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Performance of *Pleurotus ostreatus* mushroom grown on maize stalk residues supplemented with various levels of maize flour and wheat bran

Senzosenkosi Surprise MKHIZE^{1*}, Jacques CLOETE², Albertus Kotze BASSON¹, Godfrey Elijah ZHARARE³

Abstract

Improving the performance of mushroom in terms of high production and fast growth rate is essential in mushroom cultivation. In the present study the performance of *Pleurotus ostreatus* was evaluated using varying levels of wheat bran (WB) and maize flour (MF). The results indicated that *Pleurotus ostreatus* was highly influenced by different levels of supplementation, with 8% WB, 18% WB and 2% MF having higher contamination rate. The low levels of supplementation gave significantly better mycelial growth rate (MGR) and shorter colonisation period as observed that the control had highest MGR whereby 20% MF had lowest MGR. The pinning time (TP) was shortest at the first flush with minimum of 3 days (12% MF). The higher levels of supplementation showed maximum biological efficiency (BE) such as 14% MF, 12% WB and 14% WB. The yield was also higher at high levels of supplementation such as 20% MF and 8% MF being the exception in the lower levels. Based on the results it was observed that for fast production of oyster mushroom there is no need to supplement the maize stalk substrate but for improved productivity supplements can be added up to certain limits such as 14% MF and 12 WB.

Keywords: biological efficiency; *Pleurotus ostreatus*; supplement; maize stalk

Practical Application: The study will add to the pool of scientific knowledge relating to optimum substrate formulations (supplements) involving maize residues for *Pleurotus ostreatus* mushroom production. The results observed can be useful to small scale farmers who are willing to obtain good yield within minimal time at minimal cost with reduced contamination problems. Based on the results obtained, it is recommended that for fast production there is no need to add supplements on maize stalks but for improved production supplements can be added up to certain limits.

1 Introduction

Oyster mushrooms, such as *Pleurotus ostreatus*, are not usually attacked by both diseases and pests and their cultivation does not require sophisticated control of the growing environment, hence they are cultivated in a cheap and easy way (Kües & Liu, 2000; Sánchez, 2010). The *Pleurotus ostreatus* mushroom has gained popularity within the Southern African Development community (SADC) (Earnshaw et al., 2012), because of its pleasant flavour and taste (Khan et al., 2012). In addition, the mushroom has potential for livestock feed and for extraction of enzymes and medicinal compounds (Patil et al., 2010).

According to Garg & Gupta (2009) plant derived agro-industrial waste cause environmental and health problems. This waste can be managed through mushroom cultivation, since mushrooms have the ability to grow on a variety of raw lignocellulosic substrates and under a wide range of temperatures (Jandaik & Goyal, 1995; Sánchez, 2010). Oyster mushrooms (*Pleurotus* spp.) are able to perform such duties, because they contain a number of nonspecific lignocellulosic enzymes (Zhang et al., 2002) which have a major impact in the development and growth of the mushroom (Kuforiji & Fasidi, 2008). The nature and nutrient constituent of the mushroom substrate also have an effect on the

mycelium growth, mushroom quality and crop yield (Baldrian & Val'a'skov'a, 2008; Kües & Liu, 2000). One of the limiting factors in the cultivation of mushrooms is that the availability of a good substrate is essential in order to promote satisfactory yield of the mushrooms (Ueitele et al., 2014). A good substrate should consist of nitrogen supplement and carbohydrates in order to promote rapid growth of the mushroom (Ogundele et al., 2014). In most cases the substrate does not have the nitrogen required by the mushroom to grow optimally, hence the need for nitrogen supplementation of the substrate in order to gain an improved growth of the mushroom (Choi, 2004).

Although supplementing the substrate material leads to improved growth, there are limitations involved. For instance, high supplementation of substrate may lead to contamination (Yildiz et al., 2002) together with reduced yield of the mushrooms (Fanadzo et al., 2010). A variety of substrate supplements have been recommended for oyster mushrooms. These supplements include, but are not limited to, urea, ammonium sulphate, gram flour, molasses, mustard cake, soy bean meal and cotton seed cake (Ralph & Kurtzman, 1994). Wheat bran supplements are effective in improving mushroom productivity and also

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the inclusion of maize additive shows an increase in both the nutritional value and productivity of mushrooms (Oei, 2005).

