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The effect of the 1,2,3-triazolo[5,1-*b*][1,3,4]thiadiazines on *Solanum lycopersicum* L. seed germination

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Abstract. In recent years chemical pesticides are being replaced by environmentally friendly and universal means of plant protection that are able to exert a complex effect on the plant (stimulate growth and development, improve metabolic processes, develop resistance to pathogens, etc.). The effect of new synthetic growth regulators of the 1,2,3-triazolo[5,1-b][1,3,4]thiadiazine class and trade phytohormones (6-BAP, GA and TDZ) on the growth processes, growth energy and vitality of tomato seeds, as well as morphological parameters of seedlings was researched in this article. It was revealed that the effect of synthesized compounds on seed vigor and viability of seedlings are superior to commercial phytohormones. In the early stages of germination the seed vigor of tomato seeds treated with the compounds TT1-TT5 were superior to one in treatments with phytohormones and in control experiment. As a result of the experiment, the most viable seedlings were formed into the treatments **TT1, TT2** and **TT3** (in all studied concentrations). Tomato seedlings treated with 6-BAP and GA at a concentration of 5 mg L^{-1} produced the worst results.

Key words: seed germination, plant grow regulators, *Solanum lycopersicum*, tomato seeds, 1,2,3-triazolo[5,1-*b*][1,3,4]thiadiazine, 6-benzylaminopyrine, gibberellic acid, thidiazurone, leaf tissue, development/growth, root, shoot.

INTRODUCTION

Both seed germination and the earliest stages of seedling development are crucial for the establishment of viable plantlets of agricultural plants and are important processes that determine the competitiveness and fitness of the plants in various environments, such as lighting, temperature, humidity, etc (Luo et al., 2019). The establishment of seed dormancy, subsequent germination and other developmental processes in plants, are heavily influenced by hormones. Abscisic acid is required for the establishment of seed dormancy and inhibits germination. Gibberellins, on the other hand, counteract abscisic acid responses and are considered a promoting factor in seed germination. Cytokinins also appear to act positively on germination, possibly by stimulating ethylene synthesis (Toh et al., 2011).

Both natural and synthetic phytohormones are used for regulation of seed germination and plant growth. In addition, regular fertilization, and/or growth stimulants are required for normal growth and development of plants as well as increasing yields and increasing resistance to pathogens of various types (Senberga et al., 2018).
After a long period of uncontrolled use of chemical fertilizers, strict measures have been taken to regulate and use the latter. Crop producers are interested in the emergence of new natural and synthetic compounds that do not harm the environment (Pal & McSpadden Gardener, 2006).

Currently, growth regulators and elicitors are a vast group of natural and synthetic organic compounds that influence the metabolism which stimulate plants' immunity to many bacterial and viral diseases, as well as adverse environmental factors (Pekarskas & Sinkevičienė, 2015; Nugmanova, 2017).

The requirements for the properties of chemically synthesized growth regulators are rather high: physiological activity, rapid disintegration in tissues after their action, and the absence of harmful effects on the environment and humans (Harman, 1991). A special property of a physiologically active compound should be its ability to act in low concentrations (a low number of milligrams per liter of solution).

Plant seeds are a convenient test system in the study of certain properties of substances or the study of completely new chemical compounds because the growth and development of the seed can be used as an integral indicator of the state of the plant, which reflects the physiological processes at the level of the whole organism (Taiz & Zeiger, 2002). The seeds of agricultural crops are the best for studying stimulation effects.

This article is a continuation of a study on the growth-regulating properties of newly synthesized chemical compounds of the 1,2,3-triazolo[5,1-b][1,3,4]thiadiazine class (Fig. 1) on plants of the Solanaceae family. Previously, some of the studied substances showed a stimulating effect on the growth and development of seeds of common pine and animal cells (Kalinina et al., 2015; Kalinina et al., 2018). In this paper, we studied the growth-regulating properties of five synthesized compounds and three commercial phytohormones (gibberellic acid, 6-benzylaminopurine, thiadiazuron) on tomato seeds. The aim of this work was to investigate the influence of tested compounds on seed germination and seedling development.

MATERIALS AND METHODS

The objects of study were 8 chemical compounds. 3 commercial phytohormone were used (gibberellic acid (GA), 6-benzylaminopurine (6-BAP), thiadiazuron (TDZ)), as were 5 synthesized substances: **TT1, TT2, TT3, TT4, TT5** (Fig. 1).

The tested compounds (TT1-TT5) were produced according to the previously described method (Kalinina et al., 2017). The spectroscopic characteristics of the compounds **T1**–**T4** are in accordance with the previously reported data (Kalinina et al., 2017). Compound **T5** was synthesized for the first time according to the procedure described in (Kalinina et al., 2017).

^{1}H and ^{13}C NMR spectra were acquired on a Bruker Avance II spectrometer (400 and 100 MHz, respectively) in DMSO-d_6, with TMS as the internal standard. Mass spectra were recorded on a GCMS-QP2010 Plus gas chromato-mass spectrometer (EI ionization at 70 eV). Elemental analysis was performed on a Perkin Elmer PE 2400 CHNS-analyser. Melting points were determined on a Stuart SMP3 apparatus.

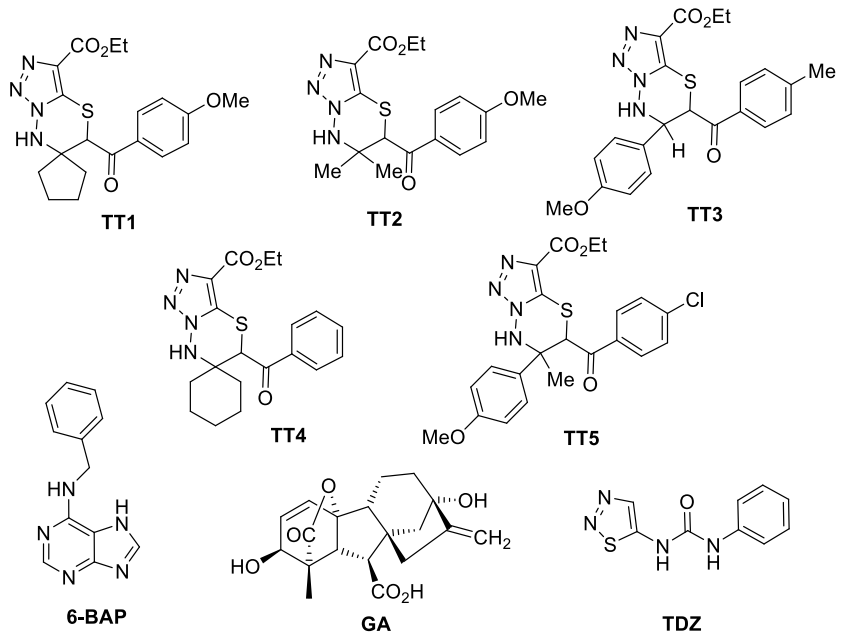


Figure 1. The structure of the studied compounds and commercial phytohormones.

