

ECOTOXICITY STUDY ON THE INFLUENCE OF GLYPHOSATE UPON THE *T. AESTIVUM L. DELABRAD 2* CULTIVAR (OECD 208) AND *F. CANDIDA* (OECD 232)

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INTRODUCTION

Wheat (*T. aestivum L.*) is among the most cultivated cereals with vital importance for human food. In Romania, the cultivation of wheat is done on an area of 2,109.0 thousand ha according to the data published by INS in the Statistical Yearbook of Romania.

Pesticides, as defined by the United States Environmental Protection Agency (EPA), are synthetic chemicals or mixtures of substances that contain biologically active ingredients against pests and are used to prevent, destroy, attract or repel them (www.epa.gov). The umbrella of pesticides includes the following product categories: zoocides, fungicides, bactericides, viricides, herbicides but also those based on other strategies e.g., repellents, desiccants and growth regulators (Pricope, 2007).

The side effects of pesticides can be grouped (Prisecaru, 2008) according to: the interaction of plant, animal and abiotic environment, being affected the biological balance of the ecosystem; the presence of residues in food and their ingestion by humans with harmful effects; the degree of development of resistance to plants and animals.

According to the degree of toxicity of the active substance expressed in the average lethal dose (DL-50), pesticides are divided into 4 groups: extremely toxic (DL-50 under 50 mg/kg body weight), highly toxic (DL-50 between 50 and 200 mg/kg body weight), moderately toxic (DL-50 between 200 and 1000 mg/kg body weight) and with low toxicity (DL-50 over 1000 mg/kg body weight).

Of the world consumption of pesticides used in agriculture, about 46% are herbicides, 31% insecticides, 18% fungicides, and the remaining other categories of pesticides (Pricope, 2007). There are general herbicides, which eliminate all vegetation, and selective herbicides that destroy certain species; according to the physiological action, the herbicides can be contact and systemic.

Glyphosate is part of the systemic herbicide category, being one of the most used herbicides in the world. Glyphosate (N-posphonomethyl-glycine) is an analog of glycine, a natural proteinogenic amino

acid. Discovered by chemist Henry Martin as early as 1950 and exploited thirty years later by chemist John E. Franza, this substance penetrates plants and is transported to growing areas. It is absorbed mostly at the leaf level and very little at the root level. It is a total herbicide, which destroys any plant except resistant plants and those genetically modified to be resistant.

Currently, there is corn, canola, alfalfa, sugar beet, cotton that has been genetically modified to be resistant to glyphosate. Although Romania has allowed the cultivation of soybean genetically modified for resistance to glyphosate since 1998, this was eventually banned after the country joined the EU. The only genetically modified product to be resistant to glyphosate that is authorized in the EU, cultivated in Romania, is corn (Information Center on genetically modified organisms, <http://infomag.ro>).

In resistant species, glyphosate becomes concentrated in the leaves instead of being translocated to other areas of the plant. Glyphosate has the property of inhibiting the enzyme 5-enolpyruvylsikmate-3-phosphate (EPSPS) which is involved in the synthesis of certain amino acids such as tyrosine, tryptophan, and phenylalanine. This enzyme is produced only by plants and certain microorganisms, but not in the mammalian genome, which has led to the idea that glyphosate is not dangerous to humans (Daruich J., et al. 2001; Davis A. et al., 2006).

Used not only for weed removal but also for desiccation, many crops that are treated with glyphosate before harvest to speed up ripening or optimize threshing cause major human health problems. Consumption of foods obtained from these cereals that have high concentrations of glyphosate generates imbalances in the intestine, disrupts the activity of cytochrome P450, and enzymes that catalyze the oxidation reactions of organic substances in the body, in the biosynthesis and transformation of vitamins and fatty acids.

In the world, 76% of glyphosate is used in agriculture, being widely used in forestry, urban areas, and gardening. 72% of the total volume of

glyphosate applied worldwide between 1974 and 2014 was sprayed only in the last 10 years (European Parliament Resolution of 24 October 2017).

Glyphosate is the substance that has divided Europe into two camps, one for and one against. On 27 November 2017, the European Parliament voted in favor of the use of this herbicide for another five years: 18 of them (65.71% of the EU population) voted in favor of renewal, 9 (32.26%) voted against and 1 (2.02%) abstained. A European Citizens' Initiative (ECI) was also organized, which collected more than one million signatures from European citizens in less than a year and aimed at banning glyphosate and protecting people and the environment.

Glyphosate generates long-term negative effects on the soil, being adsorbed by electrically positively charged surfaces, favoring the increase of concentrations of heavy metals and phosphates in the soil and aquifer.

Folsomia candida is one of the intensively studied springtail invertebrates because it reflects the condition of the soils, while improving microbial activity, intervening in decomposition and humus formation processes (Borggaard, O.K., Gimsing, A.L., 2008). Taxonomically classified in the Class *Collembolla*, Order *Entomobryomorpha*, Family *Isotomidae*, Subfamily *Proisotomidae*, these invertebrates are widely found in terrestrial ecosystems being used in a wide range of ecotoxicity tests, according to OECD 232.

One of the oldest studies was conducted by Sheals (1956), which highlighted the effects of organochlorine compounds on microarthropod communities and tested various species to observe susceptibility to DDT. Collembolans are widely used as test organisms for measuring the toxicity of many chemicals. Collembolans possess many attributes that make them suitable organisms for use in soil assessment (Carson R., 2002; Ramesh, E., 2013).

