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# Effects of Various Substrates on Growth and Yield of Oyster Mushroom (*Pleurotus ostreatus*)

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**Abstract:** Mushrooms are increasingly becoming an important component of diets worldwide and it is of paramount importance to choose appropriate substrates in a given place to grow them. The experiment was conducted at the University of Swaziland, Faculty of Agriculture, in the Crop Production Department Mushroom Laboratory. The objective was to determine the effects of some of the locally available substrate materials on the growth and yield of oyster mushroom (*Pleurotus ostreatus* Jacq. Et Fr). Banana leaves, sugarcane tops, common thatch grass (*Hyparrhenia hirta*) and cattle manure were milled, bagged and autoclaved for 5 h at 120ºC, cooled and then inoculated with actively growing mushroom culture on sorghum grain. The bags were incubated until mycelium had fully colonized the substrate and then taken to the cropping house. Sugarcane tops had significantly ($p<0.05$) lower number of contaminated bags and in increasing order of contamination followed by banana leaves, thatch grass and lastly kraal manure. Kraal manure in all bags was contaminated and was subsequently discarded. There were significant ($p<0.05$) differences in total mushroom yield, marketable yield, mushroom stalk length and mushroom cap diameter. Sugarcane tops produced the highest total mushroom yield, marketable yield and mushroom cap diameter, followed in decreasing order by banana leaves and thatch grass. However thatch grass produced the longest mushroom stalks followed in decreasing order by banana leaves and lastly sugarcane tops. The experiment showed that, in decreasing order, sugarcane tops, banana leaves and thatch grass can be used as one of the best locally available substrate for mushroom production in Swaziland, for the growth and yield parameters measured.

**Key words:** Locally available substrate, mushroom cap diameter, *Pleurotus ostreatus*, yield

## INTRODUCTION

Mushrooms have been used by mankind since prehistoric times. They are macro-fungi which have a soft delicate distinct fruiting body, large enough to be seen by the naked eye and picked by hand (Zadrazil, 1974; Anonymous, 2004; Flegg *et al*., 1985). They grow under the soil or any suitable material called substrate or medium. At maturity the fruiting body looks like an umbrella structure. Mushrooms are used all over the world as a delicacy from pizza toppings in urban areas to a highly cherished side dish in many rural parts of southern Africa. The Egyptians considered mushrooms as a delicacy reserved for Pharoahs while the Romans ate mushrooms at feasts and believed that mushrooms provided strength for warriors in battle (Jahan *et al*., 2010) and in the far East mushrooms are venerated for their medicinal value (Chang and Miles, 2004). Mushrooms can be picked from the wild during the latter wettest part of the rainy season, where they are found growing on deeply decomposing organic matter. However not all mushrooms found growing in the wild are good for human consumption. Some are edible, but other species are poisonous making people sceptical about their consumption in general (Oei, 1996). Growing of safe known mushrooms therefore, presents a window of opportunity. The market for mushrooms continues to grow due to their interest in their culinary, nutritional, health benefits and their potential for use in waste management (Zadrazil, 1980; Beetz and Kustidia, 2004). Various agricultural wastes can be made useful as mushroom substrate or media for mushroom growth in entrepreneurial projects.

In Swaziland a pilot project, initiated by His Majesty King Mswati III was started in 2001 by the then Ministry of Agriculture and Co-operatives (MOAC) on mushroom

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production particularly oyster mushroom (*Pleurotus ostreatus*). The project was meant for poverty alleviation through job creation in rural Swaziland (Anonymous, 2004) and the idea was to end up with industrial production (Zadrazil, 1974). Strong follow up studies pertaining to use of locally available mushroom substrates in the various agro-ecological zones of the country have lagged behind for such a noble national project.

Mushrooms are a good protein source, with protein content ranging from 3-7% when fresh and up to 25-40% when dry (Wang *et al*., 2000) and contains a variety of vitamins and minerals (Chang and Miles, 2004; Beetz and Kustidia, 2004; Adejumo and Awosanya, 2005). Achieving food security and eradication of malnutrition are some of the goals enshrined in the Milenium Development Goals (MDGs). By mushroom cultivation, it is possible to alleviate poverty and create employment opportunity for educated unemployed youths, adolescents and women (Jahan *et al*., 2010). After satisfying domestic demands there is potential for the country to export mushrooms in the region and to other markets such as European Union (EU), America and countries of the far East such as Japan. Therefore a well managed mushroom project can generate cash income for the household and there exists a big potential of an advanced mushroom industry in the country.

