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Copper accumulation & carpophoroids/sclerotial bodies formation at elevated copper level in *Boletus edulis* Bull. ex Fr.

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Abstract: ***During these investigations, studies were carried out to know the cultural characteristics & tolerance of Boletus edulis at different Cu concentration. Remarkably, this edible species has shown tolerance to high concentration of Cu. The fungus was found to require 400 ppm of Cu for optimum growth and also showed tolerance upto 1000 ppm of Cu. The fungus gave optimum growth and production of carpophoroids at 400 ppm and multiple sclerotial bodies at 600 ppm of Cu. Thereafter both of these gradually declined. The fungus did not form any carpophoroids/sclerotial bodies at 1000 ppm of Cu. Therefore, on the positive side, these results indicate that this fungus could be used for the biosorption in Cu contaminated sites as an alternate method to high cost metal absorption technologies being used for eco-restoration. Also, carpophoroids/sclerotial bodies formation at high Cu concentration might be a positive sign to evolve certain methodology for the commercial cultivation of this fungus. Otherwise on the negative side, high Cu tolerance could be limiting factor involving the potential risk for human health due to consumption of metal contaminated mushroom, but phyto-chelation stores these metals in detoxified form in mushroom.***

Keywords: trace element requirement, Cu tolerance, heavy metal accumulation, bioaccumulation, biosorption.

1. Introduction

Boletus edulis was first described in 1782 by the French botanist Pierre Bulliard [1], is a cosmopolitan genus of ectomycorrhizal fungi widely represented in the warmer parts of the Northern Hemisphere. As *Boletus edulis* constitutes a food source which contains vitamins, minerals and dietary fiber. Although environmental factors could affect the nutritional value to some extent during storage [3], but fresh mushrooms contain over 80% moisture [2]. Carbohydrates constitute the bulk of the fruit bodies, comprising 9.23% of the fresh weight and 65.4% of the dry weight. Chitin, hemicellulose, and pectin-like carbohydrates—all indigestible by humans—contribute to the nutritionally desirable high proportion of insoluble fibre in *B. edulis* [4]. Past studies have shown that most of the trace elements are required for the growth of mushrooms [5-12]. Though Fe, Zn and Cu are generally required for the growth of majority of the fungi investigated so far, yet the higher accumulation of Cu & other toxic metals in edible mushrooms is worrisome from human health point of view. Trace element concentrations in mushrooms are significantly higher than those in agricultural crop plants, vegetables or fruits [13]. Metal intake also reduces the nutritive content of mushrooms [14]. In the present investigations, *Boletus edulis* has shown tolerance to high Cu concentration that is highly toxic in nature. Though this, particular species could be used as a biosorbent in Cu-contaminated sites, yet a question arises whether this species could be used as a food without any apprehension. This paper focuses on the Cu tolerance & cultural characterstics of *Boletus edulis* after carrying out various experiments in laboratory.

2. Materials & Methods

2.1 Material

The pure culture of *Boletus edulis* was procured from Microbial Type Culture Collection (MTCC - M 981), Institute of Microbial Technology, Chandigarh, India. The culture of this fungus was maintained on MEA (Malt extract 10.0g, Peptone 3.0g, Agar 15.0g, distilled water to make 1000ml).

2.2 Methodology

2.2.1 Trace elements for the growth and sporulation of *Boletus edulis*

In this experiment, a comparative account of growth (average mycelial dry weight - mg) of *Boletus edulis* was studied with selected graded concentrations of trace elements (0.000001 – 400 ppm). Water of specific conductance 1.05 x 10^{-7} mhos was used and was obtained by passing the distilled water through columns of ion-exchange resins followed by twice distilling in an all glass pyrex still containing 0.5 g/l disodium salt of EDTA each time. The basal medium for *Boletus edulis* consisted of g/l: Glucose 10.0, Ammonium acetate/Sodium nitrite 2.0, Potassium dihydrogen orthophosphate 1.0, Magnesium sulphate 0.5. The trace element contaminants were removed from sugar solution (4 times its concentration) by passing it through columns of ion-exchange resin after [15] modified by [16-17] from ammonium acetate and sodium nitrite by adsorption with Al_2O_3 (BDH chromatograhic analysis material) [18]. The trace element contaminants from remaining ingredients of the basal medium were removed as a whole by $CaCO_3$ adsorption [19]. Copper was specially removed from the basal medium as a whole further by co-perception of sulphide with H_2S [20]. The contaminants of trace elements were removed from the glassware by chelation with

disodium salt of EDTA before finally rinsing out with water (specific conductance1.05 x 10^{-7} mhos) [20] modified by [16-17]. The different contaminants of trace elements from mycelial inoculum were minimized by the following procedure: -The mycelium was grown in a complete medium deficient of a single trace element at a time whose concentration were to be studied, for 4 days and thereafter transferring it four times after every 4 days each to the same medium prepared a fresh.

