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Full Length Research paper

The effects of the interaction of various oil types and rates on the mycelial wet and dry weights of *Lentinus squarrosulus* (Mont.) Singer and *Psathyrella atroumbonata* Pegler in submerged liquid cultures

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***Lentinus squarrosulus* and *Psathyrella atroumbonata*, two edible indigenous mushroom species, were cultured in various different media supplemented with coconut, cotton, groundnut, butterfat, palm kernel and palm oil respectively, at 5 different rates. The interaction of the various oil types with different rates produced highly significant differences ($p<0.01$) in the mean mycelial wet and dry weights. The heaviest mean wet and dry mycelial weight of *L. squarrosulus* was induced by butterfat x 0.007 ml/ml while for *P. atroumbonata* it was cotton and coconut x 0.003 ml/ml, respectively.**

Key words: *Lentinus squarrosulus*, *Psathyrella atroumbonata*, supplemented medium, submerged liquid medium, oil type and rate.

INTRODUCTION

Mushrooms attracted the attention of mycologists because of their big size but now occupy a unique position because of their innumerable economic advantages. In rubber tree plantations for example, *Lentinus squarrosulus*, an edible indigenous mushroom species, is grown at the base of rubber trees such as *Hevea brasiliensis* as a biological control against *Rigidoporus lignosus*, a fungal pathogen of rubber trees (Sudirman et al., 1992, 1994). In addition to their medicinal uses mushrooms are also eaten in many parts of the world because of their nutritional value (Che et al., 2001; Shofuyi, 2002; Nwanze et al., 2004).

Some investigators have examined the possibility of growing mushroom mycelia in a wide range of submerged liquid cultures as a potential source of fungal biomass and aromatic compounds, both of which may be usedá in the food industries (Berger et al., 1987; Sastry et al., 1980a,b). The dried mycelia are used to make soup ingredients while the aromatic compounds are used to produce different flavours in the food industry (Gallois et al., 1990). In addition, cellulolytic enzymes have also been obtained from submerged liquid cultures (Cai, et al., 1999; Shide et al., 2004; Velázquez-cedenoá et al., 2004).

Nwanze et al. (2005) have shown that various media, oil type and rate all have a highly significant effect on the mean mycelial dry weights of *P. atroumbonata* and *L. squarrosulus*. The present work seeks to examine the effect of the interaction of two of the above parameters, i.e., oil type and rate, on the mycelial wet and dry weights of *L. squarrosulus* and *P. atroumbonata*.

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Table 1. Different submerged liquid culture media.

Media	Components	Method of preparation
Submerged liquid culture media 1 (SLCM1) (Schisler and Volkoff, 1977)	10.0 g dextrose 2.5 g malt extract 1.5 g yeast extract 2.5 g soytone 0.50 g NH_4Cl 0.50 g $MgSO_4 \cdot 7H_2O$ 0.50 g KH_2PO_4 50.0 mg $CaCl_2$	All the above components were suspended in 1 litre of distilled water and autoclaved at 121°C for 15 min.
Submerged liquid culture media 2 (SLCM2) (Nwanze, 1996)	10.0 g dextrose 2.0 g peptone 2.0 g malt extract 2.0 g yeast extract 1.0 g K_2HPO_4 0.5 g KH_2PO_4 0.5 g $MgSO_4 \cdot 7H_2O$ 0.5 g NH_4Cl 2.0 mg thiamine hydrochloride	Same as above
Submerged liquid culture media 3 (SLCM3) (Verhagen et al., 1996)	20.0 g glucose 5.0 g peptone 2.0 g yeast extract 1.0 g KH_2PO_4 0.5 g $MgSO_4 \cdot 7H_2O$ 0.06 g NaCl	Same as above
Submerged liquid culture media 4 (SLCM4) (Kuek, 1996)	10.0 g glucose 10.0 g peptone 10.0 g yeast extract 2.0 g NH_4PO_4 3.0 g KH_2PO_4 2.38 g K_2HPO_4 5.56 g $MgSO_4 \cdot 7H_2O$ 1.0 g $CaSO_4 \cdot 5H_2O$ 6.4 mg $FeSO_4 \cdot 7H_2O$ 1.1 mg $MnCl_2 \cdot 4H_2O$ 1.9 mg $ZnSO_4 \cdot 7H_2O$	Same as above

