RESEARCH ARTICLE OPEN ACCESS

The Influence of the Metals Citrates, Obtained Using Aquanano Technologies, On the Biomass Production of Medicinal Mushroom *Ganaderma Lucidum (Curtis) P. Karst*

Al-Maali Galeb[1], Bisko Nina[1], Mustafin Kairat[2], Akhmetsadykov Nurlan[2], Suleimenova Zhanara[2], Rakhmetova Zhanar[2]
[1]N. G. Kholodny Institute of Botany, National Academy of Sciences of Ukraine, Kiev, Ukraine
[2]LLP Antigen, Almaty, Kazakhstan

Abstract
A comparative study of the impact of citrates and sulfates of zinc and manganese, obtained with the help of nanotechnology, on the growth of mycelium of medicinal fungus *Ganoderma lucidum* at their cultivation in a liquids media has been carried out. It was demonstrated that sulfates and citrates of used metals have dramatically different effects on the growth of mycelium *Ganoderma* lucidum depending on which media they were added to.
***Key words*:** Ganoderma lucidum, citrate, sulfate, zinc, manganese, cultivation, nanotechnologies.

I. 1 Introduction

Ganoderma lucidum or *Reish*i is a basidiomycete white rot fungus which is traditionally used in China, Japan and other countries in the Asiatic region to treat numerous diseases, such as cancer, immunological disorders, inflammation e.t.c. [1,2]. The effectiveness of Reishi has been attributed to polysaccharide fractions and triterpenes, which have various positive effects on diseases with little side effects [3-5]. Moreover, G. lucidum has been used as an excellent source for lignocellulose degrading enzymes, such as laccase and Mn-peroxidase [6-7].

Macro-and micronutrients, especially essential biometals are often used to increase the yield of medicinal mushroom biomass and their biological activity in the modern industrial cultivation. It`s to be noted that basidiomycetes are widely known for their ability to accumulate toxic mineral elements (Mg, Cd, As). However, the minerals used in nutrient media for growing mushrooms should be free of toxic substances, because fungal degree of absorption of toxic elements (Hg, Cd) is much higher than degree of absorption of essential metals. Besides essential biometals must also have a chemical form which mushroom mycelium can accumulate well. To this day, mycelium of the fungus is grown in culture medium using inorganic salts of essential biometals. It`s know that the mushrooms readily absorb soluble forms of elements and their chelate compounds. Organic (carboxylate) acid salts and chelate compounds of biometals, are approved for use in the food industry and are very promising in this regard. However, traditional chemical technology of carboxylate synthesis is laborious, energy- and materials-consuming, and it can`t ensure a high chemical purity of product. Intensive development of nanotechnology resulted in a new method of synthesis of high purity and relatively inexpensive carboxylates of essential metals. There is an aquananotechnology that obtains solutions of citrate, succinate, lactate and carboxylates of almost all essential elements. Use of essential carboxylates of biometals, obtained by means of aquananotechnology in growth media for edible and medicinal mushrooms, presents a real-term opportunity for modifications of chemical compounds of mushroom mycelium. Previous studies have shown a positive effect of citrate biogenic metals on the yield of some crops and increase in body weight of animals.

The aim of our research was to study the influence of zinc citrate and manganese citrate on growth of mycelium of medicinal fungi *Ganaderma lucidum*.

II. Materials and Methods

The studied strain of *Ganaderma lucidum 1900* was obtained from the N. G. Kholodny Institute of Botany, National Academy of Sciences of Ukraine, Kiev. Manganese citrate and zinc citrate was obtained from Institute nanobiotechnologies and resource conservation of Ukraine, Kiev.

Mycelium of this strain was grown in a stationary culture at a temperature of 26 ^{o}C in 250 ml Erlenmeyer flasks, containing 50 ml of liquid media. In this study we used several types of media. The first kind of medium (GPY) has a following composition of (g/L): glucose – 25; pepton – 3; yeast extract – 3; K_2HPO_4 – 1; KH_2PO_4 – 1; $MgSO_4 \cdot 7H_2O$ – 0,25; distilled water – 1000 ml; pH 6,5. The second medium (GAsn) has a following composition of

(g/L): glucose – 25; asparagine – 1; K_2HPO_4 – 1; KH_2PO_4 – 1; $MgSO_4 \cdot 7H_2O$ – 0,5; CaCl – 0,1; $FeSO_4$ – 0,02; $CuSO_4 \cdot 7H_2O$ – 0,005; $MnSO_4 \cdot 7H_2O$ – 0,005 or Zn SO_4 – 0,02; distilled water – 1000 ml; pH 6,5. Various concentrations of metal citrate (Mn or Zn) and metal sulfate (Mn or Zn), containing equivalent content of metal, were added to both media. Control sample was a liquid media that did not contain metals in question. Inoculation material was produced in a Petri dish with agar medium. We used cut disks (5 mm in diameter) with seven-days micelium for inoculation flasks with liquid media (5 disks per flask). The biomass was harvested after 7 days of cultivation in the liquid medium, filtered, washed off with distilled water, dried to a constant weight at 105 ^oC and weighted.

