CYTOGENETIC EFFECTS INDUCED BY POTASSIUM FERRICYANIDE ON MITOTIC DIVISION AT *CUCUMIS SATIVUS* L.

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Abstract

The paper presents the influence of potassium ferricyanide on the cellular division at *Cucumis sativus*. The treatments with potassium ferricyanide was used in three concentrations: 0,5%, 0,25%, 0,1% and the time of action of the respectively solutions was 2 hours, 4 hours, 6 hours, 24 hours and 48 hours, fifteen experimental variants have resulted. The treatments actioned of on cucumber radicular meristems and was noted a inhibitory effect on mitotic division of *Cucumis sativus*, diminish the mitotical index, in correlation with the concentration and time of action by potassium ferricyanide. Moreover were expressed by chromosomial mutations, particular in ana-telophases, but in metaphases. The types of chromosomial aberrations in cucumber radicular meristems, induced by potassium ferricyanide are very varied: chromosomial bridges, retardatary chromosomes, chromosomial fragments, simple and complex multi-polar ana-telophases. 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. 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 diminish in compareson by control. The experiment proved that potassium ferricyanide known as an aggressive environmental polluting agent is a potent inhibitor of cell mitogenic and a real mutagenic potential upon mitotical cells. The experiment demonstrates the harmful effect of potassium ferricyanide on vegetable orgnisms. Therefore, in this experiment is evidently the inhibitor effects to the mutations, what confirm the speciality literature.

Key words: *cell, potassium ferricyanides, mitotic division, chromosomial aberration, picnotic chromosomes*

There are numerous studies demonstrating the toxic effects of cyanides. ATP level in a rat heart during exposure of cyanide is maintained by activation of anaerobic glycolysis in compensation for cellular oxygen utilization (Honma M. et al., 1992). The effects of cyanide on dopamine receptors at rat are probably in part due to the effect of cyanide on the release of dopamine (Cassel, G.E. et al., 1993). Cyanide is a mitochondrial poison. Cyanide induced oxidative stress is known to play a key role in mediating the neurotoxicity and cell death in rat pheochromocytoma (PC12) cells. Treated cells with various concentrations (0.625-1.25 mM) of potassium cyanide (KCN) for 4 hours were marked by notable effects. Cyanide caused marked decrease in the levels of cellular antioxidants like superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase **(**Satpute, RM. et al., 2010). Exposure of juvenile male rainbow trout (*Salmo gairdneri*), to cyanide (0,01 şi 0,03 mg/L HCN) in laboratory flow-through aquaria at 12.5°C, for 18 days, consisted in reducing the number of dividing spermatogonia by 13 and 50% respectively. The exposure to cyanide led to an increase in the prophase stage spermatogonia which appeared to be due to a blockage of mitotic progress.

Cyanide also affected the formation of the mitotic spindle at the lower cyanide concentration as evidenced by the presence of multipolar spindles and multinucleate interphase cells. Cellular damage was evident in spermatogonia in all phases of the cell cycle accompanied by a high incidence of necrosis at the higher cyanide concentration (Ruby M. Sylvia et al., 2010). The results demonstrate that exposure to 50, 100, 300, and 600 mg/l of cyanide ion (CN) for 1–2 min cyanide causes mortality to corals and anemones. Even brief exposure to cyanide caused slow-acting and long-term damage to corals and their zooxanthellae (Cervino J.M. et al., 2003). Other expeiments have examined the effects of cyanide (NaCN) from cyanide fishing on photosynthesis of the symbiotic algae (zooxanthellae) located within the tissues of the zooxanthellate hard coral. The results suggest that cyanide causes the dissociation of the symbiosis (bleaching) by affecting photosynthesis of the zooxanthellae. Specifically, cyanide is an inhibitor of the dark reactions of the

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Calvin cycle, specifically as an inhibitor of ribulose-l,5-bisphosphate carboxylase/oxygenase (Ross J.J., Ove H.G., 1999).

In case of plants, cyanides reduces also mitotic index and induces the chromosomial aberattions (Pădureanu S., 2004, Pădureanu S., 2006, Pădureanu S., 2006; Abu N.E., Mba K. C., 2011). Cytogenetic study can be used to monitor biological wastewater contamined with heavy metals and cyanides (Staykova T.A. et al., 2005). Stimulation of chloroplast at *Spinacia oleracea* fatty acid synthesis by either exogenous coenzyme A is almost completely abolished in the presence of cyanide (Roughan G. P., Beevers H., 1981).

