CYTOGENETIC EFFECTS INDUCED BY LEAD NITRATE ON MITOTIC DIVISION IN *LYCOPERSICUM ESCULENTUM* MILL.

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This scientific paper shows the influence of lead nitrate on cellular division in Lycopersicum esculentum Mill. Lead nitrate treatments were carried out at three concentrations: 5%, 1% and 0.1%, for 2 and 4 hours, thus, resulting six experimental variants. Treatments were applied on tomato root meristems, resulting in chromosome mutations, expressed by metaphases and ana-telophases. After lead nitrate treatments, chromosomes from metaphases suffered high condensations, becoming pycnotic. In ana-telophases, chromosome bridges, chromosome fragments and multipolar ana-telophases appeared. In interphases, micronuclei appeared. The frequency of these chromosome aberration types differed according to concentration and action time of the chemical agent. Next to the above-mentioned chromosome aberrations, picnotic nuclei, which are inert from genetic viewpoint, appeared at supraunitary rates. Picnotic chromosomes and picnotic nuclei can be considered as a specific feature of lead nitrate influence on mytogene cells. This assessment is argued by the fact that chromosome and nuclei picnotisation was found frequently in the meristems of other genotypes (Allium cepa and Triticum aestivum), at the same concentrations and action time of lead nitrate. Furthermore, lead nitrate has a high inhibiting effect on mitotic division from tomato root meristems, diminishing significantly the value of mitotic index, proportionally correlated to lead nitrate concentration and action time. Cells reacted differently to the chemical agent action, at every mitotic stage. We found an extremely low frequency of cells in anaphase. This experiment has shown that lead nitrate, known as an very aggressive polluting agent, has a certain mutagen and inhibiting potential on mytogene cells.

***Ke ywords**: root meristem, mitotic division, chromosomial aberration, picnotic nuclei*

Polluting agents, generally, and lead, particularly, are modern mutagen and aggressive risk factors, generating a complex of biological implications, culminating in humans with cancer [1, 5]. Mutations induced by polluting agents affect the genetic fund of all life forms, with dramatic consequences on the offspring.

Lead is the most dangerous polluting agent for humans, animals and plants. Saturnism is the most characteristic disease caused by lead [2].

On the other side, nitrates, which toxicity is relatively low, are used in food industry as additives.

The goal of this scientific paper was to point out the impact of lead nitrate on cell division from root meristems of *Lycopersicum esculentum*. The cytogenetic effect of lead nitrate has been already tested on root meristems of *Allium cepa* and *Triticum aestivum* [3, 4].

MATERIAL AND METHOD

The biological material used in the experiment is represented by seeds of *Lycopersicum esculentum* Mill. Seeds were put to germinate under laboratory conditions. When roots reached 15-17 mm in length, they were treated with lead nitrate - $Pb(NO_3)_2$.

Lead nitrate was used as watery solutions at three concentrations: 5%, 1% and 0.1%. The action time of these solutions on the root meristems was differentiated as it follows: 5%, 1% and 0.1% solutions that acted for 4 hours and 2 hours.

Taking into account concentration and action time of solutions, six variants have resulted. In addition, a control plot was also used and, in this case, no treatments were applied to root meristems.

For further cytogenetic investigations, treated and non-treated roots (control) were fixed in Carnoy's fixing solution for 24 hours at 4°C, then were hydrolysed with HCl and coloured with the Carr basic colouring. The root meristem was displayed by using the squash technique. A number of 20 preparations and 10 microscopic fields/preparation were examined for all variants and for control. The microscopic examination was carried out using the Hund Wetzlar microscope. Microphotographs were taken with the microscope camera.

RESULTS AND DISCUSSIONS

Analysis of the mitotic index

The first investigated aspect, correlated to the mutagen capacity of lead nitrate, is represented by the effect of this chemical agent on stages of mitotic division. The proportion of meristematic cells found in division diminishes significantly once with the increase in concentration and action time of lead nitrate (*fig. 1, 2*).