This species is found in most regions of the world. Its original biogeographical locations are difficult to ascertain, as they were transported all over the world in small portions of soil. *F. candida* is found in a variety of habitats including caves, mines, agricultural systems, soils rich in organic matter, forests, greenhouses. It is a species well adapted to soil drying conditions. It has physiological adaptations to avoid dehydration and can absorb water vapor. Oxygen absorption is achieved through the cuticle and it can survive for up to 18 hours under completely anaerobic or high CO₂ conditions. Feeding is based on fungi, fungal hyphae (Fountain M., Hopkin S. P., 2005).

They are unpigmented arthropods with no eyespots, with a body consisting of a head provided with a pair of antennae, a thorax with three pairs of legs and an abdomen consisting of 6 segments. They have a characteristic organ called a furcula, which consists of a basal part (handlebar), which supports a

pair of distal arms, each finished in the form of a club. These little creatures are similar to fleas because of the furcula that gives them the ability to jump. Even if they lack eyes, they possess some internal photoreceptors that help them especially in choosing the place where they lay their eggs (Ramesh E., 2013). They have a high reproduction rate, and the populations are composed exclusively of parthenogenetic (asexual) females, which lay unfertilized eggs from which viable offspring develop, and the males are completely absent from the population. The eggs are laid in small batches or next to those laid by other females forming aggregates that can be seen with the naked eye in laboratory cultures. A female lays an average of 30-60 eggs from which juveniles hatch that do not undergo metamorphosis but develop directly into adults and differ only in size. Individuals can reach up to 3 mm at maturity (Fountain M., Hopkin S. P., 2005; Ramesh E., 2013).

MATERIAL AND METHODS

In this study we evaluated:

- ✓ the effect of glyphosate on *Folsomia candida* (OECD 232);
- ✓ the effect of glyphosate on plants - the effect generated by different concentrations of glyphosate on the test plant – wheat – was monitored (OECD 208);

Materials:

- Glyphosate -Roundup Classic Pro (Monsano), water-soluble concentrate in the form of a clear, slightly yellowish liquid with a characteristic odor, with active substance 360 g/l glyphosate;
- Florasol soil (pH 7.0 ± 0.5) with a level of nutrition that is suitable for all types of plants. Composition: eutrophic peat, oligotrophic peat enriched with complex fertilizers. Mandatory quality requirements: nitrogen content (N) (m/m%) min. 0.1, phosphorus (P2O5) (m/m%) min. 0.1, potassium (K2O) (m/m%) min. 0.1;
- Common winter wheat (*T aestivum L.*) Delabrad2 cultivar;
- Collembolans of the species *Folsomia candida* obtained in the laboratory in plastic boxes, on activated carbon medium. They were kept at a temperature of 20 ± 2°C and fed dry yeast.
- ✓ **Working method for evaluating the effect of glyphosate on *Folsomia candida* (OECD 232)**

The glyphosate concentrations were chosen following the indications on the package, namely 1.5 liters of product per hectare dissolved in 80-250 l/water, applied before sunrise. According to the package leaflet, if the product is to be used before harvest, the used dose is 4 l/ha.

In this experiment, five concentrations were used: two below the recommended limits, one reference concentration, and two above the permitted limits (Table 1). The concentrations were chosen in

this way to highlight the impact of the different doses on the reproduction of Collembolans. Two replicates were performed for each concentration and the control sample.

Plastic containers with a diameter of 5.6 cm and a volume of 120 ml were used in the experiment, in which 25g of soil and 5ml of solution (water and glyphosate) were placed. In each container, there were introduced 10 individuals (*F. candida*) which were kept at a temperature of $20 \pm 2^{\circ}\text{C}$ for 28 days (Figure 1a-c). The containers were kept in the Sanyo growing room (Figure 1d) and were ventilated twice a week. The 28-day breeding test was based on OECD 232 standards (OECD 232, 2009).

Table 1. Glyphosate concentration used in the reproduction and mortality test for *Folsomia candida*

Pesticide concentration (ml/ 0.25 l water)	
C ₁	0.6
C ₂	1.25
C ₃	2.5
C ₄	5
C ₅	7.5

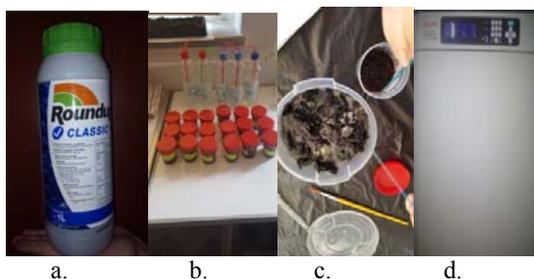


Figure 1 a-d. Preparation and assembly of the experiment to evaluate the effect of glyphosate on *F. candida* (OECD 232)

✓ **Working method for evaluating the effect of glyphosate on common winter wheat (*T aestivum L.*) Delabrad 2 cultivar**

For this experiment there were used:

- plastic containers 7.5 cm in diameter and 4.5 cm high. For each concentration of glyphosate, 6 containers (3 replicates) were prepared in which

5 seeds were added. 25g of soil and 5ml of solution were placed in each container. The waiting period after the application of glyphosate was one day after which the seeds were sown according to the instructions on the package. The samples were watered every two days with distilled water (Figure 2a-c).

✓ **Working method for evaluating the effect of glyphosate on the germination of common winter wheat (*T. aestivum L.*) Delabrad2 cultivar**

For this experiment there were used:

Plastic Petri dishes with a diameter of 9 cm in which 10 wheat seeds were placed on moist paper (Figure 3 a-c).

