

Full Length Research Paper

Cultivation of *Pleurotus ostreatus* on *Grevillea robusta* leaves at Dilla University, Ethiopia

Fekadu Alemu

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

Received July 23, 2014; Accepted September 3, 2014

Mushrooms consumption has generated interest in man from early civilization. Mushrooms have a unique texture and flavour that are not found in other food crops. In addition, mushrooms cultivating is a promising new industry, with many new businesses developing every year. Cultivation of saprophytic edible mushrooms may be the currently economical biotechnology for lingo-cellulose organic waste recycling that combines the production of protein rich food with the reduction of environmental pollution. Therefore, the present study was undertaken to assess the growth of *Pleurotus ostreatus* on the *Grevillea* leaves of solid waste disposal in order to reduce environmental pollution by bioconversion of this waste into health food. *Grevillea* leaves were good substrate for oyster mushroom cultivation. The fruit bodies produced on this substrate were large in size and many in number. Therefore, cultivation of oyster mushrooms on this substrate can contribute to solving the food supply scarcity and quality problem beside removing solid waste pollutant from the environment.

Key words: Fruit body, *Grevillea robusta*, mushroom cultivation, *Pleurotus ostreatus*, spawn.

INTRODUCTION

The word mushroom is used in all part of world to describe the fruiting bodies of saprophytic, mycorrhizal and parasites fungi, belonging to the order of Basidiomycetes or Ascomycetes. Basidiomycetes or Ascomycetes can be found in soils rich in organic matter and humus, moist wood, animal waste after heavy rain or a sudden change of temperature and soon after a few hours or days they disappear, leaving no sign, but vegetative mycelium (Zied et al., 2011). Oyster mushrooms (*Pleurotus ostreatus*) are the easiest and least expensive commercial mushrooms to grow because they are well known for conversion of crop residues to

food protein (Banik and Nandi, 2004). Oyster mushroom is an edible mushroom having excellent fragrant and taste and its cultivation on crop residues is considered as potential source of income, an alternative food production, provision of employment, and for recycling of agricultural wastes.

Mushrooming or mushroom cultivation refers to the intentional and directed production of mushrooms, substituting wild collection in the fields and forests with a harvest in defined conditions of growing, resulting in strict quality control, food safety without risk of consumption of poisonous or toxic species, and with guarantees on the

*Corresponding author. E-mail: fekealex@gmail.com. Tel: +251920839215.

benefits generated by these fungi (Zied et al., 2011). The cultivation of edible mushrooms is actually an alternative biotech which is fast, environmentally friendly and feasible to recycle organic by-products from agribusiness into high nutritional and medicinal quality food both with respect to the amount of protein or minerals and selected substances with medicinal and pharmacological properties, for example the presence of β -glucans like lentinan, and thus it can contribute significantly to food for humans. The most cultivated mushrooms in the world are *Agaricus bisporus* (Champignon or button mushroom), *Lentinula edodes* (Shiitake) and *P. ostreatus* (oyster mushroom) because of its oyster like shape and other *Pleurotus* species. World-wide mushroom cultivation is dominated by the production of *A. bisporus* which is followed by *L. edodes* and *P. ostreatus* (Chang, 1999).

The mushroom production is a global and expanding industry, its world production is 6535542 ton in 2009 (FAO, 2011). Oyster mushroom is the second most popular mushrooms after button mushroom all over the world and its cultivation has increased rapidly during the last decade (Royse, 2002; Shelly et al., 2009; Adejoye et al., 2006). Oyster mushroom is rich in proteins, vitamin, and minerals and popularly called the vegetarian's meat because it has the same nutrients with meat. Mushroom proteins are considered to be intermediate between that of animals and vegetables (Kurtzman, 1976) as they contain all the nine essential amino acids required for human body (Hayes and Haddad, 1976). Cultivation of *Pleurotus* spp. as edible mushrooms is becoming important throughout the world because of their ability to grow at temperatures of 10-35°C (Zadrazil, 1978; Yildiz et al., 1998) and on various lignocellulosic materials such as rotten wood, wood residues and most of agricultural wastes (Stamet, 2000; Straatsma et al., 2000).

