ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

DEPARTMENT OF BIOLOGY

CULTIVATION AND YIELD PERFORMANCE OF *PHOLIOTA NAMEKO* ON DIFFERENT AGRO INDUSTRIAL WASTES



BY

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SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN APPLIED MICROBIOLOGY
AT
ADDIS ABABA UNIVERSITY
ADDIS ABABA, ETHIOPIA
April, 2010

Acknowledgements

It gives me a great pleasure to acknowledge my research advisor Dr. Dawit Abate for his unreserved guidance, advice, encouragement, and support in providing me the necessary equipments and critical comments during the course of my study.

I am extremely grateful to my friends Ashenafy Kassay, Kider Emiwodih, Sintayhu Demisse, Tefferi Gessese, Abyu Abera, Daniel Bayessa as one of my next of kin, for their encouragement, contribution and invaluable support in solving my private cases. My due thanks go also my father Gizaw Tegene, all my sisters and brothers for their support and encouragement. I am indebted to my junior Lakew Gizaw for he has paid the love care and devotion to me throughout my study.

Lastly my appreciation goes to my mother Ketsela Wondimu who had offered me great care and support which enabled me come so far to this level of education.

Above all I thank the almighty God, who made me all my way comfortable and do everything to me previously through and in the future through out my life.

Table of contents

List of figures

List of tables

Abbreviations and acronyms

MEA----------Malt extract agar

PDA ----------Potato dextrose agar

SDYA----------Sabourdinat dextrose yeast agar

GLM ----------General linear model

Lac ----------Laccase

Mnp ---------- Manganase oxidizing peroxidase

BE-- ----------Biological effeciency

LEM ---------- Lentinus edodes mycelium

FAO---------- Food and agriculture organization

HDP ---------- ----------Host defense potentiators

PNPS-1 ---------- *Pholiota nameko* polysaccharide

SSF ---------- Solid state fermentation

PSK ---------- Polysaccarid kerastin

PSP ---------- Polysaccarid phosphate

NK ----------Natural killer

ROS ----------Reactive oxygen species

ES ----------Eucalyptuse shaving

CAS ----------*Cordia africana* shaving

CH ----------Coffee husk

CS---------- ----------Cotton seed

TS ----------Teff straw

Abstract

Pholiota nameko (T.Ito) S.Ito) *is white rot wood inhabiting ligninolytic mushroom species belonged to genus Pholiota, widely distributed through out Far East which has been used as food and medicinal purpose. The research experiment was carried out to investigate the yield and the biological efficiency of Pholiota nameko grown on different agro industrial wastes in Ethiopia. For the cultivation of Pholiota nameko 6 kinds of substrates, namely eucalyptuse shaving (ES), cordia shaving(CAS), coffee husk(CH), Pinus shaving(PS), cotton seed (CS) and teff straw(TS) were used as the main material or substrates. Wheat bran (WB) was used an additive material 100:10 and 100:30 w: w of the main material. Moisture content of the substrate was maintained to50-65 %. Only three substrates, these are eucalyptuse shaving (ES), cotton seed and cordia shaving(CAS) show remakable production of fruiting body. The highest mean yield and biological efficiency were 797.33 g, 53.27% respectively on eucalyptuse shaving supplemented with 30% wheat bran. The higher harvest 732.33g, 48.98% mean yield and biological efficiency respectively obtained from cotton seed supplemented with 30 % wheat bran. While the lowest mean yield and biological efficiency were obtained from Cordia africana shaving supplemented with 10% wheat bran 550.8g, 36.80% respectively. There was no statistical difference observed between substrates supplemented with 10% and 30% wheat bran on yield and the biological efficiency. But substrate supplemented with 30% wheat bran showed a little better quality of fruiting body and cropping time than substrate supplemented with 10% wheat bran. In general the yield of Pholiota nameko mushroom harvested was significantly ($P<0.05$) greater in eucalyptuses shaving than Cordia africana shaving. The use of eucalyptus shaving as raw material was found better for the production of Pholiota nameko in this study, in fact it is an abundant and chip lignin rich material in Ethiopia.*

Key Words: Bio conversion effeciency , basdiomycetes, contamination, *Lentinus edodes* , lignocelluloses, mushrooms, media, mycelium, *Pholiota nameko,* substrate, shiitake, spawn

1. Introduction

Mushrooms are macro-fungi with distinctive fruiting bodies either epigeous or hypogeous (Chang and Miles, 1992). Which have a texture, appearance and manner of growth all their own, belonged to basidiomycota and ascomycota division. They include edible, non edible, medicinal and poisonous species (Stamets and Chilton, 2005). 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).

Mushrooms are eukaryotic heterotrophy organisms; nutritionally classified as saprophytes, that obtain nutrients from dead organic materials; pathogen which depends 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 (Chang, 2008).

For millennia, mushrooms have been valued by humankind as an edible and medicinal resource (Wasser, 2002). 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). 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 one. It is usually taken as part of the food supplement (Berch *et al*., 2007). A highly nutritious and

energetic soup can also be prepared from dried mushroom powder by blending with mixed vegetable, chicken and tomato. Dried mushroom powder can also be utilized in preparing weaning food. Fast food like spring rolls, mushroom burger, mushroom chow, mushroom cutlets, and pizzas. These days, mushroom can also be taken as pill. The other common product is powdered mushrooms and mushroom extracts, alcoholic and aqueous, in liquid or dried form. These products are described both as flavoring additives, enhancers and health promoting ingredients (Zivanovic, 2006).

Apart from their nutritional potentials, they are important medicinally for cholesterol reduction, immune enhancement, cancer fighting, anti allergic activities, antimicrobial and cardiovascular treatment. They also have a long history of use as traditional medicine in China. Their legendary effects on promoting good health and increasing adaptive abilities have been also supported by recent studies (Wasser, 2000).

In addition to their edibility & health benefits, their mycelia can produce a group of complex extra cellular enzymes which can degrade and utilize the lignocelluloses wastes in order to reduce pollution (Vostrovsky and Jablonska, 2007). It has been revealed recently that their mycelia can play a significant role in the restoration of damaged environments. Saprotrophic, endophytic, mycorrhizal, or even parasitic mushrooms can be used in mycorestoration, which can be performed in four different ways. These are myco filteration (using mycelia to filter water), mycoforestry (using mycelia to restore forests), mycoremediation (using mycelia to eliminate toxic waste) and mycopesticides (using mycelia to control insect pests). This ability has potential to create the clean ecosystem (Stametes and Chlinton, 2005).

Due to its nutritional, medicinal and ecological advantages, mushroom attracted the attention of many people in the world. Cultivation of edible mushrooms may be the only currently economical biotechnology for lignocelluloses organic waste recycling that combines the production of protein rich food with the reduction of environmental pollution (Beteez and Kustudia, 2004).

Currently about 35 mushroom species have been cultivated commercially, and of these around 20 are cultivated on an industrial scale. The mushroom cultivated most worldwide is *Agaricus bisporus* (button mushroom), followed by *Lentinus edodes*(Shiitake), *Plerutus ostreatuse* (Oyster), *Auricula auricular*, *Flammulina velutipes* (Winter mushroom) and *Volveriella volvovacea* (straw mushroom), *Grifola frondosa*, and *Pholiota nameko* (Chang, 1999).

Mushroom cultivation and consumption culture is more developed in China, Japan, Koria, Thailand, America (Feeney and Beelman, 2004). But, least known in Africa, how ever country like Nigeria, Egypt, Kenya, Zimbabwe, and South Africa, relatively made good trial. In Ethiopia it is a very recent activity, almost no mushroom consumption and cultivation techniques are known except few trials in small scale on *Agarics bisporus, Lentinula edodes, Pleurotus ostreatus* with few people under known substrate formula (Dawit, 1998).

The present study is aimed to explore the cultivation possibilities of *Pholiota nameko* in Ethiopia on different substrates obtained from agricultural, industrial and forest waste. *Pholiota nameko* is a medium size mushroom produce cluster of fruit body with small glossy cap that develops an orangish slimy layer on the cape resembling jelly which peels able. This slimy layer disappear

once cooked (Vostrovsky and Jablonska, 2007). It is one of the most popular cultivated mushrooms in Japan and China closely ranking behind *Lentinus edodes* and *Flammulina velutipes*. This mushroom has an excellent flavor, texture, very delicious and best tasting mushrooms in the world (Arita, 1978).

The cultivation history of *Pholita nameko* is similar to that of many other wood rooting edible mushrooms. In 1921 in Japan, fresh cut wood logs were used, for growing of *Pholiota nameko*, Most fungi can not use freshly cut wood as effectively as wood has been cut, but *Pholiota nameko* can utilize wood containing live cells, then, saw dust spawn was first used in 1931 and 1960 saw dust pluses wheat bran and rice bran were used for commercial cultivation of *Pholiota nameko* (Neidleman, 2004).

Pholiota nameko is a low temperature mushroom and can grow especially on temperate deciduous plants like, *Fagus crenata, Fagus japonica, Querccus mangnolca*, This research is mainly conducted, to compare the yield and biological efficiencies of *Pholiota nameko* mushroom on different substrate obtained from forest and agro industrial lignocelluloses wastes in Ethiopia, and to promote the species for small scale as well as large scale cultivation.

2. Objectives

2.1. General objectives

- To compare the yield and biological efficiencies of *Pholiota nameko* mushroom grown on different substrates.

2. 2. Specific objectives

- To select substrate mixture that provides high biological efficiency.
- To select the suitability of available substrate for growth of *Pholiota nameko.*
- To elucidate choice of substrates and substrate preparation methods for small scale cultivation of *Pholiota nameko* mushroom in Ethiopia.
- To compare biological efficiencies of *Pholiota nameko* mushroom on different substrate.

3. Literature review

3.1. Morphology and classification

The genus Pholiota consists of approximately 362 species which are characterized by its brownish red, membranous annulus, smooth basdiospores (Neda, 2008). The cap has glutinous slime covering, round speckled to about 7/8 inch in diameter. The genus Pholiota was placed in the phylum Basidiomycota, order Agaricales and family, Strophariaceae (Singer, 1986). The family Strophariacea is classified mostly on the basis of morphological characters (Matsumoto *et al*., 2003).

Morphologically the Nameko is a medium size mushroom produce cluster of fruit body with small glossy cap, when grows up its center sink and looks flat. People sometimes call this a honey mushroom. The cap has a smooth surface with mucilage and a diameter of 5cm-8.5cm (Arita, 1978). The gill grow vertically and densely, white or yellow in early time, and turn to rust or ochre color when mature, at the same time it will be changed from weak yellow to brown. Its stipe is a column length of 5cm -7cm (Neda, 2008). Unlike to *Lentinus edodes* and other mushrooms, it has softer edible stem (Tabasso, 2006).

3.2. Life cycle of *Pholiota nameko*

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, 1998).

Pholiota nameko is heterothallic and bipolar species. Fruit bodies are normally formed from dikaryotic mycelia, following nuclear fusion and division in each basidium, four basidiospores are produced. One generation requires about 60 days under favorable conditions. The dikaryotic mycelia can sometimes produce monokaryotic hyphal cells, which can lead to very poor production of fruit bodies (Hui *et al*., 1999).