Gold mining is done with cyanide solutions in high concentrations (Li Q. et al., 2010). In this way, environment is an ecological disaster.

It turned out that the cyanide used to extract gold from Coronation Hill region, the South Alligator River river springs, significantly affect local aquatic animals (Rippon, G.D. et al., 1992). Therefore, any entry of cyanide in the environment is a source of toxicity.

MATERIAL AND METHOD

The biological material used in the experiment is represented by seeds of *Cucumis sativus* L. Seeds were put to germinate under laboratory conditions. When embryonary roots reached 15-17 mm in length, they were treated with potassium ferricyanide – $K_3[Fe(CN)_6]$.

Potassium ferricyanide was used as watery solutions at three concentrations: 0.5%, 0.25% and 0.1%. The action time of these solutions on the root meristems was differentiated as it follows: 0.5%, 0.25% and 0.1% solutions that acted for 2 hours, 4 hours, 6 hours, 24 hours and 48 hours.

Taking into account concentration and action time of solutions, fifteen 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 5744, and between 4550 and 5800 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. The exposure to potassium ferricyanide determined significant modifications of the mitotic index (fig. 1).

At maximum (0.5%), medium (0.25%) and minimum concentrations of tested potassium ferricyanide 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 21,1% and 42%. 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 0.5% concentration, 48 hours (0.29 cells) and in 0.25%, 48 hours. In anaphase, the lowest cells proportion was found at the variants with 0.5% concentration, 48 hours (0.2% cells) and 0.25% concentration 48 hours (0.62% cells). The minimum concentration in potassium ferricyanide (0.1%) allowed 1.3-2.31% cells in anaphasis. In telophase, the cells proportion was diminished over the control in all the experimental cases, especially at 0.1% concentration, 48 hours (0.97%cells) (fig. 2).

Frequency of aberrant mitotic phases

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

High rates of aberrant metaphases were found at 0.5% concentration, 4 and 6 hours time of action, followed by 0.1% concentration, 6 and 24 hours time of action (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 0.1% concentration, 2, 4, 6, 24 and 48 hours time of action, followed by 0.25% concentration (all times of action) and 0.5% concentration (fig. 4).

Frequency 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 (fig. 5).

The chromosome bridges are found at all the variants. The bridges induced by potassium ferricyanide were of different types: simple, double, seldom multiple, continuous, frequently interrupted, thin. The highest proportions of bridges were induced to 0.1% concentration (all the time for action), because this concentration of potassium ferricyanide allowed the highest frequency of ana-telophases of all experimental variants.

The chromosome fragments lack the four variants with the concentration of 0.5% and 0.25%, when times of action were 24 and 48 hours.

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The associations between bridges and fragments are present only in variants with 0.25% and 0.1% concentration. In variants with 0.5% concentration, such chromosomal aberrations were not identified.

The retardatary chromosomes occured in all concentrations (0.5%, 0.25%, 0.1%), except for the variant with 0.1% concentration, 48 hours time of action.

The multipolar ana-telophases were induced by all concentrations. The presence of asymmetrical ana-telophases is a proof of disturbing action of on potassium ferricyanide good function of division spindle, the chromosomes migration to poles being strongly affected. We mention that the variant with 0.5% concentration, 2 hours time of action, such multipolar ana-telophases were not identified.

At the control, bridges and chromosome fragments in subunitary proportions (0.14%, respective 0.07%) appeared spontaneously.

The limit differences of effects of potassium ferricyanide at *Cucumis sativus* is represented in table 1.

Different aspects of chromosomial aberrations induced by potassium ferricyanide at *Cucumis sativus* are presented in figures 6-11.

