CYTOGENETIC EFFECTS INDUCED BY HEAVY METALS SALTS AT *LENS ESCULENTA* MOENCH.

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Keywords: lead acetate, ferrous sulphate, copper sulphate, aberrations, *Lens esculenta* Moench.

Abstract: The aim of this paper, is to evaluate the cytogenetic effects induced by lead acetate, ferrous sulphate, copper sulphate (heavy metals salts), on meristematic root tips cells of *Lens esculenta* Moench. The different treatment variants, induced significant changes regarding cells division frequency (showing a decrease) and an increase of mitotic ana-telophases with aberrations.

INTRODUCTION

The aim of the study was to estimate the effects of some heavy metals salts on germinated seeds of lentil (*Lens esculenta* Moench), at the level of cells and more specifically at genetic level. Lead acetate is a chemical compound, a white crystalline substance with a sweetish taste and high toxicity (Berdan, 1992). Applied on the seedlings roots, produce frequent changes on both cellular or molecular level. Ferrous sulphate is most commonly encountered as the blue-green heptahydrate, In horticulture it is used as a lawn conditioner and moss killer. Copper sulphate is used as an herbicide, fungicide, pesticide, inhibits growth of bacteria such as *E. coli*..

MATERIAL AND METHODS

As biological material it was used germinable seeds of lentil (*Lens esculenta* Moench), from 2003-2005 harvest. Seeds were sawn in Petri dishes, on filter paper moisturized with distillated water. Seeds started to germinated after 2 days, and germination percentage was 90%.. For the controlle variant, roots of 10-15mm were prelevated after germination and preserved in ethanol. Germinated seeds for the different treatment variants, were further placed in Petri dishes with filter paper moisturized with lead acetate, or ferrous sulphate, and respectively copper sulphate solutions on different concentrations: 0,01%, 0,02% şi 0,05%, for 12, 24 or 48h.

There were finally 27 treatment variants and a controlle one (not treated with chemicals). The Squash technique was used to prepare the microscopical slides (Câmpeanu, 2002).

RESULTS AND DISCUSSIONS

Mitotic Index

In the case of treatment with lead acetate solution, it could be noticed (Fig. 1), that comparing with controlle, for 12 h treatment time, there is a significant decrease of Mitotic Index, for all tested concentrations : 0,01%, 0,02% şi 0,05%.

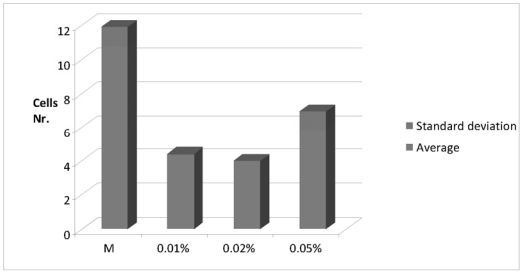

Fig. 1. Mitotic Index in the case of Lens treated for 12h with different lead acetate solution concentrations

0,01%, 0,02%, 0,05% - concentration of the substance used;

Similar results were obtained for the 24h treatment time, for all tested concentrations of lead acetate solutions (Fig. 2) :

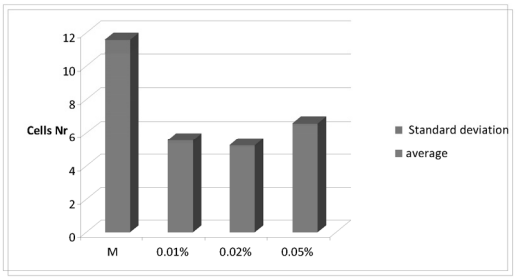

Fig. 2. Mitotic Index in the case of Lens treated for 24h with different concentrations of lead acetate solution

0,01%, 0,02%, 0,05% - concentration of the substance used;

Similar results (decrease of mitotic index with the increase of concentration of substance) were obtained for the 48h treatment time, for all tested concentrations of lead acetate solutions (Fig. 3) :

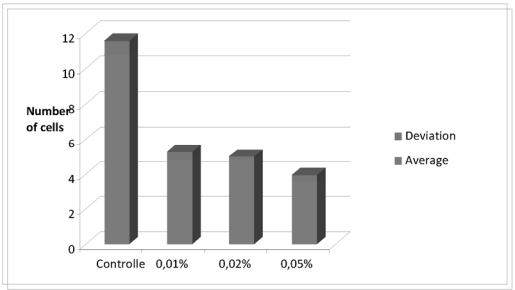

Fig. 3. Mitotic Index in the case of Lens treated for 48h with different concentrations of lead acetate solution

0,01%, 0,02%, 0,05% - concentration of the substance used;

Comparing the results got after the treatment with the tested three concentrations of lead acetate solution, regarding the cellular division frequency, we may speak of an influence of the chemical agent, depending both: solutions concentration and treatment duration. By increasing lead acetate solution concentration, and time of treatment, cells division frequency decrease comparing with controlle.

