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Research Article

PHYSICOCHEMICAL STUDIES ON *LENTINUS TUBERREGIUM* (FR)., A INDIAN EDIBLE FUNGUS

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ABSTRACT

Lentinus tuberregium (Fr)., edible higher fungus is valued for its high nutritional composition. The major limitation to its availability and utilization is that it is seasonal in nature. The fungus was obtained from dead wood and maintained on potato dextrose agar supplemented with 0.5% yeast extract. We observed the effects of temperature, pH, vitamins carbon, nitrogen, vitamins and amino acids followed by different ratio of C:N showed significant increment on biomass of mycelium with the amendment of dextrose, yeast extract, thiamine and glycine. Different rational supplement of dextrose and yeast extract confirmed the effective mycelia formation with 1:3 and 1:5 ratio, optimum growth at 25°C, pH-6.5.

Keywords: Physicochemical - *Lentinus tuberregium*- radial growth – carbon, nitrogen, amino acid, vitamin, metal ions source

INTRODUCTION

Lentinus tuberregium belongs to the Basideomycotina sub-division and to the Agaricaceae family. Basideomycotina are known as mushrooms and their taxonomy has been found out by its macro and micro morphology. The species from Agaricaceae family are saprophyte, causing the degradation of wood and lignin/cellulosic matter. Some of them are popular because of their importance in food industry (*Agaricus cretáceus, Agaricus silvicole, Clitocybe phyllphilla, Collybia longipes, Collybia radicata, Coprinus comatus, Lepiota gracilenta, Omphalia campanella, Panus torulos, Pleurotus columinus, Pleurotus pometi, Tricholoma georgii, Tricholoma jonides, Tricholoma nudum, Tricholoma personatum* and *Lentinus edodes*) All these edible fungi are seasonal; they are not available all year round. It is with this background knowledge of making the fungus available for consumers all through the year that this study underscore.

MATERIALS AND METHODS

Sample collection and preparation

Lentinus tuberregium was collected from the Keeriparai forest of Kanyakumari District, Tamil Nadu, India. The fungal culture was maintained through periodic transfer onto potato dextrose agar (PDA) plates, at 25ºC and pH 6.5± 0.2

Effect of temperature on growth of *Lentinus tuberregium*

To determine optimum temperature for mycelia growth of *Lentinus tuberregium*, the basal medium used in the experiment was prepared as described by[1] . The basal medium contained $FeSO_4$.$7H_2O$ (0.01 g), $MgSO_4$.$7H_2O$ (0.05 g), KH_2PO_4 (0.05 g), KCl (0.05 g), yeast extract (2.50 g), KNO_3 (1.55 g) and D-glucose (10.0 g) in 1000 ml of deionized water. The pH of the medium was adjusted to 6.5. Thirty millilitres of the liquid medium was dispensed into 100 ml flasks and 10 mg/100 ml of streptomycin added to suppress bacteria growth. The flasks were covered with aluminium foil, autoclaved at 121°C for 15 min, inoculated with 9-10 days old actively growing culture of the mushroom using a 7 mm diameter disc and incubated for 28 days at 0, 10, 15, 25, 30, 35, 40, 45 and 50°C. Each treatment was replicated three times. The mycelia produced were harvested by filtration through pre-weighed filter paper. Mycelia dry weight and pH of culture filtrate were determined from three replicates and the average recorded.

Effect of pH on growth of *Lentinus tuberregium*

The pH requirement of this fungus was determined by the mycelia dry weight method of using a liquid medium[1]. Thirty milligram of liquid medium was poured into each of the 250 ml flasks and the medium adjusted to different pH of 4.0, 4.5, 5.0, 5.5, 6.0,6.5, 7.0 and 8.0 with 1 M NaOH or 1 M HCl that was added in drops. Each pH medium was dispensed into 100 ml conical flask and all flasks autoclaved at 121°C for 15 min and 10 mg/100 ml of streptomycin added to suppress bacteria growth. The flasks were then inoculated with 7 mm disc mycelia plug of 9-10 days old actively growing culture of the mushroom. The flasks were incubated at 26±2°C for 28 days. Mycelia dry weight and pH of culture filtrate were determined from three replicates and the average recorded.

