# GENOTOXIC EFFECTS OF SODIUM NITRATE IN ONION ROOTS

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**Abstract.** The scope of this paper is to assess cyto- and genotoxic effects of sodium nitrate on *Allium cepa* root tips by using different concentrations (i.e. 0,1%; 1% and 5%) for treating uniform healthy onion bulbs for three different periods of time: 6, 24 and 72 hours. In the end of the experiment the harvested root tips were prepared according to Feulgen's squash technique using Schiff reagent and the investigations were realized according to *Allium* test. The cytotoxic and genotoxic effects of nitrate were investigated by calculating the mitotic index and observing all chromosomes' complement alterations during the mitosis. The phase rate of cells undergoing mitosis is also studied. For microscopy investigations a Novex Holland B microscope with digital camera included was used. The cytogenetic analysis of nitrate effects revealed a strong decrease in the mitotic index which is more intense with the concentration and time of exposure. Moreover, this effect is associated in case of the variant treated with 5% sodium nitrate acting for more than 24 hours, with the appearance of genotoxic effects such as chromosomal alterations, highly condensed chromatin expression easily identified during mitosis stages, sticky chromosomes and chromosomal bridges and laggards.

**Keywords:** *Allium cepa*, mitosis, phase ratio, sodium nitrate, mitotic index.

## INTRODUCTION

Nitrogen availability is often considered in agriculture as a limiting factor for plant growth and crops' productivity and therefore it is generally considered that is a great need to add nitrogen based fertilizers. Still, nitrates and ammonium are considered as the most important forms of inorganic nitrogen sources supporting plant growth and development [23]. However, today it is well documented that such amendments to the soil can have negative human health, economic and environmental effects [20] and as a consequence nitrate became today a monitored polluter. Due to its negative effects on human health and environment, in 2008, in Romania it was officially adopted a list of 1963 localities proved to be vulnerable to nitrate pollution [19]. Starting with the same year it was officially adopted a national programme for monitoring the nitrate level in soils and in the ground and fresh waters. As an example in the fresh waters the nitrates pollution is ranging for 11 monitored rivers between 0.25 and 10 mg/l and for 6 lakes between 0.01 and 6 mg/l. From official point of view it is considered that the major cause for this high pollution is due primarily to agricultural sector [2]. Based on these serious environmental and healthy issues [18] it is obviously that in order to maintain the balance between nitrogen availability to crops and the need to supply soils with nitrate based fertilizers it is important to first evaluate risks to nitrate pollution.

Moreover, in order to understand the relationship between the nitrogen sources into the soil and plant physiology a lot of scientific literature treated this subject after 1980. As it is well known the nitrate concentrations in soil considerably vary due to various factors such as microbial processes, mineralization and nitrification that are highly sensitive to any change into environmental conditions [18]. Measuring $NO_3^-$ ions concentration by using isotopes ($^{15}N$ and $^{18}O$) in plants may provide today a unique insight into ecosystem availability and tolerance to nitrate dynamics creating a scientific base for applying the best solutions in ecological restoration [16]. Regarding the molecular mechanism of cellular up-take of nitrates it is well known that in order to cope with highly variable nitrate concentrations in soil, plants have developed high and low-affinity transport systems known as HATS and LATS [10]. Generally it is considered that when the external nitrate concentration is higher (>1 mM), LATS is preferentially used and when nitrate availability is limited, HATS is activated and takes over the nitrate uptake process [3, 6, 11, 15, 21]. Furthermore, once taken up into the root cytoplasm, nitrate is either translocated across the tonoplast followed by storage in vacuoles, either it is reduced to nitrite and then partitioned to plastids where it is further assimilated to organic nitrogen [19]. Based on recent hypothesis it is also possible that nitrate to be loaded into xylem vessels and subsequently unloaded (moved from the xylem sap into xylem parenchyma cells) in plant aerial tissues where it undergoes processes similar to those in roots [5, 16], an idea already developed more than ten years ago [18]. At higher concentrations in the soil solution, these compounds will accumulate and affect the cells, tissues and organs such as the roots – the primary entrance of such compounds into the crop body. Nitrate concentrations in soils generally range from very low levels (i.e. a few hundred μM to around 20 mM) up to 70 mM and the meristematic mitotic cells in the roots are appropriate indicator cells for the detection of clastogenicity of environmental pollutants, especially for water and soil contaminants monitoring [16]. Onion is recognized to contain the lowest level of nitrate among legumes and it is considered that shows a moderate tolerance to this compound [3]. Based on the peculiarities of onion such as small number of chromosomes which are easy to be studied it was considered that it can be used as a model in testing

different pollutants for their genotoxic effects [1, 4, 7, 8, 9, 12, 13, 17, 22].

The scope of this study was to set a reliable laboratory method for evaluate the genotoxic effect of sodium nitrate as a nitrogen source by applying the *Allium* test that has been proposed as a standard method in environmental monitoring and toxicity screening of wastewater and fresh waters [9, 20]. For this purpose three different doses of sodium nitrate solutions have been used ranging between 11 mM and 0.5 M acting on the onion root tips for 6, - 24 and 72 hours. In the end of experiment the mitotic index, phase ratio of the cells undergoing mitosis as well as different genotoxic effects have been investigated.

