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Using of [1,3]oxazolo[5,4-d]pyrimidine and N-sulfonyl substituted of 1,3-oxazole to improve the growth of soybean seedlings

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Abstract The effect of the chemical low molecular weight heterocyclic compounds, derivatives of [1,3]oxazolo[5,4-*d*]pyrimidine and N-sulfonylsubstituted of 1,3-oxazole on stimulation of root and shoot growth of soybean seedlings (*Glycine max* L.) cultivar Valuta was studied. The growth regulating activity of the heterocyclic compounds derivatives of [1,3]oxazolo[5,4-*d*]pyrimidine and N-sulfonyl substituted of 1,3-oxazole was compared with the growth regulating activity of the plant hormone auxin NAA (1-Naphthylacetic acid). It was found that the chemical heterocyclic compounds used in the concentration of 10^{-9}M revealed the high auxin-like stimulating effect on the growth of soybean seedlings during 20 days. The biometric indices of the 20th-day-old soybean seedlings grown on the water solution of the chemical heterocyclic compounds used in the concentration of 10^{-9}M were similar or higher of the biometric indices of the control soybean seedlings grown on the distilled water or soybean seedlings grown on the water solution of auxin NAA used in the same concentration of 10^{-9} M. The obtained data confirmed the possibility of application of the chemical low molecular weight heterocyclic compounds, derivatives of [1,3]oxazolo[5,4-*d*]pyrimidine and N-sulfonyl substituted of 1,3-oxazole for intensification of vegetative growth of soybean (*Glycine max* L.) cultivar Valuta.

Keywords *Glycine max* L., [1,3]oxazolo[5,4-*d*]pyrimidine, N-sulfonyl substituted of 1,3-oxazole, NAA.

1. Introduction

Soybean (*Glycine max* L.) is the most economically valuable crop cultivated all over the world [1-4]. Soybean belongs to important grain legumes and oilseed crop, which is the source of more than 40 % of proteins and 50 % of oil used in the world food industry [5, 6]. Rhizobium-legume symbiosis enhances fixation of atmospheric nitrogen and increases soil fertility [7, 8]. Biodegradable soy protein isolate is an important basic material for food industry, agriculture and biotechnology [9]. Soybean seed contains biologically active compounds used in medical practice such as isoflavones, lectins, saponins, peptide lunasin and protease inhibitors such as Bowman-Birk protease inhibitor (BBI) and Kunitz trypsin inhibitor (KTI) that revealed cytotoxic activities against cancer [10-13].

Unfortunately, the adverse environmental factors have a negative influence on growing and productivity of soybean crop [14]. Nowadays, plant hormones, natural and synthetic plant growth regulators, biostimulants, fertilizers and micronutrients are widely used for improvement of growth and development of soybean plants [4, 15-18]. The elaboration of new effective ecologically safe plant growth regulators created on the base of the chemical low molecular weight heterocyclic compounds derivatives of pyrimidine, pyrazole, and oxazole for improving growth and increasing productivity of soybean crop is an alternative strategic approach. It is known these classes of the chemical compounds are widely used in the agricultural industry as plant growth regulators, herbicides, fungicides and antibacterial agents [19-25].

At the Institute of Bioorganic Chemistry and Petrochemistry of National Academy of Sciences of Ukraine the new chemical low molecular weight heterocyclic compounds belonging to the different classes of chemical compounds are synthesized. Our previous studies showed that the chemical compounds, derivatives of pyridine, pyrimidine, pyrazole and isoflavones revealed high stimulating effect on seeds germination and vegetative growth in the various agricultural crops [26-31].

Therefore, the possibility of application of the new heterocyclic compounds has a great theoretical and practical interest to intensify the vegetative growth of soybean crop.

This work is devoted to the study of a stimulating effect of the chemical low molecular weight heterocyclic compounds, derivatives of [1,3]oxazolo[5,4-*d*]pyrimidine and N-sulfonyl substituted of 1,3-oxazole on the growth of root and shoot system of soybean (*Glycine max* L.) cultivar Valuta.

