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Studies on *Psathyerella atroumbonata* (Pegler), a Nigerian edible fungus

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Abstract

The effects of temperature, pH, vitamins and phytohormones on growth *of Psathyerella atroumbonata* (Pegler), a Nigerian edible mushroom were investigated. This fungus was able to grow at a temperature range of 15–45 °C (optimum = 30 °C) and pH range of 5.0– 8.0 (optimum = 6.5). Exogenous supply of vitamins significantly enhanced the growth of the fungus ($P = 0.01$). Similarly, biotin was found to be the most stimulatory vitamin while nicotinic acid was the least utilised. The best phytohormone that sustained good growth was 1.0 ppm NAA while 20.0 ppm 2,4-D inhibited growth of this mushroom. The relevance of these findings in relation to cultivation of *P. atroumbonata* in Nigeria is considered.

Keywords: Psathyerella atroumbonata; Temperature; pH; Variations and phytohormones

1. Introduction

Psathyerella atroumbonata (Pegler), is one of the well known edible Nigerian mushroom. It grows wildly in the forests during the mid and late summer and has a short shelf-life of 24 h. This edible macro fungus grows at the base of decaying wood or dung and it occasionally parasitizes other giant basidomycetes, especially the agarics (Zoberi, 1972).

The cap which is often brown in colour is between 1.5 and 4.5 cm in diameter. It is conico-campanulate, becoming expanded umbonate and distinctively hygropharus (Zoberi, 1972). The veil consists of loosely interwoven hyphae. Gills are attached broadly to the stripe which is between 5.0 and 9.0 cm in length. The stripe is cylindrical, hollow, white and smooth. The spores, which are brown, are between 5.5 and 8.5 μm along the major axis, and between 3.2 and 5.2 μm along the minor axis.

Apart from its documented morphological and preliminary physiological description (Jonathan & Fasidi, 2001a; Zoberi, 1972), very little is known about *P. atroumbonata*. People in Nigeria still depend largely on mushrooms collected from the wild because commercial cultivation of mushroom is not popular. Mushrooms which are cultivated commercially in Nigeria are exotic species. It is no surprise that some of the mushroom farms have collapsed.

However, indigenous mushrooms, growing in the wild, have been found to be nutritious and very important for medicinal purposes (Oso, 1977). Mannose, malt extract, calcium and copper were found to be required for the vegetative growth of *P. atroumbonata* (Jonathan & Fasidi, 2001a). This present study, however, investigates the pH, temperature, vitamins and phytohormone requirements of this mushroom. This information will shed light on the cultivation of *P. atroumbonata* in Nigeria.

2. Materials and methods

2.1. Source of organism

The inoculum used in this study was obtained from tissue culture of *P. atroumbonata* sporophores collected from a decaying wood at the University of Ibadan Botanical Gardens. The pure mycelial culture was established on the yeast extract enriched-potato dextrose agar (YPDA).

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2.2. Temperature and pH requirements

This experiment was carried out by mycelial dry weight method (Jonathan & Fasidi, 2000). The mycological basal medium used was that described by Alofe (1985). For the temperature requirement of *P. atroumbonata*, the ingredients required to form the basal medium and streptomycin sulphate (50 mg) were dissolved in 1000 ml of deionised water and pH regulated to 6.5.

This liquid medium was dispensed in 30 ml lots into 250 ml bottles and sterilized in the autoclave at 1.02 kg cm^{-2} pressure and temperature of 121 °C for 15 min. After cooling, each bottle was inoculated with a 7 mm (diameter) mycelia mat of 5-day-old actively–growing culture of *P. atroumbonata* and incubated at 10, 15, 20, 25, 30, 35, 40 and 45 °C for 7 days. Each treatment was thrice replicated. The mycelia were harvested using a pre weighed Whatman filter paper and oven–dried at 80 °C until constant weight was obtained (Chandra & Purkayastha, 1977).

For the pH study, the basal liquid medium was dispensed into 250 ml bottles (30ml per bottle) and pH regulated (4.0–8.5), and autoclaved. On cooling, each bottle was inoculated with a vigorously growing 7 mm mycelial mat of *P. atroumbonata* and incubated at 30±2 °C for 7 days. Mycelia were then harvested and dried using the method of Fasidi and Olorunmaiye (1994).

