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OPTIMIZATION OF COFFEE WASTES FOR THE CULTIVATION OF *PLEUROTUS OSTREATUS*

BY

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Abbreviations

BE……Biological efficiency

CB……Coffee bean

CD……Cow dung

ChM……Chicken manure

CH (p) …… Coffee hulls or parchment

CHs ……. Coffee husks

C/N ……Carbon to Nitrogen ratio

CP ……Coffee pulp

FAO……Food and Agriculture Organization

MEA……Malt extract agar

NG……...No growth

NM……Nug meal

PDA ……Potato dextrose agar

SC…… Standard caffeine

SDYA……...Sabouruad dextrose yeast

SSC ……Solid state cultivation agar

WB……...Wheat bran

Wt……weight

Abstract

Solid state cultivation (SSC) was carried out to evaluate the feasibility of using coffee waste as substrates for the production of the edible mushroom, Pluerotus ostreatus. Coffee husk and coffee pulp powder media were used as a good culture media for Pleurotus ostreatus. Addition of supplementary substrate such as, Wheat bran (WB), Chicken manure (ChM), Cow dung (CD), Noug meal (NM) and Ash improved yield (production) of the mushroom. Composted coffee waste gave better yield than fresh coffee waste. Highest yield (1361.74 and 1232.18g) was obtained from aerobic composted (for eight (8) days) coffee parchment and husk when it was supplemented with 18% cow dung and 8% chicken manure, respectively. With aerobic composted coffee parchment as a substrate the biological efficiency reached 90.74% with four flushes after 90 days. With coffee husk as a substrate the biological efficiency reached 98.57% in 90 days. In the case of fresh substrate the highest biological efficiency 61.48% and 74.73% was obtained from coffee parchment and coffee husk, respectively. In all cases low biological efficiency was recorded from the substrate supplemented with 18% nug meal and 2% ash. There was significant difference (at $P<0.05$) observed between fresh and aerobic composted coffee parchment and coffee husk as well as different supplementary substrates on yield and the biological efficiency. In both cases with different supplementary substrate first flash gave high yield.

The result of these experiments has showed that the feasibility of using composted coffee husk and coffee parchment as substrates with different supplementary substrate for cultivation of edible mushroom in SSC.

Key words: Biological efficiency, coffee husk, coffee parchment, lignocellulosic wastes mushroom cultivation

1. Introduction

Coffee is one of the most important beverages (popular drink) in the world and its yearly production is about seven million tons in more than 60 countries (Mutua, 2000). The coffee plant is an evergreen shrub with a straight trunk which can survive for about 70 years. Coffee classify as a member of the *Rubiaceae* family (Mutua, 2000). Among different species of coffee plant, two alone dominate world trade - the Coffee *Arabica* and *Robusta* (Mutua, 2000). 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 needing less than other species (Mutua, 2000).

Coffee is the most important cash or export crop in Ethiopia. Ethiopia is the seventh largest coffee producer worldwide and ranked ninth in coffee export (Anwar Abasanbi, 2010). In fact the word "coffee" comes from the name of a region of Ethiopia where coffee was first discovered – 'Kaffa'. Ethiopia is the home of biodiversity of Arabica coffee (Bayetta Belachew, 2001). In Ethiopia, coffee is produced in four production systems, namely: forest, semi-forest, garden and plantation coffee in the Western, Southern, and Southwestern parts of the country (Anwar Abasanbi, 2010).

Coffee berries are processed through wet and dry processing. During the wet processing coffee pulp and parchment produced while the dry process resulting in coffee husk. The dry processing is natural, simplest and cheapest method of obtaining coffee bean. The wet method is more expensive than the dry method, but the coffee it produces has better quality properties. From these two processes more than one million tons of coffee waste generated yearly (Fan *et al.*, 2000 and 2003).

Coffee waste is 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 (Pandey *et al.*, 2000). Consequently, most of the husk remains unutilized, or poorly utilized. These lead to the problem of environmental

pollution. If the toxic constituents could be removed, or at least degraded to a reasonably low level, it would open new avenues for their utilization (Bressani, 1979; Shankaranand and Lonsane, *1994*; Soccol *et al*., 2006).

In recent years, there has been an increasing interest towards efficient utilization and value-addition of agro-industrial residues such as coffee wastes. Application of agro-industrial residues in bioprocesses on one hand provides alternative substrates for solid-state fermentation and on the other side helps solving pollution problem (Pandey *et al., 2000;* Shankaranand and Lonsane, *1994*; Soccol, 1996; Soccol *et al*., 2003).

Solid-state fermentation has emerged as an appropriate technology for the management of agro-industrial residues and for their value addition. These substrates are used under SSF for the production of bulk chemicals and value-added fine products such as ethanol, single cell protein , mushrooms, enzymes, organic acids, amino acid, aroma compounds, biologically active secondary metabolites (Fan *et al*., 2000; Medeiros *et al.,* 2006; Murthy and Manonmani 2008; Murthy *et al*., 2009).

Among the bioconversion processes mushroom cultivation is an appropriate technology for the management of agro-industrial residues (Chang and Miles, 1992). Mushroom which is a fleshy saprophyte fungus are found growing in nature on damp rotten log of wood trunk of trees, decaying organic matter and in damp soil rich in organic substances. It is cultivated for its food value world-wide. More than 2000 species of edible mushrooms exist in nature, but only approximately 22 species are intensively cultivated (Manzi *et al*., 2001).

In most countries, there is a well-established consumer acceptance for cultivated mushrooms such as *Agaricus bisporus*, *Pleurotus* spp., *Lentinus edodes*, *Volvariella volvacea* and *Auricularia* spp. (Diez and Alvarez, 2001). The common name “Oyster mushroom” refers to several species of edible mushroom belonging to the genus *Pleurotus*. *Pleurotus spp*. represents the second largest group of cultivated edible mushrooms in the world.

Like other edible Mushrooms, *Pleurotus* species are cultivated on various waste products of human, agricultural, forestry and industrial activities and effectively utilize these wastes,

thus, the growth of the fungi on these substrates help to prevent environmental and health hazards posed by indiscriminate dumping of these materials (Rajapakse *et al.*, 2007).

Several reports showed that coffee has a great potential for cultivation of different types of mushrooms. It has been established that treated coffee husk is suitable for the growth of *L.edodes* (Fan *et al.*, 2000). *Flammulina velutipes* LPB 01 (Fan.*et al*., 2001) and *pluerotus* species (Fan *et al.,* 2003) using solid state fermentation without or with nutrient supplementation. Soccol *et al.*, (2006) demonstrated that the strain *pluerotus ostreatus* LPB 09 was capable of producing the fruiting body on coffee husks, even in the presence of high concentrations of caffeine and tannin in the medium. Fan *et al.,* (2003) showed that toxic compounds decreased and the protein concentration increased after cultivation of *pluerotus ostreatus.*

The present study was undertaken to optimize coffee waste as substrate for cultivation of the oyster mushroom (*Pleurotus ostreatus*) and determination of biological efficiency and to pave the way for effective utilization of coffee waste and reduction of environmental pollution.

2. Objectives

2.1. General objective:

- To optimize different types and components of coffee wastes for production of the oyster mushroom (*Pleurotus ostreatus*).

2.2. Specific objectives:

- To evaluate the feasibility of using coffee husks and parchment as substrate for mushroom cultivation.
- To determine the biological efficiency and yield of all substrate with different supplementary substrate.
- To select the substrates mixture that provides high biological efficiency.

3. Literature review

3.1. World coffee production

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. The world annual coffee production is around 7 million tons, of which Brazil produces one-third (Stanculescu, 2011).

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. The production of coffee in Brazil in the last 5 years ranged from 2.0 to 2.7 million tons. Thus, this commodity is quite relevant to the country's economy (Gouvea *et al*., 2009).

Viet Nam entered the scene in the late 1980s. It increased production from around 500,000 bags in 1986 to about 12 million bags yearly since 2000 — around 11% of world supply. Almost all coffee from Viet Nam is Robusta. Colombia also produces about 12 million bags per year, all of it Arabica (Scholer, 2004).

About 60% to 65% of the world supply of coffee is Arabica, primarily from Latin America. Grown at high altitudes, this fine-flavoured, aromatic type of coffee usually fetches the highest prices. The largest suppliers outside Latin America are Ethiopia, Kenya, India and Papua New Guinea. Robusta makes up more than 35% of world supply (Petit, 2007).
This variety is easier to produce, more resistant to diseases and can be grown at lower altitudes. Robusta is traded at about half the price of Arabica and is often used as filler in blends. Arabica contains 1% to 1.5% caffeine and Robusta approximately 2% (Petit, 2007 and Scholer, 2004).

World coffee production in 2006/2007 is forecast at 123.6 million bags and world coffee export is forecast at 92.8 million bags. World consumption in 2006 is estimated at around 117

million bags. The top five consumers are (in order) the USA, Brazil, Germany, Japan and France (USDA, 2006).

3.2. Coffee Production in Ethiopia

The word "coffee" comes from the name of a region of Ethiopia where coffee was first discovered – 'Kaffa'. The name 'Kaffa' is inherited from the hieroglyphic nouns 'KA' and 'AfA'. 'KA' is the name of God; 'AFA' is the name of earth and all plants that grow on earth. So the meaning of Koffee (Coffee) from its birth-place bells on as the land or plant of God. Ethiopia is the home of biodiversity of Arabica coffee seeds. More genetically diverse strains of *C. arabica* exist in Ethiopia than anywhere else in the world, which has lead botanists and scientists to agree that Ethiopia is the centre for origin, diversification and dissemination of the coffee plant (Bayetta Belachew, 2001).

Ethiopia is the seventh largest coffee producer worldwide and ranked ninth in coffee export. And also the largest coffee producer in Africa: Around 400,000 tons per annual – all of it Arabica which is processed in both method (Mutua, 2000). Coffee production is mainly in South West, South and East of the country, around 90% based on smallholders. An estimated 1.2 million smallholder farmers are engaged in coffee production. Ethiopia and Brazil are the only coffee producing countries that consume a significant portion of their production; around 50% of the production for Ethiopia (Scholer, 2004 and Mutua, 2000).

Coffee is Ethiopia's number one source of foreign exchange. Annual coffee export from Ethiopia is around 200,000 tons valued at around US$ 500 million (Mekuria *et al.*, 2004).
The quality of Arabica from Ethiopia is generally good. Some regions (Sidamo, Yirgacheffe and Haraar) receive very high prices (Mekuria *et al.*, 2004).