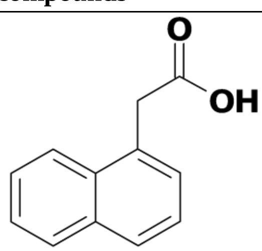
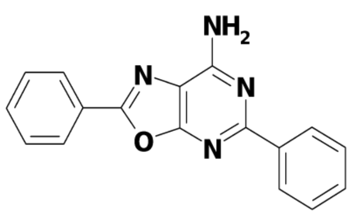
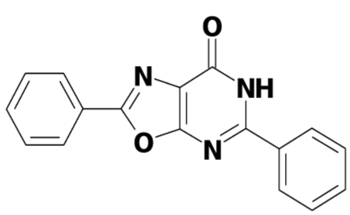
2. Materials and methods

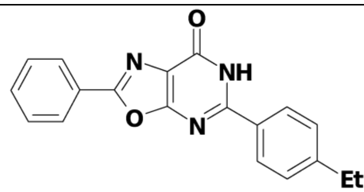
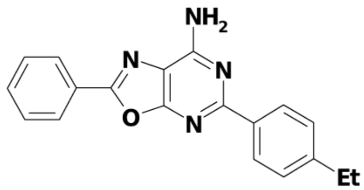
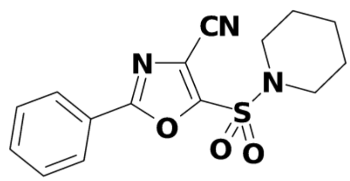
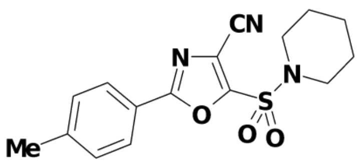
2.1. Chemical structure of heterocyclic compounds and plant hormones used for bioassays

The comparative analysis of the plant growth regulating activity of [1,3]oxazolo[5,4-*d*]pyrimidine (compounds 1 - 4) and N-sulfonyl substituted of 1,3-oxazole (compounds 5 and 6) derivates, and plant hormone auxin NAA (1-Naphthylacetic acid) was conducted.

The chemical structure, chemical name and molecular mass (MM) of the tested compounds and plant hormone NAA are shown in the Table 1.

Table 1: Chemical structure of plant hormone NAA and chemical heterocyclic compounds

No.	Chemical structure of compounds	Chemical name and relative molecular mass of compounds
NAA		NAA (1-Naphthylacetic acid) MM 186.21
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

2.2. Plant treatment and growing conditions

Soybean seeds (*Glycine max* L.) cultivar Valuta were surface sterilized by 1 % $KMnO_4$ solution for 3 min followed by treatmenting with 96 % ethanol solution for 1 min, and then washed three times with sterile distilled water. After this procedure soybean seeds were placed in the cuvettes (each containing 20-25 seeds) on the perlite moistened with distilled water (control), or with the chemical heterocyclic compounds, derivatives of [1,3]oxazolo[5,4-*d*]pyrimidine and N-sulfonyl substituted of 1,3-oxazole used in the concentration of 10^{-9}M or with the plant hormone auxin NAA used in the same concentration of 10^{-9}M. Afterward, the control and experimental soybean seeds were placed in the thermostat for their germination in the darkness at the temperature 25 °C during 48 hours. Sprouted soybean seedlings were placed in the plant growth chamber where seedlings were grown for 20 days at the 16/8 h light/dark conditions, at the temperature 24 °C, light intensity 3000 lux and air humidity 60-80 %. The comparative analysis of the biometric indices of the soybean seedlings (i.e. number of germinated seeds (%), length of shoots (cm), total number of roots (pcs), total length of roots (mm)) was carried out on the 20 day after their sprouting according to the guideline [32].

2.3. 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 $P \leq 0.05$, the values are mean ± SD [33].