MATERIALS AND METHODS

Collection, procurement and storage of spore prints

L. squarrosulus and *P. atroumbonata* were collected from Zaria and its environs, wrapped in newspapers and brought to the laboratory for further studies. The spores were obtained as described by Ainsworth (1995), placed in envelopes and stored in a laboratory refrigerator at 4°C as recommended by Agro and Shattock (1999).

Production of mycelia cultures

The pure cultures were obtained as described by Watling (1981). A nichrome wire was flamed over a Bunsen burner and allowed to cool. The wire was then moistened with sterile water and used to streak spores from a spore print on to malt extract agar contained in slant bottles. The bottles were loosely capped and incubated in a water bath at high humidity and 37°C for 72 hours (Agosin, 1985; Gordon et al., 2002). The resultant pure cultures were then subcultured on malt extract agar and kept in a refrigerator at 4°C until required.

The effect of various oil types and rates on mycelia production

For this purpose four submerged liquid culture media were prepared and arbitrarily named as SLCM1, SLCM2, SLCM3 and SLCM4 for the sake of convenience (Table 1). These four submerged liquid media were supplemented with four different rates each (0.001, 0.003, 0.005 and 0.007 ml/ml) of different lipid sources viz. groundnut, coconut, palm kernel, butterfat, palm and

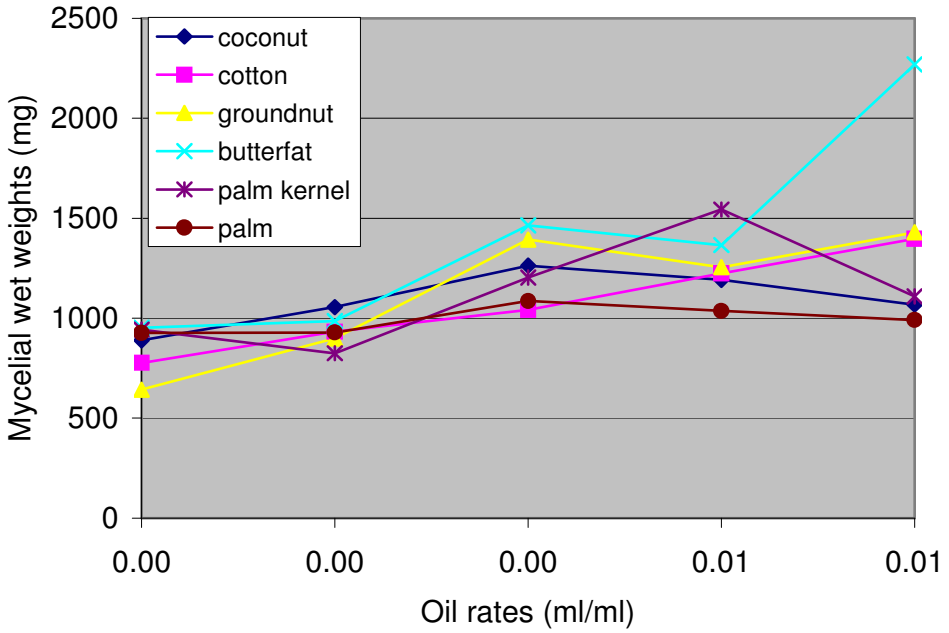

Figure 1. Mycelial wet weigths (mg) of *L. squarrosulus* as affected by the interaction of oil type and rate in submerged liquid culture.

