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RESEARCH PAPER

Conversion of Wanza (*Cordia africana*) leafs-litter and Different Proportion of Cotton Seed Waste and Wheat Bran to Oyster Mushroom *(Pleurotus ostreatus*) Biomass

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Abstract

At present conversion of agricultural and forestry by products into resources has been given priority from governmental and nongovernmental organizations. Production of mushroom by using locally available organic wastes is one of the microbial biotechnologies which able to produce nutritionally and medically valuable food items from those refuses. The present study was under taken to evaluate the bioconversion ability of oyster mushroom *(Pleurotus ostreatus)* of substrate composed from wanza (*Cordia africana)* leaves litters with the different proportion of cotton seed waste and wheat bran to the nutrient rich and medically important mushroom biomass. The oyster mushroom culture was developed on potato dextrose agar and the spawn was prepared on yellow colored sorghum grain. The substrate was mixed, sterilized and inoculated with 5% mother spawn and inoculated bags were arranged in completely randomized design in triplicate in the mushroom house. All the quantitative data collected were analyzed using ANOVA by using by the help of SPSS computer software for window version 20. The substrate composed from *Cordia africana* dry leaves: wheat bran: cotton seed waste (80:10:10) and *Cordia africana* dry leaves: wheat bran (70:30) showed the fastest mycelial extension (0.45 cm/day) and the substrate composed from *Cordia africana* dry leaves: cotton seed waste (70:30) showed slowest mycelial extension (0.34 cm/day) and the same treatments showed shortest incubation periods (75 days) and the substrate composed from *Cordia africana* dry leaves: cotton seed waste (70:30) had longer (105 days) for overall cycle of the mushroom production.. Substrate received *Cordia africana dry* leaves: wheat bran (70:30) showed highest fresh weight in 1st flush (255g)) and substrate composed from *Cordia africana* dry leaves: cotton seed waste (70:30) gave least fresh weight (50g).. Maximum number (11) of bunches was recorded on *Cordia africana* dry leaves: wheat bran (70:30) and the least on *Cordia africana dry* leaves: cotton seed waste (70:30) (3). Pilus diameter was maximum from *Cordia fricana* dry leaves: wheat bran (90:10) (7.0cm) and the minimum (4.5cm) was noticed from *Cordia africana* dry leaves:cotton seed waste(70:30). The highest numbers of fruiting bodies were collected from *Cordia africana* dry leaves: wheat bran (70:30) (57) and the least from *Cordia africana* dry leaves: cotton seed waste (70:30) (20). Higher number of aborts was recorded on substrate composed from *Cordia africana* dry leaves: wheat bran (70: 30) (57) and the lowest on *Cordia africana* dry leaves: cotton seed waste (70:30) (20). The highest total wet/fresh weight of matures and biological efficiency were recorded from *Cordia africana* dry leaves: wheat bran (70: 30) 34068% respectively and the least on *Cordia africana* dry leaves: cotton seed waste (70:30) 100 20%, respectively. The results observed in this study clearly showed that the *Cordia africana* dry leaves supplement with wheat bran and cotton seed waste together or alone supported the growth of oyster mushroom but maximum total fresh weight and biological efficiency were recorded from *Cordia africana* dry leaves: wheat bran (70: 30) 34068% so then this combination of substrate mixture recommended for commercial production of oyster mushroom.

Keywords: *Biological efficiency, Cordia africana, cotton seed waste, Wheat bran, Oyster mushroom*

Introduction

Mushrooms are defined as a “macrofungus” that have a fleshy and used throughout the world to describe the fruiting bodies of saprophytic, mycorrhizal and parasitic fungi belongs to the order of Basidiomycota (Zeid *et al*., 2011). Production of mushroom by using locally available organic wastes is one of the microbial biotechnologies which able to produce

nutritionally and medically valuable food items from those organic wastes. As it is waste bioconversion to food, mushroom production technology is given priority by scientists in the area and from governmental and nongovernmental organization. Besides, mushroom production can be carried out throughout the year and there is the possibility of vertical increasing rather than horizontal unlike other agricultural crops. Furthermore, the nutritional content of mushroom is well above cereal crops and vegetables, as a result it contributes in mitigating the food insecurity of most developing nations. .

