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Study of Auxin, cytokinin and gibberellin-like activity of heterocyclic compounds derivatives of pyrimidine, pyridine, pyrazole and isoflavones

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Abstract
The phytohormone-like activity of synthetic low molecular weight heterocyclic compounds derivatives of pyrimidine, pyridine, pyrazole, and isoflavones was studied. Testing of stimulating effect of synthetic heterocyclic compounds on seed germination and seedling growth of flax (*Linum usitatissimum* L.) of cultivar Ukrainian 3 showed expressive auxin-like activity of these compounds. The growth parameters of 15th-day-old flax seedlings grown on the solution of heterocyclic compounds used at concentration 10^{-8}M/l of distilled water were higher or similar to growth parameters of flax seedlings grown on the water solution of auxins IAA and NAA used at the same concentration as compared to lower growth parameters of control flax seedlings grown on the distilled water (control). The specific bioassay on auxin-like activity showed high stimulating effect of heterocyclic compounds derivatives of pyrimidine, pyridine, pyrazole, and isoflavones used at concentration 10^{-8}M/l of distilled water on rhizogenesis on the 14th-day-old leaf petioles isolated from seedlings of haricot bean (*Phaseolus vulgaris* L.) of cultivar Belozernaya. The specific bioassay on cytokinin-like activity conducted on the 16th-day-old cotyledons isolated from seeds of muscat pumpkin (*Cucurbita moschata* Duch. et Poir.) of cultivar Gilea, showed that heterocyclic compounds derivatives of pyrimidine, pyridine, pyrazole, and isoflavones used at concentration 10^{-8}M/l of distilled water demonstrated high stimulating activity on growth of biomass of isolated cotyledons of pumpkin, which was similar or higher than the activity of cytokinin Kinetin. The specific bioassay on gibberellin-like activity conducted on the 3rd- day-old seedlings of lettuce (*Lactuca sativa* L.) of cultivar Berlin testified that some heterocyclic compounds derivatives of pyrazole, isoflavones, and pyridine used at concentrations ranging from 10^{-4}M to 10^{-9}M/1 of distilled water revealed minor stimulating effect on elongation of hypocotyl of lettuce seedlings in relation to control**,** but their activity was significantly lower than activity of gibberellic acid GA3. This bioassay showed also that the some heterocyclic compounds derivatives of pyrazole and isoflavones used at concentration 10^{-7}M/l of distilled water demonstrated nonspecific for this bioassay auxin-like activity, which manifested in stimulation of formation and growth of root system on the seedlings of lettuce. Obtained results confirmed specific auxin-like and cytokinin-like, and minor gibberellin-like effect of synthetic heterocyclic compounds on cell division, cell proliferation, cell elongation and cell differentiation that are the basic processes of plant growth and development.

Keywords: pyrimidine, pyridine, pyrazole, isoflavones, IAA, NAA, Kinetin, GA3, *Phaseolus vulgaris* L., *Linum usitatissimum* L., *Cucurbita moschata* Duch. et Poir., *Lactuca sativa* L

1. Introduction
The successful development of modern biotechnological industry is based on using of high-intensive technologies of plant growing with application of phytohormones, synthetic plant growth regulators, natural biostimulants, herbicides, fungicides, organic and inorganic fertilizers to protect plants from abiotic and biotic stress factors causing adverse effects on plant growth and yield [1-18]. During the last decades the five major classes of natural phytohormones such as auxins, cytokinins, gibberellins, abscisic acid and ethylene are widely used in the agricultural practice as plant growth regulators [2, 4, 5, 19-31]. The newly identified plant growth substances such as brassinosteroids, jasmonates and salicylic acid have already received hormonal status [2, 4, 5, 19, 31-35]. Beside phytohormones the nontraditional compounds of natural origin such as polyamines, oligosaccharins, sterols, systemin, turgorins are known to regulate plant growth and development, but their manufacture for practical purposes is not provided until now [19, 35-37].

Nowadays the various classes of synthetic analogues of plant hormones having phytohormone-like activity are widely used in agriculture practice as plant growth regulators, herbicides and means for plant protection against pests and pathogens [1, 3, 6, 7, 19, 20, 23-25, 38, 39]. The new promising approach is elaboration of new classes of plant growth substances having high physiological activity at low concentrations, broad specificity of action on the different agricultural crops and lack of toxic effect for environment, animal and human health. 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 derivatives of pyridine, pyrimidine, pyrazole, triazine, oxazole, oxazole

pyrimidine and isoflavones have already found practical application in the agriculture as effective substitutes of traditional plant growth regulators, herbicides, fungicides and antimicrobial agents [40-57].

The great theoretical and practical interest for biotechnologists is study of specificity of plant growth stimulating activity of bioactive compounds of chemical or natural origin. For this aim the various bioassays are used, to them belong: bioassays on seed germination and seedling growth [4, 58-63], the specific for auxin-like, cytokinin-like and gibberellin-like activity bioassays [4, 59, 64-74], and bioassays on inducing phytohormone-like effect of bioactive compounds on callusogenesis, somatic embryogenesis, shoot and root organogenesis in the isolated plant tissue culture *in vitro* [19, 75-78]. It is known that bioassays on phytohormone-like activity are based on key role of phytohormones or their synthetic analogues in control of cell division, cell proliferation, cell elongation, cell differentiation, and plant metabolism that are basic processes of formation and growth of plant vegetative and reproductive organs such as leaf, stem, root, flower, fruit and seed [2, 4, 19-39, 59, 79-88]. To the most used bioassays belong: bioassays on seed germination and seedling growth [4, 58-63]; the specific bioassays on auxin-like activity based on the stimulation of adventitious root formation on the isolated stem and leaf cuttings, bioassays on elongation of hypocotyl or coleoptile of seedlings, and bioassays for the study of plant morphogenesis in the isolated plant tissue culture *in vitro* [4, 19, 64-67, 75-84]; the specific bioassays on cytokinin-like activity based on the stimulation of growth and increase of biomass of isolated cotyledons of plant seeds, leaf senescence delaying bioassays, bioassays on elongation of hypocotyl or coleoptile of seedlings, and bioassays for the study of plant morphogenesis in the isolated plant tissue culture *in vitro* [4, 19, 64, 68, 69, 75-78, 84]; the specific bioassays on gibberellin-like activity based on the stimulation of elongation of hypocotyl, coleoptile, stem and internode of seedlings, bioassays on seedlings of dwarf mutants, bioassays on endosperm tissues, leaf senescence delaying bioassays, and bioassays for the study of plant morphogenesis in the isolated plant tissue culture *in vitro* [4, 19, 70-78, 84-86].

Today the new perspective plant growth stimulators elaborated on the base of low molecular weight five and six-membered heterocyclic compounds are synthesized in the Institute of Bioorganic Chemistry and Petrochemistry of National Academy of Sciences of Ukraine. Most these compounds show high biologically activity on human and animal cells and could be used as perspective therapeutic agents for treatment of cancer, viral, bacterial, fungal, infectious, and inflammatory diseases [89-95]. Our previous researches devoted to testing of phytohormone-like activity of different classes of heterocyclic compounds showed high auxin-like and cytokinin-like stimulating activity of derivatives of pyridine, pyrimidine, pyrazole, and isoflavones on shoot organogenesis in the isolated tissue culture of flax (*Linum usitatissimum* L.) cultivar heavenly *in vitro* [96] and on germination of seed and vegetative growth of maize (*Zea mays* L.) cultivar Odesskaya 10 [97], as well as auxin-like and cytokinin-like stimulating activity of derivatives of [1, 3]oxazolo[5,4-*d*]pyrimidine and N-sulfonyl substituted of 1,3-oxazole on germination of seed and vegetative growth of soybean (*Glycine max* L.) of cultivar Valuta, wheat (*Triticum aestivum* L.) of cultivar Zimoyarka, flax (*Linum usitatissimum* L.) of cultivar Svitanok, and on growth of isolated cotyledons of muscat pumpkin (*Cucurbita moschata* Duch. et Poir.) of cultivar Gilea [98]. The obtained results confirmed high growth stimulating activity of heterocyclic compounds which was varied depending on plant species and different substituents in the chemical structure of heterocyclic compounds. At the same time the determination of specificity of phytohormone-like activity of heterocyclic compounds of different classes as effective substitutes of phytohormones is a very important task. The objective of this work was study of phytohormone-like activity of derivatives of pyrimidine, pyridine, pyrazole, and isoflavones using seed germination and seedling growth bioassay and specific bioassays on auxin-like, cytokinin-like and gibberellin-like activity.

2. Materials and Methods

2.1 Chemical structures of heterocyclic compounds and phytohormones used for bioassays

In our experiments we tested phytohormone-like activity of low molecular weight five and six-membered heterocyclic compounds derivatives of pyrimidine (compounds № V-VII), pyridine (compound № VIII), pyrazole (compounds № IX-XI), and isoflavones (compounds № XII-XIV) synthesized at the Department for chemistry of bioactive nitrogen-containing heterocyclic compounds of Institute of Bioorganic Chemistry and Petrochemistry of NAS of Ukraine [99-102]. The phytohormone-like activity of chemical heterocyclic compounds was compared with activity of phytohormones auxins IAA (compound № I), NAA (compound № II), cytokinin Kinetin (compound № III) and gibberellic acid GA3 (compound № IV) (Fig. 1).

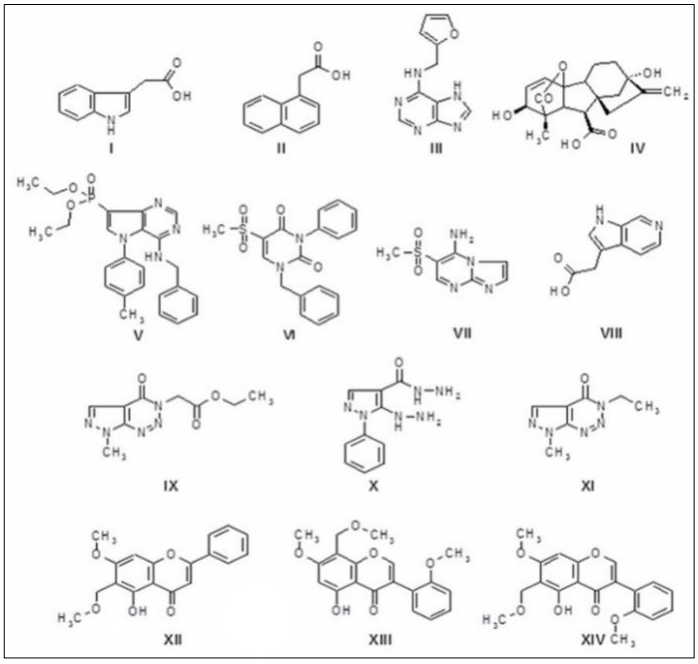

Fig 1: Chemical structures of phytohormones and heterocyclic compounds used for bioassays

i) IAA (1*H*-Indol-3-ylacetic acid), Molecular weight =175.19 g/mol
ii) NAA (1-Naphthylacetic acid), Molecular weight =186.21 g/mol
iii) Kinetin (*N*-(2-Furylmethyl)-7*H*-purin-6-amine), Molecular weight=215.22 g/mol