cotton oils prior to autoclaving at 121ºC. 100 ml of each of the supplemented media was transferred into different 250 ml flasks replicated thrice. These were later sterilized at 121°C as recommended by Hidalgo et al. (1999). Two pieces of 1 cm^2 of mycelium with agar were cut from two-week-old cultures with the help of a sterilized cork borer and introduced into axenic cultures that were incubated statically at 37°C for three weeks under continuous darkness (Karaoglanidis et al., 2000; Minussi et al., 2001). After three weeks of incubation, all the flasks were autoclaved at 121°C for 10 min (Schisler and Volkoff, 1977). The mycelia were filtered through Whatman No. 1 filter paper in a Büchner funnel and washed thrice with ethyl ether to remove excess lipids (Wardle and Schisler, 1969). The mycelial wet weight was obtained by subtracting the weight of a control wet filter paper from the weight of the experimental filter paper plus the mycelium. The filter paper plus mycelia were then dried at 70°C for 24 h and transferred to a desiccator. The mycelia and dry filter paper were re-weighed on a Mettler balance (Lalaoui et al., 2000).

Statistics

The experimental design was a split plot arrangement with media as the whole plot and oil type and rate as the subplot (Coviella et al., 2002). In order to test the main and interactive effects of media, oil type and rate of lipid amendment on both wet and dry mycelial weights, the data were subjected to factorial analysis of variance (Kluth et al., 2001). A p value of 0.05 or less was considered significant and treatment means were separated using Duncan's multiple range test (Snedecor and Cochran, 1987).

RESULTS

Mycelial wet and dry weights of *L. squarrosulus* as affected by the interaction of oil type and rate in different submerged liquid cultures is depicted in Figures 1 and 2, respectively. The controls for butterfat, palm kernel and palm oil produced mycelial wet weights of *L. squarrosulus*, which were at par, but statistically heavier than the comparable wet weights produced by the controls for cotton, coconut and groundnut oils. At an oil rate of 0.001 ml/ml, coconut oil induced a mycelial wet weight that was statistically superior to the comparable wet weights induced by the remaining oils. At an oil rate of 0.003 ml/ml, the comparable wet weights of *L. squarrosulus* induced by coconut, groundnut, butterfat, palm kernel and palm oil were significantly heavier than the weight induced by cotton. Similarly, coconut, cotton, groundnut, butterfat and palm kernel oil introduced at a rate of 0.005 ml/ml produced mycelial wet weights, which were at par, but significantly heavier than the wet weight induced by palm oil. At the highest oil rate butterfat induced a wet weight that was statistically superior to the comparable weights induced by cotton and groundnut, which were heavier than the similar weights induced by coconut and palm oils.

The controls for butterfat and palm oil produced mean mycelial dry weights of *L. squarrosulus* which were statistically similar, but significantly heavier than the dry weights produced by the remaining controls, which were also at par. Coconut oil introduced at a level of 0.001 ml/ml produced a mean mycelial dry weight, which was significantly heavier than the comparable dry weights induced by cotton, butterfat and palm that were superior to the similar dry weights induced by groundnut and palm kernel oil. Coconut, butterfat and groundnut oils introduced at a level of 0.003 ml/ml gave rise to mycelial

