

Cultivation of *Pleurotus ostreatus* Mushroom on *Ficus vasta* Leaves (Solid Waste of Plant) at Dilla University, Ethiopia

Fekadu Alemu*¹ and Melaku Fisseha²

¹Department of Biology, College of Natural and Computational Sciences,
Dilla University, P.O. Box. 419, Dilla, Ethiopia

²Department of Horticulture, College of Agriculture, Dilla University,
P.O. Box. 419, Dilla, Ethiopia

ARTICLE INFO

Received 01 Jan. 2015

Received in revised form 26 Jan. 2015

Accepted 31 Jan. 2015

Keywords:

Ficus vasta,
Mushroom cultivation,
Oyster mushroom,
Pleurotus ostreatus,
Spawn,
Sorghum.

Corresponding author: Department of
Biology, College of Natural and
Computational Sciences, Dilla
University, P.O. Box. 419, Dilla,
Ethiopia.
E-mail address: fekealex@gmail.com

ABSTRACT

Background: Mushroom cultivation is a relatively new applied technology and the mushroom industry is still small compared to many plant crops. In addition, the investment on mushroom production in Ethiopia is low. Solid waste materials are useful in the cultivation of mushrooms used as substrate. Mushroom cultivation is an excellent method of increasing the profitability of an agricultural disposal waste.

Objective of this study: The present study was designed to use *Ficus vasta* leaves of agricultural waste disposal as substrate for cultivation of the oyster mushroom (*Pleurotus ostreatus*) in order to effective utilization of solid waste residue through bioconversion into food.

Method of this study: *Ficus vasta* leaves used as substrate for composting was collected from Dilla University at Main Campus from 2013 October-2014 June. In order to prepare, aerobic composted substrate, about 80% of *Ficus vasta* leaves were thrashed manually into small pieces through using mortar and pestle. After chopping, mixing the chopped *Ficus* leaves with Wood ash, Wheat bran, Cow dung and Chicken manure, then the water was added until moisture content was between 40-60%. After two weeks of composting, these substrates were distributed equally into plastic bags of 40x60 cm size at the rate of 3.5kg substrate in triplicates and sterilized for three hours in barrel by fire (figure 4). After cooling; they were inoculated with the spawn (one glass bottle per bag) and mixed thoroughly to facilitate rapid and uniform mycelia growth.

Results of this study: As the result indicated, *Ficus* leaves were one of the substrate which supports the growth of edible mushroom (oyster mushroom). The fruiting body on the substrates that contains wheat bran and manure, the number and size of fruit body was high and larger when it was compare with alone substrate (*Ficus vasta* leaves only).

Conclusion: Therefore, Oyster mushroom can be converted these solid waste into vitamin and some essential mineral rich food beside of removal solid wastes from the environments.

INTRODUCTION

Mushroom cultivation is an appropriate technology for the management of agro-industrial residues¹ through bioconversion processes. 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 cultivated².

Mushrooms are eaten by people for their favour, texture as well as for the health benefits that they accord. Mushrooms are healthy foods, poor in calories and in fat, rich in proteins, chitin, vitamins (niacin, riboflavin, vitamin D, C, B1, B5, B6 and K and sometimes vitamins A and C)³, folic acid and pro-vitamin D ergosterol^{4,5} and minerals (potassium, phosphorus, calcium, sodium)^{2,6} and some essential amino acids, fiber and have low cholesterol and fat levels⁷. Mushroom normally ranges between 20 and 40% protein which is better than many legume sources like soybeans and peanuts, and protein-yielding vegetable foods^{8,9}.

Moreover, mushroom proteins contain all the essential amino acids needed in the human diet and are especially rich in lysine and leucine which are lacking in most staple cereal foods^{8,10}. Increasing consumption of mushroom is good for preventing malnutrition, although mushrooms cannot be an alternative protein source for of meat, fish, and egg¹¹. Edible mushrooms are recommended by the FAO as food, contributing to the protein nutrition of developing countries dependent largely on cereals. The acceptance of cultivated mushrooms such as shitake mushroom (*Lentinus edodes*), oyster mushroom (*Pleurotus ostreatus*) and button mushroom (*Agaricus bisporus*) are well-established worldwide as a delicacy. Due to their unique and subtle flavour, these mushrooms have been used as food and food flavouring material in soups for centuries¹².