Ethyl 5-(4-chlorobenzoyl)-6-methyl-6-*p*-tolyl-6,7-dihydro-5*H*-[1,2,3]triazolo[5,1-*b*][1,3,4]thiadiazine-3-carboxylate (TT5). Reaction yield is 76%, white powder, melting temperature 217–218 °C. IR υ (cm^{-1}): 3234 (NH), 2964 (CH), 2834 (CH), 1738 (CO), 1712 (CO). ^{1}H NMR (*J*, Hz): δ 1.29 (3H, t, *J*=6.5, Me), 1.47 (3H, s, Me), 3.73 (s, 3H, OMe), 4.22 (q, 2H, *J*=6.5, OCH_2), 5.75 (s, 1H, CH), 6.82 (d, 2H, *J*=7.9, ArH), 7.52 (d, 2H, *J*=7.9, ArH), 7.63 (d, 2H, *J*=7.8, ArH), 7.96 (s, 1H, NH), 8.31 (d, 2H, *J*=7.8, ArH). Attached proton test (APT) NMR: δ 13.9 (CH_3), 27.8 (CH_3), 40.6 (OCH_3), 54.9 (CH_{TDZ}), 59.7 (C_{TDZ}), 60.4 (CH_2), 113.8 (CHAr), 126.5 (CHAr), 127.1, 129.0 (CHAr), 130.8 (CHAr), 133.9, 134.8, 139.4, 158.4, 159.8, 195.7 (CO). MS-spectrum (m/z relative intensity): 472 $[M]^+$ (5), 416 (7), 400 (4), 285 (3), 251(2), 148 (100), 141 (11), 139 (35), 134 (59), 119 (18), 113 (5), 111 (14), 107 (13), 105 (5), 103 (4), 92 (18), 77 (32), 65 (7), 51 (3). Found, %: C 57.78; H 4.70; N 12.08. $C_{22}H_{21}ClN_4O_3S$. Calculated, %: C 57.83; H 4.63; N 12.26.

Biological study

The effect of the synthesized chemical compounds on seed germination was studied in 3 concentrations: 0.5, 1 and 5 mg L^{-1}. Negative control was administered with water. We used seeds of the 'Siberian early' tomato variety (Russia).

The experiment on the effect of the studied substances on the germination of tomato seeds was run in the Binger climate chamber for full control of the temperature and humidity conditions, as well as creating conditions close to natural ones: 20 °C, 55–60% humidity, and 16 daylight hours. In the experiment, we used mature tomato seeds with a stated commercial germination rate of 90%. The seeds were placed in Petri dishes on filter paper, 100 pcs per dish. For each concentration of the substance, the experiment was run three times. A total of 7,000 'Siberian early' tomato seeds were sown. To each

Petri dish 3 mL of solutions of the studied substances were added (distilled water for negative control). The measurement of the weight of the Petri dishes for humidity control was carry out every day, the difference from the starting weight was determined and the necessary amount of water to return to the starting weight was added.

The measurement of seed vigor parameters was carried out at 3 time control points: 5, 10 and 15 days (FAO, 2010). The experiment was over on 15th day. Germination (%) is percent of germinated seeds from total seeds. Seeds were counted as germinated when the length of the radicle/root was more than twice the length of the seed. To assess the quality of the seedlings, the following parameters were selected: the total length and thickness of the root, the length and width of the cotyledons, the thickness of the stem at the base of the cotyledons, the elongation of the cotyledons and the root, and the branching of the root system. These features give an estimate of the vitality of the seedling and the quality of the assimilation apparatus being formed. Simultaneously with the measurement of the linear parameters of the seedlings, a visual assessment of the condition of the seedlings was carried out on plant health scale of 0 to 5, where 0 is unsatisfactory (signs of wilting, drying out, lodging, underdevelopment, etc.), and 5 is excellent (all seedlings are viable, crops do not lodge, the cotyledons are open and have a rich green colour, etc.). The fungal contamination was evaluated visually. For this, the Petri dish was separated into 4 sectors and infested area determined in percent. The average for all experimental treatments are presented in Table 1. The experimental results were processed using the STATISTICA 8.0 software package (ANOVA, Kruskal-Wallis test, Tukey test; graph is based on the calculated Mahalanobis distances in the discriminant analysis module).

The variability of the linear parameters and the shape of the cells were studied on the first true leaf in triplicate for each treatment. To study the effect of the substances under investigation on the growth of individual cells, the tissue maceration method was used. Maceration was carried out in KOH solution on pre-dried and cut (FastPrep-24) leaflets, which were taken three times from the middle of the crown. After the solution with leaf tissues was evaporated, the tissues were additionally crushed on a glass slide with a dissecting needle, covered with a slide and examined under a CarlZeizz microscope (15 × 40) in five visual fields for each concentration. The photographs were measured in the program AxioVizion CarlZeizz.

RESULTS AND DISCUSSION

The effect of 1,2,3-triazolo[5,1-b][1,3,4]thiadiazine derivatives and trade phytohormones (6-BAP, GA and TDZ) on the growth processes, growth energy and vitality of tomato seeds was evaluated.