In all the four stages of mitosis, the cell proportion is much diminished compared to the control (*fig. 3, 4, 5, 6*). Anaphasic cells have the lowest frequency, sub-unitary in all the experimental cases, not exceeding 0.24%, compared to the control that recorded 4.32% of anaphasic cells. Telophases are found in most of cases, at the terminal stage, as late telophases (having already formed nuclei).

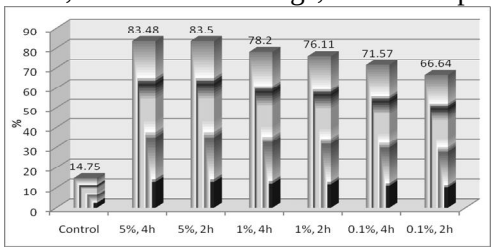

Figure 1 **Proportion of cells in interphase**

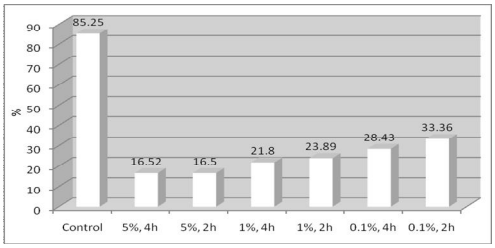

Figure 2 **Proportion of cells in division**

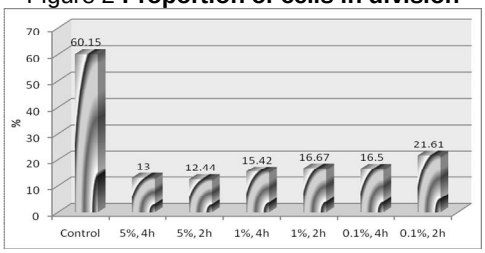

Figure 3 **Proportion of cells in prophase**

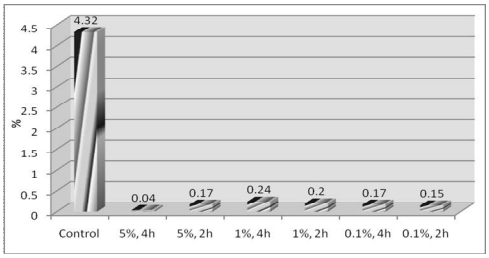

Figure 5 **Proportion of cells in anaphase**

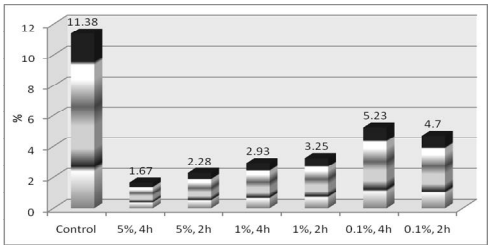

Figure 4 **Proportion of cells in metaphase**

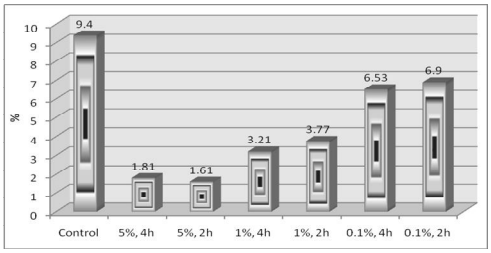

Figure 6 **Proportion of cells in telophase**

Analysis of cell proportion in aberrant metaphase and ana-telophase

Simultaneously to the inhibiting effect of the mitotic index, lead nitrate is also responsible of inducing some chromosome mutations identified at optic microscope. These chromosome mutations (aberrations) have been identified in metaphases and ana-telophases.

