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Studies on the requirements for vegetative growth of *Pleurotus tuber-regium* (Fr.) Singer, a Nigerian mushroom

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Growth requirements of *Pleurotus tuber-regium* (Fr.) Singer, a Nigerian edible mushroom, were studied. Among the carbohydrates tested, glucose was the most utilised. This was followed in order by mannitol, maltose, and dextrin, which significantly enhanced mycelial growth ($P < 0.01$). Cellulose was the least stimulatory. Of the nitrogen compounds tested, yeast extract supported the greatest growth, which was comparable with that induced by glucose. This was followed in order by asparagine, casein, glycine, and calcium nitrate. Sodium nitrate, potassium nitrate, and ammonium sulphate supported the poorest growth. The best C/N ratio that sustained good growth was 1 : 4. This was followed by 4 : 1. Similarly, thiamine, pyridoxine, GA_3 (1 and 10 ppm), 2,4-D (10 ppm), Ca, K, Cu, and Zn supported relatively good mycelial growth. The implication of these results is discussed in relation to the cultivation of *P. tuber-regium* in Nigeria.

INTRODUCTION

Pleurotus tuber-regium (Fr.) Singer is an edible mushroom that is found growing in tropical and subtropical regions of the world (Zoberi, 1973). In Nigeria, it colonises wood and produces sclerotis, which are buried in the soil. Such sclerotis are lifted out of the soil by farmers while cultivating their farms (Oso, 1977). When cropped in a warm and humid atmosphere, sclerotis produce fruitbodies, which are consumed as food or used as condiments to add flavour to food (Zoberi, 1972, 1973; Oso, 1977). *Pleurotus tuber-regium* has great medicinal value among the native doctors in the treatment of many ailments, including asthma, smallpox, and high blood pressure (Oso, 1977).

Fasidi and Ekuere (1993) cultivated the sclerotis of *P. tuber-regium* on local cellulosic wastes and obtained 30% yield. More research work needs to be done on the physiology of this fungus to make its cultivation a profitable venture. Oso (1977) reported that the fungus can utilise fructose, mannose, glucose, cellobiose, mannitol, and xylose as the carbon sources for good mycelial growth. In the present work, the effects of carbohydrates, nitrogen compounds, mineral elements, phytohormones, and vitamins on the growth of the fungus were investigated.

MATERIALS AND METHODS

Pleurotus tuber-regium mycelium was obtained by the method described by Fasidi and Ekuere (1993) and maintained on PDA. The growth of *P. tuber-regium* was determined by a mycelial-dry-weight method. The basal medium used was that described by Chandra and Purkayastha (1977). The basal medium, supplementary compounds, and streptomycin (0·05 g) were dissolved in 1 litre of distilled water. This was dispensed (30 ml) into each 250-ml milk bottle and its pH adjusted to 6·5. The mouth of each bottle was sealed with aluminium foil before sterilisation at 1·02 kg/cm^2 (10·0 Pa) pressure at 121°C for 10 min. On cooling, each bottle was inoculated with a 7-mm-diameter disc of vigorously growing mycelium and incubated for eight days at 30 ± 2°C. The mycelium in each bottle was filtered through a pre-weighed 9-cm-diameter filter paper, oven dried at 85°C for 10 h, and weighed. Each experiment was replicated three times.

Carbohydrates

The basal medium consisted of peptone (2·0 g), KH_2PO_4 (0·5 g), $MgSO_4 \cdot 7H_2O$ (0·5 g), and distilled water to make 1 litre (Chandra & Purkayastha, 1977). This medium was supplemented separately with carbo-

hydrates. The concentration of carbon in each compound is equivalent to that present in 1% glucose. For dextrin, malt extract, and starch, the amount used was 10 g/litre distilled water. The basal medium without a carbon compound served as a control.

Nitrogen compounds

The basal medium consisted of fructose (10·0 g), KH_2PO_4 (0·5 g), $MgSO_4 \cdot 7H_2O$ (0·5 g), thiamine hydrochloride (500 μg), and distilled water (1 litre) (Chandra & Purkayastha, 1977). Each nitrogen compound was supplemented at a concentration of nitrogen equivalent to that in 2·0 g of sodium nitrate. For casein, peptone, and yeast extract, each was added at the same rate as sodium nitrate (2 g/litre) (Chandra & Purkayastha, 1977).