A number of mushrooms are considered not only as nutritionally rich food but also beneficial from the standpoint of medicinal purposes. Button mushroom (*Agaricus* spp) and Shitake (*Lentinus* spp) are widely accepted for commercial production but recently oyster mushroom (*Pleurotus* spp.) cultivation has stepped up in second position after the button mushroom as per its consumption around the globe (Gyorfi and Hajdu, 2007; Sanchez 2010). At present, cultivation of oyster mushroom has increased tremendously worldwide because of their abilities to grow at a wide range of temperature and utilizing various agro-based residues. *Pleurotus* species are efficient lignin degraders, which can grow on different agricultural wastes with broad adaptability to varied agro-climatic conditions. Growing oyster mushrooms is converting a high percentage of the lignocellulosic substrate to fruiting bodies and resulted in increasing profitability (Ortega *et al*. 1992). Of them, *Pleurotus ostreatus* demands few environmental controls, and their fruiting bodies are not often attacked by diseases and pests, and they can be cultivated in a simple and economic way (Kues and Liu 2000). It requires a short growth time in comparison to other edible mushrooms. All this makes *Pleurotus ostreatus* cultivation an excellent alternative for production of mushrooms when compared to other mushroom species (Kausar 1998).

Mushrooms with their flavor, texture, nutritional value and high productivity per unit area have been identified as an excellent food source to alleviate malnutrition in developing countries (Eswaran and Ramabadran 2000). *Pleurotus osteratus* are rich source of proteins, minerals and vitamins (Sharma *et al*., 2013). Apart from food value, its medicinal value for diabetics and in cancer therapy has been emphasized (Synylsya *et al*. 2008; Wasser 2002). *Pleurotus* species contain high potassium to sodium ratio, which makes this mushrooms an ideal food for patients suffering from hypertension and heart diseases (Wasser 2002).

The practice of mushroom cultivation is not only produces medicinal and nutritious food, but also improves the straw quality. This takes place by reducing lignin, cellulose, hemicelluloses, tannin and crude fiber content of straw making it ideal for animal feed (Ortega *et al*. 1992). Strengthening mushroom production sector could be essential in order to enable the rural economy to keep its vibrancy and development, increasing and diversifying business and employment opportunities in the rural areas, and providing income opportunities for disadvantageous groups and small family farms. Also, mushroom production gives additional/alternative income to farmers looking for a value-added product and a way to supplement farm income while making use of by products or co-products from other crops. Since oyster mushrooms can be grown on nearly any type of agricultural and forest residues, they are an ideal crop for rural areas with large amounts of cultivated hectare and residue from field crops. Organic matters containing cellulose, hemicellulose and lignin can be used as mushroom substrate i.e. rice and wheat straw, cottonseed hulls, corncob, sugarcane baggase, sawdust, coffee leaves and husks, waste paper, leaves, and so on (Yildiz *et al*., 2002). The demand of mushroom has been mounting day by day due to population growth, market expansions, changing of consumer behavior, and developments (Dhar 2014**)**.

Wanza (*Cordia africana*) is a dichotomously branched broad leaved deciduous tree which has been widely grown throughout the country. REFERENCE It is a multipurpose tree which used as a wind break, and also used for making of various house and office furniture like doors, windows, boxes etc. The leaves of the *Cordia africana* is used as animal fodder, in addition, in some localities its fruits also eaten by human. A single tree of *Cordia africana* can produce huge amount of leaves litter in a season. If not wisely disposed the piles of the leaves litters will be negatively affect the soundings for example, by making the garden unclean and untidy since the

dry leaves is very light, and easily carried by wind current a long distance from the mother tree. So, bio-conversion of this wastes in to nutrient rich and medicinally useful mushroom fruiting bodies is paramount important. The usability of different substrate for production of mushroom biomass has been reported by different investigators (Asmamaw Tesfew *et al.*, 2015; Beje Gume *et al.,* 2013; Zinabu Hamsalu *et al*., 2015). But to our knowledge production of oyster mushroom by using wanza (*Cordia africana)* leaves litters together with the cotton seed waste and wheat bran is not yet reported in Ethiopia or elsewhere in the world. Then, this article reports the bioconversion efficiency of oyster mushroom *(Pleurotus ostreatus*) from different proportion of wanza (*Cordia africana)* leafs litters, cotton seed waste and wheat bran in to mushroom fruiting bodies which is a nutritionally rich and medicinally useful food items produced from the wastes that otherwise negatively affect the environment as a result this technology of cultivation enhances sustainable utilization of natural resources besides improving the food security of most developing nations.

