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Full Length Research Paper

The effect of spawn grains, culture media, oil types and rates on carpophore production of *Lentinus squarrosulus* (Mont.) Singer

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***Lentinus squarrosulus*, an indigenous mushroom species commonly found growing on dead logs in the Zaria environ of Kaduna State was cultured on six different media which were inoculated separately with three different spawn grains and amended with six different oils at five different rates. The various media, oil type and rate all had a highly significant effect ($p<0.01$) on the stipe length, stipe and pileus diameters and carpophore wet and dry weights of *L. squarrosulus*. Spawn grains, however, had a significant effect on all the above parameters except pileus diameter. The results reveal that corn and millet spawn induced comparable carpophore wet weights which were superior to that induced by wheat spawn. Corn spawn induced the longest stipe length and heaviest carpophore dry weight while millet induced the widest stipe diameter. The oil rate of 0.014 ml/g induced the longest stipe length, heaviest carpophore wet weight and widest pileus diameter while the highest oil rate (0.028 ml/g) induced the widest stipe diameter and heaviest carpophore dry weight. Coconut oil induced superior results for all the parameters tested except stipe diameter. Animal bedding and rice media induced optimum results for all the parameters.**

Key words: *Lentinus squarrosulus*, spawn grain, carpophore production, non-composted media, polypropylene heat resistant bags, supplemented media, flushes, oil type and rate.

INTRODUCTION

Mushrooms are a ubiquitous group of fungi with many uses. They reduce agricultural solid wastes, are highly efficient in bioremediation and have many medicinal applications (Saparrat et al., 2002; Daba and Ezeronye, 2003; Howard et al., 2003; Milliken et al., 2004; Magingo et al., 2004). In addition, they have high economic value because of their edibility and the enzymes they produce are very interesting for both basic research purposes and industrial applications (Ullrich et al., 2004; Velázquez-cedeno et al., 2004; Zervakis et al., 2004).

Optimization of industrial mushroom production depends on improving the culture process (Larraya et al., 2003). A range of abiotic parameters including temperature, light, carbon dioxide concentration, humidity and pH have been shown to influence carpophore production (Wessels et al., 1987). Fruiting may also be stimulated by mechanical injury and chemical treatments (Hibbitt et al., 1994).

There are also 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 (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,

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Table 1. Different carpophore production media.

Media	Components
Sawdust (Carey, 1974)	62.5 g sawdust, 62.5 g wood chips, 125.0 g brown rice
Animal bedding and rice (Roxon and Jong, 1977)	125.0 g wood chips, 125.0 g brown rice
Lime 1 (Cangy, 1994)	195.0 g sawdust, 50.0 g rice bran, 2.5 g $CaSO_4$, 2.5 g $CaCO_3$
Lime 2 (Oei, 1991)	235.0 g sawdust, 10.0 g rice bran, 2.5 g corn meal, 2.5 g $CaCO_3$
Lime 3 (Oei, 1991)	182.5 g sawdust, 62.5 g corn cobs, 5.0 g $CaCO_3$
Formulated (Nwanze, 1996)	175.0 g sawdust, 70.0 g rice bran, 2.5 g $CaCO_3$, 2.5 g oatmeal

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 litre distilled water	1.0 kg of wheat grains was boiled in 1.5 litre of water for 15 min and left to cool for an additional 15 min. The water was poured off and 900.0 g of the cooked grains was mixed with 12.0 g gypsum and 3.0 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.

lipids such as vegetable oils may also be used to stimulate fruiting (Schisler and Sinden, 1962). Schisler (1967) used both crude and refined vegetable oils while Martin and Patel (1991) used fish oil in order to obtain similar results.

Previous work has shown that various spawn grains, culture media, oil types and rates all have a highly significant effect on the dimensions and weights of *P. atroumbonata* carpophores (Nwanze et al., 2005; 2004). The present work focuses on the effect of the above factors on the culture of *Lentinus squarrosulus*.

MATERIALS AND METHODS

The effect of 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 media were supplemented with different amounts (0.007, 0.014, 0.021 and 0.028 ml/g) of lipids. The lipids investigated are groundnut, coconut, palm kernel, butterfat, palm and cotton oils. Two hundred and fifty gram of dry substrate from each of the above six different supplemented and non-supplemented media were placed in separate polypropylene heat resistant bags (Kadiri, 1999a,b). 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 (Kadiri, 1999a; Caten and Newton, 2000).

