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CLASTOGENIC POTENTIAL OF SOME CHEMICALS USED IN AGRICULTURE MONITORED THROUGH THE ALLIUM ASSAY

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Abstract

Many of the higher plants can be genetic models for the detection of environmental mutagens. One of the species often used in monitoring environmental pollution with pesticides is onion (Alium cepa). The Allium assay (Aa) is used with a high frequency in many studies to evaluate the cytotoxic and genotoxicity effects of various chemical substances in agricultural plants. This is based on the fact that compared to using animals for testing the Aa is more cost-effective and provides a large amount of data using a simple cultivation protocol without ethical concerns. In this context, the purpose of this study was to evaluate the clastogenic potential of two pesticides (Rancona fungicide and Mospilan insecticide) in plants through the Allium assay, using onion meristematic roots as biological material. The meristematic roots were exposed for 24 h to three different concentrations of pesticides as follows: 0.5, 1, and 2 μg/mL (Rancona) and 0.05, 0.1, 0.2 μg/mL (Mospilan). The obtained results showed that the tested pesticides induced the decrease, in variable percentages, of the mitotic index (MI %) in the onion meristematic cells, in all tested variants. Thus, compared to the control variant, the values of MI in the variants exposed to the pesticide treatment were between 38-54% (Rancona), respectively 30-48% in the variants treated with Mospilan. At the same time, there was a direct correlation between the pesticides concentration and the clastogenic effect observed in onion cells, through the appearance of several types of chromosomal and nuclear aberrations: sticky, fragments, bridges and chromosomal loss; nuclear dissolution and ring chromosomes. These results suggest caution when using the tested pesticides and mandatory compliance with the concentrations recommended by the producers, to avoid negative impact on plants and environment.

Key words**:** *pesticide, Allium assay, clastogenic, aneugenic, mitotic index, chromosomal aberrations*

INTRODUCTION

Conventional agriculture uses chemicals to protect plants and fertilizers to stimulate their growth and production. In organic farming, they are heavily restricted. However, in both types of agriculture, the EU is taking steps to make products safer for consumers. In this respect, EU chemicals and pesticides legislation aims to protect human health and the environment, as well as prevent barriers to trade. This includes rules governing the marketing and use of certain categories of chemical products, a set of harmonized restrictions on the placing on the market and use of specific dangerous substances, as well as rules governing major accidents and the export of dangerous substances [8]. The European Food Safety Authority (EFSA) assesses pesticides from a risk point of view and provides the European Commission and Member States with scientific support in the decision-making process [8].

Many studies show that pesticides are potentially carcinogenic and harmful to human health [4, 20, 22, 24] and to environment [3, 19]. Therefore, it is necessary to extend the assessment of cyto-genotoxicity of these chemicals by using different test systems. One of these test systems is the biological one, which also includes the *Allium* assay (Aa). Plants are effective indicators for the assessment of cytotoxicity and genotoxicity of chemical compounds in both plant and animal cells, as well as for in situ monitoring of environmental pollutants. Onion (*Allium cepa*), one of the frequently used indicator plants in biological tests, is a bulbous plant that belongs to the family *Amaryllidaceae*, genus *Allium*. Its efficiency as a toxicity bioindicator has been proven by the results of many studies [2, 5, 6, 7].

Rancona is a fungicide used for plant protection, which contains two active substances: 20 g/liter ipconazole + 50 g/liter imazalil. The fungicide is presented in the

form of microemulsion with contact and systemic action, for the treatment of cereal seeds (wheat and barley). The dose recommended by the producerer: 1 liter/to of seeds (wheat) and 1.3 liters/to of seeds (barley). According to product information, Rancona provides excellent control of *Tilletia spp*. disease with both seed and soil transmission. It provides protection and safety to the seeds, favoring the complete and rapid emergence of the crop, without the need to increase the sowing dose [21].

Mospilan (active substance acetamiprid, 200 g/kg) is a systemic insecticide from the group of neonicotinoid products, with a broad spectrum of control. According to product information, it has a rapid effect, affecting the nervous system of insects that paralyze and die. This insecticide affects all development stages (egg-larva-adult); it is not affected by temperature, and its action lasts over three weeks. After application, the insecticide quickly penetrates the plants and is not washed away by rain or irrigation water. The dose recommended for onion by the producerer: 0.2 Kg/Ha, with a break time of 14 days [17]. Although their use brings an obvious profit to farmers, pesticide residues pollute the environment in a worrying way, especially in developing countries. Moreover, the danger extends to ecosystems and human population health. In the specialized literature there are many studies that use different biological tests to evaluate the cytotoxic and genotoxic potential of pesticides [9, 14, 15, 16]. In this context, the objective of this study was to evaluate the cyto-genotoxicity, via clastogenic potential, of two frequently used pesticides (Rancona and Mospilan) in plants, through the *Allium* assay, using onion meristematic roots exposed for 24 h to different concentrations of pesticides.

