THE EFFECT OF THE INTERACTION OF DIFFERENT MEDIA WITH VARIOUS OIL RATES ON BIOMASS PRODUCTION OF *LENTINUS SQUARROSULUS* (MONT.) SINGER AND *PSATHYRELLA ATROUMBONATA* PEGLER IN SUBMERGED LIQUID CULTURE

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Abstract

Lentinus squarrosulus and *Psathyrella atroumbonata*, two edible indigenous mushroom species, were cultured in four different liquid culture media supplemented with coconut, cotton, groundnut, butterfat, palm kernel and palm oil respectively, at rates of 0.000, 0.001, 0.003, 0.005 and 0.007 ml/ml respectively. The interaction of the different media with various oil rates produced highly significant differences ($p \leq 0.01$) in the mean mycelial dry weights of both mushrooms. The heaviest mean mycelial dry weight for both mushroom species was produced by the interaction of SLCM3 with various oil rates. The heaviest mycelial dry weight for *L. squarrosulus* was produced by SLCM3 x 0.007ml/ml while the corresponding value for *P. atroumbonata* was induced by SLCM3 x 0.001 ml/ml and SLCM3 x 0.005 ml/ml.

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

Introduction

Gourmet chefs, health conscious individuals and food connoisseurs appreciate the fine culinary taste produced by mushrooms (Vetter, 2005; Leonardi, Paolocci, Rubini, Simonini and Pacioni, 2005; Wang, Hu, Liang and Lee, 2005; Kimura, Nukina, Igarashi, and Sugawara, 2005; Inglet, Song, Hansen and Hwang, 2006). In view of this new species are constantly being discovered (Vizzini, Antonin and Noordeloos, 2007; Wang, 2007; Halling, Baroni and Binder, 2007). Mushroom mycelia are ubiquitous in forest soils where they fulfill a range of key ecological functions (Nwanze, Khan, Ameh, and Umoh, 2005a; Cairney, 2005) but in the industries they are used to produce various seasonings and aromatic flavour compounds (Shiga, Yoshi, Ohe, Yasuda, Furuta, Kuwahara and Linko, 2004; Garcia-Pascual, Sanjuán, Carreres and Mulet, 2005; Nwanze, Khan, Ameh, and Umoh, 2005b). The process for the production of mushroom mycelium for food purposes has great potentiality and offers a simple, mechanical and inexpensive method for producing a nutritious food (Block, 2004). Both free and immobilized mycelia are able to sequester ions and treat raw wastewaters and contaminated soil (Ragunathan and Swaminathan, 2004; Nwanze, Khan, Ameh and Umoh, 2004a; Wingate, 2005; Estévez, Veiga and Kennes, 2005; Adenipekun and Fasidi, 2005). In addition, growth of mushroom

mycelia in liquid medium is an alternative for commercial spawn production, antimicrobial and medicinal substances and enzymes (Hatvani and Mécs, 2001; Che, Araujo, Gloer, Scott, and Malloch, 2005; Saito and Kuwahara, 2005). The mycelial-free culture of *Lentinus edodes* for example, exhibits greater antimicrobial effect against gram-positive than gram- negative bacteria with *Bacillus subtilis*, *Streptococcus pyogenes* and *Staphylococcus aureus* among the most highly inhibited (Hatvani, 2000; Ishikawa, Kasuya and Vanneti, 2001).

Factors such as culture medium components (carbon, nitrogen, and minerals) and cultivation conditions (temperature, water potential, pH and light) have been reported to increase mycelial growth (Kawagishi, Hamajima, Takanami, Nakamura, Sato, Akiyama, Sano and Tanaka, 2004; Vahidi, Kobarfard and Namjoyan, 2004; Joo, Lim, Kim, Kim, Hwang, Choi, and Yun, 2004; Ikehata, Pickard, Buchanan and Smith, 2004; Xiao and Sitton, 2004; Kim, Xiao and Rogers, 2005). In addition, amendment with various lipids, rice bran as well as thinned apples, pears and peaches also produce similar results (Yang, Ke and Kuo 2000; Jung, Ju, Yu, Ryu, Choi and Choi, 2003; Hanai, Ishida, Saito, Maita, Kusano, Tamogami, and Noma, 2005; Nwanze, Khan, Ameh and Umoh, 2004b; 2005b). Other researchers, however, have noted the importance of studying the interactions of parameters rather than optimizing individual parameters (Deshpande, Sarnaik, Paranjpe and Kanekar, 2004; Nwanze *et al.*, 2005b). Nwanze *et al.* (2004b; 2005a), have previously examined the synergistic effects of the interactions of media X oil type, and oil rate X type on biomass production. The current investigation, however, is concerned with the interaction (synergistic effect or lack there of) of culture medium and oil rate on mycelial production of *Lentinus squarrosulus* and *Psathyrella atroumbonata* in liquid culture.

