50% khat left over, 100% cotton seed, 100% coffee ground and 100% khat left over) were filled into 36 sterile polyethylene bags for two species. Thus, six substrate preparations in triplicates were used (6x3), for *Pleurotus ostreatus and* for *Pleurotus sajor-caju*) and each bag was filled with

substrates weighing 500 g of dry weight. The bags were labeled and arranged according to substrate type and mushroom species they contained (Table 5).

Table 5. Substrate and substrate mixtures

Mushroom species	Substrates	Substrate Composition
PO	sub1	50% CS +50% CG
	sub2	50% CS + 50% KL
	sub3	50% CG+50% KL
	sub4	100% CS
	sub5	100% CG
	sub6	100% KL
PS	Sub1	50% CS +50% CG
	Sub2	50% CS + 50% KL
	Sub3	50% CG+50% KL
	Sub4	100% CS
	Sub5	100% CG
	Sub6	100% KL

Note: CS = Cotton seed; CG = Coffee ground; KL = khat leftover; PO=*Pleurotus ostreratus;* PS= *Pleurotus sajor-caju*

3.6. Spawning

After the substrate preparation was over, the wet substrate was spread on a clean alcohol swabbed polyethylene sheet. Then after, 50 g (which was equal to 10% of the weight of the substrate mixed) of the edible mushroom (*Pleurotus ostreatus, Pleurotus sajor-caju)* spawns were added and thoroughly mixed with the substrate using sterile spoons under the laminar flow hood. Then, rubber bands tied the open ends of the bags and nine small holes were made using sterile scissor to allow air exchange (Dawit, 2008).

3.7. Incubation, Control of the Environment and Cropping

All spawned bags were transferred to a clean and disinfected incubation room and incubated in a complete darkness for 2 weeks at ambient temperatures (24-30 ^{0}C). After full colonization of the bags, sunlight was allowed through the improvised windows. The temperature and humidity were kept at 28^{0}C and 75-85%, respectively, by spraying water to the walls and floors of the cropping room. During the cropping period the bags were sprinkled with water twice a day.

3.8. Data Collection

3.8.1. Number effective fruiting bodies

Small and deformed fruiting bodies were discarded

3.8.2. Time taken (days) for Number effective fruiting bodies

The time taken (days) to grown each batch of effective fruiting bodies were recorded

3.8.3. Biological efficiency

The biological efficiency (BE) of the mushroom species was calculated using the formula recommended by Chang and Miles (1989) as follows:

$$\% \, \mathbf{BE} = \frac{W_2}{W_1} = \frac{weight\ of\ fresh\ musroom\ harvested\ per\ bag}{weight\ of\ dry\ substrate\ \ before\ inoculation\ per\ bag} * 100$$

3.8.4. Chemical analysis

3.8.4.1. Determination of protein content of cultivated mushroom

The crude protein content of the sample was determined by the Kjeldahl method (James, 1995 and Chang, 2003) in which the nitrogen content was priory determined and then multiplied

with 6.25 to obtain the crude protein content. Sampled weight (1g) was added into a Kjeldahl digestion flask. One gram of a mixture of catalysts (Na_2SO_4 mixed with $CuSO_4$ in the ratio of 10:1) was also added into sample. Then 10 ml of concentrated H_2SO_4 was added into the mixture. After this, the digestion flask was placed in the digester and the temperature was brought to 350^oC. Ultimately, the mixture was heated until a clear solution was obtained. Then it was allowed to cool appropriately. After cooling, 30 ml of distilled water was added into the solution. Then 25 ml of 45% NaOH solution was added into the digestion flask. The contents were distilled immediately by inserting the digestion tube line into the receiver flask that contains 25 ml of 4% boric acid solution in which 3 drops of a mixture of indicators, i.e. methyl red indicator and bromocressol green, were added and about 150 ml of distillate was collected. The collected distillate was titrated using a standard acid (0.1N HCL). Finally, percentage of nitrogen was calculated using appropriate formula and the value was converted to percentage protein by multiplying with 6.25 (AOAC, 1995).

Nitrogen (%) = V HCL in lit X N HCL (0.1) X 14(mass of Nitrogen) / Ws X 100

Where: Ws= Weight of sample in g on dry matter basis.

Therefore, % Protein (Crude protein content) = 6.25 X % of Nitrogen

3.8.4.2. Determination of Fat Content of Cultivated Mushroom

To determine of fat content, soxhlet solvent extraction method of James (1995**)** was employed. A 2g of each sample was separately wrapped in a porous filter paper and put in a thimble. The thimble was then placed in a soxhlet reflux flask and mounted into a weighed extraction flask containing 200 ml of petroleum ether. The upper end of the reflux flask was converted to a condenser. When heating, the solvent condenses into the reflux flask and covers the sample until the flask was filled up and siphoned over carrying oil (fat) extract down to the boiling flask. The process was allowed to go on repeatedly for about 4 hours before the defatted sample was removed and kept for crude fat content analysis. The solvent was recovered and the flask with its oil extract was dried in the oven at 60°C for 30 minutes, cooled in desiccators

and re-weighed to obtain the weight of the oil extract (fat), which was then expressed as percentage of the sample. The percentage fat content was calculated using the following formula:

(%) Crude Fat Content= W2-W1/ Sample mass in g on dry matter basis (db) X 100

Where: W2= Weight or mass of flask and fat (oil) extract, W1= the mass of dried flask.

