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Cultivation of Three Pleurotus spp., on Khat (Catha edulis) Leftover

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ABSTRACT

This study was carried out to evaluate the growth and performance of three oyster mushroom species: Pleurotus oestreatus, Pleurotus oestreatus-florida and Pleurotus sajor-caju on khat leftover substrate. The moisture content of khat solid medium was adjusted to 55-60% and was filled in plastic bags (16×33 cm in size). The plastic bags were then autoclaved at 121°C for 90 min. After inoculation, the bags were kept in laboratory in absence of light and ventilation. The treatments were laid out in Complete Randomized Design (CRD) with eight replications. The result of the experiment indicated that Pleurotus sajor-caju gave significantly higher Number of Primodial Formation (NPF), fresh (FWFB) and Dry Weight Fruiting Body (DWFB) production than the rest of tested oyster mushrooms. The highest FWFB was obtained from *Pleurotus saior-caiu* (422.36 g kg⁻¹ of dry substrate). The lowest FWFB (221.20 g kg⁻¹) was observed from Pleurotus oestreatus-florida. A decline of FWFB, DWFB and NPH was observed time of harvesting. Biological Efficiency (BE) of Pleurotus sajor-caju was significantly higher than those obtained from other two oyster mushrooms, although the data indicated lowest BF when compared with previously reported for oyster mushroom. For this result, we can conclude that khat leftover is the relatively suitable substrate for Pleurotus sajor-caju cultivation. Further research study would be recommended on different methods of pretreatments of khat leftover and optimal formulation of suitable substrate by adding different high nitrogen containing materials to improve the productivity on khat leftover.

Key words: Biological efficiency, khat leftover, oyster mushroom, Haramaya

INTRODUCTION

Khat (Catha edulis) is one of the most important cash crops which fetch foreign currency for the economy of Ethiopia. The total area of land under khat cultivation in Ethiopia in the year 1997/98 was estimated at 78,570 h (CSA., 1998). Within a decade, the area occupied by the crop has doubled to 163,227 ha (CSA., 2008). Oromia region of Ethiopia, particularly East and West Hararghe zone, is the most important production and trade center for khat. Despite its importance in the economy, it is not difficult to observe thousands of tons khat left-over polluting the region. The

leftover consists of over-matured leaves, twigs, stems and shoots that are discarded as not chewable by humans. These tons of khat left-over remains to be a serious environmental hazard due to the tannins and other toxic materials it contains (Mekasha *et al.*, 2007). The presence of tannins, especially the condensed ones, is toxic to soil microbial population and as a result decrease net soil nitrogen mineralization (Bradley *et al.*, 2000; Fierer *et al.*, 2001; Nierop *et al.*, 2006).

White rot fungi are currently being extensively studied for bioremediation purposes (Gao *et al.*, 2010). Of which, mushroom which are the good sources proteins, sugars, glycogen, lipids, vitamins, amino acids and crude fibers, are

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able to grow on wide variety of agro-industrial lignocellulosic wastes due to their production of ligninolytic and hydrolytic enzymes (Mikiashvili et al., 2006). Enzyme complexes in certain mushroom strains from the genus Pleurotus among others include: Cellulase, cellobiase, hemicellulase, ligninase, laccase (Platt et al., 1984; Tsang et al., 1987). Of which, Pleurotus ostreatus and is macrofungi which utilize polysaccharides (cellulose and hemicelluloses) from various lignocelluloses to produce the popularly known oyster mushroom for human consumption (Chahal, 1989). Pleurotus sajor-caju has also been known to have the ability to degrade lignin to some extent (Bourbonnais and Paice, 1988). Sobal et al. (2003) also reported that Pleurotus ostreatus degraded up to 75.4% of condensed tannins within 24h of cultivation. The present study deals with the cultivation of *Pleurotus* spp., of mushroom on khat which is common and abundantly available waste in the region and to produce edible mushroom for human food. Therefore, the objective of this study was to evaluate the suitability of khat leftover as substrate for oyster mushroom (Pleurotus oestreatus, Pleurotus oestreatus-florida and Pleurotus sajor-caju) cultivation.

MATERIALS AND METHODS

Source of species of mushroom and khat-leftover: Pleurotus oestreatus and Pleurotus sajor-caju were obtained their slant cultures which are maintained on Potato Dextrose Medium (PDA) medium, from Addis Ababa University, Department of Biology, Mycology Laboratory. While spawn form of Pleurotus oestreatus-florida was obtained from Haramaya University, soil microbiology research project laboratory.

