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Full Length Research Paper

Effect of carbon, nitrogen and trace elements on growth and sporulation of the *Termitomyces striatus* (Beeli) Heim

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Nutritional studies namely carbon, nitrogen and trace element requirements of *Termitomyces striatus* have been carried out. Amongst all the carbon compounds used, the most favourable in order of effectiveness are D (+) glucose, D (+) sucrose, maltose and D (+) raffinose. The fungus showed poor growth with lactose. Sodium nitrite served as the best inorganic nitrogen source for the growth of this fungus. Ammonium acetate, ammonium phosphate, ammonium oxalate, potassium nitrate and sodium nitrate supported fairly good growth of the fungus. Among different amino acids tested, maximum average mycelial dry weight was obtained with L-arginine followed by glycine and DL-tryptophan. The fungus showed poor growth with L-α-amino-n-butyric acid, L-cystine, L-cysteine HCl and DL-serine. The selected concentrations of trace elements also affected the mycelial growth of this fungus to a significant level. There is a gradual increase in growth (average mycelial dry weight) from control to optimum concentration of required trace elements beyond which it decreases. None of the trace elements required for growth are found to be completely fungistatic for the growth of this fungi. The fungus formed asexual conidia similar to that formed in the sporodochial stage in the termite garden of termites of the subfamily *Macrotermitinae*.

Key words: *Termitomyces,* edible mushrooms, cultural studies, C, N nutrition, mycelial growth.

INTRODUCTION

Edible mushrooms are very rich source of various active substances. Nutritional requirements for mushroom mycelium are relatively simple but various nutrient supplements play an essential role in metabolism as their coenzymes. Therefore, mycelial growth is a very much important factor that is responsible for fruiting in mushrooms (Pokhrel and Ohga, 2007; Pokhrel et al., 2009). The species of the paleotropical genus *Termitomyces* Heim are edible agarics, obligately symbiotic with termites belonging to the subfamily, Macrotermitinae. These are usually characterized by the termite association, prominent perforatorium on the pileus and the subterranean pseudorhiza connected to the comb in the termite nest. The sporodochia in the termitarium give rise to basidiome on the onset of rainy season. Review of literature reveals that a very little work has been done on nutritional requirements of this fungus.

Though this fungus is edible in nature and has been used as a food in most part of the world but it cannot be grown under laboratory conditions. This fungus grows only under natural conditions. Despite various *in vitro* investigations, only anamorphs formation has been recorded on some species on routine synthetic media (Botha and Eicker, 1991). For successful commercial cultivation is to determine the nutritional factors that are very much essential for mycelial growth and fruiting. Fruit body formation is influenced by different factors, including genetic make-up of the strain, environmental parameters and the nutrition of the medium (Pascoal et al., 2011). Therefore, studies were initiated to find out the optimum physical factors for the growth and sporulation of *Termitomyces striatus* (Beeli) Heim, to be employed later on for commercial exploitation of this fungus. Further investigations are in progress regarding this.