Grevillea robusta (Proteaceae), commonly known as "silky oak", is native to Australia (Ritchie et al., 1965; Cannon et al., 1973). So far, alkyl resorcinols, macro cyclic phenols and cinnamic acid derivatives have been reported to be constituents of this plant. *G. robusta* is popular among farmers due to its fast growth, ability to tolerate heavy pollarding and pruning of branches and because it mixes well with other crops (Muchiri et al., 2002). Furthermore, the species has a proteoid root system (cluster of roots that develop in soils deficient of phosphorus) and hence is believed to compete less for mineral with crops making it ideal for planting on small farms sizes (Akycampong et al., 1999). The species has important uses including construction material, fuel wood, shade, fodder, soil erosion control and soil fertility improvement (FAO, 2001). It has been every well adopted and forms a near monoculture in central Kenya highlands, particularly in Kirinyaga district where it was reported to be grown on about 96% of the farms (Tyndall, 1996). Currently, it is a major timber species in small-scale farms where it significantly contributes to household income (Holding et al., 2006).

Pleurotus species are characterized by a white spore print attached to decurrent gills, often with an eccentric (off center) stipe, or no stipe at all. They always grow on wood in nature, usually on dead standing trees or on fallen logs. *Pleurotus* species have been used by human cultures all over the world for their nutritional value, medicinal properties and other beneficial effects. Oyster mushrooms are a good source of dietary fibre and other valuable nutrients. They also contain a number of biologically active compounds with therapeutic activities. Oyster mushrooms modulate the immune system, inhibit tumour growth and inflammation, have hypoglycaemic and antithrombotic activities, lower blood lipid concentrations, prevent high blood pressure and atherosclerosis, and have antimicrobial and other activities (Gunde-Cimerman, 1999).

Composting is a solid-waste fermentation process, which exploits the phenomenon of microbial degradation and mineralization (Mckinley and Vestal, 1984). Unlike undeveloped countries where mushrooms food consumption is increasing (Kurtzman, 2005; Gregori et al., 2007; Neyrinck et al., 2009), in Ethiopia, mushroom eating habit is very poor (Dawit, 1998). 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. Mushroom cultivation is a useful method of environmental waste management and waste disposal.

Moreover, *P. ostreatus* are good fungi for cultivation in Ethiopia because of they are efficient degraders of lignocellulosic materials, easy to grow with simple technology, can complete a full cycle in three to four weeks and the raw materials are abundantly available. *Grevillea* leaves in Ethiopia particularly in Dilla town and surrounding areas are removed as solid waste. Cultivation of *P. ostreatus* mushroom on this substrate should have a good acceptance from the consumer and could be 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. It is very important to do more research to develop a system that can be adapted completely to by the rural producers and urban of Ethiopians. Large amounts of freely available *Grevillea* leaves from trees as solid waste offer a potential alternative substrate source for mushroom cultivation in the Ethiopia. As a result, it is possible to convert through cultivation this waste into highly nutritious mushrooms with medicinal properties. Therefore, the present study was undertaken to assess the growth of *P. ostreatus* on the *Grevillea* leaves of solid waste disposed in order to reduce environmental pollution and obtain a bioconversion of this waste into health food.