The common name “**oyster mushroom**” comes from the white shell-like appearance of the fruiting body. Oyster mushrooms are a diverse group of saprotrophic fungi belonging to

the genus *Pleurotus*¹³. According to Croan¹⁴ these mushrooms are a good source of non-starchy carbohydrates, with high content of dietary fiber and moderate quantity of proteins, including most amino acids, minerals, and vitamins. The protein content varies from 1.6 to 2.5%, and the niacin content is about ten times higher than that of any other vegetable. Moreover, Randive¹⁵ reported that oyster mushrooms are rich in Vitamin C, B complex, and mineral salts required by the human body. *Pleurotus ostreatus* is a mushroom of pleasant flavour and possesses several proteins, minerals (Ca, P, Fe, Mg), and low carbohydrate quantities and fat, constituting excellent dietary food¹⁶. Oyster mushroom powder rich in protein and low in fat contents can be incorporated into various recipes for improving the nutritional status of vulnerable population in developing countries¹⁷. Beta-glucan has been shown to decrease blood LDL-cholesterol concentration in animal models and clinical intervention studies¹⁸.

Oyster mushrooms grow over a wide range of temperature of 15-30°C and hence are ideally suitable for cultivation under both temperate and tropical climatic conditions. Oyster mushroom can be grown on various substrates including paddy straw, maize stalks/cobs, vegetable plant residues, bagasse etc.¹⁹. The oyster mushrooms can be cultivated successfully under semi controlled conditions in a small space by using agricultural as well as industrial waste and other refuse as substrate. 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.*²⁰. The most well known species of *Pleurotus* are *P. ostreatus*, *P. florida*, *P. eryngii*, *P. cystidiosis*, *P. flabellatus*, *P. cornucopie*, and *P. sajor-caju*. *P. sajor-caju* is recognized as an excellent mushroom.

Mushrooms have become attractive as a functional food and as a source for the development of drugs and nutraceuticals²¹ responsible with their antioxidant, antitumor²² and antimicrobial properties. A nutraceuticals can be defined as a substance that may be considered a food or part of a food that provides

medical or health benefits like the prevention and treatment of disease. There are many more species of cultivated and wild edible and non-edible mushrooms that have been analysed for both their nutritional and nutraceuticals components²¹.

The active constituents found in mushrooms are polysaccharides, dietary fibres, oligosaccharides, triterpenoids, peptides and proteins, alcohols and phenols, and mineral elements²³ such as zinc, copper, iodine, selenium and iron, vitamins, amino acids etc. These have been found to boost the immune system, have anti-cancerous properties, act as anti-hypercholesterolemia and hepato-protective agents, show anti-HIV activity and anti-viral activity, and ameliorate the toxic effect of chemo- and radiotherapy. Many of the species are known to be aphrodisiacs. Oxidation is essential for all living organisms for the production of energy to fuel biological processes.

Mushroom is an excellent source of folic acid, the blood building vitamin that prevents anaemia^{24,25}. Mushroom protein is comparable to muscle protein in terms of nutritive value²⁴. Various bioactive compounds isolated from *P. ostreatus* culture extracts of Ethiopian higher fungi showed other biological properties such as antiprotozoal, anthelmintic, phytotoxic and brine shrimp lethality activities²⁶. Also Inchausti²⁷ investigated Leishmanicidal and Trypanocidal activity of the extracts and secondary metabolites of some Basidiomycetes.