iv) GA3 (3S,3aR,4S,4aS,7S,9aR,9bR,12S)-7,12-dihydroxy-3-methyl-6-methylene-2-oxoperhydro-4a,7-methano-9b,3-propenoazuleno[1,2-*b*]furan-4-carboxylic acid), Molecular weight=346.38 g/mol
v) Compound 4-Benzylamino-5-*p*-tolyl-5*H*-pyrrolo-[3,2-*d*] pyrimidin-7-yl)-phosphonic acid diethyl ester, Molecular weight=450.48 g/mol
vi) Compound 1-Benzyl-5-methanesulfonyl-3-phenyl-1*H*-pyrimidine-2,4-dione, Molecular weight =356.48 g/mol
vii) Compound 6-Methanesulfonyl-imidazo[1,2-*a*] pyrimidine-5-ylamine, Molecular weight =212.23 g/mol
viii) Compound ((1H-pyrrolo[2,3-*c*]pyridin-3-yl)-acetic acid), Molecular weight=176.175 g/mol
ix) Compound 7-Methyl-4-oxo-4,7-dihydropyrazolo[3,4-*d*] [1,2,3]triazin-3-yl)-acetic acid ethyl ester, Molecular weight = 237.22 g/mol
x) Compound 5-Hydrazino-1-phenyl-1*H*-pyrazole-4-carbohydrazide, Molecular weight =232.25 g/mol
xi) Compound 3-Ethyl-7-methyl-3,7-dihydro-4*H*-pyrazolo[3, 4-*d*][1,2,3]triazin-4-one, Molecular weight=179.18 g/mol
xii) Compound 5-Hydroxy-7-methoxy-6-(methoxymethyl)-2-phenyl-4 *H*-chromen-4-one, Molecular weight =312.32 g/mol
xiii) Compound 5-Hydroxy-7-methoxy-8-(methoxymethyl)-3-(4-methoxyphenyl)-4*H*-chromen-4-one, Molecular weight = 342.35 g/mol
xiv) Compound 5-Hydroxy-7-methoxy-6-(methoxymethyl)-3-(2-methoxyphenyl)-4*H*-chromen-4-one, Molecular weight = 342.35g/mol

2.2 Study of phytohormone-like activity of synthetic heterocyclic compounds on seed germination and growth of flax seedlings

In the laboratory conditions we studied phytohormone-like activity of heterocyclic compounds derivatives of pyrimidine, pyridine, pyrazole, and isoflavones used bioassay [4, 58-63] on seed germination and seedling growth of flax (*Linum usitatissimum* L.) of cultivar Ukrainian 3. With this aim seeds were surface sterilized successively in 1 % $KMnO_4$ solution for 3 min and 96 % ethanol solution for 1 min, and then washed three times with sterile distilled water. After this procedure seeds were placed in the cuvettes (each containing 25 seeds) on the perlite moistened with distilled water (control) or with water solution of each heterocyclic compound used at concentration 10^{-8}M/1 of distilled water or water solution of auxins IAA or NAA used in the same concentration (experiment). Control and experimental seeds were placed in the thermostat for their germination in darkness at the temperature 23-25 ^{0}C during 48 hours. Sprouted seedlings were placed in the plant growth chamber in which seedlings were grown for 15 days at the 16/8 h light/dark conditions, at the temperature 24 ^{0}C, light intensity 3000 lux and air humidity 60-80 %. Comparative analysis of biometric indexes of seedlings (i.e. number of germinated seeds (%), seedlings height (cm), root number (pcs), root length (mm)) was carried out at the 15th day after their sprouting according to the guideline [103].

2.3 Study of auxin-like activity of synthetic heterocyclic compounds

To determine auxin-like activity of heterocyclic compounds derivatives of pyrimidine, pyridine, pyrazole, and isoflavones we used specific bioassay [65, 66] conducted on the leaf petioles isolated from haricot bean (*Phaseolus vulgaris* L.) seedlings of cultivar Belozernaya. With this aim seeds of haricot bean were surface sterilized in 1 % $KMnO_4$ solution for 3 min and 96 % ethanol solution for 1 min and then washed three times with sterile distilled water. After this procedure seeds were placed in the cuvettes (each containing 15–20 seeds) on the perlite moistened with distilled water and placed in the thermostat for their germination in darkness at the temperature 23 ^{0}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 ^{0}C, light intensity 3000 lux and air humidity 60-80 %**.** To stimulate rhizogenesis 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 25 cm in length containing either distilled water (control), or water solution of each heterocyclic compound used at concentration 10^{-8}M/1 of distilled water, or water solution of auxins IAA and NAA used at the same concentration (experiment). After 14th days the indexes of total number of roots (pcs) and total length of roots (mm) calculated per one experimental haricot bean leaf petiole were determined and compared with the analogical indexes of control leaf petiole on which root formation should not be observed.

2.4 Study of cytokinin-like activity of synthetic heterocyclic compounds

To determine cytokinin-like activity of heterocyclic compounds derivatives of pyrimidine, pyridine, pyrazole, and isoflavones we used specific bioassay [4, 21, 64, 68] conducted on the cotyledons isolated from seeds of muscat pumpkin (*Cucurbita moschata* Duch. et Poir.) of cultivar Gilea. With this aim seeds of pumpkin were surface sterilized in 1 % $KMnO_4$ solution for 3 min and 96 % ethanol solution for 1 min and then washed three times with sterile distilled water. After this procedure seeds were placed in the cuvettes (each containing 20-25 seeds) on the filter paper moistened with distilled water. After this procedure seeds were placed in the thermostat for their germination in darkness at the temperature 25 ^{0}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 each heterocyclic compound used at concentration 10^{-8}M/1 of distilled water or with water solution of phytohormone cytokinin - Kinetin used at the same concentration (experiment). Control and experimental isolated cotyledons were placed in the plant growth chamber in which they were grown at above mentioned conditions. To determine index of average biomass (g) of the 30 isolated 16th-day-old cotyledons of pumpkin, they were washed by sterile distilled water and weighted.

2.5 Study of gibberellin-like activity of synthetic heterocyclic compounds

The specific bioassay on gibberellin-like activity [70, 71, 74] of synthetic heterocyclic compounds derivatives of pyrazole, isoflavones, and pyridine was conducted on the seedlings of lettuce (*Lactuca sativa* L.) of cultivar Berlin. With the aim seeds of lettuce were surface sterilized successively in 1 % $KMnO_4$ solution for 3 min and 96 % ethanol solution for 1 min

and then washed three times with sterile distilled water. After this procedure seeds were germinated in the darkness at the temperature 25 ^{0}C during 36 hours on the filter paper moistened with distilled water. Then seedlings having hypocotyl length between 3.0 and 3.4 mm were placed on the Petri dishes with diameter of 8 cm (each containing 10 seedlings) on the filter paper moistened either with distilled water (control) or with the solution of each heterocyclic compound (3 ml per one Petri dish) used at concentrations ranging from 10^{-4}M to 10^{-9}M/1 of distilled water. Then seedlings of lettuce were placed in the plant growth chamber in which they were grown during 72 hours at the 16/8 h light/dark conditions, temperature 25 ^{0}C, light intensity 3000 lux and air humidity 90 %. The indexes of average hypocotyl length (mm) as well as average root length (mm) calculated per one seedling were determined and compared with similar indexes of control seedlings of lettuce. The growth stimulating activity of synthetic heterocyclic compounds derivatives of pyrimidine, pyridine, pyrazole, and isoflavones was compared with activity of GA3 (the total content of gibberellins accounts more than 90 %) used as a standard in the concentrations ranging from 10^{-4}M to 10^{-9}M/l of distilled water.

2.6 Statistical Analysis

Each test was performed in triplicate. 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 [104].

3. Results and Discussion

3.1 Impact of synthetic heterocyclic compounds and auxins IAA and NAA on germination of seeds and growth of flax seedlings

It is known that bioassay on seed germination and seedling growth is widely applied to study plant growth regulating activity of synthetic or natural bioactive compounds [4, 58-63].

In this work we used this bioassay to study plant growth stimulating activity of heterocyclic compounds derivatives of pyrimidine, isoflavones, and pyridine as compared to activity of phytohormones auxins IAA and NAA. As an object of our investigation we used an economically important oilseed and fiber crop - flax (*Linum usitatissimum* L.) of cultivar Ukrainian 3 [105-109].

Taking into account our previously obtained data confirmed high stimulating effect of low molecular weight heterocyclic compounds derivatives of pyridine, pyrimidine, pyrazole and isoflavones on shoot organogenesis of *Linum usitatissimum* L. cultivar heavenly *in vitro* conditions [96], the main task of this work was study of stimulating activity of some from previously tested heterocyclic compounds on vegetative growth of this agricultural important crop in the laboratory conditions.

Thus we conducted researches and obtained results that testified that all tested heterocyclic compounds used at concentration 10^{-8}M/l of distilled water demonstrated high stimulating activity on seed germination and vegetative growth of flax. The activity of heterocyclic compounds was similar or higher than the activity of phytohormones auxins IAA and NAA, and manifested in considerable acceleration of growth and development of shoots and roots on the 15th-day-old flax seedlings (Fig. 2 and Fig. 3).

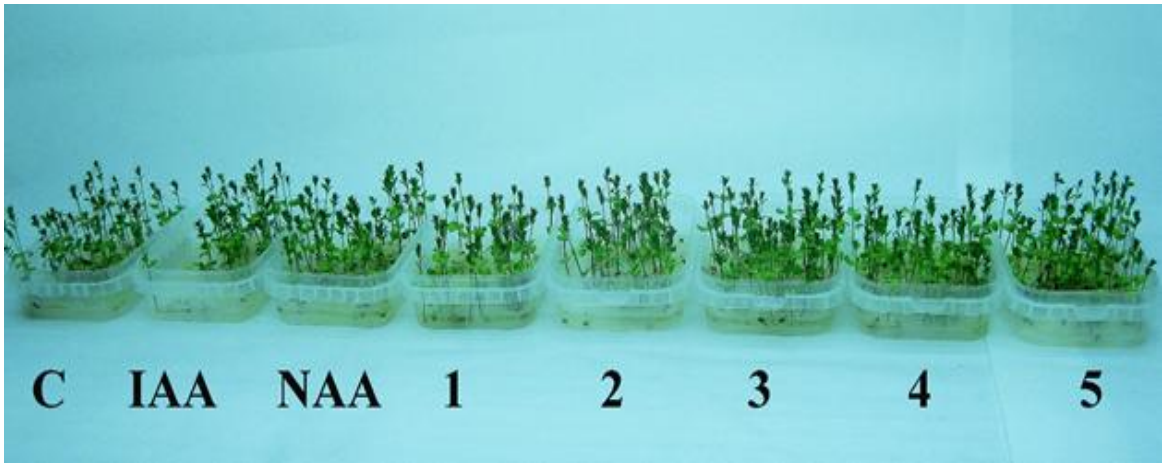

Fig 2: Impact of chemical heterocyclic compounds derivatives of pyrimidine, pyrazole, isoflavones, pyridine, and auxins IAA and NAA on the growth and development of the 15th-day-old flax seedlings as compared to control (**C**) flax seedlings. **C** – Control (distilled water), **IAA** – Compound 1*H*-Indol-3-ylacetic acid, **NAA** – Compound 1-Naphthylacetic acid, **1** – Compound 6-Methanesulfonyl-imidazo[1,2-*a*]pyrimidine-5-ylamine, **2** – Compound 5-Hydrazino-1-phenyl-1*H*-pyrazole-4-carbohydrazide, **3** – Compound 3-Ethyl-7-methyl-3,7-dihydro-4*H*-pyrazolo[3,4-*d*][1,2,3]triazin-4-one, **4** – Compound 5-Hydroxy-7-methoxy-8-(methoxymethyl)-3-(4-methoxyphenyl)-4*H*-chromen-4-one, **5** – Compound ((1H-pyrrolo[2,3-*c*]pyridin-3-yl)-acetic acid)

The comparative analysis of biometric indexes of 15th-day-old flax seedlings (i.e. number of germinated seeds (%), length of seedlings (cm), total number of roots (pcs), total length of roots (mm)) showed that the biometric indexes of seedlings grown on solution of chemical heterocyclic compounds used at concentration 10^{-8}M/l of distilled water were similar or higher than the biometric indexes seedlings grown on the solution of phytohormones auxins IAA and NAA used at the same concentration as compared to lower biometric indexes of flax seedlings grown on the distilled water (control). The results of stimulating effect of the heterocyclic compounds on the biometric indexes of 15th-day-old flax seedlings are shown on the Fig 4.

