CITOGENETIC EFFECTS INDUCED BY SODIUM NITRITE ON MITOTIC DIVISION AT *LYCOPERSICUM ESCULENTUM* MILL.

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Abstract

The paper presents the influence of sodium nitrite on the cellular division at *Lycopersicum esculentum*. The treatments with sodium nitrite was used in three concentrations: 5%, 1% and 0.1%. The time of action of the respectively solutions was 4 hours and 2 hours, six experimental variants have resulted. The treatments actioned of on tomato radicular meristems, were expressed by chromosomial mutations, particular in ana-telophases, but in metaphases. The types of chromosomial aberrations in tomato radicular meristems, induced by sodium nitrite are very varied: chromosomial bridges, chromosomial fragments, associations between bridges and fragments, retardatary chromosomes, simple and complex multi-polar ana-telophases, micronuclei. Aberrant metaphases consisted in genetically inert picnotic chromosomes, which are spread in the entire mixoplasma. The rate of this types of chromosomial aberrations was differentiated depending on the concentration function and time of action of respective chemical agent. Beside of these types of chromosomial aberrations appear chromatin bridges very thick known with denomination cytomixy, also picnotic nuclei in high rate, genetic inert. Last types of cromosomial aberrations are considered special effects of sodium nitrite. Moreover, sodium nitrite has a inhibitory effect on mitotic division of *Lycopersicum esculentum*, diminish the mitotical index, in correlation with the concentration and time of action by sodium nitrite. The cells reacted differently in each phase of mitotic division to the action of the chemical agent: the proportion of cells in prophase, metaphase, anaphase and telophase is very diminish in compareson by control. The experiment proved that sodium nitrite known as a polluting agent has a real mutagenic potential and inhibitory upon mitotical cells.

Key owrds: cell, sodium nitrite, mitotic division, chromosomial aberration, picnotic chromosomes

Sodium nitrite know as E250 is synthetic preservatives you find in canned meat, sausages, bacon, pressed meat, smoked meat, frozen pizza. This food additive is responsible for these adverse effects: carcinogenic, affect the kidneys, cardiovascular system, gastrointestinal and nervous system, causing allergies, nausea, headaches. For all these, sodium nitrite is considered very dangerous. (Knekt P., Järvinen R., Dich J., Hakulinen T., 1999; Larsson S.C., Wolk A., 2006; Larsson S.C., Orsini N., Wolk A., 2006; Rashmi Sinha & coll., 2009). The concentration of sodium nitrite the European Union allows is 0.6% (Rashmi Sinha & coll., 2009).

The goal of this paper was to investigate the influence of sodium nitrite on tomato meristematic cells. Such investigations were also carried out on onion and wheat meristematic cells (Pădureanu Silvica, 2006; Pădureanu Silvica, 2008).

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 embryonary roots reached 15-17 mm in length, they were treated with sodium nitrite - $NaNO_2$.

Sodium nitrite 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. Number of cells analysed for control was 5872, and between 4880 and 5601 for each experimental variant.

The microscopic examination was carried out using the Hund Wetzlar microscope. Microphotographs were taken with the microscope camera.

RESULTS AND DISCUSSIONS

The main analysed parameters were: mitotic index, frequency of aberrant mitotic phases, frequency and type of chromosome aberrations.

The dynamics of mitotic index

As show in *figure 1*, the exposure to sodium nitrite determined significant modifications of the mitotic index.

At maximum (5%) and medium (1%) concentrations of tested sodium nitrite induced the mitotic index decrease, the frequency of dividing cells is much inferior to the control, the value of this parameter being, in respective variants between 13,38% and 15,85%. The variants with 0.1% concentration induced the mitotic index also inferior to the control, namely 29,9% and 30,3%.

