Cytological Effects of Herbicidal and Fungicidal Pesticides on Root Apex Meristem Cells of Triticum Aestivum

Natalia Menzyanova
Sibirskij federal'nyj universitet Institut fundamental'noj biologii i biotehnologii
Ekaterina Shishatskaya (shisatskaya@inbox.ru)
Sibirskij federal'nyj universitet Institut fundamental'noj biologii i biotehnologii https://orcid.org/0000-0001-7967-243X
Svetlana Pyatina
Sibirskij federal'nyj universitet Institut fundamental'noj biologii i biotehnologii
Tatiana Volova
Sibirskij federal'nyj universitet Institut fundamental'noj biologii i biotehnologii

Research article

Keywords: Triticum aestivum, pesticides, inhibition of root development, mitotic index, chromosome aberrations

Posted Date: June 30th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-35256/v1

Abstract

Using the example of 2-day-old *Triticum aestivum* seedlings, grown in hydroponic culture, the cytological effects of pesticides with various mechanisms of the damaging were studied: the herbicides metribuzin, tribenuron-methyl, fenoxaprop-P-ethyl and the fungicides tebuconazole, epoxiconazole, and azoxystrobin. All pesticides showed a dose-dependent inhibitory effect on the development of the roots of *Triticum aestivum*, but did not affect the mitotic activity and did not reduce the mitotic index of root apex meristem cells. Herbicides and fungicides affected the duration of metaphase, anophase, prophase, and cytokinesis activity in the root apex meristem of the wheat, and these changes were specific for the six studied preparations. Under the influence of all pesticides, the number of abnormal cells increased significantly, to the greatest extent (10–12 times compared with control - distilled water) under the influence of herbicides tribenuron-methyl and phenoxaprop-ethyl and fungicides azoxystrobin and epoxiconazole; to a lesser extent - under the influence of metribuzin and tebuconazole (6–7 times). For all studied pesticides the manifestation of cyto- and genotoxicity, including both clastogenic and aneugenic effects, was revealed in relation to the non-target plant *Triticum aestivum*. Under the influence of pesticides, structural anomalies, caused by chromosome breakdown (bridges, fragments, micronuclei) and spindle damage (lagging chromosomes and their fragments, uneven chromosome separation) were detected in the cells of the *Triticum aestivum* root apex meristem.

1. Background

Intensive farming technologies require the use of a huge variety of chemicals to control pests, weeds and pathogens of cultivated species. In this case, no more than 10% of applied and introduced into the environment pesticides reaches the goal; most of these substances accumulate in biological objects, pollute soils, water bodies, cause the death of beneficial organisms and upset the balance in natural ecosystems. Pesticides are the most dangerous product of human civilization, causing multiple damage to beneficial biota and threatening human health. The use of pesticides for processing crops leads to the inclusion of pesticides in the food chain, which determines the high risk of these pollutants for ecosystems in general, as well as for human health. Pesticides have reproductive toxicity [1, 2], teratogenic activity [3, 4, 5], are endocrine disruptors [1, 6], reduce the activity of humoral immunity [7], have epigenetic activity [8, 9]. Long-term direct contacts with pesticides of people, working in agriculture, significantly increase the risk of developing cancer, diseases of the immune and nervous systems [10, 11, 12]. This determines the relevance of the study of the damaging properties of pesticides, due to their manifestations of mutagenic, carcinogenic and many other negative effects, that pose a danger to biota and human health. Moreover, due to the huge variety of pesticides, used in agriculture, the available literature data on the genotoxicity of chemical plant protection products are far from an exhaustive picture.

The widespread use of pesticides also potentially poses a threat to economically important crops due to cytological damage to plants and a number of side effects [13]. Systemic herbicides not only cause plant death, but also exhibit a chronic toxic effect, exerting a versatile effect on important vital processes: cell division, tissue development, chlorophyll synthesis, photosynthesis and respiration. The mechanism of action of herbicides is based on their diversified influence on the growth and development of the whole plant, its individual organs, tissues and cells, on cell organelles, physiological and biochemical processes. Approximately 25% of all sold herbicides are genotoxic to plants and belong to the group of herbicides with mitotic disorders [14, 15]. This determines the relevance of studying of the biological activity of herbicides, in particular their cyto- and genotoxicity against weeds, as well as non-target higher plants.

Pesticides have selective and targeted activity against mycopathogens and crop weeds. However, the selectivity of pesticide activity is not absolute: the evolutionary conservatism of many molecular-cellular events determines the activity of pesticides with respect to non-target organisms [16, 17, 18, 19, 20, 21, 22]. In this regard, cytogenetic studies of pesticides with respect to cereal crops, which are the main source in the nutritional structure of the world's population, are very important. It is known, that a number of pesticides to one degree or another induce genetic disorders of these valuable crops.

For example, the systemic fungicide Tricyclazole, used on irrigated rice crops, has genotoxic activity: increases the frequency of chromosomal abnormalities [23]. The systemic herbicide Roundup, used to control weeds in cereal crops, increases the number of metaphase disorders in barley, which is due to the negative effect on the mitotic spindle formation [24].

The negative effects of pesticides on wheat are described in a number of publications. This refers to chlorpropham [25]; imazethapyr [26, 27]; butachlor [13]; 2,4-D-isoproturon [14, 28] and others. In these studies, performed on the apical root meristem of wheat seedlings, the cytotoxic and genotoxic effects of these pesticides were identified. However, these fragmentary data do not allow a qualitative picture of the cytogenetic effects of pesticides with a different mechanism of action for wheat, as well as other crops, to be obtained.

This determined the purpose of this work - to study the cyto- and genotoxicity of herbicidal and fungicidal pesticides with a different mechanism of action in relation to a non-target higher plant – wheat, *Triticum aestivum*.

2. Results

The cytogenotoxicity of herbicides and fungicides, differing in the mechanism of action towards biological objects [29], was investigated. The fungicides epoxiconazole (EPO) and tebuconazole (TEB) (fungicides of the triazole group) disrupt the synthesis of sterols, inhibiting demethylation at position C-14. The fungicide azoxystrobin (AZO) (a group of strobilurins) inhibits mitochondrial respiration, disrupting the processes of electron transport in the chain of cytochromes *b* and c_1.

The herbicide metribuzin (MET) (a group of triazines) reduces photosynthesis activity, inhibiting the Hill reaction (water photolysis) and photosynthetic electron transfer between the primary and secondary electron acceptor of photosystem II. The Tribenuron-methyl (TBM) (sulfonylurea group) inhibits acetolactate synthase, disrupting the synthesis of amino acids and proteins. The fenoxaprop-P-ethyl (FEN) hydrolysis products inhibit the key fatty acid synthesis enzyme, acetyl-CoA-carboxylase, which leads to disruption of the biogenesis of cell membranes.

A common feature of the studied pesticides is the presence of planar cyclic structures in the molecule with a double bond system (Table 2), which determines the ability of pesticides to participate in π-π-stacking interactions. Stacking interactions, non-covalent interactions, arise between plane-parallel structures and play an important role in the stabilization of bioorganic molecules. In DNA stacking interactions between parallel nitrogenous bases make a significant contribution to the stabilization of the double helix. In proteins stacking interactions between aromatic amino acid residues also play an important role in maintaining the stability of their 3D structure. The ability of pesticide molecules to participate in the formation of stacking interactions implies their ability to form protein adducts and DNA adducts, and, as a result, to exhibit cyto- and genotoxicity [30, 31, 32, 33, 34, 35, 36, 37].

2.1. The influence of pesticides to the formation of roots of seedlings of Triticum aestivum

The classic bioanalysis, used to determine the amount of pesticides in the soil, uses a single “standard” dose-response curve. This standard curve shows the plant's response to various concentrations of pesticides and provides information on various concepts, related to their effectiveness. A typical dose-response curve has a sigmoid shape; one example of such a curve is a logistic curve [38].

