ESTIMATION OF ROUNDUP ACTION ON GENETIC MATERIAL OF *TRIGONELLA FOENUM GRAECUM* L.

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Abstract: In the present study, the cytogenetic effects of the herbicide roundup on division of root meristematic cells of *Trigonella foenum graecum* and influence of this treatment on height plants in early ontogenetic phase were studied. The seeds of plants were treated with various concentrations of roundup (0.1%, 0.5%, 1.0 %, and 2.0 %), for 3 and 6 h. Roundup had an inhibitory effect on plantlets height in early ontogenetic phases at all tested concentrations in 6 h treatment, comparatively with control, and at maximum tested concentration applied for 3 h. The mitotic index was significantly reduced by roundup 6h, for all tested concentrations. Roundup 3h, 0.1% and 1.0% variants, and roundup 6h, 0.5% and 2.0% variants induced the most numerous ana-telophase chromosomal aberrations. The presence of micronuclei and fragments suggests the potential clastogen effect of roundup.

INTRODUCTION

The uncontrolled presence of chemicals in ecosystems, the diversification of synthetic drugs, the use of pesticides in agriculture, the increased number of food preservatives and additives, the use of synthetic dyes are factors increasing the mutation incidence. Many different types of pesticides have been introduced in order to increase the crop production fact that increases the expositions of population to them due to the residues persistence in the soil and in the animal and plant tissues. Several methods are available to determine the genotoxic effects of chemical, one of these being the establishment of incidence and types of mitotic chromosomal aberrations. Till now, the studies on mutagenic potential of pesticides showed the ability of some of these to induce chromosome damage, sister chromatid exchange or point mutations. Generally, their genotoxic properties have been evidenced in plant systems.

Trigonella foenum-graecum L. (*Fabaceae*) is a species of pharmaceutical, industrial and culinary importance that can constitute, as other plant species, monitoring systems in the evaluation of possible genetic risk of the pesticide use. Trigonelline alkaloid has shown potential for use in cancer therapy (*http://www.pfaf.org/database/plants.php?Trigonella+foenum-graecum*), the fenugreek seeds and leaves have anticholesterol, antiinflamatory, antitumour, carminative, emollient, laxative, uterotonic effects, while the plant extracts are heart tonic, diuretic, antiphlogistic, hypotensive. The species was also investigated from cytogenetically point of view but sometimes – as it happens in the case of satellite presence – the data are contradictory (Căpraru et al., 2006). Some of authors established 2n=16 chromosomes, included in three morphotypes – with median centromere, submedian centromere, respectively subtelocentric (this later type identified only in early metaphases). Roundup, a systemic postemergent herbicide, assures an excellent control of a great number of mono- and dicots, some of these difficult to prevent. Its action is exerted by inhibition of aromatic aminoacids by the plant. At small doses, it can act as growth regulator. These are reasons for which we analysed the potential negative effect of roundup pesticide on fenugreek, by studying the modifications induced in mitotic cell cycle development and on genetic material structure.

MATERIAL AND METHOD

Roundup ($C_3H_8NO_5P$) is an organophosphonate herbicide having the relative molecular mass of 169.01 and the following structural formula:

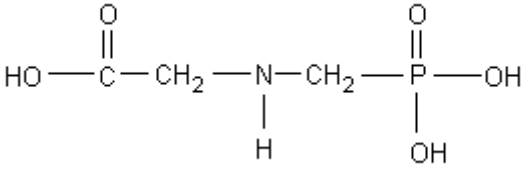

Figure 1. Structure of roundup

Roundup has as active ingredient glyphosate, 360 g/l; it is a postemergent, systemic herbicide with no soil residual activity. Glyphosate is an aminophosphonic analogue of the natural amino acid glycine; it inhibits the plant and microorganism enzyme that is essential to formation of specific amino acids. It was first discovered to have herbicidal

activity in 1970 by John Franz, a scientist who worked for the Monsanto company. Roundup is classed as a "moderately toxic" herbicide in Environmental Protection Agency toxicity class II (*www.inchem.org*) or, in other opinions, III-IV.

Seeds of fenugreek were treated with 0.1%, 0.5%, 1.0 %, and 2.0 % roundup solutions, for 3 and 6 hours. The preparations were analysed at Nikon Eclipse 600 microscope and photographed at 100x objective, with a Cool Pix Nikon digital camera. Each variant consisted in 50 seeds. Five preparations/variant and 10 microscopic fields/slide were scored, in view of calculus of mitotic index and chromosomal aberrations.