A number of organic substrate material are available for mushroom production, e.g., pine sawdust, corncobs, cotton waste and seed hulls, and wheat/rice/barley straw (Zadrazil, 1980; Oei, 1996; Labuschagne *et al*., 2000; Yamashita *et al*., 2002; Onyango *et al.*, 2011 ). However various places are endowed with different substrates in varying amounts. What is important is to as asses availability of a given substrate and its ability to grow the highest yield of relatively good quality attributes in a given locality. The general objective is to contribute knowledge on mushroom production in the Kingdom of Swaziland to achieve food and nutritional security using locally available substrate materials. This particular study was aimed at investigating the effects of different substrates, i.e., kraal manure, banana (*Musa* cvs) leaves, sugarcane (*Saccharum officinarum*) tops and common thatch grass (*Hyparrhenia hirta*) on oyster mushroom growth parameters and yield.

## MATERIALS AND METHODS

**Experimental site:** The experiment was conducted during the period between October 2007 and April 2008, at the University of Swaziland, Faculty of Agriculture, Luyengo campus, at the Crop Production Mushroom Laboratory. Luyengo is located at latitude 26º34′S and longitude 31º34′E. The average altitude of this area is 750 m above sea level. The mean annual precipitation is 980 mm where most of the rain falls between October and April. The average summer temperature is 27ºC while for winter it is about 15ºC. Drought hazard is about 40%. In the mushroom house temperature was maintained between 28 and 30ºC.

**Sorghum preparation:** Untreated sorghum seeds, weighing 1.5 kg were first soaked in water overnight, and then boiled for 30 min the following day. After cooling the sorghum seeds, 30 g glucose (at a rate of 20g /kg of sorghum seeds) and 7.5 g of 0.1% dolomite limestone were added and mixed thoroughly. Bottles of 375 mL volume were half-filled with the sorghum and where autoclaved for 2 h and then cooled.

**Spawn making:** Fungal slants, on Potato Dextrose Agar (PDA), that had been prepared previously and stored in a refrigerator at 4ºC were used (Khare *et al*., 2010). The slants were used to inoculate 90 mm Petri dishes containing PDA, which were then incubated for a period of seven days at 24ºC in the dark. After the incubation period, the actively growing mycelium was cut from the periphery of a Petri dish into plugs as culturing material. The plugs were inserted in bottles containing autoclaved sorghum seeds. All processes described were done under near sterile conditions of the lamina air flow chamber to minimise potential contamination. The bottles were incubated at 25ºC until sorghum seeds were fully colonised.

**Substrate preparation:** Organic substrate materials used were: kraal manure and banana leaves collected from the departmental farm, sugarcane tops collected from nearby sugarcane fields, and common thatch grass obtained at Pine valley. Sterilisation of substrate material was done at Malkerns Research Station. Ten kg of each substrate was weighed, to which 2 kg (20%) wheat bran was added, using a ratio of 1:5 wheat bran to substrate respectively. The mix was wetted to about 65-70% moisture, mixed and bagged into autoclaveable bags which were fastened using elastic rubber bands and then sterilised. The bags were then autoclaved at 120ºC for 5 h and cooled.

**Culturing:** The cooled bags were inoculated using the sorghum grain spawn under a lamina air flow to minimise contamination. The bags were incubated to attain full mycelium colonisation, at temperatures between 28 and 30ºC.

**Design of experiment and data collection:** Treatments consisting of four substrates were laid in a Randomised Complete Block Design (RCBD), each treatment replicated 4 times with 10 bags making a replicate, giving a total of 40 bags per treatment. Data were collected over a period of four weeks on the following parameters: number of contaminated bags, number of days to full colonisation, total mushroom yield, total marketable yield, mushroom cap diameter and stalk length.

**Number of contaminated bags:** Each treatment contained 40 bags, which were randomly distributed into 4 replicates of 10 bags per replicate. The number of contaminated bags were counted in each replicate, and then added up to form the total number of contaminated bags in that treatment.

