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RESEARCH PAPERS

Effect of (+) and (–) Usnic Acid on Physiological, Biochemical, and Cytological Characteristics of *Allium fistulosum* Seeds

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Abstract—The effect of (+) and (–)-usnic acid (UA) on the physiological, biochemical, and cytological characteristics of *Allium fistulosum* L. seedlings was studied. It was shown that germination of seeds in the medium supplemented with both enantiomers of UA at concentrations of 62.5–1000 μM led to a decrease in laboratory germination, an inhibition of growth processes, a slowing of the mitotic activity of root meristems, and tissue depigmentation. A dose-dependent increase in the frequency of chromosomal aberrations and the degree of damage to nuclear DNA in cells was shown, which indicates the potentially genotoxic and mutagenic effect of the studied UA enantiomers. However, (–)-UA induced a greater number of atypical DNA comets than the (+)-enantiomer, which may indicate its stronger effect on DNA fragmentation in cells. An increase in the activity of antioxidant enzymes and a decrease in the content of flavonoids were observed under the action of both UA enantiomers against the background of the accumulation of lipid peroxidation products in seedlings' cells, which indicates the development of oxidative stress. At the same time, no significant differences between the activity of (+) and (–)-UAs at the level of physiological and biochemical parameters of seedlings were revealed.

Keywords: Allium fistulosum, usnic acid, phytotoxicity, genotoxicity, oxidative stress

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INTRODUCTION

Lichens synthesize a number of specific compounds of varying chemical nature named lichen substances. Usnic acid (UA), which is found in lichens in the form of two enantiomers, differing in the *R* and *S* configurations of the chiral C^{9b} atom, is the most common (Fig. 1). It is believed that the main biological function of the UA is to protect the lichen photobiont by absorbing excess ultraviolet radiation [1]. In addition, high antibacterial activity of enantiomers of UA in relation to gram-positive microorganisms and mycobacteria, including strains resistant to antibiotics, is known [1, 2]. In this case, (+)-UA has a greater antibacterial activity compared to (–)-UA [2].

Many lichen substances exhibit phytotoxic properties that are expressed in the inhibition of seed germination and seedling growth. At the same time, information about the phytotoxicity of UAs is contradictory. It was shown that UA had an inhibitory effect on the germination of mung and wheat seeds [3]. However, other authors have shown the absence of the toxic effect of UA on seedlings of lettuce and pine [4].

In addition to seeds and seedlings, UA has an impact on the growth and development of already formed plants. A slowdown in the growth rate and a decrease in the leaf area and biomass of tomato shoots were observed when (+)-UA was introduced into the medium in the form of sodium salt at concentrations of 1–40 μM [5]. In addition, UA at a concentration of 50 μM decreased the transpiration rate and pathological changes in the morphology of the root system in sunflower and maize [6].

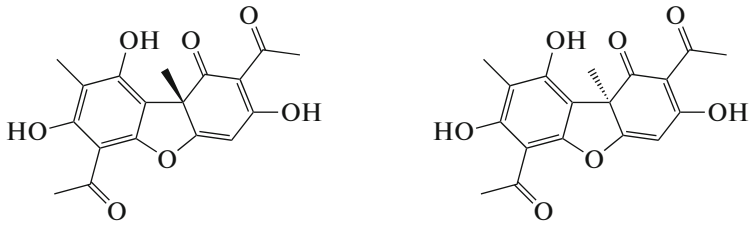
The purpose of the study is to investigate the effect of various concentrations of usinic acid enantiomers on the physiological and cytogenetic characteristics of *Allium fistulosum* seedlings, the content of photosynthetic pigments and flavonoids, and the activity of enzymatic antioxidants.

MATERIAL AND METHODS

The seeds and seedlings of *Allium fistulosum* L. (Aprelskyi variety, harvested in 2017, $n = 16$) were the objects of study. The model object was chosen because of the widespread use of onions for assessing the toxicity of various physical and chemical factors [7].

Stereoisomers of UA, (+) and (–) enantiomers, were isolated from the lichens *Cladonia arbuscula* and *C. stellaris,* respectively, based on their predominant

Abbreviations: CAT—catalase; MI—mitotic index; POX—peroxidase; SOD—superoxide dismutase; UA—usnic acid.