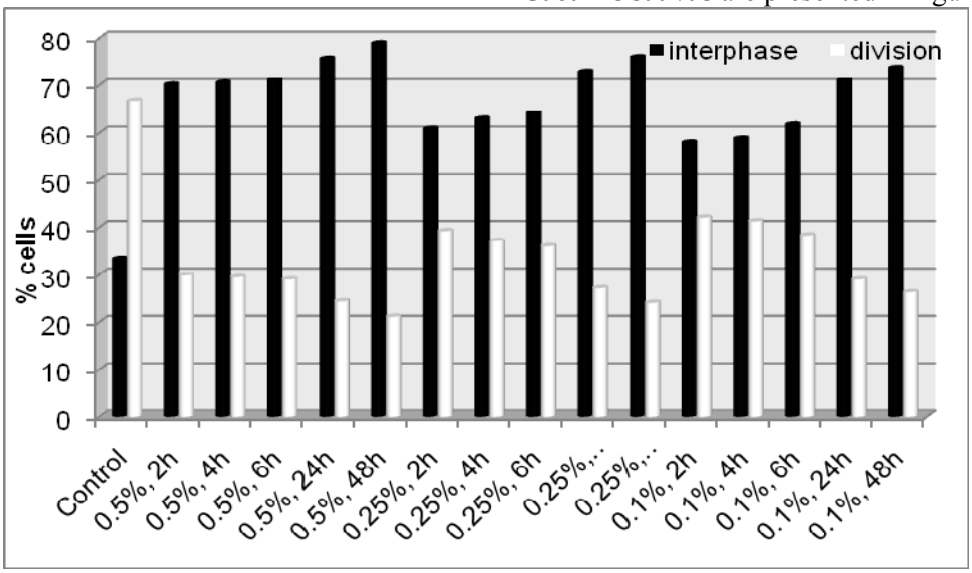

Figure 1 **Mitotic index in *Cucumis sativus*, after the treatment with potassium ferricyanide**

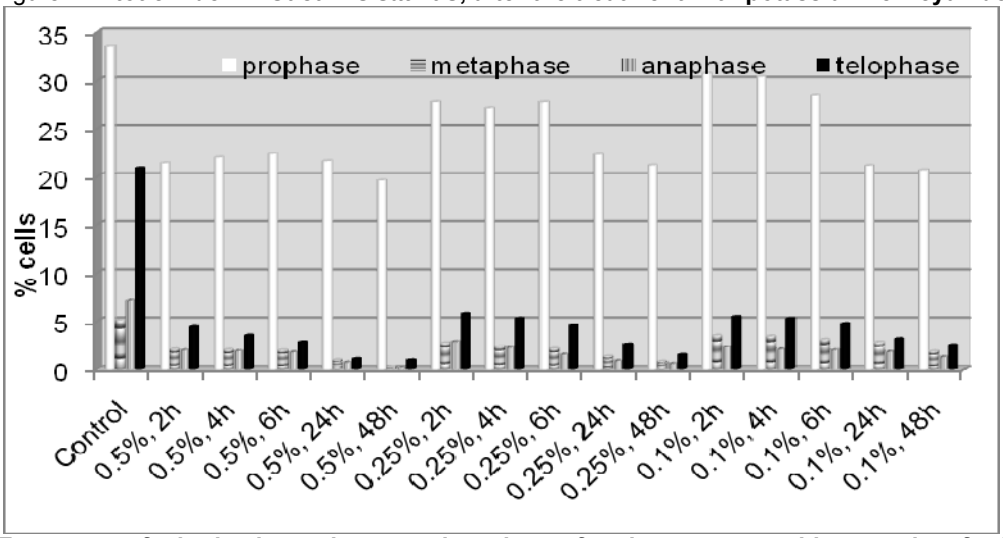

Figure 2 **Frequency of mitotic phases in *Cucumis sativus*, after the treatment with potassium ferricyanide**

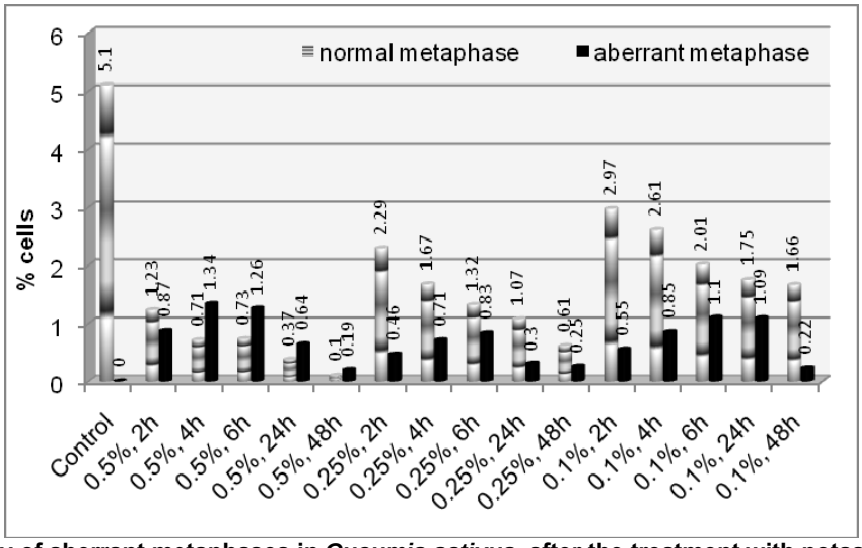

Figure 3 **Frequency of aberrant metaphases in *Cucumis sativus*, after the treatment with potassium ferricyanide**

