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AN ONION (*ALLIUM CEPA* L.) AS A TEST PLANT

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ЦИБУЛЯ РІПЧАСТА (*ALLIUM CEPA* L.) ЯК ТЕСТ-РОСЛИНА

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ABSTRACT

Phytotesting has long been used to determine the quality of seeds, agricultural soil fertility, in biomedical research, and recently in the field of environmental protection to assess the ecological quality of natural environment (water, soil) in phytotesting of toxicants. Various plant representatives are used as model test organisms, in particular, onion (*Allium cepa L.*) is considered as a standard test plant for the determination of toxicants.

The purpose of the work is to generalize methodological approaches of using onion (*Allium cepa L.*) in phytotesting of toxicants.

Methodology. During the research, the following methods were used: 1) general scientific methods (methods of theoretical research of available information); 2) analytical and generalized methods (for the analysis of scientific and literature sources on the given problem); 3) empirical (for accumulating facts); 4) methods of argumentation (to prove one's own judgments).

Scientific novelty - methodical approaches of using onion in phytotesting of toxicants are summarized, formulas for calculating phytotoxic indices (root length index, phytotoxic effect of solutions, toxicity index of solutions for each test function, average toxicity index of the tested solutions) are presented.

Conclusions - phytotesting is a commonly used method of assessing the quality of natural environment (water, soil) which is based on the sensitivity of plants to external chemical influences and is reflected in growth and morphological characteristics. The standard test plant for the determination of toxicant influence is the onion (*Allium cepa*), which can be used both in the growth test and in the *Allium*-test. The most sensitive characteristics of onion are the mitotic activity of the apical meristem cells and the frequency of cells with chromosomal aberrations.

Key words: *Allium cepa*, *Allium*-test, phytotesting, phytotoxic indices.

АНОТАЦІЯ

Фітотестування застосовується здавна для визначення якості насіння, родючості ґрунтів сільськогосподарського використання, в біомедичних дослідженнях та не так давно у природоохоронній сфері для оцінки екологічної якості природних середовищ (води, ґрунтів). Різні представники рослин використовуються як модельні тест-організми, зокрема, цибуля ріпчаста (*Allium cepa* L.) розглядається як стандартна рослина для визначення токсикантів.

Мета роботи – узагальнення методичних підходів використання цибулі ріпчастої (*Allium cepa* L.) у фітотестуванні токсикантів.

Методологія. У ході дослідження використано методи: 1) загальнонаукові методи (методи теоретичних досліджень доступної інформації); 2) аналітичний та узагальнений методи (для аналізу наукових і літературних джерел з поставленої проблеми); 3) емпіричний (для накопичення фактів); 4) методи аргументування (для доведення власних суджень).

Наукова новизна – узагальнено методичні підходи використання цибулі ріпчастої у фітотестуванні токсикантів, представлено формули для розрахунків фітотоксичних індексів (індексу довжини корінців, фітотоксичного ефекту розчинів, індексу токсичності розчинів для кожної тест-функції, середнього індексу токсичності досліджуваних розчинів).

Висновки – фітотестування є загальновживаним методом оцінки екологічної якості природних середовищ (води, ґрунтів), природних та синтетичних біоактивних сполук, який базується на чутливості рослин до зовнішнього хімічного впливу, що може викликати зміни фенотипічних та генотипічних характеристик. Стандартною тест-рослиною для визначення токсикантів є цибуля ріпчаста (*A. cepa*), яка використовується у ростовому тесті та *Allium*-тесті. Найбільш чутливими показниками цибулі при дослідженнях токсикантів є мітотична активність клітин апікальної меристеми та частота клітин з хромосомними аберраціями.

Ключові слова: *Allium cepa*, *Allium*-тест, фітотестування, фітотоксичні індекси.