MATERIALS AND METHODS

The experiments were carried out in the Genetics laboratory of the Faculty of Agronomy, University of Craiova.

The two types of pesticides were purchased from a local phytosanitary store and the onion bulbs were purchased from the central market Craiova (Dolj County).

The biological material consisted of onion roots obtained from bulbs of medium size and weight put to germination in water (the control group) respectively in the pesticide solution with various concentrations, to determine the effective concentration value (EC_{50}), through the method described by Ozkara [18]. First of all was determined the inhibition of the onion meristematic roots growth. The range of concentrations for establishing the EC_{50} value was chosen starting from values that vary between three times higher and three times lower than the concentration recommended by the producers. Then, the lengths of roots were plotted against pesticides concentrations and the point showing 50 % growth was considered EC_{50} concentration. Thus, it was found that the dose that caused a 50% shortening in root length compared to the control group (i.e the EC_{50} values) were 1 μg/mL (Rancona) and 0.1 μg/mL (Mospilan).

The meristematic roots were exposed for 24 h to three different pesticide concentrations as follows: 0.5, 1, and 2 μg/mL (Rancona fungicide) and 0.05, 0.1, 0.2 μg/mL (Mospilan insecticide). These concentrations were established based on the EC_{50} value for each pesticide, namely: $1/2EC_{50}$; EC_{50} and $2xEC_{50}$.

A number of 10 onion bulbs were used for each treatment variant. After the 24 hours of exposure to pesticides, the onion roots were harvested and processed so that they could be studied under a microscope by going through the fixation stage (for 24 hours in Carnoy's fixative); hydrolysis (in 1N HCl for 5 minutes followed by 50% HCl for 15 minutes) and staining with Schiff's reagent.

Microscopic analyses were performed on Optika digitale microscope at 1000x magnification and 1,000 cells were counted in each microscopic slide.

To evaluate the cytotoxic and clastogenic potential of pesticides, the following calculation formulas were used:

Mitotic Index (MI%) = (Number of mitotic cells/Total number of cells) x 100;

Mitotic index of prophase (MIP%) = (Number of cells in prophase/Number of dividing cells) X 100;
Mitotic index of metaphase (MIM%) = (Number of cells in metaphase/Number of dividing cells) x 100;
Mitotic index of anaphase (MIA%) = (Number of cells in anaphase/Number of dividing cells) x 100;
Mitotic index of telophase (MIT%) = Number of cells in telophase/Number of dividing cells) x 100;
Chromosomal and nuclear aberrations (CNA%) = Total number of aberrant cells/Total number of cells in division x 100.
Analysis of variance (ANOVA) and statistical analyses were performed using SPSS package program. Also, the standard deviation (SD) was used at a probability level of $p \leq 0.05\%$ subsequent to the ANOVA analysis.

RESULTS AND DISCUSSIONS

The mitotic index (MI) is a parameter that allows estimating the frequency of cell division, and the inhibition of mitotic activity in certain percentages suggests the phenomenon of cytotoxicity in plants [12].

Table 1. The cyto-genotoxic potential of the tested fungicides throught Aa

Pesticide /Variant /Conc. (µg/mL)	MI (%)±SD	MIP (%)	MIM (%)	MIA (%)	MIT (%)
Rancona					
Control	71.23±1.52	36.29	18.28	9.52	7.14
V_1/0.5	38.15±3.92*	25.31	7.24	3.18	2.42
V_2/1	31.17±4.38*	18.12	8.93	2.15	1.97
V_3/2	27.31±1.68*	12.54	7.23	4.40	3.14
Mospilan					
Control	83.31±2.19	40.11	20.33	11.85	11.02
V_1/0.05	40.15±4.12*	19.02	9.12	6.18	5.83
V_2/0.1	35.95±3.68*	21.37	7.15	4.01	3.42
V_3/0.2	24.65±2.51*	9.45	6.28	5.96	2.96

Aa=*Allium* assay; MI%=Mitotic Index; SD=Standard deviation; MIP%=Mitotic index of prophase; MIM%=Mitotic index of metaphase; MIA%=Mitotic index of anaphase; MIT%=Mitotic index of telophase.
*Mean statistically significant at $p \leq 0.05$ subsequent to the ANOVA analysis.
Source: Own calculation.

