THE EFFECTS OF THE INTERACTION OF VARIOUS OIL TYPES AND RATES ON CARPOPHORE WET AND DRY WEIGHTS AND STIPE AND PILEUS DIAMETERS OF *LENTINUS SQUARROSULUS* (MONT.) SINGER

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Abstract

Lentinus squarrosulus, an indigenous mushroom specie 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 results revealed that the interaction of oil type x rate produced a highly significant effect on carpophore wet and dry weights and stipe and pileus diameters of *L. squarrosulus*. The interaction of coconut oil x 0.014 ml/g induced the heaviest carpophore wet weight and widest pileus diameters respectively, while the interaction of groundnut oil x 0.028 ml/g and coconut oil x 0.028 ml/g induced the widest stipe diameter and heaviest carpophore dry weight, respectively.

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

Introduction

There is great commercial interest in edible mushrooms world wide with *Agaricus bisporus* and *Lentinus edodes* being the two most popular species (Simsek *et al.*, 2008; Komon-Zelazowska *et al.*, 2007; Nwanze *et al.*, 2005a). In addition, New and faster methods are constantly being developed to identify new species (Yang *et al.*, 2007). Mushrooms produce novel enzymes with industrial applications, serve as agents of bioremediation, have numerous medicinal uses, can be used to reduce agricultural solid waste products and may be used as a biological control against certain nematodes (Okamura-Matsui *et al.*, 2003; Moeder *et al.*, 2005; Pointing *et al.*, 2005; Tsai *et al.*, 2006; Luo *et al.*, 2007; Anh *et al.*, 2007; Lee *et al.*, 2008.

Fruiting initiation and stimulation in mushrooms can be triggered by a variety of environmental and biochemical stimuli, including substances of natural or synthetic origin (Berne *et al.*, 2007; Magae *et al.*, 2005). Additives that stimulate fruiting include rice bran, cassava peels, carbohydrates such as glycogen, natural extracts like yeast and malt extract, as well as cell-free extracts, glucose derivatives and substances such as osteolysin (Magae *et al.*, 2005). In addition, grains used in making spawn and various lipid sources may also be used to stimulate fruiting (Schisler and Sinden, 1962; Royse and May 1982).

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

Materials and Methods

The Effect of Various Grains, Culture Media, Oil Types and Rates on Carpophore Production of *Lentinus 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 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 media were placed in separate polypropylene heat

resistant bags (Kadiri, 1999a; 1999b). After thoroughly wetting the substrates, the bags were autoclaved for 15 minutes 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).

Table 1: Different Carpophore Production Medium

Medium	Components	Method of preparation
Sawdust	62.5g sawdust 62.5g wood chips 125.0g brown rice	All the components were thoroughly mixed, moistened and sterilized for 15 minutes at 121°C
Animal bedding and rice	125.0g wood chips 125.0g brown rice	Same as above
Lime 1	195.0g sawdust 50.0g rice bran 2.5g $CaSO_4$ 2.5g $CaCO_3$	Same as above
Lime 2	235.0g sawdust 10.0g rice bran 2.5g corn meal 2.5g $CaCO_3$	Same as above
Lime 3	182.5g sawdust 62.5g corn cobs 5.0g $CaCO_3$	Same as above
Formulated	175.0g sawdust 70.0g rice bran 2.5g $CaCO_3$ 2.5g oatmeal	Same as above

Experimental Design

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 grain as the sub-subplot treatment (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 (Malone 2002).

Statistics

In order to test the main and interactive effects of spawn grain, medium, 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 (Kluth *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). The present results were analyzed by analysis of variance as a 5X6 factorial, with 3 replicates, using Genstat.

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 most excellent 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) (Insert Table 2 here).

Table 2: Spawn Preparation

Spawn	Components	Method of preparation
Wheat	1.0kg wheat grains 12.0g $CaSO_4 \cdot 2H_2O$ 3.0g $CaCO_3$ 1.5 litre distilled water	1.0kg of wheat grains was boiled in 1.5 litre of water for 15 minutes and left to cool for an additional 15 minutes. The water was poured off and 900.0g of the cooked grains was mixed with 12.0g gypsum and 3.0g $CaCO_3$. The grains were then filled into bottles and sterilized for 20 minutes 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 minutes

Results

Oil type x rate interaction

Mean stipe and pileus diameters and wet and dry weights of *L. squarrosulus* as affected by the interaction of oil rate and type is presented in Table 3. The control for cotton oil produced a statistically wider stipe diameter than the control for groundnut oil, which was superior to the comparable stipe diameters induced by coconut and palm oils that were wider than those of palm kernel and butterfat, respectively. Butterfat introduced at a level of 0.007 ml/g produced a significantly wider stipe diameter than coconut, which was superior to the comparable stipe diameters induced by cotton, groundnut, palm kernel or palm oil. At the oil rate of 0.014 ml/g coconut and cotton oils, though statistically similar, produced significantly wider stipe diameters than butterfat, which produced a wider diameter than groundnut oil. However, the mean stipe diameter induced by groundnut oil was statistically wider than the stipe diameter produced by palm kernel and palm oil, which were at par. Coconut, groundnut, butterfat and palm oil introduced at a level of 0.021 ml/g induced similar mean stipe diameters that were statistically wider than the diameters induced by cotton and palm kernel oils, respectively. However, the pileus diameters induced by groundnut and butterfat were at par. When the oil rate was raised to 0.028 ml/g, groundnut oil produced a mean stipe diameter that was wider than the comparable stipe diameters induced by coconut and butterfat, which were superior to the similar diameters induced by cotton, palm or palm kernel oil.