Assessment of the morphological and physiological parameters. On the 5th day of the experiment, an assessment of seed germination energy was performed. In general, the seed vigor of tomato seeds ranged from 2% (GA treatment at a concentration of 5 mg L^{-1}) to 77.6% (**TT3** treatment at a concentration of 1 mg L^{-1}). Inhibition of growth processes was observed in one Petri dish in the TDZ treatment at a concentration of 0.5 mg L^{-1}. Influence of concentration of substance on the germination process was not detected for compound TT2. Seed vigor of tomato seeds treated TT2 was more than in the control and phytohormone treatments for all concentration of compound TT2 and

ranged from 41 to 51% (Table 1). By the 10th day of the experiment, the proportion of germinated seeds reached maximum values in almost all treatments and concentrations, as well as in the control (water), with the exception of GA at a concentration of 5 mg L^{-1}, which demonstrated 35.7% (Table 1). By the 15th day of the experiment, the proportion of germinated seeds in the GA treatment, regardless of the concentration, increased slightly. It was noted that with increasing GA concentration, a general inhibition of seed germination rate was observed (Gupta & Chakrabarty, 2013).

Table 1. The share of germinated seeds of the 'Siberian early' tomato variety

Concentration, mg L^{-1}	5th day		10th day			15th day		
	Germinatio, %	Fungi infected, %	Germinatio, %	Fungi infected, %	Plant health scale	Germinatio, %	Fungi infected, %	Plant health scale
TT1								
0.5	27.6 ± 4.5	0	92.7 ± 2.6	0	5.0	100 ± 0.0	9.0 ± 3.3	5.0
1	30.0 ± 9.5	10.0 ± 7.6	94.7 ± 2.0	5.0 ± 2.9	3.8	100 ± 0.0	9.0 ± 2.1	4.5
5	40.6 ± 3.5	3.3 ± 1.7	97.7 ± 0.3	10.0 ± 5.8	4.2	97.7 ± 0.3	10.7 ± 0.3	4.5
TT2								
0.5	51.3 ± 3.8*	23.3 ± 7.3	97.4 ± 0.9	8.3 ± 1.7	3.7	97.0 ± 1.5	8.3 ± 1.7	4.0
1	41.3 ± 3.8*	11.7 ± 73	94.0 ± 0.6	8.3 ± 2.9	3.7	97.4 ± 0.9	8.3 ± 2.9	4.0
5	49.0 ± 7.8*	16.7 ± 8.3	94.7 ± 0.3	9.0 ± 3.3	3.0	97.4 ± 0.3	10.0 ± 2.9	4.0
TT3								
0.5	48.0 ± 24.2*	16.7 ± 3.3	94.4 ± 2.7	3.3 ± 1.7	4.3	97.0 ± 0.6	5.0 ± 0.0	4.0
1	77.6 ± 1.9*	0	98.4 ± 1.2	5.0 ± 2.9	4.0	97.4 ± 0.9	6.7 ± 1.7	4.0
5	37.6 ± 19.6	8.3 ± 4.4	92.0 ± 3.0	5.0 ± 3.0	5.0	97.4 ± 2.3	6.7 ± 1.0	4.0
TT4								
0.5	65.6 ± 6.4*	8.3 ± 3.3	92.7 ± 0.7	3.3 ± 3.3	4.3	95.0 ± 1.2	10.0 ± 5.8	4.0
1	64.3 ± 4.8*	21.7 ± 3.3	92.7 ± 2.4	8.3 ± 1.7	4.0	95.4 ± 1.5	11.7 ± 1.6	3.8
5	47.0 ± 6.7*	10.0 ± 5.8	91.7 ± 2.4	8.3 ± 1.7	3.3	94.0 ± 2.4	21.7 ± 7.3	3.3
TT5								
0.5	59.6 ± 1.2*	36.7 ± 1.7	92.0 ± 2.1	6.7 ± 1.7	4.3	97.4 ± 0.3	15.0 ± 5.0	3.0
1	36.3 ± 1.7*	3.3 ± 3.3	95.4 ± 0.7	8.3 ± 3.3	4.7	95.4 ± 0.9	20.0 ± 1.7	3.0
5	64.0 ± 12.0*	21.7 ± 7.3	94.4 ± 1.2	18.3 ± 4.4	4.7	94.0 ± 9.2	25.0 ± 5.6	3.0
TDZ								
0.5	0	–	84.4 ± 8.4	16.7 ± 4.4	1.3	83.4 ± 14.3	18.3 ± 8.3	1.8
1	11.3 ± 9.4	26.7 ± 26.7	78.4 ± 14.5	18.3 ± 9.3	1.8	77.4 ± 3.8	21.7 ± 11.7	2.5
5	25.6 ± 2.3	53.3 ± 3.3	89.4 ± 3.3	18.3 ± 5.3	2.8	80.4 ± 3.0	21.7 ± 1.7	2.5
GA								
0.5	20.6 ± 7.5	31.7 ± 4.4	93.0 ± 0.6	18.3 ± 7.3	3.2	93.0 ± 0.3	33.3 ± 12.0	3.0
1	17.0 ± 9.5	23.3 ± 12.0	79.0 ± 9.0	20.0 ± 7.3	2.5	89.0 ± 9.6	40.0 ± 10.1	3.0
5	5.0 ± 2.0	16.7 ±	35.7 ± 23.7	30.0 ± 10.0	1.0	66.3 ± 21.0	50.0 ± 26.5	1.3
6-BAP								
0.5	19.0 ± 4.0	13.3 ± 3.3	88.4 ± 0.7	23.3 ± 3.3	3.0	95.0 ± 2.5	36.7 ± 11.7	1.0
1	29.0 ± 9.0	16.7 ± 3.3	84.0 ± 0.6	18.3 ± 8.3	2.8	93.7 ± 0.7	39.7 ± 13.0	1.0
5	15.0 ± 5.1	40.0 ± 5.8	83.4 ± 5.5	23.3 ± 9.3	2.8	97.4 ± 1.2	40.0 ± 5.0	0.8
water								
–	34.0 ± 4.0	45.0 ± 0.1	91.5 ± 2.5	20.0 ± 10.0	3.3	94.0 ± 1.8	10.0 ± 5.0	4.0

Note: * – significant differences from water (Tukey test, ANOVA, $p < 0.001$).