C:N ratio

The basal medium was similar to that used for testing nitrogen compounds except that glucose was omitted. Varying ratios of glucose and yeast extract (found to be the best in the previous experiments) were used. A concentration of 0·15 g/litre of glucose and yeast extract in the basal medium served as the 1 : 1 ratio; other ratios were prepared proportionately.

Macroelements

The basal medium used consisted of glucose (10·0 g), alanine (1·0 g), $NaNO_3$ (2·0 g), KH_2PO_4 (2·0 g), $MgSO_4 \cdot 7H_2O$ (0·2 g), $CaCl_2$ (0·3 g), thiamine hydrochloride (500 μg), and distilled water 1 litre (Steinberg, 1946). To determine the effect of potassium, KH_2PO_4 in the basal medium was replaced by an equal amount of $(NH_4)H_2PO_4$. Similarly, $MgSO_4$, $CaCl_2$, and $NaNO_3$ were replaced by $(NH_4)_2SO_4$, NH_4Cl, and NH_4NO_3, respectively.

Trace elements

The trace elements tested (Fe, Zn, Mn, and Cu) were added in their sulphate forms to give 1 ppm in the basal medium (glucose (10·0 g), alanine (1·0 g), KH_2PO_4 (0·5 g), $MgSO_4 \cdot 7H_2O$ (0·5 g), thiamine hydrochloride (500 μg), and distilled water (1 litre)). The trace element to be tested was excluded from the medium. Two sets of control were employed: one contained the four trace elements, whereas the second did not contain any trace element.

Vitamins

The basal medium was similar to that used for investigating the effect of nitrogen compounds. Each vitamin was added to the basal medium to give 500 μg/litre. Two control experiments were set up: one contained all the vitamins and the other no vitamins. The media were filter-sterilised.

Phytohormones

Each hormone was added to the basal medium (similar to that used for testing nitrogen compounds) to give 0·1-, 1·0-, or 10-ppm concentration.

Analysis of data

The data obtained were analysed by ANOVA and tests of significance determined by Duncan's multiple-range tests.

RESULTS AND DISCUSSION

In this study, carbohydrates were utilised to a great extent for the growth of *P. tuber-regium*, and glucose was the most stimulatory. Growth on mannitol (the second best) and maltose was not significantly different from that on glucose ($P < 0.05$) (Table 1). This result is similar to that reported by Hashimoto and Takahashi (1976), Hong (1978) (on *P. ostreatus*), Jandaik and Kapoor (1976) (on *P. sajor-caju*), and Oso (1977) (on *P. tuber-regium*). Glucose and mannitol have been reported as good respiratory substrates (Hammond, 1978). Cellulose was the least utilised of the carbohydrates tested. This is in agreement with the result of Styer (1930) and implies that, in the presence of simple sugars, the utilisation of cellulose may be restricted (Chang & Quimio, 1982).

Yeast extract, a complex nitrogen, sustained the greatest growth among the nitrogen compounds tested ($P < 0.05$) (Table 2). It has been reported that combined amino acids stimulate greater growth than single

Table 1. Effect of different carbon compounds on mycelial growth of *P. tuber-regium*

Carbon compound	Mycelial dry weight (mg/30 ml)	Final pH
Control (basal medium)	20·0 *d*	7·0
Monosaccharides		
Sorbose	33·0 *d*	6·6
Glucose	110·0 *a*	5·2
Galactose	40·0 *cd*	6·6
Oligosaccharides		
Lactose	33·3 *d*	6·6
Maltose	83·3 *ab*	5·4
Raffinose	36·7 *d*	5·9
Polysaccharides		
Dextrin	66·7 *bc*	5·2
Starch	56·7 *cd*	4·9
Malt extract	56·7 *cd*	4·8
Cellulose	23·3 *d*	7·7
Sugar alcohols		
Mannitol	86·7 *ab*	5·7
Inositol	33·3 *d*	7·1
Sorbitol	53·3 *cd*	6·2

Means followed by the same letter(s) are not significantly different ($P > 0.01$) by Duncan's multiple-range test.

