

Yield and mushroom size of *Pleurotus ostreatus* grown on rice straw basal substrate mixed and supplemented with various crop residues

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1 SUMMARY

Two crops of *Pleurotus ostreatus* were grown on rice straw as the basal substrate. In crop I, rice straw was mixed at spawning with 0%, 25%, 50%, 75% and 100% of banana leaves or *Leucaena leucocephala* or maize bran or maize cobs. In crop II, rice straw was supplemented at spawning with 0%, 1%, 2%, 3%, 4%, and 5% of sunflower or cotton seed cake. Mushroom yield (1,040.0 g) and Biological efficiency (BE) (98.5%) were greater on a 50/50 mixture of rice straw and banana leaves. Rice straw supplemented with 2% sunflower seed hulls (yield 1,087.5 g, BE 103.3%) gave similar yield and BE to rice straw supplemented with 2% cotton seed hulls (yield 1,073.8 g, BE 101.8%), and were significantly greater than ($p < 0.001$) other supplement ratios. By comparison, mushroom yield on banana leaves were 786.5 g, on rice straw were 582.5 g, on *Leucaena leucocephala* were 534.5 g, on maize cobs were 468.5 g, on rice bran were 406.0 g and on maize bran were 305.3 g. The largest mushrooms (21.0 g) were obtained from non-supplemented rice straw.

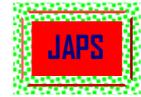
2 INTRODUCTION

Tanzania is endowed with a rich diversity of wild edible mushrooms and people gather them for household consumption and for sale to generate income. More than 60 edible mushroom species were found gathered for consumption in Tanzania and have been identified (Härkönen *et al.*, 1995, 2003). Availability of wild mushrooms is seasonal and unreliable. While mushrooms require sufficient moisture to grow, with climate change, rains are getting less and less and more forest land is brought under cultivation, therefore depriving wild mushrooms growth. To meet the increasing demand of mushrooms, its cultivation was first introduced in Tanzania in 1993 (Kivaisi, 2007) and the focus was on oyster mushrooms (*Pleurotus* spp.) because they are well known for conversion of crop residues to protein food. Crop residues are used as substrates in the production of oyster mushrooms. Oyster mushrooms are also the easiest and least

expensive commercial mushrooms to grow (Banik and Nandi, 2004).

National and international demand for oyster mushroom is increasing. Currently, Tanzania is producing 960 t oyster mushrooms worth 3,840 million Tanzanian shillings (USD 2.6 million) and are produced by 4,000 small scale farmers (Kivaisi, 2007). The Peoples' Republic of China is the major producer and consumer of oyster mushrooms, accounting for about 90% of total world production. Oyster mushroom production accounted for 14.2% of the total world yield (6,161,000 t) of all edible mushrooms in 1997 (Chang, 1999).

Pleurotus spp. can be grown on a number of substrates. Crop residues such as wheat straws, rice straws, banana leaves, corn cobs, sawdust, and bean straws can be utilized as substrates to grow oyster mushrooms (Poppe, 2000). Large quantities of crop



residues are generated by more than 80% of Tanzanian's who live in rural areas and depend on agriculture for crops such as sugarcane, tea, coffee, maize, cotton, horticultural crops and livestock keeping as the main source of livelihood. An annual production of crop residues from sisal, sugar cane and cereal processing was estimated to be 615,000 t and cereal straws was estimated to be 7.0 million tons (Kivaisi and Magingo, 1999). It has been observed that over 70% of agricultural and forest crop residues are conceived as waste materials (Chang and Mshigeni, 2001). Most of these crop residues are set on fire and cause environmental degradation because of fire going wild and destroying untargeted flora and fauna.

Oyster mushrooms can utilize a wide range of crop residues due to their great adaptability and has short growth cycle. The cultivation of oyster mushrooms on crop residues is considered as potential source of income, an alternative food production, provision of employment, and for recycling of agricultural wastes. Oyster mushrooms can be grown in small scale space of a farmer's house and yet generate income that aids in family support. Oyster mushrooms are good source of cheap protein, vitamins and minerals. The use of crop residues as substrates is important, because most of them remain unused.