Ficus vasta is a very large tree from Moraceae family growing over 25 m tall. *F. vasta* is a tree of dry north and eastern Africa, Sudan, Ethiopia, Saudi Arabia and Tanzania²⁸. In traditional medicine, *F. vasta* used in rheumatism, pains, intestinal worms²⁹. It was already studied for the extract from the fruits of *F. vasta* antibacterial³⁰, mainly against Gram-positive bacteria, and antihelmintic³¹ activities.

Composting is a fertilizing mixture of partially decomposed organic matter from plant and animal origin³². Composting is a solid-waste fermentation process, which exploits the phenomenon of microbial degradation and mineralization³³. The main purpose of composting to a mushroom grower is to

prepare a substrate in which the growth of mushroom is promoted to the practical exclusion of other microorganisms. Fermor³⁴ reported that a composted substrate improved mushroom fruit body yield but, reduced infestation by insects, fungi and bacteria pathogens. Microorganisms colonizing mushroom compost during composting process are regarded as active agents, which determine the chemical composition and mineralization thereby making it possible for mushroom growth³⁴.

Unlike in developed countries where mushrooms food consumption is increasing^{4, 35,36} in Ethiopia, mushroom eating habit is very poor³⁷. Information on nutritive value and sensory properties of edible oyster mushroom foods cultivated on agricultural residues in Ethiopia is limited. Such information is important to facilitate the popularization of mushroom cultivation, processing, marketing and consumptions.

Pleurotus species are good candidates for cultivation in Ethiopia because of the following reasons is some are efficient degraders of lingo-cellulosic materials and the raw materials are abundantly available. *Ficus vasta* leaves larger removed as solid waste in Dilla University and collected into garbage in order to maintain the environment from pollutants. Cultivation of *Pleurotus ostreatus* mushroom on this substrate has a good acceptance from the consumer and also a good opportunity for small producers to embark in an enterprise. It is necessary to keep on promoting the benefits of this product, one of the main problems to start this project was the resistance to change the traditional ways of production so a new culture of sustainable agriculture needs to be developed. Therefore, the present study was designed to use *Ficus vasta* of agricultural waste disposal as substrate for cultivation of the oyster mushroom (*Pleurotus ostreatus*) in order to effective utilization of this residue and reduction of environmental pollution.

MATERIALS AND METHODS

Pure culture collection and maintain

Pleurotus ostreatus was obtained from Mycology Laboratory, Department of Biology

from Addis Ababa University. The pure culture of *Pleurotus ostreatus* was inoculated onto Malt extract agar. The pure culture was maintained on Malt extract agar slants at -4°C for one month, then sub-culturing subsequently after one month transferred (inoculated) onto fresh slant of Malt extract agar.

Substrate collection

Ficus vasta leaves used as substrate for composting was collected in Dilla University from Main Campus from 2013 October -2014 April as indicated in figure 1. Other nutrient supplement such as wheat bran and pH adjustment of Wood ash was obtained from the Dilla town. Beside this Cow dung and Chicken manure was obtained from Allege Research centre. (See figure 1.)

Compost preparation

The compost was prepared by outdoor single-phase solid-waste fermentation³⁸. In order to prepare, aerobic composted substrate, about 80% of *Ficus vasta* leaves were thrashed manually into small pieces through using mortar and pestle (figure 2). After chopping, mixing the chopped *Ficus* leaves with Wood ash, Wheat bran, Cow dung and Chicken manure, then the water was added until moisture content was between 40-60%. This is usually being determined by the 'rule of thumb' method³⁹. Then supplement with 20% of three different supplements on 80 % of *Ficus* leaves as follows:-

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

Substrate B. 10% Cow dung, 8% Wheat bran 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% Chicken manure and 2% wood ash on dry weight basis with some Modification of³⁷. The substrates were then added into hole of about 1.5 m wide, 1.5 m high and 1.5 m long which was under shadow area at Dilla University. This was covered with banana leaves and left for 2 weeks with turning and restacking every 3-4 days to produce homogenous compost. (See figure 2.)