Particularly it was found that the biometric indexes of flax seedlings grown on the 10^{-8}M water solution of compound №1 -6-Methanesulfonyl-imidazo[1,2-*a*]pyrimidine-5-ylamine were

as generally higher than the biometric indexes of flax seedlings grown either on the distilled water (control) or on the 10^{-8}M water solution of auxins IAA and NAA as follows: according with length of seedlings – at the 6 % compared with control; according with total length of roots – at the 58 %, 20 %, and 15 % as compared with control, IAA and NAA, respectively; according with total number of roots – at the 67 % as compared with control seedlings (Fig. 4).

Fig 3: Impact of chemical heterocyclic compounds derivatives of pyrimidine, pyridine, pyrazole, isoflavones, and phytohormones auxins **IAA** and **NAA** on the root formation of the 15th-day-old flax seedlings as compared to control (**C**) flax seedlings. **C** – Control (distilled water), **IAA** – Compound 1*H*-Indol-3-ylacetic acid, **NAA** – Compound 1-Naphthylacetic acid, **1** – Compound 6-Methanesulfonyl-imidazo[1,2-*a*]pyrimidine-5-ylamine, **2** – Compound 5-Hydrazino-1-phenyl-1*H*-pyrazole-4-carbohydrazide, **3** – Compound 3-Ethyl-7-methyl-3,7-dihydro-4*H*-pyrazolo[3,4-*d*][1,2,3]triazin-4-one, **4** – Compound 5-Hydroxy-7-methoxy-8-(methoxymethyl)-3-(4-methoxyphenyl)-4*H*-chromen-4-one, **5** – Compound ((1H-pyrrolo[2,3-*c*]pyridin-3-yl)-acetic acid)

The biometric indexes of flax seedlings grown on the 10^{-8}M water solution of compound №2 – 5-Hydrazino-1-phenyl-1*H*-pyrazole-4-carbohydrazide, were as generally higher than the biometric indexes of flax seedlings grown either on the distilled water (control) or on the 10^{-8}M water solution of auxins IAA and NAA as follows: according with length of seedlings – at the 7 % as compared with control; according with total length of roots – at the 53 %, 16 %, and 11 % as compared with control, IAA and NAA, respectively; according with total number of roots – at the 58 % as compared with control seedlings (Fig. 4).

The biometric indexes of flax seedlings grown on the 10^{-8}M water solution of compound №3 – 3-Ethyl-7-methyl-3,7-dihydro-4*H*-pyrazolo[3,4-*d*][1,2,3]triazin-4-one, were as generally higher than the biometric indexes of flax seedlings grown either on the distilled water (control) or on the 10^{-8}M water solution of auxins IAA and NAA as follows: according with length of seedlings – at the 5 % as compared with control; according with total length of roots – at the 56 %, 19 %, and 13 % as compared with control, IAA and NAA, respectively; according with total number of roots – at the 61 % as compared with control seedlings (Fig. 4).

The biometric indexes of flax seedlings grown on the 10^{-8}M water solution of compound №4 – 5-Hydroxy-7-methoxy-8-(methoxymethyl)-3-(4-methoxyphenyl)-4*H*-chromen-4-one, were as generally higher than the biometric indexes of flax seedlings grown either on the distilled water (control) or on the 10^{-8}M water solution of auxins IAA and NAA as follows: according with length of seedlings – at the 5 % as compared with control; according with total length of roots – at the 36 % and 3 % as compared with control and IAA, respectively; according with total number of roots – at the 39 % as compared with control seedlings (Fig. 4).

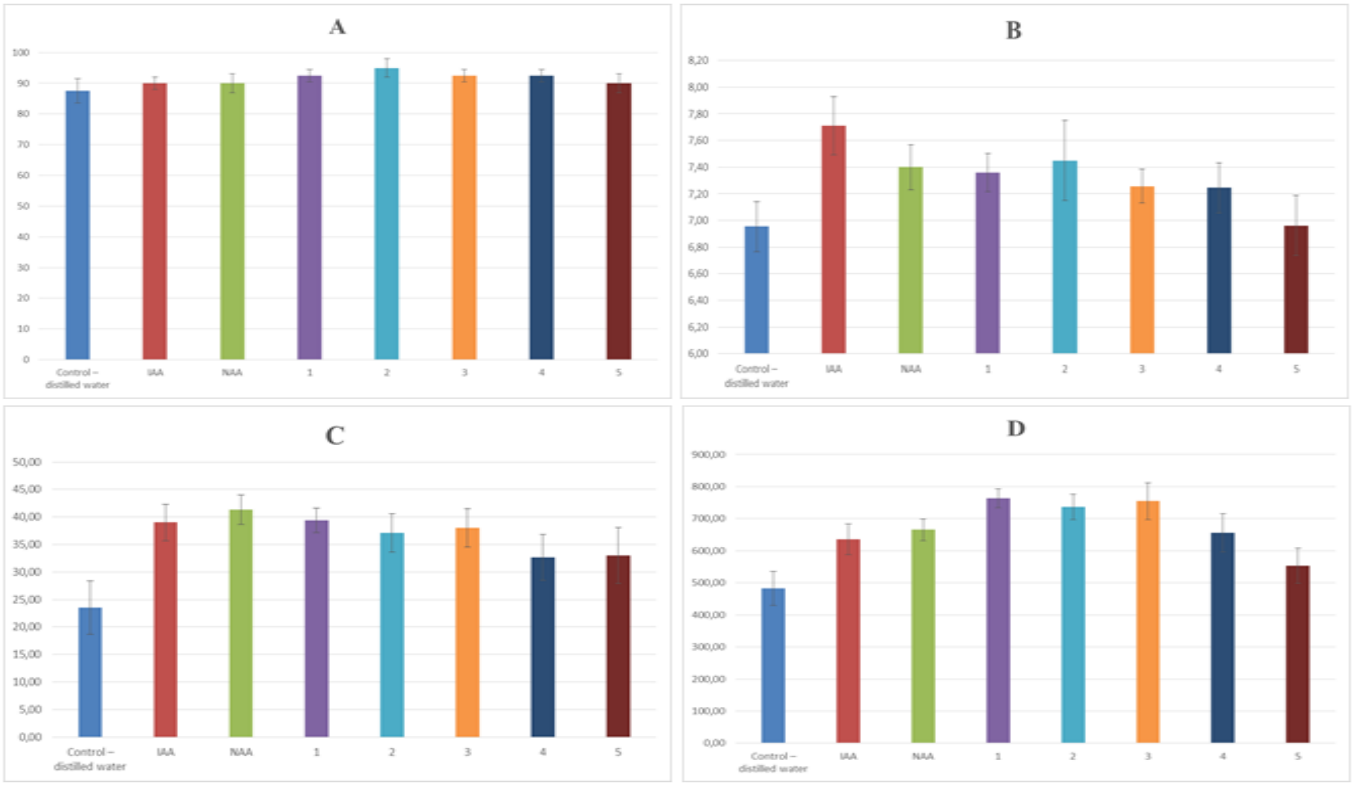

Fig 4: Impact of chemical heterocyclic compounds derivatives of pyrimidine, pyridine, pyrazole, isoflavones, and phytohormones auxins **IAA** and **NAA** on the biometric indexes of 15th-day-old flax seedlings. Control - distilled water, **IAA** – Compound 1*H*-Indol-3-ylacetic acid, **NAA** – Compound 1-Naphthylacetic acid, **1** - Compound 6-Methanesulfonyl-imidazo[1,2-*a*]pyrimidine-5-ylamine, **2** – Compound 5-Hydrazino-1-phenyl-1*H*-pyrazole-4-carbohydrazide, **3** – Compound 3-Ethyl-7-methyl-3,7-dihydro-4*H*-pyrazolo[3,4-*d*][1,2,3]triazin-4-one, **4** – Compound 5-Hydroxy-7-methoxy-8-(methoxymethyl)-3-(4-methoxyphenyl)-4*H*-chromen-4-one, **5** – Compound ((1H-pyrrolo[2,3-*c*]pyridin-3-yl)-acetic acid. **A** – Number of germinated seeds (%), **B** – length of seedlings (cm), **C** – total number of roots (pcs), **D** – total length of roots (mm)

The biometric indexes of flax seedlings grown on the 10^{-8}M water solution of compound №5 – ((1H-pyrrolo[2,3-*c*]pyridin-3-yl)-acetic acid), were as generally higher than the biometric indexes of flax seedlings grown either on the distilled water (control) or on the 10^{-8}M water solution of auxins IAA and NAA as follows: according with total length of roots – at the 15 % as compared with control; according with total number of roots – at the 40 % as compared with control seedlings (Fig. 4). Thus, the obtained results testify that all tested chemical heterocyclic compounds derivatives of pyrimidine, pyridine, pyrazole, and isoflavones revealed expressive auxin-like activity on stimulation of vegetative growth of 15th-day-old seedlings of flax (*Linum usitatissimum* L.) of cultivar Ukrainian 3.

Taking into account the obtained results it is possible to assume that high growth stimulating activity of synthetic heterocyclic compounds, which is similar to activity of phytohormones auxins, may be explained by their inducing effect on the plant cell division, cell proliferation, cell elongation and cell differentiation, and on the increase of rate of plant cell metabolism. These processes result in acceleration of plant growth and improvement of plant development.

3.2 Stimulating effect of synthetic heterocyclic compounds on rhizogenesis on the isolated leaf petioles of haricot bean seedlings

Auxins are the major plant hormones involved in control of plant embryogenesis, seed germination, cell elongation and cell division in 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, as well as morphogenesis in plant tissue cultures *in vitro* [2, 4, 5, 19, 21-23, 30, 31, 38, 39, 75-78, 87, 88].

In this work we used specific bioassay conducted on the leaf petioles isolated from seedlings of haricot bean (*Phaseolus vulgaris* L.) of cultivar Belozernaya to study auxin-like activity of synthetic heterocyclic compounds. It is known that this bioassay is based on key role of phytohormones auxins in stimulation of adventitious root formation on stem and leaf cuttings [4, 64-66, 79-84].

Our researches showed that heterocyclic compounds derivatives of pyrimidine, pyridine, pyrazole, and isoflavones used at concentration 10^{-8}M/l of distilled water revealed expressive auxin-like activity (Fig. 5). 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 root formation on the isolated 14th-day-old leaf petioles of haricot bean seedlings showed heterocyclic compounds derivatives of pyrazole and isoflavones (Fig. 5, compounds № 7, 8, 10 - 12).