Table: 1. Area, production and yield of coffee for private peasants from 2007-2011

years	No of holders	Area in hectors	Production in quintals	Yield quintals / hector
2006/7[a]	2,948,665	295,237.96	2,432,760.79	8.24
2007/8[b]	3,499,219	407,147.07	2,736,028.31	6.72
2008/9[c]	3,223,355	391,296	2,602,118.4	6.65
2009/10[d]	2,959,093	395,003	2,654,420.16	6.72
2010/11[e]	3,854,931	498,617.85	3,704,730.63	7.43

Source:

(CSA, 2007) [a]
(CSA, 2008) [b]
(CSA, 2009) [c]
(CSA, 2010) [d]
(CSA, 2011) [e]

3.3. Processing of coffee cherries

Once the cherries are harvested, the beans have to be extracted by using either the dry or the wet method (fig. 1). The wet method is more expensive than the dry method, but the coffee it produces has better quality properties (Pandey *et al.*, 2000).

3.3.1. Dry method

The dry method (also called the natural method) is the oldest, simplest and requires little machinery. The method involves drying the whole cherry. There are variations on how the process may be carried out, depending on the size of the plantation, the facilities available and the final quality desired. The three basic steps, cleaning, drying and hulling, are described below (Bressani, 1979).

Firstly, the harvested cherries are usually sorted and cleaned, to separate the unripe, overripe and damaged cherries and to remove dirt, soil and leaves. This can be done by winnowing,

which is commonly done by hand, using a large sieve. Any unwanted cherries or other material not winnowed away can be picked out from the top of the sieve.

The ripe cherries can also be separated by flotation in washing channels close to the drying areas. The coffee cherries are then spread out in the sun, either on large concrete or on matting raised to waist height wire mesh tables to dry. As the cherries dry, they are raked or turned by hand to ensure even drying. It may take up to 4 weeks before the cherries are dried to the optimum 11.5% moisture content, depending on the weather conditions. On larger plantations, machine drying is sometimes used to speed up the process after the coffee has been pre-dried in the sun for a few days (Mutua, 2000).

The drying operation is the most important stage of the process, since it affects the final quality of the green coffee. A coffee that has been over dried will become brittle and produce too many broken beans during hulling (broken beans are considered defective beans). Coffee that has not been dried sufficiently will be too moist and prone to rapid deterioration caused by the attack of fungi and bacteria (Jacquet *et al.*, 2008).

The dried cherries are stored in bulk in special silos or in bags until they are sent to the mill where hulling, sorting, grading and bagging take place. All the outer layers of the dried cherry are removed in one step by the hulling machine (USDA, 2006).

The dry method is used for about 95% of the Arabica coffee produced in Brazil; most of the coffee's produced in Ethiopia, Haiti and Paraguay, as well as for some Arabica produced in India and Ecuador. Almost all Robusta are processed by this method. It is not practical in very rainy regions, where the humidity of the atmosphere is too high or where it rains frequently during harvesting (Shankaranand and Lonsane, 1994)

Coffee husks, which are produced through these methods, are the major solid residues from the handling and processing of coffee, since for every kg of coffee beans produced, approximately 1 kg of husks are generated. Proposed alternative uses for coffee husks include employing this solid residue as a supplement for animal feed, direct use as fuel, fermentation for the production of a diversity of products (enzymes, citric acid and flavoring substances), use as a substrate for growth of mushrooms (Gouvea *et al*., 2009; Boccas *et al.*, 1994).

3.3.2. Wet Method

The wet method requires the use of specific equipment and substantial quantities of water. When properly done, the qualities of the coffee beans are better preserved, producing a green coffee which is homogeneous and has few defective beans. Hence, the coffee produced by this method is usually regarded as being of better quality and commands higher prices.

Even after careful harvesting, a certain number of partially dried and unripe cherries, as well as some stones and dirt, will be present among the ripe cherries. As in the dry method, preliminary sorting and cleaning of the cherries is usually necessary and should be done as soon as possible after harvesting. This operation can be done by washing the cherries in tanks filled with flowing water. Screens may also be used to improve the separation between the ripe and unripe, large and small cherries (Bekalo and Reinhardt, 2009).

After sorting and cleaning, the pulp is removed from the cherry. This operation is the key difference between the dry and the wet methods, since in the wet method the pulp of the fruit is separated from the beans before the drying stage. The pulping is done by a machine which squeezes the cherries between fixed and moving surfaces. The flesh and the skin of the fruit are left on one side and the beans, enclosed in their mucilaginous parchment covering, on the other. The clearance between the surfaces is adjusted to avoid damage to the beans. The pulping operation should also be done as soon as possible after harvesting to avoid any deterioration of the fruit which might affect the quality of the beans (Stanculescu, 2011).

The pulped beans go on to vibrating screens which separate them from any un pulped or imperfectly pulped cherries, as well as from any large pieces of pulp that might have passed through with them. From the screens, the separated pulped beans then pass through water-washing channels where a further flotation separation takes place before they are sent to the next stage (Gouvea *et al.*, 2009).

Because the pulping is done by mechanical means it normally leaves some residual flesh as well as the sticky mucilage adhering to the parchment surrounding the beans. This has to be completely removed to avoid contamination of the coffee beans by products resulting from the degradation of the mucilage. The newly pulped beans are placed in large fermentation tanks in which the mucilage is broken down by natural enzymes and can easily be washed

away. Unless the fermentation is carefully monitored, the coffee can acquire undesirable, sour flavours (Mutua, 2000).

When the fermentation is complete, the coffee is thoroughly washed with clean water in tanks or in special washing machines. The wet parchment coffee at this stage consists of approximately 57% moisture. To reduce the moisture to an optimum 11% the parchment coffee is dried either in the sun, in a mechanical dryer, or by a combination of the two. Sun drying should take from 8 to 10 days, depending upon ambient temperature and humidity (Daniels, 2009).

The final stages of preparation of the coffee, known as "curing", usually take place at a special plant just before the coffee is sold for export. The coffee is hulled, to remove the parchment, then passes through a number of cleaning, screening, sorting and grading operations which are common to both wet- and dry-processed coffee. Electronic sorting machines may be used to remove defective beans; including those known as "stinkers" which cannot be distinguished by eye (Mutua, 2000). The wet method is generally used for all the Arabica coffees. It is rarely used for Robustas.

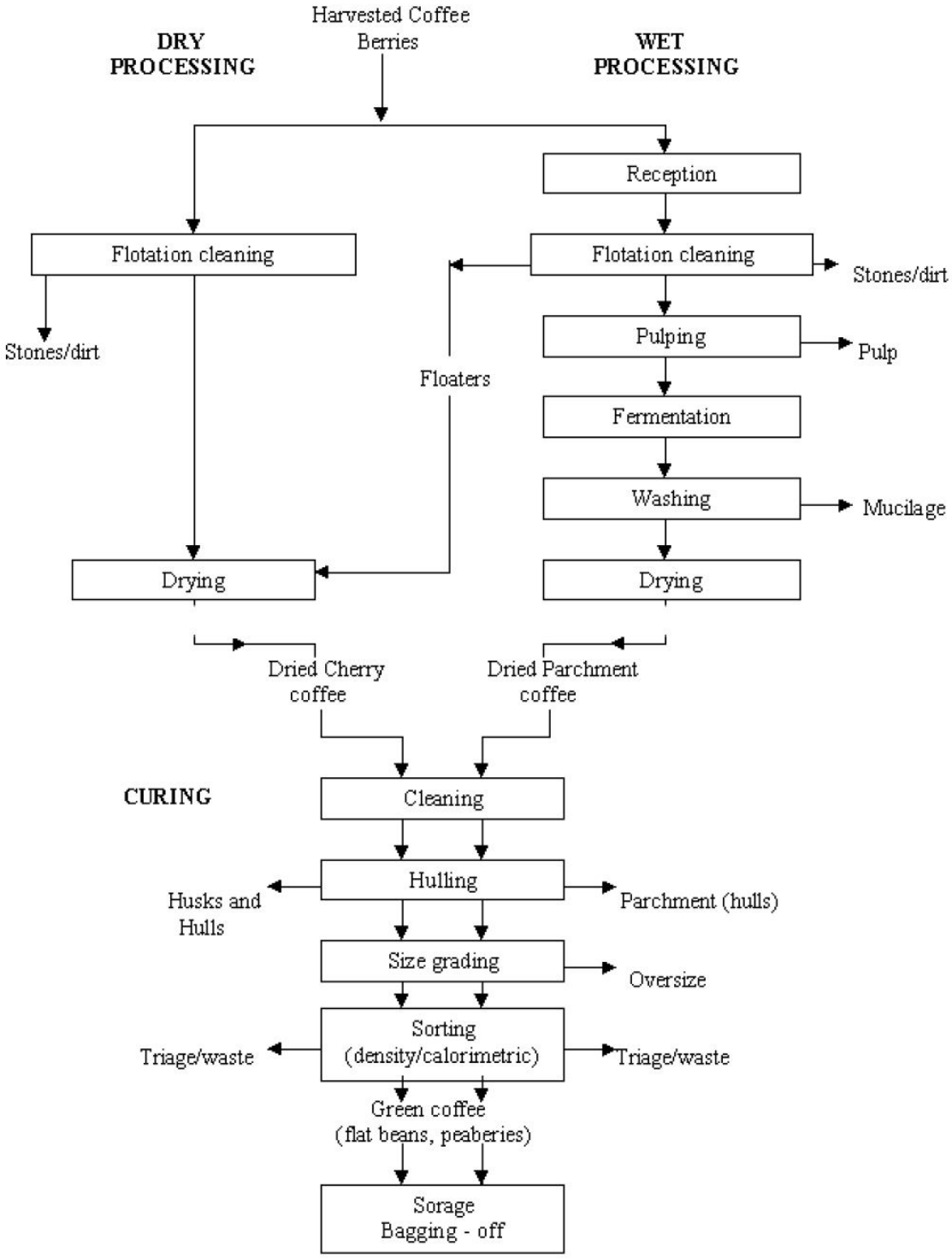

Figure:1 Flow sheets illustrating the stages of wet and dry processing of coffee.

Source: Mutua, (2000).

3.4. Chemical composition of coffee waste

3.4.1. Coffee husks

Coffee husks are the major solid residues from the handling and processing of coffee, since for every kilo grams of coffee beans produced, approximately one kilo grams of husks are generated. Proposed alternative uses for coffee husks include employing this solid residue as a supplement for animal feed, direct use as fuel and fermentation for the production of a diversity of products (enzymes, citric acid and flavouring substances), use as a substrate for growth of mushrooms and use as adsorbents. Such residue consists mainly of the pulp and hull of the coffee fruit; it presents a high concentration of carbohydrates and thus can be viewed as a potential raw material for bio-ethanol production (Franca and Oliveira, 2009).

3.4.2. Coffee pulp

Coffee pulp is some of the most abundantly available agro industrial waste produced during the pulping operation of the coffee cherries to obtain coffee beans in many coffee-producing areas of the tropics. For every 2 tons coffee cherries processed, nearly 1 ton pulp is generated (Roussos *et al.*, 1994).