Spawn production

Spawn is the vigorous mycelia growth of a single fungus on a chosen substrate material (liquid media, grains, saw dust substrate, wooden sticks⁴⁰). Sorghum was used for mother spawn. About 20 kg of sorghum was washed and dead floating removed then soaked overnight in 15L water and rinsed three times in distilled water. The excess water was drained off and 20% wheat bran, 12% gypsum ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$), and 3% limes (CaCO_3) were added as shown in figure 3. 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 bottles and autoclaved for 121°C to 1 hour. After cooling, each bottle was inoculated with 7 days old culture which grown on Malt extract agar and incubated for 25 days at 25°C until the substrate fully colonized; at ten days interval mycelia invasion and contamination were recorded. (See figure 3.)

Sterilization of substrates and cultivation of mushrooms

After two weeks of composting, these substrates were distributed equally into plastic bags of 40x60 cm size at the rate of 3.5kg substrate in triplicates and sterilized for three hours in barrel by fire (figure 4). 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°C and mycelia development in the bag was observed and noted within 5 days. (See figure 4.)

Cultivation conditions

The bags were subsequently placed, long side down, into a spawn running room at $20 - 25^{\circ}\text{C}$ in the dark and 65 - 70% relative humidity until completion of spawn running. After completion of spawn running the temperature and relative humidity was changed to $19 - 20^{\circ}\text{C}$ and 80 - 90% RH, respectively. The bags were slit and the cut portions folded back. Water was sprayed for maintaining moisture up to the desired level in the form of fine mist from a nozzle.

Watering

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

Harvesting of mushroom

The first primordia appear 2-4 days after scratching depending upon types of substrate, which were recorded. The harvesting date also varied depending upon types of substrate. Matured mushroom identified by curl margin of the cap was harvested by twisting to uproot from the base. Mushroom matured generally 48 hours after appearing the primordia. Data were recorded periodically during culture.

Statistical analysis

The data of actively mycelium growth during spawn making and formation of full morphology of *Pleurotus ostreatus* mushroom and fruiting body were observed during cultivation on substrate. The data were expressed qualitatively in the form of figure.

RESULT AND DISCUSSION

Pleurotus ostreatus) was cultured on malt extract agar for 7 days at 28°C and mycelium covered the plate. It was fully grow on plates as shown in figure 5. 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 mushrooms⁴¹. Mushrooms cultivation also enables farmers to utilize organic substrates that would otherwise be regarded as waste products^{42,43}. Healthy nutrition and diet are gaining importance, not only in the everyday 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 nutrition⁴⁴. (See figure 5.)

Spawn production

Sorghum is important crop for spawn production of oyster mushroom. Sorghum based spawn took 25 days to colonize the substrate completely (figure 6). The moisture content of the sterile moist sorghum (55-60%) was found to be

suitable for growth of mycelium of oyster mushroom. (See figure 6.)

Substrate sterilization and spawn inoculation

The substrate was sterilized by in boiling water for three hours. Mycelium running rate on the substrates was observed after 7 days inoculation of spawn (figure 7). Therefore, mycelium running was required high humidity, temperature and cultivation room should be dark. (See figure 7.)

Primordial formation of *Pleurotus ostreatus* mushroom

The first primordia appear after 20 days after scratching depending upon types of substrate. The primordial formation and number of primordia per plastic bag (substrate) was affected by humidity, aeration and the substrate itself. Beside of this the supplement such as wheat bran and manure also factor for either high or low number of primordial formation (figure 8). An ever increasing human population and diminishing farm sizes have resulted in declining soil fertility associated land productivity and increasing poverty levels⁴⁵. However, mushrooms products are solved both problem through cultivation in small size area finally by consumed. (See figure 8.)

Fruiting body production

The effect of supplemented ingredients on *Ficus vasta* leaves were investigated and found to influence the number of fruit body and size of fruit body. Fruiting body is the edible part of mushroom *Pleurotus ostreatus*. The fruiting body on the substrates that contains wheat bran and manure, the number and size of fruit body was high and larger when it was compare with alone substrate (*Ficus vasta* leaves only). The mature fruit body became curl margin of the cap of *Pleurotus ostreatus* as indicated in figure 9. 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 states⁴⁶. 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 acids⁴⁷. (See figure 9.)

