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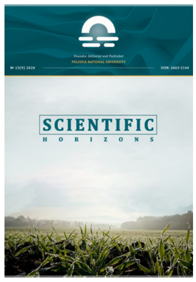

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Growth Intensity of *Trichoderma Viride* at Different Doses and Sources of Copper in the Medium

Larysa Mitiohlo[1], Serhii Merzlov[2], Halyna Merzlova[2*], Oleksandr Dudnyk[2], Oleksandr Rozputnii[2]

[1]State enterprise "Experimental Farm "Niva" of the V. Zubets Institute of Animal Breeding and Genetics of the National Academy of Agrarian Sciences of Ukraine"
20009, 1 Sadova Str., v. Hrystynivka, Cherkasy region, Ukraine

[2]Bila Tserkva National Agrarian University
09117, 8/1 Soborna Sq., Bila Tserkva, Ukraine

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Abstract. The biological properties of Trichoderma Viride fungi allow them to be effectively used in the technologies of crop waste disposal, production of organic, environmentally friendly fertilisers, and prevention of soil diseases of plants. The growth and development of these fungi depend on a number of trace elements that are found in the medium. Copper is of great importance among trace elements. The establishment of the ability to use copper as a stimulator of biomass accumulation of Trichoderma Viride fungi is of scientific and practical interest for national economic needs, which indicates the relevance of the study. The purpose of the study is to establish the effectiveness of the effect of various copper compounds on the growth and development of Trichoderma Viride. To investigate the effect of the metal-biotic on the growth of microorganisms, 0.5 to 10.0 mg/100 cm^3 of the element in the form of copper sulphate, mixed ligand complex and copper glycinate were added to the medium with potato dextrose agar (PDA). In the control variant, the element was not added to the nutrient medium. The media were inoculated with the Viridin preparation. Trichoderma Viride growth was recorded on days 4 and 7 of the experiment. It has been experimentally established that the fungal population is affected by the content and source of copper in the medium. It was found that the presence of a biotic metal in the medium in mineral or chelated form is up to 1.0 mg/100 cm^3 stimulates the build-up of fungal biomass. It was proved that on the 4^{th} day of cultivation for the introduction of copper into the medium in the amount of 1.0 mg/100 cm^3 in the mixed-ligand form, the population of Trichoderma Viride increased by 75.0%. On the 7^{th} day, the increase in the number of fungal cells was 58.3%. It was found that with the introduction in the medium of more than 5.0 mg/100 cm^3 copper in any form, the population growth of Trichoderma Viride fungi decreased. The higher the copper content in the medium, the greater the inhibition of fungal cell reproduction. It was generalised that comparing the action of the mixed ligand complex copper, copper glycinate, and copper sulphate, it was found that the latter compound is the most toxic against Trichoderma Viride

Keywords: fungal growth, mixed ligand complexes, copper glycinate, copper sulphate, metal biotic

*Corresponding author

INTRODUCTION

Among the wide variety of fungi, Trichoderma Viride is widely used in crop production and bioconversion technologies. Trichoderma Viride promotes the control of plants against soil diseases and to a certain extent stimulates their growth and yield. The effect of fungal action on the accumulation of chlorophyll and carotenoids in onions has been established (Aleksandrova & Velikanov, 2000; Metwally & Al-Amri, 2020).

Trichoderma is one of the microorganisms that are promising for biological control, as it has different mechanisms of action on plant pathogens (Aleksandrova *et al.*, 2004; Aleksandrova *et al.*, 2006). This includes competition for nutrients, antibiosis by hydrolytic enzymes (β-glucanases, chitinases, and proteinases) and mycoparasitism. Fungal preparations are used to accelerate the utilisation and bioconversion of organic waste (straw, chaff, damaged silage, haylage, sawdust, shavings, sunflower and corn crop residues) into organic fertiliser. Trichoderma spp. is one of the methods of controlling phytopathogenic fungi (Reino *et al.*, 2008). This is possible due to the fact that Trichoderma species have lytic activity against phytopathogens, synthesise antibiotics, and transmit plant resistance to phytopathogenic fungi (Mendez-vilas, 2010; Valencia *et al.*, 2011).

