

STUDY OF A MICROBIAL INOCULUM ON SEVERAL BIOCHEMICAL INDICES IN SUNFLOWER (*HELIANTHUS ANUUS* L.)

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Abstract: The study of the complex interaction between rhizospheric bacteria and plant roots represents a very important and actual problem in microbiology. The use of bacteria that stimulate plant growth – PGPR (plant growth promoting rhizobacteria) – as biofertilizers is one of the most promising biotechnologies used for the increase of the primary production with reduced amounts of chemical fertilizers (Lemanceau, Alabouvette, 1993).

INTRODUCTION

It is well known that by their roots, plants secrete in the environment an important diversity of organic substances. These products are used by microorganisms as important sources of nutrients. On their part, the microbial populations adapted to rhizospheric environment influence plant development and production quality in a significant manner. (Whipps and Lynch, 1980). Lately it was shown that several rhizospheric bacteria could stimulate the plant metabolic process and implicitly the plant growth (Merbach and Ruppel, 1992). The microorganisms' activity can produce these effects by:

- Secretion of vitamins and phytohormones (Polonskaya, 1995);
- Production of antibiotics which inhibit the growth of the pathogen fungi
- Conversion of some minerals to more accessible forms for the plants.

In this context, the goal of our paper is the study of the influence of several rhizospheric bacteria on both the catalase activity and the assimilating pigment content in the sunflower plants.

MATERIALS AND METHODS

Six bacterial strains (conventionally marked as F1, F2, F3, F4, F5, and F6) isolated from *Helianthus annuus* rhizosphaera were used to prepare a suspension in distilled water = the inoculum. Biopreparate concentration was calculated by counting the colonies in plates and estimated at 64×10^6 UFC/ml.

After the initial sterilization of all sunflower caryopses, the inoculation by immersion in biopreparate was performed only on the treated group of caryopses. Both groups (control and treated) were sown by using a mini experimental sower. The experiment was done in 2004 in the Didactic Farm Ezăreni, Didactic Station of USAMV „Ion Ionescu de la Brad” Iași on a cambic chernozim with argilo-sandy texture and middle to good fertility, with a moderate content in humus and a relatively high amount of nitrogen, well enriched in potassium and a slightly acid to neutral pH.

The testing of catalase activity and assimilating pigment content in plant leaves was performed on the foliar tissue of both control and treated plants in two stages: 29.06.2004 (prior to the bloom) and 19.07.2004 (when the heads were formed completely).

Determination of the catalase activity was performed by using the iodometric titration (V. Artenie, Elvira Tănase, 1981), the results being displayed in catalase units (CU)/ g vegetal material.

Chlorophyll and carotene concentration determination in leaves was performed by using photocolometric technique (V. Artenie, Elvira Tănase, 1981) and the results were shown in mg/100 g vegetal material. 90% acetone was used for extraction, while the readings were performed on a Metertek SP-830 spectrophotometer using the following wavelengths: chlorophyll a –662 nm, chlorophyll b 644 nm, and carotene – 440.5 nm.

Statistical analysis

The results expressed as mean \pm standard error were statistically analyzed by using T-test (Student). Theoretical probability of the test was set at $p < 0.05$.

RESULTS AND DISCUSSIONS

Catalase activity

The results obtained showed that the studied biopreparate influenced the catalase activity in the foliar tissues of *Helianthus annuus* plants. Thus, Fig. 1 shows that catalase activity prior to the bloom is bigger in the treated group (363.41 CU/100 g vegetal matter) than that in the control (259.41 CU/100 g vegetal matter), with a significant t-test ($p < 0.03$).

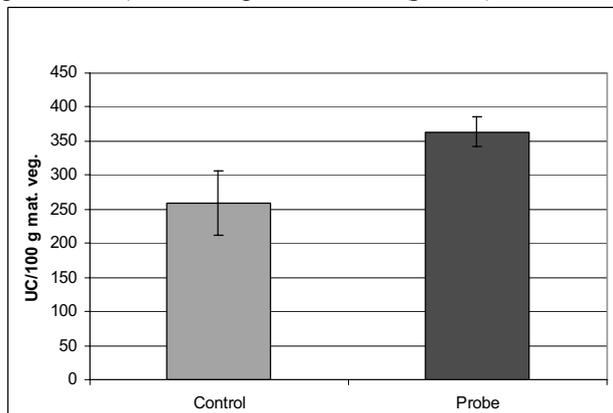


Fig. 1 - Catalase activity of control and treated groups of *Helianthus annuus* prior to the bloom period (29.06.2004)

This difference observed is the result of the rhizospheric microorganism activity (the initial inoculum) because the other factors that could take place (neighbor influence, non-uniformity of the soil, climatic factors, etc) have no influence in this case. Having in consideration the fact that the probes were taken from physiologically normal plants we suggest that an increased catalase activity in the treated group can eventually be correlated with an intensification of respiratory processes that metabolically underlie the growth process.