(–)-usnic acid (+)-usnic acid

Fig. 1. Structural formulas of usnic acid enantiomers.

content in thalli according to [8]. Identification of UAs was carried out by comparing the obtained IR and ^{1}H-NMR spectra with published data [9]. The optical activity of the UA's isolated enantiomers was determined polarimetrically. The purity of the isolated enantiomers according to thin layer chromatography was 95–97%.

Low solubility of UA in water is one of the obstacles that impede the study of its biological activity [10]. UA is titrated as a monobasic acid. Therefore, the enantiomers of UA are mixed with an equimolar aqueous solution of potassium hydroxide to obtain water soluble salts. If necessary, the excess alkali was neutralized with hydrochloric acid to pH 7.5. The pH was measured using an Ecotest 2000 ionometer (Russia). The obtained aqueous solutions of (+) and (–) UA salts were used for further studies.

The seeds of *A. fistulosum* were divided into 12 experimental and one control group to determine the effect of different concentrations of (+) and (–) UA potassium salts on the physiological, biochemical, and cytological characteristics of seedlings. In the experimental groups, solutions of UA enantiomers were put into Petri dishes in a 1000–31.2-μM concentration range before sowing the seeds. The control group sprouted on distilled water. The choice of concentrations was based on the data obtained in [3, 11], in which it was shown that there was a significant decrease in laboratory germination of seeds and inhibition of growth of seedlings of wheat, oats, watercress, and mung beans in this concentration range of potassium salt of usnic acid.

Seeds were germinated on filter paper (50 pcs. in each dish, in four replicates) at a 16-h illumination period and a temperature of 20–25°C. Germination energy was determined on the fifth day of the experiment. Laboratory germination and the length of the aerial part and the root of the seedlings was determined on the 12th day of observation.

Cytological studies were carried out on the 0.3–0.8-cm-long roots of seedlings (n = 10 for each variant), which were fixed on the third day with a mixture of 96% ethyl alcohol and glacial acetic acid in a 3 : 1 ratio for 12 h. Then they were stained with acetoorcein. Pressed preparations were examined under an Axiostar plus light microscope (Carl Zeiss, Germany). Chromosome aberrations were taken into account by the anaphase-telophase method. Bridges and fragments were distinguished into separate groups. Violations of cell divisions, represented by lagging, "ejection," running of chromosomes, chromosomes at the equator, multipolar anaphases, etc. belonged to the group with an atypical arrangement of chromosomes—other abnormalities. The mitotic index (MI), which was determined by the ratio of the number of cells in mitosis from their total number expressed as a percentage, was used to determine the activity of cell division [12].

The degree of DNA fragmentation in the roots of seedlings was determined using the alkaline version of the DNA-comet method (pH > 13) [13], which allows a quantitative measuring damaged DNA, including single-stranded breaks, double-stranded breaks, and alkaline labile sites. All operations to isolate cell nuclei were performed under dim yellow light. Using a sharp razor blade, the roots were neatly sliced in a Petri dish on ice. The tips of the roots with the apical meristem were cut off. The preparations were stained with SYBR Green I fluorescent dye (Sigma-Aldrich, United States) (20 μg/mL) for 30 min immediately prior to microscopy. The analysis was performed on a fluorescence microscope (LabMed-2L, Russia) using excitation and cut-off filters at 490 and 530 nm, respectively. The images of DNA comets obtained from micropreparations were analyzed using CASP 1.2.2 software. The percentage of DNA in the tail of comets (% of DNA in the tail of the total amount of DNA in the comet) was used as an indicator of DNA damage. Atypical DNA comets, characterized by an absent or practically absent head and a wide diffuse tail, were put into a separate category, and the percentage was calculated for every 100 pcs. [14].

All spectrophotometric measurements were performed on a UV-2600 instrument (Shimadzu, Japan). We studied the samples of 0.1–0.3 g of crude tissue of 12-day-old seedlings and homogenized them in a ceramic mortar in the presence of 0.1 M Na-phosphate buffer, pH = 7.4, to extract the enzymes and

96% ethanol with 1% Triton X-100 for the extraction of flavonoids and TBA-reactive products. The homogenate was centrifuged for 10 min at 6000 *g*; the obtained supernatant was used for further studies.

Superoxide dismutase (SOD; EC 1.15.1.1) activity was measured according to Giannopolitis and Ries [15]. The reaction was initiated with light from a fluorescent lamp for 5 min. The enzymatic activity was evaluated by inhibiting the formation of light-colored product of the restoration of nitro blue tetrazolium ($\varepsilon = 3.98/(\text{mM cm})$, $\lambda = 560$ nm).