Coffee pulp is essentially rich in carbohydrates, proteins and minerals (especially potassium) and it also contains appreciable amounts of tannins, polyphenols and caffeine (Bressani, 1979).
Owing to the presence of anti-nutritional factors such as caffeine, tannins and polyphenols, its use as an animal feed has been restricted to a large extent. Hence, coffee pulp has to follow a preliminary treatment before is used. Moreover, this by-product can occur in the nature and spoiling hardly the environment (Roussos *et al.*, 1998).

3.4.3. Coffee Hulls

Coffee hulls are characterized chemically by a high concentration of crude fibre and in this respect they are similar to various other by-products used as fillers in animal feeds. The cellular contents of coffee hulls amount to about 12%, while the cellular wall components,

that are the neutral and acid detergent fibres, are found in amounts of 88 and 67%, respectively (Bressani, 1979).

Cellulose can be utilized by ruminants as a source of energy; however, the utilization of coffee hulls is limited by lignin, silica, and other compounds. Lignin content runs as high as 18% and insoluble ash about 5%. To increase the metabolic utilization of coffee hulls it is necessary to hydrolyze cellulose and similar compounds. Because of its structure and chemical composition, coffee hulls do not offer many other possibilities for use, although it is considered a good fuel (Bekalo and Reinhardt, 2010).

Table: 2. Chemical composition of coffee wastes (% dry basis)

Components	Coffee husks [a]	Coffee pulp [b]	Coffee hulls [c]
Carbohydrates	58-85	44-50	66.65
Proteins	8-11	8.5-12.1	-
Fibres	-	18-21	62.1
Fat	0.5-3	1.5-2.0	0.6
Caffeine	1.3	1 .3	-
Tannins	4.5- 5.4	1.8-2.4	-
lignin	20	17.5	34.2
Cellulose	19–26	17.7	46.1
Pectins	12.4 -13	12.4	-

Source: (Gouvea *et al.*, 2009) [a]
(Bressani, 1979) [b]
(Bekalo and Reinhardt, 2010) [c]

4.5. Chemical composition of supplementary substrates

4.5.1. Chicken manure

Poultry manure (chicken in particular) is the richest animal manure in N-P-K. Chicken

manure must be composted before adding it to the garden. Otherwise, it will burn any plants it comes in contact with (Dikinya and Mufwanzala 2010).

Chicken manure contains about 2.91% of total phosphorus and 2.12% of total potassium which is higher than cow dung (Naher *et al.*, 2004).

4.5.2. Wheat bran

It is a by product of flour making industries. Wheat bran consists of a high amount of starch and other nutrients. Thus it is an important additional substrate in mushroom cultivation. It is also a common supplement in spawn preparation (Dawit, 1998).

4.5.3. Cow dung

The main constituent of cow dung is debris from cells within the digestive tract and secretions from the body such as salts, sloughing of animal cells and mucus. Faeces also include undigested diet comprising cellulose and lignin, originating from the cell walls of the plants. In the analysis of dung, it should also be considered that cowpats and slurry often contain urine as well as faeces. Cow dung is a nitrogen rich material and is of economic importance as fertilizer or as energy sources. Cow dung has been collected and used to supply nitrogen, potassium, phosphorous and calcium to the soil for plant production. Cow dung has a relatively high carbon to the nitrogen ratio. The cow dung facilitated the early maturity of the compost and also contributed to the nutritional content of the composts (Adegunloye *et al.*, 2007).

4.5.4. Noug meal

Niger seed provides 50 to 60% of Ethiopia's indigenous edible oil. The protein-rich meal known as *fagulo* (noug meal) which remains after oil extraction is used as a fuel and animal feed supplement in Ethiopia (Ramadan and Morsel, 2002).

It has appreciable amount of carbohydrates, proteins and oil. It is oil rich waste, as extraction methods often do not exhaustively extract the oil (Dawit, 1998).

4.5.5. Ash

Wood ash is the inorganic and organic residue remaining after the combustion of wood. Wood ash contains calcium carbonate as its major component, representing 25 or even 45%. Less than 10% is potash, and less than 1% phosphate; there are trace elements of iron, manganese, zinc, copper and some heavy metals. In terms of commercial fertilizer, average wood ash would probably be about 0-1-3 (N-P-K). Ash is an alkaline material with a pH ranging from 9-13. However these numbers vary as combustion temperature is an important variable in determining wood ash composition (Mandre, 2006).

3.6. Potential uses of coffee waste residue

The tropical agro-industrial residues such as coffee pulp and coffee husk are generated in large amounts during the processing and their disposal rather causes serious environmental problems. In recent years, there has been an increasing trend towards efficient utilization and value-addition of agro-industrial residues such as coffee pulp and husk. Application of these agro-industrial residues in bioprocesses on one hand provides alternative substrates for solid-state fermentation (SSF) and on the other side helps solving pollution problem (Martinez *et al.*, 2000; Pandey *et al., 2000;* Shankaranand and Lonsane, *1994*; Soccol, 1996 and Soccol *et al* ., 2003).

Several processes have been developed that utilize these as raw material. Solid-state fermentation (SSF) has emerged as an appropriate biotechnology for the management of these agro-industrial residues and for their value addition. These substrates are used under SSF for the production of bulk chemicals and value-added fine products such as ethanol, single cell protein (SCP), mushrooms, enzymes, organic acids, amino acid, aroma compounds, biologically active secondary metabolites (Fan *et al*., 2000; longo and Sanroman, 2006; Medeiros *et al.*,2006; Murthy and Manonmani 2008; Murthy *et al*., 2009).

Coffee by-products are suitable substrates for the production of α- amylase by SSF.
Coffee wastes were transformed into value-added products by fermentation using *Neurosprora crassa* CFR 308 (Murthy *et al*., 2009).

According to Murthy and Naidu, (2010) coffee cherry husk was found to be suitable substrate for protease production. Maximum protease production of 12234U/gds was obtained on pre-treated coffee cherry husk by steam.

Coffee husk has been investigated with solid-state fermentation techniques for their potential to be used as substrates for citric acid production (Nigam, 2009).

Fruity aroma was detected in the cultures of *Ceratocystis fimbriata* using coffee husk as substrate (Medeiros *et al., 2006*). Feasibility of ethanol production from coffee husks also studied by Gouvea *et al*., (2009).

Fan.*et al.,* (2000) reported that the feasibility of using coffee waste without any pre-treatment for cultivation of *pluerotus.* After cultivation, the mushroom spent might be useful for feeding of ruminant as toxic compounds significantly decrease, while the protein concentration was shown to increase to (9.62%). The feasibility of using coffee husk without any nutrients supplementation for cultivation of *Flammulina velutipes* LPB 01 in solid state cultures was studied by Fan *et al.,* (2001).

The feasibility of using the treated coffee husk suitable for the cultivation of *L. edodes* LPB 02 in solid state cultivation was studied by Fan *et al.,* (2000). Fan *et al.,* (2006) demonstrated that the strain *pluerotus ostreatus* LPB 09 was capable to produce the fruiting body on coffee husks, even with high concentrations of caffeine and tannin in the medium.

3.7. Morphology and classification

3.7.1. Morphological description

Species identification within the genus *Pleurotus* is difficult because of the morphological similarities and possible environmental effects. Mating compatibility studies have demonstrated the existence of eleven discrete intersterility groups in *Pleurotus* to distinguish one species from the others. *P. columbinus, P. florida, P. salignus,* and *P. spodoleucus* are the synonyms or subspecies taxa for the species of *P. ostreatus*. Cap: 40-250mm broad, oyster-shape, spatulate to lingulate when young, convex then later becoming conchate to flabellate, surface smooth. Gills: crowded, whitish to cream or pale greyish, edge smooth,

later somewhat undulating, lamellulae 1- or 3-tiers. Stipe: 10-20×10-25mm, rudimentary, usually lateral, severa concrescent, surface longitudinally striate (OECD, 2005).

3.7.2. Classification

P.ostreatus is in Phylum Basidiomycota, Class Agaricomycetes, Order Agaricales family *Pleurotaceae* (Fr.) Fr., belonging to the genus *Pleurotus*, is widely distributed throughout the Northern Hemisphere, such as Europe, North Africa, Asia and North America (Singer,1986). To date, approximately as many as 70 species of *Pleurotus* have been recorded and new species are discovered more or less frequently although some of these are considered identical to previously recognised species (Inamulhaq *et al*., 2010).

Several closely related species of mushroom are commonly known as oyster mushrooms: *Pleurotus ostreatus*, *Pleurotus pulmonaris* and *Pleurotus populinus*. These three types are similar in many respects but are different enough to be classified as different species. Mating experiments and DNA analysis demonstrate these differences in the laboratory, while morphological and ecological differences are also apparent in the field (OECD, 2005).

3.8. Life cycle of Pleurotus ostreatus

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 (Dawit, 1998). Four basidiospores form at the end of each basidum on the gill of a fruitbody (Fig. 2). Each spore has one nucleus. Spores germinate to become primary mycelia, and then form secondary mycelia by plasmogamy. Chances are 25% that a primary mycelium will meet with a compatible one. Secondary mycelia of oyster mushroom can be distinguished by the clamp connections and each cell has two nuclei. Only secondary mycelia can produce fruitbodies under the proper conditions. In the basidia of a mature fruitbody, the two nuclei fuse into one, then pass through meiosis, and produce 4 haploid nuclei. The four haploid nuclei are then made into four new basidiospores *(*Kang, 2004).

