



Oyster mushroom (*Pleurotus* spp.) cultivation technique using re-usable substrate containers and comparison of mineral contents with common leafy vegetables

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ABSTRACT

Alternative re-usable substrate containers for fructification are required because plastic bags currently used suffocate soil biotic entities. They are a cost for mushroom farmers who purchase plastic bags in every oyster mushroom crop and they are left as non-biodegradable wastes, which are disposed of to the environment after every oyster mushroom cropping. On the other hand, oyster mushroom contains essential nutritional elements comparable to leafy vegetables.

Objectives: The objectives of this study were to determine the effects of substrate types and substrate containers on yield, biological efficiency, size and solid content of oyster mushroom *Pleurotus ostreatus*; and to compare mineral content of mushrooms produced from different types of substrates to common leafy vegetables.

Methodology and results: Substrate containers: clear plastic bags, re-usable substrate containers (RSC), coloured plastic bags, shelved clear plastic bags and substrates: banana leaves, rice straws and maize cobs were used to grow oyster mushrooms. The experiment was carried out in complete randomized block design (CRBD) in a factorial arrangement. The mineral content of oyster mushroom grown on rice straws, *Leucaena leucocophala*, sorghum grains, banana leaves and maize cobs substrates were compared in a CRBD to those of pumpkin leaves (*Curcubita* spp.), narrow-leaved African night shade (*Solanum villosum*), broad-leaved African night shade (*Solanum scabrum*), cowpea leaves (*Vigna unguiculata*), cabbage (*Brassica* spp.), sweet potato leaves (*Ipomea batatas*), amaranth (*Amaranthus* spp.) and cassava leaves (*Manihot esculentus* L.). Mushroom yields and BE in RSC were comparable to non-RSC. The highest yields (1,116.25 g/g) and BE (105.75%) were produced from rice straws substrate in coloured bags followed by RSC (yield 694.6 g/g, BE 65.6%). Mushroom solids content (19.4%) was highest from banana leaves substrates. The highest Fe, Zn, Se and Ca were obtained from amaranth, oyster mushrooms produced from rice straws, narrow- and broad-leaves African nightshade and pumpkin leaves respectively.

Conclusion and application of results: The utilization of RSC to produce oyster mushrooms protects the environment from disposed plastic bags which are non-biodegradable and if burned may cause ill-health effect to the human. Additional research is needed to specify quality of material to be used in the manufacture of RSC and specifications to suit cultivation of oyster mushrooms. In addition, consumption of oyster mushrooms in combination with other vegetables complements availability of various essential dietary elements such as Fe, Zn, Se and Ca.

Key words: biological efficiency, mushroom yield, mushroom size, mushroom solids content

INTRODUCTION

Cultivation of oyster mushrooms (*Pleurotus* spp.) was initiated in Germany in 1917 on tree stumps and wood logs (Upadhyay, 2010). Since then growing technologies (Chang & Miles, 1989; FAO, 1990;) have been improved and vary considerably around the world. Like other mushrooms, *Pleurotus* spp. can be grown on various crop residues with the use of different substrate containers (Bisaria *et al.*, 1989). Some substrate containers are such as plastic tubs and trays of various descriptions for *Agaricus bisporus*, open straw bales and plastic bags and bottles for *Pleurotus* spp. Suitable substrate containers for *Pleurotus* spp. should have the following properties:

- It should enclose substrate during the spawn run;
- It should not allow the light to the spawned substrate to avoid initiation of mushroom primordia in inaccessible places;
- It should not break or puncture easily during handling;
- It should not allow excessive loss of moisture; it should avoid excessive self-heating and
- It should allow the maximum production in the space used for production of oyster mushrooms.

