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Growth and Yield Response of Oyster Mushroom (*Pleurotus ostreatus*) Grown on Different Locally Available Substrates

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Abstract: Oyster mushroom (*Pleurotus ostreatus*) production is low despite its high demand in Swaziland. Most communal farmers dispose of their agricultural waste while it can be used usefully as substrates for the production of mushrooms. The objective of this study was to compare the effect of different agricultural wastes used as mushroom substrates on growth, development and yield of mushroom. The substrates investigated were banana leaves, sugarcane tops, maize stover and maize stover and cobs (1:1 dry mass/dry mass). The study was conducted at the University of Swaziland, Faculty of Agriculture-Luyengo Campus, at the Crop Production's mushroom laboratory. Sterilization of substrates was done at the Malkerns Research station. *Pleurotus ostreatus* was evaluated for growth and yield using four replicate bags of sugarcane tops, maize stover, maize stover and cobs and banana leaves as substrates. The moist substrates were sterilised, packed in heat-resistant plastic bags, seeded with 2-4% spawn and incubated for 3-3.5 months. Yield of each mushroom flush, marketable yield, pileus diameter and stipe length were measured and recorded. For the first flash the significantly ($p<0.05$) highest yield was obtained from maize stover and cobs followed in decreasing order by banana leaves, sugarcane tops and lastly maize stover gave the least yield. The trend was similar for the second and third flash except that in the third flash sugar cane tops produced mushroom of higher yield than banana leaves, similar trends were measured for the other mushroom attributes. The maize stover and cobs substrate gave the highest yield which was 221.7, 189.2 and 107.9 g in the first, second and third flashes, respectively.

Keywords: Marketable yield, mushroom flush, oyster mushroom, pileus diameter, stipe length, substrate

INTRODUCTION

Oyster mushroom (*Pleurotus ostreatus* Jacq.et Fr) production in Swaziland is low despite the high demand in the market; this can be attributed to the lack of knowledge on how to grow and nurture mushrooms (Shongwe, 2007). Mushroom production in Swaziland is not a common enterprise among small scale farmers. This can be explained by the fact that most farmers grow maize, however maize requires relatively high rainfall yet rain has become a scarce resource of late (Oseni and Masarirambi, 2011). Mushrooms are not dependent on weather conditions such as rainfall and can be grown all year round in a cropping house. A mushroom is a macro fungus, which has a distinct fruiting body, which can be either epigeous or hypogenous and large enough to be seen by the naked eye and picked by hand (Zadrazil, 1974; Flegg *et al*., 1985; Chang and Miles, 1992).

Mushroom cultivation has been reported as an alternative way of alleviating poverty in developing countries due to its possibility of low cost of production, high profit and quick returns (Masarirambi *et al*., 2011). Farmers can utilise agricultural wastes, such as dried sugar cane leaves, saw dust, maize stover and banana leaves as substrates for mushroom production (Beetz and Kustida, 2004; Lourdes *et al*., 2008). Fresh mushrooms have traditionally been imported into Swaziland (Anon, 2007).

Oyster mushroom contains 20-35% protein in dry weights which makes its protein higher than that of vegetables and fruits and is good as ingredients of functional foods (Wermer and Beelman, 2002; Chandy, 1997). Mushrooms are considered ideal for patients of hypertension, renal effects and diabetics (Yip *et al*., 1987; Chandy, 1997), their immunomodualatory and antitumor activities of Polysaccharide-Protein Complex (PSPC) from mycelial cultures (Liu *et al*., 1995, 1996; Wang *et al*., 1995, 1996b, 1997) and their immunomodualatory and antitumor activities of lectins from edible mushrooms (Wang *et al*., 1996b, 1996a, 1997) gives them valued medicinal value. Other mushrooms are known to have medicinal properties, for an example bracket mushroom (*Ganoderma lucidum*)

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has been reportedly used for disease management of patients with HIV and AIDS and can be justified by the increase in body weight (Chang and Buswell, 1999; Chang and Miles, 2004; Anon, 2007).