Aberrant metaphases consisted in genetically inert picnotic chromosomes, which are spread in the entire mixoplasma. Such metaphases were recorded in all the six experimental variants, exceeding as frequency the normal metaphases. The proportions of tomato meristematic cells, which are found in aberrant metaphases, are supra-unitary in all the experimental cases. We noticed the reverse proportional relationship between the number of aberrant metaphases, on the one hand, and the concentration, corroborated to the action time of lead nitrate, on the other hand (*fig. 7*). The chromosome picnotisation in metaphase, as influenced by lead nitrate, was found clearly in case of other genotypes: *Allium cepa* and *Triticum aestivum*. In *Allium cepa*, they found that lead nitrate had an identical effect induced by colchicines on chromosomes. Therefore, the picnotic chromosomes of a cell are no longer spread to the two cellular poles in anaphase, resting together during mitosis, resulting a single self-tetraploid cell. The proof was the big nuclei, found in onion

root meristems treated with lead nitrate. Therefore, we demonstrated that lead nitrate had a self-polyploidization potential in *Allium cepa* [3]. It results that inducing the chromosome picnotization is a special effect of lead nitrate on mitogene cells.

Ana-telophases where chromosome aberrations appeared were at a lower sub-unitary proportion, compared to aberrant metaphases and normal ana-telophases (*fig. 8*). In two variants: 1% $Pb(NO_3)_2$, 2 hours and 0.1% $Pb(NO_3)_2$, 4 hours, aberrant ana-telophases were absent. The extremely low rate of aberrant ana-telophases in the other four variants may be explained by the great inhibition of anaphasic cells by lead nitrate.

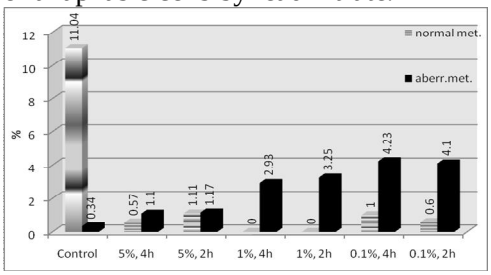

Figure 7 **Proportion of cells in normal and aberrant metaphases**

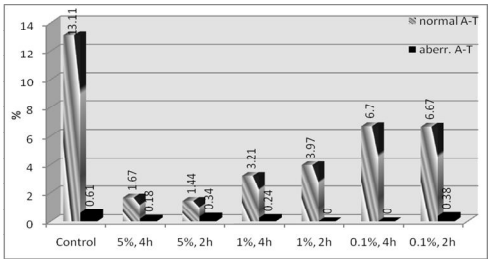

Figure 8 **Proportion of cells in normal and aberrant ana- telophases**

Analysis of chromosomal aberration types

Aberrant ana-telophases were pointed out by the appearance of four types of chromosomal mutations (aberrations) in sub-unitary values: chromosomal bridges, chromosomal fragments, multipolar ana-telophases and micronuclei (*fig. 9*).

The chromosomal bridges have been identified in four experimental variants. The appeared bridges were of many ways, irrespective of the experimental variant: broken, thick and very thick, the latest case being known in literature as cytomixy.

The chromosomal fragments appeared only in case of the variants with the highest concentration - 5% $Pb(NO_3)_2$.

Multipolar ana-telophases were identified only in the variant with 5%, 2 hours, and a low frequency. Micronuclei were found in the entire experiment, except the variant of 5%, 4 hours. They are associated to telophasic and interphasic nuclei. The highest micronuclei frequency was recorded in the variant with 1% $Pb(NO_3)_2$, 2 hours.

In the control, chromosomal fragments and insignificant rate micronuclei appeared spontaneously.

Next to the above-mentioned aberrations, genetically inert picnotic nuclei were found. They appeared in all the experimental variants, with supra-unitary high frequencies (3.27-6.12%), compared to the other chromosomal aberrations (*fig. 10*). We noticed clearly the direct proportional relationship between the number of cells with picnotic nuclei, on the one hand, and lead nitrate concentration and action time, on the other hand.