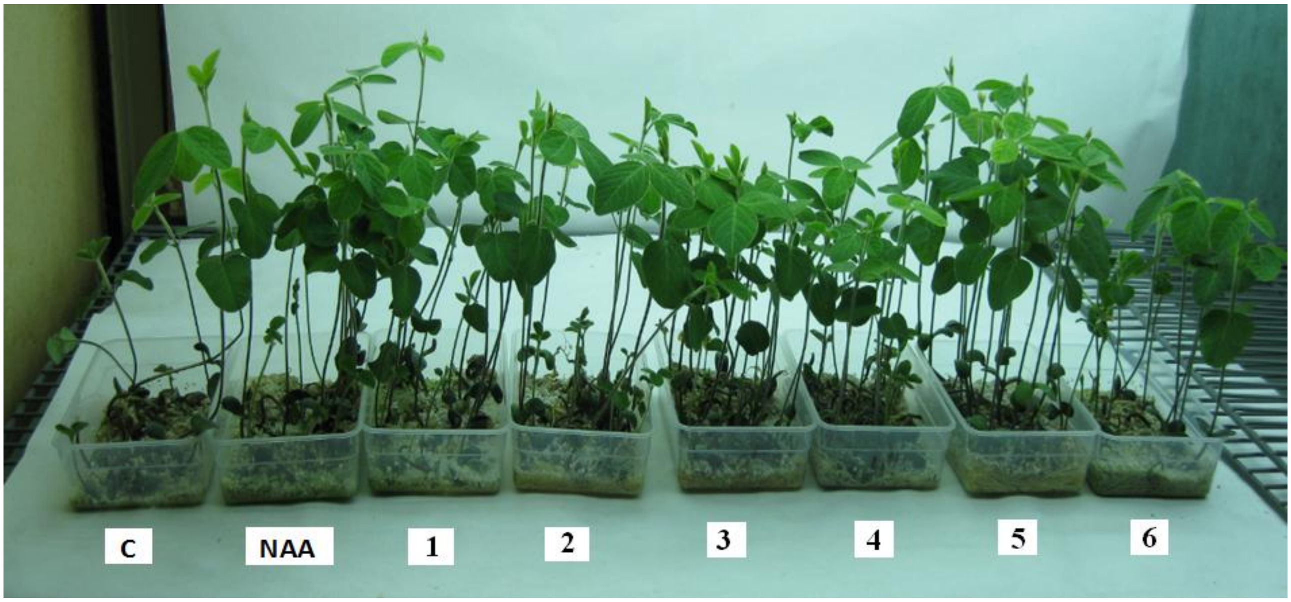
3. Results

3.1. Effect of chemical heterocyclic compounds on growth of soybean seedlings

In the laboratory conditions we studied the stimulating effect of the chemical heterocyclic compounds, derivatives of [1,3]oxazolo[5,4-*d*]pyrimidine and N-sulfonyl substituted of 1,3-oxazole, and plant hormone auxin NAA on seeds germination and growth of root and shoot system of the soybean (*Glycine max* L.) seedlings cultivar Valuta.

It was shown that all tested compounds used in the concentration of 10^{-9}M revealed the high stimulating effect on the growth of root and shoot system of the soybean seedlings during 20 days (Fig. 1).

A



B

*Figure 1: Effect of chemical heterocyclic compounds, derivatives of [1,3]oxazolo[5,4-d]pyrimidine (**№1** - 7-amino-2,5-diphenyl[1,3]oxazolo[5,4-d]pyrimidine, **№2** - 2,5-diphenyl[1,3]oxazolo[5,4-d]pyrimidin-7(6H)-one, **№3** - 5-(4-ethylphenyl)-2-phenyl[1,3]oxazolo[5,4-d]pyrimidin-7(6H)-one, **№4** - 7-amino-5-(4-ethylphenyl)-2-phenyl[1,3]oxazolo[5,4-d]pyrimidine), compounds derivatives of N-sulfonyl substituted of 1,3-oxazole (**№5** - 2-phenyl-5-(piperidin-1-ylsulfonyl)-1,3-oxazole-4-carbonitrile and **№6** - 2-tolyl-5-(piperidin-1-ylsulfonyl)-1,3-oxazole-4-carbonitrile), and plant hormone auxin NAA on growth of shoot system of the 20th-day-old soybean seedlings as compared to control (**C**) soybean seedlings (**A**); and growth of root system of the 20th-day-old soybean seedlings as compared to control (**C**) soybean seedlings **(B)***

Obviously, the obtained results are explained by auxin-like stimulating effects of the chemical compounds on plant such as cell elongation, proliferation and differentiation, which are the basic processes of plant growth and development [34-39].