In the other four phases of mitosis, cell reacted differently. Thus, in prophase, the cell proportion was below the control level in all the tested variants *(fig. 2)*. The metaphases registered a percentual decrease in comparison with the control, especially in 5% concentration (0.77-0.9% cells). In anaphase, the lowest cells proportion was found at the variants with 1% (0.05% cells) and 5% (0.9-0.99% cells) concentrations. The minimum concentration in sodium nitrite (0.1%) induced 0.75-1.48% cells in anaphasis. In telophase, the cells proportion was diminished over the control in all the experimental cases, especially at 1% concentration (0.43-0.75% cells) *(fig 2)*.

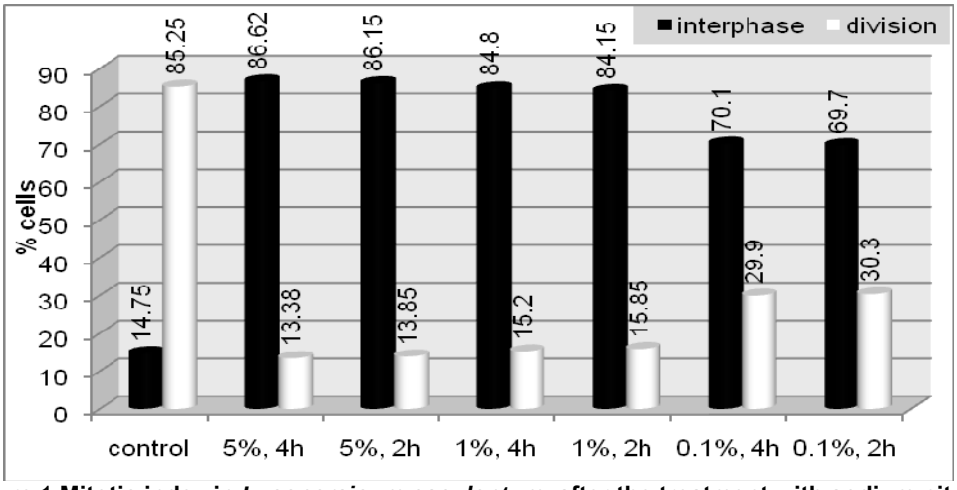

Figure 1 **Mitotic index in *Lycopersicum esculentum*, after the treatment with sodium nitrite**