Swaziland is faced with the problem of the prevailing dry weather conditions which makes it difficult to grow field crops like maize (Oseni and Masarirambi, 2011). Most communal farmers dispose of their agricultural wastes while it could be used as substrates for the production of mushrooms (Shongwe, 2007). Swaziland imports large quantities of mushrooms while less research has been done on locally available substrates to avoid the loss of revenue through mushroom importation (Shongwe, 2007). Not enough information is available on the potential of locally available agricultural wastes for use as substrates in mushroom production.

The purpose of this study aimed at investigating the growth and yield response of Oyster mushroom (*Pleurotus ostreatus*) grown on locally available substrates. The substrates were; banana (*Musa* cvs) leaves, sugarcane (*Saccharum officinarum*) tops, maize (*Zea mays* L.) stover and maize stover mixed with maize cobs (1:1 dry weight/dry weight).

MATERIALS AND METHODS

Project site: The project was conducted at the University of Swaziland (UNISWA), Faculty of Agriculture-Luyengo Campus, at the Crop Production's Mushroom Laboratory and sterilisation of substrate was done at the Malkerns Research Station. The Luyengo campus is located at 26°34′S and 31°21′E while Malkerns Research Station is about 3 km NW of Luyengo.

Substrates: The four organic substrates used for the production of the oyster mushrooms (*Pleurotus ostreatus*) were collected around the Crop Production Farm while sugarcane tops were obtained from Illovo Sugarcane Company, Big Bend. To increase the surface area, the substrates were milled at the UNISWA Farm using a tractor powered mill. Maize cobs were not milled but broken into small pieces of about 2 mm in length using a hammer to reduce chances of spoilage through contamination. This was done because it was noted in experiments done previously that once the maize cobs were too fine they were easily contaminated.

Culture preparation: The spawn was cultured from a potato dextrose agar slant that had previously been prepared and stored at 4°C. Such slant (about 1x1 cm) was used to inoculate newly prepared potato dextrose agar in Petri dishes and were sealed using parafilm (BRAND GMBH + CO KG, Wertheim, Germany) to avoid contamination. They were then incubated (in the dark) for a period of 14 days at temperatures around 24°C.

Sorghum preparation: Untreated sorghum seed (sourced from Malkerns Research Station) weighing 2 kg were soaked overnight in water, with the water discarded 3 times and fresh water added upon every decantation. The following day, the soaked sorghum seeds were cooked at 96°C using a cooker for 2 h and then cooled and dolomitic lime was added to separate the grain at the ratio of 1kg substrate: 65% (dry substrate mass) lime. The dolomitic lime was added so as to allow the seeds to be friable so that it could be easy to inoculate. The sorghum seeds were then put into 350 mL bottles which were then covered with cotton wool and plain paper. The bottles were then autoclaved (TICA, Bangkok, Thailand) for 30 min and then after autoclaving they were allowed to cool under the lamina airflow (Vivid Air, Durban, South Africa) without opening the bottles.

Spawn culturing: The culturing of the spawn was done under the lamina airflow. The actively growing mycelium obtained from the incubated Petri dishes was then cut into plugs (1x1 cm) and then 3 to 4 were inserted into the bottles with the autoclaved sorghum seeds, with the part with the actively growing mycelium touching the sorghum seeds. After covering them with cotton wool and a plain sheet of paper, the bottles were incubated at temperatures around 28°C for ease of colonisation.

Substrate preparation: Sixteen kgs of the substrates were weighed, wetted to about 65-75% moisture content and 10% wheat bran added. The squeeze method was used to determine the moisture content. When squeezing the substrate between fingers, small amounts of water oozed from the substrate. Having done that 1kg of the substrate was packed into an autoclaving bag and fastened using rubber bands. The substrates were then sterilized by autoclaving.

Culturing: The bags of the different substrates were marked and were sterilised at 102°C for a period of 4 h at the Malkerns Research Station. After sterilisation, the bags were inoculated using the spawn under the lamina air flow to reduce contamination. The bags were then placed in the incubation room (24-28°C) for colonisation to take place. Optimum colonisation takes place at temperatures around 24-28°C.

Design of the experiment: The experimental design was a single factor experiment which was laid in a Randomised Complete Block Design (RCBD). Each treatment was replicated 4 times with four bags per replicate adding to16 bags per treatment.

