STUDIES CONCERNING THE INFLUENCE OF SOME MINERAL COMPOUNDS ON THE DYNAMICS OF SOME OXIDOREDUCTASES ACTIVITY AT *MONILINIA LAXA* (ADERH.& RUHL.) HONEY PARASITE ON PLUM TREES

STUDII PRIVIND INFLUENȚA UNOR COMPUȘI MINERALI ASUPRA DINAMICII UNOR OXIDOREDUCTAZE LA *MONILINIA LAXA* (ADERH.& RUHL.) HONEY PARAZITĂ PE PRUN

***CIORNEA Elena*[1], *TUTU Elena*[1], *COJOCARU Sabina Ioana*[1]**
e-mail: ciornea@uaic.ro

Abstract. *This study, orientated in two directions, aimed, on one hand, to evaluate the Monilinia laxa (Aderh.&Ruhl.)Honey fungus response to oxidative stress generated by the action of inorganic compounds, knowing the concept that enzymes are involved in pathogenically manifestation induced by biotic agents in microorganisms and, on the other hand, the estimation of antifungal effect „in vitro" of the mineral compounds. We used H_3BO_3, $CuSO_4 x 5H_2O$, $MnCl_2 x 4H_2O$, $Na_2MoO_4 x 2H_2O$, $FeCl_3 x 6H_2O$, $ZnSO_4 x 7H_2O$ and a mixture of them and also, a control sample. The experimental determinations were made at two time intervals and the results showed the clear fungistatic effect of copper and zinc salts, and of the mix of mineral substances, but also the significant differences in the activity of catalase and peroxidase*

Key words: *Monilinia laxa*, mineral compounds, catalase, peroxidase

Rezumat. *Studiul de față, orientat în două direcții, a urmărit pe de o parte evaluarea răspunsului fungului Monilinia laxa (Aderh.&Ruhl.)Honey la stresul oxidativ generat de acțiunea compușilor anorganici, ținând cont de conceptul conform căruia enzimele sunt implicate în manifestările patogenice induse de către agenții biotici în microorganisme, iar pe de altă parte, estimarea efectului fungistatic „in vitro" asupra patogenului a compușilor minerali. S-au folosit în acest sens, H_3BO_3, $CuSO_4 x H_2O$, $MnCl_2 x 4H_2O$, $Na_2MoO_4 x 2H_2O$, $FeCl_3 x 6H_2O$, $ZnSO_4 x 7H_2O$, o mixtură a acestor compuși precum și o variantă martor. Determinările experimentale s-au desfășurat la două intervale de timp, iar rezultatele au demonstrat efectul net fungistatic al sărurilor de cupru, de zinc și al amestecului de substanțe minerale, dar și diferențe semnificative în activitatea catalazei și a peroxidazei.*

Cuvinte cheie: *Monilinia laxa,* compuși minerali, catalaza, peroxidaza

INTRODUCTION

Brown rot caused by *Monilinia laxa* (Aderh.&Ruhl.) Honey is considered one of the most important diseases which affects the species of the *Prunus* genus, being able to generate significant damage by destroying flowers and fruit, and finally, the entire tree (Ogawa J.M., English H., 1991; Four P.H., Holz G., 2003; Holb I., 2008; Michailides T.J. *et al*, 2011). Known as an effective resource, nontoxic and affordable to control diseases caused by fungi, inorganic salts are widely accepted today as an alternative method for

[1] "Alexandru Ioan Cuza" University of Iași, Romania

their control techniques. The main effects are fungistatic and/or fungicides, but, while pure water sometimes inhibit spore germination, such as *Monilinia fructicola*'s spores (Kirk P.M. *et al*., 2008), very low concentrations of some fungicides, even if they are of mineral nature can stimulate germination of spores or even maintain on the other hand, the mycelium growth. Moreover, the data from literature shows that certain nutritional fungal communities react differently to the presence of toxic metals in the environment - some groups (e.g. micorises) decreased for other groups of fungi when the environment is contaminated with metals (Kelly J.J. *et al*., 1999). The fungi can also be highly efficient bio-accumulators of the soluble and particulate forms of metals (Baldrian P., 2003; Gadd G.M., 2010). The behaviour of the fungi towards the mineral compounds and implicitly, towards the metals is dictated by abiotic environmental factors - pH of the medium but also by genetic background and phenotypic expression of the fungus – for example, the cell wall, with is content of melanin (Gadd G.M., 2006). Some researches considers that the anionic composition of the environment influences the toxicity of heavy metals (eg. zinc). Some studies have shown interaction between Zn^{2+} and other divalent cations (e.g. Cd^{2+}, Mg^{2+}, Cu^{2+}) and their combined effects on fungi (Laborey F., Lavollay J., 1973).

