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Study of auxin-like and cytokinin-like activities of derivatives of pyrimidine, pyrazole, isoflavones, pyridine, oxazolopyrimidine and oxazole on haricot bean and pumpkin plants

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Abstract: The auxin-like and cytokinin-like activities of new chemical low molecular weight heterocyclic compounds, derivatives of pyrimidine, pyrazole, isoflavones, pyridine, oxazolopyrimidine and oxazole were studied. The specific bioassay on auxin-like activity showed high stimulating effect of the chemical heterocyclic compounds, derivatives of pyrimidine, pyrazole, isoflavones, and pyridine used at the concentration 10^{-8}M on the formation of adventitious roots on the 14[th]-day-old leaf petioles isolated from seedlings of haricot bean (*Phaseolus vulgaris* L.) cultivar Belozernaya, which was similar or higher of the effect of plant hormones auxins IAA and NAA used at the same concentration 10^{-8}M. The specific bioassay on cytokinin-like activity showed the high stimulating effect of the chemical heterocyclic compounds, derivatives of pyrimidine, pyrazole, isoflavones, pyridine, oxazolopyrimidine and oxazole used at the concentrations 10^{-8}M and 10^{-9}M on the growth of biomass of 16[th]-day-old cotyledons isolated from seeds of muscat pumpkin (*Cucurbita moschata* Duch. et Poir.) cultivar Gilea, which was similar or higher of the effect of plant hormone cytokinin Kinetin used at the same concentrations 10^{-8}M and 10^{-9}M. The obtained results proved the inducing auxin-like and cytokinin-like effect of synthetic heterocyclic compounds on plant cell elongation, division, and differentiation that are the basic processes of plant growth.
Keywords: plant hormones, IAA, NAA, Kinetin, pyrimidine, pyrazole, isoflavones, pyridine, oxazolopyrimidine, oxazole, *Phaseolus vulgaris* L., *Cucurbita moschata* Duch. et Poir.

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1. Introduction

The successful development of modern agriculture is based on practical application of high-intensive technologies of plant growing. Today, natural plant growth regulators, such as plant hormones or their synthetic analogs having phytohormone-like activity, biofertilizers and biostimulants are widely used in the agricultural practice to accelerate plant growth, increase plant productivity, and to protect plant against abiotic and biotic stress factors causing adverse effects on plant growth and yield[1-25].

A very promising approach is the elaboration of new classes of plant growth regulating substances having high physiological activity at low concentrations, broad specificity of action in various agricultural crops and lack of toxic effect for environment, animal and human health. The great theoretical and practical interest for plant biologists is study of specificity of plant growth regulating activity of new bioactive compounds of chemical or natural origin, for this purpose, various bioassays specific for the plant hormone-like activity are used. As is known, these bioassays are based on the key role of plant hormones or their synthetic analogues in control of plant cell division, elongation, and differentiation that are basic processes of formation and growth of plant vegetative and reproductive organs such as leaf, stem, root, flower, fruit and seed[26-29].

The most well-known bioassays include bioassays specific for auxin-like and cytokinin-like activities based on a stimulating effect of natural or chemical compounds on the basic processes of plant growth such as acceleration of the germination of plant seeds and growth of plant seedlings, elongation of hypocotyl or coleoptile on the plant seedlings, formation of the adventitious roots on the plant seedlings or on the isolated stem and leaf cuttings, increasing of growth of biomass of cotyledons isolated from plant seeds, delaying of leaf senescence, and *in vitro* morphogenesis in plants[30-45].

In recent years the considerable attention is focused on study of plant growth regulating activity of different classes of low-molecular weight heterocyclic compounds, some of them, belonging to derivatives of pyrimidine, pyrazole, isoflavones, pyridine, oxazolopyrimidine and oxazole, have already found practical application in the agriculture as effective substitutes of traditional plant growth regulators, herbicides, fungicides and antimicrobial agents[46-58].

Today the new classes of the plant growth regulating substances are elaborated on the base of chemical low molecular weight five and six-membered heterocyclic compounds synthesized in the Institute of Bioorganic Chemistry and Petrochemistry of National Academy of Sciences of Ukraine. Our numerous researchers showed that different classes of chemical low molecular weight heterocyclic compounds, derivatives of pyrimidine, pyrazole, isoflavones, pyridine, oxazolopyrimidine and oxazole revealed a high stimulating auxin-like and cytokinin-like effect on seed germination and vegetative growth of various agricultural crops, and *in vitro* morphogenesis in plants[59-67].

The main task of our present work was study of the auxin-like and cytokinin-like activities of new chemical low molecular weight heterocyclic compounds, derivatives of pyrimidine, pyrazole, isoflavones, pyridine, oxazolopyrimidine and oxazole using specific bioassays on the isolated organs of haricot bean and pumpkin plants.

2.Materials and methods

2.1. Plant treatment and growing

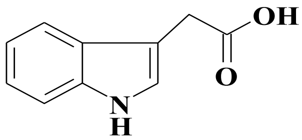
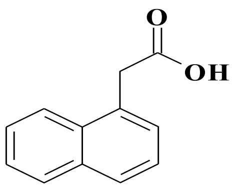
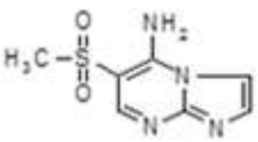
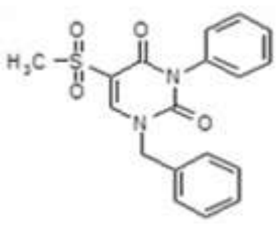
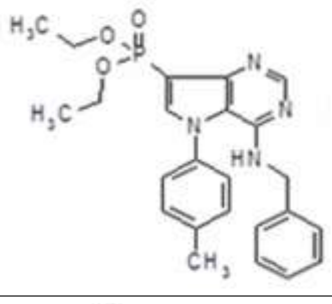
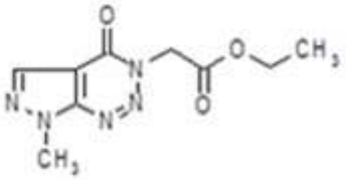
2.1.1. Bioassay on auxin-like activity of chemical low molecular weight heterocyclic compounds

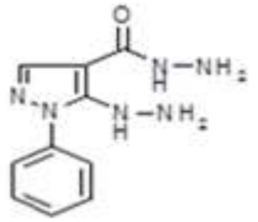
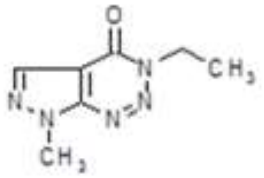
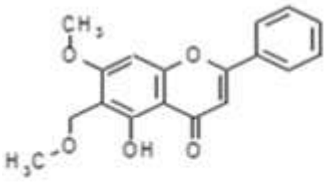
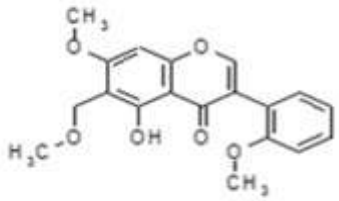
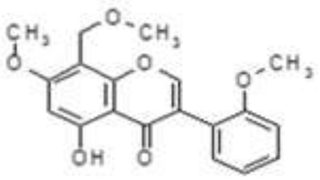
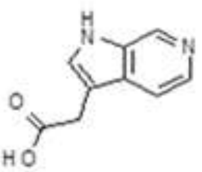
As is known, the major plant hormones auxins are involved in control of plant embryogenesis, seed germination, cell elongation and division in plant hypocotyls and coleoptiles, apical dominance, cambium cell division, plant tropisms, growth and development of root system, promotion of fruit setting, prevention of leaf abscission, plant-pathogen interactions, and *in vitro* morphogenesis in plants[30-41].