In addition to sexual cycle there are 5 different asexual cycles observed in *Pholiota nameko*. (Fig. 1)

(1) The homokaryotic mycelium, derived from a single haploid basdiospores, is able to form fruit bodies bearing monokaryotic basidia, which may produce spore when grown on a suitable substrate and under favorable conditions. The spores produced by homokaryotic fruit bodies germinate normally, and develop into homokaryotic mycelia again with exactly the same incompatibility factor as the parental homokaryon. Both homokaryotic and dikaryotic mycelia can produce two kind of asexual spores, i.e., arthroconidia and aleuriocondia (Artia, 1968). (2) All conidia born from homokaryotic mycelia can form fresh homokaryotic mycelia again upon germination. (3) The monokaryotic and homokaryotic conidia formed by dikaryotic mycelia gives rise to homokaryotic mycelia upon germination i.e., dedikaryotitization.

(4) The heterodikaryotic conidia from dikaryoitic mycelia redevelop into dikaryiotic mycelium bearing clamp connection (Artia, 1968). (5) The dikaryotic mycelia may be resolved naturally into component homokaryons, i.e., dedikryotization, during mycelial growth without spore formation (Chang and Hayes, 1978).

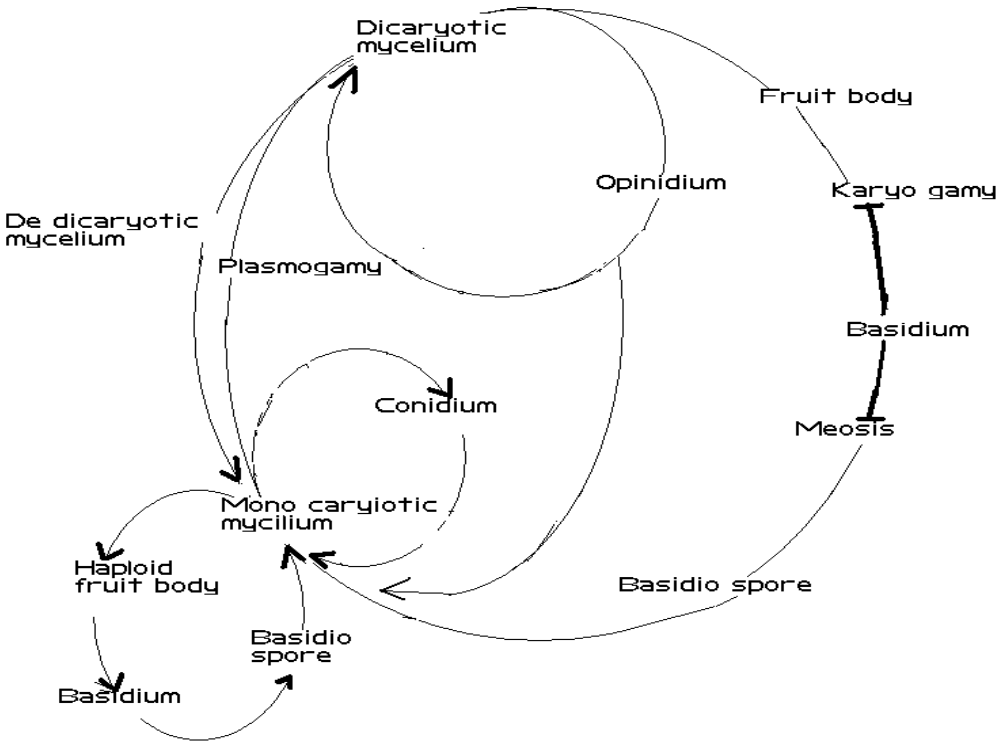

Figure 1. The life cycle of *Pholiota nameko* (Arita, 1968)

3.3. Growth condition

3.3. 1. Mycelia Growth

Immediately following inoculation, whitish mycelia begin to grow on the supplemented substrate, or grain until colonization is completed. This is an active assimilation phase with high fungal metabolic rate. Enzymes are activated to break down complex substrate components (e.g. cellulose, hemicelluloses and lignin) into simpler molecules which can be absorbed by mycelia as nutrients for growth and propagation (Chen, 2001).

The mycelia characteristics of *Pholiota nameko* is whitish, longitudinally radial, becoming light orangish or tawany from the center as the mycelium ages. On sterilized grain the mycelium is densely cottony white and become speckled with yellowish to orangish zone at maturity (Stametes, 2005).

Pholiota nameko mushroom requires 70-80 days for mycelia growth, and the main work in mycelia growth is the management of temperature and moisture. The moisture content is much more important at the time of mycelia growth (Chang, 1978). The mycelia grow well under 4-32°C, the optimum temperature is 25-26°C. It stops growing at 32°C and dies under over 40°C in a long period. The mycelia have tolerance to low temperature, up to -5°C (Nakazawa and Tochigi, 1989).

Mycelia growth and mushroom production of Nameko are generally related to the kind of sawdust and log woods used. *Quercus mangolica, Fagus crenata, Fagus japonicas are* satisfactory for both mycelia growth and fruiting (Chang and Hayes, 1978).

3.3. 2. Fruiting body production

Pholiota nameko is one of the hygrophilous fungi and needs more moisture for fruiting compared with other cultivated mushrooms, such as *Lentinus edodes, Flammulina velutipes,* and *Pleurotus ostreatuse* (Chang and Hayes, 1978). Aeration also plays an important role in frutification. The fruiting body formation was triggered by shifting the environmental variables namely moisture, air exchange, temperature and light in the cropping room (Stamets, 2000). The appearance of fruiting bodies or mushroom varies according to the species, but all have a vertical stalk (stipe) and a head (pileus or cap). This mushroom produces a cluster of yellowish and creamy fruit body, also cinnamon brown spores. Frutifification requires 30 days (Marshall and Nair, 2009).

3.4. Wild and cultivated Nameko mushroom

Fungi described as being ubiquitous, they are found just about everywhere. The mushrooms are rather more selective than other fungi in the size of the fruiting body requires the availability of more nutrients than are required by micro fungi. The distribution of mushroom is worldwide although their production is seasonal. Plentiful moisture leads to mushroom formation, and their growth abundance frequently follows rain (Royse, 1997). In temperate regions, there is particular flora of mushroom species associated with the season fall, summer, and spring. Relatively few mushroom species are produced during the cold winter months although there are perennial fruiting bodies that persist during winter, such mushroom *Flammulina velutipes* can survive freezing temperature. Mushrooms are also produced in tropical and subtropical regions. A good example of this is edible straw mushroom, *Volveriella volvacea* that grows optimally at 32-35 ^{0}C. Habitats in which mushrooms are found include grassy meadows, deciduous hardwoods,

woodlands where they grow up lingo cellulosic, (hemi) cellulose substrates, such as straw, hard, & soft woods of the temperate as well as tropical region (Chang and Miles, 1997). Their relative adaptability to various substrate species and forms (*e.g*., stumps, logs, wood particles, leaf litter, *etc*.) and their preferences with respect to the microbiological condition of their substrates (Bruhn, 1998).

3.4.1. Host range of *Pholiota nameko*

Nameko was originally used for several species of Pholiota and similarly looking fungi, including *Flammulina velutipes*, in which the cap surface was sticky or slimy. Today, however, Nameko refers only to *Pholiota nameko*, a species that is restricted to the Far East (Pegler, 2003). It is cosmopolitan like a closely related species of Pholiota, such as *Pholiota aurivella* (Batsch: Fr.), *Pholiota adipose*(Fr.) kummer, *Pholiota lubirica*(Fr.) Sing , *Pholiota mutabilis* (Schaeff.: Fr) , distributed throughout Far east that found growing in there wild on dead organic matter ,trunks or stumps of deciduous trees , and mostly on decayed logs of Salicaceae trees. The host range of this Nameko mushroom includes especially *Fagus crenata, Fagus japonica, Querccus mangnolca*. Its natural distribution is almost coincident with that of *Fagus crenata* in Japan (Chang and Hayes, 1978). Nameko can also utilize wood containing live cells especially Salicaceae trees, for the reason some have considered *Pholiota nameko* to be a semi- parasitic fungus (Stamets, 1993). The reason why this mushroom is restricted to Far East, its glutinous aspect of the cap has prevented these mushrooms from being developed in other countries, as it is a characteristic that offers little appeal to the western gourmet. Nevertheless, the slime on the Nameko mushroom disappears completely on cooking (Pegler, 2003).

3.4.2. Cultivated *Pholiota nameko* mushroom

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). *Pholiota nameko* is one of the four important wood inhabiting cultivated mushrooms in Japan, China together with *Lentinus edodes, Flammulina velutipes*, and *Pleurotus ostreatuse* (Chang and Hayes, 1978).

The history of *Pholiota nameko* cultivation is similar to that of many wood rooting edible mushrooms. In Japan cut wood logs were used in 1921 for growing *Pholiota nameko.* In 1931 for the first time saw dust spawn was used, and then 1960 saw dust pluses wheat bran was used for commercial cultivation of *Pholiota nameko.* Gradually Nameko become most popular in Japan, closely ranking behind *Lentinus edodes* and *Flammulina velutipes* (Atsushi and Takashi, 1999). Until about ten years ago, *Pholiota nameko* had been cultivated mainly outdoors in winter by implanting the spawns on dead trees. However, at present, exclusively cultivated indoors in all seasons. It is also cultivated in a small windowless building equipped with an air conditioner .They are easy to grow in large quantities by using fresh hard wood as substrate in form of sawdust, wood chips and a mixture of grain (Ishii *et al*., 1994). Recently Japan came in advances to bottle cultivation technology where, this mushroom is now available in the domestic market of Japan throughout the year (Tachikawa *et al*., 2009).

Japan cultivated 21,738 t of *Pholiota nameko* in 1991 and increase of only 1,700 t (8% increases) from 1986 levels .World-wide production increases averaged 60% over the same time. In 1991,

Japan produced about 54% of the total world production of Nameko compared to 80% of total production in 1986. Its annual volume of commercial production was 1000 tons (fresh weight) in 2000 followed by *Lentinula edodes* (Berk.) Pegler, *Hypsizygus marmoreus* (Peck) Bigelow, *Flammulina velutipes* (Curt. Fr.) Sing., and *Grifola frondosa* (Dicks. Fr.) S.F. Gray. Because *Pholiota nameko* now a days has great economic importance in Japan (Obatake *et al*., 2002).

Thus, production of Nameko rapidly is gaining popularity in other Asian countries. Preparation of the medium for Nameko production is similar to that of *Flammulina velutipes* except that a higher moisture content of the substrate is desirable. A substrate of broad leaf tree sawdust is preferred but research has shown that saw dust's from conifers such Pinus species, and *Cryptomeria japonica* is suitable for growth. Rice bran usually is added as a supplement in the ratio of 15% for conifer sawdust and 10% for broad-leaf sawdust (Stamets, 1993).