To determine the activity of peroxidase (POX; EC 1.11.1.7), 0.1 mL of a 4.3-mM solution of (o)-dianisidine and 0.7 mL of a 0.1-M Na-phosphate buffer pH = 7.4 were added to 0.1 mL of the supernatant. The reaction was initiated by adding 0.1 mL of 0.45-mm hydrogen peroxide. The enzymatic activity was determined by increasing the optical density during the formation of the colored oxidation product of (o)-dianisidine for 1 min ($\varepsilon = 30/(\text{mM cm})$, $\lambda = 460$ nm) [16].

To determine the activity of catalase (CAT; EC 1.11.1.6), 0.8 mL of 0.1 M Na-phosphate buffer pH = 7.4 was added to 0.1 mL of the supernatant. The reaction was initiated by adding 0.1 mL of 0.45-mM hydrogen peroxide. The enzymatic activity was determined by the decrease in optical density during the decomposition of H_2O_2 for 1 min ($\varepsilon = 39.4/(\text{mM cm})$, $\lambda = 240$ nm) [17].

To determine the total content of flavonoids in the seedlings, 0.8 mL of borate citrate was added to 0.2 mL of the supernatant (ethanol solution of 2.5% boric and 10% citric acids). The optical density of the solutions was measured after 15 min. The content of flavonoids was determined by the accumulation of the colored complex with boric citrate ($\varepsilon = 4.4/(\text{mM cm})$, $\lambda = 420$ nm) [18].

The content of chlorophylls *a* and *b* and carotenoids in the seedlings (without roots) was determined based on fresh weight in extracts with 80% acetone at absorption maxima of 663, 647 and 470 nm, respectively [19].

The intensity of lipid peroxidation (LPO) was determined by the accumulation of carbonyl compounds forming colored complexes with thiobarbituric acid (TBA-reactive products), the amount of which was expressed in terms of the concentration of malondialdehyde-TBA complex ($\varepsilon = 155/(\text{mM cm})$, $\lambda = 532$ nm) [20].

All measurements were performed on fresh samples in four biological and analytical replicates. The experimental results are presented as arithmetic mean and its standard error. The mean values of the samples were compared by the ANOVA. The significance of differences between the means was determined using the Newman-Keuls and Dunnet tests for multiple comparisons at $P < 0.05$. The calculations were performed using the AnalystSoft package, StatPlus (Statistical Analysis Software, v. 2007).

RESULTS AND DISCUSSION

The effect of various concentrations of UA (+) and (–) enantiomers on the physiological characteristics of *A. fistulosum* seedlings was studied. It was shown that a significant decrease in germination energy (1.6 and 2.7 times) and laboratory germination of seeds (1.4 and 2.6 times) relative to the control was observed at concentrations of 500 and 1000 μM (Table 1). In this case, the indicators of shoot and root length began to significantly decrease already at a UA concentration of 125 μM. Earlier, inhibition of growth processes in seedlings of watercress and oats under the action of (–)-UA in a concentration above 100 μM was indicated in [11], which is consistent with our data.

We studied the mitotic activity of root meristems of *A. fistulosum* seedlings under the influence of various concentrations of UA enantiomers. It was shown that the mitotic activity of cells began to significantly decrease relative to the control at a UA concentration of 125 μM. The maximum effect was observed at 1000 μM, at which the MI value was 1.6 and 2.2 times lower relative to the control, respectively (Table 2). It was shown earlier that (+)-UA shows an antimitotic effect against plant cells of *A. cepa* starting from a concentration of 580 μM [21]. Our data indicate that the cells of *A. fistulosum* seedlings were more sensitive to the action of UA enantiomers than the cells of *A. cepa*.

The effect of UA enantiomers on the frequency of chromosomal aberrations in dividing cells of the roots of *A. fistulosum* seedlings was studied. It was shown that an increase of 1.8–4.1 and 2.1–4.6 times, respectively, in the frequency of formation of pathological mitoses as compared with the control, which indicates a mutagenic effect of the studied compounds, was observed in a 62.5–1000-μM concentration range of (+) and (-)-UAs (Table 2). The chromosome lagging, single (chromatid dicentrics) and double bridges (asymmetric chromosome exchanges), and single fragments (chromatid deletions) were the main types of violations. This indicates dose-dependent disturbances both in the chromosomes themselves and in the achromatin division spindle.

