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Effect of Wheat Bran Supplement on Growth and Yield of Oyster Mushroom (*Pleurotus Ostreatus)* on Fermented Pine Sawdust Substrate

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Abstract

This study evaluated the effect of supplementing fermented pine sawdust substrate with different levels of wheat bran on the yield of oyster mushroom (*Pleurotus ostreatus*). The fermented pine sawdust was mixed at spawning with 0%, 5%, 10%, 15% and 20% of wheat bran supplement and arranged in a randomized complete block design with three replications and there were 10 bags per treatment. Result showed significant ($P<0.05$) differences in the number of contaminated bags which ranged from 5 to 30, with the number of contaminated bags increasing with increasing wheat bran level up to 20% supplementation. Number of days to full colonization decreased with increasing wheat bran supplementation, and substrate supplemented with 20% wheat bran took shorter time (33 days) to fully colonize the substrate while it took 43 days in substrate supplemented with 5% wheat bran to full colonization, and the non supplemented substrate failed to colonize due to contamination. The growth of pileus diameter and stipe length were significantly ($P<0.05$) different, being highest on 15% and lowest on 5% wheat bran supplemented substrates, respectively. Significantly ($P<0.05$) higher yields of oyster mushroom (683.9g/500g substrate) and biological efficiency (136.8%) were obtained on substrate supplemented with 15% wheat bran compared to other treatments. Thus, 15% wheat bran supplementation of fermented pine sawdust proved to be a viable option for oyster mushroom and can therefore be recommended for commercial use while any supplementation above this level might reduce the yield of oyster mushroom significantly.

Keywords: biological efficiency, Oyster mushroom, pine, *Pleurotus ostreatus*, sawdust, substrate supplementation, wheat bran

1. Introduction

The cultivation of edible mushrooms has become an attractive economic alternative over past few years, mainly due to increase in its demand and market value (Chang, 2006). Oyster mushrooms are the easiest and least expensive commercial mushrooms to grow because they are well known for conversion of crop residues to food protein (Banik and Nandi, 2004). Oyster mushroom (*Pleurotus ostreatus*) is an edible mushroom having excellent fragrant and taste and its cultivation on crop residues is considered as potential source of income, an alternative food production, provision of employment, and for recycling of agricultural wastes. The market for mushrooms has been reported to be on a continuous growth due to the interest in their culinary, nutritional, health benefits and

their potential for use in waste management (Beetz and Kustidia, 2004). Shah et al. (2004) reported that oyster mushrooms are one of the most delicious foods due to their high nutritional value, very good taste and medicinal value. Mushroom cultivation has been shown by Tripothi and Yadar (1992) to exploit the natural ability of fungi to bio-divert solid waste generated by industry and agriculture into food and therefore many agricultural and industrial wastes can be utilized as substrates for production of *Pleurotus species* (Zadrazil and Brunnert, 1981).

Oyster mushroom is popular and considered a nutritious food in Swaziland, while its cultivation has long been neglected, because most of the mushrooms consumed locally are picked from the wild. However, awareness in oyster mushroom cultivation in Swaziland increased significantly in 2001, when the Queen mother initiated a pilot project on mushroom production in collaboration with the Ministry of Agriculture and Cooperatives through the technical assistance from the government of Thailand, which was aimed at poverty alleviation and women empowerment through job creation in the rural areas (Earnshaw, personal communication). Mushroom cultivation in Swaziland was initially established using sugarcane bagasse from the Royal Swaziland Sugar Company (RSSC) as the basic substrate due to its abundant availability as a waste product of the sugar processing industries until late 2002 when its supply was suspended and was no longer made available to mushroom growers because of its use in energy generation by the sugarcane processing mills. Thus, the search for an alternative agro industrial substrate became very imperative to allow for the continuous cultivation of mushroom. Sawdust which is a by-product of the saw-mill industries is also largely available and has been considered a possible alternative for mushroom cultivation. However, reports from studies in Swaziland have shown that low yields and bad odor in oyster mushroom often result when fresh sawdust is used as substrate.