Spawn preparation: Sorghum grains were used as substrate and support for growth. The grains were washed in flowing water and then cooked during 10 min (after boiling) in deionized water at the ratio of 1:2 (sorghum grains:water, w.v). The extract obtained, was drained and the grains were supplemented with 0.35% CaCO₃ and 1.3% CaSO₄. The sorghum grains, cooked and supplemented, were then packed a glass jar of 500 mL capacity, closed and sterilized in an autoclave at 121°C, for 1 h. Once cooled, each glass jar was inoculated with 6 agar disks of 8 mm diameter containing mycelium of respective oyster species (Pleurotus oestreatus and Pleurotus sajor-caju). For Pleurotus oestreatus-florida, glass jar containing sterilized sorghum grain was inoculated with spawn of Pleurotus oestreatus-florida which was previously prepared. The inoculated jars were incubated at 25°C in a dark room until mycelia had completely covered the bags. The spawn were ready to be used for the inoculation of the solid substrate when the mixture turn totally to white. This process took approximately 2-3 weeks to complete.

Mushroom cultivation: The mushroom growing process was carried out in the Experimental Mushroom House of the Haramaya University, School of Natural Resources

Management and Environmental Sciences in which the temperature, ventilation and relative humidity could be manually controlled. The substrate (Khat leftover) was collected from central market place of khat which is located in Aweday Khat market, Eastern Ethiopia. The major chemical properties of khat leftover is indicated as follows: Organic matter (%) = 92.2, crude protein (%) = 12.6, lignin (%) = 7.8, phosphorus (%) = 0.1, sodium (Na) (ppm) = 56.5, potassium (K) (%) = 0.7, calcium (Ca) (%) = 2.4, magnesium (Mg) (%) = 0.4, Mn (ppm) = 18. Khat left over was cut into small pieces. The substrate was soaked in the tap water for overnight. The excess water in the substrates was allowed to run off until the moisture content reached to 70% (± 5). The moistened substrate (1500 g for each bag) was packed into polypropylene bags (16×33 cm), sealed plugs held in place with plastic collars, then autoclaved at 121°C for 90 min.

This experiment included three treatments. These three treatments corresponded to three species of oyster mushroom species. Eight bags each weighing 1500 g of wet substrate were used for each treatment. Each treatment was replicated eight times. The treatments were laid out in Complete Randomized Design (CRD). All bags were assigned at random places in the cropping room. Cooled substrates were then under aseptic conditions inoculated 3% (w/w) spawn by placing the spawn on the substrate surface through the opening at the top of the bag. Bags were incubated for 25 days at 25°C. During mycelial growth phase, the bags were neither aerated nor illuminated. After the complete colonization of substrate with mycelia, the bags were transferred to cropping room set at 85-90% relative humidity and 12 h light/12 h dark cycle using white fluorescent bulbs.

After primordial formation, two large slashes were made on the bag surfaces to allow development of fruit bodies. After the primordial emerged, the holes were manually made adjacent to emerged primordial. Mushrooms were manually harvested when matured and lasted seven weeks. At the end of the 7 weeks harvesting period, yield (fresh and dry biomass of mushroom), days required for mycelia coverage, Number of Primordial Formation (NPF) and Biological Efficiency (BE) were calculated.

Yield and Biological Efficiency (BE): Total weight of all the fruiting bodies harvested from all the three pickings were measured as total yield of mushroom. The biological efficiency (yield of mushroom per kilogram substrate on dry wt. basis) was calculated by the following formula (Chang and Miles, 1991) and expressed as percentage.

BE (%) =
$$\frac{\text{Fresh weight of mushroom}}{\text{Dry weight of substrate } (1000 \text{ g})} \times 100$$

Moisture content: The moisture content of mushroom were expressed in percent and calculated by the following formula:

$$\label{eq:Moisture content (\%) = } \frac{\text{Weight of fresh sample - Weight of dry sample}}{\text{Fresh weight of sample}}$$

Statistical analysis: All the data obtained were analyzed by one-way ANOVA and test of significant differences were determined by Turkey-HSD at p<0.05. Data were analyzed using the statistical package SAS 9.2.