CONCLUSION

Mushroom cultivation required knowledge as well as skill for growing fungi on plant solid waste materials that are not necessary consumed by humans. But left as solid waste through collecting them and removed into garbage. However, Oyster mushrooms can be converted this solid waste in to vitamin and some essential mineral rich food and low with fat. Commercial production of oyster mushrooms is largely determined by the availability and utilization of cheap solid waste products, which are agricultural solid waste that are the most promising substrates for cultivation.

ACKNOWLEDGEMENTS

The authors greatly acknowledge the Departments of Biology, College of Natural, and Computational Sciences of Dilla University for the kind assistance in providing the laboratory facilities and all the required consumables and equipment during the whole period of this research work.

REFERENCES

1. Chang ST, Miles PG. Mushrooms: Cultivation, Nutritional Value, Medicinal Effect and Environmental Impact, 2nd edn., Crc Press, Boca Raton 2004; pp: 451, ISBN: 0849310431.
2. Manzi P, Gambelli L, Marconi S, Vivanti V, Pizzoferrato L. Nutrients in edible mushrooms: An inter-species comparative study, *Food Chem.* 1999; 65(4): 477-482.
3. Syed AA, Kadam JA, Mane VP, Patil SS, Baig MMV. Biological efficiency and nutritional contents of *Pleurotus florida* (Mont.) Singer cultivated on different Agro-wastes, *Natural Science* 2009; 7(1): 44- 48.
4. Kurtzman RHJr. A review mushrooms: sources for modern western medicine, *Micologia Aplicada International* 2005; 17: 21–33.
5. Moharram HA, Salama MF, Hussien AA. Characterization of oyster mushroom mycelia as a food supplement, *Australian Journal of Basic and Applied Sciences* 2008; 2: 632–642.
6. Stamets P. Notes on nutritional properties of culinary-medicinal mushroom, *J. Inter. Med. Mushrooms* 2005; 7: 103-110.
7. Rafique AN. Studies on the Cultivation of Mushroom *Pleurotus* species in Gujarat. Ph. D. Thesis, Department of Microbiology, M.G. Science Institute, Navrangpura, Ahmedabad, India 1996.
8. Chang ST, Buswell JA. Mushroom nutraceuticals, *World J. Microb. Biotechnol.* 1996; 12: 473-476.
9. Chang ST, Mshigeni KE. Mushroom and their human health: their growing significance as potent dietary supplements, The University of Namibia, Windhoek 2001; pp. 1-79.
10. Sadler M. Nutritional properties of edible fungi, *Br. Nutr. Found. Nutr. Bull.* 2003; 28: 305-308.
11. Çağlarırmak N, Ünal K, Ötles S. Nutritive value of edible wild mushrooms grown in Black Sea region of Turkey, *Myco Appl. Int.* 2002; 14 (1): 1-5.
12. Shin CK, Yee CF, Shya LJ, Atong M. Nutritional Properties of Some Edible Wild Mushrooms in Sabah, *J. Applied Sci.* 2007; 7: 2216-2221.
13. Kong W-S. Descriptions of commercially important *Pleurotus* species. In: Mushroom world (Ed.). Oyster mushroom cultivation. Part II. Oyster mushrooms. Seoul: Heineart Incorporation, 2004; p.54-61. (Mushroom growers' handbook, 1).
14. Croan SC. Conversion of conifer wastes into edible and medicinal mushrooms, *Forest Products Journal* 2004; 54:68-76.
15. Randive SD. Cultivation and study of growth of oyster mushroom on different agricultural waste substrate and its nutrient analysis, *Advances in Applied Science Research* 2012; 3 (4):1938-1949.
16. Silva SO, Costa SMG, Clemente E. Chemical Composition of *Pleurotus pulmonarius* Substrates and Residue after Cultivation, *Brazilian Archives Biology Technol.* 2002; 45: 531-535.
17. Dunkwal V, Jood S, Singh S. Physico-chemical properties and sensory evaluation of *Pleurotus sajor caju* powder as influenced by pre-treatments and drying methods, *British Food Journal* 2007; 109: 749-759.
18. Queenan KM, Stewart ML, Smith KN, Thomas W, Fulcher G, Slavin JL. Concentrated oat β -glucan, a fermentable fiber, lowers serum cholesterol in hypercholesterolemic adults in a