In the USA, oyster mushrooms have been produced on a substrate containing cottonseed hulls, wheat or oat straws, sawdust or combination of these (Royse

et al., 2004). These substrates were sometimes found unavailable or available at high prices. Locally available crop residues such as banana leaves, banana juice pulp, rice straws, bean trash, finger millet straws, maize stover and peels, cotton seed oil waste, sunflower seed hulls and sawdust among others were reported to be used in Tanzania (Kivaisi, 2007). Price of substrates varies depending on locality, season, type of substrates and transport but generally, in most places crop residues are free. Most farmers use single type of substrate and they don't use supplements. Some of advantages of using supplements was reported to increasing mushroom yields and hasten the production process (Royse, 2002).

Very few studies have used various ratios of crop residues and locally available organic supplements in the practice of oyster mushroom cultivation. Substrate mixture has got complementary advantages over single type substrate. Commercial delayed release nutrients (the types of supplements commercially manufactured to slowly release required elements to the substrate) were reported to increase oyster mushroom yield significantly (Royse *et al.*, 2002).

Thus the present work was carried out with objectives of using various ratios of crop residues as a substrate for oyster mushroom cultivation, and the use of rice straw as a basal substrate supplemented with various ratios of cotton and sunflower seed hulls to produce oyster mushrooms.

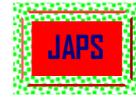
3 MATERIALS AND METHODS

3.1 Substrates: Dry rice straws were obtained from various paddy fields around Morogoro suburban areas and were chopped to 6 cm (FAO, 1990). For crop I, rice straw was mixed with other agricultural waste materials such as rice bran, maize bran, maize cobs, *Leucaena leucocephala* leaves, and banana leaves at rates of 100%, 75%, 50%, 25% and 0%. Leafy materials were chopped to size as for rice straw. For crop II, the rice straw was supplemented with 5%, 4%, 3%, 2%, 1% and 0% of either sunflower or cotton see hulls. Sunflower and cotton see cake were milled and sieved using 0.1 mm mesh before mixing with basal substrate.

3.2 Spawn: *Pleurotus ostreatus* was selected because it is more acceptable to Tanzanian communities. Spawn was prepared by placing 500 g of sorghum (*Sorghum bicolor* L.) grains in a beaker (1000 ml) and 750 ml of tap water was added to soak the sorghum grains for 3 h. Two hundred

grams (w/w) of soaked grains were placed in five jam bottles (350 ml), and autoclaved at 121°C at 15 psi for 20 min then allowed to cool in a lamina hood. This was repeated four times to obtain 20 jam bottles full of spawn. Each bottle was inoculated with three 5 cm² diameter *P. ostreatus* culture. Inoculated jam bottles were thoroughly shaken by hand to distribute the mycelia to the grains, placed on a laboratory bench at room temperature (29±2°C), and shaken again every 2-4 days, to enhance uniform mycelial growth. The mature sorghum spawn were stored at 5°C in the refrigerator until use.

3.3 Experimental design and data analysis: Two cropping experiments were conducted (crops I and II). Both cropping experiments were designed as a two-factor (5 x 5 for crop I, 2 x 6 for crop II) factorial in a randomized complete block design (RCBD) where treatment combinations were



randomly assigned to the units within each block with 4 replicates per treatment (Kuehl, 2000). Both cropping experiments contained rice straw as a basal substrate and other agricultural waste materials at various rates as substrate for mixing or as supplements. For both cropping experiments, rice straws and agricultural waste materials were mixed before pasteurization. All experiments were repeated two times (blocks) and the mushrooms were harvested for three flushes (60 – 70 days from day of first harvest). The general linear model (SAS, 2001) procedure was used for an analysis of variance. Treatment means were separated according to Fisher's least significant difference test ($p < 0.05$) and whenever necessary, treatment means comparisons with the controls were made according to Dunnett's procedure (Kuehl, 2000).

