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Growth and Yield of Oyster Mushroom (*Pleurotus ostreatus*) Grown on Different Substrates Ammended with Varying Levels of Wheat Bran

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ABSTRACT

Oyster mushroom (*Pleurotus ostreatus* Jacq.et Fr) is gaining popularity in Swaziland. Agricultural wastes disposal can pose a challenge to communal farmers, however the organic waste can be used productively as substrates for the production of mushrooms. Substrate supplementation with wheat bran (WB) can boost mushroom yields. The objective of this study was to compare the effect of different agricultural wastes used as substrates on growth and yield of mushroom [banana leaves, sugarcane tops, maize stover, and maize stover and cobs (1:1 dry mass/dry mass)], with different levels of wheat bran amendments of. 0%, 10% and 15%. The moist substrates were sterilised, packed in plastic bags, seeded with 2-4% spawn and incubated for 3-3.5 months. The bags were incubated to allow full colonization of the various substrates and then taken to the cropping house. Yield of each mushroom flush, marketable yield, pileus diameter and stipe length was measured and recorded. Banana leaves amended with 10% WB and sugar cane tops amended with 15% WB significantly ($P < 0.05$) took the highest number of days to be fully colonized by mushroom mycelia compared to the other substrates. Maize stover amended with 10% WB had significantly ($P < 0.05$) the highest number of contaminated bags followed in decreasing order by sugar cane tops amended with 10% or 15 % WB and lastly the other substrates which had similar contamination. Mushroom yields were significantly ($P < 0.05$) higher in substrates amended with 15% WB, followed by 10 % WB and lastly straight substrate (0% WB). Maize stover and cobs to which 15% wheat bran was added gave the highest yield which were 382.2g, 360.7g, and 278.4g in the first, second and third weeks, respectively.

Keywords: Oyster mushroom, substrate plus wheat bran level, mushroom flush, marketable yield, pileus diameter and stipe length.

INTRODUCTION

Oyster mushroom (*Pleurotus ostreatus* Jacq.et Fr) is increasing in popularity in the Southern African Development Community (SADC) countries including Swaziland. Its 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). The economy of Swaziland is agro-based, with considerable amount of crop production. Crop residues are largely abundant as agricultural waste after harvest. It is important to dispose agricultural waste in a green way which is environmentally friendly in this era of climate change. Extreme environmental conditions due to climate change are already being experienced (Manyatsi et al., 2010). An alternative way of use of agricultural residues/wastes is in the use of the organic material in mushroom production (Chang and Miles, 2004; Khare et al., 2010).

A mushroom is a macro fungus, which has a distinct fruiting body, which can be either hypogeous or epigeous and large enough to be seen by a naked eye and picked by hand (Zadrazil, 1974; Flegg et al., 1985; Chang & Miles, 1992). Mushroom cultivation has been reported as other effective way of alleviating poverty in developing countries (Masarirambi et al., 2011). Oyster mushroom contains 20-35% protein in dry weight which makes it higher than that of vegetables and fruits (Yao, 1998; Anon., 2008a). Mushrooms are ideal for consumption by patients of hypertension and diabetics (Wermer and Beelman, 2002; Anon., 2008a). Other mushrooms are known to have medicinal properties (Yip et al., 1987; Chang and Buswell, 1999;) for example bracket mushroom (*Ganoderma lucidum*) has been used for disease management of patients with HIV and AIDS (Anon., 2007). Their immunodulatory and anti-tumor activities of polysaccharide – protein complex from mycelial cultures (Liu et al., 1995, 1996; Wang et al., 1995a, 1995b, 1996a, 1996b, 1996c, 1997) gives those valued medicinal properties.

Swaziland is faced with the problem of prevailing dry weather conditions which makes it difficult to grow field crops like maize (Oseni & Masarirambi, 2011). Mushrooms are not dependent on weather conditions such as rainfall and can be grown all year round through environmental manipulation. Swaziland imports large quantities of mushrooms while less research has been done to avoid the loss of revenue through **mushroom** importation (Shongwe, 2007) especially during the current financial challenges. Not enough information is available on the potential of using locally available agricultural residues/wastes ammended with wheat bran for mushroom production under local conditions.