Currently, biotechnological studies are being conducted to develop effective methods for growing Trichoderma Viride fungi and obtaining stable preparations from them that are convenient for use in various sectors of the economy. The biological properties of biotic metals, including copper, in the fungal medium, can affect the course of metabolic processes in Trichoderma Viride. The amount of copper in the medium depends on the increase in the biomass of the fungus, its development and reproduction. With large doses of biotic metal in the medium, the growth of the fungus and even its death can stop. Low doses can stimulate the processes of anabolism in cells (Sidorenko, 2012; Tiwari *et al.*, 1989). However, currently in the available literature, there are no clear data on the use of organic compounds of copper (compounds with amino acids, compounds with organic acids, compounds with vitamins, compounds in combination with vitamins and amino acids) as stimulants of fungal biomass growth, including Trichoderma Viride. By investigating the optimal doses of biotic metal in the medium, it is possible to activate the metabolic processes in Trichoderma Viride, thereby, accelerating the growth of fungal biomass, increasing the efficiency of using a unit of mass of the medium and obtaining stable preparations for further bioconversion technologies.

The purpose of the study is to establish the optimal source and optimal doses of organic compounds of copper in the medium for the cultivation of Trichoderma Viride.

LITERATURE REVIEW

A number of researchers (Tiwari *et al.*, 1989; Samuels, 2006; Gonzalez *et al.*, 2010) proved that most species of the genus Trichoderma accelerate the fermentation time of organic parts in compost due to the ability to synthesise a significant amount of cellulolytic enzymes that hydrolyse lignin-cellulose complexes of plant residues. This leads to the enrichment of the finished compost with nutrients. In soils and compost, Trichoderma Viride persists as long as there are nutrients for it. On plant residues there is a significant amount of culture getting into the soil fungi continue the process of hydrolysis of this waste (Tiwari *et al.*, 1989; Druzhinina *et al.*, 2011).

The process of growth and development of fungi, including Trichoderma Viride, in the medium is affected by the concentration and shape of trace elements (heavy metals). A number of researchers (Sidorenko, 2012; Benitez *et al.*, 2004; Chaverri *et al.*, 2003) proved that a significant amount of mineral elements inhibit the growth rate of fungi that cause plant diseases (Hanada *et al.*, 2009). These elements include heavy metal – copper (Sidorenko, 2012; Hryhora *et al.*, 2003).

Copper as a biotic metal is an important trace element found in organisms of all life forms from microorganisms to mammals. According to the computational algorithm, about 0.3% of bacterial proteomes are copper-binding proteins (especially metalloenzymes). Linder & Hazegh-Azam, 1996; Andreini *et al.*, 2008).

Copper and its various complexes are an effective agent for inhibiting the growth of fungi and yeast (Aspergillus fumigates, Candida albicans, Aspergillus niger, Aspergillus fumigates, Saccharomycesel cerevisiae, Aspergillus carbonarius, Cryptococcus neoformans, Aspergillus oryzae) (Kumbhar *et al.*, 1991; Zatcoff *et al.*, 2008).

New copper compounds – metal nanoparticles – are also used to fight fungal diseases. It has been experimentally proven that the antifungal effect of copper nanoparticles on Saccharomycesel cerevisiae (baker's yeast) depends on its concentration in the medium (Cioffi *et al.*, 2004; Sydorenko, 2012). The harmful effect of nanomaterials was also found in relation to fungi Candida sp. (Lipovsky *et al.*, 2011; Monteiro *et al.*, 2011).

The effect of copper (II) ions on the growth and development of white and brown rot fungi was established (Guillén & Machuca, 2008). N. Poitou and J.M. Olivier investigated the effect of various copper salts on the mycelium growth of several species of edible ectomycorrhizal fungi: Lactarius deliciosus, Suillus granulates, and truffle. It was found that with an increased dose of metal in the medium, the growth of fungal mycelium significantly decreases and the absorption of potassium, calcium, and magnesium is disrupted (Poitou & Olivier, 1990). In addition to inorganic compounds (chlorides, sulphates), and metal-biotic nanoparticles, chelated copper complex is widely used in the national economy (Merzlov, 2009; Merzlov & Gerasymenko 2005). Currently, the effect of various compounds and doses of copper on the growth rate of Trichoderma Viride in the nutrient medium has not been investigated.

MATERIALS AND METHODS

Potato dextrose agar (PDA) medium was used for the study. The medium was pre-autoclaved (temperature 122-124°C for 25 minutes). Penicillin – sterile benzylpenicillin sodium salt (Benzylpenicillin 1,000,000 OD O.L.KAR.) 0.5 g/dm^3 and streptomycin sulphate 0.4 g/dm^3 were added to the PDA medium 5 minutes before bottling to suppress possible bacteria that may have entered the drug and copper sources. Various doses and sources of copper were added to the nutrient medium. Copper mixed ligand complex, copper glycinate, and copper sulphate were used as a source of biotic metal. In the control variant, no metal was introduced to the nutrient medium. The experimental nutrient media contained copper according to Table 1.