RESULTS AND DISCUSSIONS

The effect of roundup on plant height growth (Table 1, Figure 2) in early ontogenetic phases was different, depending on treatment duration and herbicide concentration. For 3h treatment, the pesticide had stimulant effect at the first three tested concentrations, while at the maximum concentration an inhibition of growth was registered. A 6h treatment had an inhibitory action for all concentrations, especially for 2% roundup, where the average value of length growth was more than 2 times smaller comparatively to control.

Table 1. Average value of height of plantlets 7 days old, depending on roundup concentration and treatment duration

Variant	roundup - 3h		roundup - 6h	
	x±Sx	SD	x±Sx	SD
Control	36.86±2.5	13.69	50.61±2.42	22.5
0.1%	41.87±2.73	15.41	40.88±2.07	14.71
0.5%	47.9±2.5	13.69	44.12±1.86	13.0
1.0%	39.38±2.51	15.64	31.61±1.89	13.3
2.0%	29.60±2.49	15.35	23.77±1.58	6.26

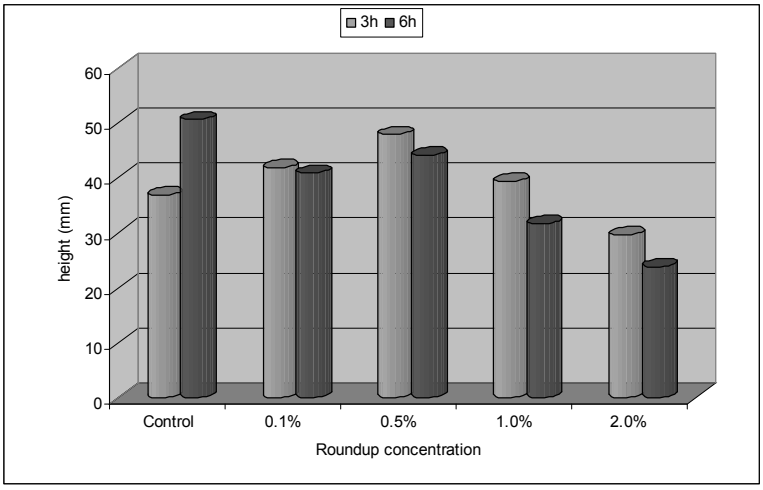

Figure 2. Influence of roundup on fenugreek height plantlets

The dynamics of mitotic index (Table 2, Figure 3) was different, depending on concentration and duration of pesticide treatment. For 1.0 and 2.0% roundup - 3h, an unsignificant stimulation of cell division appeared, by comparing with control, while at 0.5% - 3h a decrease of mitotic index was noted (MI=7.58%, for 0.5% variant, MI=9.46%, for respective control). When the pesticide was applied for 6h, the roundup effects were more pronounced for all tested

concentrations, especially for 0.5% roundup (the number of dividing cell is 2.2 times smaller than control) and for 2,0% roundup (MI is 2.5 times smaller than control).

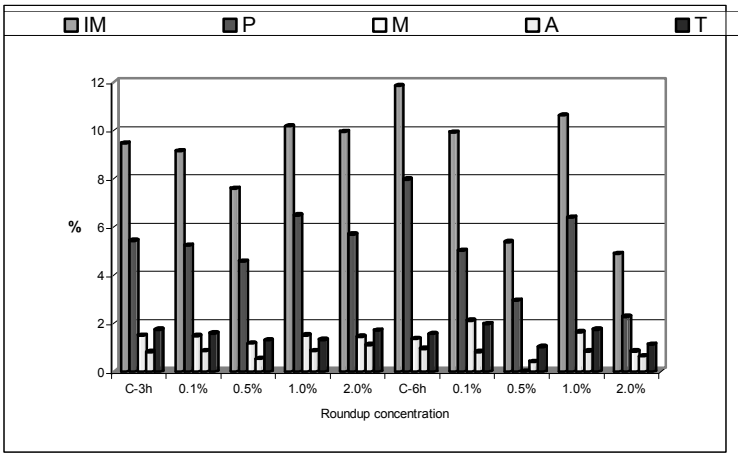

Figure 3. Evolution of mitotic index and cell division phases in fenugreek, depending on roundup concentration and treatment duration

The effect of Roundup on frequency and type of A-T chromosomal aberrations. The aberrant ana-telophases (Table 2) are more numerous than in control (21.45%) in 0.1% variant (27.44%) and especially in 1.0% variant (32.32%), in 3h treatments. For 6h treatment, Roundup induced a greater number of abnormal ana-telophases in all tested variants (for example, at 0.5% concentration, their number was 3.2 times higher than in control). The bridges, multipolar ana-telophases, the expulsed chromosomes and lagging chromosomes were the most frequent aberrations identified in ana-telophases (Table 2, Figure 4 - 7). A high number of lagging chromosomes have been evidenced in 0.1% - 3h and 0.5% - 6h variants. These variants also displayed the highest number of ana-telophases with expulsed chromosomes.

