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Studying the Effect of Plant Growth Regulators Ivin, Methyur and Kamethur on Growth and Productivity of Sunflower

Tsygankova V.A.*, Voloshchuk I.V., Kopich V.M., Pilyo S.G., Klyuchko S. V., Brovarets V.S.
Department for Chemistry of Bioactive Nitrogen-Containing Heterocyclic Compounds, V.P. Kukhar Institute of Bioorganic Chemistry and Petrochemistry, National Academy of Sciences of Ukraine, 1, Academician Kukhar str., 02094, Kyiv-94, Ukraine.
*Corresponding author: Tsygankova Victoria Anatolyivna, Dr. Biol. Sci., Principal researcher, Senior Staff Scientist, e-mail: vTsygankova@ukr.net

Abstract.
In field conditions, the effect of plant growth regulators Ivin (N-oxide-2,6-dimethylpyridine), Methyur (sodium salt of 6-methyl-2-mercapto-4-hydroxypyrimidine), Kamethur (potassium salt of 6-methyl-2-mercapto-4-hydroxypyrimidine) on growth and productivity of sunflower (*Helianthus annuus* L.) variety Bastion was studied. The growth-regulating activity of plant growth regulators Ivin, Methyur and Kamethur was compared with the growth-regulating activity of the auxin IAA (1*H*-Indol-3-yl)acetic acid). It was found that the treatment of seeds before planting in the soil with water solutions of Ivin, Methyur and Kamethur, used at a concentration of 10^{-7}M, contributes to an increase in morphological parameters (length of shoot and root, fresh weight of plant and basket) and biochemical parameters (content of chlorophylls a and b, and carotenoids) of sunflower (*Helianthus annuus* L.) variety Bastion grown in field conditions for 3 months. The growth-regulating activity of plant growth regulators Ivin, Methyur and Kamethur was higher than the growth-regulating activity of the auxin IAA used at the same concentration of 10^{-7}M. The obtained results confirmed the possibility of using plant growth regulators Ivin, Methyur and Kamethur to increase the length of shoot and root, fresh weight of the plant and basket, and the content of chlorophylls in sunflower (*Helianthus annuus* L.) variety Bastion.

Keywords: sunflower, growth, productivity, Ivin, Methyur, Kamethur, IAA.

1. Introduction

Sunflower (*Helianthus annuus* L.) is one of the most important agricultural crops that are widely grown in the world [1]. Sunflower oil obtained from its seeds is among the five most consumed oils in the world [2]. Sunflower oil, seeds, shoots and roots are a source of biologically active compounds that have anti-inflammatory, antipyretic, laxative, diuretic, expectorant, antidiabetic, anthelmintic and other medicinal properties, yellow pigments from the petals are used as dyes in the cosmetic industry [3 - 9].

Global climate change and environmental pollution are the most adverse environmental factors affecting sunflower growth and productivity. Today, plant growth regulators of natural or synthetic origin are widely used in the agricultural industry to improve the growth and yield of sunflower [10 - 12]. However, the creation of new effective plant growth regulators capable of increasing sunflower productivity and resistance to stress factors is a very urgent task.

Currently, new effective and environmentally friendly plant growth regulators Ivin (N-oxide-2,6-dimethylpyridine), Methyur (sodium salt of 6-methyl-2-mercapto-4-hydroxypyrimidine), Kamethur (potassium salt of 6-methyl-2-mercapto-4-hydroxypyrimidine) are developed in the V.P. Kukhar Institute of Bioorganic Chemistry and Petrochemistry of National Academy of Sciences of Ukraine. These synthetic plant growth regulators are used in low, non-toxic to human and animal health concentrations in the range from 10^{-5}M to 10^{-8}M to increase productivity and adapt important agricultural crops to adverse environmental factors [13 – 20].

The use of these plant growth regulators makes it possible to reduce or eliminate the negative impact of chemical pesticides, herbicides and fungicides used to protect plant that are unsafe for human health, animals and the environment [17, 21 - 23].

The purpose of this work is to study the effect of plant growth regulators Ivin, Methyur and Kamethur on the growth and productivity of sunflower (*Helianthus annuus* L.) variety Bastion.

