CYTOGENETIC ASPECTS HIGHLIGHTED IN MERISTEMATIC TISSUES OF CUCUMBERS (*CUCUMIS SATIVUS*) UNDER ACTION OF COPPER SULPHATE

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ABSTRACT

Folosirea frecventă a pesticidelor în agricultură, precum şi creşterea alarmantă a poluării ecosistemelor justifică evaluarea citotoxicităţii acestor agenți poluatori asupra plantelor. Sulfatul de cupru este folosit pentru combaterea manei la legume, pomi fructiferi și viță de vie. Din acest punct de vedere, este foarte des utilizat în culturile de castraveţi, pentru combaterea manei cucurbitaceelor, produsă de ciuperca Pseudoperonospora cubensis. Scopul cercetărilor a fost acela de evaluare a influenţei sulfatului de cupru asupra activităţii citologice din țesuturile meristematice radiculare la specia Cucumis sativus (castravetele), una dintre legumele foarte preferate de consumatori, pentru prospetimea sa.

Studiul relevă efectul mitodepresiv al sulfatului de cupru precum și o corelaţie directă între concentraţia acestuia şi frecvența modificărilor citogenetice (cromozomi de tip sticky, vagrants, micronuclei, C-mitoze) identificate în țesuturile meristematice de Cucumis sativus. Acest lucru sugerează prudenţă în folosirea excesivă a sulfatului de cupru pentru protecția plantelor şi mai ales, respectarea cu stricteţe a concentraţiilor la tratamente.

The frequent use of pesticides in agriculture, as well as alarming increase of pollution of the ecosystems, justify the assessment of the cytotoxicity of these polluting agents on plants. Copper sulphate is used for the control of downy mildew to vegetables, fruit trees and vines. From this point of view, it is very often used in cucumber crops to combat downy mildew produced by the fungus Pseudoperonospora cubensis. The purpose of research was to assess the influence of copper sulphate on cytological activity in radicular meristematic tissues to Cucumis sativus (cucumber), one of the most popular vegetables for its freshness.

The study reveals the mitodepresive effect of copper sulphate as well as a direct correlation between its concentration and the frequency of cytogenetic changes (sticky chromosomes, vagrant chromosomes, micronucleus, C-mitosis) identified in the meristematic tissues of Cucumis sativus. This suggests caution in the excessive use of copper sulphate for plant protection and, in particular, strict adherence to treatment concentrations.

INTRODUCTION

Vegetables are part of the usual food category, belonging to a segmented binary market, in regular food and festive foods. The cucumber (*Cucumis sativus*) is a vegetable plant of the family *Cucurbitaceae* which includes pumpkin, watermelon and green, etc.; cucumber is original from India and is widely grown throughout the world. Due to its popularity, cucumber is cultivated in all areas of Romania, but only irrigated [9]. In Romania and especially in Oltenia region, drought is one of the major problems that affect crops [2,6].

The diseases of the plants, along with the pests and weeds, cause significant damage to all crops. An important role in diminishing their unfavourable activity is the integrated control. In this way can be obtained seeds with appropriate quality indices and in the planned quantities [8]. *Downy mildew*, caused by *Pseudoperonospora cubensis*, is one of the most important foliar diseases of *Cucurbits*. It has been reported worldwide in production areas where humidity and temperature favour its establishment. Without adequate control measures, downy mildew can result in major crops losses in cucurbits in both open fields and greenhouses. Control of *downy mildew* on cucurbits is achieved by planting resistant cultivars, early planting of crops, and/or fungicide sprays. One of the best known fungicides for combating this disease of the cucumbers is copper sulphate. In general, is used the product called Bouille Bordelaise, but also other fungicides commercial based on copper: tribasic copper sulphate, copper hydroxide, etc., substances which are authorized for use to plant protection on the territory of Romania. Heavy metals (of which copper is part) is the term that is generally used for metals that have a density greater than 5kg / dm^3 and are generally toxic, their residues producing environmental pollution [11].

Soil pollution, as well as its degradation, can severely affect its operation, both the essential support of life on earth, and as a filter for water in its crossing to the water groundwater or its leakage to surface water. In addition, soil pollution can affect or inhibit the plant growth or can introduces some toxic elements into the nutrient chain by absorbing them by plants and their accumulation in organic tissue, the consequence being the appearance of many types of chromosomal aberration in the meristematic cells of the exposed plants.