Generally in the class Basdiomycetes the cultivated members belong to 10 families placed in 5 orders and 2 sub class. By far the greatest numbers of edible species that are cultivated members of the order Agarcales and subclass Holobasidiomycetidae. No ascomycetous mushroom has been completely commercially cultivated yet, except morel and truffle (Chang and Miles, 1997).

3.5. Importance of *Pholiota nameko* and other mushrooms

3.5. 1. Nutritional value

The food for human beings comes from three different sources, i.e. land, water, and microbes. Among microbes used as human food, the fungi comprise the largest and the most important group containing edible mushrooms (Singh, 2008). Healthy nutrition and diet are gaining importance, not only in the every day life of human beings, but also in the treatment of chronic

diseases. Medical practitioners of worldwide are recognizing that mushrooms are medicinal foods rich in nutrition (Stamets, 2005).

Fauzia showed that mushrooms have higher nutritional values than fish or beef (Fauzia *et al.,* 2003*)*. Matila also suggested that a diet rich in mushrooms provides all the essential amino acids usually available in fruits and vegetables (Matila *et al*., 2002).

Mushrooms are low calorie food, rich in protein, very low in simple carbohydrates, rich in high-molecular weight polysaccharides, high in antioxidants, and very low in fat. They lack cholesterol, starch, and vitamin C. They are a good source of vitamin B complex ,riboflavin (B_2), niacin (B_3), pantothenicacid (B_5), ergo sterols (provitamin D_2), other substances are found such as selenium, calcium, phosphorus and potassium in fair quantity along with copper, and iron. Also high potassium to sodium ratio present in mushrooms is desirable for the patients with hypertension, also high in dietary fiber important for immune function, for producing antioxidants that reduce free radicals, and helpful in excretion of waste and prevention of constipation (Onokpise *et al*., 2008).

In general mushrooms on dry weight bases, composes of , 10%-40% proteins, 2%-8% fat, 3%-28% carbohydrates, 3%-32% fibers, 8%-10% mineral (Stamets, 2005). The high nutritional value of mushrooms is due to the presence of 8 essential amino-acids, polyunsaturated fatty acids (linoleic and arachidonic acids) and reduced quantities of saturated fatty acids (Fortes *et al*., 2006).

In Particular the *Pholiota nameko* mushroom is rich in protein and carbohydrate. According to Paul Stamets the protein continent of *Pholiota nameko* is 33.65%, fat 3.91%, polyunsaturated fat

1.01%, saturated fat 0.17%, carbohydrate 48.36% (Stamets, 2005) (Table. 1). Minamekawa also suggest that fresh canned *Pholiola nameko*, consists of crude protein 35.0%, carbohydrate 31.6%, crude fiber 13.5%, ash 9.0%, crude fat 3.5%, mannitol 14.3%, trehalose 4.3%, ergo sterol 0.217% and moisture 95.8% (Minamekawa *et al*., 2006). The amount of vitamin in dry weight bases that thiamin 18.8mg/100g, riboflavin 14.6mg/100g, niacin, 72.9mg/100g and minerals like , calcium 42mg/100g, potassium 2.083mg/100, iron 22.9mg/100g, sodium 33. 017mg/100g (Wasser, 2002).

At present, about 800 million people in the world are living in poverty. On the other hand, it has been observed that over 70 % of agricultural wastes and forest products have not been put to total productivity, and have been wasted in processing. Mushrooms not only can convert these huge lignocelluloses biomass wastes into human food, but can also produce notable immune enhanced products, which have many health benefits (Chang *et al*., 1993).

In Ethiopia hunger and malnutrition are devastating problems, particularly for the poor and unprivileged society. About 50 percent of the population are living below the food poverty line and can not meet their daily minimum nutritional requirement of 2200 calories (FAO, 1998). The most important forms of malnutrition in Ethiopia are protein energy malnutrition (PEM), -

Iodine; vitamin A deficiency disorders (Edris, 2004).

An ever increasing human population and diminishing farm sizes have resulted in declining soil fertility associated land productivity and increasing poverty levels (Sanchez, 2009). Elsewhere in the world, mushrooms are consumed widely. Wide spread malnutrition with ever increasing protein gap in our country has necessitated the search for alternative source of protein because

the production pluses has not kept pace with our requirement due to high population growth. Animal protein is beyond the reach of the most people in different countries (FAO, 1998).

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 through out the world (Chang and Mshignei, 2000).

	Calorie	Protein g /100g	Fat g/100g	Polyunsaturated g/100g	Total unsaturated g/100g	Saturated fat g/100g	Carbohydrate g/100g	Complex carbohydrate g/100g	sugar g/100g	Dietary fiber g/100g	Cholesterol mg/100g	Vitamin A mg/100g	Thiamine mg/100g	Riboflavin mg/100g	Niacin mg/100g	Vitamin C mg/100g	Vitamin D mg/100g	calcium mg/100g	Copper mg/100g	Iron mg/100g	Potassium mg/100g	Selenium mg/100g	Sodium mg/100g
Agarics bisporus portobella	355	34.4	3.10	1.43	1.46	0.30	47.38	24.68	22.30	20.8	0	0	0.27	4.13	69.20	0	2.35	23	4.33	2.1	4500	0.415	52
Agarics bisporus crimini	340	33.8	2.39	o.41	0.44	0.26	46.17	24.27	21.90	19.10	0	0	0.23	3.49	38.5	0	26	9	20.8	4.8	4800	0.066	3
Agarics braziliancise	362	35.9	3.39	1.51	1.72	0.37	47.70	26.50	21.20	21.00	0	0	0.26	2.40	58.50	0	737	16	4.28	1.9	5200	0.35	43
Flammunila populicola	346	26.59	3.06	1.08	1.22	0.23	52.95	30.55	22.40	25.80	0	0	0.35	1.69	60.60	0	113	14	0.61	8.3	3100	0.054	19
Gandroma lucidium Reishi	376	15.05	3.48	0.50	1.20	0.27	71.00	69.30	1.70	66.80	0	0	0.06	1.59	12.40	0	66	37	1.3	13	760	0.014	6
Gandroma oregonensis	367	13.27	2.52	0.21	0.48	00.1	72.79	72.09	0.70	72.00	0	0	0.20	1.49	20.90	0	12	8	1.1	4.3	8.50	0.039	2
Grifola frondosa	377	25.51	3.83	1.12	2.08	0.34	60.17	41.37	18.80	28.50	0	0	0.25	2.61	64.86	0	440	31	1.88	7.6	2300	0.056	14
Hericium erinancius	375	20.45	5.06	0.83	1.85	0.76	61.80	40.90	20.90	39.20	0	0	0.16	2.26	11.80	0	17	8	1.66	6	2700	0.091	4
Lentinus edodes	356	32.93	3.73	1.30	1.36	0.22	47.60	31.80	15.80	28.90	0	0	0.25	2.30	20.40	0	110	23	123	5.5	2700	0.076	18
Pholiota nameko	**364**	**33.65**	**3.91**	**1.01**	**1.29**	**0.17**	**48.36**	**29.26**	**19.10**	**28.10**	**0**	**0**	**0.28**	**3.06**	**106.0**	**0**	**18**	**10**	**1.6**	**16**	**2500**	**0.103**	**4**

Table 1. Nutritional profile of diverse mushrooms (Stametes, 2005)

3.5. 2. Health benefit

During the past 50 years, several major advancements in medicine came from lower organisms such as molds, yeast, and mushrooms. Of approximately 140,000 known species of mushroom, 2000 are safe for people's health, and about 300 of them possess medicinal properties. Of about 300 mushroom species with known medicinal properties, only about 20 species are in use at the present. Most of traditional knowledge about medicinal properties of mushrooms came from the Far East (China, Japan, Korea, Siberia), where such mushrooms as Reishi, Shiitake and others were collected, cultivated and used for thousands of years (Wasser, 2002) .

Of all cultures, mushrooms were least valued in the West, especially in regard to their use as medicine (Halpern, 2007). But now the western countries have just started to ponder into diversity and great potential of mushrooms. Many pharmaceutical substances with potent and unique properties were recently extracted and made their way all around the world (Wasser, 2002). They are known to contain pharmacologically active components which cause no harm nor place additional stress to the body (Oyetayo, 2002). They are also probiotic, help our body strengthen itself and fight off illness by maintaining physiological homeostasis, restoring our bodies balance and natural resistance to disease. Mushrooms 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).

Problems in the immune system come in two varieties. When the immune system is underactive, it makes the body susceptible to infections, cancer, and other illnesses. When it is overactive, it may create allergies and autoimmune reactions. Autoimmune means the immune system is over stimulated and mistakenly attacks the body. Diseases such as diabetes, lupus, and lymphoma are autoimmune diseases. AIDS, hepatitis, flu, and colds, on the other hand, are associated with a weakened, underactive immune system (Halpern, 2007). In Japan, Russia, China, and the U.S.A several different polysaccharide anti-tumor agents have been developed from the fruiting body, mycelia, and culture medium of various medicinal mushrooms *Lentinus edodes, Ganoderma lucidum, Schizophyllum commune, Trametes versicolor, Inonotus obliquus, and Flammulina velutipes.* Both cellular components and secondary metabolites of a large number of mushrooms have shown an effect on the immune system of the host and can be used to treat a variety of disease states (Wasser, 2002).

Some kinds of mushroom polysaccharides such as Galacto-β-glucan (*Pholiota nameko*), Lentinan (*Lentinus edodes*), Schizophyllan (*Schizophyllum commune*), and Krestin (*Coriolus versicolor*, are currently available to the pharmaceutical industry (Wasser, 2002).Polysaccharides or peptideglycan, pharmaceutically active mushroom compounds, continue to be the subject of most researches, including isolation, chemical structures and experiments in vitro or in vivo. These polysaccharides extracted from mushrooms have extraordinarily low toxicity, even at high doses, now it has been known that they can profoundly improve the quality of human health (Daba and Ezeronye, 2003).

Polysaccharides from higher basidiomycetes mushroom have a promising effect for cancer treatment, anti microbial action, blood pressure lowering, liver protection, anti inflammatory, anti diabetic, and cholesterol lowering (Smith *et al*., 2002). Those polysaccharides present in mushroom have the highest capacity for carrying biological information than protein and nucleic acids because of their greatest potential for structural variability (Smith *et al*., 2002).

The polysaccharides of mushrooms occur mostly as glucans. Some of which are linked by β-(1-3), (1-6) glycoside bonds and α-(1-3) glycoside bonds but many are true heteroglycans (Fortes *et al*., 2006). Most often there is a main chain, which is either β-(1-3), β-(1-4) or mixed β-(1-3), β-(1-4) with β-(1-6) side chains (Wasser, 2002).

Generally mushrooms contain numerous medicinal compounds such as triterpenoids, glycoprotein, polysaccharides, natural antibiotics, enzymes and enzyme inhibitors and also there are many biologically active polysaccharides in mushrooms that are not beta-glucans, such as beta-mannans, cyclo-furans, and the alpha-bound varieties that fortify health (Halpern, 2007).