When studying the cells of peripheral blood lymphocytes, we found a lack of an increase in the frequency of chromosomal aberrations in the cells when they were exposed to (+)-UA in the concentration range of 3–580 μM [22]. In addition, a study of the effect of both UA enantiomers on lymphocytes using a micronuclear test also did not reveal an increase in the frequency of micronucleus formation in cells [23]. It can be assumed that the genetic apparatus of plant cells is more sensitive to the action of UA in comparison with animal cells.

The study of the genotoxic effect of UA in relation to plant cells has not been conducted. Genotoxicity of (+) and (–)-UAs was evaluated using the alkaline version of the DNA-comet method. It was revealed that the degree of damage to nuclear DNA (% of DNA in

Table 1. Physiological parameters of *Allium fistulosum* seedlings under the action of varying concentrations of usnic acid enantiomers

Concentration of UA, μM	Germination energy, %	Germination, %	Shoot length, cm	Root length, cm
Control	55.3 ± 4.7	66.0 ± 9.2	3.7 ± 0.2	1.5 ± 0.2
(+)-usnic acid				
31.2	50.0 ± 4.0	62.7 ± 1.7	3.1 ± 0.2	1.2 ± 0.2
62.5	47.3 ± 6.4	58.7 ± 6.4	2.5 ± 0.1	0.8 ± 0.1
125	46.0 ± 2.3	50.7 ± 4.4	1.6 ± 0.1*	0.2 ± 0.1*
250	45.3 ± 5.8	54.7 ± 7.0	1.6 ± 0.1*	0.4 ± 0.1*
500	34.7 ± 6.8*	47.3 ± 7.4*	1.5 ± 0.1*	0.3 ± 0.1*
1000	22.0 ± 3.1*	25.3 ± 4.4*	1.0 ± 0.1*	0.4 ± 0.1*
(–)-usnic acid				
31.2	54.0 ± 3.5	62.0 ± 4.2	3.2 ± 0.2	1.1 ± 0.2
62.5	44.0 ± 5.1	61.8 ± 5.1	2.9 ± 0.1*	0.9 ± 0.1*
125	47.3 ± 7.9	59.1 ± 4.8	2.3 ± 0.1*	0.5 ± 0.1*
250	47.3 ± 4.8	58.0 ± 7.0	1.7 ± 0.1*	0.3 ± 0.1*
500	36.0 ± 3.1*	40.7 ± 2.7*	1.5 ± 0.1*	0.3 ± 0.1*
1000	20.7 ± 5.2*	24.7 ± 3.5*	1.5 ± 0.1*	0.4 ± 0.1*

* Differences are statistically significant compared to control ($P < 0.05$, ANOVA, Dunnett test).

Table 2. Cytological characteristics of *Allium fistulosum* seedlings under the action of varying concentrations of usnic acid enantiomers

Concentration of UA, μM	Mitotic index, %	Chromosomal aberrations, %			
		bridges	fragments	other abnormalities	total
Control	7.9 ± 0.6	7.5 ± 2.3	1.6 ± 0.6	4.0 ± 1.0	13.1 ± 2.4
(+)-usnic acid					
31.2	8.2 ± 1.3	8.0 ± 1.8	2.5 ± 0.9	6.8 ± 2.4	17.3 ± 1.7
62.5	7.5 ± 0.8	10.2 ± 2.8	4.7 ± 1.5*	8.2 ± 2.3*	23.1 ± 4.1*
125	6.4 ± 0.6*	14.8 ± 4.3*	5.4 ± 1.9*	9.3 ± 2.4*	29.5 ± 7.0*
250	6.0 ± 1.0*	15.1 ± 2.9*	5.5 ± 1.9*	16.7 ± 4.7*	37.3 ± 8.6*
500	5.7 ± 0.9*	15.2 ± 3.9*	5.1 ± 1.2*	20.1 ± 7.6*	40.4 ± 8.2*
1000	4.8 ± 1.0*	16.1 ± 3.1*	7.8 ± 3.2*	29.7 ± 11.1*	53.6 ± 11.1*
(–)-usnic acid					
31.2	8.8 ± 0.8	10.6 ± 2.0	2.5 ± 0.8	7.7 ± 1.5	20.8 ± 2.2
62.5	8.6 ± 1.2	13.8 ± 4.3*	6.4 ± 1.9*	7.9 ± 2.3*	28.1 ± 4.9*
125	5.8 ± 0.9*	14.4 ± 3.7*	7.6 ± 2.7*	14.2 ± 3.3*	36.2 ± 6.3*
250	5.2 ± 1.6*	16.1 ± 5.3*	7.9 ± 3.6*	15.2 ± 2.9*	39.2 ± 4.8*
500	4.1 ± 0.8*	17.0 ± 6.3*	8.3 ± 2.3*	26.4 ± 7.7*	51.7 ± 4.2*
1000	3.5 ± 1.0*	24.3 ± 9.9*	8.6 ± 3.6*	27.7 ± 10.3*	60.6 ± 10.3*