3.8.4.3. Determination of moisture content (%) of cultivated mushroom

The moisture content (MC) of the harvested mushroom was determined by the gravimetric method (AOAC, 1995). One gram of a mushroom grown on each substrate mix ratio was measured separately into a porcelain dish. Then it was dried in an oven at 105^0C for 3 hours, cooled in desiccators and reweighed. The cooled sample was returned to the oven for further drying. Drying, cooling and weighing were done repeatedly at 1 hr interval until no further reduction in the weight (constant weight) was obtained. The weight of moisture loss was determined and expressed as percentage of the sample analyzed.

The percentage moisture content (%MC) was generally calculated using the following formula

% MC = Wet Weight-Dry Mass/ Wet Weight X 100

3.8.4.4. Determination of Fiber Content of cultivated mushroom

Fiber content was determined through digestion of 3 g of each dried, ground (using mortar and pestle) and fat free sample by boiling in a weak solution of 1.25% H_2S0_4 for 30 minutes. The sample was boiled again in a weak solution of 1.25% NaOH for 30 minutes. Then the residue was washed with 25 ml near boiling water and filtered onto a filter paper containing no ash after each washing and drying had taken place. The dried residue was then transferred to the ash dish and ignited at 550°C in a muffle furnace (AOAC, 1990). The fiber content in percentage was calculated using the formula shown below:

(%) Fiber Content= W_3-W_2/ W_1 X 100

Where, W_3= Weight of crucible with dry residue before ashing, W_2 = Weight of crucible with ash after ignition and W1= Weight of sample used in g

3.8.4.5. Determination of Ash Content

Two grams of each processed mushroom sample collected from each substrate mix ratio were dried at 120 ^{0}C for 1 hour in drying oven. Sample dish was removed from the oven and carbonized by a blue flame of Bunsen burner. Ash content was determined by subjecting the carbonized sample at 5500C for 8 hours in a muffle furnace until ashing is complete. At this temperature all the organic matter have been burned off as CO_2, Oxides of Nitrogen and water vapor and the remaining matter was recorded as ash content (AOAC, 1995).

Total ash content was determined the following method:

(%) (Ash) AC= W_2 (g)-W_1 (g) / W (g) X 100

Where: W_1 = Weight of empty crucible, W_2 = Weight of crucible + ash, and W = Weight of sample.

3.8.4.6. Determination of Carbohydrate Content of Cultivated Mushroom

The available carbohydrate content was determined using the following equation (Raghuramulu *et al*., 2003):

% Carbohydrate = 100 − (moisture +crude fat + crude protein + total ash + crude fiber) /100

3.9. Data Analysis

The collected data was subjected to Analysis of Variance (ANOVA) according to Gomez (1984) with three replications using GenStat, Fifteenth edition. Means were compared using Duncan`s Multiple Range Test (DMRT) for significant difference at $p<0.05$.

4. RESULTS AND DISCUSSION

4.1. Number Effective Fruiting Bodies (*Pleurotus ostreatu*)

Table 6 indicates the number effective fruiting bodies obtained from *Pleurotus ostreatus.* There was highly significant ($p< 0.01$) effect of growth substrates on number of effective fruiting bodies. The highest number of 1st effective fruiting bodies (37.00) was grown on 100% CS followed by (18.33) which was grown on 50% CS and 50% KL, while, the lowest number of 1st effective fruiting bodies (10.50) was recorded on 100% CG. Salmones *et al,* (2005) reported that caffeine high concentration of caffeine might have inhibitory effect on invasion of oyster mycelia.

The 2nd number of effective fruiting bodies was also highly significantly ($p< 0.01$) affected by type of growth substrates. The highest 2nd number effective fruiting bodies (26.83) recorded on 100% cotton seed, while, the lowest number (9.50) was obtained on coffee ground. Similarly, the number of 3rd effective fruiting bodies showed highly significant ($p< 0.01$) difference with growth substrates. The highest 3rd number of effective fruiting bodies (13.67) were grown on 100% CS followed by (9.50) grown on 50% CS and 50% CG, while, the lowest number (1.00) recorded on 100% CG.

The highest total number of effective fruiting bodies (77.50) were recorded on 100% CS followed by (48.17) grown on 50% CS +50% CG. The lowest total number of effective fruiting bodies (21) recorded on 100% CG. Mshandete (2011) reported that variations of the number of fruiting bodies could be because of the type of substrate, spawn rate, type and level of supplements and type of mushroom species. Highest number of flash might happen due to nature of the species, utilization of the substrate, type of the substrate or aeration during fructification while the lowest number of flash might be the phenolic contents of the substrate (Cherney, 1989).

The number of effective fruiting bodies through batches decreased on cotton seed, this might be due to exhaustion of nutrient in the substrate, contamination or fluctuation in environmental

condition. However, Masarirambi *et al*. (2011) reported that the highest yield generally obtained at the second flash, this was so probably of maximum metabolism pertaining to substrate breakdown was attained during this time.