## MATERIAL AND METHODS

**Plant material.** Onion bulbs (*Allium cepa* L.) the Romanian cultivar "Diamant" have been used as a biological material. Before starting the experiment the onion chives of more or less than 1 cm in diameter where selected based on their appearance and uniformity in size and healthy status. Petri dishes of 10 cm in diameter were used filled with 10 ml boiled and cooled tap water in which the onion bulbs were placed. The water was changed daily and the rooting process was stimulated under a photoperiod of 16h light/8 h dark at 18-20°C.

**Experiment description.** Only bulbs with new emerged roots ranging between 4 and 5 cm in length have been selected for testing the sodium nitrate effects. Three series of sodium nitrate solutions have been prepared as following 0.1% (11.76 mM), 1% (0,17 M) and 5% (0.58 M) which based on the proposed time of action (6, 24 and 72 hours) all experimental variants were named according the table no. 1. It was used the anhydrous sodium nitrate extra pure produced by Merck: no 106535. The control was represented by onion maintained in the boiled and cooled tap water. The selected rooted bulbs were directly placed in 10 ml solution of sodium nitrate as a genotoxic agent and maintained for 6, - 24 and 72 hours, starting with the 10:00 hour in the morning. In this experiment the Test Levan (1938) was applied because it is economic, requires short time and gives the possibility to work on excellent prepared slides. Moreover, the chromosomes from *Allium cepa* are large enough, in a low number (2n=16) making easily their observation under the microscope and the root meristems contain a huge number of cells under division [8, 19]. In the end of the experiment for each variant, according to the time of exposure the roots tips are taking out and fixed in a solution of absolute ethanol and anhydrous acetic acid in a volume ratio of 3:1 for 16 hours at refrigerator followed by a gentle acidic hydrolysis using HCl 1 N for 5 min at 60°C. The roots tips staining was realized following the Feulgen technique and using Schiff reagent for 90 min followed by water immersion for 20 min.

**Table 1.** Experimental variants used in the treatment of onion roots tips with sodium nitrate solutions

| Sodium nitrate solution concentration | Variant | Exposure time (hours) |
|---|---|---|
| 0,1% | $V_1$ | 6 |
| 1% | $V_2$ | |
| 5% | $V_3$ | |
| 0,1% | $V_4$ | 24 |
| 1% | $V_5$ | |
| 5% | $V_6$ | |
| 0,1% | $V_7$ | 72 |
| 1% | $V_8$ | |
| 5% | $V_9$ | |

The ready stained root tips of onion where squashed on slides and used for microscopy analysis under a Novex Holland B microscope with digital camera included. The mitotic index (MI) was calculated based on each slide analysis and observations have been made for each mitosis stage regarding the presence of chromosomes abnormalities.

In order to determine the MI there have been counted cells for each of five replica of each experimental variant trying to cover the entire slide. Five repetitions for each variant have been analyzed and the optical microscopy fields was magnified x1000 for investigating chromosomal alterations. The mitotic index (MI) was calculated using the formula:

$$MI = \frac{\text{no. of cells in mitosis}}{\text{no. of total investigated cells}} \cdot 100$$

**Statistical analysis.** The MI was compared using analysis of variance (ANOVA) to confirm the variability of the data and validity of results. The differences between variants have been analyzed for their significance based on Duncan's multiple range (DMRT) test (pb0.05) and differences between corresponding controls and exposure treatments were considered statistically significant at pb0.05.

## RESULTS

Cytotoxicity was estimated by analysing cytological parameters such as the mitotic index and phase ratio and and genotoxicity by observing the appearance of chromosome abnormalities, including laggards, chromosome breaks, abnormal anaphases and bridges, sticky chromosomes. This study is developed at the laboratory level as a first stage for further investigating the genotoxic effects of inorganic pollutants present in fresh waters or soils. As it was already mentioned nitrates are very toxic compounds which may pollute fresh and ground waters and very often concentrations of 10 mM are reported according to the Romanian State of Environment published in 2011 [2]. In this case, when the onion root tips reached 4-5 cm it was started the experiment for analyzing the effects of sodium nitrate at three different concentrations (i.e. 0.1%, 1% and 5%) and three different period of time (i.e. 6, 24 and 72 hours). In the end of the experiment all root tips have been treated according to Feulgen method and investigated through microscopic analysis. In these investigations have been registered all encountered cells, for each five replica of