In our work to study auxin-like activity of chemical heterocyclic compounds, derivatives of pyrimidine, pyrazole, isoflavones, and pyridine we used specific bioassay conducted on the leaf petioles isolated from seedlings of haricot bean (*Phaseolus vulgaris* L.) cultivar Belozernaya. As is known, this bioassay is based on a key role of auxins in regulation of formation of adventitious roots on the stem and leaf cuttings[34-41]. The activity of chemical low molecular weight heterocyclic compounds, derivatives of pyrimidine, pyrazole, isoflavones, and pyridine was compared with the activity of plant hormones auxins IAA and NAA.

The chemical structure, chemical name and molecular mass (MM) of plant hormones auxins IAA (1*H*-Indol-3-ylacetic acid) and NAA (1-Naphthylacetic acid), and tested chemical heterocyclic compounds, derivatives of pyrimidine (compounds № 1-3), pyrazole (compounds № 4-6), isoflavones (compounds № 7-9), and pyridine (compound № 10) are shown in the Table 1.

Table 1: Chemical structure of plant hormones auxins and chemical heterocyclic compounds, derivatives of pyrimidine, pyrazole, isoflavones, and pyridine

№	Chemical structure of compounds	Chemical name and relative molecular mass of compounds
IAA		IAA (1*H*-Indol-3-ylacetic acid), MM 175.19
NAA		NAA (1-Naphthylacetic acid), MM 186.21
1		6-Methanesulfonyl-imidazo[1,2-*a*]pyrimidine-5-ylamine, MM 212.23
2		1-Benzyl-5-methanesulfonyl-3-phenyl-1*H*-pyrimidine-2,4-dione, MM 356.48
3		4-Benzylamino-5-*p*-tolyl-5*H*-pyrrolo-[3,2-*d*]pyrimidin-7-yl)-phosphonic acid diethyl ester, MM 450.48
4		Ethyl 2-(4-oxo-7-methyl-4,7-dihydro-3*H*-pyrazolo[3,4-*d*]-[1,2,3]triazin-3-yl)acetate, MM 237.22

5		5-Hydrazino-1-phenyl-1*H*-pyrazole-4-carbohydrazide, MM 232.25
6		3-Ethyl-7-methyl-3,7-dihydro-4*H*-pyrazolo[3,4-*d*][1,2,3] triazin-4-one, MM 179.18
7		5-Hydroxy-7-methoxy-6-(methoxymethyl)-2-phenyl-4*H*-chromen-4-one, MM 312.32
8		5-Hydroxy-7-methoxy-6-(methoxymethyl)-3-(2-methoxyphenyl)-4*H*-chromen-4-one, MM 342.35
9		5-Hydroxy-7-methoxy-8-(methoxymethyl)-3-(4-methoxyphenyl)-4*H*-chromen-4-one, MM 342.35
10		((1H-pyrrolo[2,3-*c*]pyridin-3-yl)-acetic acid), MM 176.175

To study auxin-like activity of chemical low molecular weight heterocyclic compounds, seeds of haricot bean (*Phaseolus vulgaris* L.) cultivar Belozernaya were sterilized in 1 % $KMnO_4$ solution for 3 min and 96 % ethanol solution for 1 min and washed three times in the sterilized distilled water. After this procedure seeds were placed in the cuvettes (each containing 15–20 seeds) on the perlite moistened with distilled water. Then seeds were placed in the thermostat for their germination in darkness at the temperature 23°C during 48 hours. Sprouted seedlings were placed in the plant growth chamber in which seedlings were grown for 10 days at the 16/8 h light/dark conditions, at the temperature 23-25°C, light intensity 3000 lux and air humidity 60-80 %**.** To stimulate the formation of roots on the leaf petioles isolated from haricot bean seedlings they were cut at a distance of 4.3 mm from their base and then were placed immediately to a depth of 3 cm in separate glass test-tubes 30 cm in length containing either distilled water (control), or water solution of chemical heterocyclic compounds used at the concentration 10^{-8}M, or water solution of plant hormones auxins IAA and NAA used at the same concentration 10^{-8}M. After 14^{th} days the indices of total roots number (pcs) and total length of roots (mm) calculated per one experimental haricot bean leaf petiole were determined and compared with the analogical indices of control leaf petiole on which the formation of adventitious roots should not be observed.

2.1.2. Bioassay on cytokinin-like activity of chemical low molecular weight heterocyclic compounds

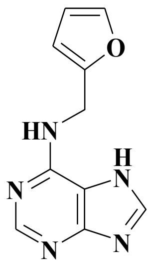
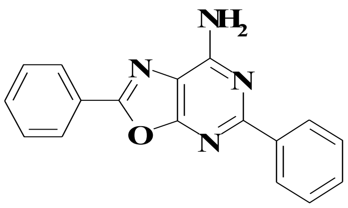
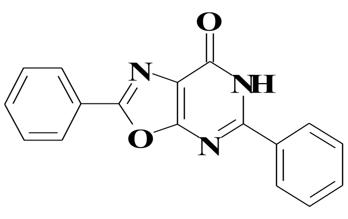
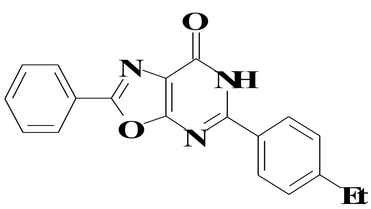
As is known, the major plant hormones cytokinins take an important part in control of embryo patterning, seed germination, de-etiolation, cell cycle control and protein synthesis, chloroplast differentiation, overcoming of apical dominance, releasing of lateral buds from dormancy, flower and fruit development, delaying of leaf senescence, plant-pathogen interactions, and *in vitro* morphogenesis in plants[40-44].

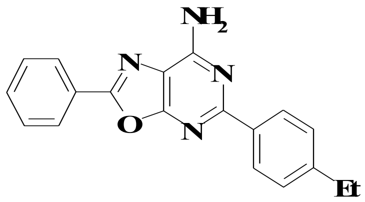
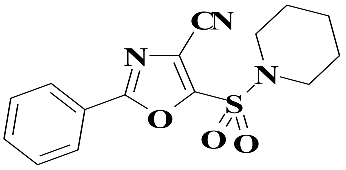
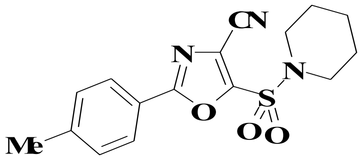
In our work to study cytokinin-like activity of chemical heterocyclic compounds, derivatives of pyrimidine, pyrazole, isoflavones, and pyridine we used specific bioassay conducted on the cotyledons (i.e. food-storage organs) isolated from seeds of muscat pumpkin (*Cucurbita moschata* Duch. et Poir.) cultivar Gilea. As is known, this bioassay is based on key role of cytokinins in regulation of cell division in isolated plant organs, which leads to an increase in their biomass[40,45]. The activity of chemical heterocyclic compounds was compared with the activity of plant hormone cytokinin Kinetin.