The introduction into the culture medium of the studied pesticides in concentrations of 0.01; 0.10 and 1.00 μg/mL, did not affect the activity of germination of the grains. At a concentration of 1 μg/mL, a significant inhibition of the growth of the roots of wheat seedlings occurred when 3 fungicides were added (Fig. 1a). Inhibition of root growth by pesticides was also accompanied by a dose-dependent decrease in their biomass (Fig. 1b). Among fungicides, the inhibition effect was more pronounced for AZO, under the action of which the absolutely dry root biomass was 2.5 times lower than under the action of TEB and EPO. This is almost 4 times lower than the root biomass in the control (distilled water, no pesticide in the medium).

Under the action of the herbicides TBM and FEN at a concentration of 1 μg/ml, a pronounced inhibition of root growth and a decrease in their biomass were also observed (1.7 times and 2 times, respectively, compared with the control variant) (Fig. 1c, d). MET in the studied concentrations did not significantly affect the root growth of 2-day-old seedlings (Fig. 1c, d).

In the early stages of seedling development, root growth in length is provided by cell elongation. In this regard, the observed inhibition of root growth on media with pesticides can be associated with a disruption in the processes of biogenesis of cell walls, and, as a result, with a blocking of the processes of cell elongation. It is known, that pesticides can inhibit cellulose synthesis. Inhibition of cellulose synthesis leads to remodeling of cell walls (reducing their thickness, changes in chemical composition and changed the distribution of cellulose microfibrils) and disruption of cell elongation processes [39, 40, 41].

Thus, with the exception of metribuzin, inhibition of wheat root growth occurred under the influence of all studied herbicides and fungicides, starting from a concentration of 0.10 μg / mL. At the same time, pronounced changes were recorded at drug concentration of 1.00 μg / mL, which was used for further research.

2.2. The effect of pesticides to the mitotic index of Triticum aestivum root apex meristem cells.

The mitotic index (MI), which is characterized by the total number of dividing cells in the cell cycle [42], serves as a parameter for assessing of the level of cytotoxicity for various chemical agents, including pesticides. The level ofagent cytotoxicity can be determined by an increase or decrease in MI. If MI is significantly lower, than the negative control, then this indicates changes, resulting from chemical effects on the growth and development of the organisms exposed. On the other hand, a MI higher than the negative control, is the result of increased cell division, which can be harmful to cells also, leading to impaired cell proliferation and even to the formation of tumor tissues [43].

The studied pesticides (in concentration in the medium 1.0 μg/mL) had practically no effect on the MI of the root apex meristem of 2-day-old wheat seedlings (Fig. 2).

Therefore, pesticides under the experimental conditions during the study did not exhibit cytotoxicity towards the root apex meristem of 2-day-old wheat seedlings. As noted above, in the early stages of seedling development (2–3 days), the length of the root increases mainly due to the extension of the cell wall and increasing in cell size, while the number of cells does not change, in contrast to later dates. This, in our opinion, is the reason for the absence of a change in the value of MI.

The ambiguity of the experimental data suggests that the cytotoxicity of pesticides depends not only on the dose, processing time, chemical class, but also on the biological characteristics of the test systems: a low basal level of proliferative activity does not reveal a significant decrease in MI under the influence of pesticides, while in actively proliferating systems the cytotoxic effects of pesticides are more pronounced.

2.3. The effect of pesticides to the duration of cell division phases in Triticum aestivum meristem cells.

Herbicides are known to act as mitotic poisons by blocking mitosis in meristems. The plants treated with herbicides causing disruption of cell division have mitotic stages present, but sometimes one or more stages, that are normally present, will be absent or aberrant. The inhibition of cell division is a secondary effect caused by the disturbance of a plant's metabolic process [44].

The studied pesticides had different effects on the duration of metaphase, anaphase, and cytokinesis activity (number of cells in one or another phase of mitosis) in the root apex meristem of *Triticum aestivum* (Fig. 3a, b). So, the fungicide AZO led to an increase in the duration of the metaphase: the number of cells in the metaphase was 1.8 times greater than in the control version. A delay in the division processes at the metaphase stage led to a sharp decrease in the relative number of cells at the telophase stage (Fig. 3a). The fungicide TEB reduced the number of cells at the metaphase stage by 1.6 times, compared with the control. This is probably due to an increase in the duration of prophase, for which a tendency toward an increase in the number of cells was recorded.

The studied herbicides did not affect the duration of prophase; the number of cells in this phase of mitosis did not significantly differ from the control variant. But, at the same time, changes in the duration of other phases of mitosis were observed. Thus, MET reduced the number of cells in metaphase by 1.6 times, compared with the control (Fig. 3b). A decrease in the duration of metaphase was accompanied by an increase in the number of cells at the anaphase stage (1.8 times) and cytokinesis (2 times), compared with the control.

It should be noted, that a characteristic feature of herbicides (in contrast to fungicides) was an increase in the number of cells at the stage of cytokinesis (stage with an incompletely formed intermediate plate, a new cell wall between two daughter cells) (Fig. 3b). For MET, TBM, and FEN, the number of cells at the stage of cytokinesis was 2.0, 3.4 and 3.0 times, respectively, more, than in the control. An increase in the duration of cytokinesis may be associated with herbicide-induced disturbances in cell wall biogenesis.

The toxic effect of pesticides, estimated by the number of abnormal cells, manifested itself in different ways.In the control (germination without herbicides), the number of cells with mitosis abnormalities было (0.68%). The numberof cells with abnormalities increased significantly under the influence of all three herbicides: to 5.43% for MET; and to 8.54 and 9.18%, respectively, for FEN and TBM (Table 1).

A common feature of the action of fungicides was also a significant increase in the number of cells with various mitosis anomalies in the population of proliferating cells of the wheat root apex meristem (Table 1). Moreover, for TEB, the total number of cells with anomalies was almost 2 times less than for AZO and EPO.

In experimental variants with AZO and EPO, the number of cells with anaphase-telophase anomalies (8.92% and 8.74%, respectively) was significantly higher, than for TEB (4.61%). This suggests a most pronounced effects of azostrobin and epoxypron.

2.4. The effect of pesticides on chromosomal abnormalities of Triticum aestivum root apex meristem cells

The results of the revealed chromosomal abnormalities and DNA damage in the meristem cells of the root apex of wheat seedlings during cultivation in a hydroponic culture on media with herbicides are presented in Fig. 4 and Table 2. The criteria for mitosis disorders under the influence of mutagens are aberrations: chromatid type, when the chromosome is doubled (single fragments, single bridges) and the chromosomal type, when the chromosome is not doubled (double fragments, double bridges).

In the course of studies structural anomalies were identified in root apex meristem cells:

a) caused by chromosome breaks (bridges, fragments, micronuclei), clastogenic effects; b) caused by damage of the mitotic spindle (lagging chromosomes and their fragments, uneven chromosome separation), aneugenic effects.

It should be noted, that for each fungicide and herbicide, a specific percentage of cells with various anomalies was formed (Table 1). Presumably, the spectrum of cyto- and genotoxic anomalies is determined by the peculiarities of the chemical structure of pesticide molecules and the relatively selective their interaction with specific molecular targets in plant cell. Among the studied pesticides the fungicide TEB and the herbicide MET had the most pronounced effect on the formation of lagging chromosomes: 0.77 and 1.02% of cells with this damage (Table 1; Fig. 4a, b).

Table 1
The number of cells with chromosomal abnormalities and DNA damage in the root apex meristem 2-day-old seedlings of *Triticum aestivum* when cultured on pesticide media (1.0 μg/mL).