The purpose of this study was to investigate the effect of different locally available substrates amended with different levels of wheat bran on Oyster mushroom (*Pleurotus ostreatus*) growth, development and yield.

MATERIALS AND METHODS

Project site

The study was conducted at the University of Swaziland, Faculty of Agriculture-Luyengo Campus, at the Crop Production's Mushroom Laboratory, while sterilisation of substrates 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 three km NW of Luyengo.

Culture preparation

The spawn was cultured from a potato dextrose agar slant that had previously been prepared and stored at 4°C. The slant (about 1x1cm) 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 any contamination and subsequently incubated in the dark for a period of 14 days at temperatures around 24°C (Dlamini et al., 2012) .

Sorghum preparation

Untreated sorghum seed (sourced from Malkerns Research Station) weighing 2kg were soaked overnight in water, with the water discarded three times and fresh water added upon every discarding. The following day, the soaked sorghum seeds were cooked using a cooker for 2 hours and then cooled and dolomitic lime was added to separate the grain at the ratio of 1 kg substrate: 65% (dry substrate mass) lime. The dolomitic lime was to allow the seeds to be friable so that it could be easy to inoculate. The sorghum seeds were then put into 350mL bottles which were then covered with cotton wool and plain paper. The bottles were then autoclaved (Gemmy Industries Co-operation, Taiwan) for 30 minutes and then after autoclaving they were allowed to cool under the lamina airflow (Vivid Air, Durban, South Africa) without opening the bottles (Dlamini et al., 2012).

Spawn culturing

Culturing of the spawn was done under the lamina airflow. The actively growing mycelium obtained from the previously incubated petri dishes was cut into plugs (1x1cm), and then three to four pieces were inserted into bottles with the autoclaved sorghum seeds. After covering them with cotton wool and plain sheet of paper, the bottles were incubated at temperatures around 28°C for ease of colonisation (Dlamini et al., 2012).

Substrate supplementation

The four organic substrates used for the production of the oyster mushrooms (*Pleurotus ostreatus*) were collected around the Crop Production Farm with sugarcane tops obtained from Illovo Sugarcane Company, Big Bend. Substrate preparation was according to Dlamini et al. (2012).

For substrates with 0% level of wheat bran 16 kg of the substrates were weighed and wetted to about 65-75% moisture content. The squeeze method was used to determine the moisture content. One kilogramme (kg) of substrate was packed into an autoclaving bag and fastened using rubber bands. For substrates with 10% level of wheat bran the 16 kg of the substrate was mixed with 1.6 kg of the wheat bran and further wetted to 65-75% moisture content while for the 15% level of wheat bran 16 kg substrates were mixed with 2.4 kg of wheat bran and were wetted to about 65-75% moisture content. The squeeze method was, also, used to determine the moisture content. After that 1kg of the substrate was packed into an autoclaving bag and fastened using rubber bands. All the substrates were then sterilized by autoclaving (Dlamini et al., 2012).

Colonisation

The bags of the different substrates were marked and were sterilised at 100 °C for a period of four hours at the Malkerns Research Station. After sterilisation, the bags were inoculated using 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 (Dlamini et al., 2012). Optimum colonisation takes place in temperatures around 24-28°C.

Design of the experiment

The experimental design was a factorial experiment (substrates X wheat bran levels) which was laid in a randomised complete block design (RCBD). The substrates which were not amended with wheat bran (0%) acted as the control to compare with substrates amended with 10% or 15% wheat bran. Each treatment was replicated four times with four bags per replicate adding up to16 bags per treatment. Data collected were analysed using MSTAT-C (Nissen, 1989) statistical package (version 2.0). Analysis of variance (ANOVA) was performed and where significant differences were detected at the 5% probability level mean separation was done by LSD (Gomez and Gomez, 1984).

