# Cultivation of Shiitake Mushroom on Corn Bran (Agricultural by-Product) at Dilla University, Ethiopia

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#### ABSTRACT

Mushroom production can convert the huge lingo-cellulosic waste materials into a wide of edible products or medicinal food, protecting and regenerating the environment. Moreover, the mushroom production can generate equitable economic growth that has already had an impact at community, national and regional levels. Edible mushrooms are the easiest and least expensive commercial mushrooms to grow because they are well known for conversion of crop residues to food protein. The present study was undertaken to evaluate locally available Corn bran of agricultural waste disposal used as substrate for cultivation of the Shiitake mushroom to pave the way for effective utilization of this residue and reduction of environmental pollution. Generally, study was revealed to cultivate other edible mushrooms on this substrate in order to improve food quality and supply.

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#### **INTRODUCTION**

Mushroom cultivation represents the only current economically viable biotechnology process for the conversion of waste plant from forests and agriculture<sup>1</sup>. residues Cultivation of saprophytic edible mushrooms the only currently economical may be biotechnology for lingo-cellulose organic waste recycling that combines the production of protein rich food with the reduction of environmental pollution<sup>2</sup>. Mushroom production in rural communities can alleviate poverty and improve the diversifcation of agricultural production<sup>3</sup>. Sawdust and sugarcane bagasse were the best substrates for growing of Oyster Mushroom than other agro-based substrates<sup>4</sup>.

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),<sup>5</sup> folic acid and pro-vitamin D ergosterol<sup>6,7</sup> and minerals (potassium, phosphorus, calcium, sodium)<sup>8,9</sup> and some essential amino acids, fiber and have low cholesterol and fat levels<sup>10</sup>. Mushroom normally ranges between 20 and 40% protein which is better than many legume sources like soybeans and peanuts, and protein-yielding vegetable foods<sup>11,12</sup>.

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<sup>11,13</sup>. Increasing consumption of mushroom is good for preventing malnutrition, although mushrooms cannot be an alternative protein source for of meat, fish, and  $egg^{14}$ . 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), ovster mushroom Pleurotus ostreatus, Kumm 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 centuries<sup>15</sup>.

Lentinus edodes shiitake mushroom, accounts for 17 % of world production in terms of weight<sup>16,17</sup>. It can grow in winter season and also it can grow all the year in controlled condition. After the button mushroom (Agaricus bisporus), shiitake is the most cultivated mushroom in the world <sup>16</sup>. The fungus is saprophytic and grows on dead material<sup>16</sup>. Mushrooms depend substrates on for nutrition and the substrate is normally a source of lignocellulose material which supports growth, development and fruiting of mushroom<sup>16</sup>.

Shiitake mushrooms have been attributed with many medical properties by both eastern and western medicine. They range from reducing cholesterol, lowering blood pressure, strengthening the immune system against diseases including viral ones, fighting tumors, and improving liver function<sup>18-21</sup>. Many of the shiitake health benefits come from chemical compounds these mushrooms produce, these include: lentinan, eritadenine, L-ergothioneine <sup>21-24</sup>.

Lentinan has shown some effect on bowel cancer, liver cancer, stomach cancer, ovarian cancer and lung cancer. Lentinan stimulates the production of T lymphocytes and natural killer cells and can potentiate the effect of AZT in the anti-viral treatment of AIDS. Shiitake is rich in several antioxidants (selenium, uric acid, vitamin A, E, C) as well as vitamin  $D^{20,25,26}$ . Shiitake *Lentinula edodes* mushrooms may also lower blood pressure in those with hypertension, lower serum cholesterol levels, stimulate the production of interferon which has antiviral effects, and has proven effective against Hepatitis in some cases<sup>20,23,27</sup>.

Corn (Zea mays) is a major crop in the USA, with  $329 \times 106$  tons produced domestically in 2008<sup>28</sup>. Corn bran, however, currently has low value and is often used for animal feed alone or in combination with corn germ cake or meal (germ after oil has been pressed or extracted). Corn bran is produced in yields of about 60-70 g kg-1. Corn bran is dietary fiber, which is nearly completely insoluble. The insoluble dietary fiber in corn bran is composed of cellulose ( $\sim 280 \text{ g kg}$ -1) and hemi-cellulose ( $\sim$ 700 g kg-1), with only a small amount of lignin ( $\sim 10$  g kg-1). The remainder of corn bran comprises residual starch, lipid, protein, ash, phenolic compounds and other trace phyto-chemicals<sup>29</sup>.