Obodai et al. (2002) reported that sawdust substrate for mushroom production should undergo a period of composting to breakdown the cellulose and lignin components of the wood in order to release the essential materials for the establishment of mushroom mycelium. The ligno-cellulosic materials in sawdust are generally low in protein content and thus insufficient for the cultivation of mushrooms, and therefore require additional nitrogen, phosphate and potassium. Carvalho et al. (2010) reported that supplementation of substrate is a practice used in Pleurotus sp. production to obtain satisfactory yield and development. The supplements or additives supply extra nitrogen and/or easily degradable carbohydrates to increase mushroom yields and hasten the production process (Royse, 2002). Cereal bran rich in protein is usually added to the substrate in *P. ostreatus* cultivation to stimulate mycelia growth and increase the yield of mushroom (Kinugawa et al., 1994; Siddiqui and Khan, 1989). The mushroom mycelia requires specific nutrients for its growth and the addition of supplements increases mushroom yield by providing specific nutrients for the mycelium growth (Oei, 1996). There is paucity of information on mushroom substrate supplementation in Swaziland and the objective of this work, therefore was to evaluate the effects of pine sawdust substrate fermentation and wheat bran supplementation on the growth and productivity of oyster mushroom, with a view to eliminating the bad odor and low yields attributable to fresh sawdust substrate.

2. Materials and Methods

The experiment was conducted in the mushroom research unit of Malkerns Research Station (MRS), Swaziland during November 2011 to February 2012. *Pleurotus ostreatus* (strain-PN), the most cultivated oyster mushroom in Swaziland was used in this study.

2.1 Spawn Preparation

The spawn was prepared using the method of spawn preparation outlined by Stamets and Chilton (1983). The sorghum grains were washed and steeped overnight in water. After washing, the grains

were allowed to drain, tied in a wire mesh and steamed for 45 min in an autoclave at 105 ^{o}C to ensure that the steamed grains were cooked but intact. Thereafter, they were air-dried to cool on a wooden frame with a wire mesh. After cooling, 3 percent (w/w) of calcium carbonate ($CaCO_3$) was added and thoroughly mixed manually to ensure that the grains did not stick together after sterilization and to make them friable for easy inoculation.

Two hundred grams (200g) of the grains were weighed into transparent 350 ml glass bottles, plugged with cotton wool and covered with plain sheet of paper held in place with rubber band. The bottled grains were sterilized in an autoclave at 121 ^{o}C for 1hr and then cooled under lamina airflow without opening the bottles.

2.2 Inoculation and Incubation of Grains

The bottled sterilized grains was aseptically inoculated with 1cm^3 of pure cultures of *Pleurotus ostreatus* strain-PN, obtained from mushroom research unit of Malkerns Research Station using a flamed and cooled scalpel in a laminar flow hood. The spawns were incubated without illumination in an incubator set at 28°C according to Garcha (1994).

2.3 Sawdust Substrate Preparation and Supplementation

Fresh hard wood sawdust from pine trees (*Pinus* subg. *Strobus*) were collected from the timber sawmill. The sawdust was heaped to about 0.5 m in height and water in the ratio of 1: 2 (v/v) was added onto the heap on a cement platform and mixed thoroughly. The heap was covered with black plastic polyethylene sheet and left to ferment for 28 days with regular turning once a week. After 28 days, when the moisture content had dropped to 72 %, it was then supplemented with varying levels of wheat bran. Before spawning, five levels of wheat bran 0, 5, 10, 15 and 20 % were added and mixed thoroughly with the fermented sawdust substrate. The mixing area was disinfected with sodium hypochlorite to ensure a sterile environment and no water was added during the mixing process. After proper mixing, 500 g of each mixed substrate were filled in plastic bag (12.5 cm wide and 18.5 cm long). The bags were sealed using cotton plugged polyvinyl chloride pipe ring and sterilized at 100 °C for 3 h and allowed to cool.

