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GROWTH CHARACTERISTICS OF *COPRINUS COMATUS*

Botany

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ABSTRACT

BACKGROUND: Agarics, commonly known as gill- fungi, mushroom or toadstools produce conspicuous basidiocarps. Agarics may be edible, poisonous or unpalatable. Edible agarics are commonly known as mushrooms and poisonous ones the "toad stools". They are mostly saprophytic, growing commonly in lawns, pasture and gardens.
AIM: To study the growth characteristics of *Coprinus comatus.*
OBJECTIVE: To investigate the growth performance of *Coprinus comatus* under conditions of different nutritional sources, inorganic salts, growth factors, pH and temperature.
METHODS: In the present study the growth characteristics of edible mushrooms *Coprinus comatus* was studied under conditions of different carbon and nitrogen, inorganic salts, temperature and pH.
RESULTS: The results revealed that among different carbon sources sucrose, fructose, maltose, lactose and glucose favoured maximum mycelia growth rate. Soybean meal, bran and yeast extract were found to be the best nitrogen sources which favoured maximum growth rate of mycelium. The three inorganic salts KH_2PO_4, MgSO4 and KNO3 promoted high mycelia growth rate and vigority. The vitamins and natural components promoted high mycelia growth rate. The optimum temperature and pH for maximum growth rate were found to be 30^0C and 8.0 respectively.
CONCLUSION: *Coprinus comatus* exhibited luxuriant mycelia growth in solid media supplemented with monosaccharide and disaccharides as carbon sources, organic nitrogen sources like Soybean meal, bran and yeast extract, and growth factors (Vitamin C, Vitamin B1and Vitamin E) and natural components of plant origin at temperature 30^0C and pH 8.0.

KEYWORDS

Mycelial growth, *Coprinus comatus*, Carbon and Nitrogen sources, Growth factors, Vitamins

INTRODUCTION

Coprinus comatus, commonly known as Shaggy Ink cap is a large conspicuous edible mushroom which occurs naturally in meadows, woods and roadside vergers all over the world[1]. They deliquesce from the bottom of the cap upwards and eventually turn into black ink. *Coprinus comatus* contains (1→3)-β-glucan[2] and possesses anti-oxidant activity[3].This is a delicious and nutritious agaric[4]. The optimum temperature for the growth of this fungus is 25^0C[5]. Dong *et al*[6] have successfully grow *C. comatus* in liquid medium consisting of sucrose 3%, wheat bran 4%, KH_2PO_4 0.1% and $MgSO_4.7H_2O$ 0.05% and at pH 8.0. Two methods of cultivation of *C. comatus* have been designed viz. bag cultures[7, 8] and bed cultures[8]. The substrates used for its cultivation include cotton waste, corn cobs, rice straw, urea, ox manure, lime etc[9]. In the present investigation the effect of physic-chemical parameters on growth characteristics of *Coprinus comatus* was studied.

MATERIALS AND METHODS

Coprinus comatus was cultured in malt yeast extract agar medium. The spawn was prepared and planted in well prepared culture media in the laboratory.

Culture media preparation: Five different culture media were prepared for laboratory cultivation of *C. comatus*. The composition of these media was as follows:

Medium A: yeast extract 2 g, KH_2PO_4 1 g, $MgSO_4$ 0.5 g, agar 20 g, distilled water 1000 mL; **Medium B:** glucose 20 g, KH_2PO_4 1 g, $MgSO_4$ 0.5 g, agar 20 g, distilled water 1000 mL; **Medium C:** glucose 20 g, yeast extract 2 g, VB1100 mg, agar 20 g, distilled water 1000 mL; **Medium D:** glucose 20 g, yeast extract 2 g, agar 20 g, $MgSO_4$ 0.5 g, KH_2PO_4 1 g, distilled water 1000 mL; **Medium E:** potato 200 g, sugar 20 g, soybean meal 2 g, KH_2PO_4 0.5 g, agar30 g, distilled water 1000 mL.

Effect of Carbon nitrogen sources: The PDA (Potato Dextrose Agar) medium was used for the present work. This medium consisted of potato 200 g, glucose 20 g, agar 20 g, pH neutral (7.0) per litre of distilled water. When mycelia had grown all over Petri dishes, it was punched into 5 mm homogeneous pieces at the periphery of colonies by the puncher. These pieces of mycelia were used for the next experiment. In order to study the effect of carbon sources on growth 2% of different carbon sources viz., glucose, sugar, maltose, starch, lactose, CMC–Na, fructose was added individually to the basal medium A. A medium containing no carbon source was used as a control. These media were autoclaved at 121°C for 30 min. Mycelium was inoculated into the test medium under aseptic operation. Culture dishes were inoculated and incubated at 25°C in a BOD incubator. All experiments were performed in sextuple. The diameter of colonies was measured every 24 hours and the mycelia growth was observed until the mycelium completely covered the Petri dishes[10]. In order to study the effect of nitrogen source on growth performance 2% addition level of yeast extract, peptone, beef extract, carbamide, KNO_3, bran, soybean meal was added individually to the basal medium B. No added medium served as a control.