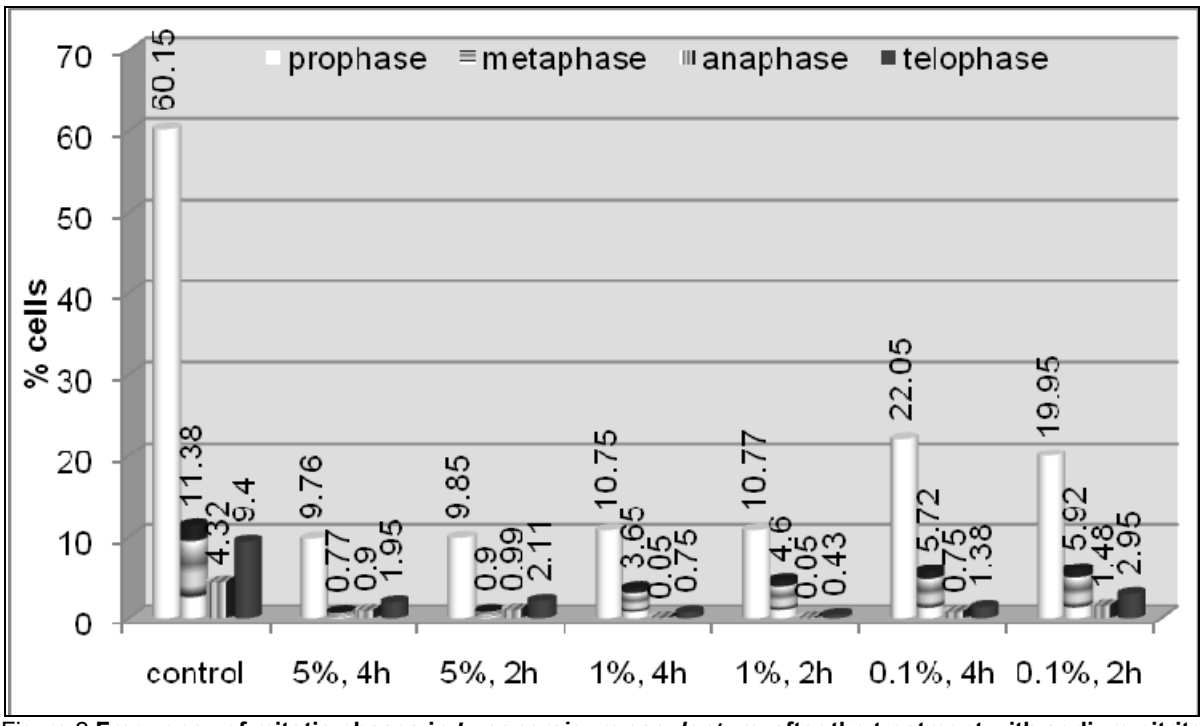

Figure 2 **Frequency of mitotic phases in *Lycopersicum esculentum*, after the treatment with sodium nitrite**

Frequency of aberrant mitotic phases

By analysing *figures 3* and *4*, it is obvious that exposure of biologic material to the tested sodium nitrite determined beside mitotic index decrease, a high increase of aberrant cells. Sodium nitrite induced aberrant cells in metaphases and in ana-telophases.

High rates of aberrant metaphases were found at 1% concentration, followed by 0.1% concentration *(fig. 3)*. The aberrant metaphases consist in picnotic chromosomes, responsible for lowest cells proportion in ana-telophases.

High rates of aberrant ana-telophases were found at 5% concentration, followed by 0.1% and 1% concentrations *(fig. 4)*.

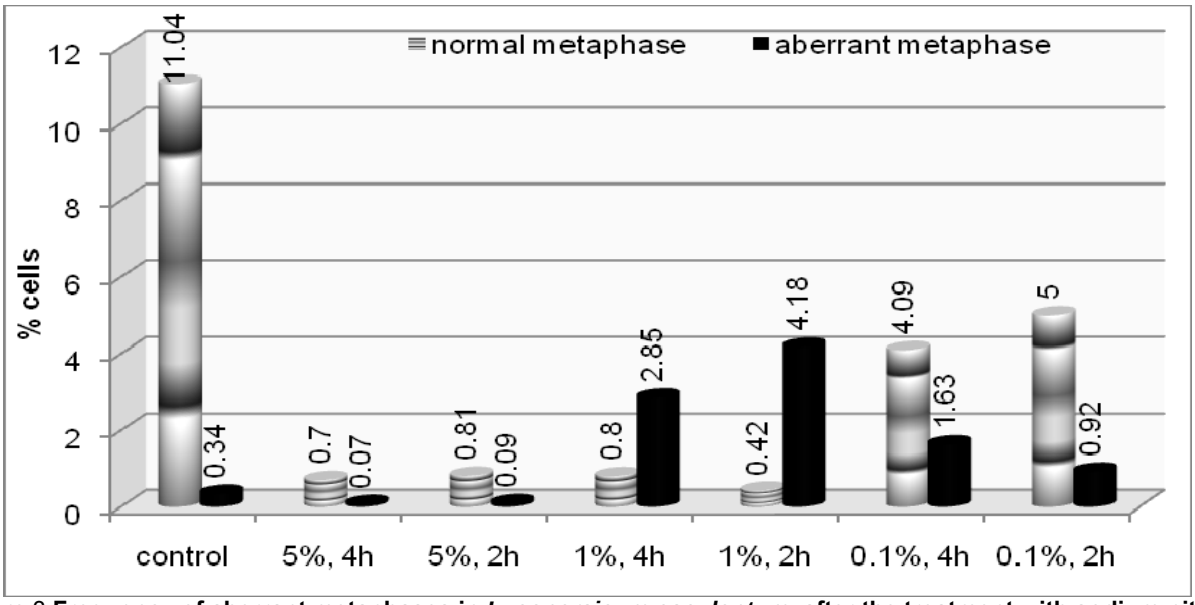

Figure 3 **Frequency of aberrant metaphases in *Lycopersicum esculentum*, after the treatment with sodium nitrite**

