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Optimization of *Pleurotus* Mushroom Cultivation on Saline Sisal Solid Waste

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Abstract: Mushrooms are a primary part of managing solid waste, building organic soil and returning minerals to the soil. Sisal processing in Kenya generates an estimated 611,875 tonnes of solid waste annually, which is discarded leading to environmental pollution. Sisal waste contains; lignin, cellulose and hemicelluloses 4.5 %, 76.5 and 21.6 % respectively, which are ideal for mushroom cultivation. However, the waste from Kilifi Kenya has high concentrations of salts such as chlorides and sodium in the range of 31,857.12 and 20,660.28 mg/l, respectively, which are inhibitory to mycelia vegetative growth. The aim of this study was to evaluate pre treatment of saline sisal waste for mushroom production Two *Pleurotus* mushrooms commercials strains, *Pleurotus.* HK 37 and *P. sapidus* 969 were cultivated. The highest recorded biological efficiencies for *P.* HK 37 and *P. sapidus* 969 were 39.4% and 26.3% in cold water soaked substrate as well as 40.05% and 38.2% in a 1:1 co-substrate combination of grass and sisal waste, this can result to annual generation of 159,088 - 244,750 tonnes of fresh mushrooms. The results from this study indicate that saline sisal solid waste can be utilised for commercial mushroom production.

Key words: *Pleurotus* · Spawn · Biological efficiencies · Pre treatment · Sisal waste

INTRODUCTION

Oyster mushrooms (*Pleurotus*) are becoming increasingly important and common in human diets, due to their nutritional composition as well as their medicinal characteristics [1]. The nutritional advantages of mushrooms include a low content of calories and a high content of proteins, minerals and dietary fiber [2]. The Oyster mushroom has also been found to exhibit strong anti-inflammatory and immune-modulatory properties due to their chemical composition [3].

Sisal, *Agave sisalana is* a drought tolerant crop that thrives in semi-arid, marginal and saline soils and does not compete for farmland. It is also an ideal feedstock for an integrated biorefinery that produces bioproducts such as food and bioenergy [4]. The sisal has been has been principally utilized in production of hard fiber which is only 2.7-7.3% of the whole plant, depending on the age of the plant as well as the efficiency of the decortications process; the remaining 97.3-92.7% [5] is discarded as waste leading to serious environmental pollution [6]. The estimated annual production of clean sisal fibre in Kenya alone in the year 2010 stood at 35,119 tonnes resulted in generation of 611,875 tones sisal solid sisal waste and 3,511,900m^3 of sisal leaf decortications waste water.

Oyster mushroom can be grown on various substrates [7]. According to [8], the mushroom cultivation substrate influences its growth, yield and composition. Mshandete and Cuff [9] reported successful cultivation of wild edible *P. flabellatus* mushrooms on solid sisal decortications wastes in Tanzania. Nevertheless, attempts in Kenya at Kilifi County to utilize the solid sisal waste as a substrate for *Pleurotus* mushroom cultivation revealed scanty mycelia colonization of the substrate during vegetative

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growth phase, which lead to failure to produce pinheads which could had developed into primordia and finally into mushrooms during reproductive phase. The preliminary results revealed presence of relatively high concentrations of salts such as chlorides and sodium in the range of 31,857.12 and 20,660.28 mg/l, respectively. Such salt concentrations were higher 45-69 times than 461ppm (461mg/l), which have been reported to be inhibitory to mycelia vegetative growth subsequently to mushroom production [10]. Therefore, in order utilize the abundant saline solid sisal waste; evaluation of various pretreatment methods of the waste was conducted. This study will identify the best pre treatment for *Pleurotus* cultivation and help to reduce the underutilized agro industrial resource.

MATERIALS AND METHODS

Source of Commercial Strains of Pleurotus and Sisal Waste: P.HK-37and *P. sapidus* (P 969) were obtained from the strain bank of Department of Molecular Biology and Biotechnology, University of Dar es Salaam in Tanzania. The sisal leaf decortications waste (SLDW) used in this study was obtained from Kilifi Plantation factory in Kilifi County, a sisal farming estate along the Indian Ocean coast line in Kenya.