MATERIALS AND METHODS

Methods of morphological studies

For detailed microscopic examinations, the dried specimen of the

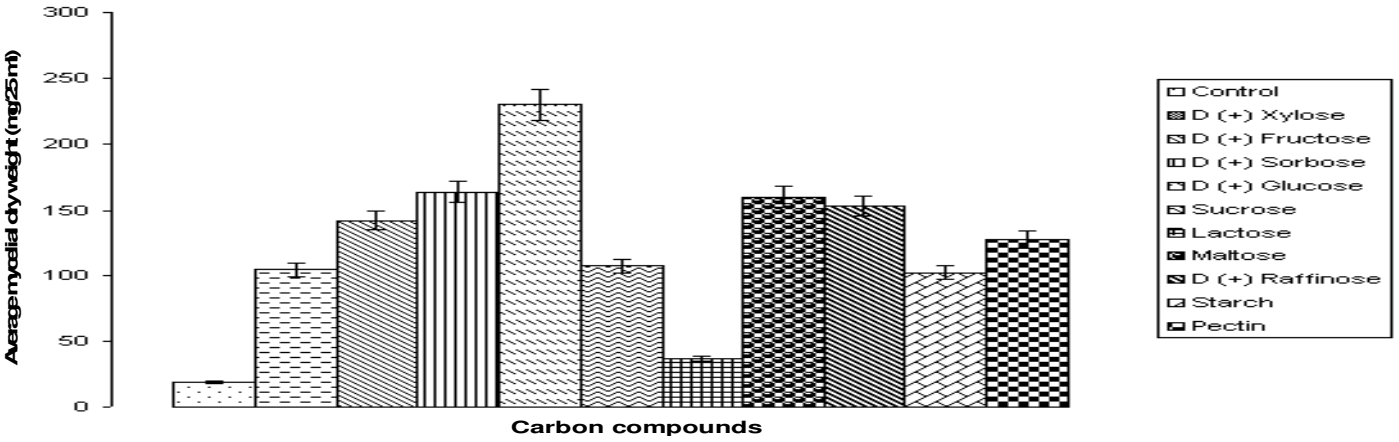

Figure 1. Growth (average mycelial dry wt.-mg/25ml) of *Termitomyces striatus* with different carbon compounds at optimum temperature (32°C), pH (7.0) and days (12) of incubation. S.E.- 1.7490737, S.Ed.- 2.58732716, C.D (at 5% level)-5.11746543.

fungus was revived in 2% KOH for 10 to 15 min. The specimen was microscopically studied from macerations and free hand sections for various structures like basidiospore, basidia and cystidia. For determining the presence or absence of clamps and septation of hyphae, 1% aqueous solutions of Congo red in 5% ammonia and 2% aqueous solutions of phloxine were used. Cotton blue (lactic acid 50% 30 ml, cotton blue 0.05 g) was used to study the cyanophilous nature of the spores or hyphae after Le Gal (1947). To note the amyloidity of the spores, Melzer's reagent (Chloral-hydrate 22.0 g, potassium iodide 1.5 g, iodine 0.5 g, distilled water 20 ml) was used after Singer (1962). For studying the cystidia, sulphobenzaldehyde (water 1.5 ml, pure H_2SO_4, 5.0 ml and benzaldehyde 4.5 ml) was used after Slysh (1960).

Methods of physiological studies

The fungus was isolated from the pileus of the basidiome by single hyphal tip isolate technique and maintained on PDA (Potatoes 200 g, Dextrose 20 g, Agar 15 g and distilled water to make 1 L). Glucose–peptone medium (glucose 10 g, peptone 2 g, KH_2PO_4 1.5 g, $MgSo_4.7H_2O$ 0.5g/L), adjusted to pH 7.0 was used in all the experiments. To study the effect of carbon sources on mycelial growth, the quantity of carbon sources calculated on the basis of carbon present in 10 g of glucose was added to the basal medium but in the experiments on nitrogen sources utilization, the nitrogen sources were added in amount equivalent to that of peptone present in the basal medium.

The solution of each carbon and nitrogen sources was sterilized separately after adjusting to pH 7.0 (neutral) in order to avoid the possibility of their breakdown during autoclaving. Each of the solution was then added aseptically and proportionately to get the normal strength of the basal medium. A medium without carbon and nitrogen sources was used as control in the respective experiments. In the experiments to study the effect of trace elements on growth and sporulation of *T. striatus*, the peptone was replaced by sodium nitrite. Eight trace elements (Fe, Zn, Mn, Ca, Cu, Co, Mo and B) with selected concentrations (0.000001 to 400 ppm) were tested in the experiments on trace element requirements. A medium devoid of trace elements was used as control. The "materials and methods" are same as described by Prasher and Rawla (1988).

An aliquot of 25 ml of the medium was dispensed in each 250 Erlenmeyer flask, plugged and sterilized at 15 lbs psi steam pressure for 15 min. Each flask was seeded with 1 ml of standardized mycelial suspension having 2.5 mg dry weight/ml and incubated at optimum temperature (32°C) for optimum days (13). At the termination of the experiment, the data were recorded on average mycelial dry weight (mg/25 ml), microscopic deficiency symptoms and biochemical deficiency symptoms (in terms of total sugars, soluble proteins and free amino acids) of each treatment.