**Table 2.** The effect of sodium nitrate on the mitotic index and cell division in *Allium cepa*

| Experimental variant | Total cells in interphase | Total cells in mitosis | Total cells in prophase | Total cells in metaphase | Total cells in anaphase | Total cells in telophase | Total cells in cytokinesis | Mitotic index (mi) |
|---|---|---|---|---|---|---|---|---|
| **Control** | 282±4.71 | 218±4.12 | 93±2.13 | 33±3.23 | 29±2.32 | 33±2.21 | 30±2.62 | 43.6±0.22 |
| **V1** | 344±2.60* | 156±2.15 | 76±2.22 | 16±2.89 | 14±2.81* | 25±2.53 | 25±2.56 | 31.2±0.44* |
| **V2** | 391±3.01* | 109±2.95 | 56±2.71 | 10±2.73 | 8±1.33* | 19±2.22* | 16±2.36 | 21.8±0.39** |
| **V3** | 429±2.53** | 71±2.83 | 45±3.07 | 4±2.61 | 2±2.67** | 12±3.43* | 8±2.71* | 14.2±0.32** |
| **V4** | 374±2.51* | 126±3.23 | 61±3.34 | 14±3.67 | 11±3.51* | 23±2.87 | 17±2.26 | 25.2±0.23* |
| **V5** | 412±3.23* | 88±2.03 | 55±2.88 | 7±3.12 | 3±3.22** | 14±2.98* | 9±2.23* | 17.6±0.31* |
| **V6** | 436±4.02** | 64±4.09 | 43±3.33 | 4±3.66 | 0 | 9±3.56* | 8±3.67* | 12.8±0.23* |
| **V7** | 390±5.86* | 110±3.31 | 56±4.21 | 11±4.44 | 11±2.89* | 18±3.36* | 14±4.56* | 22.0±0.32* |
| **V8** | 436±2.72* | 64±2.98 | 41±2.40 | 3±2.58 | 0 | 12±2.61* | 8±2.82* | 12.8±0.21** |
| **V9** | 453±3.47** | 47±3.08 | 33±1.55 | 0 | 0 | 7±3.32* | 7±3.97* | 9.4±0.19** |

Legend: Numbers in each column labelled with asterisk are significantly different from control values at p>0.05 according to DMRT.

the 10 variants. During such investigations cells in different cell cycle phases were separately counted (i.e. interphase, prophase, metaphase, anaphase, telophase and cytokinesis). The results of these investigations are summarized in table no 2.

**Cell division and phase ratio.** It can be observed that in the control case, cells undergoing mitosis, express a specific phase ratio defined as the proportion between cells undergoing mitosis' phases. In this analysis the phase ratio is calculated by dividing the higher number of cells per phase to the lowest. For control it appears that this ratio is 3:1:1:1:1 or: prophase (3): metaphase (1): anaphase (1): telophase (1): cytokinesis (1). In other words, there is a balance between different groups of cells undergoing mitosis for onion. By applying sodium nitrate it can be observed that the number of cells in division is decreasing and this is associated with the change in the ratio of cells undergoing mitosis (Fig. 1). In this case the lowest number of cells is constant for anaphase and based on this observation the entire phase ratio will be further calculated.

For a concentration of 0.1% sodium nitrate which is equivalent with 11.76 mM, it was observed that there is an increase of cells ratio in prophase starting with 49% for 6 hours to 52% for 24% up to 63% for 72 hours (Fig. 1 b-d). Such an increase of the proportion of cells in prophase may support the direct impact of nitrate on the root meristem functioning. Moreover, it was observed that this effect is associated with a decrease in the proportion of cells in metaphase (10% for 6h, 9% for 24h and 6% for 72h compared to control: 15%) and anaphase (9% for 6h, 7% for 24h and 3% for 72h compared to control: 13%). Instead it is obviously that the proportion of cells in anaphases is slightly increased (16% for 6h, 17% for 24h and 17% for 72h compared to control: 15%). For cytokinesis appears that the process become affected mostly after 72 hours of action when a significant decrease of 11% is observed compared to the control (i.e. 14%). This result may support the idea that this change in the phase ratio should be a secondary effect of the decrease in the ratio of cells undergoing metaphase and anaphase. Using a nitrate concentration of 1% on root tips resulted in the increase of the cells ratio in prophase compared to the control and more obvious compared to the concentration of 0.1% for 24 and 72 hours (Fig. 1 e-g). This response is associated with a significant decrease of cells ratio in metaphase (11% for 6 h, 8% for 24h and 6% for 72h compared to control: 15%). Continuing this analysis, it was observed the decrease of cells in anaphase to 9% at 6 hours to 3% at 24 hours and apparent absent at 72 hours (Fig. 1 j). This response may also be interpreted as an artefact of these analysis due to the low number of cells undergoing mitosis on one hand but also it can be a secondary effect of nitrates on the cells to complete their division at the root level because the ratio for cells in telophase and cytokinesis is not significantly modified compared to the control. For a concentration of 5% nitrate and only 6 hours of treatment it can be identified a similar pattern of phase ratio trend such as for 1% nitrate. Still, phase ratio in prophase is increased up to 51% which is much higher for 24 hours (63%) and for more than 70% at 72 hours (Fig. 1 h-k). This is associated with a drastic reduction of metaphase ratio compared to the control (i.e. 6% compared to 15% of the control) and the disappearance of this phase at 72h. In the case of anaphase it looks to be the most affected as it wasn't recorded for 24 and 72 h of treatment.