This study, conducted in two directions aims, on the one hand, to evaluate the response to oxidative stress of the fungus *Monilinia laxa* (Aderh.&Ruhl.) Honey, stress caused by direct action of „in vitro" ~~i~~norganic compounds hypothesis sustained by the concept that some enzymes are involved in pathogenic events induced by the biotic agents in microorganisms and, on the other hand, to estimate the fungistatic and /or fungicide „in vitro" effect of the mineral compounds as are H_3BO_3, $CuSO_4$ x $5H_2O$, $MnCl_2$ x $4H_2O$, Na_2MoO_4 x $2H_2O$, $FeCl_3$ x $6H_2O$, $ZnSO_4$ x $7H_2O$ or their cumulative effect on the fungus.

MATERIAL AND METHOD

The isolates of the *Monilinia laxa* (Aderh.&Ruhl) Honey was collected from mummified fruit of *Prunus domestica* harvested from the Research and Development Station for Fruit Tree Growing Iasi, Miroslava farm, and pure cultures were selected after qualitative screening in the Laboratory of Microbiology Research Institute of Biological Sciences. The fungus was cultivated „in vitro" on malt medium acidified with 2% streptomycin (Malvárez G. *et al.,* 2001) in Erlenmeyer flasks containing 100 ml of medium. In order to study the effects that in exces metals ions have on the activity of oxidoreductases, we used seven medium variants added after Constantinescu's formula (Constantinescu, 1974). The mineral salts that increased the concentrations were H_3BO_3 – 0,01%, $CuSO_4$ x $5H_2O$ – 0,1% , $MnCl_2$ x $4H_2O$ – 0,02%, Na_2MoO_4 x $2H_2O$ – 0,02%, $FeCl_3$ x $6H_2O$ – 0,02%, $ZnSO_4$ x $7H_2O$ – 0,2%. Each was added separately, one in the culture medium and in one variant, all compounds were added together. We used in parallel a control variant whose culture medium was not supplemented with any of these mineral compounds. This eight medium were seeded with slices of 8 mm in diameter from a *Monilinia laxa* culture at the age of 7 days and cultivated under similar conditions for "in vivo" development: light/dark photoperiod and variable temperature. The practical study consisting of three consecutive experimental measurements was conducted in two intervals, at 7 days and respectively 14 days after inoculation of the culture and the catalase and peroxidase activity was determined both from the fungus mycelium and from the culture liquid. The catalase activity was assayed by iodometric method whose principle

is based on the determination of hydrogen peroxide that remained un- decomposed after stopping the enzyme activity with sodium thiosulfate, in the presence of starch as an indicator and for the determination of the peroxidase activity the o-dianisidine method was used (Cojocaru, 2009).

RESULTS AND DISCUSSIONS

A first objective of this study was the determination of catalase activity in both the mycelium and the culture liquid of the fungus *Monilinia laxa (*Aderh. &Ruhl.) Honey parasite on plum. In the initial analysis, we can see that this oxidoreductase activity varies depending on the age of the fungus and the type of mineral compound that was used to supplement the culture medium.

As shown in figure 1, the activity of catalase in the mycelium of the fungus at 7 days after inoculation for the control variant present value of 3.7143 CU/g /min. Because $CuSO_4$ x$5H_2O$, $ZnSO_4$ x $7H_2O$ and respectively, the mixture of mineral compounds inhibited completely the growth of the fungus mycelium, the value of the catalase activity in these variants was noted 0. All medium variants showed, reported to the control variant, lower values of the endoenzyme, except $FeCl_3$ x $6H_2O$ version, which activity peaked at 4.6304 CU/g/min., followed by the variants $MnCl_2$ x $4H_2O$ - 1.0054 CU/g/min., H_3BO_3 - 0.5777CU/g/min., respectively Na_2MoO_4 x $2H_2O$ - 0.2720 CU/g/min.