The standardized mycelium suspension containing mycelial bits upto upto 100 µm long, having 2.5 mg of dry weight/ml of the suspension was prepared as usual. After the elimination of trace elements from different ingredients of the basal medium as well as from the basal medium as a whole, it was supplemented with the 8 trace elements: Fe, Zn, Mn, Ca, Co, Cu, Mo and B (1ppm concentration of each, taken tentatively). The salts of these trace elements used were: $FeSO_4.7H_2O$, $ZnSO_4.7H_2O$, $MnCl_2.7H_2O$, $CaCl_2.2H_2O$, $CoCl_2.6H_2O$, $CuSO_4.5H_2O$, $(NH_4)_6Mo_7O_{24}.4H_2O$ and H_3BO_3. All these elements were of BDH AnaLaR, E. Merk GR or S. Merk GR grade. This experiment has been computed in 3 different sets. In each set of experiments, concentrations of 8 different elements were studied for the growth of one fungus only. The basal medium without added trace elements served as control. Twenty-five ml of the basal medium were apportioned in each 100ml Erlenmeyer conical flask aseptically. Extra care was taken to avoid the falling of lint from cotton plugs into basal medium; the plugs were wrapped in muslin cloth used for bandages. A separate pipette was used for each concentration and for each element to avoid the interference of the other elements. The diluted concentrations of the elements were prepared afresh after a month to avoid the fall in concentration due to adsorption of the elements on to the walls of the containers. Each flask containing 25ml of the basal medium was seeded with 1 ml of standardized mycelial suspension and incubated at the corresponding optimum temperature for their optimum days. At the termination of this experiment, optimum concentration of each trace element was recorded. During the experiment, *Boletus edulis* showed increased growth upto 400 ppm of Cu. The data on average mycelial dry weight analyzed statistically at 5% level of significance. Therefore, further experimentation (2.2.2) was designed to study the effect of copper beyond this concentration.

2.2.2 Growth of *Boletus edulis* with different concentrations of copper (Cu) and morphology of carpophoroid

The basal medium with optimum concentrations of the required trace elements (Fe,1; Zn,100; Mn,10; Ca,1; Cu,400; Mo,1; B,1) as determined in the previous experiment was solidified with 2% agar agar (A/R). It was supplemented with different concentrations of Cu (100-1000 ppm) to study its effect on mycelial growth/fructification formation if any. Fifty ml of the medium supplemented with different concentrations of Cu were apportioned in 250 ml Erlenmeyer conical flasks and autoclaved at 15 lbs psi steam pressure for 15 minutes. The medium was then allowed to solidify and kept for overnight. Three replicates were kept for each variable and treatment. At the termination of the experiment the data were recorded on average mycelial dry weight & cultural characteristics were recorded. The data on average mycelial dry weight analyzed statistically at 5% level of significance.

3. Results & Discussion

It has been found out that *Boletus edulis* is known to be flourish on soil that is contaminated with toxic heavy metals, such as soil that might be found near metal smelters. The mushroom's resistance to heavy metal toxicity is conferred by a biochemical called a phytochelatin—an oligopeptide whose production is induced after exposure to metal. Phytochelatins are chelating agents, capable of forming multiple bonds with the metal; in this state, the metal cannot normally react with other elements or ions and is stored in a detoxified form in the mushroom tissue [23]. *Boletus edulis* bioaccumulates different elements to varying degrees, and the element concentration in the fruit bodies is often a reflection of the element concentration of the soils from which they were picked [24]. Even during these investigations, there is a gradual increase in growth (average mycelial dry weight) from control to the optimum concentrations of required elements, beyond which it starts decreasing (Figure 1).

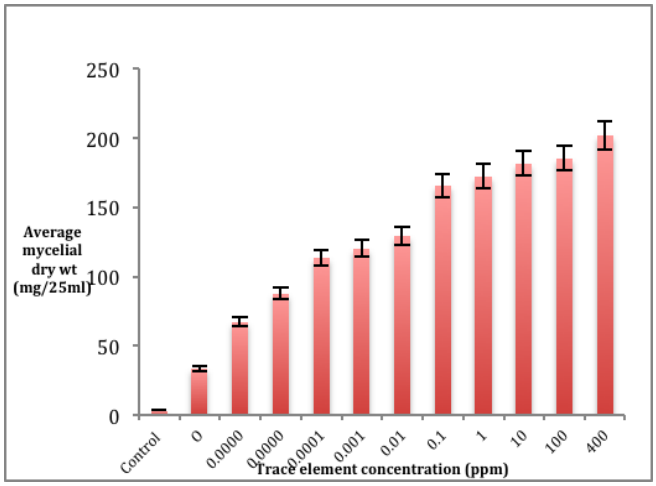

Figure 1: Growth (average mycelial dry weight –mg / 25ml) of *Boletus edulis* with different concentrations of trace elements