This study focussed on utilizing maize stubble as substrate for oyster mushroom (*Pleurotus ostreatus*) production in the Northern Kwa-Zulu Natal (South Africa) areas. The information scarcity on supplements required to improve yields of *Pleurotus ostreatus* mushroom with minimum contamination guided the research in order to determine the performance of *Pleurotus ostreatus* on maize stalks with varied levels of wheat bran and maize flour supplement. The results of the study will be crucial to poor rural communities which utilizes local maize residue material for *Pleurotus ostreatus* mushroom production for both food and income generation.

2 Materials and methods

2.1 Supplements and substrates collection

The maize residues (leaves and stalks) were obtained from farms in and around the northern Kwa-Zulu Natal area (University of Zululand and local community farms) and stored at room temperature. The vitamin free maize flour supplement was obtained from Empangeni millers and the wheat bran was obtained from Coastal Farmers (local market).

2.2 The test mushroom strain

The test oyster mushroom (*Pleurotus ostreatus*) was obtained from Cedara College of Agriculture at Pietermaritzburg in Kwa-Zulu Natal (South Africa). The mushroom strain was pre-cultured on potato dextrose agar (PDA) (MERCK, 1.10130) and incubated at 25 °C. These were maintained as working spawn cultures in a refrigerator at 4 °C in order to preserve the mushroom in its active state.

2.3 Preparation of mushroom spawn

A modified method outlined by Fritsche (1978) was used to prepare the spawn, whereby 1 kg of sorghum grain was soaked overnight in 1.5 litres of water and the excess water was thereafter drained from the grains. A quantity of 900 g of the grains was mixed with 12 g of the gypsum and 3 g CaCO₃. The mixture was packed halfway into 250 ml bottles. The grain in the 250 ml glass bottles were sterilised at 121 °C in an autoclave (Labtech, LAC5060S) for 15 minutes and allowed to cool to room temperature. The sterilised sorghum grain mixture was inoculated with 10 mm² of previously grown pure cultures of *Pleurotus ostreatus* strain using a flamed and cooled scalpel in a laminar flow hood. The inoculated bottles were incubated in the dark at room temperature (25 °C) for two weeks until the mycelium had fully colonised the grain.

2.4 Bulk substrate preparation

The substrate was prepared using a modified version of the method proposed by Bano & Srivastava (1962). The maize stalks were milled using the milling tractor machine and thereafter the dry weight of maize stalks was measured. This assisted in the analysis of the results (especially, biological efficiency). Tap water was added to the substrate to achieve 65% moisture

content using the rule of thumb (1-2 droplets of water must be released when the substrate is squeezed). Thereafter, the maize stalk substrates were separately supplemented with 8 levels of maize flour and wheat bran, viz: 0%, 2%, 4%, 8%, 12%, 14%, 18% and 20% respectively. After the supplements were added and thoroughly mixed with the base substrates, 1 kg of the resultant substrate was packed into polypropylene bags (22.5 cm × 30 cm) in four replicates and compressed by hand until compactness was achieved. The bagged substrates were pasteurised at 60-65 °C for six hours (Jang et al., 2003) and allowed to cool to room temperature.

2.5 Substrate inoculation, spawn running and fruiting

After cooling, all the bagged substrates were inoculated with previously prepared pure grain spawn of the *Pleurotus ostreatus* mushroom at the rate of 2% of wet substrate. The *Pleurotus ostreatus* was grown in four replicates for every level of supplemented substrate. Inoculation was strictly conducted in a sterile environment (laminar flow hood) using one spoonful of mother culture placed aseptically into the top surfaces of each bag with substrates. The inoculated substrates were tightly closed with cotton wool and rubber bands to hold it in place. The cotton wool was used as plugs that prevented contamination into bags but allowed gaseous exchange. The inoculated bags were incubated in a dark room at 25-27 °C until they became fully colonised. Thereafter the bags were transferred to a fruiting room which was constructed from plastic film supported by gum poles and covered by a double layer of 60% shade cloth on the outside. The fruiting room was under the canopy of a Zygium tree, which is an evergreen tree and hence provided shade throughout the year. The fruiting room was fitted with timed micro-jet sprinklers that watered the mushrooms in a fine spray at least three times a day in order to obtain 80-90% relative humidity. The mushrooms were allowed to fruit under ambient temperatures. The experiment was terminated after 3 fruiting flushes.