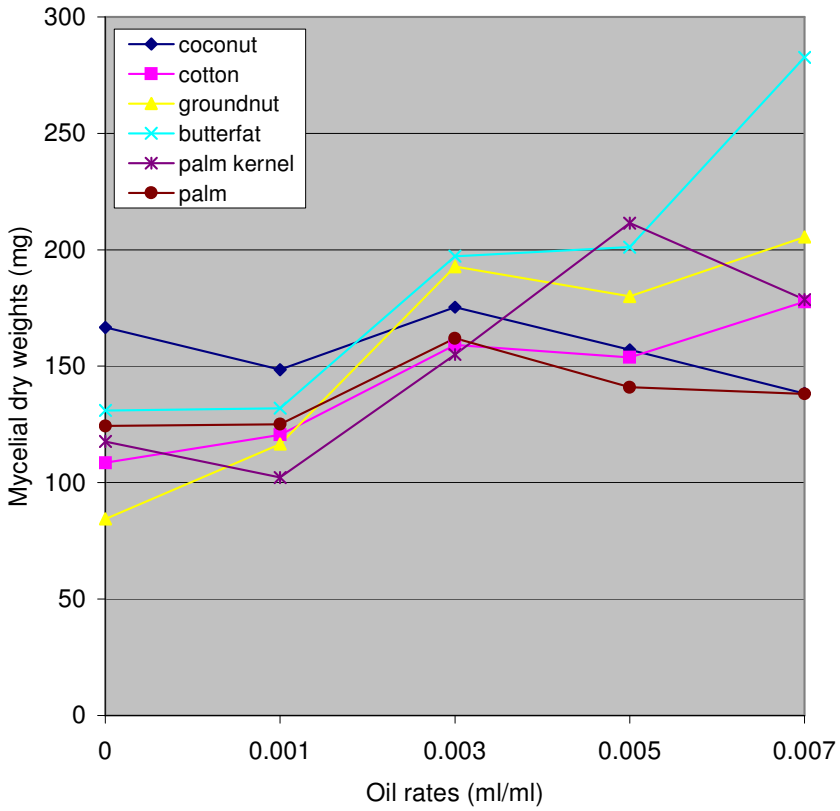

Figure 2. Mycelial dry weights (mg) of *L. squarrosulus* as affected by the interaction of oil type and rate.

dry weights which were at par, but significantly heavier than the weights induced by palm oil, which was superior to the comparable weights induced by cotton and palm kernel oils. The introduction of palm kernel, butterfat and groundnut oil at a rate of 0.005 ml/ml gave rise to comparable mean mycelial dry weights, which were significantly heavier than the dry weights produced by coconut and cotton oils that were at par. Butterfat introduced at a rate of 0.007 ml/ml produced a mean mycelial dry weight that was significantly heavier than the comparable dry weights produced by cotton, groundnut and palm kernel oils, which were statistically superior to the similar weights induced by coconut and palm oils.

Mycelial wet and dry weights of *P. atroumbonata* as affected by the interaction of oil type and rate in different submerged liquid cultures are presented in Figures 3 and 4, respectively. The results show that the control for butterfat produced a mycelial wet weight which was statistically heavier than the wet weights produced by the remaining controls which were at par. Coconut, butterfat and palm oil introduced at a rate of 0.001 ml/ml produced mycelial wet weights of *P. atroumbonata* which were comparable, but significantly heavier than the wet weights produced by palm kernel oil, which was superior to the similar weights produced by cotton and groundnut oils. Cotton oil introduced at a rate of 0.003 ml/ml produced a mycelial wet weight, which was at par with coconut, but significantly heavier than the weight produced by palm oil. In addition, butterfat and palm kernel oils produced mycelial dry weights that were at par, but significantly heavier than the weights produced by groundnut oil. Coconut, groundnut, butterfat and palm kernel oils introduced at a level of 0.005 ml/ml produced comparable mycelial weights which were statistically heavier than the weight produced by palm oil which was superior to cotton. The introduction of groundnut, butterfat and palm oil at a rate of 0.007 ml/ml resulted in the production of mycelial wet weights which were similar, but statistically heavier than the comparable weights induced by coconut, cotton and palm kernel oils.

The control for butterfat produced a mean mycelial dry weight of *P. atroumbonata* that was significantly heavier than the comparable weights induced by the remaining oil types. Coconut, butterfat and palm oil introduced at a rate of 0.001 ml/ml induced similar dry weights, which were statistically heavier than the comparable weights induced by cotton, groundnut and palm kernel oils. The introduction of coconut, cotton and palm oil at a rate of 0.003 ml/ml produced similar dry weights that were statistically heavier than the comparable dry weights induced by butterfat and palm kernel oil. Coconut, groundnut, butterfat and palm kernel oils introduced at a

