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The effect of substrate and substrate formulation on the yield of Oyster mushroom (*Pleurotus ostreatus)*

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ABSTRACT

Developing countries like Ethiopia, should strive to solve acute protein deficiencies in the diets of their population by cultivating mushrooms in the urban areas, without heavily depending on agricultural lands and capital. This experiment was conducted to identify the suitable basic substrate for yield and yield contributing attributes for oyster mushroom in wolayta zone of Ethiopia. Seven combinations of substrates *viz.* maize, sorghum, soybean, maize+sorghum, maize+soybean, sorghum+soybean and maize+sorghum+soybean stacks were evaluated for oyster mushroom production efficiency in a completely randomized design (CRD) with three replications. Percent biological efficiency (% BE), fresh yield and yield related components of oyster mushroom was analyzed using SPSS (version 20.0). Highest fresh yield (1012g/kg) with percent BE (101.2%) and the lowest fresh yield (52g/kg) with percent BE (5.2%) were achieved on, maize + Sorghum + Soybean stack and Soybean stack, respectively. During 1st flush, maximum yield was also obtained in substrate comprising maize + sorghum + soybean stack compared with other treatments. Therefore, producers should be encouraged to use this substrate to maximize oyster mushroom yield and use agricultural wastes for food production. Oyster mushroom could play a pivotal role in supporting the food and nutritional self-sufficiency in Ethiopia and should be included as a component of food security assurance strategy for the country.

Keywords: Oyster Mushroom, agricultural residues, substrate formulation, biological efficiency

One of the world's biggest challenges is food and nutritional insecurity. This problem is largely common in low- and middle-income group countries, which mainly have poor food production system and hence, suffer from serious malnutrition. Such countries must find ways of improving food production so as to feed fast increasing human population. Mushroom cultivation could be a possible option to alleviate poverty and develop the life style of vulnerable people (Bobek *et al.*, 1999). Mushroom, a macro fungus with a distinctive fruiting body, is a unique biota, which assembles its food by secreting degrading enzymes. It decomposes the complex organic materials on which it grows (the substrate) to generate simpler compounds for its nutrition (Chang and Miles, 1992).

The substrates used for mushroom production are usually by-products from industry, households and agriculture, and are usually considered as wastes. These wastes, if carelessly disposed of in the surrounding environment by dumping or burning, will lead to environmental pollution and consequently cause health hazards. However, they are actually resources in place at a particular time and mushroom cultivation can harness these resources (wastes) for beneficial purpose. Mushroom cultivation represents one of the

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economically viable bio-technological process for conversion of waste plant residues from forests and agriculture (Wood and Smith, 1987) to food and manure.

Mushroom cultivation technology is environment-friendly. The mushroom mycelia can produce a group of complex extracellular enzymes, which can degrade and utilize the recalcitrant lignocellulosic wastes reducing pollution. Mushrooms can not only convert lignocellulosic waste materials into human food, but also can produce notable nutraceutical products, which have many health benefits. They provide people with an additional vegetable of high quality, and enrich the diet with high quality proteins, minerals and vitamins, which can be of direct benefit to the human health (Tam *et al*., 1986; Yip *et al*., 1987). Edible mushrooms are highly nutritious and can be compared with eggs, milk and meat (Chang and Miles, 1989). The extractable bio-active compounds from medicinal mushrooms would modulate human immune systems and improve their quality of life. A few of the health benefits are anti-hypertensive (Tam *et al*., 1986; Yip *et al*., 1987), immuno-modulator, antitumor activities of polysaccharide-protein complex (PSPC) and lectins (Liu *et al*., 1995, 1996; Wang *et al*., 1995, 1996a, 1996b, 1997). Several other bioactive compounds such as Type I Ribosome-Inactivation protein from *Volvariella volvacea* (Yao *et al*., 1998) and ganoderic acids from *Ganoderma lucidum* (Chang and Buswell, 1996; Chang and Miles, 1989) have been reported with varieties of health benefits.