2. Materials and methods

2.1. Chemical compounds studied in the experiment

Plant growth regulators Ivin (N-oxide-2,6-dimethylpyridine), Methyur (sodium salt of 6-methyl-2-mercapto-4-hydroxypyrimidine), Kamethur (potassium salt of 6-methyl-2-mercapto-4-hydroxypyrimidine)

were synthesized in the Department for Chemistry of Bioactive Nitrogen-Containing Heterocyclic Compounds, V. P. Kukchar Institute of Bioorganic Chemistry and Petrochemistry of the National Academy of Sciences of Ukraine. Their growth-regulating activity was compared with the growth-regulating activity of the phytohormone auxin IAA (1*H*-Indol-3-yl)acetic acid) manufactured by Sigma-Aldrich, USA (Fig. 1).

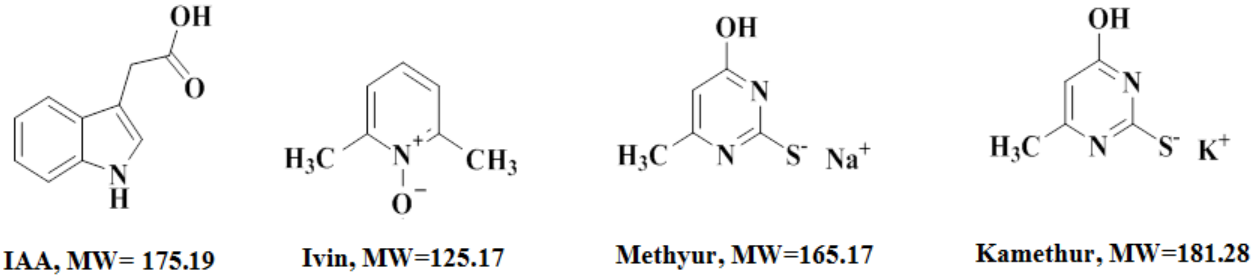

Figure 1. Chemical structure and relative molecular mass of IAA, Ivin, Methyur and Kamethur.

2.2. Sunflower growing conditions

Field experiments were carried out on sunflower (*Helianthus annuus* L.) variety Bastion. Before sowing, seeds were superficially sterilized with 1% $KMnO_4$ solution for 15 min., washed three times with sterile distilled water, and then treated with distilled water (control) or water solutions of auxin IAA or plant growth regulators: Ivin, Methyur, or Kamethur at a concentration of 10^{-7}M for 24 hours. After this procedure, the treated seeds were dried and planted in the soil where sunflower plants were grown for 3 month.

2.3. Determination of morphological parameters of sunflower plants

Comparative analysis of morphological parameters of sunflower plants grown for 3 month in field conditions (average length of shoot and root (in cm), average fresh weight of plant and basket (in gram)) was carried out according to the guidelines [24]. The morphological parameters of sunflower plants (in %) were calculated as the ratio between the morphological parameters of experimental plants obtained from seeds treated with water solution of auxin IAA or plant growth regulators: Ivin, Methyur or Kamethur at a concentration of 10^{-7}M and morphological parameters of control plants obtained from seeds treated with distilled water.

2.4. Determination of biochemical parameters of sunflower plants

Comparative analysis of biochemical parameters of sunflower plants grown for 3 month in field conditions (content of chlorophyll a, chlorophyll b, and carotenoids) was carried out according to the guidelines [25, 26]. To carry out the extraction of photosynthetic pigments from sunflower leaves we homogenized a sample (500 mg) of sunflower leaves in the porcelain mortar in a cooled at the temperature 10 °C 96 % ethanol at the ratio of 1: 10 (weight: volume) with addition of 0,1-0,2 g CaCO3 (to neutralize the plant acids). The 1 ml of obtained homogenate was centrifuged at 8000 g in a refrigerated centrifuge K24D (MLW, Engelsdorf, Germany) during 5 min at the temperature 4 °C. The obtained precipitate was washed three times, with 1 ml 96 % ethanol and centrifuged at above mentioned conditions. After this procedure, the optical density of chlorophyll a, chlorophyll b and carotenoids in the obtained extract was measured using spectrophotometer Specord M-40 (Carl Zeiss, Germany). The content of chlorophyll a, chlorophyll b, and carotenoids was calculated in accordance with formula [25, 26]:

Cchl a = 13.36×A664.2 – 5.19×A648.6,
Cchl b = 27.43×A648.6 – 8.12A×664.2,
Cchl (a + b) = 5.24×A664.2 + 22.24×A648.6,
Ccar = (1000×A470 – 2.13×Cchl a – 97.64×Cchlb)/209,

Where,
Cchl a – concentration of chlorophyll a (µg/ml), Cchl b – concentration of chlorophyll b (µg/ml), Cchl (a+b) – concentration of chlorophylls a and b (µg/ml), Ccar – concentration of carotenoids (µg/ml), A – absorbance value at a proper wavelength in nm.