In the case of treatment with ferrous sulphate, if the concentration of the chemical substance applied is raised, the mitotic index decrease, demonstrating an inhibitory effect on cells division (Fig. 4, Fig. 5, Fig. 6).

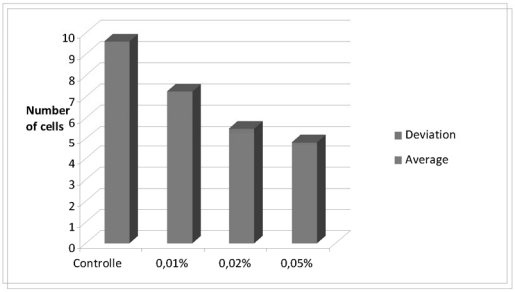

Fig. 4. Mitotic Index in the case of Lens treated for 12h with different concentration of ferrous sulphate solutions

0,01%, 0,02%, 0,05% - concentration of the substance used;

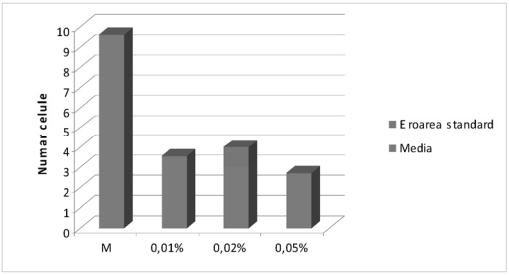

Fig. 5. Mitotic Index in the case of Lens treated for 24h with different concentration of ferrous sulphate solutions

0,01%, 0,02%, 0,05% - concentration of the substance used;

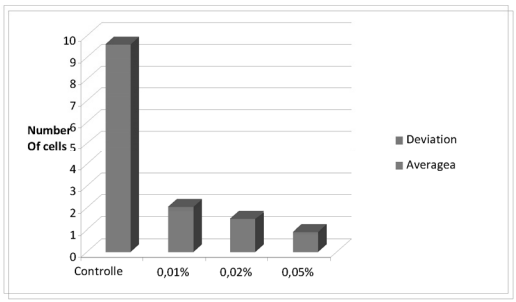

Fig. 6. Mitotic Index in the case of Lens treated for 48h with different concentration of ferrous sulphate solutions

0,01%, 0,02%, 0,05% - concentration of the substance used;

The analysis of the radicular apex of the lens shows big modifications as a result of using copper sulphate. In comparison to the control, for all concentration, there is a decrease of the mitotic index, almost to completely inhibition of cells division.

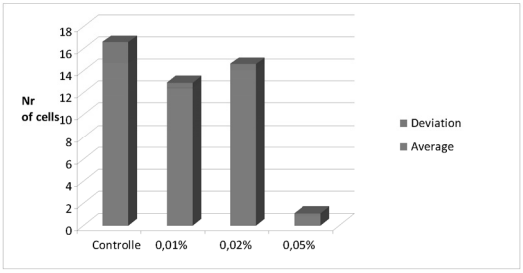

Fig. 7. Mitotic Index in the case of Lens treated for 12h with different concentration of copper sulphate solutions

0,01%, 0,02%, 0,05% - concentration of the substance used;

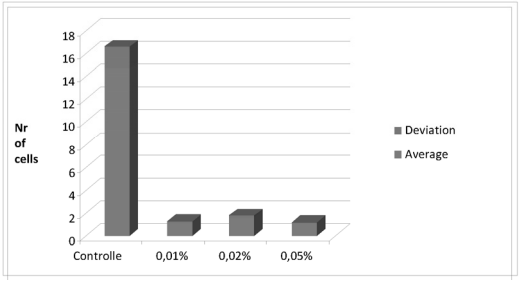

Fig. 8. Mitotic Index in the case of Lens treated for 24h with different concentration of copper sulphate solutions

0,01%, 0,02%, 0,05% - concentration of the substance used;

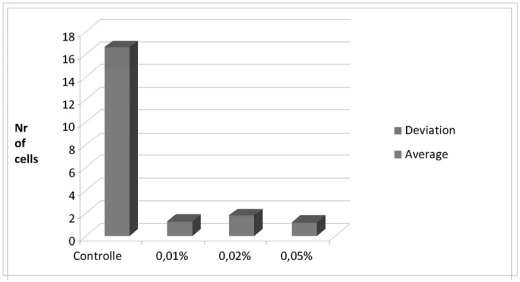

Fig. 9. Mitotic Index in the case of Lens treated for 48h with different concentration of copper sulphate solutions

0,01%, 0,02%, 0, 0,01%, 0,02%, 0,05%- concentration of the substance used;

The frequency of cells with chromosomial aberrations

If we consider the values obtained after 12 hours of treating with lead acetate, we notice that, compared to the control, the frequency of the aberrant ana-telophases (double or single bridges, late or expulsed chromosomes) increases progressively very much, proportionally with the increase of the concentrations of the chemical agent.

