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Studies on the Growth Requirements of *Lentinus tuberregium* (Fr.), An Edible Mushroom

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Abstract: Growth requirements of *Lentinus tuberregium* (Fr.), an Indian edible mushroom as optimized using different carbon, nitrogen, vitamins and aminoacids 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.

Key words: Growth · Optimization · *Lentinus tuberregium* · Edible mushroom

INTRODUCTION

Lentinus tuberregium (Fr), is highly nutritious and compare favourably with other foreign edible species. The cost effectiveness of meat and fish people is turning to mushrooms as an alternative source of protein. Local people collect the mushroom in the wild and consumed as food or used as condiments to add to their food. Mushrooms are used extensively, especially by the local people as food item and for medicinal purposes [1-7]. Inspite of the importance of this mushroom in India it has never been cultivated in Nigeria and else where in the world. The mushroom is usually collected in the wild and consumed when a large collection is made, they are either sun-dried or smoked and then stored for longer use.

In the present work, studies on the requirements for vegetative growth of *L. tuberregium* were carried out. This will serve as a base or provide information on nutrients that can help in the vegetative growth and substrates formulation for cultivation of *L. tuberregium*.

MATERIALS AND METHODS

The fruitbody of *L. tuber-regium* were collected in keeriparai forest Kanyakumari district, Tamil Nadu, India. The culture was maintained on PDA for further investigation. The mycelial growth was determined by a mycelial dry weight method. The basal medium used in this study was that described by [8,9]. The basal medium and supplementary compounds were dissolved in 1 litre of distilled water. 100 ml was dispensed into 250 ml flat bottom flasks and the pH was adjusted to 6. The mouth of each flask was sealed with cotton wool and covered with aluminium foil paper before sterilization. The media after cooling were then inoculated with 5 mm diameter agar block of 7-9 days old mycelium of the mushroom. The flask was incubated at room temperature for 28 days. Each experiment was replicated three times. The mycelium in each flask was filtered through a pre-weighed 9 cm diameter filter paper and dried at 85°C for 10 h and recorded the fresh weight.

Carbon Source: Each five g of six different carbon sources of dextrose, lactose, sucrose, maltose, manitol 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 the results.

Nitrogen Source: 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 [8]. Sterilization, inoculation and assessment of dry weight were carried out as described for the carbon sources above. Basal medium alone was used as control.

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Vitamin Source: Vitamins were selected to this study such as, biotin, ascorbic acid, thiamine and 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 liter. The set up was treated the same way as for carbon, nitrogen and amino acids. Basal medium alone was used as control.

Aminoacid Source: 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 to 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 [9]. Other ratio was prepared proportionately i.e., 1:1, 1:3, 1:5 and 5:1, 3:1.

RESULTS AND DISCUSSION

L. tuberregium shows different preferences for carbon sources for its metabolism. According to [10], 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. This shows that *L.tuber-regium* produces enzymes that utilize dextrose better than any other carbon source. [11] also reported that *Volvoriella volvacea* utilizes glucose and starch better than other carbon sources. [12] obtained more growth of *V. volvacea* with glucose than starch. [13] also reported that fructose, glucose and maltose were the most suitable carbon sources for *Auricularia auricular*. [14] 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. [15] reported that glucose has been good respiratory substrate.

L.tuber-regium utilises organic nitrogen better than inorganic nitrogen. This observation is in line with the report of [9] who observed that yeast extract which is a complex nitrogen source sustained the greatest growth of *P. tuberregium*. [14] reported peptone as the best nitrogen source for *L. subnudus*. [16] also reported that *V. volvacea* frequently responds better to organic nitrogen than inorganic nitrogen. [11] reported that the best yield of *Volvoriella* were obtained on media containing peptone or potassium nitrate. In the same vein, [13] 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 togoferrol. According to [17] who found that thiamine stimulates mycelial growth of *Cercospora arachidicola* in liquid culture. [18], also reported that thiamine is required for good growth in mushrooms. [13] reported that, different vitamins produce different effects on myelial growth within a certain concentration range. [19] reported that combined amino acids stimulate greater growth than single amino acids.The least effective vitamin in this study was ascorbic acid. [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, this is followed by L-ornithine mono hydrochloride. [8], reported that asparagine and aspartic acid have been employed in increasing the mycelial growth and fruit body production in *Agaricus bisporus*. [20] reported that higher and lower concentrations of these amino acids are found to be either ineffective or inhibitory for the mycelial growth of mushrooms.