The treatments were made up of the following fermented sawdust substrate and wheat bran supplementation:

T0 =100 % fermented sawdust substrate + 0 % wheat bran (Control)

T1= 95 % fermented sawdust substrate + 5 % wheat bran

T2= 90 % fermented sawdust substrate + 10 % wheat bran

T3= 85% fermented sawdust substrate + 15 % wheat bran

T4= 80 % fermented sawdust substrate + 20 % wheat bran

After sterilization, the bags were inoculated with the spawns of oyster mushroom (strain-PN) at the rate of 5% per bag according to the dry weight of substrates and kept at room temperature of (25 ± 2 °C) and relative humidity of 70 % for mycelium colonization.

The supplemented and unsupplemented (control) substrate treatments were arranged in a randomized complete block design with three replications and there were 10 bags per replication, giving a total of 30 bags per treatment. After about 30 days, when the mycelia had fully colonized about 70% of the bags, they were cut open and transferred to the cropping house for fructification (sporulation). The relative humility (RH) inside the growing house was kept between 85-90% by watering the sand floor and spraying the substrate bags with water twice a day in the morning and evening during cropping. When mushroom pin heads grew into mature fruit bodies, harvesting was done with sharp knife from each treatment. The mushrooms were harvested in four flushes over 42-day period. The parameters measured were number of contaminated bags, number of days for

substrate colonization, mushroom pileus diameter (mm), stipe length (mm), total mushroom yield (g) and biological efficiency (BE). The biological efficiency (BE) values were calculated in accordance to Royse et al., (2004) as:

Bio-efficiency (BE) % = Fresh weight of mushroom (g) / Dry weight of substrate (g) × 100

The data collected were analysed by one-way analysis of variance (ANOVA) using MSTAT-C (Nissen, 1989) and Duncan's new multiple range test was used to separate means where significant differences ($P < 0.05$) were detected among the treatment means.

3. Results

3.1 Number of Contaminated Bags

There were significant (P<0.05) differences in the number of contaminated bags which ranged from 5 to 30 (Table 1) during incubation. The unsupplemented sawdust (T0) recorded the highest number of contaminated bags (30 bags) while the sawdust substrate supplemented with 5 % wheat bran had the least number of contaminated bags (5 bags) though not significantly different from sawdust supplemented with 10% wheat bran (6 bags). However, the number of contaminated bags increased with increasing wheat bran supplementation up to the highest level of 20% wheat bran supplement.

Table 1 Effect of wheat bran supplement on the number of contaminated bags and time of colonization of oyster mushroom on fermented sawdust substrate

Treatment	Number of contaminated bags	Days to full colonization
T0	30^{a}*	0**
T1	5^{d}	43^{a}*
T2	6^{cd}	37^{b}
T3	8^{bc}	37^{b}
T4	10^{b}	33^{c}

*Means within the same column followed by the same letter are not significantly different at P<0.05

**No colonization was obtained in this treatment

3.2 Number of Days Taken for Full Colonization of Substrate

The number of days taken to full colonization in fermented sawdust substrate supplemented with different levels of wheat bran differed significantly (P<0.05) and varied from 33 to 43 days (Table 1). The mycelium failed to colonize the unsupplemented fermented sawdust substrate (T0) and hence no fruiting bodies presumably due to substrate contamination. This might probably due to the inhibitory effects of pathogens present in the substrate as well as the poor activity of enzymes such as cellulases, hemicellulases, and ligninases which bring about hydrolysis of the macro molecules of cellulose in wheat supplemented sawdust as compared to the unsupplemented substrate. The number of days to full colonization in the fermented sawdust decreased with increasing wheat bran supplementation. Fermented sawdust supplemented with 20% wheat bran took the shortest period (33 days), while sawdust supplemented with 5% wheat bran took the longest period (43 days) to full colonization. Number of days to substrate colonization in 10 and 15 % wheat bran supplementation were similar and not significantly (P<0.05) different.