The maximum number of effective fruiting bodies of the current study agreed with the study of Mostak and Noorlidah, (2016), they reported the number of fruiting body (34.00) gown on wheat bran supplemented with sawdust. Effective fruiting bodies are important for estimating the yield, biological efficiency and productivity (Royse *et al*, 2004).

4.2. Number of Effective Fruiting Body (*Pleurotus sajor-caju*)

The growth substrates highly significantly ($p< 0.01$) affected the number of effective fruiting bodies in this study (Table 6). The highest (32.00) number of 1st effective fruiting bodies recorded on 100% CS, followed by (18.33) which were grown on 100% KL, while the lowest(1.00) number of 1st effective fruiting bodies on 50% KL and 50% CG (Table 6)

The highest 2nd number effective fruiting bodies (25.00) were recorded on 50% CS and 50% CG, while, the lowest number (4.33) on 100% CG. Similarly the highest 3rd number of effective fruiting bodies (10.00) recorded on 100% CS, while, the lowest (2.00) were grown on 100% CG.

The highest total number of effective fruiting bodies (64.33) were recorded on 100% CS followed by (47) grown on 50% CS +50% CG. The lowest total number of effective fruiting bodies (15) recorded on 100% CG According to Oei (2003) the key factors to induce fruiting bodies of mushroom are changing temperature, high humidity, deficiency of a nutrient, carbon dioxide concentration, light and physical shock.

Table 6. Number of effective fruiting bodies (*Pleurotus ostreatus and Pleurotus sajor-caju)*

Substrates	**1st NEFB**		**2nd NEFB**		**3rd NEFB**		**Total NEFB**	
	PO	PS	PO	PS	PO	PS	**PO**	**PS**
50% CS +50% CG	15.67^{bc}	15.67^{b}	23.00^{ab}	25.00^{a}	9.50^{b}	6.33^{a}	48.17	47
50% CS + 50% KL	18.33^{b}	16.00^{b}	19.33^{bc}	17.33^{abc}	7.50^{bc}	6.00^{a}	45.16	39.33
50% CG+50% KL	13.00^{bc}	1.00^{b}	12.00^{de}	8.67^{cd}	4.83^{cd}	3.67^{a}	29.83	13.34
100% CS	37.00^{a}	32.00^{a}	26.83^{a}	22.33^{ab}	13.67^{a}	10.00^{a}	77.50	64.33
100% CG	10.50^{c}	8.67^{b}	9.50^{e}	4.33^{d}	1.00^{d}	2.00^{a}	21	15
100% KL	17.83^{b}	18.33^{b}	15.83^{cd}	13.33^{bcd}	3.50^{cd}	3.67^{a}	37.16	35.33
CV (%)	7.2	9.3	2.9	6.1	11.1	10.2		
LSD (5%)	5.66	7.557	4.42	6.483	4.02	6.917		

CV= Coefficient Variation, LSD= Least Significant Difference, CG = Coffee Ground. CS = Cotton Seed, KL = Khat Left over. Values with different superscripts on the same column are significantly different ($p<0.05$).

4.3. Time (Days) Taken for Effective Fruiting Bodies (*Pleurotus ostreatus*)

As indicated in table 7, there were highly significant ($p<0.01$) effect of growth substrates on number of days of 1st and 2nd effective fruiting bodies. There was significant($p<0.05$) differences between 100% CS, 50% CS + 50% KL and 50% CS + 50% CG for 1st number effective fruiting bodies The earliest day (5.00) for 1st effective fruiting bodies was recorded on cotton seed followed by (6.33) days on 50 CS + 50% KL after full spawn running. The latest day (8.83) for 1st effective fruiting bodies was recorded on 100% CG similarly for the

2nd effective fruiting bodies the earliest day (9.00) were recorded on 100% CS, while, latest(14.02) days on 100% CG(Table 7)

For the 3rd effective fruiting bodies the earliest day (13.33) also recorded on 100% CS followed by (14.00) day on 50% CS + 50% KL, while, the latest (16.50) day which were recorded on 100% KL. The latest effective fruiting body formation could be due to phenolic content, low cellulose content or low hemicelluloses content.

The earliest total days (27.33) were recorded on 100% CS followed by (30.50) grown on 50% CS +50% KL. The latest total (39.04) recorded on 100% CG. The differences in the time taken between the 1st, 2nd and 3rd effective fruiting bodies might be due to the presence of different factors affecting the growth of fruiting bodies. According to Oei (2003) the key factors are changing temperature, high humidity, deficiency of a nutrient, carbon dioxide concentration, light and physical shock while mushrooms took longer mean maturation periods in the first flush than the three other consecutive flushes could be the effect of light during the incubation period.

4.4. Time (Days) taken for Effective Fruiting Bodies (*Pleurotus* sajor-*caju*)

As indicated in Table 7, the time taken for 1st effective fruiting bodies showed significant ($p<0.05$) difference between 50% CS + 50% KL and 50% CG+50% KL, 50% CS + 50% KL and 100% CS, 100% CS and 100% CG, 100% CS and 100% KL however, number of day for 3rd effective fruiting bodies were not significantly varied ($p>0.05$) (table 7). The lowest time from full spawn running to 1st effective fruiting bodies was observed in 5.33day on cotton seed and the highest time (9.00 day) from full spawn running to 1st effective fruiting bodies was recorded on 100% CG. Bugarski *et al.,* (1994) reported that the first fruiting bodies occurred on different days depending on substrates. Similarly for the 2nd effective fruiting bodies the earliest time(9.00 day) which was recorded on cotton seed, while the latest(13.33 days) was

recorded on 100% KL. For the 3rd effective fruiting bodies, the earliest (13.00 day) was also recorded on 100% CS, while the latest day (17.14) was recorded on 100% CG.