Experimentaloptions	The number of cells with various abnormalities of mitosis, in%					Total number of cells with anomalies (%)
	Laggingchromosomes	Sticking of chromosomes	Anaphasebridges	Chromosomalaberration	Micronuclei	
Thecontrol, distilled water	0	0	0.12	0.56	0	0.68 ± 0.11
Herbicides:						
Metribuzinum	1.02	1.03	1.02	2.36	0	5.43 ± 0.95
Tribenuron-methyl	0.41	2.44	0.81	4.47	0.41	8.54 ± 1.35^
Fenoxaprop-p-ethyl	0.54	5.94	0.54	1.62	0.54	9.18 ± 1.65^
Fungicides:						
Azoxystrobin	0.39*	6.59*	0	1.94*	0	8.92 ± 1.46*
Epoxiconazole	0.23*	4.83*	0.69*	2.07*	0.92	8.74 ± 1.63*
Tebuconazole	0.77	0.77	0.38	2.69	0	4.61 ± 0.86

* - values for azoxystrobin and epoxiconazole, significantly different from the values for tebuconazole ($p \leq 0.05$).

^ - values for tribenuron-methyl and fenoxaprop-p-ethyl, significantly different from the values for metribuzinum ($p \leq 0.05$).

Sticking of chromosomes (Table 1; Fig. 4c) and disruption of the processes of divergence of chromosomes to the poles of a dividing cell can be associated with the formation of disulfide bridges or non-covalent bonds between non-histone chromosome proteins. Among the studied pesticides, the fungicides AZO and EPO and the herbicide FEN had the most pronounced effect on the separation of chromosomes (Fig. 4c): the sticking of chromosomes for these pesticides was the main class of cell division anomalies, from 4.83 to 6.59% (Table 1).

The formation of anaphase bridges is associated with the appearance of dicentric chromosomes. In the root apical meristem the number of cells with dicentric chromosomes was the highest when cultured on media with herbicides, MET and TBM (Figure 4g; Table 1).

Chromosomal aberrations and micronuclei (Fig. 4d - k, l) are the result of double-stranded DNA breaks. Micronuclei are small, surrounded by their own membrane structures, containing fragments of chromosomes, or acentric chromosomes, that were not included in the nucleus of daughter cells in the process of cell division. No micronuclei were detected under the influence of MET and AZO; however, in the case of TBM and FEN, the amount was 0.41 and 0.54%, respectively; under the influence of fungicide EPO this value was maximal (0.92%) (Table 1).

The main class of aberrations, that were observed in the cells of the apical meristem of wheat, when cultured on pesticidal media, are small fragments of chromosomes, that retained their connection with chromatids (Fig. 4e, g). The highest number of cells with chromosomal aberrations was observed during the cultivation of grains on media with the herbicide TBM. This determines the highest level of genotoxicity (the highest activity of the formation of double-stranded breaks in DNA) for this pesticide.

3. Discussion

The work is devoted to the study of the cyto- and genotoxicity of herbicidal and fungicidal pesticides to a non-target higher plant. Object of study - samples of 2-day-old *Triticum aestivum* seedlings, grown in hydroponic culture.

Pesticides are the most dangerous products of the technosphere. At the same time, modern agriculture cannot be imagined without their application. Intensive farming involves the use of enormous amounts of various pesticides to control weeds, pests, and patho-gens of crops. However, most of these substances are accumulated in biological objects, contaminate soil and water environments, harm living organisms and up-set the balance in natural ecosystems.

Systemic herbicides not only cause plant death, but also exhibit a chronic toxic effect, exerting a versatile effect on important vital processes: cell division, tissue development, chlorophyll synthesis, photosynthesis and respiration. The pesticides with a different mechanism of action in

relation were studied: the herbicides metribuzin (MET), tribenuron-methyl (TBM), fenoxaprop-P-ethyl (FEN) and the fungicides tebuconazole (TEB), epoxiconazole (EPO), and azoxystrobin (AZO). Markers of cytotoxicity were the mitotic index and anaphase-telophase anomalies; genotoxicity – chromosomal aberrations and micronuclei.

When studying the effect of pesticides of various concentrations on the growth of wheat roots, it turned out, that with the exception of metribuzin, all fungicides and herbicides significantly inhibited root growth, starting from a concentration of 0.10 μg / mL.It should be noted, that the analysis of literature data on the effect of pesticides on the cytogenetic rearrangements of higher plants, showed a huge (by orders of magnitude) spread of data on the studied concentrations from very high: 0.5-2.0 mg/mL [20] and 5.0–15.0 mg/ml [45] to very low: 0.002–0.4 μg/mL [46]. The authors of the works did not motivate the choice of concentrations, but noted the cytotoxic effects of pesticides, which were detected for both, high and low concentrations.

The mitotic index (MI), which is characterized by the total number of dividing cells in the cell cycle, serves as a parameter for assessing of the level of cytotoxicity for various chemical agents, including pesticides. The studied pesticides had practically no effect on the MI of the root apex meristem of 2-day-old wheat seedlings. A similar lack of cytotoxic effect of the tricyclazole fungicide tricyclazole used in rice cultivation is described by Wandscheer et al. [23]. Using the *Allium cepa* test, the authors showed, that tricyclazole causes chromosomal abnormalities, but does not significantly affect the MI. In a research of Mahapatra et al. [20] examined the cytotoxicity, genotoxicity, and prooxidant activity of herbicides at a concentration of 0.5 to 2.0 mg/mL in fenugreek culture. A high yield of cells with various chromosomal abnormalities was found under the influence of all, including low, concentrations of herbicides. However, herbicides did not affect the MI, which changed only slightly for the highest concentration (2.0 mg/mL). The absence of a decrease in the MI was described by Akhter et al. [47], when studying the cytological effects of herbicides on hexaploid wheat *Triticum aestivum L*. At the same time, a number of studies showed, that individual pesticides in high concentrations negatively affect MI. So, in the *Allium cepa* test herbicides quizalofop-p-ethylandcycloxydimin concentrations 0.5, 1.0% and 1.5%, showed a strong cytotoxic effects. For 1.5% quisalofop-p-ethyl MI decreased from 30.2% (control) to 9.6% [45]. Roundup (glyphosate herbicide) reduced MI in the root meristem of barley seedlings: depending on the dose of the preparation (from 0.36 to 7.2 mg/ml) and the treatment time (3 h and 6 h), MI decreased by 3–5 times [24]. In the root meristem of wheat, a dose-dependent decrease MI was revealed under the influence of the herbicides chlorpropham and imazethapyr [25, 26]; 2.4-D-isoproturon [28]; butachlor [13]. Herbicide dithiopyr caused a cessation of root elongation results in swelling of root tips in wheat, MI decreased as the concentration of the herbicide increased and mitotic cells were arrested in late prometaphase [48]. The MI reduction effect was recorded on germinated corn grains, that were treated with Royal Flo fungicide in various concentrations for 20, 24, and 48 hours [49].

Herbicides are known to act as mitotic poisons by blocking mitosis in meristems. The inhibition of cell division is a secondary effect caused by the disturbance of a plant's metabolic process [44]. The studied pesticides had different effects on the duration of metaphase, anaphase, and cytokinesis activity (number of cells in one or another phase of mitosis) in the root apex meristem of *Triticum aestivum*. So, the fungicide AZO enlarged metaphase period and amount of cells initin 1.8 times compared with the control samples. On thecontrary, the fungicide tebuconazole reduced the number of cells at the metaphase stage by 1.6 times, compared with the control. A characteristic feature of herbicides was an increase in the number of cells at the stage of cytokinesis (stage with an incompletely formed intermediate plate, a new cell wall between two daughter cells) from 2.0 to 3.4 times, respectively, more, than in the control. The effect of pesticides on the duration of various phases of mitosis may be due to the epigenetic activity of pesticides. It has been demonstrated, that pesticides can affect the activity of methylases and rearrange gene expression patterns [9, 50]. It could be possible, that epigenetic rearrangements, induced by pesticides, lead to alterationsof proliferative signaling and to a change in the activity of molecular events in different phases of mitosis. The effect of pesticides on the phases of the cell cycle is described in a number of works. Thus, a study of the effect of butachlor on wheat meristem cells showed, that in root tip cells of wheat, butachlor arrested the mitosis at metaphase and anaphase [13]. In control sets prophase was 50.72%, while it was 55.63% in 1 ppm treatment. However, there was no much significant difference in prophase between control and 0.15 ppm concentration of butachlor. Among the treatment the percentage of anaphase and telophase were decreased, as the concentration of the herbicide increased, when compared to control. On the other hand, the percentage of prophase and metaphase were increased, as the concentration of the herbicide increased, when compared to control. In study Rad et al. [26] on the somatic cells of wheat, the imazethapyr inhibited mitosis and blocked it at the prometaphase, as well. In the control plants, the prophase was 53.40%, while it was 73.22% in the 5 ppm treatment. Among the treatments, frequency of metaphase, anaphase, and telophase decreased.