The chemical structure, chemical name and molecular mass (MM) of tested chemical heterocyclic compounds, derivatives of pyrimidine, pyrazole, isoflavones, pyridine are shown in the Table 1.

The chemical structure, chemical name and molecular mass (MM) of plant hormone cytokinin Kinetin (*N*-(2-Furylmethyl)-7*H*-purin-6-amine), and tested chemical heterocyclic compounds, derivatives of oxazolopyrimidine (compounds № 1-4) and oxazole (compounds № 5 and 6) are shown in the Table 2.

Table 2: Chemical structure of plant hormone cytokinin and chemical heterocyclic compounds, derivatives of oxazolopyrimidine and oxazole

№ Compound	Chemical structure of compounds	Chemical name and relative molecular mass of compounds
Kinetin		*N*-(2-Furylmethyl)-7*H*-purin-6-amine, MM 215.22
1		7-Amino-2,5-diphenyl[1,3]oxazolo[5,4-*d*]pyrimidine, MM 288.31
2		2,5-Diphenyl[1,3]oxazolo[5,4-*d*]pyrimidin-7(6*H*)-one, MM 289.30
3		5-(4-Ethylphenyl)-2-phenyl[1,3]oxazolo[5,4-*d*]pyrimidin-7(6*H*)-one, MM 317.35

4		7-Amino-5-(4-ethylphenyl)-2-phenyl[1,3]oxazolo[5,4-*d*]pyrimidine, MM 316.37
5		2-Phenyl-5-(piperidin-1-ylsulfonyl)-1,3-oxazole-4-carbonitrile, MM 317.37
6		2-Tolyl-5-(piperidin-1-ylsulfonyl)-1,3-oxazole-4-carbonitrile, MM 331.40

To study cytokinin-like activity of chemical low molecular weight heterocyclic compounds, seeds of muscat pumpkin (*Cucurbita moschata* Duch. et Poir.) cultivar Gilea were sterilized in 1 % $KMnO_4$ solution for 3 min and 96 % ethanol solution for 1 min and washed three times in the sterilized distilled water. After this procedure seeds were placed in the cuvettes (each containing 20-25 seeds) on the filter paper moistened with distilled water. Then seeds were placed in the thermostat for their germination in darkness at the temperature 25°C during 96 hours. The 4th-day-old pumpkin seedlings were separated from cotyledons using sterile scalpel. The isolated cotyledons were weighted and placed in the cuvettes (each containing 20 seeds) on the filter paper moistened with distilled water (control) or with water solution of chemical heterocyclic compounds, derivatives of pyrimidine, pyrazole, isoflavones, and pyridine used at the concentration 10^{-8}M or with water solution of derivatives of oxazolopyrimidine and oxazole used at the concentration 10^{-9}M, or with water solution of plant hormone cytokinin - Kinetin used at the same concentrations 10^{-8}M or 10^{-9}M. Then isolated cotyledons were placed in the plant growth chamber in which they were grown during16 days at above mentioned conditions. To determine indices of growth of biomass (g) of cotyledons isolated from seeds of pumpkin, they were washed with sterilized distilled water and weighted.

2.2. Statistical analysis

All experiments were performed in three replicates. Statistical analysis of the data was performed using dispersive Student's-t test with the level of significance at P≤0.05, the values are mean ± SD[68].

3. Results and Discussion

3.1. Study of auxin-like activity of chemical low molecular weight heterocyclic compounds, derivatives of pyrimidine, pyrazole, isoflavones, and pyridine

The conducted researches showed that chemical heterocyclic compounds, derivatives of pyrimidine, pyrazole, isoflavones, and pyridine used at the concentration 10^{-8}M revealed the expressive auxin-like activity on the formation of adventitious roots on the 14th-day-old leaf petioles isolated from seedlings of haricot bean (*Phaseolus vulgaris* L.) cultivar Belozernaya (Figure 1).

Conversely, the formation of roots on the control haricot bean leaf petioles treated with distilled water was not observed. Among all heterocyclic compounds the highest stimulating effect on the formation of adventitious roots on the 14th-day-old leaf petioles isolated from haricot bean seedlings showed the chemical heterocyclic compounds, derivatives of pyrazole and isoflavones, the compounds № 7, 8, 10 - 12 (Figure 1).