**Mitotic index** is significantly decreased compared to control in all experimental variants and in a direct and logarithmic correlation with the concentration of the genotoxic agent and the exposure time (fig. 2). For control variant of onion root tips the mitotic index was 43,6 and for all variants treated with sodium nitrate the lowest mitotic index was for the highest concentration in this agent and for the longer exposure time of 72 hours. This is consistent with the results in interpreting phase ratio.

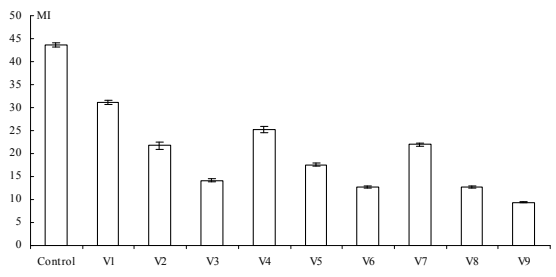


**Figure 2.** The mitotic index (MI) in onion root tips treated with sodium nitrate. V1-V9 are the variants according to table no 1

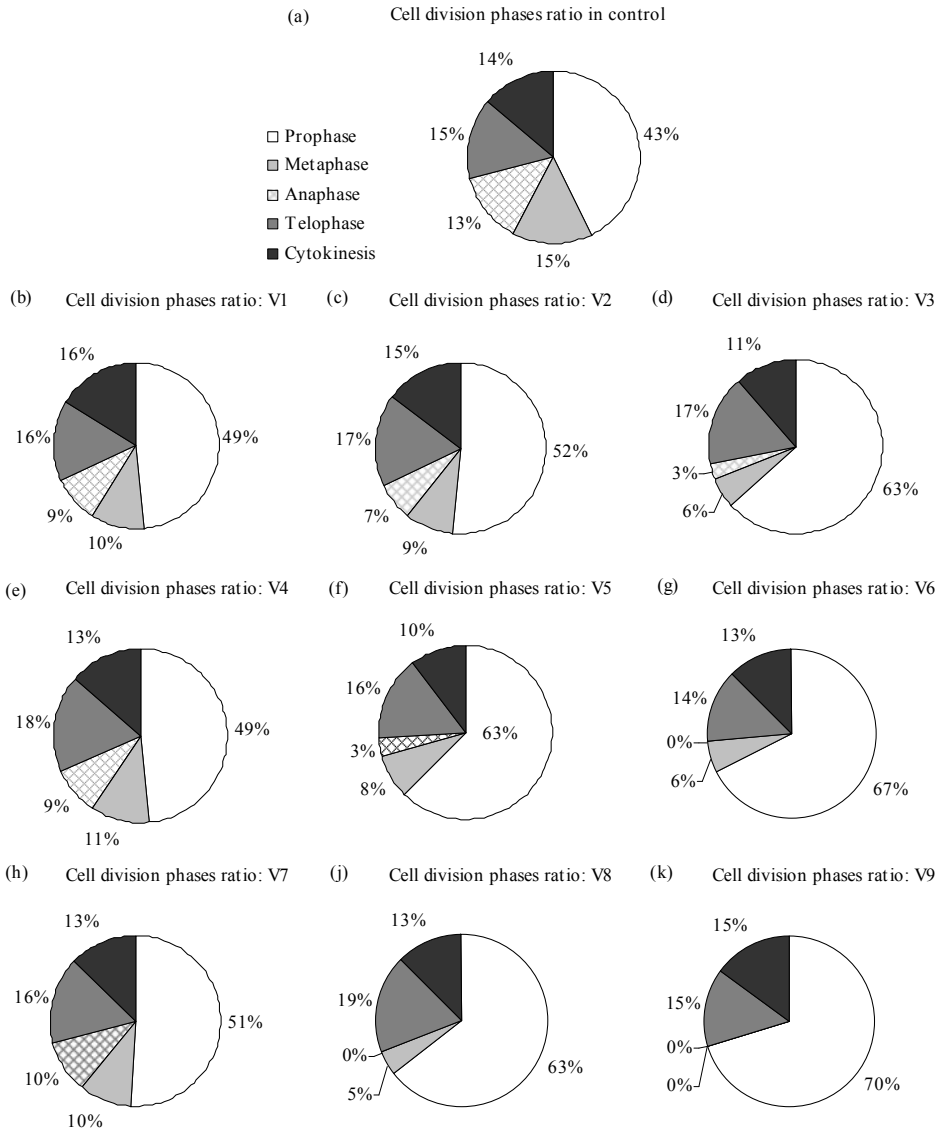


**Figure 1** The phase ratio for cell undergoing mitosis in onion root tips treated with sodium nitrate. V1-V9 according to table no 1

