CYTOGENETIC EFFECTS INDUCED BY PHENOLIC COMPOUNDS IN *LYCOPERSICON ESCULENTUM* MILL

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Abstract: The aim of this paper is to evaluate the effects of the phenolic compounds extracted from spruce bark on cells from the radicular apex of *Lycopersicon esculentum* Mill. We found that different concentrations of polyphenols and the time of treatment modified the frequency of cells division and the number of mitotic ana-telophases with aberrations.

INTRODUCTION

In recent years, a special concern is manifested toward the environment protection and the use of new waste sources to obtain biological active substances applied in different fields (medicine, agriculture, food industry). Approximately 18 % of the mass of logs from conifers is bark, with a high content of phenolic compounds (Hemingway, 1997). Previous results proved the biostimulative effect of bark extracts on seed germination and plant development (Popa et al., 2002, Bălaş et al., 2005).

MATERIALS AND METHODS

Plant material Spruce bark was provided by Ambro S. A. Suceava, obtained from logs processing.

Extraction Spruce bark, was air-dried and ground in a mill. The lipophilic compounds were extracted in a Soxhlet apparatus with benzene. The phenolic compounds were extracted with NaOH 1.5%, for three hours, at 90°C, and a liquor-to-wood ratio of 10. The global phenolic extract was neutralized with 10% HCl to pH 7. The global extract contained 18.05 g/L polyphenolic substances.

Bioassay Seeds of *Lycopersicon esculentum* L. cv. A106/25, collected during 2004, had commercially provenience. Germination and growth were conducted in aqueous solutions of the global extract at different concentrations: 0.12, 0.16, 0.24, 0.28 and 0.36 g/L. The control tests were performed in the same conditions using distilled water. After adding 25 seeds on filter paper and 10 mL test solutions, the Petri dishes were placed at 22±2°C, in darkness. Samples were collected for cytogenetic investigations when the roots plantlets measured between 10-15 mm. This was considered time 0 and was followed by further sampling after 3, 6, 12 and 24 hours. The division’s phases were observed according to literature protocols (Cîmpeanu et al., 2002).

RESULTS AND DISCUSSIONS

The effects of phenolic compounds at time 0

The presence of phenolic compounds has different effects on the mitotic index, depending on the concentrations tested. At concentrations 0.12 and 0.36 g/L, the value of mitotic index is higher with 1% compared to the control, while the minimum value is obtained at 0.16 g/L (figure 1A). The frequency of prophases is higher in all variants and control, comparing with other division phases (figure 1B). At concentration 0.12 g/L, the growth of the mitotic index is correlated with the increase of the number of cells in prophases. At 0.16 and 0.24 g/L concentrations, the number of aberrations is numerous compared to control (figure 1C).