The earliest total days (27.33) were recorded on 100% CS followed by (31.66) grown on 50% CS +50% KL. The latest total (39.37) recorded on 100% CG. Robert (1996) who reported that long period of fruiting body formation is related to high nitrogen content of a different lignocellulosic substrate.

Table 7. Time (days) taken for effective fruiting bodies (*Pleurotus ostreatus Pleurotus sajor-caju*)

Substrates	**1st EFB (days)**		**2nd EFB (days)**		**3rdEFB(days)**		**Total Days**	
	PO	**PS**	**PO**	**PS**	**PO**	**PS**	**PO**	**PS**
50% CS +50% CG	7.33^{bc}	7.67^{ab}	10.83^{cd}	12.33a	14.78a^{b}	16.82^{a}	32.94	36.82
50% CS + 50% KL	6.33^{c}	6.33^{bc}	10.17^{de}	10.33^{a}	14.00^{b}	15.00^{a}	30.50	31.66
50% CG+50% KL	7.83^{ab}	8.67^{a}	11.83^{bc}	12.67^{a}	15.93^{a}	16.64^{a}	35.61	37.98
100% CS	5.00^{d}	5.33^{c}	9.00^{e}	9.00^{a}	13.33^{b}	13.00^{a}	27.33	27.33
100% CG	8.83^{a}	9.00^{a}	14.02^{a}	13.23^{a}	16.19^{a}	17.14^{a}	39.04	39.37
100% KL	8.50^{ab}	8.67^{a}	12.83^{ab}	13.33^{a}	16.50^{a}	17.04^{a}	37.83	39.04
CV (%)	3.7	3.3	2.1	4.4	8.2	9.4		
LSD (5%)	1.205	1.228	1.526	2.633	1.710	3.424		

CV= Coefficient Variation, LSD= Least Significant Difference, CG =Coffee Ground. CS = Cotton seed, KL = Khat left over. Values with different superscripts on the same column (are significantly different ($p<0.05$).

4.5. Effect of Growth Substrates on Biological Efficiency of *Pleurotus ostreatus*

As indicated in Table 8, the growth substrates and combination of substrates was highly significantly ($p<0.01$) affect biological efficiency of *Pleurotus ostreatus* and there was no significant ($p<0.05$) difference between two species on 50% CG+50% KL and 100% CG. The highest biological efficiency (95.18%) was obtained from cotton seed which followed by 50%

CS + 50% KL (77.37%). Cotton seed has fast decomposition rate and hence, it was accepted as a superior substrate over other lignocellulosic wastes (Quinio *et al*. 1990). Zakia Bano *et al*., (1993) also observed Cotton seed supplementation, in trace amounts, doubles the mushroom yield through an activated secretion of cellulolytic and ligninolytic enzymes, favouring active biodegradation of other substrates. On the other hand the lowest (42.27%) biological efficiency of *P.ostreatus* was obtained from 100% CG. Salmones *et al*. (2005) reported that caffeine might have inhibitory effect on invasion of oyster mycelia. The current study was closely related to the finding of Alam *et al.* (2007) who reported the biological efficiency (45.21% to 125.70 %) of *Pleurotus* species grown on saw dust and rice straw while Tsegaye (2015) and Asefa and Geda (2017) reported 79 % and 114% biological efficiency respectively which were grown on combination of cotton waste and coffee pulp and cotton seed waste alone. The fresh mushroom yield or biological efficiency of a species is directly related to strain, substrate nutrition and growth conditions (Upadhyay *et al*., (2002)

4.6. Effect of Growth Substrates on Biological Efficiency of *Pleurotus sajor-caju*

Biological efficiency of *Pleurotus sajur caju* was significantly ($p<0.01$) affected by the substrate type and combination of substrates however, there was no significant ($p>0.05$) difference between two species on 50% CS +50% CG, 50% CS + 50% KL, 100% CS and 100% KL but there was significant ($p<0.05$) difference on50% CG+50% KL and 100% CG across the species (Table 8). The highest biological efficiency (93.47%) of *Pleurotus sajur caju* was obtained on cotton seed followed by 50% CS + 50% KL (73.47%). The lowest (38.97%) biological efficiency of *pleurotus sajur caju* was obtained from 100% CG .The first two highest values of the current study are lies within the range of values reported by Salmons *et al* (2005) for biological efficiency (71.6 - 86.5 %). On the other hand Anteneh (2015) recorded 42.34 % biological efficiency for the same species grown on khat left over. The fresh mushroom biological efficiency was directly related to nutritional composition of the substrate used for growing mushrooms (Khan *et al*., 2001). *Pleurotus sajor-caju* requires relatively high temperature from the group of *pleurotus* (oyster) mushrooms for fructification (Chang and

Miles, 2004) the difference could be capability of the species for utilization and colonization of substrates.