After 14 days of incubation, the catalase activity in the fungus mycelium has reached 5.0038 CU/g/min in the control variant and, for the remaining samples the intracellular enzyme activity is inhibited. Lowest catalase activity was present for variant V4 - 1.4026 CU/g/min., followed by variant V1 - 1.7739 CU /g/min., variant V3 - 1.0054 CU/g/min and for variant V5 - 2.2019 CU/g/min.

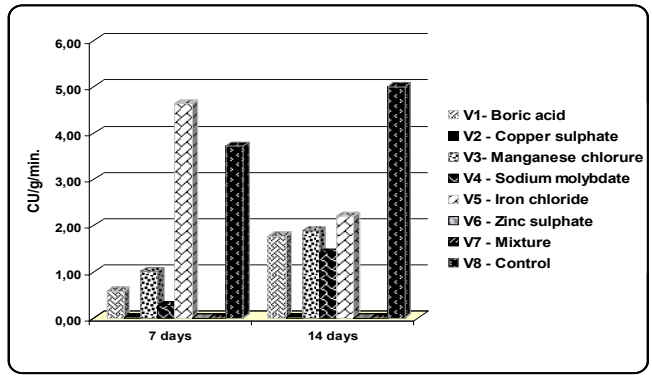

Fig. 1 - The catalase activity in the mycelium of *Monilinia laxa* (Aderh.&Ruhl.) Honey specie's cultivated on the medium supplemented with various mineral compounds

A critical analysis on the dynamics of the enzyme activity shows an increase of the catalase activity with the aging of the culture medium to all variants, except variant $FeCl_3$ x $6H_2O$, in which the enzyme activity decreased very slightly (from 4.6304 CU/g/min. at 2.2019CU /g/min).

The experimental data regarding the influence exercised by the boric acid and mineral salts used in this study on the extracellular catalase activity are plotted in

figure 2, and, in their analysis it could be found that all the samples studied had inferior levels of the exoenzyme activity of the control variant, which registered a value of 1.3260 CU/ml /min. Compared with that, more diminished activity was recorded for variant containing $FeCl_3$ x $6H_2O$ - 1.1560 CU/ml/min., while Na_2MoO_4 x $4H_2O$ and $MnCl_2$ x $2H_2O$ recorded almost identical values - 0.8250 CU/ml/min. respectively, 0.8500 CU/ml/min. Minimum point of the activity of this biochemical parameter was noted in the medium variant containing H_3BO_3 - 0.6120 CU/ml/min. and to the working variants V2, V6 and V7, the enzyme activity was null.

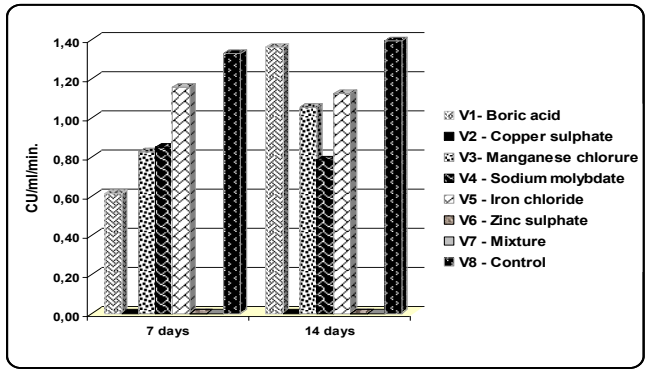

Fig. 2 - Catalase activity in the liquid culture of the *Monilinia laxa* (Aderh.&Ruhl.) Honey specie's cultivated on the medium supplemented with various mineral compounds

The aging of the culture entailed a moderate increase in the catalase activity in liquid culture medium at all options. The control recorded the value of extracellular catalase 1.3940 CU/ml/ min. and the minimal activity was found in the medium variant containing Na_2MoO_4 x $2H_2O$- 0.7820 CU/ ml/min. this time Boron had the most intense effect (although less than that of the control sample) - 1.3600 CU/ml/min. followed in descending order of variant with $FeCl_3$ x $6H_2O$ - 1.1220 CU/ml/min. and variant with $MnCl_2$ x $4H_2O$ - 1.0540 CU/ml/min. Detailed examination of the dynamics of catalase activity in the liquid culture revealed that all medium variants have been stimulated by this enzyme, less variant V5, which decreased from 1.1560 CU/ml/min. to 1.1220 CU/ml/min. and V4, which decreased from 0.8500 CU/ml/min to 0.7820 CU/ml/min., all medium variants having lower values than the control sample.