***Table 1**. Application of copper to the medium, mg/100 cm^3, n = 5*

Group	Copper source		
	Copper sulphate	Copper mixed ligand complex	Copper glycinate
Control	–	–	–
I experimental	0.1	0.1	0.1
II experimental	0.5	0.5	0.5
III experimental	1.0	1.0	1.0
IV experimental	2.5	2.5	2.5
V experimental	5.0	5.0	5.0
VI experimental	10.0	10.0	10.0

Nutrient media were poured into Petri dishes of 15 cm^3. Each petri dish was labelled considering the number of the experimental group and the dose of copper added. The media were inoculated with the Viridin preparation. Incubation was carried out in a thermostat at a constant temperature of 26 ± 0.5°C for 7 days. Counting was performed on the 4th and 7th days of incubation. The experimant was repeated three times.

RESULTS AND DISCUSSION

Experimentally, it was found that the development of Trichoderma Viride is affected by both the dose of copper in the medium and the form of its compound. On the 4th day of cultivation on a medium without additional application of copper (control group), the indicator of colony-forming units (CFU) of the fungus was 2.0*104. With the additional application of copper to the medium in mixed ligand form and glycinate form in the amount of 0.1 mg/100 cm^3, the amount of fungi increases by 10.0% relative to the control. At this dose of metal biotic, there was no difference in the growth of Trichoderma Viride between the variants where copper was used in mineral and organic form. Increase in the copper content in the medium to 0.5 mg/100 cm^3 promoted the growth of CFU of Trichoderma Viride in the experimental groups, respectively, by 20.0% (medium containing copper sulphate), 45.0% (medium containing copper mixed ligand complex), and 50.0% (medium containing copper glycinate) compared to the control (2).

***Table 2**. Amount of Trichoderma Viride in the medium at the 4th day of cultivation, CFU/g*

Group	Medium without adding copper	Copper source		
		Copper sulphate	Copper mixed ligand complex	Copper glycinate
Control	$2.0 * 10^4$	–	–	–
I experimental	–	$2.1 * 10^4$	$2.2 * 10^4$	$2.2 * 10^4$
II experimental	–	$2.4 * 10^4$	$2.9 * 10^4$	$3.0 * 10^4$
III experimental	–	$2.4 * 10^4$	$3.5 * 10^4$	$3.2 * 10^4$
IV experimental	–	$0.7 * 10^4$	$2.8 * 10^4$	$2.4 * 10^4$
V experimental	–	$0.5 * 10^3$	$5.5 * 10^3$	$4.9 * 10^3$
VI experimental	–	$3.5 * 10^2$	$1.0 * 10^3$	$9.5 * 10^2$

The greatest stimulating effect of copper was found when adding this metal in mixed ligand form at a dose of 1.0 mg/100 cm^3. In terms of the number of CFU, the difference with the control was 75.0%. The addition of cobalt glycinate to the medium at the same dose increased the amount of fungi by 60.0%. The use of a mineral form of biotic metal at a dose of 1.0 mg/100 cm^3 of the medium also had a positive effect on the growth and development of the fungus relative to the control data, but for the variants with copper, the CFU index of Trichoderma Viride was lower, respectively, by 31.4 and 25.0%. The reduced effect of copper exposure in the form of copper sulphate is conditioned by the lower bioavailability of the metal to biotics and its inclusion in the metabolic processes of fungi compared to its organic complexes.

According to the dose of copper in mixed ligand form 1.0 mg per 100 cm^3 of the medium, the fungus biomass absorbs the optimal amount of metal, which is included in metabolic processes, thereby stimulating growth and development. Redox processes and the virulence property of Trichoderma Viride are optimised. The virulence of fungi depends on the action of a number of copper-containing proteins synthesised by their own body. This biotic metal is a critical determinant of their optimal functions. Cuproproteins associated with fungal virulence and redox processes have been identified in these processes, including superoxide dismutase (SOD) and laccase. Laccase (EC 1.10.3.2, para-benzenediol: oxygen oxidoreductase, *P* – diphenol oxidase) is an enzyme that belongs to oxidases and performs a number of oxidation reactions of aromatic and non-aromatic compounds in fungal biomass and contains copper bound to imidazole groups of histidines. SODFC 1.15.1.1) is an enzyme belonging to the group of antioxidant biocatalysts. Together with other antioxidants, it protects against highly toxic oxygen radicals. The active centres of the enzyme include copper. Due to the mixed ligand complex of copper at a dose of 1.0 mg per 100 cm^3 of medium, the metal-biotic assimilated in an accessible biological form in Trichoderma Viride stimulates the synthesis of laccase and SOD and increases their catalytic activity, thereby improving metabolic processes in fungi.