Formulation of the problem

Phytotesting has long been used to determine the quality of seeds, agricultural soil fertility, in biomedical research, and recently in environmental protection to assess the quality of natural environment (water, soil) [23; 25; 40; 50]. Various plant representatives are used as model test organisms, in particular, onion (*Allium cepa L.*) is considered as a standard test plant for the determination of toxicants [11; 13; 38]. Thus, *A. cepa* was used to study the toxic properties of synthetic organic compounds, e.g. derivatives of 2,4- and 2,6-dinitroanilines [39], two new complexes of silver(I) with sulfachloropyridazine [36], N-nitrosodiethylamine [15], drugs [1], pesticides [18; 38]. *The aim* of this work was to generalize the methodological approaches of using *A. cepa* in the phytotesting of toxicants.

Methodology

During the research, the following methods were used: 1) general scientific methods (methods of theoretical research of available information); 2) analytical and generalized methods (for the analysis of scientific and literature resources on the given problem); 3) empirical (for accumulating facts); 4) methods of argumentation (to prove one's own judgments).

Presentation of the main material

The concept of phytotesting. Test plants and their selective sensitivity

Phytotesting as a soil assessment method has long been used to determine the quality of seeds, agricultural soil fertility, in biomedical research, and recently in the field of environmental protection to assess the quality of natural environment (water, soil) [23; 25; 40; 51]. Phytotesting is based on the sensitivity of plants to external chemical influences, that can cause changes of phenotypic and genetic characteristics. Speed, accessibility and simplicity of experiments, reproducibility and reliability of the obtained results, economical and cost-effective value, low requirement for laboratory equipment as well as objectivity of the obtained data are the main benefits for the implementation of the phytotesting methods [25; 51].

There are many recommendations for using one or another species of plant as a test-culture. Thus, wheat seeds (*Triticum* spp.) [12; 32] are used for ecotoxicological assessment. Seeds of oats (*Avena* spp.) [46], radish (*Raphanus sativus* L.) [47], cress (*Lepidium sativum* L.) [3; 14; 24; 33; 46; 48] are recommended for using in laboratory phytotests.

The international ISO 11269-2 standard regulates the choice of at least two species of plants, while one must be monocotyledonous and the other dicotyledonous [26].

Seeds of different species respond selectively to certain classes of pollutants. In particular, the sensitivities of lettuce, sorghum and mustard seeds were studied on soils contaminated with a complex of heavy metals and petroleum products, including polycyclic aromatic hydrocarbons [14]. The researchers established the following order of increasing sensitivity of several plants to soil toxicity: *Lepidium sativum* L. < *Sinapis alba* L. < *Sorghum saccharatum* (L.) Moench.

Biotesting of well water using seed germination, and size and weight of the stem and root of plants of the *Poaceae* family (*Triticum aestivum* L., *Avena sativa* L., *Hordeum vulgare* L.) as the test indicators revealed its low quality, which was confirmed by chemical analysis. At the same time, the seed germination of *T. aestivum* L. (1-2 days) and *H. vulgare* L. (3-4 days), as well as the stem size of *T. aestivum* L. seedlings [45] were effective test indicators.

The manufacturer of the biotest recommend selected plant species included both monocots (*Sorghum saccharatum*) and dicotyledonous plants (*L. sativum* and *Sinapis alba*) [51]. *Sinapis alba* and *Sorghum saccharatum* had less germination than *L. sativum* in soil pollution from railway tracks [51]. At the same time, a monocot plant, sugar sorghum (*Sorghum saccharatum*), is more sensitive to oil-polluted soils [7]. Also, monocot species *Triticum aestivum* L., stimulation was visible in both root length and root number at two or one highest doses, respectively, but dicot species *Lepidium sativum* L. and *Raphanus sativus* L. were generally not sensitive to applied doses of essential oil although a little stimulation effect at some concentrations prevailed over inhibition effect [34].

The estimation of pollution level of aqueous solutions of surfactant-containing dishwashing detergents was performed on the basis of the following characteristics of *Lepidium sativum*: seed germination energy, seed germination and biometric-morphometric parameters (length of aboveground part and roots of seedlings) with further statistical analysis [49].