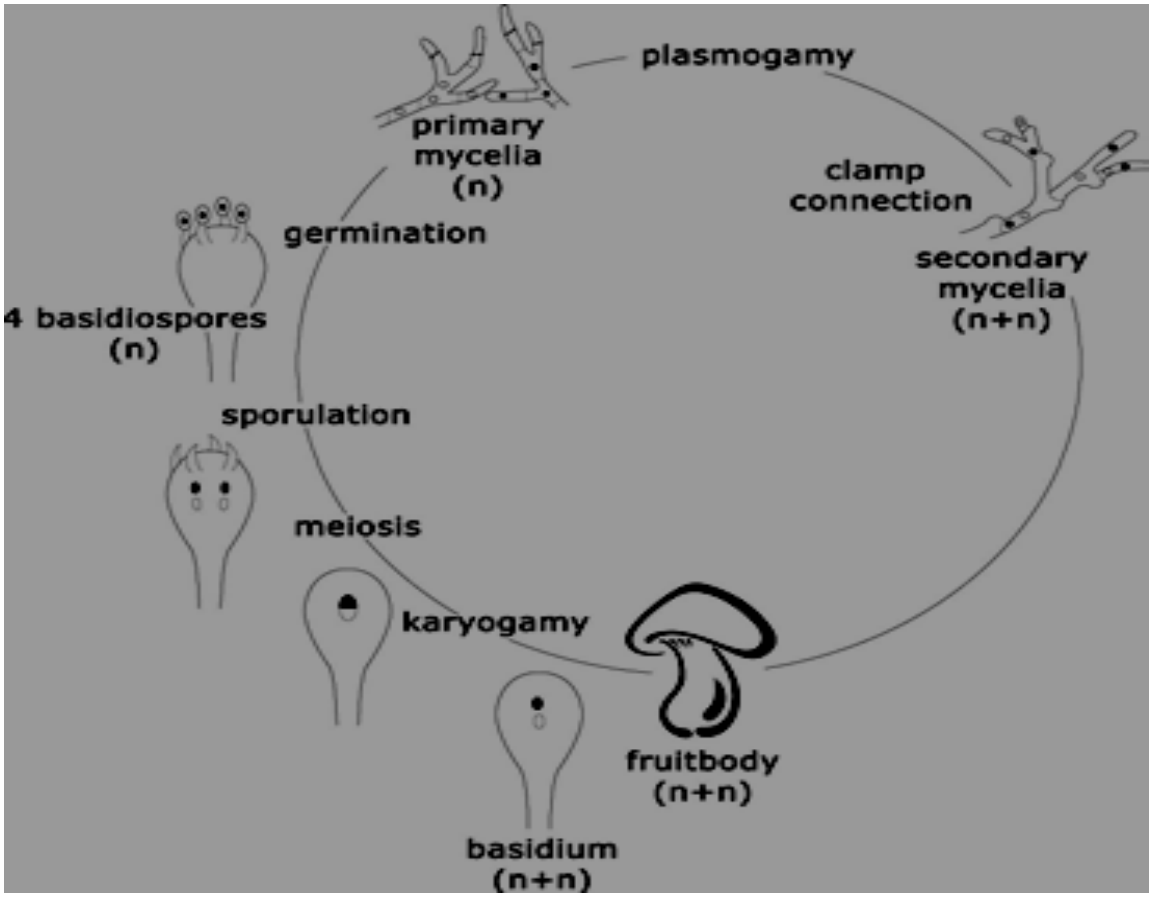

Figure: 2 Life cycles of *Pleurotus ostreatus*

Source: Kang, (2004)

3.9. Growth requirement and condition

3.9.1. Mycelia Growth

The carbon sources suitable for mycelia growth are starch, glucose, fructose, maltose, mannose, sucrose, pectin, cellulose and lignin. Ethanol is also a source of carbon for mycelia growth; however, citrate, oxalate and other organic acids are not beneficial to the growth of the mycelium. The nitrogenous sources utilised by *Pleurotus* spp. are peptone, corn steep liquor, soybean cake powder, yeast powder, ammonium sulfate, asparagine, serine, alanine and glycine (Nwokoye *et al*., 2009).

The optimal temperatures for growth of the mycelium are around 25-28 ^{0}C and the range of pH is about 5.5 to 6.5. The tolerance of mycelia for carbon dioxide is rather strong. The mycelia of *Pleurotus* spp. can still grow flourishingly at the carbon dioxide concentration of 15 to 20%. Only when the concentration of carbon dioxide is raised to 30%, the growth of mycelia rapidly decreases (Chang and Miles, 1989).

3.9.2. Fruit body Production

For fruiting body formation, Co_2, light and temperature is key environmental factors. When the Co_2 concentration in the mushroom house or growing bags is higher than 600 ppm (0.06%), the stipe elongates and the growth of the caps will be prevented.
The requirements for light are different for the various stages of growth. The growth of mycelium does not need any light and cultivation of the oyster mushroom in a dark place is better than in a bright place (OECD, 2005).

The formation of primordia and the growth of fruiting bodies require light. The former requires light of 200 lux intensity for over 12 hrs. The growth of the fruiting body requires light of 50 to 500 lux intensity. The colour of the caps is closely related to the intensity of light, and if it is low, then the colour will be pale. The optimal temperatures for the development of fruiting bodies can range from 10 to 18 ^{0}C. (Growers can choose a suitable strain for their own natural environment. Each *Pleurotus* species needs different environmental conditions for fruit body development (Chang and Miles, 1989).

3.10. Wild and cultivated Pleurotus ostreatus

During the rainy season, different species of both edible and non edible species usually grow on various natural substrates such as garden soil, decaying wood, termite nest, palm wastes, leaf litters, under the shade provided by cocoa, teak, coffee and rubber plantations. Mushrooms growing in the wild have been found to be nutritious and important for medicinal purposes. They have been considered as rich food because they contain protein, sugars, glycogen, lipids, vitamins, amino acids and crude fibres. They also contain important mineral nutrients, which are required for normal functioning of the body (Gbolagade *et al.*, 2006).

In most rural African village communities, indigenous edible mushrooms are highly treasured. Since they start growing soon after the first rains and become very handy vegetables long before the agricultural crops planted are ready for harvesting (Masamba and Mwale, 2010).

3.10.1. Cultivated *pleurotus ostreatus* mushroom

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).
Cultivated mushroom are generally saprophytes, utilizing substrate as primary or secondary decomposers. *Pleurotus oyster* is one of the four important wood inhabiting cultivated mushrooms in Japan, China together with *Lentinus edodes, Flammulina velutipes*, and *Pholiota nameko* (Chang and Hayes, 1978; Cohen *et al*., 2002).

The Chinese were pioneers in the development of fungi culture techniques, being shiitake the first mushroom produced, by using tree logs (Kues and liu, 2000). Later, the culture spread to several countries of North America and Europe. Now a day the world production of mushrooms was around 3.4×10^6 tons in 2008, the largest producers being China, with 1.5×10^6 tons and the USA with 0.38×10^6tons. Although there are more than 2000 species of edible mushrooms, nowadays only approximately 22 species are intensively cultivated (Manzi *et al.*, 2001). Among these only the champignon (*Agaricus bisporus*), the giant mushrooms (*Pleurotus ostreatus and Pleurotus ostreatoroseus*) and shiitake (*Lentinula edodes*) are among the most cultivated ones and are well appreciated (Carvalho *et al.*, 2010).

Interestingly, among commercially cultivated mushrooms, the oyster mushroom is the thrid most important mushroom in production in the world. The genus *Pleurotus* has gained much popularity world over and is presently believed to be potential rival of commonly cultivated button mushroom (*Agaricus bisporus*). In genus *Pleurotus*, more number of species has been reported as cultivated than in any other edible fungus. *Pleurotus ostreatus* (Jacq: Fr.) Kummer is the most cultivated species among the oyster mushroom. It contributes about 24.1

percent (0.909 million metric tons) of the total mushroom production of 3.772 million metric tons (Munshi and Ghani, 2003 cited in Sheikh *et al*., 2010).

P. ostreatus was first cultivated in the USA in 1900 and several other species of the oyster mushroom such as *Pleurotus sajor-caju* were initially cultivated in India after the late of 1940s. Oyster mushroom is grown worldwide, and China is the major producer. It has been regarded as one of the most profitable cash crops in Korea, accounting for 65% of total domestic mushroom production (OECD, 2005).

3.11. Importance of pleurotus ostreatus and other mushroom

3.11.1. Nutritional value

Mushrooms have been used as food and medicine in many parts of the world since time immemorial. Although mushrooms are often grouped with vegetables and fruits, they are actually fungi. They are macro-fungi which belong either to Basidiomycetes or Ascomycetes and they are very distinct from plants, animals and bacteria (Mushigeni and Chang, 2001).

It is evidently clear that the growing interest in the cultivation of mushrooms can help in solving many problems of global importance such as protein shortage as well as improving the health and well being of people, considering that mushrooms are valuable health foods which are low in calories and provide essential minerals (Masamba and Mwale, 2010).

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 and Oei, 2003).

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*., 2009).

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)**.**

3.11.2. Medicinal Properties of *Pleurotus* Species

The fungus is widely used in Asian medicine to treat all types of diseases. Some mushrooms contain compounds, which can make a contribution to the general health of man. As mushrooms are widely distributed all over the world, some of them have been used in traditional medicine as anti inflammatory, analgesics, homeostatic, diuretic, nourishment, antibiotic and anti tumour agents (Shittu *et al.*,2005 ; Chang, 1999).

Most of the medicinal extracts from mushrooms are different forms of polysaccharides and all of them are strengtheners of the immune system with little or no side effects. For example, a sizofiran, antitumour polysaccharide extracted from the culture broth of *Schizophyllum commune* is an effective immuno-therapeutic agent for cervical carcinoma because it stimulates a rapid recovery of the immunological status impaired by radiotherapy (Oei, 1991).

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).

Pleurotus ostreatus (oyster mushroom) is a commercially important edible mushroom highly acclaimed for its nutritional and medicinal properties.

Recent studies on Various *Pleurotus* species possess a number of beneficial medicinal properties, such as antitumour, immunomodulatory, antigenotoxic, antioxidant, anti-inflammatory, antiallergic, hypocholesterolaemic, antihypertensive, antihyperglycaemic, antimicrobial and antiviral activities. These activities have been reported for various extracts and isolated compounds, such as polysaccharides, polysaccharide- protein complexes, proteoglycans, proteins and DNA from oyster mushroom fermentation broth, mycelia or fruiting bodies. In particular, polysaccharides appear to be potent antitumour and immunomodulating substances, besides possessing other beneficial activities (Gregori *et al*., 2007; Khan *et al.*, 2011).

Various bioactive compounds isolated from culture extracts of Ethiopian higher fungi showed other biological properties such as antiprotozoal, anthelmintic, phytotoxic and brine shrimp lethality activities (Ermias and Dawit, 1995).

3.11.3. Waste recycling

Most of the fungi have strong enzyme and are capable of utilizing complex organic compounds which occur as agricultural wastes and industrial by products. Mushroom fungi also belong to this group. Thus agricultural wastes can also be used as bedding material for mushroom cultivation (Baysal and Peker, 2001).

The importance of edible mushrooms has increased due to the advances in cultivation technology, which makes the use of agricultural and industrial residues possible by recycling them as substrates for cultivation, consequently resulting in low-cost production and a continuous market (Pandey *et al*., 2000).

Moreover, they represent an excellent alternative for discarding several residues, helping in reducing pollution caused by the presence of these materials in the environment (Carvalho, 2010).

Large volumes of lignocellulose agricultural residues (fish waste, vegetable materials) are generated annually through agricultural and food processing industries. These are either

disposed of by burning or dumping in landfills, thus posing hazard to the environment and human health; and which would otherwise be used in the cultivation of edible and medicinal mushrooms (Atipko *et al*., 2008).