The experiment was conducted in a split-split plot design replicated thrice, with media as the main plot, oil type and rate as the sub-plot and grain as the sub-subplot treatment (Norwood, 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 (Qasem, 2001; 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 (Arraiano et al., 2001). When significant differences were determined for the main effects or their interactions (a “p” value of 0.05 or less), comparisons among means were made using Duncan’s multiple range test (Snedecor and Cochran, 1987). The values 0.01, 0.1 and 1.0 were added to dry weights; stipe and pileus diameters; and wet weight and stipe length values respectively, prior to analysis (Cowger et al., 2000).

Spawn preparation

Three different types of grain, including 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 (Belewu and Adeniyi, 2001; Gordon et al., 2002).

Table 3. The effect of grain, oil type and rate and media on carpophore production of *L. squarrosulus.*

Treatments	Stipe length(cm)	Stipe diameter(cm)	Wet weight (g)	Dry weight (g)	Pileus diameter(cm)
Grains					
wheat	3.61c	0.27c	1.17b	0.14c	1.98
corn	4.1a	0.28b	1.37a	0.23a	2.05
millet	3.76b	0.31a	1.35a	0.18b	1.98
SE±	0.03	0.002	0.03	0.01	0.03
Significance	**	**	**	**	NS
Oil Rate (ml/g)					
0.000	2.86d	0.21e	0.72d	0.09d	1.22d
0.007	3.69c	0.27d	1.06c	0.14c	1.95c
0.014	4.67a	0.33b	1.68a	0.22b	2.60a
0.021	3.91b	0.28c	1.52b	0.19b	2.11b
0.028	3.96b	0.34a	1.49b	0.26a	2.11b
SE±	0.04	0.003	0.03	0.01	0.04
Significance	**	**	**	**	**
Oil					
coconut	4.38a	0.31b	1.71a	0.29a	2.55a
cotton	4.00c	0.28d	1.14c	0.17bc	1.99d
groundnut	4.18b	0.36a	1.51b	0.20b	2.23b
butterfat	4.04c	0.29c	1.43b	0.19bc	2.10c
palm kernel	2.95e	0.22f	0.85d	0.10d	1.39f
palm	3.38d	0.25e	1.13c	0.16c	1.75e
SE±	0.05	0.003	0.04	0.01	0.04
Significance	**	**	**	**	**
Media					
sawdust	4.29b	0.32b	1.70b	0.25b	2.51b
animal bedding + rice	5.48a	0.44a	2.24a	0.36a	3.33a
lime 1	3.52c	0.28c	1.14c	0.17c	1.85c
lime 2	3.15d	0.22e	1.06c	0.11de	1.37d
lime 3	2.96e	0.21f	0.53d	0.07e	1.18e
formulated	3.52c	0.24d	1.09c	0.13d	1.77c
SE±	0.05	0.003	0.04	0.01	0.04
Significance	**	**	**	**	**
Interactions					
OxM	**	NS	**	NS	**
RxM	NS	**	**	NS	**
RxO	NS	**	**	**	**
GxM	NS	**	NS	**	**
GxO	**	**	NS	**	**
GxR	**	**	**	NS	**
RxOxM	NS	NS	NS	NS	NS
GxOxM	NS	NS	NS	NS	NS
GxRxM	NS	NS	NS	NS	NS
GxRxO	NS	NS	NS	NS	NS

Means followed by the same letter(s) within a treatment group are not significantly different statistically at 5% level of probability using DMRT.
* and ** = significant at 5% and 1% levels, respectively; NS = not significant.

RESULTS

The effect of spawn grain, media, oil type and rates on carpophore production of *L. squarrosulus*

Mean stipe length, stipe and pileus diameter, and carpophore wet and dry weight as affected by grain, media, oil type and rate is shown in Table 3. The differences in the means due to grain were highly significant ($p<0.01$) for mean stipe length, stipe diameter, and carpophore wet and dry weight, but not significant for pileus diameter. The stipe length and dry weight of *L. squarrosulus* were significantly increased in media containing corn, millet and wheat. Corn and millet induced heavier mean wet weights than wheat.

Oil rates had a highly significant effect ($p<0.01$) on stipe length, carpophore wet and dry weight and pileus and stipe diameter of *L. squarrosulus*. Each increase in oil concentration significantly increased stipe length, carpophore wet and dry weight, and pileus and stipe diameter of *L. squarrosulus* up to 0.014 ml/g. A further increase in oil concentration decreased stipe length, dry weight and pileus diameter. However, the concentration of 0.028 ml/g was superior in stipe diameter and dry weight of *L. squarrosulus* compared to the other concentrations.

