Cytogenetic assessment of the quality of media treated with *Hermetia Illucens* zoocompost components

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Abstract. The influence of black soldier fly maggots vermiculture zoocompost water extract components on the proliferative activity of root apexes' meristem and the root system growth of *Allium cepa L* was studied. Allium-test was used as a biological analysis, approved by International Program on Chemical Safety (IPCS) for monitoring environmental contaminants, substances, and chemicals. The cytogenetic effects, resulting from the action of zoocompost's water-soluble components and manifesting themselves in abnormalities of mitosis stages, were identified. The «dose-effect» relationship between the water extract components' content and the mitotic activity of *Allium cepa L* meristem was determined. The influence of zoocompost water extract components on the elongation zone of root apexes, which affects root system growth, is demonstrated. It is revealed that the biogenic elements of zoocompost water extract components produce a stimulating effect at certain concentrations. The evaluated doses inhibit the proliferative activity of meristem and the growth of the root system. The inhibiting effect is probably due to the increased salts content in the water extract, which causes plasmolysis of cells' cytoplasm, and alkalosis, caused by the presence of ammonia. It is recommended to take into account the dosage of biologically active substances in zoocompost during the formulation development of fertilizing mixes for plant cultivation.

1 Introduction

Nowadays one of the most widely used integral methods of environmental quality control is biotesting.

Traditionally biotesting is based on monitoring a reaction, registered with instruments, or a quantitatively measurable reaction – a test reaction, of a biological object, sensitive to certain classes of pollutants – a test object [1].

Biotesting allows evaluating both the quality of various media (water, soil, air) and the direct action of various toxicants – mineral and organic, natural and those of artificial origin – on living organisms.

At the same time, when evaluating the environmental quality with biotesting methods one can identify not only the adverse effect of toxicants on biological objects but also the stimulating effect of several factors (physical or chemical) on physiological, cytogenetic, and biochemical processes of living organisms.

Nowadays significant experience is accumulated in applying a wide variety of biological objects for evaluating the environmental quality or a possible influence of certain chemical substances. Such organisms are called test objects, and they can be animal and plant cell cultures, microorganisms, fungi, protophytes and protozoans, and higher plants. The main advantage of biotesting on living organisms is the possibility of in-life observations over responses to action [2–11].

Among higher plants, *Allium cepa L.* (phylum *Angiospermae*, class *Liliopsida*, subclass *Lilidae*, order *Liliales*, family *Alliaceae*, genus *Allium L.*) has been of interest lately for researchers as a test object for evaluating the genetic effect of chemical compounds in water media (natural and sewage waters, etc.) [12–13].

2 Materials and Methods

The result of carrying out the *Allium*-test can appear in form of two effects: the microscopic effect, based on determining the mitotic activity in onion roots apexes meristem, and the macroscopic effect, based on the inhibition of root growth under the action of environmental factors.

To determine the microscopic effect the following parameter was studied: the ratio of cells in mitosis to the total number of cells in the tissue under study. This parameter is denoted as a mitotic index (MI). Mitotic index is expressed per mille, i.e. in the number of cells in the stage of mitosis per one thousand cells of the population.

$$MI = \frac{P+M+A+T}{I+P+M+A+T} \cdot 1000, ‰$$

where *I, P, M, A, T* – different stages of mitosis (interphase, prophase, metaphase, anaphase, telophase).

To determine the possible influence of biologically active substances (BAS), contained in black soldier fly zoocompost, on the mitotic activity of *A. cepa*, a water extract was prepared.

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Zoocompost is a substrate, which contains castings of black soldier fly maggots. The researched zoocompost sample is characterized by brown color, loose texture, and faint ammonia smell.

Extraction of zoocompost BAS with water is explained by the fact that the root system of onion would uptake only water-soluble components. For BAS extraction the tap water was used, and dechlorinated by settling for 24 hours. Then the water was filtered through the folded filter to remove possible mechanical impurities.

Before performing the water extraction of BAS, zoocompost was dried to constant weight at a temperature of 100–105°C. The water extraction was prepared at the ratio zoocompost: water = 1:10. The suspension (zoocompost + water) was placed onto a stirring device LS 110, and the extraction was carried out for 24 hours at room temperature. The substrate was separated by filtration, and the water extraction pH was determined by using an ionometric converter I-500. Hydrogen activity (pH) was 8.32±0.2.

For performing the *Allium*-test procedure a dilution series of zoocompost water extraction was prepared. In the obtained dilutions, the dry residue was gravimetrically determined. As a reference sample, settled dechlorinated tap water was used.

The flasks were filled with water extracts, and the bulbs of *A. cepa* were planted. Healthy undamaged bulbs were selected for the test and graded by size and weight before planting.