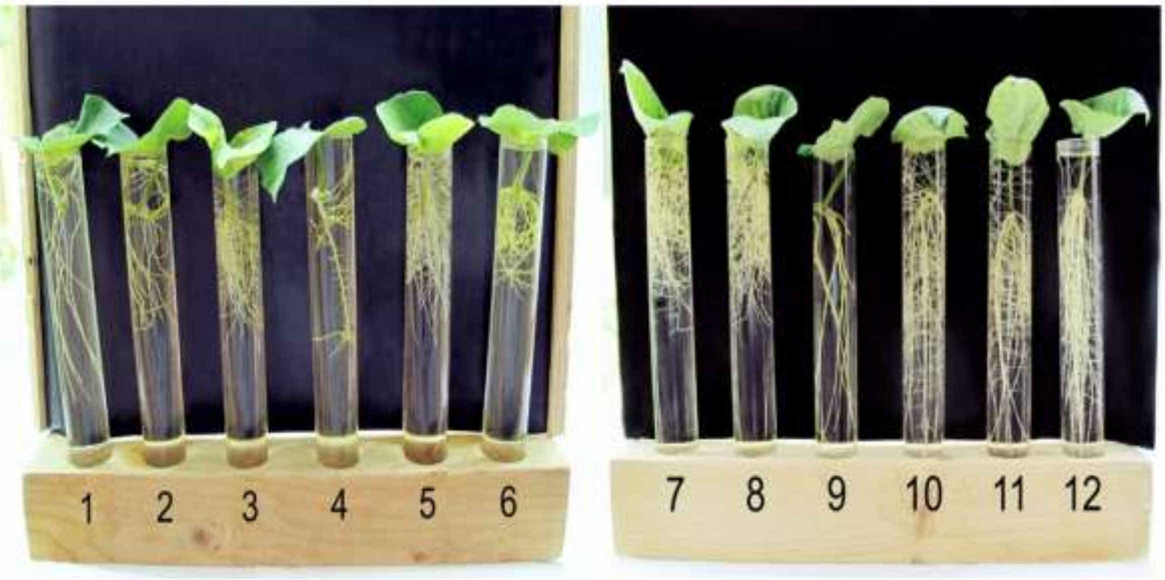


Figure 1: The auxin-like effect of chemical heterocyclic compounds, derivatives of pyrimidine, pyrazole, isoflavones, pyridine, and auxins IAA and NAA on the formation of adventitious roots on the 14th-day-old leaf petioles isolated from seedlings of haricot bean (*Phaseolus vulgaris* L.) cultivar Belozernaya. 1 - Compound 6-Methanesulfonyl-imidazo[1,2-*a*]pyrimidine-5-ylamine, 2 – Compound 1-Benzyl-5-methanesulfonyl-3-phenyl-1*H*-pyrimidine-2,4-dione, 3 – Compound 4-Benzylamino-5-*p*-tolyl-5*H*-pyrrolo-[3,2-*d*]pyrimidin-7-yl)-phosphonic acid diethyl ester, 4 – Compound Ethyl 2-(4-oxo-7-methyl-4,7-dihydro-3*H*-pyrazolo[3,4-*d*][1,2,3]triazin-3-yl)acetate, 5 – NAA (1-Naphthylacetic acid), 6 – IAA (1*H*-Indol-3-ylacetic acid), 7 – Compound 5-Hydrazino-1-phenyl-1*H*-pyrazole-4-carbo hydrazide, 8 – Compound 3-Ethyl-7-methyl-3,7-dihydro-4*H*-pyrazolo[3,4-*d*][1,2,3]triazin-4-one, 9 – Compound (1H-pyrrolo[2,3-*c*]pyridin-3-yl)-acetic acid), 10 - Compound 5-Hydroxy-7-methoxy-6-(methoxymethyl)-2-phenyl-4*H*-chromen-4-one, 11 - Compound 5-Hydroxy-7-methoxy-6-(methoxymethyl)-3-(2-methoxyphenyl)-4*H*-chromen-4-one, 12 - Compound 5-Hydroxy-7-methoxy-8-(methoxymethyl)-3-(4-methoxyphenyl)-4*H*-chromen-4-one

The data of the statistical analysis of the indices of average total root number (pcs) and average total root length (mm) calculated per one experimental 14th-day-old haricot bean leaf petiole treated with water solution of chemical heterocyclic compounds at the concentration 10^{-8}M or with water solution of auxins IAA and NAA used at the same concentration 10^{-8}M as compared to indices obtained on the control haricot bean leaf petiole treated with distilled water are shown in the Table 3.

Table 3: The auxin-like effect of chemical heterocyclic compounds, derivatives of pyrimidine, pyrazole, isoflavones, and pyridine on the average total root number (pcs) and average total root length (mm) formed on the 14th-day-old haricot bean leaf petioles

№ Compound	The average total root number per one leaf petiole (pcs)	The average total root length per one leaf petiole (mm)
Control (distilled water)		
1	29±0.76*	138±1.22*
2	43±0.31*	165±1.97*
3	67±1.18*	288±0.35*
4	23±1.48*	34±2.79*
5	79±0.64*	476±2.87*
6	62±0.47*	172±0.39*
7	96±0.62*	579±1.95*
8	83±1.66*	645±1.57*
9	35±0.44*	526±2.13*
10	129±0.32*	845±0.76*
11	117±1.19*	734±2.31*
12	146±1.55*	918±0.53*

Note. *Significant differences from control values, p ≤0.05, n =3, (-) decreasing; (+) – increasing

Compound №1 - 6-Methanesulfonyl-imidazo[1,2-*a*]pyrimidine-5-ylamine, Compound №2 – 1-Benzyl-5-methanesulfonyl-3-phenyl-1*H*-pyrimidine-2,4-dione, Compound №3 – 4-Benzylamino-5-*p*-tolyl-5*H*-pyrrolo-[3,2-*d*]pyrimidin-7-yl)-phosphonic acid diethyl ester, Compound №4 – Ethyl 2-(4-oxo-7-methyl-4,7-dihydro-3*H*-pyrazolo[3,4-*d*][1,2,3]triazin-3-yl)acetate, Compound №5 – NAA(1-Naphthylacetic acid), Compound №6 – IAA (1*H*-Indol-3-ylacetic acid), Compound №7 – 5-Hydrazino-1-phenyl-1*H*-pyrazole-4-carbo hydrazide, Compound №8 – 3-Ethyl-7-methyl-3,7-dihydro-4*H*-pyrazolo[3,4-*d*][1,2,3]triazin-4-one, Compound №9 – (1H-pyrrolo[2,3-*c*]pyridin-3-yl)-acetic acid), Compound №10 – 5-Hydroxy-7-methoxy-6-(methoxymethyl)-2-phenyl-4*H*-chromen-4-one, Compound №11 – 5-Hydroxy-7-methoxy-6-(methoxymethyl)-3-(2-methoxyphenyl)-4*H*-chromen-4-one, Compound №12 – 5-Hydroxy-7-methoxy-8-(methoxymethyl)-3-(4-methoxyphenyl)-4*H*-chromen-4-one

It was found that the chemical heterocyclic compound №12 revealed the highest auxin-like stimulating effect on the formation of roots on the 14^{th}-day-old haricot bean leaf petioles, the indices of the total root number increased at the 146 % and total root length increased at the 9.18 times as compared to control haricot bean leaf petioles treated with distilled water (Table 3).

The high auxin-like stimulating effect on the formation of roots on the 14^{th}-day-old haricot bean leaf petioles revealed also the chemical heterocyclic compounds: the compound №10, the indices of the total root number increased at the 129 % and total root length increased at the 8.45 times as compared to control haricot bean leaf petioles; the compound №11, the indices of the total root number increased at the 117 % and total root length increased at the 7.34 times as compared to control haricot bean leaf petioles; the compound №7, the indices of the total root number increased at the 96 % and total root length increased at the 5.79 times as compared to control haricot bean leaf petioles; the compound №8, the indices of the total root number increased at the 83 % and total root length increased at the 6.45 times as compared to control haricot bean leaf petioles; the compound №3, the indices of the total root number increased at the 67 % and total root length increased at the 2.88 times as compared to control haricot bean leaf petioles (Table 3).

The high auxin-like stimulating effect on the formation of roots on the 14^{th}-day-old haricot bean leaf petioles revealed also plant hormones auxins: the compound №5 (NAA), the indices of the total root number increased at the 79 % and total root length increased at the 4.76 times as compared to control haricot bean leaf petioles, and the compound №6 (IAA), the indices of the total root number increased at the 62 % and total root length increased at the 1.72 times as compared to control haricot bean leaf petioles (Table 3).

The lower auxin-like stimulating effect on the formation of roots on the 14^{th}-day-old haricot bean leaf petioles revealed the chemical heterocyclic compound №2, the indices of the total root number increased at the 43 % and total root length increased at the 1.65 times as compared to control haricot bean leaf petioles; the compound №9, the indices of the total root number increased at the 35 % and total root length increased at the 5.26 times as compared to control haricot bean leaf petioles; the compound №1, the indices of the total root number increased at the 29 % and total root length increased at the 1.38 times as compared to control haricot bean leaf petioles; the compound №4, the indices of the total root number increased at the 23 % and total root length increased at the 34 % as compared to control haricot bean leaf petioles (Table 3).

