STUDIES CONCERNING THE INFLUENCE OF TRACE ELEMENTS ON THE DYNAMICS OF SOME BIOCHEMISTRY MARKERS ACTIVITY OF OXIDATIVE STRESS AT *MONILINIA LAXA* (ADERH.& RUHL.) HONEY PARASITE ON PLUM TREES

STUDII PRIVIND INFLUENŢA OLIGOELEMENTELOR ASUPRA DINAMICII ACTIVITĂŢII UNOR MARKERI BIOCHIMICI AI STRESULUI OXIDATIV LA *MONILINIA LAXA* (ADERH. &RUHL.) HONEY PARAZITĂ PE PRUN

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Abstract. *Because some microorganisms like fungi owe their pathogenicity, aggressivity and virulence to their rich enzyme equipment, we have to monitor their biological activity by analyzing some biochemical markers like oxidoreductases. This paper synthesizes some experimental results concerning the influence that some oligoelements like B, Cu, Mn, Mo, Zn, Fe and a mixture between them, have on the time activity of catalase and peroxidase at Monilinia laxa parasite on different types of plum tree species. „In vitro" culture of the fungus was made on Leonian medium and the experimental determinations involved some samples from the fungus mycelium and the liquid used for the culture, at intervals of 7, respective 14 days from the inoculation. The evaluation of catalase activity was made using the method of Sinha and the peroxidase activity was determined by Moller's method with an o-dianisidine. The experimental data analysis indicated that the variation of the two oxidoreductases is correlated directly with the age of the culture and that there are some substantial differences between the types of oligoelements used in the culture medium.*

Key words: *Monilinia laxa,* trace elements, catalase, peroxidase, *Prunus domestica*

Rezumat. *Dat fiind faptul că unele microorganisme de genul fungilor îşi datorează atât patogenicitatea cât şi agresivitatea şi virulenţa unui bogat echipament enzimatic, se impune pentru monitorizarea activităţii lor biologice studiul unor markeri biochimici de genul oxidoreductazelor. Lucrarea de faţă sistematizează o serie de rezultate experimentale privind influenţa exercitată de oligoelemente de tipul B, Cu, Mn, Mo, Zn, Fe, dar şi a unei mixturi din acestea asupra activităţii în timp a catalazei şi peroxidazei la specia Monilinia laxa parazită pe diferite soiuri de prun. Cultivarea "in vitro" a ciupercii s-a făcut pe mediul Leonian, iar determinările experimentale s-au realizat atât din miceliul ciupercii cât şi din lichidul de cultură, la 7, respectiv, 14 zile de la însămânţare. Evaluarea activităţii catalazei s-a făcut utilizându-se metoda Sinha, iar cea a peroxidazei prin metoda Moller cu o-dianisidină. Analiza datelor experimentale a relevat faptul că, variaţia activităţii celor două oxidoreductaze este direct corelată cu vârsta culturii şi că, există diferenţe substanţiale în funcţie de tipul oligoelementului cu care a fost suplimentat mediul de cultură.*

Cuvinte cheie*: Monilinia laxa,* oligoelemente, catalază, peroxidază, *Prunus domestica*

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INTRODUCTION

One of the most significant effects that prokaryotes have on the living environment is their ability to play a crucial role in processing and recycling the essential chemical elements, in the total biomass of microbial cells in the biosphere, their metabolic diversity and persistence in all habitats constitutes the argument for this idea. In recent years there has been an increasing realization of the importance of the role that micronutrients play in biological systems. Needed in small quantities, the trace elements are used by filamentous fungi for growth and development and their physiology. The modern classification of the minerals of life, classifies the vast majority in trace elements (Fraústo da Silva and J.J.R. and Williams R.J.P, 2001, Gârban Z., 2005, Nikolaevich Baskin, V., 2006), others authors considering that only Fe is an oligoelement, and the rest are micronutrients (Şoldea C.and Mocanu, M. 2011) and other studies fits in addition Fe, Cu and Zn in the group of essential trace elements, and the rest, B, I, F, Se, Si, As, Mn, Mo, Co, Cr, V, Ni and Cd in the essential of the ultratrace elements group (Reddy H., 2007).