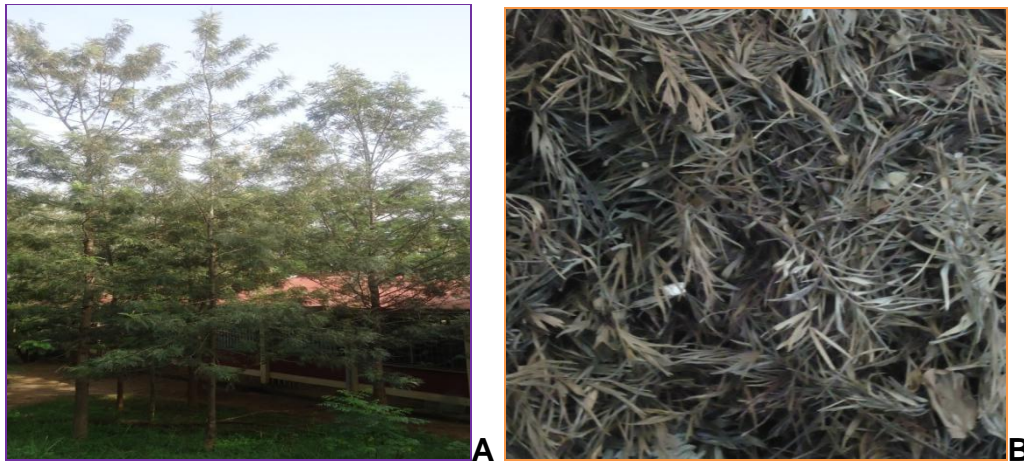


Figure 1. A, *Grevillea robusta* plant; B, its leaves after dry.



Figure 2. A, composting area and observation; B, filling compost substrate (*G. robusta* leaves) into plastic bags for sterilization.

MATERIALS AND METHODS

Pure culture collection and maintain

P. ostreatus was obtained from Mycology Laboratory, Department of Biology from Addis Ababa University, where it was brought from China. The pure culture of *P. 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-cultured subsequently after one month and transferred (inoculated) onto fresh slant of malt extract agar.

Substrate collection

G. robusta leaves used as substrate for composting were collected in Dilla University from Main Campus from in October -2014 April as shown in Figure 1. Other nutrient supplement such as wheat bran and wood ash was obtained from the Dilla Town. Beside this, cow dung and chicken manure were obtained from Allege Research

Centre.

Compost preparation

The compost was prepared by outdoor single-phase solid-waste fermentation (Nair and Price, 1991). In order to prepare aerobic composted substrate, about 80% of *G. robusta* leaves were chopped manually into small pieces by using hammer mill. After chopping, the chopped *Grevillea* leaves with wood ash, wheat bran, cow dung and chicken manure were mixed, then water was added until moisture content was between 40-60% (Figure 2). This is usually being determined by the 'rule of thumb' method (Buswell, 1984). Then supplemented with 20% of three different supplements on 80% of *Grevillea* leaves as follows: Substrate A, 80% of *Grevillea* leaves with 10% chicken manure, 8% wheat bran and 2% wood ash; Substrate B, 80% of *Grevillea* leaves with 10% cow dung, 8% wheat bran and 2% wood ash; Substrate C, 80% of *Grevillea* leaves with 18% wheat bran and 2% wood ash; Substrate D, 80% of *Grevillea* leaves with 18% cow dung and 2% wood ash;



Figure 3. A, Weighing of sorghum; B, adding of tap water for overnight soaking; C, supplement of wheat bran and pH adjustment; D, addition of formulated sorghum into glass bottle.

Substrate E, 80% of *Grevillea* leaves with 18% chicken manure and 2% wood ash on dry weight basis with some modification of Dawit (1998). 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.

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). Sorghum was used for mother spawn. About 20 kg of sorghum was washed and dead floating sorghum removed then soaked overnight in 15 L 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 h. After cooling, each bottle was inoculated with 7 day old cultures grown on malt extract agar and incubated for 25 days at 25°C until the substrate was fully colonized; at 10 days interval mycelia invasion and

contamination were recorded.

Sterilization of substrates and cultivation of mushrooms

After two weeks of composting, these substrates were distributed equally into plastic bags of 40 x 60 cm size at the rate of 3.5 kg substrate in triplicates and sterilized for three hours in barrel by fire. 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.

Cultivation conditions

The bags were subsequently placed, long side down, into a spawn running room at 20 - 23°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 to 20°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 *P. 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 h after appearance of the primordia. Data were recorded periodically during culture.

Biological efficiency

The biological efficiency (yield of mushroom per kg substrate on dry wt. basis) was calculated by the following the formula of Chang et al. (1981):

$$\text{B. E (\%)} = \frac{\text{Fresh weight of mushroom}}{\text{Dry weight of substrate}} \times 100$$

Moisture content

The moisture content of mushroom was also expressed in percent and calculated by the formula:

$$\text{Moisture content (\%)} = \frac{\text{Weight of fresh sample} - \text{weight of dry sample}}{\text{Weight of fresh sample}} \times 100$$

Data analysis

The data of actively mycelium growth during spawn making and formation of full morphology of oyster mushroom and fruiting body were observed during cultivation on substrate. Analysis was performed for all data with triplicates for each. The data were expressed qualitatively in the form of picture as well as quantitatively. The data groups were analyzed using Statistical Package for Social Sciences (SPSS) for windows 16.0.