The waiting period after the application of glyphosate concentrations was one day after which the seeds were placed. The Petri dishes were placed in the Sanyo chamber and were moistened daily with 1 ml of glyphosate concentrations and the control sample with distilled water (Figure 3a-c).

✓ **Method for dosing assimilative pigments in the hypocotyl and cotyledon of wheat (*T aestivum L.*) Delabrad2 cultivar**

The quantitative determination of the assimilating pigments was performed according to the Mayer-Bertenarth method with modifications brought by Ştirban and Fărcaş. The extraction of pigments is done in 85% acetone by grinding the plant material.

Quartz sand and a small amount (0.05 g) of CaCO₃ were added to this process to prevent the conversion of chlorophylls to pheophytins. 1 gram of plant material was ground with quartz sand, CaCO₃, and washed repeatedly with 85% acetone until the obtained extract remains colorless (Figure 4 a-d). The homogenate is filtered with filter paper, at the vacuum pump and brought to a volume of 25-30 ml, in a volumetric flask, with acetone. The extracts obtained are read on the Libra SS spectrophotometer. Each sample is placed in the spectrophotometer cuvette and read in parallel with the solvent used as a control (85% acetone), at wavelengths corresponding to: $\lambda = 663 \text{ nm}$ for chlorophyll "a"; $\lambda = 645 \text{ nm}$ for chlorophyll "b"; $\lambda = 472 \text{ nm}$ for carotenoid pigments.

Calculation of the results:

$$\text{Chlorophyll "a" (mg/g plant material)} = \frac{12,3 \times E(663) - 0,85 \times E(645)}{dx \times 1000 \times W} \times V$$

$$\text{Chlorophyll „b" (mg/g plant material)} = \frac{19,3 \times E(645) - 3,6 \times E(663)}{dx \times 1000 \times W} \times V$$

$$\text{Carotenoid pigments} = \frac{E(472) \times V \times 10}{dx \times 1000 \times W \times b}$$

where: d = cuvette thickness; W = weight of plant material; V = extraction volume; b = coefficient (2485)

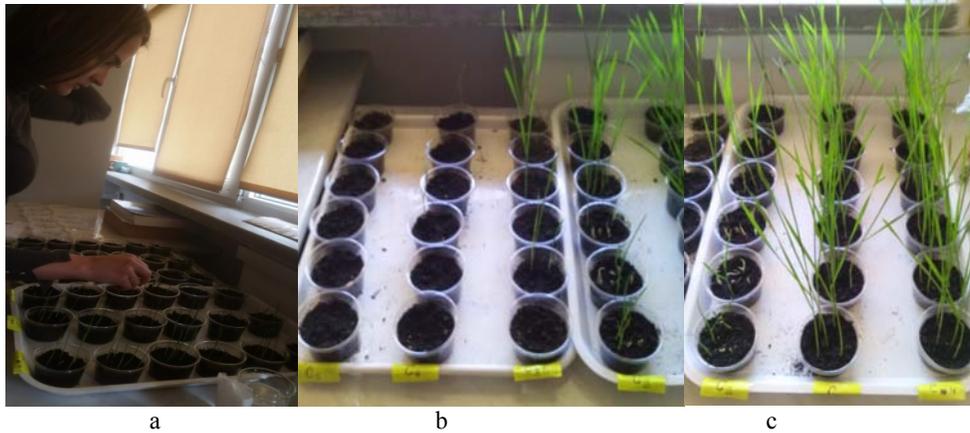


Figure 2 a-c. Preparation and assembly of the experiment to evaluate the effect of glyphosate on common winter wheat (*T aestivum L.*) Delabrad 2 cultivar



Figure 3 a-c. Preparation and installation of the experiment to evaluate the effect of glyphosate on the germination of common wheat (*T aestivum L.*) Delabrad2 cultivar

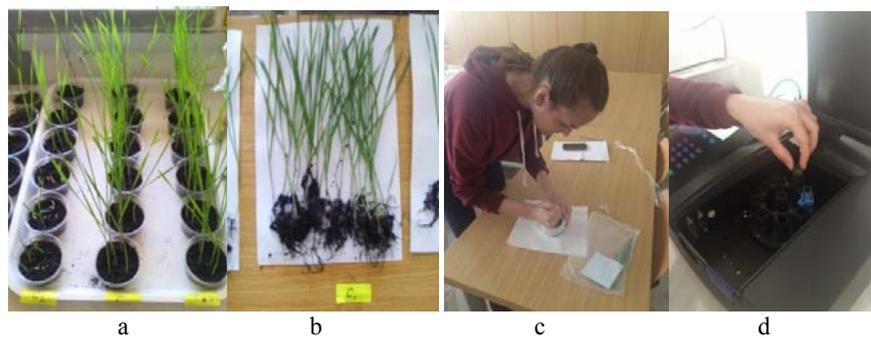


Figure 4a-d. Dosing the assimilating pigments in the hypocotyl and cotyledon of wheat (*T aestivum L.*), cultivar Delabrad 2

RESULTS AND DISCUSSIONS

✓ Results on the influence of glyphosate on the species *Folsomia candida* (OECD 232)

Following the experiment, a low degree of mortality was found among adults, but there were changes in the number of juveniles as glyphosate levels increased (Table 2).

There was a reduction in the average number of individuals of *Folsomia candida* (juveniles, adults)

by approx. 7% compared to the control at C5 concentration and by 5% at C1.