The superior plants can serve as genetic tests for screening and monitoring environmental pollutants. On the other hand, other plants (e.g. maize) can serve as test plants to determine the allelophathic effect induced by certain species on germination and seedlings growth [1]. One of the plant species susceptible to the genotoxicity of chemicals and other types of pollutants (heavy metals) is *Cucumis sativus*. This species is used less frequently in cytotoxicity tests, unlike other plants, such as *Allium cepa*, for example.

MATERIALS AND METHODS

The biological material consisted of cucumber seeds (*Cucumis sativus*, 2n = 14) purchased from a specialized store in Craiova. The copper sulphate (CuSO4) used is on the list of chemical substances from the Genetics Laboratory of the Faculty of Agronomy, Craiova.

Experience has been established in 3 treatment variants, each with 3 repetitions. The biological material was exposed to copper sulphate for 72 hours, while the untreated control was spraying with plain water. The treatment scheme was as follows:

Ct = spraying with plain water;

V_1 = 1% $CuSO_4$;

V_2 = 1.5 % $CuSO_4$;

V_3 = 2.5 % $CuSO_4$.

The meristematic roots, as a biological material, were obtained by germinating cucumber seeds in Petri dishes on wet filter paper in the above treatment variants. Thus copper sulphate was dissolved in water to obtain different dilution and different concentrations, taking into account the fact that, on the market, there are several types of fungicides based of copper sulphate, each applied at different concentrations. 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. When the meristematic roots were about 2 cm, they were harvested and processed according to a preset protocol for colouring the meristematic peaks using the Feulgen Rossenbeck method. After colouring, the biological material was processed into temporary squash microscopic preparations and studied using the Kruss microscope from the Genetics Laboratory. It was studied 500 cells for each variant, calculated the mitotic index, and it was recorded any chromosomal aberrations.

To determine the mitotic index, the following formula was used:

$$Im\% = Nm \times 100 / Nt$$

(Nm = the number of cells in mitosis; Nt = total number of cells).

To determine the frequency of the chromosomal aberrations, the following formula was used:

$$CA\% = Nab \times 100 / Nt$$

(Nab = number of aberrant cells; Nt = total number of cells).

Figure 1. Aspects from laboratory

RESULTS

The mitotic index is a parameter that allows estimation of the frequency of cell division and the inhibition of mitotic activity is often the indicator of the cytotoxicity effect to plants. Influence of copper sulphate on cytological activity in meristematic tissues of *Cucumis sativus* is shown in table 1.

Table 1.

Evaluation of the mitotic index and chromosomal aberrations to *Cucumis sativus* after seeds treatment with copper sulphate

Variant	$CuSO_4$ conc. (%)	I_m (%)	CA (%)	Total number of aberrant cells	Type of chromosomal aberrations			
					Sticky	Vagrants	C-Mitosis	Micro nucleus
Ct	0	36.5	2	10	8	2	0	0
V_1	1	31.6	6	30	15	7	8	0
V_2	1.5	24.5	9	45	27	6	8	4
V_3	2.5	19.2	23	115	48	20	19	28

Number of examined cells = 500
I_m = Mitotic index
CA = Chromosomal aberrations

Exposure to various concentrations of copper sulphate had different effects on the mitotic index to *Cucumis sativus*. The analysis of the results demonstrates a decrease in the percentage of the mitotic index, in direct correlation with the increase of the concentration of this fungicide (figure 2). Thus, the untreated control variant recorded the mitotic index value of 36.5%; variant V1, 31.6%, variant V2, 24.5%, while in case of variant V3, the mitotic index decreased even more, compared of the control, having a value of only 19.2%. The cells reacted differently in each phase of mitotic division to the action of the chemical agent: the proportion of cells in prophase, metaphase, anaphase and telophase is diminishing compared to the control variant.