Data collection

Each treatment contained 16 bags, which were randomly distributed into 4 replicates of 4 bags per treatment. Data 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) as previously described (Dlamini et al., 2012). .

Cropping house management

The cropping house had to be free from any contaminants. A footbath was used which contained a solution of Jayes fluid (Cambridge, England) disinfectant containing carbolic acid. The sand floor was watered daily to keep the environment as humid as possible to about 70-85% relative humidity (RH) needed for the mycelium to make a fruiting body (Dlamini et al., 2012). .

RESULTS

Number of days to full colonisation of substrates

The number of days taken to fully colonise the different substrates and wheat bran levels was significantly ($P < 0.05$) different depending on substrate and wheat bran combination. The mycelium failed to colonise maize stover + 10% wheat bran. The number of days taken to colonise the substrates ranged from 60 days to 85 days (Table 1). The number of days taken to fully colonise the banana leaves + 10% wheat bran and sugarcane + 15% wheat bran was significantly ($P < 0.05$) longer than those taken by the other substrates. However there were no significant differences in the number of days taken to fully colonise the other substrates. There was no significant

difference in the number of days taken to fully colonise the banana leaves + 10% wheat bran and sugarcane + 15% wheat bran (Table 1).

Table 1. Number of days taken to fully colonise the various substrates and wheat bran levels

Substrate and wheat bran level	Number of days to full colonisation
Maize stover and cobs + 0% wheat bran	60 a
Sugarcane + 0% wheat bran	63 a
Maize stover and cobs + 10% wheat bran	74 a
Maize and cobs + 15% wheat bran	76 a
Maize stover + 0% wheat bran	78 a
Maize stover + 15% wheat bran	78 a
Banana leaves + 15% wheat bran	78 a
Sugarcane + 10% wheat bran	80 a
Banana leaves + 0% wheat bran levels	84 a
Banana leaves + 10% wheat bran	85 b
Sugarcane + 15% wheat bran	85 b
C.V(%)	18.78
LSD (P < 0.05)	24.752

Means followed by the same letter are not significantly different from each other by LSD, P < 0.05.

Number of contaminated bags of the different substrates and wheat bran levels

During the incubation period, there were no bags that were contaminated by fungi (no mycelial growth observed), but there were bags which were contaminated by bacteria. There were significant (P < 0.05) differences in the number of contaminated bags. The number of bags varied from 0 to 4 bags per substrate + wheat bran level (Figure 1). Maize stover + 10% wheat bran level had the highest number of contaminated bags (4 bags) followed by sugarcane tops + 10% or 15% WB.

