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Research Article

# EFFECT OF DIFFERENT GRAIN SPAWNS AND SUBSTRATE STERILIZATION METHODS ON YIELD OF OYSTER MUSHROOM IN BOTSWANA

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**Abstract:** The effect of three different grain spawns and two substrate sterilization methods on the spawn quality and yield of a hybrid of *Pleurotus ostreatus* x *P. florida* (Po x Pf) was studied on maize cobs supplemented with 20% wheat bran and 2% gram flour in a low technological mushroom house at Botswana College of Agriculture, Sebele. Sorghum grains proved to be better mycelium carriers (5.25, 7 days, 12 days and 75.4%) over wheat (2.45, 17 days, 16 days and 53.55%) and barley grains (3.46, 14 days, 13 days and 55.24%) in terms of mycelium growth vigor, colonization time, spawn running time of the substrate and yield of the oyster mushroom. Hot water treatment and steaming of substrate significantly reduced substrate contamination and improved mushroom yield as compared to the untreated control. Mushrooms grown on steamed substrates had significantly higher yield (BE: 69.4%) than those grown on substrates treated with hot water (BE: 53.3%).

**Keywords:** *Pleurotus ostreatus* x *P. florida*, Grain Spawns, Sterilization Methods, Yield

## INTRODUCTION

Oyster mushroom (*Pleurotus* species) is now cultivated in the most tropical countries because of its desirable attributes. These include unique flavor, exotic taste, rich in protein, vitamins and minerals, and more importantly its high ability for degrading lingo-cellulosic farm wastes, simple and cheap cultivation techniques and the wide choice of species under different climatic conditions (4, 6, 16, P17).

With the prevailing poverty status affecting rural areas in Botswana, there is a need to develop technologies of oyster mushroom cultivation that may be used by less privileged to help themselves earn their livelihood. Oyster mushroom cultivation has great potential since it requires limited space, low initial investment and the raw materials used are cheap and easy to acquire (3, 5, 15, 16). Thus, its cultivation is the most affordable to small scale farmers in order to generate extra income during the dry months.

Oyster mushroom cultivation in Africa has become more popular in recent years, and works in regards to its cultivation were summarized by Khare *et al.,* (5). In Botswana, several species of *Pleurotus* were screened on un-supplemented and supplemented locally available crop residues and a hybirid of *Pleurotus ostreatus* and *P. florida* (Po x Pf) and *P. ostreatus* were found to perform better than other species while pearl millet stalk was the best substrate (4,9,10,11). No other published works other than these are traceable in literature from Botswana in regards to cultivation of oyster mushroom. Moreover these studies specifically did not include the best type of spawn carriers and method of substrate sterilization which eventually affect yield of oyster mushroom. The first step in mushroom cultivation is the production of good quality spawn and a stable strain which can fulfill the expectation of the growers. The quality spawn may be achieved by selection of a suitable spawn carrier which influences the growth habit of the mycelium and subsequent yield (2). Different types of sterilization methods of substrates have been reported in the literature (1, 2). The objective of this study was to stabilize the standard protocol for these two parameters which farmers, unemployed youth and other growers can use them in maximizing the production of oyster mushroom. The present paper reports the effect of type of spawn carriers and different substrate sterilization methods on spawn quality and yield of oyster mushroom.

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## MATERIALS AND METHODS

The study was conducted at the Botswana College of Agriculture, Sebele (Latitude 24 34'S, Longitude 25 57'E, Altitude 994 m above sea level) from August 2008 to July 2009. The experiment was conducted during the warm season (August, 2008 to March, 2009) and repeated during the winter season (April, 2009 to July, 2009). Weather data were collected at the nearby Department of Agriculture Research, Sebele weather station.

Spawn running and mushroom productions were carried out in a low technology mushroom house with minimal environmental control. The house was 6 m long, 4 m wide and 2.5 m high. The walls and the roof

were made of gum poles and timber covered with black plastic for the maintenance of humidity and thatched with grass for insulation. The house was fitted with windows on each width of the house and a door. The house was used for spawn running and production. A hybrid strain of P. *ostreatus* x *P. florida* (Po x Pf) used in this research was acquired through the courtesy of Prof E. B. Khonga (Botswana College of Agriculture, Gaborone, Botswana) who obtained it from the Belgian Coordinated Collections of Microorganisms in Belgium.