By day 10, the tomato seedlings treated with compounds **TT1** and **TT2** at a concentration of 1 mg L^{-1} were in the lead in terms of the parameter of growth processes (Table 2, Fig. 2). The maximum linear dimensions of the root, which were noted in the **TT2** treatment at a concentration of 0.5 mg L^{-1}, were 49.07 ± 2.55 mm. The maximum linear dimensions of the cotyledons (7.78 ± 0.55 mm) were noted in the **TT1** treatment at a concentration of 1 mg L^{-1}. The maximum thickness of the young spine was noted in the 6-BAP treatment in two concentrations – 0.5 mg L^{-1} and 1 mg L^{-1} (1.42 ± 0.23 mm and 0.99 ± 0.12 mm), more than 2.5 times the size of the control. Earlier, it was noted that high concentrations of 6-BAP (more than 1 mg L^{-1}) are able to inhibit the growth of cells following stretching, which leads to the formation of short internodes and highly branched plant forms (Pan et al, 2013). In the **TT2** and **TT4** treatments, the formation of lateral roots was noted in all concentrations. Probably, these substances, like cytokines, are capable of removing the apical dominance, causing inhibition via the growing apical bud of lateral shoots (Engelbrecht, 1971).

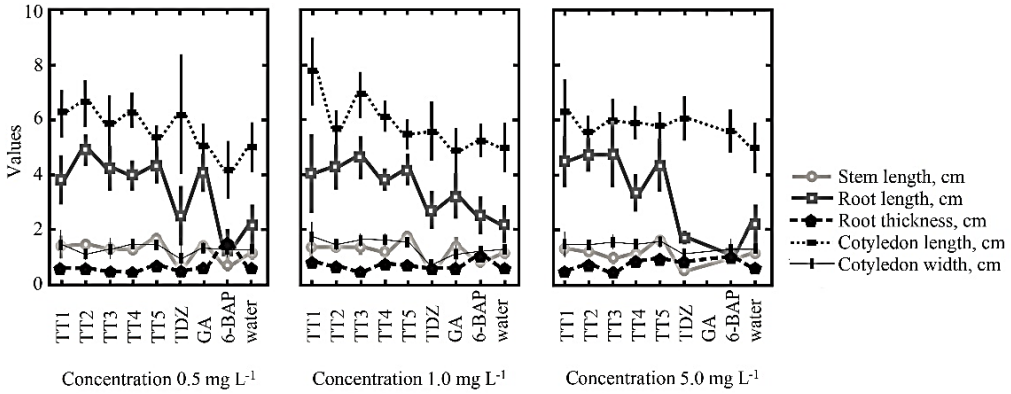
Table 2. Linear parameters of the seedling of the 'Siberian early' tomato variety on the 10th experimental day

Concentration, mg L^{-1}	Stem length, cm (M ± m)	Root length, mm (M ± m)	Root thickness, mm (M ± m)	Root elongation (M ± m)	Cotyledon length, mm (M ± m)	Cotyledon width, mm (M ± m)	Cotelydon elongation (M ± m)
TT1							
0.5	1.36 ± 0.17	38.22 ± 3.79	0.52 ± 0.01	0.014 ± 0.003	6.22 ± 0.36	1.48 ± 0.22	0.23 ± 0.04
1	1.33 ± 0.14	40.56 ± 6.21	0.79 ± 0.16	0.024 ± 0.006	7.78 ± 0.55$^{I, III}$	1.70 ± 0.23	0.23 ± 0.03
5	1.26 ± 0.11	44.83 ± 4.13	0.46 ± 0.07$^{II, III}$	0.011 ± 0.002$^{II, III}$	6.29 ± 0.53	1.48 ± 0.19	0.26 ± 0.04
TT2							
0.5	1.45 ± 0.07$^{I, II, III}$	49.07 ± 2.55^{II}	0.59 ± 0.04	0.012 ± 0.001	6.60 ± 0.40	1.13 ± 0.07	0.18 ± 0.01
1	1.37 ± 0.06^{II}	42.87 ± 3.69	0.59 ± 0.03	0.016 ± 0.002	5.73 ± 0.28	1.46 ± 0.09	0.26 ± 0.02^{II}
5	1.24 ± 0.03	47.40 ± 2.88	0.59 ± 0.02	0.013 ± 0.001	5.60 ± 0.29	1.44 ± 0.03^{II}	0.27 ± 0.02
TT3							
0.5	1.33 ± 0.08	42.18 ± 3.56	0.39 ± 0.04$^{III, V}$	0.010 ± 0.001	5.91 ± 0.46	1.33 ± 0.16	0.24 ± 0.04
1	1.39 ± 0.12^{II}	46.36 ± 3.58	0.41 ± 0.04^{III}	0.010 ± 0.001$^{I, II}$	6.93 ± 0.39	1.64 ± 0.09^{III}	0.24 ± 0.01
5	0.99 ± 0.09^{II}	47.70 ± 5.21$^{I, II}$	0.36 ± 0.04$^{II, III,V}$	0.009 ± 0.001$^{I, II,III}$	6.00 ± 0.33	1.55 ± 0.12^{II}	0.26 ± 0.02
TT4							
0.5	1.27 ± 0.07	39.67 ± 2.49	0.41 ± 0.04$^{III, V}$	0.011 ± 0.001	6.33 ± 0.30	1.49 ± 0.07	0.24 ± 0.02
1	1.23 ± 0.09	38.29 ± 1.87	0.69 ± 0.09	0.019 ± 0.003	6.14 ± 0.25	1.62 ± 0.09	0.27 ± 0.01^{II}
5	1.12 ± 0.07	33.79 ± 3.03	0.79 ± 0.10^{VI}	0.028 ± 0.006	5.93 ± 0.27	1.46 ± 0.08	0.25 ± 0.02
TT5							
0.5	1.61 ± 0.08$^{I, III}$	43.53 ± 3.01$^{I, III}$	0.60 ± 0.02	0.015 ± 0.002	5.40 ± 0.19	1.47 ± 0.04	0.28 ± 0.01
1	1.67 ± 0.09^{II}	42.07 ± 2.65$^{I, III}$	0.62 ± 0.02^{VII}	0.015 ± 0.001	5.47 ± 0.26	1.56 ± 0.05	0.30 ± 0.02
5	1.61 ± 0.10	43.71 ± 4.33	0.84 ± 0.11^{VI}	0.022 ± 0.003^{III}	5.79 ± 0.24	1.56 ± 0.09^{II}	0.28 ± 0.02