✓ Results regarding the germination percentage of common winter wheat (*T aestivum L.*) Delabrad 2 cultivar, under the influence of glyphosate

In agriculture, it is accepted that a certain percentage of seeds do not germinate. The maximum limit of the germination faculty is 100% and the minimum for wheat and rice is 85%, and for vegetables, it is 50%.

Following the observations, it was found that the control sample had a germination rate of 95%, a percentage that decreased with increasing glyphosate concentrations (Table 3).

The germination process is a spectacular and vulnerable phenomenon to internal or external disruptors. We found that although many of the seeds

sprouted, the growth process was stopped. This explains why in the case of the last concentrations there is still a percentage of germination and in the case of the last concentrations in the experiment performed with seed sowing in the soil the percentage of emergence is very small or non-existent (Figure 6a-b).

Table 2. Number of *Folsomia candida* (juveniles, adults) observed after 28 days (OECD 232)

	CM	C1	C2	C3	C4	C5
R1	1300	992	720	621	618	610
R2	1324	987	894	672	673	598
R3	1220	995	791	746	702	710
Media	1281.3	991.3	801.6	679.6	664.3	639.3

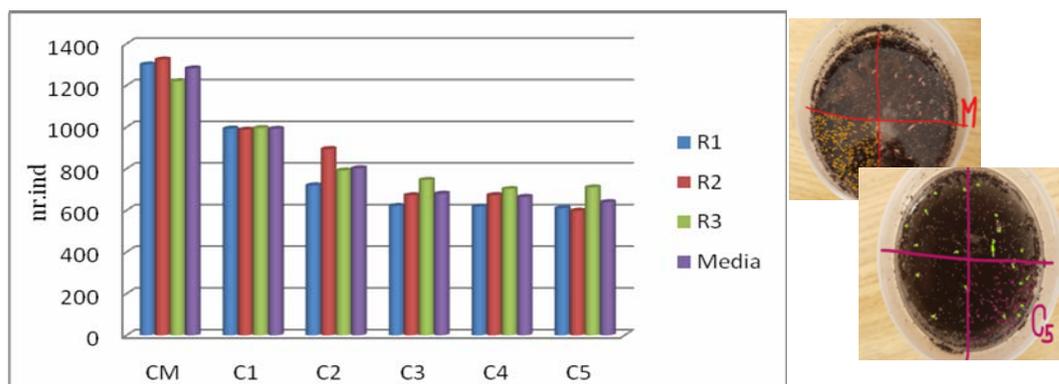


Figure 5. Number of *Folsomia candida* (juveniles, adults) obtained by exposure to glyphosate

Table 3. Percentage of germination of common wheat (*T aestivum L.*) Delabrad2 cultivar

	C _M		C ₁		C ₂		C ₃		C ₄		C ₅	
	V ₁	V ₂										
No. of germinated seeds	10	9	8	8	6	6	6	6	5	5	5	6
Total	19		16		12		12		10		11	
Germination percentage (%)	95%		80%		60%		60%		50%		55%	

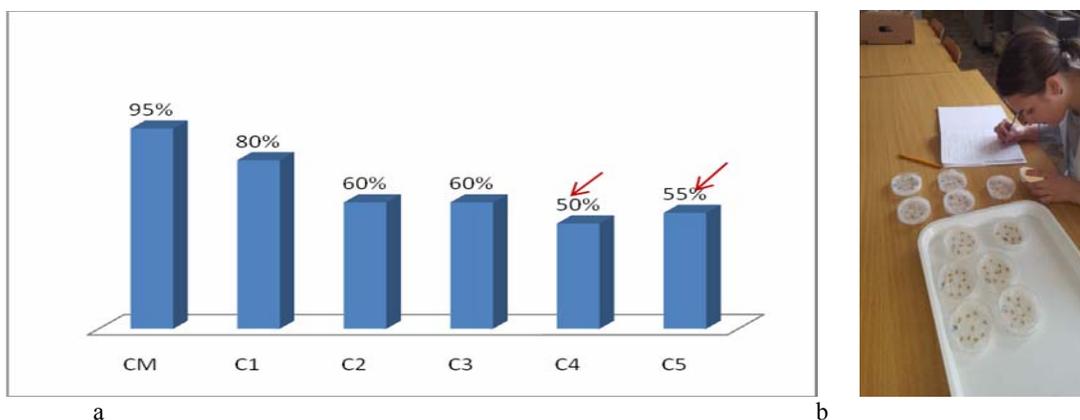


Figure 6. Percentage of germination of common wheat (*T aestivum L.*) Delabrad2 cultivar under the influence of glyphosate

✓ **Results regarding the percentage of the emergence of common winter wheat (*T aestivum* L.)Delabrad2 cultivar under the influence of glyphosate.**

To be profitable, wheat crops must have an optimal density. Many factors can influence the emergence of wheat, whether or not independent of human action. Following the determination of the percentage of emergence, it was found that significant differences appeared with the increase of the concentrations used. If in the control variant the percentage of emergence was 100%, in the case of the last concentration, the one with the highest amount of glyphosate, the percentage of emergence was 0% (Table 4). If there are errors in the application of glyphosate that lead to the administration of larger amounts of product, some of the negative effects can be mitigated by supplementing the number of germinating grains per square meter corresponding to the density of plants we tend to reach.

Wheat sprouting was inhibited by different concentrations of glyphosate. Thus, in the case of the concentration of 0.6 ml the percentage of emergence was 96%, in the case of the concentration of 1.25 ml the percentage of emergence was 90%, in the case of the concentration of 2.5 ml the percentage of emergence was 36%, in the case of the concentration of 5 ml the percentage of emergence was 10%, and in the case of the last concentration, i.e. that of 7.5 ml the percentage of emergence was 0% (Figure 7a-b).