In addition, when the metal is optimally absorbed from the mixed ligand complex, copper affects transcription regulators (transfer of genetic information, as a result of which RNA is synthesised from DNA as a matrix) of Trichoderma Viride, thus activates protein synthesis and fungal reproduction (Ding *et al*., 2011; Ding *et al*., 2013; Strong & Claus, 2011).

Increase in the metal content in the medium in the form of copper sulphate to 2.5 mg/100 cm^3 had a negative effect on the growth of Trichoderma Viride. The CFU score was 2.85 times lower than in the control. At this dose of biotic metal in the fungal medium, the greatest negative effect of copper was found in the variant where the element was in mineral form. Compared to the variants where the mixed ligand complex and copper glycinate were added to the medium, the CFU rate was 4.0 and 3.4 times lower, respectively.

Application of metal in the form of sulphate, mixed ligand form, and glycinate at a dose of 5.0 mg/100 cm^3 helped to reduce the increase in the amount of fungus in the medium, respectively, by 40.0, 3.6, and 4.1 times relative to the control. At this dose of copper, the medium continued to maintain a pattern regarding the toxic effect of the biotic metal on Trichoderma Viride. The growth and development of the fungus was preferable in the variant where a mixed ligand complex of copper was used in relation to samples of the medium where copper sulphate was introduced 11 times. The obtained experimental data confirm the lower toxicity of the copper mixed ligand complex against Trichoderma Viride.

The copper content in the form of sulphate 10.0 mg/100 cm^3 of the medium maximally reduced the growth of Trichoderma Viride on the 4th day of cultivation. At this dose, the mixed ligand form of copper had the least negative effect on the growth of Trichoderma Viride. The CFU index was 2.85 times higher than in the variant with the sulphate content (VI experimental group).

On the 7th day of the experiment, the number of microorganisms in the control increased to 1.2*109. The increase in Trichoderma Viride in the first group with copper sulphate content in the medium was 8.3% higher than in the control group. For using copper at a dose of 0.1 mg/100 cm^3 in organic forms, the increase in culture was higher relative to the variant where copper sulphate was added to the medium by 7.7%. There is a pattern of increasing the increase in the amount of fungus with an increase in the content of copper in sulphate form in the medium to a concentration of 1.0 mg/100 cm^3 compared to the control. A similar phenomenon was observed with the use of copper in the mixed ligand form and glycinate form. Copper doses of 0.5 and 1.0 mg/100 cm^3 of the medium in the form of a mixed ligand complex and glycinate stimulated the increase in the mass and amount of Trichoderma Viride better than its mineral form. The CFU index in these variants was higher than in the medium with copper sulphate, respectively, by 21.4 and 14.3%, and 18.7 and 12.5 % (Table 3).

***Table 3**. Amount of Trichoderma Viride in the medium for 7 days of cultivation, CFU/g*

Group	Medium without adding copper	Copper source		
		Copper sulphate	Copper mixed ligand complex	Copper glycinate
Control	$1.2*10^9$	–	–	–
I experimental	–	$1.3 * 10^9$	$1.4 * 10^9$	$1.4 * 10^9$

Table 3, Continued

Group	Medium without adding copper	Copper source		
		Copper sulphate	Copper mixed ligand complex	Copper glycinate
II experimental	–	1.4 * 10^9	1.7 * 10^9	1.6 * 10^9
III experimental	–	1.6 * 10^9	1.9 * 10^9	1.8 * 10^9
IV experimental	–	0.1 * 10^9	0.5 * 10^9	0.6 * 10^9
V experimental	–	0.3 * 10^3	5.8 * 10^3	5.3 * 10^3
VI experimental	–	3.2 * 10^2	1.2 * 10^3	9.7 * 10^2

The highest CFU of fungi was found in the group where the medium contained 1.0 mg/100 cm^3 of mixed ligand complex of copper. The indicator was higher than in the control group by 58.3%. Comparing the effect of organic forms of copper on the growth of the fungus with each other, it is proved that the mixed ligand complex of the metal is more effective against glycinate by 5.6%.

