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Effect of Substrate Pre-treatment Methods on Oyster Mushroom (*Pleurotus ostreatus*) Production

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ABSTRACT

Among the various cultivated mushrooms, oyster mushroom is easy to cultivate due to its strong enzymatic action towards the utilization of various kinds of organic substrates. However, pre-treatment of substrates is not an easy job for the cultivation of mushroom especially in the rural areas. The objective of this study was to devise an easy handy procedure for organic substrate pre-treatment in the production of oyster mushroom. Substrate pre-treatments by autoclaving at 121°C, hot water dipping (pasteurisation) in steel drum at 60°C for 2 h and hot water dipping (pasteurisation) in steel drum at 60°C for 3 h were evaluated. The percent contamination was significantly higher in horse manure compost (70%) compared to sugarcane bagasse (12.5%). The oyster mushroom took significantly less time to colonise the autoclaved sugarcane bagasse (36 days) compared to sugar cane bagasse pasteurised for 2 h (64 days). Autoclaved horse compost manure was fully colonised in 42 days, while those pasteurised with hot water at 2 and 3 h failed to colonise due to heavy contamination by *Trichoderma harzianum* presumably due to insufficient sterilisation. Despite the shortest days to full colonisation, there was no significant difference in the yield (410.4 g) and bio-efficiency (82.10%) of autoclaved sugarcane bagasse compared to the yield (301.1 g) and bio-efficiency (60.22%) of sugarcane bagasse pasteurised in hot water for 3 h. Although, autoclaving was the best method for substrate pre-treatment, however hot water pasteurisation at 60°C for 3 h of sugarcane bagasse proved to be a viable and promising method of substrate pre-treatment, which can be adopted to produce a good yield of oyster mushroom especially in rural areas, where autoclave sterilisation is not feasible. © 2012 Friends Science Publishers

Key Words: Bio-efficiency; Oyster mushroom; *Pleurotus ostreatus*; Substrate pasteurisation; Yield

INTRODUCTION

The Oyster mushroom (*Pleurotus ostreatus*), is one of the most appreciated mushroom due to its very good taste, high nutritional and medicinal value. It has also been found to exhibit strong anti-inflammatory and immune-modulatory properties due to their chemical composition (Lavi *et al*., 2010). Mushrooms are a good source of protein, vitamins and minerals (Khan *et al*., 1981) and are known to have a broad range of uses both as food and medicine (Alice & Kustudia, 2004). Oyster mushroom is popular and considered a nutritious food in Swaziland but 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 has significantly increased since 2001, when the Queen mother initiated a pilot project on mushroom production in conjunction with the Ministry of Agriculture and Cooperatives aimed at poverty alleviation and women empowerment through job creation in the rural areas (Earnshaw, personal communication).

Oyster mushroom can be grown on various substrates including paddy straw, maize stalks/cobs, vegetable plant residues, bagasse etc. (Hassan *et al*., 2011) and this has been reported to influence its growth, yield and composition (Iqbal *et al*., 2005; Kimenju *et al*., 2009; Khare *et al*., 2010). However, an ideal substrate should contain nitrogen (supplement) and carbohydrates for rapid mushroom growth (Anonymous, 2008). The cultivation of oyster mushroom is gaining importance in tropical and subtropical regions due to its simple way of cultivation and high biological efficiency (Singh *et al*., 1990).

Oyster mushrooms have been traditionally produced using the out - door log technique (Anonymous, 2008), thereby eliminating substrate sterilisation. The technology of large scale mushroom production is a recent innovation and the establishment of laboratories for research on mushroom growing and the use of pure spawn culture resulted in rapid and increased production of mushrooms worldwide (Flegg *et al*., 1985). The substrates for cultivating edible mushrooms e.g. *Pleurotus ostreatus*, has been reported to require varying degrees of pre-treatment in order to promote growth of the mushroom mycelium to the exclusion of other microorganisms (Chang, 2008). The methodology for

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substrate preparation described in several studies consists of composting agricultural residues, followed by pasteurisation, which can be carried out in different ways (Stamets, 1993; Balasubramanya & Kathe, 1996).