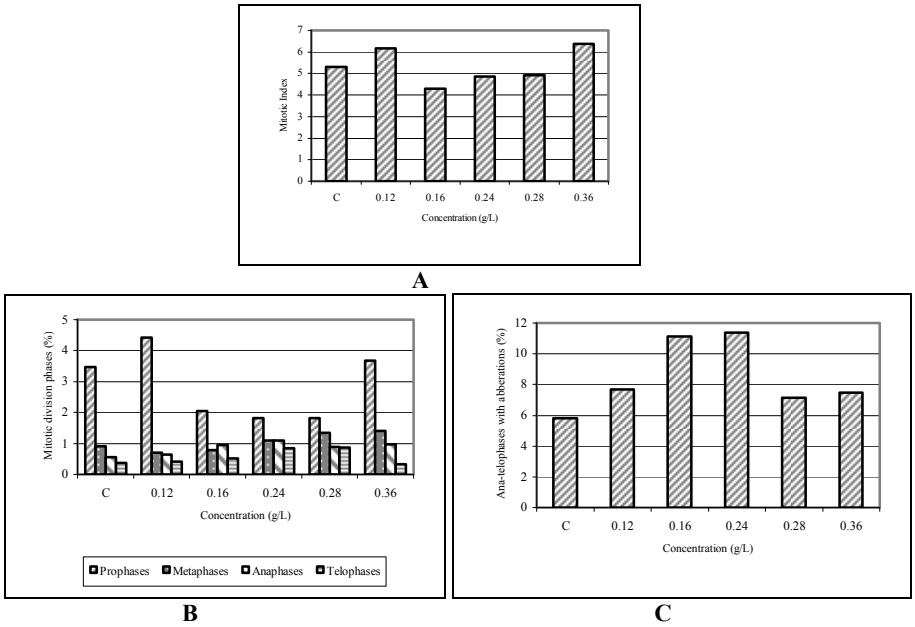


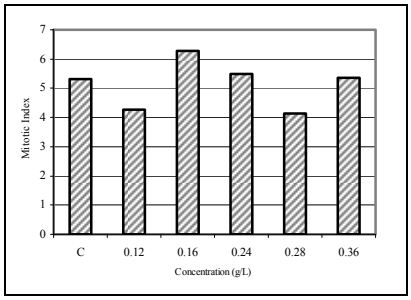
Figure 1. Cytogenetic characteristics at time 0
A. Mitotic index, **B**. Division phases, **C.** Ana-telophases with aberrations

The effects of phenolic compounds after 3 hours

The mitotic index has increased at the medium concentrations: 0.16, 0.24 and 0.36 g/L (figure 2A), while at 0.12g/L, the mitotic index has reduced. The increase of the bioactive compounds concentrations modifies the rate between metaphases, anaphases and telophases. At concentrations of 0.16 and 0.28 g/L bioactive compounds, the anaphases have higher percentage than metaphases and telophases, while at 0.24 g/L the three division phases have similar weight (Figure 2B).

The growth of the mitotic index for 0.16 g/L is associated with the numerous cells in prophases, while at the maximum treatment, the value of the mitotic index is due mainly to the increase of the cells in metaphases and telophases.

At concentrations 0.12 and 0.36 g/L, the frequency of aberrations is lower with 2%, while at concentrations of 0.16 and 0.28 g/L, the same characteristic increase with 7 % comparatively to control (figure 2C).



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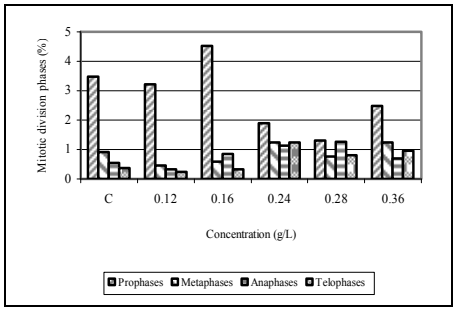
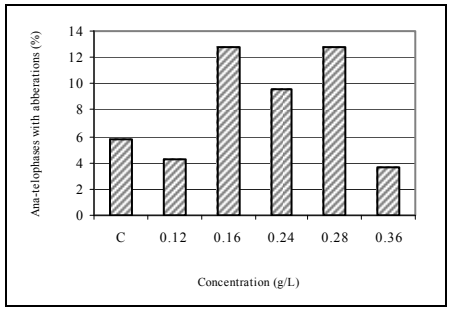
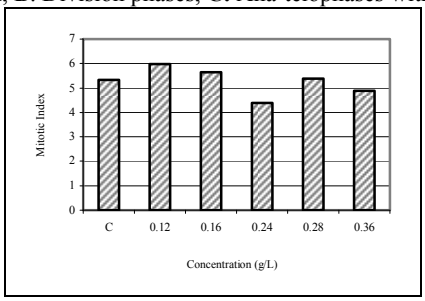




B **C**

Figure 2. Cytogenetic characteristics after 3 hours
A. Mitotic index, **B**. Division phases, **C.** Ana-telophases with aberrations



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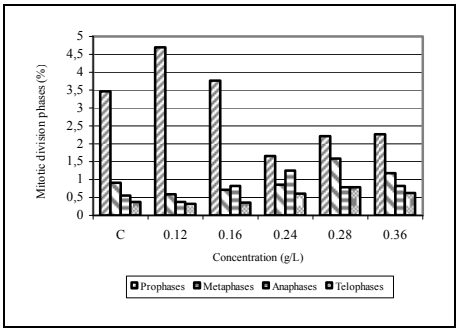
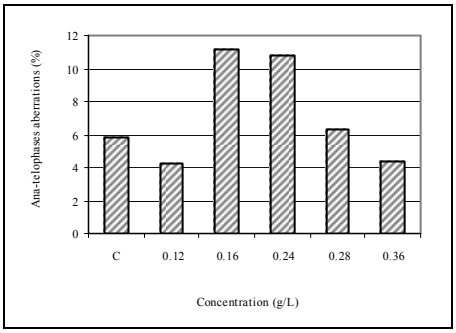