III. Results and Discussion

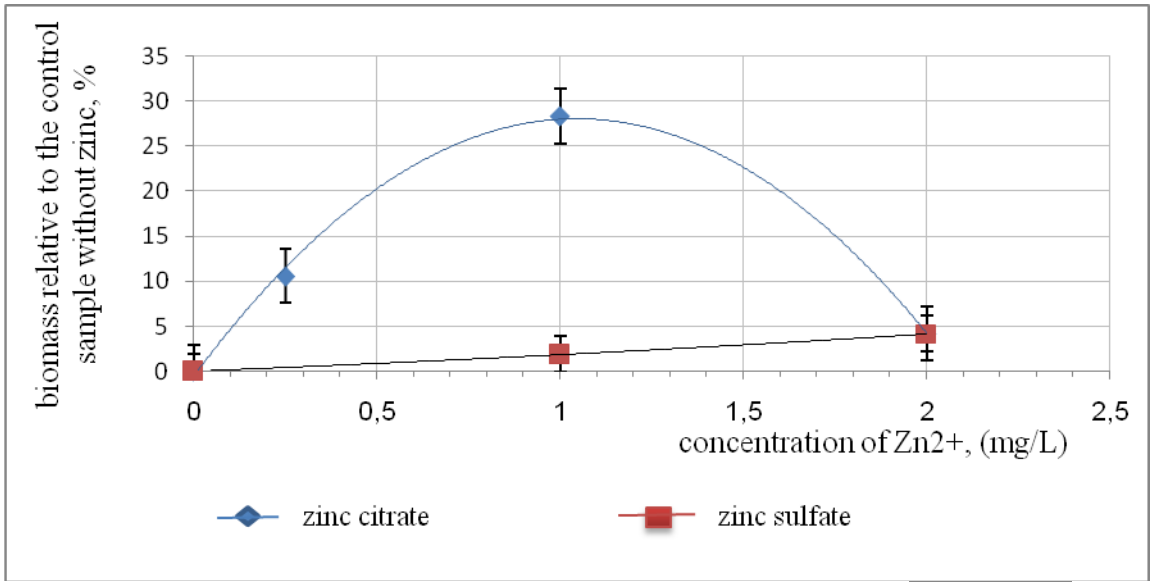

Figure 1. The influence of zinc citrate and zinc sulfate on the synthesis of biomass of G. lucidum on GPY medium

The results obtained indicate the increase of mycelia biomass that is much more significant on the GPY-citrate medium then on the GPY-sulfate medium (Fig. 1). Thus, mycelium of *G.lucidum* on GPY-citrate medium with concentration 1 mg/L of Zn^{2+} increased the biomass by 28.3% relative to the control sample. Whereas the amount of biomass harvested from the GPY-sulfate medium was the same as in the control medium without zinc.

