PHYTO-BIOLOGICAL TESTING OF SOME FLAVONOID COMPOUNDS OF VEGETAL ORIGIN
Note 3. PHYTO- BIOLOGICAL TESTING OF SOME FLAVONOID COMPOUNDS- BASED PRODUCTS

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Key words: flavonoids compounds, cytogenetic effects

Abstract. Some flavonoid compounds- based products were tested in order to evaluate the possible phytotoxic and cytogenetic effects. The tests were done on *Triticum aestivum* L. (*Dropia* cultivar). We have analized the following parameters: the germination percent, root and stem growth, fresh and dry weight of root and stem and fresh/dried mass ratio respectively, ana- telophasis frequency from root meristem with chromosomal aberrations. These products includ vegetal extracts of *Medicago herba*, *Glycine semen* and *Trifolii rubri flos* and other vegetal powders.

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)

Important sources of flavonoids compounds, antioxidants respectively, are the extracts from *Medicago herba*, *Glycine semen* and *Trifolii rubri flos*. The dried extracts were tableted in combination with other vegetal powders and are indicated as adjuvants in different disease generated by the oxidative processes induced by free radicals.

For the phyto- biological testing of the obtained tablets we realized some experimental variants depending on their administration recommended by the producer.

MATERIALS AND METHODS

In our experiments, we used seeds of *Triticum aestivum* L. (*Dropia* cultivar). For the phyto-biological testing of flavonoids compounds- based products we have dissolved a number of 1, 2, 4 and 6 tablets in 100 ml distilled water. The obtained aqueous solutions were applied on wheat seeds.

Previously, we have weight the tablets in each set test 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.

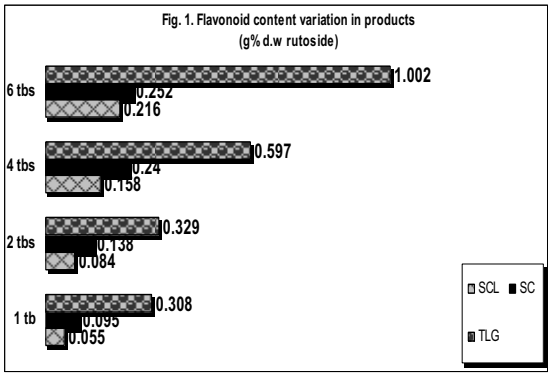
RESULTS AND DISCUSSIONS

We have analyzed the following experimental variants treated with: *SCL*- 1 tb (24 h); 2 tbs (24 h); 4 tbs (24 h); 6 tbs (24 h); *SC*- 1 tb (24 h); 2 tbs (24 h); 4 tbs (24 h); 6 tbs (24 h); *TLG*- 1 tb (24 h); 2 tbs (24 h); 4 tbs (24 h); 6 tbs (24 h); Control (24 h in distilled water).

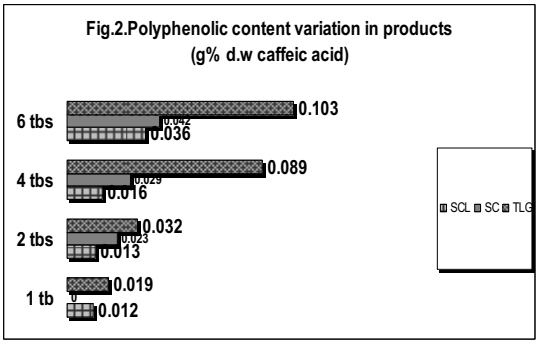
Analyzing the phytochemical results we observ that *SCL* product has the lower flavonoids contens (0,0554 g% in 1 tb and 0,216 g% in 6 tbs) (fig. 1).

It is worthy to note that depending on vegetal components in each set tablets, polyphenolic and flavonoid content is increasing. In case of *SC* tablets, flavonoids are about 1.5 times higher compared to *SCL*.

In case of the experiments with *TLG* tablets, we have noticed a minimum concentration of flavonoid compounds in 1 tb (0,308 g%) and a maximum in 6 tbs (1,002 g%) (fig.1).