Data collection: The data that were collected on the following parameters: Number of contaminated bags,

number of days to full colonisation, total mushroom yield (g), marketable mushroom yield (g), mushroom pileus diameter (mm) and mushroom stipe length (mm).

Number of days to full colonisation: The date at which bags were put into the incubation room was marked. The date at which each bag attained full colonisation in each treatment was, also marked and the average number of days was then calculated for each replicate and the calculation of the total number of days to full colonisation was calculated using the averages of each replicate.

Number of contaminated bags: Each treatment contained 16 bags, which were randomly distributed into 4 replicates of 4 bags per treatment. The number of contaminated bags was counted in each replicate and then added up to form the total number of contaminated bags in a given treatment.

Total mushroom yield: The mushrooms were harvested in a bundle when the outer margin of the fruiting body had only just rolled inwards, on the verge of becoming horizontal and care was taken so as to reduce any disruption of the substrate during harvesting. The mushrooms were then weighed using a scale taking the mass in grams (g).

Total marketable mushroom yield: While some were harvested with deformities, unwanted materials and part of the substrate material which remained at the end of the stipe, trimming was done to give the total marketable portion which was then weighed on a scale in grams (g).

Mushroom pileus diameter: The mushroom pileus diameter was taken from one end of the pileus to the other passing through the centre of the pileus and measured in millimetres (mm). This was done using a string which was then placed along a rule to get the diameter. The pileus diameter was obtained on 5 randomly picked mushrooms, from the harvest and then the average pileus diameter was calculated for a given harvest.

Mushroom stipe length: To effectively get the right stipe length the readings were taken before trimming the stipe to get the total marketable yield. The stipe length was taken on the 5 mushrooms chosen to take the pileus diameter, using a string. The length was measured by placing the string from one end where it was attached to the substrate to the point where the gills on the pileus start on the stipe. Like the pileus diameter the string was placed along a rule to get the length in millimetres (mm). The average for that day's harvest was then calculated using the 5 readings.

Table 1: Number of days to full colonisation of substrates

Substrate	Number of days to full colonisation
Maize stover	58a
Maize stover and cobs	70a
Sugarcane	76a
Banana leaves	83 b
C.V.	18.78%
LSD ($p<0.05$)	24.3962

Means followed by the same letter are not significantly different from each other at $p<0.05$

Cropping house practice: The cropping house had to be free from any contaminant, so to achieve this footbath was used which was drenched with a solution of Jayes fluid (Cambridge, England) disinfectant containing carbolic acid. To reduce inoculums one had to deep shoes into the solution of Jayes fluid disinfectant. To promote optimum conditions for fruiting the sand floor was watered daily to keep the environment as humid as possible to about 85% Relative Humidity (RH) to near saturation, needed for mycelium to make a fruiting body.

Data analysis: Data collected were analysed using MSTAT-C statistical package (version 2.0) developed by Nissen (1989) at Michigan State University, East Lansing, Michigan, USA. Analysis of Variance (ANOVA) was done and were significant differences were detected mean separation was by LSD at the 5% level of significance (Gomez and Gomez, 1984).

RESULTS

Number of days to full colonisation of substrate: There was a significant ($p<0.05$) difference in the number of days taken by the fungus to fully colonise the substrates and the days ranged from 58 to 83 days (Table 1). The number of days taken to colonise the banana leaves was significantly ($p<0.05$) longer than those taken by maize stover, maize stover and cobs and sugarcane tops which were not different from each other.

Number of contaminated bags for substrates: During the incubation period, there were no bags that were contaminated by fungi, but there were bags which were contaminated by bacteria hence there was no mycelia growth. There was significant ($p<0.05$) difference in the number of contaminated bags. The number of bags varied from one to 2 bags per substrate (Fig. 1). Maize stover had the highest number of contaminated bags (2 bags) and was significantly ($p<0.05$) different from the other substrates. Banana leaves and maize stover and cobs were not significantly different from each other in terms of contamination. Sugarcane tops substrate had significantly ($p<0.05$) higher number of contaminated bags together with maize stover and cobes when compared to maize stover and banana leaves.

