PHYTO-BIOLOGICAL TESTING OF SOME FLAVONOID COMPOUNDS OF VEGETAL ORIGIN
Note 2. PHYTO-BIOLOGICAL TESTING OF VEGETAL EXTRACTS FROM *MEDICAGO HERBA*, *GLYCINE SEMEN* AND *TRIFOLII RUBRI FLOS*

RUXANDRA CRETU [*1], DOINA DANILA[2], ELVIRA GILLE[2], FLORIN FLORIA[2], ROXANA MIHAILESCU[1]

Key words: vegetal extracts, cytogenetic effects

Abstract. Vegetal extracts from *Medicago herba*., *Glycine semen* and *Trifolii rubri flos* were tested in order to evaluate the possible phytotoxic and cytogenetic effects. The tests were done on *Triticum aestivum* L. (*Dropia* cultivar). We have analyzed the following parameters: the germination percent of the seeds, the length of root and stem, fresh and dry weight of root and stem, ana- telophasis frequency from root meristem with chromosomal aberrations.

INTRODUCTION

The recent research area regards the reactive oxygen species (ROS) also named free radicals, responsible for a large range of degenerative processes that can lead to human disease progression. Excessive generation of ROS in biological systems include membrane lipids peroxidation, nucleic acids and carbohydrates oxidative damage. All these lead to important long- term dysfunctions and ageing. (Ohshima *et al., 1998*). There is a correlation between ROS level in human organism and health, which is why it is believed that most of human diseases are ROS determined. To cancer also belongs to this category. (Jovanovic *et al.,* 1997).

The enzymatic system involved in ROS inactivation, capable of preventing the oxidative damage, it is composed of three components: superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPO) (Van Acker *et al.,* 1997).

These oxidative processes can be controlled or reduced with exogenous antioxidants.

Recently, it has been identified a group of so-called “phytochemicals” active principles with free radical-scavenging activity. The most important are polyphenols and flavonoids which act against the peroxidative effect of ROS. Both groups protect the unsaturated fatty acids from peroxidative degradation or initiated by oxygen singlet and also inactivate the oxidative enzymatic systems (lipoxygenases, xanthine oxidases and mono oxidases) which lead to oxidative stress (Kähkönen *et al.,* 1999)

The extracts from *Medicago herba*, *Glycine semen* and *Trifolii rubri flos* are important sources of flavonoids compounds who can act as antioxidants.

MATERIALS AND METHODS

We used seeds of *Triticum aestivum* L. (*Dropia* cultivar) in our experiments. We have diluted 5 ml from each initial extract to 100 ml with distilled water. The final solutions were used for the seeds immersion for 24 hours.

Previously, we have analyzed the initial extracts in order to evaluate their polyphenolic and flavonoid content. We used the phytochemical methods according to Romanian Pharmacopeea, X edition.

The following parameters were analyzed: the frequency of ana-telophases in root meristems with chromosomal aberrations; caryopsis germination percent; root and stem length; the fresh and dried weight of roots and stems, respectively.

RESULTS AND DISCUSSIONS

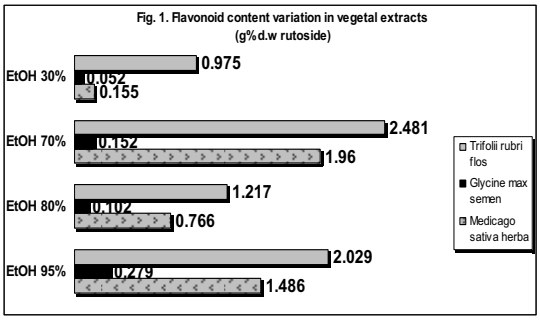
We have analyzed the following experimental variants treated with: hydro-alcoholic extracts of *Medicago herba*: 1. EtOH 95% (24 h); 2. EtOH 80% (24 h); 3. EtOH 70% (24 h); 4. EtOH 30% (24 h); hydro-alcoholic extracts of *Glycine semen*: 5. EtOH 95% (24 h); 6. EtOH 80% (24 h); 7. EtOH 70% (24 h); 8. EtOH 30% (24 h); hydro-alcoholic extracts of *Trifolii rubri flos*: 9. EtOH 95% (24 h); 10. EtOH 80% (24 h); 11. EtOH 70% (24 h); 12. EtOH 30% (24 h); 13. Control (24 h in distilled water).