Another objective of this study was the determination of the peroxidase activity in the mycelium of the fungus *Monilinia laxa* (Aderh.&Ruhl.) Honey at 7 days after seeding, the values obtained being systematized in figure 3. A first observation indicates that both minerals used in the experiment and boric acid also showed inhibitory effect, the strongest being recorded at medium variant containing $FeCl_3$ x $6H_2O$ - 0.0893 PU/g/min., versus control sample, an activity that has been of 0.4119 PU/g/min. V4 variant showed a value of 0.2768 PU/g/min. while the version with boric acid had the most intense activity of all mineral compounds (0.3418 PU/g /min.). At 14 days after seeding inoculation, the culture with mycelium peroxidase activity has registered in the control version values of 0.43907 PU/g/min., the minimum point of the endoenzyme activity at this time being found in the medium

variant containing $FeCl_3$ x $6H_2O$ (0.0616 PU/g/min.). Intermediate values were recorded as follow: $MnCl_2$ x $4H_2O$ - 0.3178 PU/g/min., H_3BO_3 - 0.2703 PU/g/min., Na_2MoO_4 x $2H_2O$ - 0.2528 PU/g/min.

Extracellular peroxidase (fig. 4) reached in the control variant at 7 days after inoculation of the culture, the value of 0.2229PU/ml/min.

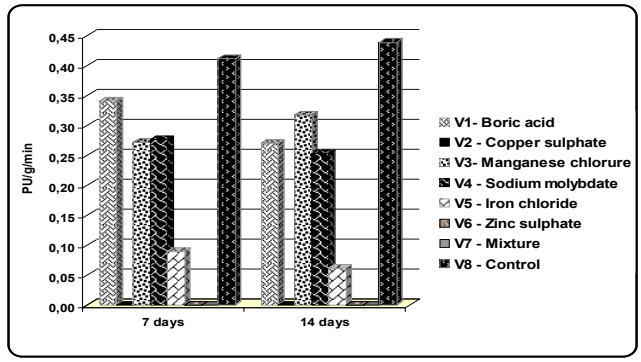

Fig. 3 - Peroxidase activity in the *Monilinia laxa* (Aderh.&Ruhl.) Honey specie's mycelium cultivated on the medium supplemented with various mineral compounds

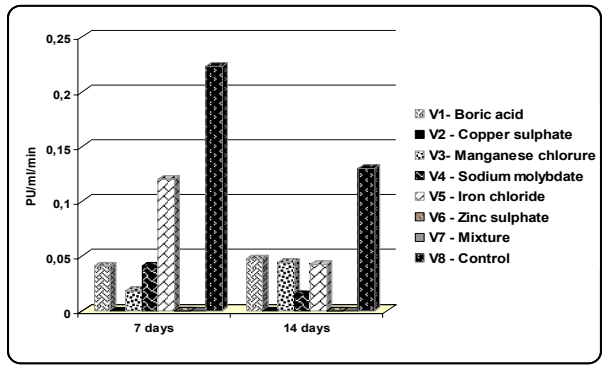

Fig. 4 - Peroxidase activity in liquid culture of the *Monilinia laxa* (Aderh.&Ruhl.) Honey specie's cultivated on the medium supplemented with various mineral compounds

Boric acid induced a peroxidase activity outside of the mycelium, from 0.0406 PU/ml/min. having like the rest of mineral salts, an inhibitory action on the exoenzyme. Minimum point was highlighted in the medium variant containing $MnCl_2$ x $4H^2O$ (0.0182 PU/ml/min) and the variant with Na_2MoO_4 x $2H_2O$ peroxidase activity was given an identical amount of boric acid (0.0406 PU/ml/min). In liquid culture, the peroxidase activity was relatively uniform, after 14 days of incubation in the medium used in all variants, except variant V4 (Na_2MoO_4 x $2H_2O$) who detected the minimum value (0.01458 PU/ml/min), the control having the highest threshold of enzyme activity in the liquid culture (0.1302 PU/ml/min). The results confirm data from literature according to which $CuSO_4$ x $5H_2O$ is fungicide (Krieger 2001; Nasim G., 2008) as $ZnSO_4$ x $7H_2O$ (Matolcsy D.G. et al, 1988), its effect being, however, lower to copper, confirmed by the Horsfall order of metal toxicity: Ag> Hg> As> Cd> Ni> Pb> Co> Zn> Fe> Ca.