The toxic effect of pesticides, estimated by the number of abnormal cells, manifested itself in different ways. The number of cells with abnormalities increased significantly under the influence of all three herbicides; from 5.8 times,compared to control,for MET to 8–9 times for FEN and TBM. The number of abnormal cells under the action of fungicides also increased, wherein TEB was twice as high as for AZO and EPO.

The criteria for mitosis disorders under the influence of mutagens are aberrations: chromatid type, when the chromosome is doubled (single fragments, single bridges) and the chromosomal type, when the chromosome is not doubled (double fragments, double bridges). In the course of studies structural anomalies were identified in root apex meristem cells: a) caused by chromosome breaks (bridges, fragments, micronuclei), clastogenic effects; b) caused by damage of the mitotic spindle (lagging chromosomes and their fragments, uneven chromosome separation), aneugenic effects. It should be noted, that for each fungicide and herbicide, a specific percentage of cells with various anomalies was formed.

The main class of aberrations in the cells of the apical meristem of wheat under the influence of pesticides was the appearance of small fragments of chromosomes. Chromosomal aberrations and micronuclei are the result of double-stranded DNA breaks. Under the influence of TBM and FEN, and, especially EPO, the appearance of micronuclei was observed in the apical meristem of wheat, which was not the case with the testing of MET. The number of cells with dicentric chromosomes was highest under the action of the MET and TBM herbicides. The most pronounced effect on the formation of lagging chromosomes was exerted by the fungicide TEB and the herbicide MET. This violation is associated with anomalies in the formation of the spindle of the division, since pesticides can disrupt the polymerization of tubulin [51, 52]. It's known, that the herbicide butachlor act as potent spindle inhibitor. The herbicides bind to tubulin, a major microtubule protein. The herbicide – tubulin complex inhibits the polymerization of microtubule, leading to loss of microtubule structure and function. As a result spindle apparatus is absent, thus preventing the alignment and separation of the chromosome during mitosis. In addition to this cell plate cannot be formed [53].

The fungicides AZO and EPO, as well as the FEN herbicide, had the most pronounced effect on chromosome separation processes. Chromosome sticking for these pesticides was a major class of anomalies. The formation of disulfide bonds can be the result of oxidative modifications under conditions of oxidative stress, which fungicides and pesticides induce in cells [20]. However, direct chemical interactions of pesticides with chromatin, which lead to disruption of the molecular architectonics of chromosomes and their sticking, are not excluded [20, 54].

An analysis of the literature testifies, that pesticides are capable of causing various types of chromosomal aberrations in higher plants. Already in early studies of the genotoxicity of pesticides, it was shown, that many pesticides are clastogenes: they cause chromosome breaks and fragmentation, which may result to anaphase bridges and destructions [55, 56, 57];the appearance of micronuclei as well as induce chromosome sticking, adhesions, fragmentation and dissolution of chromosomes, the appearance of micronuclei and binuclear cells [58].The spectrum of intracellular anomalies that induce pesticides is largely determined by the dose, the frequency of exposure and the chemical class, as well as the species characteristics of the plant meristem.In the barley meristem, after exposure to triazine herbicides propazine, atrazine and simazine, the spectrum of aberrations was mainly represented by isolocus breaks, microfragments and chromatid changes. Chromosomal type disorderswere not detected [59]. The fungicides Vitavax (group of dithiocarbamates) and Topsin-Methyl (group of benzimidazoles) induced chromatid and chromosome aberrations in the barley meristem and their number increased depending on the number of treatments and the time interval between them [60].In somatic wheat cells the herbicides butachlor (group of acetanilides) and imazethapyr (group of imidazole) induced dose-related disorders included stickiness of chromosomes, chromosome bridges, nuclear damage, scattered chromosomes, multipolar mitoses and micronuclei [13, 26]. Stickiness of chromosomes in wheat cells was observed under the influence of herbicides 2,4-dichlorophenoxy acetic acid (a derivative of phenoxyacetic acid, a herbicide from the group of synthetic auxins) and isoproturon (class of phenylureas) [28]. But these two herbicides were characterized by the absence of chromosomal bridges.

The genotoxic effects of pesticides are associated not only with chromatid and chromosomal aberrations, but also with impaired microtubule biogenesis [25]. Cells of control wheat roots contained abundant microtubules both in interphase and mitotic arrays. In chlorpropham (carbamate herbicide) treated roots no microtubules could be detected neither in dividing nor in differentiating cells. Dividing cells became binucleate, polyploid or contained incomplete cell walls as the result of inhibition of cytokinesis. It is assumed that the chlorpropham disorganized directly microtubules in addition to irreversibly affecting microtubule organizing centres, which failed to further support microtubule arrays.Literature data and our results indicate dose-dependent cytogenetic effects of pesticides in plants.

4. Conclusion

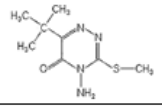
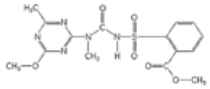
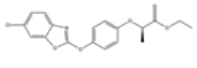
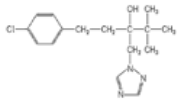
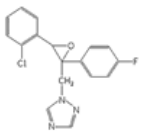
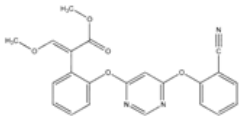
The studied herbicidal and fungicidal pesticides showed a dose-dependent inhibitory effect towards development of the roots of *Triticum aestivum*, grown in hydroponic culture, without negatively affecting of the mitotic indexin all cases. Herbicides and fungicides influenced the duration of mitosis phases (metaphase, anophase, prophase) and cytokinesis activity in the meristem of the root apex of wheat, and these changes were specific for all six studied drugs. All pesticides in the experimentation increased the number of abnormal cells in culture, to the greatest extent under the influence of herbicides Tribenuron-methyl and Fenoxapron-ethyl and fungicides Azoxystrobin and Epoxiconazole (10–12 times, compared with the control - distilled water); to a lesser extent - under the influence of Metribuzin and Tebuconazole (6–7 times). For the studied herbicides and fungicides, regardless of the mechanism of their damaging effect, the manifestation of both, clastogenic and aneugenic effects, was characteristic. Structural abnormalities, suchchromosome breaks, asbridges, fragments and micronuclei, and damage of the fission spindle - lagging chromosomes and their fragments, uneven chromosome separation, caused by the application of herbicidal and fungicidal preparations, were revealed in *Triticum aestivum* meristematic cells.

5. Materials And Methods

5.1. Materials

Six systemic pesticides, differing in the mechanism of the damaging effect, were investigated (Table 1).