Table 2. Effect of different nitrogen compounds on mycelial growth of *P. tuber-regium*

Nitrogen compound	Mycelial dry weight (mg/30 ml)	Final pH
Control (basal medium)	43·3 *c*	5·1
Inorganic nitrogen compounds		
$NaNO_3$	36·7 *c*	6·6
KNO_3	36·7 *c*	5·8
NH_4NO_3	50·0 *b*	3·0
$Ca(NO_3)_2$	60·0 *b*	5·0
$(NH_4)_2SO_4$	36·7 *c*	2·6
Amino acids		
Glutamic acid	53·3 *b*	5·8
Leucine	56·7 *b*	4·5
Glycine	60·0 *b*	4·7
Aspartic acid	63·3 *b*	5·7
Asparagine	70·0 *b*	5·2
Tryptophan	40·0 *c*	6·0
Phenylalanine	46·7 *b*	4·8
Methionine	50·0 *b*	5·5
Citrulline	50·0 *b*	5·5
Complex nitrogen compounds		
Peptone	56·6 *b*	5·0
Urea	56·7 *b*	5·1
Yeast extract	103·3 *a*	5·2
Casein	60·0 *b*	5·8

Means followed by the same letter(s) are not significantly different ($P > 0.01$) by Duncan's multiple-range test.

amino acids (Cochrane, 1958; Nolan, 1970). Hence the stimulatory effect of yeast extract is due to its protein, amino-acid, and vitamin contents (Fergus, 1952; Bolton & Blair, 1982). Asparagine promoted the second-best growth while peptone, casein, and urea substantially enhanced mycelial growth (Table 2). Hashimoto and Takahashi (1976), Khanna and Garcha (1985), and Madunagu (1988) obtained substantial growth of various species of *Pleurotus* with asparagine, peptone, casein, and urea. Of the inorganic nitrogen compounds tested, NH_4NO_3 and $Ca(NO_3)_2$ supported good growth, whereas $NaNO_3$, KNO_3, and $(NH_4)_2SO_4$ depressed growth (Table 2). This result implies that *P. tuber-regium* has a greater preference for organic than inorganic nitrogen compounds.

The carbon : nitrogen ratio affected mycelial growth ($P < 0.05$). Growth increased from 1 : 1 until it reached the optimum at 4 : 1. Growth also increased from 1 : 2 to 1 : 4 (Table 3). Growth at 1 : 4 was appreciably greater than that at 4 : 1, but the difference was not statistically significant ($P < 0.05$). This result suggests that *P. tuber-regium* is able to utilise substrates that are rich in carbon or nitrogen (within C/N tolerable limits). This result also agrees with that obtained by Chandra and Purkayastha (1977) for *Agaricus campestris* and *volvariella volvacea*.

Supplementation of basal medium with macroelements significantly improved growth ($P < 0.05$). The complete medium minus Na produced the greatest growth ($P < 0.05$) (Table 4(a)). This implies that Ca, Mg, and K are required for good growth, whereas Na

Table 3. Effect of different carbon/nitrogen ratios (C : N) on mycelial growth of *P. tuber-regium*

C : N ratio	Mycelial dry weight (mg/30 ml)	Final pH
Control (basal medium)	30·0 *b*	6·3
1 : 1	33·3 *b*	6·3
2 : 1	46·7 *b*	6·5
3 : 1	53·3 *ab*	6·2
4 : 1	60·0 *ab*	6·4
5 : 1	56·7 *ab*	6·3
1 : 2	33·3 *b*	6·4
1 : 3	36·7 *b*	6·4
1 : 4	76·7 *a*	6·2
1 : 5	76·7 *a*	6·1

Means followed by the same letter(s) are not significantly different ($P > 0.01$) by Duncan's multiple-range test.

is slightly inhibitory, Hawker (1950) reported that Na was not required by most fungi in culture or is already available in sufficient quantity as an impurity from other chemicals or glassware. The complete medium minus Ca supported the lowest growth, which was not significantly different from that sustained by the K-free medium (Table 4(a)). This result emphasises that Ca and K are important for growth of *P. tuber-regium* and agrees with the findings of Humfeld and Sugihara (1952) and Chandra and Purkayastha (1977). Kadiri and Fasidi (1990) have reported that K was the most abundant mineral element in *P. tuber-regium* fruitbody and Ca was more preponderant in the stipe than pileus. Calcium and potassium are therefore important in the metabolism of *P. tuber-regium*.

Trace elements are also required for the growth of *P. tuber-regium* because the basal medium supplemented with Cu, Fe, Mn, and Zn produced greater growth than the basal medium ($P < 0.05$) (Table 4(b)). When

Table 4. Effect of mineral elements on mycelial growth of *P. tuber-regium*

	Mycelial dry weight (mg/30 ml)	Final pH
(a) Macro elements		
Control (basal medium minus macro elements)	16·7 *c*	6·0
Complete medium minus Na	103·3 *a*	5·8
Complete medium minus Ca	56·7 *b*	5·9
Complete medium minus K	63·3 *b*	5·8
Complete medium minus Mg	70·0 *b*	5·4
(b) Trace elements		
Control I (basal medium + all trace elements)	20·0 *a*	6·2
Control II (basal medium − all trace elements)	13·3 *b*	6·2
−Cu	16·7 *ab*	3·4
−Zn	16·7 *ab*	6·3
−Fe	20·0 *a*	6·1
−Mn	20·0 *a*	6·1

Means followed by the same letter(s) are not significantly different ($P > 0.01$) by Duncan's multiple-range test.