Composting is a fertilizing mixture of partially decomposed organic matter from plant and animal origin<sup>30</sup>. Composting is a solid-waste fermentation process, which exploits the phenomenon of microbial degradation and mineralization<sup>31</sup>. 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. Fermor reported that a composted substrate improved mushroom fruit body yield but, reduced by insects, fungi and bacteria infestation pathogens<sup>32</sup>. 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 growth<sup>32</sup>.

Unlike in developed countries where mushrooms food consumption is increasing, <sup>6,33,34</sup> in Ethiopia, mushroom eating habit is very poor<sup>35</sup>. 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. Beside, shiitake mushroom are good candidates for cultivation in Ethiopia because of the following reasons are some are efficient degraders of lingo-cellulosic materials, easy to grow with simple technology, can complete a full cycle in 3-4 weeks and the raw materials are abundantly available. In Ethiopia lingo-cellulose waste residues are mostly used in the domestic sector for feeding animals as well as for cooking and baking using very low efficiency devices. Corn bran in Ethiopia particularly in Dilla town and surrounding areas was left as solid waste. However, cultivation of shiitake 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 undertaken to evaluate locally available corn bran of agricultural waste disposal used as substrate for cultivation of the Shiitake mushroom to pave the way for effective utilization of this residue and reduction of environmental pollution.

# MATERIALS AND METHODS

### **Pure Culture Collection and Maintain**

*Lentinus edodes* was obtained from Mycology Laboratory, Department of Biology from Addis Ababa University. The pure culture of *Lentinus edodes* was inoculated onto Malt extract agar. The pure culture was maintained on Malt extract agar slants at -40C for one month, then sub-culturing subsequently after one month transferred (inoculated) onto fresh slant of Malt extract agar.

### **Substrate Collection**

Corn bran used as substrate for composting was collected in Dilla University from Main Campus from 2013 October -2014 April after sun drying 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.

# **Compost Preparation**

The compost was prepared by outdoor single-phase solid-waste fermentation<sup>36</sup>. In order to prepare, aerobic composted substrate, about 80% of Corn bran was chopped manually into small pieces through using hammer mill (Figure 2). After chopping, mixing the chopped Corn bran 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' method<sup>37</sup>. Then supplement with 20% of three different supplements on 80 % of Corn bran 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<sup>35</sup>. 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<sup>38</sup>. 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 (CaSO<sub>4</sub>. 2H<sub>2</sub>0), and 3% limes (CaCO<sub>3</sub>) were added (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  $25^{\circ}$ C until the substrate fully colonized; at ten 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 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  $27^{0}$ 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 -  $25^{\circ}$ 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 *Lentinus edodes* 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. The data of actively mycelium growth during spawn making and formation of full morphology of shiitake mushroom species and fruiting body were observed during cultivation on substrate. The data were expressed qualitatively in the form of figure.

# **RESULT AND DISCUSSION**

Lentinus edodes was cultured on malt extract agar for 7 days at 28°C and mycelium covered the medium as indicated in Figure 5. During mycelium growth, there is a circular pattern of mycelium growth observed on plates. 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<sup>39</sup>. 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<sup>40</sup>.

# **Spawn Production**

Sorghum is important cereals for spawn production of mushroom species. 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.

# 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). This is occurring when the humidity and cultivation room maintain at high humidity and room at dark. Spent mushroom substrate has been as animal feed, since its degradation by the mushroom can improve its nutritional quality and digestibility<sup>41</sup>. Chang found that when maize straw generated after mushroom cultivation was added to the diets of sheep, the weight gain of the sheep increased<sup>42</sup>.

### **Fruiting Body Production**

Fruiting body is the edible part of mushroom (Lentinus edodes). The fruiting body was observed after 20 day of spawn inoculated later. The fruiting body grown double or more than two fruit body together as in shown in figure 8. The harvesting date also varied depending upon types of substrate. Mushroom matured generally 48 hours after appearing primordia. Fruiting body (forming) the formation was triggered by shifting the environmental variables namely moisture, air exchange, temperature and light in the cropping room<sup>43</sup>. 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 vellowish and creamy fruit body, also cinnamon brown spores. Frutifification requires 30 days<sup>44</sup>.