The content of essential amino acids in mushroom is high and close to the need of the human body. Mushroom is also easily digestible and it has no cholesterol content (Oei, 2003). The spent substrate left after harvesting the mushrooms, which is entangled with innumerable mushroom threads (collectively referred to as mycelia), can also be used as animal feed (more palatable), bio-fertilizer for soil fertility enrichment and biogas. Furthermore, mushroom cultivation can be a labor-intensive agro-industrial activity, thus can help generate income and employment, particularly for women and youth in developing countries.

Mushrooms are relatively fast-growing organisms. Thus, mushroom cultivation as a short return agricultural business can be of immediate benefit to the community. While land availability is usually a limiting factor in most types of primary production, mushroom cultivation requires relatively little space; they can be stacked using shelf-like culture systems. It is, therefore, hoped that the avocation of mushroom farming will become a very important cottage industry in integrated rural development programs. This will lead to the economic betterment of not only small land holding farmers but also of landless laborers and other sections of communities (Alam and Raza, 2001; Shah *et al*., 2004; Sher, 2006; Flores, 2006).

Consequently, Ethiopia has not been benefited from mushrooms as the rest of the world (Kiflemariam, 2008). Moreover, the lack of appreciation about the food and dietary importance of mushrooms, and the monotonous traditional diets and the conservative eating habit of Ethiopian people are among the main impediments constraining to mushroom cultivation in Ethiopia. As a result, promoting technology transfer concerning mushroom cultivation is urgently required intervention option. The implication of this study is to facilitate technology adoption of oyster mushroom cultivation using agricultural wastes, and thereby identify the feasibility of mushroom cultivation in the study area for the betterment of the life of the local community. Oyster mushroom cultivation can play an important role in managing organic materials considered as waste (Beetz and Kustudia, 2004). The demand of mushroom has now been increasing in Ethiopia due to population growth, market expansion, changing of

consumer behavior, and developments in the manufacturing industries, storage, transportation, and retailing. Despite its contribution to the urban economy and environment, mushroom has been underestimated and unrecognized, unassisted or discriminated and in some cases outlawed because of the supposed hazards associated with it. Researches on mushroom has been scanty in Ethiopia. It has been disregarded by researchers and little understood by urban planners, Ministry of Agriculture, NGO's and decision makers.

Mushrooms can generate additional employment and income through local, regional and national trade offering opportunities through processing enterprises (FAO, 2009). The ability of fungus to degrade agricultural materials and the readily availability of many agricultural by-products may contribute in addressing unemployment and protein deficiency. In line with this, the yield and quality of mushrooms depends on the type of substrate used, the method of preparation and the suitability of environmental condition (temperature and humidity) for growth and fruit-body formation. Oyster mushroom can be cultivated in any type of lignocellulotic material like maize stack (*Zea mays*), sorghum stack (S*orghum bicolor*), common bean stack (*Phaseolus vulgaris* L), etc. Most communal farmers dispose of their agricultural wastes while it could be used as substrates for the production of mushrooms (Shongwe, 2007). Less research has been done on locally available substrates for utilization as substrate for mushroom cultivation (Shongwe, 2007).

Viewing the potential of mushroom as food source, nutritional treasure, role in in environment protection, animal feeds and organic manure, this study has been undertaken to standardize the oyster mushroom growing practices using locally available substrate so that local people can take up mushroom production at lower level for upliftment of their socio-economic and nutritional status.

MATERIALS AND METHODS

Descriptions of the study area

Kindo Koysha woreda (district) is in the Wolita Zone in Southern Ethiopia. The woreda is located at 329 Km from the national capital city of Addis Ababa and 36 Km from the wolayita Sodo. Its absolute location extends between the coordinates of 6^0 50'N-6^0 59'N latitude and 37^0 52'-38^0'E longitude. Currently the woreda comprises of 23 Kebele (villages) administration and 2 towns. The topography of the woreda varies from place to place and significant difference in altitude can be observed in a short distance. Its altitude ranges from 1100-1900 amsl.

