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Full Length Research Paper

The effect of the interaction of various spawn grains with different culture medium on carpophore dry weights and stipe and pileus diameters of *Lentinus squarrosulus* (Mont.) Singer

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***Lentinus squarrosulus*, an indigenous mushroom specie commonly found growing on dead logs in the Zaria environ of Kaduna State was cultured on six different medium which were inoculated separately with three different spawn grains and amended with six different oils at five different rates. The interaction of spawn grains x culture medium had a highly significant effect on carpophore dry weight and stipe and pileus diameters of *L. squarrosulus*. The results reveal that the interaction of millet spawn x animal bedding and rice medium induced the widest stipe diameter while the interaction of corn spawn x animal bedding and rice medium induced the heaviest carpophore dry weight as well as the widest pileus diameter.**

Key words: *Lentinus squarrosulus*, spawn grain, carpophore production, non-composted culture medium, polypropylene heat resistant bags, flushes, stipe and pileus diameter, fruiting bodies.

INTRODUCTION

Mushrooms are consumed by connoisseurs because of their exceptional flavour and nutritional content and may be cultured for commercial purposes (Ogbonda, 2000; Shofuyi, 2002; Nwanze and Adamu, 2004a; 2004b). In addition they have a varied range of applications in bioremediation of soil, bioconversion of wastewater, medicine, and agricultural waste disposal (Vinciguerra et al., 1995; Daba and Ezeronye, 2003; Ullrich et al., 2004; Magingo et al., 2004).

Optimization of industrial mushroom production depends on improving the culture process (Larraya et al., 2003). There are various additives that are known to stimulate fruiting. They include rice bran, cassava peels, carbohydrates such as glycogen, natural extracts like yeast and malt extract, as well as cell-free extracts (Uno and Ishikawa, 1971; Brunt and Moore, 1989; Fasidi and Kadiri, 1993). Highly proteinaceous materials such as ground pigeon pea and soybean have been reported to stimulate high fruit yield. Wheat, rye and millet that are used in making spawn also belong to this genre (Royse and May, 1982). In addition, refined and crude vegetable oils, as well as fish oil may also be used to stimulate fruiting (Schisler and Sinden, 1962; Schisler, 1967; Martin and Patel, 1991).

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Table 1. Different carpophore production media.

Media	Components	Method of preparation
Sawdust (Carey, 1974)	62.5 g sawdust 62.5 g wood chips 125.0 g brown rice	All the components were thoroughly mixed, moistened and sterilized for 15 min at 121°C
Animal bedding and rice (Roxon and Jong, 1977)	125.0 g wood chips 125.0 g brown rice	Same as above
Lime 1 (Cangy, 1994)	195.0 g sawdust 50.0 g rice bran 2.5 g $CaSO_4$ 2.5 g $CaCO_3$	Same as above
Lime 2 (Oei, 1991)	235.0 g sawdust 10.0 g rice bran 2.5 g corn meal 2.5 g $CaCO_3$	Same as above
Lime 3 (Oei, 1991)	182.5 g sawdust 62.5 g corn cobs 5.0 g $CaCO_3$	Same as above
Formulated (Nwanze, 1996)	175.0 g sawdust 70.0 g rice bran 2.5 g $CaCO_3$ 2.5 g oat meal	Same as above

Nwanze et al. (2004a; 2004b; 2005) earlier reported on the effect of factors such as spawn grain, culture media, oil type and rate on the culture of *Psathyrella atroumbonata* and *Lentinus squarrosulus*. The current investigation is interested in the interaction of two of the above factors, spawn grains and culture medium, on the culture of *L. squarrosulus*.

MATERIALS AND METHODS

The effect of various spawn grains, culture media, oil types and rates on carpophore production of *L. squarrosulus.*

Various non-composted media including sawdust (Carey, 1974), animal bedding and rice (Roxon and Jong, 1974), formulated (Nwanze 1996) and lime were used for these studies. To distinguish among three lime media, they were arbitrarily named as lime 1 (Cangy, 1994), lime 2 (Oei, 1991) and lime 3 (Oei, 1991) (Table 1). These six different medium were supplemented with different rates (0.007, 0.014, 0.021 and 0.028 ml/g) of different lipid sources viz. groundnut, coconut, palm kernel, butterfat, palm and cotton oils, respectively, in order to study the effect of lipids on carpophore production. Two hundred and fifty gram of dry substrate from each of the above six different supplemented and non-supplemented medium were placed in separate polypropylene heat resistant bags (Kadiri, 1999).

After thoroughly wetting the substrates, the bags were autoclaved for 15 min at 121°C and allowed to cool (Bhandari et al., 1991). The substrates were then separately inoculated with 10 g (4% on dry weight basis) of three different types of spawn separately (wheat, corn and millet) (Bahukandi and Munjai, 1990). All the bags were incubated in total darkness at 30 ± 2°C for three weeks after which the bags were aerated and exposed to light (Caten and Newton, 2000).

The experiment was conducted in a split-split plot design replicated thrice, with medium as the main plot, oil type and rate as the sub-plot and spawn grain as the sub-subplot treatment (Sheaffer et al., 2001; Jefferson et al., 2001). The fruiting bodies from different flushes (1-3) in the different experiments were collected and the pileus and stipe diameters as well as the stipe lengths measured (Largent, 1986; Bhandari et al., 1991). In addition, fresh and dry weights were also taken (Raggi, 2000; Malone, 2002).