The chlorophyll and carotenoids content per 1 g of fresh weight (FW) of extracted from plant leaves was calculated by the following formula (separately for chlorophyll a, chlorophyll b and carotenoids):

$A_1=(C\times V)/(1000\times a_1)$,
Where, A_1 – content of chlorophyll a, chlorophyll b, or carotenoids (mg/g FW),
C - concentration of pigments (µg/ml),
V - volume of extract (ml),
a_1 - sample of plant tissue (g).

The content of chlorophyll a, chlorophyll b, and carotenoids (in %) was calculated as the ratio between the biochemical parameters of experimental sunflower plants obtained from seeds treated with water solution of auxin IAA or plant growth regulators: Ivin, Methyur or Kamethur at a concentration of 10^{-7}M and similar parameters of control plants obtained from seeds treated with distilled water.

2.5. 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 $P\leq0.05$, the values are mean ± SD [27].

3. Results and Discussion

3.1. Effect of Ivin, Methyur, Kamethur on morphological parameters of sunflower plants

The results of field studies showed that the morphological parameters of experimental sunflower plants exceeded those of control plants obtained from seeds treated with distilled water. The average length of shoot increased: by 8,2603 % - in plants obtained from seeds treated with Ivin, by 6,383 % - in plants obtained from seeds treated with Methyur, by 8,1352 % - in plants obtained from seeds treated with Kamethur, compared to the control plants (Fig. 2).

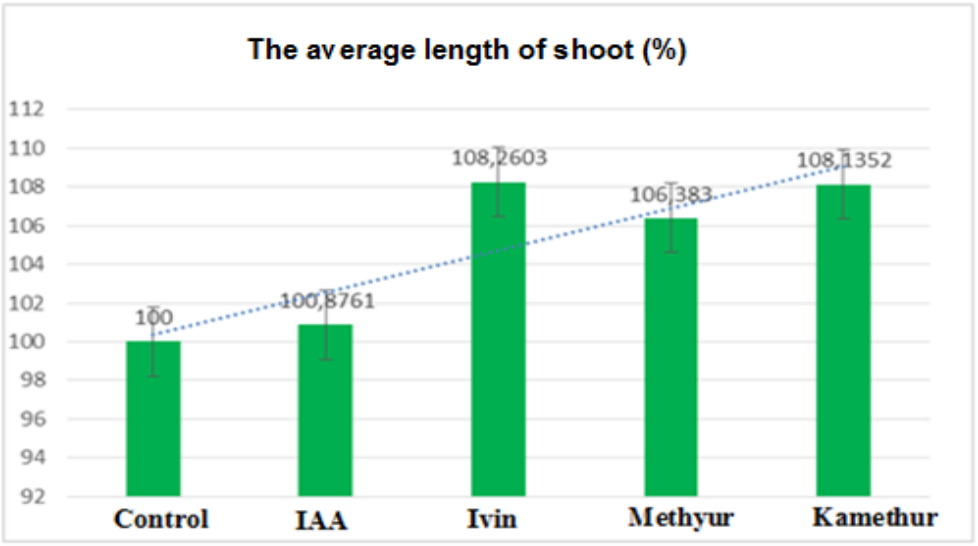

Figure 2. The average length of shoot (%) of control and experimental sunflower (*Helianthus annuus* L.) plants variety Bastion grown for 3 month in field conditions

The average length of root increased: by 3,2231 % - in plants obtained from seeds treated with Ivin, by 20,5638 % - in plants obtained from seeds treated with Methyur, by 9,259 % - in plants obtained from seeds treated with Kamethur, compared to the control plants (Fig. 3).