Pholiota nameko has medicinal properties including immune boosting and cancer fighting. According to Tabasso. (2006) Galacto- β - glucan is water and sodium hydroxide extracts of Nameko mushroom, which has 90% efficiency against cancer. Nameko can also prevent the infection of staphylococcus, coli form, and Pneu-mobacillus (Ying, 1987). Further more the ethanolic extract of Nameko show significant anti allergic effect in mice (Oxazolone-induced type IV allergy (Fan *et al*., 2006).

3.5.2.1. Anti oxidative and anti inflammatory activities of *Pholiota nameko*

Free radicals and oxygen species presents in biological system from a wide variety of sources could oxidize nucleic acid, proteins, lipid, DNA, that leads to oxidative stress bring chronic disease includes cancer, heart disease, aging and other degenerative diseases (Fig. 2) (Shin *et al*, 2007). Anti oxidants able to trap free radicals and reactive oxygen species (ROS) presents in many biological system. The free radical scavenging activities of anti oxidant in food has been substantially investigated. Primary sources of naturally occurring antioxidants are whole grain, fruits and vegetables. Plant sourced food anti oxidant like vitamin C, E, carotenes, phenolic acid, phytoestrogen are recognized as having the potential to reduce chronic disease (Lindequist *et al*, 2005).

Recently, *Pholiota nameko* mushroom anti oxidant extract has played an important role in trapping of free radicals and reactive oxygen species (ROS) that lead to diseases of aging and cancer (Yerra *et al*., 2005). *Pholiota nameko* also has the potential to serve as effective therapeutic agent for inflammatory diseases, *Pholiota nameko* polysaccharide (PNPS-1) is another type of extract, which has a significant role in anti inflammatory activities. This PNPS-1 isolated and purified by enzymatic hydrolysis, hot water extraction, ethanol precipitation, and ion-exchange and gel-filtration chromatography.

Now day's different types of inflammatory diseases are identified. Recently, arteriosclerosis closely related to inflammation, the hypochlorousacid produced by neutrophil through inflammatory response forms lysinechloamine to react with amino-group of lysine moiety in a protein, and then produces 3-chloro-tyrosine by reacting with tyrosine. Since 3-chloro-tyrosine

produced during inflammation process that is detected in the blood or tissue of arteriosclerosis patients. Therefore the *Pholiota nameko* polysaccharide (PNPS-1) able to inhibit the chlorination of protein with hypochlorousacid (HOCl), and possesses significant anti-inflammatory activity (Fig. 3). The *Pholiota nameko* polysaccharide (PNPS-1) suggesting it's potential as an anti-inflammatory agent for use in the treatment of various inflammatory-related diseases especially the inhibitors of inflammation induced tumor (Haiping *et al.*, 2007)

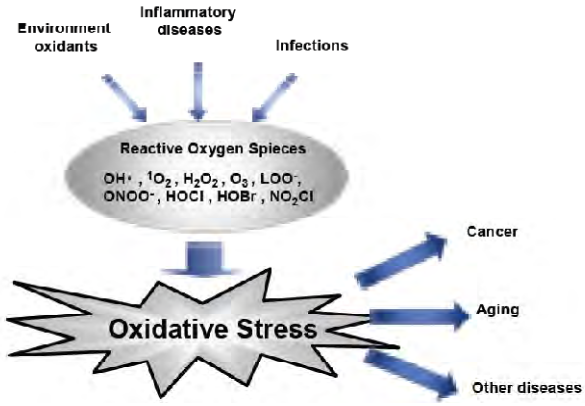

Figure 2. Reactive oxygen species bring oxidative stress (Lindequist *et al*, 2005).

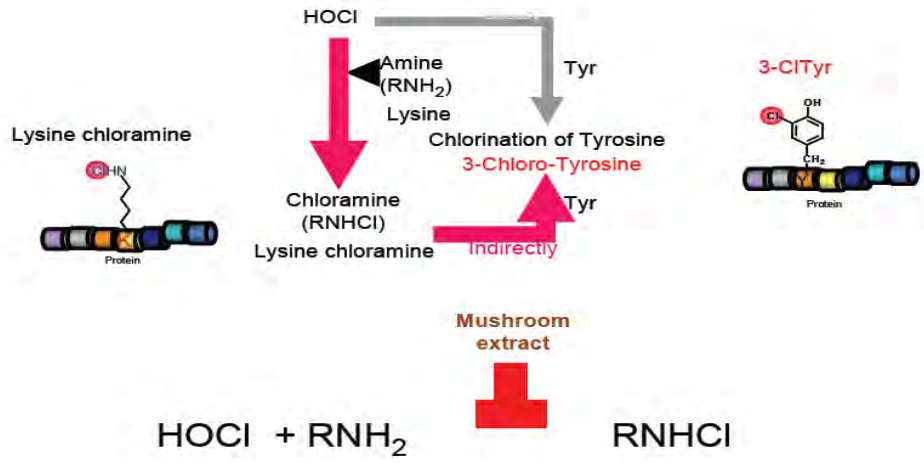

Figure 3. Anti-inflammatory activity mushroom extracts (Shin *et al*, 2007)

3.5.2.2. Myo -inositol mono phosphates in mycelia of *Pholiota nameko*

Inositol is an essential element in many organisms, plays a vitamin like function. Its deficiency causes incomplete development, fatty liver and alopecia. It is a free sugar alcohol contained abundantly in vegetables, fruits and in the pileus of 13 edible mushrooms. *Pholiota nameko* mycelia contain abundant amount of inositol mono phosphate, where it is the precursor of inositol serve as the treatment of fatty liver and alopecia (Malick *et al*., 1996).

3.5.3. Enzyme production by *Pholiota nameko*

Enzyme production is an increasing field of biotechnology. 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).

Solid-state fermentation (SSF) is a microbial process occurring mostly on the surface of solid materials, which can absorb or contain water, in the presence or absence of soluble nutrients (Gonzalez *et al*., 1994). The application of agro-industrial residues in solid-state fermentation bio-processes not only provides an alternative substrate for enzyme production but also reduces the pollution problems caused by their accumulation (Suess and Curtis, 2006). The food, agricultural and forestry industries produce large volumes of wastes annually which cause a serious disposal problem world wide. 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-catalysed reaction where a diphenol is oxidized to form a free radical, which can further undergo a second enzymatic catalysis to form a quinone (Tavares, 2006) (Fig. 4).

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).

Pholiota nameko, *Pleurotus ostreatus, Pleurotus cystidiosus* and *Pleurotus pulmonarius* produce an ample amount of laccase enzyme when grown on cotton wastes under solid-state fermentation. But, other white rot fungi have different agricultural waste preference for lacase enzyme production (Jaszek *et al.*, 1998). In addition to laccase production, Glucose -1-phosphatase enzyme is extracted from the mycelia of *Pholiota nameko*. An acid phosphatase is a key enzyme in the regulation of phosphate metabolism (Joh *et al*., 1998).

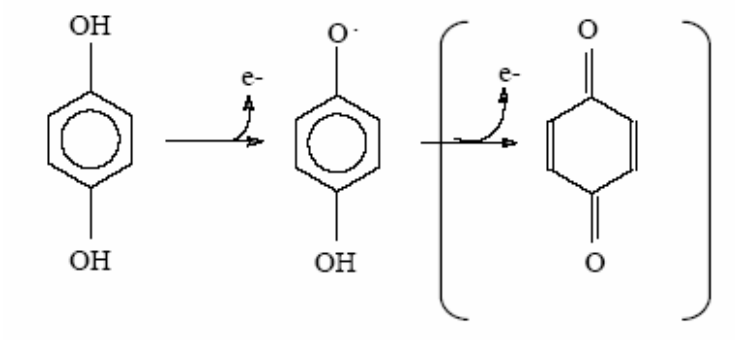

Figure 4. Typical laccase-catalysed reaction for a diphenol (Tavares, 2006)

3.5.4. *Pholiota nameko* spent substrate as animal feed and environmental protection

At the end of several mushroom harvests, the growing material is considered as spent (Danny *et al*., 2004). Agricultural crop residue, especially cereal straw is produced in high amount and contributes to a major part of the diet of ruminants in developing countries. However, the use of straw as animal feed is limited due to its low available energy as well as its low nitrogen content and poor palatability (Jalc *et al*., 1999). 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). Chang found that when maize straw generated after mushroom cultivation was added to the diets of sheep, the weight gain of the sheep increased (Chang, 1999). The fresh spent substrate was eaten at 700-800 g/head/day without any additional concentrates (Danny *et al*., 2004).

The spent substrate after Nameko mushroom cultivation there was an increase in the amount of lactic acid produced when both lactic acid bacteria and molasses were added; the moisture was retained for the palatability and improves protein feeds for ruminants (Yokita *et al*., 2006).

Danny *et al*. (2004) have suggested that the organic wastes from a coffee farm contain a biochemical's which do not permit their reuse as cattle feed. However, after Nameko mushroom cultivation on coffee farm, the waste becomes an excellent additive to cattle and pig feed.

In addtion to animal feed, the Nameko mushroom spent substrate has physical properties that may enhance its desirability as a soil amendment and can improve the structure of clay soils, reduce surface crusting and compaction, promote drainage, increase microbial activity and provide nutrients to turf grasses. This promotes faster turf establishment, improved density, soil color and recycles in the environment. Spent mushroom substrate contains a diverse range of soil microorganisms this has been proven by its disease suppressing properties and its effectiveness in bioremediation (Royse, 1992).

4. Materials and methods

4.1. Organism and culture conditions

Fungal strain; *Pholiota nameko* was obtained from Mycology Laboratory, Department of Biology in Addis Ababa University. The pure culture of *Pholiota nameko* was transferred to three types of nutrient media, these are, potato dextrose agar (PDA), malt extract agar (MEA), and subourdinate dextrose yeast agar (SDYA). Potato dextrose agar supplemented with, 100g fresh potato/250ml, 10g glucose/250ml, 10 g agar/250ml, 0.05 chloramphenicol/250ml. Malt extract agar(MEA),having 8g/400ml, 2g peptone/400ml, 8g agar/400ml,0.025g chloramphenicol/ 250ml, and 20g SDYA/400ml , 20 plates were prepared for each, a total of 60 culture plate are kept in incubator at 25 ^{0}C. Mycelia growth in terms of diameter on culture plate were measured using ruler and contamination were recorded at 3 day intervals.

4.2. Grain Spawn production

Spawn is the vigorous mycelia growth of a single fungus on a chosen substrate material (liquid media, grains, saw dust substrate, wooden sticks (Jiskani, 2000).

In this study 6 kinds of grain were used, barely, wheat, rice, shredded maize, sorghum and finger millet. Sorghum was selected best for mother spawn and later propagation depends on its mycelia invasion. About 20 kg of sorghum was washed and dead floating removed then soaked over night in 15L water and rinsed three times in distilled water. The excess water was drained off and 20% wheat bran, 12% gypsum ($CaSo_4$. $2H_20$), and 3% limes ($CaCO_3$) were added. The ingredients were thoroughly mixed; moisture was maintained at the level of 55 %, and distributed equally in to 500 ml glass bottle at the rate 370.66 g seed per jar for a total of 128 bottles and autoclaved for 121 ^{0}C to 1 hour. After cooling, each bottle was inoculated with 16

agar blocks (1 cm x 1 cm) of 18 days old agar culture and incubated for 30 days at 25 ^{0}C until the substrate fully colonized, at ten days interval mycelia invasion and contamination were recorded.