For the analysis, it is recommended to take a sampling of 3 bulbs, 3 roots of each one, for each set of experiments [14].

The monitoring of mitotic activity was started at a root length reaching 10 mm and finished at a root length reaching 100 mm on average. Throughout the experiment, the solutions were replaced every two or three days, and the roots were rinsed with distilled water, to prevent spoilage.

Mitotic count was performed in squash preparations, using a microscope Levenhuk 850B.

Fixation, maceration, and staining of onion roots apexes meristem were carried out according to the procedure, described in the work [15].

3 Results and Discussions

The effect of zoocompost BAS on the proliferative activity of onion roots apexes meristem was evaluated by the mitotic index, as well as by the absence or presence of cytogenetic abnormalities in comparison with the reference sample in squash preparations (Figures 1–2, Table 1).

The obtained findings analysis has revealed the progressive increase of mitotic activity with the decrease of BAS dry residue concentration in the black soldier fly zoocompost water extract.

According to the data in Tab. 1, on the fifth day of the experiment the number of mitoses, exceeding those in the reference sample, was observed in samples № 1 and № 2 and had increased by 1.3 and 1.1 times respectively versus the reference sample. No cytogenetic abnormalities were registered in these samples.

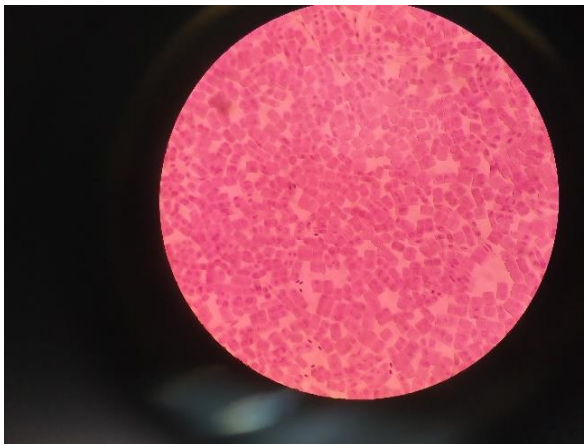

Figure 1. Squash preparation for counting cells in the mitosis stage. Cell fission zone in onion roots apexes. Reference sample.

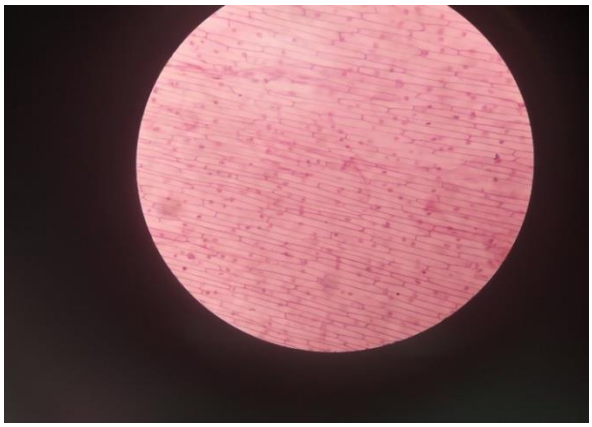

Fig. 2. Squash preparation**.** Cells stretching zone in onion roots. Reference sample.

Table 1. Dependence of mitotic activity in onion roots apexes meristem on biologically active substances contained in the water extract (dry residue).

№	Dry residue content, g/l	Mitotic index, MI, ‰	
		5 days of exposure	10 days of exposure
	Reference sample (tap water)	144±0.1	108±0.12
1	0.035	184±0.12	143±0.15
2	0.05	156±0.2	118±0.2
3	0.11	112±0.15	96±0.1
4	1.58	89±0.1	76±0.15
5	2.18	71±0.12	63±0.12
6	4.45	59±0.4	45±0.2

In samples №№ 4–6 on the fifth day of the experiment, a significant decrease of mitotic activity in *A. cepa* roots apexes meristem in comparison with the reference sample was registered. The proliferative activity was inhibited by 1.3 times in sample №3; by 1.6 times in sample № 4; by 2.03 times in sample № 5 and by 2.4 times in sample № 6 versus the reference sample.

Among the cells of the above-mentioned samples, abnormalities in chromosome disjunction in the metaphase stage were observed. The destruction of

chromatin in the interphase stage, apparently caused by damage to nonproliferating cells, was also registered. In microscopic cells of samples №№ 5–6, the plasmolysis phenomena were observed. The mitotic activity inhibition, as well as the parietal cytoplasm separation from the cell wall, is caused by the action of zoocompost water extract components.

