INFLUENCE OF SUMIDAN ON MITOTIC DIVISION IN *TRIGONELLA FOENUM GRAECUM* L. SPECIES

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Abstract: This paper includes the cytogenetic effects induced by sumidan insectofungicide in meristematic cells of *Trigonella foenum graecum* L. root tips. The increase of pesticide concentration determined the decrease of mitotic index, while the frequency and the type of chromosome aberrations are much greater in treated variants, comparatively with control.

INTRODUCTION

In agriculture, the treatments with pesticides sometimes represents a very aggressive intervention of man in biosphere equilibrium, therefore is necessary a thoroughgoing knowledge of the substances applied to prevent or to diminish the crop destruction. Insectofungicides, group including sumidan, control both the insects and the fungi that are pathogens for plants. Used in non adequate doses, these substances also present a certain degree of toxicity on crops. To take in consideration the importance of fenugreek as medicinal plant and the possible negative effects of pesticide use, we proposed to evidence the modifications induced by sumidan at the level of mitotic cell cycle.

MATERIALS AND METHODS

As biologic material, seeds of *Trigonella foenum graecum* L. (2003 harvest, I.C.C.P.T. Fundulea) were used. The germination was assured in Petri dishes, on moistened filter paper, at 22 ± 2^0C. The treatment was performed at a 10-15 root length, as follows:

- Control: seeds with embryonary roots for 3 hours were maintained in distilled water;
- Variants: the tested solutions (0.0001%; 0.001%; 0.01% sumidan) were prepared in distilled water. Each variant had 25 seeds.

To remove the pesticide, the roots were kept in distilled water, for 2 hours, at room temperature. As fixative, the mixture absolute ethyl alcohol:glacial acetic acid, 3:1, was used, for 20 hours. The roots are kept in 70% ethyl alcohol, before making preparations. The microscopic preparations were realized by squash method (Cîmpeanu et al., 2002). For this, the roots are subjected to hydrolysis in 50% HCl (v/v), for 8 minutes. The Carr solution (10%) was used as staining reactive. Five preparations were analysed for each variant. The photos were effectuated at Nikon Eclipse 600 microscope, 100x immersion objective, and Nikon Eclipse 600 digital camera.

Sumidan is an insectofungicide with systemic action produced by OLTCHIM S.A. Romania, OLTQUINO S.A. Romania, and SUMITOMO Japan. The compound is a pink, fine, homogenous suspension, containing 10 g/l diniconazole, and 500 g lindane. It is effective against the phytopathogenic fungi (*Tilletia* spp., *Fusarium* spp., *Ustilago nuda*, *Pyrenophora* spp.) and against some species of insects (*Zabrus tenebrioides*, *Agriotes* spp.)

RESULTS AND DISCUSSIONS

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

a) Mitotic index

The increasing concentrations of sumidan determined a reduction of dividing cell frequency, in root apex of fenugreek. The smallest mitotic index (3.50%) was registered at maximum tested concentration. In this case, the value of mitotic index was approximately 4.5 times smaller than that of control (15.69%) (Fig. 1).

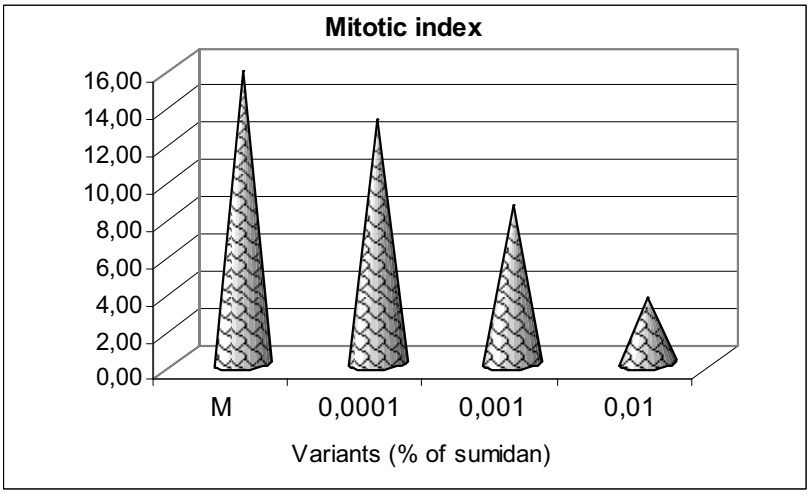

Figure 1. Mitotic index in fenugreek, after the treatment with sumidan

b) Frequency of mitotic phases

The study of the frequency of cells in different phases of mitosis evidences a predominance of metaphases, followed by prophases. Anaphases represent approximately half from whole of prophases and metaphases, while the telophases have the smallest incidence (Fig. 2). In sumidan treated variants, the higher frequency is for prophases, followed by metaphases, anaphases and telophases.

The decrease of mitotic index, after sumidan treatment, was realized by the decrease of all four division phases, therefore the pesticide affects the good development of whole mitotic process.