**Total mushroom yield:** The mushrooms were harvested in bundle form, when the outer margin of the fruiting body had just rolled inwards, on the verge of becoming horizontal. Care was taken to minimise tearing of the substrate material when harvesting was done. The bundles were then weighed on a scale, the mass taken in grams (g) which then represented the total mushroom yield per treatment.

**Total marketable mushroom yield:** During harvesting, part of the substrate material remained at the end of the mushroom stalk. Some of the mushrooms were still small, while others were deformed. All undesirable material was removed and discarded. The mushroom stalk was trimmed. Grading of the good marketable mushroom was done subjectively by visual assessment of the harvester, and subsequently weighed.

**Mushroom cap diameter:** The distance from one end to the other of the mushroom cap, going through the center of the cap was measured in millimeters (mm) using a string, which was then put against a graduated rule to take the measured length. Five mushrooms were taken at random from each harvest for each treatment, and measurements were done. The average cap diameter was then calculated for a given harvest per treatment.

**Mushroom stalk length:** The stalk is the 'stem-like' part on which the cap rests on. The stalk length was measured before mushroom grading. A string was used to measure the length of the stalk from the point of attachment on the substrate to the point where the gills of the cap started on the stalk. The string was then put against a rule to get the reading of the measurement in mm as previously described. The average mushroom stalk length was then calculated for that days harvest for each treatment.

**Cropping house practice:** To avoid contamination in the cropping house, a footbath solution of Jay's fluid disinfectant was used. Watering of the sand floor was done daily to keep a humid environment, ideal for fruiting of the mushrooms. The relative humidity (RH) of the cropping house ranged between 80% to near saturation.

**Data analysis:** Collected data were analysed using MSTAT-C (Nissen, 1989) statistical package (version 2.0). Analysis of variance (ANOVA) was done, where significant differences were detected, mean separation was done by LSD at the 5 % probability level.

## RESULTS AND DISCUSSION

**Contaminated bags:** During the incubation period, some bags were contaminated (Fig. 1). Green mould (*Trichorderma harzianum*) was the major contaminant of the various substrates. Contamination of bags significantly ($p<0.05$) differed among substrates and ranged from 4 to 40 bags (Fig. 1). All the cattle manure bags were contaminated, thus this treatment failed and was therefore abandoned. In decreasing order of contamination, thatch grass was significantly ($p<0.05$) different from banana leaves and sugarcane tops exhibited the least contamination. All the cattle manure bags were contaminated probably because it may not have been sufficiently sterilised. Low nitrogen level necessary for bioconversion of lingo-cellulose material (Anonymous, 2007) may have attributed to more contaminated bags. Several causes of contamination of mushroom substrate have been reported and ways of avoiding potential contamination are suggested (Kurtzman, 2010).

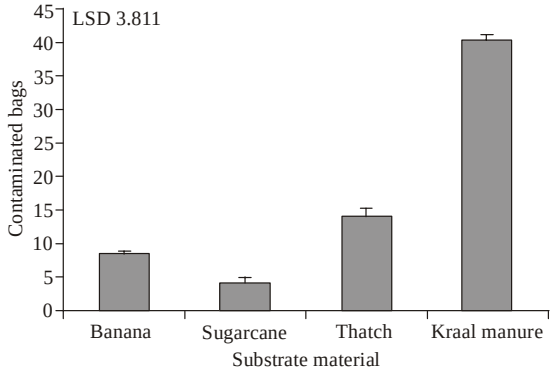


Fig. 1: Number of contaminated bags of the various substrates intended to grow mushroom (*Pleurotus ostreatus*)

Table 1: Number of days to full colonisation of mushroom (*Pleurotus ostreatus*) substrate

| Treatment | Days to full colonisation |
|---|---|
| Banana leaves | 98a* |
| Thatch grass | 61b |
| Sugar cane tops | 52b |
| CV 22.18 % | |
| Significance | * |
| LSD (p<0.05) | 35.363 |

*: Maens followed by different letters in a column are significantly different at p<0.05