3.4 Mushroom cropping trials: Cropping trials were conducted at Sokoine University of Agriculture, Morogoro, Tanzania in March to July 2010. Substrate mixtures were soaked overnight for moisture absorption; allow draining to until no water was dripping from them and then were packed into transparent poly-propylene (Simba Plastics, Dar es Salaam) bags (height 20.0 cm, diameter 15.0 cm). Each bag was kept open on both

ends, but tied loosely with collar necks and rubber band, and plugged by a piece of news paper before steam pasteurization at 98°C for 4 h in a 200 L steel drum. The bags were placed in the disinfected lamina-flow hood, cooled and spawned with 2% (w/w) of the substrate mixture in each bag. Non-mixed rice straw substrates were used as controls. After spawning, the bags were placed in dark room for spawn run (28 – 30°C, 20 -25 days). The bags were then placed in a light room and the plugs removed. 'Light hold' (time between lighting and mushroom primordial formation) lasted for 20 – 25 days at 28 – 30°C and during this period tap water in the form of mist by using knap sack sprayer was applied daily or as needed on the bags and air. Relative humidity in the production room was 85 – 95%.

3.5 Harvesting, determination of yield, BE and size: Oyster mushrooms were harvested when the in-rolled margins of the basidiomata began to flatten. Mushrooms were harvested, counted and weighed. At the end of the third flush, yield and biological efficiency (BE) were determined. Average mushroom size was calculated as fresh mushrooms harvested divided by the number of mushrooms per plastic bag as shown by the formula:

$$\text{Average mushroom size} = \frac{\text{Weight of fresh mushrooms (g)}}{\text{Number of mushrooms per container}}$$

BE was determined as the ratio of fresh mushrooms harvested (g) per g dry substrate and expressed as a percentage as shown by the formula:

$$\text{BE} = \frac{\text{Weight of fresh mushrooms (g)}}{\text{Weight of dry substrate (g)}} \times 100$$

Where: BE = biological efficiency

Yield was the weight of the mushrooms harvested per unit production (plastic bag) and was expressed as g/g.

$$\text{Yield} = \frac{\text{Weight of fresh mushrooms (g)}}{\text{Weight of dry substrate (g)}}$$

4 RESULTS

4.1 Yield and BE (biological efficiency):

Substrate types and substrate mixture significantly influenced yield and BE in crop I (Table 1). Yields ranged as low as 50 g from rice bran to as high as 1,040 g from substrate mixture of 50% banana leaves and 50% rice straws (Table 2). In general, yields were highest from banana leaves and lowest

from maize bran (Table 4). Yields were also higher when mixture ratio of 25% was used, although 100% rice straws was equally high yielder (Table 5). No mushrooms were produced when 100% maize bran were used.

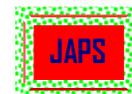


Table 1: Probabilities greater than Fisher's (*F*) test from analysis of variance for two crops (I, II) for (*Pleurotus ostreatus*) yield, biological efficiency and mushroom size

Source	Probability > <i>F</i> ^a			
	df	Yield	BE ^b	Mushroom size
<i>Crop I</i>				
Substrate type (SBT)	4	0.0001	0.0001	0.0049
Mixture ratio (MR)	4	0.0001	0.0001	0.0006
Block	1	0.0032	0.0036	0.1658
SbTxMR	16	0.0001	0.0001	0.0001
<i>Crop II</i>				
Supplement type (SPT)	1	0.1623	0.1594	0.3382
Supplement level (SPL)	5	0.0001	0.0001	0.0001
Block	1	0.0032	0.0033	0.3336
SPTxSPL	5	0.4966	0.4985	0.4993

^aValues of less than 0.05 were considered significant according to Fisher's LSD.

^bBiological efficiency (kg fresh mushrooms/kg dry substrate x 100).

