

Research Article

Protective Effect Of Synthetic Azaheterocyclic Compounds, Pyrimidine Derivatives On Maize Growth Under Heat And Drought.

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Abstract

The regulatory effect of synthetic low-molecular-weight azaheterocyclic compounds, pyrimidine derivatives on the growth of maize (*Zea mays* L.) variety Twist during the vegetation phase under heat and drought stress conditions was studied. The average parameters of maize plants grown in a solution of synthetic compounds, pyrimidine derivatives at a concentration of 10^{-6} M (i.e., length of the shoots (mm), length of the roots (mm), number of the roots (pcs), biomass of 10 plants (g), content of chlorophylls and carotenoids (mg/g fresh weight), total soluble protein (g/100 g FW), and catalase activity (mmol of decomposed H_2O_2 /min per 1 mg of protein) in plant leaves), were measured at the end of the 4-week period and compared with similar parameters of maize plants grown in a solution of auxin IAA at the same concentration of 10^{-6} M, or maize plants grown in distilled water (control). The conducted study showed that the regulatory effect of pyrimidine derivatives on the parameters of maize plants is similar to or exceeds the regulatory effect of auxin IAA and is differentiated depending on their chemical structure. The most active synthetic compounds, pyrimidine derivatives, have been identified that improve the growth and development of maize plants, increase the content of chlorophylls, carotenoids, total soluble protein and catalase activity in the leaves of maize plants growing under conditions of heat and drought stress. The use of the synthetic compounds, pyrimidine derivatives, in agricultural practice is proposed to improve the growth and development of maize, increase productivity and tolerance to heat and drought stress.

Keywords : Maize, Auxins and Cytokinins, Pyrimidine Derivatives, Heat and Drought Stress.

INTRODUCTION

Improving crop cultivation in the context of global climate change, which negatively affects plant growth and leads to loss of yield, is a priority task for modern agriculture [1, 2]. The problem of growing one of the important grain and oil crops, such as maize (*Zea mays* L.), whose seeds and oil are traditionally used as a source for the dietary food and pharmaceutical industries due to their high content of nutrients (proteins, vitamins, lipids, dietary fiber, carbohydrates, etc.), as well as a raw material for the biofuel industry and animal feed, is quite relevant [3-7].

As is known, auxins and cytokinins play a key role among different classes of phytohormones in regulating plant growth

and development during ontogenesis, starting from seed germination and subsequent organogenesis of roots, shoots, flowers, fruits and seeds, controlling photosynthesis in leaves, and plant adaptation to abiotic and biotic stresses [8 - 15]. In agriculture, phytohormones auxins and cytokinins are widely used to improve maize growth, increase photosynthesis in plant leaves, increase maize productivity and resistance to biotic and abiotic stresses, among which heat and drought are the most negative for maize yields [15 - 20].

Heat and drought are the most important abiotic stresses, characterized by extreme temperatures and water deficits that disrupt morphophysiological and metabolic processes in plants, inhibit root growth and nutrient uptake, inhibit respiration and photosynthesis, reduce chlorophyll and

water content in plant leaves, and disrupt the stability of cell membranes, which leads to reduced plant productivity [19 - 23]. Heat and drought induce oxidative stress, which promotes the formation of reactive oxygen species (ROS), including superoxide radical ($O_2^{\cdot-}$), singlet oxygen (1O_2), hydroxyl radical ($\cdot OH$), peroxy radical ($ROO\cdot$), alkoxy radical ($RO\cdot$) and hydrogen peroxide (H_2O_2), leading to autocatalytic peroxidation of membrane lipids, causing loss of membrane semipermeability and altered functionality [21 - 25].