Effect of carbon compounds

Each five g of six different carbon sources of dextrose, lactose, sucrose, maltose, mannitol and starch were amended in the basal medium having the pH of 6. The mycelia disc of 5mm was cut from 9-10 days old mycelia mat and these blocks were aseptically inoculated in the flasks including the control flask i.e. basal medium without any carbon source. All the flasks were kept undisturbed for 28 days of incubation. After the incubation period the mycelia mat collected and recorded.

Effect of nitrogen compounds

The following nitrogen sources were selected such as, sodium nitrate calcium nitrate, ammonium nitrate, peptone, yeast extract, beef extract, and urea. Each sources were added 2g. The basal medium containing fructose (10g) KH_2PO_4 (0.5 g), $MgSO_4.7H_2O$ (0.5 g), thiamine hydrochloride (500 µg) and made up to 1 litre with distilled water Chandra *et al* [2] Sterilization, inoculation and assessment of dry weight were carried out as described for the carbon sources above. Basal medium alone was used as control.

Effect of vitamin compounds

Vitamins were selected to this study such as, biotin, ascorbic acid, thiamine, and α-tocoferrol. The basal medium was the same for determination of nitrogen sources. Each vitamin was added (500 µg) to the basal medium and made up to 1 litre. The set up was treated the same way as for carbon, nitrogen and amino acids. Basal medium alone was used as control.

Effect of aminoacid compounds

The selected amino acids of aspartic acid, cysteine, phenyl alanine, tyrosine, methionine, L-glutamic acid, DL-leucine, histidine, L-leucine, tryptophan, DL-dopa, proline, DL-2-amino butric acid, DL-threonine, Isoleucine, hydroxy proline, glycine, DL-valine, L-cysteine, L-lysine mono hydrochloride, DL-serine, L-ornithine mono hydro chloride, L-arginine monohydro chloride, and DL-alanine. The basal medium was used the same as that of nitrogen source. For each amino acid (500 µg) was added to the basal medium and made up to 1 litre and dispensed into the flasks which were treated as described above. Basal medium alone without amino acid was used as control.

Carbon :Nitrogen ratio

The basal medium was similar to that used for nitrogen compounds but glucose was varied composition with yeast extract as sources of

C/N. Concentration of 0.15 g/litre of dextrose and yeast extract in the basal medium serve as 1:1 ratio[3]. Other ratio was prepared proportionately i.e., 1:1, 1:3, 1:5 and 5:1, 3:1.

RESULTS AND DISCUSSION

The result obtained in this study revealed that the best radial mycelia extension (96.12 mm) was observed at 25°C among all the temperature tested (Table 1). This is the optimum temperature for the growth of *L.tuberregium*. There was considerable growth at 30°C, but growth was minimal at 40°C, respectively. Temperature is found to be an important environmental factor that controls the growth of most microorganisms Fasidi IO [4] eported that *Volvariella esculenta* was able to tolerate temperature range of 20-40°C. At low temperature (0 and 10°C), growth of *L.tuberregium* was not observed. Also, the growth of the mushroom was inhibited at 45 and 50°C (Table 1).

Table 1: Effect of temperature on radial mycelial extension of *L. tuberregium*

Temperature (°C)	Mycelia dry weight(mg/30 cm3), mean of three replicates
0	0.00
10	0.00
15	0.03
25	96.12
30	89.45
35	76.94
40	21.74
45	15.79
50	0.00

Analysed on dry weight basis, (mean ±SD)

The optimum temperature of 30°C reported for *V. volvacea* agree with this report[5]. The optimum pH of growth of *L.tuberregium* was found to be 6.5 where the highest vegetative growth was observed (81.12 mg/30 cm3) Fasidi IO [5] reported the pH range for the growth of *V. Volvacea* to be 5.5-8.5, while ,Fasidi IO [4] obtained an optimal pH range of 5-7 for *Pleurotus tuberregium.* The growth of 73.69 mg/30 cm3, which was the second best, was recorded in the slightly acidic medium of 5.5 while the least growth (14.57 mg/30 cm3) was observed in the acidic medium of 4.0 (Table 2).