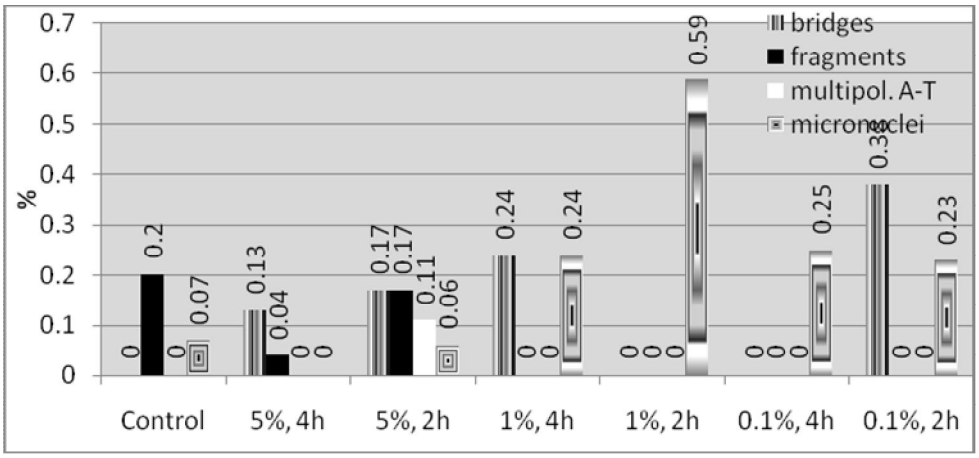

Figure 9 **Proportion of chromosomal aberration types**

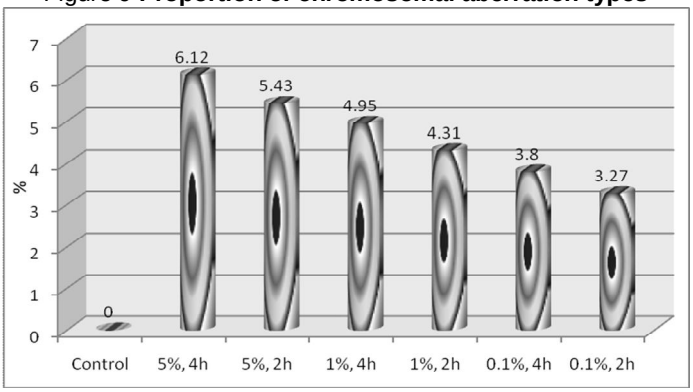

Figure 10 **Proportion of picnotic nuclei in root meristem in tomato, treated with lead nitrate**

The limit differences of the effects of lead nitrate in *Lycopersicum esculentum* Mill. is shown in *table 1.*

Table 1

Differences found after the treatment with lead nitrate upon mitotic division in *Lycopersicum esculentum*

variant	Aberrant metaphases		Aberrant ana-telophasaes	
	average value (%)	significance of difference	average value (%)	significance of difference
control	0.34	-	0.61	-
5%, 4 h.	1.10	***	0.18	000
5%, 2 h.	1.17	***	0.34	000
1%, 4 h.	2.93	***	0.24	000
1%, 2 h.	3.25	***	0.00	000
0.1%, 4 h.	4.23	***	0.00	000
0.1%, 2 h.	4.10	***	0.38	000
DL 5% = 0.135 DL 1% = 0.189 DL 0.1% = 0.267			DL 5% = 0.042 DL 1% = 0.059 DL 0.1% = 0.083	

Figures 11-14 show some aspects of the chromosomal aberrations induced by lead nitrate.