**Time of treatment with sodium nitrate.** The treatment for 6 hours with sodium nitrate in different concentrations (i.e. 0.1%, 1% and 5%) induced a logarithmic decrease in cells numbers for all groups of cells undergoing prophase, metaphase, anaphase, telophase and cytokinesis (Fig. 3). These responses may be associated with the stress expressed in the root tips which should cope with higher levels of nitrate. Considering that $R^2$ values for logarithmic trend is varying very tide with the exception of those registered for prophase it can be concluded that the stress is already expressed (Fig. 3, a-f). Increasing the treatment to 24 hours it can be observed the same logarithmic decrease in the cell numbers undergoing mitosis following the same nitrate treatments (Fig. 3 g-l). In this case the $R^2$ values differ with 0.2-0.3 units with the exception of telophase ($R^2$=0.99). Another abnormal event is that for a concentration of 5% of sodium nitrate it is not recorded any anaphase which is consistent with the idea that the stress might be higher for this period of treatment compared to 6 hours (Fig. 3 i). In the last case after 72 hours of treatment the logarithmic decrease is following the same pattern like above described but this time $R^2$ values range between 0.3 and 0.5 and again with the exception of telophase ($R^2$=0.99) (Fig. 3 m-s). This time no cells in anaphase have been recorded for 1%. Moreover, no cells in anaphase and metaphase have been recorded for sodium nitrate at 5% (Fig. 3 n and o) supporting the idea that this is a response to a higher stress induced by nitrates on the root tips.

**Cytological analysis.** As a general view of a microscopic filed in control is presented in fig. 4 A) where it can easily be observed cells undergoing prophase, metaphase, anaphase, telophase and transversal cyokinesis. Instead clear changes appear for cells undergoing division or even in interphase (Fig. 4 B-J). Thus, considering the treatment for 6 hours it appears that this process is associated with the dominance of cells in prophase in the detriment of cells in anaphase for all three variants (Fig. 4 B, E, H) and as a genotoxic effect very often there can be seen abnormal stellar metaphases at a concentration of 5% for 6 and 24 hours (Fig. 4 H, Ji). On the other hand

these characteristics are associated with the hypertrophy of nuclei belonging to cells in interphase (Fig. 4 B-J). The genotoxic negative effects are more obviously after a 24 hours of treatment (Fig. 4 D, G, J). Still, if for 0.1% and 1% nitrate concentration the cytological effects are similar with those already mentioned at 6 hours. However for a concentration of 1% and 5% in 24 hours aside the normal transversal cytokinesis appears also abnormal anaphases, anaphase bridges (Fig. 5 E), and longitudinal cytokinesis which may be a side effect of the negative influence on the mitosis which also should be correlated with the change in phase ratio and the apparent absence of anaphases (Fig. 4 I). For 5% sodium nitrate it was also observed that cells in anaphase are missing. After 72 hours of treatment the meristematic cells are seriously affected both for 1% and 5% concentrations (Fig. 4 G and J and Fig. 5 A). Vacuolisations appear to be associated with an obvious expression of heterochromatin packaging in nuclei for cells in interphase (Fig. 5 B), cells in prophase are dominant and the abnormal longitudinal cytokinesis become more often associated with abnormal telophase and micronuclei (Fig. 5D) late anaphase bridges (Fig. 5 E) and sticky chromosomes (Fig.5).

## DISCUSSIONS

A series of studies, starting with 1985, demonstrated the efficiency of using *Allium* tests for investigating the genotoxic activity of a series of inorganic polluters. Sodium nitrate is considered as a substance which in overdoses is inducing both cytotoxic and genotoxic effects in living organisms and therefore it is studied especially following treatments of the root tips as they are easily produced, all over the year and are not costly. By applying *Allium* test in the laboratory for investigating nitrates effects on root meristematic cells it was proved to be a reliable method in terms of time and expenses [7, 8, 9, 22].

Considering the results obtained in this experiment, and discussed above, it may be generally concluded that mitotic index is significantly decreased under the treatment of all used sodium nitrate concentrations for all three periods of time and this is in line with other scientists' results [17]. Moreover, this result is associated with significant changes in the phase ratio of the cells undergoing mitosis for the above described experimental conditions if the control phase ratio is a reference. Based on these observations we consider that this may become a tissue marker to be associated to mitotic index analysis as it is well known that meristems comprises a specific number of cells undergoing mitosis and it was already shown in the presented results that this may be statistically significant when it is applied in specific conditions. For these experimental conditions we mention that the reference phase ratio is 3:1:1:1:1 of cells in different mitosis phases such as prophase (3): metaphase (1): anaphase (1): telophase (1): cytokinesis (1) and this may be considered as the control balance between different groups of cells undergoing mitosis in onion. Such a phase ratio changed under the sodium nitrate treatments, but as a constant observation, it was registered that the lowest proportion of cells is counted for anaphase. It appears that the anaphase ratio is more sensitive in these analyses and this is significantly expressed for all variants. In supporting this, by increasing the dose of sodium nitrate at 1% for 72 hours it induced the disappearance of cells in anaphase which appear not to be registered too for 5% nitrates acting for 24 and 72 hours (Fig. 3 i and o). In the later case, at 72 hours the lack of cells in anaphase is associated with the apparent disappearance of metaphase which is further associated with the presence of an important cells ratio in telophase and cytokinesis as it was discussed above (Fig. 3 n). Such a phenomenon is difficult to be interpreted: on one hand it can be an artefact of preparation but on the other hand it can be a response of the root tissue to the stress of living under such high concentrations of sodium nitrate and supporting the results of other scientists [17, 22]. Moreover as a general observation it can be underlined that sodium nitrate is generally affecting the ratio between cells undergoing different mitosis phases.