Table 8. Biological efficiency (*Pleurotus ostreatus and Pleurotus sajor-caj*) cultivated on different substrates & substrate combination.

Substrates	**Species**	
	Pleurotus ostreatus	***Pleurotus sajor-caju***
50% CS +50% CG	67.93^{Cc}	67.13^{Cc}
50% CS + 50% KL	77.37^{Bb}	73.47^{Bb}
50% CG+50% KL	60.00^{Ee}	55.47^{Ee}
100% CS	95.18^{Aa}	93.47^{Aa}
100% CG	42.27^{Ff}	38.97^{Ff}
100% KL	63.57^{Dd}	62.57^{Dd}
CV = 7.22		
LSD = 7.31		

CV= Coefficient Variation, LSD= Least Significant Difference, CG =Coffee Ground, CS = Cotton seed, KL = Khat left over. Means with different small letters in column represent significant difference, whereas means with different capital letters in row show significance difference ($p<0.05$).

4.7. Effect of Growth Substrates on Proximate Composition of *Pleurotus ostreatus*

4.7.1. Moisture content

The effect of growing substrates in relation to moisture content of the for *Pleurotus ostreatus* were not significant (p> 0.05) on 50% CS +50% CG, 50% CS + 50% KL and 100% KL but moisture content of *pleurotus ostreatus* grown on 100% CS, 100% CG and 100% KL were significantly (P<0.05) different from each other (Table 9). The highest moisture contents (93.42%) was obtained from 100% CS followed by (90.12%) from 50% CS + 50% KL whereas, the least moisture content (75.08%) was recorded in *Pleurotus ostreatus* grown on 100% CG. Low moisture content might be due to environmental factor (temperature and relative humidity) during growth and storage.

The current result (75.08 – 93.42%) disagreed with those of Hamid *et al.* (1996) who reported that moisture contents of most *Pleurotus* species lie between 80-90% but closely related to Chang and Miles (1989) who reported the moisture content of fresh mushrooms varies within the range of 70 - 95% depending upon the harvest time and environmental conditions. The highest moisture content(93.42%) obtained from 100% CS of the current study was higher than obtained from Ashraf *et al.* (2013) by which the moisture content of *Pleurotus ostreatus* he recorded was 86.27% on cotton seed. As well the moisture content obtained from coffee ground (75.08%) was higher than the result of FAN (2000) who recorded moisture content 66-65% form spent coffee ground. Moisture content obtained from 50% CS + 50% KL was higher than Anteneh (2015) who recorded (89.70 %) grown on khat left over.

4.7.2. Crude fat

The effect of growing substrates were not significantly (p>0.05) different on the crude fat content *Pleurotus ostreatus.*(Table 9). The highest (1.87%) crude fat contents recorded from 50% CS +50% CG and 50% CG+50% KL. The second highest (1.85) crude fat contents was recorded on 100% CS while lowest crude fat content (1.73%) was recorded on 100% CG.

These results agreed with Crisan and Sand (1978) and Anthony (2007) who reported that the crude fat content ranged from 1.6-2.2% and 0.6-3.1%, respectively for *Pleurotus ostreatus.* In another study, Jawad *et al* (2013) reported that the total fat content in *Pleurotus ostreatus* (2.37%) grown on cotton waste, paddy straw and wheat straw. The dissimilarity in crude fat content could be due to the growing substrates or nature of the species of mushrooms even though the factors that influences fat content have not been completely described (Kurtzman, 1997).

4.7. 3. Total ash

As in table 9 indicated effect of 100% CS was significantly ($p<0.05$) different from the other substrates and substrate combinations on the total ash content and the rest growing substrates were not significantly ($p>0.05$) different from each another. The highest (7.97%) values of the total ash contents for *Pleurotus ostreatus* grown on the 100% CS followed by (7.22%) obtained from 50% CS + 50% KL, while, the lowest value (6.77%) of the total ash content was obtained from 100% KL.

The current study closely related to Oei (2003) who reported 8.8% total ash content for *Pleurotus ostreatus* grown on agricultural wastes but lower than reported by Debu (2015) highest value (13.00%) from *pleurotus ostreatus* grown on different saw dusts. According to Anthony (2007), a number of factors such as the site of growth, the type of substrate used and the developmental stage usually influence nutritional composition of the mushrooms.

4.7.4. Crude fiber

As table 9 indicates the effect of growing substrates (50% CS +50% CG, 50% CS + 50% KL and 100% CS) were not significantly ($p>0.05$) difference on crude fiber content in *pleurotus ostreatus* however, these growing substrates were significantly ($p<0.05$) different from 50% CG+50% KL and 100% CG. The highest crude fiber content (11.95%) was obtained from *pleurotus ostreatus* which grown on 100% CS followed by (11.28%) on 50% CS +50% CG while the least (8.68%) crude fiber content was obtained from 100% CG.

These results are in agreement with that of Obodai (1992) who reported 7.5-16.5% crude fiber content for *pleurotus* species. In another study, Jawad (2013) recorded highest (24.53%) crude fiber content for *p. ostreatus* grown on combination of cotton waste, paddy straw and wheat which is lower than combination of cotton seed and khat left over of the current study. The difference could be due to the variation in the nutritional composition of the substrates used by the mushroom species.