The oyster mushroom *Pleurotus* sp. is cultivated in many countries both in sub-tropical and temperate regions of the world. Like other edible mushrooms, *Pleurotus* species too can be grown on various agricultural waste products without the addition of enrichment materials (Rajapakse, 2007).

An attractive feature of this group of mushroom is due to the capacity of secreting spectrum of enzyme 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).

3.12. Uses of spent

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. 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 and 2007).

Spent after mushroom cultivation on different substrates such as cottonseed waste, cereal straw and bagasse serve as animal feed supplements (Dawit, 1998).

Analyses of the banana leaf straw after its use in the culture of *P. ostreatus* (spent substrate) has shown that the degradation of this lignocellulosic material promoted by the action of lignocellulolytic enzymes excreted by *Pleurotus* makes this spent substrate desirable for diverse uses, such as animal feed, mulch in agriculture and substrate for growing fungi (Gern, *et al*., 2010).

Agaricus blazei is a secondary decompositor and cannot grow directly on cellulose and lignin present in the straw, requiring a previous degradation of the substrate through a composting process (Oei, 1996).

Mushroom spent used as organic fertilizer. The spent is made of degraded and undegraded plant polymers. Spent from already composted substrates such as *Agaricus bisporus* could be applied directly to fertilize the gardens and horticulture plants (Dawit, 1998).

4. Materials and methods

4.1. Sample collection

Coffee hulls or parchment were obtained from coffee-processing factories (BUNA BOARD) located in Nifasilk (Addis Ababa) and coffee husks were obtained from Jimma. Cow dung and chicken manure used for the study were collected from local animal and poultry farm, respectively found in Sendafa. Wheat bran and Nug meal were collected from local market.

4.2. Preparation of culture media

4.2.1. Cultivation of *Pleurotus* on PDA

The mushroom species, pure cultures of *Pleurotus ostreatus* maintained on PDA (Oxoid) slant at 4^0C were obtained from Mycology Laboratory, Faculty of Life Science. Thirty nine (39) grams of PDA (potato extract- 4.0g, Dextrose- 20.0g and Agar- 15g) was added to 1 litre distilled water into 1litre flask. Then it was placed on Bunsen burner to dissolve agar. It was autoclaved at 121 ^{0}C for 15 min. 15 ml of the medium was then dispended into 9-cm diameter petridishes. These were inoculated with the *Pleurotus ostreatus* cultures by using cork borer and incubated at 25 ^{0}C. Mycelia growth in terms of diameter on culture plate was measured using ruler to compare with mycelia growth on coffee waste agar medium (Thomas *et al.*, 2010).

4.2.2. Cultivation on other media

Five types of agar media, these are Coffee husk (CHs), Coffee parchment or hulls (CH (p)), Coffee pulp (CP), Coffee bean (CB) and pure caffeine (PC) were used as media. These were prepared by drying and powdering all coffee waste, and then 40 g/l in distilled water was taken for each sample (Fan.*et al*., 2001and 2006). In the case of pure caffeine, which obtained from chemistry laboratory, 20mg/l was taken in distilled water. By using its natural pH (CHs= 5.17, CH (p) = 5.55, CP = 4.94, CB = 5.68 and PC =6.50) all media were sterilized at 121 ^{0}C for 15 min after 2% agar was added. 15 ml of the medium was then dispended into 9-cm diameter Petri dishes. These were inoculated with the pure culture of *Pleurotus ostreatus*

by using cork borer and incubated at 25 ^{0}C. Mycelia growth in terms of diameter on culture plate was measured using ruler in two days of interval.

4.3. Spawn preparation

In order to prepare spawn, about 15 kg of sorghum was soaked overnight (for 12hr) in water. The excess water was drained off and (2%) wheat bran and (1%) gypsum was added. The ingredients were thoroughly mixed; moisture was adjusted to 55- 60% with some modification of determination of moisture content according to Fan *et al*. (2000a). Then the mixture was distributed equally in to 500 ml glass bottle, at the rate of 350 g seed per jar for a total of 100 bottles and autoclaved, at 121 ^{0}C for 30 min. After cooling, each bottle was inoculated with fungal culture (one plate to one glass jar). After 20 days of incubation (when the mixture was totally invaded by mycelium), at 25 ^{0}C, the spawn was ready to be used for the inoculation of the solid substrate (Fan *et al*., 2000a).

4.4. Substrate preparation and evaluation

4.4.1. Aerobic compost preparation

In order to prepare, aerobic composted substrate, about 80% of coffee husk or parchment Alone was soaked overnight (for 12hr) in water. The excess water was drained off and the moisture was adjusted to 55- 60% (Fan *et al*., 2000b).Then supplemented with 20% four different substrates individually as follows.

Substrate A. 10% Wheat bran, 8% chicken manure and 2% wood ash

Substrate B. 18% Wheat bran and 2% wood ash

Substrate C. 18% Cow dung and 2% wood ash

Substrate D. 18% Noug meal and 2% wood ash on dry weight basis with some modification of (Dawit , 1998).Then the mixture were filled in wooden boxes of 1m x 0.5m size and incubated outside the house under the shade for eight (8) days. After eight days of composting, these substrates were distributed equally in to plastic bags of 40x60 cm size at the rate of 3.5kg substrate in triplicates and autoclaved at 121^{o}C for 30min. After cooling; they were inoculated with the spawn (one glass bottle per bag) and mixed thoroughly to facilitate rapid and uniform mycelia growth. The mouth of the bags was tied using a cotton plug and thread and holes were made over the Polythene bags for aeration.

Then they were incubated in the dark at 27 ^{0}C and mycelia development in the bag was observed and noted within 5 days. In the case of fresh substrate the same trend was followed as above except aerobic composting.

4.5. Measurement of growth

The growth was measured qualitatively and quantitatively through

- **a.** Colony diameter measuring
- **b.** Mycelia invasion(visual observation of mycelia growth)
- **c.** Biological efficiency

4.6. Biological efficiency

Biological efficiency (BE) was calculated as follows (Fan *et al*., 2003).

$$BE = \frac{\text{Fresh weight of the mushroom}}{\text{Dry weight of the substrate}} \times 100$$

4.7. Data analysis

The data on mycelia growth rate on 6 types of media with Triplicates for each and biological efficiency of *Pleurotus ostreatus* on 2 kinds of substrates were determined. Analysis was performed for all data with Triplicates for each. The data were analyzed by comparing the mean weights, and % biological efficiency through ANOVAs. The data groups were analyzed using Statistical Package for Social Sciences (SPSS) for windows 16.0. Biological efficiency was compared using LSD test.

5. Results

5.1. Culture production and mycelia growth rate on different agar media

During the present investigation, 6 types of agar media were used and obtained different resulted. The result revealed that the length of mycelia growth in diameter on CH (p) media was reached (9cm) after 8 days of incubation, at 25^{o}C. Full mycelium invasion of *Pleurotus ostreatus* on culture plate took 10 days on CHs, 12 days on CP, PDA and PC, 14 days on CB relatively (table 3). As shown in table 3 even though, full mycelium growth of *Pleurotus ostreatus* took 8 days on CH (p), mycelia invasion on culture plate was very thin within 12 days when compared to growth on other media (Fig. 3). Mycelia growth diameter and invasion on CHs (9.0cm) and CP (8.87cm) medium was almost comparable with PDA (8.9cm) which is used as standard, at 10 days of growth. The least very thin mycelia invasion was observed on PC agar medium (Fig 3).

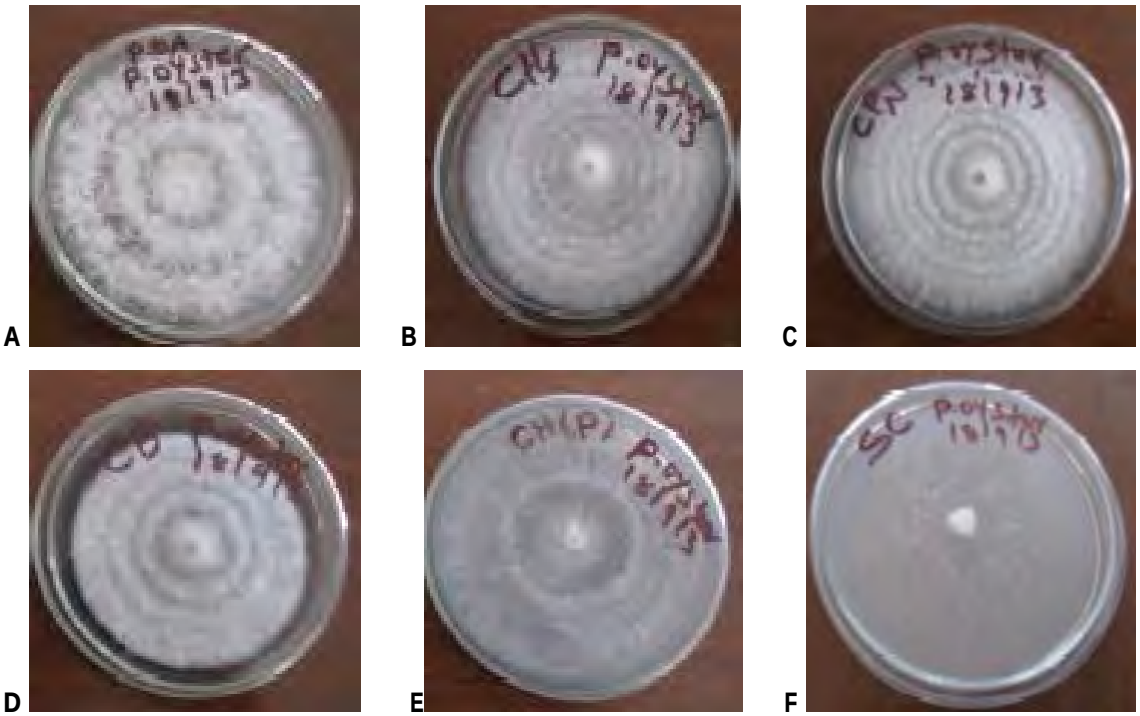

Figure: 3.The mycelia growth diameter of *Pleurotus ostreatus* on different agar media.

Table: 3. Mycelia growth diameter of *Pleurotus ostreatus* on different media

	Measurement (cm)					
Culture media	2nd day	4th day	6th day	8 th day	10 th day	12 th day
CP	1.00	2.80	4.63	7.13	8.87	9.13
CHs	0.97	2.90	4.93	7.27	9.00	9.13
CB	1.17	2.53	4.17	5.87	7.30	8.83
CH(p)	1.37	3.90	6.33	9.00	9.10	9.10
SC	0.70	2.67	4.73	6.40	8.47	9.17
PDA	1.50	3.37	5.17	7.73	8.90	9.16

Data repesented by mean value of triplicate

CP=Coffee pulp agar medium, CH= Coffee husks agar medium, CB=Coffee bean agar medium, CH (P) = Coffee hulls or parchment agar medium, PC=Standard caffeine agar medium, PDA= Potato dextrose agar medium

5.2. Preparation of Spawn

Fully white mycelia invasion of *Pleurotus ostreatus* was observed on sorghum after 20 days of incubation as in Fig. 4. It was ready to be used for the inoculation of the solid substrate.