Materials and Methods

Organism and culture conditions

The pure culture of *Pleurotus ostreatus* originally obtained from Addis Ababa University, department of Biology Mycology Laboratory was maintained on Potato Dextrose Agar (PDA) slant prepared in the laboratory using fresh potato 250g; glucose (Dextrose) 20g; agar 20g and chloramphenicol 0.2g in 1000 mL of water and inconsistently sub-cultured in order to maintain the genetic stability of the strain. For this experiment the fungal strain was grown on the same medium prepared as described above. The medium was poured into the Petri dishes and allowed to cool under aseptic condition in a laminar flow chamber. The cooled and solidified medium was inoculated with 1cm×1cm agar block of the fungal strain and incubated at 25 ^{o}C. The growth of the culture and presence of contamination were visually inspected at three days intervals.

Grain Spawn production

In this study, the spawn (mushroom seed) of *Pleurotus ostreatus* was produced on yellow colored sorghum grain, wheat bran and calcium sulfate (gypsum) in the ratio of 88:10:2, respectively (Dawit, 1998). The required amount of sorghum grain was weighed and soaked overnight in a sufficient amount of water. The grains were washed and drained to remove the dead and floating seeds with water. After removing the excess water from the grain, the required amount of wheat bran and gypsum ($CaSO_4$ $2H_20$) were added and transferred to 1000 mL glass bottles (75% level) leaving a head space over the grain and autoclaved at 121 ^{0}C for 45 minutes. After cooling, each bottle was inoculated with 20 agar blocks (1 cm x 1 cm) of a 15 days old mushroom culture from the Petri dish and incubated for 21 days at 28±2 ^{o}C until the substrate was fully colonized and the mycelia invasion and contamination were inspected at five days intervals.

Treatments substrate composition and preparation

Five different treatments comprising different proportions of the major substrates wanza (*Cordia africana*) dry leaves and different proportion of wheat bran, cotton seed and lime stone (Calcium Carbonate 1%) on dry weight basis were used as shown in Table 1. Wanza (*Cordia africana*) dry leaves were collected from the main campus of the Ambo University and brought to microbiology laboratory, department of Biology and the leaf blade and pitole were broken down to smaller size of 2cm×2cm by hand and the required amount of wanza dry leaves was weighed by using balance and socked in sufficient amount of water over night. The required amount of cotton seed waste was also weighed and socked in sufficient amount of water as described above. The other additive of the substrate was weighed and mixed with the leaves and cotton seed waste before sterilization of the substrate.

Table 1. The composition and ratio of different substrates used in this experiment

Treatment	Treatment ratio	*Cordia africana* leaves (g)	Supplement type in (g)	
T1	90:10	450	Wheat bran (50)	-
T2	90:10	450	-	Cotton seed waste (50)
T3	80:10:10	400	Wheat bran (50)	Cotton seed waste (50)
T4	70:30	350	Wheat bran (150)	-
T5	70:30	350		Cotton seed waste (150)

While the *Cordia africana* dry leaves were mechanically broken down into small pieces by hand weighed and, socked in sufficient amount of water over night. On the next day the excess water was removed and appropriate amount of wheat bran and cotton seed waste were weighed and mixed with it to constitute the treatment types.

Excess water present in the substrates was drained thoroughly and mixed with 10 % wheat bran and one percent calcium carbonate and filled in sterilized yellow colored polyethylene bags (Kurtu pestal). The substrates were autoclaved at 15Psi pressure at 121^{o}C temperatures for 1h. After sterilization the substrates were transferred to transparent polyethylene cultivation bags for easy supervision of the growth of the mycelia and presence of contamination. Each substrate (500 g) with 70% moisture was mixed with 10 % spawn (dry weight/wet weight basis) and the inoculated polythene bags were then tightly tied with string made from polyester/cotton cloth. Pin holes were made through the bags (1/100 cm2) for drainage and aeration. Then the inoculated bags were kept in a spawn running room at room temperature in the dark until primordia were formed. After primordial formation started the bags were transferred in mushroom production house and large holes were made in the polythene bag to allow normal development of fruiting bodies.

In the mushroom house the bags were kept under normal environmental conditions and relative humidity of the room was maintained at 85–90% by keeping water in open containers at different corners of the room. The cultivating bags were irrigated using tap water every morning and evening until all flushes of *Pleurotus ostreatus* fruiting bodies were harvested. Adequate ventilation was provided to prevent increased CO_2 concentration in the room by opening the door and windows of the room for half an hour in the morning and in the evening. The mushrooms were manually harvested at maturity which was indicated by upward curving of the edges of the cap. The overall oyster mushroom production steps in this study are presented by the figure (1).