* Differences are statistically significant compared to control ($P < 0.05$, ANOVA, Dunnett test).

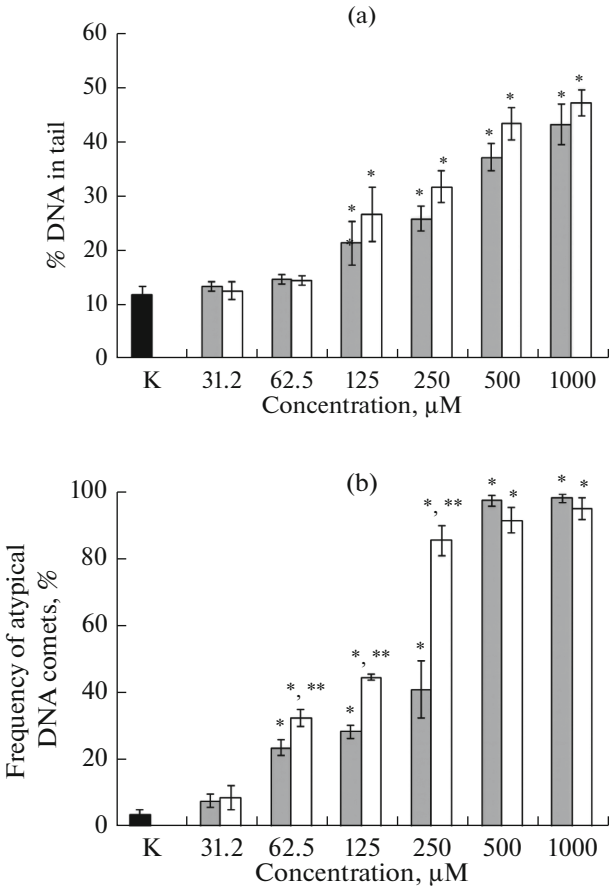


Fig. 2. Genotoxic effect of varying concentrations of usnic acid enantiomers in relation to *A. fistulosum* seedlings: (a) percentage of DNA in the tail of a comet; (b) frequency of atypical DNA comets. Gray columns are (+)-enantiomer and white columns are (–)-enantiomer; * statistically significant differences from control (C) at $P \leq 0.05$ (ANOVA, Dunnett *t*-test), ** statistically significant differences between enantiomers at $P \leq 0.05$ (ANOVA, Newman-Keuls test).

the tail of the comet) at concentrations of (+) and (–) enantiomers of the UA of 125–1000 μM was 1.8–3.6 and 2.2–3.9 times higher, respectively, than in the control (Fig. 2).

In addition to the formed DNA comets, we identified comets with an absent or practically absent head and a wide diffuse tail, called ghost cells, or hedgehogs. According to existing ideas, the appearance of such atypical DNA comets is a consequence of the process of cell death associated with a high level of oxidative stress or the formation of apoptotic cells at the stage of chromatin fragmentation [14].