Currently, in the developing countries, oyster mushrooms (*Pleurotus* spp.) are produced by using plastic bags as substrate containers for fructification. Type of plastic bags used are made out of polyethylene (for pasteurized substrates), polypropylene or polyvinyl chloride (for sterilized substrate) (Oei, 2003; Mushworld, 2004). After harvest of oyster mushrooms the waste substrate containers are most often burnt. While some farmers' burn plastic wastes after removal of spent mushroom compost (SMC) from the mushroom

growing rooms, others pile them near mushroom growing houses and become source of non-biodegradable solid wastes and breeding places for mosquitoes especially in the tropics. If polyvinyl chloride is burned at temperature below 600°C, it forms dioxins, which are extremely toxic substance (Andersen & Knapp, 1993; Alcock & Jones, 1996; Thorntom, 1997). Plastic bags should be removed before the SMC is applied to the soil, but quite often, the oyster mushroom producers do not know the importance of this practice (Oei, 2003; Mushworld, 2004). Plastic bags suffocate soil biotic entities and are used only once, hence a cost for mushroom farmer to purchase plastic bags in every mushroom crop. Alternative re-usable substrate containers such as plastic tubs and clay pots for fructification may be utilized. Alternative re-usable substrate containers for fructification in the cultivation of *Pleurotus* spp. would both conserve the environment and save money for mushroom grower to which otherwise would be used purchase plastic bags at every oyster mushroom spawning time. Very few studies have used re-usable substrate containers in the production of *Pleurotus* spp. King Oyster (*Pleurotus eryngii* (DC.) Qul) has been produced by using re-usable plastic bottles (Rodrigues-Estrada, 2008). The information thus obtained from this study would be relevant for oyster mushroom producers for cleaner environment and cost reduction of purchasing plastic bags every oyster mushroom cropping. Thus, the present work was carried out with an objective of testing alternative substrate containers to plastic bags for fructification in oyster mushrooms [*Pleurotus ostreatus* (Jacq. Ex. Fr.) Kumar] production in small-scale farming and comparison of mushroom minerals with those from leafy vegetables.

MATERIALS AND METHODS

Determining the effects of substrate types and substrate containers on yield, biological efficiency, size and solid content of oyster mushroom: The primary inoculum was prepared from the fresh fruiting

bodies of the oyster mushroom through tissue culture method and was maintained and multiplied by sub-culturing on sterilized potato dextrose yeast extract agar medium in Petri dishes incubated at room

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temperature ($28\pm 2^{\circ}\text{C}$). Spawn was prepared as described by Mamiro & Mamiro (2011) whereby 750 ml of tap water was added to 500 g of white sorghum (*Sorghum bicolor* L.) grains in 1000 ml-beaker and allowed to soak for 3 h. Two hundred grams (w/w) of soaked grains were placed in 350 ml bottles, capped and autoclaved at 12°C for 20 min. The sorghum grains were allowed to cool over night before inoculation with

0.5 mm diameter *P. ostreatus* culture under aseptic conditions. Inoculated sorghum grains were placed on a laboratory bench at room temperature ($29\pm 2^{\circ}\text{C}$), and shaken after every 2-4 days, depending on rate of mycelial growth to enhance uniform growth. The mature sorghum spawn was stored at 5°C until use. Two oyster mushroom cropping trials were conducted at Sokoine University of Agriculture.



Figure 1: Production of *P. ostreatus* in various substrate containers (a) Clear bags - *vipete* in Kiswahili, (b) Re-usable substrate containers- *visado* in Kiswahili, (c) Hanging clear plastic bags and (d) coloured plastic bags as substrate containers - *rambo* in Kiswahili

Known weight of hand-chopped substrates were soaked overnight; allow draining to until no water was dripping from them when hand squeezed. They were placed in the respective containers, steam pasteurized

at 98°C for 4 h, aseptically cooled and spawned with 40 g spawn per 3 kg substrate (1.3%, w/w). After spawning, the bags were placed in dark room for spawn run ($28-30^{\circ}\text{C}$, 20-25 days). The substrate

containers were placed in light room. 'Light hold' (a time between lighting and mushroom primordial formation) lasted for 20–25 days at 28–30°C; during this period tap water was applied daily or as needed and misting the air with knap sack sprayer. Relative humidity in the production room was kept at 85–95%. The first oyster mushroom cropping experiment treatments were arranged in factorial in complete randomised block design (CRBD) with four replications. There were two sources of variation; substrate type viz: rice straws

(*Oryza sativa* L.), banana leaves (*Musa* spp.) and maize cobs (*Zea mays* L.) while substrate containers were: shelved-clear plastic bags: height 47.5 cm, length 25.3 cm (Fig. 1a); re-usable substrate containers (RSC): height 18.8 cm, diameter 19.0 cm (Fig. 1b and 2); hanging clear plastic bags - height 20.0 cm, diameter 15.0 cm (Fig. 1c) and coloured plastic bags - height 59.0 cm, length 27.0 cm known as "rambo" (Fig. 1d).