Obviously, that the high auxin-like activity of tested chemical heterocyclic compounds, derivatives of pyrimidine, pyrazole, isoflavones, and pyridine may be explained by their inducing auxin-like effect on plant cell elongation, division, and differentiation that are the basic processes of the formation of the adventitious roots on the leaf petioles isolated from seedlings of haricot bean (*Phaseolus vulgaris* L.) cultivar Belozernaya.

3.2. Study of cytokinin-like activity of chemical low molecular weight heterocyclic compounds, derivatives of pyrimidine, pyrazole, isoflavones, and pyridine

The obtained results showed that, according to the indices of growth of biomass of cotyledons isolated from seeds of muscat pumpkin (*Cucurbita moschata* Duch. et Poir.) cultivar Gilea during 16 days all tested compounds, derivatives of pyrimidine, pyrazole, isoflavones, and pyridine used at the concentration 10^{-8}M revealed the expressive cytokinin-like activity, which was similar or higher of the activity of plant hormone cytokinin Kinetin used at the same concentration 10^{-8}M (Figure 2).

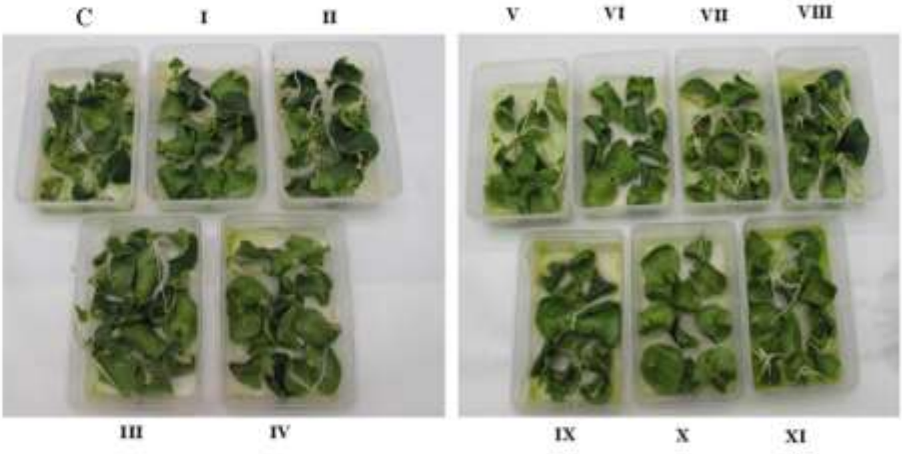


Figure 2: The cytokinin-like effect of chemical heterocyclic compounds, derivatives of pyrimidine, pyrazole, isoflavones, pyridine, and plant hormone cytokinin Kinetin on the growth of biomass of 16th-day-old cotyledons isolated from seeds of muscat pumpkin (*Cucurbita moschata* Duch. et Poir.) cultivar Gilea. C – Control (distilled water), I - Compound 6-Methanesulfonyl-imidazo[1,2-*a*]pyrimidine-5-ylamine, II - Compound 1-Benzyl-5-methanesulfonyl-3-phenyl-1*H*-pyrimidine-2,4-dione, III - Compound 4-Benzylamino-5-*p*-tolyl-5*H*-pyrrolo-[3,2-*d*]pyrimidin-7-yl)-phosphonic acid diethyl ester, IV - Compound Ethyl 2-(4-oxo-7-methyl-4,7-dihydro-3*H*-pyrazolo[3,4-*d*][1,2,3]triazin-3-yl)acetate, V - Compound 5-Hydrazino-1-phenyl-1*H*-pyrazole-4-carbo hydrazide, VI - Kinetin (*N*-(2-Furylmethyl)-7*H*-purin-6-amine), VII - Compound 3-Ethyl-7-methyl-3,7-dihydro-4*H*-pyrazolo[3,4-*d*][1,2,3]triazin-4-one, VIII - Compound 5-Hydroxy-7-methoxy-6-(methoxymethyl)-2-phenyl-4*H*-chromen-4-one, IX - Compound 5-Hydroxy-7-methoxy-6-(methoxymethyl)-3-(2-methoxyphenyl)-4*H*-chromen-4-one, X - Compound 5-Hydroxy-7-methoxy-8-(methoxymethyl)-3-(4-methoxyphenyl)-4*H*-chromen-4-one, XI - Compound (1H-pyrrolo[2,3-*c*]pyridin-3-yl)-acetic acid)

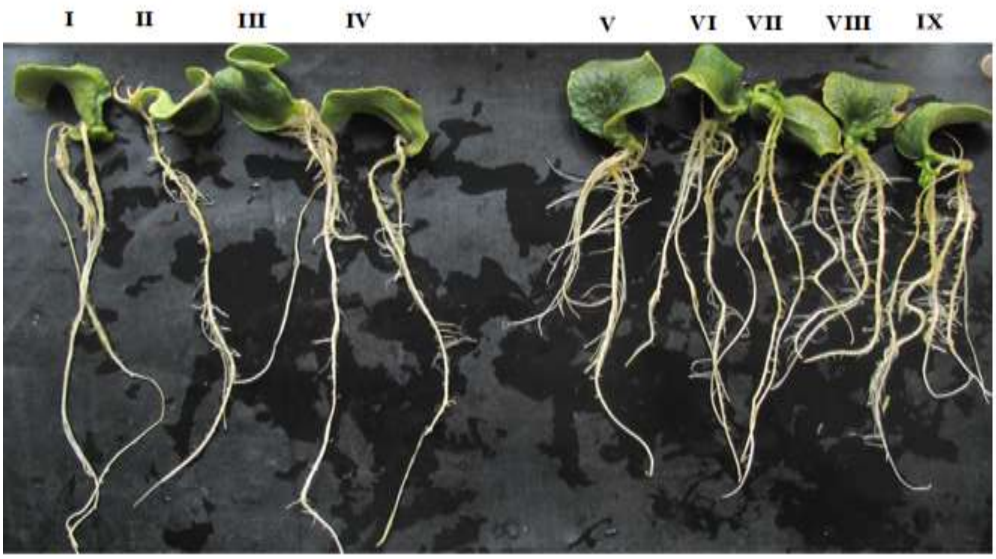


Figure 3: The auxin-like effect of chemical heterocyclic compounds, derivatives of pyrimidine, pyrazole, isoflavones, and pyridine on the formation of roots on the six-week-old cotyledons isolated from seeds of muscat pumpkin (*Cucurbita moschata* Duch. et Poir.) cultivar Gilea. I - Compound 6-Methanesulfonyl-imidazo[1,2-*a*]pyrimidine-5-ylamine, II - Compound 1-Benzyl-5-methanesulfonyl-3-phenyl-1*H*-pyrimidine-2,4-dione, III – Compound Ethyl 2-(4-oxo-7-methyl-4,7-dihydro-3*H*-pyrazolo[3,4-*d*][1,2,3]-triazin-3-yl)acetate, IV - Compound 5-Hydrazino-1-phenyl-1*H*-pyrazole-4-carbo hydrazide, V - Compound 3-Ethyl-7-methyl-3,7-dihydro-4*H*-pyrazolo[3,4-*d*][1,2,3]triazin-4-one, VI - Compound 5-Hydroxy-7-methoxy-6-(methoxymethyl)-2-phenyl-4*H*-chromen-4-one, VII - Compound 5-Hydroxy-7-methoxy-6-(methoxymethyl)-3-(2-methoxyphenyl)-4*H*-chromen-4-one, VIII - Compound 5-Hydroxy-7-methoxy-8-(methoxymethyl)-3-(4-methoxyphenyl)-4*H*-chromen-4-one, IX - Compound (1H-pyrrolo[2,3-*c*]pyridin-3-yl)-acetic acid)

It was found that some chemical heterocyclic compounds used at the concentration 10^{-8}M revealed nonspecific for this bioassay auxin-like activity, which was manifested in formation of the roots on the six-week-old cotyledons isolated from seeds of pumpkin (Figure 3).