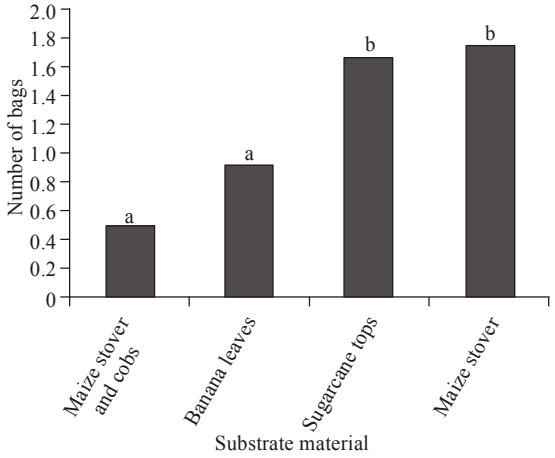

Fig. 1: Number of contaminated bags for the different substrates
Means followed by the same letter are not significantly different from each other at $p<0.05$

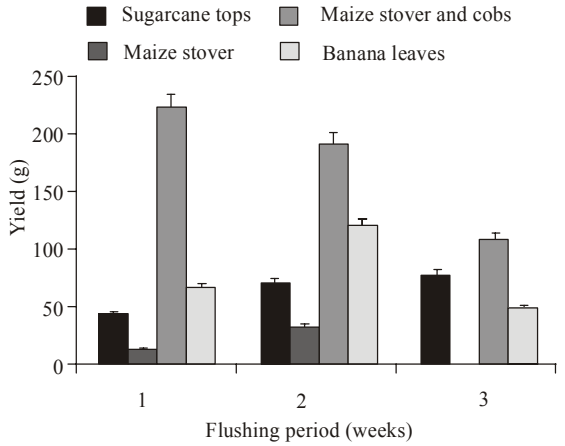

Fig. 2: Total oyster mushroom yield from the four substrates
Vertical bars represent standard deviation above and below the mean

Total mass of mushroom yield for the four different substrates: The total yield of the mushroom harvested was measured before grading was done. The yield of mushroom from the four different substrates is shown in Fig. 2.

In the first week, the mushroom yield for the 4 substrates ranged from 13.0 g in maize stover to 221.7 g in maize stover and cobs. There was significant ($p<0.05$) difference in the mushroom yield among the four different substrates. The mushroom yield in maize stover was lower than maize stover and cobs. There was significant ($p<0.05$) difference in the yield of mushroom from sugarcane tops, maize stover, maize stover and cobs and banana leaves.

During the second week, the mushroom yield from the four substrates ranged from 33.8 g in maize stover to 189.1 g in maize stover and cobs. There were significant ($p<0.05$) differences in the mushroom yield among the four different substrates. Maize stover and cobs had the highest yield of 189.2 g followed by banana leaves which had 119.9 g, then sugarcane tops which had 70.9 g and lastly maize stover with the lowest yield of 32.8 g.

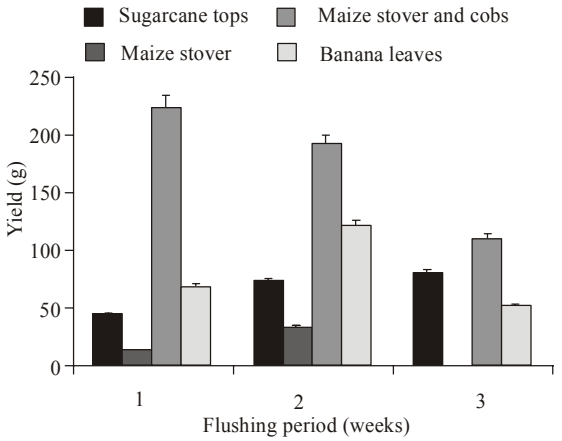

Fig. 3: Total marketable mushroom yield for the four substrates
Vertical bars represent standard deviation above and below the mean

In the third week, there were significant ($p<0.05$) differences in mushroom yield among the four different substrates. Maize stover and cobs substrate gave mushroom yield which was significantly higher than the rest of the substrates. The mushroom yield for the four substrates ranged from 0.0 g in maize stover to 107.9 g in maize stover and cobs.