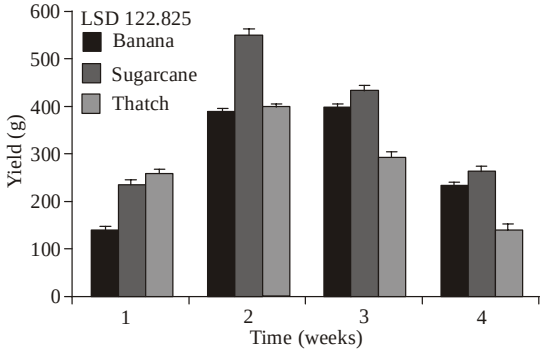


Fig. 2: Total mushroom (*Pleurotus ostreatus*) yield during four weeks of growth on various substrates

**Number of days to full colonization:** The number of days to full colonisation of the different substrates was not the same. The mycelium failed to colonise the cattle manure substrate. The number of days taken to colonise various substrate materials ranged from 52 days to 98 days (Table 1). There were a significant (p<0.05) differences in days taken to colonise the various substrates as previously mentioned. Days taken to colonise banana leaves were significantly (p<0.05) longer (98 days) than those taken to colonise thatch grass (61 days) and sugarcane tops took the least number of days to be colonised (52 days). Sugarcane tops have high polysaccharides, which are carbohydrates in nature. Carbohydrates hasten the growth of fungus, therefore much faster colonisation (Oei, 1996). These results are in agreement with those of Kimenju *et al*. (2009) and Onyango *et al*. (2011).

**Total mushroom yield:** Yield over the four week period of the experiment is shown in Fig. 2. Yield on sugarcane tops and thatch grass were significantly (p<0.05) higher than mushroom yield from banana leaves at week 1. Mushroom yield on sugarcane tops was significantly (p<0.05) higher than yield of mushroom from other substrates which were similar at week 2. Total mushroom yield difference was not significant at week 3 for banana leaves and sugarcane tops which however showed

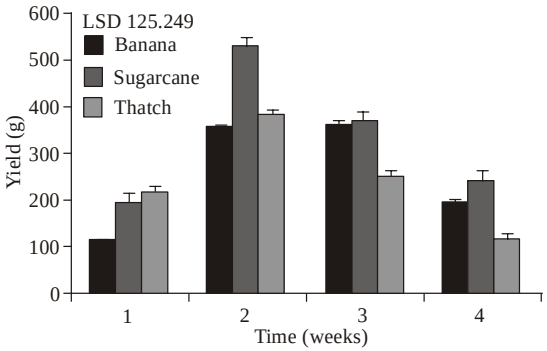


Fig. 3: Total marketable yield of mushroom (*Pleurotus ostreatus*) during four weeks of growth on various substrates

significantly (p<0.05) higher yield than thatch grass. Overall, mushrooms grown on sugarcane tops were larger and total yield was higher than mushrooms grown on other substrates during the four weeks of the experiment. These results are in agreement with results previously obtained using several substrate materials (Shah *et al*., 2004; Onyango *et al*., 2011) which performed differently in terms of mushroom yield obtained. Banana psedostems did not perform well in a study reported by Khare *et al*. (2010), however in other studies they were reported to be suitable substrates for cultivation of oyster mushroom (Quimio *et al*., 1990; Jandaik, 1997). In our study banana leaves derived substrate gave intermediate results. The importance of this study was to establish suitability of locally available substrates in oyster mushroom production. Evaluation of locally available substrates for production of oyster mushroom has been reported (Shah *et al*., 2004; Kimenju *et al*., 2009; Khare *et al*., 2010).

**Marketable mushroom yield:** Sugarcane tops and thatch grass yielded significantly (p<0.05) higher marketable mushrooms than banana leaves in week 1 (Fig. 3). Yield on sugarcane tops was significantly (p<0.05) higher than on other substrates at week two. There was no significant (p>0.05) difference in marketable mushroom yield at week three for banana leaves or sugarcane tops grown mushroom. The mushroom yield on banana leaves and sugarcane tops was significantly (p<0.05) higher than that of mushrooms from thatch grass at week three. At week four sugarcane tops significantly produced mushrooms of higher marketable yield followed in decreasing order by banana and lastly thatch derived substrate. Generally, mushrooms grown on sugarcane tops were of higher yield and more marketable than mushroom from the other substrates. Trends, causes and effects observed for total