On the 7th day of cultivation, with the content of copper at a dose of 2.5 mg/100 cm^3 (copper glycinate and mixed ligand form), a decrease in the population growth activity of Trichoderma Viride was observed. The content of microorganisms in the medium was 2.0 and 2.4 times lower compared to the control group, respectively. The presence of copper sulphate in the medium (IV experimental group) led to a 12-fold decrease in the CFU of Trichoderma Viride compared to the control. In addition, a decrease in the amount of Trichoderma Viride was observed for the action of copper at a dose of 2.5 mg/100 cm^3 in mineral form, a mixed ligand complex and copper glycinate were used as part of the medium in relation to the variants. The difference was 5 and 6 times, respectively.

According to the content of copper, in the amount of 5.0 mg/100 cm^3 of the medium in mineral and organic form, the CFU index of Trichoderma Viride was lower compared to the control group. For the use of biotic metal in a mixed ligand complex, the difference with the control was more than 206 thousand times. The presence of a high dose of copper in the form of glycinate also led to almost suspension of growth and development of the fungus. In comparison with the values for the 4th day of cultivation, the CFU indicator increased by only 8.1%.

A significant inhibition of the reproduction of the fungus and its neutralisation was found when using a mineral form of metal-biotic in the medium. Comparing with the data obtained on the 4th day of fungus cultivation, the CFU index of Trichoderma Viride on the 7th day for the action of copper sulphate decreased by 40.0%.

Comparing different sources of metal, it was found that copper sulphate at a dose of 10.0 mg/100 cm^3 has the most toxic effect on Trichoderma Viride compared to chelated metal compounds. The CFU index (VI experimental group) for cultivating the fungus on a medium containing copper sulphate was 3.75 and 3.0 times lower, respectively, compared with the variant where the metal biotic was in mixed ligand form and in the form of glycinate. It was found that due to the accumulation of copper by fungi from the medium, the toxic effect of metal increases over time. This phenomenon is confirmed by the data obtained at the end of the experiment. Thus, on the 7th day of cultivation, the CFU indicator in the variant with copper sulphate decreased by 8.6% compared to the indicator obtained on the 4th day.

Thus, it was found that copper at a low dose of up to 1.0 mg/100 cm^3 of the medium stimulates the growth of the fungus Trichoderma Viride. The most effective source of copper was its mixed ligand form. The explanation for this may be that the metal biotic in this form has a high bioavailability and actively enters into a compound with the corresponding proteins (metalloenzymes), which take an active part in the metabolism of the fungus (Linder & Hazegh-Azam, 1996). The stimulating effect of copper on the growth of microorganisms in low doses has also been established (Ding *et al*., 2011; Ding *et al*., 2013). The authors state that the expression of the CTR1 and CTR4 genes responds to a copper-deficient medium. At critically low levels of copper in the medium, the growth of microbial cells slows down (Ding *et al*., 2013; Ding *et al*., 2011).

Increase in the content of copper in the medium to 10.0 mg/100 cm^3 in mineral and organic form has a negative effect on the growth and reproduction of Trichoderma Viride, because copper in mineral form is more toxic to microorganisms. The negative effect of copper on fungal cells is explained by the generation of free radical stress due to the Fenton reaction. In addition, excess metal biotics alter the structures and metabolic functions of fungal cells. Copper can also affect the DNA of microorganisms and disrupt its replication and transcription, which, in turn, leads to partial and complete death of fungal organisms (Cioffi *et al*., 2044; Garcia-Santamarina *et al*., 2017).

Data (Druzhinina *et al*., 2011) argue that Trichoderma fungi have the biological property of interacting with other fungi, bacteria, plants, and animals, the ability to counteract plant-pathogenic fungi and stimulate plant growth and protective reactions confirm our claim of the feasibility of finding ways to stimulate the accumulation of Trichoderma Viride biomass for use for national economic purposes.

The results obtained by the authors regarding the detection of the stimulating effect of copper on the growth and development of Trichoderma at low doses of metal biotic in the form of copper sulphate, mixed ligand complex, and copper glycinate are confirmed by other researchers (Benitez *et al*., 2004; Hanada *et al*., 2009, Kalatehjari *et al*., 2015), who argue that different types of Trichoderma, although found almost everywhere and not very demanding of the medium, still prefer quite different substrates and are exposed to different ranges of temperature, humidity, and copper content in the medium.

The study found that an increase in the content of copper to 2.5 mg/100 cm^3 of the medium and above in mineral and organic form leads to inhibition of the growth and development of Trichoderma Viride. These results are supported by data (Cioffi *et al*., 2004), where it is noted that the possible mechanisms of action of various copper compounds are based on changes in the structure and function of fungal cells. In addition, the metal can affect DNA and disrupt its replication and transcription, which can eventually cause the death of fungal microorganisms (Cioffi *et al*., 2004).