4.3. Substrate preparation and formulation

4.3.1 Collection of Substrates

To study the influence of different substrates for the growth and yield of *Pholiota nameko* mushroom, 6 substrates, namely, Pinus shaving, eucalyptus shaving, cordia shaving, teff straw, coffee husk, cotton seed and the additive material, wheat bran were collected from local market and saw mill in Addis Ababa. All these industrial and agricultural wastes were cheap and locally available substrates stored abundantly without any significant use.

4.3.2. Substrates formulation

Two types of substrate formulation were done, at first; 3200g from 6 types of substrates (80%) for each were soaked in water for 24 hours to moisten them thoroughly and were stalked on the steep cemented floor so as to remove the excessive moisture from the substrates to get 55-65% moisture level. About 400g wheat bran (10%), 200g $CaCO_3$ (5%), 200g $CaSo_4$. $2H_20$ (5%) were mixed thoroughly by hand.

Secondly, 2400g substrates from each type of substrates (60%), 1200g wheat bran (30%), 300g $CaCO_3$ (5%), and 300g $CaSo_4$. $2H_20$ (5%) were done in the same procedure. A total of 36 polyethylene bags of 5 kg sizes were used for substrate medium. 2993.3g prepared substrate were filled into each plastic bag, tighten with an elastic band, and put the bags flat wise in the autoclave for sterilization (1- hour). The bags were autoclaved at 121^{o}C at high pressure and allowed to cool.

4.3.3. Moisture content determination of the substrates

The moisture content of the substrates that was suitable for the growth of *Pholiota nameko* was adjusted prior to inoculation. The essential amount of a dry weight substrate was weighed from each substrates and were soaked in water for 24 hours, then squeezed and drain off excess water. Dry weight is subtracted from wet weight amount divided to its wet weight, multiplied by hundreds.

Moisture level (%) = (Wet weight of the substrate – dry weight of the substrate) x 100

of substrate (Wet weight of the substrate)

4.4. Bag cultivation and fruiting management

4.4.1. Inoculation

After sterilization, the substrate bags were cooled to less than 30°C, and moved into the inoculation hood. Using the bags, opened and 8-10 holes for 2-3 Kg substrate were made. Then, the spawn transferred into the holes, 2 pieces of spawn of horse bean size for one hole, and scatter a layer of spawn on the substrate surface. Put the plastic ring on the plastic bag, turn the inside bag out of the ring, after inoculation the bags are moved into the growing room for spawn running.

4.4.2. Measuring spawn running in substrate bags

In order to promote the rapid development of mycelia and to bring forwards the fruiting date as well as to inhibit the contamination. The substrate bags were kept in clean growing room under room temperature of about 25°C. Natural ventilation was done to avoid too high temperature by

opening the door within 3 days intervals. The mycelia continue growing, absorbing and accumulating nutrition. Until the coat of rust color forms on the surface of mycelia clump, and the spawn running finish. The spawn run and cropping were performed during months from November 2009 to February 2010 in a locally made growing room in Addis Ababa University. The length or extension of mycelium run in each calibrated substrate bags within 15 days interval were measured using ruler and contamination were recorded.

4.4.3. Breaking bag and cutting coat

Get rid of the plastic bag when the mycelia mature, when the coat is too thick to be helpful for fruit body coming out, the coat was cut with a bamboo knife vertically and horizontally in breath of 2 cm and deep of 1 cm. Put the mycelia clump on the bed flatly or upright, then water sprinkled and kept it under room temperature of about 25°C to promote the fruit body formation.

4.4.4. Ventilation

The introduction of fresh air into the growing room supplies oxygen to the fungus enhances the evaporation of moisture from the surface of the sporocarp and removes CO_2. In fruiting phase the mycelia enhance the respiration and the oxygen requirement increase obviously, so it is necessary to keep the indoor air fresh. Proper ventilation were done by opening the door within 3 days intervals and let air through wire bar of the growing room.

4.4.5. Watering

Each cultivating bags were irrigated using tap water every morning and evening until 2 flushes of *Pholiota nameko* fruiting bodies appears.

4.4.6. Mushroom yield (Bioconversion Efficiency)

With proper management, 2-3 flushes mushroom can be harvested and the biological efficiency measured. Biological efficiency (BE), which is defined as the ratio of weight of fresh fruiting bodies to the weight of substrate multiplied by 100 (Fan *et al*., 2004). Yield performance and biological efficiency *Pholiota nameko* on the 6 kinds of substrates were calculated for two flushes.

$$\text{Biological Efficiency} = \frac{\text{Weight of fresh fruiting bodies}}{\text{Dry weight of substrate}} \times 100$$

4.4. 7. Isolation of fungal contaminant during Nameko cultivation

Fungal contaminants were recorded depending on their morphological characters, during the 3 phase of Nameko cultivation, i.e. culture production, spawn, and bag cultivation.

4.5. Method of data analysis

The data on mycelia growth rate on 3 types of nutrient media with 5 replicates for each, degree of mycelium running on each substrates type, yield and biological efficiency of *Pholiota nameko* on 6 kinds of substrates and fungal contamination were determined. Analysis was performed for 6 kinds of substrate with 3 replicates for each. The data were analyzed by comparing the mean weights, and % biological efficiency through two ways ANOVAs. The data groups were analyzed using Statistical Package for Social Sciences (SPSS) for windows 15.0.Treatment mean were compared using Turkey's t- test.

Figure 5. Important substrate during *Pholiota nameko* production

A=Eucalyptuse shaving, B=*Cordia africana shaving*, C=Coffee husk, D=Teff straw ,E=Pinus shaving, F= cotton seed .

Figure 6. Substrate in plastic bags after sterilization ready for inoculation

5. Results

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

During the present investigation, 3 types of nutrient media i.e., malt extract agar medium (MEA), subourdinate dextrose yeast agar medium (SDYA), potato dextrose agar medium (PDA) were used for tissue culturing of *Pholiota nameko* mushroom. The result revealed that the length of mycelia growth in diameter of culture plate was high on MEA media as compared to PDA and SDYA with in 3 day interval measures for 18 days. Full mycelium growth of *Pholiota nameko* took 18 days on MEA, 23 on PDA, 28 on SDYA relatively (Fig. 7, 8 & 9). AS shown in figure 9, 30% mycelia growth was seen on MEA, where as 18.2% & 13.11% on SDYA and PDA respectively at the third day. Great difference in mycelia growth was recorded at 12th day between MEA and PDA. After 15th days mycelia growth became rapid on PDA and it is almost parallel with SDYA. At the end of 18th day 97.33% mycelia growth was seen on MEA, 84.22% on SDYA and 78.66% growth on PDA.

Figure 7. Mycelia growth in length across the diameter of the culture plate

Figure 8. The mycelia growth rate of *Pholiota nameko* on MEA, SDYA, and PDA respectively within 18 days

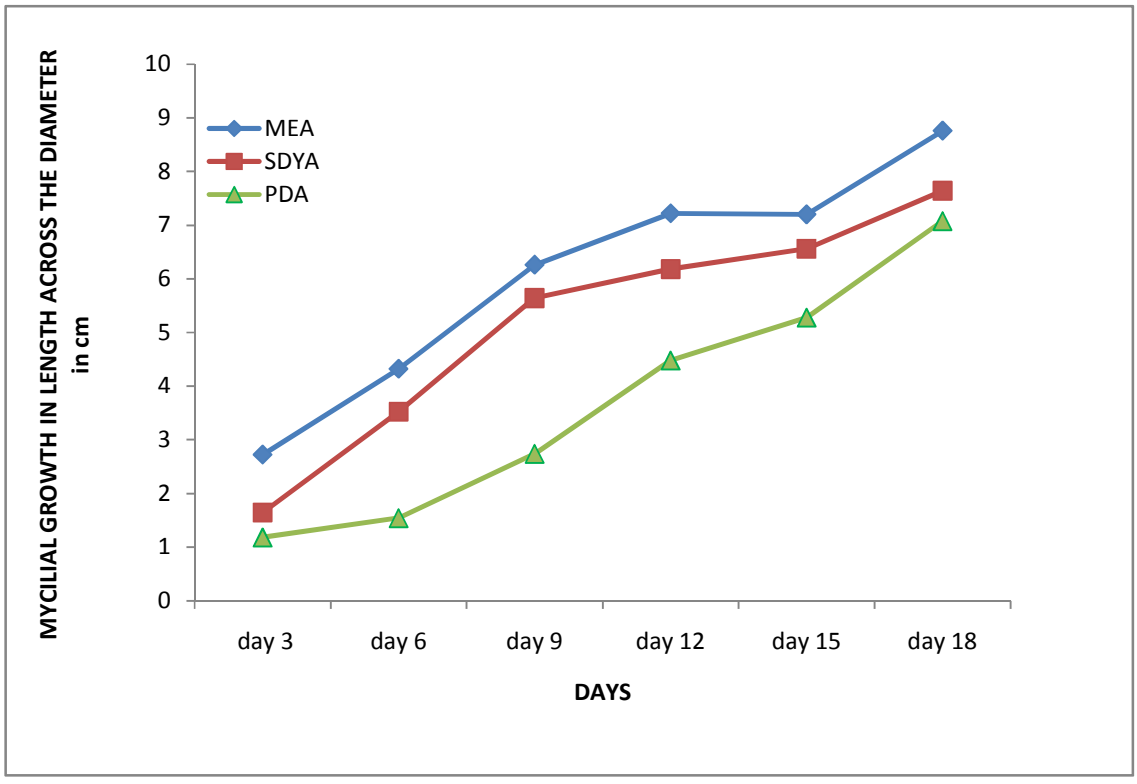

Figure 9. Mycelia growth of *Pholiota nameko* in terms of increase in diameter on different nutrient media.

Malt extract agar medium (MEA), Potato dextrose agar medium (PDA), Subourdinate dextrose yeast agar medium (SDYA)

5.2. Spawn production

Barely, wheat, rice, shredded maize; sorghum and finger millet are important cereals for spawn production of different mushroom species and were tested for *Pholiota nameko*. How ever, a better mycelia invasion is seen on sorghum and wheat (Fig. 10 & 11). Comparatively sorghum support good mycelia invasion and selected as the best. But the rest four namely shredded maize, rice, barely and finger millet did not show any mycelia growth of *Pholiota nameko*. Generally sorghum based spawn took 1 month to colonize the substrate completely. The moisture content of the sterile moist sorghum (55-60%) was found to be suitable.

