# ANTIOXIDANT ACTIVITY OF SAFFLOWER LEAVES AND ITS MODIFICATION BY ABIOTIC FACTORS

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**Abstract**

The purpose of this research was to study the changes in antioxidant activity of safflower leaves extracts under influences of various abiotic factors: a) meteorological conditions of vegetation season; b) time of sowing (fall or spring); c) pre-sowing treatment of seeds with different doses of γ-radiation. The antioxidant activity was determined by potentiometric methods against peroxyl free radicals and expressed as gallic acid equivalent (μMGAE) per g of dry residue of extract. Obtained data showed that the antioxidant activity of extracts from leaves of safflower growing in the same vegetation season had no differences and did not depend on time of sowing (fall or spring). If to compare the different vegetation seasons the differences in antioxidant activity indices was approximately by 2.0 times (in season of 2015-2016 years the antioxidant activity of leaves extracts was in medium 32-34μMGAE/g, but in season of 2016-2017 this index was equal 61-62μMGAE/g. The extracts of leaves collected from safflower, seeds of which were pre-sowing treated with different doses of γ-radiation (50, 100, 150Gy), possessed higher antioxidant activity than the extracts of safflower leaves derived from untreated seeds.

**Key words**: safflower, leaves, antioxidant activity, γ-radiation

Exposure to different abiotic stresses such as environmental factors (temperature, drought, and salt), time of sowing (fall or spring) and pre-sowing seed irradiation often leads to modification in accumulation of secondary metabolites and changes in redox state of plants (Gorkal P. *et al*, 2009; Sajedi N.A. *et al*, 2012; Jan S. *et al*, 2012). The results of numerous studies suggest that increased resistance to abiotic stress is correlated with more efficient antioxidant systems. Under water deficit the antioxidant properties of safflower extracts against different free radicals increase by 1.5-2.0 times (Salem N. *et al*, 2014). The increase in tolerance to drought stress was associated in safflower leaves with antioxidant enzymes activity, proline and abscisic acid contents (Sajedi N.A. *et al*, 2012). The salt stress also attributed to changes in antioxidant activity of safflower leaves. Abdallah S. B. *et al* (2012) found that the ability to scavenge hydroxyl radicals was sharply increased especially in leaves of the salt-sensitive cultivar.

The gamma rays are the most efficient ionizing radiation, often utilized for creating the mutant modifications in plants and improving accumulation of essential metabolites (Moghaddam S.S. *et al*, 2011). The free radicals formatted in plant cells by gamma radiation affect the morphology, anatomy, biochemistry, and physiology of plants (Jan S. *et al*, 2012). Depending on the dose of irradiation the changes in photosynthesis, and antioxidative system, variation of malondialdehyde levels as marker of free radicals and enhancement of phenolic compounds were reported (Kovacs E., Keresztes A., 2002; Kim J.H. *et al*., 2004).

Secondary metabolites, especially phenolic compounds, play specific roles in the plant protection from abiotic stress as well as the adaptation of plants to the environmental changing and in overcoming stress constraints (Di Ferdinando M. *et al*, 2012; Edreva A. *et al*, 2008). The accumulation of secondary metabolites in safflower depends on eco-geographical area and various technological approaches of plant cultivation (Treutter D., 2010), and in particular on the time of sowing and pre-sowing treatments of seeds. The safflower seeds can be sown in the fall as well as in the spring gratitude to their tolerance to low temperatures. Petrie S. *et al*. (2010) reported that the fall seeding resulted in earlier flowering and maturity and increased yield compared to spring seeding.

The differences in accumulation of phenolic compounds in leaves and flowers of safflower sown in fall and spring were shown earlier (Ivanova *et al*, 2016). In flowering phase the total phenolic content and special the flavonoid glycosides in leaves and flowers of spring sown

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safflower was 20-30% more than in leaves of fall sown plants. Antioxidant activity of extracts from leaves and flowers of spring sown safflower was more but no significantly than antioxidant activity of extracts from fall sown plants.

There are no published investigations focusing on impact of associated abiotic factors such as meteorological conditions of vegetation season; time of sowing (fall or spring); and pre-sowing treatment of seeds with different doses of γ-radiation on phenols accumulation and biological potentials of safflower. The main objective of this study was to evaluate the changes in antioxidant activity of safflower leaves extracts under influences of various abiotic factors.

## MATERIALS AND METHODS

Experiments were carried out at the research field station of the Institute of Genetics and Plant Protection in Chisinau area of the Republic of Moldova (lat. 47°01', long. 28°75', alt. 85 m above sea level), in the seasons of 2015-2016 and 2016-2017. Safflower seeds have been sowed in fall in the first decade of November and in spring in the last decade of March by using following planting patterns: row spacing 50cm, intra-row spacing 15 cm. Moreover, in fall of 2016 the part of seeds was subjected to pre-sowing treatment by gamma radiation of 50, 100, 150Gy.