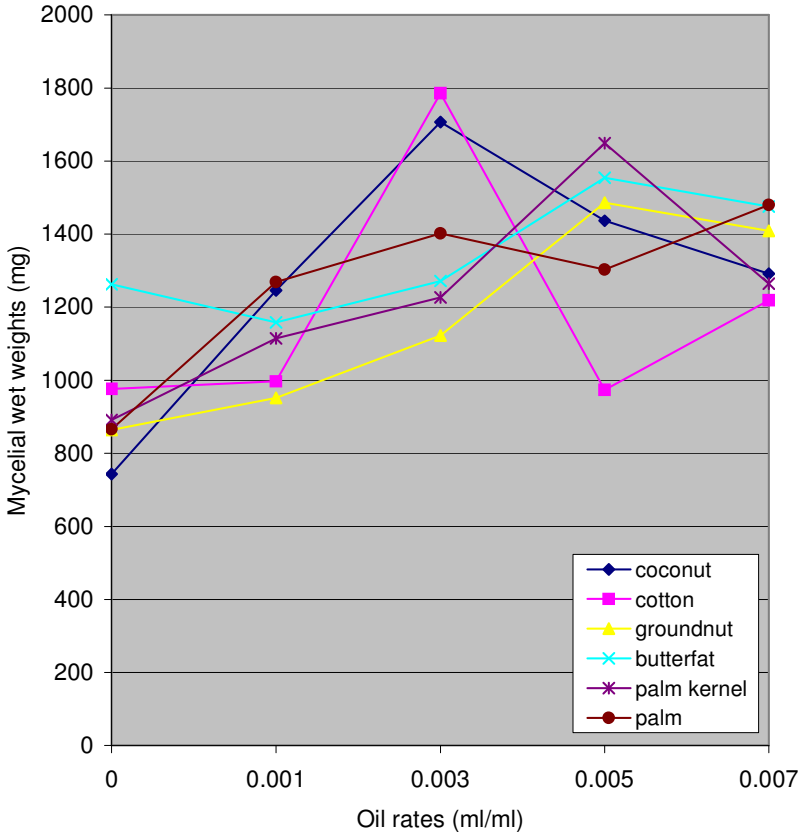

Figure 3. Mycelial wet weights (mg) of *P. atroumbonata* as affected by the interaction of oil type and rate in submerged liquid culture.

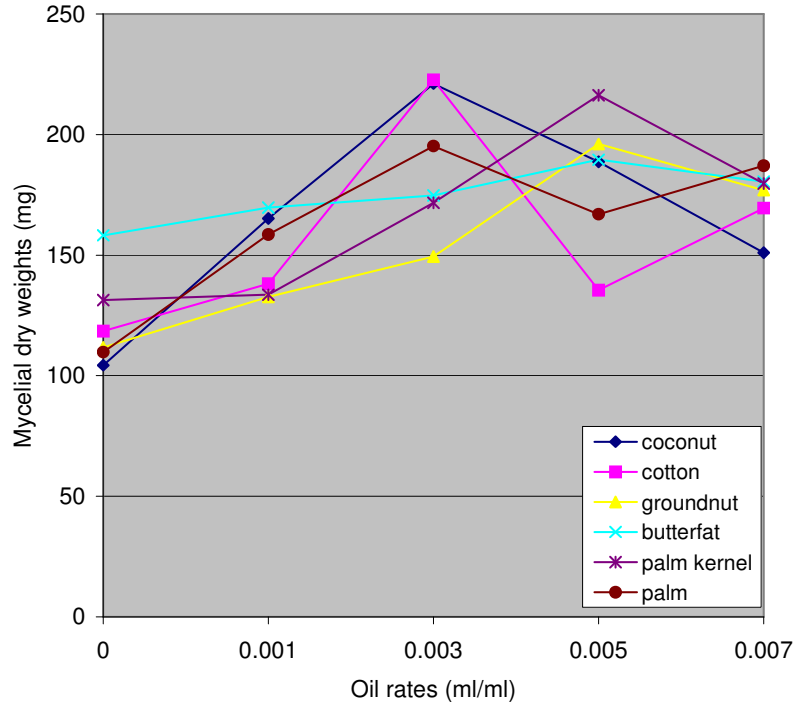

Figure 4. Mycelial dry weights (mg) of *P. atroumbonata* as affected by the interaction of oil type and rate.

rate of 0.005 ml/ml induced dry weights which were statistically at par, but significantly heavier than the weight induced by palm oil. The introduction of butterfat, palm kernel and palm oil at a rate of 0.007 ml/ml produced statistically similar mean mycelial dry weights, which were significantly heavier than the weight induced by groundnut that was superior to the comparable weights induced by coconut and cotton oils.