RESULTS

P. ostreatus cultured on malt extract agar for seven days at 28°C and mycelium covered the medium. Full mycelium invasion of *P. ostreatus* on culture plate took seven days. It was fully grown on plates as shown in Figure 4. It was ready to be used for the inoculation into sorghum for spawn preparation.

Spawn production

Sorghum is important cereals for spawn production of

mushroom species (*P. ostreatus*). Sorghum based spawn took 25 days to colonize the substrate completely (Figure 5). The moisture content of the sterile moist sorghum (55-60%) was found to be suitable. It was ready to be used for the inoculation of the solid substrate.

Substrate sterilization and spawn inoculation

The substrate was sterilized by soaking into the boiled water for three hours in the barrel. Mycelium running rate on the substrates was observed after seven days inoculation of spawn (Figure 6). Therefore, mycelium running required high humidity and cultivation room should be dark.

Primordia formation of *P. ostreatus*

The first primordia appeared 20 days after scratching depending on types of substrate. The primordia formation and number of primordia per plastic bag (substrate) was affected by humidity and the substrate itself. The supplements such as wheat bran and manure also caused either high or low number of primordial formation as indicated in Figure 7.

Fruiting body production

The effect of supplemented ingredients on substrates (*G. robusta* 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 *P. ostreatus*. On the substrates that contains wheat bran and manure as supplements, the number and size of fruit bodies was higher and larger than that in the substrate alone (*G. robusta* leaves only) (Figure 8).

Biological efficiency

Considerable variation was found in yield of oyster mushroom using different supplements on *G. robusta* leaves. The maximum biological yield (555 g/3.5 kg) was found with supplements of 18% cow dung on substrate which gives 15.86% of biological efficiency. The maximum moisture content was found with supplement of 18% chicken manure (94.05%) and the lowest was found with supplements of 10% cow dung and 8% wheat bran (91.88%) (Table 1).

DISCUSSION

The harvesting date of mature fruit body varied depending upon types of substrate. Oyster mushroom (*P.*

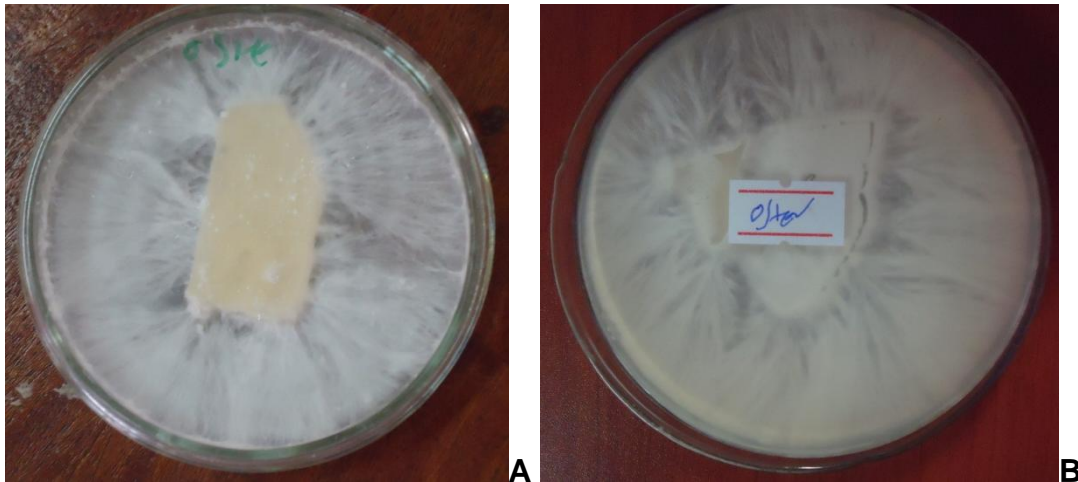


Figure 4. *P. ostreatus* mycelium grown on malt extract agar: A, Front observation of mycelium growth on plate; B, back observation of mycelium growth on plate.