Table 2: Effect of pH on vegetative growth of *L. tuberregium*

pH	Mycelia dry weight (mg/30 cm3), mean of three replicates	Final pH of culture filtrate
4.0	14.57	3.68
4.5	19.86	3.77
5.0	26.23	4.23
5.5	73.69	4.57
6.0	69.36	4.96
6.5	81.12	5.33
7.0	62.18	5.97
8.0	22.90	6.29

Analysed on dry weight basis, (mean ±SD)

L. tuberregium shows different preferences for carbon sources for its metabolism. According to[4], the ability of an organism to utilize the carbohydrate depends on type of enzyme produced by the organism. In this study, dextrose was best source of carbon for this mushroom (Table-3).

Analysed on dry weight basis, (mean ±SD)

This shows that *L.tuber-regium* produces enzymes that utilize dextrose better than any other carbon source. Ofosu-Asiedu A.O *et al* [6] also reported that *Volvoriella volvacea* utilizes glucose and starch better than other carbon sources. Kadiri *et al* [7] obtained more growth of *V. volvacea* with glucose than starch. Luo *et al* [8] also reported that fructose, glucose and maltose were the most suitable carbon sources for *Auricularia auricular*. Kadiri *et al* [9] reported that the best utilizable carbon sources for *Lentinus subnudus* were fructose, maltose, dextrin and glucose. This study showed that *L.tuber-regium* utilizes dextrose better than maltose, manitol, lactose and starch. The least carbon sources were lactose. Hammond *et al* [10] reported that glucose has been good respiratory substrate. *L.tuber-regium* utilises organic nitrogen better than inorganic nitrogen (Table-4).

Table 3: Effect of different carbon source on vegetative growth of *L.tuberregium*

Carbon sources	Mycelia dry weight (mg/30 cm3), mean of three replicates	Final pH of culture filtrate
Glucose	92.88	6.22
Fructose	69.56	6.98
Lactose	30.28	6.51
Dextrose	97.45	5.98
Maltose	89.90	6.71
Soluble starch	81.12	5.67
Glycerol	32.26	6.45
D – Sorbitol	71.14	6.23
Mannitol	77.63	5.97
Xylose	45.32	5.54

Table 4: **Effect of different nitrogen source on vegetative growth of *L. tuberregium***

Nitrogen sources	Mycelia dry weight (mg/30 cm3), mean of three replicates	Final pH of culture filtrate
Malt Extract	61.54	6.35
Beef Extract	64.56	5.81
Yeast Extract	68.23	5.90
Soya bean casein	51.22	6.89
Hiveg peptone	56.43	6.58
Sodium Nitrate	23.26	5.51
Potassium Nitrate	27.45	4.40
Casein	37.49	5.90
Urea	31.12	5.98
Ammonium Molybdate	21.36	5.72

Analysed on dry weight basis, (mean ±SD)

This observation is in line with the report of Fasidi *et al* [4] who observed that yeast extract which is a complex nitrogen source sustained the greatest growth of *P. tuberregium*. Kadiri *et al* [9] reported peptone as the best nitrogen source for *L. subnudus.* Kuforiji *et al* [11] also reported that *V. volvacea* frequently responds better to organic nitrogen than inorganic nitrogen. Ofosu-Asiedu *et al* [6] reported that the best yield of *Volvoriella* was obtained on media containing peptone or potassium nitrate. In the same vein Luo *et al* [8] reported that organic nitrogen sources such as yeast extract and peptone are the preferred nitrogen sources for *A. auricular.* In this study, *L.tuber-regium* showed preference for organic nitrogen than inorganic nitrogen. Thiamine proved best among the vitamins followed by biotin and tocoferrol (Table-5).