Table 2 (continued)

TDZ							
0.5	0.62 ± 0.06	25.00 ± 3.91^{V}	0.50 ± 0.10	0.021 ± 0.003	6.20 ± 0.80	0.93 ± 0.26	0.14 ± 0.02
1	0.57 ± 0.06	27.20 ± 3.03^{VI}	0.55 ± 0.06	0.024 ± 0.005	5.60 ± 0.48	0.72 ± 0.04^{V}	0.14 ± 0.01^{V}
5	0.58 ± 0.04^{I}	16.73 ± 1.06	0.81 ± 0.06	0.052 ± 0.007	6.07 ± 0.36	1.10 ± 0.07^{V,VI}	0.19 ± 0.02
GA							
0.5	1.39 ± 0.09	40.69 ± 3.19	0.53 ± 0.02	0.014 ± 0.001	5.08 ± 0.37	1.28 ± 0.08	0.27 ± 0.03
1	1.38 ± 0.14	31.89 ± 3.30	0.54 ± 0.04	0.019 ± 0.002	4.89 ± 0.35^{IV}	1.12 ± 0.14	0.23 ± 0.03
6-BAP							
0.5	0.74 ± 0.17^{IV,V,VI}	13.20 ± 2.46	1.42 ± 0.23^{IV}	0.128 ± 0.041^{IV}	4.20 ± 0.37	1.32 ± 0.13	0.32 ± 0.02
1	0.88 ± 0.09^{II}	25.17 ± 3.06	1.03 ± 0.11^{VI,I}	0.054 ± 0.014^{V,VI,VIII}	5.25 ± 0.28	1.17 ± 0.06^{VII}	0.23 ± 0.01
5	0.96 ± 0.07^{V}	11.10 ± 1.21$^{I, III}$	0.99 ± 0.12^{VI,VII}	0.106 ± 0.023^{V,VI}	5.60 ± 0.34	1.30 ± 0.15	0.24 ± 0.03
water							
–	1.15 ± 0.07^{II}	21.90 ± 3.03^{VI}	0.53 ± 0.03	0.033 ± 0.009	5.00 ± 0.39	1.30 ± 0.04	0.28 ± 0.03

Note: M – mean value; m – standard error of mean.
^{I} – significant differences from water (Kraskall-Wallis test, ANOVA, $p < 0.001$); ^{II} – significant differences from TDZ (Kraskall-Wallis test, ANOVA, $p < 0.001$); ^{IV} – significant differences from TT4 (Kraskall-Wallis test, ANOVA, $p < 0.001$); ^{V} – significant differences from TT5 (Kraskall-Wallis test, ANOVA, $p < 0.001$); ^{VI} – significant differences from TT3 (Kraskall-Wallis test, ANOVA, $p < 0.001$); ^{VII} – significant differences from TT1 (Kraskall-Wallis test, ANOVA, $p < 0.001$).



Note: ⋆ – mean value; │– standard error of mean.

Figure 2. The visualisation of some of the linear parameter of the seedling of the "Siberian early" tomato variety on the 10th day of the experiment.

The maximum length of a young root on the 15th day is seen in seedlings treated with the **TT2** compound (5.24 ± 0.49 cm) at a concentration of 1 mg L^{-1}, which is almost 1.5 times more than in the control (3.64 ± 0.69 cm), while the minimum length was demonstrated by seedlings treated with the TDZ compound (1.92 ± 0.20 cm) in the same concentration, which is 1.9 times less than in the control. In the remaining treatments **TT2, TT3, TT4,** and **TT5**, the seedlings were characterized by some increase in the length of the linear parameters (with respect to the control) (Table 3, Fig. 3).

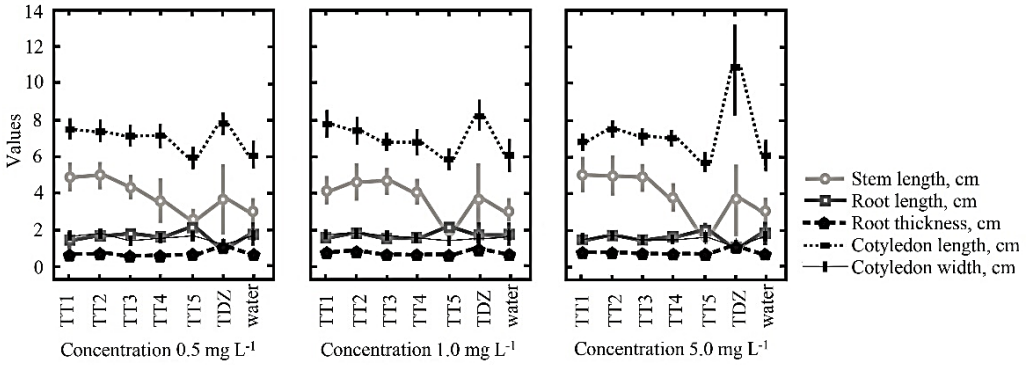
Table 3. Linear parameters of the seedlings of the "Siberian early" tomato variety on the 15th experimental day