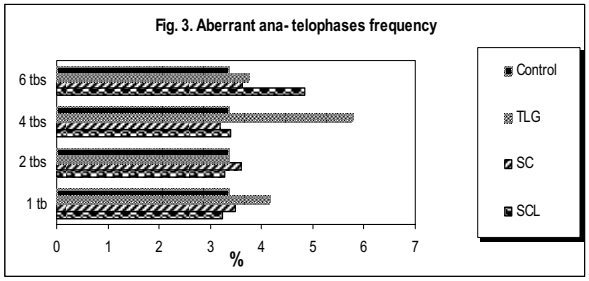
We have to mention that in the analyzed vegetal powders and extracts the polyphenolic content is lower than those of flavonoids. Quantitative values of polyphenols are about 10 times lower in analyzed tablets. The higher values belong to *TLG* tablets (0,019 g%/1 tb and 0,103 g%/6 tbs) (fig. 2).



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

In our experiment on *Triticum aestivum* (*Dropia* cultivar), it has been recorded 3.36% root meristem ana-telophases with chromosomal aberrations in control variant.

In experimental variants, vegetal powders and extracts from each tablet set do not induce any major genotoxic effect (clastogenic). The frequency of ana-telophases with chromosomal aberrations does slightly oscillate compared to control: 3,19% in 4 tbs of *SC* and 5,76% in 4 tbs of *TLG* (fig. 3).



Var.1 (*SCL*, 1 tb), var. 3 (*SCL*, 2 tbs) and var. 10 (2 tbs of *TLG*) are slightly under control value with about 3,24%, 3,29% and 3,34% aberrant cells, respectively. So, in ¼ of our experimental variants the frequency of aberrant ana- telophases is inferior to those of control, but only at minimum and medium concentrations (1-4 tbs) of the analyzed phytotherapeutical products (*SCL*, *SC* and *TLG*). As for ¾ experimental variants, the number of aberrant root cells is slightly increased compared to control: from 3,40% in var.3 (4 tbs, *SCL*) to 5,76% in var.11 (4 tbs of *TLG*) (fig. 3).

The highest values of wheat cells with chromosomal aberrations are beyond control`s at most 2-3% in var.11 (5,76%) and 4 (4,85%) and that is why we do not consider them very significant for any clastogenic potential in case of using these vegetal powders and extracts in some phytotherapeutical products (fig. 3).

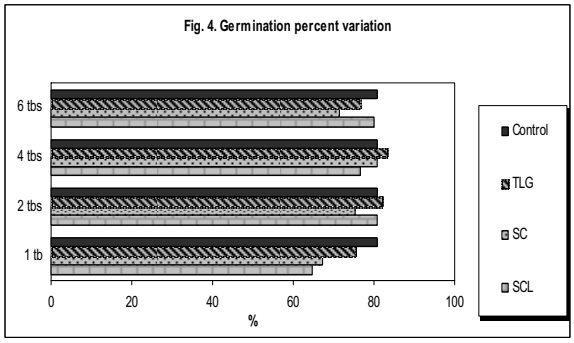
It seems that the analyzed powders and extracts with flavonoid compounds do not numerically or structurally affect the chromosomes, at least on wheat and in this experiment. However, the testing of these natural products on microorganisms, animal and human cell *in vivo* and *in vitro* could provide us more certain information in the context of a specific respond of every living system on the applied treatments.

2. Caryopsis germination percent

In thea treated variants with powders and/ or vegetal extracts the germination percent has oscillated compared to those of control: 64,67% in var.1 (*SCL* – 1 tb) and 83,33% in var.11 (4 tbs of *TLG*) compared to 80,67 % germinated caryopsis in control (fig. 4).

Considering that control`s germination percent is 100%, we observ that in 8 of 12 variants (2/3 of total) the number of germinated seeds is lower, at least 1% in var.4 (6 tbs of *SCL*) to at most 20% in var.1 (1 tb of *SCL*).

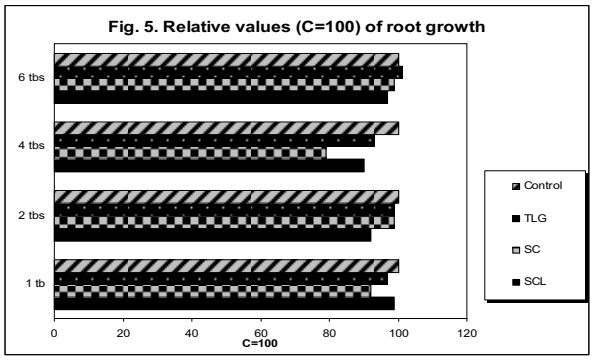
A slightly stimulation of germinative capacity about 2-3% it was recorded only in two variants: var.10 and 11 (2 and 4 tbs respectively, of *TLG*).