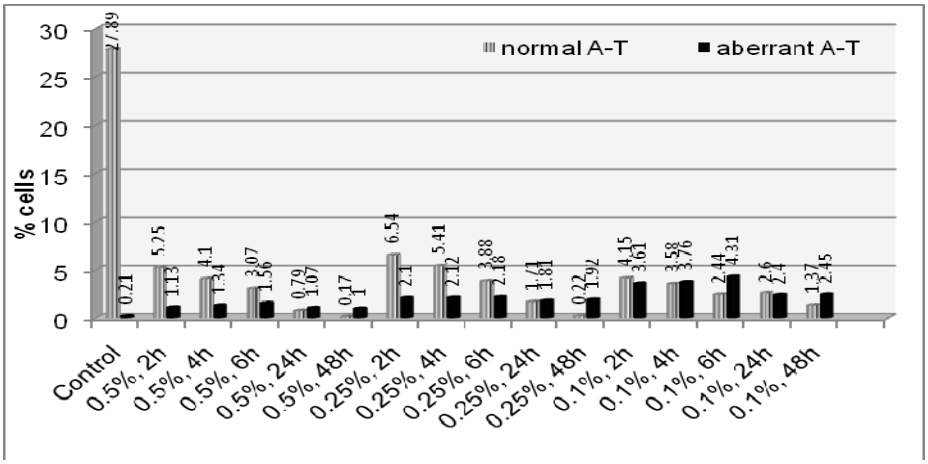

Figure 4 **Frequency of aberrant ana-telophases in *Cucumis sativus*, after the treatment with potassium ferricyanide**