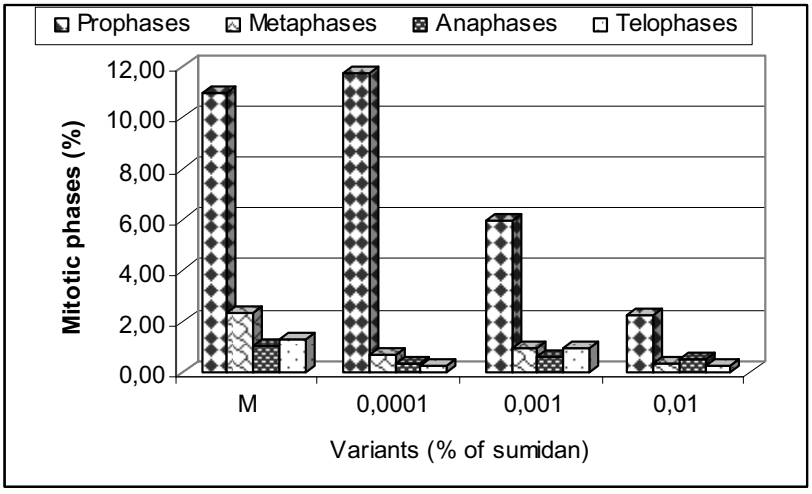

Figure 2. Phases of mitotic division in fenugreek, after the treatment with sumidan

c) Frequency and type of chromosome aberrations

As shown in Figure 3, in control, the frequency of aberrant ana-telophases is much reduced but in treated variants their incidence is significant increased. A direct relationship appears between the frequency of aberrant cells and pesticide concentration increase. If the control has 0.9% aberrant cells, in 0.001% sumidan variant 6.0% aberrant cells exists, this value representing a 6 times increase. At maximum pesticide concentration, the frequency of aberrant cells is more reduced than in previous case, fact that permit us to suppose the existence of repair processes. A similar reactivity to pesticides was cited for some graminaceous (Căpraru et al., 2004) and vegetable cultivars (Grama et al, 2004).

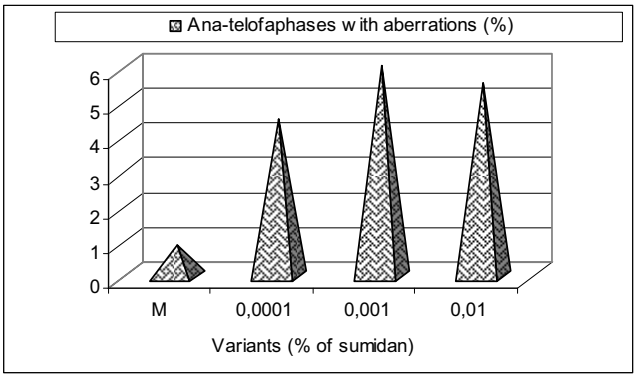

Figure 3. Frequency of aberrant ana-telophases in fenugreek, after the treatment with sumidan

The spectrum of chromosome aberrations identified in mitotic ana-telophases was enough large: ana-telophases with simple, double, triple, even multiple bridges; lagging chromosomes; fragments, as well as a reduced number of complex aberrations (ana-telophases with bridges and fragments, ana-telophases with bridges and lagging chromosomes). Micronucleuses and binucleate cells were observed in interphases (Fig. 4 and 5). The most frequent aberrations were ana-telophases with bridges. Sumidan affects the normal function of mitotic spindle, so that the chromosome migration to the poles is disturbed. The identification of anaphase fragments and interphase micronucleuses evidences the clastogen effect of this insectofungicide.

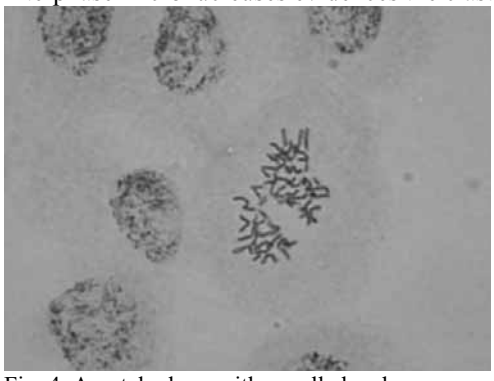

Fig. 4. Ana-telophase with expelled and lagging chromosomes

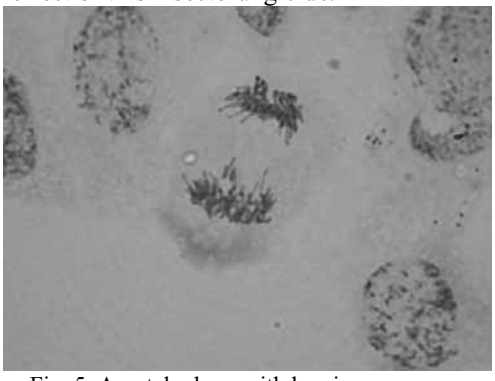

Fig. 5. Ana-telophase with lagging cromosomes

CONCLUSIONS

The sumidan increasing concentrations induce a significant reduction of frequency of dividing meristematic cells in fenugreek root tips.

The incidence of aberrant cells increases proportional to increase of pesticide concentration.

The main aberrations types were ana-telophases with bridges, lagging chromosomes, and fragments, binucleate cells, and interphases with micronucleuses.

Sumidan has both mitotic and clastogenic effect.

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