Sanchez (2010) reported that substrate used for the oyster mushroom cultivation do not require sterilisation, but only pasteurisation, which is less expensive to diminish the damages produced by different pathogens (bacteria, moulds or insect pests) on mushroom development and yield. Diana *et al*. (2006) recommended disinfection of the substratum before spawning, which should only destroy the competitive fungi and not the useful micro organisms. Quimio *et al*. (1990) observed that substrate sterilisation is not ideal since both beneficial and harmful organisms in the substrate are killed, while Miroslawa (1991) recommended maintaining the substrate for 24 h at 70°C. Similarly, sterilisation of substrates is not an easy job for the cultivation of mushroom and the right sterilisation time and temperature depend on the possible pathogens in a given substrate material (Kwon & Sik Kim, 2004).

The mushroom project embarked upon by the Swaziland World Vision in National Care Points (NCPs) around the country in an attempt to provide food for the orphans and vulnerable children (OVC) was not successful due to lack of financial resources to prepare the substrates for mushroom production. Therefore, the objective of this study was to compare the effectiveness of substrate pre-treatment methods using autoclave sterilisation and hot water pasteurisation on oyster mushroom growth and yield.

MATERIALS AND METHODS

The study was conducted at the Mushroom laboratory of the Crop Production Department, University of Swaziland, Luyengo Campus and Malkerns Research Station, Malkerns, Swaziland between October, 2010 and January, 2011. Two locally available substrates namely sugarcane bagasse and horse manure compost were used in this study. To each of the substrate, 20% of wheat bran supplement was added and thoroughly mixed. The substrates were soaked in clean water for 8-10 h and allowed to imbibe water. The substrates were separately subjected to a short composting procedure using the method of Sinden and Hauser (1980). The substrates were divided into batches of 500 g each and packed into heat resistant autoclaveable bags of 18 × 14 cm in size, which were fastened at the end using plastic rings. The substrates were then pre-treated using the following methods viz., steam sterilisation in autoclave at 121°C for 4 h, hot water dipping in steel drum at 60°C (pasteurisation) for 2 h and hot water dipping in steel drum at 60°C (pasteurisation) for 3 h and allowed to cool. The treatments which consisted of two substrates and three pre-treatment methods were arranged in a randomised complete block design with four replications and there were 10 bags per replicate, giving a total of 40 bags per treatment.

The cooled bags were inoculated using the prepared planting grain spawn of oyster mushroom (*Pleurotus ostreatus*) under a lamina air flow to minimize contamination at the rate of 5% per bag according to the dry weight of the substrates. The bags were incubated in dark chamber for spawn running at a temperature of 28 - 30°C until full colonisation by mycelia was attained. The bags were later transferred to the mushroom cropping room where they were opened to trigger fructification. The relative humility inside the growing room was kept as high as 85-95% by watering the sand floor and spraying the substrate bags with water twice a day in the morning and evening.

Mushrooms were harvested as soon as the fruiting bodies developed and attained their full size above the substrate with sharp knife from each treatment bag. Mushrooms were harvested in three flushes over a 42-day period. The parameters recorded were number of contaminated bags, days taken for the completion of substrate colonisation, mushroom pileus diameter (cm), stipe length (cm), total mushroom yield (g) and bio efficiency (BE). The bio efficiency (BE) of mushroom was calculated by using the formula recommended by Chang and Miles (1989) as:

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

The data collected were subjected to analysis of variance (ANOVA) using MSTAT-C (Nissen, 1989). Mean separation was done using LSD, where effect showed significant difference at 5% level of probability.

RESULTS

The analysis of variance for stipe length, pileus diameter, bio-efficiency and mean yield of oyster mushroom indicated significant differences among the various substrate pre treatment methods (Table I).

Number of contaminated bags: There was a significant ($P<0.05$) difference in the number of contaminated bags, which ranged from 2 to 40 by green mould (*Trichoderma harzianum*) and other bacteria during incubation (Table II). The horse manure compost pasteurised either for 2 or 3 h had the highest number of contaminated bags (40 bags) and was significantly ($P < 0.05$) higher than the other treatments and thus abandoned. The autoclaved sugarcane bagasse exhibited the least contamination (3 bags), which was not significantly different from autoclaved horse manure compost (4 bags) and sugarcane bagasse pasteurised for 3 h (7 bags).

Number of days taken for full colonisation of substrate: The number of days taken to full colonisation for the substrate pre-treatments (Table II) differed significantly ($P<0.05$). The mycelium failed to colonise the horse manure compost substrates that were pasteurised for 2 and 3 h, respectively presumably due to contamination.