4.7.5. Crude protein

Crude protein contents of *Pleurotus ostreatus* were highly significantly ($p<0.01$) affected by the type of growth substrate and substrate combinations (Table 9). The highest (34.61%) crude protein content was obtained from *pleurotus ostreatus* grown on 100% CS followed by (28.08%) obtained from 50% CS +50% CG, while, the least (23.27 %) was recorded from 100% CG.

The values of crude proteins obtained from *Pleurotus ostreatus* grown on 50% CS +50% CG, 50% CS + 50% KL and 100% CS were agreed with the values observed by Chang *et al*. (2003) which is found in the range of 26.9-37.2%. In another study Jawad et *al* (2013) reported the maximum protein (27.23%) grown on combination of cotton waste, paddy straw and wheat straw. This report is also similar with current study that found (28.08 and 27.44%) on 50% CS +50% CG and 50% CS + 50% KL respectively. Difference in crude protein content could be differences in the efficiency of the *Pleurotus ostreatus* in nitrogen utilization or differences in C: N ratio of substrates.

4.7.6. Total carbohydrate

There was highly significant ($p<0.01$) effect of growth substrates on carbohydrate contents of *pleurotus ostreatus* (Table 9). Carbohydrate contents of *Pleurotus ostreatus* obtained from 50% CS +50% CG and 50% CS + 50% KL, while, 50% CG + 50% KL and 100% CG were not significantly ($p>0.05$) different from each other. The highest (49.62 %) carbohydrate content was recorded for *Pleurotus ostreatus* grown on 100% CS and the second highest

(35.88%)carbohydrate content was recorded from *Pleurotus ostreatus* grown on CS + 50% KL. The least carbohydrate content (22.80%) was recorded from 100% CG.

The current study agreed with Bernas *et al*. (2006) which were reported as carbohydrate contents ranged between 16-85%, on the other hand Jawad *et al* **(**2013) reported the highest carbohydrate content(36.74%) for the same species. Sangwan & Saini (1995) reported that difference in carbohydrate content could be due to differences in the carbon content of the substrates.

Table 9. Effect of growth substrate on proximate composition of *Pleurotus ostreatus*

	Proximate Compositions (%)					
Substrates	**Moisture %**	**Crude fat %**	**Ash %**	**Fiber %**	**Protein %**	**Carbohydrate %**
50% CS +50% CG	86.33^{b}	1.87^{a}	7.13^{b}	11.28^{a}	28.08^{b}	32.74^{b}
50%CS + 50% KL	90.12^{ab}	1.82^{a}	7.22^{b}	10.98^{a}	27.44^{bc}	35.88^{b}
50% CG+50% KL	75.17^{c}	1.87^{a}	6.87^{b}	9.63^{b}	23.07^{d}	25.52^{cd}
100% CS	93.42^{a}	1.85^{a}	7.97^{a}	11.95^{a}	34.61^{a}	49.62^{a}
100% CG	75.08^{c}	1.73^{a}	6.80^{b}	8.68^{b}	23.27^{d}	22.80^{d}
100% KL	86.96^{b}	1.79^{a}	6.77^{b}	9.80^{b}	24.85^{cd}	28.40^{c}
CV (%)	1.8	2.8	1.6	4.6	0.8	1.9
LSD (5%)	5.352	0.2743	0.4356	1.097	2.752	3.947

CV= Coefficient Variation, LSD= Least Significant Difference, CG =Coffee Ground .CS = Cotton seed, KL = Khat left over. Values with different superscripts on the same column (are significantly different ($p<0.05$).

4.8. Effect of Growth Substrates on Proximate Composition of *Pleurotus sajor-caju*

4. 8 .1. Moisture content

As indicated in table 10, the effect of 50% CS +50% CG, 50% CS + 50% KL, 100% KL and 100% CS were not significantly (P>0.05) different from each other on moisture content but significantly (P<0.05) different from 50% CG +50% KL and 100% CG for *Pleurotus sajor-caju*. The highest moisture contents (92.87%) were obtained from *Pleurotus sajor-caju* which grown on 100% CS whereas, the second highest moisture contents (90.19%) was recorded on 50% CS +50% CG. The least moisture content (73.77%) was recorded in *Pleurotus sajor-caju* grown on the 100% CG.

These results are agreed with the result of Mckeller and Khorma (1990) in which mushroom moisture contents in the range of 70-90 % but dissimilar with Johnsy *et al*. (2011) who recoded the moisture content of collected mushroom samples including *Pleurotus sajor caju* was in the range of (87.13% to 95.17%). Alam *et al*. (2007) reported (87–87.5%) for *Pleurotus sajor -caju.* On the other hand Anteneh *et al* (2015) reported 91.7% moisture content on khat left over for the same species. Moisture content in mushroom depends on the species, maturity of fruiting bodies and storage conditions during processing or packaging (Guillamón et al., 2010).

4. 8 .2. Crude fat

As presented in table 10, the effect of growth substrates on crude fat were not significantly (p>0.05) different for *Pleurotus sajor-caju*. The highest value of crude fat (2.00%) obtained from 50% KL +50% CG and 100% CG followed by (1.97%) obtained from 50% CS +50% CG While the least (1.76%) from 100% CS. The range of crude fat (1.76 - 2%) obtained in this study disagreed with Stanley (2011) and Anthony (2007 reported a crude fat content of oyster mushrooms were in the range of (2-5%) and (4.30 to 4.41%) respectively however

closely similar with Alam *et al*. (2007) reported crude fat from 0.6-3% for specific species of *Pleurotus sajor -caju*.

