DISTURBANCE OF MITOTIC ACTIVITY TO SUNFLOWER UNDER INFLUENCE OF PROPAQUIZAFOP HERBICIDE

BONCIU ELENA[1*], ROSCULETE ELENA[1], OLARU L.A[1], ROSCULETE C.A.[1]
[1]University of Craiova, Faculty of Agronomy, 19 Libertatii Street, Craiova, Romania
*Corresponding author email: elena.agro@gmail.com

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ABSTRACT

Weed control is one of the very important technological stages for sunflower, executed both pre-emergence and post-emergence. However, excessive use of herbicides may have some adverse effects on the normal development of cell division. One of the active substances used post-emergence frequently to sunflower crops is Propaquizafop (commercial name Agil).

In order to determine the mitotic activity to sunflower under the influence of this herbicide, three treatment variants (V1/10 ppm; V2/20 ppm; V3/50 ppm) were performed along with an untreated control. Cytogenetic analysis revealed the mitodepresive effect of the herbicide at the same time with the increased of concentrations; as well as its genotoxic effect, quantified by the occurrence of several types of mitotic anomalies (metaphase and telophase stickiness, ring chromosomes and multinucleated cells).

The results suggest genotoxicity *and mutagenic potential of the Propaquizafop herbicide and the need to apply the integrated methods for crops protection to protect the environment and preserve biodiversity.*

INTRODUCTION

Sunflower (Helianthus annuus) is a dicotyledonated plant belonging to family Compositae, the genus Helianthus. Sunflower species have North American origin, namely Mexico (Bonea D., 2009). Sunflower continues to be (after soybean) a world leader of the plants with high nutrition's value, as it is considered a miraculous source of food and a therapeutic miracle in the treatment of many diseases.

The elements of agro-production process can be of a general character, with mandatory application to all agricultural crops, or a particular character with application for certain crops; an important role is due by amendments and chemicals (Pandia O. et al., 2018; Pandia O. et al., 2017). The sunflower successful cropping depends on the yielding ability of genotypes, as well as on the reliability of production systems. Numerous studies have been conducted to evaluate the agronomic performances of commercially available sunflower genotypes in different areas of Romania. If the technological elements are to a great extend firmly and correctly applied, and the climatic conditions practically incontrollable, the genotype is the most dynamic factor of influence of the productivity of the sunflower. Numerous researchers recommend the biological weed control, as a safe alternative, by the use of aqueous extracts of allelopathic plants that release allochimics to inhibit the growth of certain weeds. Also, these extracts can stimulate or inhibit the germination and growth of some agricultural species. Bonea D. (2016) reported that the aqueous extracts of horseradish could help germination and seedling growth of maize reducing the negative effect of drought stress.

Sunflower crops are sensitive to weeds, so they must be eliminated as early as they because can compromise about 50% of the production. Propaquizafop (the commercial name is Agil) is an herbicide of the Aryloxyphenoxy propionates family. It is used for the post emergence control of a wide range of annual and perennial grasses to many crops, including sunflower.

The farmers use often excessively the herbicides and therefore a cellular who compete with the plants for nutrients, level study of the active substance effects on mitosis and chromosomes was considered necessary.

The appearance of chromosomal anomalies in plants is generally regarded as a potential danger for environment, even if mutagens can create variability (Bonciu E., 2012; Bonea D. et al., 2018; Bonea D. and Bonciu E., 2017).

MATERIALS AND METHODS

The biological material consisted of sunflower seeds clean, dry and free from pathogen attack. The herbicide chosen was Propaquizafop (Agil), as it is often used in the post-emergence control of weeds to sunflower crops. Experience has been established in 3 treatment variants, each with 4 repetitions.

For obtain dilutions with different concentrations for treatment, the herbicide was dissolved in water as follows: 10 ppm (V1); 20 ppm (V2) and 50 ppm (V3). With these dilutions, the seeds of the three variants were periodic sprayed for 72 hours, while the untreated control was maintained in wet medium by repeated sprayed with tap water to obtain radicular meristems. The meristematic roots were obtained by germinating sunflower seeds in Petri dishes on wet filter paper in the above treatment variants. When these were about 2 cm, they were harvested and processed according to Feulgen-Rossenbeck method. The effective colorized operation was the introduction to 3-4 cc colorant solution (Schiff reactive, prepared in the laboratory) from biological material placed in glass ampoules, at room temperature. After maximum 30 minutes, the meristematic tissues were coloured in purple-red.

