THE CYTOGENETIC EFFECTS OF TREATING THE HOT CHILLI PEPPER (*CAPSICUM ANNUUM L.*)WITH SALTS OF HEAVY METALS

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Key words: lead acetate, iron sulphate, , mitotic division, *Panicum miliaceum* L..
Abstract: this article presents the cytogenetic effects that the lead acetate and ferrous iron sulphate have on the mitotic division and on the growth and development of the plant after being treated with such chemical substances. The applied treatment determined significant alterations on the mitotic index and on the frequency of the cells in various stages of division.

INTRODUCTION

By the term “heavy metals” , we understand a series of elements with high density (over 5 kg/dm^3) with quite complex chemical properties, especially a high level of oxidation. A noticeable toxic action was was observed in the case of copper(d = 8,9), mangane (d = 7,43), lead(d = 11,34), mercury (d = 13,6) etc (Hătărăscu, 1982).

The biogeodynamic characteristics of each heavy metal has a very important ecologic significance within the framework of the relationships among the individual components of the ecosystems. The transfer of metals in the interior of trophic chains air – soil – plant – animal is not to be neglected. The heavy metals produce a series of profound changes in the metabolism of plants, most of the plants being sensitive to the effects of heavy metals: stomas’ opening is compromised, the photosynthesis diminishes considerably, the breathing process is disturbed, growth is slowed down, etc.

MATERIALS AND METHODS

As a biological material, we used seeds of hot chilli pepper (*Capsicum annuum* L.) from the harvest 2003-2005. The seeds were placed to germinate in Petri dishes on filter paper soaked with distilled water. The germination took place 4 days later, in a proportion of 90%. From the Petri dishes with control-seeds, we harvested the roots that were 10-15mm long. From the rest of the Petri dishes, we harvested the seeds and replanted them in new Petri dishes in which the paper was soaked with lead acetate and ferrous sulphate, with concentrations of de 0,01%, 0,02% şi 0,05%, for 12, 24, 48 hours.

In the end, we got 27 samples for this species, plus the control-variant, on which no chemical substances had been applied. Once the roots obtained, we used the Squash method (Câmpeanu, 2002) to emphasise the chromosome aberrations. Also, we took digital camera pictures of various stages of division for all three concentrations and times.

RESULTS AND DISCUSSIONS

Mitotic index

Applying the lead acetate in the three concentrations determined the reduction in the values of the mitotic index. From the table 1, fig. 1, we can draw the conclusion that the mitotic index in the radicular apex is diminished. Thus, in the case of the control (with no chemicals applied), the value is approximately 5,85 ori higher than the value obtained by applying the substance(lead acetate) in a concentration of 0,01%, while for the la other concentrations (0,02 and 0,05%), this parameter is 9,56 times lower.

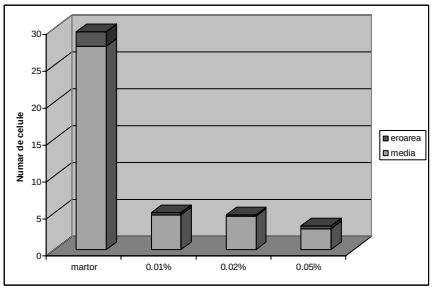

Fig. 1 The mitotic index of the hot chilli pepper after being treated with lead acetate for12 hours

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

For the action time of 24 hours, we can also see (fig. 2), a decrease in the mitotic index corresponding to a growth in the concentration of the chemical agent. The drop can be seen from the lowest concentration (0,01%) to the highest

(0,05%). Therefore, the elevation of the concentration of the lead acetate is directly proportional with the drop of the mitotic index.

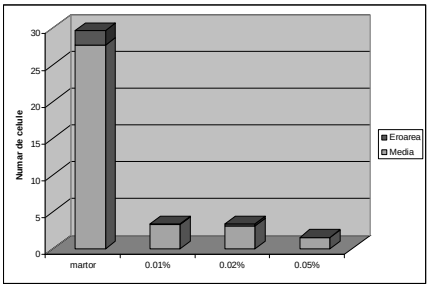

Fig. 2. The mitotic index of the hot chilli pepper after being treated with lead acetate for 24 hours

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

As a result of prolonged exposure of the hot chilli pepper to lead acetate for 48 hours, (fig. 10), there is a drop of the mitotic index for the concentration of 0,01 %. Surprisingly, when the concentration of the chemical substance grows (0,02%), there is a slight raise of the mitotic index but, when the concentration is still higher, the mitotic index diminishes again.