It was shown that the proportion of cells with atypical DNA comets in seedlings under the action of (+) and (–)-UAs in concentrations of 65.2–1000 μM was 6–25 and 8–24 times higher, respectively, compared with the control. It was revealed that the action of (–)-UA in the concentration range of 62.5–250 μM

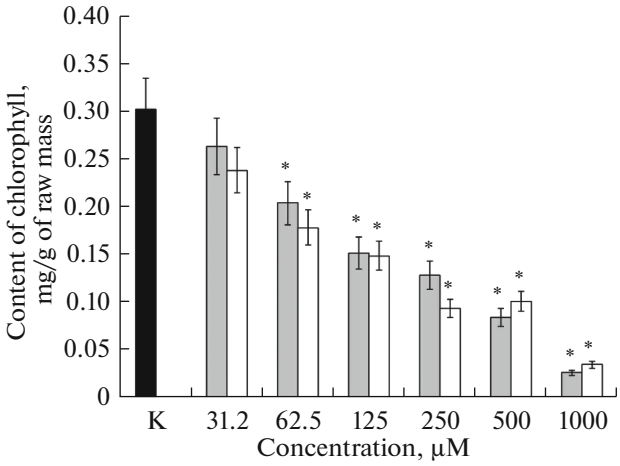


Fig. 3. Content of chlorophylls in the tissues of *A. fistulosum* seedlings under the action of various concentrations of (+) and (–) enantiomers of usnic acid. Gray columns are (+)-enantiomer and white columns are (–)-enantiomer; * statistically significant differences from control (C) at $P \leq 0.05$ (ANOVA, Dunnett *t*-test).

led to the formation of a greater number of atypical DNA comets (1.4–2.1 times) than the (+) enantiomer, which may indicate its stronger ability to induce cellular processes leading to DNA fragmentation.

We have earlier shown that both enantiomers of UA show genotoxic properties against human lymphocytes, while (–)-UA induces more atypical comets than its (+) enantiomer [8], which is confirmed by the data obtained for plant cells in this study.

We have studied the effect of UA enantiomers on the total content of chlorophylls (*a* + *b*) in *A. fistulosum* seedlings. It was shown that the content of chlorophylls in tissues decreased under the action of (+) and (–)-UA in the concentration range of 62.5–1000 μM by 1.4–10.0 and 1.7–7.5 times, respectively, relative to the control, leading to almost complete discoloration at concentrations of 1000 μM seedlings (Fig. 3).

A dose-dependent discoloration of cotyledonous tissues, which was associated with a decrease in the number of chlorophylls and carotenoids in plants treated with only (–)-UA was revealed when studying the action of UA enantiomers on the seedlings of *Lactuca sativa* and *A. cepa*. Meanwhile, (+)-enantiomer had hardly any effect on the content of pigments [24], which is not confirmed by our data.

The irreversible inhibition of the enzymes 4-hydroxyphenylpyruvate dioxygenase and protoporphyrinogen oxidase involved in the biosynthesis of photosynthetic pigments is one of the possible mechanisms of plant depigmentation under the action of UA enantiomers [24].

The mechanism of biological activity of UA is associated with its effect on the functional activity of chloroplasts and mitochondria. The suppression of photo-

Table 3. Biochemical characteristics of *Allium fistulosum* seedlings under the action of varying concentrations of usnic acid enantiomers

Concentration of UA, μM	SOD activity, mM/(g fr wt min)	POX activity mM/(g fr wt min)	CAT activity, mM/(g fr wt min)	Content of flavonoids, mg/g fr wt	Content of TBA-reactive products, nM/g fr wt
Control	2.2 ± 0.1	0.8 ± 0.2	0.1 ± 0.0	2.6 ± 0.1	17.2 ± 2.7
(+)-usnic acid					
31.2	2.0 ± 0.2	1.2 ± 0.1	0.1 ± 0.0	2.5 ± 0.1	17.4 ± 2.2
62.5	2.7 ± 0.2*	1.5 ± 0.1*	0.3 ± 0.1	2.5 ± 0.1	18.3 ± 2.2
125	3.7 ± 0.3*	1.8 ± 0.2*	0.4 ± 0.1*	2.1 ± 0.2*	21.5 ± 2.8*
250	3.5 ± 0.4*	1.8 ± 0.1*	0.4 ± 0.1*	2.0 ± 0.2*	25.6 ± 2.3*
500	5.1 ± 0.5*	1.9 ± 0.5*	0.8 ± 0.2*	1.3 ± 0.1*	30.2 ± 3.4*
1000	8.5 ± 0.9*	2.5 ± 0.2*	0.6 ± 0.1*	0.7 ± 0.2*	32.5 ± 6.3*
(–)-usnic acid					
31.2	2.1 ± 0.1	1.3 ± 0.2	0.3 ± 0.1	2.4 ± 0.1	17.2 ± 2.2
62.5	3.0 ± 0.2*	1.7 ± 0.1*	0.2 ± 0.1	2.0 ± 0.1	19.8 ± 2.2
125	5.2 ± 0.6*	1.6 ± 0.2*	0.4 ± 0.1*	1.8 ± 0.2*	23.4 ± 2.8*
250	5.3 ± 0.5*	1.9 ± 0.3*	0.4 ± 0.1*	1.6 ± 0.1*	29.1 ± 3.3*
500	7.4 ± 0.6*	2.3 ± 0.5*	0.8 ± 0.2*	1.4 ± 0.2*	35.0 ± 4.9*
1000	9.1 ± 1.4*	3.2 ± 0.6*	0.7 ± 0.1*	0.4 ± 0.1*	39.6 ± 5.6*