2.3. Effect of phytohormones on growth of P. atroumbonata

The basal liquid medium used was made up of fructose (10.0 g), peptone (2.0 g), KH_2PO_4 (0.5 g), $MgSO_4 \cdot 7H_2O$ (0.5 g), thiamine hydrochloride (500 μg), and 1000 ml of de-ionised water (Jonathan & Fasidi, 2000). The phytohormones used include 2,4 dichlorophenoxy acetic acid (2, 4D), gibberellic acid (GA_3) and naphthalene acetic acid (NAA). These plant hormones were added separately to the basal medium to give concentrations of 0.1, 1.0, 10.0, 15.0 and 200 ppm. The basal medium without any phytohormone source was used as the control experiment. The phytohormone were filter-sterilised and each treatment was replicated thrice.

2.4. Effect of vitamins on growth of P. atroumbonata

The same basal medium as used for the phytohormone investigation was employed, except that thiamine hydrochloride was omitted. The vitamins used were ascorbic acid, biotin, cobalamine, folic acid, nicotinic acid, pantothenic acid, pyridoxine, riboflavin and thiamine. Two control experiments were set up; one contained all the vitamins and the other lacked all vitamins. The medium was sterilised by millipore filtration. Vitamins were added to the basal medium, separately, to give a concentration of 500 μg per 1000 ml and each set-up was replicated thrice.

3. Results and discussion

In this study, *P. atroumbonata* was found to grow fairly well in acidic, neutral and alkaline environments (pH 5.0–8.0). The best vegetative growth (133 mg/ml) was observed in the acidic medium (pH 6.5). The vegetative growth of 76.7 mg/30 ml, which was the second best, was recorded in the neutral medium (pH 7.0), while the least growth (12.0 mg/30 ml) was found at the alkaline pH of 8.0 (Table 1). This result suggests that *P. atroumbonata* thrives best within a narrow range of pH. This finding agrees favourably with that obtained by Humfeld and Sugihara (1952), for *Agaricus campestris*. Fasidi (1996), also obtained the best mycelial growth of *Volvariella esculenta* at pH 6.0. The changes observed in the final pH of the culture medium at the end of the experiment (Table 1) may be due to the waste products of metabolism produced in the medium by the growing fungus.

P. atroumbonata grew fairly well between the temperatures 25 and 35 °C but, best growth was observed at 30 °C (Table 2). The ability of this fungus to grow well

Table 1
Effect of pH on vegetative growth of *Psathyerella atroumbonata*

pH	Dry weight of mycelia (mg)	Final pH
4.0	–	4.0
5.0	20.3d	5.6
5.5	55.0c	6.2
6.0	70.0b	6.4
6.5	133.3a	6.7
7.0	76.7b	7.3
7.5	53.3c	7.0
8.0	12.7d	7.2
8.5	–	8.5

Values followed by the same letters are not significantly different by Duncan's multiple range test ($P = 0.01$).

Table 2
Effect of temperature on the vegetative growth of *Psathyerella atroumbonata*

Temperature (°C)	Dry weight of mycelia (mg)	Final pH
10	–	6.5
15	17.0f	5.8
20	50.0d	5.7
25	96.7b	6.2
30	144.0a	6.3
35	70.0c	5.9
40	30.0e	5.7
45	10.7f	6.1

Values followed by the same letters are not significantly different by Duncan's multiple range test ($P = 0.01$).

between 25 and 35 °C enables it to survive in the tropical climate. Chang and Chu (1969) reported 30–35 °C as the optimum range for *Volvariella volvacea* mycelial growth with 32 °C as the most suitable temperature. Temperature and PH are the two most important environmental factors that support the growth of fungi (Garraway & Evans, 1984).