4. 8 .3. Total ash

Total ash content of *Pleurotus sajor-caju* mushroom were presented in the table 10.There were significantly ($p<0.05$) differences between 100% CS and 100% CG and 100% CS and 100% KL on total ash content however, the effect of other growth substrates were not significantly ($p>0.05$) different, one from another for the *Pleurotus sajor-caju.*. The highest (8.10%) total ash content was recorded from 1000% CS followed by (7.17%) obtained from50% CS +50% CG while the least one (6.633%) obtained from 100% CG.

The results of current study are somewhat closer to values reported from Dawit (1998) that was (7.2- 8.8%) but different from Alam *et al* (2007) reported (8 - 13%). Kidane (2006) reported higher ash content (12.32%) for *Pleurotus sajor-caju* grown on khat leaves this result also different from the current study that obtained from 100%KL(6.7%) and 50% CS + 50% KL(6.933%). The difference could be due to site of growth, the type of substrate used or developmental stage of the fungal species as described by (Anthony, 2007).

4. 8. 4. Crude fiber

The crude fiber content in *Pleurotus sajor-caju* grown on 50% CS +50% CG and 100% CG and 100% CS and 100% CG were Significantly ($p<0.05$) different on crude fiber content, while there were no significantly ($p>0.05$) difference one from the other 50% CS +50% CG, 50% CS + 50% KL 50% CG+50% KL, 50% CS +50% CG and 100% KL. The highest fiber content (11.40%) obtained from 50% CS +50% CG and 100% CS followed by (10.47%) obtained from 50% CS +50% KL (table 10). The least fiber content (8.43%) was obtained from 100% CG for *Pleurotus sajor-caju*.

The range of crude fiber content (8.43 - 11.4%) obtained from *Pleurotus sajor-caju* in the current study were agreement with the results of Obodai (1992) who reported that the crude

fiber content of *Pleurotus* mushrooms range from 7.5-16.5% similarly, Kidane (2006) reported 10.6% crude fiber content for *Pleurotus sajor–caju* which grown on khat leaves which is closely related with the result of crude fiber obtained from 50% CS + 50% KL of the current study.

4. 8 .5. Crude protein

As in table 10 indicated, the effect of 100% CS on crude protein was statistically highly significantly ($p<0.01$) difference for *Pleurotus sajor-caju* but the effect of 50% CS +50% CG, 50% CS + 50% KL, 50% CG+50% KL, 100% CG and 100% KL were not statistically significantly ($p>0.05$) different one from another. The highest (32.97%) values of protein contents was recorded from *Pleurotus sajor-caju* grown on 100% CS on the other hand, the lowest values of crude protein content (23.71%) obtained from 100%CG.

The current study disagreed with Chang *et al*. (1981) how reported that the fruiting bodies of mushrooms contain 26.6-34.1% crude protein. Jawad *et al* **(**2013) found (25.24%) crude protein in *Pleurotus sajor-caju* which grown on combination of cotton waste, paddy straw and wheat straw, this result closely related to the result of 50% CS +50% CG (27.08%) and 50% CS + 50% KL(26.25%) of the current study. Patil *et al*. (2010) reported that increase in protein content discovered in mushrooms may be attributed to decomposition of total carbohydrate, cellulose, hemicellulose and fiber during growth stage in addition Ragunathan and Swaminathan (2003) also described that, the crude protein of mushrooms is dependent on biological, chemical and the C: N ratio differences of substrates.

Table 10. Effect of growth substrate on proximate composition of *Pleurotus sajor-caju*

Substrate	Proximate Compositions (%)					
	Moisture %	**Crude fat %**	**Ash%**	**Fiber%**	**Protein %**	**Carbohydrate %**
50% CS +50% CG	90.19[a]	1.97[a]	7.17[ab]	11.40[a]	27.08[b]	37.19[b]
50% CS + 50% KL	87.90[a]	1.97[a]	6.93[ab]	10.47[ab]	26.25[b]	31.52[bc]
50%CG+ 50% KL	74.10[b]	2.00[a]	6.97[ab]	9.47[ab]	24.23[b]	25.50[c]
100% CS	92.87[a]	1.76[a]	8.10[a]	11.40[a]	32.97[a]	46.93[a]
100% CG	73.77[b]	2.00[a]	6.63[b]	8.43[b]	23.71[b]	23.93[c]
100% KL	86.15[a]	1.78[a]	6.70[b]	8.93[ab]	24.79[b]	26.00[c]
CV (%)	1.4	3.0	2.3	5.1	2.5	1.0
LSD (5%)	5.154	0.2416	0.7239	1.596	4.506	6.502

CV= Coefficient Variation, LSD= Least Significant Difference, CG =Coffee ground, CS = Cotton seed, KL = Khat left over. Values with different superscripts on the same column (are significantly different ($p<0.05$)