The meteorological data in experimental field of Chisinau area in growing seasons of 2016 and 2017 is presented in table 1.

Table 1

**Meteorological data in Chisinau, R. Moldova**

| Year | Month | Monthly precipitation,mm | Monthly temperatures, °C | | |
|---|---|---|---|---|---|
| | | | **medium** | max | min |
| 2017 | March | 38 | +7.6 | +23.1 | -3.8 |
| | April | 120 | +9.5 | +27.0 | -3.0 |
| | May | 52 | +16.0 | +30.7 | +1.0 |
| | June | 85 | +21.2 | +34.0 | +6.9 |
| | July | 78 | +21.7 | +37.1 | +8.0 |
| 2016 | March | 54 | +6.0 | +24.4 | -7.2 |
| | April | 65 | +13.2 | +30.5 | -2.0 |
| | May | 65 | +15.5 | +29.7 | +2.0 |
| | June | 75 | +20.9 | +35.7 | +3.5 |
| | July | 20 | +22.5 | +37.0 | +9.0 |

The leaves were collected in the full flowering stages (in second decade of July), when the extracts from leaves possessed the highest antioxidant activity. The extracts were prepared from medium sample of identical plant material in 5 replicates by method of maceration extraction with 70% water-ethanol solution in ratio 1/10 (leaves /water-ethanol). After 7 days of extraction, the extracts were filtered through paper filter and stored at 4–6°C.

**Total phenolic content** was determined by Folin-Ciocalteu method (Singleton V.L. *et al,* 1999). The results of spectrophotometric evaluation at λ=760 nm were expressed as gallic acid equivalent, using the calibration curve over the range of 0…200µg/mL ($r^2$=0.9972) and recalculated per one gram of dry residue (DR) of leaves extract.

**Antioxidant activity** determination was performed by potentiometric method (Sano M. *et al,* 2004) in our modification (Ivanova R., 2016) which evaluates the peroxyl radical scavenging activity of tested samples (reference antioxidants and plant extracts). Peroxyl radicals were generated by 2,2'-azobis-(2-amidinopropane)-dihydrochloride (AAPH). The gallic acid was used as reference antioxidant and the antioxidant activity of leaves extract was expressed as gallic acid equivalent (µMGAE) per g of dry residue of extract.

## RESULTS AND DISCUSSIONS

Safflower leaves possessed high antioxidant activity. Antioxidant activity of extracts from leaves of both fall and spring sowing safflower in the same growing season had no significant differences (*figure 1*). The time of sowing did not influence on antioxidant activity of leaves extracts. The indexes of antioxidant activity of leaves extracts in season of 2017 varied from 55.01 to 69.49µMGAE/g. Take into account that the antioxidant activity is predetermined by accumulation of secondary metabolites, special phenolic substances, it was studied the total phenolic content tested extracts. The total phenolic content of these extracts also varied in limited diapason (0.21…0.25 mg/ml). The ratio of total phenolic content in dry residue of extracts from safflower leaves grown and collected in season 2017 was 4.12…4.50%. It is important to indicated that the extracts of safflower leaves obtained by soxhlet extraction method contained by two times more phenolic compounds (Kusoglu E. & Kahraman S., 2015).

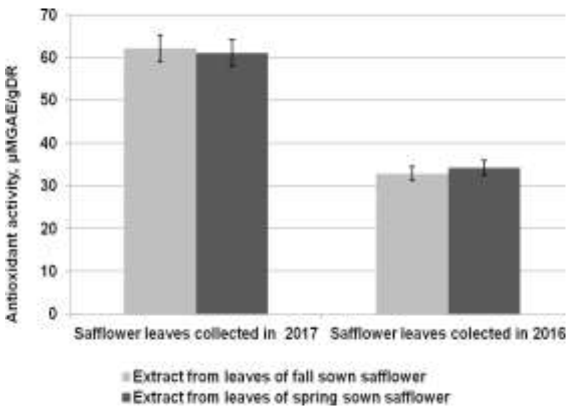


Figure 1 **Antioxidant activity of leaves extracts**

Comparing the different vegetation seasons it was detected significant differences in values of antioxidant activity indexes (*figure 1*). Thus in growing season of 2016 the antioxidant activity of leaves extracts modified in limits from 29.65 to 43.50µMGAE/g, approximately by 1.5…2.0 times less than in season of 2017.

This effect can be explained by differences in meteorological conditions (relatively humidity, monthly precipitation, averages of maximum and minimum temperatures), and the accumulation of active phenolic substances as results to weather stress responses. The seasons of 2016 and 2017 had significant differences of weather condition in mounts of April (*table 1*). In 2017 the seedlings were affected by brisk modification of temperatures and weather phenomenon of snowing in 20-21 April, when approximately 1.0 m of snow fall down during these days. To mention, that the safflower seedlings in this period were in rosette stages. Before of snowfall (19 April, 2017) the seedlings from fall sown seeds had rosette of 6-8 leaves, and the spring sown safflower had first two leaves.