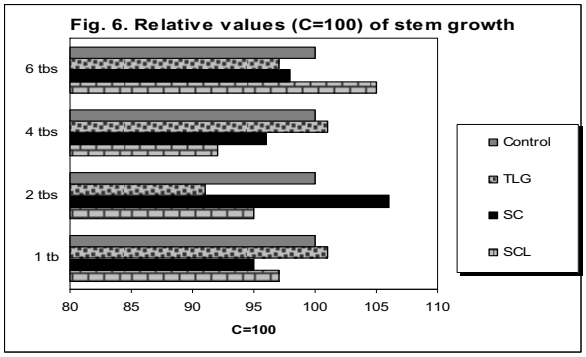


3. Root and stem length

After the treatment with these three products - *SCL* (var. 1 – 4), *SC* (var. 5 – 8) and *TLG* (var. 9 – 12) respectively, we also have observed minimum and maximum values compared to control

No metter what the used product and its dose in the applied treatment, there are some disturbances (cytotoxic effects) of roots length growth. Apart from var. 12, there are inhibiting processes with minimum 21% in var. 7 and only 1% in var. 1 (1 tb. *SCL*), var. 6 (2 tbs. *SC*), var. 8 (6 tbs. *SC*) and var. 10 (2 tbs. *TLG*) (fig. 5).





So, in 7 from the 12 experimental variants (2/3) the applied treatments do not have a significant cytotoxic effect on roots growth (only a reduction of 1 – 3%) (fig. 5).

Concerning the inhibitory effect on stems growth we have to mention that this took place in only 5 from 12 variants (½); the reductions were about 1– 3% compared to control. Significant reductions in stems were noted in var. 10 (2 tbs. *TLG*) and 3 (4 tbs. *SCL*) about 8 – 9% (fig. 6).

In some variants: var. 4 (6 tbs. *SCL*) and var. 6 (2 tbs. *SC*) stems growth was stimulated with about 5 – 6% compared to control. That is why we could consider that the cytotoxic effects of these products are not very significant, stems growth being less sensitive than roots growth (fig. 6).

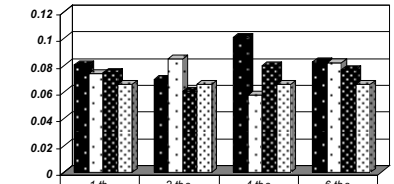
4. The fresh and dried weight of roots and stems

According to fig. 7, 8, 9 and 10 we could observe a variation of individual fresh and dried weight in wheat plants during the ten days of ontogenesis. The fresh /dried weight ratio also give us some detailed information about cell synthesis (grade, intensity), weight accumulation (substances, cell structures) despite the water content of plants treated with natural products in our experiments (fig.11 and 12).

In this manner, the individual fresh weight of roots varies between 0, 0577 g in var.7 (4 tbs of *SC*) and 0, 0852 g in var.6 (2 tbs of *SC*) compared to 0, 0656 g in control. We have observed some reductions in fresh weight accumulation about 7-12% compared to control only in two of these 12 experimental variants (var.7 and 10, especially) (fig. 7). The dried weight/ specimen oscillated between 0, 0109 and 0, 0111 in var. 2 (2 tbs of *SCL*) and var. 5 (1 tb of *SC*). It was marked a slightly reduction about 3-4% and a stimulation about 25% of mass accumulation compared to control (fig. 8).

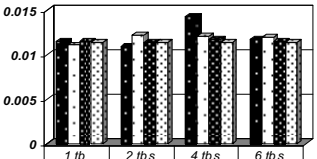
As a whole, in case of roots treated with the analyzed natural products we assist to a stimulation of fresh and dried weight accumulation.