The data from the literature awards of the nutrients the modulators role (Cojocaru D.C., 2007), these having an inductive or inhibitory action in different chemical reactions catalyzed by a variety of enzymes. Although they are known to play an important role in the production of secondary metabolites including antibiotics and mycotoxins, and acting as growth factors - Cu, Zn, Mn or inducer of the conidiogenese, as element of the mycelium pigmentation - Zn, Cu, as elements coupled with the protein- transporting ATP-ase (Saitoh Y. *et al*., 2010), concentrated in the vacuolar juice - Fe^{2+}, Zn^{2+} (Gow N.A.R. and Gadd G.M., 1996), the trace elements get into the enzymatic context depending on the needs of the fungal cell, performing different roles: some trace elements such as Cu and Fe achieves a catalytic function, their presence in the enyzmes composition stimulating their functioning. They act as a union factors between active enzyme and the substrate (metal ions), and in several enzymes acts as a powerful electronic attraction center, leading to the important oxido reduction reactions (Zn, Cu, Mn, Se and Fe) (Halliwell, B. and Gutteridge, J.M.C., 2007). Although some micronutrients are less important than others in the development of metabolism and growth of fungal cell, they must exist in a certain proportion to act synergistically. There are several essential nutrients with mineral origin which are taking part in antioxidant processes with the enzymes, delaying or completely inhibiting the oxidation of the substrate and acting at all different levels of the oxidative sequence. Possessing the transduction systems and the mechanisms to adapt to oxidative stress (Tanaka C. and Izumitsu K., 2010) materialized in an endogenous antioxidant system, the fungi releases an exoenzyme in the extracellular space to minimize the negative impact of the reactive oxygen species.

In this context, the objective of the present work, going on the line of studies that have followed the influence of trace elements on the the oxidoreductase or other environmental factors on the catalase and peroxidase activity in different fungal species (Manoliu Al. *et al.,* 2009, 2010) follows to quantify the activity of biochemical parameters in *Monilinia laxa* species grown on the medium supplemented with various micronutrients.

MATERIAL AND METHOD

The inoculum source have constituted a sporodochia from mummified fruit harvested from different varieties of *Prunus domestica* from Experimental Orchard Pomiculture Station of Mirolaslava, Iasi county. For "in vitro" cultivation of the *M. laxa* fungus was used the Leonian medium, distributed in Erlenmeyer flask, over which we added the following trace elements in the following quantities: B -10 mg, Cu - 100 mg, Mn - 20 mg, Mo - 20 mg, Fe -20 mg, Zn - 200 mg (Constantinescu, O., 1974) as H_3BO_3, $CuSO_4$ x H_2O, $MnCl_2$ x $4H_2O$, Na_2MoO_4 x $2H_2O$, $FeCl_3$ x $6H_2O$, $ZnSO_4$ x $7H_2O$. Trace elements were added one by one, separately, and in one variant we added all We used a control sample, without micronutrients. The media were seeded with disks cut-out from an7 days old culture of *M. laxa* and incubated at 28^0C. The experiment was conducted in two intervals, respectively 7 days and 14 days after inoculation of the culture, the catalase and the peroxidase activity was determined both from the fungus mycelium and from culture liquid. The catalase activity was assayed by the method Sinha and the peroxidase by Moller method (Artenie Vl., 2008).

RESULTS AND DISCUSSIONS

The first conclusion from the statistical analysis and represented graphically in figures 1 and 3 of the catalase activity in the *M. laxa* species cultivated on medium supplemented with different trace elements, indicates that the enzyme activity in the mycelium was different from that in liquid culture.

The catalase activity in the mycelium, shows in figure 1 that the control sample had the highest value in 7 days - 1599.206 μmol hydrogen peroxide/min., and the minimum point of the activity was recorded in the variant with Mo - 273.7148 μmol hydrogen peroxide/min. Between them, in descending order, the following values of the endocelulare catalase are: variant V2 - 1427.606 μmol hydrogen peroxide/min, variant V5 - 1090.383 μmol hydrogen peroxide/min, variantaV1 - 1030.233 μmol hydrogen peroxide/min, variant V7 - 817.2983 μmol hydrogen peroxide/min, variant V3 - 748.5597 μmol hydrogen peroxide/min and the V6 version - 278, 1847 μmol hydrogen peroxide/min.