RESULTS AND DISCUSSION

Number of days required for mycelium coverage: Significant difference of NMC was also observed among different species of oyster mushroom cultivate on khat leftover (Table 1). Pleurotus sajor-caju was taken significantly lower NMC than those observed in other oyster mushroom species. The lowest NMC was 16.27 days in Pleurotus sajor-caju while the highest NMC was 25.57 days in Pleurotus florida. However, the study indicated that the number of days required for primordial initiation of oyster mushroom was between 17-19 for Pleurotus oestratus (Salmones et al., 2005). Similarly, the number of days taken for mycelium colonization of Pleurotus sajor-caju ranged from 21-36 days grown on wheat straws supplied with olive mill waste water (Kalmis and Sargin, 2003). Required days to production for Pleurotus cornucopiae varied from 21-47 days depending on the amount of spawning and supplements (Royse et al., 2004). The authors found that the fewer days to production was related to higher biological efficiency and fresh weight biomass as the present study shows for Pleurotus sajor-caju.

Number of harvesting cycle: The Number of Harvesting Cycle (NHC) also exhibited significant difference among the species of oyster mushroom cultivated on khat leftover (Table 2). Significantly higher NHC was obtained from *Pleurotus sajor-caju* cultivated on khat leftover. The highest NHC was 3.857 from *Pleurotus sajor-caju* followed by 2.571 from *Pleurotus florida* and the lowest 2.143 from *Pleurotus oestratus*.

Number of prim ordial formation: The growth performances of different oyster mushroom species (Pleurotus oestreatus, Pleurotus oestreatus-florida and Pleurotus sajor-caju) were tested on khat leftover at Haramaya University experimental site. The result indicated a significant difference in all investigated parameters among oyster mushroom species (Table 1 and 2). Significantly higher Number of Primodial Formation (NPF) was obtained from Pleurotus sajor-caju compared to the remaining two oyster mushroom species. The highest NPF (5.57) was obtained from Pleurotus sajor-caju followed by 3.29, from Pleurotus florida. Figure 1 show that NPF decreased with increasing the time of cultivation regardless of the species of oyster mushroom. This is probably due to the exhaustion of the nutrients present in the substrates. In Pleurotus oestratus species, none of the pin head was produced at the 7th week of cultivation. Inconsistent reduction of NPF was also observed in Pleurotus florida in which the higher NPF was observed in 4th week as compared to those produced in the 3rd week. This is probably due to the late coverage of mycelium which consequently causes higher NPF in the late stage of cultivation. From this, it can be concluded that Pleurotus sajor-caju is the best species of oyster mush which gives sustainable higher pinhead for extended period of time than the other tested oyster mushrooms.

Fresh weight of fruiting bodies: Cumulative Fresh Weight of Fruiting Bodies (FWFB) was also displayed significant difference among difference species of oyster mushroom cultivated on khat leftover (Table 1). Table 3 also indicated positive and significant correlation among investigated traits of mushroom. The FWFB positively correlated with NPF (R² = 0.6002, at p<0.001) and Number of Harvesting Cycle (NHC) (R² = 0.5923 at p<0.001) and negatively correlated with number of days required for mycelium coverage (NMC)

Table 1: Number of days for mycelium coverage, number of pin heads, fresh and dry weight of mushroom cultivated on khat leftover at Haramaya experimental site

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Treatments	No. of pin head	No. of days for mycelium coverage	Fresh weight (g)	Dry weight (g)
PS	5.57+0.202ª	16.27+0.522 ^b	422.36+30.765°	34.04+0.915°
PO	3.14+0.508 ^b	23.00+1.024ª	266.06+4.0060 ^b	25.87+4.005ab
PF	3.29+0.360 ^b	25.57+1.043°	221.20+26.517 ^b	24.14+1.782 ^b
Mean	04.000	21.620	303.210	28.020
LSD	01.364	03.234	122.820	09.333
CV (%)	25.000	10.960	029.690	24.420
F value	13.000****	28.640***	009.630**	04.180**

*Significant at 0.05, ***Highly significant at 0.01, ***Very highly significant at 0.001, PS: Pleurotus sajor-caju, PO: Pleurotus oestreatus, PF: Pleurotus oestreatus-florida, Means in the same column followed by the same letter are not significantly different at the 5% probability level according to Tukey's test

Table 2: Number of harvesting cycles, biological conversion and moisture contents of mushroom cultivated on khat leftover at Haramaya experimental site