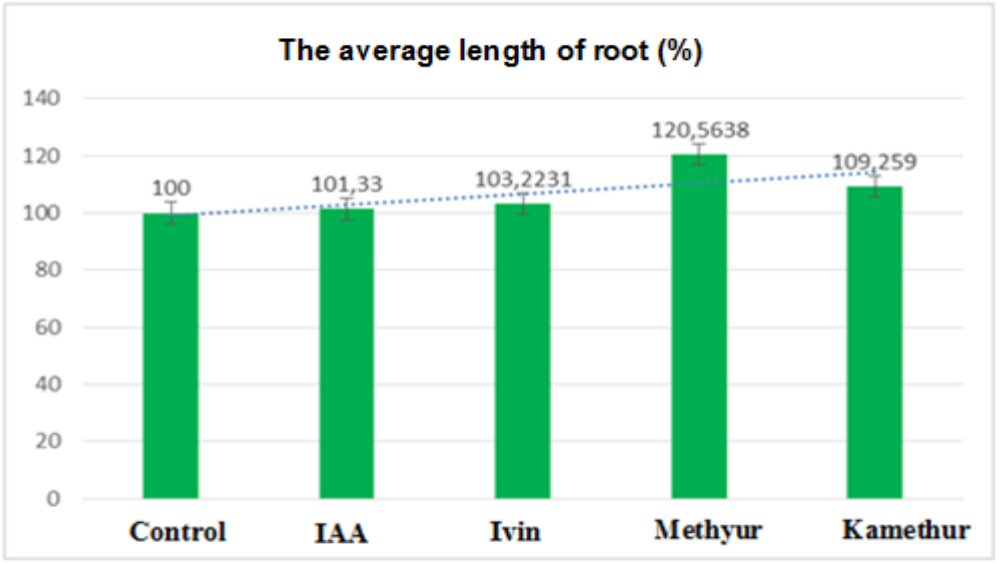

Figure 3. The average length of root (%) of control and experimental sunflower (*Helianthus annuus* L.) plants variety Bastion grown for 3 month in field conditions

The average fresh weight of plant increased: by 124,1379 % - in plants obtained from seeds treated with Ivin, by 89,6552 % - in plants obtained from seeds treated with Methyur, by 97,0443 % - in plants obtained from seeds treated with Kamethur, compared to the control plants (Fig. 4).

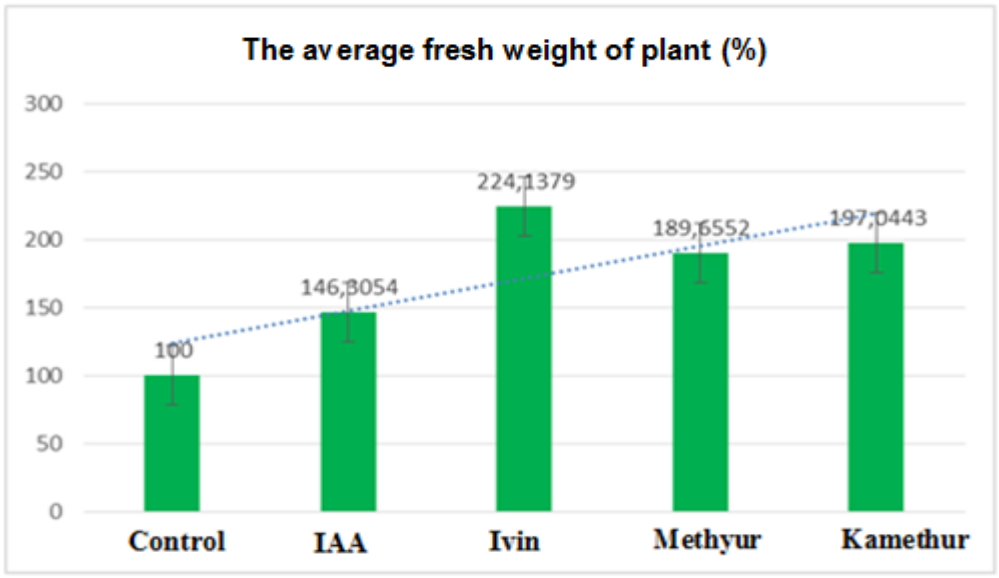

Figure 4. The average fresh weight of plant (%) of control and experimental sunflower (*Helianthus annuus* L.) plants variety Bastion grown for 3 month in field condition

The average fresh weight of basket increased: by 90,678 % - in plants obtained from seeds treated with Ivin, by 83,8983 % - in plants obtained from seeds treated with Methyur, by 72,8814 % - in plants obtained from seeds treated with Kamethur, compared to the control plants (Fig. 5).