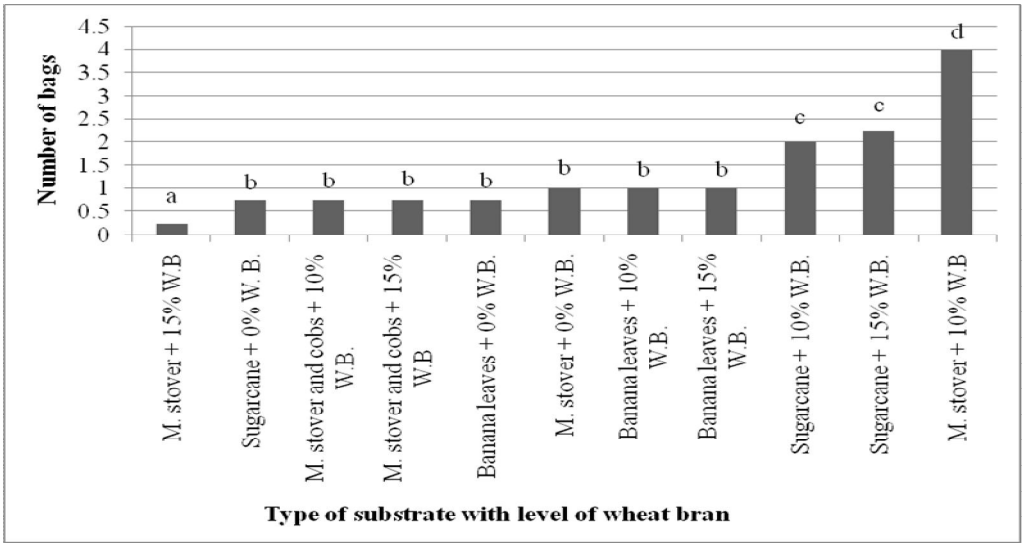

Figure 1. Number of contaminated bags for the different substrates at various levels of wheat bran ammendments. Means followed by the same letter are not significantly different from each other at P < 0.05.

Total mushrooms yield from the various substrates + WB ammendmends

After the first week, there were significant (P < 0.05) differences in the total mushroom yield (Figure 2). Mushrooms from maize stover and cobs + 15% wheat bran yielded significantly (P < 0.05) more than the other substrates + bran levels. The total mushrooms yield ranged from 0.0 g from sugarcane + 10% wheat bran to 382.2g in maize stover and cobs + 15% wheat bran. There were no significant differences in the mushroom yield from sugarcane + 10% wheat bran, maize stover + 10% wheat bran, maize stover + 15% wheat bran, banana leaves + 10% wheat bran and banana leaves + 15% wheat bran substrate.

After the second week, there were significant (P < 0.05) differences in the total mushroom yield grown in the various substrate+wheat bran levels. Maize stover and cobs + 15% wheat bran mushrooms yield was significantly (P < 0.05) higher than yield from the other substrate-wheat bran combinations. The total mushrooms yield ranged from 0.0 g in maize stover + 10% wheat bran to 360.7g in maize stover and cobs + 15% wheat bran (Figure 2). The substrate-wheat bran combinations of sugarcane + 15% wheat bran and maize stover + 10% wheat bran were not significantly different from each other.

After the third week, there were significant ($P < 0.05$) differences in the total mushroom yield among the substrate-wheat bran level combinations. The total mushrooms yield ranged from 0.0 g in maize stover + 0% wheat bran to 278.4g in maize stover and cobs + 15% wheat bran (Figure 2). The substrate+wheat bran combinations in which there were no significant difference in mushroom yield were maize stover + 0% wheat bran, maize stover + 10% wheat bran, maize stover + 15% wheat bran and banana leaves + 10% wheat bran to 278.4g in maize stover and cobs + 15% wheat bran.

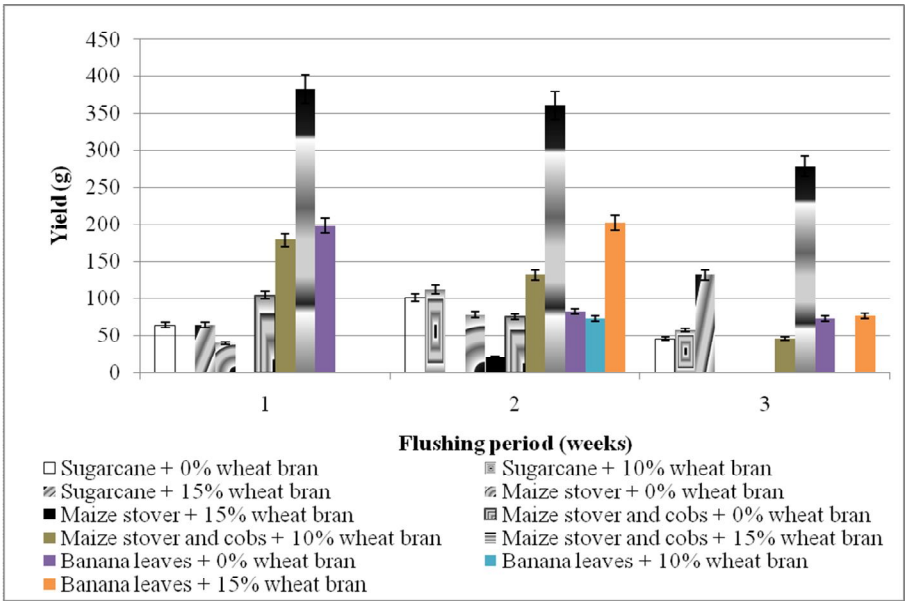

Figure 2. Total mushroom yield from the different substrate + wheat bran levels. Vertical bars represent standard deviation above and below the mean.