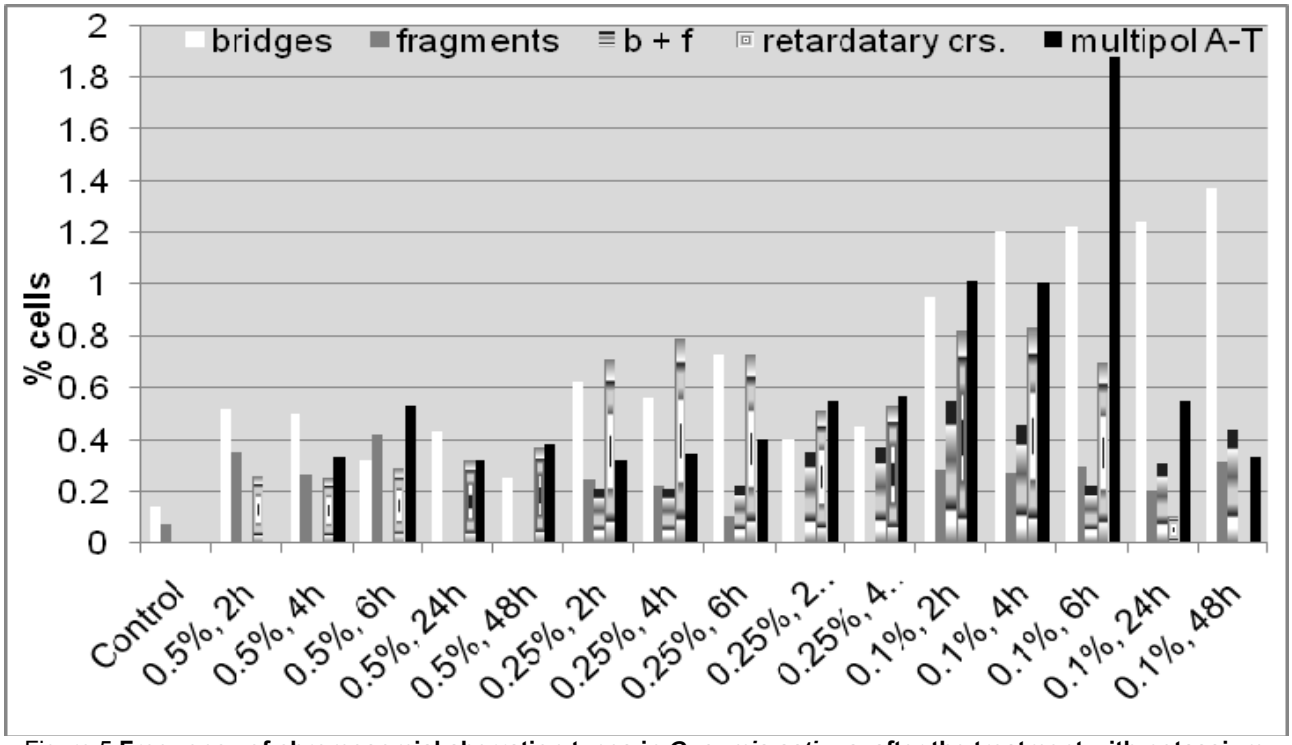

Figure 5 **Frequency of chromosomial aberration types in *Cucumis sativus*, after the treatment with potassium ferricyanide**

Table 1

Differences found after the treatment with potassium ferricyanide upon mitotic division in *Cucumis sativus*

variant	Aberrant metaphases		Aberrant ana-telophasaes	
	average value (%)	significance of difference	average value (%)	significance of difference
control	0.00	-	0.21	-
0.5%, 2 hours	0.87	***	1.13	***
0.5%, 4 hours	1.34	***	1.34	***
0.5%, 6 hours	1.26	***	1.56	***
0.5%, 24 hours	0.64	***	1.07	***
0.5%, 48 hours	0.19	***	1.00	***
0.25%, 2 hours	0.46	***	2.10	***
0.25%, 4 hours	0.71	***	2.12	***
0.25%, 6 hours	0.83	***	2.18	***
0.25%, 24 hours	0.30	***	1.81	***
0.25%, 48 hours	0.25	***	1.92	***
0.1%, 2 hours	0.55	***	3.61	***
0.1%, 4 hours	0.85	***	3.76	***
0.1%, 6 hours	1.10	***	4.31	***
0.1%, 24 hours	1.09	***	2.40	***
0.1%, 48 hours	0.22	***	2.45	***
	DL 5% = 0.03876 DL 1% = 0.05225 DL 0.1% = 0.06935		DL 5% = 0.051 DL 1% = 0.06875 DL 0.1% = 0.09125	

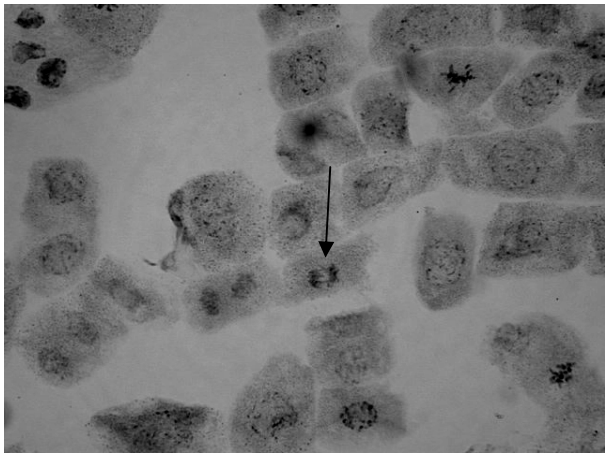

Figure 6 **Anaphase with a continuously bridge and a ragged bridge in root meristem at cucumber, treated with $K_3[Fe(CN)_6]$ 0.1%, 4 hours (400X)**

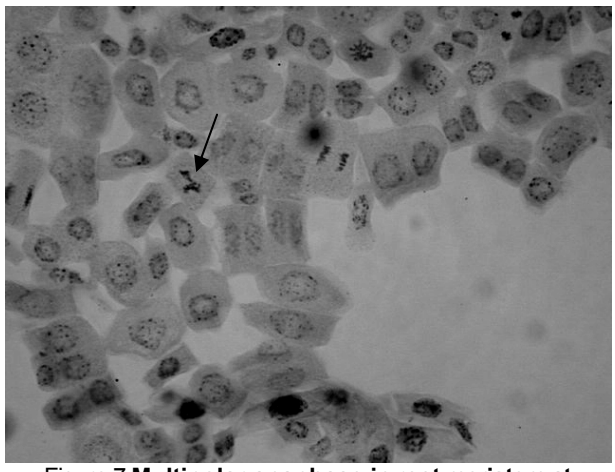

Figure 7 **Multipolar anaphase in root meristem at cucumber, treated with $K_3[Fe(CN)_6]$ 0.25%, 24 hours (400X)**