Table 2: Mean yield (g/g), percentage biological efficiency (%BE) and mushroom size (g/mushroom) of *P. ostreatus* grown on rice straws basal substrate mixed with various crop residues

Substrate type	Rice straw ratio (%)	Yield (g/g) ^a	Difference (%) ^b	BE (%) ^{ac}	Size (g) ^a
Rice bran	0	406.0efgh	-30.3	38.3efgh	8.4cd
Rice bran	25	682.5cd	17.2	64.5cd	13.2a
Rice bran	50	431.3efgh	-26.0	40.6efgh	7.2d
Rice bran	75	283.8ghi	-51.3	26.3hij	10.6abcd
Maize bran	0	305.3fghi	-47.6	28.6fghi	7.6d
Maize bran	25	505.0def	-13.3	47.6def	10.0abcd
Maize bran	50	191.3ijk	-67.2	17.9ijk	9.2abcd
Maize bran	75	247.5hij	-57.5	22.6hij	10.6abcd
<i>Leucaena leucocephala</i>	0	534.5cde	-8.2	50.4cde	11.0abcd
<i>Leucaena leucocephala</i>	25	681.3cd	17.0	64.6cd	12.2abc
<i>Leucaena leucocephala</i>	50	596.3cde	2.4	56.1cde	10.2abcd
<i>Leucaena leucocephala</i>	75	473.8efg	-18.7	44.5efg	11.4abc
Maize cobs	0	468.5efg	-19.6	44.2efg	12.2abc
Maize cobs	25	606.3cde	4.1	57.3cde	12.6ab
Maize cobs	50	460.0efg	-21.0	43.4efg	8.2cd
Maize cobs	75	488.8def	-16.1	46.1defg	12.4abc
Banana leaves	0	783.8bc	34.6	74.1bc	9.6abcd
Banana leaves	25	592.5cde	1.7	56.3cde	9.8abcd
Banana leaves	50	1,040.0a	78.5	98.5a	10.8abcd
Banana leaves	75	933.8ab	60.3	88.3ab	8.6bcd
Rice straw	100	582.5cde	0.0	55.0cde	10.6abcd

^aMeans within a column followed by the same letter are not significantly different if *p* > 0.05 according to Fisher's LSD; values are means of four replicates,

^bControl used to calculate % difference was rice straw. Difference (%) = [(a-x)/x] 100 where a = yield from substrate mixture treatment, x=yield of control (rice straw), ^c% BE = (g fresh mushrooms/g dry substrate) x 100

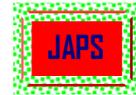


Table 3: Mean yield (g/g), percentage biological efficiency (%BE) and mushroom size (g/mushroom) of *P. ostreatus* grown on rice straws basal substrate supplemented with cotton and sunflower seed cake

Supplement type	Supplement ratio (%)	Yield (g/g) ^a	BE (%) ^{ab}	Size (g) ^a
Rice straw	0	582.5de	55.0de	8.6cd
Cotton seed cake	1	891.3abc	84.1abc	12.4ab
Cotton seed cake	2	1,073.8a	101.8a	12.2abc
Cotton seed cake	3	777.5bcd	73.6bcd	12.4ab
Cotton seed cake	4	797.5bcd	75.4bcd	10.0abcd
Cotton seed cake	5	658.8cde	62.4cde	9.8bcd
Sunflower seed cake	1	938.8ab	88.9ab	13.2ab
Sunflower seed cake	2	1,087.5a	103.3a	11.4abcd
Sunflower seed cake	3	665.0cd	62.9cd	13.6a
Sunflower seed cake	4	690.0bcd	65.4bcd	11.8abc
Sunflower seed cake	5	401.3e	37.9e	8.0d

^aMeans within a column followed by the same letter are not significantly different if $p > 0.05$ according to Fisher's LSD; values are means of four replicates, ^{b0}% BE = (g fresh mushrooms/g dry substrate) x 100

Table 4: Means and groupings from analysis of variance for two factors (substrate type and substrate mixture ratio) for *P. ostreatus* yield, percentage biological efficiency (%BE) and mushroom size of (Crop I)