3.3 Oyster Mushroom Pileus Diameter and Stipe Length

The effect of wheat bran supplementation of fermented sawdust was significant (P<0.05) on pileus diameter (Fig.1) of oyster mushroom during the four weeks of growth. The mean pileus diameter on sawdust supplemented with different wheat bran levels ranged from 57.9 to 62.3 mm, the largest being on sawdust substrate supplemented with 15% wheat bran (62.3 mm) and the smallest obtained on sawdust substrate supplemented with 5% wheat bran (57.9 mm). However, mean pileus diameter on substrate supplemented with 15% wheat bran was higher but not significantly different from those on substrate supplemented with 20% wheat bran.

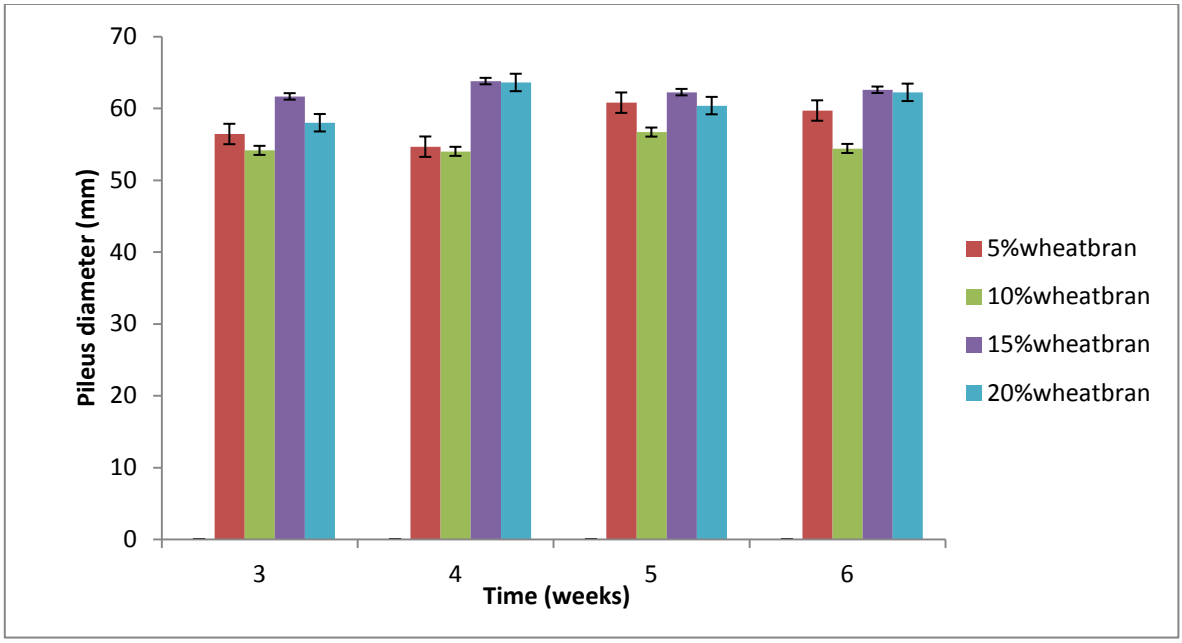

Figure 1 Pileus diameter of oyster mushroom grown on fermented sawdust substrate supplemented with different wheat bran levels

There were significant (P<0.05) effect of wheat bran supplementation of fermented sawdust on the pileus height (Fig.2) of oyster mushroom. Similarly, the stipe length of oyster mushrooms ranged from 39.4 to 59.5 mm on fermented sawdust substrate supplemented with different wheat bran levels. The highest stipe length (59.5 mm) was observed on substrate supplemented with 15% wheat bran and the smallest on substrate supplemented with 10% wheat bran (39.4 mm).