[21] 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 [22,23]. 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. [9] also reported C: N ratio of 1:3 and 1:5 for *P. tuberregium*. According to [22,23] over-supplementation of mushroom substrates with nitrogen and carbohydrates impedes mycelial growth of mushrooms. As the ratio of C: N increased, the mycelial growth of *L. tuberregium* also increased up to a point after which further increase in carbon decreased the mycelial growth. The same was applicable to nitrogen.

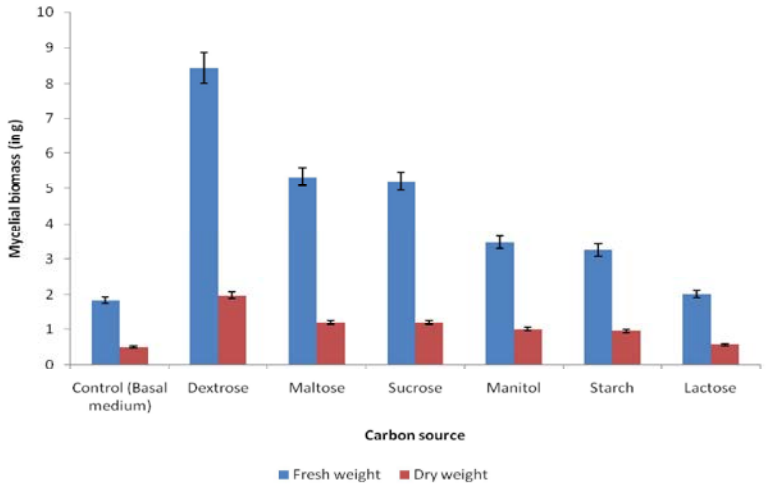

Fig. 1: Growth of *Lentinus tuberregium* on different carbon source

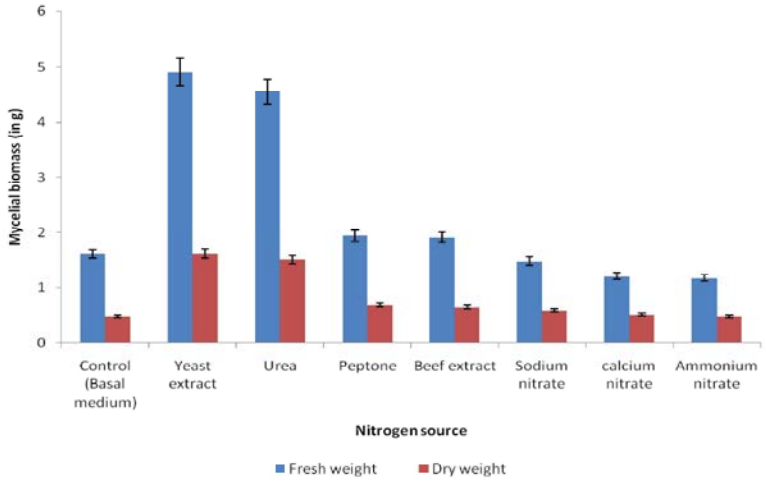