The comparative analysis of the 20th-day-old soybean seedlings biometric indices (i.e. number of germinated seeds (%), length of shoots (cm), total number of roots (pcs), total length of roots (mm)) showed that the biometric indices of seedlings grown on the 10^{-9}M water solution of the chemical heterocyclic compounds were similar or higher of the biometric indices seedlings grown on the water solution of NAA used in the same concentration as compared to lower biometric indices seedlings grown in distilled water (control).

The results of stimulating effect of the heterocyclic compounds on the biometric indices of the 20th-day-old soybean seedlings are shown on the Fig 2.

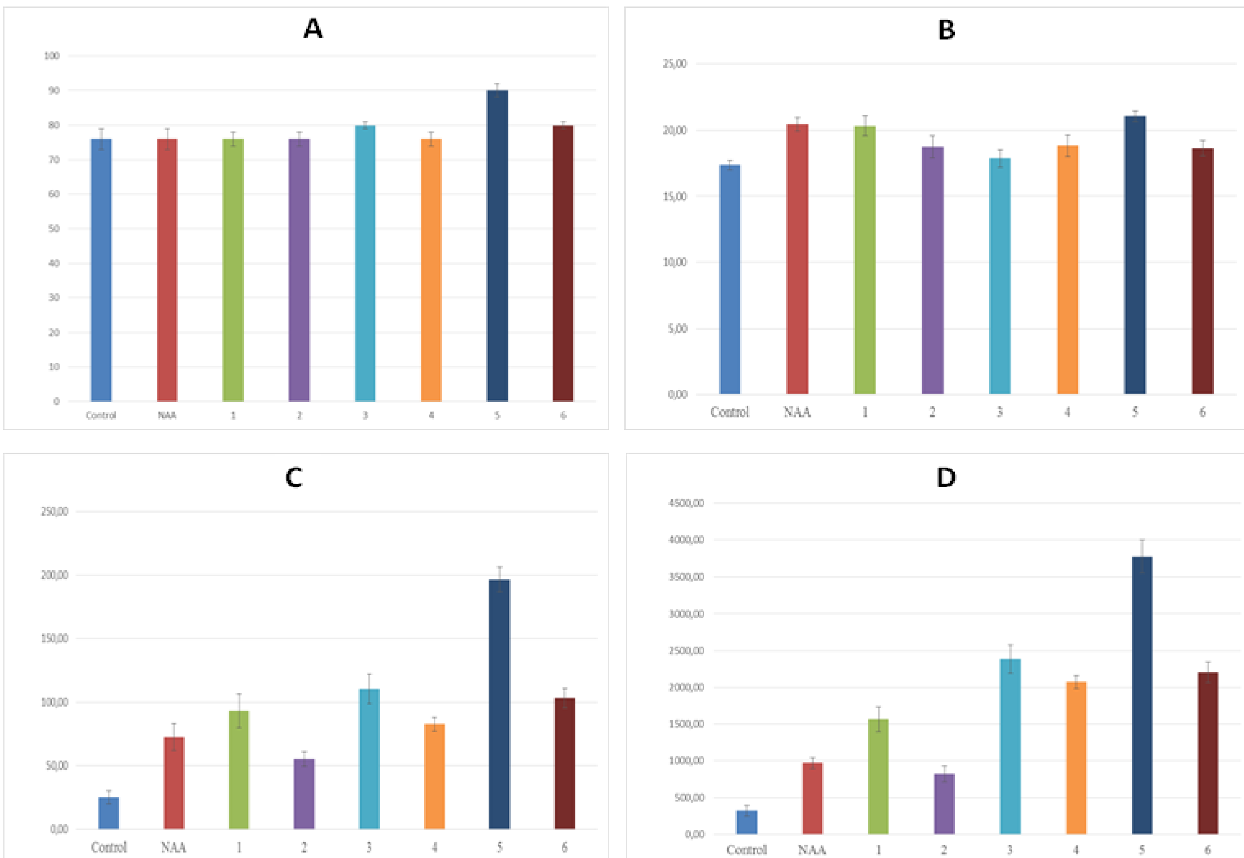


Figure 2: Effect of chemical heterocyclic compounds derivatives of [1,3]oxazolo[5,4-d]pyrimidine (№1 - 7-amino-2,5-diphenyl[1,3]oxazolo[5,4-d]pyrimidine, №2 - 2,5-diphenyl[1,3]oxazolo[5,4-d]pyrimidin-7(6H)-one, №3 - 5-(4-ethylphenyl)-2-phenyl[1,3]oxazolo[5,4-d]pyrimidin-7(6H)-one, №4 - 7-amino-5-(4-ethylphenyl)-2-phenyl[1,3]oxazolo[5,4-d]pyrimidine), compounds derivatives of N-sulfonyl substituted of 1,3-oxazole (№5 - 2-phenyl-5-(piperidin-1-ylsulfonyl)-1,3-oxazole-4-carbonitrile and №6 - 2-tolyl-5-(piperidin-1-ylsulfonyl)-1,3-oxazole-4-carbonitrile), and plant hormone auxin NAA on the biometric indices of the 20th-day-old soybean seedlings. A – number of germinated seeds (%), B – length of shoots (cm), C – total number of roots (pcs), D – total length of roots (mm)

Particularly, it was found that the biometric indices of soybean seedlings grown on the 10^{-9}M water solution of the compound **№1** were higher of the biometric indices of soybean seedlings grown either on the distilled water (control) or on the water solution of NAA in average: according to length of shoots – at the 17% as compared with control; according to total length of roots – at the 4.85 times as compared with control and at the 62 % as compared with NAA; according to total number of roots – at the 3.72 times as compared with control, and at the 28% as compared with NAA, respectively (Fig. 2).

The biometric indices of the 20th-day-old soybean seedlings grown on the 10^{-9}M water solution of the compound **№2** were higher of the biometric indices of soybean seedlings grown on the distilled water (control) in average: according to length of shoots – at the 8% as compared with control; according to total length of roots – at the 2.5 times as compared with control; according to total number of roots – at the 2.2 times as compared with control (Fig. 2).