Figure 10. *Pholiota nameko* spawn on sorghum in 30 days

Figure 11. *Pholiota nameko* spawn on wheat in 30 days

5.3. Degree of mycelium running rate in length on different substrate bags

The bags were placed on wooden shelves after inoculation; until it is fully colonized by Nameko mycelia. The window, door and the wall frame were covered with wire gauze to bar insects and rodents; mycelium running rate in substrate bag varied on different substrate types used (Fig.13). Mycelium running is an extension and colonization of fungal hypha through out the substrate. The mycelia growth rate in length in a calibrated substrate bags were measured at 15 days intervals using ruler. The highest running rate in length was observed in eucalyptus shaving (24.6cm/60days), cordia shaving (21.3 cm/60days), teff straw (19.6cm/60days), cotton seed (18cm/60days) and coffee husks (15.4cm/60 days). The lowest running rate in length of mycelium was observed in pinus shaving (13.37 cm/60days) (Table. 2). However total days

required completing mycelium running relatively in eucalyptus shaving, cordia shaving, teff straw, cotton seeds, coffee halls and pinus shaving, 60, 63, 70, 65, 75, 80 days took respectively.

Table 2. Degree of mycelium running rate in length on different substrates.

Days	Mycelia growth rate in length on ES (cm)	Mycelia growth rate in length on TS (cm)	Mycelia growth rate in length on CS (cm)	Mycelia growth rate in length on CH (cm)	Mycelia growth rate in length on PS (cm)	Mycelia growth rate in length on CAS (cm)
15 day	5.73±0.12Aa	3.70±0.25Ab	2.90±0.06Ac	2.43±0.09Ad	2.53±0.09Ae	5.20±0.06Af
30 day	12.67±0.24Ba	8.97±0.09Bb	8.80±0.06Bc	7.27±0.12Bd	6.37±0.23Be	10.07±0.37Bf
45 day	18.20±0.35Ca	17.30±0.25Cb	15.87±0.47Cc	13.17±0.13Cd	11.23±0.12Ce	15.60±0.36Cf
60 day	24.60±0.31Dae*	19.57±0.35Db	17.60±0.31Dc	15.30±0.17Dd	13.73±0.12De	21.27±0.37Df

*=Significant at $p<0.05$

Means followed by the same letter/s within a row (uppercase) on the same day and column (lowercase) on different day are not significantly different ($p<0.05$),Tukey's Studentized Range Test

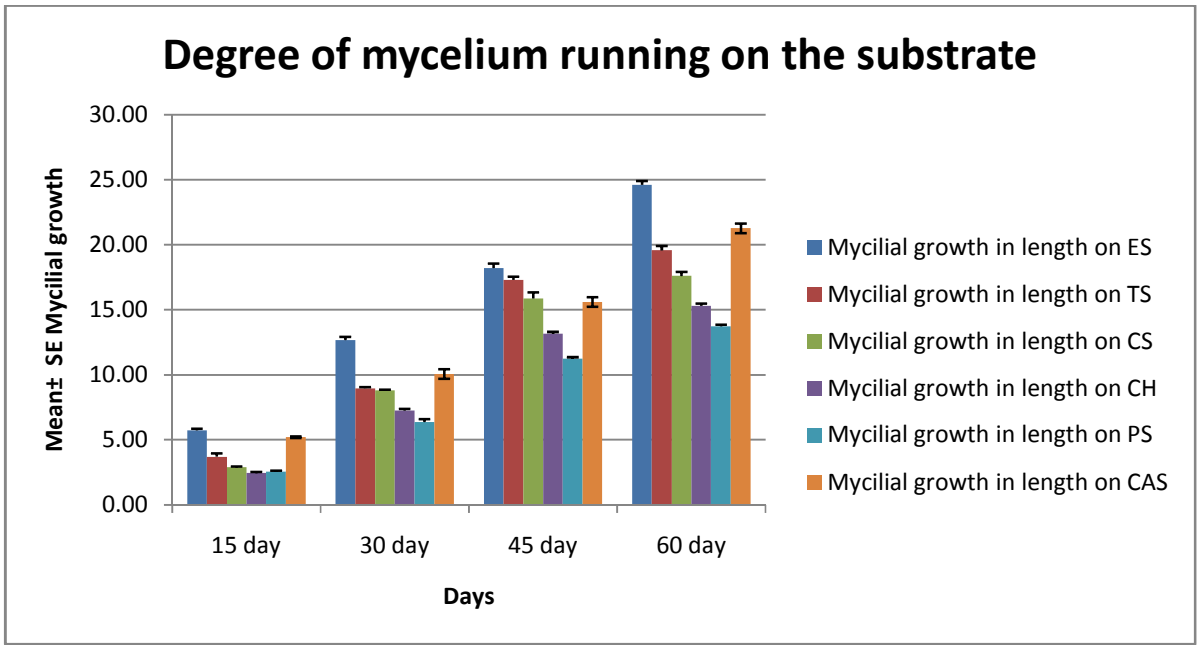

Figure 12. Degree of mycelium running rate in length on different substrates

ES=Eucalyptuse shaving , CAS= *Cordia africana* shaving , CS= Cotton seed, CH=Coffee husk, TS= Teff straw, PS=Pinus shaving

Figure 13. Mycelia (spawning) running rate in length on different substrate

A=Eucalyptuses shaving, B= *Cordia africana* shaving, C=Teff straw, D= Pinus shaving, E= Coffee husk, F= Cotton seed

5.4. Harvesting yield and bio conversion efficiency of *Pholiota nameko* on different substrate

Fruiting bodies of *Pholiota nameko* were harvested as soon as the caps have reached 5 to 7 cm in diameter for up to 2 flushes. It took two cropping cycle in 30 days, 10-14 days apart. The whole period took 90 days. 6 substrates were tested for the cultivation of *Pholiota nameko*, namely pinus shaving, eucalyptus shaving, cordia shaving, teff straw, coffee husk and cotton seed supplemented with 10% and 30% wheat bran for each (Fig. 5 or 6). Three of them, the pinus shaving, teff straw, coffee husk did not show any growth of fruiting body, how ever had mycelia invasion during spawn running. Eucalyptus shaving, cordia shaving and cotton seed show remarkable production of *Pholiota nameko* (Fig. 14). The result revealed that eucalyptus shaving gave the highest mean yield and biological efficiency of 797.33g and53.27%, respectively. The harvesting yield and bio conversion efficiency of *Pholiota nameko* grown on eucalyptus shaving, cordia shaving and cotton seed supplemented with 10% and 30% wheat bran did not show significant difference in this study (Table. 3&4). How ever quality and cropping time were better in the substrate supplemented with 30%wheat bran (Fig.14). But a significance difference on mean yield is seen between eucalyptus shaving and cordia shaving (Table. 4).

Table 3. Harvesting yield and bio conversion efficiency on different substrate supplemented with 10% Wheat bran.

Substrate	Mean Yield (g)	Biological efficiency (%)
ES	761.5^{*b}	50.88
CS	660.6^{ab}	44.14
CAS	550.8^{a}	36.80

*=Significant at p<0.05

Note; Mean in column followed by the same superscripts are not statistically different at P<0.05 according to Turkeys test.

Table 4. Harvesting yield and bio conversion efficiency on different substrate supplemented with 30% Wheat bran

Substrate	Mean Yield (g)	Biological efficiency (%)
ES	797.33*[b]	53.27
CS	732.33[ab]	48.98
CAS	629.17[a]	42.04

*=Significant at p<0.05

Mean in column followed by the same superscripts are not statistically different at P<0.05 according to Turkeys test.

ES=Eucalyptuse shaving , CS= Cotton seed, CAS= *Cordia africana* shaving

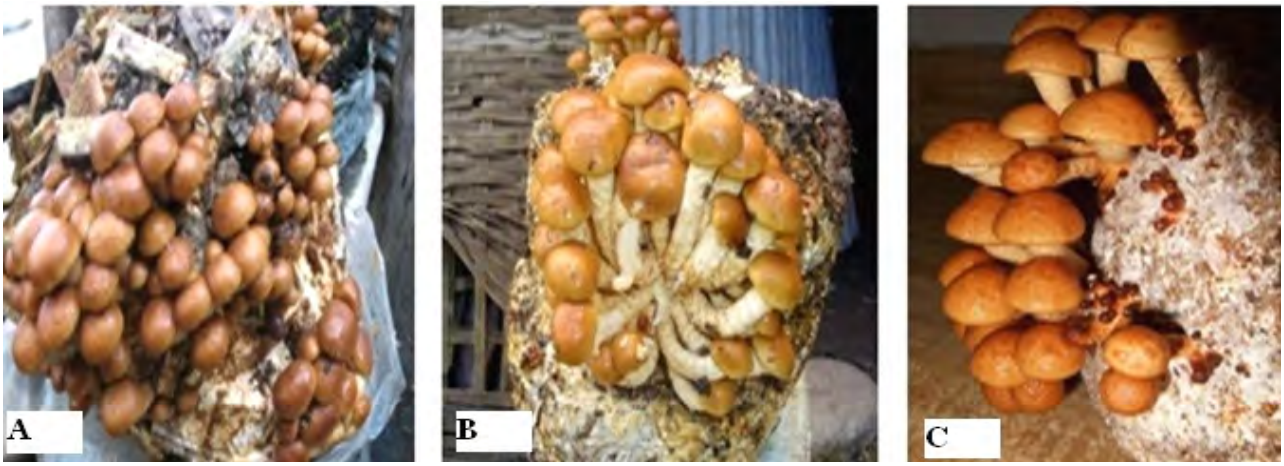

Figure 14. *Pholiota nameko* grown on eucalyptus shaving (A), cotton seed (B) and *Cordia africana* (wanza) shaving (C).

5. 5. Contamination

The mushroom, like any other cultivated crop, is subject to attack by pathogens and pests. The mushroom diseases can be caused by both fungi and bacteria. In the present investigation, the incidence of pests and diseases were high, pests like rats and insects were trouble some at each stage of *Pholiota nameko* production, how ever, the growing house wall were covered with wire gauze to bar rodents and formaldehyde was sprayed to stop insect invasion. Through their morphology and spore study under the microscope indicate that the culture plate, spawn bottle and substrate bag were repeatedly spoiled by other fungal contaminants. Culture plate dominantly contaminated by *Scopulariopsis fimicola* (white plaster mold), *Chaetomium olivaceum*, *Aspergillus niger* and *Pencillium notatum* (Fig. 16). Most of the time, *Aspergillus flavus, Aspergillus niger and* Trichoderma species were contaminating spawn bottle and substrate bags in this study (Fig. 17 &18).