Substrate type	Yield (g) ^a	BE (%) ^{ab}	Size (g) ^a
Rice bran	406.0c	38.3c	8.4bc
Rice straw	582.5b	55.0b	10.6a
Maize bran	305.3d	28.6d	7.6c
<i>Leucaena leucocephala</i>	534.5b	50.4b	10.6a
Maize cobs	468.5bc	44.2bc	10.6a
Banana leaves	786.5a	74.4a	10.2ab

^aMeans within a column followed by the same letter are not significantly different if $p > 0.05$ according to Fisher's LSD; values are means of four replicates, ^{b0}% BE = (g fresh mushrooms/g dry substrate) x 100

Mushroom yield and BE in crop II were significantly affected by supplement levels (Table 1). In general, 2% level of supplement gave highest yields (Table 6). Rice straw supplemented with 2% sunflower seed cake gave a yield of 1,087 g and BE of 103.3% and when the same substrate was supplemented with 2% cotton seed cake the yield and BE were 1,073 g and 101.8% respectively (Table 3). There was an exception to this, 2% sunflower seed cake and 2% cotton seed cake were equally effective in stimulating yield when added to rice straw.

Table 5: Means and groupings from analysis of variance for Crop I (*P. ostreatus*) yield, percentage biological efficiency (%BE) and mushroom size

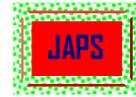
Rice straw ratio (%)	Yield (g/g) ^a	BE (%) ^{ab}	Size (g) ^a
0 ^c	275.5c	25.9c	7.4c
25	613.5a	58.1a	11.4a
50	543.8ab	51.3ab	10.4ab
75	485.5b	45.6b	9.8ab
100	582.5a	55.0a	8.6bc

^aMeans within a column followed by the same letter are not significantly different if $p > 0.05$ according to Fisher's LSD; values are means of four replicates, ^{b0}% BE = (g fresh mushrooms/g dry substrate) x 100

^cA zero percent rice straw ratio is a control

The necessity for blocking in both crops was due to big experimental room. A significant difference

($p < 0.05$) was observed in blocks (due to growing room environment). Through blocking, this



variation was accounted for in the experimental design, improving precision.

4.2 Size: Substrate types, substrate mixture (crop I) and supplement levels (crop II)

significantly influenced mushroom size (Table 1). However, the largest mushrooms (21.0 g) were obtained from non-supplemented rice straw (Table 6).

Table 6: Means and groupings from analysis of variance for Crop II (*P. ostreatus*) yield, percentage biological efficiency (%BE) and mushroom size

Supplement level (%)	Yield (g) ^a	BE (%) ^{ab}	Size (g) ^a
0 ^c	0.58bc	55.6bc	21.0a
1	0.92a	87.0a	12.8b
2	1.10a	102.8a	11.8b
3	0.72b	68.7b	14.2b
4	0.74b	70.8b	12.2b
5	0.53c	50.4c	11.4b

^aMeans within a column followed by the same letter are not significantly different if $p > 0.05$ according to Fisher's LSD; values are means of four replicates, ^b% BE = (g fresh mushrooms/g dry substrate) x 100

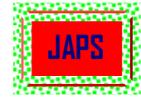
^cA zero percent rice straw ratio is a control