The quantitative analysis has revealed an important variability of active principles content in hydro-alcoholic extracts of different concentration. The results are presented in fig. 1 and 2.

We have noticed that the ethanolic *Trifolii rubri flos* have the higher content in rutoside compared to the extracts obtained from the other vegetal species. The higher rutoside values of 2,481 g% d.w. and 2,029 g% d.w are in 70% and 95% ethanolic extracts (fig. 1).

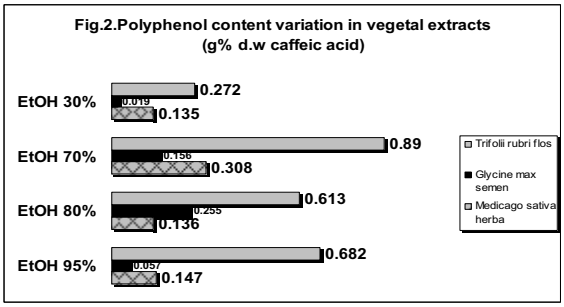
Ethanolic extracts from *Medicago herba* also have important quantities of rutoside of 1,960% and 1,486% in 70% and 95% extracts, but lower than those obtained form red clover (fig. 1).

The lower values of flavonoids belong to 30% extracts from alfalfa and soybean (0,155% and 0,052 g% rutoside, respectivelly). The 30% extract of red clover contains 0,975 g% rutoside and it is the higher value for these kind of extracts in the intire experiment (fig. 1).



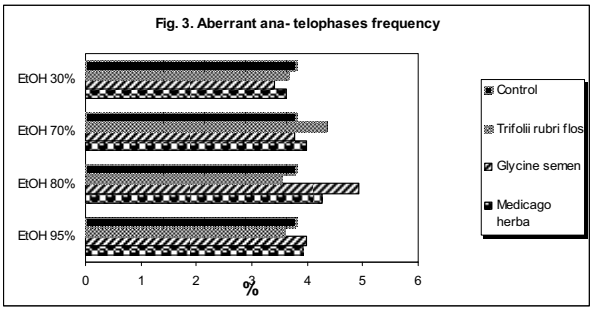
Polyphenols quantity is also variable according to extract alcoholic concentration and its vegetal origin. In this case, extracts from red clover have also high values of polyphenols determined as caffeic acid ; the higher value of 0,980 g% belongs to 30% variant, followed by 95% variant with 0,682 g% caffeic acid (fig. 2).

In Medicago ethanolic extracts the polyphenolic content is lower with 2,5-3 times compared to red clover, the maximum value being 0,308 g% in 70% extract and the minimum of 0,135 g% in 30% extract. In soybean ethanolic extracts the polyphenolic content is lowest, from 0,019 g% (var. 8) to 0,255 g% s.u. (var. 6) (fig. 2).



1. The frequency of ana-telophases in root meristems with chromosomal aberrations

The extracts used in our experiments do not induce cytogenetic modifications on wheat seeds. The ana-telophases frequency with chromosomal aberrations do not semnificantly differ if we compare the experimental variants with the control: 3,41% aberrant ana- telophases in var. 8 (30%) to 4,93% in var. 6 (80%) compared to 3,79% for control (fig. 3).