The obtained data of the statistical analysis of indices of average biomass of the 30 cotyledons (g) and average length of one root per 30 cotyledons (cm) of the six-week-old cotyledons isolated from seeds of pumpkin are presented in the Table 4.

Table 4: The cytokinin-like and auxin-like effect of chemical heterocyclic compounds, derivatives of pyrimidine, pyrazole, isoflavones, and pyridine on the growth of biomass (g) and length of roots (cm) formed on the six-week-old cotyledons isolated from seeds of pumpkin

№ Compound	The average biomass of the 30 cotyledons (g)	The average length of one root per 30 cotyledons (cm)
Control (distilled water)	45.63±0.29*	1.12±0.45*
1	55.27±0.86**	14.23±1.27**
2	58.64±0.53**	17.78±0.66**
3	54.91±1.64**	19.56±1.14**
4	56.26±1.52**	14.45±0.93**
5	59.67±0.83**	13.21±1.24**
6	50.48±1.18**	3.35±1.57**
7	57.61±0.45**	11.24±1.14**
8	55.22±0.69**	13.15±1.78**
9	61.34±1.94**	10.23±1.44**
10	66.27±1.12**	14.61±1.26**
11	57.49±1.19**	12.45±0.89**

Note. **Significant differences from control values*, $p \leq 0.05$, n =3, (-) decreasing; (+) – increasing

Compound №1 - 6-Methanesulfonyl-imidazo[1,2-*a*]pyrimidine-5-ylamine, Compound №2 - 1-Benzyl-5-methanesulfonyl-3-phenyl-1*H*-pyrimidine-2,4-dione, Compound №3 - 4-Benzylamino-5-*p*-tolyl-5*H*-pyrrolo-[3,2-*d*]pyrimidin-7-yl)-phosphonicaciddiethylester, Compound №4 - Ethyl 2-(4-oxo-7-methyl-4,7-dihydro-3*H*-pyrazolo[3,4-*d*][1,2,3]triazin-3-yl)acetate, Compound №5 - 5-Hydrazino-1-phenyl-1*H*-pyrazole-4-carbohydrazide, Compound №6 - Kinetin (*N*-(2-Furylmethyl)-7*H*-purin-6-amine), Compound №7 - 3-Ethyl-7-methyl-3,7-dihydro-4*H*-pyrazolo[3,4-*d*][1,2,3]triazin-4-one, Compound №8 - 5-Hydroxy-7-methoxy-6-(methoxymethyl)-2-phenyl-4*H*-chromen-4-one, Compound №9 - 5-Hydroxy-7-methoxy-6-(methoxymethyl)-3-(2-methoxyphenyl)-4*H*-chromen-4-one, Compound №10 - 5-Hydroxy-7-methoxy-8-(methoxymethyl)-3-(4-methoxyphenyl)-4*H*-chromen-4-one, Compound №11 - (1H-pyrrolo[2,3-*c*]pyridin-3-yl)-acetic acid)

The indices of average biomass (g) and average length of one root (cm) per 30 six-week-old cotyledons of pumpkin treated with 10^{-8}M water solution of compound №1 were in average higher of the analogical indices of cotyledons of pumpkin treated either with distilled water (control) or with 10^{-8}M water solution of cytokinin Kinetin (compound №6) as follows: according to the average biomass - at the 121 % as compared with control and at the 109 % as compared with cytokinin Kinetin; according to the average length of one root- at the 12.7 times as compared with control and at the 4.25 times as compared with cytokinin Kinetin (Table 4).

The indices of average biomass (g) and average length of one root (cm) per 30 six-week-old cotyledons of pumpkin treated with 10^{-8}M water solution of compound №2 were in average higher of the analogical indices of cotyledons of pumpkin treated either with distilled water (control) or with 10^{-8}M water solution of cytokinin Kinetin (compound №6) as follows: according to the average biomass - at the 128 % as compared with control and at the 116 % as compared with cytokinin Kinetin; according to the average length of one root- at the 15.9 times as compared with control and at the 5.3 times as compared with cytokinin Kinetin (Table 4).

The indices of average biomass (g) and average length of one root (cm) per 30 six-week-old cotyledons of pumpkin treated with 10^{-8}M water solution of compound №3 were in average higher of the analogical indices of cotyledons of pumpkin treated either with distilled water (control) or with 10^{-8}M water solution of cytokinin Kinetin (compound №6) as follows: according to the average biomass - at the 120 % as compared with control and at the 108 % as compared with cytokinin Kinetin; according to the average length of one root- at the 17.5 times as compared with control and at the 5.8 times as compared with cytokinin Kinetin (Table 4).

The indices of average biomass (g) and average length of one root (cm) per 30 six-week-old cotyledonsof pumpkin treated with 10^{-8}M water solution of compound №4 were in average higher of the analogical indices of cotyledons of pumpkin treated either with distilled water (control) or with 10^{-8}M water solution of cytokinin Kinetin (compound №6) as follows: according to the average biomass - at the 123 % as compared with control and at the 112 % as compared with cytokinin Kinetin; according to the average length of one root - at the 12.9 times as compared with control and at the 4.3 times as compared with cytokinin Kinetin (Table 4).

The indices of average biomass (g) and average length of one root (cm) per 30 six-week-old cotyledons of pumpkin treated with 10^{-8}M water solution of compound №5 were in average higher of the analogical indices of cotyledons of pumpkin treated either with distilled water (control) or with 10^{-8}M water solution of cytokinin Kinetin (compound №6) as follows: according to the average biomass - at the 130 % as compared with control and at the 118 % as compared with cytokinin Kinetin; according to the average length of one root- at the 11.8 times as compared with control and at the 3.9 times as compared with cytokinin Kinetin (Table 4).

The indices of average biomass (g) and average length of one root (cm) per 30 six-week-old cotyledons of pumpkin treated with 10^{-8}M water solution of compound №7 were in average higher of the analogical indices of cotyledons of pumpkin treated either with distilled water (control) or with 10^{-8}M water solution of cytokinin Kinetin (compound №6) as follows: according to the average biomass - at the 126 % as compared with control and at the 114 % as compared with cytokinin Kinetin; according to the average length of one root- at the 10.0 times as compared with control and at the 3.6 times as compared with cytokinin Kinetin (Table 4).