CONCLUSIONS

Addition of copper sulphate, copper mixed ligand complex, and copper glycinate at the rate of 0.1 mg of the active substance per 100 cm^3 of the medium did not significantly affect the growth and development of Trichoderma Viride fungi on both the 4^{th} and 7^{th} days of cultivation. For increasing the metal content in the medium due to its mineral and chelated forms to an amount of 0.5 mg/100 cm^3, the CFU index of the fungus increases by 16.7-41.7% relative to the variant where copper was not added to the medium (control group). The lowest indicator was observed in the variant with copper sulphate in the medium.

Use of mineral and chelated forms of biotic metal at a dose of 1.0 mg/100 cm^3 of the medium contributed to the best activation of the growth and development of the fungus Trichoderma Viride. The optimal source of copper at this dose was found to be a mixed ligand complex of the metal. In this form, copper is optimally absorbed and has a positive effect on increasing the amount of fungus, being included in the synthesis of metalloenzymes and activating the metabolic processes of Trichoderma Viride cells. For applying copper to the medium at a dose of 1.0 mg/100 cm^3 in the mixed ligand form, on the 7^{th} day of cultivation, the CFU index of the fungus increased by 58.3% relative to the control and by 18.7% and 5.6% relative to variants where, respectively, the medium contained copper sulphate and copper glycinate.

Introduction of metal into the medium in the form of copper sulphate at a dose of 2.5 mg/100 cm^3 negatively affected the growth and development of Trichoderma Viride. Fungal CFU score was 12 times lower than in the control. Increase in copper content in the medium to 5.0 mg/100 cm^3 due to the mixed ligand complex of copper and copper glycinate negatively affects the growth of the Trichoderma Viride population.

The most toxic compound for the fungus is copper sulphate at a dose of 10.0 mg/100 cm^3 of the medium. The CFU index, in this case, was 3.0 and 3.75 times lower, respectively, compared with similar doses of the biotic metal in the form of glycinate and the copper mixed ligand complex. For a dose of copper in mineral or organic form from 2.5 mg/100 cm^3 of the medium and higher, metal is absorbed by the fungus in excessive amounts, thereby causing disruption of oxidative processes (free radical stress) and the processes of replication and transcipation of fungal DNA.

REFERENCES

[1] Aleksandrova, A.V., & Velikanov, L.L. (2000). The impact of Trichoderma harzianum on the soilborne micromycetes. *Mycology and Phytopathology*, 34(3), 68-77.
[2] Aleksandrova, A.V., Velikanov, L.L., & Sidorova, I.I. (2004). Historical overview and modern system of Trichoderma. *Mycology and Phytopathology*, 38(1), 3-23.
[3] Aleksandrova, A.V., Velikanov, L.L., & Sidorova, I.I. (2006). Key for determining the Trichodermaspecies. *Mycology and Phytopathology*, 40(6), 437-468.
[4] Andreini, C., Banci, L., Bertini, I., & Rosato, A. (2008). Occurrence of copper proteins through the three domains of life: A bioinformatic approach. *Journal of Proteome Research*, 7, 209-216.
[5] Benitez, T., Rincon, A.M., Limon, M.C., & Codon, A.C. (2004). Biocontrol mechanisms of Trichoderma strains. *International Microbiology*, 7, 249-260.
[6] Chaverri, P., Overton, B.E., & Samuels, G.J. (2003). Trichoderma/Hypocrea species with conidiephore elongations and green conidia. *Mycologia*, 95, 1110-1140.
[7] Cioffi, N., Torsi, L., Ditaranto, N., & Sabbatini, L.P. (2004). Zamboni Antifungal activity of polymer-based copper nanocomposite coatings. *Applied Physics Letters*, 85(12), 2417-2419.
[8] Ding, C., Festa, R.A., Chen, Y.L., Espart, A., Palacios, O., Espin, J., Capdevila, M., Atrian, S., Heitman, J., & Thiele, D.J. (2013). Cryptococcus neoformans copper detoxification machinery is critical for fungal virulence. *Cell Host & Microbe*, 13, 265-276. doi: 10.1016/j.chom.2013.02.002.