Mass of marketable mushroom yield for the four different substrates: After each harvest, sorting and grading was done to obtain marketable mushroom. The yield of marketable mushroom from the four different substrates is shown in Fig. 3.

At week 1, there were significant ($p<0.05$) differences in marketable mushroom yield among the four different substrates of sugarcane tops, maize stover, maize stover and cobs and banana leaves.. The total marketable mushroom yield for the four substrates ranged from 12.8 g in maize stover to 220.7 g in maize stover and cobs. The marketable mushroom yield in maize stover was lower than maize stover and cobs. The trend was similar as for total mushroom yield.

At the second week, there was significant ($p<0.05$) difference in the marketable mushroom yield among the four different substrates. The marketable mushroom yield for the four substrates ranged from 32.2 g in maize stover to 188.4 g in maize stover and cobs. Finally, during the third week, there was significant ($p<0.05$) difference in the marketable mushroom yield among the four different substrates. The marketable mushroom yield for the 4 substrates ranged from 0.0 g in maize stover to 107.4 g in maize stover and cobs. Maize stover and cobs marketable mushroom yield was significantly higher than the rest of the substrates. The trend was similar as for total mushroom yield.

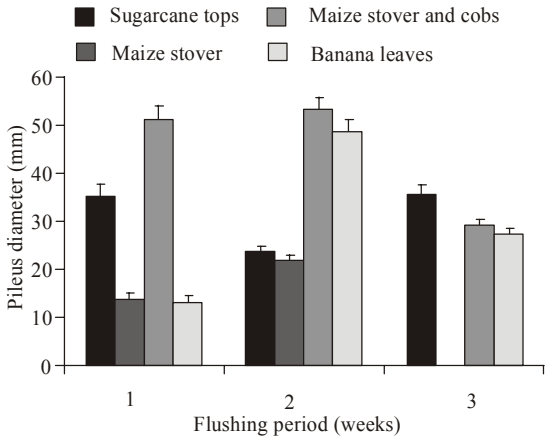

Fig. 4: Average pileus diameter for the four different substrates
Vertical bars represent standard deviation above and below the mean

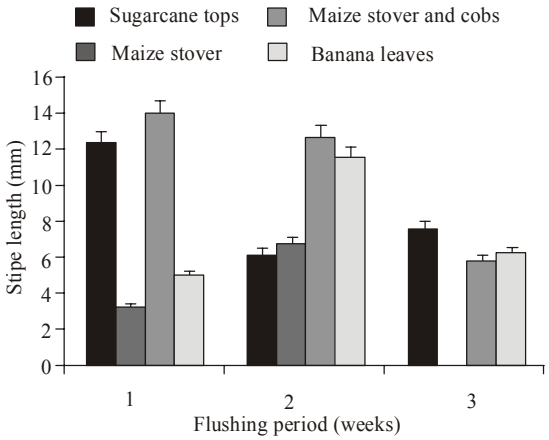

Fig. 5: Average stipe length for the four different substrates
Vertical bars represent standard deviation above and below the mean

Pileus diameter for the four different substrates: Figure 4 shows the mushroom pileus diameter, measured during the experiment for the four different substrates.

At the first week, there was significant ($p<0.05$) difference in the mushroom pileus diameter, where the mushroom pileus diameter ranged from 13.3 mm in banana leaves to 51.0 mm in maize stover and cobs. On the other hand, the pileus diameter for sugarcane tops was 35.3 mm, 13.8 mm in maize stover, 51.0 mm in maize stover and cobs and 113.3 mm in banana leaves. The pileus diameter for maize stover and cobs was significantly higher than the other substrates. There was however no significant difference in the pileus diameter of maize stover and banana leaves.

At the second week, there was significant ($p<0.05$) difference in the mushroom pileus diameter, where the mushroom pileus diameter grown on maize stover and cobs was highest followed by in decreasing order banana leaves, sugarcane tops and lastly maize stover. During the third week, there was significant ($p<0.05$) difference in the mushroom pileus diameter, where the mushroom pileus diameter ranged from 0.0 mm in maize stover to 35.2 mm in sugarcane tops. The pileus diameter for mushroom grown in sugarcane tops was higher than the rest of the substrates followed in decreasing order by mushrooms from maize stover and cobs, banana leaves and lastly maize stover.