In order to test the main and interactive effects of spawn grain, media, oil type and rate of amendment, pileus and stipe diameter, stipe length and wet and dry weights of fruiting bodies were recorded and the data subjected to factorial analysis of variance (Porter, 2001). When significant differences were determined for the main effects or their interactions ($p \leq 0.05$), comparisons among means were made using Duncan's multiple range test (Snedecor and Cochran, 1987; Sullivan and Sullivan, 2001). Values of 0.01, 0.1 and 1.0 were added to dry weights, stipe and pileus diameters,

Table 2. Spawn preparation**.**

Spawn	Components	Method of preparation
Wheat	1.0 kg wheat grains 12.0 g $CaSO_4 \cdot 2H_2O$ 3.0 g $CaCO_3$ 1.5 L distilled water	1.0 kg of wheat grains was boiled in 1.5 L of water for 15 min and left to cool for an additional 15 min. The water was poured off and 900 g of the cooked grains was mixed with 12 g gypsum and 3 g $CaCO_3$. The grains were then filled into bottles and sterilized for 20 min at 121°C. After cooling, the bottles were inoculated with pieces of agar medium colonized with mycelium and incubated for 2 weeks in total darkness.
Corn	Same as above except for use of corn as grain	Same as above
Millet	Same as above except for the use of millet as grain	Same as above except that the grains were boiled for 5 min

Table 3. Stipe diameter, dry weight (g) and pileus diameter (cm) of *L. squarrosulus* as affected by the interaction of spawn grain and culture medium.

Treatments	sawdust	animal bedding and rice	lime 1	lime 2	lime 3	formulated
Stipe diameter (cm)						
wheat	0.36c	0.32e	0.23j	0.26hi	0.18l	0.27gh
corn	0.28g	0.47b	0.27gh	0.19kl	0.25i	0.20k
millet	0.30f	0.53a	0.34d	0.22j	0.19kl	0.26hi
SE ± 0.006						
Dry weight (g)						
wheat	0.25bcd	0.23cde	0.08ij	0.11g-j	0.05j	0.14f-l
corn	0.32b	0.62a	0.16e-h	0.11g-j	0.07ij	0.09hij
millet	0.19def	0.25bcd	0.28bc	0.10g-j	0.09hij	0.17efg
SE ± 0.024						
Pileus diameter (cm)						
wheat	2.62c	2.94b	1.40h	1.68fg	1.17ij	2.07e
corn	2.40d	4.37a	1.59gh	1.37hi	1.15j	1.39h
millet	2.51cd	2.68c	2.56cd	1.07j	1.21hij	1.86f
SE ± 0.069						

Means followed by the same letter(s) within the same row or column in a treatment group are not significantly different statistically at 5% level of probability using DMRT.

and wet weight and stipe length, respectively, prior to analysis (Cowger et al., 2000).

Spawn preparation

Three different types of grains; corn, wheat and millet, were used to produce spawn in order to determine which spawn produces the best crop yield. The spawns were prepared as described by Fritsche (1978) (Table 2) and kept inside a water bath at 37°C and 70% relative humidity for two weeks in order for the spawn to run (Gordon et al., 2002).

RESULTS

Spawn grain x culture medium interaction

The mean dry weight and stipe and pileus diameter of *L. squarrosulus* as affected by the interaction of spawn

grain and culture medium is presented in Table 3.

Analysis of the data showed that wheat grain interacted with the various growth medium to induce the widest stipe diameter in sawdust, followed by animal bedding and rice, formulated or lime 2, lime 1 and lime 3 media. Corn spawn induced a stipe diameter in animal bedding and rice medium that was statistically wider than the comparable ones induced in sawdust and lime 1 medium, which were significantly wider than the similar diameters it induced in lime 2 and formulated medium. Millet spawn also induced the widest stipe diameter in animal bedding and rice medium.

The interaction of wheat spawn with sawdust and animal bedding and rice media induced similar mean dry weights of *L. squarrosulus* that were statistically heavier than the comparable weights induced in lime 1, 2, 3 and formulated media. In contrast, the interaction of corn spawn with animal bedding and rice medium induced a mean dry weight that was significantly heavier than the weight induced by its interaction with sawdust, which was superior to the similar dry weights induced in lime 1, 2, 3, and formulated media. Millet spawn induced comparable dry weights in lime 1, sawdust and animal bedding and rice medium that were significantly heavier than the similar weights it induced in formulated, lime 2 or lime 3 medium. Wheat spawn grain induced pileus diameters in the various growth medium in the decreasing order of animal bedding and rice, sawdust, formulated, lime 2, 1 and 3, while for corn spawn, the pileus diameters were widest in animal bedding and rice, followed by sawdust, lime1 or 2 or formulated and lime 3 media. The pileus diameters induced by millet spawn in sawdust, lime 1 and animal bedding and rice media were significantly wider than that of formulated, which was superior to the diameters induced in lime 2 or 3 media.

DISCUSSIONS

Spawn grains and various growth mediums have a significant effect on carpophore production (Nwanze et al., 2004a). As previously observed (Nwanze et al., 2004c), the widest stipe diameter of *P. atroumbonata* is induced by the interaction of sawdust medium x wheat spawn but *L. squarrosulus* favours animal bedding and rice medium x millet spawn. We also observed that although *P. atroumbonata* favours the interaction of both wheat and corn spawn with sawdust medium to produce the heaviest carpophore weight, *L. squarrosulus* favours corn spawn solely. However, both species are induced to produce the widest pileus diameter by corn spawn.

The above result is due to the composition of sawdust and animal bedding and rice medium, which contain brown rice. The high protein, carbohydrate, fatty acid and amino acid content of brown rice stimulate fruiting (Roux and Labarère, 1991; Shin and Godber, 1996). In addition, grains have also been known to improve mushroom yield (Royse and May, 1982).

The experimental results show that large fruiting bodies of *L. squarrosulus* can be easily cultured using simple lignocellulosic waste materials, in conjunction with readily available grains. This species is definitely fertile for commercial exploitation.

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