Marketable mushroom yield from the various substrates

After the first week, second and third weeks there were significant ($P < 0.05$) differences in the marketable mushroom yield from the various substrates and wheat bran amendments. Similar trends as with total mushroom yields were observed (Figure 3). Maize stover and cobs + 15% wheat bran yielded significantly ($P < 0.05$) more than the rest of the substrate-wheat bran combinations (Figure 3).

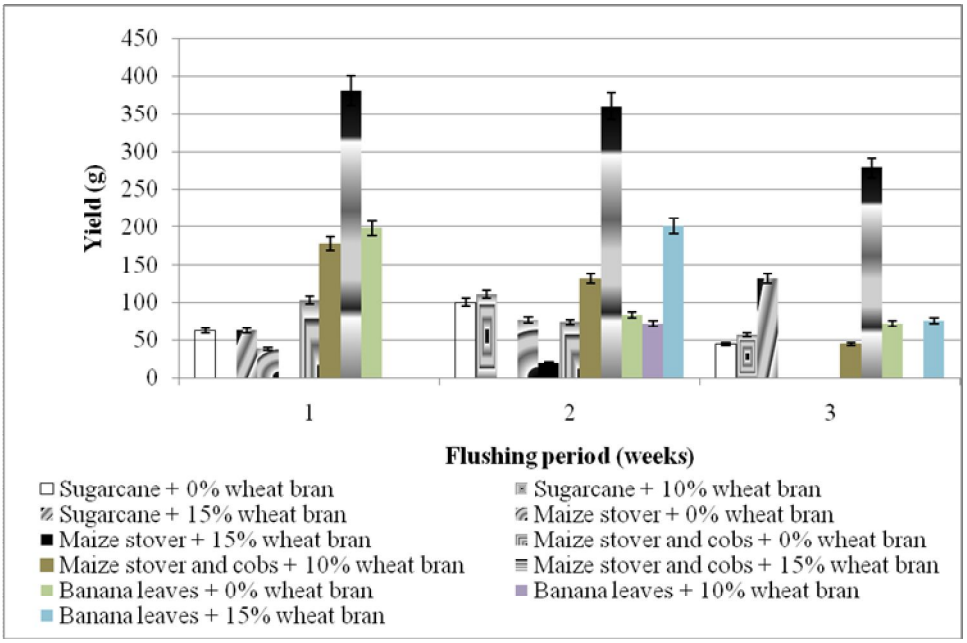

Figure 3. Total marketable mushroom yield from the different substrate-wheat bran combinations. Vertical bars represent standard deviation above and below the mean.

Pileus diameter of mushroom from the various substrates

After one week , the pileus diameter for the mushrooms ranged from 0.0 mm in sugarcane + 10% wheat bran to 55.0 mm for mushrooms from sugarcane tops + 15% wheat bran (Figure 4). There were no significant differences in the pileus diameter of mushrooms from the other substrates and wheat bran combinations.

After the second week, there were significant ($P < 0.05$) differences in the pileus diameter of mushrooms from the different substrates-wheat bran combinations. The pileus diameter for the mushrooms ranged from 0.0 mm in maize stover + 10% wheat bran to 56.5 mm from maize stover and cobs + 15% wheat bran.