Treatments	No. of harvesting cycles	Biological efficiency (%)	Moisture content (%)
PS	3.857+0.261°	42.34+3.08 ^a	91.7+1.5°
PO	$2.143 \pm 0.406^{\circ}$	26.61+4.27 ^b	89.7+1.1a
PF	2.571+0.297 ^b	22.12+2.65 ^b	88.2+1.5a
Mean	2.857	30.32	89.9
LSD	1.178	12.282	0.0414
CV (%)	30.230	29.69	3.37
E value	7.470**	9.63**	2 32ns

*Significant at 0.05, ***Highly significant at 0.01, ****Very highly significant at 0.01, PS: Pleurotus sajor-caju, PO: Pleurotus oestreatus, PF: Pleurotus oestreatus-florida, Means in the same column followed by the same letter are not significantly different at the 5% probability level by Tukey's test

Table 3: Correlation among the investigated parameters obtained from three *Pleurotus* spp., grown on khat leftover

Parameters	NHC	NMC	FWFB	BE	DWDF	MC	NPF
NHC	1.0000						
NMC	-0.5115*	1.0000					
FWFB	0.5923**	-0.6616**	1.0000				
BE	0.5923***	-0.6616**	1.0000***	1.0000			
DWDF	0.7412***	-0.5269*	0.7174***	0.7174***	1.0000		
MC	$0.2126^{\rm ns}$	-0.4259**	0.6987***	0.6987****	$0.0767^{\rm ns}$	1.0000	
NPF	0.6995***	0.6231***	0.6002***	0.6002***	0.7300****	$0.2221^{\rm ns}$	1.0000

ns: Non significant, *Significant at 0.05, ***Highly significant at 0.01, ***Wery highly significant at 0.001, NHC: No. of harvesting cycles, NMC: No. of days required for mycelium coverage, FWFB: Fresh weight of fruiting body, DWDF: Dry weight of fruiting body, MC: Moisture content, NPF: No. of primodial formation, BE: Biological efficiency, Means in the same column followed by the same letter are not significantly different at the 5% probability level by Tukey's test

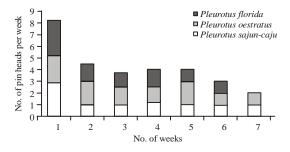


Fig. 1: Number of pin heads per week of oyster mushroom along different time (weeks) at Haramaya site

 $(R^2 = 0.6616 \text{ at p} < 0.001)$. Wang et al. (2011) indicated the different amount of fresh mushroom biomass harvest from different species of oyster mushroom. The study reported the fresh weight of mushroom harvested ranging from 72-138 g and from 199-232 g, respectively, from Pleurotus abalonus and Pleurotus geesteranus obtained from 650 g dry weight of substrate. Yildiz et al. (2002) also found that the fresh biomass of Pleurotus oestratus ranged from 15.39-467.97 cultivated on 570 g of different substrate. In the present study, Pleurotus sajor-caju gave significantly higher FWFB as compared to those obtained from other tested ovster mushroom. This result is probably related with higher NPH produced which could, in turn, leads to higher productivity of fruiting body. The highest cumulative FWFB was 422.36 g which was obtained from Pleurotus sajor-caju, followed by 266.06 g from Pleurotus oestratus, probably due high production of lascassas enzymes which oxidize phenolic compound which could consequently, improve the productivity of Pleurotus sajuncaju on high tannin containing khat leftover (Bettin et al., 2011). In the present study indicated comparable amount of FWFB from *Pleurotus sajor-caju* as that of obtained ranging from 23.2-414.18 g cultivated on different substrates (Ragunathan and Swaminathan, 2003). The lowest FWFB was 221.20 g which was harvested from *Pleurotus florida*. Figure 2 indicates the decreases of FWFB with increasing time of harvesting, irrespective of the species of oyster mushroom.

Dry weight of fruiting body: As has been indicated in FWFB, a significant difference in cumulative Dry Weight of Fruiting Body (DWFB) among different species of oyster mushroom cultivated on khat leftover was observed.