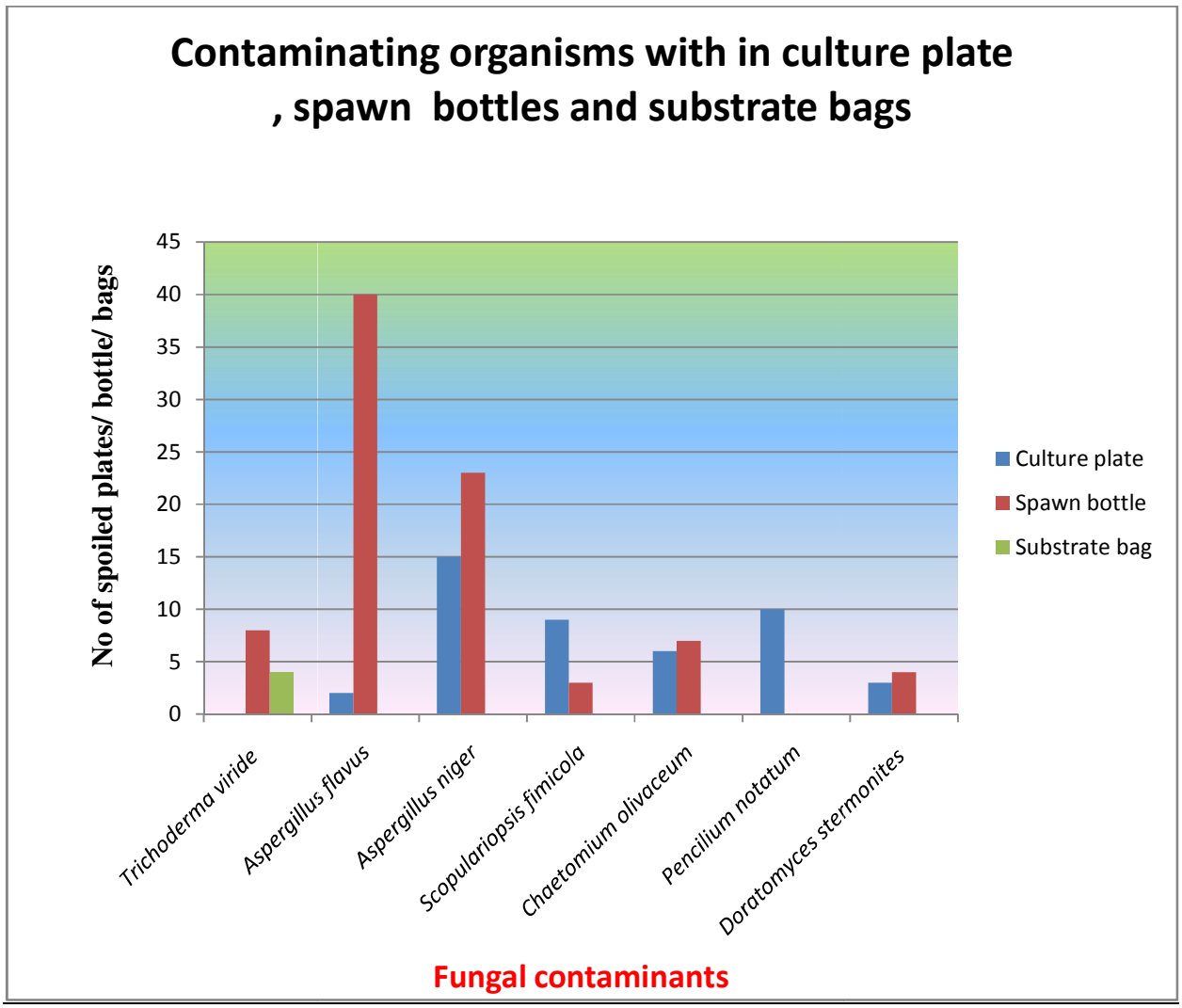

Figure 15. Fungal contaminants in culture plate spawn bottle, substrate bags

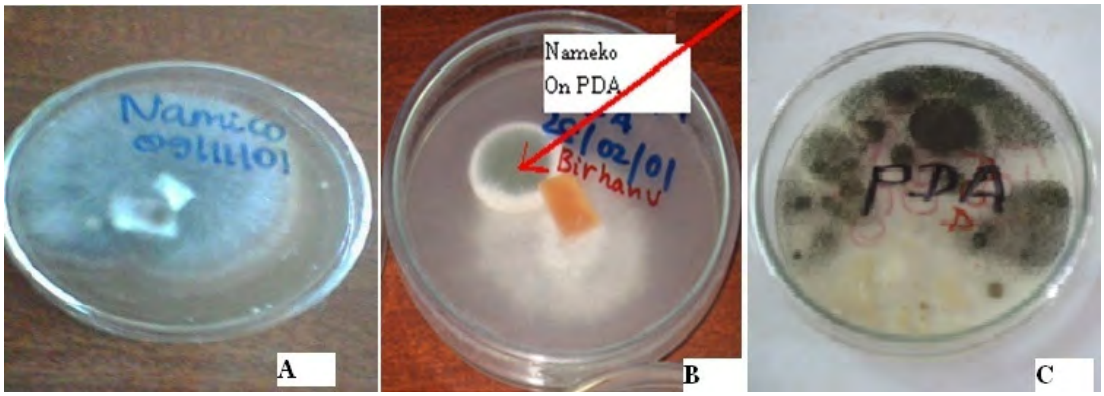

Figure 16. Cultur plate were contaminated by *Chaetomium olivaceum* (A), *Pencillium notatum* (B), *Aspergillus niger* (C).

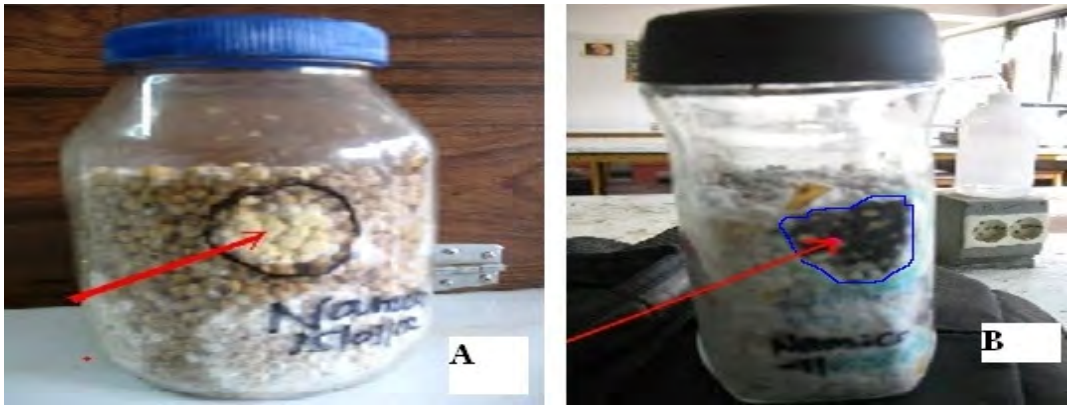

Figure 17. Spawn bottle were contaminated by *Aspergillus flavus*(A) and *Aspergillus niger*(B)

Figure 18. Trichoderma species contaminate substrate bags

6. Discussion

So far about 35 species from 140,000 naturally occurring mushrooms species are under artificial cultivation. However, the world production is still dominated by the white button mushroom, due to its rapid mycelia invasion, short fruiting time, vast substrate choice and good taste. Hence; exploitation of the new cultivable edible species is becoming more important. In this research one type mushroom species that is *Pholiota nameko*, is tried to cultivate on agro industrial waste in Ethiopia environmental condition.

Pholiota nameko is white rot wood inhabiting ligninolytic species belonged to genus *Pholiota*, widely distributed through out Far East (Pegler, 2003). The only organisms reported to degrade lignin efficiently are the white-rot fungi under natural conditions, mostly colonize dead or living wood. White-rot fungi attack the lignin component of wood and leave the cellulose and hemicelluloses less affected, also called selective degrader (Eriksson *et al.,* 1990).

Lignocelluloses wastes represent huge amounts of unutilized renewable resource (Hader *et al*., 1992). The large amount of lignocelluloses wastes are generated through forestry and agricultural practice, paper-pulp industries, timber industries and many agro –industries and they pose an environmental pollution problem (Howard *et al*., 2003). The cultivation of edible mushroom is a prime factor for the conversion of this low value inedible wastes in to a higher value commodity which can serve as food material for humans and as a source commercially important metabolites (Chang *et al*., 1993). Also their spent can be used as cattle feed, fertilizer or landfill (Cohen *et al.,* 2002). There fore cultivation of mushroom on agro industrial

lignocelluloses waste provides multi disciplinary advantages for human being, animals as well as for the ecosystem.

In Ethiopia there is big waste from saw mill and agro industrial activities. There are approximately 39 saw mills and a total of 5-10 factories involved in the production of wood. Saw mill residue is estimated to total about 25,000 tones per year (Yisehak *et al.,* 2009). Agricultural and agro-industrial residues used in the domestic sector for cooking and baking, using very low efficiency devices constitute 15% of the total energy consumed in Ethiopia. But the rest 85 % of the residues are mostly tends to be disposed of wastefully (Yisehak *et al.,* 2009). That can be easily diverted to produce the mushroom which can compensate a big problem of nutrient deficit in food of this country.

The first stage in any mushroom cultivation process is to obtain a pure mycelia culture of the specific mushroom strain. Mushrooms grow on a variety of culture media and on different agar formulas, both natural and synthetic, depending on the organism to be cultivated and the purpose of the cultivation (Chang *et al*., 1993).

In this study 3 types of nutrient media were tested for tissue culturing of *Pholiota nameko*. The result revealed that *Pholiota nameko* culture showed dense and good mycelia growth on malt extract agar (MEA) than potato dextrose agar (PDA) and subourdinate dextrose yeast agar (SDYA) in terms of mycelia growth in length across the diameter of the culture plate (Fig.7& 8). AS shown in figure 9, 30% mycelia growth was seen on MEA, where as 18.2% & 13.11% on SDYA and PDA respectively at the third day. Great difference in mycelia growth was recorded at 12^{th} day between MEA and PDA. After 15^{th} days mycelia growth became rapid on PDA and it

is almost parallel with SDYA. At the end of 18th day 97.33% mycelia growth was seen on MEA, 84.22% on SDYA and 78.66% growth on PDA. These results may be explained by the fact that the malt extract agar medium used was a good source of organic nitrogen. It has been suggested that malt extract agar (MEA) support good mycelia growth of *Pholiota nameko* than, potato dextrose yeast agar (PDYA), and dog food agar (DFA) (Stametes, 2000). Chang. (2006) found *Pholiota nameko* grew well in malt extract yeast agar and malt extract yeast peptone agar than potato-composite medium, poorly in potato dextrose agar.

The second stage in any mushroom cultivation process is spawn production. The mushroom "seed" (propagation material) is generally referred to as spawn. A number of materials, mostly agricultural wastes, can be used to prepare mushroom spawn. Some of these wastes are chopped rice straw, sawdust, water hyacinth leaves, used tea leaves, cotton wastes and lotus seed husks (Chang and Mshignei, 2000). Modern methods of spawn preparation use cereal grains (wheat, sorghum, maize, millet, rye), which are sterilized in glass jars, inoculated with a selected strain and incubated at appropriate temperatures for complete colonization (Carrera, 1989).

In the present study 6 kinds of grain were tested for spawn production of *Pholiota nameko*, and sorghum was selected best for later spawn propagation. There is dense whitish mycelia growth on the entire surface sorghum grain (Fig. 10).

Sorghum grain provide good food base for the fungal species to grow throughout the substrate also described that sorghum is a better available and cheaper than other substrates like maize in some areas in Africa including Ethiopia (Dawit, 1998).

Wheat next to sorghum also support good mycelia invasion for *Pholiota nameko* in this study. Jiskani *et al*. (2000) have reported that sorghum grains were found to be best medium for spawn growth followed by wheat, maize, and pearl millet grain respectively. Hafeez *et al*. (2000) have also reported that spawn production on sorghum grains was significantly higher than pearl millet, maize and wheat grains. There for sorghum grain showed a better product of spawn due it's full of nutrient that able to support the mycelia of *Pholiota nameko* mushroom.