Oil type had a highly significant effect ($p<0.01$) on mean stipe length, carpophore wet and dry weight and stipe and pileus diameter of *L. squarrosulus*. Oil type significantly increased stipe length in the order of coconut>groundnut>cotton or butterfat>palm>palm kernel oil while the order for stipe diameter was groundnut>coconut>butterfat>cotton or palm>palm kernel oil. Coconut oil significantly increased mean carpophore wet weight of *L. squarrosulus* over groundnut and butterfat, which were at par, but superior to cotton and palm oil that were similar. The mean dry weight that was induced by coconut oil was significantly heavier than that of groundnut, which was comparable to cotton and butterfat. However, cotton, butterfat and palm oil though statistically similar, significantly increased mean dry weight of *L. squarrosulus* over that of palm kernel.

Animal bedding and rice media significantly increased stipe length, stipe and pileus diameter, and carpophore wet and dry weight of *L. squarrosulus* over sawdust and lime 1 media, respectively. The stipe length produced by lime 1 media was comparable to that of formulated and significantly longer than those induced by lime 2 and 3, while the mean carpophore wet weight induced by lime 1 was at par with those of lime 2 and formulated, but significantly heavier than that of lime 3 media. The stipe diameter induced by lime 1 media was superior to that of formulated, lime 2, and lime 3, while the pileus diameter it produced was comparable to that of formulated, but significantly wider than those of lime 2 and 3. However, the carpophore dry weight induced by lime 1 was significantly heavier than the comparable weights induced by formulated, lime 2 and 3. In addition, the majority of the first and second order interactions were significant across the various parameters.

DISCUSSION

Spawn grains were used to introduce pure fungal cultures into different growth substrates as well as to increase mushroom yield. Kadiri (1999a) showed a preference for millet while producing spawn for *L. squarrosulus*. The present study, however, examined the effect of corn, millet and wheat spawn on some growth parameters of *L. squarrosulus*.

Nwanze et al. (2004) observed that in the case of *P. atroumbonata*, wheat and corn spawn were similar and induced the longest stipe lengths and heaviest carpophore wet weights. However, with *L. squarrosulus*, only corn spawn had that effect. In addition, wheat spawn produced the widest pileus diameters with *P. atroumbonata*, but with *L. squarrosulus*, grains had no significant effect on pileus diameter. It can be inferred from the results that Fasidi and Kadiri (1993) and Kadiri (1999a) could have gotten superior fruit body production and mycelia density of *L. squarrosulus* if they had used corn or millet as opposed to rice or sorghum.

Nwanze et al. (2004) observed that the oil concentration of 0.007 ml/g induced the best results with *P. atroumbonata* for all the parameters examined. *L. squarrosulus*, however, required high concentrations (0.028 ml/g) to induce the widest stipe diameters and heaviest carpophore dry weights. In addition, intermediate oil levels (0.014 ml/g) induced the longest stipe lengths, heaviest carpophore wet weights and widest pileus diameters. The controls produced the poorest results, thus implying that amendment with various lipid concentrations had significant effects on the parameters examined.

Coconut oil induced the best weights and dimensions of both *L. squarrosulus* and *P. atroumbonata* (Nwanze et al., 2004). However, groundnut oil induced the widest stipe diameters with *L. squarrosulus*. It is thus evident that the sterol from the various oils was responsible for the stimulated fruiting response (Haskins et al., 1963; Schisler, 1967; Nwanze et al., 2005). However, in nature oils are obtained from animal and plant tissues, microbial cells and animal secretions (Cooke and Whipps, 1993).

Media composition has a significant effect on mushroom production whether it is at the mycelia or carpophore level (Klemmer and Lenny, 1965; Schisler and Sinden, 1962). Animal bedding and rice was the best media for *L. squarrosulus*, producing superior weights and dimensions. This was in contrast to *P. atroumbonata* that favours sawdust media (Nwanze et al., 2004). The one component that the two media have in common is brown rice. This observation supports the finding that rice protein, which is rich in phenylalanine, leucine,

isoleucine, and valine is important in increasing mushroom yield (Schisler and Sinden, 1962). Lime 1 and 2 media also produced good results. This can be attributed to the rice bran contained in the two media. Rice bran contains oil, phospholipids, proteins and vitamins, all of which induce carpophore production (Barber and Barber 1980; Fasidi and Kadirir, 1993; Shin and Godber, 1996). Nonetheless, Bhandari et al. (1991) obtained heavier wet weights and wider pileus diameters of *Pleurotus sajor-caju* using grasses and wheat straw as substrates.

The results clearly show that spawn grains, media, oil type and rate all have a positive effect on carpophore production of *L. squarrosulus*. The possibilities of economic exploitation should be examined since this particular specie can easily be cultured.

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