The annual rainfall of the woreda ranges between 400-1400mm. The rainy season normally begin in March and extends to September. The minimum and maximum temperatures of the woreda are 25°C and 40°C, respectively. According to 2007E.C census, the total population of the woreda was 128,017. Agricultural system of the study area is defined by different farming systems, livestock production, crop production and other livelihood related activities.

Spawn preparation

Maize grain was used to prepare mushroom spawn for faster ramification of the substrate and ease of planting (Stanley and Awi-Waadu, 2010).

Substrates used

Substrates included maize stack (M), Sorghum stack (S), Soybean stack (B), maize stack and Sorghum stack (M+S) and maize stack+soybean (MB), Sorghum stack+Soybean stack(SB) and maize stack(M)+ Sorghum stack(S)+ Soybean stack(B) (MSB). All the treatments used in the study are enlisted in Table 1.

Table 1. Substrate and substrate Formulations

Treatment	Substrate	Code
T1	Maize stack 100%	M
T2	Sorghum stack 100%	S
T3	Soybean stack 100%	B
T4	Maize stack 50% and sorghum stack 50%	MS
T5	Maize stack 50% and soybean stack 50%	MB
T6	Sorghum stack % and soybean stack 50%	SB
T7	Maize+sorghum+soybean stacks (33% each)	MSB

Experimental design

The experiment was carried out using a completely randomized block design (CRD) with 7 treatments and three replications. Treatments were randomly assigned on shelves for mycelium running and fruiting body formation.

Substrate processing

The maize stack and sorghum stack substrates were chopped into 2-3 cm and 1-2 cm, respectively. The Soybean stack was procured in small pieces (1-2 cm), therefore, did not need chopping. The substrates were allowed to dry in the sun for 10 days and regularly (daily) weighed for five consecutive days to determine the change in the air-dry weight of substrates and mixed by hand on a clean cemented floor covered with a plastic sheet. One-kilogram air dried substrate was transferred to individual polypropylene bags (55 cm × 50 cm). After mixing, the substrates (except the maize stack) were soaked for 24 Hours to allow for moisture absorption. The maize stack was soaked for 24 hours and composted for four days by covering with polyethylene sheet. The excess water was drained until it reached 65% moisture level.

The moisture content of the substrates was determined by applying the squeeze test to determine whether the substrate is moist enough (Maheshwari, 2013). A few drops of water (2 - 3 drops) were released with some pressure. The opening of each bag was tied and were sterilized in an autoclave and allowed to cool to room temperature. Following this, each bag (with 1 kg substrate) was inoculated with 70 g of spawn. For a uniform distribution of the inocula, spawn and substrates were mixed thoroughly under aseptic condition. Several (6–8) holes were punched on the sides of the plastic bags to facilitate cross-sectional ventilation.

Cultivation conditions and cropping system

Inoculated bags were put in a dark room to initiate mycelia growth. After mycelial growth in the bags became abundant and/or pinheads emerged, portions of the bags were cut-off to create perforations to facilitate the development of fruiting bodies. Then, fully colonized substrates were transferred to growth room and placed on racks (made from wood and nylon rope) at a spacing of 15-20 cm. Proper ventilation of the growth room was assured by opening the door occasionally. Inoculated bags were watered 2-3 times a day to keep the mycelia moist. Relative humidity (RH) and room temperature were maintained between 80 and 85 % by spraying fine mist of water occasionally (Oei, 2003). Temperature was kept 25°C for spawn run and 18-20°C for fruit body development.

Data collection and analysis

The growth and development of mushroom were monitored daily. The time (number of days) required from inoculation to completion of mycelium running, time elapsed between opening the plastic bags to pinhead formation and time required from opening the plastic bags to first round harvesting was recorded.

Growth parameters including stipe length (cm) and pileus diameter (cm) were recorded with a transparent ruler before each harvest. Yield parameters, such as number of fruiting bodies, flush per bag, and total fresh weight (g) of mushroom were also recorded at harvest time.