At 14 days the catalase activity in the myelium was stimulated to all medium variants with trace elements and the control sample recorded a minimum of the activity: 112.6078 μmol hydrogen peroxide/min A strong induction at this time had on the catalase the zinc - 1766.281 μmol hydrogen peroxide/min, followed in descending order by V3 - 630.6306 μmol hydrogen peroxide/min , variant V1 - 366.8229 μmol hydrogen peroxide/min, variant V5 - 308.7956 μmol hydrogen peroxide/min, variant V2 - 282.4119 μmol hydrogen peroxide/min variant V7 (the trace elements mixture) - 258.2396 μmol hydrogen peroxide/min., respectively, variant V4 - 216.9855 μmol hydrogen peroxide/min. The analysis of the enzyme activity in mycelium indicates various reductions in the catalase activity in for all variants excepting variant V6 (Zn), in which the aging culture had induced a strong amplification of the enzyme activity – from 278.1874 μmol hydrogen peroxide/min. to 1766, 282 μmol hydrogen peroxide/min. Depending on the mean values and standard deviation for all the samples we calculated the

upper and the lower limits of the confidence intervals based on the critical value t (α, n-1), given by α = 0.05 and n-1 degrees of freedom (fig. 2, 4,6, 8).

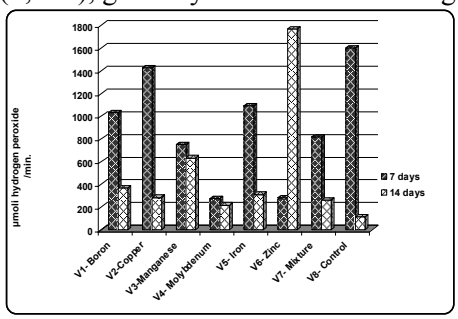

Fig. 1 - The influence of the trace elements on the catalase activity in the mycelium of the *Monilinia laxa*

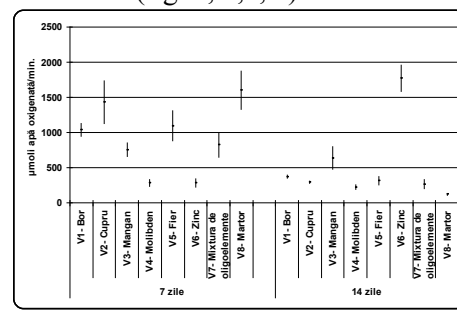

Fig. 2 - The confidence intervals of the catalase activity in the mycelium of the *Monilinia laxa*

In culture liquid, the control sample from 7 days had a value of the catalase activity of 434.5048 µmol hydrogen peroxide/min. The strongest induced effect on the enzyme at this time had zinc - 927.2237 µmol hydrogen peroxide/min, followed by Fe - 845.7711 µmol hydrogen peroxide/min, and more modest amplifications to Mo and Mn - 508.8757 µmol hydrogen peroxide/min, respectively, 451.9774 µmol hydrogen peroxide/min. A significant inhibitory effect on the catalase activity at 7 days, in culture liquid, had the trace element mixture – 123.829 µmol hydrogen peroxide/min. and from this mixture the elements with a moderate inhibitory effect was Cu - 387.0968 µmol hydrogen peroxide/min, followed by B 326.5306 µmol hydrogen peroxide/min.

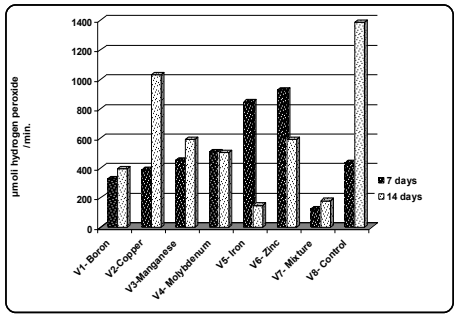

Fig. 3 - The influence of the trace elements on the catalase activity in the culture liquid of *the Monilinia laxa*

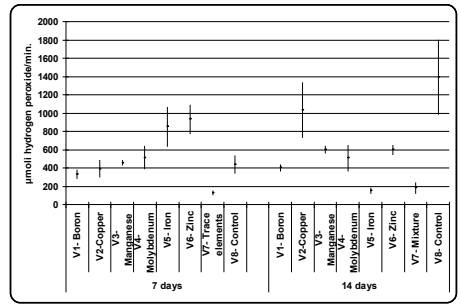

Fig. 4 - The confidence intervals of the catalase activity in culture liquid in the *Monilinia laxa*

With the aging of culture, the extracellular catalase activity decreased in all medium variants, the most significantly in the case of the iron and the mixture of the trace elements - 146.6395 µmol hydrogen peroxide/min, respectively, 178.9096 µmol hydrogen peroxide/min. Other medium variants had relatively uniform values, excepting the copper - 1031.301 µmol hydrogen peroxide/min.