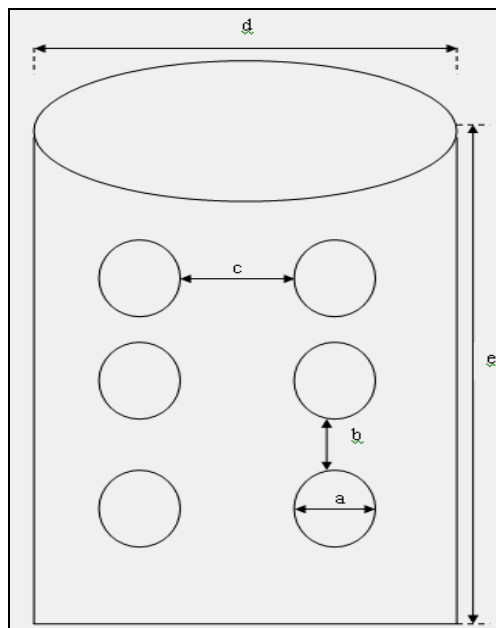


Figure 2: Specifications of re-usable substrate containers: a=0.5 cm, b=3.5-4.0 cm, c=5.0 cm, d=19.0 cm

Re-usable substrate containers were 5-L solid plastic bucket-like and they contained lids. They were modified by making a total of 6 holes on the side-surface (two rows of three-holes each) to allow pin head emergence. The holes (0.5 cm diameter) were made by piercing the side surface of container by red-hot nail; each side had 6 holes and the distances between holes were 3.5–4.0 cm. Five different types of substrates were used in the second oyster mushroom cropping experiment in CRBD with four replications. The substrates used to produce oyster mushrooms were rice straws, banana leaves, maize cobs, *Leucaena leucocophala* leaves and sorghum (*Sorghum bicolor* L.) grains. All substrates used in both crops were fresh and dry. Only shelved-clear plastic bags (height 47.5 cm, length 25.3 cm) were used as substrate containers because mushrooms harvested from this experiment were used only in the analysis of mineral content. All substrate containers were purchased and modified accordingly to

suit oyster mushroom fructification requirement. Substrates initial moisture content was determined by constant-temperature oven method. Hundred grams of each chopped substrates were placed in a metal container, replicated 4 times and oven-dried at 60°C for 24 h. All substrates were allowed to cool in the desiccators and the moisture content and percentage by weight was calculated as

$$\text{Loss of weight} / \text{initial weight} \times 100.$$

Oyster mushrooms were harvested when the lamellae were fully exposed. Mushrooms were harvested, counted and weighed daily for three flushes. Mushroom yield and BE were determined and average mushroom size was calculated as fresh mushrooms harvested divided by the number of mushrooms per substrate container. Biological efficiency was determined as the ratio of fresh mushrooms harvested (g) per gram of dry substrate and expressed as a percentage. Yield was the weight of the mushrooms harvested per unit

production weight of dry substrates and was expressed as g/g. Fresh oyster mushrooms for solids content determination were randomly sampled from each treatment (regardless of block) and were sliced into quarters and eighths depending on the original mushroom size. Samples (100 g) were placed in a paper bag and oven dried at 60°C for 48 h. Dried oyster mushrooms were placed in the desiccators to cool before weighing. The general linear model (SAS, 2001) procedure was used for analysis of variance. Treatment means were separated according to Fisher's Least Significant Difference test ($p < 0.05$) (Kuehl, 2000).