3.4 Yield and Biological Efficiency (BE) of Oyster Mushroom

There was a significant (P<0.05) effect of wheat bran supplementation on the yield and biological efficiency (BE) of oyster mushroom (Table 2) on fermented sawdust substrates. The crops of oyster mushroom were harvested in four flushes and the maximum yield was obtained in first flush than the second, third and fourth flushes, respectively. The highest total yield of mushrooms was recorded on sawdust supplemented with 15% wheat bran (683.9g/500g substrate), which was found to be significantly higher than all other treatments. The lowest yield (390.9g/500g substrate) was obtained on sawdust supplemented with 5% wheat bran over the cropping period. The variations observed in yields may, therefore be attributed to the complexity of the fermented sawdust cellulose degradation by the mushroom enzymes as a result of the different levels of wheat bran supplement added.

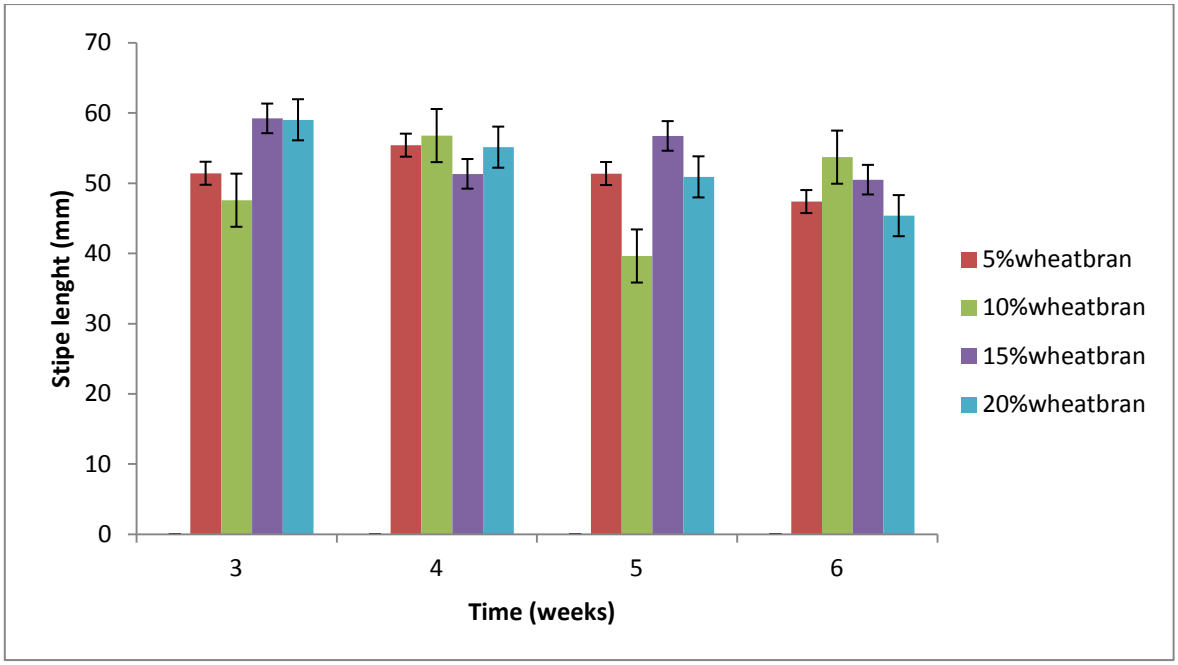

Figure 2 Stipe length of oyster mushroom grown on fermented sawdust substrate supplemented with different wheat bran levels

Biological efficiency (BE) used in evaluating the efficiency of substrate conversion in mushrooms, was variable and significantly ($P<0.05$) different among the wheat bran treatments. The result showed that all the fermented sawdust substrate supplemented with wheat bran gave BE above 100% except that supplemented with 5% wheat bran. Fermented sawdust supplemented with 15% wheat bran had the highest BE of 136.8 %, while that supplemented with 5 % had the lowest BE of 78.2 %. Generally, the total yield and BE of mushroom increased significantly with increasing wheat bran supplementation up to 15% and then decreased with further increase in wheat bran supplementation up to 20%.