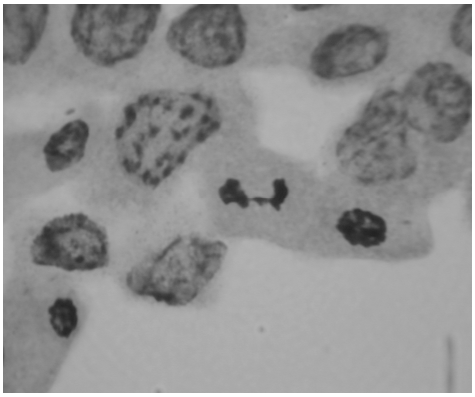

Figure 11 **Ana-telophase with bridge in root meristem at tomato treated with $Pb(NO_3)_2$ 5%, 4 hours (1000X)**

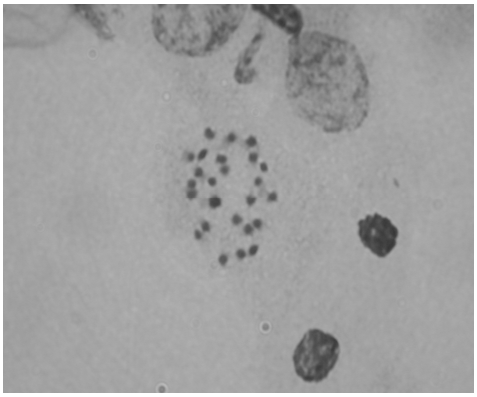

Figure 13 **Picnotic chromosomes and nuclei in root meristem at tomato treated with $Pb(NO_3)_2$ 0.1%, 4 hours (1000X)**

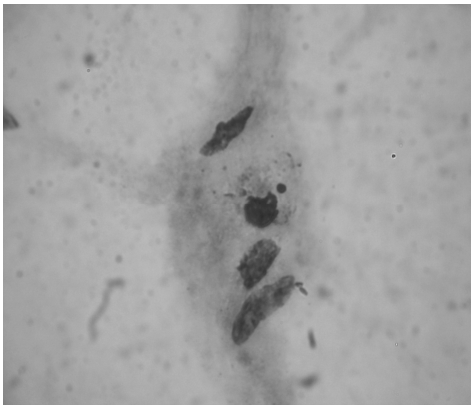

Figure 12 **Micronucleus in root meristem at tomato treated with $Pb(NO_3)_2$ 1%, 4 hours (1000X)**

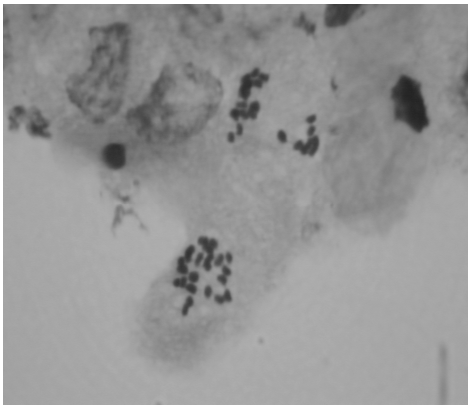

Figure 14 **Metaphases with picnotic chromosomes in root meristem at tomato treated with $Pb(NO_3)_2$ 1%, 2 hours (1000X)**

CONCLUSIONS

The trial demonstrated that lead nitrate, known as a dangerous polluting agent, has a high inhibiting effect on mytogene cells of *Lycopersicum esculentum*. The most inhibited are anaphasic cells.

Lead nitrate induces a high frequency, picnotisation of chromosomes in metaphase disturbing the normal way of mitotic division. The picnotisation of chromosomes in metaphase could be the cause of the extremely low rate of anaphasic cells.

Lead nitrate has a mutagen potential, demonstrated by installing the chromosomal mutations in ana-telophases of tomato meristematic cells. Lead nitrate is also responsive for the appearance of picnotic nuclei with high supra-unitary frequencies.

Picnotic chromosomes from metaphases and picnotic nuclei point out the effect of lead nitrate on mytogene cells belonging to plant genotypes. This conclusion is argued by the studies of the effects of lead nitrate on root meristems of *Allium cepa*, *Triticum aestivum* and *Lycopersicum esculentum*. On this occasion, we draw attention that, by the picnotisation of chromosomes, lead nitrate may determine self-tetraploidity, a phenomenon that was demonstrated in *Allium cepa,* where the chromosomes are great and easy to study.

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