The biometric indices of soybean seedlings grown on the 10^{-9}M water solution of the compound **№3** were higher of the biometric indices of soybean seedlings grown either on the distilled water (control) or on the water solution of NAA in average: according to total length of roots – at the 7.4 times as compared with control and at the 2.47 times as compared with NAA; according to total number of roots – at the 4.4 times as compared with control, and at the 52% as compared with NAA, respectively (Fig. 2).

The biometric indices of soybean seedlings grown on the 10^{-9}M water solution of the compound **№4** were higher of the biometric indices of soybean seedlings grown either on the distilled water (control) or on the water solution of NAA in average: according to length of shoots – at the 8% as compared with control; according to total length of roots – at the 6.4 times as compared with control and at the 2.15 times as compared with NAA; according to total number of roots – at the 2.59 times as compared with control, and at the 55% as compared with NAA, respectively (Fig. 2).

The biometric indices of soybean seedlings grown on the 10^{-9}M water solution of the compound **№5** were higher of the biometric indices of soybean seedlings grown either in distilled water (control) or on the water solution of NAA in average: according to length of shoots – at the 21% as compared with control; according to total length of roots – at the 11.7 times as compared with control and at the 3.9 times as compared with NAA; according to total number of roots – at the 7.7 times as compared with control, and at the 2.7 times as compared with NAA, respectively (Fig. 2).

The biometric indices of soybean seedlings grown on the 10^{-9}M water solution of the compound **№6** were higher of the biometric indices of soybean seedlings grown either in distilled water (control) or on the water solution of NAA in average: according to length of shoots – at the 7% as compared with control; according to total length of roots – at the 6.8 times as compared with control and at the 2.3 times as compared with NAA; according to total number of roots – at the 4.1 times as compared with control, and at the 73% as compared with NAA, respectively (Fig. 2).