✓ **Results of biometric measurements on common winter wheat (*T aestivum* L.)Delabrad 2 cultivar under the influence of glyphosate**

Biometrics is useful in many fields, as it provides useful information in a short time. It is also

used successfully in agriculture, especially for the selection of certain varieties, for the analysis of the variability of certain characters under the influence of certain factors. In this experiment, we looked at the influence of glyphosate on the root and stem of common wheat (Figure 8). After performing biometric measurements, we found that glyphosate had a negative impact on the length of the root and stems of common winter wheat, monitored from the needle stage until the appearance of the second leaf.

At the level of the stem, it was found that significant differences appeared with increasing glyphosate concentrations. At the control sample, the average length of the stem was 27.15 cm; at the C1 concentration of 0.6 ml glyphosate the average length of the stem was 27.4 cm; at the C2 concentration of 1.25 ml glyphosate the average length of the stem was 15.6 cm; at the C3 concentration of 2.5 ml glyphosate the average length of the stem was 14.55 cm; at the C4 concentration of 5 ml glyphosate the average length was 10.75 cm (Figure...)

Following biometric measurements at the root level, we found that some plants under the influence of glyphosate did not reach the stage of adventitious root formation. The adventitious roots begin to form with the appearance of the second leaf; until then there are only embryonic roots. At the root level, as in the case of the stem, there were significant differences with increasing glyphosate concentrations. At the control sample, the average root length was 13.03 cm; at the concentration of 0.6 ml the average root length was 12.49 cm; at the concentration of 1.25 ml the average root length was 7.85 cm; at a concentration of 2.5 ml the average length of the root was 3.62 cm; and at a concentration of 5 ml, the average length was 2 cm (Figure 9a-b)

Table 4. Percentage of the emergence of common winter wheat (*T aestivum* L.)Delabrad 2 cultivar, under the influence of glyphosate

Wheat emergence	C _M			C ₁			C ₂			C ₃			C ₄			C ₅		
	V ₁	V ₂	V ₃	V ₁	V ₂	V ₃	V ₁	V ₂	V ₃	V ₁	V ₂	V ₃	V ₁	V ₂	V ₃	V ₁	V ₂	V ₃
Day 5	10	10	10	10	9	10	9	9	7	2	3	5	1	1	1	0	0	0
Day 7	10	10	10	10	9	10	9	10	8	2	4	5	1	1	1	0	0	0
Total	30			29			27			11			3			0		
Percentage	100%			96,6%			90%			36,6%			10%			0%		

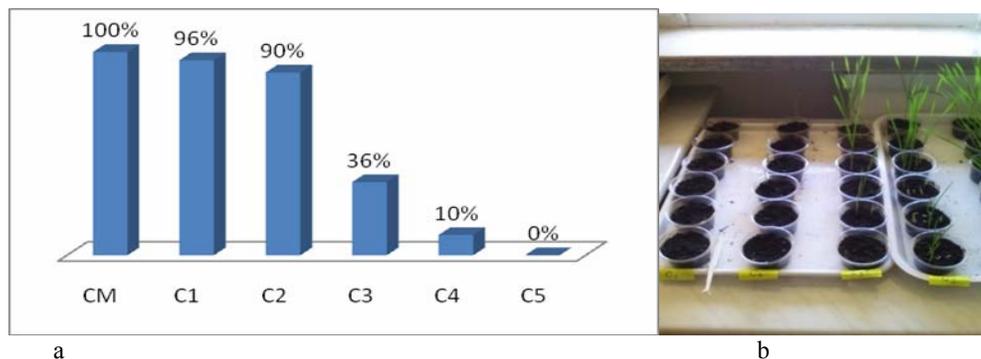


Figure 7a-b. Common winter wheat (*T aestivum* L.)Delabrad 2 cultivar under the influence of glyphosate

To have an overview, we made measurements on the whole plant. At the control sample, the average length was 40.19 cm; at the concentration of 0.6 ml the average length was 39.89 cm; at the concentration of 1.25 ml the average length was 23.44 cm; at the concentration of 2.5 the average length was 18.22 cm; and in the case of the concentration of 5 ml the average length was 12.75 cm (Figure10).

“Extremes are good neither in nature, nor in life”, according to Professor CălinPentre Rang, but they can provide us with useful information. At the level of the stem, in the case of the control sample, we had a minimum of 24 cm and a maximum of 31 cm. We obtained similar values in the case of the concentration of 0.6 ml, but with the increase of the concentrations the extreme values started to move further and further away (Figure 11).

At the root level, in the case of the control sample, we had a minimum length of 7.5 cm and a maximum of 24 cm. In the case of the lowest concentration, the minimum length was 7 cm and the maximum 20 cm,

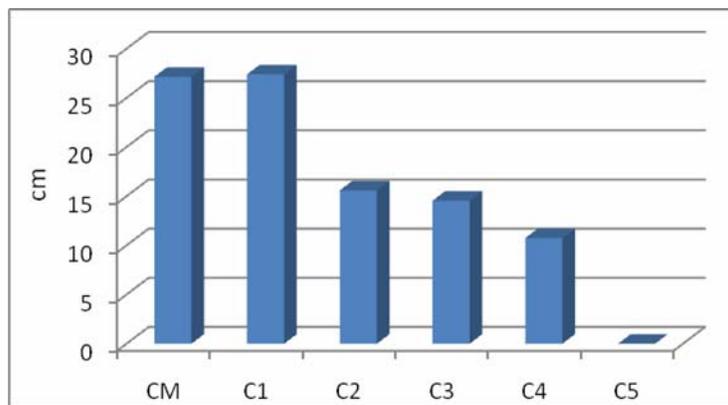
while in the case of the penultimate concentration the minimum length was 1 cm and the maximum 12.5 cm (Figure 12).