Matured fruiting bodies (white in color, with up curved pileus) were harvested by severing the base just above the surface of the substrate with a sharp blade. To evaluate the growth performance of mushroom on different substrates, yield and biological efficiency were calculated. Accordingly, biological yield (g) was determined by weighing the whole cluster of fruiting bodies without removing the base of stalks, and finally, biological efficiency (%) was calculated as follows:

Biological yield= the total weight of fresh mushroom.

$$\%\ BE = FWm/DWs * 100\%$$

Where, BE is Biological Efficiency (%); **FWm** is total fresh weight (g) of mushroom yield, **DWs** are substrate dry weight (g).

The data were analyzed using SPSS version 20.0 software. A one-way analysis of variance (ANOVA) was used to test for significance of variation in yield and yield attributes of substrates on different flushes. Means were compared using Tukey test, when F-test from ANOVA was significant at ($p<0.05$).

RESULT AND DISCUSSION

Number of days for mycelium running, pinhead and fruiting body formation

The number of days to start and complete mycelium running in spawn bags ranged from 4-11 and 13-17days, respectively (Table 2). The lowest days to start and complete mycelium running were recorded for T3 (4 days) and T2 (13 days), respectively. The maximum number of days for completion of mycelium running was 17 recorded for T5. The number of days required for starting of pinhead formation ranged from 16 to 22 days (Table 2). The maximum number of days (22) for pinhead formation was recorded for T5 while the lowest was taken in T2. Days taken to fruiting bodies development ranged between 29-39 days after inoculation. The

Table 2. The effect of different substrates and substrate formulations on mycelium running, pinhead and fruiting body formation.

Treatments	Start of mycelium running	Completion of mycelium running	Start of pinhead formation	Completion of pinhead formation	Fruiting Body
T1(M)	11^{c}	15^{c}	18^{c}	25^{b}	29^{a}
T2(S)	10^{b}	13^{a}	16^{a}	19^{a}	32^{ab}
T3(B)	4^{a}	14^{ab}	17^{b}	25^{b}	34^{c}
T4(M+S)	10^{b}	16^{bc}	21^{e}	29^{d}	37^{e}
T5(M+B)	10^{b}	17^{c}	22^{f}	30^{e}	33^{ab}
T6(S+B)	11^{c}	14^{ab}	18^{c}	24^{b}	39^{f}
T7(M+S+B)	9^{ab}	15^{c}	20^{d}	27^{c}	36^{d}

Key: The same letter within each column indicates no significant difference among treatments by ANOVA and Duncan's multiple range test at $P<0.05$ level.

lowest number of days (29 days) for fruiting bodies completion was recorded on T1 and T2. The highest number of days (39) was recorded on T6.

Stipe length and pileus diameter

The stipe length and pileus diameter were variable for different flushes. In the first flush, the highest stipe length 3.24 cm was recorded in T5 whereas the lowest stalk length 2.2 cm was recorded in T4 (Table 3). Among the three flushes the maximum stalk length (3.38cm) was found for the T7 during the second flush and the minimum (1.71 cm) was recorded from T3 during the third flush .

The highest pileus diameter (5.97 cm) was obtained from T7 and the lowest pileus diameter (2.55 cm) was recorded from T3 in first flush. Overall, the maximum pileus diameter (6.56 cm) was documented from T7 on the second flush and the minimum pileus diameter (2.0 cm) was recorded from T3 during the third flush (Table 3).

Effect of substrates and substrate formulations on yield attributes

The maximum number of fruiting body (11) was recorded in T5 and T7 during first flush while minimum number of fruiting body (5.3) was observed in T1. Overall, the maximum number of fruiting body (12.6) was counted in T4 in the second flush and the minimum number of fruiting body (3.6) was counted from T1 in the third flush (Table 4).

Effect of substrates on fresh yield and Biological efficiency

The highest yield (380g kg^{-1} of substrate) was obtained from T7 in the first flush while the minimum fresh yield (24g kg^{-1} of substrate) was obtained from T3 (Table 5). Overall, the maximum total yield (1012g) was obtained in T7 followed by T6 (720g) and T5 (580).

Biological efficiency varied significantly between the treatments (Table 5). The highest biological

Table 3. Effect of substrates and substrate formulations on the stipe size and pileus diameter.