Table 2 Effect of wheat bran supplement on the yield and biological efficiency of oyster mushroom on fermented sawdust substrate

Treatment	Mushroom yield (g) per 500 g of substrate				Total yield (g) of mushroom in 4 flushes	Biological Efficiency (%)
	1st flush	2nd flush	3rd flush	4th flush		
T0**	0.0	0.0	0.0	0.0	0.0	0.0
T1	110.0	101.4	98.5	81.0	390.9[d]*	78.2[d]*
T2	149.0	139.6	126.7	101.8	517.1[c]	103.4[c]
T3	200.0	173.0	166.7	144.2	683.9[a]	136.8[a]
T4	167.3	164.2	147.0	141.8	620.3[b]	124.1[b]

*Means within the same column followed by the same letter are not significantly different at $P<0.05$
** No colonization was obtained in this treatment (T0), and hence no yield

4. Discussion

Oyster mushroom (*Pleurotus spp.*) was successfully produced on fermented pine sawdust substrate supplemented with various levels of wheat bran. The utilization of insoluble ligno-cellulosic substrates by edible mushrooms has been reported to depend on the production of the enzymes such as cellulases, hemicellulases, ligninases which bring about hydrolysis of the macro molecules of cellulose, hemicellulose and lignin components of the substrate, thereby liberating the low molecular weight nutrients essential for mushroom growth (Buswell et al,. 1993). The addition of supplements to mushroom substrate is very important especially for substrates having low protein content to enhance the growth and yield of mushrooms. Dhanda et al. (1996) reported that substrate supplementation is a practice used in producing *Pleurotus* sp. in order to increase its productivity.

The number of contaminated bags increased with increase in rate of wheat bran supplementation up to the highest level of 20%. This may probably be due to the fact that the supplement has become too rich thereby increasing the risk of its contamination (Oei, 2003). In this study all the unsupplemented sawdust substrate (control) bags were heavily contaminated and this could probably be as a result of the materials broken down during substrate sterilization which encouraged the growth of harmful pathogens that attacked and prevented the growth of mushroom mycelium as well as causing chemical alteration of the substrate, which hinders mushroom development. Also, the inadequate nitrogen level in the fermented sawdust for bioconversion of lingo-cellulose material as well as the high pH of the sawdust substrate may also have contributed to the contamination of the bags. However, Kurtzman (2010) reported several causes of contamination of mushroom substrate and ways of avoiding potential contamination were also suggested. The most abundant contaminants were found to be *Penicillium* and *Trichoderma* sp. (Oseni et al., 2012) which were responsible for the blackening of substrate bags. Balasubramanya and Kathe (1996) reported that these microorganism species (*Penicillium* sp. and *Trichoderma* sp.) compete with *Pleurotus* sp. after pasteurisation with hot water probably due to the partial breakdown of cellulose and hemicelluloses, thus making them available to competitors. Mazumder and Rathaiah (2001) reported similar contaminants in oyster mushroom substrates. The number of days taken to full colonization of fermented sawdust supplemented with wheat bran ranged from 33 to 43 days. Fermented sawdust supplemented with 20% wheat bran were fully colonized within 33 days whereas sawdust supplemented with 5% wheat bran took longer period and were fully colonized within 43 days after inoculation. This agreed with the findings of (Royse et al., 2004; Oseni et al., 2012; Khare et al., 2010 and Mane et al., 2007). The result however, contradicts those of Shah et al. (2004) and Ponmurugan et al. (2007) who reported full colonization in *Pleurotus ostreatus* in 17 - 20 days on different substrates. Buswell et al. (1996) reported that the production of the enzymes such as cellulases, hemicellulases, and ligninases by the fungal mycelium is a crucial part of the colonization process and thus an important determinant of mushroom yield. Narain et al. (2008) reported that mushroom mycelia growth and primordial development is dependent on the ligno-cellulosic materials especially the C: N ratio. Similarly, Ayodele and Okhunya (2007) showed that supplements or inclusion of additives change the rate and sequence of decomposition of substrate components.