Figure: 4. *Pleurotus ostreatus* spawn grown on sorghum after 20 days of incubation.

5.3. Mushroom growing using coffee hulls or parchment

As indicated in Table 4 and 5 in all cases with different supplementary substrate *Pleurotus ostreatus* cultivation on aerobic composted coffee parchment gave higher yield when compared to fresh coffee parchment. Among the fresh coffee parchment which is used as a substrate, highest biological efficiency (61.48%) was obtained when coffee parchment supplemented with chicken manure and less biological efficiency (30.74%) obtained when the substrate supplemented with noug meal (Table 4). In the case of aerobic composted coffee parchment high (90.74%) and less (67.07%) biological efficiency was obtained when the substrate supplemented with 18% cow dung and 18% nug meal, respectively (table 5). *Pleurotus ostreatus* cultivation on the control (coffee parchment without any treatment) gave very less biological efficiency (27.95%) when compared to the treated once (Table 4 and 5). In both cases (fresh and composted coffee parchment) first flush gave high yield and become decrease from flush to flush.

Table: 4. Yield and biological efficiency on fresh coffee parchment supplemented with different substrates.

substrate	Content of substrate	Flushes(grams)				total	BE in %
		1st	2nd	3 rd	4th		
Fresh coffee parchment	A_1	303.83[a]	267.17[c]	216.43[e]	134.83[f]	922.26	61.48[a]
	B_1	259.43[c]	201.00[b]	176.27[d]	89.17[e]	725.87	48.39[b]
	C_1	233.53[e]	146.87[a]	105.77[f]	42.67[g]	528.84	35.25[d]
	D_1	229.27[e]	145.83[a]	89.00[a]	NG	464.1	30.74[c]
Control(CP)		206.41[h]	91.25[f]	66.33[g]	55.35[g]	419.34	27.95[c]

Means within the column under a parameter, having a common letter do not differ significantly at ($p<0.05$).
A_1 =Coffee parchment with chicken manure, B_1= Coffee parchment with wheat bran, C_1= Coffee parchment with cow dung, D_1= Coffee parchment with Noug meal, Control(CP) = Coffee parchment without any supplement , CP= Coffee parchment and NG=No growth.

Table: 5. Yield and biological efficiency obtained from composted coffee parchment with different substrates

substrate	Content of substrate	Flushes(grams)				total	BE in %
		1st	2nd	3 rd	4th		
Composted coffee parchment (8days)	A_1	485.50[b]	330.83[d]	229.67[e]	128.83[f]	1174.83	78.32[f]
	B_1	485.07[b]	325.67[d]	191.67[d]	147.33[d]	1149.74	76.64[f]
	C_1	549.67[f]	423.17[e]	227.67[e]	160.67[d]	1361.18	90.74[g]
	D_1	452.33[g]	281.50[c]	177.33[d]	95.00[e]	1006.16	67.07[e]
Control(CP)		206.41[h]	91.25[f]	66.33[g]	55.35[g]	419.34	27.95[c]

Means within the column under a parameter, having a common letter do not differ significantly at ($p< 0.05$).

A_2 =Coffee parchment with chicken manure, B_2= Coffee parchment with wheat bran, C_2= Coffee parchment with cow dung, D_2= Coffee parchment with Noug meal, Control(CP)= Coffee parchment without any supplement.

5.4. Mushroom growing using coffee husk

In general, cultivation on coffee husk provided higher biological efficiency than coffee parchment. However, higher biological efficiency was obtained from aerobic composted coffee husk just like coffee parchment (Table 7). Among fresh coffee husk the one which is supplemented with 18% cow dung and 18% noug meal gave high (74.73%) and less (61.43%) biological efficiency, respectively. In the case of aerobic composted substrates the one which is supplemented with chicken manure is a good substrate for *Pleurotus ostreatus* cultivation, biological efficiency reached 98.57% and less biological efficiency was obtained from the substrate supplemented with noug meal (68.02%). When compared to treated substrate, yield and biological efficiency of *P. ostreatus* on control (coffee husk without any treatment) was lower (Table 6 and 7). In all cases fewer yield obtained from the substrate supplemented with Noug meal but better over control. In both cases with different supplementary substrate first flash gave high yield, but from flush to flush the yield became decrease in all cases with different supplementary substrate.

Table: 6. Yield and biological efficiency on fresh coffee husk supplemented with different substrate.

substrate	Content of substrate	Flushes(grams)					Total	BE in %
		1st	2nd	3 rd	4th	5th		
Fresh coffee husks	a_1	525.00^{i}	188.33^{h}	126.66^{j}	53.33^{l}	NG	893.32	71.46^{a}
	b_1	520.50^{i}	170.00^{k}	91.00^{i}	52.66^{l}	NG	834.16	66.73^{b}
	c_1	504.33^{a}	231.83^{d}	137.66^{a}	60.33^{l}	NG	934.15	74.73^{a}
	d_1	480.00^{c}	168.00^{k}	76.66^{i}	43.33^{l}	NG	767.99	61.43^{c}
Control(CH)		359.71^{b}	185.5^{h}	93.5^{i}	73.83^{l}	NG	712.54	57.00^{f}

Means within the column under a parameter, having a common letter do not differ significantly at ($p< 0.05$).

a_1= Coffee husk with chicken manure, b_1= Coffee husk with wheat bran, c_1= Coffee husk with cow dung, d_1= Coffee husk with nug meal, Control(CH)= Coffee husk without any supplement, CH= Coffee husk and NG= No growth.

Table: 7. Yield and biological efficiency on composted coffee husk supplemented with different substrate.

substrate	Content of substrate	Flushes(grams)					Total	BE in %
		1st	2nd	3 rd	4th	5th		
Composted coffee husks (8 days)	a_1	438.66^{j}	338.50^{b}	220.03^{k}	139.33^{m}	95.66^{b}	1232.18	98.57^{d}
	b_1	401.66^{d}	280.00^{j}	166.66^{c}	110.00^{m}	83.33^{b}	1041.65	83.33^{e}
	c_1	463.33^{c}	254.00^{c}	128.83^{a}	105.00^{m}	53.33^{a}	1004.49	80.35^{e}
	d_1	395.00^{d}	206.66^{k}	105.33^{i}	86.66^{l}	56.66^{a}	850.31	68.02^{b}
Control(CH)		359.71^{b}	185.5^{h}	93.5^{i}	73.83^{l}	NG	712.54	57.00^{f}

Means within the column under a parameter, having a common letter do not differ significantly at ($p< 0.05$).

CP= Coffee husk, NG= No growth

Figure: 5. Fruiting bodies of *Pleurotus ostreatus* cultivated on fresh and aerobic composted coffee parchment supplemented with different substrate.

A=FCH (p) + chicken manure, B= FCH (p) + wheat bran, C= FCH (p) + cow dung, D= FCH (p) + nug meal, E= ACH (p) + cow dung, F= ACH (p) + chicken manure, G= ACH (p) + wheat bran, H= ACH (p) + nug meal, I= CH (p) or control.

Figure: 6. Fruiting bodies of *Pleurotus ostreatus* cultivated on fresh and aerobic composted coffee husk supplemented with different substrate

A=FCHs+ chicken manure, B= FCHs + wheat bran, C= FCHs + cow dung, D= FCHs + noug meal, E= ACHs + chicken manure, F= ACHs + wheat bran, G= ACHs + cow dung, H= ACHs+ noug meal, I= ACHs or control.

6. Discussion

In this study 6 agar media were indicated that different mycelia growth of *Pleurotus ostreatus*. The result revealed that the length of mycelia growth reach 9.00 cm within 8 days of incubation on CH (p) media as compared to the growth on other media. It has been indicated that in table 3 even though, full mycelium growth of *Pleurotus ostreatus* on culture media CH (p) took 8 days, mycelia invasion was thin within 12 days when compared to mycelia growth on other culture media. These may be due to the absence of adequate nutrients necessary for mushroom growth. The mycelial growth on CHs and CP medium was almost comparable with PDA which is used as control (standard).

Thomas *et al.,* (2010) reported that cocoa pod powder medium and coffee husk medium can be used as a substitute for PDA. It is evident that from this finding of media, coffee husk and coffee pulp powder media, which is comparable with PDA, was used as culture media for *Pluerotus ostreatus.*

In this experiment, the least and very thin mycelia invasion was observed on pure caffeine (PC) agar medium. Fan *et al.,* (2003) have observed that as the caffeine concentration increase, the mycelia growth and the biomass production decreased. It was noticed that the concentration of caffeine above 100mg/l showed a significantly negative effect, and no mycelia growth was observed when the concentration reached 2500mg/l with PDA. In present study on 20mg/l alone there is mycelia growth but it is very thin growth. So when pure caffeine used alone as a culture media for the cultivation of *Pluerotus ostreatus* the mycelia growth inhibited, at low concentration of caffeine.

In the present study the cultivation of *Pleurotus ostreatus* on two main substrates namely coffee parchment and coffee husk, supplemented with different agricultural waste (18% Wheat bran and 2% ash, 18% Nug meal and 2% ash, 18% cow dung and 2% ash, 10% wheat bran, 8% chicken manure and 2% ash individually) were investigated. The result revealed that biological efficiency was obtained from aerobic composted coffee husk and parchment than fresh substrate. One reason behind these is composting is to prepare a uniform substrate suitable for growth of mushroom but not suitable for growth of other organism. Compost favours proliferation of thermophilic microorganisms (bacteria, actinomycetes and fungi).

These microorganisms are degraded and utilized carbohydrates for growth of their microbial mass. The free ammonia is also incorporated into microbial protein. The microbial protein later becomes a nitrogen source for mushroom cells mass production (Dawit, 1998).

Preethu *et al.,* (2007) have indicated that the lignin, cellulose and phenol content of composting materials decreased with composting. This may be due to positive effect of additives such as Wheat bran, Cow dung, Chicken manure and Nug meal on decomposition by way of increased availability of Nitrogen, carbon and essential nutrients for microorganisms involved during the decomposition process. Microbial succession ensures the breaking down of such compounds over a period of time.