**The performance of *P. ostreatus* × *P. florida* mycelium on wheat, barley and sorghum a spawn carriers and on mushroom yield on maize cobs.**

The preparations of the spawn, maize cob substrates, the method of sterilization and spawning were earlier described (4). Each carrier was replicated three times and the spawn bottles were placed in an incubator in completely randomized design. The spawned grains were inoculated onto 500g dry weight equivalent maize cobs to assess yield and yield parameters. The experimental design was a CRD with three replicate bags per treatment.

**Data collection and analysis:**

Comparative growth vigor of the mushroom mycelia on each grain carrier was measured by visual assessment as shown in Table 1. Colonization time (time taken in days for each grain to be fully colonized by the mushroom mycelium), incubation period (the number of days from spawning of maize cob substrate to third flush) and total yield (g) of fresh mushrooms per bag expressed as BE% were recorded. The data were subjected to the analysis of variance(ANOVA) and if the f-value was significant p<5%, the means were separated using the Least Standard Deviation (LSD) method using MSTAC computer package.

**Effect of sterilization methods of maize cob substrate on yield and yield parameters of Po × Pf.**

The effect of dipping in boiling water and steaming of maize cob substrate for 2 hours on contamination levels and yield of Po × Pf was assessed during October to December 2008.

Four and a half kilograms of ground maize cobs was soaked in water overnight and drained to remove excess water. The final wet weight was determined in order to assess the moisture content of the substrate. The wet substrate was thoroughly mixed with 900 g of wheat bran and 90 g of gram flour. 500g (dry weight) of the substrate was packed in each of the 9 perforated polythene bags measuring 30cm x 45cm. Three replicate bags were each subjected to steaming, dipping in boiling water and unheated bags served as controls.

**Steam pasteurization:**

A slightly modified method of that described by Khare *et al.,* (7) was adopted for this treatment. A 210 liter metallic drum was used as a steam pot. The steam pot was cleaned, placed on the firewood burner and water added to a depth of 60 cm. A cage 25 cm high above the surface of the water was placed inside the steam pot. The substrate was placed in the cage. The drum was covered with double layer of synthetic (nylon) bags and tied tightly using strings and the water was maintained at boiling point for two hours.

**Hot water treatment:**

The method devised by Singh & Dwivedi (18) was used. The pot used for steaming was used for this treatment. The pot was cleaned and placed over the firewood burner. Substrates were arranged in the pot and water added to cover them all. The substrates were packed in perforated sacks to allow the water to have direct contact with the substrate and also for excess water to drain easily and efficiently after cooking. Fire was lit and the water was kept at boiling point for 2 hours.

The bags were spawned at the rate of 4% per 500g (dry weight) bag of sorghum grain spawn of Po × Pf. Substrate not subjected to heat treatment was the control. The three treatments were replicated three times and arranged in a Randomized Complete Design (RCD) in the mushroom house.

**Data collection and analysis:**

Comparative assessment of contaminants after 2 and 4 weeks on a 1-8 scale (12) as detailed in Table 2 and total yield obtained from three flushes were recorded. The data were subjected to analysis of variance(ANOVA) and if the f-value was significant at p<5%, the means were separated using the Least Standard Deviation (LSD) method using MSTAC computer package.

## RESULTS AND DISCUSSION

The type of grain used had no significant effect on Po x Pf mycelium colonization time and growth vigor of the carriers, and spawn running period and BE when different grain spawns were inoculated on maize cobs as substrate supplemented with wheat bran and gram flour (Table 1). The mean mycelium growth vigor, colonization time, spawn running period and yield (BE) of mushrooms were noted with the use of different grain spawns respectively, wheat: 2.45, 17 days, 16 days and 53.55%; barley: 3.46, 14 days, 13 days and 55.24%; sorghum: 5.25, 7 days, 12 days and 75.4%. The sorghum grains proved better spawn quality in terms of mycelia growth, colonization time of grains, spawn running period of the substrate and yield of mushrooms. The trend from the data showed that sorghum could be a better carrier than wheat and barley.