Figure 5. Spawn preparation on sorghum: A, inoculation of old culture (seven days) *P. ostreatus* on the sorghum; B, fully colonized sorghum by *P.ostreatus* mycelium after 25 days.

ostreatus) is an edible mushroom having excellent fragrant and taste and its cultivation on crop residues is considered as potential source of income, an alternative food production, provision of employment, and for recycling of agricultural wastes (Oseni et al., 2012). The market for mushrooms has been reported to be on a continuous growth due to the interest in their culinary, nutritional, health benefits and their potential for use in waste management (Beetz and Kustidia, 2004).

Shah et al. (2004) reported that oyster mushrooms are one of the most delicious foods due to their high nutritional value, very good taste and medicinal value. 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).

Mushrooms matures generally 48 h after primordial appearance. Mushrooms not only can convert these huge lignocelluloses biomass wastes into human food, but can also produce notable immune enhanced products, which



Figure 6. Sterilization and inoculation spawn: A, sterilization of substrate; B, inoculation of *P. ostreatus* spawn on *G. robusta* leaves substrate; C, mycelium colonization of the *G. robusta* leaves.

have many health benefits (Chang et al., 1993). In Ethiopia, hunger and malnutrition are devastating problems, particularly for the poor and unprivileged society. The most important forms of malnutrition in Ethiopia are protein energy malnutrition (PEM), iodine; vitamin A deficiency disorders (Edris, 2004).

The mature fruit bodies became curl margin of the cap of *P. ostreatus* as shown in Figure 8. *Pholiota nameko* is one of the hygrophilous fungi and needs more moisture for fruiting as compared to other cultivated mushrooms, such as *L. edodes*, *F. velutipes* and *P. ostreatus* (Chang and Hayes, 1978). Aeration also plays an important role in fructification. 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 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 bodies, also cinnamon brown spores. Fructification requires 30 days (Marshall and Nair, 2009).

Highest biological efficiency was found with supplements of 18% cow dung (15.86%) and the lowest biological efficiency was found without the supplements (11.94%). The yield of mushrooms was affected by different supplements (Tikdari and Bolandnazar, 2012). On another study, Alam et al. (2007) observed that the biological efficiency ranged from 45.21 to 125.70% in the case of oyster mushroom. As reported by Islam et al. (2009), the maximum biological yield (150 g) was found in mango sawdust based substrate. Coconut sawdust based substrates gave the minimum yield (83 g). The fresh mushroom yield or biological efficiency of a species is directly related to strain, substrate nutrition and growth



Figure 7. Primordial formation on the *G. robusta* leaves which contain wheat bran and manure.

conditions (Upadhyay et al., 2002).

Conclusion

Mushroom cultivation needs knowledge as well as experience to grow fungi on plant solid waste materials that are not necessarily consumed by humans. Oyster mushrooms can convert these wastes into protein and vitamin rich food. Commercial production of oyster mushrooms is largely determined by the availability and utilization of cheap solid waste products, which are agricultural and industrial waste that are the most promising substrates for cultivation. Therefore, this study demonstrate the feasibility to cultivate *P. ostreatus* on *G. robusta* leaves supplemented with wheat bran manure as

a way to solve the food supply scarcity and quality problem and also remove solid pollutant from the environment.

Conflict of Interests

The author(s) have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors greatly acknowledge the Department 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



Figure 8. Fruit body grown on the *Grevillea robusta* leaves and the mature fruit body.

equipment during the whole period of this research.

REFERENCES

Adejoye OD, Adebayo-Tayo BC, Ogunjobi AA, Olaoye OA, Fadahunsi FI (2006). Effect of carbon, nitrogen and mineral sources on growth of *Pleurotus florida*, a Nigeria edible mushroom. *Afr. J. Biotechnol.* 5:1355-1359.