Allium cepa L. is considered as a standard test object for the determination of toxicants [11, 13, 38]. In particular, *A. cepa* was used as a test organism for determining the toxic properties of such synthetic organic compounds as derivatives of 2,4- and 2,6-dinitroanilines [39], two new complexes of silver(I) with sulfachloropyridazine [36], N-nitrosodiethylamine [15], medicinal drugs [1], pesticides [18; 38]. In these studies, the root length of onion seedlings was measured (growth test), and the mitotic index and chromosomal aberrations in the cells of the root meristem of the seedlings were evaluated (*Allium*-test).

Allium-test is a plant test system for detection and evaluation the mutagenic, mitosis modifying and toxic effects caused by chemical and physical factors by using *A. cepa* plants. The *Allium*-test uses the roots of onion (*A. cepa*) seedlings, which was first proposed by the Royal Swedish Academy of Sciences as a standard test object. In modern studies, *A. cepa* is considered as a reference plant test object for the analysis of mutagenicity, mitotoxicity and toxicity of various factors [44].

There were also reports of the assessment of the toxicity of oil-contaminated soils by the seeds of flax (*Linum usitatissimum* L.) and sunflower (*Helianthus annuus* L.) [22]. Studies of chlorpyrifos (a hazardous insecticide and important pollutant of the environment) conducted on white mustard seeds (*Sinapis alba*) and maize (*Zea mays* L.) showed the sensitivity these cultures [21].

Onion in toxicant testing

Onion (*Allium cepa* L.) is considered as a standard test object for the determination of toxicants [11; 13; 38]. *Allium cepa* (division *Magnoliophyta*, class *Liliopsida*, subclass *Liliidae*, order *Liliales*, family *Alliaceae*, genus *Allium* L.) genus *Allium* L.) is widely used as a test object to assess the influence of chemical compounds, natural and wastewater on organisms' genetic potential. *Allium* has 8 (2n = 16) chromosomes which can be well defined by various dyes. The duration of the cell cycle is approximately 17.8 hours. The mitotic index can fluctuate in different roots of the same plant, but the averaged data are quite stable. The duration of mitosis in different tissues of the *A. cepa* root is the same and does not change along the root length. The ratio of different phases of mitosis does not depend on the fixation time [44].

The *Allium*-test is simple, economical, rapid and sensitive enough to determine whether a factor is «mutagenic» or «non-mutagenic», «cytotoxic» or «non-cytotoxic». The *Allium*-test is recommended for the study of almost any chemical, physical and biological factors. Since the new substances are being synthesized, the test is getting modifications and improvements that makes it one of the most popular [31]. Factors of various origin are suitable for testing, e.g. physical [different types of radiation, temperature], chemical [various chemical compounds or solutions of substances (solutions of various salts, nanoparticles, pharmaceutical and medicinal preparations, dyes, pesticides)], natural and anthropogenic environments (natural waters, industrial emissions / wastewater, mine waters, disturbed ecosystems in areas of mineral extraction), biological [products of living organisms (biotoxins, hormones, metabolic products of cyanobacteria)] [31].

Thus, Fatma et al. examined [18] phytotoxic effects of pesticides mancozeb and chlorpyrifos for A. cepa seeds, via germination percentage, survival percentage, root and shoot length, root shoot length ratio, seedling vigor index, percentage of phytotoxicity and tolerance index. At the same time, different toxicity of the new compounds-derivatives of simazine on the growth of onion roots was established. The calculated phytotoxic effect of the solutions studied substances varied from 22.7% to 29.7% [50].

Toxicants can diffusely invade the plant organism through the roots and aerial organs. Roots absorb substances less selectively than leaves because in root chemicals pass only the cell wall, while in leaf these additionally pass through the cuticle of the epidermis or stomata [44].