The pileus diameter of oyster mushrooms varied markedly in fermented sawdust supplemented with different levels of wheat bran, and the trend of variation was more or less similar to that of stipe length growth. Sawdust supplemented with 15% wheat bran produced mushrooms with the longest stipe and pileus diameter, respectively and followed by substrate supplemented with 20% wheat bran, while sawdust supplemented with 5% wheat bran produced mushrooms with least stipe length and pileus diameter. Onyango et al. (2011) reported that large sized fruit bodies is a desirable quality which is rated highly in mushroom production but considered an inferior quality by Shen and Royse (2001) because such mushroom fruit bodies break during packaging thereby reducing their quality. However, AMGA (2004) listed temperature, relative humidity, fresh air and compact

material as the major ecological factors affecting stalk height, stalk diameter and cap size in mushrooms. Previous report by Jandaik (1981) on Carbon: Nitrogen ratio in mushroom substrate showed that low N content may be a limiting factor and as such growers should use substrates that do not promote excessive growth of stalk length at the expense of marketable yield. However, substrate supplementation with various additives including nitrogen sources has been reported to improve growth, yield and quality of mushrooms (Khare et al., 2010; Onyango et al., 2011). There were significant effects of substrate supplementation with wheat bran on the total yield and B.E. of oyster mushroom. Maximum yield and highest biological efficiency (BE) were obtained in 15% wheat bran supplemented substrate compared to other treatments. Higher yields and B.E. were obtained in this study compared to that reported on sawdust by Shah et al., (2004). Nunez and Mendoza (2002) also reported BE values varying from 50.8 to 106.2 % in *Pleurotus ostreatus* on different substrates**.** Similarly, higher yields were reported in *P. sajor caju* (Cho et al., 1981) and shiitake mushrooms (Royse and Schisler, 1986) when wheat bran was included in the growing substrate. Zadrazil (1980) reported that the growth of *Pleurotus* species is favoured on substrates low in nitrogen content since high carbon and low nitrogen ratio is needed for good yield, thus the low yield obtained in this study with 5% wheat bran supplement could probably be due to carbon: nitrogen imbalance in the substrate. The type and concentration of nutrient supplement has been shown to have considerable effects on both substrate colonization and the type of hydrolytic and oxidative enzymes produced. Nikitina et al. (2007) and Silva et al. (2005) reported that mycelium extension is related to bio availability of nitrogen when they found that eucalyptus residues supplemented with cereal bran supported fast growth of L. edodes. However, the low amount of available nitrogen (N) in the ligno-cellulosic substrate of wood components is often considered as a limitation to its use as mushroom substrate. Royse et al. (1990)] and Oei (2003) suggested the inclusion of wheat bran supplement at the rate of 10-40 % and 5-10 %, respectively would serve as nutrients that will provide optimum growing medium in the substrate ingredients used for mushroom production. However, result obtained in this study indicated that wheat bran supplementation of fermented sawdust substrate above 15% had significant and depressing effects on total yield and BE of oyster mushrooms. This decrease in yield and BE could probably be explained by the fact the 20 % wheat bran might have generated a lot of heat resulting in the overheating of the substrate which subsequently affected the mushroom growth negatively thereby leading to poor yield, as the study was conducted during the peak of summer months under room temperature without adequate cooling facility in mushroom growing house.

5. Conclusion and Recommendation

The present study showed that supplementing fermented pine sawdust substrate with 15% wheat bran produced the highest yield and biological efficiency in oyster mushroom compared to the other treatments. It also proved to be better in terms of mycelia growth and development as well as in substrate colonization. Therefore, wheat bran supplementation of fermented pine sawdust substrate can be recommended to oyster mushroom growers in Swaziland for mushroom production and this will solve the problem of non availability of sugarcane bagasse and also eliminate bad odor in mushroom associated with fresh sawdust due to contamination. However, addition of wheat bran supplement beyond 15% to fermented pine sawdust substrate may result in significantly lower mushroom yield and poor performance. Similarly, the pine sawdust used in this study is economically feasible due to its abundant availability throughout the year.

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