2.6 Performance/productivity measurements

In order to determine the performance and how does the supplements affect the performance of *P. ostreatus* when grown on maize stalk different parameters were checked. The parameters that were measured for performance of *P. ostreatus* included the number of contaminated bags, mycelial growth rate, number of days to full colonisation, time to fruiting (initiation of pin heads), fresh mushroom yield and biological efficiency. The number of contaminated bags for each and every treatment was recorded or counted manually. The advancement of mycelium down the substrate bag was ringed at 6 day intervals until the substrate was fully colonised. The rate of colonisation of the substrate by the mushroom mycelia was then estimated by measuring the distance from the top down to each of the rings at four equally spaced points around the bag using a marked ruler. The number of days it took for the mycelia to fully colonise the substrate was also noted from the time it first colonised the substrate up to a point where the mycelia fully covered substrate. Immediately after mycelia fully colonised the substrate, the bags were taken to fruiting room where the bags were opened on the top part and the time taken for pin head formation after the full colonisation

of substrate and after each and every flush was recorded on each replicate.

The mushroom yield was calculated according to Morais et al. (2000), using the equation: $MY = [\text{Weight of fresh mushroom harvested (g) per fresh substrate weight}]$. The biological efficiency was calculated according to (Stamets, 2000; Royse et al., 2004), as follows $BE = [\text{weight of fresh mushroom harvested (g)/ dry substrate weight (g)}] \times 100$.

2.7 Experimental design and statistical analysis

The experiment was done in a completely randomised design with 4 replications ($n = 4$). Data was analysed and graphs were constructed using SPSS version 23 and Microsoft Excel. Tests used were Pearson's Correlation, Binomial Test for Proportions, and Repeated Measures ANOVA followed by post hoc Duncan's homogeneous groups. A 5% level of significance was used throughout.

3 Results

3.1 The number of contaminated bags

There were eight (12.5%) contaminated bags from a total of sixty four. This indicates that the *Pleurotus ostreatus* mushroom was not highly susceptible to contamination. From the total of eight contaminated bags, high contamination was observed at 18% WB, 8% WB, and 2% MF (with 50% of replicates), and the lowest contamination was observed at 12% MF, and 2% WB (with 25% of replicates) respectively (Table 1). The maize flour supplement contained a total of three (9.3%) contaminated bags whereas wheat bran supplement contained a total of five (15.6%) contaminated bags. The proportion of contaminated bags are not significantly different between the two supplements ($p = 0.4005$). Over time the contamination was overcome and only 2% WB yielded two flushes whereas the other seven yielded three flushes.

3.2 Mycelial growth rate of *Pleurotus ostreatus* on maize stalk supplemented with different levels of wheat bran and maize flour

There is a significant negative correlation between supplement level and mycelial growth rate (MGR) of oyster mushroom for MF ($r = -0.71$; $p = 0.00001$) and WB ($r = -0.59$; $p = 0.00075$) (Figure 1).

There is no significant difference between the average mycelial growth for MF and WB ($p = 0.06655$). The mycelial growth rate (MGR) did differ significantly between supplement levels for both MF and WB ($p = 0.0000$). The MGR ranged from 0.35 cm/day for 20% MF to 0.60 cm/day for 0% WB (Table 2). Supplement levels 14%, 18% and 20% of both WB and MF showed significantly lower MGR than the control.

3.3 The number of days to full colonisation of maize stalk substrate by *Pleurotus ostreatus* mushroom

The days to full colonisation displayed a significant positive correlation with the supplement level for both MF ($r = 0.58463$; $p = 0.00055$) and WB ($r = 0.53651$; $p = 0.00270$) (Figure 2). The average number of days for WB (39 ± 1) was significantly less than for MF (43 ± 1) with $p = 0.04275$. The number of

days to full colonisation of maize stalk ranged from 30 ± 5 (8% WB) to 56 ± 4 (20% MF) (Table 2). The longest periods to full colonisation were observed with 20% MF (56 ± 4), 14% MF (53 ± 4), and 20% WB (51 ± 4) respectively.