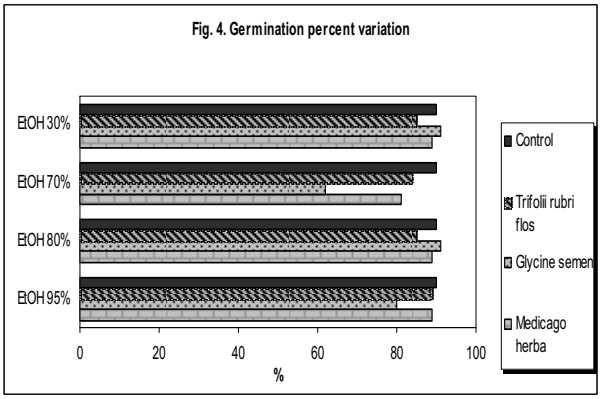


Some increases or reductions (about 1%) of root meristem cells with chromosomal aberrations have no significant importance.

2. Caryopsis germination percent

The biological extracts obtained from *Medicago herba*, *Glycine semen* and *Trifolii rubri flos* did not influenced in a significant way the seeds germination, with some exceptions. Thus, compared to control where the germination percent is 90%, for the experimental variants (1, 2, 4 and 9) the germination is 89% (a non-significant reduction with 1%). Important reductions of 6% (var. 10 and 12), 7% (var. 11), 10% (var. 3) and 31% (var. 5) respectively, does reflect a cytotoxic effect (fig. 4).

Seeds germination was slightly stimulated with 1% in case of vegetal extracts of soybean (var. 6-80% and var. 8-30%).



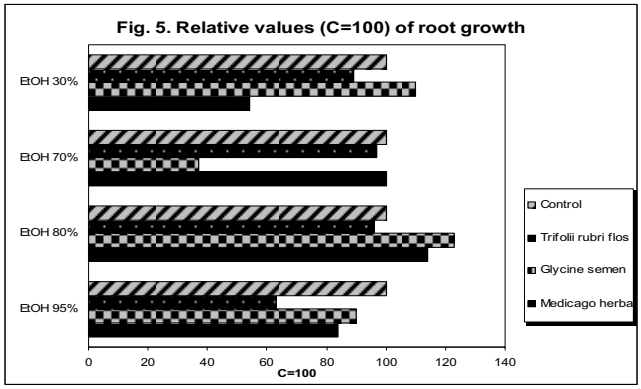
3. Root and stem length

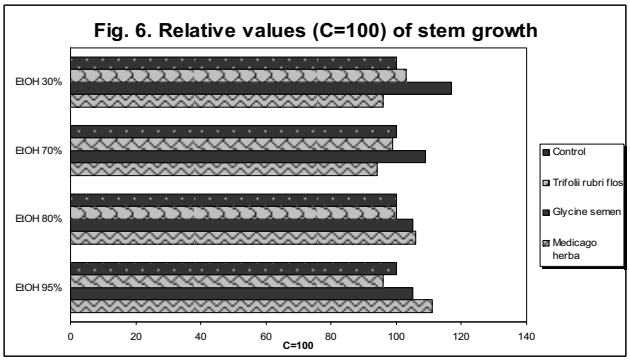
In case of this parameter we asist to a great heterogenity of values obtained from the root and stem lenght measurements. Root length is not clearly correlated with those of stem. It is very clear the fact that we asist to a specific respond of roots and stems.

We have observed for roots and stems respectively, some reductive and stimulative effects compared to control, in some experomental variants: 1-4, 5-8 and 9-12 (fig. 5 and 6).

We have noticed negative effects on root growth from 3% (var. 11-70%) to 63% (var. 7) in 8 of the 12 experimental variants 9 (fig. 5).

Stem length was stimulated by the applied treatments in 8 of the 12 experimental variants. It is worthy to note that the inhibition of stem growth for the other 4 variants is only about 1-6%, compared to roots (3-63%) (fig. 6).