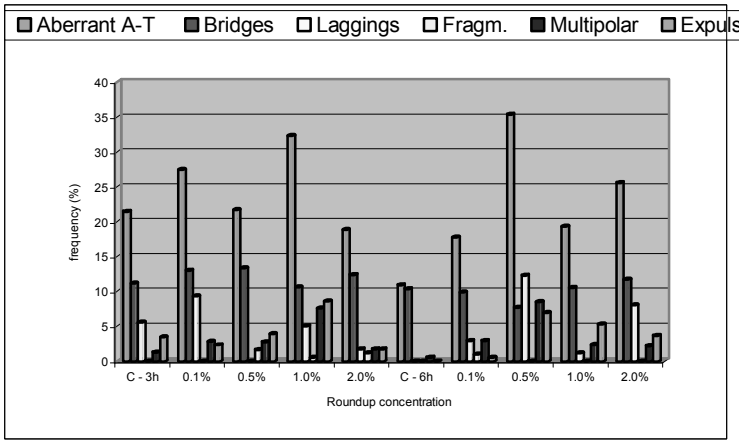

Figure 4. Frecvency and types of ana-telophase aberrations in fenugreek root meristems in the case of roundup treatments

Table 2. Effects induced by roundup on cell division, number and types of chromosome aberrations in *Trigonella foenum graecum* L.

Variant	Total analysed cells	MI (%)	Mitosis phases (%)				Aberrant A-T		Types of A-T aberrations										Types of metaphase anomalies			
									bridges		lagging chromosomes		fragments		multipolar		A-T with expulsed chromosomes		M with expulsed chromosomes		C-metaphases	
			P	M	A	T	Nr.	%	Nr.	%	Nr.	%	Nr.	%	Nr.	%	Nr.	%	Nr.	%	Nr.	%
Control - 3h	9140	9.46	5.42	1.49	0.81	1.74	50	21.45	26	11.15	13	5.57	0	0.00	3	1.28	8	3.43	3	0.03	1	0.01
0.1%	8825	9.14	5.22	1.48	0.85	1.58	59	27.44	28	13.02	20	9.30	0	0.00	6	2.79	5	2.32	7	0.08	5	0.05
0.5%	9788	7.58	4.56	1.17	0.53	1.30	39	21.66	24	13.33	0	0.00	3	1.66	5	2.77	7	3.88	6	0.06	8	0.08
1.0%	9070	10.16	6.48	1.50	0.86	1.32	64	32.32	21	10.60	10	5.05	1	0.50	15	7.57	17	8.58	12	0.13	14	0.15
2.0%	6067	9.93	5.68	1.45	1.10	1.70	32	18.82	21	12.35	3	1.76	2	1.17	3	1.76	3	1.76	0	0.00	6	0.09
Control - 6h	6905	11.83	7.96	1.35	0.95	1.56	19	10.92	18	10.34	0	0.00	0	0.00	1	0.57	0	0.00	0	0.00	0	0.00
0.1%	7293	9.91	5.01	2.11	0.81	1.97	36	17.73	20	9.85	6	2.95	2	0.98	6	2.95	1	0.49	5	0.07	8	0.11
0.5%	8970	5.37	2.95	0.96	0.41	1.03	46	35.38	10	7.69	16	12.30	0	0.00	11	8.46	9	6.92	1	0.01	5	0.05
1.0%	6604	10.61	6.39	1.63	0.84	1.74	33	19.29	18	10.52	2	1.17	0	0.00	4	2.33	9	5.26	4	0.06	14	0.21
2.0%	7776	4.88	2.27	0.84	0.64	1.12	35	25.55	16	11.68	11	8.03	0	0.00	3	2.19	5	3.65	4	0.05	2	0.02

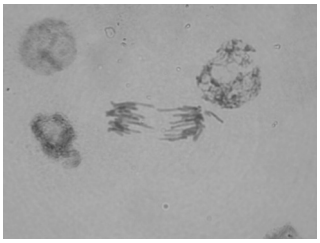

Figure 5. Tripolar anaphase, with lagging and expulsed chromosomes - roundup 2%, 3h