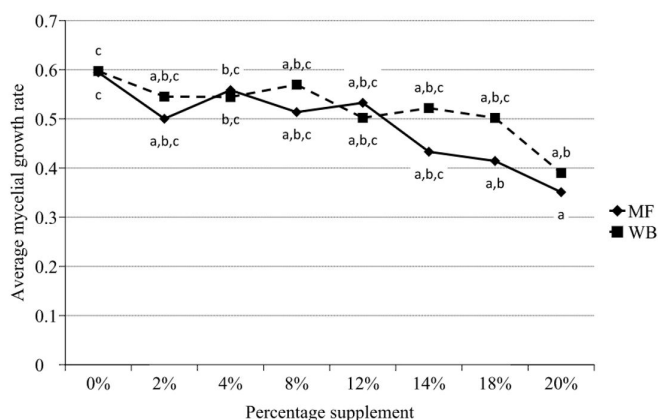


Figure 1. Average mycelial growth rate of *Pleurotus ostreatus* mushroom per supplement level. (a-e) Averages with different letters are significantly different at $p < 0.05$.

Table 1. The effect of wheat bran and maize flour supplements on the number of contaminated bags on the maize stalk base substrate.

Treatment	Level	Number of contaminated bags
Maize flour	2%	2 (50%)
Maize flour	12%	1 (25%)
Wheat bran	2%	1 (25%)
Wheat bran	8%	2 (50%)
Wheat bran	18%	2 (50%)

Table 2. Maize stalk substrate – Effect of WB and MF supplementation on time required for pin head formation (time for pinning) between flushes.

Supplement	Level	TP flush 1	TP flush 2	TP flush 3
MF	0%	4 ± 0^a	$23 \pm 1^{c,d,e,f}$	$25 \pm 1^{c,d,e,f}$
	2%	$4 \pm 0^{**}$	$24 \pm 1^*$	$31 \pm 5^*$
	4%	$8 \pm 4^{a,b}$	$20 \pm 3^{c,d}$	$24 \pm 1^{c,d,e,f}$
	8%	$8 \pm 3^*$	$22 \pm 2^*$	$25 \pm 1^*$
	12%	3 ± 0^a	$23 \pm 3^{c,d,e,f}$	$23 \pm 0^{c,d,e,f}$
	14%	$6 \pm 3^{a,b}$	30 ± 3^f	$27 \pm 7^{c,d,e,f}$
	18%	$5 \pm 1^*$	$26 \pm 2^*$	$28 \pm 5^*$
	20%	$6 \pm 1^{a,b}$	30 ± 2^f	30 ± 2^f
WB	0%	4 ± 0^a	$23 \pm 1^{c,d,e,f}$	$25 \pm 1^{c,d,e,f}$
	2%	$4 \pm 0^*$	$24 \pm 1^*$	$30 \pm 2^*$
	4%	$6 \pm 1^{a,b}$	$21 \pm 1^{c,d,e}$	$28 \pm 2^{d,e,f}$
	8%	$20 \pm 2^*$	$19 \pm 12^*$	$26 \pm 0^{***}$
	12%	12 ± 4^b	$23 \pm 3^{c,d,e,f}$	$29 \pm 1^{e,f}$
	14%	$10 \pm 3^{a,b}$	19 ± 1^c	$23 \pm 2^{c,d,e,f}$
	18%	$4 \pm 0^{**}$	$20 \pm 2^*$	$36 \pm 1^*$
	20%	4 ± 1^a	29 ± 1^f	$26 \pm 3^{c,d,e,f}$

***Based on a single value. **Based on two or more identical values. *Excluded due to ** and ***. Averages with different superscript in a single column are significantly different at $p \leq 0.05$.