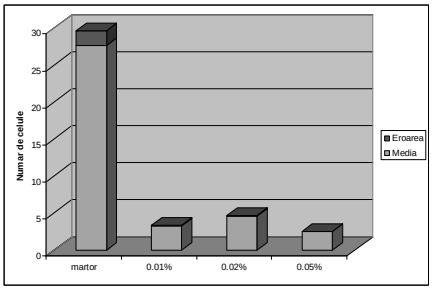

Fig. 3. The mitotic index of the hot chilli pepper after being treated with lead acetate for 48 hours

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

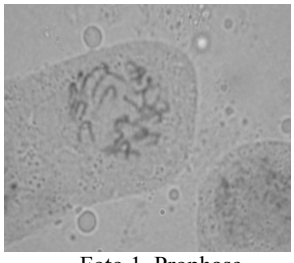

Foto 1. Prophase (0,01% lead acetate)

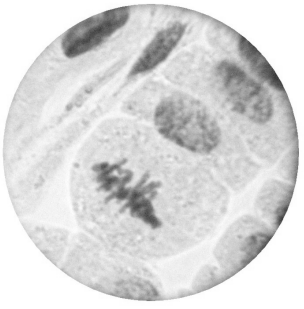

Foto 2. Metaphase (martor)

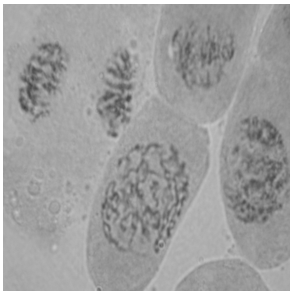

Foto 3. Telophase and prophase (0,05% lead acetate)

In the case of using the second chemical substance, that is iron sulphate, we can observe alterations of the values of the mitotic index compared to the control sample which are:

As we can see in fig. 4, the mitotic index varies greatly for the concentrations treated with ferrous sulphate, within 5,06% for the control to 3,58 % at highest concentration. There was a drop of the values compared to the control thus proving that this substance has an influence upon mitosis.

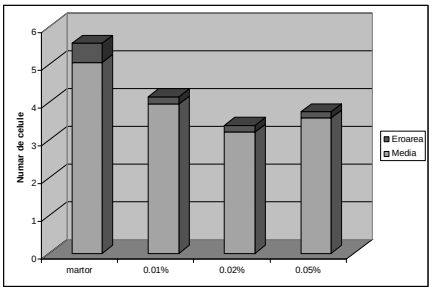

Fig. 4. The mitotic index of the hot chilli pepper after being treated with ferrous sulphate for12 hours

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

In the case of treating the seeds of hot chilli pepper with ferrous sulphate (fig. 5) for 12 hours, we can notice values lower than those of the control (5,06%) as far as the mitotic index is concerned. The values are relatively similar to those obtained in the case of the 12-hour action time; the mitotic index drops as the concentration of the chemical agent grows; but, once a new raise in the concentration occurs, the value of this parameter drops.

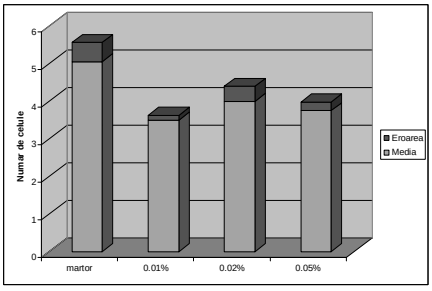

Fig. 5 The mitotic index of the hot chilli pepper after being treated with iron sulphate for 24 hours

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

After 48 hours of treatment, we notice that the lowest value was recorded for the concentration of 0,01% of the ferrous sulphate. If, until this point, the values have been directly proportional to the growth of the concentration of ferrous sulphate, for this acting time, the drop is only noticeable for the concentration of 0,01% from all three concentrations administered to the seeds.

