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RESEARCH ON THE EFFECT OF THE DICOPUR ERBICIDE ON GERMINATION, GROWTH AND CELLULAR DIVISION IN ROOT MERISTEMS AT WHEAT (*TRITICUM AESTIVUM*)

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Key words: *Triticum aestivum. DICOPUR D pesticide, biometric measurements, cell division, the mitotic index, chromosomal aberrations*

INTRODUCTION

Wheat culture is a tradition and has been written in the history of peoples since ancient times. Wheat is one of the most important agricultural crops, used to make bread, pasta, starch and glucose, providing the basis for a healthy diet.

However, plant crops are affected by quantitative and qualitative losses caused by the presence of weeds, pathogens and pests. Because of this, various chemicals are used to reduce damage. The reaction of plant organisms to various chemicals has been the subject of many researches (PANDEY RM, 2008; QIAN XW, 2004). The interest shown by researchers in the use of chemical agents has increased as methods of investigation have continued to develop (MA TH , et al., 2005). In recent decades, this research area has expanded considerably, because of the achievement of theoretical and practical results of other related sciences regarding biochemical, biophysical and physiological processes induced by pesticides to living organisms.

Chemistry of agriculture must be organized and applied in such a way that it does not interfere with the health of human beings and useful creatures, nor with the environment. When applied to plants, pesticides can evaporate, be photochemically decomposed, absorbed by plants or be decomposed by metabolic processes whose final product may be more toxic than the original product (NIKONOROW, 1981). In the case of animals, the penetration of pesticides into their bodies can be carried out accidentally or intentionally both through direct contact and the food chain. The ways of disintegration of pesticides in animals are similar to those in plants, but transformations are faster.

The application of pesticides creates the risk of harmful influence on organisms other than those treated (BABU K., 2014). This risk indirectly concerns people through pesticide residues in food. The biotransformation of the various compounds is limited to the discovery and identification of one or more of their derivatives in certain tissues or the elimination by experimental animals previously subjected to the action of these compounds.

Root tip systems of various plants have been widely used for determining the harmful effects of mutagens (TARTAR G. et al., 2006). Research on the influence of pesticides on cell division revealed their harmful effect. The cytogenetic effects of the herbicide Avenoxan, active substance 2,4-D, were investigated in both *Allium cepa L.* and *Allium sativum L.* (TARTAR G. et al., 2006). All of the concentrations of Avenoxan induced abnormalities such as c-Mitosis, chromosome stickiness, bridges, laggards, multipolar cells compared to control. Inhibition of the mitotic index was dependent on the concentration and time of treatment.

Our research aimed at highlighting the influence of treatment with three solutions obtained based on the DICOPUR D herbicide on the germination, growth and cell division of wheat root meristems (*Triticum aestivum*). DICOPUR D (active substance: 2.4 D acid of dimethyl amine salt 600 g/l) is a systemic herbicide of the long-lasting hormonal type that is absorbed partly through the leaves and partly through the roots; the herbicide causes an abnormal growth of the cells (growth becomes uncontrollable), inhibits the respiration and development of the weed root system.

MATERIALS AND METHODS

For the experiments, we used as biological material wheat seeds from the *Crina* variety, purchased from the Secuieni research resort (Neamț County, Romania). For cytogenetic investigations we used microscopic blades and lamellae, solutions needed for preparation and staining of roots, and the binocular microscope NOVEX MICROSCOPE K-RANGE (2000), an OPLYMPUS digital camera, DELL laptop and Manual Cell Counting and Marking – SOFTWARE for results interpretation.

For the research, 50 seeds were counted for each sample, including the control sample. They were distributed in 100 ml Type B Erlenmayer vessels, and pesticide solutions for the experimental variants, and distilled water for the control sample were added (Table 1).

Table 1. Experimental variants used

No.	Experimental variant	Pesticide	Concentration of pesticide solution	No. of seeds	Time of action (hours)
1	CONTROL	-	-	50	-
2	D0,5	DICOPUR	0,5	50	24
3	D1		1	50	24
4	D1,5		1,5	50	24

From the DICOPUR herbicide, three solutions of different concentrations were prepared, depending on the recommended dose for the treatment of weeds. With these solutions, the wheat seeds were treated for 24 hours.

After 24 hours of treatment, the seeds were washed with distilled water to remove pesticide solutions.

To investigate germination and growth processes, 30 seeds/sample were placed in germinators, and for cytogenetic analysis 25 seeds were placed in Petri dishes lined with filter paper wetted with water.

