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Nutritional, Temperature and pH Requirements for the Mycelial Growth of Black Poplar Mushroom, *Agrocybe aegerita*

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Influences of nutrition, pH and temperature requirements on the mycelial growth of black poplar mushroom *Agrocybe aegerita* were studied *in vitro*. Good mycelial biomass was obtained at 25°C and pH of 7. Upon six carbon and nine nitrogen sources tested, sucrose and peptone proved the best carbon and nitrogen respectively. The best carbon and nitrogen sources at 3:3 ratio of sucrose: peptone was found to increase the mycelial biomass in liquid medium.

Key words: *Agrocybe aegerita*, black poplar mushroom, pH, temperature, carbon, nitrogen sources

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Agrocybe aegerita is widely known as black poplar mushroom and grows often in clusters on stumps in the temperate regions of South Eastern United States and Southern Europe. This mushroom occurs naturally on hard woods such as cotton woods, willows, poplars, maples, box elders and tea oil trees (Stamets, 1993). Though this mushroom is found to have very good medicinal and antimicrobial properties such as anti-tumor (Kiho *et al*., 1989), hypoglycemic (Kiho *et al*., 1994), lipid peroxidation inhibitory activities (Lee *et al*., 1998), its biological efficiency is vey low (Patrick *et al.,* 2005). The exploitation and domestication of new edible fungus like *Agrocybe aegerita* is difficult as the environmental and physiological factors required for its cultivation are unknown and still in the infant stage (Suman and Sharma, 2007). Also very limited work has been done on this mushroom. In India, Sharma and Sharma (2009) attempted the cultivation of *A. aegerita* in saw dust supplemented with wheat bran.

However, the cultivation packages have to be standardised for commercial cultivation of this medicinal mushroom. Therefore, the present study was aimed for preliminary information on the physiological and nutritional requirements for the growth of *A.aegerita* for large scale cultivation of this mushroom.

Materials and Methods

Effect of temperature on biomass production of A.aegerita

The Yeast extract broth (Yeast extract, 2.5 g; KH_2PO_4, 0.05 g; $MgSO_4.7H_2O$, 0.05 g; FeSO4, 0.01 g; $KN0_3$, 1.55 g and 1000 cm_3 of de-ionised water) was used as basal medium. A mycelial disc of 9 mm diameter of *A. aegerita* was inoculated into

basal medium (25 ml) in conical flasks. The flasks were incubated at different temperatures (5°, 10°, 15°, 20°, 25°and 30°C) for 15 days. Five replications were maintained. After the incubation period, the mycelial mats were harvested, oven dried and weighed to get the biomass production (Kadiri, 1998).

Effect of pH on biomass production of A.aegerita

The basal medium, Yeast extract broth was prepared and adjusted to different pH (4, 5, 6, 7, 8, 9) levels. A mycelial disc of 9 mm diameter of *A. aegerita* was inoculated in to basal medium (25 ml) in conical flasks with different pH. The flasks were incubated for 15 days. Five replications were maintained. After the incubation period, the mycelial mats were harvested, oven dried and weighed to get the biomass production (Kadiri, 1998).

Effect of carbon compounds on biomass production of A.aegerita

The yeast extract broth used as basal medium was supplemented separately with one percent carbon (w/v). The carbon compounds viz*.,* glucose, fructose, dextrose, sucrose, cellulose and maltose were used. The medium without any carbon source served as the control. Five replications were maintained. After the incubation period, the mycelial mats were harvested, oven dried and weighed to get the biomass production (Chandra and Purkayastha, 1977).

Effect of nitrogen sources on biomass production of A.aegerita

The basal medium was made up of KH_2P0_4, 0.5 g; $MgS0_4.7H_20$, 0.5 g; glucose, 10 g; thiamine hydrochloride 0.5 mg and 1000 cm_3 of de-ionised water. The nitrogen sources *viz.*, peptone, urea, yeast extract, malt extract, casein, ammonium

chloride, calcium nitrate, sodium nitrate and ammonium per sulphate were added separately at the rate of 2 g/litre of medium.

The basal medium without nitrogen source served as the control. Five replications were maintained. After the incubation period, the mycelial mats were harvested, oven dried and weighed to get the biomass production (Fasidi and Olorunmaiye, 1994).

Effect of different carbon: nitrogen ratios on biomass production of A.aegerita

The basal medium used was the same as for the nitrogen sources but without glucose. The best performing carbon and nitrogen sources in the last two experiments, i.e. sucrose and yeast extract (0.1 g of each), were supplemented in the one litre of basal medium; this formed a ratio of 1:1. Similarly other ratios (1:2, 1:3, 2:1, 2:2, 2:3, 3:1, 3:2, 3:3) were also prepared to form different concentrations. Five replications were maintained. After the incubation period, the mycelial mats were harvested, oven dried and weighed to get the biomass production (Fasidi and Olorunmaiye, 1994).