B **C**

Figure 3. Cytogenetic characteristics after 6 hours
A. Mitotic index, **B**. Division phases, **C.** Ana-telophases with aberrations

The effects of phenolic compounds after 6 hours

In all experimental variants, the mitotic index is similar to control. The spruce bark extract had a light stimulatory effect on the number of dividing cells at lower concentrations 0.12 and 0.16 g/L, and a light inhibitory effect at 0.24 g/L (figure 3A).

At concentration of 0.12 and 0.36 g/L, the main division phases are: prophases, followed by metaphases, anaphases and telophases (figure 3B). In the case of concentrations of 0.16 and 0.24 g/L, anaphases are numerous compared to metaphases and telophases, while at 0.28 g/L the percentages of anaphases and telophases are equal (0.77%).

The presence of spruce polyphenolic extract in the medium determines the development of ana-telophases with aberrations (figure 3C). At concentrations 0.16 and 0.24 g/L, the abnormal cells are approximately with 4% numerous than the cells from the control samples. The reduction of ana-telophases with aberrations at 0.12 and 0.36 g/L can be explained by the appearance of repairing or adjustment processes during the cells division.

The effects of phenolic compounds after 12 hours

At this time sampling, the mitotic index is equal or higher to control, demonstrating the mitogenic effect of the spruce extract. In the case of concentration 0.24 g/L, this characteristic is with 1.24% higher than control (figure 4A).

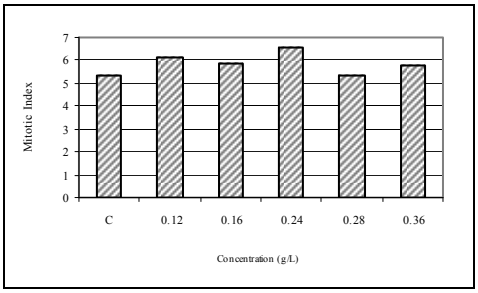
At lower concentrations, the distribution of division phases is prophases, metaphases, anaphases and telophase, while at higher concentrations, the anaphases are numerous than metaphases (figure 4B). The frequency of ana-telophases with aberrations is higher with 2.3% at concentrations of 0.12 and 0.16 g/L, while at 0.24 g/L the registered value is 1% lower than control (figure 4C).

After 12 hours, the spruce extract has a biostimulative effect on the mitotic division of radicular apex cells. These results are correlated with previous data obtained for lignin (Obreja et al., 2002).

The effects of phenolic compounds after 24 hours

The prolong exposures to phenolic treatment determine the reduction of the division cells (figure 5A). At a concentration of 0.28 g/L, the mitotic index value is with 1% higher as compared to control. The frequency of division phases is correlated with the concentration applied (figure 5B). At 0.28 g/L, the number of prophases is lower than the control. The frequency of ana-telophases with aberrations is lower at the majority of the concentrations tested (figure 5C). The number of aberrations is increased only at 0.16 g/L.

The main types of ana-telophases with aberrations are similar at all concentrations and time treatments: simple and multiple bridges. Others types of aberrations are ana-telophases with lagging chromosomes, fragments, and also a reduced number of C-mitosis, indicating that the spruce global extract has a light colchicomimetic effects (figure 6).



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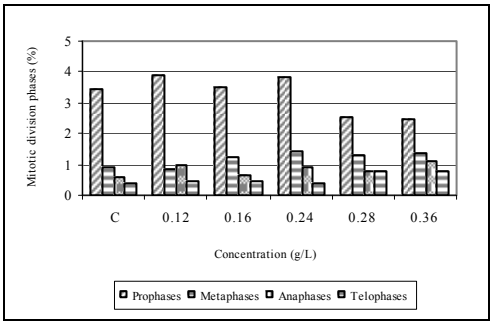
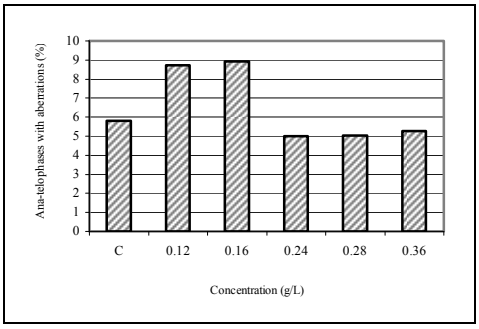
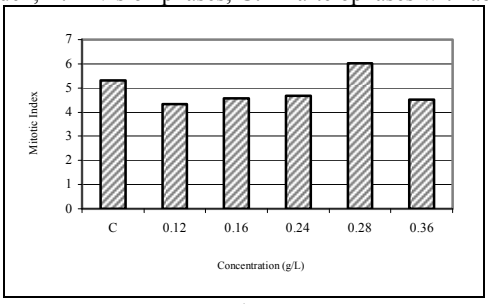