Materials and Methods

Experimental Procedure

L. squarrosulus and *P. atroumbonata* were collected from Zaria and its environs and used to produce pure cultures which were inoculated into four different media. 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 0.007ml/ml) of different lipid sources viz. groundnut, coconut, palm kernel, butterfat, palm and cotton oils prior to autoclaving at 121°C. One hundred ml of each of the supplemented media was transferred into different 250 ml flasks, replicated thrice and sterilized at 121°C. 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 (Minussi, de Moraes, Pastore and Duran, 2001). After three weeks of incubation, all the flasks were autoclaved at 121°C for 10 minutes. 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 hours and transferred to a desiccator. The mycelia and dry filter paper were re-weighed on a Mettler balance. In order to obtain the mycelial dry weight to the nearest milligram, the weight of a dried control filter paper was subtracted from the weight of the experimental mycelia and filter paper (Lalaoui, Halama, Dumortier and Paul, 2000).

Table 1: Different Submerged Liquid Culture Media

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

Section 2.01 Statistics

The experimental design was a split plot arrangement with media as the whole plot and oil type and rate as the subplot (Coviella, Stipanvic and Trumble, 2002). The data was subjected to factorial analysis of variance in order to test the interactive effect of media with various oil rates on both wet and dry mycelial weights (Snedecor and Cochran, 1987; Kluth, Kruess and Tscharntke 2001). The results were analyzed as a 4X5 factorial with three replicates, using Genstat.

(a) Results

Article III. Media x oil Rate Interaction

Mycelial dry weights of *L. squarrosulus* and *P. atroumbonata* as affected by the interaction of various media and oil rates is depicted in Figure 1 and 2 respectively. The highest dry mycelial weight of both *L. squarrosulus* and *P. atroumbonata* was produced by the interaction of SLCM3 with various oil rates. However, *L. squarrosulus* interacted best with the highest oil rate (0.007 ml/ml) while *P. atroumbonata* interacted best with low and moderately high (0.001 and 0.005 ml/ml) oil rates in order to produce optimum results.

Figure 1. Mycelial dry weights (mg) of *L. squarrosulus* as affected by the interaction of various media and oil rates in submerged liquid cultures

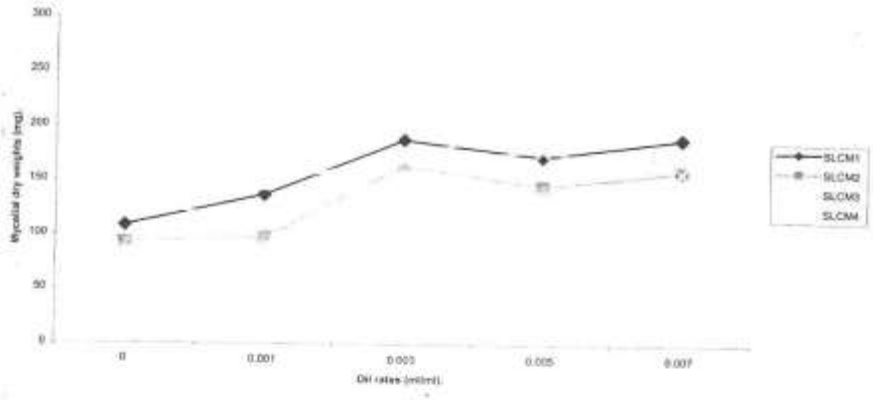
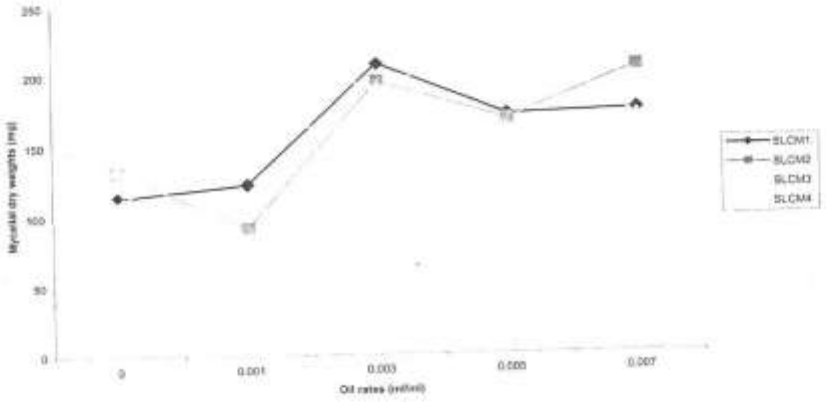


Figure 2. Mycelial dry weights (mg) of *P. atroumbonata* as affected by the interaction of various media and oil rates in submerged liquid cultures



Discussion

The interaction of media X oil type had a significant effect on the dry mycelial weight of both mushroom species just like the present interaction of media X oil rate (Nwanze *et al.*, 2004b). However, the results are different from those of the interaction of oil type X rate, which had a synergistic effect on both wet and dry mycelial weights of the mushrooms (Nwanze *et al.*, 2005a). The effectiveness of SLCM3 in increasing fungal biomass is due to its high content of glucose (Ikehata *et al.*, 2004). Joo *et al.* (2004), however, obtained optimal results with 30g/l of glucose as opposed to the present concentration of 20g/l. Kurbanoglu, Algur and Zulkadir (2004) also got similar results with 20g/l of glucose, however, Maekawa, Intabon, Sugiura, Isoda and Akazawa (2002) and Pessoni (2007) reported good results with sucrose as carbon source. The results reflect the importance of the quantity and source of carbon as well as the importance of various rates of lipid supplementation on mycelial production (Vahidi *et al.*, 2004). The relevance of these findings should, therefore, be carefully reviewed for industrial application in optimal biomass production.

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