International Scholars Journals

International Journal of Biochemistry and Biotechnology ISSN 2169-3048 Vol. 8 (11), pp. 001-006, November, 2019. Available online at www.internationalscholarsjournals.org © International Scholars Journals

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Full Length Research Paper

The effect of media, oil type and rate on the mycelia wet and dry weights of *Lentinus squarrosulus* (Mont.) singer and *Psathyrella atroumbonata* Pegler in submerged liquid culture

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Accepted 28 July, 2019

Lentinus squarrosulus **and** ***Psathyrella atroumbonata*****, two edible indigenous mushroom species, were cultured in four different submerged liquid media supplemented with coconut, cotton, groundnut, butterfat, palm kernel and palm oil at 5 different rates. The differences in the means due to the different media types were highly significant ($p<0.01$) for the mean mycelia wet and dry weights of both** ***L. squarrosulus*** **and** ***P. atroumbonata.*** **SLCM3, which contained twice the glucose level present in each of the other three liquid culture media, induced the heaviest mean wet and dry mycelia weights of both mushroom species. Oil type had a highly significant ($p<0.01$) effect on the wet and dry mycelia weights of** ***L. squarrosulus*** **as well as the mycelia wet weight of** ***P. atroumbonata*****. Butterfat induced the heaviest weights. In addition, oil rate also had a significant effect ($p<0.01$) on the mean mycelia wet and dry weights of both mushroom species. The oil rates of 0.003, 0.005 and 0.007 ml/ml produced comparable optimum mean mycelia wet and dry weights of the mushrooms.**

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

INTRODUCTI0N

Mushrooms are highly nutritive and compare favourably with meat, eggs and milk because of their high content of proteins amino acids and mineral elements (Parent and Thoen, 1977). With the present high cost of meat and fish in Nigeria, many dwellers in the rural areas are turning to mushrooms as an alternative source of protein since their protein quality approaches that of animal .protein. The proximate and amino acid composition as well as the mineral content of *Lentinus squarrosulus* and *Psathyrella atroumbonata* has been documented (Alofe, 1985; Fasidi and Kadiri, 1991; Aletor, 1995; Nwanze and Adamu 2004a; 2004b). The two species are highly nutritious and compare favourably with other foreign edible species.

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 used in the food industries (Berger et al., 1987; Sastry et

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al., 1980a; 1980b). 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; Vel zquez-cedeno et al., 2004).

It has been reported that modifications of culture conditions, especially the choice of nitrogen and carbon sources influences the composition of fungal odorous profiles (Yong and Lim, 1986). Addition of lipids in the culture media has been reported to increase mycelia growth, the production rate of various metabolites, antibiotics and lactones (Asther et al., 1988; Fasidi and Kadiri, 1993). In addition, agitation of liquid cultures and incubation at various different temperatures has also been reported to increase mycelia growth (Agosin, 1985; Gallois et al., 1990). With the above facts in mind this paper seeks to report the effect of media, oil type and rate on the mycelia wet and dry weights of *L. squarrosulus* and *P. atroumbonata* in submerged liquid cultures.

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 h (Agosin, 1985; Gordon et al., 2002). The resultant pure cultures were then sub cultured 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 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 Buchner funnel and washed thrice with ethyl ether to remove excess lipids (Wardle and Schisler, 1969). The mycelia 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. In order to obtain the mycelia dry weight to the nearest mg, the weight of a dried control filter paper was subtracted from the weight of the experimental mycelia and filter paper (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 mycelia weights, the data were subjected to factorial analysis of variance (Porter, 2001). A p-value of 0.05 or less was considered significant and treatment means were separated using Duncan's multiple range tests (Snedecor and Cochran, 1987).