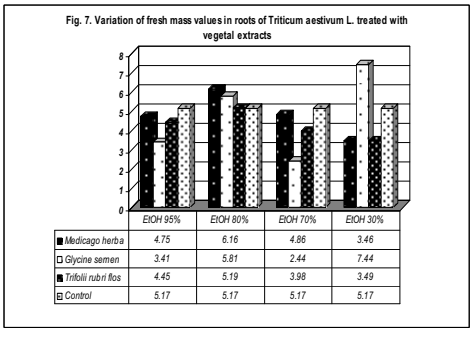


4. The fresh and dried weight of roots and stems

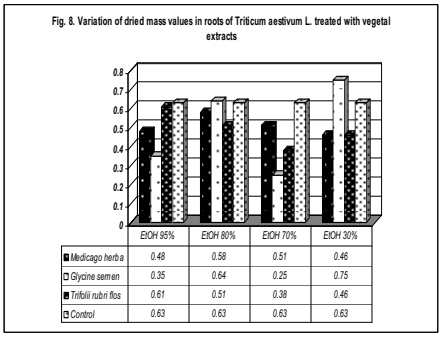
In case of this parameter we wanted to demonstrate that the same number of analyzed specimens can accumulate different quantities of fresh mass and dried mass per variant, depending on the studied vegetative organ (root or stem) and on the applied treatment.

Fresh mass is between 3,46 g and 5,19 g in roots (fig. 7) and between 7,04 g and 8,49 g in stems, respectivelly (fig. 8); dried mass values are between 0,46 g and 0,51 g in roots (fig. 9) and between 1,06 g and 1,19 g in stems respectivelly (fig. 10).

In roots, the fresh /dried mass ratio oscilated between 0.0941 (var. 2-80%) (maximum reduction of 23% compared to control) and 0.1371 (var. 9-95%) where an increase of 12% compared to control does reflect a plus regarding the cell synthesis (histo- anatomical structures, substances). We can also note 2 variants with positive values (8-9%) (fig. 11). In conclusion for roots, only in 3 from these 12 variants (about 1/3) the processes of cell synthesis are stimulated by the increase of fresh mass.

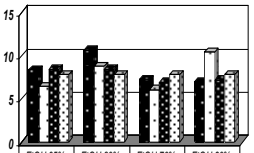


	EtOH 95%	EtOH 80%	EtOH 70%	EtOH 30%
Medicago herba	4.75	6.16	4.86	3.46
Glycine semen	3.41	5.81	2.44	7.44
Trifolii rubri flos	4.45	5.19	3.98	3.49
Control	5.17	5.17	5.17	5.17



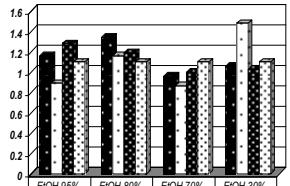
	EtOH 95%	EtOH 80%	EtOH 70%	EtOH 30%
Medicago herba	0.48	0.58	0.51	0.46
Glycine semen	0.35	0.64	0.25	0.75
Trifolii rubri flos	0.61	0.51	0.38	0.46
Control	0.63	0.63	0.63	0.63

Fig. 9. Variation of fresh mass values in stems of Triticum aestivum L. treated with vegetal extracts



	EtOH 95%	EtOH 80%	EtOH 70%	EtOH 30%
Medicago herba	8.38	10.67	7.33	7.04
Glycine semen	6.45	8.78	6.12	10.44
Trifolii rubri flos	8.44	8.49	6.94	7.21
Control	7.86	7.86	7.86	7.86

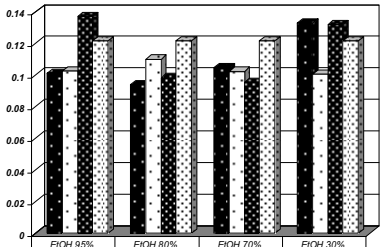
Fig. 10. Variation of dried mass values in stems of Triticum aestivum L. treated with vegetal extracts