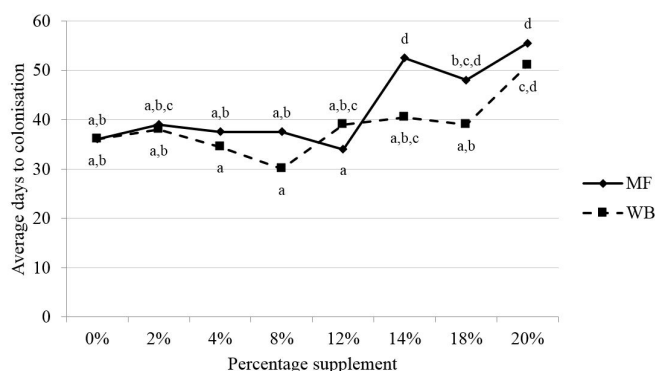


Figure 2. Average days to full colonisation of maize stalk substrate supplemented with different levels of MF and WB. (a-d) Averages with different letters are significantly different at $p < 0.05$.

3.4 Biological efficiency (BE) of *Pleurotus ostreatus* on maize stalk supplemented with different levels of wheat bran and maize flour

Biological efficiency is used as a measure of substrate conversion into mushroom (Oseni et al., 2012). There was a positive (non-significant) correlation between supplement level and BE for MF ($r_{MF} = 0.4994$, $p_{MF} = 0.1038$) and a negative (non-significant) correlation between supplement level and BE for WB ($r_{WB} = -0.0145$, $p_{WB} = 0.5136$) (Figure 3). There was significant interaction between supplements and the level of supplementation ($p = 0.0005$), since the supplements of both WB and MF caused different reaction in BE of *Pleurotus ostreatus* over different levels of supplementation.

The BE values ranged from 95 ± 3 (8% WB) to 158 ± 11 (14% MF) (Table 2). This range indicated that maize stalk is efficiently profitable for cultivation of *Pleurotus ostreatus* since it produced BE which was above 50% as stated by Patra & Pani (1995). The highest BE was observed on 14% MF (158 ± 11) and 12% WB (156 ± 6) followed by 14% WB (148 ± 3). The lowest BE was observed on 8% WB (95 ± 30) and 2% MF (109 ± 17) (Table 2). When comparing every supplement level from 2% to 20% with the control (0%), the 12% WB (156 ± 6), 14% WB (148 ± 3) and 14% MF (158 ± 11) were significantly higher than the non-supplemented maize stalk (control) which had a biological efficiency of 119 ± 4 .

3.5 Yield of *Pleurotus ostreatus* on maize stalk supplemented with different levels of wheat bran and maize flour

There was significant interaction between supplement and supplement levels ($p = 0.0020$) for *Pleurotus ostreatus*. This is due to the behaviour of 8% WB.

The minimum yield was recorded at 8% WB (0.08 ± 0.03) and this was significantly less than the average yields at all other MF and WB levels. The maximum yields were recorded at 20% MF (0.14 ± 0.01) and 8% MF (0.14 ± 0.01) (Table 2), however these were only significantly different from 2% MF and 8% WB. The 8% WB showed lowest yield due to the presence of contaminants within this level since out of four replicates two of them were heavily contaminated.

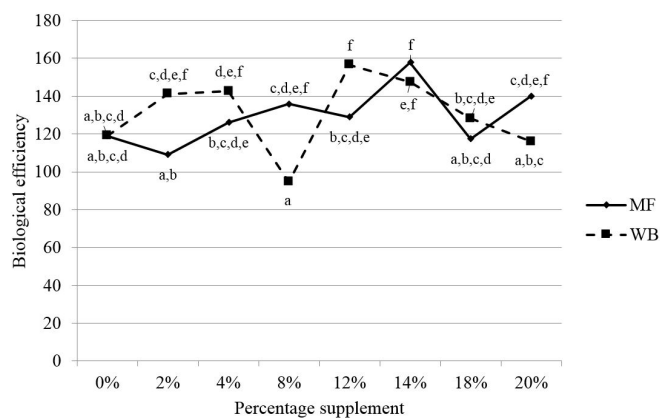


Figure 3. Average biological efficiency of *Pleurotus ostreatus* mushroom on maize stalk substrate supplemented with different levels of maize flour and wheat bran.

3.6 Effect of different levels of wheat bran and maize flour on time required for pin head formation (time for pinning) between flushes

The time for pinning (TP) ranged from 3 days to 36 days based on all flushes. There was a positive correlation between the supplement level and TP (averaged over all 3 flushes) for both MF and WB ($r_{MF} = 0.6092$, $r_{WB} = 0.4305$), however the correlations were not significant ($p_{MF} = 0.0544$, $p_{WB} = 0.1435$). The lowest TP for flush 1 was observed to be 12% MF (3 ± 0 days) which was not significantly different from 0% MF, 2% MF, 0% WB, 2% WB, 18% WB, and 20% WB (Table 3). The highest TP for flush 1 was recorded for 8% WB (20 ± 2 days) of which 50% of the replicates initially displayed contamination. The second highest TP for flush 1 is for 12% WB (12 ± 4 days).