Substrates	Mean length/diameter in cm							
	1st Flush		2ndFlush		3rdFlush		Total mean	
	Stipe length	Pileus diameter	Stipe length	Pileus diameter	Stipe length	Pileus diameter	Stipe length	Pileus diameter
T1(M)	2.65^{ab}	2.96^{ab}	2.1^{a}	3.03^{b}	3.26^{d}	3.59^{cd}	2.67^{bc}	3.34^{c}
T2(S)	2.97^{ab}	3.50^{bc}	2.45^{ab}	2.41^{a}	1.9^{ab}	2.96^{ab}	2.40^{b}	2.95^{ab}
T3(B)	2.27^{ab}	2.55^{a}	2.27^{ab}	3.03^{b}	1.71^{a}	2.0a	2.08^{a}	2.53^{a}
T4(M+S)	2.2^{a}	4.2^{d}	2.75^{ab}	4.75^{d}	2.26^{bc}	5.21^{ef}	2.40^{b}	4.62^{de}
T5(M+B)	3.24^{c}	4.82^{de}	2.7^{ab}	3.82^{bc}	2.2^{bc}	4.72^{e}	2.71^{bc}	4.44^{d}
T6(S+B)	2.67^{ab}	3.36^{bc}	3.2^{b}	4.37^{c}	2.17^{bc}	3.38^{c}	2.68^{bc}	3.87^{cd}
T7(M+S+B)	2.36^{ab}	5.97^{f}	3.38^{c}	6.56^{e}	3.12^{c}	5.86^{ef}	2.95^{c}	6.34^{e}

Key: The same letter within each column indicates no significant difference among treatments by ANOVA and Duncan's multiple range test at $P<0.05$ level.

Table 4. Effect of substrates and substrate formulations on yield attributes number of fruiting bodies, total yield and overall biological efficiency of oyster mushroom.

Substrates	Stack length(cm)			Pilus diameter(cm)			No of Fruiting body		
	1st Flush	2nd Flush	3rd Flush	1st Flush	2nd Flush	3rd Flush	1st Flush	2nd Flush	3rd Flush
T1(M)	2.65^{ab}	2.1^{a}	3.26^{c}	2.96^{ab}	3.47^{bc}	3.59^{bc}	5.3^{a}	4.3^{a}	3.6^{a}
T2(S)	2.97^{c}	2.58^{ab}	1.94^{ab}	2.55^{a}	3.05^{b}	2.0^{a}	8.3^{c}	6.3^{b}	4.3^{b}
T3(B)	2.27^{ab}	2.78^{ab}	1.71^{a}	3.50^{b}	2.41^{a}	2.96^{b}	7.3^{b}	6.3^{b}	5.6^{bc}
T4(M+S)	2.2^{a}	2.75^{ab}	3.26^{c}	5.97^{e}	4.37^{d}	3.85^{bc}	8.3^{c}	12.6^{e}	6.3^{c}
T5(M+B)	3.24^{d}	2.7^{ab}	$2.2b^{c}$	5.97^{e}	6.56^{e}	4.72^{c}	11^{e}	10.3^{d}	4.3^{b}
T6(S+B)	2.68^{ab}	3.2^{b}	2.17^{bc}	4.82^{cd}	3.82^{bc}	5.86^{de}	9.6^{d}	7^{c}	5.3^{bc}
T7(M+S+B)	2.36^{ab}	3.38^{c}	4.2^{d}	4.2^{c}	4.75^{de}	5.21^{d}	11^{e}	10.3^{d}	9.6^{d}

Key: The same letter within each column indicates no significant difference among treatments by ANOVA and Duncan's multiple range test at P<0.05 level.

Table 5. The effect of substrate and combinations on the yield and biological efficiency of mushroom.