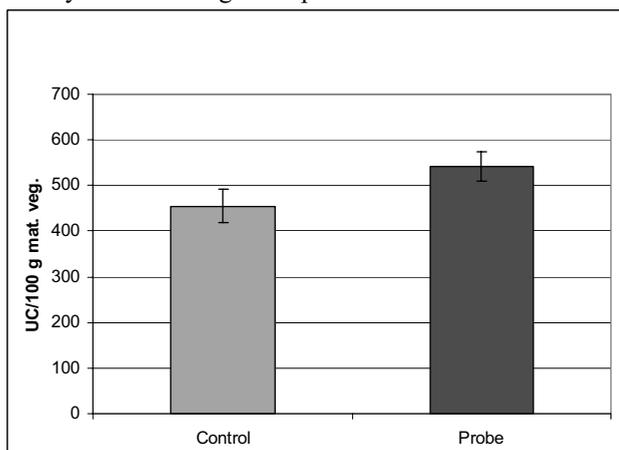


Fig. 2 - Catalase activity of control and treated group of *Helianthus annuus* after the bloom. (19.07.2004)

Fig. 2 shows that the differences between catalase activity of the control and treated group after the bloom (19.07.2004) become statistically non-significant ($0.005 < p < 0.05$). This result can be explained by the fact that, after the bloom, the decreased intensity of the majority of the plant metabolic processes is followed by a decreased exchange of substances mediated at the radicular

level by the rhizobacteria. This reduction of the positive influence of the rhizospheric microorganisms can be considered as a normal one in this situation.

Concentration of assimilating pigments

The concentration of assimilating pigments (chlorophyll a and b and carotenes) in the foliar tissue of control and treated group was determined in two stages, before and after the bloom. The results are presented in Fig. 3 and Fig. 4.

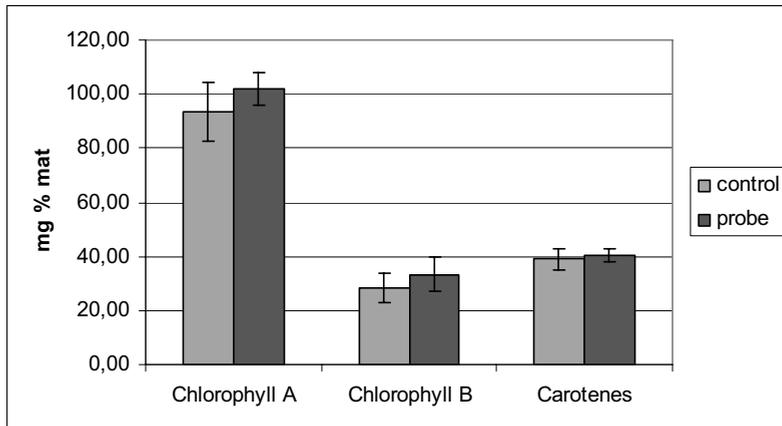


Fig. 3 Concentration of assimilating pigments in the foliar tissue of *Helianthus annuus* prior to the bloom (29.06.2004)

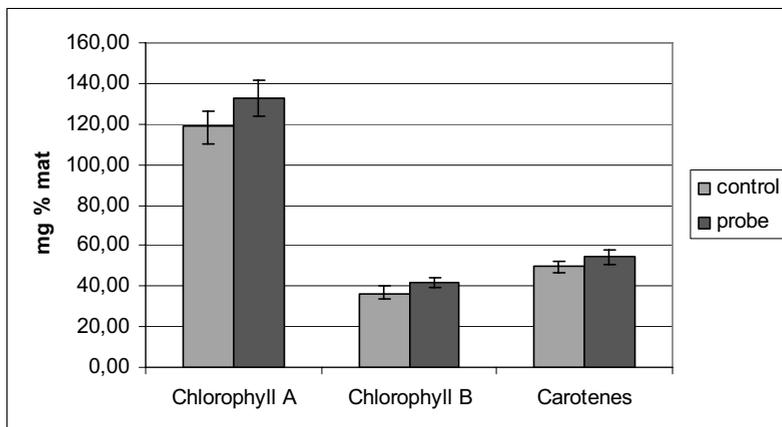


Fig. 4 Concentration of assimilating pigments in the foliar tissue of *Helianthus annuus* after the bloom (19.07.2004)