The latter was significantly different to the aforementioned minimum values. All the first flush values (except for the anomaly at 8% WB which was excluded from the comparison) were significantly different to the second flush and third flush recorded values. The lowest TP for flush 2 is at 14% WB (19 ± 1) and the highest is at 14% MF (30 ± 3) and 20% MF (30 ± 2). The highest and lowest values were significantly different from each other. The only flush 3 TP values that were not comparable with flush 2 values were at 18% WB (36 ± 1). The remaining flush 3 TP values were not significantly different from the flush 2 TP values.

4 Discussion

Supplementation of the substrate is of importance in order to increase production of *Pleurotus ostreatus* mushroom (Estrada et al., 2009) and obtain better yield and development (Carvalho et al., 2010). The lignocellulosic materials have low protein content and therefore supplements are useful in the provision of minerals as phosphorus, potassium and nitrogen for production improvement (Mangat et al., 2008; Moore-Landecker, 1982). In this study maize stalk was supplemented with different levels of wheat bran and maize flour in order to obtain higher growth and yield of mushroom. Although supplements enhanced

Table 3. Effect of different levels of supplementation of maize flour and wheat bran on growth of *Pleurotus ostreatus* mushroom.

Supplement	Level	MGR (cm/day)	Days	BE	MSY
MF	0%	0.59 ± 0.05 ^e	36 ± 4 ^{a,b}	119 ± 4 ^{a,b,c,d}	0.12 ± 0.00 ^{b,c}
	2%	0.50 ± 0.01 ^{b,c,d,e}	39 ± 5 ^{a,b,c}	109 ± 17 ^{a,b}	0.11 ± 0.02 ^b
	4%	0.56 ± 0.04 ^e	38 ± 4 ^{a,b}	126 ± 2 ^{b,c,d,e}	0.13 ± 0.00 ^{b,c}
	8%	0.51 ± 0.02 ^{c,d,e}	38 ± 4 ^{a,b}	136 ± 6 ^{c,d,e,f}	0.14 ± 0.01 ^c
	12%	0.53 ± 0.02 ^{c,d,e}	34 ± 4 ^a	129 ± 7 ^{b,c,d,e}	0.13 ± 0.01 ^{b,c}
	14%	0.43 ± 0.03 ^{a,b,c,d}	53 ± 4 ^d	158 ± 11 ^f	0.13 ± 0.01 ^{b,c}
	18%	0.41 ± 0.02 ^{a,b,c}	48 ± 4 ^{b,c,d}	117 ± 6 ^{a,b,c,d}	0.12 ± 0.01 ^{b,c}
	20%	0.35 ± 0.02 ^a	56 ± 4 ^d	140 ± 9 ^{c,d,e,f}	0.14 ± 0.01 ^c
WB	0%	0.60 ± 0.05 ^e	36 ± 4 ^{a,b}	119 ± 4 ^{a,b,c,d}	0.12 ± 0.00 ^{b,c}
	2%	0.55 ± 0.05 ^{d,e}	38 ± 4 ^{a,b}	141 ± 6 ^{c,d,e,f}	0.12 ± 0.01 ^{b,c}
	4%	0.54 ± 0.02 ^{d,e}	35 ± 4 ^a	143 ± 4 ^{d,e,f}	0.12 ± 0.00 ^{b,c}
	8%	0.57 ± 0.07 ^e	30 ± 5 ^a	95 ± 30 ^a	0.08 ± 0.03 ^a
	12%	0.50 ± 0.01 ^{b,c,d,e}	39 ± 4 ^{a,b,c}	156 ± 6 ^f	0.13 ± 0.01 ^{b,c}
	14%	0.52 ± 0.05 ^{c,d,e}	41 ± 4 ^{a,b,c}	148 ± 3 ^{c,f}	0.12 ± 0.00 ^{b,c}
	18%	0.51 ± 0.06 ^{b,c,d,e}	39 ± 5 ^{a,b}	128 ± 11 ^{b,c,d,e}	0.13 ± 0.01 ^{b,c}
	20%	0.39 ± 0.02 ^{a,b}	51 ± 4 ^{c,d}	116 ± 5 ^{a,b,c}	0.12 ± 0.01 ^{b,c}