Comparison mineral contents of oyster mushroom to commonly used leafy vegetables: The trial was conducted in a complete randomised design, replicated three times to analyse the mineral quantity of eight leafy vegetable types and oyster mushrooms produced from different five different types of substrates. The leafy vegetables were pumpkin (*Curcubita* spp.),

narrow-leaved African nightshade (*Solanum villosum*), broad-leaved African nightshade (*Solanum scabrum*), cowpea (*Vigna unguiculata* L.), cabbage (*Brassica* spp.), sweet potato (*Ipomea batatas*), amaranth (*Amaranthus* spp.) and cassava (*Manihot esculentus* L.). All vegetables were purchased from local market in Morogoro town. Mineral quantity of the samples of oyster mushrooms harvested from five different substrates (viz. and 8 common leafy vegetables obtained from local market was carried out after dry ash. The ash was dissolved in a solution of 1:1 ratio of water:hydrochloric acid ($H_2O:HCl$), in which the concentration of the final mixture was 6 Normality of Hydrochloric acid (6N HCl). To equate normality to molarity, $6N = 6 \text{ mol/l}$ L of Hydrochloric acid. Total iron, zinc, selenium and calcium were determined by atomic absorption spectrophotometer by AOAC method (AOAC, 1995). Data for each mineral type were subjected to one-way ANOVA.

RESULTS

Effect of substrate type and containers on yield, BE, size and solid content of oyster mushroom:

Results show that there were significant differences on the interaction of substrate types and substrate

containers on mushroom yield ($p \leq 0.05$, $n = 96$, $df = 80$, Fisher's $LSD = 0.1121$) as indicated in Figure 3 and BE ($p \leq 0.05$, $n = 96$, $df = 80$, Fisher's $LSD = 9.7198$) as shown in Figure 4.

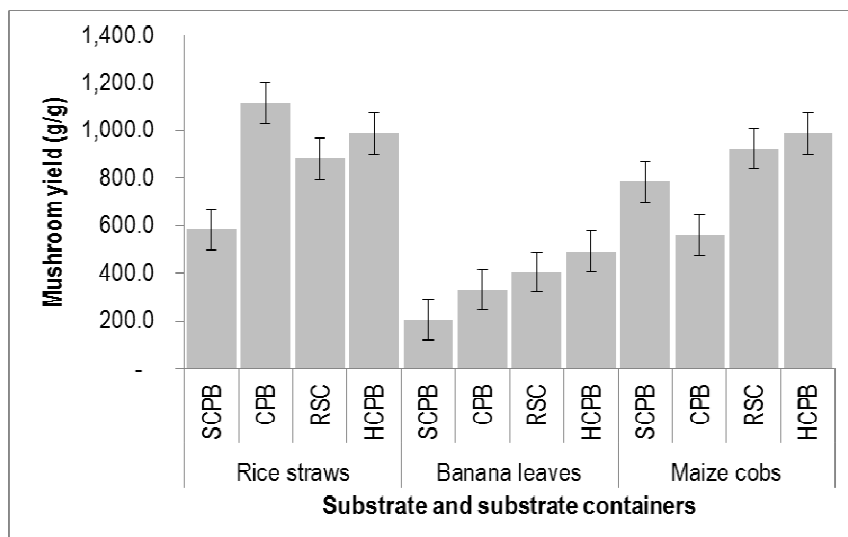


Figure 3: Effect of treatments (substrates X substrate containers [SCPB=shelved clear plastic bags, CPB=coloured plastic bags, RSC=re-usable substrate containers, HCPB=hanging clear plastic bags]) combination on yield of *P. ostreatus* ($p \leq 0.05$, $n = 96$, $df = 80$, Fisher's $LSD = 0.1121$)