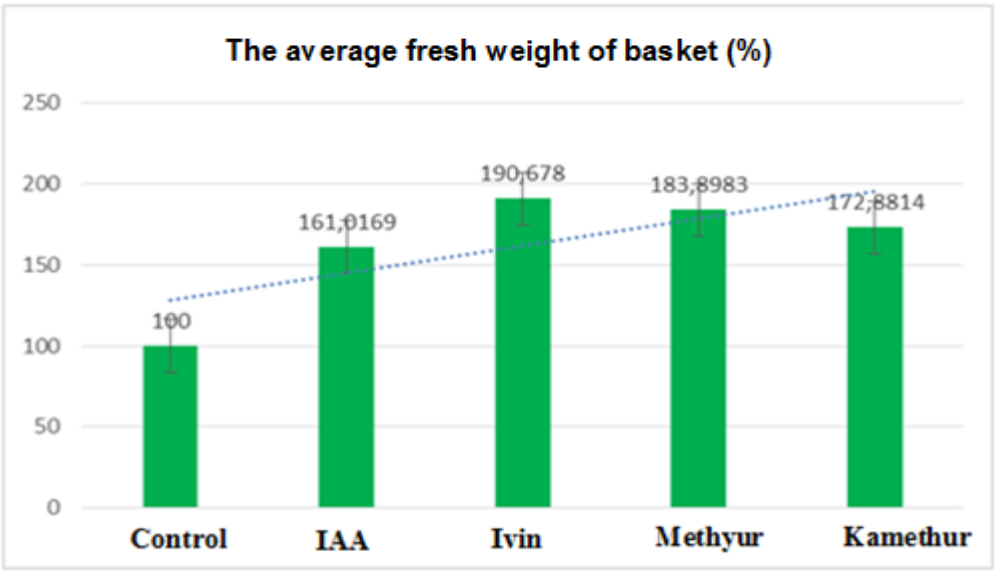

Figure 5. The average fresh weight of basket (%) of control and experimental sunflower (*Helianthus annuus* L.) plants variety Bastion grown for 3 month in field condition

A comparative analysis showed also that the morphological parameters of experimental sunflower plants exceeded those of plants obtained from seeds treated with IAA. The average length of shoot increased by 0,8761 %, the average length of root increased by 1,33 %, the average fresh weight of plant increased by 46,3054 %, the average fresh weight of baskets by 61,0169 % - in plants obtained from seeds treated with IAA, compared to the control plants (Fig. 2, 3, 4 and 5).

It is possible to assume that the high growth-regulating activity of Ivin, Methyur and Kamethur is explained by their specific auxin-like stimulating effect on the proliferation, elongation and differentiation of plant cells, which are the main processes of the formation and development of plant shoots and roots, as well on the biosynthesis, metabolism and signaling of endogenous auxins in plant cells [28 - 32].

Molecular mechanisms of action of new plant growth regulators based on synthetic low molecular weight heterocyclic compounds directly, through the network of signaling pathways of plant hormones, or indirectly, that is, affecting the biosynthesis, metabolism and signaling of endogenous auxins and cytokinins in plant cells, were discussed in our previously published works [17, 31, 32].

3.2. Effect of Ivin, Methyur, Kamethur on biochemical parameters of sunflower plants

The results of field studies showed that the main indicator of plant productivity - content of chlorophylls in the leaves of experimental sunflower plants exceeded those of control plants obtained from seeds treated with distilled water. The content of chlorophyll a increased by 11,1659 % - in plants obtained from seeds treated with Kamethur, compared to the control plants (Fig. 6).