Moreover considering the analysis of mitotic index a treatment with sodium nitrate 0.1% for 6 hours induced a MI of 31.2 (71% of the control), for 24 hours induced a MI of 21.8 (50% of the control value) and for 72 hours a MI of 14.2 (32% of the control). In direct correlation the phase ratio in different mitosis phases for V1, V2 and V3 as it is presented in Figs. 1 b-d, was slightly modified after 6 hours and seriously affected after 24 and 72 hours of treatment. It appears that the increase in the proportion of cells in interphase and prophase is associated with the disruption of the normal ratio of the cells in different mitosis' phases. Based on a treatment with 1% sodium nitrate the MI is considerably affected following o similar model but more drastic compared to 0.1%. At 6 hours the MI is decreased to 57% of the control (i.e. 25.2), at 24 hours is 40% (i.e. 17.6), and at 72 hours it becomes 29% of the control (i.e. 12.8). By increasing the sodium nitrate concentration up to 5%, the pattern in the decrease of MI is higher but similar this time as following: at 6 h the MI is only 50% of the control (i.e. 22). After 24 hours at the same concentration MI is decreasing up to 29% (i.e. 12.8) and after 72 hours it becomes only 21% of the control (i.e. 9.4). As a general remark based on the above discussed results it can be concluded that MI is negatively influenced by rising the sodium nitrate concentration and time of treatment of the root tips and it is consistent with previous results [4, 7, 8, 22].

The normal appearance of mitosis phases are already well documented from the scientific literature (Fiskejo 1985) and all cytological observations have been made according to the method already proposed for applying *Allium* test. In the control it can be easily observed the abundance of cells in different phases of mitosis with a normal appearance of chromosomes in line with previous studies [7]. Sodium nitrate action for more than 24 hours produced cells' plasmolysis and the