RESULTS

The effect of media, oil type and oil rate on the mycelia wet and dry weights of *L. squarrosulus* and *P. atroumbonata* in liquid culture

The mean mycelia wet and dry weights of *L. squarrosulus* and *P. atroumbonata* as affected by media, oil type and rate in submerged liquid culture is depicted in Table 2. The differences in the means due to media types were highly significant ($p< 0.01$) for the mycelia wet and dry weights of both *L. squarrosulus* and *P. atroumbonata*. Submerged liquid culture media 1 (SLCM1) 3 and 4 induced mean mycelia wet weights of *L. squarrosulus* that were significantly heavier than those produced by SLCM2. SLCM3 gave rise to mean mycelia dry weights of *L. squarrosulus* which were significantly heavier than the ones produced by SLCM1 and 4 that were at par, but none the less significantly heavier than those produced by SLCM2. SLCM3 gave rise to mean mycelia wet and dry weights of *P. atroumbonata* that were significantly heavier than those produced by SLCM1, 2 or 4, which were at par.

Oil type had a highly significant ($p<0.01$) effect on the wet and dry mycelia weights of *L. squarrosulus* as well as the mycelia wet weights of *P. atroumbonata*. Oil type, however, did not have a significant effect on the mean mycelia dry weight of *P. atroumbonata*. Butterfat influenced the production of mean mycelia wet and dry weights of *L. squarrosulus*, which were statistically heavier than those induced by coconut, cotton, groundnut, palm kernel or palm oil, all of which were at par. In addition, butterfat gave rise to mean mycelia wet

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 $MgSO_4 . 7H_2O$ 0.50 g KH_2PO_4 50.0 mg $CaCl_2$	All the above components were suspended in 1L 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 $MgSO_4 . 7H_2O$ 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 $MgSO_4 . 7H_2O$ 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 $MgSO_4 . 7H_2O$ 1.0 g $CaSO_4 . 5H_2O$ 6.4 mg $FeSO_4 . 7H_2O$ 1.1 mg $MnCl_2 . 4H_2O$ 1.9 mg $ZnSO_4 . 7H_2O$	Same as above

weights of *P. atroumbonata* that were at par with those produced by coconut, palm kernel and palm, but significantly heavier than the comparable weights produced by cotton and groundnut oils.

Oil rate had a highly significant effect ($p<0.01$) on the mean mycelia wet and dry weights of both *L. squarrosulus* and *P. atroumbonata*. Increasing lipid rate to 0.001 ml/ml did not have any significant effect on the mean mycelia wet weight of *L. squarrosulus*. A further increase to 0.003 ml/ml resulted in a significant decrease in the wet weight that was comparable to that of 0.005 ml/ml, but significantly lighter than the mycelia wet weight induced by the highest oil rate (0.007 ml/ml).

Increasing oil rate to 0.001 ml/ml had no significant effect on the mycelia dry weight of *L. squarrosulus*. However, a further increase to 0.003 ml/ml resulted in a significant increase in the mycelia dry weight, which was comparable to that of 0.005 or 0.007 ml/ml, respectively.

The mycelia wet and dry weights of *P. atroumbonata* increased significantly when the oil rate was increased to 0.001 ml/ml. Increasing the oil rate further to 0.003 ml/ml produced mean mycelia wet and dry weights of *P. atroumbonata* that were comparable to weights induced by 0.005 or 0.007 ml/ml, respectively. In addition, the majority of the first order interactions were significant across the various parameters.

Table 2. The effect of media, oil type and rate on the mycelia wet and dry weights of *L. squarrosulus* and *P. atroumbonata* in submerged liquid cultures.

Treatments	*L. squarrosulus*		*P. atroumbonata*	
	wet weight (mg)	dry weight (mg)	wet weight (mg)	dry weight (mg)
Media				
SLCM1	1179.61a	158.70b	1204.42b	157.70b
SLCM2	946.34b	131.41c	1242.57b	158.10b
SLCM3	1243.23a	178.41a	1418.76a	189.16a
SLCM4	1157.28a	149.41b	1124.27b	149.02b
SE±	38.80	4.70	40.66	4.95
Significance	**	**	**	**
Oils				
coconut	1093.48b	147.18b	1284.83ab	166.08
cotton	1074.28b	143.95b	1190.30b	156.88
groundnut	1123.77b	155.87b	1166.57b	153.37
butterfat	1407.25a	188.82a	1350.18a	174.57
Palm kernel	1124.72b	153.00b	1229.25ab	166.52
Palm	993.20b	138.08b	1263.88ab	163.55
SE±	47.52	5.76	49.80	7.65
Significance	**	**	**	**
Oil rate				
0.000ml	854.67c	113.76b	933.96c	122.33c
0.001ml	937.51c	124.14b	1127.38b	149.67b
0.003ml	1241.83b	173.63a	1419.21a	189.17a
0.005ml	1269.51ab	174.08a	1400.38a	182.19a
0.007ml	1377.06a	186.81a	1356.60a	174.11a
SE±	43.38	5.26	45.46	5.54
Significance	**	**	**	**
Interactions				
MxO	NS	**	NS	**
MxR	NS	**	NS	**
OxR	**	**	**	**
MxOxR	**	**	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.