* Differences are statistically significant compared to control ($P < 0.05$, ANOVA, Dunnett test).

synthesis in thylakoids, inactivation of PS II reaction centers, and destabilization of thylakoid membranes were noted studying the effect of UA on chloroplasts [25]. Dissociation of oxidative phosphorylation and suppression of ATP synthesis were observed in mitochondria [26]. It is believed that the mentioned processes can lead to hyperproduction of ROS in cells [27]. Although it is known that UA has antioxidant activity, according to available experimental data, it is characterized by concentration-dependent inversions of effects associated with the transition of antioxidant activity to prooxidant [1, 28]. Thus, UA in relatively low concentrations (0.01–1 μM) showed antioxidant properties on human lymphocytes under the influence of UV radiation. Meanwhile, it showed prooxidant properties, enhancing the damaging effect of UV radiation, at a concentration of 100 μM [28]. Similar results were obtained studying the effect of UA on the DNA-damaging effects of the dioxidine prooxidant [29]. With respect to human lymphocytes, it was shown that both enantiomers in concentrations up to 10 μM reduced the toxic effects caused by dioxidine, and, on the contrary, they increased the toxic effects of dioxidine at a concentration of 100 μM.

Hyperproduction of ROS can initiate the mobilization of responses that can significantly increase the antioxidant potential of plants [27]. We found that an increase in the activity of antioxidant enzymes (SOD, CAT, and POX) was observed in the 62.5–1000 μM concentration range of UA of both enantiomers (Table 3). At the same time, the content of flavonoids (which also perform the function of low molecular weight antioxidants) in the tissue cells of seedlings decreased by 1.2–3.7 and 1.4–6.5 times, respectively, at 125–1000 μM of (+) and (–)-UAs. The accumulation of TBA-reactive products in seedling cells increased by 1.3–1.9 and 1.4–2.3 times, respectively, at concentrations of (+) and (–)-UAs from 125 to 1000 μM. An increase in the activity of the catalase and the content of malondialdehyde (the main LPO product) was also recorded earlier under the action of 30 μM (+)-UA on the roots of tomato seedlings, which is generally consistent with our data [30].

It can be assumed that ROS overproduction in the concentration range of 31.2–62.5 μM was compensated by the activation of protective antioxidant systems, which ensure a balanced occurrence of redox reactions in the tissues of *A. fistulosum* seedlings. At the same time, a shift in the prooxidant-antioxidant balance towards the activation of lipid peroxidation and the development of oxidative stress was observed under the action of higher concentrations of UA.

Thus, micromolar concentrations of (+) and (–)-UA caused phytotoxic effects, expressed in a decrease in laboratory germination, inhibition of growth processes, slowdown of mitotic activity of root meristems, and depigmentation of tissues of *A. fistulosum* seedlings. A dose-dependent increase in the frequency of chromosomal aberrations and the degree of damage to nuclear DNA in cells has been shown, which indi-

cates the potentially genotoxic and mutagenic effect of the studied enantiomers of UA. Moreover, (–)-UA induced a greater number of atypical DNA comets than (+)-enantiomers, which may indicate a stronger effect on DNA fragmentation in cells. An increase in the activity of antioxidant enzymes and a decrease in the content of flavonoids were observed in the concentration range of enantiomers of 62.5–1000 μM against the background of an increase in the concentration of lipid peroxidation products in cells, which indicates the development of oxidative stress. At the same time, we found no significant differences between the activity of (+) and (–)-UA at the level of physiological and biochemical parameters of seedlings.

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COMPLIANCE WITH ETHICAL STANDARDS

This article does not contain any research involving humans or animals as research objects.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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