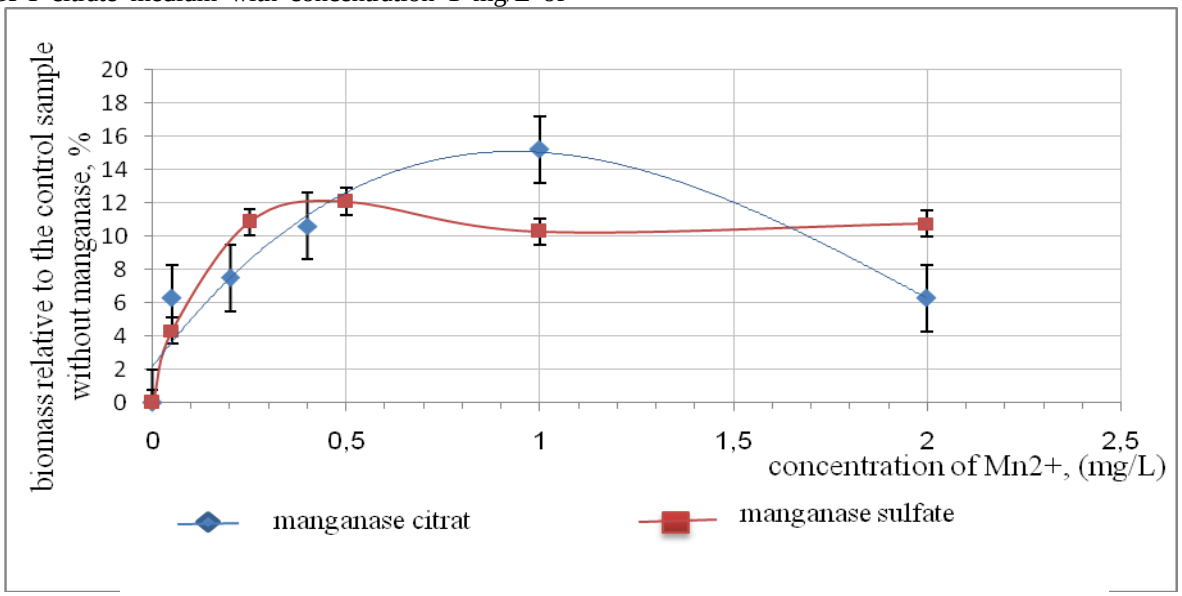

Figure 2. The influence of manganese citrate and manganese sulfate on the synthesis of biomass of G. lucidum on GPY medium

The data obtained in our work indicates that biomass of *G. lucidum* grew slightly better on the GPY medium with manganase citrate than on the same medium with manganese sulfate (Fig. 2). Thus, the maximum biomass was obtained on the GPY-citrate medium with 1 mg/L of Mn^{2+} and the growth of biomass was 15.2% relative to control sample without manganase. The most effective concentration of Mn^{2+} on the GPY-sulfat medium was 0.5 mg/L of Mn^{2+}, in this case the increase of biomass was 12.1% relative to control sample without manganase.

Due to the fact that our medium initially contained the impurities of zinc and manganese, we decided to repeat the experiment using a synthetic medium. We assumed that in this experiment, the effect of metal citrates on the mycelia growth will be more expressed. But we obtained unexpected results.

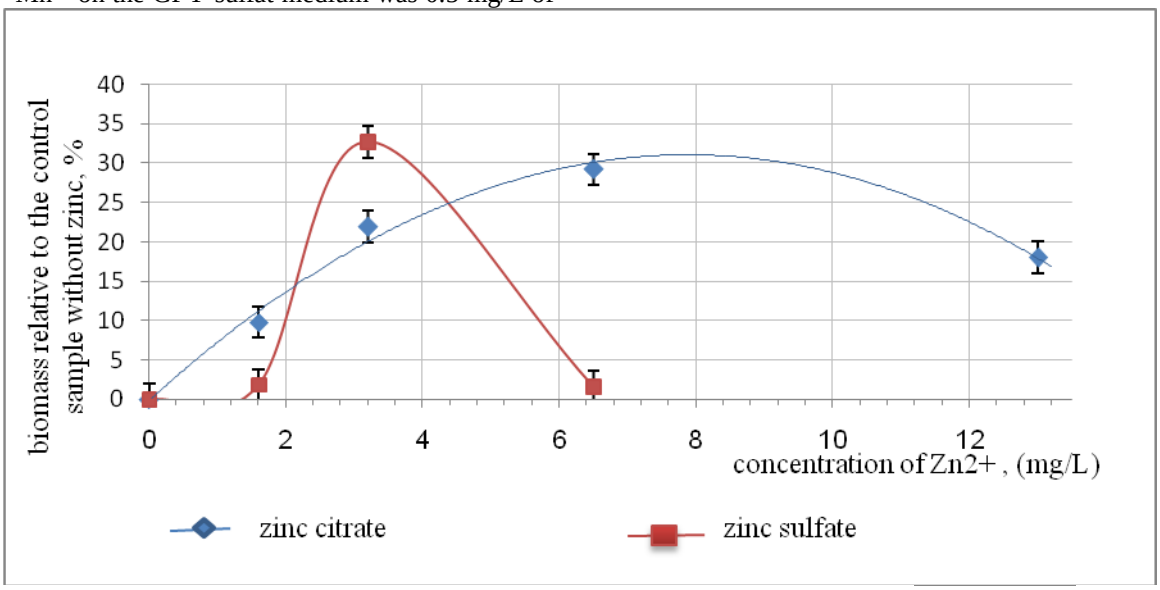

Figure 3. The influence of zinc citrate and zinc sulfate on the synthesis of biomass of G. lucidum on GAsn medium

Those results indicate that zinc sulfate and zinc citrate on the GAsn medium stimulate the growth of mycelium similarly (Fig. 3). Those results are radically different from the results on the GPY media with zinc. So the biomass growth was 32.7% in the GAsn-sulfate medium and 29.2% in the GAsn-citrate medium relative to control sample without zinc. But it must be emphasized that the optimum concentration of zinc sulfate (3.2 mg/L) is two times less than the optimum concentration of zinc citrate (6.5 mg/L).