[9] Ding, C., Yin, J., Tovar, E.M., Fitzpatrick, D.A., Higgins, D.G., & Thiele, D.J. (2011). The copper regulon of the human fungal pathogen Cryptococcus neoformans H99. *Molecular Microbiology*, 81, 1560-1576. doi: 10.1111/j.1365-2958.2011.07794.x.
[10] Druzhinina, I.S., Seidl-Seiboth, V., Herrera-Estrella, A., Horwitz, B.A., Kenerley, Ch.M., Monte, E., Mukherjee, P.K., Zeilinger, S., Grigoriev, I.V., & Kubicek, Ch.P. (2011). Trichoderma: The genomics of opportunistic success. *Nature Reviews Microbiology*, 10, 749-759.
[11] Garcia-Santamarina, S., Uzarska, M.A., Festa, R.A., Lill, R., & Thiele, D.J. (2017). Cryptococcus neoformans iron-sulfur protein biogenesis machinery is a novel layer of protection against Cu stress. *ASM Journals*, 8(5). doi: 10.1128/mBio.01742-17.
[12] Gonzalez, I., Infante, D., Peteira, B., Martinez, B., & Arias, Y. (2010). Biochemical characterization of isolates of Trichoderma spp. promising biological control agent. *Journal of Plant Protection Research*, 25, 58-63.
[13] Guillén, Yu., & Machuca, A. (2008). The effect of copper on the growth of wood-rotting fungi and a blue-stain fungus. *World Journal of Microbiology and Biotechnology*, 24, 31-37.
[14] Hanada, R.E., Pomella, W.V., Soberanis, W., Loguercio, L.L., & Pereira, J.O. (2009). Biocontrol potential of Trichoderma martiale against the black-pod disease (Phytophthora palmivora) of cacao. *Biological Control*, 50, 143-149.
[15] Hryhora, I.M., Aleynikov, I.M., Lushpa, V.I., Shabarova, S.I., & Yakubenko, B.E. (2003). C*ourse of general botany*. Kyiv: Phytosocial Center.
[16] Kalatehjari, P., Yousefian, M., & AliKhalilzadeh, M. (2015). Assessment of antifungal effects of copper nanoparticles on the growth of the fungus *Saprolegnia* sp. on white fish (*Rutilus frisii kutum*) eggs. *The Egyptian Journal of Aquatic Research*, 41, 303-306
[17] Kumbhar, A.S., Padhye, S.B., Saraf, A.P., Mahajan, H.B., Chopade, B.A., & West, D.X. (1991). Novel broad-spectrum metal based antifungal agent correlations amongst the structural and biological properties of copper (II). *Journal of Biology and Medicine*, 4, 141-143.
[18] Linder, M.C., & Hazegh-Azam, M. (1996). Copper biochemistry and molecular biology. *The American Journal of Clinical Nutrition*, 63(5), 797-811. doi: 10.1093/ajcn/63.5.797.
[19] Lipovsky, A., Nitzan, Y., Gedanken, A., & Lubart, R. (2011). Antifungal activity of ZnO nanoparticles – the role of ROS mediated cell injury. *Nanotechnology*, 22(10), article number 105101. doi: 10.1088/0957-4484/22/10/105101.
[20] Mendez-vilas, A. (2010). A review on contributions presented at the BioMicroWorld 2009. Conference. *American Journal of Agricultural and Biological Sciences*, 5(4), 486-487. doi: 10.3844/ajabssp.2010.486.487.
[21] Merzlov, S.V. (2009). Assessment of complexation technology in cobalt-ligand compounds using IR spectroscopy. *Bulletin of the Bila Tserkva National Agrarian University*, 60(2), 79-81.
[22] Merzlov, S.V., & Gerasimenko, V.G. (2005). Effectiveness of using the drug Orgmet-1 in growing broiler chickens. *Agricultural News*, 4, 13-15.
[23] Metwally, R.A., & Al-Amri, S.M. (2020). Individual and interactive role of Trichoderma viride and arbuscular mycorrhizal fungi on growth and pigment content of onion plants. *The American Journal of Clinical Nutrition*, 70(2), 79-86.
[24] Monteiro, D., Group, L.F., Silvia, S., Negri, M., Camarqo, E.R., Oliveira, R., Barbosa, D.B., & Silver, H.M. (2011). Colloidal nanoparticles: Antifungal effect against adhered cells and biofilms of Candida albicans and Candida glabrata. *Biofouling*, 27(7), 711-720.
[25] Poitou, N., & Olivier, J.M. (1990). Effect of copper on mycelium of three edible ectomycorrhizal fungi agriculture. *Ecosystems & Environment*, 28(1-4), 403-408.
[26] Reino, J.L., Guerriero, R.F., Hernandez-Gala, R.I., & Collado, G. (2008). Secondary metabolites from species of the biocontrol agent Trichoderma. *Phytochemistry Reviews*, 7, 89-123.
[27] Samuels, G.J. (2006). Trichoderma: Systematics, the sexual states, and ecology. *Phytopathology*, 96, 195-206.
[28] Strong, P.J., & Claus, H. (2011). Laccase: A review of its past and its future in bioremediation. *Critical Reviews in Environmental Science and Technology*, 41, 373-434.
[29] Sydorenko, M.L. (2012). The effect of trace elements on the growth of laricifomes officinalis (vill.) Kotl. et Pouzar. *KAU Bulletin*, 1, 76-81.
[30] Tiwari, S.C., Tiwari, B.K., & Mishra, R.R. (1989). Microbial populations, enzyme activities and nitrogen-phosphorus-potassium enrichment in earthworm casts and in the surrounding soil of a pineapple plantation. *Biology and Fertility of Soils*, 8(2), 178-182.
[31] Valencia, G.B., Vargas, V.H., Soto, J.N.U., Jimenez, N.N., & Corral, J.H. (2011). Trichoderma sp native from chili region of Poanas, Durango, Mexico antagonist against phytopathogen fungi. *American Journal of Agricultural and Biological Sciences*, 6(2), 185-188.
[32] Zatcoff, R.C., Smith, M.S., & Borkow, G. (2008). Treatment of tinea pedis with socks containing copper impregnated fibers. *Foot*, 18, 136-141.