DISCUSSION

The heaviest mycelial wet weight for *L. squarrosulus* was produced by butterfat x 0.007 ml/ml. Other good mycelial wet weights were produced by butterfat x 0.003 ml/ml and palm kernel x 0.005 ml/ml.

The heaviest mycelial dry weight for *L. squarrosulus* was also produced by butterfat x 0.007 ml/ml. This value was higher than the dry mycelial weight of *Sullius lakei*, *S. punctipes*, *Fuscobletinus ochraceoroseus* and *Leccinum aurantiacium* obtained with 0.003 ml/ml of safflower oil by Schisler and Volkoff (1977). According to Wardle and Schisler (1969), however, 0.01 ml/ml of cottonseed and corn oils produced the heaviest mycelial dry weights of *A. bisporus* over groundnut, safflower, linseed, wheat germ, olive and soybean oils respectively. The results of the experiments portray the importance of oil rate in mycelial culture. The mycelial dry weights obtained in the present experiments using an oil of rate of 0.007 ml/ml was heavier than the results obtained by Schisler and Volkoff (1977) using 0.003 ml/ml, but less than that obtained by Wardle and Schisler (1969) who used 0.01 ml/ml.

Although heavy mycelial dry weights of *L. squarrosulus* were produced by groundnut x 0.005 ml/ml, butterfat x 0.005 ml/ml and palm kernel x 0.005 ml/ml, heavier weights might have been obtained if the rate was increased to 0.01 ml/ml as suggested by Wardle and Schisler (1969). However, comparable mycelial dry weights were also obtained with groundnut x 0.007 ml/ml and butterfat x 0.003 ml/ml.

Esters of oleic and linoleic acid at a rate of 0.0001 ml/ml produced mean mycelial dry weights of *A. bisporus* which were comparable to the value produced by butterfat x 0.007 ml/ml. According to Wardle and Schisler (1969) and Alais and Linden (1999) the increased growth from the lipid addition is due to oleic and linoleic acid components in the lipids.

The heaviest mycelial wet weights of *P. atroumbonata* were produced by cotton x 0.003 ml/ml and coconut x 0.003 ml/ml. However, comparable weights were also produced by coconut x 0.005 ml/ml, groundnut x 0.005 ml/ml, butterfat x 0.005 ml/ml and palm kernel x 0.005 ml/ml. These values support 0.005 ml/ml as the optimum level of amendment for the various lipid sources as reported by Wardle and Schisler (1969).

The mycelial dry weights of *P. atroumbonata* followed the same basic patterns as the wet weights. The heavier mycelial dry weights were produced by coconut x 0.003 ml/ml and cotton x 0.003 ml/ml. However, as in the case of the mycelial wet weight, comparable mycelial dry weights were also produced by coconut x 0.005 ml/ml, groundnut x 0.005 ml/ml, butterfat x 0.005 ml/ml and palm kernel x 0.005 ml/ml. The present investigations reveal that butterfat x 0.007 ml/ml, palm kernel x 0.007 ml/ml and palm x 0.007 ml/ml produced mycelial dry weights that were comparable to those produced by 0.005 ml/ml, but less than the values which may have been obtained with higher oil rates (Wardle and Schisler, 1969).

The results reveal that butterfat used at the highest rate (0.007 ml/ml) produced optimum wet and dry weights of *L. squarrosulus* while the heaviest corresponding wet and dry weights of *P. atroumbonata* were obtained with 0.003 and 0.005 ml/ml each of groundnut and coconut oils, respectively. The results thus support the importance of esters of oleic and linoleic acids in the promotion of mycelial growth.

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