Substrates	Weight (Yield) per Flush							
	1st Flush		2nd Flush		3rd Flush		Total yield (g)	B.E (%)
	Yield (g)	BE (%)	Yield (g)	BE (%)	Yield (g)	BE (%)		
T1(M)	60	5.54	35	3.4	20	2.36)	115	11
T2(S)	28	2.58	22	2.14	20	2.36	70	6.89
T3(B)	24	2.2	16	1.56	12	1.42	52	5.18
T4(M+S)	240	22.2	260	25.29	120	14.2	720	61.6
T5(M+B)	150	13.86	200	19.46	230	27.23	580	60.55
T6(S+B)	200	18.48	170	16.54	135.5	16.04	505.5	51.06
T7(M+S+B)	380	35.12	325	31.61	307	36.35	1012	103.08

efficiency. The total biological efficiency in all the three flushes was maximum in T7 (103.08 %), which proved this treatment to be the superior substrate followed by T4 (61.6%), T5 (60.55%) and T6 (51.06%).

DISCUSSION

The mycelium running took 2-3 weeks after inoculation. The length of days taken to complete mycelium running of oyster mushroom on different substrates might be due to a variation in the chemical composition of substrates (Jenkins and Zwieten, 2003; Shroomery, 2011; Chen *et al*, 2011; Ibekwe *et al.,* 2008). The peak mycelial growth rate attained (1.9 cm/day) might be due to the differences in the prevailing environmental conditions at the experimental site (Musieba, 2012; Poppe, 2004 and Siqueira, 2012). The poor mycelial growth of bean stack observed in the present study is in contrast with the observations made by Musieba (2012), Poppe (2004) and Siqueira (2012) who noted that soybean stack is one of the best substrates for mushroom production. The findings of this research indicate that the soybean stack responds well when used with other substrates but not alone. The typical growth of the mycelia on maize

stack and the mixed substrates might be in response to the availability of nutrients and their timely release to the developing mycelia. This confirms the work of Ibekwe *et al.* (2008).

The result of the pileus diameter from this study differed from that of Mondal *et al*. (2010) as they observed the highest (7.21cm) pileus diameter in Maize+sorghum+soybean (33% each) in second flush. The pileus diameter affected the fresh yield. The number of fruiting body significantly affect the yield of oyster mushroom. The yield difference of more than 69% in the yield may be explained by variation in the number of fruiting body. Temperature and relative humidity have been considered to play a significant role that affected production of fruit bodies of mushroom (Musieba, 2012; Poppe, 2004 and Siqueira, 2012).

The mixed substrates gave the highest yield in grams of mushrooms per kilogram of substrates (g/kg) among the seven substrate types studied, This higher yield observed in the mixed substrates may be due to good physical and chemical qualities of these substrates that ensured a smooth transition from vegetative phase to reproductive phase. This agrees with reports of Shashirekha and Rajarathnam (2007) and Kapoor *et al*. (2009) who observed that supplementing the soybean stack with maize stack and sorghum stack increased the enzyme activities.

Biological efficiency is the yield of mushrooms per kg of substrates on dry weight basis (Chang *et al.,* 1981). The mixed substrates were gave more biological efficiency than the other substrates. Differences in biological efficiencies of the various substrates were due to different substrate compositions (Ajonina and Tatah, 2012). The efficiency of the mixed substrate was comparatively constant and higher among the substrates. The mixed substrates were appropriate chemical properties needed for oyster mycelia growth and fruiting (Jenkins and Zwieten, 2003; Shroomery, 2011; Chen *et al*, 2011; Ibekwe *et al.,* 2008). The variations observed in the yield were related to the complexity of substrates in terms of their cellulose content resulting from the difference in the rate of degradation by the mushroom enzymes (Ibekwe *et al.,* 2008).

CONCLUSION

The maize stack (M) 33% + Sorghum stack (S) 33%+ Soybean stack (B) 33% substrate is found to be the most productive substrate for the cultivation of *P. ostreatus* at small-scale level compared to the other substrates. Further research must be conducted on biological treatment (composting of substrate) to obtain better yield. Therefore, producers should be encouraged to use this substrate for maximizing the yield in utilizing agricultural waste to produce food in the form of mushroom. Oyster mushroom can play a pivotal role in promoting food self-sufficiency; therefore, it should be included as one component of food security assurance strategy of the country.

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