Heat treatment of substrate (boiling and steaming) significantly reduced substrate contamination and improved mushroom yield compared to the unheated control (Table.2). Mushrooms grown on steamed substrates had significantly higher yield than those grown on substrates dipped in boiling water or unheated substrates.

**Table.1:** Performance of Po × Pf on wheat, barley and sorghum as mycelium carriers and mushroom yield on maize cobs

| Trial | Treatment | Yield Parameters | | | |
|---|---|---|---|---|---|
| | | Mycelium Growth vigor rating** | Colonization time (days) | Spawn running period (days) | E[§] (%) |
| 1 | Wheat | 2.43 | 17.33 | 15.67 | 53.33 |
| | Barley | 3.43 | 13.33 | 15 | 55.07 |
| | Sorghum | 5.33 | 7 | 12.07 | 74.3 |
| 2 | Wheat | 2.47 | 16 | 16 | 53.77 |
| | Barley | 3.5 | 14.33 | 11.67 | 55.4 |
| | Sorghum | 5.17 | 7.33 | 11.67 | 76.53 |
| CV[α] (%) | | 6.83 | 11.67 | 4.83 | 1.71 |

BE=biological efficiency; CV= coefficient of variation

*Means are not significant (NS) at P=0.05 according to LSD Test.

**1-1.9 (No growth), 2-2.9 (Slow growth), 3-3.9 (Moderately fast growth), 4- 4.9 (Fast growth), 5-5.9 (Vigorous growth) and 6-6.9 (very vigorous growth).

**Table.2:** Comparison of Steaming and boiling as methods of pasteurizing substrates on mushroom yield and yield parameters

| Substrate Treatment | Rate of mycelium contamination* | | Biological Efficiency (%) |
|---|---|---|---|
| | After 2 weeks | After 4 weeks | |
| Steaming | 1b | 2.33b | 69.4a |
| Boiling | 1.67ab | 2b | 53.3a |
| Unheated control | 2.33a | 8a | 0b |
| LSD* | 0.926 | 2.56 | 18.75 |
| CV** (%) | 2.51 | 5.46 | 17.39 |

BE=biological efficiency; * LSD= least Standard deviation (p=0.05); ** CV= coefficient of variation

*1. No visible contaminants. 2. Less than 5% of surface area covered by contaminants 3.5.1-10% of surface covered by contaminants, 4. 10.1-20% surface area covered by contaminants 5.20.1- 30% of surface area covered by contaminants. 6. 30.1 – 40% surface area covered by contaminants. 7. 40.1 -50% of surface area covered by contaminants. 8. More than 50 .1 % of surface area covered by contaminants.

The type of grain used to carry the mycelium had no significant difference in the yield and yield parameters. The yield of mushrooms when sorghum and barley spawns used was 76.53 and 55.4% respectively. The results are in line with those of Khonga (2001, unpublished) who found sorghum grains to be the best carriers of the mycelium. Barley and wheat can be good carriers if the pre-treatment methods used favor them i.e. the soaking period has to be reduced so that they do not take in a lot of moisture since their outside coats are soft (6). However Khare *et al.,* (2010a) recommended wheat, barley, sorghum and millet grains could be equally used in the production of good quality spawn for the cultivation of oyster mushrooms.

Heat treatment of substrates significantly reduced contaminants in the substrate as compared to the control. As shown in table 2, BE of 69.4% and 53.3 was obtained from substrates that were steamed and hot-water treated respectively, but there was no yield from substrates that were un- heated control because of heavy contamination. In mushroom cultivation, substrate contamination is economically important as yield can be reduced. The most common contaminants observed were species of *Aspergillus, Penicillium, Rhizopus and Trichoderma.* Similar contaminants were reported by others (13, 17). *Trichoderma* species is one of the organisms that, when present, have the potential of inhibiting the growth of *Pleurotus* mushrooms; therefore it is important that pasteurization methods are used to eliminate the contaminants.

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