Table I: Analysis of Variance for stipe length, pileus diameter, yield and bio-efficiency of oyster mushroom as affected by substrate pre treatment

Sources of variation		Stipe length		Pileus diameter		Yield		Bio-efficiency	
	d.f.	M.S.	F	M.S	F	M.S	F	M.S	F
Replication	3	404.70	2.83	475.40	3.59	1670.13	1.01	417.53	0.94
Treatment	3	1335.71	9.42*	821.80	6.21*	7947.74	4.84*	1986.78	4.47*
Error	9	142.06		132.27		1684.20		444.7	

Significant at 5% level

Table II: Effect of substrate pre-treatment on the number of contaminated bags and time of colonisation of oyster mushroom

Substrate pre-treatment	Number of contaminated bags	Days to full colonisation
Autoclaved Bagasse	3c	36c
Autoclaved Compost	4c	42c
Bagasse pasteurised for 2 h	7b	64a
Compost pasteurised for 2 h	40a	0**
Bagasse pasteurised for 3 h	5bc	52b
Compost pasteurised for 3 h	40a	0**

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

**No colonisation was obtained in these treatments

Table III: Effect of substrate pre-treatment on the stipe length and pileus diameter of oyster mushroom

Substrate pre-treatment	Stipe length (cm)	Pileus diameter (cm)
Autoclaved Bagasse	5.48ab*	4.78a
Autoclaved Compost	5.94a	4.84a
Bagasse pasteurised for 2 h	4.78bc	4.70a
Bagasse pasteurised for 3 h	4.53c	4.37b

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

Substrate pre-treatment had variable effects on the duration of full colonisation ranging from 36 to 64 days for autoclaved sugarcane bagasse and sugarcane bagasse pasteurised for 3 h, respectively. The days taken to colonise the sugarcane bagasse sterilised for 2 h were significantly longer (P<0.05) than those taken to colonise the sugarcane bagasse, which was autoclaved and those pasteurised for 3 h. However, full colonisation was completed 42 days after inoculation in the autoclaved horse manure compost and was not significantly different from autoclaved sugar cane bagasse (36 days).

Stipe length and pileus diameter growth: The stipe length and pileus diameter development in oyster mushroom were significantly (P<0.05) affected by substrate pre treatment as shown in Table III. The mean stipe length ranged from 4.53 cm in sugarcane bagasse pasteurised for 3 h to 5.94 cm in autoclaved compost manure, but there was no significant difference in mean stipe length of oyster mushroom in both autoclaved bagasse and horse compost manure. The pileus diameter ranged from 4.37 cm in pasteurised bagasse for 3 h to 4.84 cm in autoclaved horse compost manure. However, there was no significant difference (P<0.05) among the pileus diameter in autoclaved horse compost manure, autoclaved sugarcane bagasse and pasteurised bagasse for 2 h, all of which were significantly larger than in pasteurised bagasse for 3 h.

Yield and bio-efficiency of oyster mushroom: There were significant (P<0.05) effects of the substrate pre-treatment methods on the average yield of oyster mushroom (Table IV). The crop of oyster mushroom was harvested in three flushes and the maximum yield was obtained in first flush than the second and third flush, respectively. Maximum average yield of 410.4 g was obtained from autoclaved bagasse, while the lowest yield was obtained from bagasse pasteurised for 2 h (118.9 g). The yield from bagasse pasteurised for 3 h (301.1 g) was higher but not significantly different from autoclaved compost manure (209.2 g). The variations observed in yield may, therefore be attributed to the complexity of substrates in terms of their cellulose content resulting from the difference in the rate of degradation by the mushroom enzymes as a result of the pre treatment methods. The Bio-Efficiency (BE) was calculated to determine how the mushrooms utilized nutrients present in the substrates efficiently. The average bio-efficiency was variable and significantly (p<0.05) different among the substrate pre-treatments. Autoclaved sugarcane bagasse had the highest BE of 82.10%, which was not significantly different from sugarcane bagasse pasteurised for 3 h (60.22%), but significantly higher than autoclaved horse manure compost (41.85%). Sugarcane bagasse pasteurised for 2 h had the lowest BE of 23.78% compared to others.