The results obtained in this study indicated a decrease of the MI values in all tested variants for both pesticides, which suggests a considerable cyto-genotoxic potential of the two pesticides (Table 1). Thus, following the treatment with Rancona fungicide, MI recorded values of 38.15% (V_1), 31.17 (V_2) and 27.31% (V_3).
In according to Sharma and Vig (2012), a significant decrease in MI suggests genotoxicity effect in cells. When this decrease reaches 50% it indicates a lethal effect on the cells [23].
It can be appreciated that at least in two variants (V_2 and V_3) the Rancona fungicide showed a strong genotoxic effect, even possibly lethal in plant cells. In these variants, %MI compared to Control was only 44% and 38% respectively.
Similar results were reported by other authors who tested other pesticides through Aa, namelly: Mancozeb [10], Imidacloprid and Iprodione [11], mixture of Imidacloprid, Imazalil and Tebuconazole [13], etc.
Regarding the indices of mitosis stages, the values recorded in treated variants were between 12.54-25.31% (MIP); 7.23-8.93% (MIM); 2.15-4.40% (MIA) and 1.97-3.14 (MIT).
As for Mospilan insecticide, MI recorded values of 40.15% (V_1), 35.93 (V_2) and 24.65% (V_3). It can be observed that all three showed a strong genotoxic and even possibly lethal in plant cells. In these variants, %MI compared to Control was reached between 30-48%.
Regarding the indices of mitosis stages, the values recorded in treated variants were between 9.45-19.02% (MIP); 6.28-9.12% (MIM); 4.01-6.18% (MIA) and 2.96-5.83 (MIT). The results obtained suggest a direct correlation between the increase of pesticides concentration and the decrease of MI (Figure 1).

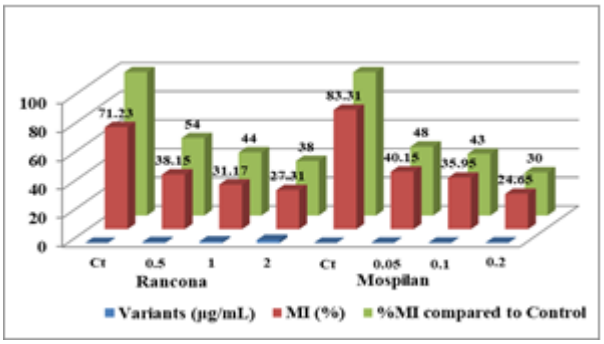

Fig. 1. Correlation between the increase of pesticides concentration and the decrease of MI.
Source: Own design.

Results reported by Adesuyi et al. (2018) suggest that the cytotoxic effects of pesticides on plants may be the direct effect of chromosome aberrations in mitosis [1].

Generally, the chromosomal aberrations occurrence reflects the clastogenic effect of some stressors on plant genome. In this study, the clastogenic and aneugenic potential of Rancona and Mospilan pesticides at the tested concentrations was manifested by the identification of a large number of different chromosomal and nuclear aberrations, such as: sticky, fragments, bridges and chromosomal loss; ring chromosomes, nuclear dissolution, etc. (Figure 2 and Figure 3).

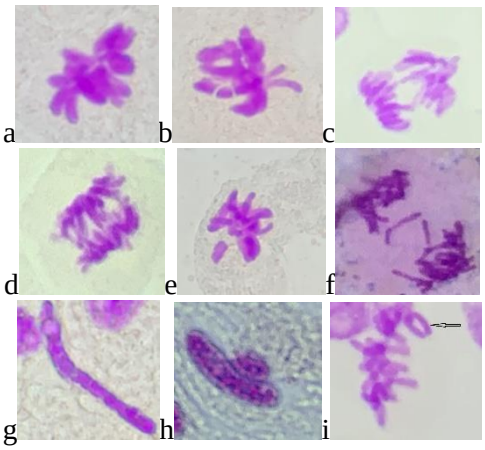

Fig. 2. The chromosomal and nuclear aberrations induced by Rancona and Mospilan pesticides in plant cells through the *Allium* assay: (a) sticky methaphase; (b) fragmented chromosomes; (c, d) bridges; (e, f) chromosomal loss; (g, h) nuclear dissolution; (i) ring chromosome.
Source: Own identification and cuantification.

It was also calculated the chromosomal and nuclear aberrations index (CNA%), the results obtained showing significant and distinctly significant statistical differences from the control (Figure 3).