B **C**

Figure 4. Cytogenetic characteristics after 12 hours
A. Mitotic index, **B**. Division phases, **C.** Ana-telophases with aberrations



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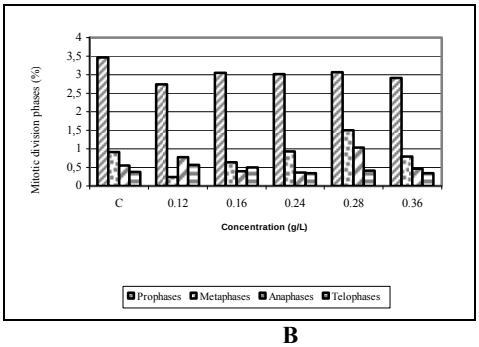

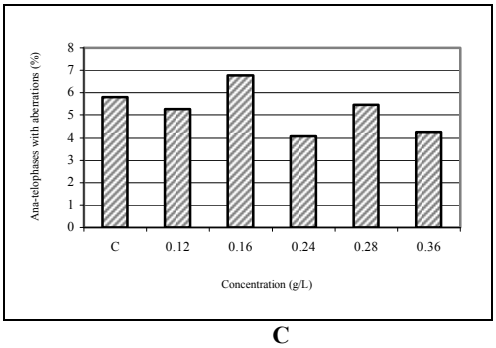

B **C**

Figure 5. Cytogenetic characteristics after 24 hours
A. Mitotic index, **B**. Division phases, **C.** Ana-telophases with aberrations

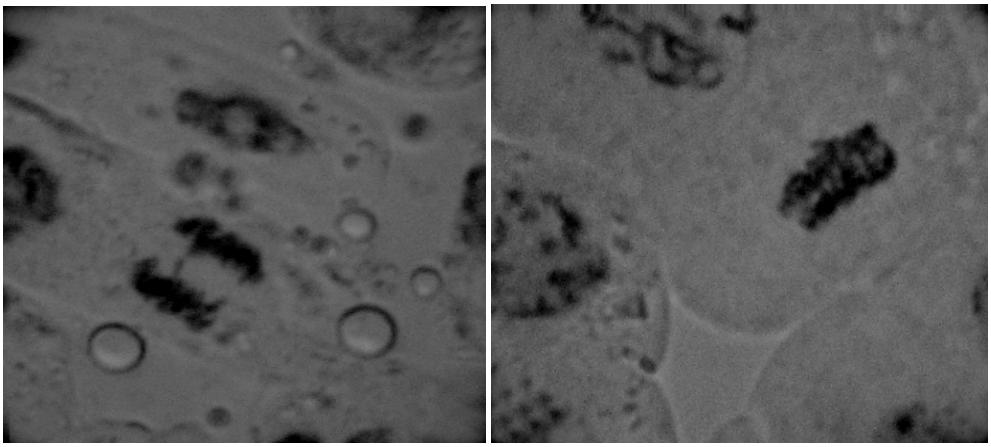

Figure 6. Ana-telophases with aberrations at *Lycopersicon esculentum* Mill: lagging chromosome (left) and multiple bridges (right)

CONCLUSIONS

Under the influence of phenolic compounds, the cellular division from the radicular apex of *Lycopersicon esculentum* Mill is modified in accordance with the concentrations used and the period of analysis. The most representative effects are obtained after 12 hours from the beginning of the sampling, the mitotic index is increased at all concentrations and the ana-telophasic aberrations are reduced compared to the control. A long time of treatment of polyphenolic extract has a mitoclastic effect of reduction or inhibition of cellular division.

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