Fig 5: Impact of chemical heterocyclic compounds derivatives of pyrimidine, pyridine, pyrazole, isoflavones, and phytohormones auxins IAA and NAA on the root formation on the 14th-day-old leaf petioles of haricot bean seedlings. 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 7-Methyl-4-oxo-4,7-dihydropyrazolo[3,4-*d*][1,2,3]triazin-3-yl)-acetic acid ethyl ester, 5 – NAA (1-Naphthylacetic acid), 6 – IAA (1*H*-Indol-3-ylacetic acid), 7 – Compound 5-Hydrazino-1-phenyl-1*H*-pyrazole-4-carbohydrazide, 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 comparative statistical analysis of indexes 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 10^{-8}M water solution of either chemical heterocyclic compounds or auxins IAA and NAA used at the same concentration as compared to control are shown in the Table1.

It was found that the heterocyclic compound №12 revealed the highest auxin-like stimulation effect on the root formation on the 14th-day-old haricot bean leaf petioles according to indexes of total root number – at the 146 % and total root length – at the 9.18 times as compared to control haricot bean leaf petioles treated with distilled water.

Table 1: Impact of chemical heterocyclic compounds derivatives of pyrimidine, pyridine, pyrazole, isoflavones on the average total root number (pcs) and average total root length (mm) formed on the14th-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, $p \leq 0.05$, 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)-phosphonic acid diethyl ester, Compound **№4** – 7-Methyl-4-oxo-4,7-dihydropyrazolo[3,4-*d*][1,2,3]triazin-3-yl)-acetic acid ethyl ester, 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-carbohydrazide, 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-methoxy phenyl)-4*H*-chromen-4-one

The high auxin-like stimulation effect on the root formation on the 14th-day-old haricot bean leaf petioles revealed also heterocyclic compounds: the compound №10 according to indexes of total root number – at the 129 % and total root length – at the 8.45 times as compared to control haricot bean leaf petioles; the compound №11 according to indexes of total root number – at the 117 % and total root length – at the 7.34 times as compared to control haricot bean leaf petioles; the compound №7 according to indexes of total root number – at the 96 % and total root length – at the 5.79 times as compared to control haricot bean leaf petioles; the compound №8 according to indexes of total root number – at the 83 % and total root length – at the 6.45 times as compared to control haricot bean leaf petioles; the compound №3 according to indexes of total root

number – at the 67 % and total root length – at the 2.88 times as compared to control haricot bean leaf petioles.

The high auxin-like stimulation effect on the root formation on the 14th-day-old haricot bean leaf petioles revealed also phytohormones auxins: the compound №5 (NAA) according to indexes of total root number – at the 79 % and total root length – at the 4.76 times as compared to control haricot bean leaf petioles and the compound №6 (IAA) according to indexes of total root number – at the 62 % and total root length - at the 1.72 times as compared to control haricot bean leaf petioles.

The lower auxin-like stimulation effect on the root formation on the 14th-day-old haricot bean leaf petioles revealed heterocyclic compounds: the compound №2 according to indexes of total root number – at the 43 % and total root length – at the 1.65 times as compared to control haricot bean leaf petioles; the compound №9 according to indexes of total root number – at the 35 % and total root length – at the 5.26 times as compared to control haricot bean leaf petioles; the compound №1 according to indexes of total root number – at the 29 % and total root length – at the 1.38 times as compared to control haricot bean leaf petioles; the compound №4 according to indexes of total root number – at the 23 % and total root length – at the 34 % as compared to control haricot bean leaf petioles.

Obviously, that high auxin-like stimulating root growth activity of tested heterocyclic compounds may be explained by similarity of some fragments of their chemical structure to phytohormones auxins IAA or NAA and to biologically active substances containing in the plant cells such as flavonoids, as It is known that 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, leaf senescence delaying, plant-pathogen interactions, as well as morphogenesis in plant tissue cultures *in vitro* [2, 4, 5, 19, 21, 22, 24, 26, 27, 30, 31, 38, 75-78, 87, 88].

3.3 Stimulating effect of synthetic heterocyclic compounds on the growth of biomass of isolated cotyledons of pumpkin

To one of the most known bioassays on cytokinin-like activity belongs bioassay on growth of biomass of cotyledons (i.e. food-storage organs) isolated from seed of muscat pumpkin (*Cucurbita pepo* L.) [4, 21, 64, 68]. In this work we studied cytokinin-like activity of heterocyclic compounds derivatives of pyrimidine, pyridine, pyrazole, and isoflavones using cotyledons isolated from seed of muscat pumpkin (*Cucurbita moschata* Duch. et Poir.) of cultivar Gilea (Fig. 6).

The obtained results showed that according to indexes of growth of biomass of the isolated cotyledons of pumpkin during 16 days the all tested compounds used at concentration 10^{-8}M/l of distilled water revealed expressive cytokinin-like activity, which was similar or higher than the activity of phytohormone cytokinin Kinetin used at the same concentration (Fig. 6).

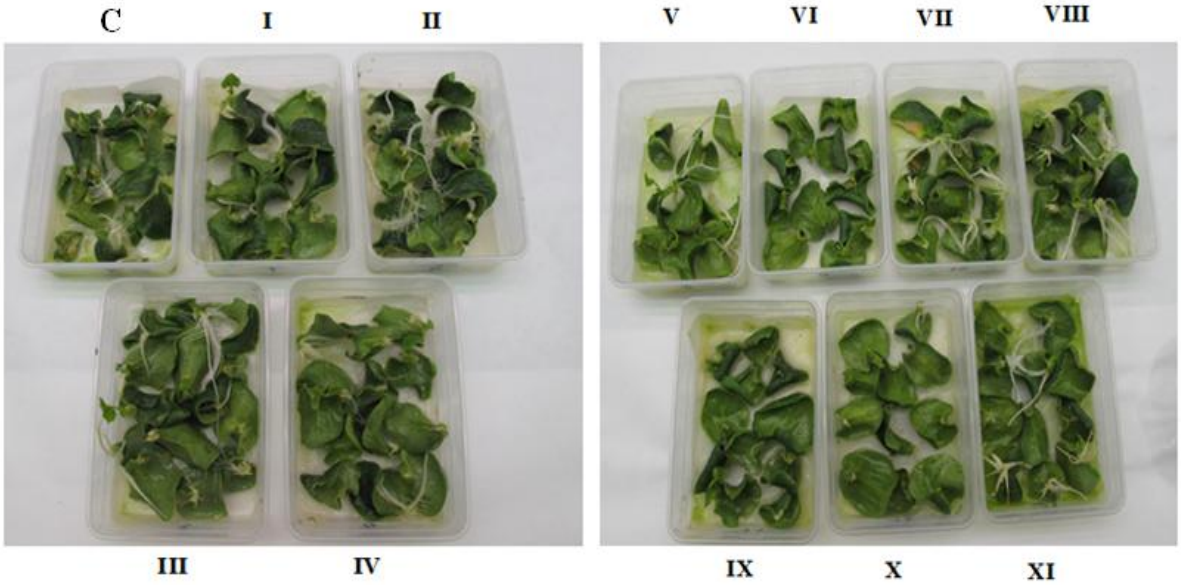

Fig 6: Impact of chemical heterocyclic compounds derivatives of pyrimidine, pyridine, pyrazole, isoflavones, and phytohormone cytokinin **Kinetin** on the growth of biomass of the isolated 16th-day-old cotyledons of pumpkin. **C** – Control (distilled water), **I** - Compound 6-Methanesulfonyl-imidazo[1,2-*a*]pyrimidine-5-ylamine, **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 7-Methyl-4-oxo-4,7-dihydropyrazolo[3,4-*d*][1,2,3]triazin-3-yl)-acetic acid ethyl ester, **V** - Compound 5-Hydrazino-1-phenyl-1*H*-pyrazole-4-carbohydrazide, **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)

At the same time it was found that some heterocyclic compounds used at concentration 10^{-8}M/l of distilled water revealed also nonspecific for this bioassay auxin-like activity, which was manifested in stimulation of root formation on the isolated six-week-old cotyledons of muscat pumpkin (Fig. 7).

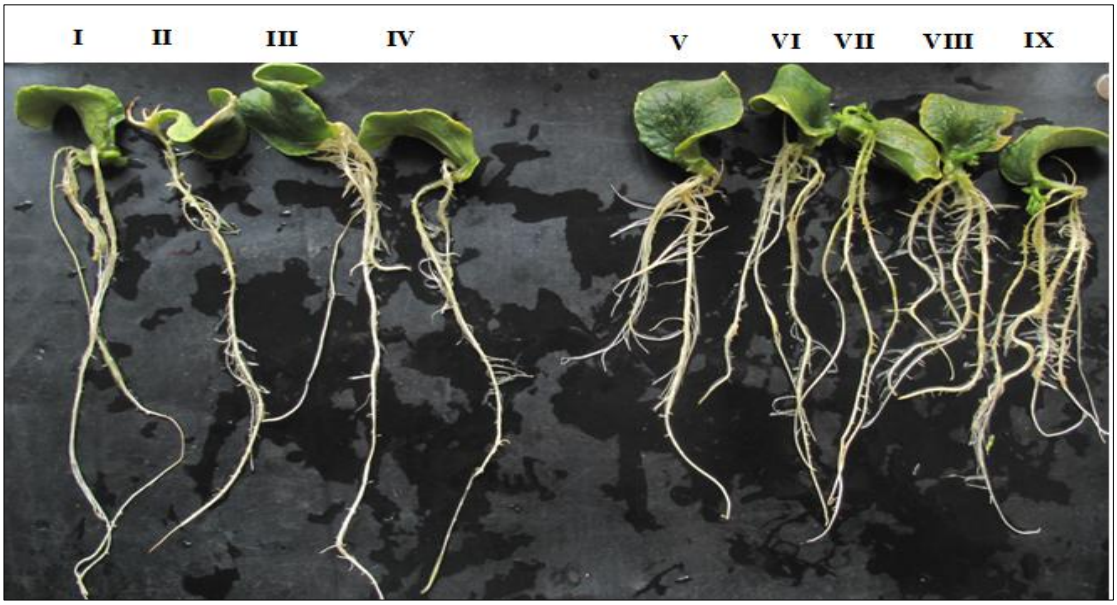

Fig 7: Impact of chemical heterocyclic compounds derivatives of pyrimidine, pyridine, pyrazole, isoflavones on the root formation on the isolated six-week-old cotyledons of pumpkin. **I** - Compound 6-Methanesulfonyl-imidazo[1,2-*a*]pyrimidine-5-ylamine, **II** - Compound 1-Benzyl-5-methanesulfonyl-3-phenyl-1*H*-pyrimidine-2,4-dione, **III** – Compound 7-Methyl-4-oxo-4,7-dihydropyrazolo[3,4-*d*][1,2,3]triazin-3-yl)-acetic acid ethyl ester, **IV** - Compound 5-Hydrazino-1-phenyl-1*H*-pyrazole-4-carbohydrazide, **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)

The data of comparative statistical analysis of indexes of average biomass of 30 cotyledons (g) and average length of one root per 30 cotyledons (cm) of isolated six-week-old cotyledons of pumpkin are shown in the Table 2.