4. 8 .6. Total carbohydrate

Table 10 presents the results of the total carbohydrate obtained from the *Pleurotus sajor-caju.* The effects of growth substrates showed significantly ($p<0.01$) difference on carbohydrate content of *Pleurotus sajor-caju*. The effect of 100% CS was significantly higher than other growing substrates however, there were no significant ($p>0.05$) difference on 50% CS + 50% KL, 50% CG+50% KL, 100% CG and 100% KL among each other on carbohydrate content of *Pleurotus sajor-caju*(Table 10). The highest (46.93%) carbohydrate content was obtained from

Pleurotus sajor-caju grown on 100% CS followed by (37.19%) which recorded on 50% CS + 50% CG while the lowest (23.93%) carbohydrate content was recorded on 100% CG. The result of current study was agreed with Stanley (2011) who reported 17-47% carbohydrate content for oyster mushrooms but lower than Alam *et al* (2007) reported 39.82-42.83% in *Pleurotus spp*, the difference in carbohydrate content could be due to differences in the carbon content of the substrates. In another study in addition Crisan and Sands (1978) reported (50.7%) carbohydrate content for *Pleurotus sajor-caju*.

5. SUMMARY, CONCULSIONS AND RECOMMENDATIONS

5.1. Summary

This study was conducted at the Mushroom Research, Production and Training Center of Haramaya University with the main objective of cultivating oyster mushrooms (*Pleurotus ostreatus and Pleurotus sajor-caju)* using cotton seed, khat left over and spent coffee ground as substrates. The study carried out with six types of substrate and substrate combinations for each species with three replications.

These growth substrates and substrate combinations highly significantly ($p<0.01$) affected the number of effective fruiting bodies, the time required to grown effective fruiting bodies and biological efficiency for both species (*Pleurotus ostreatus and Pleurotus sajor-caju*). The highest number effective fruiting bodies were recorded on 1st batch of effective fruiting bodies which grown on 100% CS taken lowest time (day) and the lowest number of effective fruiting bodies were recorded from 3rd batch of effective fruiting bodies on 100% CG which taken highest time (day) after full spawn running for both species (*Pleurotus ostreatus and Pleurotus sajor-caju*). The highest and lowest biological efficiency were recorded on 100% CS and 100% CG respectively for both species (*Pleurotus ostreatus and Pleurotus sajor-caju*).

Crude protein and carbohydrate contents were highly significantly ($p<0.01$) affected by substrate and substrate combinations for both species (*Pleurotus ostreatus and Pleurotus sajor-caju*) and moisture contents, crude fiber content, total ash content also significantly ($p<0.05$) affected by substrate and substrate combinations. The highest moisture contents obtained from *Pleurotus ostreatus* and *pleurotus sajor-caju* were grown on 100% CS. Maximum crude protein obtained from *pleurotus ostreatus and sajor-caju* were grown on 100% CS while, the minimum were grown on 100% CG similarly the maximum crude fat from *Pleurotus ostreatus* and *Pleurotus sajor-caju* were recorded from 50% CS +50% CG, 50% CG+50% KL and 50% KL +50% CG, 100% CG respectively. Maximum crude fiber was

obtained in *Pleurotus ostreatus* and *Pleurotus sajor-caju* grown on 100% CS and 50% CS +50% CG, 100% CS respectively. The maximum total ash content from *Pleurotus ostreatus and Pleurotus sajor-caju were grown* on 100% CS. The minimum total ash content was recorded on 100% KL and 100% CG for the respective species. The highest carbohydrate content recorded from *pleurotus ostreatus and Pleurotus sajor-caju were* grown on 100% CS while the minimum were on 100% CG.

5.2. Conclusions

In conclusion, both pure substrates and substrate combinations can affect growth and nutritional composition of oyster mushrooms (*Pleurotus ostreatus and Pleurotus sajor-caju*). In comparison of the two species (*Pleurotus ostreatus and Pleurotus sajor-caju*), *Pleurotus ostreatus* gives better growth and nutritional composition. Supplementation of khat left over and coffee ground with cotton seed can give better growth and nutritional composition than the substrates alone for both species while the combination of khat left over and coffee ground given better growth and nutritional composition than coffee ground alone. Despite the results obtained from coffee ground minimum, in the view of its availability and cost, it is not as such a minimum. Uses of these substrates (khat left over and coffee ground) also control environmental pollution resulting from the uncontrolled disposal of these wastes.

5.3. Recommendations

Based on the findings of the study, the following recommendations are made:

- ✓ The combination of khat left over and coffee ground with cotton seed is more suitable for *Pleurotus ostreatus*.

- ✓ The combination of khat left over and coffee ground with cotton seed provides better yield biological efficiency and nutritional composition than khat left over and coffee ground alone for both species.

- ✓ Further study should be made to supplement coffee ground with other cheaper substrate than cotton seed(cotton seed is commercial substrates)
- ✓ Further detailed studies should be made to investigate which part of khat left over (only leaf or leaf and stalk part) gives better production and nutritional composition.
- ✓ Further detailed studies must be made to investigate vitamin and mineral composition of the selected mushroom species on these substrates and substrates combination

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7. ANNEX

During spawn preparation (1), Prepared spawn (2), drying substrates (3), Chopping khat left over (4), Sterilizing substrates (5) and Cultivated *Pleurotus ostreatus* on combination khat left over and cotton seed (6).