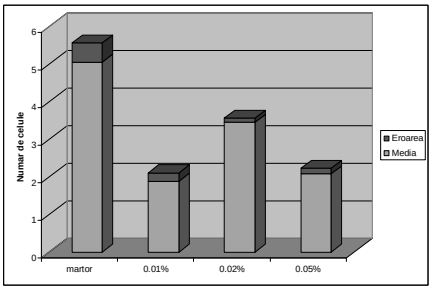

Fig. 6. The mitotic index of the hot chilli pepper afterbeing treated with iron sulphate for 48 hours

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

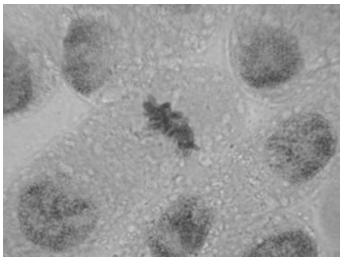

Foto 4. Metaphase (0,02 % ferrous sulphate)

Foto 5. Metaphase with an expulsed chromosome (0,05 % iron sulphate)

The frequency of cells in mitotic division

The repartition of the four phases of the mitotic division shows us (fig. 4) a decrease of it as the concentration of the chemical agent grows. The highest percentage was registered for the cells in prophase, followed by the ones in metaphase, anaphase and telophase, proportionally to the increase of the concentration of the chemical agent. Thus, it was determined that this substance has an influence upon the celular division.

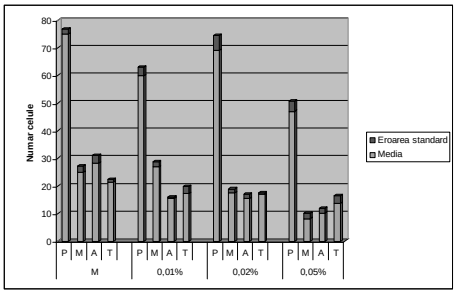

Fig. 4 The dynamics of the phases of the cell division in hot chilli pepper after treating it with lead acetate for 12 hours

- *M – control; 0,01%, 0,02%, 0,05% - concentration of the substance used;*
- *P – prophase; M – metaphase; A – anaphase; T – telophase..*

The frequency of cell division is lower after 24 hours of treatment compared to the previous acting time (12 hours), the highest concentration of the lead acetate resulted in the lowest level of cells in division. Otherwise, the frequency of the other phases of division (metaphase, anaphase and telophase) decreases progressively with the rate of lead acetate concentration increase.

This time again, after treating the seeds for 24 hours, we could notice a perturbing influence upon the mitosis. (fig. 5).

Capsicum annuum L.), after treating with lead acetate for 24 hours

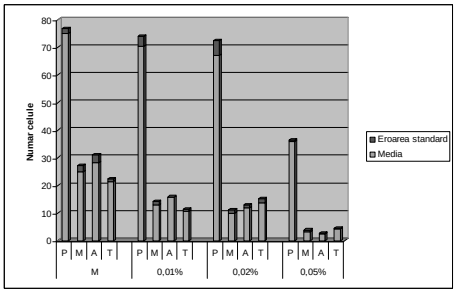

Fig. 5. The dynamics of the phases of the cell division in hot chilli pepper after treating it with lead acetate for 24 hours

- *M – control; 0,01%, 0,02%, 0,05% - concentration of the substance used;*
- *P – prophase; M – metaphase; A – anaphase; T – telophase..*

As far as the percentual repartition of the four phases of mitotic phases is concerned, surprisingly, after 24 hours of treatment, (fig. 6) there occurs a decrease of the cells in division for the maximum concentration of lead acetate (0,05%), compared to the values obtained for the prevous acting time, but also for the control sample.