After the third week, there were significant ($P < 0.05$) differences in the pileus diameter among the substrate-wheat bran combinations. The pileus diameter of mushrooms ranged from 0.0 mm from maize stover + 0% wheat bran to 56.3 mm in maize stover and cobs + 15% wheat bran. The substrate-wheat bran combinations in which there were no significant difference in mushroom pileus diameter were maize stover + 0% wheat bran, maize stover + 10% wheat bran, maize stover + 15% wheat bran and banana leaves + 10% wheat bran and maize stover and cobs + 15% wheat bran (Figure 4).

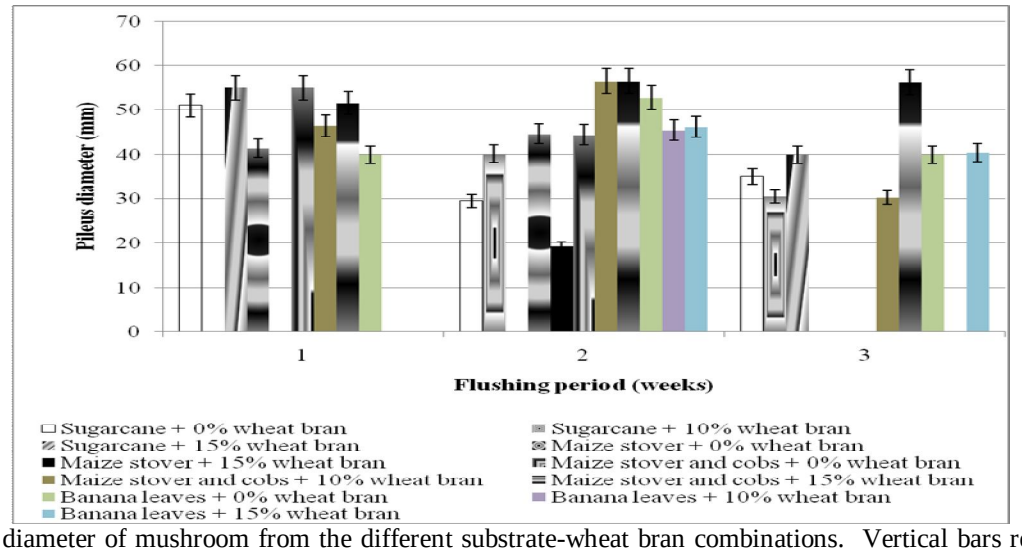

Figure 4. Pileus diameter of mushroom from the different substrate-wheat bran combinations. Vertical bars represent standard deviation above and below the mean.

Stipe length of mushroom from the various substrates

After the first week there were significant ($P < 0.05$) differences of mushroom stipe length from the different treatments. The stipe length of mushrooms ranged from 0.0 mm from sugarcane + 10% wheat bran to 20.3 mm for mushrooms from sugarcane tops + 15% wheat bran (Figure 5). There were no significant differences in the pileus diameter of mushrooms from the following: sugarcane + 10% wheat bran, maize stover + 10% wheat bran, maize stover + 15% wheat bran, banana leaves + 10% wheat bran and banana leaves + 15% wheat bran.

After the second week, there were significant ($P < 0.05$) differences in the stipe length of mushrooms from the different substrates-wheat bran combinations. The stipe length for the mushrooms ranged from 0.0 mm in maize stover + 10% wheat bran to 28.8 mm for mushrooms from maize stover and cobs + 10% wheat bran.

After the third week, here were significant ($P < 0.05$) differences in the stipe length among the substrate-wheat bran level combinations. The stipe length ranged from 0.0 mm for mushrooms from maize stover + 0% wheat bran to 10.3 mm for mushrooms from sugarcane + 15% wheat bran, The substrate-wheat bran combinations in which there were no significant difference in stipe length were maize stover + 0% wheat bran, maize stover + 10% wheat bran, maize stover + 15% wheat bran and banana leaves + 10% wheat bran.