All the phytohormones used in this study significantly enhanced the growth of *P. atroumbonata* ($P = 0.01$). The most utilizable hormone was naphthalene acetic acid (NAA) at 1.0 ppm concentration, followed in order by 10.0 and 15.0 ppm of 2, 4-D and GA_3 respectively (Table 3). Similar utilizations of these hormones were reported by Fasidi and Olorunmaiye (1994), for *Pleurotus tuberregium*; and by Jonathan and Fasidi (2001b) for *Lentinus subnudus* and *Schizophyllum commune*. Hayes (1981), also reported the stimulation of mycelial growth and fruit body yield when he incorporated NAA and GA_3 into the growing medium of *Agaricus bisporus*.

Generally, it was observed that high concentrations of these phytohormones reduced the vegetative growth of *P. atroumbonata* (Table 3). The poorest growth (38.3 mg/30 ml) was observed at 20.0 ppm of 2,4-D. This value is lower than that obtained for the basal medium (control). This result is contrary to the report of Voltz (1972), who observed that, at 20.0 and 30.0 ppm of phytohormones, growth of *Volvariella volvacea* was unaffected. The difference in these results may be attributed to different genera of basidomycetes used.

Table 3
Effect of phytohormones on growth of *Psathyerella atroumbonata*

Phytohormones (ppm)	Dry weight of mycelia (mg)	Final pH
Gibberellic acid (GA_3)		
0.1	50.0e	5.6
1.0	53.3e	5.8
10.0	61.7e	5.9
15.0	86.7d	6.0
20.0	71.6de	6.2
Naphthalene acetic acid (NAA)		
0.1	98.3c	6.5
1.0	151.7a	6.9
10.0	80.0d	6.1
15.0	70.0de	5.9
20.0	53.0e	5.8
2,4 Dichlorophenoxy acetic acid (2,4D)		
0.1	58.3e	6.1
1.0	85.0d	6.3
10.0	123.3b	6.6
15.0	70.0de	5.9
20.0	38.3ef	5.5
Basal medium (Control)	50.0e	5.3

Values followed by the same letters are not significantly different by Duncan's multiple range test ($P = 0.01$).

Table 4
Effect of vitamins on the growth of *Psathyerella atroumbonata*

Vitamins	Dry weight of mycelia (mg)	Final pH
Ascorbic acid	100.0b	6.8
Biotin	133.3a	7.2
Cobalamine	60.0cd	6.0
Folic acid	88.3bc	6.1
Nicotinic acid	53.3cd	5.3
Pantothenic acid	76.7c	5.9
Pyridoxine	70.0c	5.7
Riboflavin	101.7b	6.8
Thiamine	103.3b	7.0
All vitamins	48.3d	5.9
Basal medium (control)	50.0d	5.6

Values followed by the same letters are not significantly different by Duncan's multiple range test ($P = 0.01$).

All the vitamins employed in this investigation also stimulated the growth of *P. atroumbonata* (Table 4). Biotin was the most stimulatory vitamin source, followed in order by thiamine and riboflavin ($P = 0.01$). Similar utilization of biotin was reported by Jonathan and Fasidi (2001b) for *L. subnudus*; and by Madunagu (1988), for *Pleurotus squarrosulus*. Rao and Modi (1968), reported that biotin acts as a co-factor for acetyl co A carboxylase, an enzyme which is important in lipid metabolism. Likewise, thiamine has been implicated as a co-enzyme for several enzymes of intermediary metabolism (Gounaris, Turkenkopf, Averchia, & Greenlie, 1975).

The ability of *P. atroumbonata* to utilise riboflavin for its growth supports the assertion of Garraway and Evans (1984), that this vitamin stimulates enzymes which function in the oxidative degradation of pyruvate during the Krebs cycle and E.T.C which leads to ATP production and growth enhancement. The medium that contained all the vitamins (Control 1) was observed to sustain the poorest growth (Table 4). This may be due to antagonistic effect of some of the vitamins. Besides, the medium may be concentrated and toxic to the cells of *P. atroumbonata*.

In conclusion, the growth of *P. atroumbonata* was greatly improved at pH 6.5 and a temperature of 30 °C. An exogenous supply of NAA, biotin, thiamine and riboflavin was also required to sustain good mycelial growth. These could be incorporated into the mycological growth media for the multiplication of mycelia for spawn and fruit body production. This would improve the growing technology of this indigenous mushroom in Nigeria.

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