Table 2. Structural formulas of the herbicides and fungicides

Pesticides:	Structural formula
Metribuzin (MET) (4-amino-6-tert-butyl-3-methylthio-1,2,4-triazin-5(4H)-one)	
Tribenuron-methyl (TBM) (methylesterof2-(6-methyl-4-methoxy-1,3,5-triazin-2-yl(methyl) carbamoylsulfamoyl) benzoicacid)	
Fenoxaprop-P-ethyl (FEN) (R)-2-(4-(6-chloro-2-benzoxazolyl)-phenoxy-propanoate)	
Tebuconazole (TEB) (RS)-1p-(4-chlorophenyl)-4,4-dimethyl-3-(1H-1,2,4-triazol-1-ylmethyl)pentan-3-yl	
Epoxiconazole (EPO) (2RS,3SR)-1-(3-(2-chlorophenyl)-2,3-epoxy-2-(4-fluorophenyl)propyl)-1H-1,2,4-triazole)	
Azoxystrobin (AZO) (Methyl(2E)-2-(2-{[6-(2-cyanophenoxy)pyrimidin-4-yl]oxy}phenyl)-3-methoxyacrylate	

5.2. Methods

5.2.1. Wheat cultivation

Wheat *Triticum aestivum*was used as tested crops. The short-cycle spring wheat variety Novosibirskaya 15 was bred at the Siberian Research Institute of Plant Cultivation and Breeding (Russia); the variety was registered in the National Registry of Plant Varieties and approved for use in the Ural, West Siberian, and East Siberian Regions in 2013.

The roots of 2-day-old seedlings, obtained in hydroponic culture, were used. For this, the seed grain was washed for 5–6 hours under running water and soaked for 24 hours in distilled water at room temperature. Hatching grains were laid out in Petri dishes of 50 pieces each.

Wheat sprouts were grown as follows: the seeds were washed in running water for 5–6 h and soaked in distilled water for 24 h at room temperature. Germinated seeds were placed into Petri dishes, 50 seeds per dish.In the control variant, 7 ml of distilled water were added to the plates. 7 ml of solutions of pesticides of various concentrations (0.01; 0.10; 1.00 µg/mL) were added to the experimental variants. Seeds were germinated at room temperature under round-the-clock lighting conditions. The length of the main root and the absolutely dry root biomass were determined in the seedlings.

5.2.2. Cytogenetic analysis

Markers of cytotoxicity were the mitotic index (MI) and anaphase-telophase anomalies; genotoxicity – chromosomal aberrations and micronuclei.

The 2-day-old roots of wheat seedlings were fixed in a mixture of ethanol and glacial acetic acid (as 3:1, by volume), for 24 hours at room temperature. The roots were washed 3 times from the fixative with distilled water and stored in 70% ethanol. Before staining, the roots were washed 3 times in distilled water and incubated in 1N HCl on a water bath for 9 min at 60 °C. After hydrolysis, the roots were washed, 3 times with distilled water and were stained with Schiff's reagent for 2 hours at room temperature in the dark. After staining, the roots were washed once again. The top of the root apex (1.0–1.5 mm) was cut off with a sharp scalpel, a drop of 45% acetic acid was applied. After 5–10 minutes, a squeezed preparation was prepared and analyzed under a light microscope. In each experimental variant, 300 cells in various mitotic phases were analyzed.

To determine the mitotic index (MI), 900–1700 cells were analyzed in each preparation. In total, 5000–12000 cells were counted for the evaluation of MI for each pesticide. MI was determined as the percentage of cells in different mitotic phases in relation to the total number of cells analyzed (at least five roots were used).

Chromosomal abnormalities were investigated in ana-telophase cells. In each sample, were analyzed dividing cells in all fields of vision (in all preparation of root apex meristem).

The percentage of abnormal cells was calculated, as the number of ana-telophase cells, containing the number of abnormal chromosomes, to the total number of ana-telophase cells, and expressed in%. In the control, 300–400 ana-telophase cells were analyzed, under the action of different pesticides –by 150–300 ana-telophase cells.

5.2.3. Statistical analysis

Statistical analysis of obtained results was performed, using the standard software package of Microsoft Excel, STATISTICA 8. Arithmetic means and standard deviations were determined using Student's t test. Results are given as X±m.

Abbreviations

mitotic index (MI)

Azostrobin (AZO)

Tebuconazole (TEB)

Epoxinazole (EPO)

Tribenuron-methyl (TBM)

Fenoxapron (FEN)

Metribuzin (MET)

Declarations

Ethics approval and consent to participate

Research without the use of living objects doesn't need the approval of the ethical committee of our research organization.

All authors are consent for the participation in this research work.

Consent for publication

All authors are consent for the participation in this publication.

Competing interests

All authors declare no conflict of interest.

The funders had no role in the design of this study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Authors' contributions

Conceptualization - Volova, methodology - Menzyanova; validation - Menzyanova and Shishatskaya; formal analysis and data curation - Volova, investigation - Menzyanova and Pyatina, resources - Menzyanova, writing (original draft preparation) - Menzyanova and Pyatina, writing (review and editing) - Shishatskaya, supervision and project administration - Shishatskaya. All authors have read and agreed to the published version of the manuscript.

Funding

This study was financially supported by Project "Agropreparations of the new generation: a strategy of construction and realization" (Agreement No 074-02-2018-328) in accordance with Resolution No 220 of the Government of the Russian Federation of April 9, 2010, "On measures designed to attract leading scientists to the Russian institutions of higher learning".

Acknowledgements

Krasnoyarsk Regional Center of Research Equipment of Federal Research Center «Krasnoyarsk Science Center SB RAS».

Availability of data and materials

Data available within the article and on request from the authors.