According to Lander *et al* [12], who found that thiamine stimulates mycelial growth of *Cercospora arachidicola* in liquid culture. Madunagu *et al* [13] also reported that thiamine is required for good growth in mushrooms. Luo *et al* [8] reported that, different vitamins produce different effects on mycelial growth within a certain concentration range Nolan *et al* [14] reported that combined amino acids stimulate greater growth than single amino acids. The least effective vitamin in this study was ascorbic acid. Fasidi *et al* [9] who reported that ascorbic acid, folic acid and riboflavin did not support good growth of *P. tuberregium*. Glycine proved to be the best amino acid,(Table-6)

Table 5: Effect of different vitamin source on vegetative growth of *L. tuberregium*

Vitamin sources	Mycelia dry weight (mg/30 cm3) mean of three replicates	Final pH of culture filtrate
Thiamin	76.90	5.98
Ascorbic Acid	62.34	6.71
Pyridoxin	71.67	5.67
Biotin	68.78	6.45
Tocoferrol	45.71	5.51
Nicotinic acid	41.12	4.40
Inositol	43.38	5.90
Folic Acid	51.14	5.98
Riboflavin	76.90	5.72

Analysed on dry weight basis, (mean ±SD)

Table 6: Effect of different aminoacid source on vegetative growth of *L. tuberregium*

Aminoacid sources	Mycelia dry weight (mg/30 cm3), mean of three replicates	Final pH of culture filtrate
DL – Aspartic Acid	32.35	6.10
DL - Phenyl Alanine	38.52	6.67
L–Cysteine Monohydrochloride	54.24	5.89
DL – Methionine	23.26	5.59
Leucine	27.45	6.65
DL – nor – Leucine	37.49	5.51
DL – Threonine	31.12	4.40
L – Glutamic Acid	21.36	5.90
L-Ornithine Monohydrochloride	56.18	5.98
DL – Dopa	48.65	5.72
L - Valine	39.89	6.22
DL – 2 Amino – n butyric acid	25.63	6.38
L-Histidine Monohydrochloride	34.44	5.67
L – Hydroxy Proline	24.52	6.34
L – Isoleucine	26.18	5.86
Glycine	58.23	5.18
L – Proline	14.57	5.33
L – Tryptophan	19.86	5.97
L – Lysine Monohydrochloride	26.23	6.35
DL – Serine	27.54	5.81
L-Argenine Monohydrochloride	32.16	5.90
L – Cysteine	42.23	6.89
L – Tyrosine	32.26	6.58
DL - Alanine	24.18	6.45

Analysed on dry weight basis, (mean ±SD)

Table 7: Effect of different carbon:nitrogen ratio on vegetative growth of *L. tuberregium*

Carbon:nitrogen ratio	Mycelia dry weight (mg/30 cm3), mean of three replicates	Final pH of culture filtrate
1:1	14.76	5.70
1:3	18.53	5.96
1:5	39.47	6.62
5:1	68.56	6.43
3:1	41.32	5-90

Analysed on dry weight basis, (mean ±SD)

This is followed by L-ornithine mono hydrochloride. Chandra *et al* [2] ,reported that asparagine and aspartic acid have been employed in increasing the mycelial growth and fruit body production in *Agaricus bisporus.* Hayes *et al* [15] reported that higher and lower concentrations of these amino acids are found to be either ineffective or inhibitory for the mycelial growth of mushrooms. [Stamets *et al* [16] reported that the ratio of carbon to nitrogen (C: N) balance in mushroom substrate is very important. A well balanced carbon to nitrogen ratio enhances the growth and development of mushrooms while an imbalance of C: N impedes their growth[17] . In this study the C: N ratio of 1:3 and 1:5 supported best growth of the mushroom, growth was reduced above or below this levels. Fasidi *et al* [9] also reported C: N ratio of 1:3 and 1:5 for *P. tuberregium*. According[18] over supplementation of mushroom substrates with nitrogen and carbohydrates impedes mycelial growth of mushrooms. As the ratio of C: N increased, the mycelia growth of *L. tuberregium* also increased up to a point after which further increase in carbon decreased the mycelial growth. This work has shown that significant improvement in the mycelia growth of *L.tuberregium* can be attained through cultivation of the fungus on pH 6.5 and temperature of 25°C. Thiamine and riboflavin promoted good vegetative growth dextrose and glucose were the best utilizable carbon sources most suitable for its cultivation. This result may provide a sustainable means of adding value to *L.tuberregium* cultivation which will result in increasing human protein

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