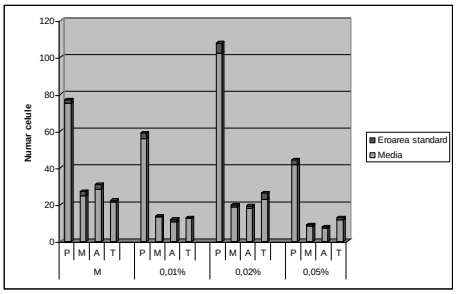

Fig. 6. The dynamics of the phases of the cell division in hot chilli pepper after treating it with lead acetate for 48 hours

- *M – control; 0,01%, 0,02%, 0,05% - concentration of the substance used;*
- *P – prophase; M – metaphase; A – anaphase; T – telophase..*

The ferrous sulphate produced some alterations that are:

Compared to the control (fig. 7.), the frequency of the cells in division is reduced considerably from the lowest to the highest concentration. Most cells are in prophase, followed by the ones in meta-, ana- and telophase. The number of cells in metaphase is smaller than in the case of the control, so the control has the highest percentage. the decrease of the mitotic index for the three concentrations is owed to the increase of the cells in prophase.

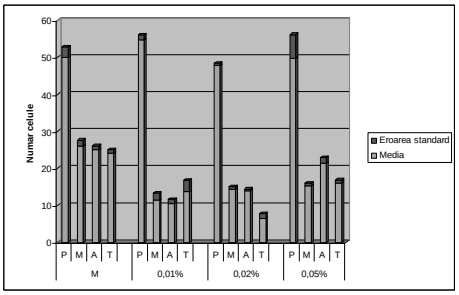

Fig. 7. The dynamics of the phases of the cell division in hot chilli pepper after treating it with ferrous sulphate for 12 hours

- *M – control; 0,01%, 0,02%, 0,05% - concentration of the substance used;*
- *P – prophase; M – metaphase; A – anaphase; T – telophase..*

If we look at the values for the mitotic division for 24-hour acting time, we can notice they are relatively constant for the three concentrations of the chemical agent, but lower than in the case of the control. (fig. 8).

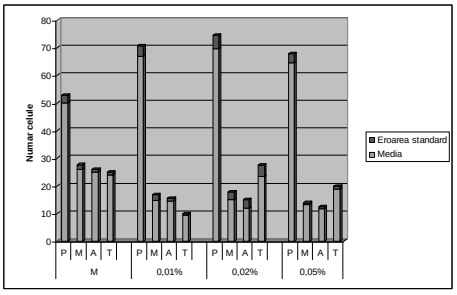

Fig. 8. The dynamics of the phases of the cell division in hot chilli pepper after treating it with ferrous sulphate for 24 hours

- *M – control; 0,01%, 0,02%, 0,05% - concentration of the substance used;*
  P – prophase; M – metaphase; A – anaphase; T – telophase..

We can notice (fig. 9) a decrease in the frequency of the cells in division after being exposed for 48 hours, compared to the other acting times. (12, 24 hpours). The number of cells in the other phases (metaphase, anaphase and telophase) also decreases for this particular acting times.

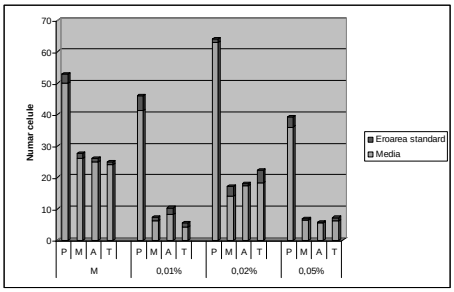

Fig. 9 The dynamics of the phases of the cell division in hot chilli pepper after treating it with ferrous sulphate for 48 hours

- *M – control; 0,01%, 0,02%, 0,05% - concentration of the substance used;*
- *P – prophase; M – metaphase; A – anaphase; T – telophase..*

On the grouds of the results, we can draw the conclusio that the ferrous sulphate has no perturbating effect as far as the frequency of the mitotic is concerned compared to the ones obtained in the case of applying lead acetate.

CONCLUSIONS

The application of the salts of the two heavy metals in the concentrations of 0,01%, 0,02% and 0,05%, determined the alteration of some parameters: in the case of the hot chilli pepper, (*Capsicum annuum* L.), the lead acetate proved to have a highly perturbing effect, effect noticeable in the values of the parameters taken under observation.

Tthe mitotic index registered very low values compared to the control, the frequency of the cells in division also dropped, but the number and the types of chromosomial aberrations increased.

By applying ferrous sulphate on the hot chilli pepper, we noticed that it does not determine as great modifications as in the case of the lead acetate. There were noticed alterations in the mitotic division, so this plant is not particularly sensitive to this substance..

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