Effect of inorganic salt and growth factor on growth performance: The effect of inorganic salts on growth performance was studied in the medium B. Three different concentrations viz., 0.5%, 1% and 1.5% were set for the three inorganic salts (KH_2PO_4, $MgSO_4$, KNO_3).Similarly, the effect of growth factors like Vitamin C, Vitamin B1 and Vitamin E, natural components (potato, bean sprout, malt extract, hay, mushroom, corn) on growth performance was evaluated. About 20 g/L of the natural components was extracted with distilled water and was individually added to the basal medium C. Unsupplemented basal medium served as control[11].

Single factor test of temperature and pH: The culture of *Coprinus comatus* was incubated for 10 days at 5 different temperature viz., 15^0C, 20^0C, 25^0C, 30^0C and 35^0C in a BOD incubator. Medium E was used as the basal medium. The effect of different pH (5.0-8.5) on the growth of *C. Comatus* was studied in the basal mediumE at 25°C[12].

The results obtained have been presented in Table1- 3.

Table-1: Effect of different carbon and nitrogen sources on mycelia growth of Coprinus comatus (Mycelial growth in mm/d of average value of six replicates ±SE)

Carbon sources	Mycelial growth rate in mm/d Vigority		Nitrogen sources	Mycelial growth rate in mm/d	Vigority
Sucrose	4.85±0.26	+++	Soybean meal	4.95±0.24	+++
ructose	4.65±0.32	+++	Bran	4.75±0.32	+++
Maltose	4.87±0.17	+++	Yeast extract	4.81±0.15	+++
Lactose	4.25±0.21	+++	KNO_3	4.97±0.23	+++
Glucose	4.35±0.24	+++	Peptone	1.65±0.14	

CMC-cellulose	3.75±0.23	++	Beef extract	1.95±0.13	++
Starch	2.67±0.24	+	Carbamide	0.15±0.07	+

Table-2: Effect of inorganic salts, growth factors and natural products on growth performance *Coprinus comatus* (Mycelial growth in mm/d of average value of six replicates ±SE)

Salt	Concentration (%)	Growth in mm/d Vigor		Growth factors and natural products	Growth in mm/d Vigor	
KH_2PO_4	0.5	4.35±0.31	+++	Vitamin C	3.98±0.18	+++
	1.0	4.45±0.41	+++	Vitamin B1	3.95±0.29	+++
	1.5	4.75±0.31	+++	Vitamin E	4.15±0.27	+++
$MgSO_4$	0.5	4.25±0.27	+++	Potato	4.17±0.31	+++
	1.0	4.75±0.28	+++	Bean sprout	4.25±0.41	+++
	1.5	4.95±0.15	+++	Malt extract	4.31±0.24	+++
KNO_3	0.5	3.55±0.17	+++	Hay	4.17±0.23	+++
	1.0	3.75±0.19	+++	Mushroom	4.47±0.14	+++
	1.5	4.21±0.23	+++	Corn	4.35±0.16	+++

Table-3: Effect of different temperature and pH on growth performance of Coprinus comatus (Mycelial growth in mm/d of average value of six replicates ±SE)

Temperature ^{0}C	Growth in mm/d	Vigor	pH	Growth in mm/d	Vigor
15	1.65±0.17	+	5.0	2.12±0.18	++
20	3.75±0.19	++	6.0	3.21±0.29	++
25	3.85±0.17	++	7.0	4.37±0.27	+++
30	4.77±0.32	+++	8.0	4.85±0.31	+++
35	3.55±0.21	++	8.5	4.35±0.41	+++

Degree of vigourity is represented by + sign
+++ = high mycelia growth
++ = intermediate growth
+ = poor mycelia growth

RESULTS

From the results (Table-1) it is evident that Sucrose, fructose, maltose, lactose and glucose favoured maximum mycelia growth rate in the range of 4.25 mm/d to 4.87 mm/d. The mycelial vigority was high in all these soluble sugars. Among insoluble carbohydrates the CMC-cellulose favoured growth rate of mycelia to 3.75 mm/d. The growth rate of mycelia was less in media supplemented with starch (2.67 mm/d). The mycelia vigority was intermediate in CMC-cellulose but poor in media supplemented with starch.