The indices of average biomass (g) and average length of one root (cm) per 30 six-week-old cotyledons of pumpkin treated with 10^{-8}M water solution of compound №8 were in average higher of the analogical indices of cotyledons of pumpkin treated either with distilled water (control) or with 10^{-8}M water solution of cytokinin Kinetin (compound №6) as follows: according to the average biomass - at the 121 % as compared with control and at the 109 % as compared with cytokinin Kinetin; according to the average length of one root- at the 11.7 times as compared with control and at the 3.9 times as compared with cytokinin Kinetin (Table 4).

The indices of average biomass (g) and average length of one root (cm) per 30 six-week-old cotyledons of pumpkin treated with 10^{-8}M water solution of compound №9 were in average higher of the analogical indices of cotyledons of pumpkin treated either with distilled water (control) or with 10^{-8}M water solution of cytokinin Kinetin (compound №6) as follows: according to the average biomass - at the 134 % as compared with control and at the 122 % as compared with cytokinin Kinetin; according to the average length of one root - at the 9.1 times as compared with control and at the 3.0 times as compared with cytokinin Kinetin (Table 4).

The indices of average biomass (g) and average length of one root (cm) per 30 six-week-old cotyledons of pumpkin treated with 10^{-8}M water solution of compound №10 were in average higher of the analogical indices of cotyledons of pumpkin treated either with distilled water (control) or with 10^{-8}M water solution of cytokinin Kinetin (compound №6) as follows: according to the average biomass - at the 145 % as compared with control and at the 131 % as compared with cytokinin Kinetin; according to the average length of one root- at the 13.0 times as compared with control and at the 4.4 times as compared with cytokinin Kinetin (Table 4).

The indices of average biomass (g) and average length of one root (cm) per 30 six-week-old cotyledons of pumpkin treated with 10^{-8}M water solution of compound №11 were in average higher of the analogical indices of cotyledons of pumpkin treated either with distilled water (control) or with 10^{-8}M water solution of cytokinin Kinetin (compound №6) as follows: according to the average biomass - at the 125 % as compared with control and at the 113 % as compared with cytokinin Kinetin; according to the average length of one root- at the 11.1 times as compared with control and at the 3.7 times as compared with cytokinin Kinetin (Table 4).

The obtained results suggest that high cytokinin-like and auxin-like activities of chemical heterocyclic compounds, derivatives of pyrimidine, pyrazole, isoflavones, and pyridine may be explained by their inducing cytokinin-like and auxin-like effect on plant cell division and elongation resulting in increasing growth of biomass of the cotyledons isolated from seed of muscat pumpkin (*Cucurbita moschata* Duch. et Poir.) cultivar Gilea and formation of the adventitious roots on the isolated cotyledons.

3.3. Study of cytokinin-like activity of chemical low molecular weight heterocyclic compounds, derivatives of oxazolopyrimidine and oxazole

It was found that according to the indices of growth of biomass of cotyledons isolated from seeds of muscat pumpkin (*Cucurbita moschata* Duch. et Poir.) cultivar Gilea during 16 days all tested chemical compounds, derivatives of oxazolopyrimidine and oxazole used at the concentration 10^{-9}M showed the expressive cytokinin-like activity, which was similar or higher of the activity of plant hormone cytokinin Kinetin used at the same concentration 10^{-9}M.

The obtained data of the statistical analysis of indices of growth of biomass of isolated cotyledons of pumpkin showed that the highest cytokinin-like activity revealed the compounds, derivatives of oxazolopyrimidine: the compound №2 - 2,5-diphenyl[1,3]oxazolo[5,4-*d*]pyrimidin-7(6*H*)-one and compound №4 - 7-amino-5-(4-ethylphenyl)-2-phenyl[1,3]oxazolo[5,4-*d*]pyrimidine, as well as the compound, derivative of oxazole: the compound №6 - 2- tolyl-5-(piperidin-1-ylsulfonyl)-1,3-oxazole-4-carbonitrile (Figure 4).

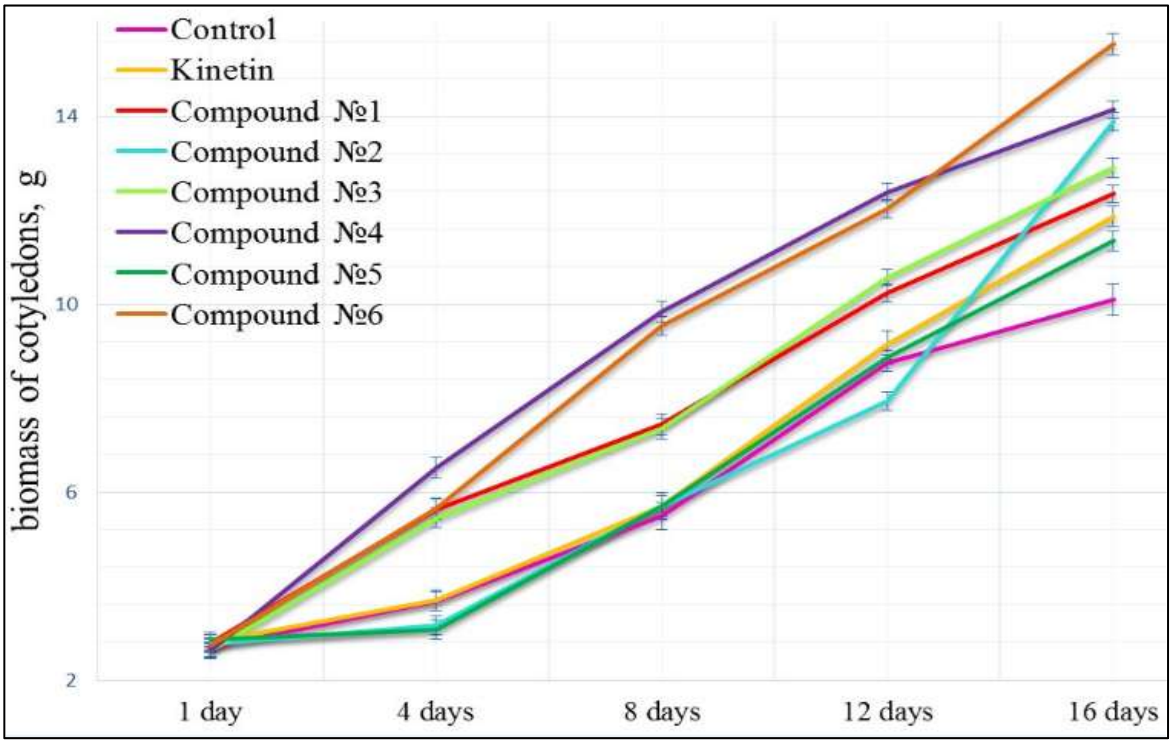


Figure 4: The cytokinin-like effect of chemical heterocyclic compounds, derivatives of oxazolopyrimidine (compound №1 - 7-amino-2,5-diphenyl[1,3]oxazolo[5,4-*d*]pyrimidine, compound №2 - 2,5-diphenyl[1,3]oxazolo[5,4-*d*]pyrimidin-7(6*H*)-one, compound №3 - 5-(4-ethylphenyl)-2-phenyl[1,3]oxazolo[5,4-*d*]pyrimidin-7(6*H*)-one, compound №4 - 7-amino-5-(4-ethylphenyl)-2-phenyl[1,3]oxazolo[5,4-*d*]pyrimidine), and derivatives of oxazole (compound №5 - 2-phenyl-5-(piperidin- 1-ylsulfonyl)-1,3-oxazole-4-carbonitrile and compound №6 - 2-tolyl-5-(piperidin-1-ylsulfonyl)-1,3-oxazole-4-carbonitrile)), and plant hormone cytokinin Kinetin (*N*-(2-Furylmethyl)-7*H*-purin-6-amine) on the growth of the biomass of cotyledons isolated from seeds of muscat pumpkin (*Cucurbita moschata* Duch. et Poir.) cultivar Gilea (the biomass was weighted with the interval of each 4 day)