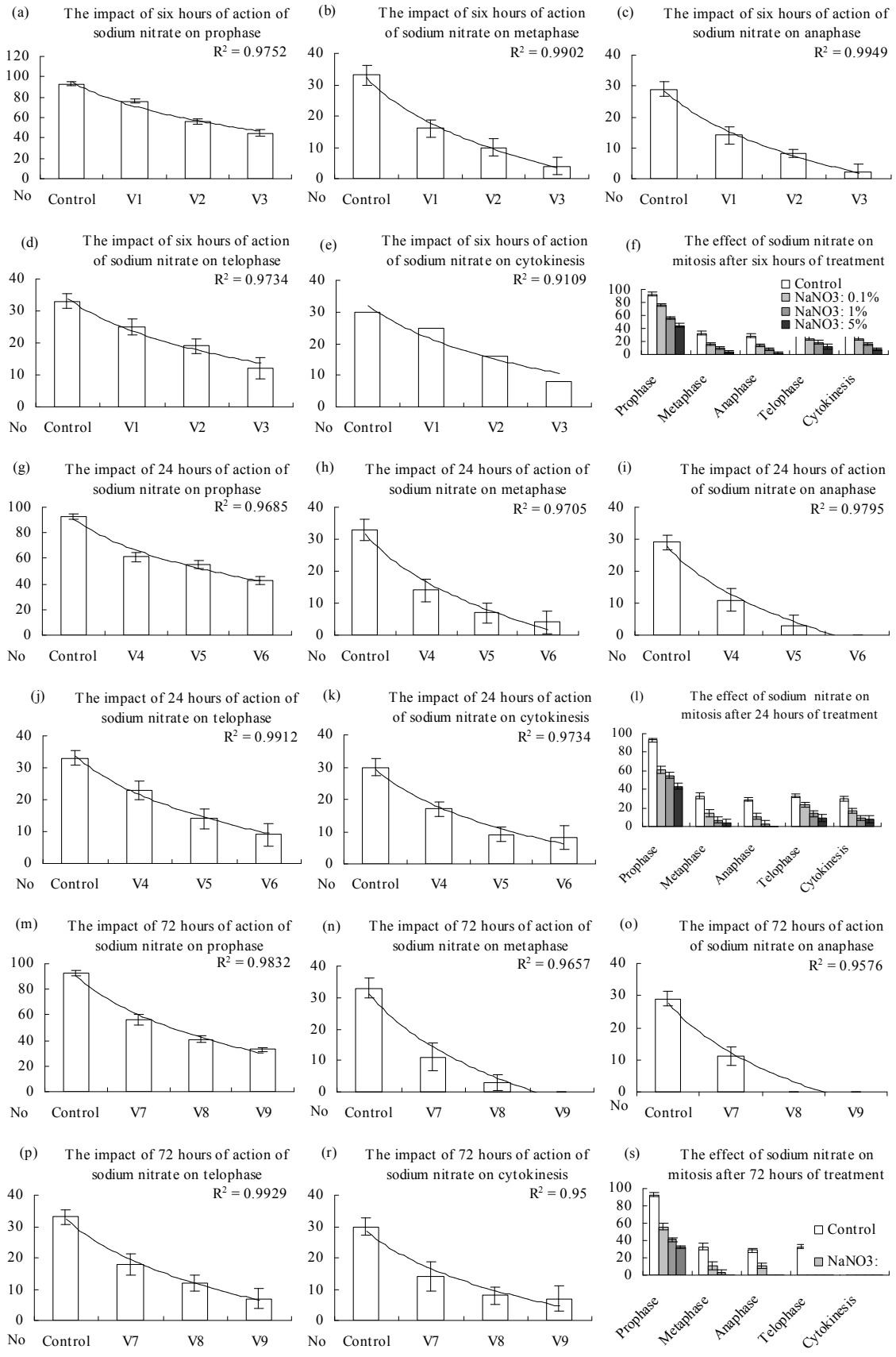


**Figure 3.** The mitosis phases analysis in onion root tips treated with sodium nitrate. V1-V9 are the variants according to table no 1

