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CULTIVATION OF *LENTINUS EDODES* ON TEFF STRAW (AGRICULTURAL RESIDUE) AT DILLA UNIVERSITY, ETHIOPIA

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ABSTRACT

Shiitake mushroom is worldwide one of the most widely cultivated mushrooms. Edible fungi are natural recycler which converts lignocelluloses wastes into protein rich health food and medicinal value of food. Mushroom cultivation represents the current economically viable biotechnology process for the conversion of waste plant residues from agriculture. As the result, mushrooms are increasingly becoming an important component of diets worldwide and it is of paramount importance to choose appropriate substrates in a given place to grow them. Therefore, study was undertaken to find suitable abundantly waste such as Teff straw used as substrate for cultivation of mushroom. As the result revealed, this agricultural residue was the best for cultivation of Lentinus edodes which help to collect large amount of fruit body. As conclusion, Lentinus edodes fungi can be converted this solid waste in to delicious foods.

Keywords: Fruit body, Lentinus edodes, Mushroom cultivation, Spawn, Substrate, Teff straw.

Contribution/ Originality

This study will help for scale up of mushroom cultivation on other substrate. Moreover, this study will help to cultivate the edible of wild mushroom this waste or other waste products.

1. INTRODUCTION

Mushroom cultivation has been reported as other effective way of alleviating poverty in developing countries [1]. *Lentinus edodes* is a very popular food in Asia and the raw materials can be stably supplied by cultivation of the mycelia, the extract is a promising candidate for use as an antioxidant and hepatoprotective agent [2, 3]. Shiitake mushroom has been reported to boost the immune system, lower cholesterol, function as an anticoagulant and may have use in treatment of some cancers [4-8]. *Lentinus edodes* can lower both blood pressure and free cholesterol in plasma, as well as accelerate accumulation of lipids in liver by removing from circulation. Ten years ago the researches were concentrated on the four mushrooms, *Lentinus (Lentinula) edodes*, *Schizophyllum commune*, *Grifola frondosa*, and *Sclerotinia sclerotiorum*, particularly their respective β-glucans, lentinan, schizophyllan (also called SPG, sonifilan, or

sizofiran), grifolan, and SSG. Most of them, β-(1-6)-branched β-(1-3)-linked glucans, were found to exhibit significant antitumor activity [9].

Some mushrooms contain compounds, which can make a contribution to the general health of man. As mushrooms are widely distributed all over the world, some of them have been used in traditional medicine as anti inflammatory, analgesics, homeostatic, diuretic, nourishment, antibiotic and anti tumour agents [10, 11]. Mushrooms are also used for chronic catarrh diseases of the breast and hinges, lower the cholesterol level of blood, improves circulation, remedy for night sweating in tuberculosis, rheumatism, gout, jaundice, dropsy, intestinal worms and have anti-tumor, anti-viral and anti-cancer agents. Mushrooms are considered ideal for patients of hypertension, renal effects and diabetics [12, 13], their immune-modualatory and antitumor activities of Polysaccharide-Protein Complex (PSPC) from mycelial cultures [14],[15],[16-18] and their immune-modualatory and antitumor activities of lectins from edible mushrooms [16-18] gives them valued medicinal value.

Other mushrooms are known to have medicinal properties; Bracket mushroom (*Ganoderma lucidum*) has been reportedly used for disease management of patients with HIV and AIDS and can be justified by the increase in body weight [19-21]. Scientists have discovered that the polysaccharide compound lentinan, found in shitake mushrooms, possess immune-stimulant and anti tumour properties. Lentinan can also prevent platelet adhesion, which causes the clots responsible for coronary artery disease and stroke. The water extract of *Lentinus edodes* demonstrated growth-enhancing effects on colon inhabiting beneficial lactic acid bacteria, *Lactobacillus brevis* and *Bifidobacteria brevis* and *Bifidobacteria brevie.* The effective factor in the extract is considered to be the disaccharide sugar, tehalose. The *L. edodes* extract can improve the beneficial intestinal flora of the gut and reduced harmful effects of certain bacterial such as β-gucosidase, β-glucorinidase and tryptophase as well as reducing colon cancer formation [22]. It is clear from the results that mushrooms also have antimicrobial properties. Oxalic acid is an agent responsible for the antimicrobial effect of *Lentinula edodes* against *S. aureus* and other bacteria [9]. Ethanolic mycelial extracts from *L. edodes* possess antiprotozoal activity against *Paramecium caudatum* [9].

Composting is a fertilizing mixture of partially decomposed organic matter from plant and animal origin [23]. Composting is a solid-waste fermentation process, which exploits the phenomenon of microbial degradation and mineralization [24]. 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, et al. [25] 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 growth [25].

Compost or un-compost wheat and paddy straw, banana leaves, sugarcane bagasses and leaves, wheat barn, rich husk, sawdust etc can be used as substrate for growing mushroom [26]. Large amounts of freely available teff straw from different agricultural waste offer a potential alternative substrate source for mushroom cultivation in Ethiopia, particularly around Dilla town. As the result, it is possible to convert for highly nutritious and medicinal aspects of mushroom through cultivation of *Lentinus edodes*. Therefore, study was undertaken to find suitable agricultural residue such as Teff straw used as substrate for growing of mushroom.

2. MATERIALS AND METHODS

2.1. 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 -4^{0}C for one month, then sub-culturing subsequently after one month transferred (inoculated) onto fresh slant of Malt extract agar.