Among the compounds, derivatives of oxazolopyrimidine the compound №4 - 7-amino-5-(4-ethylphenyl)-2-phenyl[1,3]oxazolo[5,4-*d*]pyrimidine, which contains amino group at the 7th position of pyrimidine fragment, showed the highest cytokinin-like activity; the indices of growth of biomass of the isolated cotyledons of pumpkin grown on the 10^{-9}M water solution of compound №4 were higher at the 40% and 19% of the indices of growth of biomass of the isolated cotyledons of pumpkin grown either on the distilled water (control) or on the 10^{-9}M water solution of cytokinin Kinetin, respectively (Figure 4).

The high cytokinin-like activity demonstrated also the compound №2 - 2,5-diphenyl[1,3]oxazolo[5,4-*d*]pyrimidin-7(6*H*)-one, which contains phenyl substituent at the 5th position of pyrimidine fragment; the indices of growth of biomass of the isolated cotyledons of pumpkin grown on the 10^{-9}M water solution of compound

№2 were higher at the 38% and 17% of the indices of growth of biomass of the isolated cotyledons of pumpkin grown either on the distilled water (control) or on the 10^{-9}M water solution of cytokinin Kinetin, respectively (Figure 4).

The lower cytokinin-like activity showed the compound №3 - 5-(4-ethylphenyl)-2-phenyl[1,3]oxazolo [5,4-*d*]pyrimidin-7(6*H*)-one, which contains 4-ethylphenyl substituent at the 5th position and oxygen at the 7th position of pyrimidine fragment; the indices of growth of biomass of the isolated cotyledons of pumpkin grown on the 10^{-9}M water solution of compound №3 were higher at the 28% and 9% of the indices of growth of biomass of the isolated cotyledons of pumpkin grown either on the distilled water (control) or on the 10^{-9}M water solution of cytokinin Kinetin, respectively (Figure 4).

The lower cytokinin-like activity showed also the compound №1 - 7-amino-2,5-diphenyl[1,3] oxazolo[5,4-*d*]pyrimidine, which contains phenyl substituent at the 5th position and amino group at the 7th position of pyrimidine fragment; the indices of growth of biomass of the isolated cotyledons of pumpkin grown on the 10^{-9}M water solution of compound №1 were higher at the 22% and 4% of the indices of growth of biomass of the isolated cotyledons of pumpkin grown either on the distilled water (control) or on the 10^{-9}M water solution of cytokinin Kinetin, respectively (Figure 4).

Among the compounds, derivatives of oxazole the compound №6 – 2-tolyl-5-(piperidin-1-ylsulfonyl)-1,3-oxazole-4-carbonitrile, which contains tolyl substituent at the 2th position of oxazole, showed the highest cytokinin-like activity; the indices of growth of biomass of the isolated cotyledons of pumpkin grown on the 10^{-9}M water solution of compound №6 were higher at the 54% and 31% of the indices of growth of biomass of the isolated cotyledons of pumpkin grown either on the distilled water (control) or on the 10^{-9}M water solution of cytokinin Kinetin, respectively (Figure 4).

At the same time the compound №5 - 2-phenyl-5-(piperidin-1-ylsulfonyl)-1,3-oxazole-4-carbonitrile that contains phenyl substituent at the 2th position of oxazole revealed lower cytokinin-like activity; the indices of growth of biomass of the isolated cotyledons of pumpkin grown on the 10^{-9}M water solution of compound №5 were higher at the 23% of the indices of growth of biomass of the isolated cotyledons of pumpkin grown on the distilled water (control) (Figure 4).

Thus, the specific bioassay on cytokinin-like activity showed that among heterocyclic compounds, derivatives of oxazolopyrimidine and oxazole the highest activity on the growth of biomass of cotyledons isolated from seed of muscat pumpkin (*Cucurbita moschata* Duch. et Poir.) cultivar Gilea demonstrated the compounds: the compound №2 - 2,5-diphenyl[1,3]oxazolo[5,4-*d*]pyrimidin-7(6*H*)-one, which contains phenyl substituent at the 5th position of pyrimidine fragment, the compound №4 - 7-amino-5-(4-ethylphenyl)-2-phenyl[1,3]oxazolo[5,4-*d*]pyrimidine, which contains amino group at the 7th position of pyrimidine fragment, and the compound №6 - 2-tolyl-5-(piperidin-1-ylsulfonyl)-1,3-oxazole-4-carbonitrile, which contains tolyl substituent at the 2th position of oxazole.

It is obvious that cytokinin-like activity on the growth of the biomass of cotyledons isolated from seed of muscat pumpkin (*Cucurbita moschata* Duch. et Poir.) cultivar Gilea of chemical compounds, derivatives of oxazolopyrimidine may depend on substituents at the 5th and 7th positions of pyrimidine fragment, while as activity of compounds, derivatives of oxazole may depend on substituents at the 2th position of oxazole.

4. Conclusion

The auxin-like and cytokinin-like activities of chemical low molecular weight heterocyclic compounds, derivatives of pyrimidine, pyrazole, isoflavones, pyridine, oxazolopyrimidine and oxazole were studied. With this aim the specific bioassay on auxin-like activity conducted on the leaf petioles isolated from seedlings of haricot bean (*Phaseolus vulgaris* L.) cultivar Belozernaya and specific bioassay on cytokinin-like activity conducted on the cotyledons isolated from seeds of muscat pumpkin (*Cucurbita moschata* Duch. et Poir.) cultivar Gilea were used. It was shown that chemical low molecular weight heterocyclic compounds used at the concentrations 10^{-8}M and 10^{-9}M demonstrated the high auxin-like and cytokinin-like activities, which were manifested in intensification of growth of isolated plant organs. The obtained results suggested the expressive auxin-like and cytokinin-like inducing effect of chemical heterocyclic compounds on plant cell division, elongation, and differentiation that are the basic processes of plant growth. This study confirmed the perspective of practical application of chemical low molecular weight heterocyclic compounds, derivatives of pyrimidine, pyrazole, isoflavones, pyridine, oxazolopyrimidine and oxazole as a new effective plant growth regulating substances.

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