The data analysis has pointed out that although in the maximum activity period (prior to the bloom) the amount of the assimilating pigments is bigger in the treated group (101.98 mg chlorophyll a /100 g vegetal matter, 33.34 mg chlorophyll b /100 g vegetal matter, 40.43mg carotenes /100 g vegetal matter), the difference is not statistically significant (T test is non-significant $p=0.24>0.05$, $p=0.28>0.05$ respectively $p=0.39>0.05$).

The same situation was also observed after the bloom when as a result of the decreased influence exerted by the rhizospheric microorganisms, the difference regarding the content of assimilating pigments between the control and treated group become non-significant.

The lack of statistical significance of the values obtained as a result of quantitative determination of assimilating pigments should not be exclusively interpreted as a consequence of the positive non-influence of rhizospheric microorganism on plant metabolism. A possible explanation of this result could relay on the fact that the positive influence of rhizospheric microorganisms weakly manifests at the level of photosynthetic pigment metabolism maybe because of the complexity of the metabolic chains involved in this process. The more obvious manifestation of the positive action exerted by the rhizospheric microorganisms on the catalase activity level comparatively with the action on photosynthetic pigment biosynthesis can also be associated with a larger involvement of catalase in energetic processes associated with the plant growth and development, the photosynthetic pigments representing end products of several metabolic chains compared to catalase which is directly involved in more metabolic chains.

CONCLUSIONS

Catalase activity of treated group is higher (statistically significant) than that observed in the control group both before and after the bloom period, with a smaller difference between the groups after the bloom.

The amount of photosynthetic pigments in the foliar tissue of treated group is smaller but statistically non-significant compared to that in the foliar tissue of control group.

Taking in consideration the intensification of catalase activity and the increase of assimilating pigments in treated group as a result of biopreparate administration, we suggest that the metabolic modifications induced by the rhizobacteria prior to the bloom could lead to a stimulation of plant growth and development.

After the bloom, this beneficial influence becomes non-significant, maybe because of the normal physiologic decrease in the intensity of metabolic processes.

REFERENCES

1. Artenie, V., Elvira Tănase, 1981 – *Practicum de biochimie generală*, Ed. Univ. „Al. I. Cuza” Iași, 110-111, 135-138.
2. Burzo, I. Toma, S., Dobrescu, A., Ungurean, L., Ștefan, V., 1999 - *Fiziologia plantelor de cultură*, vol. 2, Fiziologia culturilor de câmp, Întreprinderea Editorial-Poligrafică Știința, Chișinău.
3. Merbach, W., Ruppel, S., 1992 – Influence of microbial colonization on $^{14}\text{CO}_2$ assimilation and amopunts of rootborne 14C compounds in soil, *Photosynthetica*, vol. 26, no. 4, 551-554.
4. Orazova, M.K., Polyanskaya, L.M., Zviagintsev, D.G., 1999 – The structure of the microbial community in the barley root zone, *Microbiology*, vol. 68, No. 1, 109-115.
5. Polonskaya, J.E., 1995 – The influence of epiphytic bacteria on growth and nitrogen absorbtion of pine seedling roots (sterile conditions and field test), *Abstracts of papers of Inst. Long Ashton Int. Symp.* Bristol, England, 13-15 Sept., 100.
6. Thuar, A.M., Olmedo, C.A., Bellone, C., 2000 – Greenhouse studies on growth promotion of maize inoculated with plant growth-promoting rhizobacteria (PGPR), *Proceedings of the Fifth International PRPR Workshop*, Cordoba, Argentina.
7. Whipps, J.m., Lynch, J.M., 1986 – The influence of the rhizosphere on crop productivity, *Adv. Microb. Ecol*, vol. 9, 187-244.

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