Our result once again confirmed that the abiotic factors provoked by meteorological conditions had significant effect on plants and their responses to stress by increasing antioxidant capacity.

The extracts of leaves collected in 2017 from fall sown safflower, seeds of which were pre-sowing treated with different doses of γ-radiation (50, 100, 150Gy), possessed higher antioxidant activity than the extracts of safflower leaves derived from untreated seeds (*figure 2*). An increase in the dose of seeds irradiation contributed to enhancing the antioxidant activity of leaves extracts in direct proportional dependences on doses (coefficient of linearity was equal 0.9932).

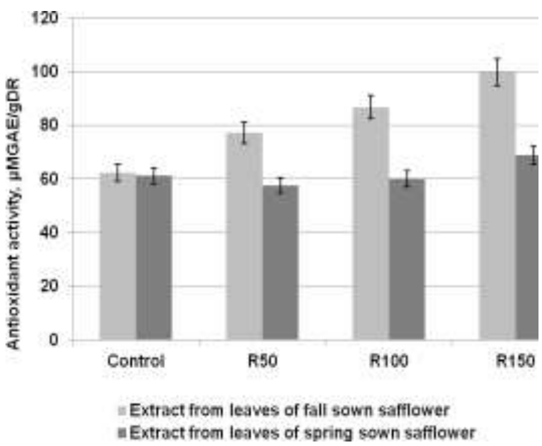


Figure 2 **Antioxidant activities of leaves extracts: control - safflower derived from untreated seeds, R50, R100 and R150 - from seeds irradiated by 50Gy, 100Gy, 150Gy, respectively.**

The seeds irradiated in fall were sown on fifth day after treatment, but the long time of seeds dormancy from fall sowing to spring germination affected significantly on metabolic processes of safflower seeds and plants.

Despite the fact (*table 2*), that the total phenolic content of extracts from leaves of fall sowing safflower seeds did not differences in all variants their antioxidant activities had big differences (*figure 2*). The antioxidant activities of tested extracts increased significantly with rising of radiation dose on examples of fall sowing seeds, and the lightly increased in extracts from leaves of safflower sown in the spring. This effect could be attributed to the accumulation of phenolic compounds possessed different levels of antioxidant activities in dependence on environmental and radiation stresses. The responsible for the synthesis of diverse array of phenolic metabolites is phenylpropanoid pathway, which could be different induced by stresses ((Di Ferdinando M. *et al.*, 2012). The complexity of chemical types, interactions and structure stabilization by polymerisation and condensation of phenols lead to different their biological activities (Edreva *et al*, 2008).

Table 2

**Characteristics of leaves extracts**

| Sample | Dry residue of extract, mg/ml | Yield of extracted compounds, % | Total phenolic content in extract, mg/ml |
|---|---|---|---|
| Extracts from leaves of fall sown safflower | | | |
| Control | 4.98±0.20 | 4.94 | 0.24±0.01 |
| R50 | 5.03±0.10 | 4.99 | 0.23±0.02 |
| R100 | 4.80±0.18 | 4.72 | 0.23±0.01 |
| R150 | 5.54±0.20 | 5.45 | 0.24±0.01 |
| Extracts from leaves of spring sown safflower | | | |
| Control | 5.66±0.22 | 5.66 | 0.25±0.01 |
| R50 | 6.22±0.15 | 6.22 | 0.25±0.01 |
| R100 | 6.50±0.21 | 6.47 | 0.29±0.01 |
| R150 | 6.11±0.28 | 6.01 | 0.29±0.01 |

Changes in antioxidant activities of leaves extracts from safflower of spring sown seeds after irradiation were more lightly (*figure 2*). The antioxidant activities of leaves extracts from untreated and treated by 50-100Gy safflower possessed the approximately identical values. The leaves extracts of spring sown safflower pre-sowing treated by 150Gy showed the significantly more antioxidant activity in comparison with untreated safflower, but by 1.5 times less than the leaves extracts of fall sown safflower (*figure 2*).

However the extracts from leaves of fall sowing seeds were characterized by the lower yields of extracted compounds and contents of phenols in comparison with the extracts from leaves of spring sown seeds. The total phenolic contents of leaves extracts from fall and spring sown safflower pre-sowing treated by 150Gy were 0.24±0.01 and 0.29±0.01mg/ml, respectively. In this case, probably, influence on antioxidant activity had not only total content but the qualitative components of phenolic compounds provoked by irradiation. Differences in total phenols and antioxidant activities of leaves from safflower of different time of sowing can be also explained by some differences in phenological stage of these plants growth. The collection of leaves samples was carried out in the same days, but the phenological stages of plants differed by 5-7 days.

## CONCLUSIONS

The increase of antioxidant activities under influences of different abiotic factors may facilitate the stability of the safflower plants to survive in the adverse environmental conditions.

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