After colouring, the biological material was processed into temporary squash microscopic preparations and studied to optic microscope. It was studied 500 cells for each variant, calculated the mitotic index, and it was recorded any chromosomal aberrations.

Mitotic index ($I_{m\%}$) was calculated using this formula:

$I_{m\%} = N_m \times 100/N_t$ (N_m - Total number of cells in mitotic division; Nt - Total number of cells).

The mitotic index of each stages of mitosis was calculated using these formulas:

I_m prophase% = $N_{prof.} \times 100/N_m$ ($N_{prof.}$ – Number of cells in prophase; N_m - Total number of cells in mitosis);

I_m metaphase% = $N_{met.} \times 100/N_m$ ($N_{met.}$ - Number of cells in metaphase);

I_m anaphase% = $N_{anaf.} \times 100/N_m$ ($N_{anaf.}$ - Number of cells in anaphase);

I_m telophase% = $N_{tel.} \times 100/N_m$ ($N_{tel.}$- Number of cells in telophase).

The frequency of chromosomal aberrations was calculated using this formula:

CA% = Nab x 100 / Nt (Nab - number of aberrant cells; Nt - total number of cells).

RESEARCH RESULTS

Experimental results concerning the disturbance of the mitotic activity to sunflower treated with Propaquizafop are reported in table 1. In this respect, the mitodepresive effect, expressed by decrease of mitotic index, was variable

from 27.01% to 9.08% limits. Thus, untreated control variant recorded the highest mitotic activity (27.01%), followed by V1/10 ppm (21.35%), V2/20 ppm (16.60%) and V3/50 ppm (9.08%). The analysis of the results demonstrates a decrease of the mitotic index, in direct correlation with the increase of concentration of herbicide (Figure 1). The sunflower meristematic cells reacted differently in each phase of mitotic division to the action of the Propaquizafop (Figure 2). In this context, mitotic index of prophase ($I_mP\%$) recorded values between 48.1% (V1), 46,7% (Control), 44.8% (V3) and 43.7% (V2). Mitotic index of metaphase ($I_mM\%$) recorded values between 22.1% (V2), 20.9% (Control), 20.3% (V1) and 19.8% (V3). As regards the anaphase mitotic index (I_m A%), its values ranged by 14.3% (V3), 14.1% (Control), 13.8% (V2) and 12.8% (V1). Mitotic index of telophase (I_m T%) has registered variable values: 21.1% (V3), 20.4% (V2), 18,8% (V1) and 18.3% (Control).

Chromosomal aberrations are the changes occurring in the structure of the chromosome; the appearance of these aberrations indicates the harmful effect of the chemical agent to the plant cells (Shilpa N. et al, 2013; Petrescu I. et al., 2015; Sărac I. et al., 2015).

Concentration of the herbicide has determined the appearance of some chromosomal aberrations to sunflower. Thus, while in the case of control variant it was not recorded mitotic abnormalities, on the contrary in the treated variants were identified aberrant cells with a frequency of 8% (V1), 11% (V2) and respectively 21% (V3) (Figure 3). The main mitotic anomalies were metaphase and telophase stickiness, ring chromosomes and multinucleated cells (Figure 4).

Klasterskii et al. (1976) and McGill et al. (1974) quoted by Grant W. (1978) suggested that chromosome stickiness appear from improper folding of the chromosome fiber into single chromatids. As a result there is an intermingling of the fibers, and the chromosomes become attached to each other by means of subchromatid bridges.

Ring chromosome formation may occur through breaks in the chromosome arms and fusion of the proximal broken ends, which leading to loss of distal material. On the other wise, binucleated cells appear as a consequence of the inhibition of cell plate formation and, in case of already binucleated cells may give rise to the multinucleate condition. Mitotic irregularities, such as incomplete anaphases or unequal distribution of the chromosomes to the daughter cells can result in aneuploid or even euploid cells (Grant W., 1978).