Results and Discussion

Temperature and pH are found to be an important environmental factor that controls the growth of most microorganisms and specific pH is required for each fungus for effective utilisation of the substrates. Most mushrooms display best vegetative growth slightly on acidic pH (6.5-6.8) which varies between different species and strains. (Philip and Shu-ting

Table 1. Effect of temperature and pH on the biomass production of *A. aegerita*

Temperature (°C)	Mycelial dry weight of *A.aegerita* (mg/25 ml)	Different pH levels	Mycelial dry weight of *A.aegerita*(mg/ 25 ml)
5	04.50_{f}	4	19.16_{d}
10	22.01_{d}	5	32.50_{c}
15	52.85_{c}	6	64.85_{b}
20	78.30_{b}	7	84.05_{a}
25	84.50_{a}	8	12.55_{e}
30	14.20_{e}	9	4.98_{f}
SE(d)	1.0755	SE(d)	0.9642
CD(P=0.05)	2.2197	CD(P=0.05)	1.9901

Mean of five replications
Means followed by common letter are not significantly different at 5% level by DMRT

chang ,1997). The role of temperature is correlated with enzyme activity and thereby leads to better utilisation of lignocellulosic substrates (Chang and Quimio, 1989). The present study conducted on the pH requirements of *A.aegerita* showed that the maximum dry mycelial weight was obtained in a pH of 7 (84.05 mg/25ml) followed by pH of 6 (64.85mg/ 25ml). Similarly, Song Ai Rong and Liutong Bao (2004) observed better growth at a pH range of 5-7. The studies on the temperature requirement of *A.aegerita* showed that maximum dry mycelial weight (84.50 mg/25ml) was produced at a temperature of 25°C followed by temperature of 20°C (78.30 mg/ 25ml). Least mycelial growth was observed at temperature of 5°C and pH of 4 (Table 1). The results are in agreement with the findings of Sharma and Satish Kumar (2006) and Zervakis *et al* (2001) reported that temperature of 25°C is best suited for the growth of *A. Aegerita*.

Many kinds of mushrooms frequently require carbon and nitrogen for their growth as nutrition. Hossein vahidi *et al*. (2006) reported that in *Gymnopilus spectabilis*, addition of sucrose in the medium increased the antifungal inhibition capacity. A well balanced carbon to nitrogen ratio enhances the growth and development of mushrooms while an imbalance of C: N impedes their growth (Okhuoya *et al*., 2000). The present study on carbon and nitrogen requirements of *A. aegerita* revealed that sucrose as best carbon source produced maximum dry mycelial weight (64.54 mg) of *A. aegerita* (Table 2). Similarly, peptone as best nitrogen source produced

Table 2. Effect of carbon sources on the biomass production of *A. aegerita*

Carbon source	Mycelial dry weight of *A.aegerita* (mg/25ml)
Sucrose	64.54_{a}
Fructose	61.30_{c}
Glucose	62.42_{b}
Dextrose	49.59_{d}
Cellulose	40.18_{e}
Maltose	30.09_{f}
Control	28.07_{g}
SE(d)	1.1939
CD(P=0.05)	2.4456

Mean of five replications
Means followed by common letter are not significantly different at 5% level by DMRT

maximum dry mycelial weight (97.12 mg) of *A. aegerita* (Table 3) . Zadrazil (1994) stated that the better mycelium growth of *A. aegerita* can be

Table 3. Effect of nitrogen sources on the biomass production of *A. aegerita*

Nitrogen sources	Mycelial dry weight of *A.aegerita* (25mg/ml)
Ammonium chloride	15.63_{i}
Calcium nitrate	78.54_{b}
Sodium nitrate	21.08_{g}
Ammonium per sulphate	10.09_{j}
Yeast extract	65.68_{c}
Peptone	97.12_{a}
Urea	22.54_{f}
Casein	46.83_{d}
Malt extract	37.43_{e}
Control	20.19_{h}
SE(d)	1.1152
CD(P=0.05)	2.2539

Mean of five replications
Means followed by common letter are not significantly different at 5% level by DMRT

obtained by increasing inorganic nitrogen content or by addition of protein-rich additives in the substrate. Nutrients such as Glucose and starch as better carbon sources and peptone as best nitrogen source supported the growth of *Volvariella volvacea* (Ofosu Asiedu *et al.,* 1984) where as Sodium nitrate and casein were the best nitrogen sources for the growth of *Lepiota procera* (Jonathan and Fasidi,2001) . Banerjee and Samajpati (1989) also showed that soluble starch and cellulose were

the best carbon sources for optimum production of *V.volvacea* . Experiments on the mycelial growth of *A*. *aegerita* on carbon:nitrogen sources showed that maximum mycelial dry weight (70.97 mg) was obtained in 3:3 ratio of carbon:nitrogen followed by 3:2 (52.42 mg). Control without any carbon and nitrogen sources recorded least mycelial growth of 20.55 mg (Table 4). Nirod Chandra Sarker *et al*.

Table 4. Effect of carbon: nitrogen (sucrose: peptone) ratios on the biomass production of *A. aegerita*

Carbon :Nitrogen source	Mycelial dry weight of *A.aegerita* (mg/25ml)
1:1	23.05$_i$
1:2	25.58$_h$
1:3	41.14$_d$
2:1	30.38$_g$
2:2	30.84$_f$
2:3	45.67$_c$
3:1	35.19$_e$
3:2	52.42$_b$
3:3	70.97$_a$
Control	20.55$_j$
SE(d)	0.7924
CD(P=0.05)	1.6015

Mean of five replications
Means followed by common letter are not significantly different at 5% level by DMRT

(2008) reported that the mycelial growth of *Agrocybe aegerita* also increased with increase in C: N ratio. This showed that *A. Aegerita* can effectively utilize carbon and nitrogen in the substrates as evidenced by increase in dry mycelial weight with increase in C: N ratio.

The results obtained from the above studies provide basic information on the temperature, pH, carbon and nitrogen source requirements for maximum production of mycelium. From the above results, it is clear that the black poplar mushroom, *A aegerita* although grows in temperate regions can also be cultivated in subtropical regions as it produces maximum dry mycelial weight at a temperature of 20-25°C and thereby offers scope for commercial cultivation under artificial cultivation methods.

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