### CONCLUSION

Mushroom cultivation needs knowledge as well as experience for growing fungi on crop solid waste materials that are not necessary consumed by humans. *Lentinus edodes* fungi can be converted this waste in to available health foods. Commercial production of *Lentinus edodes* mushrooms is largely determined by the availability and utilization of cheap by-products waste materials, which are agricultural wastes that are ideal and most promising substrates for cultivation. Therefore, this study was gives the way of cultivation other edible mushrooms this substrate in order to solve the food supply scarcity and quality.

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#### REFERENCES

1. Wood DA, Smith JF. The Cultivation of Mushroom, In essays in agricultural and food

microbiology editor by Norris J.R. and Pettipher G.L. John Wiley and Sons Ltd. 1987, 310-343.

- Obodai M, Cleland-Okine J, Vowotor KA. Comparative study on the growth and yield of *Pleurotus ostreatus* mushroom on different lignocellulosic by-products, *J. Ind. Microbiol. Biotechnol.* 2003; 30: 146-149.
- Gofrey EZ, Siti MK, Judith ZP (2010). Effects of temperature and hydrogen peroxide on mycelial growth of eight *Pleurotus* strains. Scientia Horticulture, 125: 95-102.
- 4. Ahmed S. Performance of different substrates on the growth and yield of Oyster Mushroom *Pleurotus sajorcaju* (Fr.) Sing. M.S. thesis, Department of Horticulture, Institute of postgraduate studies in Agriculture, Salna, Gazipur, Bangladesh, 1998.
- 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.
- 6. Kurtzman RHJr. A review mushrooms: sources for modern western medicine, *Micologia Aplicada International* 2005; 17: 21–33.
- 7. 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.
- 8. Stanley, RP. Enumerative combinatorics, Cambridge university press, 2011; 49.
- 9. 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.
- 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.
- 11. Chang ST, Buswell JA. Mushroom nutriceuticals, *World J. Microb. Biotechnol.* 1996; 12: 473-476.
- 12. Chang ST, Mshigeni KE. Mushroom and their human health: their growing significance as potent dietary supplements, *The University of Namibia, Windhoek* 2001; 1-79.
- 13. Sadler M. Nutritional properties of edible fungi, *Br. Nutr. Found. Nutr. Bull.* 2003; 28: 305-308.
- 14. Çaglarirmak 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.
- 15. Shin CK, Yee CF, Shya LJ, Atong M. Nutritional Properties of Some Edible Wild Mushrooms in Sabah, *J. Applied Sci.* 2007; 7: 2216-2221.

- Chang ST, Miles PG. Mushrooms: Cultivation, nutritional value, medicinal effect, and environmental impact, 2<sup>nd</sup> ed., CRC Press, Boca Raton, FL 2004.
- 17. Miles PG, Chang ST. Mushroom biology, concise basics and current developments, World scientific Publ., London 1997.
- Bobek P, Ginter E, Kuniak L, Babala J, Jurcovicova M, Ozdin L, Cerven J. Effect of mushroom *Pleurotus ostreatus* and isolated fungal polysaccharide on serum and liver lipids in Syrian hamsters with hyperlipoproteinemia, *Nutrition* 1991; 7: 105-108.
- 19. Wang H, Gao J, Ng TB. A new lectin with highly potent antihepatoma and antisarcoma activities from the oyster mushroom *Pleurotus ostreatus*, *Biochem. Biophys. Res. Commun.* 2000; 275: 810–816.
- Mau J, Lin H, Song S. Antioxidant properties of several specialty mushrooms, *Food Res. Int.* 2002; 3: 519-526.
- 21. Rajewska J, Bałasińska B. Związki biologicznie aktywne zawarte w grzybach jadalnych iich korzystny wpływ na zdrowie (Biologically active compounds of edible mushrooms and their beneficial impact on health), *Post. Hig. Med. Dośw.* 2004; 58: 352-357.
- Lasota W, Karmańska A. Wpływ podawania suszów twardziaka jadalnego i boczniaka ostrygowatego na poziom cholesterolu w surowicy krwi szczurów (Effect of dried shiitake and oyester mushrooms on the cholesterol level in serum), *Probl. Hig.*1994 44: 77-86.
- 23. Smith J, Rowan N, Sullivan R. Medicinal mushrooms: Their therapeutic properties and current medical usage with special emphasis on cancer treatments, Univ. Strathclyde, Glasgow, UK 2002.
- 24. Bernaś E, Jaworska G, Lisiewska Z. Edible mushrooms as a source of valuable nutritive constituents, *Acta Sci. Pol. Technol. Aliment.* 2006; 5 (1): 5-20.
- 25. Lasota W, Florczak J. Porównanie składu chemicznego i wartości odżywczej grzybów uprawowych: pieczarki dwuzarodnikowej, boczniaka ostrogowatego, twardziaka jadalnego iucha bzowego (A comparison of chemical compositions and nutrition value mushroom: field mushroom, oyster fungus, shiitake and wood ear), *Probl. Hig.* 1996; 53: 51-56.
- 26. Florczak J, Karmańska A, Wędzisz A. Porównanie składu chemicznego wybranych gatunków grzybów dzikorosnących (Comparison of the chemical contents of