Thus, the comparative analysis of biometric indices of 20th-day-old soybean seedlings grown in the 10^{-9}M water solution of the chemical heterocyclic compounds showed that the highest growth stimulating activity among tested compounds, derivatives of [1,3]oxazolo[5,4-*d*]pyrimidine revealed compounds: **№3** - 5-(4-ethylphenyl)-2-phenyl[1,3]oxazolo[5,4-*d*]pyrimidin-7(6*H*)-one and **№4** - 7-amino-5-(4-ethylphenyl)-2-phenyl[1,3]oxazolo[5,4-*d*]pyrimidine. At the same time the compounds **№1** - 7-amino-2,5-diphenyl[1,3]oxazolo[5,4-*d*]pyrimidine and **№2** - 2,5-diphenyl[1,3]oxazolo[5,4-*d*]pyrimidin-7(6*H*)-one demonstrated lower growth stimulating activity. The obtained results of the different growth stimulating activity of [1,3]oxazolo[5,4-*d*]pyrimidine obviously may be explained by the presence of phenyl substituents at the 5th and 7th positions of the pyrimidine fragment in these heterocyclic compounds.

The highest growth stimulating activity among tested compounds, derivatives of N-sulfonyl substituted of 1,3-oxazole revealed the compound **№5** - 2-phenyl-5-(piperidin-1-ylsulfonyl)-1,3-oxazole-4-carbonitrile. It is possible to suppose that the high growth stimulating activity of the compound №5 obviously may be explained by the presence of phenyl substituent at the 2nd position of oxazole. While the compound **№6** - 2-tolyl-5-(piperidin-1-ylsulfonyl)-1,3-oxazole-4-carbonitrile, which structure is similar to the compound **№5**, but different by the presence of tolyl substituent at the 2nd position of oxazole, showed lower activity than the compound **№5. A**t the same time, its activity of compound **№ 6** was higher of the activity of auxin NAA. The obtained data witness that the various substituents at the 2nd position of oxazole have an impact on the activity of N-sulfonyl substituted of 1,3-oxazole.

4. Conclusion

The stimulating effect of the chemical heterocyclic compounds, derivatives of [1,3]oxazolo[5,4-*d*]pyrimidine and N-sulfonyl substituted of 1,3-oxazole on the vegetative growth of soybean (*Glycine max* L.) seedlings cultivar Valuta was studied. The comparative analysis of the obtained biometric indices of the 20th-day-old soybean seedlings grown on the 10^{-9}M water solution of chemical heterocyclic compounds showed that the highest stimulating effect revealed the compounds, derivatives of [1,3]oxazolo[5,4-*d*]pyrimidine: **№3** - 5-(4-ethylphenyl)-2-phenyl[1,3]oxazolo[5,4-*d*]pyrimidin-7(6*H*)-one and **№4** - 7-amino-5-(4-ethylphenyl)-2-phenyl[1,3]oxazolo[5,4-*d*]pyrimidine, and the compound, derivative of N-sulfonyl substituted of 1,3-oxazole: **№5** - 2-phenyl-5-(piperidin-1-ylsulfonyl)-1,3-oxazole-4-carbonitrile. Obviously, the high growth stimulating effect of these chemical compounds may be explained by the presence of phenyl substituents at the 5th and 7th positions of the pyrimidine fragment in compounds, derivatives of [1,3]oxazolo[5,4-*d*]pyrimidine and presence of phenyl substituent at the 2nd position of oxazole in compounds, derivatives of N-sulfonyl substituted of 1,3-oxazole. The obtained data confirmed the possibility of practical application in the agriculture of chemical heterocyclic compounds, derivatives of [1,3]oxazolo[5,4-*d*]pyrimidine and N-sulfonyl substituted of 1,3-oxazole as a new effective stimulators of vegetative growth of soybean (*Glycine max* L.) cultivar Valuta.

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