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The influence of the manganese citrates, obtained using aquananotechnologies, on the biomass production of medicinal mushroom Trametes versicolor (L.) Lloyd.

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Abstract:
A comparative study of the impact of citrate of manganese, obtained with the help of nanotechnology, on the growth of mycelium of medicinal fungus *Trametes versicolor* on their cultivation in a liquid media. It was demonstrated that sulfate and citrate manganese have dramatically different effects on the growth of mycelium *Trametes versicolor* depending on which medium they were added to.
Key words: *Trametes versicolor*, citrate, sulfate, manganese, cultivation, nanotechnologies.

I. Introduction

Modern industrial cultivation of medicinal xylotrophic basidiomycetes is directed at optimization of the process of their cultivation to increase the yield of biomass and biologically active substances. Growing the mycelium of fungus on synthetic liquid nutrient media gives the opportunity to change and modify the mineral composition of the culture substance, and thereby, affecting the biomass growth and synthesis of biologically active substances. As a rule, inorganic metal salts are used in the culture medium for growth of fungi mycelium. However, aformentioned inorganic salts have several disadvantages, amongst which should be mentioned their low chemical purity and lower bioavailability as compared to the organic metallic compounds. In this sense, the most prospective are the salts of carboxylic acids, including metal citrates, that are allowed for use in the food industry. The intensive development of nanotechnology has created a number of methods by which it is possible to manufacture metal citrates of a high degree of purity. Several studies conducted with microorganisms and plants showed high biological activity of manganese citrate, obtained by aquananotechnology.

Higher basidiomycetes of the genus *Trametes* have numerous medicinal properties including antitumor, antibiotic, hepatoprotective and anti-virus properties [1-3]. The long history of use of these fungi in traditional oriental medicine is to treat inflammation of the upper respiratory tract, urinary system and digestive channel. It confirms their safety for human health. There are known products of medical purpose of *Trametes* consisting of purified polysaccharide fractions and proteins[4-6]. Moreover, T.versicolor has been used as an excellent source for lignocellulose degrading enzymes, such as laccase and Mn-peroxidase.

The aim of our research was to study the influence of manganese citrate on growth of mycelium of medicinal fungi *Trametes versicolor*.

II. Materials and Methods

The studied strain of *Trametes versicolor* 353 was obtained from the N. G. Kholodny Institute of Botany, National Academy of Sciences of Ukraine, Kiev. Manganese citrate was obtained by aquananotechnology from Institute of 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; Zn SO_4 – 0,02; distilled water – 1000 ml; pH 6,5. Various concentrations of manganese citrate and manganese sulfate, containing equivalent content of metals ion, were added to both media. Control sample was a liquid media that did not contain manganese. 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 ^{o}C and weighted.

Reducing sugars were determined in the cultural liquid of T. versicolor after stationary cultivation using the method of Hagedorn-Jensen. Economic coefficient (*Ec*) was calculated according to the following formula:

$$Ec = \frac{M}{Y1 - Y2}$$

M - mass of the harvested mycelium (mg/L), *Y1* - the amount of glucose in the medium before cultivation (mg/L), *Y2* - the amount of glucose in the medium after cultivation (mg/L).

III. Results and Discussion

The results obtained indicate the increase of micelial biomass that is much more significant on the GPY-citrate medium then on the GPY-sulfate medium (Fig. 1). Thus, micelium of T.versicolor on GPY-citrate medium with concentration 1 mg/L of Mn^{2+} increased the biomass by 28.81% relative to the control sample. Whereas the amount of biomass harvested from the GPY-sulfate medium has slightly increased relative to the control sample. Here, we were not able to determine a reliable maximum of biomass using statistics. But clearly we can speak of a higher biological activity of manganese citrate on the GPY medium.