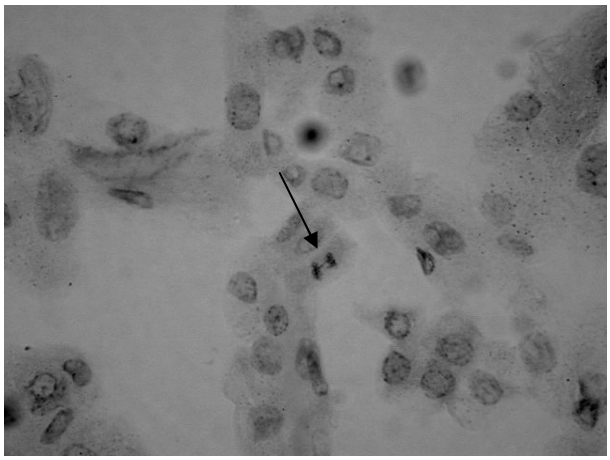

Figure 8 **Anaphase with a bridge in root meristem at cucumber, treated with $K_3[Fe(CN)_6]$ 0.5%, 48 hours (400X)**

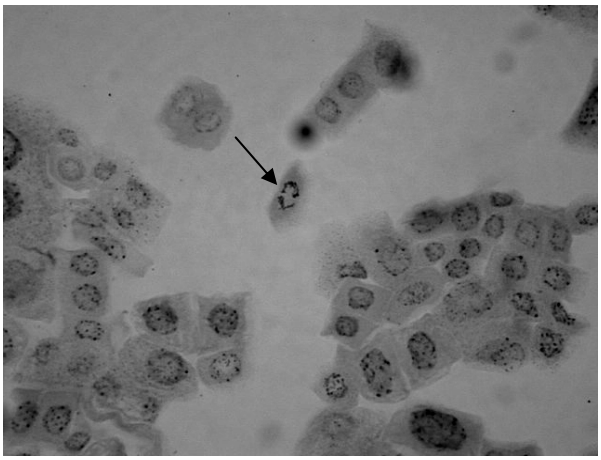

Figure 9 **Multipolar anaphase in root meristem at cucumber, treated with $K_3[Fe(CN)_6]$ 0.1%, 6 hours (400X)**

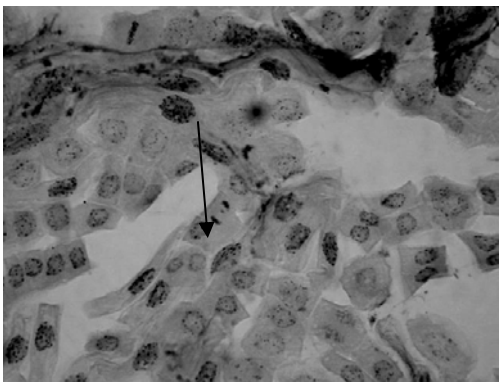

Figure 10 **Anaphase with chromosome fragment in root meristem at cucumber treated with $K_3[Fe(CN)_6]$ 0.5%, 2 hours (400X)**

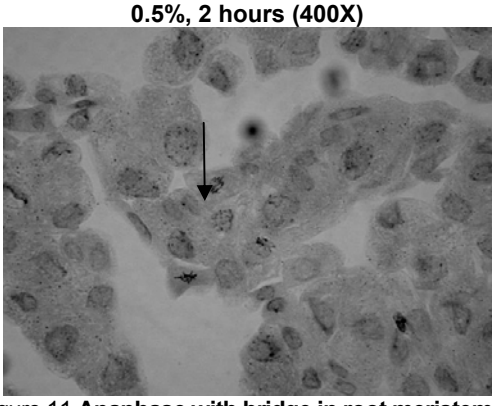

Figure 11 **Anaphase with bridge in root meristem at cucumber, treated with $K_3[Fe(CN)_6]$ 0.25%, 4 hours (400X)**

CONCLUSIONS

Potassium ferricyanide, known as a aggressive pollutant, has an strong inhibiting effect on *Cucumis sativus* mitogene cells. The most inhibited are metaphasic and anaphasic cells.

Potassium ferricyanide has a mutagen potential on *Cucumis sativus* 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. Picnotic chromosomes from metaphases, especially at 0.5% concentration (4 and 6 hours) and 0.1% concentration (6 and 24 hours), are other features of the effect of potassium ferricyanide. The experiment demonstrates the harmful effect of potassium ferricyanide on *Cucumis sativus* cells.The aggression of potassium ferricyanide is even more pronounced, the more concentration and time of action are greater.

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