CONCLUSIONS

The analysis of experimental results regarding the influence of the mineral compounds on the dynamics of some oxidoreductases activity in *Monilinia laxa* (Aderh.&Ruhl.) Honey parasite on plum has allowed us to draw the following general conclusions:

1. In all medium variants, both in fungus mycelium and in liquid culture, catalase and peroxidase were inhibited to a greater or lesser measure by the use of mineral compounds, finding values for the lower activity exhibited by the control.

2. $CuSO_4$ x $5H_2O$, $ZnSO_4$ x $7H_2O$ and the mixture of mineral compounds had an clear antifungal effect on the *Monilinia laxa* (Aderh.&Ruhl.) Honey fungus.

REFERENCES

1. **Baldrian P., 2003 –** *Interaction of heavy metals with white- rot fungi*. Enzyme Microb. Technolog. 32, pg. 72-91
2. **Cervantes C., Gutierrez - Corona, 1994 -** *Copper resistance mechanisms in bacteria and fungi.* FEMS Microbiology Reviews, volume 14, pg. 121-137
3. **Cojocaru D. C., 2009** - *Enzimologie practica*. Editura Tehnopress 458 p
4. **Constantinescu O., 1974** - *Metode si tehnici în micologie*. Ed. Ceres, Bucuresti.
5. **Fitter A.H., Raffaelli D.G., 1999 -** *Advances in Ecological Research***.** volume 30, Academic Press, pg. 73-76
6. **Fourie P.H., Holz G,. 2003** - *Germination of dry, airborne conidia of Monilinia laxa and disease expression on nectarine fruit.* Australasian Plant Pathology 32: 19-25
7. **Gadd G.M., British Mycologycal Society, 2006 -** *Fungi in biochemical cycles***.** Cambridge University Press, pg. 242-246
8. **Gadd G.M., 2010 –** *Metals, minerals and microbes. Geomicrobiology and bioremediation***.** Microbiology 156 (3) 609-643
9. **Holb I., 2008** - *Brown rot blossom blight of pome and stone fruits: symptom, disease cycle. host resistance and biological control*. International Journal of Horticultural Sc. 14 (3), pg. 15–21
10. **Kelly J**.J., **Haggblom M., Tate R.L., 1999** – *Changes in soil microbial communities over time resulting from one time application of zinc. A laboratory microcosm study.* Soil. Biol.Biochem. 31, pg. 1455 - 1465
11. **Kirk P.M., Ainsworth G.C., Cannon P.F., Minter D.W**., **2008** - *Ainsworth & Bisby's dictionary of the fungi.* CABI Press, pg. 265-266
12. **Laborey F., J. Lavollay, 1973** - *Sur la nature des antagonismes responsables de l'interaction des ions Mg++, Cd+, et Zn++ dans croissance d'Aspergillus niger.* C. R. Acad. Sci. 276, pg. 529-532.
13. **Malvárez G., Rodríguez A., Aguilar C., Silvera E., Mondino P., 2001 -** *Identificación de especies de Monilinia spp., en aislamientos obtenidos de Prunus spp. por PCR con primers específicos.* Agrociencia V(1), pg.48-53.
14. **Matolcsy D.G., Nádasy M., Andriska V., Terényi S., 1988** – *Pesticide chemistry*. vol 32, pg. 272
15. **Mehra R.K., Winge D.R., 1991** - *Metal ion resistance in fungi: Molecular mechanisms and their regulated expression,* Journal of Cellular Biochemistry, volume 45, pg. 30-40
16. **Michailides T.J., Luo Y., Ma Z., Morgan D.P., 2011** - *Brown rot of dried plum in California. New insights on an old disease.* The American Phytopathological Society
17. **Nasim G., Ilyas N., Ali A., Munawar A., 2008 –** *Effect of salt priming on "in vitro" growth of some soil fungi.* Pak. J. Bot., 40(1), pg. 427-431**.**
18. **Ogawa J. M., English H., 1991** - *Diseases of Temperate Zone Tree Fruit and Nut Crops.* University of California, Div. of Agric. and Nat. Resources, Oakland, CA. Publ. No. 3345