Biological efficiency was calculated and defined as the ratio of weight (g) of fresh mushrooms harvested to dry weight (g) of the substrate.

$$\text{Biological Efficiency} = \frac{\text{Weight of fresh fruiting bodies (g)}}{\text{Weight of dry substrate (g)}} \times 100$$

Data analysis

The data were analyzed by comparing the mean weights and percentage of biological efficiency through one way ANOVA. The data groups were analyzed using a Statistical Package for Social Sciences (SPSS) for windows 20.0. Treatments means were compared using LSD.

Figure 1. The different steps in the production of mushroom: (A) Pure plate culture of *Pleurotus ostreatus on* Potato dextrose agar, (B) oyster spawn ready for use prepared on sorghum grains; (C) *Cordina africana* dry leaves; (D) Substrate inoculated with spawn in the production plastic bag; (E) Primordial formation and differentiation into matures and (F) mature oyster mushroom fruit bodies ready for harvest.

Results

Mycelia extension

There were significant (P≤0.05) differences in the mycelial extension of oyster mushroom grown on different substrates composition. Substrate composed from *Cordia africana* dry leaves:wheat bran: cotton seed waste (80:10:10) and *Cordia africana* dry leaves:wheat bran (70:30) showed the fastest mycelial extension while the substrate composed from *Cordia africana* dry leaves:cotton seed waste (70:30) exhibited slowest mycelial extension on 7th and 14th days of incubation periods (Table 2). There were significant (P≤0.05) differences in the days equired for complete invasion of the substrates receiving different treatments. The time required for complete invasion of the, substrate were significantly (P≤0.05) less for substrate composed from *Cordia africana* dry leaves:wheat bran: cotton seed waste (80:10:10) and *Cordia africana* dry leaves:wheat bran (70:30) when compared to that of *Cordia africana* dry leaves:cotton seed waste (70:30) (Table 2).

Total days required to complete the production cycle was observed shortest for *Cordia africana* dry leaves:wheat bran:cotton seed waste (80:10:10) and *Cordia africana* dry leaves:wheat bran (70:30) while it was took more days for *Cordia africana* dry leaves:cotton seed waste (70:30).

Table 2. Mycelial extension on the different substrates composition at treatments measured on 7th and 14th days of incubation

Treatment ratios	Mycelia extension in (cm)		Mean values (cm/day)	Number of days required for complete invasion	Total days required to complete the cycle
	7th day	14th day			
90:10	2.9	4.8	0.34	30	92
90:10	3.1	6.2	0.43	24	83
80:10:10	3.6	7.2	0.45	20	75
70:30	3.6	7.2	0.45	20	75
70:30	2.2	4.4	0.32	40	105
P value (ANOVA)	0.001	0.003	0.001	0.001	0.001
STDEV	0.58	1.32	0.06	8.34	12.73

Growth rate of mushroom (Flushes)

Mean incubation periods of mushroom flushes showed highly significant differences (P≤0.05). *Cordia africana* dry leaves:wheat bran:cotton seed waste (80:10:10) and *Cordia africana* dry leaves:wheat bran (70:30) showed relatively shorter incubation to 1st flush while it took more days for *Cordia africana* dry leaves:cotton seed waste (70:30), incubation to 1st flush. In this experiment 1st -2nd flush and 2nd – 3rd flush took relatively more days for substrate composed from *Cordia africana* dry leaves: wheat bran (90:10) and *Cordia africana*: cotton seed waste (70:30), as compared to the rest of the treatment. In this experiment none of the substrate composition gave the fourth flush (Table 3).

Table 3. Incubation periods of different flushes of different substrate composition

Treatment ratios	Incubation -1st flush (days)	1st -2nd flush (days)	2nd – 3rd flush (days)
0:10	49	22	21
90:10	43	21	19
80:10:10	38	19	18
70:30	38	19	18
70:30	59	24	22
P value (ANOVA)	0.004	0.434	0.036
STDEV	8.35	2. 12	1.81

Pinning to maturation duration of oyster mushroom

The mean data taken from pinning to maturation of each treatment did not showed significant variation (P≤0.05) on pinning to maturation of oyster mushroom. The substrate composed from *Cordia africana* dry leaves:wheat bran:cotton seed waste (80:10:10) and *Cordia africana* dry leaves:wheat bran (70:30) showed relatively shorter pinning to maturation as compared to the other treatments (Table 4).