✓ **Results regarding the content of assimilative pigments in common winter wheat (*T aestivum L.*), Delabrad 2 cultivar under the influence of glyphosate**

The analysis of the chlorophyll content in the green parts of the wheat seedlings treated with glyphosate in the proposed concentrations showed values close to those of the control sample of the parameters (Table 5).

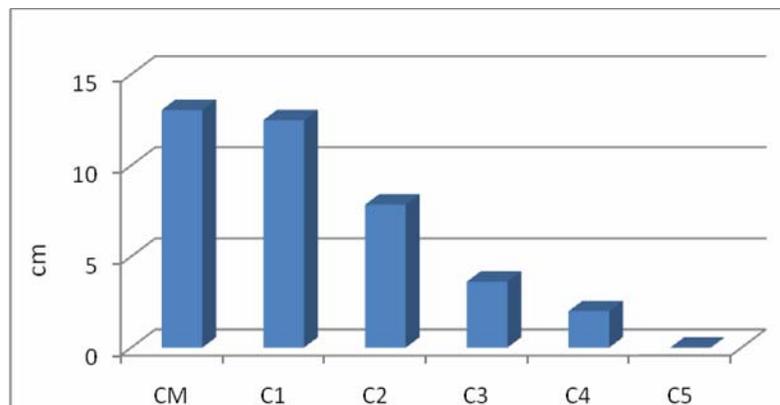
In the experimental variants tested, the chlorophyll a content recorded a maximum of 1.15 mg/g in the control and a minimum of 1.04 mg/g. Chlorophyll b recorded a maximum in the control variant of 1.13 mg/g and a minimum of 0.98 mg/g.

At the concentration of C3 chlorophyll a, it recorded a maximum of 1.04 mg/g and a minimum of 0.97 mg/g. Chlorophyll b, recorded a maximum of 1.10 mg/g and a minimum of 0.70 mg/g.



a

Figure 8 a-b. Average stem length of common wheat (*T aestivum L.*)Delabrad cultivar 2 under the influence of glyphosate



a

Figure 9 a-b. Average root length of common wheat (*T aestivum L.*) Delabrad cultivar 2 under the influence of glyphosate

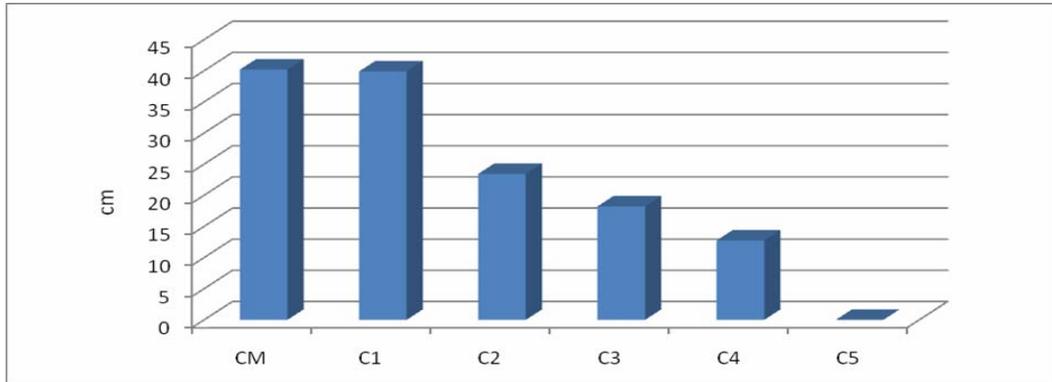


Figure 10. The average length of winter common wheat plants (*T aestivum L.*) Delabrad cultivar 2 under the influence of glyphosate

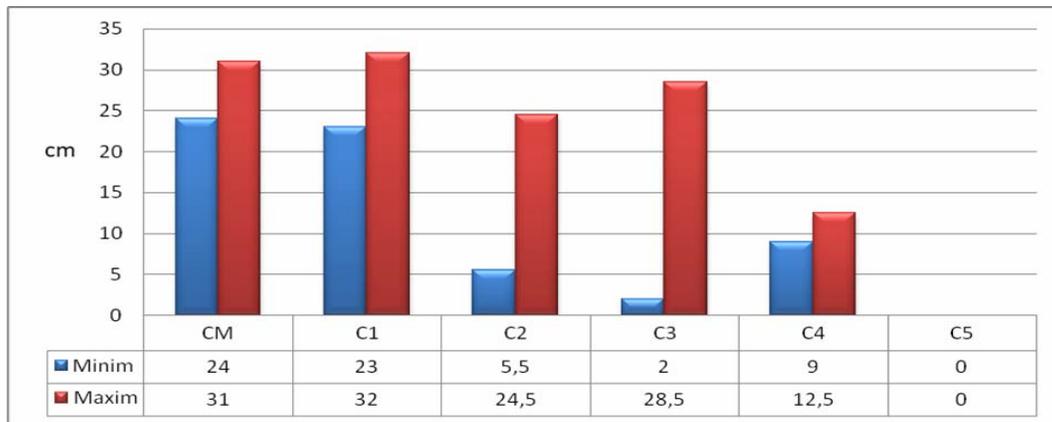


Figure 11. Extreme values obtained from measurements made on the stem (*T aestivum L.*) Delabrad cultivar 2