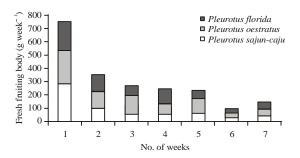


Fig. 2: Fresh fruiting body weight of oyster mushroom along different time (weeks) at Haramaya site

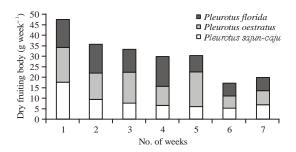


Fig. 3: Dry fruiting body weight of oyster mushroom along different time (weeks) at Haramaya site

Pleurotus sajor-caju gave significantly higher DWFB than those obtained from other species of oyster mushroom. The highest DWFB was 34.04 g from Pleurotus sajor-caju followed by 25.87 g which was harvested from Pleurotus oestratus. The lowest DWFB was 24.14 g from Pleurotus oestratus florida. Generally Fig. 3 indicates declined of DWFB productivity with increasing time of harvesting, irrespective of oyster mushroom species that were cultivated.

Biological efficiency: Biological Efficiency (BE) indirectly indicates the suitability of the substrates for cultivation of particular species of mushroom. The higher the BE, the greater will be the suitability of the substrate for cultivation of that particular strain of mushroom. Accordingly, the present study indicates significant difference in BE among tested oyster mushroom at p<0.05 (Table 2). *Pleurotus sajor-caju* gave significantly higher BE than the other tested oyster mushroom. The highest BE was 42.34% followed by 26.61 from

Pleurotus oestratus while the lowest was 22.12% which was obtained from *Pleurotus oestratus florida*. The correlation analysis indicated positive correlation between BE with DWDF ($R^2 = 0.7174$ at p<0.001), NPF ($R^2 = 0.6002$ at p < 0.001) and NHC (R² = 0.5923, p < 0.001) (Table 3). It was also negatively correlated with NMC ($R^2 = 0.6616$ at p<0.001). The observed BE was much lower than previously reported for different oyster mushrooms including Pleurotus oestratus (Salmones et al., 2005). They reported that the BE of Pleurotus oestratus was greater than 71.6% up to 86.5%. Lower BE of Pleurotus oestratus, however, were reported on the sawdust and tilia leaves substrates (less than 10% BF) (Yildiz et al., 2002). Similarly, the BE of Pleurotus oestratus cultivated on spent beer grain was ranged from 5.8-16.9 with supplement of wheat barn. Lower yield in khat substrate may probably have been observed due to the presence of higher phenolic content in khat leftover which inhibits the growth of fungal and hydrolytic enzymes which catalyse the breakdown of cellulolytic and hemicellulolytic components of plant cell walls (Buswell and Ericksson, 1994). The substrate with different carbon and nitrogen supplements improved the biological efficiency of oyster mushroom (Wu et al., 2003). However, Wang et al. (2011) found that the BE of Pleurotus abalonus and Pleurotus geesteranus ranged from 40.0-66.3% which were cultivated on Asparagus straw. It has been reported that BE of oyster mushroom in rice or wheat straw was about 40%. From this result, we can conclude that, due to higher BE with Pleurotus oestratus khat left over is the potential substrate for Pleurotus oestratus.

Fruiting body production over time: Table 4 indicates a variation of FWFB produced from three tested oyster mushroom harvested in each week. In the 1st week, significantly higher FWFB was obtained Pleurotus sajor-caju which 376.00 g followed by 254.33 g was obtained from *Pleurotus oestratus*. In contrast, Pleurotus oestratus florida gave significantly higher FWFB in 2nd week of harvesting as compared to those obtained from other species of oyster mushroom. While in 3rd week of harvesting, Pleurotus oestratus was produced significantly higher FWFB than which obtained from other species of tested oyster mushroom. These differences among the tested oyster mushroom could be related late formation of primodial in Pleurotus oestratus as compared to Pleurotus sajor-caju and Pleurotus oestratus florida. In 4th week and further harvesting cycles, the data indicated non-significant difference in FWFB obtained from tested oyster mushroom. When we see the Moisture Content (MC), the data indicate non-significant difference among the fruiting body obtained from tested oyster mushroom cultivated on khat leftover, indicating the presence of acceptable relative humidity in cropping room. The data, however, indicated higher MC in Pleurotus sajor-caju which was 91.7% followed by MC obtained the fruiting body of Pleurotus oestratus.