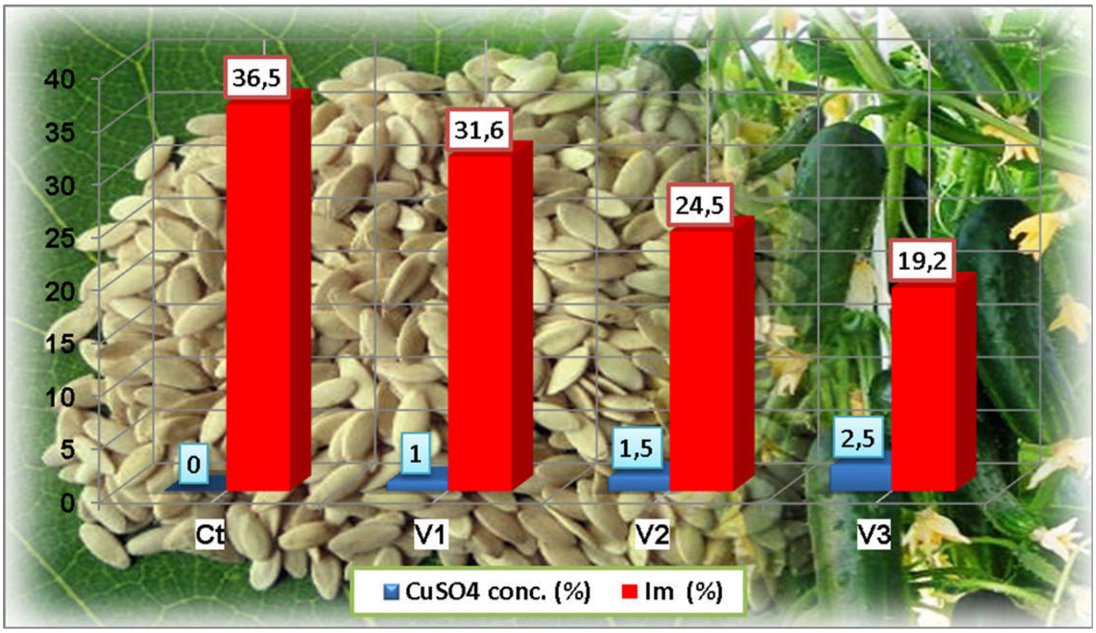

Figure 2. Highlighting the mitodepresive effect of copper sulphate to meristematic tissues of *Cucumis sativus*, after 72 hours of exposure

The concentration of copper sulphate has clearly determined the appearance of a larger one frequency of chromosomal aberrations in the meristematic tissues of *Cucumis sativus*. Thus, if to the control variant were recorded only 2% mitotic abnormalities, in the other variants were identified aberrant cells with a frequency of 6% (V1), 9% (V2) and respectively 23% (V3) (figure 3).