Results are mean ± s.e. MF – maize flour; WB – wheat bran; MGR – mycelia growth rate ^(a-e); Days – Days until full colonisation ^(a-d); BE – Biological efficiency ^(a-f); MSY – Average mushroom yield ^(a-c) per gram. Means with different superscript in a single column are significantly different at ($P \leq 0.05$).

mushroom production, they are however required in moderate quantities in order to avoid yield reduction (Fanadzo et al., 2010) as well as decreasing contamination risk (Miller & Jong, 1987; Przybylowicz & Donoghue, 1990; Yildiz et al., 2002). In this study the number of contaminated bags ranged from 1 to 2 (25-50%) for different level of supplementation. This indicated that *Pleurotus ostreatus* mushroom was not highly affected by contamination although 18% WB, 8% WB and 2% MF had slightly higher contamination rates. For 18% WB this was most likely due to the fact that high supplementation causes possibility of contamination (Yildiz et al., 2002), since it becomes too rich in nutrients and therefore gravitate the risk of contamination (Oei, 2003). As for 8% WB and 2% MF, the slightly high contamination may most likely be due to partial break down of cellulose and hemicellulose of maize stalk substrate which causes rapid growth of competitor organisms (Balasubramanya & Kathe, 1996). When comparing both supplements, the wheat bran treatment resulted in slightly more contamination of 5 bags (15.6%) than maize flour treatment which had 3 bags (9.3%). This could be a result of the presence of high nitrogen in wheat bran and also high moisture content (Andrade et al., 2007). Overall this study indicated a low rate of contamination. This means that *Pleurotus ostreatus* was able to colonise maize stalk efficiently and overcome contamination. One of the important factors in mushroom cultivation is the mycelia with exceptional growth (Pokhrel & Ohga, 2009). In this study the 0% supplemented maize stalk showed the highest mycelial growth rate (0.60 cm/day) followed by 8% WB (0.57 cm/day) and 4% MF (0.56 cm/day). These results closely corresponds to the findings by Bhattacharjya et al. (2014) who reported closely related mycelial growth rate on some of the treatment although their study dealt with sawdust as substrate. Such high mycelial growth rate indicated that these level of supplementation consisted of higher carbon to nitrogen ratio (C/N) which favoured high mycelial growth rate (Yang, 2000). Sarker (2004), established that mycelium running rate of oyster mushroom is influenced by different wheat bran levels of supplementation. In this study,

by looking at the growth patterns from Figure 1, there was gradual decline in the mycelial growth rate (MGR) as the supplement level was increased from 0% to 20%, since the lowest MGR was at 20% MF and 20% WB with respective rates 0.35 cm/day and 0.39 cm/day. This was probably due to high nitrogen content which is known to inhibit mushroom growth if it is in excessive amount within the substrate (Yang et al., 2013). Therefore the results of the current study support the findings obtained by Naraian et al. (2009) who observed that the increase in the amount of supplements added into the substrate results in the decrease of mycelium growth. The number of days to full colonisation of maize stalk supplemented with both wheat bran and maize powder ranged from 30 to 56 days. The shortest periods observed on 8% WB (30 days), 12% MF (34 days) and 4% WB (35 days). This closely corresponds to 34 days reported for *Pleurotus ostreatus* on sawdust as indicated by Sopit (2006). The longest period to full colonisation was observed at 20% MF (56 days). This was not significantly different to 14% MF (53 days) and 20% WB (51 days). These closely correspond to 58 days reported by Dlamini et al. (2012) on maize stover. These results contradicts the findings by some researchers who stipulated that *Pleurotus ostreatus* took 17-20 days to accomplish full colonisation (Ponmurugan et al., 2007; Shah et al., 2004; Vetayasuporn et al., 2006). Mushroom pinning require conditions which are different from those required for mycelial growth (Patrick et al., 2014). The shortest time for pinning (TP) for flush 1 was recorded for 12% MF (3 days) which was not significantly different to 0% supplements (control), 2% MF, 2% WB, 18% WB, and 20% WB with TP of 4 days each. The longest TP for flush 1 was recorded for 8% WB (20 days) and 12% WB (12 days). All flush 1 pinning times were significantly shorter than the pinning times for flushes 2 and 3 (Table 3). This may be attributed to the decrease in carbon and nitrogen, since the formation of primordia or pin heads is directly related to availability of carbon and nitrogen (C:N) from lignocellulose substrate (Naraian et al., 2008). The lowest TP for flush 2 is for 14% WB (19 ± 1) and the highest is for 14% MF (30 ± 3) and