Plants have developed an antioxidant detoxifying/scavenging enzyme system and several non-enzymatic antioxidants that prevent ROS damage and counteract the toxic and destructive effects of ROS, which cause oxidation of biomolecules (lipids, carbohydrates, proteins, enzymes, DNA) and lead to plant death [21 - 25]. The enzymatic antioxidant system includes superoxide dismutase (SOD), which converts $O_2^{\cdot-}$ to H_2O_2 , catalase (CAT), ascorbate peroxidase (APX), glutathione peroxidase (GPX), guaiacol peroxidase (POD), and peroxiredoxin (Prx), which then convert H_2O_2 into H_2O and O_2 molecules, as well as other antioxidant enzymes such as monodehydroascorbate reductase (MDAR), dehydroascorbate reductase (DHAR), glutathione reductase (GR), peroxidase (POX), alternative oxidase (AOX), glutathione S-transferase (GST), which are involved in maintaining redox homeostasis either by directly scavenging particular ROS and ROS-byproducts or by replenishing antioxidant reserves; while non-enzymatic antioxidants include vitamins, flavonoids, stilbenes, carotenoids, ascorbate (AsA), and glutathione (GSH), which work in concert with antioxidant enzymes to maintain intracellular steady-state level of ROS, suppress excess ROS, thereby providing protection against oxidative stress and promoting plant growth, development, cell cycles and hormone signaling, and reinforces the responses to abiotic and biotic environmental stressors [21 - 25]. However, heat and drought reduce the activity of antioxidant enzymes, so stimulation of antioxidant enzymatic systems using phytohormones auxins and cytokinins may be one of the important defense mechanisms to avoid abiotic damage to plants [26 - 29]. A large number of regulatory and functional stress-associated proteins, such as protein kinases, phosphatases, transcription factors, late embryogenesis abundant proteins, dehydrins, osmotins, aquaporins, and heat shock proteins, are also involved in sensing, signaling and defense of plant cells in response to drought and heat stress in various crop plants [30, 31].

In recent years, the most promising issue for the agricultural industry is the development of new environmentally friendly plant growth regulators based on synthetic low-molecular-weight azaheterocyclic compounds capable of exerting an effect on plant growth and development similar to the phytohormones auxins and cytokinins [32, 33]. New promising plant growth regulators are based on synthetic low-

molecular-weight azaheterocyclic compounds, derivatives of pyridine and pyrimidine, among which the most famous representatives are Ivin, Methyur and Kamethur, which improve the growth and yield of various agricultural crops, and also increase their adaptation to abiotic stresses such as salt and osmotic stress, as well as soil pollution with toxic trace elements [34 - 42]. Based on the data from previous studies, the greatest interest is the possibility of using synthetic low-molecular-weight azaheterocyclic compounds to improve maize growth and enhance adaptation to abiotic stress.

The aim of this work is to study the regulatory effect of synthetic low-molecular-weight azaheterocyclic compounds, pyrimidine derivatives, on maize growth during the vegetation phase under heat and drought stress conditions.

MATERIALS AND METHODS

Plant treatment and growing conditions

The seeds of maize (*Zea. mays* L.) variety Twist were sterilized with 1 % $KMnO_4$ solution for 10-15 min, then treated with 96 % ethanol solution for 1 min, after which they were washed three times with sterile distilled water. The sterilized seeds were then placed in the plastic cuvettes each containing 20-25 seeds on the perlite moistened with solutions of auxin IAA (1H-indol-3-yl)acetic acid or synthetic compounds, derivatives of sodium and potassium salts of 6-methyl-2-mercapto-4-hydroxypyrimidine (Methyur and Kamethur) or pyrimidine (compounds № 1-7) at a concentration of $10^{-6}M$. Seed germination was carried out in a thermostat in the dark at a temperature of 20-22°C for 48 hours. Seedling cultivation was carried out in a climate chamber, in which the plants were grown for 4 weeks under a light intensity of 3000 lux, a light/dark regime of 16/8 hours, and under conditions of abiotic stress factors: heat (at an increased temperature to 35°C) and drought (with reduced watering by 50%). Control maize plants were germinated from seeds moistened with distilled water and grown under similar conditions of abiotic stress factors: heat and drought.

Comparative analysis of average plant growth parameters (length of the shoots (mm), length of the roots (mm), number of the roots (pcs), and biomass of 10 plants (g)) was performed according to the methodical manual [43]. Plant growth parameters determined at the end of the 4-week period on experimental plants, compared with similar parameters of control plants, were expressed in %.

Extraction of chlorophylls and carotenoids and determination of their content

To extract photosynthetic pigments (chlorophylls and carotenoids) from plant leaves, we homogenized the sample (500 mg) of 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 carotenoid in the obtained extract was measured using spectrophotometer Specord M-40 (Carl Zeiss, Germany).