Table 5. Effect of different vitamins on mycelial growth of *P. tuber-regium*

Vitamin	Mycelial dry weight (mg/30 ml)	Final pH
Control I (Basal medium) + all vitamins)	60·0 *ab*	6·2
Control II (Basal medium)	60·0 *ab*	5·3
Ascorbic acid	23·3 *c*	6·7
Biotin	53·0 *ab*	6·0
Folic acid	20·0 *c*	6·2
Nicotinic acid	40·0 *bc*	5·8
Pantothenic acid	40·0 *bc*	4·2
Pyridoxine	70·0 *ab*	5·7
Riboflavin	26·7 *c*	6·7
Thiamine	73·3 *a*	6·3

Means followed by the same letter(s) are not significantly different ($P > 0.01$) by Duncan's multiple-range test.

Table 6. Effect of different phytohormones on mycelial growth of *P. tuber-regium*

Phytohormone	Mycelial dry weight (mg/30 ml)	Final pH
Control (basal medium)	33·0 *b*	5·8
GA 10 ppm	40·0 *a*	5·8
1 ppm	40·0 *a*	6·4
0·1 ppm	20·0 *c*	6·4
NAA 10 ppm	23·0 *c*	6·0
1 ppm	30·0 *bc*	5·6
0·1 ppm	26·7 *c*	6·4
2,4-D 10 ppm	40·0 *a*	5·8
1 ppm	36·6 *ab*	5·5
0·1 ppm	30·0 *b*	6·2

Means followed by the same letter(s) are not significantly different ($P > 0.01$) by Duncan's multiple-range test.

each of the trace elements was omitted from the medium, the growth produced was slightly greater than that of the basal medium (Table 4(b)). This suggests that the four trace elements are required for growth. However Cu- and Zn-free media produced slightly lower growth than Fe- and Mn-free media. This shows that Cu and Zn are more important than Fe and Mn. Zinc and copper are essential for growth and pigment production in fungi (Hawker, 1950).

Among the vitamins tested, only thiamine and pyridoxine improved mycelial growth, and thiamine was the better of these two. Ascorbic acid, folic acid, and riboflavin significantly suppressed growth (Table 5). Chandra and Purkayastha (1977) and Madunagu (1988) reported that thiamine was required for growth. In this study, the basal medium supplemented with eight vitamins supported the same growth as the vitamin-free basal medium (Table 5). These results suggest that an exogenous supply of vitamins is not absolutely required for the growth of *P. tuber-regium*. However, thiamine and pyridoxine can improve growth. Lilly and Barnett (1957) observed that some fungi synthesise the vitamins they require for growth. Perhaps *P. tuber-regium* is in this category.

Gibberellic acid (1 and 10 ppm) and 2,4-D (10 ppm) promoted the greatest growth while 0·1 ppm GA supported the least growth among the three phytohormones tested (Table 6). This result is similar to that reported by Kurancowa (1963) and Voltz (1972). NAA (0·1 and 10 ppm) slightly suppressed growth (Table 6). Gibberellin and IAA at 10^{-5}–10^{-4} M have been employed in increasing mycelial growth and fruitbody production of *A. bisporus*, while higher and lower concentrations were found to be either ineffective or inhibitory (Hayes, 1981).

In this study, the vegetative growth of *P. tuber-regium* was greatly improved by carbon and organic-nitrogen sources, and the C : N ratio 1 : 4 was the best. However, since growths at 1 : 4 and 4 : 1 were not statistically different and composts with high C : N ratio are cheaper than those with a low C : N ratio, *P. tuber-regium* can be profitably cultivated commercially on composts having a C : N ratio of 4 : 1. Yeast extract can be incorporated in the mycological media for the multiplication of mycelia in spawn production. It is also necessary to analyse ligno-cellulosic wastes to ensure that prepared composts are not deficient in Ca, K, Cu, and Zn, which are essential for the growth of *P. tuber-regium*.

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