Thus, in the cells of the meristematic roots of *A. cepa* treated with the fungicide Rancona, various chromosomal and nuclear aberrations were identified, the most frequently encountered being those of the stickiness, chromosomal loss and nuclear dissolution type, all registering CNA values significantly and distinctly significantly higher than the control variant of 29.43% in V_1 (0.5 µg/mL), 38.75% in V_2 (1 µg/mL) and respectively 62.31% in V_3 (2 µg/mL).

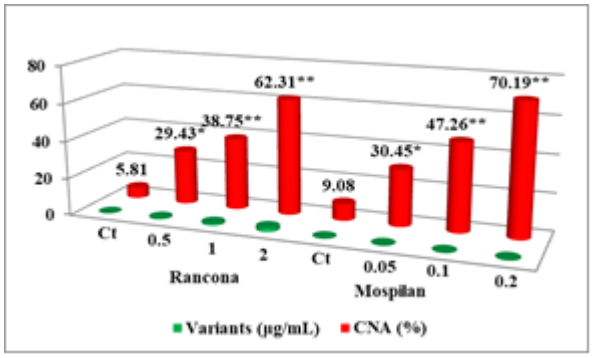

Fig. 3. The clastogenic and aneugenic potential of two pesticides in plant cells quantified by chromosomal and nuclear aberrations index (CNA%).
*Mean statistically significant and **distinctly significant compared to Control.
Source: Own design.

In the case of treatment with Mospilan, the most frequently abnormalities were fragments, bridges and ring chromosomes, all registering CNA values significantly and distinctly significantly higher than the control variant of 30.45% in V1 (0.05 µg/mL), 47.26% in V2 (0.1 µg/mL) and respectively 70.19% in V3 (0.2 µg/mL).

The results obtained indicate a high clastogenic potential of both pesticides, at the tested concentrations, and suggest caution in their use, with mandatory compliance with the doses recommended by the producers. The clastogenic potential is particularly indicated by the appearance of chromosomal aberrations such as bridges, fragments and rings.

Also, the results obtained confirm the suitability of the *Allium* assay for evaluating and monitoring the cyto-genotoxic, clastogenic and aneugenic effects of chemicals in plant and animal cells, as well as the toxicity to the environment.

CONCLUSIONS

Plants are effective indicators for the assessment of cytotoxicity and genotoxicity/clastogenity of chemical compounds in cells and, from this point of view, *Allium cepa* is one of the frequently used bioindicator, through the *Allium* assay protocol.

The results obtained showed a high decrease of the mitotic index values in all tested variants for both pesticides, which suggests

theirs cyto-genotoxic potential in plant cells. Also, the chromosomal aberrations like bridges, fragments and rings reflect the clastogenic effect of tested pesticides on plant genome.

There are not many studies into the clastogenic effects of the tested pesticides in *A. cepa*, although they are frequently used in agriculture. Therefore, further studies are needed, for providing new informations about harmful potential of these pesticides in plant cells and environment too.

However, the obtained results suggest that, at the doses recommended by the manufacturer, both pesticides can be used safely for plant protection. The issue that remains is that of excessive use, without respecting the concentrations recommended and the break time between treatments.

REFERENCES

[1]Adesuyi, A.A., Njoku, K.L., Ogunyebi, A.L., Dada, E.O., Adedokun, A.H., Jolaoso, A.O., Akinola, M.O., 2018, Evaluation of the Cytogenotoxic Effects of Emulsifiable Concentrate form of Amitraz Pesticide on *Allium cepa* L., J. Appl. Sci. Environ. Manage., 22 (11): 1837-1843.

[2]Adrovic, J., Eminovic, I., Stojko Vidovic, S., Feriz Adrovic, F., 2021, Chromosomal aberrations and nuclear anomalies in root tip cells of *Allium cepa* L. caused by radon in water, IJMBR, 9: 25-36.

[3]Alengebawy, A., Abdelkhalek, S.T., Qureshi, S.R., Wang, M.Q., 2021, Heavy metals and pesticides toxicity in agricultural soil and plants: ecological risks and human health implications, Toxicology, 9(3), 42.

[4]Bast, A., Semen, K.O., Drent, M., 2021, Pulmonary toxicity associated with occupational and environmental exposure to pesticides and herbicides, Curr. Opin. Pulmo. Med., 27, 278–283.

[5]Basu, S., Tripura, K., 2021, Differential sensitivity of *Allium cepa* L. and *Vicia faba* L. to aqueous extracts of *Cascabela thevetia* (L.) lippold, South African Journal of Botany, 139: 67–78.

[6]Bellani, L., Muccifora, S., Barbieri, F., Tassi, E., Ruffini Castiglione, M., Giorgetti, L., 2020, Genotoxicity of the food additive E171, titanium dioxide, in the plants *Lens culinaris* L. and *Allium cepa* L., Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 849, 503142.