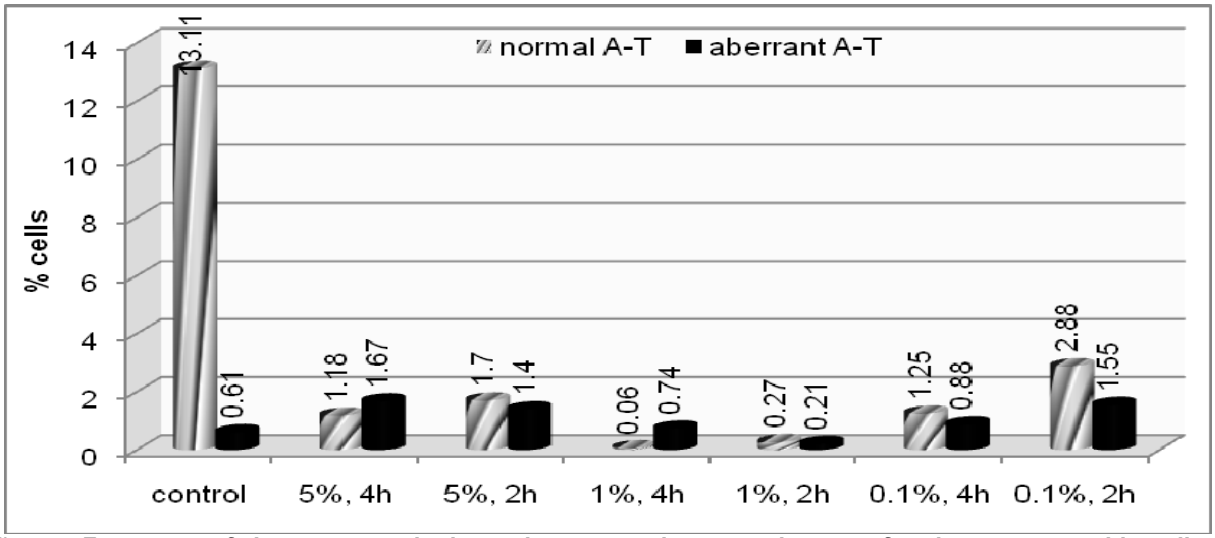

Figure 4 **Frequency of aberrant ana-telophases in *Lycopersicum esculentum*, after the treatment with sodium nitrite**

Fequency and type of chromosome aberrations

The spectrum of aberrations ana-telophases in identified in mitotic ana-telophases was enough large and was represented by: bridges, fragments, associations between bridges and fragments, retardatary chromosomes, multipolar ana-telophases. Micronuclei were observed in interphases and telophases *(fig. 5)*.

The chromosome bridges are found at all the variants. The bridges induced by sodium nitrite were of different types: simple, double, seldom multiple, continuous, frequently interrupted, thin, thick and very thick. The last one, which is responsible of cytomixy appeared at all concentrations, more at 0.1% concentration, 2 hours.

The chromosome fragments appeared at 5% and 0.1% concentrations. The associations between bridges and fragments were found at the variants with 5% and 0.1% concentrations.

The retardatary chromosomes appeared at 5%, 1% (4 hours) and 0.1% (2 hours) concentrations.

The multipolar ana-telophases were induced by 5% (2 hours), and 0.1% concentrations. The presence of asymmetrical ana-telophases is a proof of disturbing action of sodium nitrite on good function of division spindle, the chromosomes migration to poles being strongly affected.

In interphases and telophases, miocronuclei were found at 5% concentration in supraunitary proportions and 1% concentration in subunitary proportions.

At the control, bridges and micronuclei in subunitary proportions (0.2%, respective 0.07%) appeared spontaneously.

Next to the shown aberration types, sodium nitrite has also induced the formation of picnotic nuclei, which are genetically inert, at 5% and 1% concentrations, which frequency (0.4-2.85%) is in direct relation with the concentration and the action time of the chemical agent *(fig. 6)*.