Akycampong E, Hitimana E, Torquebiaus EM, Unyemana PC (1999). Multistrata agroforestry with beans; bananas and *Grevillea robusta* in the highlands of Burundi. *Exp. Agric.* 35:357-369.

Alam N, Amin SMR, Sarker NC (2007). Efficacy of five different growth regulators on the yield and yield contributing attributes of *Pleurotus ostreatus* (Jacquin ex Fr.) Kummer. *Bang. J. Mush.* 1 (1): 51-55.

Banik S, Nandi R (2004). Effect of supplementation of rice straw with biogas residual slurry manure on the yield, protein and mineral contents of oyster mushroom. *Industrial Crops and Products* 20:311-319.

- Beetz A, Kustidia M (2004). Mushroom Cultivation and Marketing. ATTRA Publication IP 087. <http://attra.ncat.org/attra-pub/mushroom.html>.
- Buswell JA (1984). Potentials of spent mushroom substrates for bioremediation purposes. *Compost* 2:31-35.
- Cannon JR, Chow PW, Fuller MW, Hamilton BH, Metcalf, BW, Power AJ (1973). Phenolic constituents of *Grevillea robusta* (Proteaceae)-structure of robustol, a novel macrocyclic phenol. *Aust. J. Chem.* 26:2257-2275.
- Chang ST (1999). World production of cultivated and medicinal mushrooms in 1997 with emphasis on *Lentinus edodes* (Berk.) Sing. In China. *Int. J. Med. Mushrooms* 1:291-300.
- Chang ST, Buswell T, Chiu SW (1993). *Mushroom Biology and Mushroom Products*. The Chinese University Press, Hong Kong. pp: 45-57.
- Chang ST, Hayes WA (1978). *The Biology and Cultivation of Edible Mushrooms*. Harcourt brace Jovanovich publishers. pp:475-493.
- Chang ST, Lau OW, Cho KY (1981). The cultivation and nutritive value of *Pleurotus sojar caju*. *European J. Appl. Microbiol. Biotechnol.* 12:58-62.
- Dawit A (1998). *Mushroom cultivation; a practical approach*. Addis Ababa: Berhanena Selam Printing Enterprise.
- Edris M (2004). *Textbook of Food and Nutrition*. University of Gondar. pp: 42-43.
- FAO (2001). Protecting plantations from pests and diseases. Reports based on the works of W.M Ciesla. Forest plantation thematic papers working paper 10. Forest Resources Development Service, Forest Resources Division. FAO, Rome.
- FAO (2011). Available on: www.FAO.com.
- Gregori A, Vagelj MS, Pohleven J (2007). Cultivation techniques and medicinal properties of *Pleurotus* spp. *Food Technology and Biotechnology* 45: 238-249.
- Gunde-Cimmerman N (1999). Medicinal value of the genus *Pleurotus* (Fr.) P. Kaest. (Agaricales S.I., Basidiomycetes). *Int. J. Med. Mushrooms* 1: 69-80.
- Hayes WA, Haddad SP (1976). The nutritive value of mushrooms. *Mush. J.* 30:204.
- Holden C, Carsan S, Nijuguna P (2006). Small holder timber and firewood marketing in the coffee and cotton/ tobacco zones of eastern Mount Kenya in proceedings of IUFRO conference hosted by Galway-Mayo institute of technology, Galway Ireland.
- Islam MZ, Rahman, MH, Hafiz F (2009). Cultivation of Oyster Mushroom (*Pleurotus flabellatus*) on Different Substrates. *Int. J. Sustain. Crop Prod.* 4(1):45-48
- Jiskani MM, Wagan KH, Pathan MA, Jamro GH (2000). Spawn growth of oyster mushroom as affected by different temperatures and grain media. *J. Farm Scie.* 6 (10):33-35.
- Kurtzman RH (1976). Nitration of *Pleurotus sapidus* effects of lipid. *Mycology* 68:268-295.
- Kurtzman RH (Jr) (2005). A review mushrooms: sources for modern western medicine. *Micologia Aplicada International.* 17:21-33.
- Marshall E, Nair NG (2009). Make money by growing mushroom. Rural Infrastructure and Agro-Industries Division Food and Agriculture Organization of the United Nations. Diversification booklet number 7, p. 42.