Concentration, g L^{-1}	Stem length, cm (M ± m)	Root length, mm (M ± m)	Root thickness, mm (M ± m)	Root elongation (M ± m)	Cotyledon length, mm (M ± m)	Cotyledon width, mm (M ± m)	Cotelydon elongation (M ± m)	Branching of the root system
TT1								
0.5	3.85 ± 0.36 VI	1.49 ± 0.11	0.66 ± 0.03	0.19 ± 0.02	7.46 ± 0.29	1.72 ± 0.08	0.23 ± 0.01	0.31 ± 0.01
1	5.24 ± 0.49	1.52 ± 0.07	0.72 ± 0.02	0.15 ± 0.01	7.77 ± 0.34 I, V	1.85 ± 0.07 II	0.24 ± 0.01	0.85 ± 0.01
5	4.37 ± 0.40	1.45 ± 0.07	0.75 ± 0.02 II	0.20 ± 0.02	6.80 ± 0.22	1.53 ± 0.09	0.23 ± 0.02*	1.00 ± 0.01
TT2								
0.5	4.88 ± 0.36	1.68 ± 0.09	0.60 ± 0.04	0.13 ± 0.02	7.40 ± 0.31	1.78 ± 0.04	0.25 ± 0.01	1.00 ± 0.01
1	4.15 ± 0.35	1.79 ± 0.08	0.72 ± 0.03	0.19 ± 0.02	7.40 ± 0.34 I, V	1.77 ± 0.07	0.25 ± 0.02	0.80 ± 0.01
5	5.05 ± 0.45	1.65 ± 0.07	0.72 ± 0.02 II, IV, V	0.16 ± 0.02	7.53 ± 0.22 II	1.63 ± 0.05	0.22 ± 0.01 II	1.00 ± 0.01
TT3								
0.5	4.95 ± 0.35	1.77 ± 0.05	0.55 ± 0.03	0.12 ± 0.01	7.13 ± 0.26	1.48 ± 0.08	0.21 ± 0.02	0.80 ± 0.01
1	4.61 ± 0.48 I, II	1.53 ± 0.07	0.56 ± 0.03	0.15 ± 0.02	6.80 ± 0.22	1.66 ± 0.05	0.25 ± 0.01	0.93 ± 0.01
5	4.99 ± 0.52	1.49 ± 0.09	0.57 ± 0.02 II, IV,V	0.14 ± 0.02	7.07 ± 0.23	1.47 ± 0.06	0.21 ± 0.01	0.73 ± 0.01
TT4								
0.5	4.30 ± 0.30	1.65 ± 0.09	0.58 ± 0.02	0.15 ± 0.02	7.13 ± 0.31	1.60 ± 0.07	0.23 ± 0.01	1.00 ± 0.01
1	4.65 ± 0.34	1.51 ± 0.07	0.59 ± 0.03	0.13 ± 0.01	6.80 ± 0.33 II	1.52 ± 0.07	0.23 ± 0.02	0.93 ± 0.01
5	4.82 ± 0.35	1.58 ± 0.05	0.63 ± 0.02	0.14 ± 0.01	7.00 ± 0.20	1.43 ± 0.08	0.21 ± 0.01	0.86 ± 0.01
TT5								
0.5	3.57 ± 0.51II	2.14 ± 0.09	0.64 ± 0.03	0.21 ± 0.03	5.90 ± 0.28 VII	1.70 ± 0.12	0.30 ± 0.03	0.70 ± 0.01
1	4.04 ± 0.33 II,VII	2.11 ± 0.11	0.58 ± 0.03	0.16 ± 0.02	5.87 ± 0.26 II	1.44 ± 0.09 II	0.25 ± 0.02	0.40 ± 0.01
5	3.76 ± 0.35	2.01 ± 0.18	0.57 ± 0.03 II, VII	0.18 ± 0.02	5.73 ± 0.23 V	1.52 ± 0.10	0.27 ± 0.02 II	0.53 ± 0.01
TDZ								
0.5	2.58 ± 0.25 I, V	1.03 ± 0.05 I	0.96 ± 0.09 I, IV,V	0.44 ± 0.07	7.85 ± 0.27 V	1.26 ± 0.09 I, V	0.16 ± 0.10I, V	0.00*
1	1.92 ± 0.20I, V, VI, VII	1.02 ± 0.05 I, VI	0.86 ± 0.05 V	0.50 ± 0.05 VI	8.23 ± 0.38 I, V	1.52 ± 0.12	0.19 ± 0.01	0.00*
5	1.53 ± 0.18 I, V, VI	1.10 ± 0.04 I, V, VI	0.99 ± 0.07 VI, VII	0.73 ± 0.10 I	10.75 ± 1.0 I, V, VII	1.23 ± 0.13	0.12 ± 0.02V	0.00*
water								
–	3.64 ± 0.69	1.80 ± 0.22	0.62 ± 0.08	0.21 ± 0.05	6.00 ± 0.32	1.71 ± 0.15	0.29 ± 0.03	0.40 ± 0.01

Note: M – mean value; m – standard error of mean.

* – significant differences from all groups (Kraskall-Wallis test, ANOVA, $p < 0.001$); I – significant differences from water (Kraskall-Wallis test, ANOVA, $p < 0.001$); II – significant differences from TDZ (Kraskall-Wallis test, ANOVA, $p < 0.001$); IV – significant differences from TT4 (Kraskall-Wallis test, ANOVA, $p < 0.001$); V – significant differences from TT5 (Kraskall-Wallis test, ANOVA, $p < 0.001$); VI – significant differences from TT3 (Kraskall-Wallis test, ANOVA, $p < 0.001$; VII – significant differences from TT1 (Kraskall-Wallis test, ANOVA, $p < 0.001$).

All seedlings treated with GA and BAP on 15th day were contaminated by fungi and most of them were putrefied. The minimal linear parameters of seedlings were noted in the TDZ treatment (Table 3) in all concentrations. The seedlings with minimal linear parameters were observed in experimental treatment TDZ in a concentration of 5 mg L^{-1}: the length of the root was 1.53 ± 0.18 cm, which is 2.4 times lower than in the control, and 1.23 ± 0.13 mm wide, which is 1.4 times lower than in the control. However, a number of authors have noted the positive effect of low concentrations of 6-BAP and TDZ (0.5 mg L^{-1}) on plant rhizogenesis (Shein et al, 2004; Laplaze et al, 2007).



Note: ⋆ – mean value; │– standard error of mean.

Figure 3. The visualisation of some linear parameter of the seedling of the "Siberian early" tomato variety on the 15th day of the experiment.

In the course of this experiment, a positive effect of TDZ at a concentration of 5 mg L^{-1} on the formation of cotyledons was revealed, the length of which was 10.75 ± 1.08 mm (maximum). This is probably due to compensatory mechanisms: blocking the development of the root and the apical meristem by a maximum concentration of TDZ provoked an increase in the linear parameters of the cotyledons, it is important for incresing of photosynthetic activity.