5 DISCUSSION

The oyster mushroom (*Pleurotus* spp) is successfully produced on various crop residues as substrates. They are known for its ability to degrade lignocellulosic (i.e. resources comprised primarily of cellulose, hemicellulose and lignin) residues from agricultural fields and forests and convert them into protein-rich biomass. Lignocellulosic plant materials include wood materials or sawdust, agricultural residues, water plant, grasses and other plant substances (Rowel *et al.*, 2000). Crop residues such as rice straw degradation is achieved by the influence of enzymes from *P. ostreatus*, particularly of cell-wall components, cellulose and lignin. The most suitable substrate mixture with highest yield (1,040.0 g) and BE (98.5%) was rice straw and banana leaves at ratio of 1:1, followed by rice straws and banana leaves at ratio of 1:3. The reason for these results may be due to the high water holding capacity of banana leaves if compared to rice straws. This suggests that banana leaves could be beneficial as a nutrient substrate mixture component as well as providing moisture to the growing system. Across all substrates, BE of oyster mushroom production in terms of overall production could be ranked as follows: maize bran (28.6%), rice bran (38.3%), maize cobs (44.2%), *Leucaena leucocephala* (50.4%) and banana leaves (74.4%). Substrates which contain low ash content are more advantageous in oyster mushroom cultivation when compared with those which have high ash content (Pant *et al.*, 2006). Banana leaves is a fibrous agricultural residue with advantageous low ash content of 1.5% (Abdul

Khalil *et al.*, 2006) when compared to wheat straw and rice straw of 11.0% and 17.5% (Pandey *et al.*, 2000) respectively for usage in bioconversion processes using microbial cultures.

The most suitable supplement level was 2% for both types of supplements (sunflower and cotton seed cake). Yield (sunflower seed cake 1,087.5 g, cotton seed cake 1,073.8 g) and BE (sunflower seed cake 103.3%, cotton seed cake 101.8%) from rice straw supplemented with 2% cotton or sunflower seed cake were higher than other supplement levels. Overall, rice straw supplemented with cotton at various levels (0%, 1%, 2%, 3%, 4% and 5%) had BE ranging from 55.0% to 101.8%. These findings are in general agreement with Royse *et al.* (2004) who showed that the mixture of 24% wheat straw and 75% cotton seed hulls ranged from 87.3% to 95.9%. The big variation in cotton seed cake and cotton seed hull ratios used may be explained to the high oil content in cotton seed cake. Yield increase of up to 90% has been observed by addition of 3% commercial delayed release nutrient at time of spawning (Royse, 2002). Supplementation levels above 2% of any of sunflower or cotton seed cake had a decreasing effect on yield and BE of oyster mushrooms. With sunflower seed cake 5% level of supplementation had yield and BE lower than control (rice straw only). Decrease of yield and BE could be explained by the fact that use of supplements may cause overheating of substrate. The crops were grown under room temperatures without cooling facility. Some substrates such as



maize cobs, maize bran and rice bran were prone to molds. The common genera which were observed includes: *Aspergillus*, *Penicillium*, *Botrytis*, *Coprinus*, *Fusarium*, *Mucor*, *Monilia* and *Trichoderma*. Some of these molds are true pathogens attacking the mushroom mycelium, while others can outcompete mushroom mycelium growth. Fungal pathogens can either affect the quality of the mushroom produced, reduce production or both but are not harmful to the consumers.

The study conducted by Kivaisi (2007) indicated that some farmers were mixing 40 kg (90.4%) basal substrate (banana leaves and or rice straws) with 2 kg (4.4%) rice bran, 1 kg (2.3%) lime or ash, 1 kg (2.3%) chicken manure and 0.25 kg (0.6%) sugar. The mixture was fermented for 3 days and were able to obtain BE as high as 150%. Yields and BE obtained from rice straw supplemented and non-supplemented with chicken manure were not

significantly different (Andrew et al., 2008). Cost effective production of oyster mushrooms depends on cost of substrate ingredients. Some ingredients like sugar are expensive and based on yield and net cost it does not appear that the use of such ingredients is an economically viable supplement to the growers. The use of more cost effective organic crop residues supplements such as cotton and sunflower seed cakes would lower the cost of production of oyster mushrooms and should ultimately lower the cost to consumers.

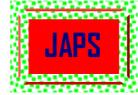
Transparent poly-propylene bags (height 20.0 cm, diameter 15.0 cm) were used as spawn run and production containers. The container size matters in the tropical warm climate. Too large substrate containers are associated with high temperatures that might kill the mycelia at the center of the substrate and especially when cooling facilities are not in place.

6 ACKNOWLEDGEMENT

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