The content of chlorophyll a, chlorophyll b, and carotenoids (mg/g fresh weight) in plant leaves was calculated in accordance with formula [44, 45]:

$$Cchl\ a = 13.36 \times A_{664.2} - 5.19 \times A_{648.6},$$

$$Cchl\ b = 27.43 \times A_{648.6} - 8.12 \times A_{664.2},$$

$$Cchl\ (a + b) = 5.24 \times A_{664.2} + 22.24 \times A_{648.6},$$

$$Ccar = (1000 \times A_{470} - 2.13 \times Cchl\ a - 97.64 \times Cchl\ b) / 209,$$

Where, Cchl – concentration of chlorophylls (µg/ml), Cchl a – concentration of chlorophyll a (µg/ml), Cchl b – concentration of chlorophyll 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 of extracted from 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, A₁ – content of chlorophyll a, chlorophyll b, or carotenoids (mg/g FW), C - concentration of pigments (µg/ml), V - volume of extract (ml), a₁ - sample of leaves (g). The ratio of chlorophyll and carotenoid content between experimental and control plants was expressed in %.

Determination of total soluble protein content

The content of total soluble protein (g/100 g FW) in plant leaves was determined by the Bradford technique [46]. To make the plant extracts, a sample (100 mg) of plant leaves was homogenized in a porcelain mortar in a 0.1 M sodium phosphate buffer (pH 6.0–8.0) at a weight-to-volume ratio of 1:5 at 4 °C for 1 h. The resulting homogenates were centrifuged at 8000 g in a refrigerated centrifuge K24D (manufactured by MLW, Engelsdorf, Germany) at 4°C for 15 min. A volume of 1.5 mL of distilled water and 1.5 mL of Coomassie Brilliant Blue G-250 reagent (manufactured by Bio-Rad, 500-0006) were added to 50 mL of the obtained supernatant. The resulting mixture was stirred for 10 min. The optical density (OD) of total soluble protein was then determined using spectrophotometer Specord M-40 at a wavelength of 595 nm. The total soluble protein content (g of protein per 100 g of fresh weight (FW) of plant material) in the sample was

quantified using a calibration curve based on the optical density (OD) measurements of standard samples containing 1.5 mL of bovine serum albumin (BSA) solution and 1.5 mL of Coomassie Brilliant Blue G-250 reagent (manufactured by Bio-Rad, 500-0006). The total soluble protein content in the leaves of experimental plants was calculated relative to that of the control plants and expressed in %.

Determination of catalase activity

The study of catalase activity in plant leaves was carried out by the spectrophotometric method [47], the principle of which is based on the ability of hydrogen peroxide to form a stable colored complex with molybdenum salts. For this purpose, a cell-free extract was obtained from plant leaves, which was prepared by grinding a sample (100 mg of plant leaves) in a porcelain mortar with the addition of 0.1 M sodium phosphate buffer (pH 7.0) in a ratio of 1:5 (weight:volume) at a temperature of 25 °C for 1 h. The obtained homogenates were centrifuged at 8000 g in a refrigerated centrifuge K24D (MLW, Engelsdorf, Germany) at 4°C for 15 min. The supernatant was used for analysis. Then, 2 ml of 0.03% H₂O₂ solution was added to 0.1 ml of the cell-free extract supernatant. In the control sample, the same amount of distilled water was added instead of the cell-free extract. The reaction was stopped after 10 min by adding 1 ml of 4% ammonium molybdate ((NH₄)₆Mo₇O₂₄·4H₂O). The color intensity was measured using spectrophotometer Specord M-40 at a wavelength of 410 nm, relative to a control sample, in which 2 ml of distilled water was added instead of H₂O₂.

Catalase activity was calculated by the formula:

$$A = (E_{\text{contr.}} - E_{\text{exp.}}) \cdot 146,04 / (t \cdot V),$$

where

A - catalase activity (µmol/min·ml);

E_{contr.} and E_{exp.} - absorbance of the control and experimental samples, respectively;

t - incubation time (10 min);

V - volume of the added sample (0.1 ml);

146.04 - conversion factor for catalase activity in µmol.

Catalase activity was expressed in mmol of decomposed H₂O₂/min per 1mg of protein.

The ratio of catalase activity determined in the leaves of experimental plants to the indicators of control plants was expressed in %.

Statistical processing of the experimental data

Each experiment was performed three times. Statistical processing of the experimental data was carried out using Student's t-test with a significance level of P ≤ 0.05; mean values ± standard deviation (± SD) [48].