Among seven different nitrogen sources Soybean meal, bran and yeast extract favoured maximum growth rate of mycelium. The mycelia growth rate was moderate in media supplemented with KNO_3 and Beef extract, but poor in presence of peptone and carbamide. *Coprinus comatus* exhibited mycelia growth rate in the range of 4.75 mm/d to 4.95 mm/d. The mycelial vigority was high in media supplemented with Soybean meal, Bran and Yeast extract (3+). KNO_3 and Beef extract favoured poor growth rate (+). Peptone and carbamide promoted very poor mycelia growth rate.

The effect of three different concentrations of inorganic salts viz. KH_2PO_4, $MgSO_4$ and KNO_3, three growth factors and six natural components on mycelia growth of *Coprinus comatus* was investigated (Table-2). From the results it is evident that all the three inorganic salts promoted high mycelia growth rate and vigority. The optimum mycelial growth rate was achieved at 1.5 % KH_2PO_4, $MgSO_4$ and KNO_3. However, growth rate was slightly less in media supplemented with KNO_3 in comparison with other two salts.

The effect of three vitamins viz. Vitamin C, Vitamin B1 and Vitamin E and six natural components on the growth rate of three edible mushrooms was studied. The results revealed that all the vitamins and natural components promoted high mycelia growth rate in *C. comatus.* The mycelia growth vigour was also high with these vitamins and natural components.

Effect of temperature and pH on mycelia growth rate of *Coprinus comatus* has been presented in Table-3.The growth rate of mycelium was poor at 15^{0}C (1.65 mm/d). The growth rate increased with increasing incubation temperature. The optimum temperature for maximum growth rate was 30^{0}C. The growth rate declined to 3.55 mm/d at 35^{0}C.

The growth rate of mycelium was poor (2.12±0.18 mm/d) at pH 5.0. The optimum pH was found to be 8.0 at which the growth rate of mycelium was high (4.85±0.31 mm/d).

DISCUSSION

Edible mushrooms possess the ability to utilize a wide spectrum of nutrients as sources of energy. Being nutritional heterotrophs, they obtain their carbon requirements from organic carbon sources, including various carbohydrate and non-carbohydrate compounds. With an effective battery of enzymes, they are able to utilize a wide array of such substances with high degree of efficiency.

Fungi exhibit carbon heterotrophy and obtain their carbon-requirement from various organic sources. In the present investigation it was found that the disaccharide sugars viz., sucrose, maltose, lactose and monosaccharide sugar glucose and fructose favoured highest growth rate in comparison to polysaccharides like CMC-cellulose and starch. It can, therefore, be concluded that disaccharides and monosaccharide's are more easily utilizable sources of carbon than the polysaccharides. The present findings gain support from the work of Xiao Yu ZHANG *et al*[13] who have studied the effect of different carbon sources, on the growth rate of a mushroom *Auricularia villosula* and observed a more or less similar results.

Nitrogen is also used both for functional as well as structural purposes by fungi. It was observed that the soybean meal, bran and yeast extract promoted highest mycelia growth rate of *Coprinus comatus* than inorganic nitrogen (KNO_3). The peptone and beef extract were found to promote poor mycelia growth. In presence of carbamide the mycelia growth rate was very poor. In the present investigation it was observed that the growth factors viz., Vitamin C, Vitamin B1 and Vitamin E, and natural components like potato, bean sprout, malt extract, hay, mushroom and corn promoted luxuriant mycelia growth of *C. comatus*. A temperature of 30^{0}C and pH in the range of 8.0-8.0 was found optimal for luxuriant growth of this mushroom. The present findings gain support from the work of Xiao Yu ZHANG *et al*[13] who have studied these parameters on the growth rate of a mushroom *Auricularia villosula* and observed a more or less similar results. Gizaw[14] studied the cultivation yield performance of *Pholitota nameko* on different agro-industrial wastes. Lu *et al*[11] have studied the similar physiological behaviour of wild edible mushroom *Leucocalocybe mongolica.* Zhu *et al*[15]have also studied the similar effect of inorganic salts and growth factors on the mycelia growth of *Morchella esculenta.* Wang Jing[16] investigated the morphological development and domestication of *Auricularia polytricha*. Wang *et al*[17] have studied the cultivation characteristics of four wild species of *Auricularia.* Shim *et al*[10]have studied the cultural conditions for the mycelia growth of *Macrolepiota procera* under similar pattern.

CONCLUSIONS:

It can be concluded that *Coprinus comatus* exhibited luxuriant mycelia growth in solid media supplemented with monosaccharide and disaccharides as carbon sources, organic nitrogen sources like Soybean meal, bran and yeast extract, and growth factors (Vitamin C, Vitamin B1 Vitamin E) and natural components of plant origin at temperature 30^{0}C and pH 8.0. Nitrate-nitrogen (KNO_3), peptone, beef extract and carbamide are less assimilable.

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