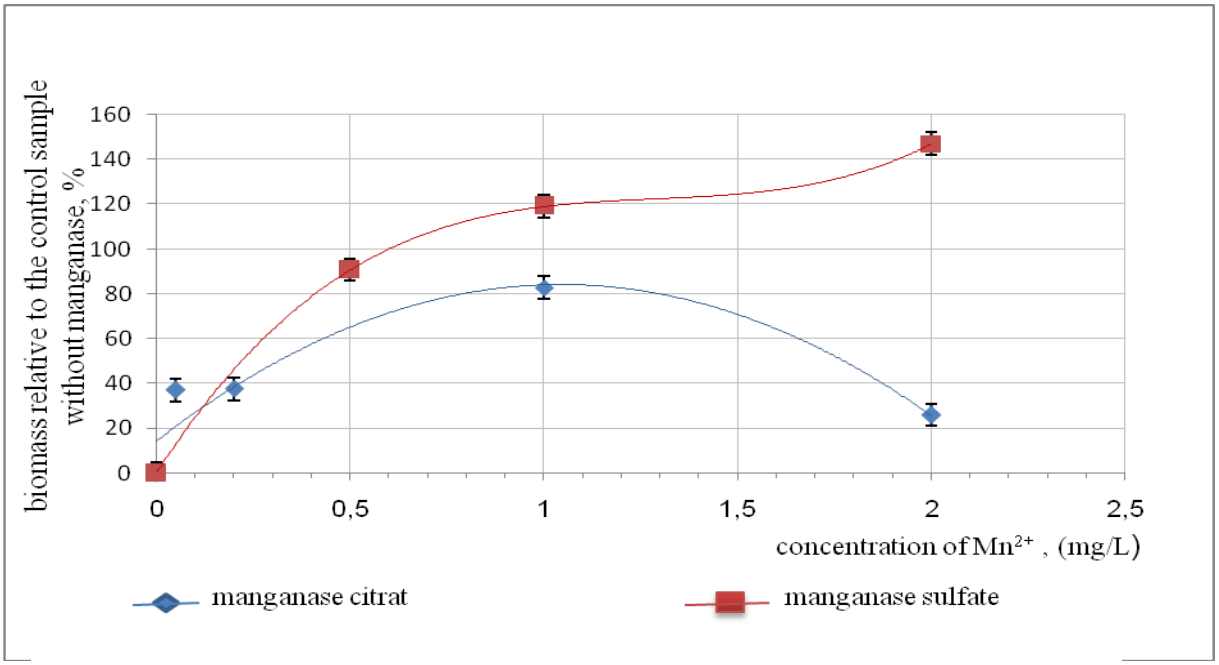

Figure 4. The influence of manganese citrate and manganese sulfate on the synthesis of biomass of *G. lucidum* on GAsn medium

The data obtained in trial with manganase indicates that the maximum increase of biomass on the GAsn-citrate medium at a concentration of 1 mg/L of Mn^{2+} was 82.8 % relative to control sample without manganase (Fig.4). But with the same concentration of zinc on the GAsn-sulfate medium, we observed the growth of biomass at 119.2%. Moreover, when we further increased the concentration of zinc to 2 mg/L on GAsn-sulfate medium, the amount of harvested biomass continued to rise. But increasing the concentration of Mn^{2+} to 2 mg/L on the GAsn-citrate medium led to a decrease in mycelia biomass growth to 26%.

Our experiment was first to show that sulfates and citrates of used metals have dramatically different effects on the growth of mycelium *G. lucidum* depending on which media they were added to. Thus, zinc citrate has much better influence on mycelia growth on GPY medium than does sulfate of zinc, which doesn't have significant effect on growth of mycelium. At the same time they have equally increased biomass of mycelium on GAsn medium, but the optimal concentration of zinc sulfate is two times less than zinc citrate. In contrast, manganese sulfate much better enhances the growth of the mycelium on the GAsn medium than does manganese citrate. But manganese sulfate and citrate on GPY medium had almost the same effect on mycelium growth.

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