However, when compare the two (aerobic composted coffee parchment and husk) high biological efficiency 98.57% was obtained from aerobic composted coffee husk when it was supplemented with 10% wheat bran, 8% chicken manure and 2% ash (Table 7). In the case of aerobic composted coffee parchment high biological efficiency 90.74% was obtained when it was supplemented with 18% cow dung and 2% ash (Table 5). This is due to C/N ratio is important factor for optimal substrate composition of oyster mushroom. Oyster mushroom requires much carbon and less nitrogen source than button mushroom (*Agaricus bisporus*). (Kang, 2004).

Coffee parchment have high C/N ratio (43.36) when compared to coffee husk (15.6). Even though C/N ratio was decreasing during composting the mixture of coffee parchment and cow dung yield high biological efficiency, due to both coffee parchment and cow dung (23.0) have high C/N ratio alone. In the case of coffee husk low C/N ratio was optimized by 10% wheat bran which has very high C/N (89.3) when compared to other supplementary substrate. As a result of this the coffee husk which is supplemented 10% wheat bran and 8% chicken manure gave high biological efficiency (98.57%).

Low biological efficiency was obtained from the substrate supplemented with 18% nug meal and 2% ash in all cases. Adesina *et al.,* (2011) also reported that oil palm fiber contain complex lipids which may hinder easy access of the fungus to simpler carbon sources, thus, the low mycelia growth recorded on oil palm fiber supplemented substrate. As extraction

methods often do not exhaustively extract the oil, noug meal also contain high amount of lipid which affect the growth of mushroom.

In both cases the yield obtained from the control (coffee parchment and husk without any treatment) lower when compared to supplemented substrate; however 27.95% and 57.00% of biological efficiency was obtained from coffee parchment and coffee husk, respectively.

Fan *et al.,* (2003) observed that the cultivation of *Pleurotus ostreatus* on coffee husk showed a remarkable yield & bio-conversion efficiency. From four flushes during 60 day cultivation 96.5% total biological efficiency were obtained without any supplement. But in the present, study *Pleurotus ostreatus* did not yield high biological efficiency on control coffee husk. These may be due to the chemical composition of different coffee waste in Ethiopia vary from other countries (Brazil) and environmental condition have also its own impact. Due to high proportion lignin (34.2) and cellulose (46.1) in coffee parchment than coffee husk (lignin (20) and cellulose (19-26)) in all cases coffee husk gave better yield.

7. Conclusion

The present study successfully demonstrated that aerobic composted substrate gave better biological efficiency than fresh substrate (coffee husk and parchment).

Aerobic composted coffee parchment and husk which are supplemented with cow dung and chicken manure respectively, were good substrates used for the cultivation of *Pleurotus ostreatus.*

Growth on coffee husk and coffee pulp powder agar medium was almost comparable with PDA which is used as standard medium.

The highest yield of *Pleurotus ostreatus* was obtained from the coffee husk, which is easily available substrates and a large biomass exist in the country.

8. Recommendations

Based on the result of this study, the following recommendations are given:

- Further study must be carried out using a combination of different agro industrial waste as a supplement to optimize coffee waste as a good substrate not only for production of *Pleurotus ostreatus* but also for others like *Lentinula edodes* and *Agaricus bisporus*.

- Further research must be conducted on biological treatment (composting of substrate) to obtain better yield.

- Further study must be done on coffee husk and pulp agar media by using different parameters to optimize as a culture media.

9. Reference

Adegunloye, D.V., Adetuyi, F.C., Akinyosoye , F.A. and Doyeni, M.O. (2007). Microbial Analysis of Compost Using Cowdung as Booster. *Pakistan J. Nutr*. **6:** 506-510.

Adesina, F. C., Fasidi, I. O. and Adenipekun, O. C. (2011). Cultivation and fruit body production of *Lentinus squarrosulus* Mont. (Singer) on bark and leaves of fruit trees supplemented with agricultural waste. *African J.Biotechnol*. **10:**4608-4611.

Anwar Abasanbi (2010). *Assessment of Coffee Quality and Its related problems in Jimma Zone of Oromia Regional State*. Msc. Thesis, Jimma University. Pp. 2-4

Atikpo, M., Onokpise, O., Abazinge, M., Louime, C., Dzomeku, M., Boateng, L. and Awumbilla, B. (2008). Sustainable mushroom production in Africa: A case study in Ghana. *African J. Biotechnol.* **7:** 249-253,

Baysal, E. and Peker, H. (2001). An alternate to waste paper recycling; mushroom cultivation. *Teknoloji.* **4:** 9-13.

Bayetta, Belachew. (2001). *Arabica Coffee Breeding for Yield and Resistance to Coffee Berry Disease (Colletotrichum kahawae sp. nov.)*. PhD. Dissertation, University of London, Imperial College, U. K.

Bekalo, S. A. and Reinhardt, H.W. (2010). Fibers of coffee husk and hulls for the production of particle board. *Materials and Structures.* **43:**1049–1060

Belewu, M. A. and Belewu, K.Y. (2005). Cultivation of mushroom (*Volvariella volvacea*) on banana leaves. *African J. Biotechnol*. **4:**1401-1403.

Bhatti, M. I., Jiskani, M.M., Wagan, K. H., Pathan, M.A. and Magsi, M.R. (2007). Growth, development and yield of oyster mushroom , *Pleurotus ostreatus* (JACQ. EX. FR.) kummer as affected by different spawn rates. *Pakistan. J. Botany*. **39**:2685-2692.

Boccas, F., Roussos, S., Gutierrez, M., Serrano, L. and Viniegra, G.G. (1994). Production of pectinase from coffee pulp in solid-state fermentation system - selection of wild fungal isolate of high potency by a simple 3-step screening technique. *Journal of Food Science and Technology.* **31:** 22-26.

Bressani, R. (1979). Antiphysiological factors in coffee pulp. **In:** *Coffee Pulp Composition,Technology and Utilization.* (Braham, J.E. and Bressani, R. eds). IDRC Publisher. Canada. pp. 83–96.

Carvalho, C. S. M., Campos, C.S. and Andrade, M. C. N. (2010). Mushrooms of the *Pleurotus* genus: A review of cultivation techniques. *Interciencia* **35:**177-182.

Chang, S.T. and Hayes, W.A. (1978). *The Biology and Cultivation of Edible Mushrooms.* Harcourt brace Jovanovich publishers. pp: 475-493

Chang, S.T. and Miles, P.G. (1989). *Edible Mushrooms and Their Cultivation.* Academic Press, London. pp: 265-275.

Chang, S.T. and Miles, P.G. (1992). Mushroom biology-a new discipline. *The Mycologist.* **6**: 64- 65

Chang, S.T., Buswell, T. and Chiu, S.W. (1993). *Mushroom Biology and Mushroom Products.*The Chinese University Press, Hong Kong. pp: 45-57.

Chang, S.T. (1999). World production of cultivated and medicinal mushrooms in 1997 with emphasis on *Lentinus edodes* (Berk.) Sing, China. *Intern. J. Med. Mushroom.* **1:**291–300.

Chang, S.T. (2004). *Production of Cultivated Edible Mushroom in China with Emphasis on Lentinula edodes.* The Chinese University press, Hong Kong. pp: 234-256.

Chang, S.T. (2007). *China Training Manual on Mushroom Cultivation Technology. United Nations economic and social commission for Asia and the pacific centre for agricultural engineering and machinery.* Chaoyang district, Beijing. pp:26-52.

Chang, S, T. (2008). *Overview of Mushroom Cultivation and Utilization as Functional Foods.* The Chines University Press, Hong kong. pp: 1-29.

Cohen, R., Persky, L. and Hader, Y. (2002). Biotechnological application and potential of wood degrading mushrooms of genus *Pleurotus*. *Appl. Microbiol. Biotechnol*. **58**: 582-594.

CSA (2007). Agricultural Sample Survey. Report on: Area and production of crops.Vol. 1. Bulletin, 388. Central Statistical Agency, Addis Ababa, Ethiopia.

CSA (2008). Agricultural Sample Survey. Report on: Area and production of crops.Vol.1. Bulletin, 417. Central Statistical Agency, Addis Ababa, Ethiopia.

CSA (2009). Agricultural Sample Survey. Report on: Area and production of crops.Vol. 1. Bulletin, 446. Central Statistical Agency, Addis Ababa, Ethiopia.

CSA (2010). Agricultural Sample Survey. Report on: Area and production of crops. Vol.1. Bulletin , 446. Central Statistical Agency, Addis Ababa, Ethiopia.

CSA (2011). Agricultural Sample Survey. Report on: Area and production of crops. Vol. 1. Bulletin, 505. Central Statistical Agency, Addis Ababa, Ethiopia.

Daniels, N. (2009). *Variations in Coffee Processing and Their Impact on Quality and Consistency*. Msc.Thesis, Michigan Technological University. Pp. 10-12.

Dawit Abate. (1998). Mushroom cultivation: A practical approach. Berhanena Selam Printing Press, Addis Ababa. pp. 16-21.

Diez, V.A. and Alvarez, A. (2001). Compositional and nutritional studies on two wild edible mushrooms from Northwest Spain. *Food Chem.* **75:** 417-422.

Dikinya, O. and Mufwanzala, N. (2010). Chicken manure-enhanced soil fertility and productivity: Effects of application rates. *J. Soil Sci. Environ. Management.* **1:** 46-54.

Ermias Dagne and Dawit Abate. (1995). Bioactive compounds from plants and higher fungi of Ethiopia. **In:** *Phytochemistry of Plants Used in Traditional Medicine.* (Hostettmann *et al.,eds.*). Oxford University Press, oxford. pp. 295-312.

Fan, L., Pandey, A., Mohan, R. and Soccol, C .R. (2000a). Use of various coffee industry residues for the cultivation of *Pleurotus ostreatus* in solid state fermentation. *Acta Biotechnol.* **20:** 41-52.

Fan, L., Pandey,A. and Soccol,C.R. (2000b). Solid state cultivation –an efficient method to use toxic agro-industrial residues. *J. Basic Mcrobiol.* **40:** 187-197.

Fan, L., Pandey,A. and Soccol,C.R. (2001). Production of *Flammulina velutipes* on coffee husk and coffee spent-ground. *Brazilian Archives of Biol. and Technol. An Inter.J.* **44:**205-212

Fan, L., Soccol,A.T., Pandey, A. and Soccol,C.R. (2003). Cultivation of *Pleurototus* mushroom on brazilian coffee husk and effects of caffeine and tanninc acid. *Micologia Aplicada Intern.* **15:**15-21.

Fan, L., Soccol, A.T., Pandey, A., Vandenberghe, L.P.S. and Soccol, C.R. (2006). Effect of caffeine and tannins on cultivation and fructification of *Pluerotus* on coffee husks. *Brazilian J. Microbiol.* **37**:420-424.

FAO, (2005). Composting of Coffee Pulp. Food and Agriculture Organization, Rome.