The germinators and Petri dishes were kept in a climatic chamber (LEEC) at 22° C under dark conditions to induce germination. Three days later, the germinators were transferred to another climatic chamber - SANYO, at 22° C under light conditions, 16-hour photoperiod, to continue plant growth and development.

Roots of about 0.8-1.2 cm, hydrolysed and coloured, were used to make microscopic preparations using the "squash" technique. Microscopic preparations were examined under the 40x and 100x lenses.

To calculate the mitotic index (MI), there were analysed 30 microscopic fields/preparations /tested treatment variants.

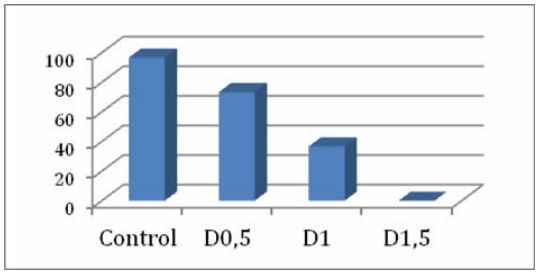
Three microscopic preparations were read for each tested experimental variant. The results were centralized in tables. For the study of chromosomal aberrations, 100 normal and aberrant A-T were observed for each preparation.

Performing the biometric measurements

Seven days later, the seedlings generated from seeds placed in germinators were harvested and subjected to biometric measurements. For each plant, the length of roots and stems was measured. We also weighed the fresh mass of stems and roots using analytical scales.

RESULTS AND DISCUSSIONS

The first observations were made regarding the percentage germination of wheat seeds treated with pesticides compared to the control sample, as highlighted in graph 1.

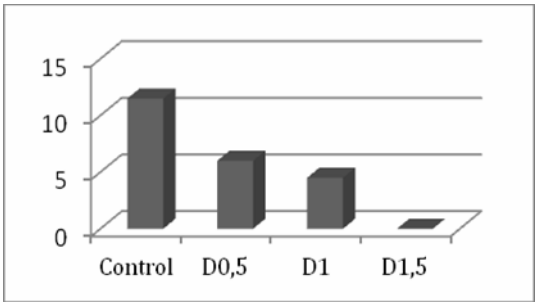


Graph 1. Percentage of germinated seeds

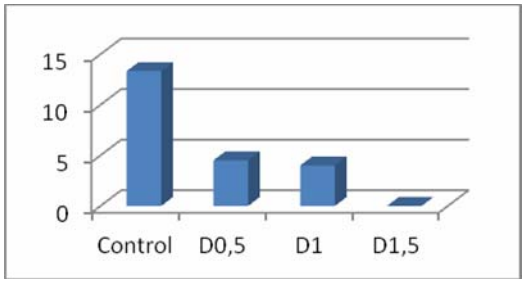
Treatment with DICOPUR pesticide solutions was harmful because the number of germinated seeds was much lower compared to the control variant. The solution D1.5 totally inhibited the germination process, and in the case of the D1 solution, the germination percentage was below 40%.

Interpretation of biometric measurement data

From the statistical calculations regarding the average length of the stems and the root of the wheat plants obtained from the seeds treated with pesticides, we noticed that the highest values were recorded at the control variant, followed by variant D0.5, and then by variant D1 (graphs 2 and 3).

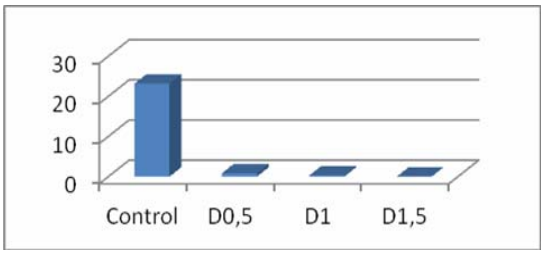


Graph 2. Stem average length

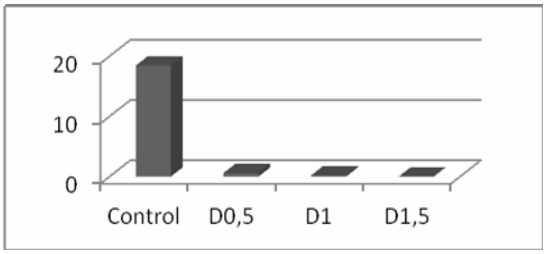


Graph 3. Root average length

Also, regarding the fresh biomass, there are clear differences in the results obtained. Thus, for both fresh stems and roots, the highest values were calculated for the control variant, followed by variant D0.5 (graph 4 and graph 5).