A large number of studies have shown that some herbicides act as mutagenic agents. From this point of view, Huseyin I. et al. (2004) states that Linuron, herbicide like any other pesticides in the environment, can be absorbed by higher plants and may adversely affect the genetic systems, thus cause damage to plants. Wide application of herbicides for the control of weeds in agricultural practices is a potential threat to genetic constitution of economically important plants like *Helianthus annuus* (Huseyin I. et al., 2004).

The level of genotoxicity is defined by the percentage of metaphase cells with damaged cells with damaged chromosomes related to all the cells studied. In our experience, most of the chromosomal aberrations were identified in the metaphase and ana-telophase stages. However, study of the any genotoxic substances has a major importance for the protection of the environment because it enables an insight into the influence of these substances to plants, by quantification of chromosomal aberrations.

Table 1

The mitotic index and the mitosis index phases to sunflower* treated with different concentration of Propaquizafop

Treatment	I_m %	The mitosis index phases			
		I_m P %	I_mM %	I_m A %	I_m T %
Control	27,01	46,7	20,9	14,1	18,3
V1/10 ppm	21,35	48,1	20,3	12,8	18,8
V2/20 ppm	16,6	43,7	22,1	13,8	20,4
V3/50 ppm	9,08	44,8	19,8	14,3	21,1