Franca, A.S. and Oliveira, L.S. (2009). Coffee processing solid wastes: current uses and future perspectives. **In:** *Agricultural Wastes*. (Columbus, F. ed.). Nova Publishers, New York.

Gbolagade, J., Ajayi, A., Oku, I. and Wankasi, D. (2006). Nutritive value of common wild edible mushrooms from Southern Nigeria. *Global J.Biotechnol.* and *Biochem.* **1:** 16-21.

Gern, R.M.M., Junior, N.L., Patricio, G.N., Wisbeck, E., Chaves, M.B. and sandra aparecida Furlan, S.A. (2010). Cultivation of *Agaricus blazei* on *Pleurotus* spp. Spent substrate. *Brazilian Archives Biol. and Technol. An Intern. J.* **53:** 939-944.

Gouvea, B. M., Torres, C., Franca, A. S., Oliveira, L. S. and Oliveira, E. S.(2009). Feasibility of ethanol production from coffee husks. *Biotechnol. Letter.* **31:**1315–1319.

Gregori, A., Vagelj, M. and Pohleven, J. (2007). Cultivation techniques and medicinal properties of pleurotus spp. *Food Technol. Biotechnol.* **45:** 238–249.

Inamulhaq, M., Nasir, A., Khan, M., Khan, M . A., Khan, N. A., Binyamin, J.R. and Irshad, G. (2010). Use of medicinal plants in different composts for yield improvement of various strains of oyster mushroom. *Pakistan . J. Bot.* **42:** 3275-3283.

Jacquet, M., Getinet Kelekle, Legesse Seyoum and Teshome Menjour(2008). Coffee sector strategy on production, productivity, quality and marketing sector final report volume IV. Coffee Improvement Program. Addis Ababa, Ethiopia.

Kang, S.W. (2004). Oyster mushroom cultivation. Mushroom Growers Hand Book 1.part II. Chapter 3. Pp.48-51.

Khan, A., Rahman, M.M., Tania, M. and Ahmed, N.U.S. (2011). *Pleurotus sajor-caju* and *pleurotus florida* mushrooms improve some extent of the antioxidant systems in the liver of hypercholesterolemic rats. *The Open Nutraceuticals J.* **4:** 20-24.

Kirbag, S. and Akyuz, M. (2008). Evaluation of agricultural wastes for the cultivation of *Pleurotus eryngii* (DC. ex Fr.) Quel. var. *ferulae* Lanzi. *African J.Biotechnol.* **7:** 3660-3664.

Longo, M.A. and Sanroman, M.A. (2006). Production of Food Aroma Compounds: Microbial and Enzymatic Methodologies. *Food Technol. Biotechnol.* **44:**335–353.

Mandre, M. (2006). Influence of wood ash on soil chemical composition and biochemical parameters of young Scots pine. *Estonian Acad. Sci. Biol. Ecol.* **55:** 91-107.

Manzi, P., Aguzzi, A. and Pizzoferrato, L. (2001). Nutritional value of mushrooms widely consumed in Italy. *Food Chem.* **73:** 321-325.

Masamba, K. G. and Mwale, K.R. (2010). Determination and comparison of nutrient and mineral contents between cultivated and indigenous edible mushrooms in Central Malawi. *African J. Food Sci.* **4:**176-179.

Martinez, C.D., Aguilar, A., Martinez, W., Bonilla, M., Morales, P. & Sobal, M. (2000). Commercial production and marketing of edible mushrooms cultivated on coffee pulp in Mexico. **In:** *Coffee Biotechnology and Quality*. (Sera, T., Soccol, C., Pandey, A. and Roussos, S. eds). Kluwer Academic Publishers, Dordrecht. pp. 471-488.

Mazzafera, P. (2002). Degradation of caffeine by microorganism and potential use of decaffeinated coffee husk and pulp in animal feeding. *Scientia Agricola*. **59:** 815-821.

Medeiros, A.B.P., Pandey, A., Vandenberghe, L.P.S., Pastore, G.M. and Soccol, C.R.. (2006). Production and recovery of aroma compounds produced by solid-state fermentation using different adsorbent. *Food Technol. and Biotechnol*. **44:** 47-51.

Mekuria, T., Neuhoff, D. and Kopke, U. (2004).The Status of Coffee Production and the Potential for Organic Conversion in Ethiopia. Conference on International Agricultural Research for Development.

Murthy, P.S. and Manonmani, H.K. (2008). Bioconversion of coffee industry wastes with white rot fungus *Pleurotus florida*. *J. Environ. Sci. Res.* **2:** 145-150.

Murthy, P.S., Naidu, M.M. and Srinivas, P. (2009). Production of α-amylase under solid-state fermentation utilizing coffee waste. *J. Chem. Technol. Biotechnol.* **84:**1246-1249.

Murthy, P.S. and Naidu, M.M. (2010). Protease production by *Aspergillus oryzae* in solid state fermentation utilizing coffee by product. *World Appl.Sci.J.* **8:**199-205.

Mushigeni, K.E. and Chang, T.S. (2001). Mushrooms: Their biology, nutritional and medicinal properties, cultivation technologies and perspectives on mushroom research and development. In: *Proceedings of the Mushroom Farming Training Workshop held in Lilongwe*, Malawi. Pp.74

Mutua, J. (2000). Post harvest handling and processing of coffee in African countries. Rome, Italy.

Naher,U.A., Hashem, M.A., Mitra, B.K., Uddin, M.K. and Saleque, M.A. (2004). Effects of rice and lime on phosphrorus and potassium mineralization from cow dung and poultry manure under covered and uncovered conditions in the tropical environment. *Pakistan J. biological sci*. **7:**45-48.

Nigam, P. S. (2009). Production of organic acids from agro-industrial residues. **In:** *Biotechnology for Agro-industrial Residues Utilisation.* (Nigam, P.S. and Pandey, A., eds.). Northern Ireland. Pp.37-60.

Nwokoye, A. I., Kuforiji, O.O. and Oni, P.I. (2010). Studies on mycelia growth requirements of *Pleurotus ostreatus* (Fr.) singer. *Intern. J. Basic Appl. Sci.* **10:**70-89.

OECD. (2005). Consensus Document on the Biology of *Pleurotus* spp. (Oyster Mushroom)-; Organisation for Economic Co-operation and Development, Paris.

Oei, P. (1991). *Manual on mushroom cultivation*. *Techniques, species and opportunities for commercial applications in developing countries*. Tool publications, Amsterdam.

Oei, P. (1996). *Mushroom cultivation. With special emphasis on appropriate techniques for developing countries.* TOOL publications, Leiden.

Oei, P. (2003). *Mushroom Cultivation, Appropriate Technology for Mushroom Growers*. Backhugs Publishers, Leiden.

Pandey, A., Soccol, C.R., Nigam, P., Brand, D., Mohan, R. and Roussos, S. (2000). Biotechnological potential of coffee pulp and coffee husk for bioprocesses. *J. Biochem. Engineering* **6:** 153–162.

Petit, N. (2007). Ethiopia's Coffee Sector: A Bitter or Better Future. *J. of Agrarian* **7:** 225–263.

Preethu, D.C., Prakash, B.N.U.H., Srinivasamurthy, C.A. and Vasanthi, B.G. (2007). Maturity indices as an index to evaluate the quality of compost of coffee waste blended with other organic wastes. *In***:** *Proceedings of the International Conference on Sustainable Solid Waste Management,* Chennai, India. pp.270-275.

Rajapakse, J. C., Rubasingha, P. and Dissanayake, N. N. (2007). The effect of six substrates on the growth and yield of American oyster mushrooms based on jancao technology**.** *The J. Agri. Sci.* **3:** 82-85.

Ramadan, M.F. and Morsel, J.T. (2002). Proximate neutral lipid composition of niger (*Guizotia abyssinica* Cass.) seed. *Czech J. Food Sci*. **20:** 98–104.

Roussos, S., Aquiahuatl, M.L.A., Trejo-Hernandez, M.R., Perraud, I.G., Favela, E., Ramakrishna, M., Raimbault, M. and Gonzalez, G.V. (1994). Biotechnological management of coffee pulp - isolation, screening, characterization, selection of caffeine degrading fungi and natural microflora present in coffee pulp and husk. *Appl. Microbio1. Biotechnol.* **42:** 756-762.

Roussos, S., Gaime, I. p. and Denis, S. (1998). Biotechnological Management of Coffee Pulp. Pp: 151-161.

Scholer, M. (2004). Bitter or Better Future for Coffee Producers. International Trade Centre, International Trade Forum.

Shankaranand, V.S. and Lonsane B.K. (1994). Coffee husk: an inexpensive substrate for production of citric acid by *Aspergillus niger* in a solid state fermentation system. *World J. Microbiol. Biotechnol*. **10:**165-168

Sheikh, P.A., Kauser, M.Y.S., Ghani, N. A., Munshi, K. A. and Fahim, A. (2010). Influence of supplements on the quality parameters of pleurotus sajor- caju (Fr.) singer. *J. Phytology* **2:** 60–65.

Shittu, O.B., Alofe, F.V., Onawunmi, G.O., Ogundaini, A.O. and Tiwalade, T.A. (2005). Mycelial growth and antibacterial metabolite production by wild mushrooms. *African J. Biol. Med. Res* .**8:**157 -162.

Singer, R. (1986). *The Agaricales in Modern taxonomy*. Koeltz scientific, Koenigstien, Germany. Pp: 1-98.

Soccol, C. R. (1996), Biotechnology products from cassava root by solid state fermentation. *J. Ind. Sci. Res.* **55:** 358-364.

Soccol, C.R., Vandenberghe, L.P.S. (2003). Overview of applied solid- state fermentation in Brazil. *J. Biochem. Eng.* **13:**205-218.

Soccol, C.R., Rodrigues, C., Vandenberghe, L.P.S. and Pandey, A. (2006). New perspectives for citric acid production and application. *Food Technol. Biotechnol.* **44:**141–149.

Stanculescu, D. (2011). Ethiopian Coffee Quality Improvement Project. 2011 aid for trade global review: case story. Africa, Ethiopia.

Thomas, D. P., Thulasi, E.P., Ravichandran, B. and Madhusudhanan, K . (2010) Mycelial Culture and Spawn Production of two Oyster Mushrooms, *Pleurotus florida* and *Pleurotus eous* on different substrates. *Intern. J. Biol. Technol*. **1:**39-42.

USDA, (2006). Tropical Products: World Markets and Trade. Washington: United States Department of Agriculture, Foreign Agricultural Service.

10. Appendices

A  B 

Appendix: 1. important substrate during *Pleurotus ostreatus* production

A= coffee husk, B= coffee hulls or parchment

Appendix: 2. Substrate in plastic bags after sterilization ready for inoculation