Along with studying the mitotic index, the root growth dynamics of all samples were also determined. As follows from Fig. 3, root system growth is also a plant's response to zoocompost components content in the water extract. Inhibition of root system growth is observed at dry residue content over 0.11 g/l, and the stimulating effect on this parameter is observed at dry residue content below 0.05 g/l.

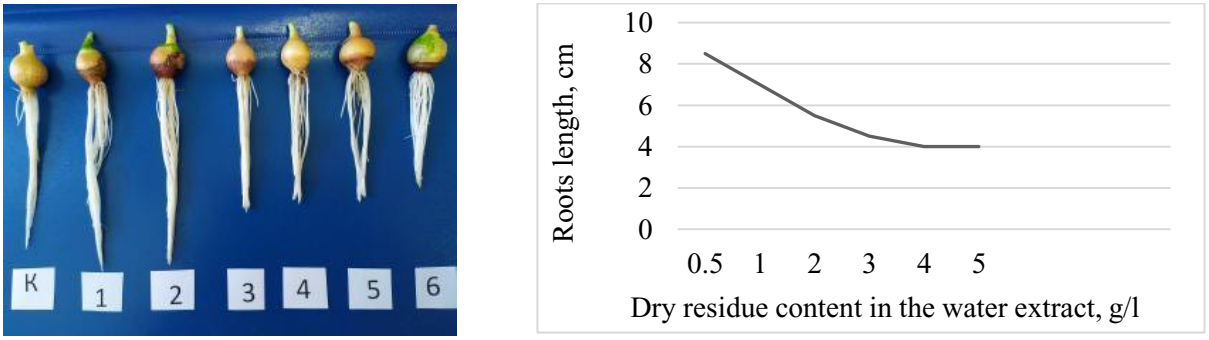

Fig. 3. Dependence of *A. cepa* root length on the BAS content (dry residue in the water extract). Exposure time 10 days. Notations: K – reference sample (settled tap water); 1-6 – dry residue content, g/l, according to data in Table 1.

Earlier, the authors [16–17] determined by chemical analysis methods the prevailing of ammonium cations among nitrogen metabolism products in zoocompost samples. There are data, indicating that the elevated concentration of ammonium ions increases intracellular pH, inhibits several enzymes, causes the formation of free radicals, and results in the ammoniac poisoning of plant cells. The same authors discovered the elevated salt content, with the prevailing sulphates and hydrocarbonates, in zoocompost samples. It can be assumed that in samples №№ 4–6, we observe a cumulative effect of a set of adverse factors, such as the elevated salt content and alkalosis. This affects biochemical and physiological processes in cells of *A. cepa,* which in its turn has a negative impact on the mitotic activity of the researched cell populations [18].

The increase of test objects' exposure to the water extracts components up to 10 days results, as follows from the data in Tab. 1, in the decrease of proliferative activity and, consequently, of the number of mitoses, both in the reference sample, and in the samples under study by 10–15% at an average. The process is accompanied by the growth of vacuoles, filling almost the whole intracellular compartment, and with the passing of young cells from the fission zone to the growth zone (Fig. 2).

At the same time, a persistent dose-positive effect is registered in samples №№ 1 and 2, expressed in the higher mitotic index in comparison with the reference sample. The multiplicity factor of exceeding the reference sample's mitotic index for sample № 1 was 1.3 and for sample № 2 – 1.1.

A persistent dose-negative effect is registered in samples №№ 3–6 (the multiplicity factor of mitotic activity decrease was by 1.2–1.3 times an average). So, samples № 1 and № 2 produce a certain stimulating effect on the mitotic activity of *A. cepa* root apexes meristem cells versus the reference sample.

The obtained experimental data are recommended to take into account at the formulation development of organo-mineral fertilizers on the basis of black soldier fly maggots' vermiculture zoocompost for improving soils fertility and at the formulation development of zoocompost-based fertilizing mixes for plants, such as onion (*A. cepa*).

4 Summary

The findings of the research have allowed establishing that *Allium*-test is characterized by high sensitivity to the action of water extract components, and allows revealing not only the stimulating but also the inhibiting effect from the action of BAS, contained in black soldier fly maggots vermiculture zoocompost. The water-soluble components of zoocompost can produce an integrated cytogenetic effect, manifesting itself in the reduction of proliferative activity of *Allium cepa* meristem cells, in an amount of 0.1–5.0 g/l. The inhibitory effect is apparently due to the suppressive action of elevated salt content and alkalosis, caused by the prevailing ammonium cations among nitrogen metabolism products in the samples.

The stimulating effect on mitotic activity is produced by biogenic elements, first of all, by nitrogen compounds. The recommended dosages for growing media, which don't inhibit the cell fission of *Allium cepa*, and, consequently, don't affect its other physiological parameters, makeup, according to the experimental data, 0.03–0.05 g/l.

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