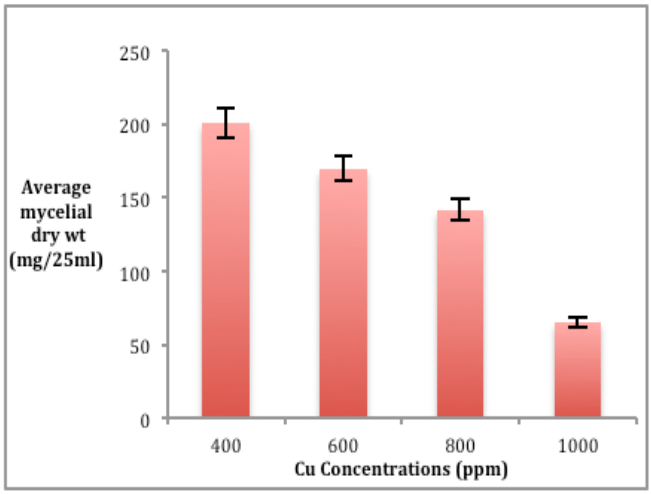

Figure 2: Growth (average mycelial dry wt. – mg/25 ml) of *Boletus edulis* with higher concentrations of Cu

Study of Figure 2, reveals that 400 ppm of Cu is optimum for the growth of *Boletus edulis*. The growth declined gradually beyond 400 ppm of Cu. The fungus showed tolerance to 1000 ppm of Cu that is the positive factor to use it at heavy metal/Cu contaminated sites.

Table 1: Mycelial growth and Carpophoroid formation in *Boletus edulis* with different concentrations of Cu in the solid medium

Cu concentrations (ppm)	Mycelial growth	Carpophoroid	Sclerotial bodies
100	++	-	-
200	++	-	-
400	+++	+++	-
600	++	++	+
800	++	++	-
1000	+	-	-

The fungus gives optimum growth and production of carpophoroids at 400 ppm and multiple sclerotial bodies at 600 ppm of Cu. Thereafter both of these gradually declined. The fungus does not form any carpophoroids/sclerotial bodies at 1000 ppm of Cu (Table 1). These findings are in accordance with accumulation of high levels of several heavy metals like copper, mercury, lead, zinc and cadmium [20-21].

4. Morphology of Fructification

A close examination of one of the mature fructification revealed it to be carpophoroid type of fructification as reported by [22]. The fruit body is short stipitate 1-4mm in size; dull brown with inrolled margin (Figure 3). The anatomical examination of it revealed it to be made up of texture intricate type of tissue without any differentiation into basidia and basidiospore.

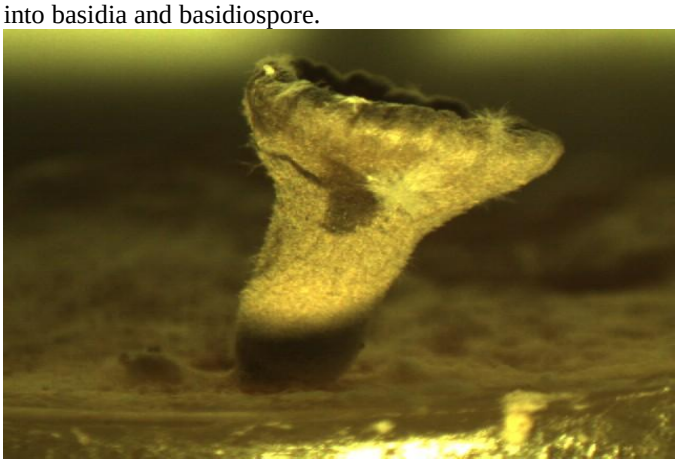

Figure 3: carpophoroid type of fructification of *Boletus edulis* with higher concentrations of Cu

5. Conclusion

It is inferred from the above findings that *Boletus edulis* has shown Cu tolerance upto 1000 ppm. Though, high Cu concentration is fungistic for most of the fungi; but the tolerance shown by this fungus could be an advantage factor in using it as an eco-friendly cleaning weapon in biosorption process. As this species has the ability to control the environmental pollution; but there are potential health risks for people due to daily intake of this mushroom contaminated with Cu. But natural process of phytochelation in mushrooms keeps the toxic metals in detoxified form. Also *Boletus edulis* grows only in natural environment, therefore the findings of optimum conditions for the growth has shown carpophoroids and multiple sclerotial bodies formation at higher Cu concentrations that could be an important factor for commercial cultivation of this mushroom. The role of Cu along with other factor/s like water relationships and constituent ingredients of the Photosynthate of the plants (with which *Boletus edulis* forms ecto-mycorrhizal relationships) in transforming these sterile structures into fertile basidiome needs further investigations.

6. Acknowledgement

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