Statistical analysis

The data on growth of all the experiments were analyzed statistically with respect to dry weight of the mycelium of individual replicate with variables by applying one-way ANOVA (SPSS 11 for windows) in terms of significance and non-significance of the data. The significance is denoted by statistical error (S.E.), statistical error of difference (S.Ed.) and critical difference (C.D.) at 5% level.

RESULTS

The study of Figure 1 reveals that out of 12 carbon sources tested for *T. striatus,* growth response of the fungus was different with various carbon sources. D (+) glucose was found to be best carbon source followed by D (+) sucrose, maltose, D (+) raffinose, D (-) fructose, pectin, sucrose, D (+) xylose and starch. The fungus showed poor growth with lactose. The study of Figures 2 and 3 reveals that inorganic sources of nitrogen had shown better growth of this fungus as compared to organic sources. Among inorganic nitrogen sources, sodium nitrite served as the best nitrogen source for the growth of this fungus. Ammonium acetate, ammonium phosphate, ammonium oxalate, potassium nitrate and sodium nitrate supplemented fairly good growth. L-arginine was the best nitrogen source followed by glycine and Dl-tryptophan. The fungus showed poor growth with L-α-amino-n-butyric acid, L-cystiene HCl, L-Cystine and Dl-serine.

The observation recorded in Figure 4 reveals that all the selected eight trace elements were required for the growth of this fungus. There was gradual increase in growth (average mycelial dry weight) from control to the optimum concentrations of the required elements, beyond

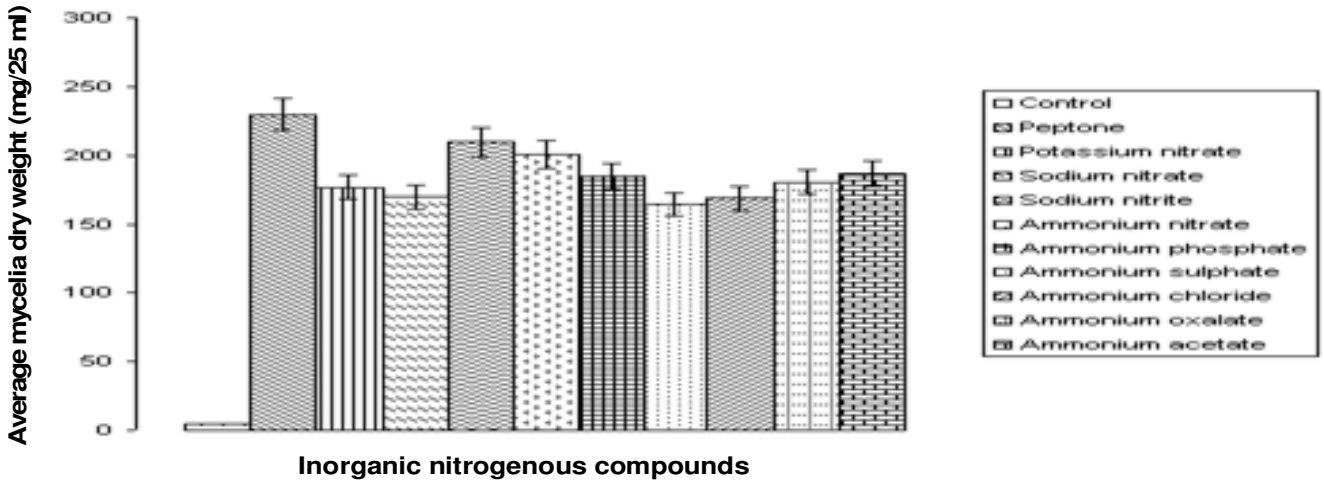

Figure 2. Growth (average mycelial dry weight-mg/25 ml) of *Termitomyces striatus* with different inorganic compounds at optimum temperature (32°C), pH (7.0) and days (12) of incubation. S.E.-1.76876524, S.Ed.-2.32379100, C. D. (at 5% level) -3.50479463.