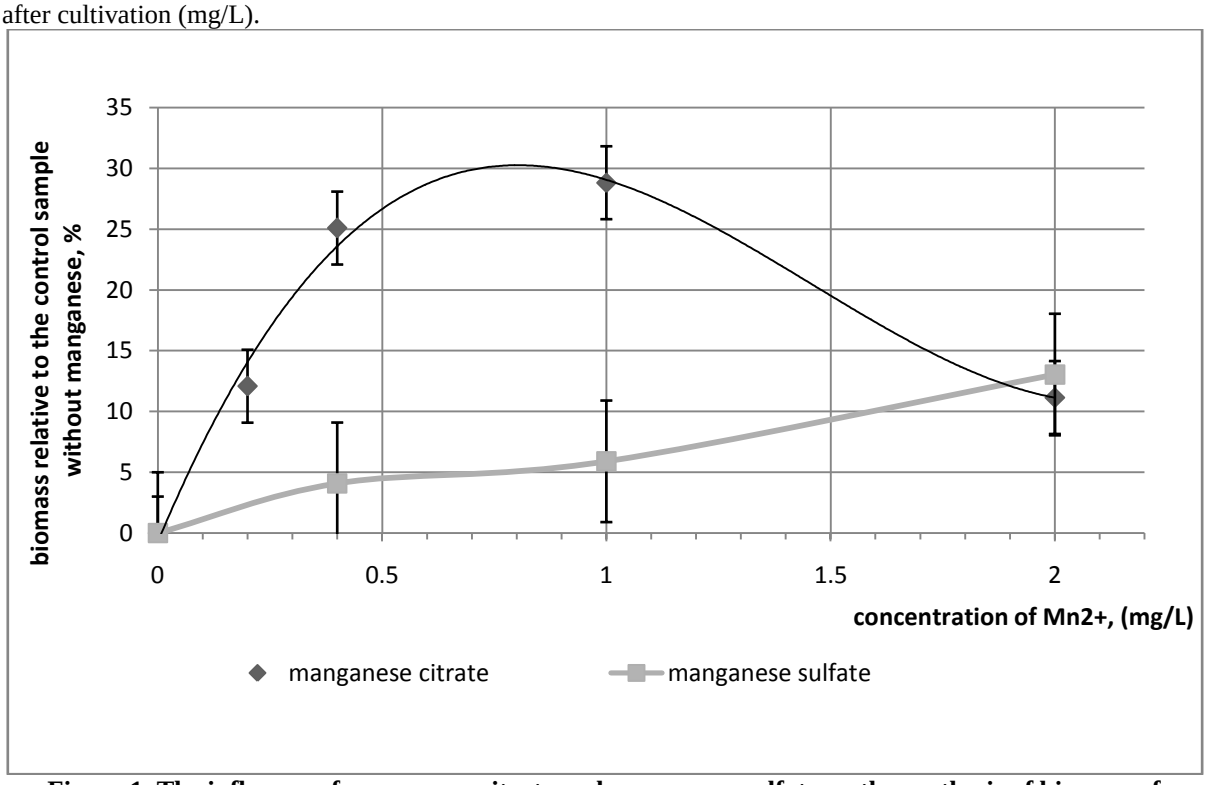

Figure 1. The influence of manganese citrate and manganese sulfate on the synthesis of biomass of T.versicolor on GPY medium.

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

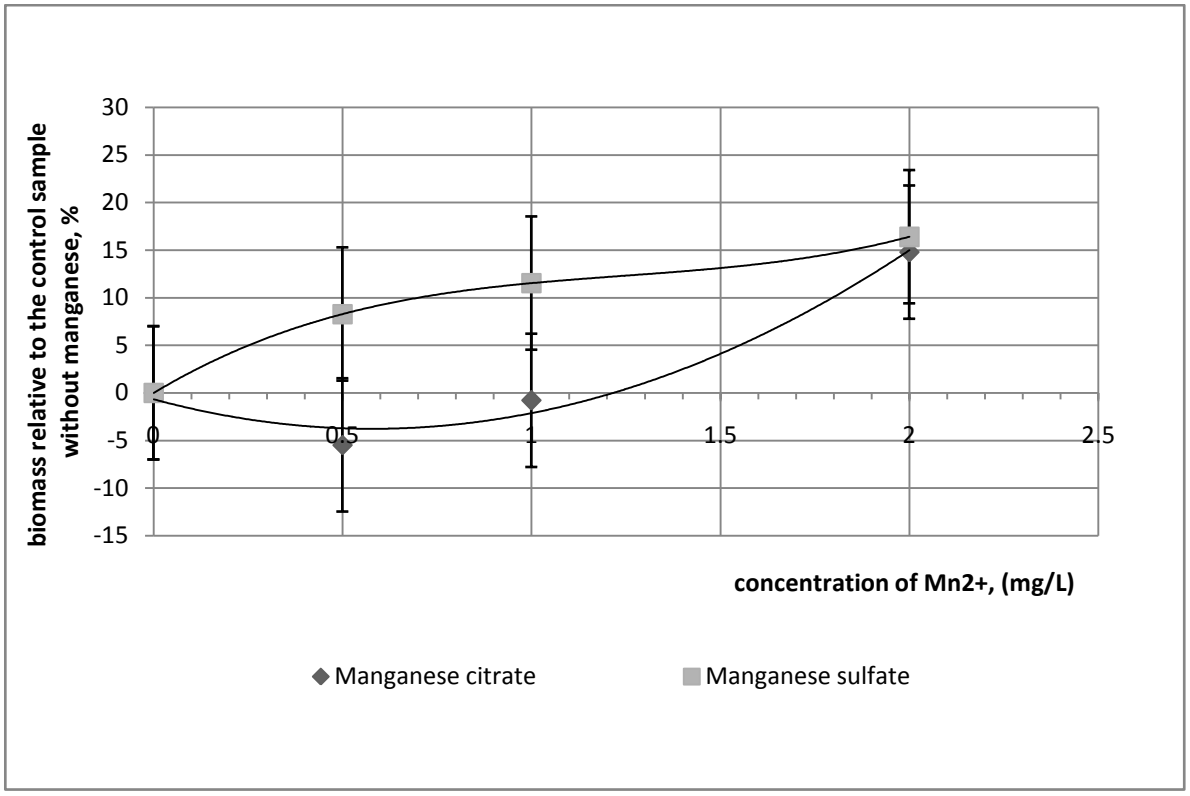

Figure 2. The influence of manganese citrate and manganese sulfate on the synthesis of biomass of T.versicolor on GAsn medium.