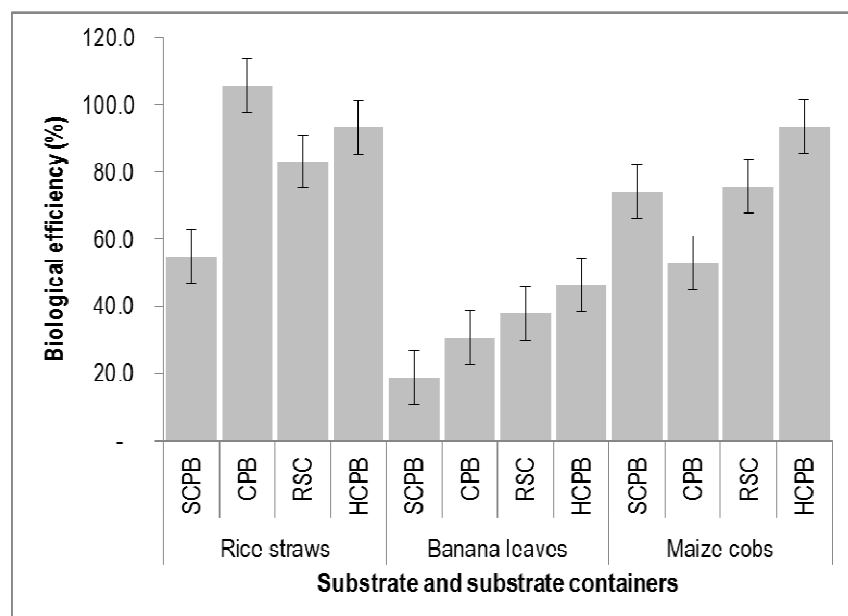


Figure 4: Effect of treatments (substrates X substrate containers [SCPB=shelved clear plastic bags, CPB=coloured plastic bags, RSC=re-usable substrate containers, HCPB=hanging clear plastic bags]) combination on biological efficiency of *P. ostreatus* ($p \leq 0.005$, $n=96$, $df=80$, Fisher's $LSD=9.7198$). BE = (g fresh mushrooms/g dry substrate) x 100.

Yields (1,116.25 g/g) and BE (105.75%) were highest in the interaction between rice straws substrate and coloured plastic bags substrate containers (Figures 3 and 4). For substrate containers, the highest mushroom yields (821.67 g/g) and BE (77.71%) were obtained from hanging clear plastic bags (Table 1). However, yields and BE from hanging clear plastic bags (821.67 g/g, 77.71%) were not significantly higher than those from RSC (694.58 g/g, 65.6%) as indicated in Table 1. For substrates, the highest mushroom yields (891.25 g/g) and BE (84.3%) were obtained from rice straws and were not significantly different from banana leaves

(778.06 g/g, 74.0%) (Table 2). Finally, significant differences ($p \leq 0.05$) in substrates on solid content of oyster mushroom were observed (Table 2). Mushrooms produced from banana leaves had highest solids contents (9.70%) when compared to the other substrate types. However, the mushroom solids produced from banana leaves were not significantly ($p \leq 0.05$) different from solids obtained from rice straws (9.45%). The lowest solids (9.20%) occurred in mushrooms produced on maize cobs and were not significantly different ($p \leq 0.05$) from rice straws.

Table 1: Mean yield, percentage biological efficiency and mushroom size of *P. ostreatus* grown on various substrate containers

Substrate container	Yield (g/g) ^a	BE (%) ^{ab}	Size (g) ^a
Hanging clear plastic bags	817.83a	77.7a	10.2a
Re-usable substrate containers (<i>Visado</i>)	694.58ab	65.6ab	11.2a
Coloured plastic bags (<i>Rambo</i>)	668.33bc	63.1bc	11.4a
Shelved clear plastic bags (<i>Vipete</i>)	523.75c	49.4c	10.8a

^aMeans within a column followed by the same letter are not significantly different at $p \leq 0.05$ according to Fisher's LSD; values are means of four replicates

^b%BE = (g fresh mushrooms/g dry substrate) x 100

Table 2: Mean yield, percentage biological efficiency, size and mushroom solids content of *P. ostreatus* grown on three types of substrates

Substrate type	Yield (g/g) ^a	BE (%) ^{ab}	Size (g) ^a	Solids (%) ^a
Banana leaves	778.06a	74.0a	10.4a	9.70a
Rice straws	891.25a	84.3a	10.8a	9.45ab
Maize cobs	357.81b	33.5b	11.4a	9.20b

^aMeans within a column followed by the same letter are not significantly different at $p \leq 0.05$ according to Fisher's LSD; values are means of four replicates

^b%BE = (g fresh mushrooms/g dry substrate) x 100

Comparison of mineral content of oyster mushroom to commonly used vegetables:

Significant differences ($p \leq 0.05$) were observed on mineral contents of mushroom grown on various substrates and those of leafy vegetables. Significantly higher Fe content were from amaranth (439.8 mg/kg) followed by cassava leaves (362.0 mg/kg) (Fig. 5). The

least Fe content was from oyster mushrooms (49.7 mg/kg) produced from *Leucaena leucocophala* substrate. Significantly higher Zn content were from mushrooms produced from rice straws substrate (113.5 mg/kg) followed by mushrooms produced from maize cobs (100.9 mg/kg) substrate (Fig. 5).