Table 3. Stipe diameter (cm), wet weight (g) dry weight (g) and pileus diameter (cm) of *Lentinus squarrosulus* as affected by the interaction of oil type and rate.

Treatments	Oil type					
Stipe diameter	Coconut	Cotton	Groundnut	Butterfat	Palm kernel	Palm
Oil rate (ml/g)						
0.000	0.21k	0.30fgh	0.26i	0.12m	0.14l	0.20k
0.007	0.29gh	0.23j	0.26i	0.34cd	0.25ij	0.24ij
0.014	0.38b	0.37b	0.32def	0.35c	0.29gh	0.28h
0.021	0.34cd	0.24ij	0.31efg	0.32def	0.19k	0.29gh
0.028	0.34cd	0.25ij	0.64a	0.33cde	0.24ij	0.25ij
SE± 0.007						
Wet weight						
Oil rate (ml/g)						
0.000	0.65o	1.36ghi	1.09j-m	0.23p	0.16p	0.84mno
0.007	1.33g-j	0.85mno	1.19i-l	1.01k-n	0.95lmn	1.06klm
0.014	2.54a	1.56fg	1.37ghi	1.69ef	1.66ef	1.25h-k
0.021	1.87de	0.68o	2.35ab	2.00cd	0.79no	1.45fgh
0.028	2.17bc	1.23h-k	1.57fg	2.24b	0.66o	1.06klm
SE± 0.081						
Dry weight						
Oil rate (ml/g)						
0.000	0.08b	0.24b	0.12b	0.02b	0.02b	0.10b
0.007	0.20b	0.10b	0.16b	0.14b	0.11b	0.15b
0.014	0.35ab	0.23b	0.21b	0.19b	0.19b	0.17b
0.021	0.20b	0.10b	0.26b	0.30b	0.08b	0.20b
0.028	0.61a	0.18b	0.26b	0.27b	0.01b	0.16b
SE± 0.099						
Pileus diameter						
Oil rate (ml/g)						
0.000	1.27jk	2.54bcd	1.61hi	0.32l	0.51l	1.10k
0.007	2.43cde	1.44ij	1.71h	2.56bcd	1.72h	1.83gh
0.014	3.65a	2.82b	2.63bcd	2.42cde	2.26ef	1.85gh
0.021	2.69bcd	1.40ij	2.81b	2.66bcd	1.03k	2.10fg
0.028	2.71bc	1.72h	2.40de	2.53b-e	1.44ij	1.88gh
SE± 0.090						

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.

The control for cotton oil produced a mean wet weight of *L. squarrosulus* that was significantly heavier than that of the control for groundnut, palm, and coconut oils, which were similar, but superior to the comparable weights induced by butterfat and palm kernel oil. Coconut oil introduced at a rate of 0.007 and 0.014 ml/g respectively, produced a mean wet weight that was statistically heavier than the similar weights induced by the remaining oils. The oil rate of 0.021 ml/g induced mean wet weights in the order of groundnut> butterfat or coconut> palm> palm kernel or cotton oil, respectively. When the oil rate was raised to 0.028 ml/g, butterfat and coconut oil induced carpophore mean wet weights that were statistically heavier than that of groundnut oil, which was superior to cotton and palm oils, respectively.

Oil levels up to the highest rate (0.028 ml/g ml) had no significant effect on the dry weight of *L. squarrosulus* produced by all the various lipid sources except coconut oil. With coconut oil the dry weights induced by 0.014 and 0.028 ml/g were at par, but significantly heavier than all other oils.

The control for cotton gave rise to a pileus diameter that was statistically wider than the control for groundnut, which was superior to the comparable pileus diameters obtained for the controls of coconut and palm oil. Butterfat and coconut oil introduced at a rate of 0.007 ml/g produced similar pileus diameters, which were statistically wider than the comparable diameters of palm, palm kernel and groundnut oils. At a rate of 0.014 ml/g coconut oil induced a pileus diameter, which was statistically wider than the similar diameters produced by cotton, groundnut, butterfat and palm kernel that were statistically wider than that of palm oil. In contrast, at a rate of 0.021 ml/g coconut, groundnut and butterfat induced pileus diameters that were at par, but significantly wider than the pileus diameter induced by palm oil, which was superior to cotton. The comparable diameters induced by coconut, groundnut and butterfat at a rate of 0.028 ml/g were statistically wider than the diameters induced by palm and cotton, which were at par, but nonetheless wider than that of palm kernel oil.

Discussions

Oil type x rate interaction

Previous work by Nwanze *et al.* (2005a; 2005b), has shown that factors such as spawn grain, culture media and various lipid sources and rates have a significant effect on carpohore production of both *Lentinus squarrosulus* and *Psathyrella atroumbonata.* Nwanze *et al.* (2005c), also emphasized the importance of examining the interactions of parameters rather than optimizing the individual parameters. With regard to this it was observed that the interaction of oil type x rate produced similar and positive results in the wet weight and pileus diameter of both *L. squarrosulus* and *P. atroumbonata* but also produced significant increases in the stipe diameter and dry weight of *L. squarrosulus* (Nwanze *et al.*, 2004a; 2005a).

It was observed that in general *P. atroumbonata* produced better results with low to moderate lipid levels (0.007-0.014 ml/g) (Nwanze *et al.*, 2004a) while *L. squarrosulus* required moderate to high lipid levels (0.014-0.028 ml/g). The results show that contrary to the views of Schisler (1967), oil affects the actual size of the carpophores. This is supported by the increased stipe and pileus diameters of *L. Squarrosulus* with elevated oil levels.

Conclusion

The results affirm the fact that various oils utilized at different rates produce a significant effect on carpophore dimensions and weights. Being that this particular specie can easily be cultured here in Nigeria, it should be exploited commercially.

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