The data on the influence of the various trace elements on the peroxidase activity in the mycelium, after 7 days of incubation in *M. laxa* are shown graphically in figure 5. It may be noted that boron had a stimulating effect on endoenzyme - 1.5500 PU/g /min.

while a strong inhibitory effect was obtained with zinc -0.0630 PU/g /min, reported for peroxidase activity value in control control - 0.6990 PU/g /min.

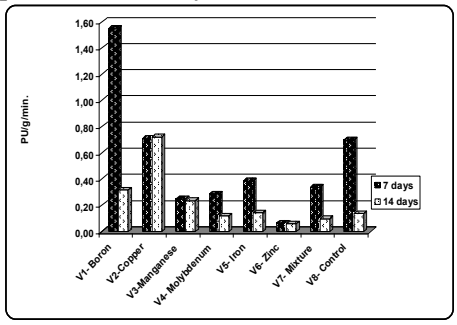

Fig. 5 - The influence of the trace elements on the peroxidase activity in mycelium of the *Monilinia laxa*

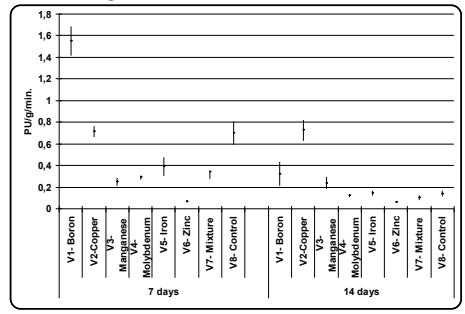

Fig. 6 - The confidence intervals of the peroxidase activity in mycelium of the *Monilinia laxa*

At 14 days after seeding the culture medium, in the mycelium, the peroxidase value for the control sample was 0.1379 PU/g/min. Zinc inhibited at this time the peroxidase activity - 0.0599 PU / g / min. The same thing happened in the medium variants with the mixture of trace elements and molybdenum -0.1990 PU/g/min respectively, 0.0973 PU/g/min. An enhancement of the activity was induced in variant containing copper - 0.7215 PU/g/min and the boron - 0.3178 PU/g /min.

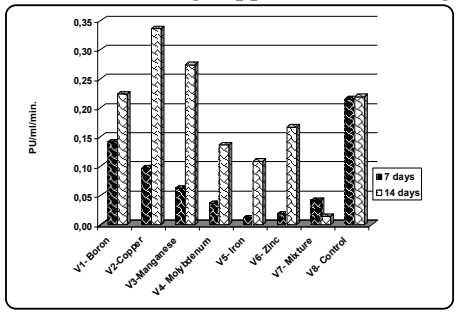

Fig. 7 - The influence of the trace elements on the peroxidase activity in culture liquid of the *Monilinia laxa*

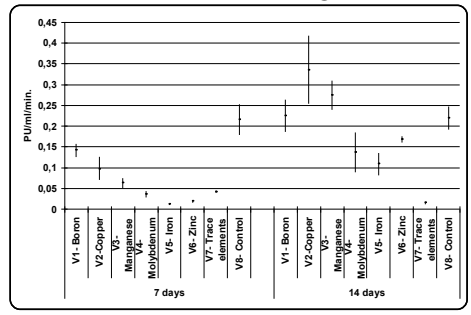

Fig. 8 - The confidence intervals of the peroxidase activity in culture liquid of the *Monilinia laxa*

So, for the case of the peroxidase also we calculated and plotted graphically the values in both mycelium and culture fluid, with the limits of the confidence intervals for each treatment option in part (fig. 6, 8). The eliberations of the peroxidase in the extracellular space for the first intervals of tests was inhibited in all medium variants with the trace elements, compared with the control sample (0.2156 PU/ml/min) the highest in variants containing zinc and iron-0.0180 PU/ ml/min and respectively, 0.0117 PU/ml/min.