By the 15th day, the share of fungal invasion in the studied treatments remained at an average level of 10–15%, which indicates a certain inhibition by these compounds of the development of the fungal mycelium. An increase in the pathogenic fungal mycelium in the **TT4** treatment at a concentration of 5 mg L^{-1} to 35% was noted once. In the comparison group (in the GA, 6-BAP, and TDZ treatments), only in the TDZ treatment at a concentration of 5 mg L^{-1} did the proportion of fungi by the end of the experiment range from 5 to 10%. In the remaining treatments, GA and 6-BAP, the share of fungi increased to 55% by the 15th day, and once amounted to 100% (GA, 5 mg L^{-1}) (Table 3).

Tomato seeds treated with these compounds formed weakened seedlings by 15th day, which later quickly died when planted into the soil substrate.

The general condition of the tomato seedlings formed was significantly different depending on the studied compounds and their concentration. By the end of the experiment, the most viable seedlings grew in treatments **TT2**, **TT1**, and **TT4** (in all concentrations), where a low percentage of cup infection with mold fungi was also noted. The seedlings treated with 6-BAP and GA in the maximum concentrations, where fungal invasion was high, produced the worst results (Table 1). In a number of works

(Yamauchi, et al, 2004; Kucera et al, 2005; de Lucas et al, 2008), growth was inhibited against a background of an increase of mitoses in the shoot meristems with high concentrations of GA, 6-BAP, and TDZ, applied both separately and in various combinations. It is known that GA plays an important role in the revival of seeds from the state of rest, has a stimulating effect during the period of cell division, and also ensures the growth of an interstice by stretching the cells. With prolonged exposure to GA, as well as high concentrations in plants, seedlings are formed with a changed ratio of cotyledons to root system: equally, the seedlings have a pale green colour and the root may lag behind or not develop at all and also effect on cell elongation and as a result in long thin hypocotyls. (Lang, 1970; Bewley, 1997; Kucera et al., 2005).

Assessment of the anatomical parameters of the leaf. Significant statistical differences in the size and shape changes of mesophyll cells of tomato leaves grown using synthesized compounds and trading phytohormones were not found. We selected three substances - gibberellic acid (GA), one synthesized compound (TT3) and water as control to illustrate cell structure of a mesophyll of treated tomato plants (Fig. 4).

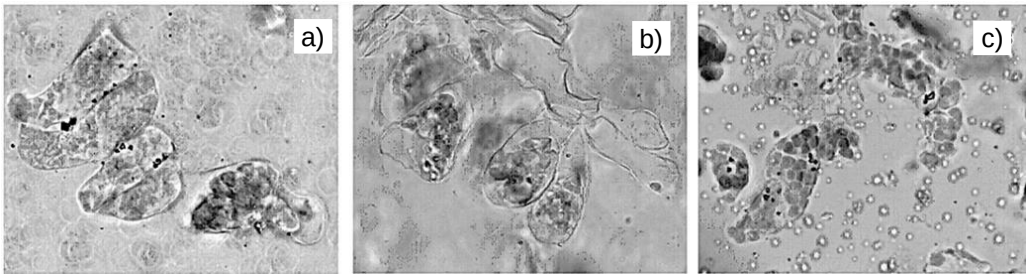


Figure 4. Cell structure of a mesophyll of tomato plants treated with: a) water, b) GA (1 mg L^{-1}), and c) TT3 (5 mg L^{-1}).

Comparison of the studied parameters of the leaf showed that the length, width, and area of cells leads in plants treated with GA at a concentration of 1 mg L^{-1} exceeds control by 56%, 43%, and 36%, respectively (Table 4). It is interesting to note that a sharp increase in the linear dimensions of cells in the GA treatment (1 mg L^{-1}) somewhat reduces their tortuosity. The treatment of GA at concentrations of 0.5 and 5 mg L^{-1} does not give such a pronounced result, although the most tortuous cells were noted at a concentration of 0.5 mg L^{-1} (Table 4). It was once again possible to confirm that one of the most important stimulation effects of GA is the acceleration of cell growth by stretching them (Gupta & Chakrabarty, 2013).

The length of the cells of plants treated with **TT3** at a concentration of 5 mg L^{-1} and GA at a concentration of 1 mg L^{-1} have a similar value. At the same time, the concentration of **TT3** 5 mg L^{-1} even exceeds that of GA (only 55.9 ± 3.22 μm). The width and area of cells in plants treated with **TT3** exceeds the control by an average of 1.4–2.1 times, but is inferior to cells of healthy plants after exposure to GA, except for the cell area at a concentration of 5 mg L^{-1} (Table 4). The mesophyll cells of plants treated with TDZ had the most minimum linear parameters in comparison with mesophyll cells of the other experiment treatments.