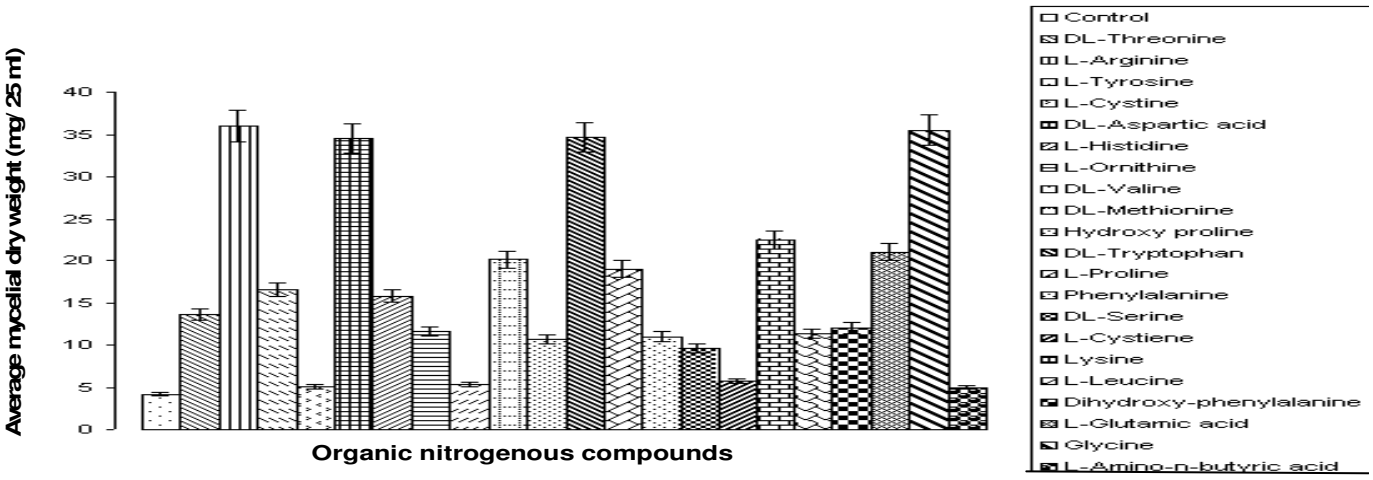

Figure 3. Growth (average mycelial dry weight-mg/25 ml) of *Termitomyces striatus* with different organic compounds at optimum temperature (32°C), pH (7.0) and days (12) of incubation. S.E.-1.4981263, S.Ed.-3.0301891, C.D. (at 5% level)-4.7484701.

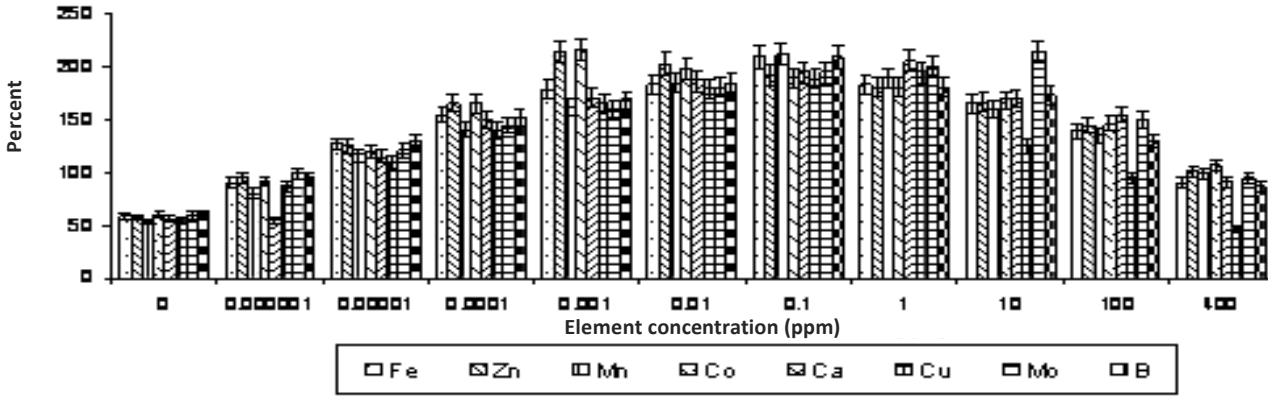

Figure 4. Growth (average mycelial dry wt.-mg/25ml) of *Termitomyces striatus* with selected concentrations of trace elements at its optimum temperature (32°C), pH (7.0) and days (12) of incubation.