Table 2: Impact of chemical heterocyclic compounds derivatives of pyrimidine, pyridine, pyrazole, and isoflavones on the growth of biomass (g) and length of roots (cm) formed on the isolated six-week-old cotyledons of muscat pumpkin

№ Compound	The average biomass of 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)-phosphonic acid diethyl ester, Compound **№4** - 7-Methyl-4-oxo-4,7-dihydropyrazolo[3,4-*d*][1,2,3]triazin-3-yl)-acetic acid ethyl ester, 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 indexes 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 as generally higher than the analogical indexes 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 with average biomass - at the 121 % as compared with control and at the 109 % as compared with cytokinin Kinetin; according with 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.

The indexes 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 as generally higher than the analogical indexes 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 with average biomass - at the 128 % as compared with control and at the 116 % as compared with cytokinin Kinetin; according with 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.

The indexes 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 as generally higher than the analogical indexes 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 with average biomass - at the 120 % as compared with control and at the 108 % as compared with cytokinin Kinetin; according with 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.

The indexes 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 №4 were as generally higher than the analogical indexes 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 with average biomass - at the 123 % as compared with control and at the 112 % as compared with cytokinin Kinetin; according with 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.

The indexes 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 as generally higher than the analogical indexes 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 with average biomass - at the 130 % as compared with control and at the 118 % as compared with cytokinin Kinetin; according with 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.

The indexes 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 as generally higher than the analogical indexes 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 with average biomass - at the 126 % as compared with control and at the 114 % as compared with cytokinin Kinetin; according with 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.

The indexes 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 as generally higher than the analogical indexes 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 with average biomass - at the 121 % as compared with control and at the 109 % as compared with cytokinin Kinetin; according with 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.

The indexes 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 as generally higher than the analogical indexes 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 with average biomass - at the 134 % as compared with control and at the 122 % as compared with cytokinin Kinetin; according with 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.

The indexes 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 as generally higher than the analogical indexes 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 with average biomass - at the 145 % as compared with control and at the 131 % as compared with cytokinin Kinetin; according with 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.

The indexes 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 as generally higher than the analogical indexes 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 with average biomass - at the 125 % as compared with control and at the 113 % as compared with cytokinin Kinetin; according with 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.

Obtained results suggest that high cytokinin-like activity and auxin-like stimulating rhizogenesis activity of synthetic heterocyclic compounds may be explained by similarity of some fragments of their chemical structure to phytohormones cytokinin Kinetin and auxins IAA and NAA, and their specific cytokinin-like and auxin-like inducing effect on cell division and cell elongation resulting in increasing growth and biomass of isolated cotyledons as well as adventitious root formation.

3.4 Impact of synthetic heterocyclic compounds on elongation of hypocotyl of lettuce seedlings

It is known that plant hormone gibberellic acid plays a major role in control of plant embryogenesis, seed germination, stem elongation, leaf expansion, trichome development, meristematic tissue development, differentiation of floral organs, another development, pollen maturation, seed and pericarp growth, plant adaptation to the environment, and morphogenesis in plant tissue cultures *in vitro* [2, 4, 5, 19, 21, 22, 25, 28-31, 38, 58, 75-78, 84-88]. One of the most known bioassay on specific gibberellin-like activity of natural or chemical compounds is based on their stimulating effect on elongation of hypocotyl of seedlings of normal lettuce or lettuce dwarf mutants [70, 71, 74].

In our experiments we used normal lettuce (*Lactuca sativa* L.) of cultivar Berlin. The obtained results work testified that some tested heterocyclic compounds derivatives of pyrazole, isoflavones, and pyridine – compounds № II, III and VI revealed minor stimulation effect which depended from their used concentrations ranging from 10^{-4}M to 10^{-9}M/1 of distilled

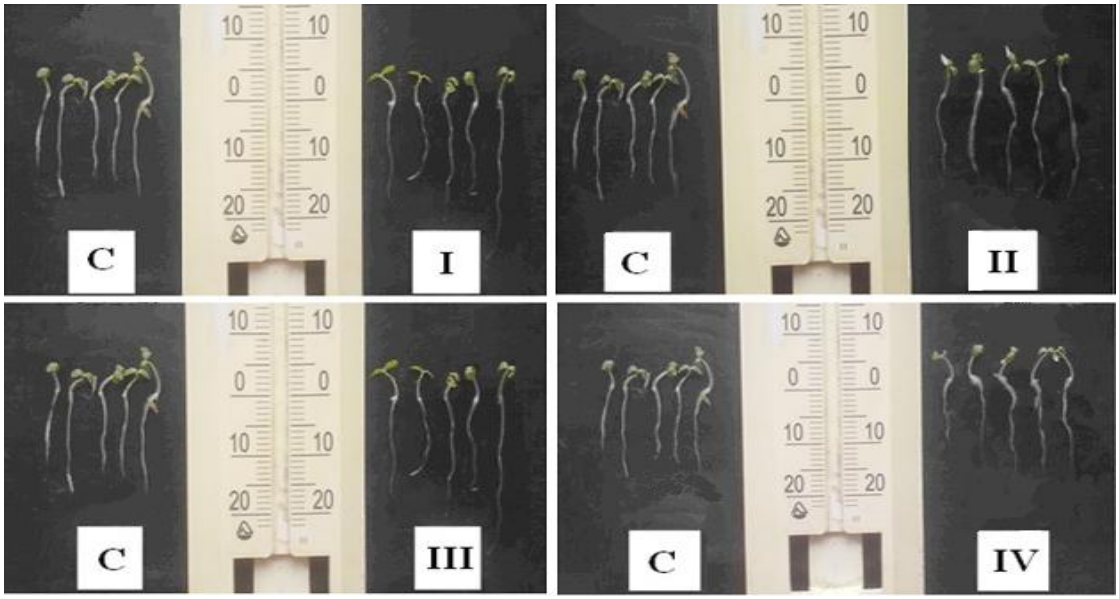

Fig 8: Impact of chemical heterocyclic compounds derivatives of pyrazole and isoflavones used in concentration 10^{-7}M/l of distilled water on the growth of hypocotyl and root on the 3rd-day-old lettuce seedling. **C** – Control (distilled water), **I** – Compound 7-Methyl-4-oxo-4,7-dihydropyrazolo[3,4-*d*][1,2,3]triazin-3-yl)-acetic acid ethyl ester, **II** - Compound 3-Ethyl-7-methyl-3,7-dihydro-4*H*-pyrazolo[3,4-*d*][1,2,3]triazin-4-one, **III** - Compound 5-Hydroxy-7-methoxy-6-(methoxymethyl)-2-phenyl-4*H*-chromen-4-one, **IV** - Compound 5-Hydroxy-7-methoxy-6-(methoxymethyl)-3-(2-methoxyphenyl)-4*H*-chromen-4-one

water on elongation of hypocotyl (i.e. stem of germinating seedling) on the 3rd- day-old seedlings of lettuce (Fig. 8 and Fig. 9A).

At the same time other heterocyclic compounds derivatives of pyrazole and isoflavones – compounds № I, IV and V used at concentrations ranging from 10^{-4}M to 10^{-9}M/1 of distilled water did not reveal any marked stimulation effect on elongation of hypocotyl on the 3rd- day-old seedlings of lettuce. This fact confirms lack of gibberellin-like activity at these heterocyclic compounds (Fig. 8 and Fig. 9A).

This bioassay showed also that the some heterocyclic compounds derivatives of pyrazole and isoflavones - compounds № II-V used in concentration 10^{-7}M/l of distilled water demonstrated nonspecific for this bioassay auxin-like activity, which manifested in stimulation of formation and growth of root system on the 3rd- day-old lettuce seedlings (Fig. 8 and Fig. 9 B).

The comparative analysis of indexes of average hypocotyl length (%) calculated per one 3rd-day-old lettuce seedling in relation to indexes of control seedling showed that activity of all tested heterocyclic compounds derivatives of pyrazole, isoflavones, and pyridine used in the concentrations ranging from 10^{-4}M to 10^{-9}M/l of distilled water was significantly lower than activity of GA3 used as a standard in the same concentrations in relation to control (Fig. 9 A).

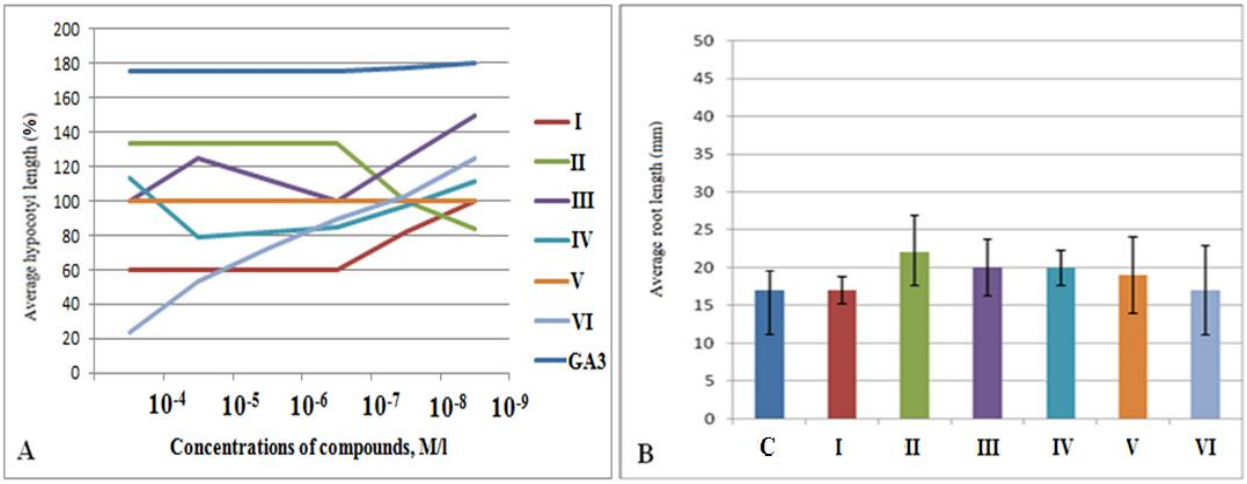

Fig 9: Impact of chemical heterocyclic compounds derivatives of pyrazole, isoflavones, and pyridine on the growth of 3rd-day-old lettuce seedlings. **C** – Control (distilled water), **I** - Compound Ethyl 2-(4-oxo-7-methyl-4,7-dihydro-3*H*-pyrazolo[3,4-*d*][1,2,3]triazin-3-yl)acetate, **II** - Compound 3-Ethyl-7-methyl-3,7-dihydro-4*H*-pyrazolo[3,4-*d*][1,2,3]triazin-4-one, **III** - Compound 5-Hydroxy-7-methoxy-6-(methoxymethyl)-2-phenyl-4*H*-chromen-4-one, **IV** - Compound 5-Hydroxy-7-methoxy-6-(methoxymethyl)-3-(2-methoxyphenyl)-4*H*-chromen-4-one, **V** - Compound 5-Hydroxy-7-methoxy-8-(methoxymethyl)-3-(4-methoxy phenyl)-4*H*-chromen-4-one, **VI** - Compound (1H-pyrrolo[2,3-*c*]pyridin-3-yl)-acetic acid). **A** - average hypocotyl length (%) in relation to control, the concentrations of phytohormone **GA3** and heterocyclic compounds - 10^{-4}M - 10^{-9}M/l of distilled water, **B** - average root length (mm), the concentrations of heterocyclic compounds - 10^{-7}M/l of distilled water

Among all tested compounds the minor stimulating effect on hypocotyl growth in relation to control showed compounds derivatives of pyrazole - compound № II and isoflavones - compound № III, their activity was various depending from their used concentrations ranging from 10^{-4}M to 10^{-9}M/l of distilled water, but it was significantly lower than activity of GA3.