Graph 4. Stems fresh biomass



Graph 5. Roots fresh biomass

The pesticide DICOPUR showed an inhibitory effect on the processes of germination, growth and development of wheat plants, and the data obtained were much lower compared to the control variant.

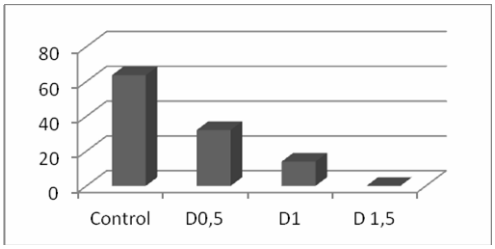
Interpretation of cytogenetic observation data

Cells were identified in all phases of the mitotic division, with different frequencies depending on the concentration of the pesticide solutions tested. In the case of seeds treated with DICOPUR solution, concentration 1.5, the seeds did not germinate, therefore, in this variant, cytogenetic studies could not be carried out. Cytogenetic investigations carried out on root meristems also identified cells with different types of chromosomal aberrations.

Their frequency varied according to the type and concentration of pesticide solutions used in our experiments.

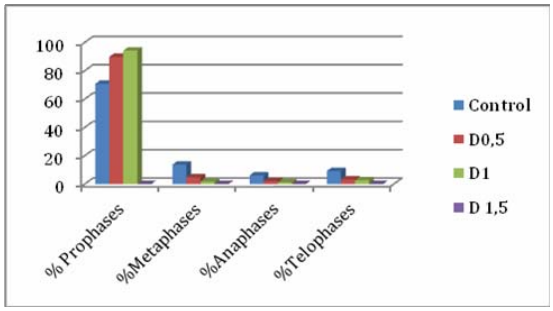
➢ *Mitotic index and cell frequency in different phases of the mitotic division*

The most intense mitotic activity was obtained in the case of seeds which constituted the control sample (MI-63.62). In the case of seeds treated with the DICOPUR pesticide, the mitotic activity was inhibited, the values of MI obtained being much lower compared to the control sample. The lowest calculated MI value was for sample D1, namely 13.96.



Graph 6. MI calculated for the analysed samples

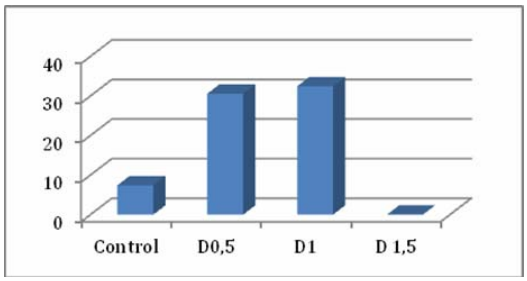
Regarding the frequency of the cells on the different phases of the mitotic division, both in the control variant and in the other analysed samples, it can be seen from graph 7 that the percentage of cells in the prophase is very high compared to the other phases of the mitotic division. In terms of percentage, the prophases are followed by metaphase cells, then by those in the telophase, and the lowest percentage was recorded for anaphase cells.



Graph 7. Frequency of cells identified in the 4 phases of mitotic division

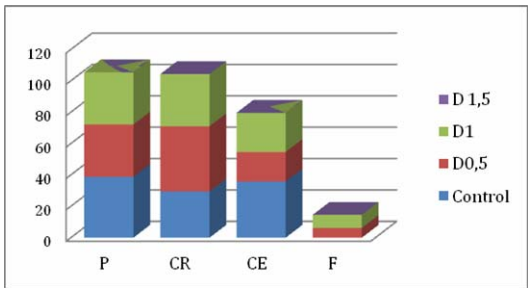
Frequency of ana-telophase cells (A-T) with chromosomal aberrations

Cytogenetic observations revealed at all experimental variants, including the control sample, cells found in different phases of the mitotic division with chromosomal aberrations. The highest percentage was represented by the ana-telophase cells, and their frequency varied according to the experimental variant tested. A high percentage of aberrant ana-telophase cells has been identified in the case of seeds treated with DICOPUR pesticide solutions (graph 8).



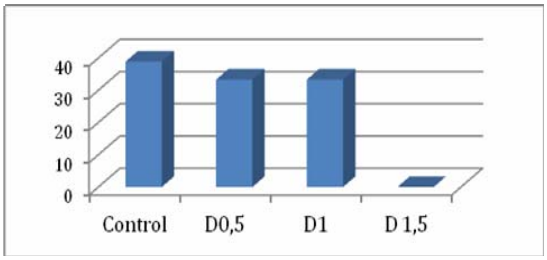
Graph 8. Percentage of ana-telophase cells with chromosomal aberrations

Of the types of chromosomal aberrations, the most frequent ones were A-T ana-telophases with bridges (B), followed by A-T with retarded chromosomes (RC), (photo 1) and A-T with expelled chromosomes (EC). And the fewest were A-T with fragments (F), (photos 5) (graph 9).