- randomized controlled trial, *Nutrition Journal* 2007; 6: 6-9.
19. Hassan S, Mohammad AY, Kiramat K. Cultivation of the oyster mushroom (*Pleurotus ostreatus* (Jacq.) P. Kumm.) in two different agroecological zones of Pakistan, *African Journal of Biotechnology* 2011; 10: 183-188.
20. Diez VA, Alvarez A. Compositional and nutritional studies on two wild edible mushrooms from northwest Spain, *Food Chem.* 2001; 75:417-422.
21. Lakhanpal TN, Rana M. Medicinal and nutraceutical genetic resources of mushrooms, *Plant Genetic Resources: Characterization and Utilization* 2005; 3: 288-303.
22. Jonse N, Janardhanan KK. Antioxidant and antitumor activity of *Pleurotus florida*, *Curr. Sci.* 2000; 79: 941-943.
23. Pardeshi BM, Pardeshi PM. The edible medicinal mushrooms as supportive natural nutrients: Study of nonvolatile mineral contents of some edible medicinal mushrooms from India; eastern remedies for modern western maladies, Proc. 5th. Int. Medicinal Mushroom Conference, Mycological Society of China, Nantong, China. 2009; pp. 514-18.
24. Kannaiyan S, Ramaswamy K. A hand book of edible mushrooms. Today and Tomorrows pub., New Delhi, 1980; pp. 39-43.
25. Bisaria R, Madan M, Bisaria VS Mineral content of the mushroom *P. sajor-caju* cultivated on different agroresidues, *Mush. J. Tropics.* 1987; 7: 53-60.
26. Ermias D, Dawit A. Bioactive compounds from plants and higher fungi of Ethiopia. In: Phytochemistry of plants used in traditional medicine. Hostettmann, K., Marston, A., Millard, M. and Hamburger, M. eds. Oxford University Press, 1995; pp. 295-312.
27. Inchausti A, Yaluff G, rojas de Arias A, Torres S, Ferreira ME, Nakayama H, Schinini A, Lorenzen K, Anke T, Fournet A. Leishmanicidal and trypanocidal activity of extracts and secondary metabolites from basidiomycetes, *Phytotherapy Research* 1997; 11: 193-197.
28. Abate D, Debella A, Urga K. Medicinal Plants and Other Useful Plants of Ethiopia. Ethiopian Health and Nutrition Research Institute, 2003; pp. 269-281.
29. Hundera K. Traditional Forest Management Practices in Jimma Zone, South West Ethiopia, *Ethiopia Journal of Education Sciences* 2007; 2(2): 1-5.
30. Al-Fatimi M, Wurster M, Schröder G, Lindequist U. Antioxidant, antimicrobial and cytotoxic activities of selected medicinal plants from Yemen, *Journal of Ethnopharmacology* 2007; 111: 657-666.
31. Raju NJ, Yesuf EA, Bekele M, Wabe NT. Investigation of In Vitro Anthelmintic Activity of *Ficus vasta* leaves, *Asian Journal of Pharmaceutical Biology* 2011; 1(4): 454-458.
32. Piet JL, Derik X, Huub JM, Drift CV, Leo JLD, Vogel D. Composting Processes, *J. Appl. Environ. Microbiol.* 1990; 3029-3034.
33. McKinley VL, Vestal JR. Biokinetic Analysis and Succession of Microbial Activity in Decomposition of Municipal Sewage Sludge, *Appl. Environ. Microbiol.* 1984; 47: 933-941.
34. Fermor TR, Randle PE, Smith JF. Compost as substrate and Its Preparation. In: Bio and Technol of Cultivated Mushrooms, Flegg PB, Spencer DM, Klood DA, Ed. 1985; 81-109.
35. Gregori A, Vagelj MS, Pohleven J. Cultivation techniques and medicinal properties of *Pleurotus* spp, *Food Technology and Biotechnology* 2007; 45: 238-249.
36. Neyrinck AM, Bindels LB, De Backer F, Pachikian BD. Dietary supplementation with chitosan derived from mushrooms changes adipocytokine profile in diet-induced obese mice, a phenomenon linked to its lipid lowering action, *International Immunopharmacology* 2009; 9: 767-773.
37. Dawit A. Mushroom Cultivation; A Practical Approach. Addis Ababa: Berhanena Selam Printing Enterprise 1998.
38. Nair NG, Price G. A Composting process to minimize odour pollution, *Mushroom Science* 1991; 13: 205-206.
39. Buswell JA. Potentials of Spent Mushroom Substrates for Bioremediation Purposes, *Compost* 1984; 2: 31-35.
40. Jiskani MM, Wagan KH, Pathan MA, Jamro GH. Spawn growth of oyster mushroom as affected by different temperatures and grain media, *J. Farm Scie.* 2000; 6 (10): 33-35.
41. Singh S. Mushrooming enterprise protein revolution through mushroom cultivation, *J. Agri. Rular Development* 2008; 1: 15-17.
42. Wood DA. Useful biodegradation of lingo-cellulose. In plant products and the new technology (K W Fuller and J R Gallan eds) Calderndon press, oxford 1985.
43. Labuschagne PM, Eicher A, Aveling TAS, Meillon S, Smith MF. Influence of wheat cultivars on straw quality and *Pleurotus ostreatus* cultivation, *Bio resource Technology* 2000; 71: 71-75.
44. Stamets P. Notes on nutritional properties of culinary-medicinal mushrooms, *J. Inter. Med. Mushrooms* 2005; 7: 103-110.