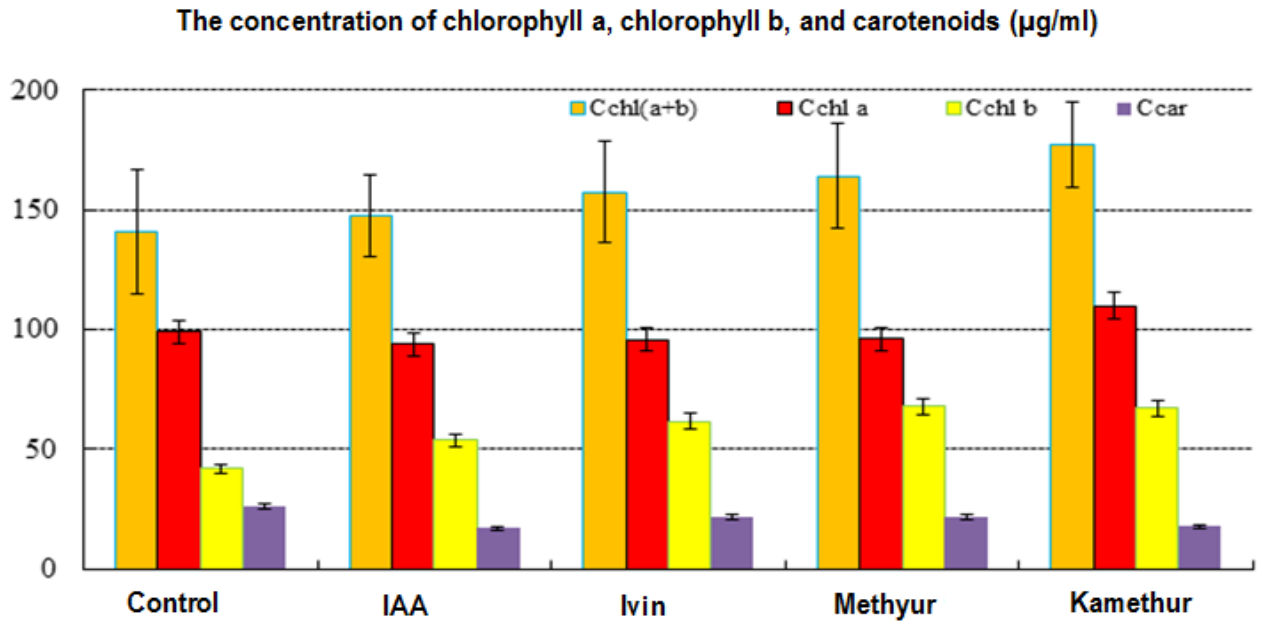

Figure 6. The concentration of chlorophylls and carotenoids (µg/ml) in the leaves of control and experimental sunflower (*Helianthus annuus* L.) plants variety Bastion grown for 3 month in field conditions: Cchl (a+b) – concentration of chlorophylls a and b (µg/ml), Cchl a – concentration of chlorophyll a (µg/ml), Cchl b – concentration of chlorophyll b (µg/ml), Ccar – concentration of carotenoids (µg/ml)

The content of chlorophyll b increased: by 47,7819 % - in plants obtained from seeds treated with Ivin, by 62,1236 % - in plants obtained from seeds treated with Methyur, by 60,5887 % - in plants obtained from seeds treated with Kamethur, compared to the control plants (Fig. 6).

The content of chlorophylls a+b increased: by 11,8394 % - in plants obtained from seeds treated with Ivin, by 16,468 % - in plants obtained from seeds treated with Methyur, by 25,841 % - in plants obtained from seeds treated with Kamethur, compared to the control plants (Fig. 6).

A comparative analysis showed also that the biochemical parameters (content of chlorophylls and carotenoids) in the leaves of experimental sunflower plants exceeded those of plants obtained from seeds treated with IAA, the content of chlorophyll b increased by 28,2853 %, the content of chlorophylls a+b increased by 4,7939 % - in plants obtained from seeds treated with IAA, compared to the control plants (Fig. 6).

A decrease in content of chlorophyll a was observed in plants obtained from seeds treated with IAA, Ivin and Methyur, and decrease in content of carotenoids was observed in plants obtained from seeds treated with IAA, Ivin, Methyur and Kamethur, compared to the control (Fig. 6). It is possible that the decrease in the concentration of chlorophyll a and carotenoids is associated with a significant increase in the fresh weight of plant.

4. Conclusion

Thus, the results obtained indicate the possibility of using plant growth regulators Ivin, Methyur or Kamethur in a concentration of 10^{-7}M for the pre-sowing treatment of sunflower (*Helianthus annuus* L.) seeds variety Bastion to increase the length of shoot and root in the vegetative phase, as well as to increase the main indicators of plant productivity - the fresh weight of the plant and basket, as well as the content of chlorophylls b and a+b in the leaves of sunflower (*Helianthus annuus* L.) variety Bastion.

5. Conflict of Interest

The authors declare no conflict of interest.

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