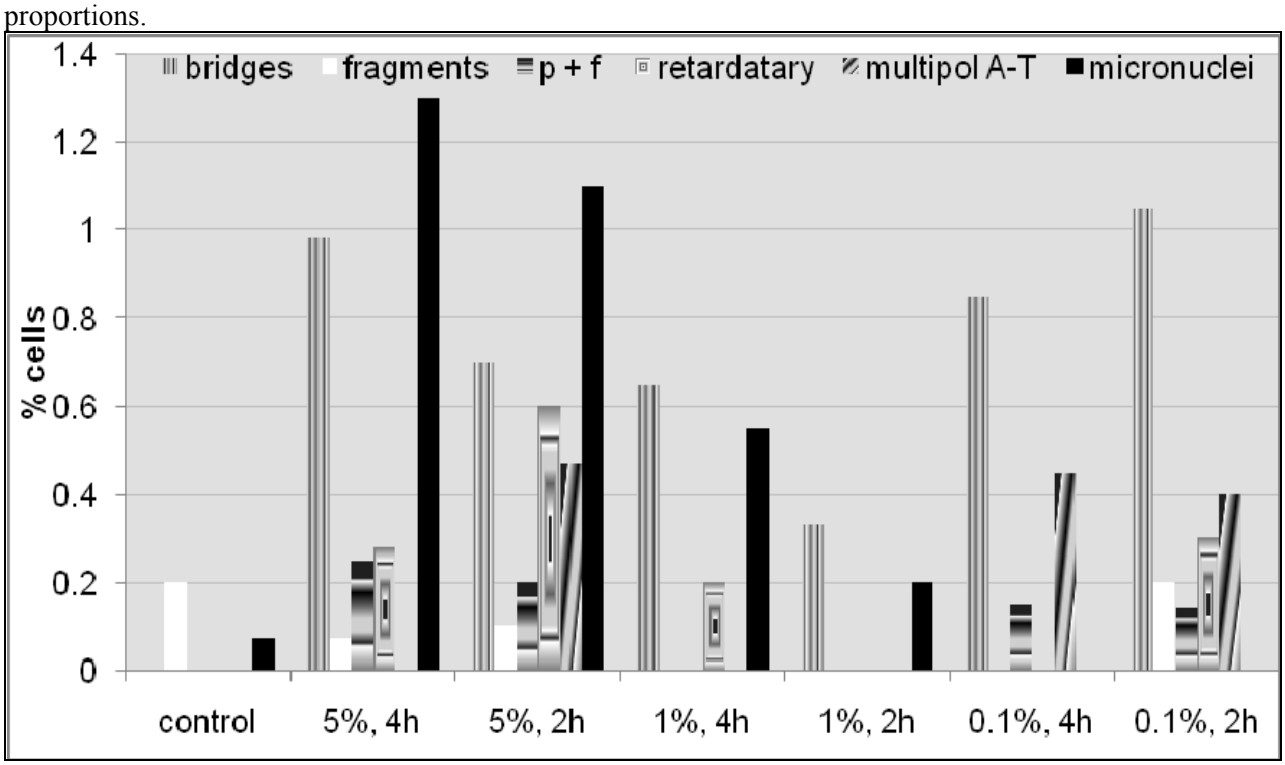

Figure 5 **Frequency of chromosomial aberration types in *Lycopersicum esculentum*, after the treatment with sodium nitrite**