The root tip is the part of the plant that first comes into contact with the environment. It contains enzymes that activate promutagens and mutagens [19], so the use of root meristems does not require activation systems in the process of studying those compounds that exert their mutagenic effect through an activated metabolite. This determines the high sensitivity of the root meristem cells to the action of mutagenic factors [30].

Apical meristems are located at the top of shoots (main and lateral) and at the tip of all young roots. This arrangement of meristems is determined already in the initial phases of ontogenesis [4]. Parenchymal cells of the primary (apical) meristem of the division zone have thin walls covered with a root cap [8].

Some authors [2; 9; 17] note that to assess cytotoxicity and genotoxicity, attention should be paid to inhibition of mitotic activity, delay of cells at the stage of prophase or metaphase of mitosis, as well as ratios of the cell number in different phases of mitosis. This can be evaluated using the *Allium*-test [19; 28]. The mitotic activity of onion apical meristem cells is used to determine toxicants, for example to analyze the contamination of agricultural soils [27].

Using *Allium*-test, phyto- and cytotoxic activities of salts of heavy metals and aluminum was evaluated [16-17], 2,4- and 2,6-dinitroaniline derivatives were screened for phytotoxicity and antimitotic activity [36]. For many years, this method remains to be one of the main test systems for evaluation genotoxicity, cytotoxicity, and general toxicity of various factors [5; 44].

Methods of phytotesting with Allium cepa

Growth test

Onion seeds are spread evenly in 50 pieces on filter paper in Petri dishes (d = 90 mm). 5 ml of the test solution (experiment) and distilled water (control) are poured into each Petri dish. The repetition is threefold. The closed Petri dishes are placed at a temperature of 23-24 °C. On the 3rd day, the roots length of the onion seedlings is measured (Fig. 1) [5] and phytotoxic indices are calculated.

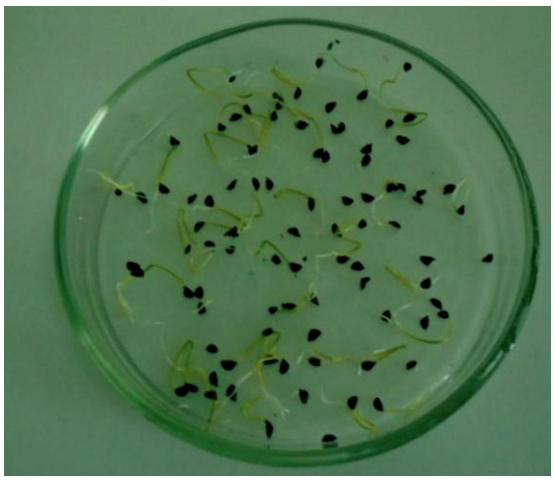

Fig. 1. Seedlings of *Allium cepa* [29]

In particular, researchers use the following phytotoxic indices [5; 6; 10; 37]: root length index (RLI), phytotoxic effect of solutions (PhE), toxicity index of solutions for each test function (TI), average toxicity index of the tested solutions (TI_{avr}), which are calculated according to formulas (1), (2), (3) and (4), respectively.

The root length index:

$$RLI = \frac{L_T(i) - L_C}{L_C}, \quad (1)$$

where RLI is the root length index, $L_T(i)$ and L_C are mean root lengths in test (i) and control, respectively. Phytotoxicity is determined by the following scale [6; 10; 37]:

weak: –0.25 ≤ RLI < 0;
average: –0.5 ≤ RLI < –0.25;
high: –0.75 ≤ RLI < –0.5;
extreme: –1 ≤ RLI < –0.75.

The phytotoxic effect of solutions:

$$PhE = (L_C - L_T) \times 100\% / L_C, \quad (2)$$

where PhE is the phytotoxic effect of solutions, L_C is the root length in control, L_T is the root length of the in experiment.