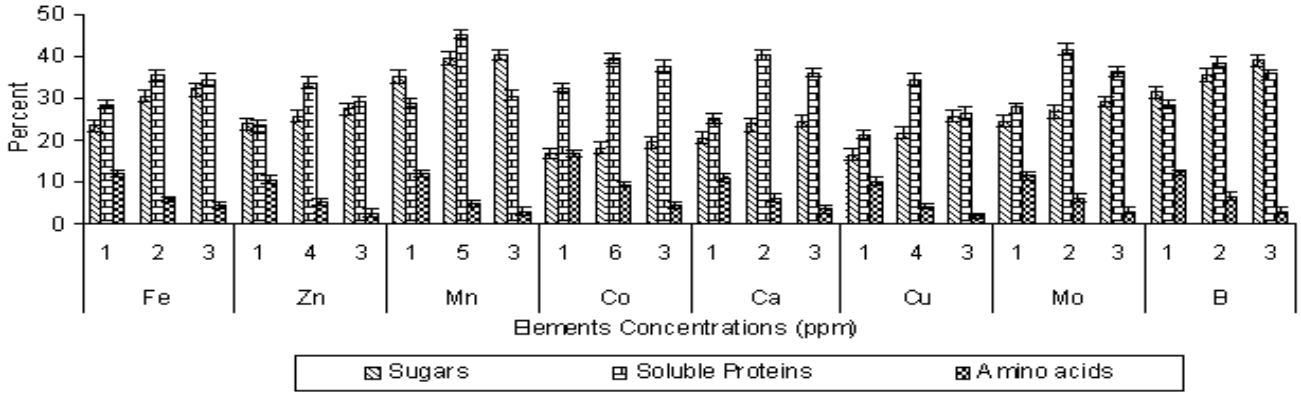

Figure 5. Relationship between the amount of total sugars, soluble proteins, free amino acids and the essential trace elements at their nil, optimum and inhibitory concentrations in the mycelium of *Termitomyces striatus*.

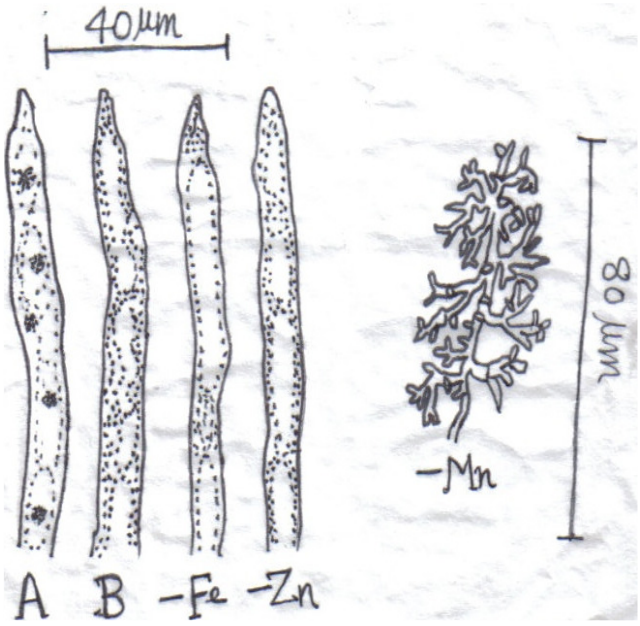

Figure 6. A) Basal medium supplemented with optimum concentration of all trace elements.B) Basal medium not supplemented with optimum concentration of all trace elements.