RESULTS AND DISCUSSION

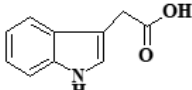
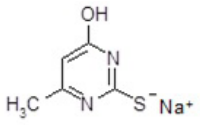
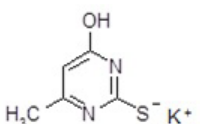
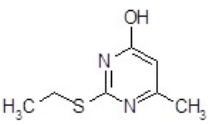
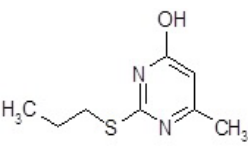
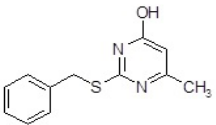
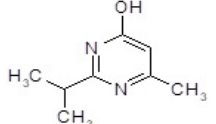
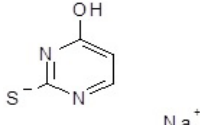
Study of the regulatory effect of pyrimidine derivatives on maize growth under conditions of heat and drought stress

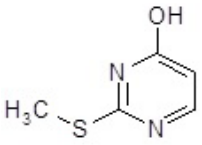
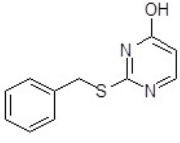
A screening of auxin- and cytokinin-like synthetic compounds among derivatives of 6-methyl-2-mercapto-4-hydroxypyrimidine sodium and potassium salts (Methyur and Kamethur) and pyrimidine derivatives (compounds № 1-7) was carried out for their regulatory effect on the growth of an important grain and oilseed crop - maize (*Zea mays* L.) variety Twist during the vegetative phase under conditions of heat and drought stress.

The plant growth-regulatory effect of derivatives of 6-methyl-2-mercapto-4-hydroxypyrimidine sodium and potassium salts (Methyur and Kamethur) and pyrimidine (compounds № 1-7), applied at a concentration of 10^{-6} M, was compared with the effect of the auxin IAA (1H-indol-3-yl)acetic acid, applied at a similar concentration of 10^{-6} M.

The chemical structure and relative molecular weight of auxin IAA (1H-indol-3-yl)acetic acid and synthetic compounds, derivatives of sodium or potassium salt of 6-methyl-2-mercapto-4-hydroxypyrimidine (Methyur and Kamethur) and pyrimidine derivatives (compounds № 1-7) are presented in **Table 1**.

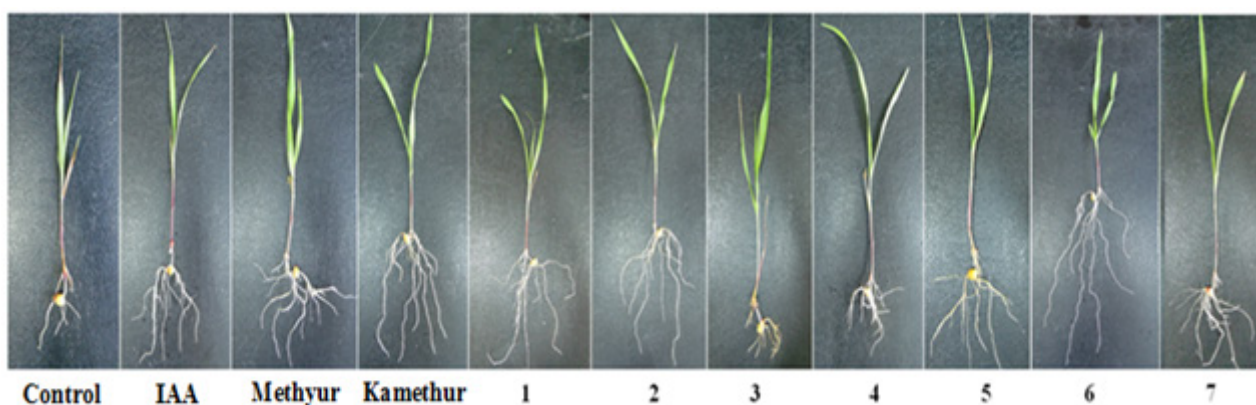
Table 1. Chemical structure and relative molecular weight of the studied compounds.