- McKinley VL, Vestal JR (1984). Biokinetic Analysis and Succession of Microbial Activity in Decomposition of Municipal Sewage Sludge. *Appl. Environ. Microbiol.* 47:933-941.
- Muchiri S, Pukkala T, Miina J (2002). Modeling tress effect on maize in the *Grevillea robusta* + maize system in Central Kenya. *Agro Forestry System* 55:113-123.
- Nair NG, Price G (1991). A Composting process to minimize odour pollution. *Mushroom Sci.* 13:205-206.
- Neyrinck AM, Bindels LB, De Backer F, Pachikian BD (2009). Dietary supplementation with chitosan derived from mushrooms changes adipocytokine profile in diet-induced obese mice, a phenomenon linked to its lipid lowering action. *Int. Immunopharmacol.* 9:767-773.
- Oseni TO, Dube SS, Wahome PK, Masarirambi M T, Earnshaw DM (2012). Effect of wheat bran supplement on growth and yield of oyster mushroom (*Pleurotus ostreatus*) on fermented pine sawdust substrate. *Experimental Agriculture and Horticulture.* pp. 30-40.
- Ritchie E, Taylor WC, Vautin STK (1965). Chemical studies of the Proteaceae. I. *Grevillea robusta* A. Cunn, and *Orites excelsa* R. Br. *Aust. J. Chem.* 18:2015-2020.
- Royse DJ (2002). Influence of spawn rate and commercial delayed release of nutrient levels on *Pleurotus conocopiae* yield, size and time to production. *Appl. Microbiol. Biol.* 17:191-200.
- Shah ZA, Ashraf M, Ishtiaq CH (2004). Comparative study on cultivation and yield performance of Oyster mushroom (*Pleurotus ostreatus*) on different substrates wheat straw, leaves, saw dust. *Pakistan J. Nutr.* 3: 158-160.
- Shelly NJ, Ruhul Amin SM, Nuruddin MM, Ahmed KU, Khandakar J (2009). Comparative study on the yield and yield related attributes of some newly introduced strains of *Pleurotus cystidiosus* with *Pleurotus ostreatus* (PO2). *Bang. J. Mush.* 3(1):67-72.
- Stamets P (2000). *Growing gourmet and medicinal mushrooms 3rd ed.* Ten Speed Press, Berkeley. pp: 574.
- Straatsma G, Gerrits PG, Thissen TN, Amsing GM, Loeffen H, Vab Griensven JLD (2000). Adjustment of the composting process for mushroom cultivation based on initial substrate composition. *Bioresour. Technol.* 72(1):67-74.
- Tikdari MM, Bolandnazar S (2012). Application of organic nitrogen supplementations increases the yield of oyster mushroom (*Pleurotus florida*). *Research in Plant Biology* 2(3):10-15.
- Tyndall B (1996). The socio-economics of *Grevillea robusta* within the coffee land use system of Kenya. AFRENA report No 109 p. 71.
- Upadhyay RC, Verma RN, Singh SK, Yada MC (2002). Effect of organic nitrogen supplementation in *Pleurotus* species. *Mushroom Biology and Mushroom Products* 105(3):225-232.
- Wasser SP (2002). Medicinal mushrooms as a source of antitumor and immune-modulating polysaccharides. *J. Appl Microbiol. Biotechnol.* 60: 258-274.
- Yildiz A, Karakaplan M, Aydın F (1998). Studies on *P. ostreatus* (Jacq.ex Fr.) Kum. var. *salignus* (Pers. ex Fr.) Konr. et Maubl.: cultivation, proximate composition, organic and mineral composition of carpophores. *Food Chem.* 61:127-130.
- Zadrazil F (1978). *Cultivation of Pleurotus: In the biology and cultivation of edible mushrooms.*(Eds) Chang, S. T., and Hayes, W. A., Academic Press, New York. pp. 521-558.
- Zied DC, Savoie J-M, Pardo-Giménez A (2011). Soybean the Main Nitrogen Source in Cultivation Substrates of Edible and Medicinal Mushrooms, Soybean and Nutrition.