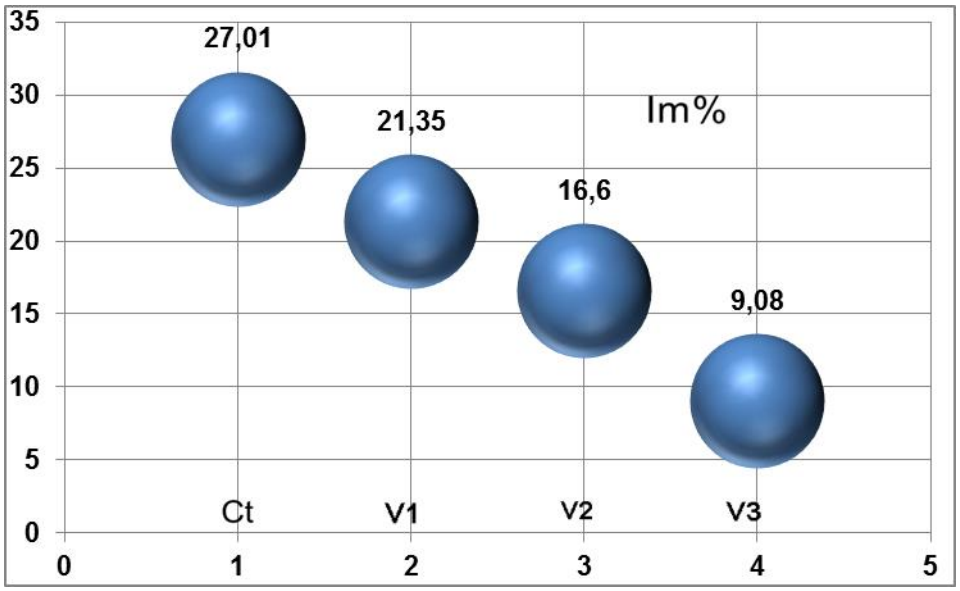

Figure 1. The mitodepresiv effect of the Propaquizafop to sunflower

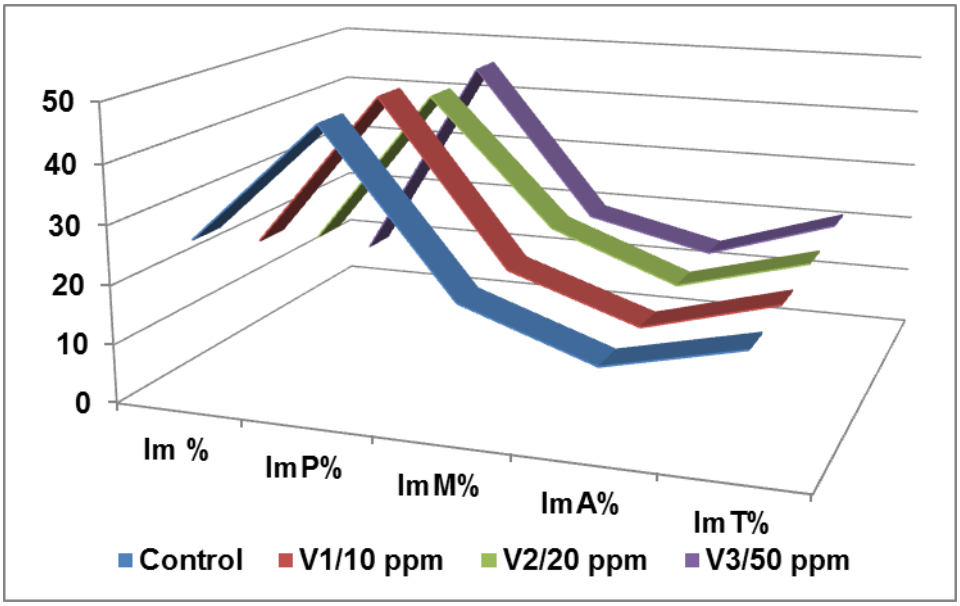

Figure 2. Disturbance of mitotic activity to sunflower under influence of Propaquizafop herbicide

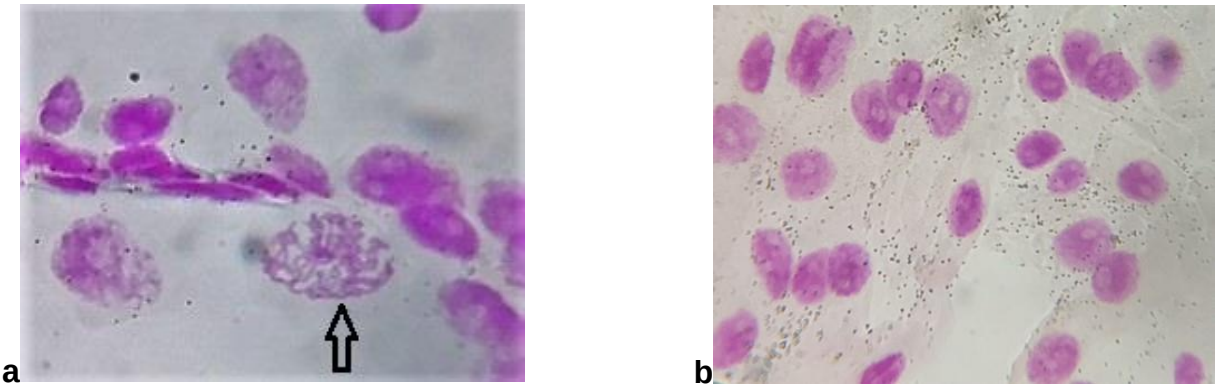

Figure 4. Some aspects of Propaquizafop genotoxicity to sunflower: sticky chained metaphase in a polyploid cell (a) and multinucleated cells (b)

CONCLUSIONS

Study of any chemical substance at cytogenetic level has a major importance for the protection of the environment because it enables an insight into the genotoxic potential of these substances to plants, by quantification of chromosomal aberrations.

The cytogenetic aspects highlighted in meristematic cells of sunflower under action of Propaquizafop were an strong mitodepresive effect at the same time with increased of concentrations; as well as its genotoxic effect, quantified by the occurrence of several types of mitotic anomalies (metaphase and telophase stickiness, ring chromosomes and multinucleated cells). Propaquizafop has induced aberrant cells especially in metaphase and thelophase. These results suggest genotoxicity and mutagenic potential of the Propaquizafop herbicide.

Combating weeds from sunflower crops is a very important action for plant protection, but chemical herbicides can affect the environment and therefore farmers need to use them in low concentrations and need to combine chemical control measures with biological ones.

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