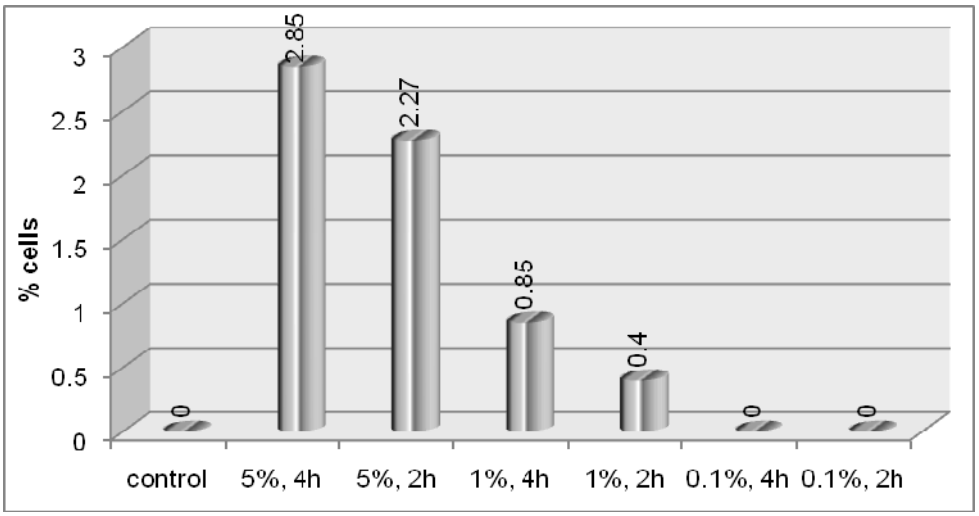

Figure 6 **Frequency of picnotic nuclei in *Lycopersicum esculentum*, after the treatment with sodium nitrite**

The limit differences of effects of sodium nitrite at *Lycopersicum esculentum* is represented in *table 1*.

Different aspects of chromosomial aberrations induced by sodium nitrite at *Lycopersicum esculentum* are presented in *fig.7-16.*

Table 1

Differences found after the treatment with sodium nitrite 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.	0.07	°°°	1.67	***
5%, 2 h.	0.09	°°°	1.40	***
1%, 4 h.	2.85	***	0.74	-
1%, 2 h.	4.18	***	0.21	°
0.1%, 4 h.	1.63	***	0.88	-
0.1%, 2 h.	0.92	***	1.55	***
DL 5% = 0.072; DL 1% = 0.101 DL 0.1% = 0.143		DL 5% = 0.298; DL 1% = 0.418 DL 0.1% = 0.590		

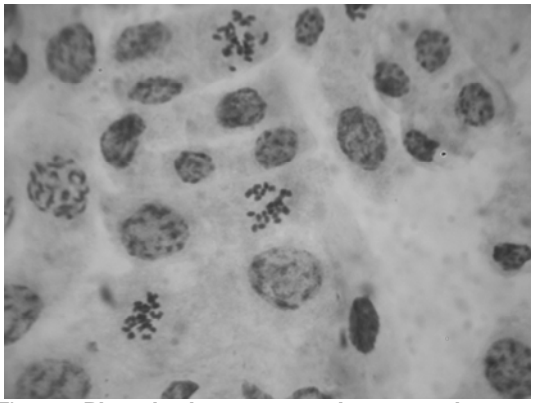

Figure 7 **Picnotic chromosomes in root meristem at tomato treated with $NaNO_2$ 1%, 2 hours (1000X)**

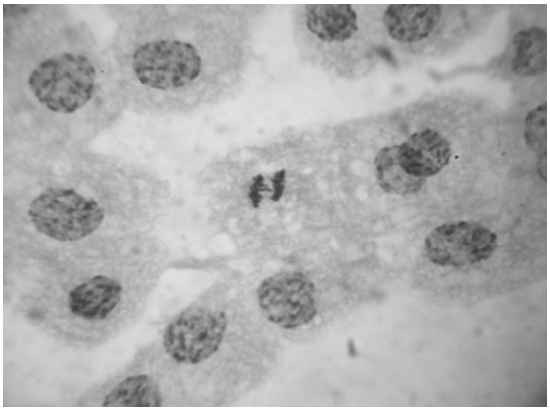

Figure 8 **Ana-telophase with ragged bridges in root meristem at tomato treated with $NaNO_2$ 0.1%, 4 hours (1000X)**