	EtOH 95%	EtOH 80%	EtOH 70%	EtOH 30%
Medicago herba	1.16	1.34	0.96	1.06
Glycine semen	0.89	1.16	0.87	1.48
Trifolii rubri flos	1.28	1.19	1	1.03
Control	1.1	1.1	1.1	1.1

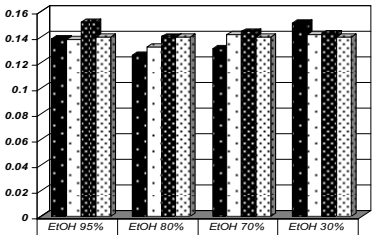
In stems, the ratio fresh /dried mass is superior to control in ½ from experimental variants (0.1418-0.1516, compared to 0.1399 in control) (fig. 12).

Fig. 11. Variation of fresh/ dried mass in wheat roots



	EtOH 95%	EtOH 80%	EtOH 70%	EtOH 30%
Medicago herba	0.101	0.0941	0.1049	0.1329
Glycine semen	0.1026	0.1101	0.1024	0.1008
Trifolii rubri flos	0.1371	0.0983	0.0955	0.1318
Control	0.1218	0.1218	0.1218	0.1218

Fig. 12 Variation of fresh/dried mass ratio in wheat stems



	EtOH 95%	EtOH 80%	EtOH 70%	EtOH 30%
Medicago herba	0.1384	0.1256	0.1309	0.1506
Glycine semen	0.138	0.1321	0.1421	0.1418
Trifolii rubri flos	0.1516	0.1402	0.1441	0.1428
Control	0.1399	0.1399	0.1399	0.1399

CONCLUSIONS

The investigations on *Triticum aestivum* seeds and plants can be included in the research area regarding the screening and use of some vegetal products as therapeutics and supplements

(extracts, specific chemical compounds). The issue can become very important in the large area of "mutagenesis and carcinogenesis" and of „functional foods", respectively, concepts that are reconsidered and accepted by the scientific community.

In case of these 12 experimental variants treated with vegetal extracts from *Medicago herba*, *Glycine semen* and *Trifolii rubri flos* we have not found any ideal situation, such as positive effects and with superior values for control plants..

That is why it is vey difficult to pressume, even in case of some positive effects, that one of this three species is indicated for its certain favorable effects on growth and development of wheat plants.

We can surely conclud that the biological extracts used in our experiments have no genetic effects. They did not increase the frequency of ana-telophases aberrations.

REFERENCES

Jovanovic, V.S., Steenken, S., Simic, G.M., Hara, J., 1997. *Antioxidant properties of flavonoids: reduction potentials and electron transfere reactions of flavonoid radicals.* Flavonoids in health and disease. Editors: Packer, L., Rice-Evans, C. Marcel Dekker, New-York, 137-157.

Kähkönen, Marja, Hopia, A., I., Vuorela, H., J., Jussi- Pekka, R, Pihlaja, K., Kujala, T., S., Heinonen, Marina. 1999. J. Agric. Food Chem, 47: 3954- 3962.

Ohshima, H., Joshie, Y., Auriol, S., Gilbert, Isabelle, 1998. Free Radical Biology & Medicine, vol. 25, No 9, 1057-1065.

Van Acker, A.B.E., Bast, A., Van Der Vijgh, J.F.W, 1997. *Structural aspects of antioxidant activity of flavonoids.* Flavonoids in heatth and disease. Editors: Packer, L., Rice-Evans, C., Marcel Dekker, New-York, 221-248.

Acknowledgments. We thank professor Ion I. Bara from the University of Iasi. We also thank Elvira Gille and Roxana Mihailescu for helpful discussions and the members of S.C "PLANTAVOREL S.A. and "STEJARUL" Research Center.

[1]"PLANTAVOREL" Research Center for Medicinal Plants Processing, Piatra- Neamt
* petrut_ruxandra@yahoo.com
[2]"STEJARU" Research Center Piatra- Neamt