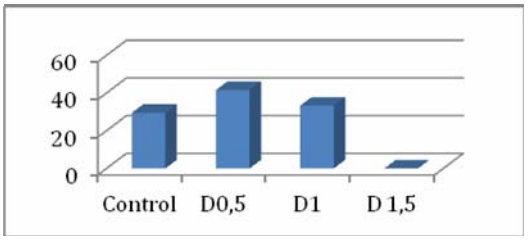
Graph 9. Types and frequency of identified chromosomal aberrations (P – bridges, CR - retarded chromosomes, CE - expelled chromosomes, F-fragments)

In the case of ana-telophase cells showing chromosomal bridges (photo 6), most were identified for control sample seeds, followed by the seeds from samples D1 and D0.5 (graph 10).



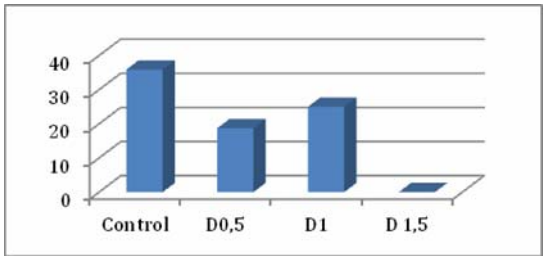
Graph 10.Percentage of A-T cells with bridges

The percentage of delayed chromosomal ana-telophases was rather high for most experimental variants, including the control sample. The highest frequency was identified for variant D05, followed by very close values for the D1 and control samples (graph 11).



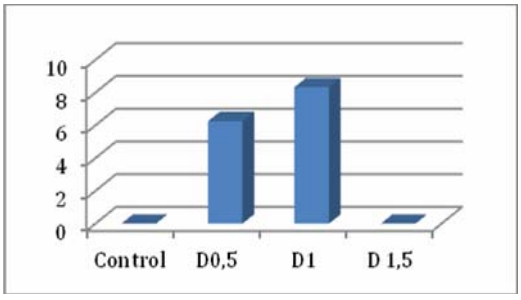
Graph 11. Percentage of A-T cells with retarded chromosomes (RC)

In the case of A-T cells with expelled chromosomes, the most numerous cells were identified in the control sample, and the fewest in the M05 variant. For the D1, M1 and M1.5 variants, the EC-cell frequency was quite high and recorded close values (graph 12), (photo 4).



Graph 12. Percentage of A-T cells with expelled chromosomes (EC)

The fewest aberrant ana-telophase cells, compared to all types highlighted, were the A-T with fragments, (photo 8). Regarding their percentage in the analysed samples, the highest weight was observed on cytogenetic preparations for sample M1, followed by the sample D1. In the control variant, A-T cells with fragments were not identified (graph 13).



Graph 13. Percentage of A-T cells with fragments (F)

Some chromosomal aberrations have also been identified in metaphase (disorganized metaphases, chromosomes expelled from the metaphase plate, fragments) (photos 2; 3), prophase (photo 7) and even interphase (where the presence of micronuclei has been reported).