DISCUSSION

The ability of a sterilisation method to eliminate substrate contaminants is shown by the presence or absence of contaminants in the substrate after sterilisation, spawning and incubation. Kurtzman (2010) reported several causes of mushroom substrate contamination. All the horse manure compost bags pasteurised either for 2 or 3 h were heavily contaminated by green mould and thus abandoned. This agreed with Kalberer (1974) who observed that compost was a poor substrate for the growing oyster mushrooms. Similarly, insufficient pasteurisation could also be attributed to the contamination of the horse manure compost. Kwom and Sik Kim (2004) observed that cattle manure contained high salt levels, which may also increase the risk of contamination. This also concurred with the result obtained by Oei (1996). According to Balasubramanya and Kathe (1996), the microorganism species that competed with *Pleurotus* sp. after pasteurisation with hot water (80°C for 2 h) were the fungi *Penicillium* sp. and *Trichoderma* sp., probably due to the partial breakdown of cellulose and hemicelluloses, thus making them available to competitors.

Table IV: Effect of substrate pre treatment method on the yield of oyster mushroom

SubstratePre-treatment Method	Yield of mushroom (g) per 500 g of substrate			Average yield (g) in three flushes per 500 g of substrate
	1st flush	2nd flush	3rd flush	
Autoclaved Bagasse	900.10	325.75	5.33	410.39a*
Autoclaved Compost	256.68	258.40	112.63	209.23bc
Bagasse pasteurised for 2 h	117.83	216.47	22.33	118.88c
Bagasse pasteurised for 3 h	487.00	360.10	56.18	301.08ab

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

The contamination of the hot water pasteurised substrate may have occurred probably due to inadequate temperature and time used during pasteurisation, since the literature is quite variable with reference to these characteristics. Chang and Miles (1989) reported that green mould competes with the mushroom for space, nutrients as well as causing chemical alteration of the substrate, which hinders mushroom development.

The substrate pre-treatment had variable effects on days to full colonisation ranging from 36 to 64 days, while the mycelium failed to colonise the horse manure compost substrate. Sugar cane bagasse contained high polysaccharides, which hastened the fungus growth resulting in faster colonisation. This concurred with the findings of Iqbal *et al*. (2005) who reported 37 days for full colonisation completion in exotic strains of *Pleurotus ostreatus* on sugarcane bagasse. Result obtained contradicts those of Shah *et al*. (2004); Vetayasuporn *et al*. (2006); Ponmurugan *et al*. (2007) who obtained full colonisation in *Pleurotus ostreatus* in 17 - 20 days on different substrates. Nutritional composition of substrates has been reported to be crucial in determining how mycelia growth initiation occurs (Stamets, 2005) and Narain *et al*. (2008) reported that mushroom mycelia growth and primordial development is dependent on the lignocellulosic materials especially the C: N ratio.

There were significant effects of substrate pre-treatment methods on the average yield of oyster mushroom and highest growth vigour, yield and B.E. were obtained in autoclaved sugarcane bagasse. Onyango *et al*. (2011) reported that large sized fruit bodies were considered to be of good quality and rated highly in mushroom production but Shen and Royse (2001) reported this as an inferior quality since such fruit bodies tend to break during packaging thereby reducing their quality. However, major ecological factors that affect stalk height, stalk diameter and cap size in mushroom are temperature, humidity, fresh air and compact material (AMGA, 2004). The crop of oyster mushroom was harvested in three flushes, while the maximum yield was obtained in first flush. Result similar to this was obtained by Iqbal *et al*. (2005) and Shah *et al*. (2004). Maximum yield and highest biological efficiency (BE) were obtained in autoclaved sugarcane bagasse, however low fresh weights were recorded for fruit bodies obtained from sugarcane bagasse in this study compared to that reported on sawdust by Shah *et al*. (2004). Thomas *et al*. (1998) observed that the very complex nature of sugarcane bagasse impedes its efficient conversion by fungal mycelium. In addition, Zervakis *et al*. (2001) opined that the mushroom received nutrition and energy from the abundant free sugars that were present in the bagasse and therefore made limited use of the cellulose fraction. Similarly, the average biological efficiency was variable and significantly different among the substrate pre-treatments. This confirms the finding of Mandeel *et al*. (2005) that B.E is highly affected by the quality of the spawn of the cultivated mushroom strain. Different substrates have been used to grow *Pleurotus* sp. with BE values varying from 32.10 - 79.18% (Dhanda *et al*., 1996).

CONCLUSION

It can therefore be concluded that hot water dipping of sugarcane bagasse in steel drum at 60°C (pasteurisation) for 3 h despite its low yield and biological efficiency compared to autoclaved bagasse, would be a viable and promising technique of substrate pre-treatment that can be adopted to produce a good yield of oyster mushroom in most rural areas, where autoclave sterilisation may not be feasible.

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