which it started decreasing. Zn, Co 0.001 ppm; Ca, Cu 1 and Mo 10; Mn, Fe and B 0.1 ppm supported optimum growth of the fungus. None of the trace elements required for the growth was found to be completely fungistatic for this fungus. The study of Figure 5 reveals that the amount of sugars decreased in *T. striatus* in the deficiency of required trace elements. It increased beyond optimum concentrations of the required trace elements. Likewise, amount of total soluble proteins also decreased in deficiency of trace elements. It however, decreased beyond optimum concentrations of trace elements required for growth. The amount of total free amino acids increased in the deficiency of trace elements individually, as well as in control and decreased otherwise. The dry weight of the replicates did not vary significantly in all the experiments. The fungus showed trace element deficiency symptoms in the deficiency of Fe, Zn and Mn (Figure 6). No deficiency symptoms were shown by the fungus at the microscopic level in the deficiency of Ca, Mo, B, Cu and Co. The deficiency of Fe and Zn made the hyphae vacuolated whereas in the deficiency of Mn there was excessive hyphal branching near apex in the fungus.

DISCUSSION

In utilizing glucose, D (+) raffinose, D (-) fructose *T. striatus* resembles *Morchella hybrida* (Sharma, 2003) and *Ustilago esculenta* (Chung and Tzeng, 2004). In utilizing nitrite source of nitrogen, it resembles with edible fungi like *Morchella esculenta* and many other fungi (Morton and MacMillan, 1954; Dutt and Bedi, 1974). Nitrite is found to be toxic for most of the fungi and the poor growth of many fungi in nitrite was attributed to the toxic effect exerted by the pyruvic acid accumulated in the mycelium (Nord and Mull, 1945). But *T. striatus* has shown maximum growth on sodium nitrite. Detailed investigations with certain fungi have previously indicated that hyphal output was maximum at alkaline pH and the fungi attained good growth on nitrite nitrogen if initial pH was adjusted to 6.5-7.5. More growth on alkaline nitrite medium suggests that it is the free unionized acid rather than nitrite ion which is toxic to fungi (Bilgrami and Verma, 1978). Varied utilization of amino acids tested during these investigations is in accordance with many other workers (Bilgrami and Verma, 1978; Sharma, 2003; Jonathan and Fasidi, 2001; Chung and Tzeng, 2004).

In requiring Fe, Zn and Mn, this fungus resembles many other fungi (Prasher and Rawla, 1988; Carlile et al., 2001; Sharma et al., 2005). The fungus shows interesting behaviour in requiring Co becausee no member of

basidiomycota, so far, has been reported to require Co for its growth, except only a few fungi belonging to other groups have been reported to require Co (Madan and Thind, 1979; Singh, 1979; Prasher and Rawla, 1988). The excessive branching in the deficiency of Mn in this fungus indirectly reflects the occurrence of factor/factors promoting, excessive branching which most probably might be concerned with softening of hyphal wall. Vacuolization of the hyphae with age or under stress is a natural phenomenon among fungi. Biochemical changes include a decrease in total sugars, soluble proteins and increase in free amino acids. The decreased sugars and soluble proteins suggest the marked effect on their synthesis and the enzymes concerned in their metabolism. This is further evidenced by the increase in total free amino acids in the trace element deficient cultures.

T. striatus reproduced asexually producing conidia with carbon sources, inorganic nitrogen sources and all the selected trace elements but it has shown nil sporulation with amino acids. Fe, Zn, Mn and Ca are essential for conidial production whereas Boron is promotary for conidial production. The role of Fe, Zn, Mn and Ca in conidial production is in agreement with earlier findings of Prasher and Rawla (1988). The development of anamorph in cultures of *T. striatus* under optimum physical conditions, which have been obtained from basidiome of the fungus in the present study and similar findings of development of anamorph in cultures obtained from basidiome context (Botha and Eicker, 1991) are indicative of the fact that factor/s other than studied during investigations may be required for the stimulation of the basidiome context cultures to revert to the perfect phase. Studies are in progress in this direction.

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