Preparation of Grain Spawn: The spawn was prepared using sorghum grains according to the method of spawn preparation outlined by Stamets [11].

Substrate Pretreatment to Remove Salts: The salt content of substrate before and after the different treatments was determined using standard methods for examination of water and waste water [12] at Polucon Services (K) Limited, Mombasa, Kenya.

Three Days Soaking in Cold Water: The chopped SLDW was weighed in a gunny bag and place in a 160 liters' container in triplicates, water at an ambient temperature of 27±2°C was added at the ratio of 1:5 (SLDW: water) and soaked for three days (72 hours).Thereafter, the excess water in substrate was drained and the substrate pasteurized.

Treatment with Hot Water: SLDW was treated by immersion in hot water at 80±5°C for 3 hrs followed by washing in water at 27±2°C to remove the leached salts.

Fermentation: The substrate was soaked in water for 10 days in a 160 liters plastic container at 1: 5 ratio (water:substrate) without water change and thereafter, the excess water in substrate was drained before packing in the cultivation bags.

Treatment with Lime: SLDW was submerged in a solution of 2% lime ($Ca(OH)_2$) at 1: 5 (solution of lime: substrate) combination for 24 h using plastic container [13].

Co-Substrate Pretreatment of SLDW: The SLDW was soaked in water at 1:5 (water:substrate) ratio for 3 days and subsequently mixed with locally available grass (*Panicum coloratum)* soaked in cold water for 1 day soaked at 1:5 (water: grass) ratio. The pretreated substrates was draining excess water to reduce the moisture content to 65-70% which was determined by the hand squeeze test method and each packed in 1kg polythene bags.

Pasteurization, Spawning and Spawn Run: The pretreated substrates were all packet in cultivation bags and steam pasteurized for 4-5 hours at 80 °C in a fabricated in metallic firewood-heated drum. When cooled to room temperature, the substrates were inoculated with previously prepared spawn at 5% (wet weight spawn/wet weight substrate) [14].

The inoculated bags were transferred to a clean and disinfected incubation room for 18 days. A dark environment and room temperature of 24-28°c was maintain during incubation. After full colonization, the bags, they were transferred to the cropping room, whose environment was kept illuminated by sunlight and a temperature and humidity of 28°c and 75-85% were maintained through adequate watering.

Mushroom Harvesting and Crop Yield: After incubation period, the bags were transferred to a cultivation chamber at 25 ± 2 °C and 90% relative air humidity in the presence of light throughout the entire harvesting period. The Fruit primordia were allowed to grow to the recommended harvesting stage and were picked and five aspects of crop yield evaluated as previous [9] (i) Total number of mushrooms harvested (ii) stipe size (iii) cap diameter (iv) Mushroom yield (MY) (v). BE values were calculated as B.E. = [Weight of fresh mushrooms harvested (g) /dry substrate weight (g)] x100. On the other hand mushroom yield (MY) values were as MY= [Weight of fresh mushrooms harvested (g) per fresh substrate weight].

Analytical Methods: Before pre treatment as well as after pre treatment, the sisal waste substrates were characterized. The Ash content, Moisture, Total solids (TS), Volatile Solids (VS) Total carbon and total organic matter, were [15]. The lignin, hemicellulose and cellulose were analyzed [16] while Sodium and Chlorides was done according to [12].

Statistical Analysis: The experiments were carried out in triplicates and the data on mushroom total number of mushrooms harvested, stipe size, cap diameter, mushroom, BE and mushroom yield (MY) were subjected to analyses of variance (one-way ANOVA) when significant differences were determined post test were made using Turkey multiple range test. The results are given as mean ± SD.

RESULTS AND DISCUSSION

The Composition of Solid Sisal Decortications Waste: The physical composition of SLDW including; the moisture content, TS, VS, total carbon, total organic matter, lignin, hemicelluloses and cellulose composition are tabulated in (Table 1). The high organic carbon content of SLDW which is evaluated as VS, organic matter and total organic matter is an indication that the sisal waste is a rich alternative to traditional substrates used in mushroom cultivation.