The indexes of average hypocotyl length (%) calculated per one 3rd-day-old lettuce seedling grown on the water solution of compound № II used at concentrations 10^{-4}M-10^{-7}M/l of distilled water were at average higher at 35 % as compared to indexes of control seedlings of lettuce treated with distilled water, whereas this compound used at lower concentrations ranging from 10^{-8}M-10^{-9}M/l of distilled water revealed inhibitory effect on hypocotyl elongation on the lettuce seedlings.

The indexes of average hypocotyl length (%) calculated per one 3rd-day-old lettuce seedling grown on the water solution of compound № III used at concentrations 10^{-5}M and 10^{-8}M-10^{-9}M/l of distilled water were at average higher at 25-45 % as compared to indexes of control seedlings of lettuce treated with distilled water.

The lowest activity showed also compound derivative of pyridine - compound № VI, its activity increased at 23 % as compared to control at decrease its concentration up to 10^{-9}M/l of distilled water, whereas this compound used at higher concentrations ranging from 10^{-4}M to 10^{-8}M/l of distilled water revealed inhibitory effect on hypocotyl elongation on the lettuce seedlings.

It was found also that some heterocyclic compounds derivatives of pyrazole and isoflavones – compounds № II-V used at concentration 10^{-7}M/l of distilled water demonstrated nonspecific for gibberellins bioassay auxin-like activity. The indexes of average length of roots (mm) obtained on the 3rd-day-old seedlings of lettuce grown on the 10^{-7}M water solution of these heterocyclic compounds were at average higher at 17-35 % as compared to indexes of control seedlings of lettuce treated with distilled water (Fig. 9 B).

Taking into account the obtained results it is possible to propose that lack of gibberellin-like effect or minor gibberellin-like effect of heterocyclic compounds derivatives of pyrazole, isoflavones, and pyridine on cell division and cell elongation in the hypocotyl and auxin-like effect on cell division and cell elongation in the roots of lettuce seedlings may be explained by difference their chemical structure from phytohormone GA3 and similarity to auxins such as IAA or NAA.

4. Conclusion

The phytohormone-like activity of synthetic low molecular weight heterocyclic compounds derivatives of pyrimidine, pyridine, pyrazole, and isoflavones using seed germination and seedling growth bioassay on flax (*Linum usitatissimum* L.) of cultivar Ukrainian 3, the specific bioassay on auxin-like activity on the leaf petioles isolated from seedlings of haricot bean (*Phaseolus vulgaris* L.) of cultivar Belozernaya, the specific bioassay on cytokinin-like activity on the cotyledons isolated from seeds of muscat pumpkin (*Cucurbita moschata* Duch. et Poir.) of cultivar Gilea, as well as specific bioassay on gibberellin-like activity on the seedlings of lettuce (*Lactuca sativa* L.) of cultivar Berlin was studied. It was shown that synthetic heterocyclic compounds used at physiologically active concentrations 10^{-7}M and 10^{-8}M/1 of distilled water demonstrated high auxin-like and cytokinin-like activity which manifested in intensification of growth of plant organs such as roots and cotyledons, as well as acceleration of plant vegetative growth. The bioassay on gibberellin-like activity showed minor stimulating effect of some heterocyclic compounds derivatives of pyrazole, isoflavones, and pyridine on elongation of hypocotyl of lettuce seedlings, their activity was various depending from their concentrations ranging from 10^{-4}M to 10^{-9}M/1 of distilled water, but it was considerably lower than activity of GA3. The obtained results suggested expressive auxin-like and cytokinin-like, and minor gibberellin-like inducing effect of synthetic heterocyclic compounds on cell division, cell elongation, cell proliferation, and cell differentiation that are the basic processes of plant growth and development. This study confirmed perspective of using of synthetic low molecular weight heterocyclic compounds derivatives of pyrimidine, pyridine, pyrazole, and isoflavones as new effective plant growth stimulators in practice of biotechnology and agriculture.

5. References

1. Altman A, Hasegawa PM. Plant Biotechnology and Agriculture: Prospects for the 21st Century. Academic Press, Elsevier, USA. 2012, 201-624.
2. Wania SH, Kumarb V, Shriramc V, Sah SK. Phytohormones and their metabolic engineering for abiotic stress tolerance in crop plants. THE CROP Journal. 2016; 4:162-176. http://dx.doi.org/10.1016/j.cj.2016.01.010 2214-5141/
3. Basra AS. (Ed). Plant Growth Regulators in Agriculture and Horticulture: Their Role and Commercial Uses. Haworth Press, Inc., New York, London, Oxford, 2000, 264. https://www.crcpress.com/Plant-Growth-Regulators-in-Agriculture-and-Horticulture-Their-Role-and/Basra/p/book/9781560228912
4. Arteca RN. Plant Growth Substances: Principles and Applications. Chapman & Hall, NY, 1996, 332. http://iauyasooj.ac.ir/my_doc/iauyasooj/Plant%20Growth%20Substances.pdf
5. Davies PJ. (Ed). Plant Hormones - Biosynthesis, Signal Transduction, Action! Kluwer academic publishers. Dordrecht, boston, London, 2004, 776. https://www.scribd.com/document/177140158/P-J-Davies-Plant-Hormones-Biosynthesis-Signal-Transduction-Action-Kluwer-Academic-Publishers-2004
6. Rademacher W. Plant Growth Regulators: Backgrounds and Uses in Plant Production. J Plant Growth Regul. 2015; 34(4):845-872. http://agris.fao.org/agris-search/search.do?recordID=US201500207845
7. Lopez-Lauri F. Plant Growth Regulators. In: Siddiqui MW, Zavala A, Hwang JF, Andy C.-A. (Eds.), Postharvest Management Approaches for Maintaining Quality of Fresh Produce, Springer International Publishing. Switzerland, 2016, 222:125-139. http://www.springer.com/it/book/9783319235813
8. Jardin P. Plant biostimulants: Definition, concept, main categories and regulation. Sci. Hortic. 2015; 196(30):3-14. https://orbi.ulg.ac.be/bitstream/2268/187492/1/PduJardin2015_Plant-Biostimulants_InPress.pdf
9. Le Mire G, Nguyen ML, Fassotte B, Jardin P, Verheggen F, Delaplace P. Implementing plant biostimulants and biocontrol strategies in the agroecological management of

cultivated ecosystems. A review. Biotechnol. Agron. Soc. Environ. 2016; 20(S1):299-313. http://www.pressesagro.be/ojs/index.php/base/article/viewFile/1720/816
10. Calvo P, Nelson L, Kloepper JW. Agricultural uses of plant biostimulants. Plant Soil. 2014; 383(1):3-41. DOI 10.1007/s11104-014-2131-8
11. Bradáčová K, Weber NF, Morad-Talab N, Asim M, Imran M, Weinmann M, *et al*. Micronutrients (Zn/Mn), seaweed extracts, and plant growth-promoting bacteria as cold-stress protectants in maize. Chem. Biol. Technol. Agric. 2016; 3:19. DOI 10.1186/s40538-016-0069-1
12. Ponomarenko SP, Hrytsaenko ZM, Tsygankova VA. Increase of plant resistance to diseases, pests and stresses with new biostimulants. Acta Horticulturae: I World Congress on the Use of Biostimulants in Agriculture. Strasburg (France). 2012; 1009:225-233. http://agris.fao.org/agris-search/search.do?recordID=US201400150177
13. Victoria Tsygankova, Elena Shysha, Yaroslav Andrusevich, Anatoly Galkin, Galina Iutynska, Alla Yemets, *et al.* Using of new microbial biostimulants for obtaining in vitro new lines of *Triticum aestivum* L. cells resistant to nematode *H. avenae*. European Journal of Biotechnology and Bioscience. 2016; 4(4):39-53. http://www.biosciencejournals.com/archives/2016/vol4issue4/4-4-26.1.pdf
14. Mostafa GG. Improving the Growth of Fennel Plant Grown under Salinity Stress using some Biostimulants. American Journal of Plant Physiology. 2015; 10(2):77-83. DOI: 10.3923/ajpp.2015.77.83
15. Bhardwaj D, Ansari MW, Sahoo RK, Tuteja N. Biofertilizers function as key player in sustainable agriculture by improving soil fertility, plant tolerance and crop productivity. Microbial Cell Factories. 2014; 13(66):1-10. DOI: 10.1186/1475-2859-13-66
16. Acton QA. (Ed.). Pesticides-Advances in Research and Application: 2013 Edition. Scholarly Editions, Georgia, 2013, 862.
17. Aktar MW, Sengupta D, Chowdhury A. Impact of pesticides use in agriculture: their benefits and hazards. Interdiscip Toxicol. 2009; 2(1):1-12.
18. Brent KJ. (Ed.) Fungicide resistance in crop pathogens: how can it be managed? University of Bristol, University Walk, Bristol, BS8 1TD, UK, 2007, 60. http://www.frac.info/docs/default-source/publications/monographs/monograph-1.pdf
19. Gaspar T. Kevers C, Penel C, Greppin H, Reid Dm, Thorpe TA. Plant hormones and plant growth regulators in plant tissue culture. *In Vitro* Cell. Dev. Biol.-Plant. 1996; 32:272-289. http://link.springer.com/article/10.1007/BF02822700
20. Kirakosyan A, Kaufman PB. Recent Advances in Plant Biotechnology. Springer Science & Business Media. 2009; XVI:412.
21. Ellenberg H, Esser K, Merxmüller H, Schnepf E, Ziegler H. (Eds.). Progress in Botany. Morphology, Physiology, Genetics, Taxonomy, Geobotany. - Fortschritte der Botanik. Morphologie, Physiologie, Genetik, Systematik, Geobotanik. Springer-Verlag, Berlin. Heidelberg, New York. 1977, 402:37. http://onlinelibrary.wiley.com/doi/10.1002/fedr.19770880407/full
22. Kende H, Zeevaart JAD. The Five Classical Plant Hormones. The Plant Cell. 1997; 9(1):197-121.
23. George EF, Hall MA, De Klerk GJ. (Eds.). Plant Growth Regulators I: Introduction; Auxins, their Analogues and Inhibitors. Chapter 5. In: Plant Propagation by Tissue Culture. 3rd Edition, Springer. 2008, 175-204.
24. George EF, Hall MA, De Klerk GJ. (Eds.). Plant Growth Regulators II: Cytokinins, their Analogues and Antagonists. Chapter 6. In: Plant Propagation by Tissue Culture. 3rd Edition, Springer. 2008, 205-226.
25. George EF, Hall MA, De Klerk GJ. (Eds.). Plant Growth Regulators III: Gibberellins, Ethylene, Abscisic Acid, their Analogues and Inhibitors; Miscellaneous Compounds. Chapter 7. In: Plant Propagation by Tissue Culture. 3rd Edition, Springer. 2008, 227-281.
26. Mok DWS, Mok MC. Cytokinin metabolism and action. Annu. Rev. Plant Physiol. Plant Mol. Biol. 2001; 52:89-118.
27. Haberer G, Kieber JJ. Cytokinins. New Insights into a Classic Phytohormone. Plant Physiol. 2002; 128:354-362.
28. Daviére JM, Achard P. Gibberellin signaling in plants. Development. 2013; 140: 1147-1151. doi:10.1242/dev.087650
29. Hedden P. Gibberellin Biosynthesis: Enzymes, Genes and Their Regulation. Annu. Rev. Plant Physiol. Plant Mol. Biol. 1997; 48:431-60.
30. Spartz AK, Gray WM. Plant hormone receptors: new perceptions. Genes & development. 2008; 22:2139-2148.
31. Wang YH, Irving HR. Developing a model of plant hormone interactions. Plant Signal Behav. 2011; 6(4):494-500. doi: 10.4161/psb.6.4.14558
32. Kutschera U, Wang ZY. Darwin review. Brassinosteroid action in flowering plants: a Darwinian Perspective. Journal of Experimental Botany. 2012; 63(10):3511-3522. doi:10.1093/jxb/ers065
33. Creelman RA, Mullet JE. Biosynthesis and action of jasmonates in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. 1997; 48:355-381. http://www.ufv.br/dbv/pgfvg/BVE684/htms/pdfs_revisao/sinais/hormonios/BIOSYNTHESISANDACTIONOFJA.pdf
34. Ahmad P, Rasool S, Gul A, Sheikh SA, Akram NA, Ashraf M, *et al*. Jasmonates: Multifunctional Roles in Stress Tolerance. Front Plant Sci. 2016; 7:813. doi: 10.3389/fpls.2016.00813. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4908892/pdf/fpls-07-00813.pdf
35. Creelman RA, Mullet JE. Oligosaccharins, brassinolides, and jasmonates: nontraditional regulators of plant growth, development, and gene expression. Plant Cell. 1997; 9(7):1211-23.
36. Didyk NP, Blum OB. Natural antioxidants of plant origin against ozone damage of sensitive crops. Acta Physiol Plant. 2011; 33(1):25-34. doi:10.1007/s11738-010-0527-5 http://link.springer.com/article/10.1007/s11738-010-0527-5
37. Hunter DC, Burritt DJ. Polyamines of Plant Origin - An Important Dietary Consideration for Human Health. In: Phytochemicals as Nutraceuticals - Global Approaches to Their Role in Nutrition and Health, Dr. Venketeshwer Rao