Table 4: Pinning to maturation of the oyster mushroom under different substrate composition

Treatment ratios	Mean duration (days)		
	1stFlush	2nd Flush	3nd Flush
90:10	7	6	5.5
90:10	6.5	6	5.5
80:10:10	7	6.5	5.0
70:30	7	6.5	5.0
70:30	7	7	6.5
P Value (ANOVA)	0.981	0.580	0.164
STDEV	0.22	0.42	0.61

Mean values with in a column sharing the same superscript letter(s) are not significantly different by using LSD test at P≤0.05.

Yield of mushroom per flushes

Yield of mushroom per flush (wet weight) showed significant variation between treatments (P≤0.05) as well as between flushes (Table 5). *Cordia africana* dry leaves:wheat bran (70:30) showed highest fresh weight in grams in 1st flushes followed by *Cordia africana* dry leaves:wheat bran:cotton seed waste (80:10:10). The result observed with *Cordia africana* dry leaves:cotton seed waste was not comparable with all the treatments. In all the treatments the second and the third flushes were very low as compared to the first flushes (Table 5).

Table 5. Mean yield per flush in the different substrate composition

Treatment ratios	Mean fresh- weight of mushroom (g)			
	1st Flush	2nd Flush	3nd Flush	Total
90:10	187.50	40	22.50	250
90:10	225.50	50	25.25	301
80:10:10	240	60	20	320
70:30	255	60	25	340
70:30	50	30	20	100
P Value (ANOVA)	0.001	0.001	0.009	0.001
STDEV	83.03	13.04	2.56	96.64

Mean values with in a column sharing the same superscript letter(s) are not significantly different by using LSD test at P≤0.05

Number of aborts, bunches and matures

More number of Aborts were recorded on *Cordia africana* dry leaves:wheat bran (70:30) followed by *Cordia africana* dry leaves:wheat bran: cotton seed waste (80:10:10) while *Cordia africana* dry leaves: cotton seed waste (70:30) showed least number of aborts. The remaining treatments showed intermediate number of aborts between the highest and lowest treatments (Figure 2). More number of mature fruting bodies were recorded on *Cordia africana* dry leaves:wheat bran (70:30) followed by *Cordia africana* dry leaves:wheat bran:cotton seed waste (80:10:10) while *Cordia africana* dry leaves: cotton seed waste (70:30) showed least number of manture fruting bodies (Figure 2). More number of bunches were recorded on *Cordia africana* dry leaves:wheat bran (70:30) followed by *Cordia africana* dry leaves:wheat bran:cotton seed waste (80:10:10) while *Cordia africana* dry leaves:cotton seed waste (70:30) gave least number of bunches (Figure 2).

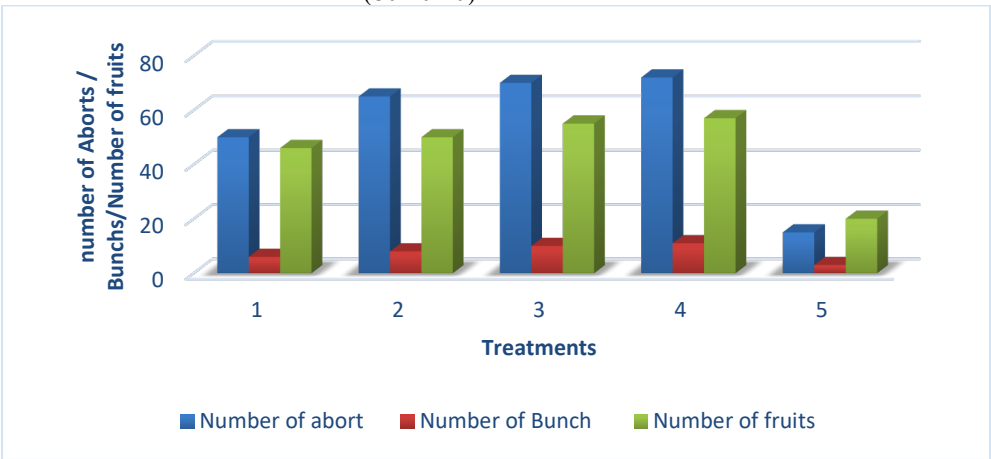

Figure 2. Number of Aborts, bunchs and fruits of oyster mushroom grown on the substrate composed from (Wanza)Cordina africana dry leaves and wheat bran and cotton seed wastes: (1) *Cardia africana* dry leaves:wheat bran (90:10), (2) *Cardia africana* dry leaves:cotton seed waste (90:10) (3) *Cardia africana* dry leaves:wheat bran:cotton seed waste (80:10;10), (4) *Cardia africana* dry leaves:wheat bran (70:30), (5) *Cardia africana* dry leaves:cotton seed waste (70:30).