Table 1: Composition of Sisal Leaf decortications waste (SLDW) used in the study

Parameter	SLDW before pre treatment
Moisture content %	20.7±4.1
Total solids (TS) %	12.1±0.1
Volatile solids (% TS)	67.3±2.9
Total carbon (%TS)	49.4±0.4
Total organic matter (% TS)	97.3±4.3
Lignin [a]	4.5±2.7
Cellulose[a]	76.7±5.6
Hemicellulose[a]	21.6±7.2

All values are averages of triplicates

[a] % of dry weight

Members of the genus *Pleurotus* are well known for the conversion of various lignocellulosic substrates into edible mushrooms. Consequently, successful cultivation *Pleurotus flabellatus* mushrooms on solid sisal decortications wastes has been reported [9]. On the other hand, mushroom cultivation using SLDW from Kilifi Kenya had not been reported previously and, several attempts to utilize the solid sisal waste as a substrate for *Pleurotus* mushroom cultivation revealed scanty mycelia colonization of the substrate during vegetative growth phase, which lead to failure to produce pinheads which could had developed into primordia and finally into fruiting bodies (mushrooms) during reproductive phase.

Table 2: Comparative analysis of SLDW in three Kenyan sisal estates

	SLDW (Kilifi)				
Parameter	Whole Leaf	Dry SLDW	3 days soaked	SLDW (Mogotio)	SLDW (Kibwezi)
Chlorides (mg/l)	23,510.18	31,857.12	2,148.59	1,180.77	929.64
Sodium (mg/l)	15,251.47	20,660.28	1,393.83	765.97	603.07

Table 3: Average number of *Pleurotus* HK 37mushroom fruit bodies, Cap diameter, Stipe height yield and biological efficiency of pre-treated substrate

Treatment of SLDW	No. of fruit bodies	Cap diameter (cm)	Stipe height (cm)	Yield (g/Kg) wet Substrate	B.E (%)
Soaked in cold water	15±3.0	6.3±0.7	4.0±0.6	161.7	39.4±6.3
Boiling in water	5.6±1.8	6.2±1.3	4.6±0.8	103.0	25.1±3.0
Lime pretreatment					
Fermentation	4.2±1.6	5.7±1.1	3.1±0.8	57.6	14.05±2.1
co-substrate-*Panicum coloratum*	13±3.0	9.5±3.0	4.9±2.0	164.2	40.05±8.8

Table 4: Average number of *Pleurotus sapidus* 969 mushroom fruit bodies, Cap diameter, Stipe height yield and biological efficiency of pre-treated substrate

Treatment of SLDW	No. of fruit bodies	Cap diameter (cm)	Stipe height (cm)	Yield (g/Kg) wet Substrate	B.E (%)
Soaked in cold water	2.1±0.6	5.6±0.9	2.4±0.5	107.8	26.3±3.9
Boiling in water	5.2±1.3	3.7±0.8	6.1±0.7	42.2	10.4±1.9
Lime pretreatment	3.2±1	7±1.0	5±0.2	42.8	10.4±2.4
Fermentation					
co-substrate *Panicum coloratum*	14±2	6.5±1.2	3.2±1.0	156.4	38.2±9.7

The preliminary results revealed presence of relatively high concentrations of salts such as chlorides and sodium in the range of 31,857.12 and 20,660.28 ppm, respectively (Table 2). Such salt concentrations were higher than 461ppm, which have been reported to be inhibitory to mycelia vegetative growth subsequently to mushroom production [10].