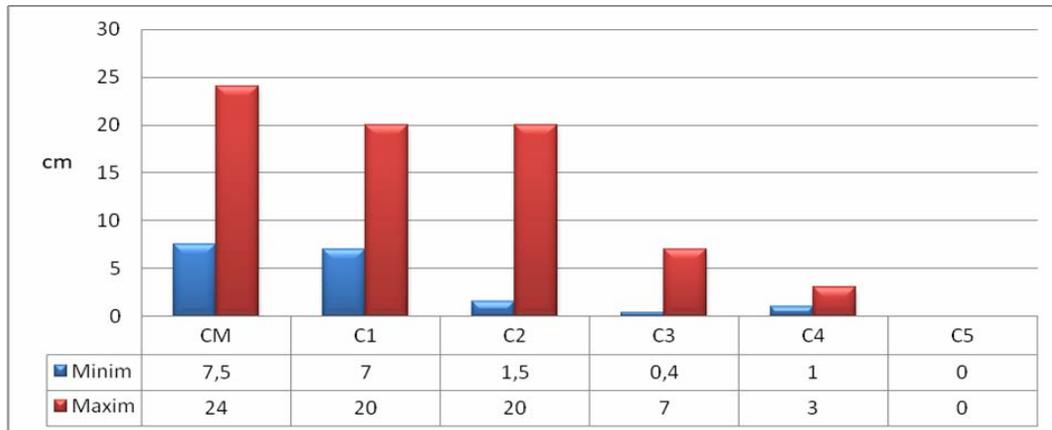


Figure 12. Extreme values obtained from measurements made at the root level (*T aestivum L.*) Delabrad cultivar 2

It can be seen in the graph in Figure 13 that glyphosate did not decisively influence the pigment content compared to the control sample. No measurements were made for the last two

concentrations as the plant material was in too small an amount and no plant emerged at the last concentration.

Table 5. Chlorophyll a and chlorophyll b content (mg/g fresh substance) in common winter wheat seedlings (*T aestivum L.*) Delabrad 2 cultivar under the influence of glyphosate

<i>Triticum aestivum L.</i>		CM	C1	C2	C3
V1	Chlorophyll a(mg/g)	1.15	0.93	1.03	1.04
	Chlorophyll b(mg/g)	0.98	0.84	1.13	1.10
V2	Chlorophyll a(mg/g)	1.04	1.04	1.05	0.97
	Chlorophyll b(mg/g)	1.13	1.14	0.98	0.92
V3	Chlorophyll a(mg/g)	1.04	1.04	1.05	1.03
	Chlorophyll b(mg/g)	1.02	1.11	0.98	0.70

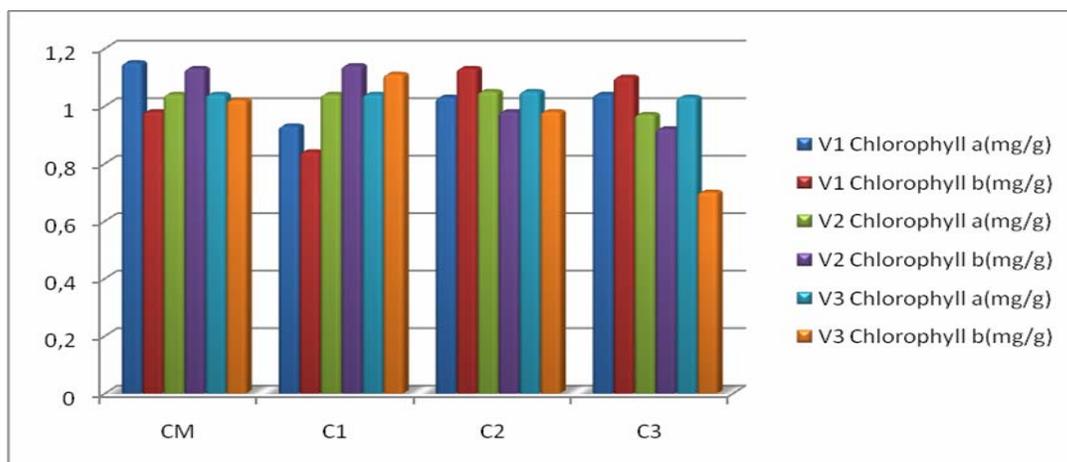


Figure 13. Chlorophyll a and chlorophyll b content of common winter wheat (*T aestivumL.*)Delabrad 2 cultivar under the influence of glyphosate

CONCLUSIONS

Following the tests, it was found that this herbicide – glyphosate – had a negative impact on seed germination, emergence, root size, and stem of common winter wheat. All these effects can significantly influence the production and productivity of wheat.

If there are errors in the application of glyphosate that lead to the administration of larger amounts of product, some of the negative effects can be mitigated by supplementing the number of germinating grains per square meter corresponding to the optimal plant density for good productivity.

Following the test performed with *Folsomia candida*, no high degree of mortality was observed among adults, but an influence on the number of juveniles was observed. Thus, identifying the impact of pesticides on soil organisms is useful, as it provides useful information from an ecological point of view and in terms of agricultural productivity. Knowing the lethal and sublethal effects of pesticides on soil organisms, we find ecologically relevant information on biodiversity conservation.

In conclusion, glyphosate alone must not be used for weed control. Excessive use of this product may promote nonspecific resistance to plants and soil organisms. Populations with multiple resistance

mechanisms can thus become much more difficult to manage.