Pilus Diameter and Stipe length

The plius diameter collected from different treatments showed significant differences at (P≤0.05) differences between treatments. Pilus diameter was found to be largest for the samples collected from *Cordia africana* dry leaves: cotton seed waste (90:10); *Cordia africana* dry leaves: wheat bran: cotton seed waste (80:10:10) and *Cordia africana* dry leaves: cotton seed waste (70:30), while it was smallest for the sample collected from *Cordia africana* dry leaves: cotton seed waste (70:30) (Figure 3). The stipe length of the samples collected from different treatments did not show significant variation among the treatments (Figure 3).

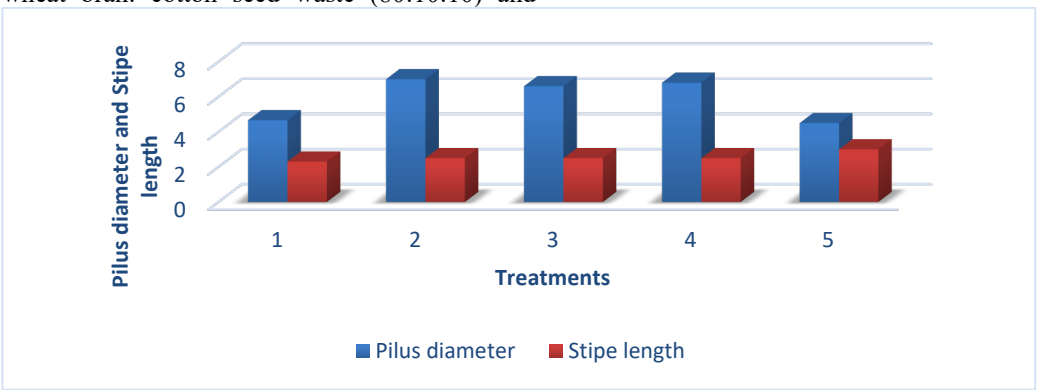

Figure 3. Pilus diameter ans stipe length of oyster mushroom grown on the substrate composed from Wanza (*Cordia africana* dry leaves) and wheat bran and cotton seed wastes: (1) *Cardia africana* dry leaves: wheat bran (90:10), (2) *Cardia africana* dry leaves::cotton seed waste (90:10) (3) *Cardia africana* dry leaves:wheat bran:cotton seed waste (80:10;10), (4) *Cardia africana* dry leaves: wheat bran (70:30), (5) *Cardia africana* dry leaves:cotton seed waste (70:30).

Total yield and biological efficiency

The highest total wet/fresh weight of matures was recorded in *Cordia africana* dry leaves:wheat bran (70:30), followed by *Cordia africana* dry leaves:wheat bran: cotton seed waste (80:10:10). The least total fresh/wet weight was recorded in *Cordia africana* dry leaves:cotton seed waste (70:30) (Table 5). The effect of different treatments on biological efficiency of oyster mushroom showed significant (P≤0.05) differences. The highest biological efficiency was recorded with *Cordia africana* dry leaves: wheat bran (70:30) followed with *Cordia africana* dry leaves:wheat bran:cotton seed waste (80:10:10). The least was recorded with the treatment *Cordia africana* dry leaves:cotton seed waste (70:30 (Figure 4).