References

1. Li S, Wu Q, Sun Q, et al. Parental exposure to tebuconazole causes thyroid endocrine disruption in zebrafish and developmental toxicity in offspring. AquatToxicol. 2019;211:116–23. doi:10.1016/j.aquatox.2019.04.002.
2. Manfo FPT, Harthé C, Nantia EA, et al. Bisphenol A differentially affects male reproductive function biomarkers in a reference population and agro pesticides users from Djutitsa. Cameroon ToxicolInd Health. 2019;35:324–35. doi:10.1177/0748233719838437.
3. Yu Y, Yang Y, Zhao X, et al. Exposure to the mixture of organophosphorus pesticides is embryotoxic and teratogenic on gestational rats during the sensitive period. Environ Toxicol. 2017;32:139–46. doi:10.1002/tox.22219.
4. Yang X, Li S, Wang Z, et al. Constraining the Teratogenicity of Pesticide Pollution by a Synthetic Nanoreceptor. Chem Asian J. 2018;13:41–5. doi:10.1002/asia.201701527.
5. Patel S, Sangeeta S. Pesticides as the drivers of neuropsychotic diseases, cancers, and teratogenicity among agro-workers as well as general public. Environ Sci Pollut Res. 2019;26:91–100. doi:10.1007/s11356-018-3642-2.
6. Piazza MJ, Urbanetz AA. Environmental toxins and the impact of other endocrine disrupting chemicals in women's reproductive health. JBRA Assist Reprod. 2019;23:154–64. doi:10.5935/1518-0557.20190016.
7. Morgan AM, Ibrahim MA, Hussien AM. The potential protective role of Akropower against Atrazine- induced humoral immunotoxicity in rabbit. Biomed Pharmacother. 2017;96:710–5. doi:10.1016/j.biopha.2017.10.028.
8. Kubsad D, Nilsson EE, King SE, et al. Assessment of glyphosate induced epigenetic transgenerational inheritance of pathologies and sperm epimutations: Generational toxicology. Sci Rep. 2019;9:6372. doi:10.1038/s41598-019-42860-0.
9. Smith CM, Vera MKM, Bhandari RK. Developmental and epigenetic effects of Roundup and glyphosate exposure on Japanese medaka (*Oryziaslatipes*). AquatToxicol. 2019;210:215–26. doi:10.1016/j.aquatox.2019.03.005.
10. Attaullah M, Yousuf MJ, Shaukat S, et al. Serum organochlorine pesticides residues and risk of cancer: A case-control study. Saudi J BiolSci. 2018;25:1284–90. doi:10.1016/j.sjbs.2017.10.023.
11. Davoren MJ, Schiestl RH. Glyphosate-based herbicides and cancer risk: a post-IARC decision review of potential mechanisms, policy and avenues of research. Carcinogenesis. 2018;39:1207–15. doi:10.1093/carcin/bgy105.
12. Kawagashira Y, Koike H, Kawabata K, et al. Vasculitic Neuropathy Following Exposure to a Glyphosate-based Herbicide. Intern Med. 2017;56:1431–4. doi:10.2169/internalmedicine.56.8064.
13. Kumar NKH, Jagannath S. Cytological effects of herbicide Butachlor 50 EC on Somatic cells of *Triticum aestivum* L. Journal of Applied Biology Biotechnology. 2015;3:030–4. doi:10.7324/JABB.2015.3206.
14. Kumar S, Arya SK, Roy BK, Singh AK. The effects of 2,4-dichlorophenoxy acetic acid and isoproturon herbicides on the mitotic activity of wheat (*Triticum aestivum L.*) root tips. Turk J Biol. 2010;34:55–66. doi:10.3906/biy-0802-5.
15. Kumar N, Willis A, Satbhai K, et al. Developmental toxicity in embryo-larval zebrafish (*Danio rerio*) exposed to strobilurin fungicides (azoxystrobin and pyraclostrobin). Chemosphere. 2019;241:124980. doi:10.1016/j.chemosphere.2019.124980.
16. Ali A, Machado VS. Rapid detection of triazine resistant weeds using chlorophyll fluorescence. Weed Res. 1981;21:191–7. doi:10.1111/j.1365-3180.1981.tb00116.x.
17. Castroa TFD, da Silva Souza JG, de Carvalho AFS, et al. Anxiety-associated behavior and genotoxicity found in adult *Danio rerio* exposed to tebuconazole-based commercial product. Environ ToxicolPharmacol. 2018;62:140–6. doi:10.1016/j.etap.2018.06.011.
18. Zhang C, Zhou T, Wang J, et al. Acute and chronic toxic effects of fluoxastrobin on zebrafish (*Danio rerio*). Sci Total Environ. 2018;610–611:769–75. doi:10.1016/j.scitotenv.2017.08.052.
19. Garanzini DS, Medici S, Moreyra LD,Menone ML. Acute exposure to a commercial formulation of Azoxystrobin alters antioxidant enzymes and elicit damage in the aquatic macrophyte *Myriophyllum quitense*. PhysiolMolBiol Plants. 2019;25:135–43. doi:10.1007/s12298-018-0603-7.
20. Mahapatra K, De S, Banerjee S, Roy S. Pesticide mediated oxidative stress induces genotoxicity and disrupts chromatin structure in fenugreek (*Trigonella foenum - graecum* L.) seedlings. J Hazard Mater. 2019;369:362–74. doi:10.1016/j.jhazmat.2019.02.056.
21. Moreyra LD, Garanzini DS, Medici S, Menone ML. Evaluation of Growth, Photosynthetic Pigments and Genotoxicity in the Wetland Macrophyte Bidenslaev is Exposed to Tebuconazole. Bull Environ ContamToxicol. 2019;102:353–7. doi:10.1007/s00128-019-02539-8.
22. Fernandez-Vidal A, Arnaud LC, Maumus M, et al. Exposure to the fungicide captan induces DNA base alterations and replicative stress in mammalian cells. Environ Mol Mutagen. 2019;60:286–97. doi:10.1002/em.22268.
23. Wandscheerg ACD, Marchesanj E, Tedesco SB, et al. Cytogenotoxicity of rice crop water after application of the tricyclazole fungicide. An Acad Bras Ciênc. 2017;89:1251–8. doi:10.1590/0001-3765201720150536.
24. Truta E, Vochita G, Rosu CM, et al. Evaluation of roundup-induced toxicity on genetic material and on length growth of barley seedlings. ActaBiol Hung. 2011;62:290–301. doi:10.1556/ABiol.62.2011.3.8.

25. Eleftheriou EP, Bekiari E. Ultra structural effects of the herbicide chlorpropham (CIPC) inroottip cells of wheat. Plant soil. 2000;226:11–9. doi:10.1023/A:1026409027223.
26. Rad MH, Aviazi AA, Jagannath S. Cytogenetic and biochemical effects of imazeyhapyr in wheat (*Triticumduram*). Turk J Biol. 2011;35:1–6. doi:10.3906/biy-1008-75.
27. Sta C, Ledoigt G, Ferjani E, Goupil P. Exposure of *Viciafaba* to sulcotrione pesticide induced genotoxicity. PesticBiochemPhysiol. 2012;103:9–14. doi:10.1016/j.pestbp.2012.02.002.
28. Kumar S. Effect of 2,4-D and isoproturon on chromosomal disturbances during mitotic division in root tip cells of *Triticum aestivum*L. Cytol Genet. 2010;44:79–87. doi:10.3103/S0095452710020027.
29. PPDB: Pesticide Properties DataBase. 2020. https://sitem.herts.ac.uk/aeru/ppdb/ Accessed 26 February 2020.
30. Zhang Y, Zhang G, Fu P, et al. Study on the interaction of triadimenol with calf thymus DNA by multispectroscopic methods and molecular modeling. SpectrochimActa A MolBiomolSpectrosc. 2012;96:1012–9. doi:10.1016/j.saa.2012.08.002.
31. Zhuang S, Zhang J, Wen Y, et al. Distinct mechanisms of endocrine disruption of DDT-related pesticides toward estrogen receptor α and estrogen-related receptor γ. Environ ToxicolChem. 2012;31:2597–605. doi:10.1002/etc.1986.
32. Santucci A, Bernardini G, Braconi D, et al. 4- Hydroxyphenylpyruvate dioxygenase and its inhibition in plants and animals: Small molecules as herbicides and agents for the treatment of human inherited diseases. J Med Chem. 2017;60:4101–25. doi:10.1021/acs.jmedchem.6b01395.
33. Ndikuryayo F, Moosavi B, Yang WC, Yang GF. 4-Hydroxyphenylpyruvate Dioxygenase Inhibitors: From Chemical Biology to Agrochemicals. J Agric Food Chem. 2017;65:8523–37. doi:10.1021/acs.jafc.7b03851.
34. Jiang Z, Zhang Z, Cui G, et al. DNA Topoisomerase 1 Structure-BASED Design, Synthesis, Activity Evaluation and Molecular Simulations Study of New 7-Amide Camptothecin Derivatives Against *Spodoptera frugiperda*. Front Chem. 2018;6:456. doi:10.3389/fchem.2018.00456.
35. Yang B, Qin C, Hu X, et al. Enzymatic degradation of extracellular DNA exposed to chlorpyrifos and chlorpyrifos-methyl in an aqueous system. Environ Int. 2019;132:105087. doi:10.1016/j.envint.2019.105087.
36. He B, Dong J, Lin HY, et al. Pyrazole-Isoindoline-1,3-dione Hybrid: A Promising Scaffold for 4-Hydroxyphenylpyruvate Dioxygenase Inhibitors. J Agric Food Chem. 2019;67:10844–52. doi:10.1021/acs.jafc.9b04917.
37. Fu Y, Zhang D, Zhang SQ, et al. Discovery of *N*-AroylDiketone/Triketone Derivatives as Novel 4-Hydroxyphenylpyruvate Dioxygenase Inhibiting-Based Herbicides. J Agric Food Chem. 2019;67:11839–47. doi:10.1021/acs.jafc.9b01412.
38. Seefeldt SS, Jensen JE, Fuerst EP. Log-logistic analysis of herbicide dose-response relationships. Weed Technol. 1995;19:218–27. doi:10.1017/S0890037X00023253.
39. Peng L, Zhang L, Cheng X, et al. Disruption of cellulose synthesis by 2,6-dichlorobenzonitrile affects the structure of the cytoskeleton and cell wall construction in *Arabidopsis*. Plant Biol. 2013;15:405–14. doi:10.1111/j.1438-8677.2012.00630.x.
40. Brabham C, Lei L, Gu Y, et al. Indaziflam herbicidal action: a potent cellulose biosynthesis inhibitor. Plant Physiol. 2014;166:1177–85. doi:10.1104/pp.114.241950.
41. Tateno M, Brabham C, DeBolt S. Cellulose biosynthesis inhibitors - a multifunctional toolbox. J Exp Bot. 2016;67:533–42. doi:10.1093/jxb/erv489.
42. Fisun K, Rasgele PG. Genotoxic Effects of Raxil on Root Tips and Anthers of. *Allium cepaL* Caryologia. 2009;62:1–9. doi:10.1080/00087114.2004.10589659.
43. Fernandes TCC, Mazzeo DEC, Marin-Morales MA. Mechanism of micronuclei formation in polyploidizated cells of *Allium cepa* exposed to trifluralin herbicide. Pest Biochem Physiol. 2007;88:252–9. doi:10.1016/j.pestbp.2006.12.003.
44. Prasad I, Pramer D. Genetics effects of ferbam on *Aspergillus* and *Allium cepa*. Phytopathology. 1968;58:1188–9.
45. Rosculete CA, Bonciu E, Rosculete E, Olaru LA. Determination of the Environmental Pollution Potential of Some Herbicides by the Assessment of Cytotoxic and Genotoxic Effects on *Allium cepa*. Int J Environ Res Public Health. 2019;16:75. doi:10.3390/ijerph16010075.
46. Felisbino K, Santos-Filho R, Piancini LDS, et al. Mesotrione herbicide does not cause genotoxicity, but modulates the genotoxic effects of Atrazine when assessed in mixture using a plant test system (Allium cepa). PesticBiochemPhysiol. 2018;150:83–8. doi:10.1016/j.pestbp.2018.07.009.
47. Akhter A, Rani R, Munira S, et al. Cytological effects of herbicides on hexaploid wheat (*Triticum aestivum* L.). Journal of Bio-Science. 2009;17:21–6. doi:10.3329/jbs.v17i0.7095.
48. Armbruster BL, Molin MW, Bugg MW. Effects of the herbicide dithiopyr on cell division in wheat root tips. PesticBiochemPhysiol. 1991;39:110–20. doi:10.1016/0048-3575(91)90131-5.
49. Bonea D, Bonciu E. Cytogenetic effects induced by the fungicide Royal Flo to maize (*Zea mays* L.). Caryologia. 2017;70:195–9. doi:10.1080/00087114.2017.1318232.
50. Yin J, Zhu F, Hao W, et al. Acylamino acid chiral fungicides on toxiciepigenetics in lambda DNA methylation. Food ChemToxicol. 2017;109:735–45. doi:10.1016/j.fct.2017.04.038.