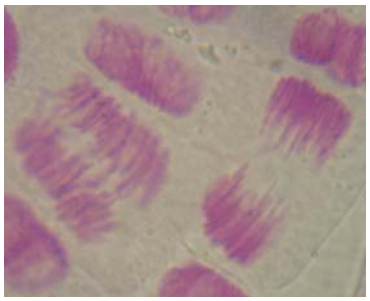


Photo 1. Anaphase with bridges and telophase with delayed chromosomes

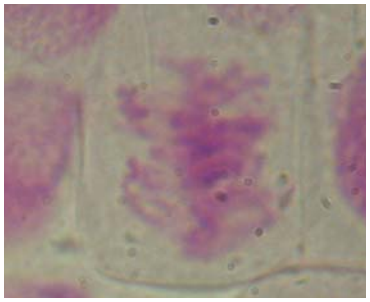


Photo 2. Metaphase with expelled chromosomes from the metaphase plate

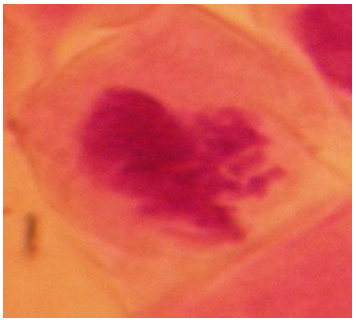


Photo 3. Disorganized metaphase

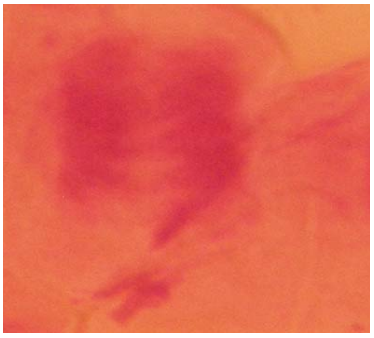


Photo 4.Anaphase with bridges and expelled chromosomes

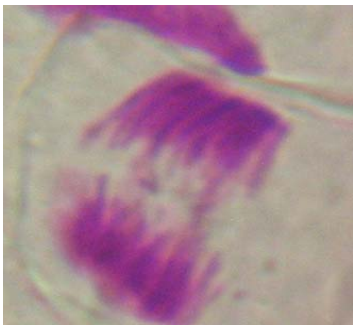


Photo 5. Anaphase with bridges and fragments

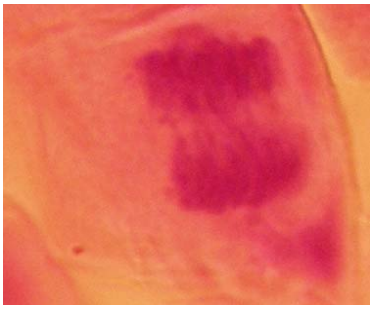


Photo 6. Anaphase with bridges

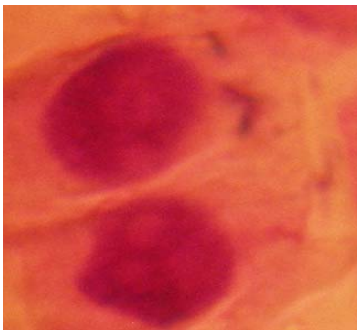


Photo 7. Prophase expelled chromosomes and fragment

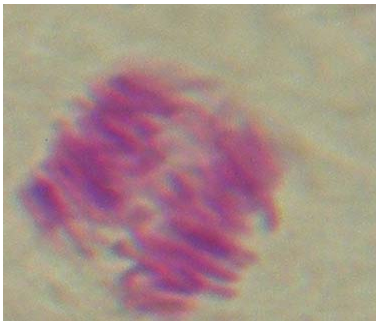


Photo 8. Ana-telophase with bridges, delayed chromosomes and fragments

CONCLUSIONS

The investigations on the effect of solutions of the DICOPUR herbicide on seed germination, plant growth and cell division at wheat enabled us to conclude that:

- ✓ The DICOPUR herbicide inhibited seed germination, even at low-concentration solutions; in the case of the highest-concentration solution, the germination phenomenon was totally inhibited;
- ✓ The mitotic activity of the cells in the root meristems treated with the three solutions of DICOPUR was much lower compared to the control sample;
- ✓ The highest value of the mitotic index was calculated for the seeds from the control sample, and the lowest value of the MI was calculated for the D1 variant;
- ✓ Cells were recorded in all phases of the division, the highest percentage being represented by prophases, followed by metaphases, telophases and anaphases, in all tested samples, including the control sample;
- ✓ A wide spectrum of chromosomal aberrations was identified, especially in cells in the ana-telophase of the mitotic division, but also in metaphase, prophase and interphase;
- ✓ The highest frequency in all tested variants was recorded for the ana-telophases (A-T) with bridges (B), followed by A-T with retarded chromosomes (RC), expelled chromosomes (EC) and A-T with fragments (F);
- ✓ The frequency of chromosomal aberrations was higher in the case of root meristems developed from wheat seeds treated with DICOPUR solutions.

ABSTRACT

In our research, the effect of the DICOPUR D pesticide on the germination of wheat seeds, plant growth, as well as on cell division in root meristems was studied. The results have shown that the pesticide used inhibits germination even at low concentrations of the test solution; plant growth and development is also negatively influenced, and in terms of cell division, the mitotic index is much lower compared to the control variant. The percentage of cells with chromosomal aberrations was higher for samples tested with DICOPUR solutions. The 1.5 concentration of the DICOPUR solution resulted in total inhibition of the germination process of the wheat seeds.

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