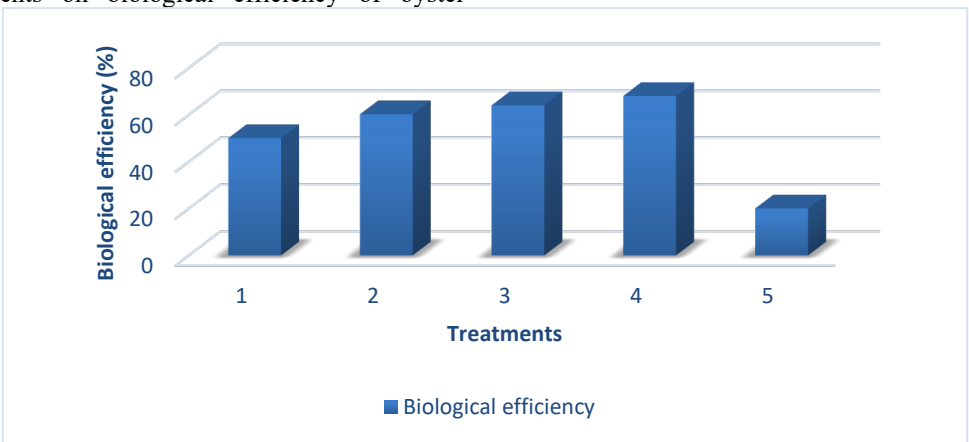

Figure 4. Biological efficiency of oyster mushroom grown on the substrate composed from Wanza (Cordina africana dry leaves) and wheat bran and cotton seed wastes:: (1) *Cardia africana* dry leaves: wheat bran (90:10), (2) *Cardia africana* dry leaves::cotton seed waste (90:10) (3)*Cardia africana* dry leaves: wheat bran :cotton seed waste (80:10;10), (4) *Cardia africana* dry leaves: wheat bran (70:30), (5) *Cardia africana* dry leaves::cotton seed waste (70:30).

Discussion

It has been well recognized that huge by products and or co-products of agricultural, agro-processing units and forestry processing by products are available specially in countries like Ethiopia whose main economic activities is based on Agriculture. If these huge wastes, will not be disposed wisely it will affect the environment negatively. With this regard the usability of Wanza (*Cordia africana*) dry leaves as a major substrate for oyster mushroom production was carried out. In this study, the complete colonization of the substrate took 20 days in the faster and 40 in the slower and then another 18 day were required after complete invasion of the mycelium to first harvest in the fastest treatment and 19 days in the slower.

In this study, the periods taken for spawn running on the different treatments showed relatively longer periods as compared to results reported in the literature. Ashraf *et al.* (2013) reported shorter periods for the substrate to completely colonized by mycelium of the different oyster mushroom species which also indicates differences among the different species and the substrate compositions. According to these authors the minimum number of days 16.20 taken by *P. ostreatus* while species *P. sajor-caju* and *P. djmor* showed same level of significance with 18.07±0.69 and 18.67±0.61. Oseni *et al*. (2012) reported periods of colonization to first harvest from 33 to 43 days on fermented saw dust supplemented with different proportions of wheat bran. In this experiment it took longer days from complete colonization to primordial formation, which is not in line with the reports in the literature. These differences may be due the differences in chemical composition of the major substrate, and the mixture composition of the different components, which may act as early physiological inhibition of the mushroom strain studied.

Ashraf *et al*., (2013) reported that all the treatments tested were did not showed variation and days taken for primordial initiation was only 3.73 to 5.13days after complete colonization. In this study, in all the treatments, the successive pinning to harvest duration was shortened by at least half day. The shortest mean duration of pinning to maturation was 6.5 days in the first, 6 days in the second, 5.5 days in third. And the longest mean duration of pinning to maturation was 7 days, in the first, 6.5 days in the second, and 5.5 days in third. The duration observed in the present study was longer periods when compared with the report of Beje Gume *et al*., (2013) which was 3.3 days in the shortest and 6.0 days in the longest. This may be due to the fact that the time cultivation of the mushroom and other environmental factors during the cultivations. Studies indicated that environmental factors affects the incubation periods of oyster mushroom. According to Zadrazil (1976) and Daba *et al*. (2008) longer period of incubation for oyster mushrooms was at lower temperatures and low relative humidity.

In this work, in all the treatments the yield (fresh/wet weight) of the mushroom harvested in the first cycle was greater than the remaining successive harvests. In addition to the reduced yield in the next consecutive harvest, all the treatments did not give harvest at the fourth cycle. Our observation on the yield of the different harvest is in line with reports in the literature. Ashraf *et al*., (2013) reported that the different treatments vary in the amount of mushroom yield harvest at different flushes and at each successive harvest the amount of the yield decline. Number of bunches formed on different treatments were significantly different. Substrate composed from *Cordia africana* dry leaves:wheat bran (70:30) produced highest number of bunches (11), followed by *Cordia africana* dry leaves: wheat bran: cotton seed waste (80:10:10) (10). The least number of bunches was recorded in substrate composed from *Cordia africana* dry leaves:cotton seed waste (70:30) (3).

The highest and nearly equal numbers of fruiting bodies were collected from the substrate composed from *Cordia africana* dry leaves:wheat bran (70:30) and *Cordia africana* dry leaves:wheat bran:cotton seed waste (80:10:10) 57 and 55, respectively. Substrate composed from *Cordia africana* dry leaves:cotton seed waste (70:30) gave the least number of fruiting bodies (20). Higher number of aborts were recorded on substrate that received *Cordina africana* dry leaves:wheat bran (70:30) (72) followed by *Cordia africana* dry leaves:wheat bran: cotton seed waste (80:10:10) (70). The least number of aborts were recorded on substrate composed from *Cordia africana* dry leaves: cotton seed waste (70:30) (15).