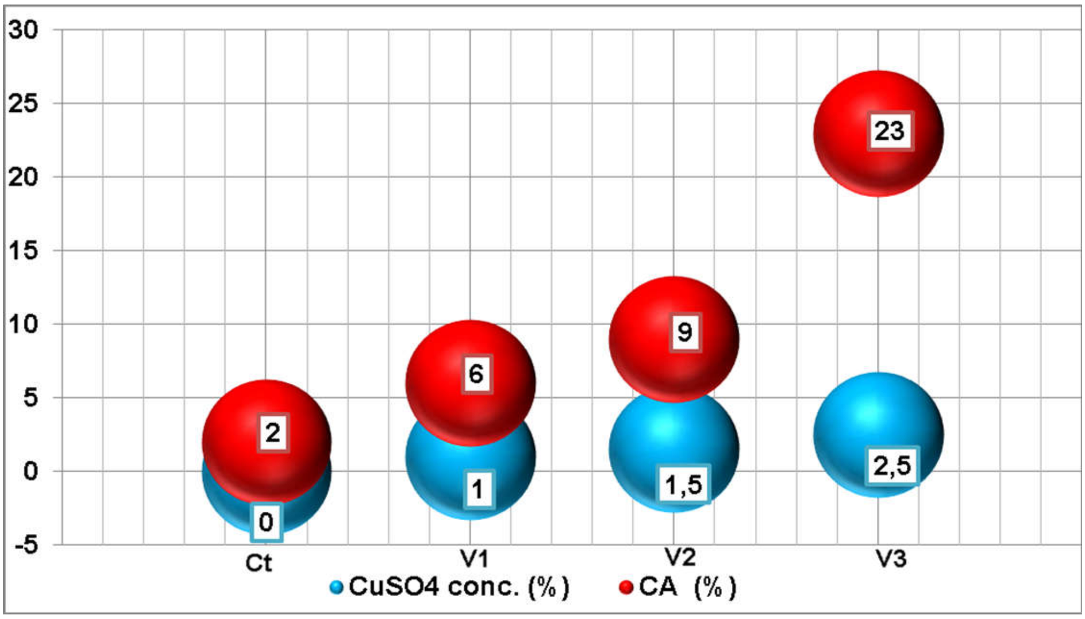

Figure 3. The frequency of chromosomal aberrations in the meristematic tissues of *Cucumis sativus* treated with different concentration of copper sulphate.

The main cytological changes were the following: sticky chromosomes, disturbed prophases with vagrant chromosomes, cells with micronucleus, C-mitosis (figure 4). The chromosomal aberrations of sticky type were observed in a number of 8 cells (Ct) to 48 cells (V3), vagrants chromosomes were observed in a number of 2 cells (Ct) to 20 cells

(V3). In terms of C-Mitosis cells, were observed only for variants exposed to copper sulphate. Micronucleus, a clear sign of cytotoxicity, were identified in V2 (4 cells) and especially V3 (28 cells). Thus, for variant V3, the number of all types of cytogenetic modification was higher than in other variants: 28% cells with micronucleus; 48 cells with sticky chromosomes; 20 cells with vagrant chromosomes and 19 C-Mitosis cells.

a b c

Figure 4. Some cytogenetic aspects highlighted in *Cucumis sativus* meristematic tissues under the influence of copper sulphate: Disturbed prophase with vagrant chromosomes (a); Disturbed metaphase with sticky chromosomes (b) and C-mitosis (c)

Exposure of biologic material to copper sulphate has determined beside mitotic index decrease, a high increase of aberrant cells. CuSO4 induced aberrant cells especially in prophases and metaphases. High rates of aberrant prophases and metaphases were found at 2.5% concentration, followed by 1.25 % concentration.

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 [7]. Generally, the aberrations commonly found in plants chromosomes are as follows: chromosome stickiness, vagrant and lagging chromosomes. Formation of C-mitosis takes place when nuclear division is aborted in the cell after the metaphase and anaphase is blocked [10].

Micronuclei (MN) are extra-nuclear bodies that contain damaged chromosome fragments and/or whole chromosomes that were not incorporated into the nucleus after cell division. MN can be induced by defects in the cell repair machinery and accumulation of DNA damages and chromosomal aberrations. Due to their rapid formation and easy detection, MN has become the most prevalent biomarker of chromosomal defects induced by genotoxic agents [4]. Their possible fate includes expulsion from the cell, reincorporation into the nucleus or retention within the cytoplasm [3].

The mitodepresive effect of some chemicals on *Cucumis sativus* has been demonstrated by other authors too. For example, the Potassium ferricyanide has a mutagen potential on *Cucumis sativus* cells, which is shown by chromosome aberrations induced in anatelophases: chromosome bridges, chromosome fragments, associations between bridges and fragments, retardatary chromosomes, multipolar ana-telophases [5].

CONCLUSIONS

The cytogenetic aspects highlighted in radicular meristematic tissues of cucumbers (*Cucumis sativus*) under action of copper sulphate have materialized by reducing the mitotic index and increasing the frequency of chromosomal aberrations as the fungicide concentration was increased.

The copper sulphate induced aberrant cells especially in prophases and metaphases and the main cytological changes were the following: sticky chromosomes, disturbed prophases with vagrant chromosomes, cells with micronucleus, C-mitosis. High rates of aberrant cells were found at 2.5% concentration, followed by 1.25 % concentration. The occurrence of cytogenetic alterations and especially of cells with micronucleus indicates the harmful effect of the copper sulphate on meristematic tissues to *Cucumis sativus*.

Repeated and especially uncontrolled use of any chemical pesticide leads, invariably, to the accumulation of phytotoxic substances in plants and soil, contaminating them in a way worrying about the environment. However, in the modern culturally technologies use chemicals for the plant protection is an indispensable link. What users must respect is applying any pesticides with great discernment, respecting the application rates and break times as recommended by manufacturers.

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