(Ed.), ISBN: 978-953-51-0203-8, In Tech. 2012; 225-244. http://www.intechopen.com/books/phytochemicals-as-nutraceuticals-global-approaches-to-their-role-innutrition-and-health/polyamines-of-plant-origin-an-important-dietary-consideration-for-human-health
38. Rigal A, Ma Q, Robert S. Unraveling plant hormone signaling through the use of small molecules. Front. Plant Sci., 2014; 373:1-20. http://dx.doi.org/10.3389/fpls.2014.00373
39. Sauer M, Robert S, Kleine-Vehn J. Auxin: simply complicated. Journal of Experimental Botany. 2013; 64(9):2565-2577. doi:10.1093/jxb/ert139
40. Taylor EC, Wipf P. (Eds.). Chemistry of Heterocyclic Compounds: A series of monographs. Series Online ISSN: 1935-4665. Series DOI: 10.1002/SERIES1079. http://onlinelibrary.wiley.com/bookseries/10.1002/SERIES1079
41. Scriven EFV, Murugan R. Pyridine and Pyridine Derivatives. Kirk-Othmer Encyclopedia of Chemical Technology. John Wiley & Sons, Inc. 2005; 20:1-53. http://onlinelibrary.wiley.com/doi/10.1002/0471238961
42. Joule JA, Mills K. (Eds). Heterocyclic Chemistry at a Glance. 2nd ed. John Wiley & Sons, Ltd. 2012, 230.
43. Dai H, Li YQ, Du D, Qin X, Zhang X, Yu HB, *et al.* Synthesis and biological activities of novel pyrazole oxime derivatives containing a 2-chloro-5-thiazolyl moiety. J Agric Food Chem. 2008; 56(22):10805-10810. http://www.ncbi.nlm.nih.gov/pubmed/18959421
44. Corsi C, Wendeborn SV, Bobbio C, Kessabi J, Schneiter P, Grasso V, *et al.* Isothiazole and pyrazole derivatives for use as plant growth regulators. EP Patent 2358699A1. 2011. http://www.google.com/patents/EP2358699A1?cl=deFl
45. Nimbalkar S, Hote SV. Pyrazole Derivatives and their Synthesis - A review. International Journal on Recent and Innovation Trends in Computing and Communication. 2015; 3(2):61-65. http://www.ijritcc.org/download/ICAET15TR061499.pdf
46. Basedia DK, Dubey BK, Shrivastava B. A Review on Synthesis and Biological activity of Heterocyclic Compounds bearing 1, 3, 5-Triazine Lead Moiety. American Journal of PharmTech Research. 2011; 1(4):174-193. http://www.ajptr.com/archive/volume-1/december-2011-issue-4/article-49.html
47. Minn K, Dietrich H, Dittgen J, Feucht D, Häuser-Hahn I, Rosinger CH. Pyrimidine derivatives and their use for controlling undesired plant growth. Patent US 8329717 B2. 2008. http://www.google.com/patents/US8329717
48. Cansev A, GÜlen H, Zengin MK, Ergin S, Cansev M, Kumral NA. Use of pyrimidines in stimulation of plant growth and development and enhancement of stress tolerance. Patent 20160000075. http://www.patentsencyclopedia.com/app/20160000075
49. Zhao Q, Liu Sh, Li Yo, Wang Q. Design, Synthesis, and Biological Activities of Novel 2-Cyanoacrylates Containing Oxazole, Oxadiazole, or Quinoline Moieties J Agric. Food Chem. 2009; 57:2849-2855.
50. Newton T, Waldeck I. Oxazole carboxamide herbicides. Patent US6096688 A. 2000. https://www.google.ch/patents/US6096688
51. Miller MJ, Moraski GC, Markley LD, Davis GE. Imidazo [1,2-a] pyridine compounds, synthesis thereof, and methods of using same. Patent US. 20120220457 A1. 2012. http://www.google.ch/patents/US20120220457
52. Xiao Sh, Guo D, Zhang M, Peng Sh, Chen F, Zhou Ya, *et al.* Two novel 2,5-diphenyl oxazole derivatives from Gymnotheca chinensis Chin. Chem. Lett. 2016; 27:1064-1066.
53. Baum JS, Chen TM. Plant growth and development modification using benzoxazole derivatives. Patent US 4659360 A. 1987. http://www.google.ch/patents/US4659360
54. Chang JH, Baum JS. Phenylmethyl-4, 4-dimethyl-3-isoxazolidinone plant regulators. Patent US 4892578 A. 1990. https://www.google.ch/patents/US4892578
55. Müller KH, Feucht D, Gesing RFE, Hacker E, Hills M, Huff HP, *et al.* Application of 2-lodo-N-[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)carbamoyl] benzol sulphonamide and/or its salts for inhibiting unwanted plant growth in selected agricultural crop cultures or non-cultivated land. Patent EP2052603A1. 2009. https://www.google.co.in/patents/EP2052603A1?cl=fr
56. Sergiev I, Alexieva V, Ivanov S, Bankova V, Mapelli S. Plant Growth Regulating Activity of Some Flavonoids. Comptes Rendus de l'Academie Bulgare des Sciences. 2004; 57(4):63-68. http://articles.adsabs.harvard.edu//full/2004CRABS.57d.63S/D000063.000.html
57. Preedy VR. (Ed). Isoflavones: Chemistry, Analysis, Function and Effects. CPI Group (UK). Ltd, Croydon, CR0 4YY, UK. 2013; 683.
58. Griffiths CM, MacWilliam IC, Reynolds T. Comparative effect of gibberellins and their derivatives on germination and malting of barley. J Inst. Brew. 1967; 73:189-193.
59. Zaerr JB. Lavender DP. Analysis of plant growth substances in relation to seedling and plant growth. NZ. J For. Sci. (New Zealand Journal of Forestry Science). 1980; 10(1):186-195.
60. Hall JW, Stout DG, Brooke BM. Alfalfa seed germination tests and stand establishment: The role of hard (water impermeable) seed. Canadian Journal of Plant Science. 1998; 78(2):295-300.
61. Tian Y, Guan B, Zhou D, Yu J, Li G, Lou Y. Responses of Seed Germination, Seedling Growth, and Seed Yield Traits to Seed Pretreatment in Maize (*Zea mays* L.). The Scientific World Journal. 2014. Article ID 834630, 1-8. http://dx.doi.org/10.1155/2014/834630
62. Wen B. Effects of High Temperature and Water Stress on Seed Germination of the Invasive Species Mexican Sunflower. PLoS one. 2015; 10(10):e0141567. doi:10.1371/journal.pone.0141567. http://journals.plos.org/plosone/article/file?id=10.1371/journal.pone.0141567&type=printable
63. Tahaei A, Soleymani A, Shams M. Seed germination of medicinal plant, fennel (Foeniculum vulgare Mill), as affected by different priming techniques. Applied Biochemistry and Biotechnology. 2016; 180(1):26-40.
64. Gyulai G, Heszky LE. Auxin and cytokinin bioassays: a short overview. Acta Agronomica Hungarica. 1995; 43(1/2):185-197. http://mkk.szie.hu/dep/genetika/pdf/Gyulai%20et%20al.%201994.%20Acta%20Agr%20Hung%2043,185-197.pdf
65. Basu RN. Effect of non-auxin chemicals on translocation