[7]Bonciu, E., Rosculete, E., Rosculate, C.A., Olaru, A.L., 2022, Optimization of soil pollution monitoring methods by use of biological tests, Scientific Papers Series Management, Economic Engineering in Agriculture and Rural Development, Vol. 22(3), 75-80.

[8]Chemicals and pesticides, 2023. Descriptive sheets about the European Union. https://www.europarl.europa.eu/factsheets/ro/sheet/78/substantele-chimice-si-pesticidele, Accessed on July 14, 2023.

[9]Datta, S., Singh, J., Singh, J., Singh, S., Singh, S., 2018, Assessment of genotoxic effects of pesticide and vermicompost treated soil with *Allium cepa* test, Sustainable Environment Research, 28(4): 171–178.

[10]Fatma, F., Verma, S., Kamal, A., Srivastava, A., 2018, Monitoring of morphotoxic, cytotoxic and genotoxic potential of Mancozeb using *Allium* assay, Chemosphere, 195: 864–870.

[11]Fioresi, V.S., de Cássia Ribeiro Vieira, B., de Campos, J.M., da Silva Souza, T., 2020, Cytogenotoxic activity of the pesticides imidacloprid and iprodione on *Allium cepa* root meristem, Environmental Science and Pollution Research, 27(22): 28066–28076.

[12]Fiskesjö, G., 1994, The *Allium* Test II: Assessment of chemical's genotoxic potential by recording aberrations in chromosomes and cell divisions in root tips of *Allium cepa* L., Environ. Toxicol. Water Qual. 9:234-241.

[13]Ilyushina, N.A., Egorova, O.V., Masaltsev, G.V., Averianova, N.S., Revazova, Y.A., Rakitskii, V.N., Goumenou, M., Vardavas, A., Stivaktakis, P., Tsatsakis, A., 2020, Genotoxicity of mixture of imidacloprid, imazalil and tebuconazole, Toxicology Reports, 7: 1090-1094.

[14]Kutluer, F., Çavuşoğlu, K., Yalçın, E., 2019, The investigation of the physiological, anatomical and genotoxic effects in *Allium cepa* L. of Deltamethrin, Duzce University Journal of Science and Technology, 7: 961-972.

[15]Mercado, S.A., Caleño, J.D., 2020a, Cytotoxic evaluation of glyphosate, using *Allium cepa* L. as bioindicator, Science of The Total Environment, 700, 134452.

[16]Mercado, S.A.A., Caleño, J.D.Q., Jhan Piero Rojas Suárez, J.P.R., 2020b, Cytogenotoxic effect of propanil using the *Lens culinaris* Med and *Allium cepa* L test, Chemosphere, 249, 126193.

[17]Mospilan, https://www.marcoser.ro/, accessed on June 21, 2023.

[18]Ozkara, A., Akyıl, D., Eren, Y., Erdoğmuş, S., 2014, Potential cytotoxic effect of Anilofos by using *Allium cepa* assay, Cytotechnology, 67(5):1-9.

[19]Pathak, V.M., Verma, V.K., Rawat, B.S., Kaur, B., Babu, N., Sharma, A., Dewali, S., Yadav, M., Kumari, R., Singh, S., Mohapatra, A., Pandey, V., Rana, N., Cunill, J.M., 2022, Current status of pesticide effects on environment, human health and it's eco-friendly management as bioremediation: A comprehensive review, Front. Microbiol., 13: 962619.

[20]Pedroso, T.M.A., Benvindo-Souza, M., de Araújo Nascimento, F. et al., 2022, Cancer and occupational exposure to pesticides: a bibliometric study of the past 10 years, Environ. Sci. Pollut. Res. 29, 17464–17475.

[21]Rancona fungicide 15 ME, https://www.pesticid.ro/fungicid-rancona-15-me, Accessed on June 21, 2023.

[22]Schwingl, P.J., Lunn, R.M., Mehta, S.S., 2021, A tiered approach to prioritizing registered pesticides for

potential cancer hazard evaluations: implications for decision making, Environ. Health, 20, 13.
[23]Sharma, S., Vig, A.P., 2012, Antigenotoxic effects of Indian mustard *Brassica juncea* (L.) Czern aqueous seeds extract against mercury (Hg) induced genotoxicity, Scientific Research and Essays, 7(13):1385-1392.
[24]Zheng, W., Luo, B., Hu, X., 2020, The determinants of farmers' fertilizers and pesticide use behavior in China: An explanation based on label effect, J. Clean. Prod. 272, 123054.