Fig. 7. Variation of individual fresh mass values in wheat roots



	1 tb	2 tbs	4 tbs	6 tbs
SCL	0.0805	0.0692	0.1011	0.0826
SC	0.0742	0.0852	0.0577	0.082
TLG	0.0749	0.0609	0.0795	0.0766
Control	0.0656	0.0656	0.0656	0.0656

Fig. 8. Variation of individual dried mass values in wheat roots

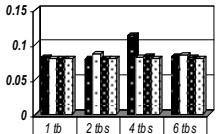


	1 tb	2 tbs	4 tbs	6 tbs
SCL	0.0115	0.0109	0.0143	0.0117
SC	0.0111	0.0122	0.0121	0.012
TLG	0.0115	0.0114	0.0117	0.0115
Control	0.0114	0.0114	0.0114	0.0114

In case of stems, fresh weight/ specimen oscillated between 0, 0793 g in var.2 (2 tbs of *SCL*) and 0, 1140 g in var.3 (4 tbs of the same product) the maximum being of 43 % compared to control (fig. 9).

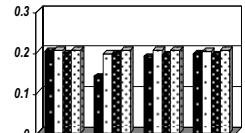
The results of dried weight/ specimen are a little different (table 3). In stems the reduction of mass accumulation took place in 5 variants (2, 9, 10, 11, and 12). The reduction is very clear for the *TLG* product where the experimental variants are lower than control (about 1-4 %). The minimum value of dried weight accumulation is in var.2 (2 tbs of *SCL*), the reduction being about 32 % compared to control (fig. 10).

Fig. 9. Variation of individual fresh mass values in wheat stems



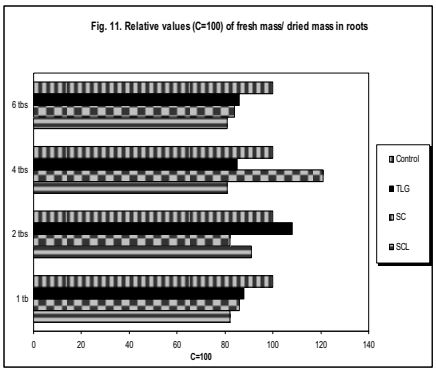
	1 tb	2 tbs	4 tbs	6 tbs
SCL	0.0821	0.0793	0.114	0.0839
SC	0.08	0.0863	0.0821	0.0848
TLG	0.0806	0.08	0.0833	0.0816
Control	0.0796	0.0796	0.0796	0.0796

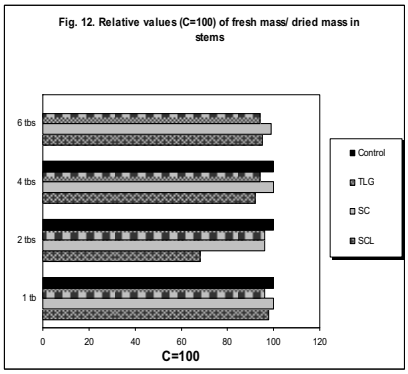
Fig. 10. Variation of dried mass values in wheat stems



	1 tb	2 tbs	4 tbs	6 tbs
SCL	0.1997	0.1387	0.1868	0.1943
SC	0.2037	0.1947	0.2034	0.2005
TLG	0.196	0.195	0.1921	0.1912
Control	0.2035	0.2035	0.2035	0.2035

In roots only in 2 cases from 12 (var.10 and 7, especially) the fresh/ dried weight is bigger than in control and in stems it is not beyond control (fig. 11 and 12).





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 „functional foods" respectively, concepts that are reconsidered and accepted by the scientific community.

About the genotoxicity we can note that the tested products (*SCL*, *SC* and *TLG*) on *Triticum aestivum* L. (wheat) do not have a clastogenic effect (induction of chromosomes and DNA alterrations) with a potential risc on their administration as phytotherapeutical products.

About the second important parameter investigated, the cytotoxicity, we have noted that in our experiments, it took places some inhibitory effects of seeds germination processes and plants growth, independently the combination of the used vegetal species. This was more obvious for roots than stems. High values of fresh mass are due to the water content increase in wheat cells. The dried weight determination proved some disturbances of cell synthesis. Fresh/ dried weight ratio does reflect an inhibitory effect of flavonoid compounds- based products on cell synthesis in wheat an also, on growth and developments processes in this experiment.

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.

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