of auxins in cuttings of *Phaseolus vulgaris* (L.), (kidney beans). J Exp. Bot. 1972; 23:357-365.
66. Oshkaya VP, Mutsenietse DK. Effect of indan-1, 3-dione derivatives on root formation in bean cuttings. Soviet Plant Physiol. 1973; 21(l):172-173.
67. Šimonová E, Henselová M, Zahradník P. Benzothiazole derivatives substituted in position 2 as biologically active substances with plant growth regulation activity. Plant Soil Environ. 2005; 51(11):496-505.
68. Chen CM, Leisner SM. Cytokinin-Modulated Gene Expression in Excised Pumpkin. Plant Physiol. 1985; 77:99-103. http://www.plantphysiol.org/content/77/1/99.full.pdf+html
69. Wilcox EJ, Selby C, Wain RL. The cytokinin activities of *6-α-alkylbenzyloxypurines*. Ann. Appl. Biol. 1981; 97:221-226. http://onlinelibrary.wiley.com/doi/10.1111/j.1744-7348.1981.tb03015.x/pdf
70. Frankland B, Wareing PF. Effect of gibberellic acid on hypocotyl growth of lettuce seedlings. Nature. 1960; 185:255-256. ttps://www.cabdirect.org/cabdirect/abstract/19600301559
71. Silk WK, Jones RL. Gibberellin Response in Lettuce Hypocotyl. Plant Physiol. 1975; 56:267-272. http://www.plantphysiol.org/content/56/2/267.full.pdf
72. Hendrx SD. Jones RL. The Activity of j3-Ecdysone in Four Gibberellin Bioassays. Plant Physiol. 1972; 50:199-200. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC367344/pdf/plntphys00244-0206.pdf
73. Koda Y, Haga T, Okazawa Y. A revised method of gibberellin assay by oat endosperm tissues. J Fac. Agr. Hokkaido Univ. 1979; 59(2):254-261. http://eprints.lib.hokudai.ac.jp/dspace/bitstream/2115/12925/1/59(2)_p254-261.pdf
74. Waycott W, Taiz L. Phenotypic Characterization of Lettuce Dwarf Mutants and Their Response to Applied Gibberellins. Plant Physiol. 1991; 95:1162-1168. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1077667/pdf/plntphys00817-0196.pdf
75. Bhojwani SS, Razdan MK. Plant Tissue Culture: Theory and Practice. The 1st Ed. Elsevier Science. 1996; 5:766. https://www.elsevier.com/books/plant-tissue-culture-theory-and-practice/bhojwani/978-0-444-81623-8
76. Kayser O, Quax WJ. (Eds). Medicinal Plant Biotechnology. From Basic Research to Industrial Applications. WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim. 2007, 604. http://doc.sciencenet.cn/upload/file/201137155444450.pdf
77. George EF, Hall MA, De Klerk GJ. (Eds). Plant Propagation by Tissue Culture. The Background, Springer, 2008; 1:501. http://www.springer.com/us/book/9781402050046
78. Smith RH. Plant Tissue Culture: Techniques and Experiments. Academic Press. 2013, 188. https://books.google.ie/books/about/Plant_Tissue_Culture.html?id=By8psdGWgbkC
79. Takatsuka H, Umeda M. Hormonal control of cell division and elongation along differentiation trajectories in roots. Journal of Experimental Botany. 2014; 65(10):2633-2643. doi:10.1093/jxb/ert485
80. Pop TI, Pamfil D, Bellini C. Auxin Control in the Formation of Adventitious Roots. Not Bot Hort Agrobot Cluj. 2011; 39(1):307-316.
81. Pandey A, Tamta S, Giri D. Role of auxin on adventitious root formation and subsequent growth of cutting raised plantlets of *Ginkgo biloba* L. International Journal of Biodiversity and Conservation. 2011; 3(4):142-146.
82. http://www.academicjournals.org/article/article1380547913_Pandey%20et%20al.pdf
83. Shimelis D, Bantte K, Feyissa T. Interaction Effect of Indole-3-Butyric Acid and α-Naphthalene Acetic Acid on *In Vitro* Rooting of Two Sugarcane (*Saccharum officinarum*) Genotypes. Adv Crop Sci Tech. 2015; S1:001. doi:10.4172/2329-8863.S1-001
84. Sheldrake AR. The production of hormones in higher plants. Biol. Rev. 1973; 48:509-559.
85. Gupta R, Chakrabarty SK. Gibberellic acid in plant, Plant Signaling & Behavior. 2013; 8:(9):e25504. DOI: 10.4161/psb.25504. http://dx.doi.org/10.4161/psb.25504
86. Coolbaugh RC. Sites of Gibberellin Biosynthesis in Pea Seedlings. Plant Physiol. 1985; 78:655-657.
87. Hedden P, Thomas SG. (Eds.), Plant Hormone Signaling. Blackwell Publishing Ltd. 2006, 339. http://samples.sainsburysebooks.co.uk/9781405173063_sample_387254.pdf
88. Tsygankova VA. Genetic Control and Phytohormonal Regulation of Plant Embryogenesis. Int. J. Med. Biotechnol. Genetics (IJMBG). 2015; 3(1):9-20. http://scidoc.org/articlepdfs/IJMBG/IJMBG-2379-1020-03-101.pdf
89. Shablykin OV, Kucharenko OP, Iakovenko IN, Yarmoluk SM, Brovarets VS. Search for specific protein kinase CK2 inhibitors and vasoactive compounds among 5-amino-1,3-oxazoles derivatives. Ukrainica Bioorganica Acta. 2008; 1:28-36 (In Ukr.). http://www.bioorganica.org.ua/UBAdenovo/pubs_6_1_08/Shablykin_2008_1.pdf
90. Kopernik IM, Blagodatnyj VM, Petrenko OV, Kalashnikova LE, Prokopenko VV, Kondratyuk KM, *et al*. Study in vitro for antimicrobic activity of new oxazole derivatives and products of its transformations. Ukr Bioorg Acta. 2011; 2:57-68 (In Ukr.). http://www.bioorganica.org.ua/UBAdenovo/pubs_9_2_11/Kopernik.pdf
91. Havrylyuk D, Zimenkovsky B, Vasylenko O, Gzella A, Lesyk R. Synthesis of New 4-Thiazolidinone-, Pyrazoline-, and Isatin-Based Conjugates with Promising Antitumor Activity. J Med Chem. 2012; 55(20):8630-8641. http://pubs.acs.org/doi/abs/10.1021/jm300789g
92. Zelisko N, Atamanyuk D, Vasylenko O, Grellier P, Lesyk R. Synthesis and antitrypanosonal activity of new 6,6,7-trisubstituted thiopyrano[2,3-d]-[1,3]thiazoles. Bioorg Med Chem Lett. 2012; 22:7071-7074. https://www.researchgate.net/publication/232530632_ChemInform_Abstract_Synthesis_and_Antitrypanosomal_Activity_of_New_667-Trisubstituted_Thiopyrano23-d13thiazoles
93. Havrylyuk D, Zimenkovsky B, Vasylenko A, Day GW, Smee DF, Grellier P, *et al*. Synthesis and biological activity evaluation of 5-pyrazoline substituted 4-thiazolidinones. European Journal of Medicinal Chemistry. 2013; 66:228-237. http://europepmc.org/abstract/med/23811085
94. Frasinyuk MS, Mrug GP, Bondarenko SP, Khilya VP, Brovarets VS. Antitumor activity of flavonoid Mannich bases. Ukrainica Bioorganica Acta. 2013; 2:3-7. (In Ukr.).

http://www.bioorganica.org.ua/UBAdenovo/pubs_11_2_13/Frasynyuk.pdf
95. Bezverkha IS, Panteleimonova TM, Sharabura LB, Frasyniuk MS, Khylia VP. Antidepressant effect of isoflavone 5/09 on anxious depression in male mice. Problems Aging and Longevity. 2014; 23(2):101-112. (In Ukr.). http://geront.kiev.ua/library/psid/2014-2.pdf
96. Tsygankova VA, Bayer OO, Andrusevich YAV, Galkin AP, Brovarets VS, Yemets AI, *et al.* Screening of five and six-membered nitrogen-containing heterocyclic compounds as new effective stimulants of Linum usitatissimum L.organogenesis in vitro. Int J Med Biotechnol Genetics. 2016; S2: 001: 1-9. http://scidoc.org/specialissues/IJMBG/S2/IJMBG-2379-1020-S2-001.pdf
97. Victoria Tsygankova, Yaroslav Andrusevich, Olexandra Shtompel, Artem Hurenko, Roman Solomyannyj, Galyna Mrug, *et al.* Stimulating effect of five and six-membered heterocyclic compounds on seed germination and vegetative growth of maize (*Zea mays* L.). International Journal of Biology Research. 2016; 1(4):1-14. https://www.academia.edu/29094174/Stimulating_effect_of_five_and_six-membered_heterocyclic_compounds_on_seed_germination_and_vegetative_growth_of_maize_Zea_mays_L
98. Victoria Tsygankova, Yaroslav Andrusevich, Olexandra Shtompel, Stepan Pilyo, Volodymyr Prokopenko, Andrii Kornienko, *et al.* Study of growth regulating activity derivatives of [1, 3] oxazolo [5,4-*d*] pyrimidine and n-sulfonyl substituted of 1,3- oxazoles on soybean, wheat, flax and pumpkin plants. International Journal of Chemical Studies. 2016; 4(5):106-120. http://www.chemijournal.com/archives/2016/vol4issue5/PartB/4-5-7-574.pdf
99. Gurenko AO, Khytova BM, Klyuchko SV, Vasilenko AN, Brovarets VS. Interaction of 5-chloro-1-phenyl-1H-pyrazole-4-carboxamide and 5-chloro-N-formyl-1-phenyl-1H-pyrazole-4-carboxamide with hydrazine hydrate. J Org Pharm Chem. 2014; 1(45):56-59. (In Russ.). http://nuph.edu.ua/wp-content/uploads/2015/04/ZhOFH1-14-56-59.pdf
100. Gurenko AO, Khytova BM, Klyuchko SV, Vasilenko AN, Brovarets VS. Synthesis of novel pyrazolo-[3, 4-d]-[1, 2, 3]-triazines. Chem Het Comp. 2014; 50(4):528-536. http://link.springer.com/article/10.1007/s10593-014-1503-6
101. Frasinyuk MS. Synthesis and Aminomethylation of 3-Substituted 6-Hydroxy-1,2-Benzisoxazoles. Chemistry of Heterocyclic Compounds. 2015; 50(11):1616-1623. http://cat.inist.fr/?aModele=afficheN&cpsidt=29051954
102. Frasinyuk MS, Mrug GP, Bondarenko SP, Sviripa VM, Zhang W, Cai X, *et al.* Application of Mannich bases to the synthesis of hydroxymethyl- ated isoflavonoids as potential antineoplastic agents. Org Biomol Chem. 2015; 13: 11292-11301. http://pubs.rsc.org/en/content/articlelanding/ob/2015/c5ob01828e#!divAbstract
103. Voytsehovska OV, Kapustyan AV, Kosik OI, Musienko MM, Olkhovich OP, Panyuta OO, *et al.* Plant Physiology: Praktykum. Parshikova T.V. (Ed.). Lutsk: Teren. 2010; 420. (In Ukr.). http://biol.univ.kiev.ua/metod/fbr/PRAKTYKUM.pdf
104. Bang H, Zhou XK, Van Epps HL, Mazumdar M. (Eds.). Statistical Methods in Molecular Biology. Series: Methods in molecular biology, New York: Humana press. 2010; 13(620):636. http://www.springer.com/gp/book/9781607615781
105. Oomah BD. Flax seed as a functional food source. J Sci Food Agric. 2001; 81:889-894.
106. Shim YY, Gui B, Arnison PG, Wang Y, Reaney MJT. Flaxseed (*Linum usitatissimum* L.) bioactive compounds and peptide nomenclature: A review. Trends in Food Science & Technology. 2014; 38(1):5-20. http://www.sciencedirect.com/science/article/pii/S0924224414000697
107. Hall LM, Booker H, Siloto RMP, Jhala AJ, Weselake RJ. Chapter 6. Flax (*Linum usitatissimum* L.). In: Industrial Oil Crops, First Edition. 2016, 157-194. http://agronomy.unl.edu/Jhala/publications/Book%20Chapter-Flax-Hall_2016.pdf
108. Czemplik M, Boba A, Kostyn K, Kulma A, Mituła A, Sztajnert M, *et al.* and Skórkowska-Telichowska K. Flax Engineering for Biomedical Application. In: Biomedical Engineering, Trends, Research and Technologies. S. Olsztynska (Ed.). InTech Publisher. 2011, 644. http://cdn.intechopen.com/pdfs/12834/InTech-Flax_engineering_for_biomedical_application.pdf
109. Janowicz J, Niemann J, Wojciechowski A. The effect of growth regulators on the regeneration ability of flax (*Linum usitatissimum* L.) hypocotyl explants in *in vitro* culture. BioTechnologia. 2012; 93(2):135-138.