Comparative Analysis of SLDW: Analysis of SLDW from two sisal estates far from the Kenyan coast; Kibwezi as well as Mogotio revealed the SLDW from the study site had three fold sodium and chloride concentration (Table 2) which are inhibitory to mycelial growth. Further survey of the study sites revealed no wild mushrooms such as *Coprinus* sp. common in sisal dump sites could be found growing naturally. The whole dried sisal leave had 23,510 and 15,251.47 mg/l of Chlorides and Sodium, respectively, which upon processing through the traditional wet decortications process and drying increased to 31,857.12 and 20,660.28mg/l, possibly due to concentration of the salts as well as the bore hole water used during processing which had 7,424.45 and 4,816.37 mg/l of Chlorides and Sodium, respectively.

Mushroom Fruit Bodies, Cap Diameter and Stipe Height: The mushroom fruit bodies, cap diameter and stipe height obtained in this study for *Pleurotus* HK 37 (Table 4) were within the reported range from *P. HK* 37 cultivated on a sisal solid substrate but with supplementation [17]. The results on the same vary slightly with those recorded by earlier research on different substrate [18, 19] supporting that variations could be as a result of the type substrate, spawn rate, type and level of supplements and type of mushroom species as well as their strains cultivated. The results obtained from *Pleurotus sapidus* 969 mushroom fruit bodies, cap diameter and stipe height (Table 4). The two species differed on these parameters significantly leading an indication of the better performance of *Pleurotus* HK 37 on the different pre treatments in this study.

Yield and Biological Efficiency of Oyster Mushroom: There were significant effects of substrate pre treatment methods on the average yield and BE of oyster mushroom (Table 3 and 4). Biological efficiency (BE) is used in evaluating the efficiency of substrate conversion in mushrooms cultivation. The crop of oyster mushroom was harvested in three flushes, while the maximum yield was obtained in first flush. Maximum average yield of 164.2 g/kg substrate and 156.4 g/kg substrate were obtained for *Pleurotus* HK 37 and *Pleurotus sapidus* 969, respectively in the co-substrate-*Panicum coloratum* experiment, however the lowest yield in *Pleurotus* HK 37 being 57.6 g/kg in the fermentation pretreatment while *Pleurotus sapidus* 969 recorded 42.2 g/kg in the boiling pretreatment. The variations observed in yield may therefore be attributed to the adaptability of the strain in the saline substrate from the study site as well as the efficiency of the pre treatment methods employed.

The BE values observed in our study were similar to those of *P*. HK 37 cultivated on solid sisal waste fractions reported by Raymond *et al*. [17] which were in the range of 8% - 43%. The agro wastes used as substrates; SDLW contains lignin and cellulose similar to other substrates such as dry plantain leave, palm oil chaff, cassava peels cotton waste, saw dust and vegetable which many basidiomycetes like *Pleurotus* are capable of degrading. The average bio-efficiency was variable and significantly different among the substrate pre-treatments and the two species cultivated. Cold water (27±2°C) pre treatment and use of a co-substrate for *Pleurotus* HK 37 had the highest BE of 39.4% and 40.05% which was statistically significant ($p = 0.05$) while, *P. sapidus* 969 cultivated on Cold water (27±2°C) pre treatment SLDW as well as SLDW with a co-substrate had a BE of 26.3% and 38.2% respectively, the BE were statistically significant ($p = 0.05$) compared to untreated as well as others pre treatments in the study.

CONCLUSIONS

From this work, it seems that, the saline sisal waste contains the necessary nutrients required for the fructification of *Pleurotus* HK and *Pleurotus*. *Sapidus* 969 and that with pre treatment to lower the salinity the substrate can support the growth of the mycelium up to fruit body formation. Mushroom growing, as an agro-industry should be encouraged in the sisal growing zones of East Africa, since the use of agricultural waste product in producing edible mushroom would improve the wellbeing as well as enhancing the income generation of the sisal estates. This will also control environmental pollution resulting from the uncontrolled disposal of these wastes.