Stipe length for the four different substrates: The stipe of the oyster mushroom was measured, in millimetres, during the experiment for the four different substrates and is shown in Fig. 5.

At the first week, there were significant ($p<0.05$) differences in the mushroom stipe length, the mushroom stipe length ranged from 3.3 mm from maize stover to 13.9 mm in maize stover and cobs. The stipe lengths for maize stover and cobs was significantly higher than that of the other substrates followed in decreasing order by mushroom from sugarcane tops, banana leaves and maize stover.

At the second week, there were significant ($p<0.05$) difference in the mushroom stipe length, where the mushroom stipe length in maize stover and cobs was significantly higher than the rest of the substrate materials followed in decreasing order by mushroom from banana leaves, maize stover and sugarcane tops.

At the third week, there was significant ($p<0.05$) difference in the mushroom stipe length, where the mushroom stipe length ranged from 0.0 mm in maize stover to 7.5 mm in sugarcane tops. The stipe length for sugarcane tops was higher than the rest of the substrates followed in decreasing order by mushroom from banana leaves, maize stover and cobs and lastly maize stover alone. However there was no significant difference between the stipe length of mushrooms from maize stover and cobs and mushrooms from banana leaves.

DISCUSSION

There was a significant difference in the number of days taken by the fungus to fully colonise the substrates. Maize stover took few days to be colonised by the fungus, which was significantly different from the other substrates. This then entails that maize stover had high amount of carbohydrates which were needed to hasten the growth of the mycelium and hence resulted in quick colonisation of the substrates (Oei, 1996).

There was significant difference in the number of contaminated bags. Maize stover had the highest number of contaminated bags (2 bags) and was significantly ($p<0.01$) different from the other substrates. Previously, similar to this study a relatively lower number of contaminated bags were observed for sugarcane tops and banana leaves (Masarirambi *et al*.,

2011). To minimise contamination, substrates for cultivating edible mushrooms (for an example *Pleurotus ostreatus*) normally require varying degrees of pretreatment in order to promote growth of the mushroom mycelium to the practical exclusion of other microorganisms (Chang, 2008; Oseni *et al*., 2012).

The marketable yield, for mushrooms grown on maize stover and cobs, was generally higher compared to those from the other substrates. The marketable yield increased in the second week from the different substrates and then decreased during the third week. The highest marketable yield, therefore, was obtained during the second week in the different substrates. Maize stover had the lowest marketable mushroom yield probably due to relatively lower amounts of carbohydrates made available to the growing mushroom. Masarirambi *et al*. (2011) reported similar results where the highest yield was generally obtained at the second week or flash. This was so probably because that was when maximum metabolism pertaining to substrate breakdown was attained.

The pileus diameter, for mushrooms grown on maize stover and cobs, was generally higher compared to those from the other substrates. Maize stover and cobs had the largest mushroom produced since the pileus diameter reflects how large the mushrooms were. The pileus diameter increased in the second week from the different substrates and then decreased during the third week. The highest pileus diameter, therefore, was obtained during the second week in the different substrates. Maize stover had the lowest pileus diameter hence smaller mushrooms.

The stipe length, for mushrooms grown on maize stover and cobs, was generally higher compared to those from the other substrates. Maize stover and cobs had the longest stipe length produced and maize stover had the shortest stipe length since it had smaller mushrooms. The longest stipe length was obtained during the second week in the four different substrates. Energy availability for mushroom growth after substrate degradation is time dependent. Measurement of bio-efficiency may be necessary (Oseni *et al*., 2012) in future studies.

CONCLUSION

Maize stover and cobs showed the best performance compared to the other substrates, in terms of growth parameters measured. High mushroom yield, which were relatively large in size, were obtained under maize stover and cobs substrate. Maize stover was the least performing substrate in terms of mushroom yield and size. Maize stover and cobs are relatively abundant in rural communities where resource poor farmers reside and are therefore recommended for use.

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