Table 5 indicates variation of DWFB harvested in different weeks obtained from *Pleurotus sajor-caju*, *Pleurotus oestratus* and *Pleurotus oestratus florida* cultivated on khat leftover. *Pleurotus sajor-caju* gave significantly higher DWFB than the other tested oyster mushroom

Table 4: Fresh weight of oyster mushrooms species grown on khat leftover harvested at one week interval

	Weeks							
Species	1st	2nd	3rd	4th	5th	6th	7th	
PS	376.00+14.57ª	103.00+3.510 ^b	50.33+4.630 ^b	66.00+17.09°	58.33+8.110°	28.33+6.66ª	42.50+15.02°	
PO	254.33+35.75 ^b	108.66+10.52ab	146.67+20.20ª	81.33+24.85°	117.67+33.49°	46.33+7.54°	44.47+5.310a	
PF	194.47+9.250 ^b	141.00+9.070°	61.33+14.89 ^b	115.67+4.670°	63.67+6.890°	52.00+2.64ª	50.00+2.640a	
Mean	274.930	117.560	86.440	87.670	79.890	42.220	45.660	
LSD	99.451	35.907	64.081	57.101	88.025	31.478	40.474	
CV (%)	14.440	12.190	29.590	26.000	43.980	29.760	35.390	
F-value	16.290***	6.140*	12.590**	3.730^{ns}	2.620^{ns}	$2.900^{\rm ns}$	$0.170^{\rm ns}$	

ns: Non significant, **Significant at 0.05, ***Highly significant at 0.01, ****Very highly significant at 0.001, PS: Pleurotus sajor-caju, PO: Pleurotus oestreatus, PF: Pleurotus oestreatus-florida, Means in the same column followed by the same letter are not significantly different at the 5% probability level by Tukey's test

Table 5: Dry weight of oyster mushrooms species grown on khat leftover harvested at one week interval

Species	Weeks								
	1 st	2nd	3rd	4th	5th	6th	7th		
PS	24.00+0.58ª	9.60+0.21 ^b	7.33+0.88 ^b	8.17+1.42°	5.40+0.97 ^b	5.70+0.36°	6.57+1.10a		
PO	16.33+0.67 ^b	10.33+1.36 ^b	14.47+0.74°	9.40+2.55°	15.97+2.61a	6.30+0.51°	6.07+0.79°		
PF	13.20+0.61°	15.67+1.20a	6.67+1.30 ^b	13.60+1.25°	7.47+1.37 ^b	5.90+1.35°	9.27+0.47ª		
Mean	17.840	11.870	9.490	10.390	9.610	5.970	7.300		
LSD	2.688	4.575	4.356	7.954	7.783	3.719	3.590		
CV (%)	6.010	15.390	18.320	30.560	32.330	24.880	19.630		
F-value	80.460***	9.860*	18.550**	$2.410^{\rm ns}$	9.750*	$0.130^{\rm ns}$	$4.330^{\rm ns}$		

ns: Non significant, *Significant at 0.05, ***Highly significant at 0.01, ****Very highly significant at 0.001, PS: Pleurotus sajor-caju, PO: Pleurotus oestreatus, PF: Pleurotus oestreatus-florida, Means in the same column followed by the same letter are not significantly different at the 5% probability level by Tukey's test

in the 1st week of harvesting. The highest DWFB harvested in 1st week was 24.00 obtained from *Pleurotus sajor-caju*, whereas 13.20 was the lowest DWFB harvested from *Pleurotus oestratus florida*. While in the 2nd week of harvesting, *Pleurotus oestratus florida* cultivation on the khat leftover resulted in significantly higher DWFB than those obtained from other tested species. Significantly higher DWFB was obtained from *Pleurotus oestratus* cultivated on khat leftover in the 3rd week of harvesting. In 4th, 6th and 7th weeks of harvesting, the data indicated non-significant difference in DWFB produced from three tested oyster mushroom. However, it was noted that significantly higher DWFB was produced from *Pleurotus oestratus* than that of other tested oyster mushroom.

CONCLUSION

From the results of this study, the following conclusions can be obtained. The khat leftover can be used to cultivate the oyster mushrooms namely Pleurotus oestreatus, Pleurotus oestreatus-florida and Pleurotus sajor-caju, consequently reducing this organic pollutant in Eastern part of Ethiopia. The otherwise waste khat left over can be used to increase bulk of food produced impacting food security. The FWFB and BE of Pleurotus sajor-caju fruit bodies are superior to that of the other tested ovster mushroom. Khat left over substrate has higher BE than substrates like wheat bran, even though, its productivity and BE was lower than those Pleurotus sp., cultivated on other types of substrates. Therefore, further research on enhancing the suitability of khat leftover and increases the yield of oyster mushroom, using different methods of pretreatments and optimal formulation by adding different high nitrogen containing substrates would be recommended.

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