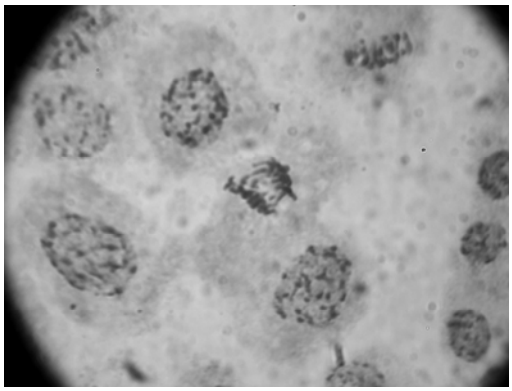

Figure 9 **Anaphase with multiple bridges in root meristem at tomato treated with $NaNO_2$ 0.1%, 2 hours (1000X)**

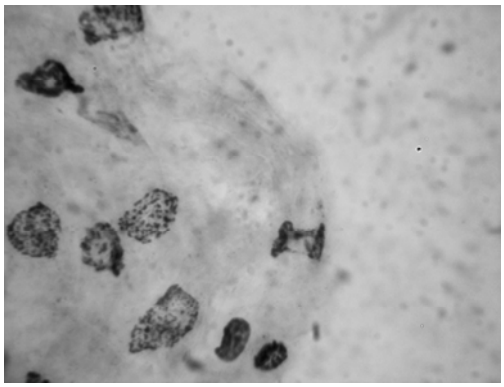

Figure 10 **Ana-telophase with bridges in root meristem at tomato treated with $NaNO_2$ 5%, 2 hours (1000X)**

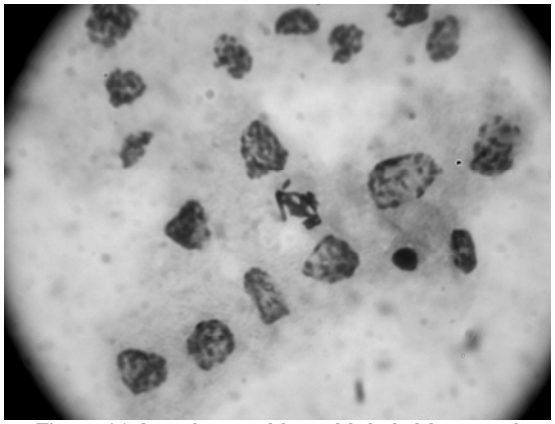

Figure 11 **Anaphase with multiple bridges and retardatary chromosomes in root meristem at tomato treated with $NaNO_2$ 5%, 2 hours (1000X)**

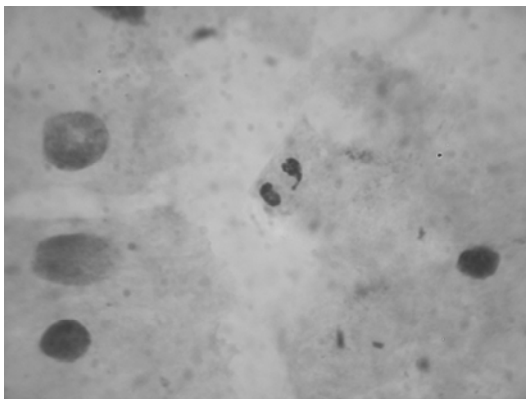

Figure 12 T**elophase with ragged bridge in root meristem at tomato treated with $NaNO_2$ 5%, 2 hours (1000X)**

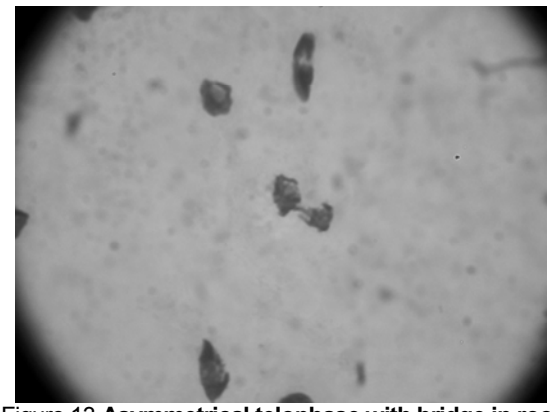

Figure 13 **Asymmetrical telophase with bridge in root meristem at tomato treated with $NaNO_2$ 5%, 4 hours (1000X)**