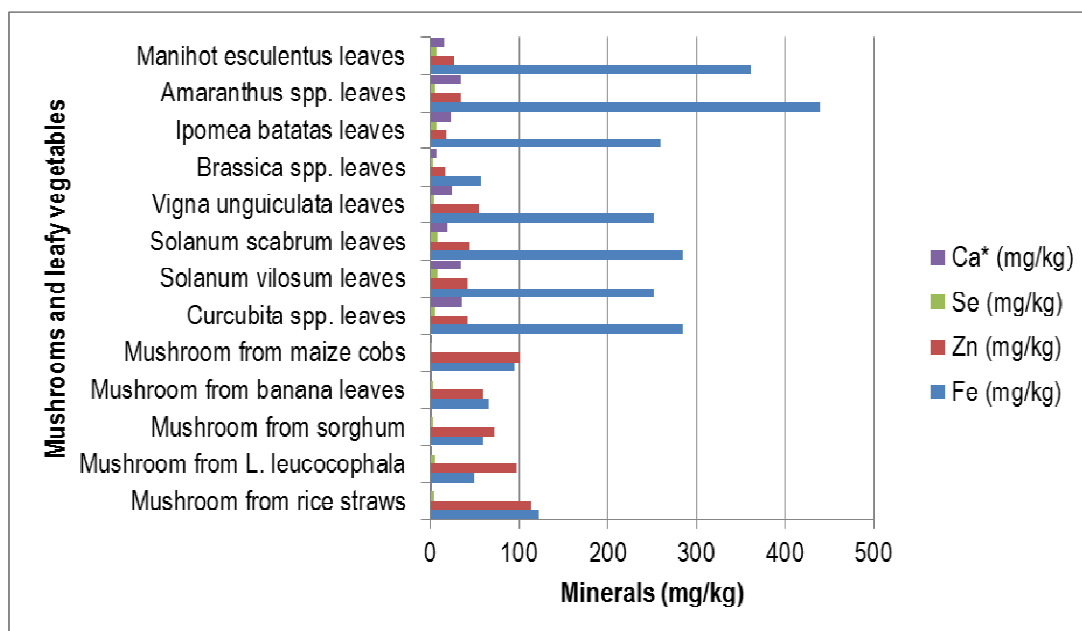


Figure 5: Mineral content of mushrooms and leafy vegetables

The least Zn content was from cabbage (16.3 mg/kg). Significantly, higher Se content was from narrow-leaved and broad-leaved night shades (8.0 gm/kg) followed by cassava leaves and sweet potato leaves (7.0 mg/kg). The least Se content was from oyster mushrooms (1.0 mg/kg) produced from maize cobs substrate. Significantly higher Ca content were from pumpkin

leaves (35.50 g/kg) followed by amaranth leaves (34.6 g/kg). The least Ca content was from oyster mushrooms (0.93 g/kg) produced from banana leaves substrate. Calcium content of mushrooms from any type of substrate was not significantly different and was the lowest ranging from 1.14 g/kg to 0.93 g/kg (Fig. 5).