To obtain comparable data from the test results, the toxicity index of solutions for each test function is calculated according to the formula:

$$TI = (TF_T/TF_C), \quad (3)$$

where TI is the toxicity index of solutions for each test function, TF_T and TF_C are the values of the registered test-respond in the experiment and in control, respectively.

TI below 80% or more, relative to the control, indicates tendency to inhibit growth and development. If these indicators are reduced by two times, then the solution has an inhibitory effect. The tendency to stimulation is determined from an indicator of 120% to the control, an excess of two times indicates a clear stimulating effect [41].

The value of the average toxicity index of the tested solutions is determined by the formula:

$$TI_{avr} = (TI_1 + TI_2 + TI_3 + TI_4) / 4, \quad (4)$$

where TI_{avr} is the average toxicity index of the tested solutions, TI_1, TI_2, TI_3, TI_4 are toxicity indices calculated for each test function: germination energy, germination, root length and aerial part, respectively; 4 – the number of test responses involved in the experiment.

Allium-test (study of the mitotic index, the duration of the mitosis phases and the frequency of cells with aberrant chromosomes in the onion root meristem)

Onion seeds are placed in Petri dishes of 50 pieces on filter paper, which are moistened with distilled water (control) or the investigated solution (experiment). Petri dishes with seeds are placed for 3-4 days in a thermostat at a temperature of 23-24 °C and moistened daily with the same amounts of solutions. The repetitions of experiment and control are threefold [5].

For analysis, seedlings with roots of 0.7-0.9 cm long are selected, fixed in acetic alcohol (3:1), stained in acetofuxin and washed from the dye in a 30% solution of acetic acid [42]. Temporary pressed preparations are made from the root meristem according to the generally accepted method and the mitotic index (‰) is calculated according to the formula (5):

$$MI, ‰ = \frac{P + M + A + T}{I + P + M + A + T} \times 1000, \quad (5)$$

where MI, ‰ is the mitotic index;
I – the number of cells in interphase;
P – the number of cells in prophase;
M – the number of cells in metaphase;
A – the number of cells in anaphase;
T – the number of cells in telophase.

The relative duration of each phase of mitosis (prophase index, metaphase index, anaphase index, telophase index, %) is also calculated using the formula (6):

$$P, \% = \frac{P}{I + P + M + A + T} \times 100 \quad (6)$$

where P, % is the prophase index;
I – the number of cells in interphase;
P – the number of cells in prophase;
M – the number of cells in metaphase;
A – the number of cells in anaphase;
T – the number of cells in telophase [10].

The duration of other phases of mitosis is calculated similarly.

Cells in different phases of mitosis are presented in Fig. 2.

The study of genotoxicity of derivatives is carried out by the ana-telophase method, deter-

mining the frequency of cells with aberrant chromosomes (%) according to the formula (7):

$$FA, \% = \frac{A\,(ab) + T\,(ab)}{A + T} \times 100 \quad (7)$$

where FA, % is the frequency of cells with aberrant chromosomes;

A(ab) is the number of cells in anaphase with aberrant chromosomes;

T(ab) is the number of cells in telophase with aberrant chromosomes;

A is the number of cells in anaphase;

T is the number of cells in telophase [20].

At the same time, it should be taken into account that the total number of viewed anaphases and telophases should be at least 200 [35]. Light microscopy at magnification (×400) is used in research (Fig. 2).