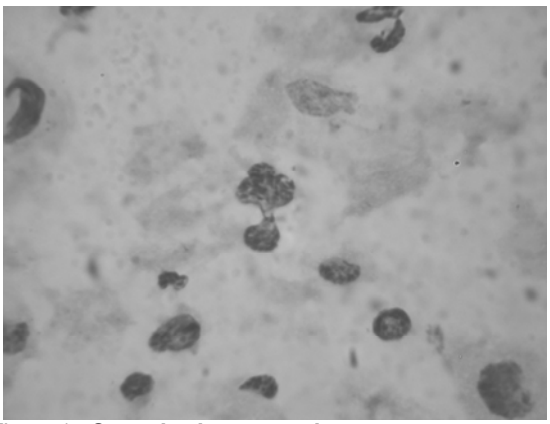

Figure 14 **Cytomixy in root meristem at tomato treated with $NaNO_2$ 0.1%, 2 hours (1000X)**

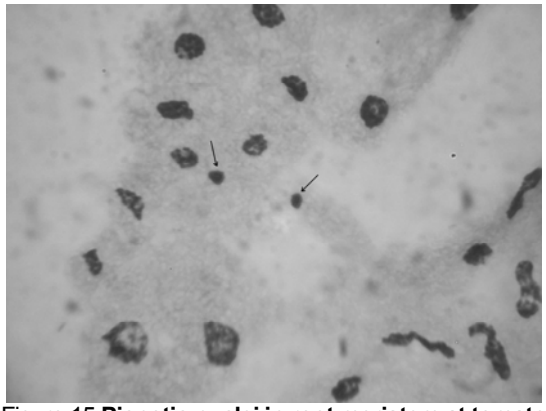

Figure 15 **Picnotic nuclei in root meristem at tomato treated with $NaNO_2$ 5%, 4 hours (1000X)**

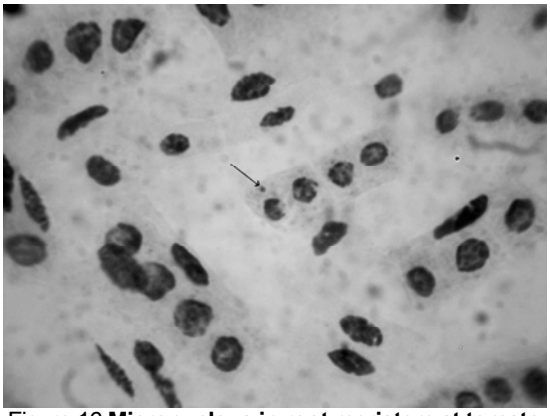

Figure 16 **Micronucleus in root meristem at tomato treated with $NaNO_2$ 1%, 4 hours (1000X)**

CONCLUSIONS

Sodium nitrite, known as a food additive, has an strong inhibiting effect on *Lycopersicum esculentum* mitogene cells. The most inhibited are anaphasic cells.

Sodium nitrite has a mutagen potential on *Lycopersicum esculentum* cells, which is shown by chromosome aberrations induced in ana-telophases: chromosome bridges, chromosome fragments, associations between bridges and fragments, retardatary chromosomes, multipolar ana-telophases and micronuclei.

Cytomixy and asymmetrical ana-telophases might be considered as characteristics of the effect of sodium nitrite, especially at small concentration (0.1%).

Picnotic chromosomes from metaphases, especially at 1% and 0.1% concentrations, are other features of the effect of sodium nitrite.

Picnotic nuclei represents as characteristic of the effect of sodium nitrite at 5% and 1% concentrations.

Undertook the study revealed that sodium nitrite in concentration below 0.6% (concentration allowed as a food additive by the EU) has mutagenic properties and inhibition of cell division. This statement is supported by other similar purpose of the studies conducted on other two vegetable genotypes: onion and wheat.

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