Table 4. Average parameters of the mesophyll cells of tomato leaves

Compound	Concentration, mg L^{-1} (M ± m)	Length, µm (M ± m)	Width, µm (M ± m)	Area, µm ^{2} (M ± m)	Perimetr, µm (M ± m)
TT1	0.5	62.01 ± 6.80	29.28 ± 1.90	1,382.97 ± 92.42	132.77 ± 11.60
	1	59.34 ± 4.90	27.80 ± 1.45	1,295.24 ± 98.33	148.39 ± 12.80
	5	64.12 ± 8.01	22.45 ± 2.09	1,404.67 ± 101.08	139.99 ± 10.42
TT2	0.5	52.13 ± 6.44	22.69 ± 1.88	1,136.23 ± 136.08	142.83 ± 12.45
	1	55.06 ± 4.81	25.07 ± 1.95	1,354.98 ± 104.73	125.70 ± 10.80
	5	59.86 ± 5.05	25.33 ± 1.64	1,476.65 ± 128.12	149.64 ± 13.17
TT3	0.5	56.18 ± 3.20	24.66 ± 1.14	1,143.53 ± 87.65	154.12 ± 9.12
	1	**69.27 ±** 6.17	25.62 ± 1.86	1,544.36 ± 160.40	180.49 ± 11.77
	5	68.01 ± 7.97	**31.81 ±** 2.36	**1,638.25 ±** 197.81	155.85 ± 28.08
TT4	0.5	54.72 ± 4.99	21.86 ± 1.90	1,451.22 ± 142.08	150.33 ± 14.98
	1	63.97 ± 4.01	27.77 ± 2.23	1,539.17 ± 127.84	171.45 ± 17.80
	5	67.90 ± 5.62	25.90 ± 2.56	1,595.38 ± 160.55	163.98 ± 11.23
TT5	0.5	53.81 ± 5.29	26.38 ± 2.43	1,206.87 ± 109.70	134.66 ± 10.65
	1	58.32 ± 6.87	27.53 ± 2.53	1,387.90 ± 129.58	139.43 ± 13.99
	5	55.43 ± 5.33	27.84 ± 1.69	1,364.73 ± 111.17	154.87 ± 14.04
TDZ	0.5	51.06 ± 4.02	19.15 ± 1.96	983.72 ± 231.56	105.44 ± 9.52
	1	59.34 ± 5.12	21.22 ± 1,55	1,108.56 ± 126.90	138.33 ± 10.20
	5	49.74 ± 5.33	20.68 ± 2.49	949.01 ± 195.40	122.84 ± 11.15
GA	0.5	65.42 ± 6.41	31.43 ± 4.31	1,501.62 ± 241.13	**421.38 ±** 254.84
	1	**70.08** ± 7.09	**41.74** ± 2.59	**2,191.54 ±** 300.30	193.2 ± 16.12
	5	55.95 ± 3.22	37.49 ± 2.02	1,622.78 ± 123.96	283.29 ± 124.99
6-BAP	0.5	55.82 ± 4.65	25.62 ± 1.96	1,254.38 ± 121.40	138.59 ± 26.97
	1	58.21 ± 6.01	24.06 ± 1.54	1,304.25 ± 98.65	129.31 ± 19.55
	5	56.93 ± 3.44	25.22 ± 2.07	1,298.03 ± 109.21	140.50 ± 10.39
water	–	39.55 ± 3.38	17.93 ± 2.54	789.19 ± 227.90	122.47 ± 11.12

Note: M – mean value; m – standard error of mean.
No significant differences (Kraskall-Wallis test, ANOVA, $p \geq 0.001$).

Fig. 5 shows the results of discriminant analysis. The scatter plot is based on the calculated values of the Mahalanobis square of the studied treatments. In terms of linear parameters, the tomato seedlings formed from seeds treated with **TT1–TT4** treatments are close. An exception is the **TT5** treatment, where the maximum dimensions of the young stalk (2.14 ± 0.09 cm) and the minimum length of the cotyledons (5.90 ± 0.27 mm) are noted. The differences of the linear dimensions of the cotyledons is well illustrated in the graph (Fig. 4). Along the Y-axis, the separation of the treatments passed the branch parameter of the root system. In the **TT1–TT4** treatments, the appearance of well-developed lateral roots was observed by the 15th day in all concentrations, with the exception of the **TT5** treatment. In the comparison group (6-BAP, TDZ, GA) branching was not observed. In the control, the beginning of lateral root growth was observed in 30% of cases.

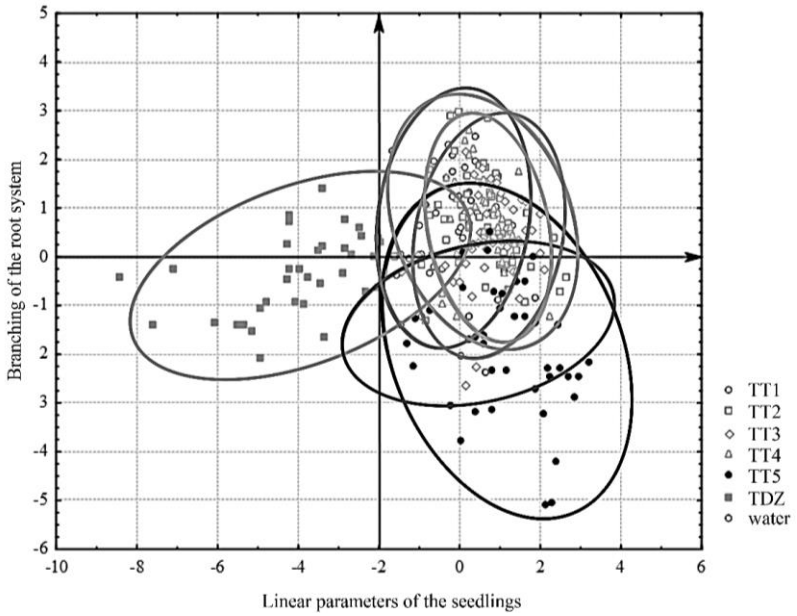


Figure 5. Visualisation of discriminant analysis results.

CONCLUSIONS

Thus, seed treatment with TT series compounds positively affected seed vigor and viability of seedlings (plant health scale on the 10th and 15th day of the experiment, Table 1) compared with treatments with commercial phytohormones (GA, 6-BAP, TDZ). Among the tested treatments of the **TT** series, the best properties for stimulating germination, subsequent removal of the apical blockade, the formation of the photosynthetic apparatus, and the suppression of the development of pathogenic fungal mycelium are possessed by compounds **TT1** and **TT4**. It should be noted that, in terms of their properties, these compounds are close to the cytokine group (triggering embryogenesis, stimulating the development of meristem, removing the apical dominant, accelerating the growth of the axial root and the development of lateral roots, etc.) (Kim et al, 2005; Osugi, A. & Sakakibara, 2015). From the compounds **TT2** and **TT3** in individual concentrations on 5^{th} day, up to 77.6% showed good stimulation in the early stages of germination.

Despite the high concentrations (5 mg L^{-1}), there was not only inhibition of growth processes (as opposed to TDZ, GA, and 6-BAP), but also a reduction in the linear parameters of the roots and cotyledons of tomato seedlings of the 'Siberian early' variety.

Synthetic compounds **TT1–TT4** can be recommended, after checking their safety, for stimulating the germination and subsequent development of crops with a low percentage of seed germination or low viability of young seedlings, as well as for increasing the quantity and quality of planting material.

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