After 24 hours of treatment, the frequency of aberrant ana-telophases continue to increase, for all tested concentration of the substance, reaching in the case of 0,05% lead acetate solution concentration, the double of aberrant ana-telophase number, comparing with the number got for control variant.

After 48 hours of treatment, for 0,05% lead acetate solution concentration, the number of aberrant ana-telophases, was six time higher compared to the number got for control variant. It can be concluded that by increasing the treatment period with lead acetate, it increase proportionally the frequency of cells with aberrant ana-telophases.

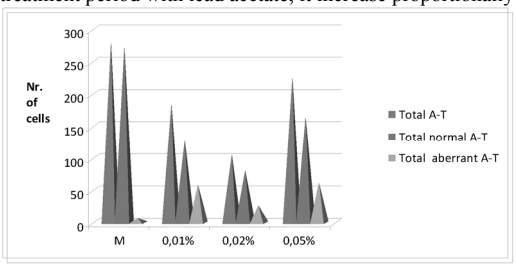

Fig. 10. The frequency of the ana-telophases with aberrations at lentil, after 12h treatment with different concentration of lead acetate solutions

0,01%, 0,02%, 0,05% - concentration of the substance used;

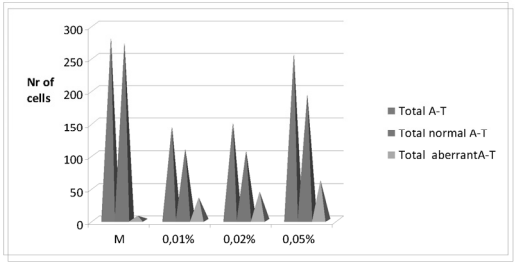

Fig. 11. The frequency of the ana-telophases with aberrations at lentil, after 24 h treatment with different concentration of lead acetate solutions

0,01%, 0,02%, 0,05% - concentration of the substance used;

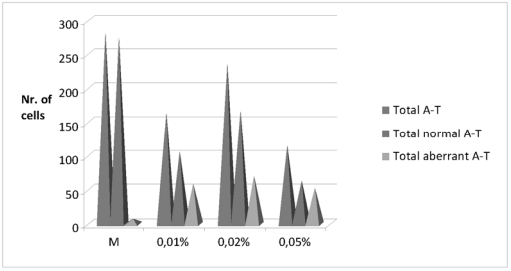

Fig. 12. The frequency of the ana-telophases with aberrations at lentil, after 48 h treatment with different concentration of lead acetate solutions

0,01%, 0,02%, 0,05% - concentration of the substance used;

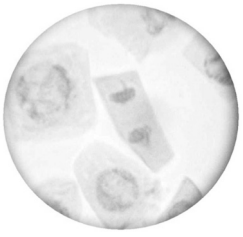

Fig. 13. A-T with expolsed chromosomes

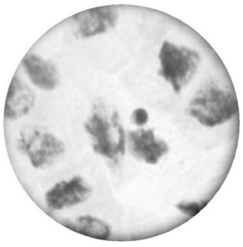

Fig. 14. A-T with micronucleus

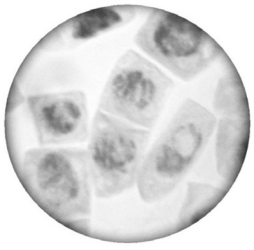

Fig. 15. Telophase (0,01% lead acetate)

In the case of treatment with ferrous sulphate, if the time the substance applied is raised, the frequency of the aberrant ana-telophases increases progressively, but the types of aberrations decrease (Fig. 16, Fig. 17, Fig. 18).