45. Sanchez C. Cultivation of *Pleurotus ostreatus* and other edible mushrooms, Review *Appl Micro. Biotechnol.* 2009; 9: 343-347.
46. Wasser SP. Medicinal mushrooms as a source of antitumor and immune-modulating polysaccharides, *J. Appl Microbiol. Biotechnol.* 2002; 60:258-274.
47. Fortes RC, Taveira VC, Rita M, Novaes CG. The immunomodulator role of β -D-glucans as co-adjuvant for cancer therapy, *J. Biotech.* 2006; 21(2): 163-168.



Figure 1. During collection of *Ficus vasta* leaves at Dilla University



(A)



(B)

Figure 2. A, thrashed the sample in to pieces; B, compost preparation area



(A)



(B)



(C)



(D)

Figure 3. A, weighing of sorghum; B, after overnight soaked in to tap water, washed and drainage of water ; C, addition of the supplement (wheat bran and pH adjustment) on the sorghum; D, addition of formulated sorghum into glass bottle for sterilization



Figure 4. Substrate sterilization for three hours by fire



(A)



(B)

Figure 5. Oyster mushroom mycelium grown on malt extract agar: A, Front observation of mycelium growth on plate; B, back observation of mycelium growth on plate



(A)



(B)

Figure 6. Spawn preparation on sorghum: A, inoculation of old culture (7 days) oyster mushroom on the sorghum; B, fully colonized sorghum by oyster mushroom mycelium after 25 days



(A)



(B)



(C)

Figure 7. Sterilization and inoculation spawn: A, sterilization of substrate; B, inoculation of oyster mushroom spawn on *sterilized Ficus vasta* leaves substrate; C, mycelium colonization of the *Ficus vasta* leaves



Figure 8. Primordial formation on the *Ficus vasta* leaves contain supplement ingredients



Figure 9. Fruit body grown on the *Ficus vasta* leaves and the mature fruit body