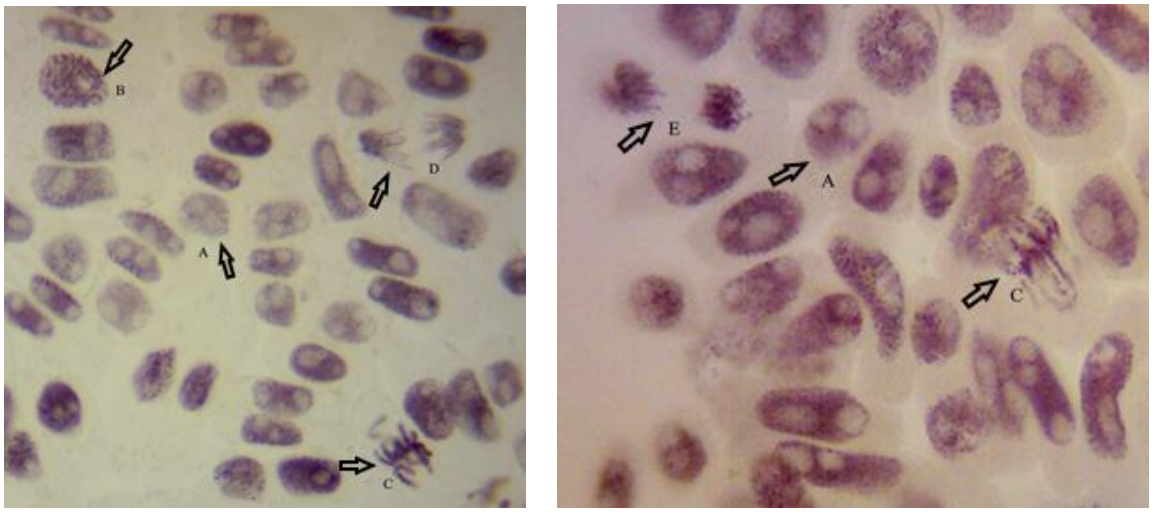

Fig. 2. Microphotographs of mitosis in onion roots (light microscopy, acetofuxin staining, magnification ×400): A – interphase; B – prophase; C – metaphase; D – anaphase; E – telophase (cells in the corresponding phase are indicated by arrows)

At the ana-telophase stage, mutations associated with a gross violation of the structure of chromosomes (a significant damage of chromosome structure), as well as damage to the mitotic spindle (division spindle) or a change in the behavior of chromosomes on the division spindle (Fig. 3) [43] are registered:

- chromosome lag,
- aberrant mitoses:
  1) tripolar mitoses,
  2) quadrupole mitoses,
  3) asymmetric mitoses.

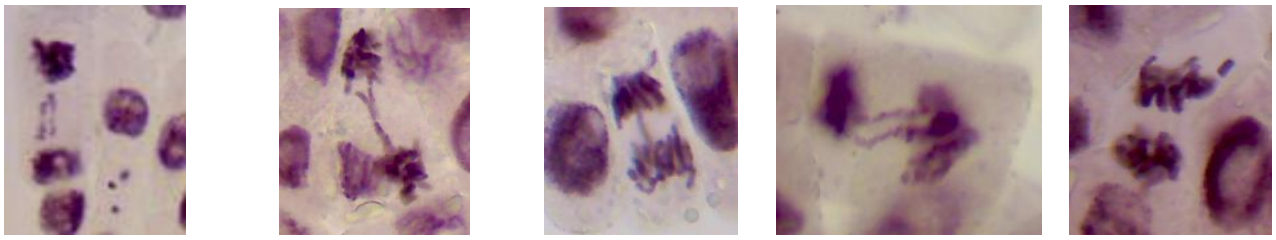

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Fig. 3. Microphotographs of some types of chromosomal aberrations found in cells of the apical meristem of *A. cepa* (light microscopy, acetofuxin staining, magnification ×400): a – fragments in telophase and micronuclei in interphase; b – the bridge and lag of chromosomes in telophase; c – bridge in anaphase; d – double bridge in telophase; e – fragment in telophase

Conclusions

Phytotesting is a commonly used method of assessing the quality of natural environment (water, soil) which is based on the sensitivity of plants to external chemical influences and is reflected in growth and morphological characteristics. The standard test plant for the determination of toxicant influence is the onion (*Allium cepa*), which can be used both in the growth test and in the *Allium*-test. The most sensitive characteristics of onion are the mitotic activity of the apical meristem cells and the frequency of cells with chromosomal aberrations.

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