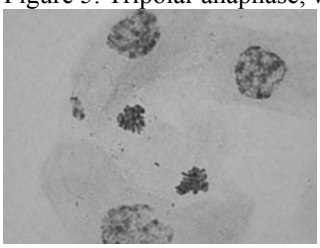

Figure 6. Telophase with fragment - roundup 0.1% - 6h

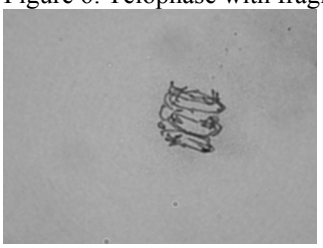

Figure 7. Multipolar anaphase with multiple bridges - roundup 0.1% - 6h

The metaphase abnormalities. It must be noted some metaphase abnormalities (Table 2, Fig. 8 – 10), such as metaphases with expulsed chromosomes or C-metaphases. The presence of C-metaphases confirms the data on colchicine – like effect of some groups of these pesticides. C-mitoses are the result of inactivation of division spindle, followed by chromosome scattering in cell. Delayed centromere division can induce colchicine-like chromosome configurations (Grant, 1978).

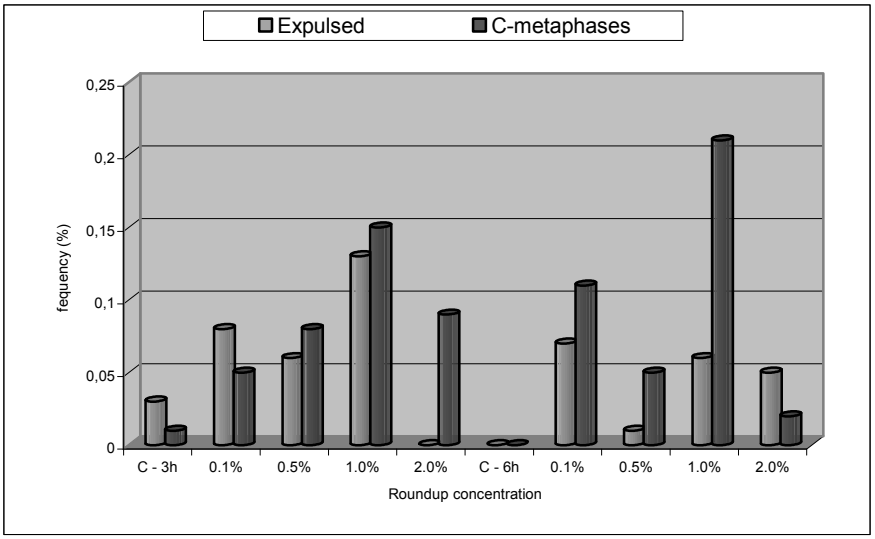

Figure 8. Frequency and types of metaphase abnormalities appeared in fenugreek root meristems under the roundup influence

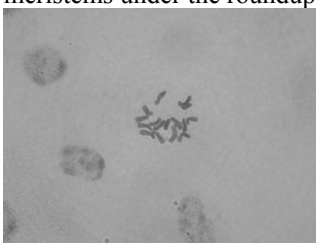

Figure 9. C-metaphase - roundup 2%, 3h

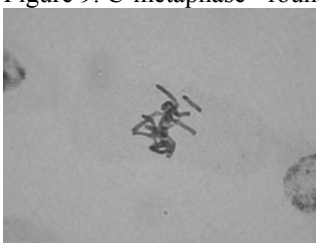

Figure 10. Metaphase with expulsed chromosome - roundup 0.1% - 6h

As the results show, like other pesticides, roundup influences the normal function of mitotic spindle, so that the chromosome movement to the cell poles is disturbed (Truță et al., 2007). Identification of micronuclei or fragments presence, although not in high number, evidences the clastogen potential of this pesticide.

CONCLUSIONS

Concerning the height growth in early ontogenetic phases, the 3h roundup treatment had – excepting the maximum tested concentration – stimulant effect, while the 6h roundup treatment inhibited this parameter at all tested concentrations.

Mitotic index was not significantly modified at 3h, but the 6h treatment induced MI decrease at all concentrations.

The most numerous ana-telophase aberrations were registered at 1.0% and 0.1% roundup 3h, respectively 0.5% and 2.0% roundup 6h.

Numerically predominant were the bridges, multipolar ana-telophases and ana-telophases with expulsed chromosomes and lagging chromosomes.

Roundup also induced C-metaphases (especially at 0.1% - 3h and 1.0% - 6h).

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