DISCUSSION

Of the 4 different liquid culture media used SLCM3 produced the best results. This particular media produced the heaviest mean wet and dry weights of both mushroom species. The media was originally formulated to grow basidiomycetes such as *Psathyrella fluvescens*, *P. conopilus*, *Clitocybe marginella*, *Laccaria laccata* and *Amanita muscaria* (Verhagen et al., 1996) and in addition, it was the simplest of all the media in terms of constituents. Carbon and nitrogen were supplied by glucose and peptone, respectively. This is of interest since Alofe (1985) found glucose to be a good carbon source for both *L. squarrosulus* and *P. atroumbonata* while Hendrix and Apple (1964) reported peptone to be a good nitrogen source.

Submerged liquid culture media 1, 2 and 4 produced mean dry and wet mycelia weights of *P. atroumbonata* which were at par. SLCM1 was originally formulated to grow *Boletaceae*, SLCM4 to grow *Laccaria laccata* while SLCM2 was formulated to grow *L. squarrosulus* and *P. atroumbonata* (Schisler and Volkoff, 1977; Nwanze,

1996). Submerged liquid culture media 1, 3 and 4 produced mean mycelia wet weights of *L. squarrosulus* which were at par, but significantly heavier than the mean wet weight produced by SLCM2. After SLCM3, it was SLCM4 and 1 that produced the heaviest mean dry mycelia weights of *L. squarrosulus*. The differences observed in the mean dry mycelia weight of *L. squarrosulus* and *P. atroumbonata* produced in the different media are not unexpected. The various media have different nutrient compositions, which may result in different growth responses (Schisler and Sinden, 1962; Raper et al., 1972; Xu et al., 1993).

Out of the six different lipid sources tested, butterfat induced the heaviest mean wet and dry mycelia weights of *L. squarrosulus* as well as the heaviest mean wet weight of *P. atroumbonata*. However, the oils had no significant effect on the mean mycelia dry weights of *P. atroumbonata*. Wardle and Schisler (1969), however, observed the heaviest dry mycelia weight with cottonseed and corn oil while Schisler and Volkoff (1977) observed similar findings with safflower oil. However it should be mentioned that the ether rinses used to remove excess lipid from the filter papers may have biased mycelia weights downward since some, or most of the mycelia lipid fraction was removed (Hendrix and Apple, 1964; Wardle and Schisler, 1969). Nonetheless, there is no disputing the fact that the sterols (- sitosterol) contained in the various oils had a significant effect on mycelia growth (Haskin et al., 1964; Hendrix, 1965).

Lipid rates of 0.003, 0.005 and 0.007 ml/ml produced comparable optimum mean mycelia wet and dry weights respectively, of *L. squarrosulus* and *P. atroumbonata*. Wardle and Schisler (1969), however, reported 0.005 ml/ml as the optimum lipid rate for the dry mycelia weight of *A. bisporus.*

The results confirm the fact that in addition to oil type and rate, media constitution also has a significant effect on mycelia production. The results show that optimal production of fungal biomass may be obtained for both mushroom species in liquid culture by using glucose and butterfat as carbon and lipid sources, respectively. Attempts should be made to exploit the current findings in the Nigerian food industries.

ACKNOWLEDGMENT

The authors would like to thank Dr. Pegler of Kew Gardens, Kew for his immense help in the identification of the specimens.

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