Fig. 2: Growth of *Lentinus tuberregium* on different nitrogen source

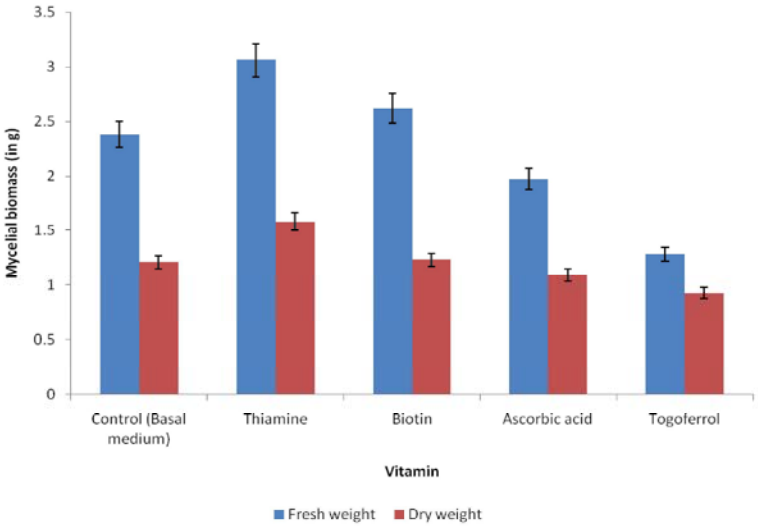

Fig. 3: Growth of *Lentinus tuberregium* on different vitamin source

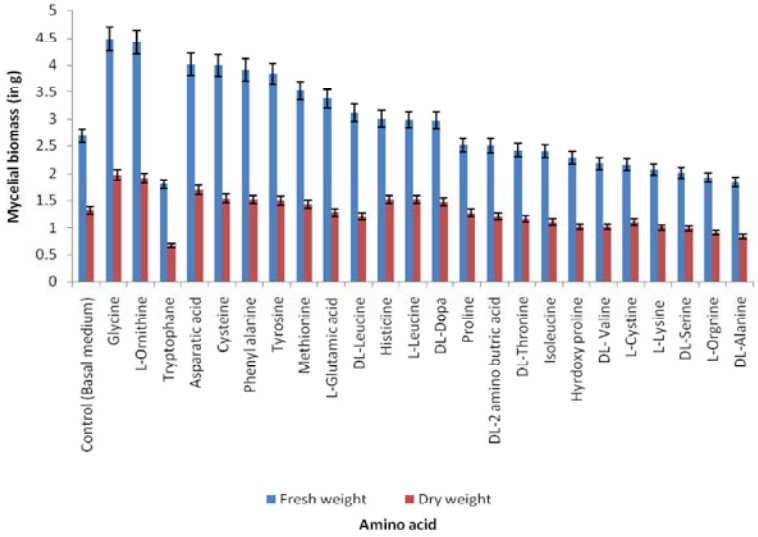

Fig. 4: Growth of *Lentinus tuberregium* on different amino acids source

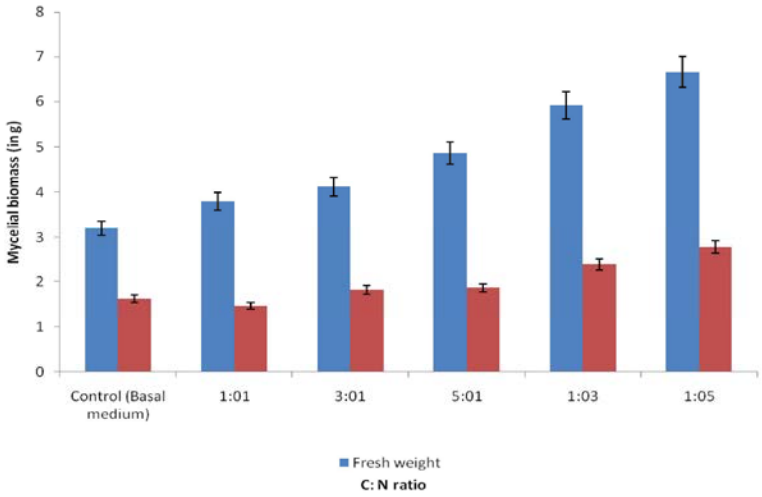

Fig. 5: Growth of *Lentinus tuberregium* on different carbon to nitrogen ratio

In this study, the vegetative growth of *L. tuber-regium* was greatly improved by carbon, nitrogen, vitamins and amino acids. Carbon to nitrogen of 1:3 and 1:5 was best and however, *L.tuber-regium* can be cultivated on substrates containing C: N ratio of 1:3 or 1:5.

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