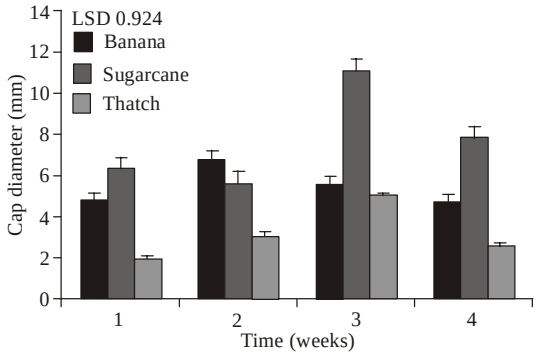


Fig. 4: Cap diameter of mushroom (*Pleurotus ostreatus*) during four weeks of growth on various substrates

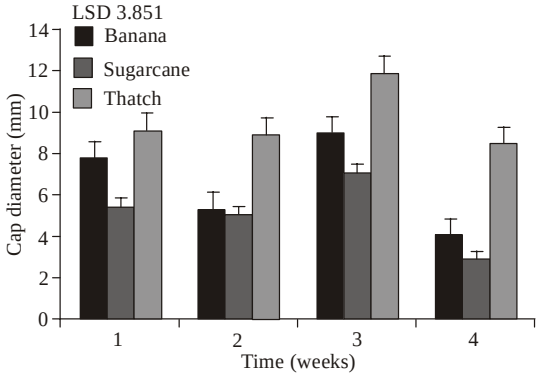


Fig. 5: Stalk length of mushroom (*Pleurotus ostreatus*) during four weeks of growth on various substrates

mushroom yield were generally similar to those of marketable yield. This is expectable (Oei, 1996).

**Mushroom cap diameter:** Substrate treatments resulted in significantly ($p<0.05$) different mushroom cap diameter from each other depending on treatment. The mushrooms grown on sugarcane tops had significantly ($p<0.05$) larger mushroom cap diameters over the 4 week period and in decreasing order followed by banana leaves and lastly thatch grass (Fig. 4). The sugarcane tops derived medium had the most amount of nutrients required by oyster mushroom followed in decreasing order by banana leaves and lastly thatch grass. Such nutrients include carbon found in cellulose, nitrogen (N), potassium (K) phosphorus (P) and micronutrients among others (Oei, 1991, 1996). Relatively larger mushroom cap diameter is desirable and growing media or substrate that promote such should be sought after coupled with favorable addatives (Onyango *et al*., 2011) and environmental conditions.

**Mushroom stalk length:** There were significant ($p<0.05$) differences in the mushroom stalk length among treatments at weeks 1, 2, 3 and 4 (Fig. 5). The stalk length for mushrooms grown on thatch grass was significantly ($p<0.05$) longer than those of mushrooms from banana leaves and sugarcane tops in decreasing order. The trend of results of mushroom stalk length are kind of opposite other growth parameters measured. Relatively long mushroom stalk length is an undesirable characteristics at the expense of marketable quantity. Nutritional inadequacies can potentially lead to this situation (Oei, 1996). Previous studies on carbon ©/ nitrogen (N) ratio have shown that low N content may the limiting factor in mushroom production (Jandaik, 1997). Growers should use substrates that do not promote excessive growth of stalk length at the expense of marketable yield. Supplementaion of substatrates with various addatives including nitrogen sources has been shown to improve growth, yield and quality of mushrooms (Khare *et al*., 2010; Onyango *et al*., 2011). In our study the only supplement used was wheat bran.

## CONCLUSION AND RECOMMENDATION

Sugarcane tops showed the least number of days (time) to full colonisation by mycelium and to produce mushrooms of relatively superior attributes and higher yield than the other substrate material (banana leaves and thatch grass) used in this experiment. Sugarcane tops could be one of the best substrate for mushroom (*P. ostreatus*) growing in Swaziland based on the results of this experiment, and fortunately sugarcane tops are found in abundance in the Kingdom. Repeating this experiment in other regions would be appropriate as it was only done under the Luyengo conditions, of particular substrate availability. Other substrates like sugarcane bagasse, wood shavings/swadust and pineapple plant remains which are available in abundance in various parts of the Kingdom can also be explored in future trials coupled with benefit cost analysis.

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