51. Zhou Y, Xu J, Zhu Y, et al. Mechanism of Action of the Benzimidazole Fungicide on Fusarium graminearum: Interfering with Polymerization of Monomeric Tubulin But Not Polymerized Microtubule. Phytopathology. 2016;106:807–13. doi:10.1094/PHYTO-08-15-0186-R.
52. Taggi AE, Stevenson TM, Bereznak JF, et al. Tubulin modulating antifungal and antiproliferativepyrazinone derivatives. Bioorg Med Chem. 2016;24:435–43. doi:10.1016/j.bmc.2015.08.038.
53. Boger P, Matthes B, Schamalfub J. Review-Towards the primary target of chloroacetamides –new findings pave the way. Pest ManagSci. 2000;56:497–508. doi:10.1002/(SICI)1526-4998(200006)56:6<497::AID-PS169>3.0.CO;2-W.
54. Trivedi AK, Ahmad I. Genotoxicity of chrysotile asbestos on *Allium cepa* L. meristematic root tip cells. Curr Sci. 2013;105:781–6.
55. Wuu KD, Grant WF. Morphological and somatic chromosomal aberrations induced by pesticides in barley (*Hordeum vulgare*). Can J GenetCytologia. 1966;8:481–501. doi:10.1139/g66-058.
56. Wuu KD, Grant WF. Chromosomal aberrations induced in somatic cells of *Viciafaba* by pesticides. Nucleus. 1967;10:37.
57. Georgian L. The comparative cytogenetic effects of aldrin and phosphamidon. Mutat Res. 1975;31:103–8. doi:10.1016/0165-1161(75)90072-2.
58. Grant WF. Chromosome Aberrations in Plants as a Monitoring System. Environ Health Perspect. 1978;27:37–43. doi:10.1289/ehp.782737.
59. Bozchataeva G, Ospanova GS, Tyrabaeva G, et al. Studying of mutagen effect of triazinovyherbicides by cytogenetic tests on barley seeds. International journal of applied fundamental research. 2014;3:47–9.
60. Pusztai T. Chromosomal aberrations and chlorophyll mutations induced by some pesticides in barley. Acta Bot Hungarica. 1983;29:55–66.

Figures

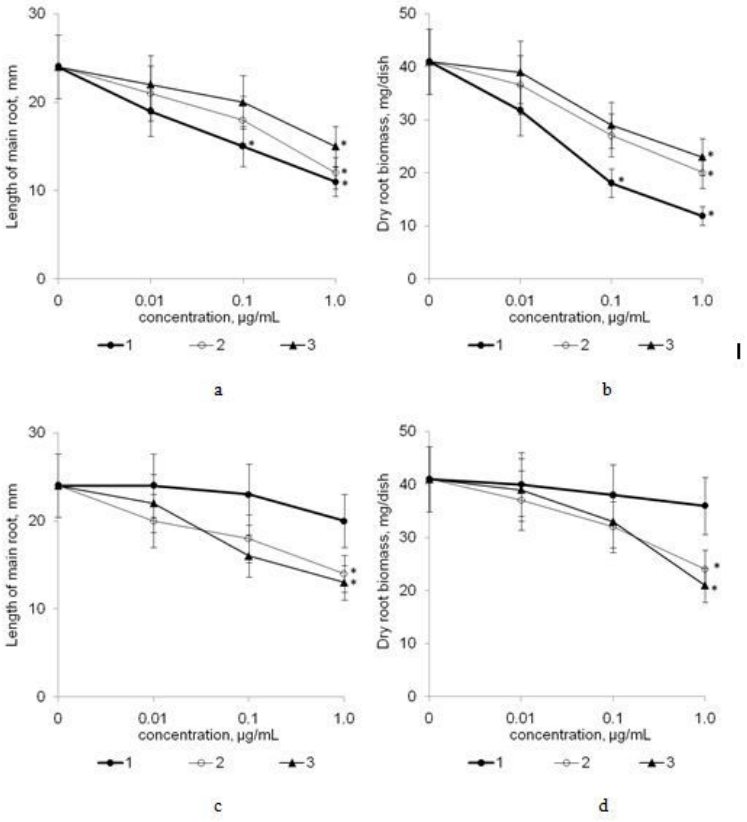


Figure 1

The length of the main root (A, C) and dry biomass (B, D) of the roots of 2-day-old seedlings of Triticum aestivum when cultivated on media with fungicides (A, B) and herbicides (C, D) For A, B: 1 - azoxystrobin, 2 - epoxiconazole, 3 - tebuconazole. For C, D: 1 - metribuzin, 2 - tribenuron-methyl, 3 - phenoxapron-p-ethyl. * - values, significantly different from the control variant (distilled water), $p<0.05$.