Those results indicate that manganese sulfate and manganese citrate on the GAsn medium stimulate the growth of mycelium equally low (Fig. 2). But at the same time, we found a significant difference in glucose consumption (Fig. 3). So, the mycelium on GAsn medium used the 2-2.5 g of glucose in all concentrations of citrate, while the economic coefficient was 0.8-0.9. High economic coefficient can be explained by the presence in the medium another carbon source – asparagine. Whereas, the mycelium cultivated on medium manganese sulfate used the 8.5 to 10,5 g of glucose in all concentrations of sulfate, while the economic coefficient was 0,2-0,23.

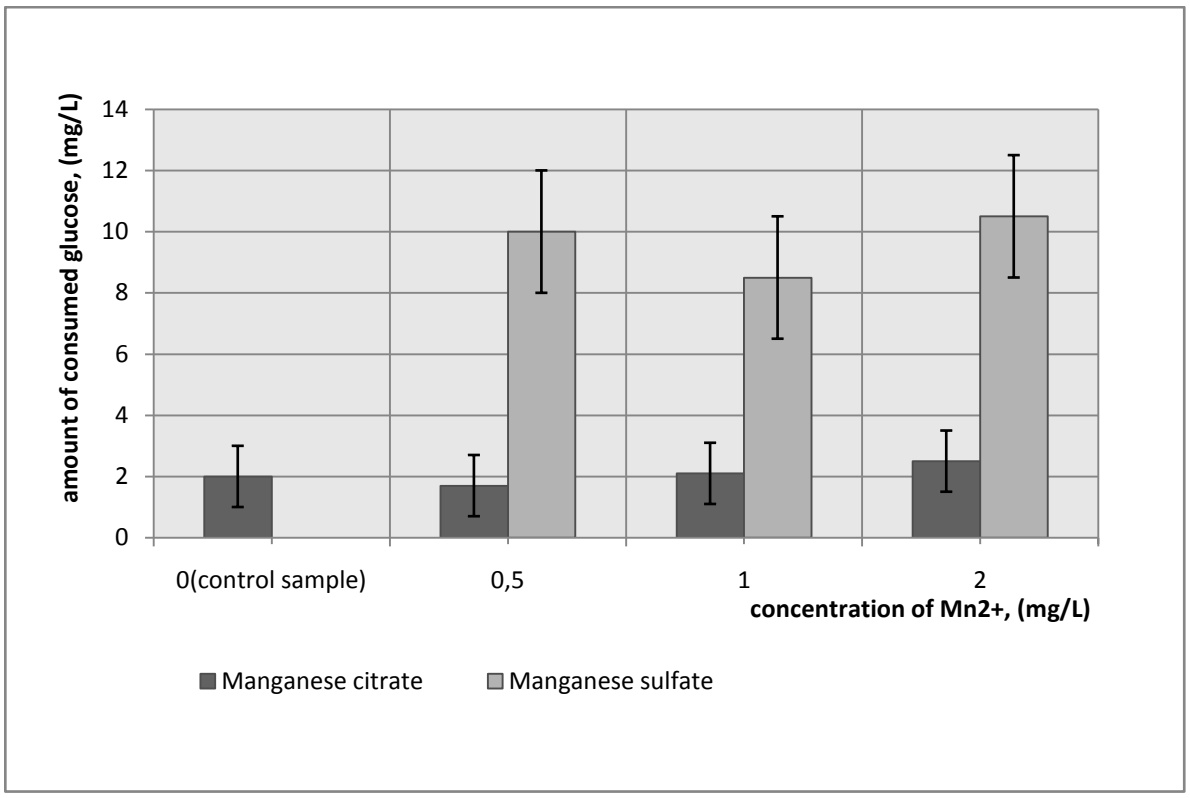

Figure 3. The influence of manganese citrate and manganese sulfate on glucose consumption by T.versicolor on GAsn medium.

Our experiment was first to show that sulfates and citrates of manganes have dramatically different effects on the growth of mycelium T. versicolor depending on which media they were added to. Thus, manganase citrate has much better influence on mycelial growth on GPY medium than does sulfate of manganase, which doesn't have significant effect on growth of mycelium. At the same time they have equally low increase of mycellial biomass on GAsn medium. But we demonstrated that citrate and sulfate of manganese have different effects on the adsorption of nutrients from the culture medium. We assume that these data may indicate that manganese sulfate and manganese citrate have different affects on the ability of the mycelium to absorb carbon from nitrogen containing compounds such as asparagine or peptone.

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