Immediately following inoculation by the mature spawn, whitish nameko mycelia begin to grow on the substrate, until colonization is completed. This is an active assimilation phase with high fungal metabolic rate. Enzymes are activated to break down complex substrate components (cellulose, hemicelluloses and lignin) into simpler molecules which can be absorbed by mycelia as nutrients for growth and propagation. Mycelium running rate of each type of substrates was observed after 10 days of inoculation in most mushrooms (Chen, 2001).

In the present study the length of spawn run in the substrate bags were measured at 15 days intervals. The highest running rate in length was observed in eucalyptus shaving (24.6 cm/60days), cordia shaving (21.3 cm/60days), teff straw (19.6cm /60days), cotton seed (18cm/ 60days) and coffee husks (15.4cm/60 days). The lowest running rate in length of mycelium was observed in pinus shaving (13.37 cm/60days). There is significant difference in mycelia running rate in length between eucalyptus shaving and pinus shaving (Table. 2)

However total days required completing mycelium running relatively in eucalyptus shaving, cordia shaving, teff straw, cotton seeds, coffee halls and pinus shaving, 60, 63, 70, 65, 75, 80 days took respectively.

According to Chen. (2001) the spawn run on the eucalyptus substrate could be observed after 12 days of incubation in the growing room, with the formation of white tawny around the spawn, indicating the beginning of degradation of the substrate by the fungus. The natural induction of primordial on the substrates occurred after 60 days. In this study significance difference is seen between eucalyptus and pinus shaving that could be the amount of lignin higher in eucalyptus than pinus shaving. Evtuguin *et al*. (2001) have suggested that eucalyptus lignin content is reached to 82-86 %. Robert. (1986) also reported that the pinus plant consists of 26% lignin.

The last stage in mushroom cultivation is fruiting body production. Edible mushrooms can be commercially cultivated on a variety of hard wood substrates, depending on the characteristics of the mushroom (Chang, 2003). The cultivation of *Pholiota nameko* on different hard wood chips showed a remarkable yield & bio-conversion efficiency. Fruiting on the first flush give an average of slightly more than 1.2 kg from 5kg of hard saw dust supplemented from rice bran (Stamets, 2002). According to Chang and Hayes. (1978) with proper management, 3-4 flushes of *Pholiota nameko* mushroom can be harvested and the biological efficiency can reach 60%-70 % on hard wood substrates.

Paddy straw and sawdust from oil free plants were tried and saw- dust amended with rice bran and calcium carbonate was found to be the best substrate for the domestication of *Pholiota*

nameko, six hundred eleven grams of fresh sporophores were harvested from one kilo of dry sawdust of Populus tree (Krishna and Sharma, 1989).

In the present study the cultivation of *Pholiota nameko* on 6 substrates namely pinus shaving, eucalyptus shaving, cordia shaving, teff straw, coffee husk, cotton seed, supplemented with 10% and 30% wheat bran were investigated. The result revealed that only 3 substrates show production of *Pholiota nameko*, these are eucalyptus shaving, cotton seed and cordia shaving (Fig. 14). Substrate supplemented with 30% wheat bran showed a little better yield in the quality and cropping time than substrate supplemented with 10% wheat bran (Fig. 14). But there is no significance difference between them in the bioconversion efficiency.

According to Chen. (2001) addition of wheat bran in sawdust substrates is one of the most important factors both, increased productivity and, often times, improved mushroom quality (Chen, 2001). Kirchhoff. (1996) has indicated that wheat bran is commonly added substrate at various ratios to encourage formation of high quality sporocarps. Because wheat bran contain bout 2.24 to 2.72%, nitrogen content.

Pinus shaving, coffee husk and teff straw did not show any production of *Pholiota nameko* in the present study, how ever they had mycelia invasion. But Chang. (1999) suggested that sawdust's from conifers such Pinus species and *Cryptomeria japonica* are suitable for growth *Pholiota nameko*. Rice bran or wheat bran usually is added as a supplement in the ratio of 15% for pinus sawdust and 10% for broad-leaf sawdust. These results may be from the quality of spawn used, temperature, pH of the substrate or the proportion of lignin and cellulose in the pinus plant.

Recent study indicated that several strain of mushroom have adapted to grow very well on straw and coffee pulp, even the biological efficiency reached up to 63% (Salamon and Mata, 2003). Danny *et al*. (2004) also suggested that *Pholiota nameko* is cultivated on coffee wastes. Even after Nameko mushroom cultivation on coffee farm, the waste becomes an excellent additive to cattle and pig feed. But in the present study *Pholiota nameko* did not grow on coffee husk and teff straw. This may be due the high proportion of cellulose than lignin in coffee and teff straw as well as the coffee species present in Ethiopia.

In general eucalyptus shaving gave the highest mean yield and biological efficiency of 797.33g and53.27%, respectively. Cotton seed showed the second higher mean yield and bioconversion efficiency 732.33g, 48.98%. The use of eucalyptus sawdust as raw material was found better for the production of *Pholiota nameko*. Since *Pholiota nameko* is white rot fungi degrade mostly lignin. It has been found that eucalypts lignin content ranges from 82-86 % (Evtuguin, 2001). The result also conforms to the report of (Chang and Miles, 1984) that the biological efficiency of *Pholiota nameko* on eucalyptus is 52.8%. The Cotton waste gave a higher mean yield of *Pholiota nameko* mushrooms than any other agro industrial wastes which could be due to high proportion of lingo cellulose and compactness on wetting. (Joh *et al*., 1998) also found that 78% cottonseed nut, 20% wheat bran, 1% sugar, 1% gypsum are important for *Pholiota nameko* cultivation for lacase enzyme production. Cotton waste is a better substrate for the cultivation of *Pholiota nameko* (Tan, 1981). This is probably due to higher nitrogen content in cotton waste. Anyakorah and Olatunji (2001) have reported that higher nitrogen content about (5.67%) found

in cotton waste. This is because nitrogen is an important basic nutrient for microorganisms, being required for protein, nucleic acid and chitin synthesis (in the case of fungi).

The lowest mean yield and bio conversion bioconversion efficiency were recorded 550.8 g, 36.80 % by Cordia shaving. The low bioconversion efficiency could be attributed to the quality of spawn used (Kalmis and Sargin, 2004). (Bhatti, 1987) has suggested that the variation of biological efficiency and incubation period of nameko mushroom on different substrates may be due to their different composition in the substrate used.

The yield of *Pholiota nameko* mushroom harvested was significantly ($P<0.05$) greater in eucalyptus shaving than Cordia shaving (Table 4). But there is no significant difference between eucalyptus shaving and cotton seed as well as cotton seed and cordia shaving (Table. 3&4).

In this study fungal contaminants were observed in different stage of *Pholiota nameko* cultivation. Particularly the molds, Trichoderma, Penicillum, and Aspergillus species are the common competent micro organisms. Twenty five percent of the culture plate was contaminated by *Aspergillus niger,* 31.25% and 25% of the spawn bottle were contaminated by *Aspergillus flavus* and *Aspergillus niger* respectively. 11.2 % of substrate bags contamination was caused by the Trichoderma spp (Fig. 16). This is because the white rot fungi are selective lignin degrader and leave the cellulose and hemicelluloses less affected (Eriksson *et al.,* 1990). This partial break down of cellulose and hemi cellulose making them available to competitors whom often grow faster. In addition to that the laboratory setting for many individuals who are working together on different fungal stain could be the source of contamination in this study.

Lastly among many factors for economic production of mushroom, one is the availability and cost of substrates (Obodai *et al*., 2003), as well as the cost of grain for spawn's production. Sorghum is a better available and cheaper than other substrates like maize in some areas in Africa including Ethiopia for spawn production (Dawit 1998). Substrate like eucalyptus shaving, cotton seed and cordia shaving also abundant in Ethiopia. It has been argued that in Ethiopia the introduction of eucalyptus species was a great success. Many species of Eucalyptus grow quickly and produce large quantities of wood (Henery, 1973). And is undeniable fact that there is a high biomass of eucalyptus plantations in Ethiopia. That is why eucalyptus has played and will play a tremendous role in alleviating the fuel and construction material problems of the community in Ethiopia (Teshome, 2007). In addition to that the eucalyptus shaving beside with cotton seed and cordia shaving could be an alternative energy source for production of *Pholiota nameko* and will solve nutrition problem of the society.

7. Conclusion

- This study successfully demonstrated that eucalyptus shaving (*Eucalyptus globules*), cordia shaving (*Cordia africana*), and cotton seed (*Gossypium. spp*) were good substrates used for the cultivation of *Pholiota nameko.*

- The pinus shaving, teff straw, coffee husk did not show any growth of fruiting body, how ever had mycelia invasion during spawn running.

- Addition of wheat bran in the substrate improved cropping time and quality of fruiting body of *Pholiota nameko.*

- Malt extract agar is an important nutrient medium for tissue culturing of *Pholiota nameko.*
- Sorghum grain is best for spawn production of *Pholiota nameko*.

- The highest yield of *Pholiota nameko* was obtained from the eucalyptuses shaving where readily available substrates from saw mills, and a high biomass exist in the country. In Ethiopia currently estimated that about 24 million cubic meters of eucalyptus wood is produced annually, of which 60 % is used for industrial and building purpose. Shaving from this wood could be an alternative energy source for the production of *Pholiota nameko* mushroom.

- In Ethiopia ligno cellulose waste residues are mostly used in the domestic sector for cooking and baking, using very low efficiency devices. But it is possible to convert for highly nutritious and medicinal aspects of mushroom through cultivation of *Pholiota nameko.*

- Most of the time agricultural residues may be left on the ground or burned in the field to recycle soil nutrients; some parts are used as animal feed, as building materials but also possible for production of *Pholiota nameko* and compensate protein gap of this country as well as in keeping of the environmental clean.

- Pests and mould contaminants were great problem during the cultivation of *Pholiota nameko.*

- In conclusion, I believe this work has led to improve small scale production potential of *Pholiota nameko*. I expect that my work will be adopted by small scale growers in Ethiopia

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8. Recommendation

- Further research must be done using a combination of two or more type of substrates together to optimize *Pholiota nameko* production.

- Small scale mushroom production is a profitable business. That can solve unemployed youth and women problems in terms of economy, time and nutrition. There fore the ministry of agriculture & government should give great emphasis in this sector.

- It is highly recommended for the city and other towns of Ethiopia to support and encourage mushroom cultivation to avoid plant waste materials and other lignocelluloses waste around the city and towns from saw mill.

- The bulk of the lignocelluloses biomass is, to a large extent, considered insignificant or of no commercial value and certainly of no food value, at least in its original form. It should be noted that large amounts of research funds have been set aside to search for increased productivity of the core product, treated as raw materials for the production *Pholiota nameko.*

- Well trained mushroom scientist and mushroom growers must give training activities (workshops and courses) and that need to be supported by governments and agriculture sector.

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