In a secondary series of tests, after 14 days, in both culture liquid and control sample, the peroxidase had a value of the activity of - 0.2195 PU / ml / min. The cumulative effect of the trace elements in the mixture has found expression in the strong inhibitory effect on the extracellular peroxidase - 0.0141

PU/ ml / min, while the variant containing copper stimulated its activity up to a peak of 0.3356 PU/ ml / min, followed by that of manganese variant - 0.2742 PU / ml / min and boron - 0.2242 PU / ml / min.

CONCLUSIONS

In young mycelia, at 7 days, all trace elements inhibited the catalase activity and for the liquid culture the exoenzyme activity was stimulated by manganese, molybdenum, iron and zinc. At 14 days after seeding the culture medium, the intracellular catalase activity was stimulated by boron, copper, manganese, molybdenum, iron, zinc and trace elements mixture and the extracellular catalase was inhibited at this time by all trace elements, including their mixture.

After 7 days of incubation, the peroxidase activity was stimulated by boron in the mycelium and for the culture fluid it was inhibited by all trace elements and their mixture and at 14 days after sowing, in the mycelium, the peroxidase activity was stimulated by boron, copper, manganese and iron, while in liquid culture, was stimulated, this time, by boron, copper and manganese.

REFERENCES

1. **Artenie V., Ungureanu E., Negură A. M.**, 2008 - *Metode de investigare a metabolismului glucidic si lipidic – manual de lucrări practice*. Editura Pim, 182 p.
2. **Cojocaru D.C., Olteanu Z., Ciornea E., Oprică L., Cojocaru S.I., 2007** - *Enzimologie generală*. Editura Tehnopress Iaşi, pag. 273
3. **Constantinescu O.,1974** - *Metode şi tehnici în micologie.* Editura Ceres, Bucureşti
4. **Fraústo da Silva, J.J.R., Williams, R.J.P., 2001**- *The biological chemistry of the elements: the inorganic chemistry of life*, Oxford University Press, 2001, 575 pages
5. **Gow N.A.R., Gadd G.M., 1996 -** *The growing fungus*. Chapman &Hall., pag. 82.
6. **Gârban Z**., **2005** – *Xenobiochimie: Tratat comprehensiv*, IV. Editura Eurobit, Timişoara.
7. **Halliwell B., Gutteridge J.M.C., 2007** - *Free radicals in Biology and Medicine*. Oxford University Press, 851 pages.
8. **Manoliu Al., Bălan M., Oprică L., 2009 -** *Studies on catalase and peroxidase activity in Phanerochaete chrysosporium Burds. cultivated on spruce sawdust media.* An. Șt. ale Univ Alexandru Ioan Cuza, Secţiunea Genetică şi Biologie Moleculară, TOM X, pp: 35-39
9. **Manoliu Al., Tutu E., Oprică L., Ciornea E., Grădinariu P., 2010** - *Influence of the culture medium pH on the activity of some oxidoreductases in Monilinia laxa (Aderh.&Ruhl.) Honey parasite on plum*. An. Şt. ale Universităţii "Alexandru Ioan Cuza", Secţiunea Genetică şi Biologie Moleculară, Tom XI, Fasc.4, 41 - 46.
10. **Nikolaevich Baskin V., 2006 –** *Modern biochemistry: envinronmental risk assessment*. Springer, page 95
11. **Reddy H., 2007 –** *Bioinorganic chemistry.* New Age International, pages 65-66
12. **Saitoh Y., Izumitsu K., Morita A., Tanaka C., 2010** - *A copper-transporting ATPase BcCCC2 is necessary for pathogenicity of Botrytis cinerea,* Molecular genetics and genomics, Volume 284, Number 1, 33-43
13. **Sarikurkcu C., Tepe T., Karslı Semiz D., Solak M.H., 2010** - *Evaluation of metal concentration and antioxidant activity of three edible mushroom from Mugla Turkey*. Food and Chemical Toxicology, 48, 1230-1233
14. **Şoldea C. and Mocanu A.M., 2011** – *Biomolecule. Biotransformări*. Edit. Matrixromn, p. 338
15. **Tanaka C., Izumitsu K., 2010 -** *Two-Component Signaling System in Filamentous Fungi and the Mode of Action of Dicarboximide and Phenylpyrrole Fungicides*. Fungides, Intech. Publishing, page 523-538.