selected wildgrowing mushrooms. Bromatol, *Chem. Toksykol.* 2004; 37 (4): 365-371.

- 27. Lasota W, Karmańska A, Sylwestrza J. Skład chemiczny twardziaka jadalnego (Chemical composition of shiitake mushroom), *Probl. Hig.* 1992; 36: 77-81.
- 28. http://www.ers.usda.gov/Data/FeedGrains/Yearb ook/FGYear
- 29. Rose DJ, Inglett GE, Liu SX. Utilisation of corn (Zeamays) bran and corn fiber in the production of food components, *J. Sci. Food Agric.* 2010; 90: 915–924.
- Piet JL, Derik X, Huub JM, Drift CV, Leo JLD, Vogel D. Composting Processes, J. Appl. Environ. Microbiol. 1990; 3029-3034.
- 31. McKinley VL, Vestal JR. Biokinetic Analysis and Succession of Microbial Activity in Decomposition of Municipal Sewage Sludge, *Appl. Environ. Microbiol.* 1984; 47:933-941.
- 32. 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.
- 33. Gregori A, Vagelj MS, Pohleven J. Cultivation techniques and medicinal properties of Pleurotus spp *Food Technology and Biotechnology* 2007; 45: 238–249.
- 34. 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.
- 35. Dawit A. Mushroom Cultivation; A Practical Approach, Addis Ababa: Berhanena Selam Printing Enterprise, Ethiopia 1998.
- Nair NG, Price G. A Composting process to minimize odour pollution, *Mushroom Science* 1991; 13: 205-206.
- Buswell JA. Potentials of Spent Mushroom Substrates for Bioremediation Purposes, *Compost*1984; 2: 31-35.
- 38. 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.
- 39. Wasser SP. Medicinal mushrooms as a source of antitumor and immune-modulating polysaccharides, *Appl. Microbiol. Biotechnol.* 2002; 60: 258-274.
- 40. Singh S. Mushrooming enterprise protein revolution through mushroom cultivation, *J. Agri. Rular Development* 2008; 1: 15-17.
- 41. Suzuki Y, Okano K, Kato S. Characteristics of white rotted woody materials obtained from

Shiitak mushroom (*Lentinus edodes* ad nameko mushrooom(*Pholiota nameko*) cultivation with in vitro rumen fermentation, *J. Animal food sci. Tech.* 1994; 54: 227-236.

- 42. Chang ST. World production of cultivated edible and medicinal mushrooms in 1997 with emphasis on Lentinus edodes (Berk) Sing in China, *Int. J. Med. Mushr*.1999; 1: 291–300.
- Stamets P. Growing gourmet and medicinal mushrooms 3<sup>rd</sup> ed., Ten Speed Press, Berkeley 2000, 574.
- 44. Marshall E, Nair NG. Make money by growing mushroom. Rural Infrastructure and Agro-Industries Division Food and Agriculture Organization of the United Nations, Diversification booklet number 2009, 42.



Figure 1. Corn bran collection during winter season at Dilla University in Main campus



Figure 2. A, Corn chopping; B, Composting area

IJAS [1][1][2014] 037-048



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**Figure 3**. A, weighing of sorghum; B, soaked in tap water overnight; C, supplement of wheat bran and pH adjustment; D, addition of formulated sorghum into glass bottle

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Figure 4. Sterilization of substrate



(A) (B) Figure 5. Lentinus edodes mycelium grown on malt extract agar: A, Front observation of mycelium on plate; B, back observation of mycelium on plate

# Alemu





(C)

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





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Figure 8. Fruit body grown on the corn bran, the fruit body was grown double by forming fork like structure