In this study more number of bunches result in more number of fruiting bodies. This observation was in line with the results reported by Beje Gume *et al*., (2013) who reported that substrates that gave higher yield also contained higher number of propagating fruit bodies per bunches. Kimenju *et al*. (2009) reported that more than 50% of pinheads emerged did not grow into marketable products. Beje Gume *et al*.(2013) also observed high rate of pinhead abortion from low-yield substrates such as *sd1C* (*cordial africana* saw dust) and *Zc*Ch (maize comb with mixed coffee bean husks).

In this study the largest pilus diameter was measured with the substrate received *Cordina africana* dry leaves:cotton seed (90:10) (7 cm) followed by *Cordina africana* dry leaves:wheat bran (70;30) (6.8 cm) and the smallest with *Cordia africana* dry leaves:cotton seed waste (70:30) (4.5 cm). The rest of the treatments gave pilus diameter between the largest and the smallest. Larger pilus diameter significantly increased the total fresh/wet weight of oyster mushroom. Oseni et *al*., (2012) reported highest mean pilus diameter 57.9 to 62.3 mm on sawdust supplemented with different levels of wheat bran. According to these authors, the largest pilus diameter was obtained from sawdust substrate supplemented with 15% wheat bran (62.3 mm) and the smallest obtained on sawdust substrate supplemented with 5% wheat bran (57.9 mm).

The stipe length of all the 5 treatments did not vary significantly (2.3-3.0 cm). This observation is in agreement with the results of Beje Gume *et al*. (2013) whose reported stipe length ranging from 1.4-1.9 cm in different treatments. Oseni *et al.* (2012) observed stipe length of oyster mushrooms ranging from 39.4 to 59.5 mm (3.94-5.95 cm) on fermented sawdust substrate supplemented with different wheat bran levels and highest stipe length (59.5 mm) (5.95 cm) was observed on substratum supplemented with 15% wheat bran. The total fresh weight of the mushroom was highest in substrate that received *Cordia africana* dry leaves:wheat bran (70;30) (340 g of mushroom per 500 g of the substrate) followed by *Cordia africana* dry leaves:wheat bran: cotton seed waste (80:10:10) (320 g of mushroom per 500 g of substrate) and the least total fresh weight of the mushroom was recorded with the substrate composed from *Cordia african* dry leaves: cotton seed waste (70:30) 100 g per 500 g substrate).

The ability of the mushroom strain conversion of the substrate in a usable mushroom biomass is one of the most important criteria for that substrate to be used for mass production of the mushroom under the consideration. In this study, the biological efficiency of the mushroom grown on the different substrate composed from different substrate mixture was greatly varied. The highest biological efficiency was recorded for the substrate composed from *Cordia africana* dry leaves:wheat bran (70: 30) (68%) followed by the substrate composed from *Cordia africana* dry leaves:wheat bran:cotton seed waste (80:10:10) (64%) and the least was from the substrate composed from *Cordia african* dry leaves:cotton seed waste (70:30) (20%). Similar biological efficiency was reported for oyster mushroom grown on wheat straw, sinar straw and barely straw, 68.16%, 66.02% and 65.52%, respectively (Asmamaw *et al*. 2015). Relatively more biological efficiency was reported by Zinabu *et al*., (2015) for oyster mushroom grown on sugar cane baggase (70.5%).

Conclusion

Productions of edible mushroom, has been considered as diversification of food production and also contribute, in the struggle for food self-sufficiency and attaining food security particularly in the developing world in which most of the communities suffer from deficiencies of proteins and minerals. The results observed in this study clearly showed that the *Cordia africana* dry leaves supplement with wheat bran and cotton seed waste together or alone supported the growth of oyster mushroom indicating the possibility of conversion of these waste biomasses into nutrient rich and medically important mushroom fruiting bodies. In this study, two of the trials *Cordia africana* dry leaves:wheat bran (70: 30) and *Cordia africana* dry leaves: wheat bran:cotton seed waste (80:10:10) were gave highest total biomass and biological efficiencies which were indicative of the usability of this combination of the substrate mixture for the farm, pilot and industrial scale production of oyster mushroom. .

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