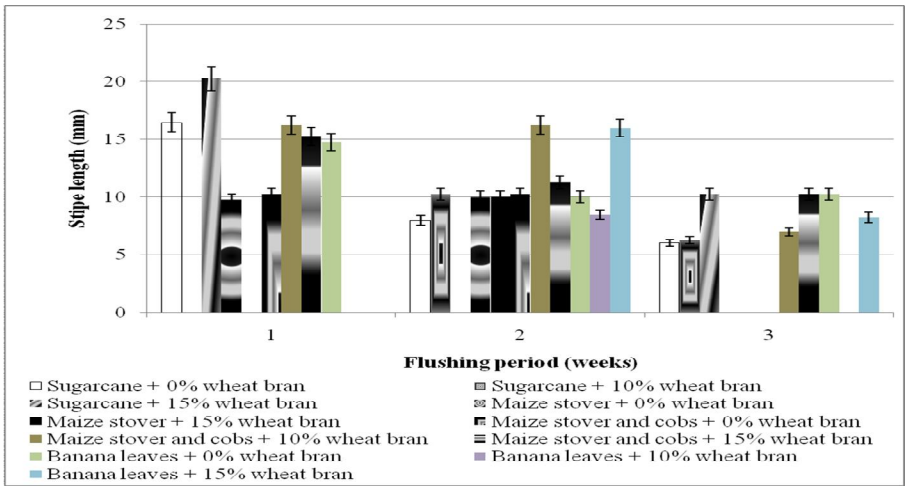

Figure 5. Stipe length of mushrooms from the different substrate-wheat bran combinations. Vertical bars represent standard deviation above and below the mean.

DISCUSSION

The number of days taken to fully colonise the different substrate and wheat bran level was not the same. The mycelium failed to colonise maize stover + 10% wheat bran. The number of days taken to colonise the substrate and wheat bran level combinations ranged from 60 days to 85 days. These results are consistent with previous findings (Khare et al., 2010; Masarirambi et al., 2010) The failure in the mycelium growth may be attributed to the low amount of spawn to use to propagate the fungus or contamination by bacteria.

There were significant differences in the number of contaminated bags. There was low conversion of lignocelluloses material which might have caused the contamination. 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 yield of mushroom from the three different wheat bran levels increased as the amount of wheat bran increased.

Similar results were reported where wheat bran supplementation of substrates resulted in increased yield of white mushroom (*C. indica*) by Tandon and Sharma (2006) & Pani (2011). Contrasting results where reported by Ayodele and Okhuoya (2007) who reported that the highest mushroom yield was on sawdust supplemented with wheat bran at 5% and not higher. An ideal substrate will contain enough nitrogen (supplement) and carbohydrate for rapid mushroom growth (Oei, 1996; Ayodele and Okhuoya, 2007; Anon., 2008a).The yield increased in the second week from the different wheat bran levels and then decreased during the third week. The highest yield, therefore, was obtained during the second week in the different wheat bran levels. By the second week substrates had generally been degraded to the maximum hence maximum energy provision resulting in relatively more growth per given time. The low yield in the control substrates could have been due to carbon to nitrogen imbalance (Oei, 1996).

The marketable mushroom yield showed similar trends to total mushroom yield. For mushrooms grown on 15% wheat bran level, was generally higher compared to those from the other wheat bran levels. The marketable yield increased in the second week from the different wheat bran levels and then decreased during the third week. The highest marketable yield, therefore, was obtained during the second week in the different wheat bran levels.

The harvest indices of mushroom stipe length and pileus diameter were similarly affected as mushroom yield by the different substrate combinations. Our work has shown that the yield of Oyster mushroom grown on some agricultural waste substrates may be increased by substrate supplementation with wheat bran

CONCLUSION

The yield, of mushrooms grown in substrates amended with 15% wheat bran, were generally higher compared to those from the other wheat bran level additions followed by 10 % wheat bran addition and lastly substrates which were not amended with wheat bran. Maize stover and cobs amended with 15% wheat bran produced relatively higher yields of marketable mushrooms and thus is recommended for farmers to use.

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