Інтенсивність росту *Trichoderma Viride* за вмісту різних доз і джерел Купруму в середовищі

Лариса Василівна Мітіогло[1], Сергій Віталійович Мерзлов[2], Галина Вікторівна Мерзлова[2], Олександр Кирилович Дудник[2], Олександр Іванович Розпутній[2]

[1]Державне підприємство «Дослідне господарство «Нива» Інституту розведення і генетики тварин імені М. В. Зубця Національної академії аграрних наук України»
20009, вул. Садова, буд. 1, с. Христинівка, Черкаська обл., Україна

[2]Білоцерківський національний аграрний університет
09117, 8/1 Соборна пл., м. Біла Церква, Україна

Анотація. Біологічні властивості грибів Trichoderma viride дозволяють їх ефективно використовувати за технологій утилізації відходів рослинництва, виробництва органічних, екологічно чистих добрив та профілактування ґрунтових хвороб рослин. Ріст і розвиток цих грибів залежить від ряду мікроелементів, які знаходяться у середовищі. Важливе значення серед мікроелементів має Купрум. Науково-практичний інтерес має встановлення здатності використання Купруму як стимулятора нарощування біомаси грибів Trichoderma viride для народно-господарських потреб, що зумовило актуальність дослідження. Метою роботи є встановлення ефективності впливу різних сполук Купруму на ріст і розвиток Trichoderma viride. Для вивчення впливу металу-біотику на ріст мікроорганізмів до середовища із картопляним декстрозним агаром (PDA) вносили від 0,5 до 10,0 мг/100 см^3 елементу у формі купрум сульфату, змішанолігандного комплексу та гліцинату купруму. У контролі до поживного середовища елемент не вносили. Середовища засівали підготовленим препаратом «Viridin». Облік росту Trichoderma viride проводили на 4 та 7 добу експерименту. Експериментально встановлено, що на популяцію грибів впливає вміст і джерело Купруму у середовищі. Було встановлено, що присутність у середовищі металу-біотику у мінеральній чи хелатній формі до 1,0 мг/100 см^3 стимулює нарощування біомаси гриба. Було доведено, що на 4 добу культивування за внесення у середовище Купруму у кількості 1,0 мг/100 см^3 у змішанолігандній формі популяція Trichoderma viride підвищується на 75,0 %. На 7 добу збільшення кількості клітин грибів становило на 58,3 %. Було досліджено, що за включення до середовища Купруму у будь якій формі більше 5,0 мг/100 см^3 нарощування популяції грибів Trichoderma viride знижувалось. Виявлена тенденція чим вищий вміст Купруму у середовищі тим гальмування розмноження клітин грибів більше. Було узагальнено, що порівнюючи дію змішанолігандного комплексу Купруму, гліцинату купруму та купрум сульфату виявлено, що остання сполука є найбільш токсичною щодо Trichoderma viride

Ключові слова: ріст грибів, змішанолігандні комплекси, гліцинат купрум, купрум сульфат, метал-біотик