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REFERENCES

1. Jedinak, A., S. Dudhgaonkar, J. Jiang, G. Sandusky and D. Sliva, 2010. *Pleurotusostreatus*inhibits colitis-related colon carcinogenesis in mice. Int. J. Mol. med., 26: 643-650.
2. Beluhan, S. and A. Ranogajec, 2011. Chemical composition and non-volatile components of Croatian wild edible mushrooms. Food Chem., 124: 1076-1082.
3. Lavi, I., D. Levinson, I. Peri, Y. Hadar and B. Schwartz, 2010. Orally administered glucans from the edible mushroom (*Pleurotuspulmonarius)* reduce acute inflammation in dextransulfatesodium induced experimental colitis. British J. Nutr., 103: 393-402.
4. Export Processing Zone Kenya report, 2010. Assessment on potential for agro-industrial biogas in Kenya.
5. Lock, G.W. Sisal, 1969. Longmans Green, Second edition, pp: 365-60.
6. Mshandete, A.M. and J. Cuff, 2008. Cultivation of three types of indigenous wild edible mushrooms: *Coprinus cinereus, Pleurotus flabellatus* and *Volvariella volvocea* on composted sisal decortications residue in Tanzania. Afr. J. Biotech., 7(24): 4551-4562.
7. Hassan, S., A.Y. Mohammad and K. Kiramat, 2011. Cultivation of the oyster mushroom (*Pleurotusostreatus* (Jacq.) P. Kumm.) in two different agroecological zones of Pakistan. African J. Biotechnol., 10: 183-188.
8. Khare, K.B., J .M. Mutuku, O.S. Achwania and D.O. Otaye, 2010. Production of two oyster mushrooms, *Pleurotussajor-caju*and *P. florida*on supplemented and un-supplemented substrates, Bots. J. Agric. Appl. Sci., 6: 4-11.
9. Mshandete, A.M. and J. Cuff, 2008. Cultivation of three types of indigenous wild edible mushrooms: *Coprinus Cinereus,Pleurotus flabellatus* and *Volvariella volvocea* on composted sisal decortications residue in Tanzania. Afr. J. Biotech. *7*(24): 4551-4562.
10. Zizzo, A., 2009. The effects of road salt on mushroom-forming saprotrophic fungi Undergraduate Thesis: Carthage College, Kenosha, Wisconsin USA.
11. Stamets, P., 2000. Growing Gourmet and Medicinal Mushrooms, 3rd edition, pp: 201-325, Berkley, California: Ten Speed Press, ISBN 1-58008-175-4.
12. APHA., 1995. Standard Methods for examination of water and wastewater. 19th ed. American Public Health Association, Washington DC., USA.
13. Bernabé-González, T. and M. Cayetano-Catarino, 2009. Cultivation of *P. pulmonarius*on substrates treated by immersion in alkaline water in Guerrero, Mexico. Micologia Aplicada International, 21(1): 19-23.
14. Oei, P., 2003. Manual on mushroom cultivation: techniques, species and opportunities for commercial applications in developing countries. TOOL Foundation, Amsterdam, The Netherlands, pp: 1-429.
15. Association of Official Analytical Chemists (AOAC), 1990. Official methods of analysis of the AOAC, 15thed. Association of Official Analytical Chemists, Arlington, Va., U.S.A.
16. Goering, H.K. and P.J. Van Soest, 1970. Forage fibre analysis (apparatus, reagents, procedures and some applications). Agricultural Handbook, vol. 379. Agricultural Research Service, United States Department of Agriculture, Washington DC, USA.
17. Raymond, P., A.M. Mshandete and A.K. Kivaisi, 2013. Cultivation of Oyster Mushroom *Pleurotus*HK-37) On Solid Sisal Waste Fractions Supplemented With Cow Dung Manure J. Biol. Sci., 4(1): 273-286.
18. Mshandete, A.M., 2011. Submerged and solid-state fermentation of the mycelium of *Coprinus cinereus* (Schaeff.) S. Gray s. lato mushroom on sisal bole wastes supplemented with human urine in Tanzania. Int. J. Res. in Pure and Appl. Microbiol., 1(3): 40-55.
19. Mamiro, D.P. and D.J. Royse, 2008. The influence of spawn type and strain on yield, size and mushrooms solid content of *Agaricus bisporus* produced on non-composted and spent mushroom compost. Bioresour. Technol., 99: 3205-3212.