DISCUSSION

Effects of substrate types and substrate containers on yield, biological efficiency, size and solid content of oyster mushroom: *Pleurotus* spp. is produced commercially and at small scale on plastic bag substrate containers (Royse, 2003; Chang & Quimio, 1982). Although different modifications and arrangement in the growing rooms for substrate containers has been applied, no grower has successfully eliminated the use of disposable plastic bags as substrate containers in small-scale oyster mushroom production. A number of substrate containers used in the production of oyster mushrooms has been described by Kurtzman (2006). They include such substrate containers as open straw bales, trays of various descriptions, plastic bags and plastic tubs that cannot be re-used. In Japan, re-usable bottle in the production of oyster mushroom is common and is becoming popular in the United States of America (Royce, 2003). This study has shown that it is possible to produce oyster mushrooms by using RSC for fructification and therefore successfully eliminate the use of disposable plastic bags. While yields obtained from RSC were not as high as those obtained from hanging clear plastic bags were, they were significantly higher than those obtained from coloured plastic bags and shelved clear plastic bags. The study results show that it is possible to obtain substantial oyster mushroom yields by using RSC. Re-usable substrate containers used in our experiment were locally manufactured 5 L plastic bucket modified by researchers to be suitable RSC without manufactures' specification for use in oyster mushroom cultivation. Although results from this study are promising, more work is required by manufacturers to obtain heat-resistant RSC specifically to be used in oyster mushroom cultivation and frequent usage. Re-usable substrate containers are ideal technology for small-scale oyster mushroom producers in the developing countries where re-cycling of plastic materials is not in place. On the other hand, the results are based on relatively small containers and may not reflect the experiences of growers who use large plastic bags that contain more than 3 kg of substrate on w/w basis and bales in small-scale oyster mushroom production industry in the developed countries. Three-kilogram RSC are ideal in the warm tropics to avoid heating up of substrate at the centre, and this is the reason for the selected container size in our study. The use of RSC may also help to reduce the problem of environmental contamination or degradation by plastic bag waste disposal. Oyster mushrooms harvested from

banana leaves had the highest solids while those from maize cobs had the lowest. The solids content reported in this study were slightly higher if compared to those of *Agaricus bisporus* ranged from 7.2-8.7% (Weil *et al.*, 2004; 2006) and 7.5-8.6% (Schroeder & Schisler, 1981). Oyster mushrooms may contain more nutrients other than those reported in this study, which might have contributed to their slightly higher solids content.

Comparison of mineral content of oyster mushroom to commonly used vegetables: Overall Fe from common vegetables except cabbages were higher than from oyster mushrooms produced from any type of substrates. The highest Fe content were from amaranth followed by cassava leaves while the least were from oyster mushrooms produced from sorghum grains followed by cabbages. This trend was closely similar to Ca and Se contents. However, Ca contents for oyster mushrooms from any substrate were similar and were 1140 mg/kg from rice straws, 1030 mg/kg from sorghum grains, 930 mg/kg from banana leaves and 1100 mg/kg from maize cobs. The highest Se content were from narrow-leaved African night shade and broad-leaved African night shade, and had the same value (8.0 mg/kg) while the least (2.7 mg/kg) were from oyster mushrooms produced from sorghum grains and banana leaves substrates. Overall Zn from oyster mushrooms was higher regardless of substrate types than from common vegetables. Cabbages had the least zinc content. The highest Zn content was from oyster mushrooms produced from rice straws followed by oyster mushrooms produced from maize cobs. Compared with vegetables, oyster mushrooms produced from any substrate were found to be good sources of Zn in ranges of 113.5-59.8 mg/kg while in vegetables the range 54.1-16.3 mg/kg. This is in agreement with the study by Mattila *et al.* (2001) where they also found that compared with the vegetables, both white and brown button mushrooms (*Agaricus bisporus*), *Lentinus edodes*, and *Pleurotus ostreatus* were good source of many mineral elements (K, P, Zn, and Cu). The mineral content of the basidiomata of *Pleurotus ostreatus* were found to be different (Vetter *et al.*, 2005) on different substrates (Bisaria *et al.*, 1987b). Iron was significantly higher in mushrooms produced from rice straw substrate followed by maize cobs substrate, then banana leaves, sorghum grains and finally *Leucaena leucicophala*. Variation of other mineral contents in the fruiting bodies (Zn, Se, Ca) was also found to vary from substrate types. Vetter *et al.*, (2005) obtained different mineral compositions in

Agaricus bisporus, *Pleurotus ostreatus* and *Lentinus edodes* produced from different types of substrates. We were able to demonstrate the utilization of RSC to produce oyster mushrooms. The utilization of RSC conserve environment from dispose of plastic bags which are non-biodegradable and if burned may cause ill-health effect to the human. Additional research is needed to specify quality of material to be used in the

manufacture of RSC and specifications to suit cultivation of oyster mushrooms. It was evident also that consumption of oyster mushrooms in combination with other vegetables would complement availability of various essential dietary elements. However, additional work is required to explore more range of elements and more vegetable species.

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