2.2. Substrate Collection

Teff straw used as substrate for composting was collected from around Dilla town from 2013 October -2014 April after sun drying (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.

Figure-1. Teff straw

2.3. Compost Preparation

The compost was prepared by outdoor single-phase solid-waste fermentation [27]. In order to prepare, aerobic composted substrate, about 80% of Teff straw was chopped manually into small pieces through using hammer mill. After chopping, mixing the chopped Teff straw 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 [28]. Then supplement with 20% of three different supplements on 80 % of Teff straw as follows:-

Substrate	Type of supplements		
Teff straw 80 %	10% Chicken manure	8% Wheat bran	2% wood ash
Teff straw 80 %	10% Cow dung	8% Wheat bran	2% wood ash
Teff straw 80 %	18% Wheat bran	2% wood ash	
Teff straw 80 %	18% Cow dung	2% wood ash	
Teff straw 80 %	18% Chicken manure	2% wood ash	
Teff straw 98 %	2% wood ash		

It was on dry weight basis with some Modification of [29]. 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.

2.4. 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 [30]. 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 ($CaSo_4$. $2H_20$), and 3% limes ($CaCO3$) were added (figure 2). 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^{0}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^{0}C until the substrate fully colonized; at ten days interval mycelia invasion and contamination were recorded.

Figure-2. A, soaked in tap water overnight; B, supplement of wheat bran and pH adjustment; C, addition of formulated sorghum into glass bottle

C

2.5. 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. 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.

2.6. Cultivation Conditions

The bags were subsequently placed, long side down, into a spawn running room at 20 - 25° 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.

2.7. Watering

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

2.8. 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.

2.9. Statistical Analysis

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.

3. RESULT AND DISCUSSION

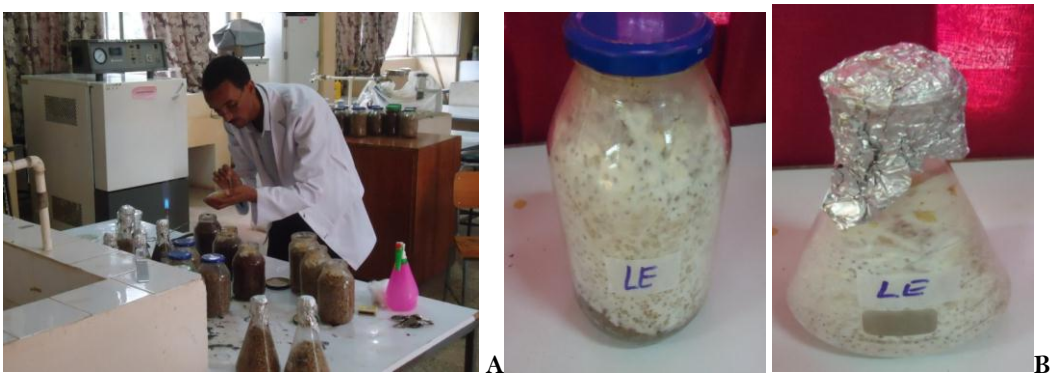
Lentinus edodes was cultured on malt extract agar for 7 days at 28°C and mycelium covered the medium. There is a circular pattern of mycelium growth observed on plates as shown in figure 3. An ever increasing human population and diminishing farm sizes have resulted in declining soil fertility associated land productivity and increasing poverty levels [31]. In addition 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. However, mushrooms products are solved both problem through cultivation in small size area finally by consumed.

Figure-3. *Lentinus edodes* mycelium grown on malt extract agar: A, Front observation of mycelium growth on plate; B, back observation of mycelium growth on plate

3.1. Spawn Production

Sorghum is important cereals for spawn production of mushroom species. Sorghum based spawn took 25 days to colonize the substrate completely figure 4. The moisture content of the sterile moist sorghum (55-60%) was found to be suitable for growth of *Lentinus edodes*.

Figure-4. 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



3.2. 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 5). Therefore, mycelium running was required high humidity and cultivation room should be dark.

Figure-5. Sterilization and inoculation spawn: A, sterilization of substrate; B, inoculation of *Lentinus edodes* spawn on Teff straw substrate; C, mycelium colonization of the teff straw

3.3. 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 was appeared as indicated in figure 6. Matured mushroom fruit body identified by curl margin of the cap was harvested by twisting to uproot from the base. Mushroom matured generally 48 hours after appearing (forming) the primordia. Cultivated mushroom are generally saprophytes, utilizing substrate as primary or secondary decomposers [32]. 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, and soft woods of the temperate as well as tropical region [33]. Their relative adaptability was to various substrate species and forms, (stumps, logs, wood particles, leaf litter) and their preferences with respect to the microbiological condition of their substrates [34].

Figure-6. Fruit body grown on the teff straw, the fruit body was grown double by forming fork like structure

4. CONCLUSION

Mushroom cultivation is the recent technology which had multiple advantages over the world. Among those advantage are one is as a source of food and medicinal value, secondary used as removal of solid waste pollutant and recycling of mineral through decaying process. Therefore, *Lentinus edodes* fungi can be converted this solid waste in to delicious foods. On the present study also *Lentinus edodes* was easily cultivated on the teff straw which can be converted in food form as the result of decaying by this fungus. Therefore, study was gives clue for cultivation of other edible mushrooms for human being consumption on this substrate.

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