Also, glyphosate is a non-selective herbicide that covers a wide range of plants which makes glyphosate residues found in many types of food and non-food products (flour, beer, drinking water, animal feed, meat, and meat products, dairy products, etc.) that have as a final link the consumer – man.

ABSTRACT

The side effects of pesticides have been constantly studied, and are the subject of books such as the one written by biologist and journalist Rachel Carson who talked about the negative effects of DDT (Dichlor-Diphenyl-Trichloroethane) on the environment in the book "Silent Spring" (Rachel Carson, 1962), a book that would destroy the fame of DDT. Due to undesirable side effects in 1970, DDT was banned in industrialized countries and since 2004 the "Stockholm Convention" has banned its use in agriculture. The human attitude towards pesticides has two aspects: the protection of consumers against pesticide residues in food and the training of those who produce or handle pesticides in the sense of proper use. Whether it is insecticides, fungicides, or herbicides, we must keep in mind that *the dose is what makes the poison*.

The objective of this paper is to test glyphosate in two groups of test organisms, according to the recommendations of the OECD-Organization for Economic Cooperation and Development, respectively on *Folsomia candida* (OECD 232) and wheat- (OECD 208).

The results obtained after laboratory testing have revealed the harmful effect of this herbicide on wheat at high concentrations (of 5mL/0.25mL water, respectively 7.5mL/0.25mL water), inhibiting the seed germination process as well as the plant growth processes and development and their content in assimilating pigments. Although the mortality rate for adults of *F. candida* was low, the number of juveniles decreased in direct proportion to the concentration of glyphosate used in the experiment.

REFERENCES

1. ACKERMANN, W., COENEN, M., SCHRODL, W., SHEHATA, A., KRUGER, M., 2015 - *The Influence of Glyphosate on the Microbiota and Production of Botulinum Neurotoxin During Ruminant Fermentation*, Current Microbiology, Vol. 70, No 3, p. 374-382,
2. BRUGGEN, V., 2018 - *Environmental and health effects of the herbicide glyphosate*, Science of The Total Environment, Vol 616-617, p.255-268,
3. DARUICH J., ZIRULNI, F., GIMENEZ, MS., 2001 - *Effect of herbicides glyphosate on enzymatic activity in pregnant rats and their fetuses*, Environmental Research Vol 85, No 3, p. 226-231,
4. DAVID, A., 2006 - *Glyphosate And The Eradication Of Due Process In Colombia*, Sound Chemicals Management, Vol 6, No 3, p. 42-45,
5. FOUNTAIN, M., HOPKIN SP., 2005 - *Folsomia candida (Collembola): a standard soil arthropod*. Annual Review of Entomology 50 (1), DOI: 10.1146/annurev.ento.50.071803.130331,
6. GERALD M. DILL și colab., 2010 - *Glyphosate: Discovery development, application, and properties*, DOI:10.1002/9780470634394,
7. JASKULSKI, D., JASKULSKA, I., 2014 - *The effect of pre-harvest glyphosate application on grain quality and volunteer winter wheat*, Romanian Agricultural Research, Vol 31, p.283-289,
8. MAILIN GAUPP-BERGHAUSEN, HOFER, M., REWALD, B., ZALLER, GJ., 2015 - *Glyphosate-based herbicides reduce the activity and reproduction of earthworms and lead to increased soil nutrient concentrations*, DOI: 10.1038/SREP12886,
9. PRICOPE, F., PRICOPE LAURA, 2007 - *Poluarea mediului și conservarea naturii*, Rovimed Publisher, Bacău,973-7719-21-8, p.189,
10. PRISECARU MARIA et al. 2008 - *Ecotoxicologie - curs universitar*, Editura Alma Mater, Bacău, ISBN – 978-973-1833-85-8, 300p.
11. RAMESH, E, 2013 - *Feeding behaviours of Folsomia candida as influenced by diet switching in the presence of live maize roots*. Dalhousie University Halifax, Nova Scotia,
12. RACHEL CARSON, 2002 - *Sient Spring*, Editie Aniversară New York Mariner Book, accesatianuarie 2018 la adresa [<https://books.google.ro>],
13. RAȚI, I.V., BĂDĂLUȚĂ NICOLETA, 2008 – *Fiziologia plantelor- note de curs*, Bacău: Editura Alma Mater,ISBN 978-973-1833-72-9 , p. 269,
14. SAMSEL, A., SENEFF, S., 2013 - *Glyphosate, pathways to modern diseases II: Celiac sprue and gluten intolerance*, Interdisciplinary Toxicology, Vol 6, No 4,p. 159-84,
15. ***Directiva 2009/128/CE a Parlamentului European și a Consiliului din 21 octombrie 2009 de stabilire a unui cadru de acțiune comunitară în vederea utilizării durabile a pesticidelor
16. ***Directiva Consiliului din 27 noiembrie 1990 privind stabilirea conținuturilor maxime de reziduri de pesticide de pesau din anumite produse de origine vegetală, inclusive fructe și legume 90/642/CEE
17. ***Centru de informare asupra organismelor modificate genetic accesatmartie 2018 la adresa [<http://infomag.ro>],
18. ***www.epa.gov accesatmartie 2018,
19. ***Test No.232: *Collembolan Reproduction Test in Soil*- OECD accesatfebruarie 2018 la adresa [<https://www.oecd.org>],
20. ***Test No. 208: *Terrestrial Plant Test-OECD* accesat februarie 2018 la adresa [<https://www.oecd.org>]

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