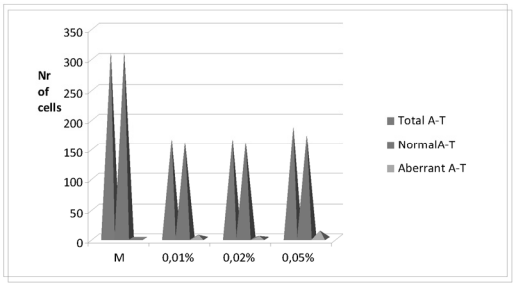

Fig. 16. The frequency of the ana-telophases with aberrations at lentil, after 12h treatment with different concentrations of ferrous sulphate solution

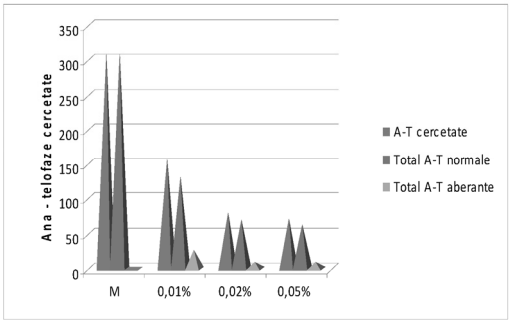

Fig. 17. The frequency of the ana-telophases with aberrations at lentil, after 24h treatment with different concentrations of ferrous sulphate solutions

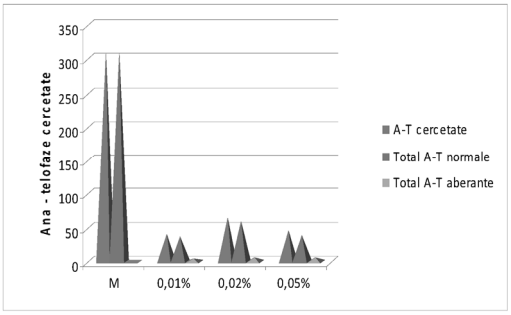

Fig. 18. The frequency of the ana-telophases with aberrations at lentil, after 48h treatment with different concentrations of ferrous sulphate solutions

In the case of treatment with copper sulphate, if the time and the concentration of the substance applied is raised (12h; 24h; and respectively 0,01%, 0,02%, 0,05% - concentration of the substance used), the frequency of aberrant ana-telophase decrease to no cell with aberration for 48h of treatment (Fig. 19, Fig. 20, Fig. 21.).

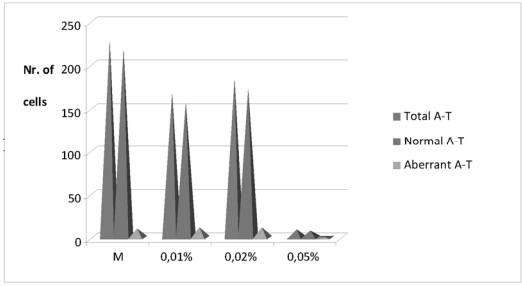

Fig. 19. The frequency of the ana-telophases with aberrations at lentil, after 12h treatment with different concentrations of copper sulphate solutions

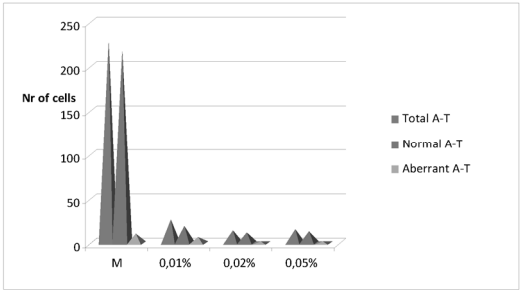

Fig. 20. The frequency of the ana-telophases with aberrations at lentil, after24h treatment with different concentrations of copper sulphate solutions

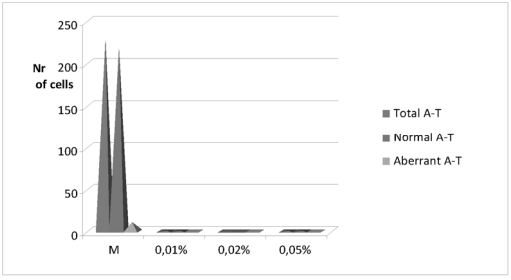

Fig. 21. The frequency of the ana-telophases with aberrations at lentil, after 48h treatment with different concentrations of copper sulphate solutions

CONCLUSIONS

Lentil *(Lens esculenta* Moench.) proves an increased sensitivity to lead acetate, showed by the decrease of mitotic index compared to control, for all tested treatment times, and respectively for all tested concentrations of the solution.

In the case of treatment with ferrous sulphate at *Lens esculenta* Moench., if the time and the concentration of the substance applied is raised, the frequency of the aberrant ana-telophases increases progressively, but the types of aberrations decrease.

In the case of treatment with copper sulphate, if the time and the concentration of the substance applied are raised, the frequency of aberrant ana-telophase decrease to no cell with aberration for 48h of treatment.

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