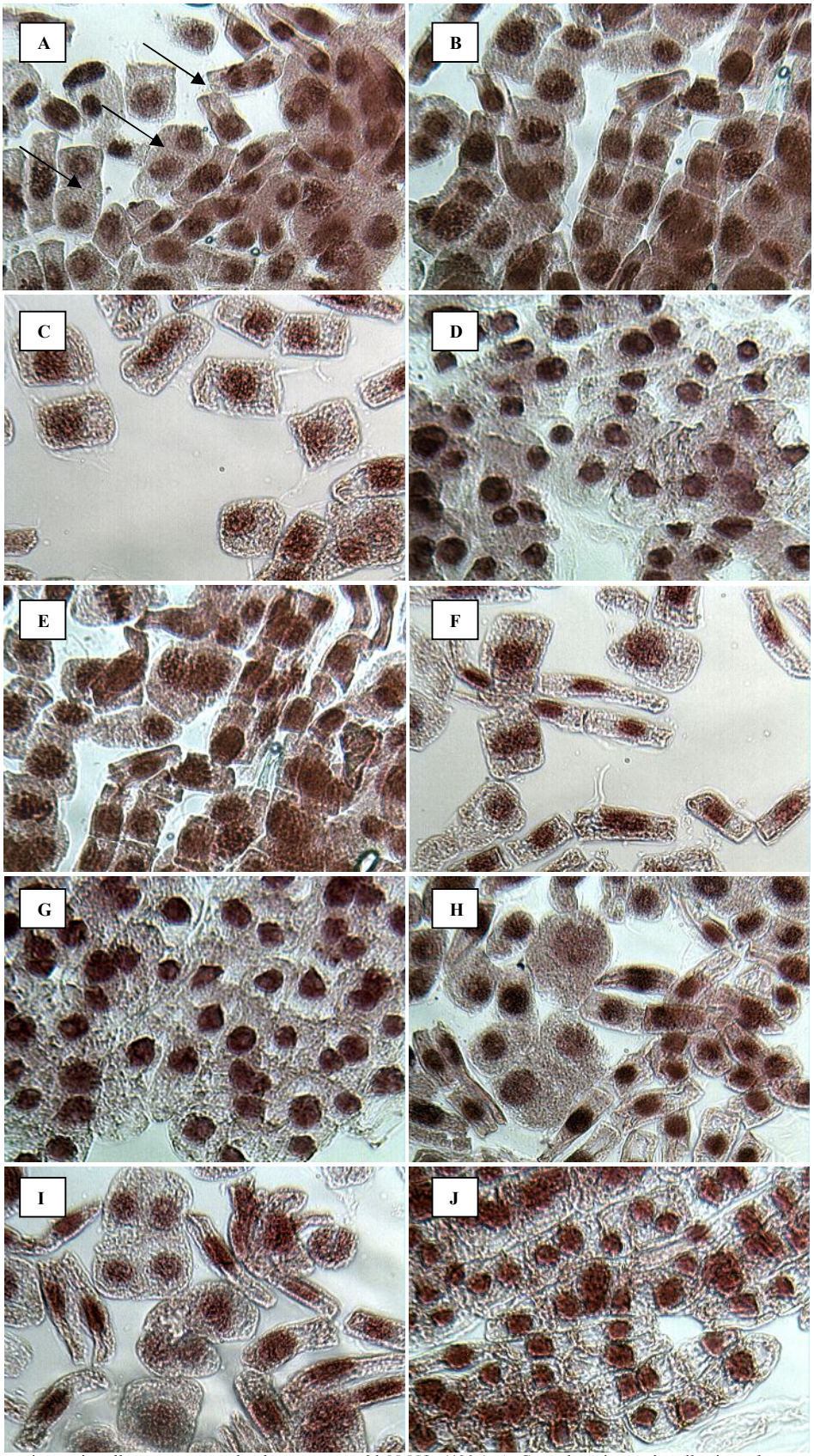


**Figure 4.** Onion root meristematic cells appearance under the treatment with $NaNO_3$ (400x): **A.** Control meristematic cells - it can be seen numerous prophases, two metaphases, one anaphase, a telophase and one cytokinesis; **B.** $NaNO_3$ 0,1%: 6 h, the dominance of prophase is observed and a stellar metaphase with sticky chromosomes; **C.** $NaNO_3$ 0,1%: 24 h, the dominance of prophases is observed and hypertrophic nuclei as well as normal cytokinesis; **D.** $NaNO_3$ 0,1%: 72 h, The dominance of prophases associated with nuclei revealing large vacuolisations and hyperchromatinisations can be observed; **E.** $NaNO_3$ 1%: 6 h, the dominance of prophase is observed, normal and stellar metaphases with sticky chromosomes; **F.** $NaNO_3$ 1%: for 24 h, The dominance of prophases is observed and hypertrophic nuclei as well as normal cytokinesis; **G.** $NaNO_3$ 1% for 72 h, The dominance of prophases associated with nuclei revealing large vacuolisations and hyperchromatinisations are observed; **H.** $NaNO_3$ 5%: 6 h, The dominance of prophases is observed and hypertrophic nuclei; **I.** $NaNO_3$ 5%: 24 h, The dominance of prophases is observed as well as telophases and longitudinal cytokinesis; **J.** $NaNO_3$ 5%: 72 h, The dominance of prophases and telophase with chromatin unbalanced nuclei can be observed

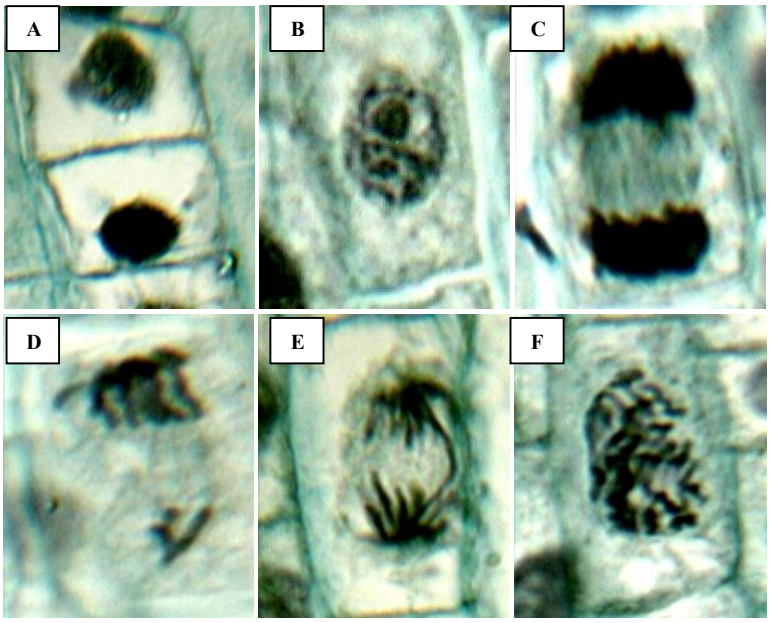


**Figure 5.** Sodium nitrate effect on chromosomal complement in onion root tips (1000x): **A.** Plasmolysis ($NaNO_3$ 1%: 72h); **B.** Strong heterochromatinisation of the nuclei ($NaNO_3$ 5%: 24h); **C.** Sticky telophase ($NaNO_3$ 5%: 24h); **D.** Abnormal telophase and micronuclei ($NaNO_3$ 5%: 24h); **E**. Late anaphase bridges ($NaNO_3$ 1%: 24h); **F**. Sticky chromosomes ($NaNO_3$ 5%: 24h)

abundance of stellar metaphases for a nitrate concentration of 1% which may be associated with the significant change in phase ratio correlated with the disappearance of anaphase for 72 hours. Still, cells in metaphase and anaphase appear to be absent for 5% sodium nitrate which also can be correlated to the phase ratio change. Sodium nitrate at a 5% concentration appears to seriously affect the root meristematic tissue from cytological point of view compared to the control which can be associated with a five time decrease of the mitotic index. The chromosomes sets appear to be affected by a concentration of 1% acting over 72 h and 5% sodium nitrate concentration acting for over 24 h when no metaphases or anaphases heve been observed. Still for concentrations of 1% at 24 h or 5% at 6 and 24h have been observed laggards, chromosomes bridges, unbalanced distribution of the chromosome after metaphases visible in telophases, sticky chromosomes, micronuclei and these results are consistent with previous studies [7, 8, 9, 12, 17, 21].

As general conclusions based on the results of this experiment it should be underline that the *Allium* test can be easily implemented into the laboratory and the scientific interpretation of the data may support the genotoxic effect of sodium nitrate on the mersitematic cells. Mitotic index can be correlated with phase ratio and the observation of genotoxic effects making the phase ratio a reliable indicator to be used in studying the cyto- and genotoxic effects of chemicals when *Allium* test is applied.

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