20% MF (30 ± 2). Flush 3 TP values are not significantly different from the flush 2 TP values except 18% WB for flush 3 (36 ± 1). The current findings corresponds to the results observed by (Obodai & Vowotor, 2002) who reported that fruiting bodies of *Pleurotus eous* strain Kapak became apparent within 4 days. These also corresponds to findings by Rühl et al. (2008) who reported 4 days for pinhead formation of *Pleurotus ostreatus*. This corresponds to our lowest TP for the first flush. Obodai and Vowotor (2002) reported that the *Pleurotus quebeca* strain PQB took 35 days. This corresponds closely to 18% WB (36 days) for flush 3. Shah et al. (2004) reported almost similar results as they reported that pin head formation ranged from 6-7 days. The largest biological efficiency (BE) was observed on 14% MF (158 ± 11) and 12% WB (156 ± 6) followed by 14% WB (148 ± 3) which was significantly higher than the control with 119 ± 4 . The lowest biological efficiency (BE) was observed on 8% WB (95 ± 30) and 2% MF (109 ± 17). The reason for lower BE on 8% WB was the presence of contaminants which competed for space and nutrients. The BE tend to increase with increase in the level of supplementation, however supplementation above 14% MF and 12% WB may cause decrease in BE (Figure 3). This may be caused by overheating of substrate which effects the mushroom negatively (Oseni et al., 2012). The obtained BE closely corroborates with the findings of Jafarpour et al. (2011) who obtained BE of 96.13% on lawn cut complemented with rice bran, 118.20% on Barley straw with rice bran, and 156.40% on chop maize complemented with soybean powder and rice bran. The system of adding supplements to the substrates proved to be useful as research have indicated that addition of certain supplements into the substrates improved yield (Kadiri & Fasidi, 1993; Ukoima et al., 2009). The current study indicated that there is significant interaction between supplement and supplement level ($p = 0.0020$) with yield ranging from lowest of 0.08 g/g (80 g/kg) to the highest of 0.14 g/g (140 g/kg). The 8% wheat bran recorded the lowest yield, but this is most likely due to the contamination in this level of supplementation which might affected the cellulosic content of the substrates. The results obtained indicate that the yield at 8% MF and 20% MF levels of supplementation is only significantly higher than the yield at 8% WB and 2% MF. The low yield may be attributed to factors such as contamination and carbon and nitrogen balance within the substrate. Zadrazil (1980) states that substrate low in nitrogen content favours the growth of *Pleurotus* species and this might have caused the highest yield which was obtained on 8% MF and 20% MF. The yield for this study corresponds favourably with the findings of Pokhrel et al. (2013) who obtained the yield of 153.53 g on banana leaves with rice bran, 131.28 g banana leaves with chicken manure, and a lowest yield of 94.90 g.

5 Conclusion

The results of the study stipulate that the performance of *Pleurotus ostreatus* mushroom was highly affected by addition of supplements (wheat bran and maize flour) into the substrate. It was observed that the addition and increase of supplements results in a decrease of the mycelial growth for both wheat bran and maize flour. In addition, the days to full colonisation of the maize stalk tend to increase significantly with an increased level of supplement for both wheat bran and maize flour. The time for

pin head (primordia) formation behave in the same way as the level of supplementation is increased. Therefore the mushroom production period is said to be delayed by increasing the level supplementation within the maize stalk substrate. In terms of productivity (biological efficiency and yield), it is clear that an increase in level of supplementation leads to an improved performance of *Pleurotus ostreatus* mushroom.

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