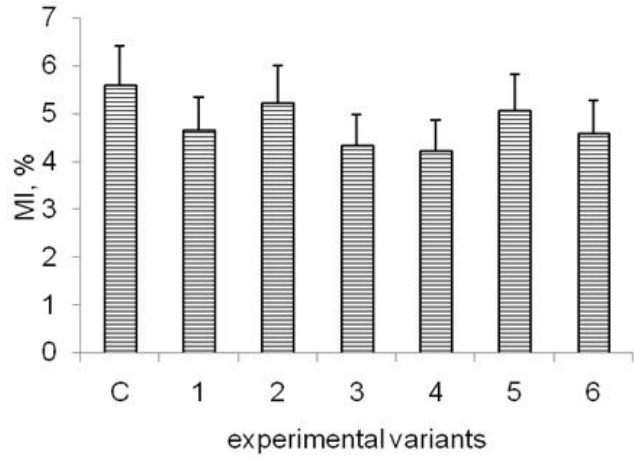


Figure 2

The mitotic index for root apex meristem of 2-day-old seedlings of Triticum aestivum when cultured on pesticidal media Herbicides: 1 - metribuzin, 2 - tribenuron-methyl, 3 - phenoxaprop-p-ethyl. Fungicides: 4 - azoxystrobin, 5 - epoxinazole, 6 - tebuconazole. C - control option, cultivation in distilled water.

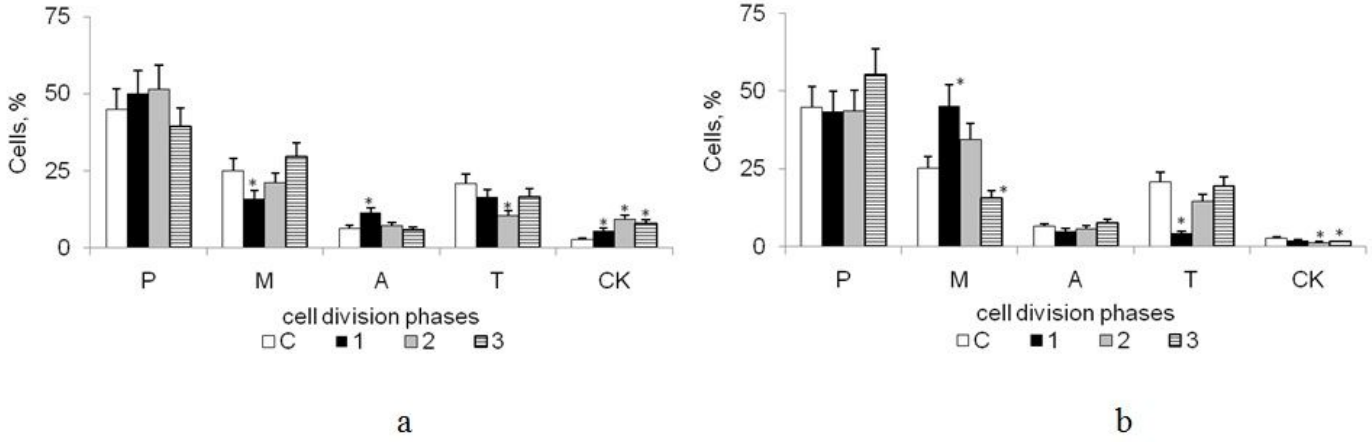


Figure 3

The relative number of cells (in%) at different stages of mitosis in the root apex meristem of 2-day-old Triticum aestivum seedlings when cultivated on media with pesticides A - herbicides: 1 - metribuzin, 2 - tribenuron-methyl, 3 - phenoxaprop-p-ethyl. B - fungicides: 1 - azoxystrobin, 2 - epoxiconazole, 3 - tebuconazole. C - control option, cultivation in distilled water. Cell division phases : P - prophase, A - anaphase, M - metaphase, T - telophase, CK - cytokinesis. * - marked values are significantly different from the number of cells in the corresponding phases of the cell cycle in the control variant (cultivation in distilled water), $p < 0.05$.

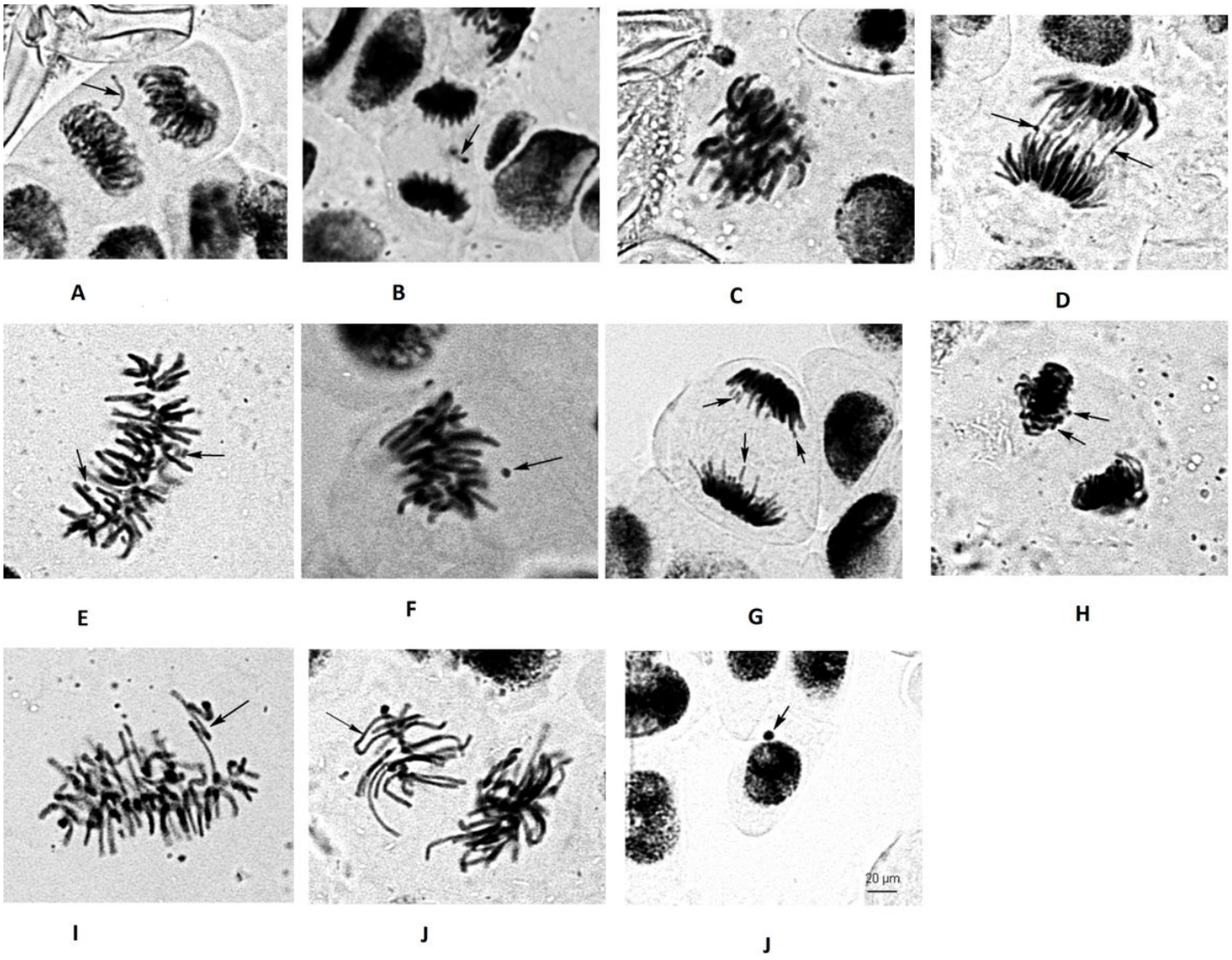


Figure 4

Anaphase-telophase anomalies, chromosome aberrations and micronuclei in the meristem cells of 2-day-old seedlings of Triticum aestivum a, b - lagging chromosomes in the telophase; c - sticking of chromosomes; d - chromosome bridges in anaphase. e – k - chromosomal aberrations: e - single chromosomal fragments, that retain a connection with chromatids; f - free single chromosomal fragment in metaphase; g - single chromosome fragments in anaphase; h - single chromosome fragments in the telophase; i - dicentric chromosome; j - ring chromosome; k – micronucleus.