Chemical compound	Chemical structure	Chemical name and relative molecular weight (g/mol)
IAA		1H-indol-3-ylacetic acid MW=175.19
Methyur		Sodium salt of 6-methyl-2-mercapto-4-hydroxypyrimidine MW=165.17
Kamethur		Potassium salt of 6-methyl-2-mercapto-4-hydroxypyrimidine MW=181.28
1		2-ethylsulfanyl-6-methylpyrimidin-4-ol MW=170.23
2		6-methyl-2-propylsulfanyl-pyrimidin-4-ol MW=184.26
3		2-benzylsulfanyl-6-methylpyrimidin-4-ol MW=232.31
4		2-isopropyl-6-methyl-pyrimidin-4-ol MW=152.20
5		Sodium salt of 4-hydroxypyrimidine--2-thiolate MW=149.14

6		2-methylsulfanylpyrimidin-4-ol MW=142.18
7		2-benzylsulfanylpyrimidin-4-ol MW=218.28

The conducted studies showed that synthetic compounds, derivatives of 6-methyl-2-mercapto-4-hydroxypyrimidine sodium and potassium salts (Methyur and Kamethur) and pyrimidine derivatives (compounds № 1-7), applied at a concentration of 10^{-6} M, exhibit a growth-regulatory effect similar or higher than the effect of auxin IAA on the formation and development of roots and shoots of maize plants during the vegetative phase under conditions of heat and drought stress (**Figure 1**).

Figure 1. Regulatory effect of auxin IAA, synthetic compounds Methyur, Kamethur and pyrimidine derivatives (№ 1-7) at a concentration of 10^{-6} M on the growth of shoots and roots of 4-week-old maize (*Zea mays* L.) variety Twist under conditions of heat and drought stress, compared to control maize plants.

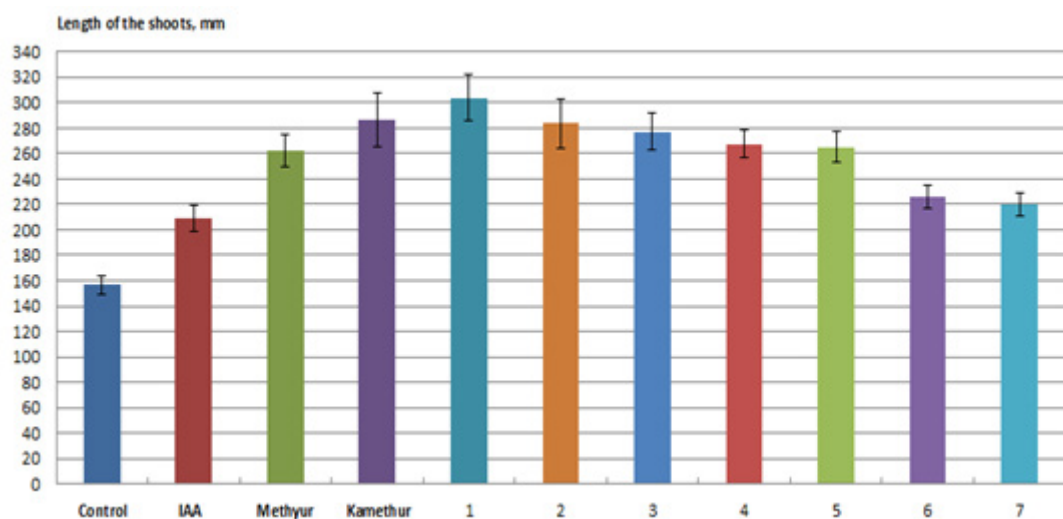


The average maize growth parameters (length of the shoots (mm), length of the roots (mm), number of the roots (pcs), biomass of 10 plants (g)), measured on the 4th week of plant growth on solutions with auxin IAA and synthetic compounds Methyur, Kamethur and pyrimidine derivatives (№ 1-7) at a concentration of 10^{-6} M, exceeded similar indicators of control maize plants grown on distilled water (**Figures 2 - 5**).

The highest regulatory effect on the length of the shoots (mm) was found in synthetic compounds Methyur, Kamethur and pyrimidine derivatives № 1-5, under the influence of which these indicators increased: by 67.87% - under the influence of Methyur, by 82.96% - under the influence of Kamethur, by 69.37-94.29% - under the influence of compounds № 1-5, respectively, compared to the same indicator in the control maize plants (**Figure 2**).

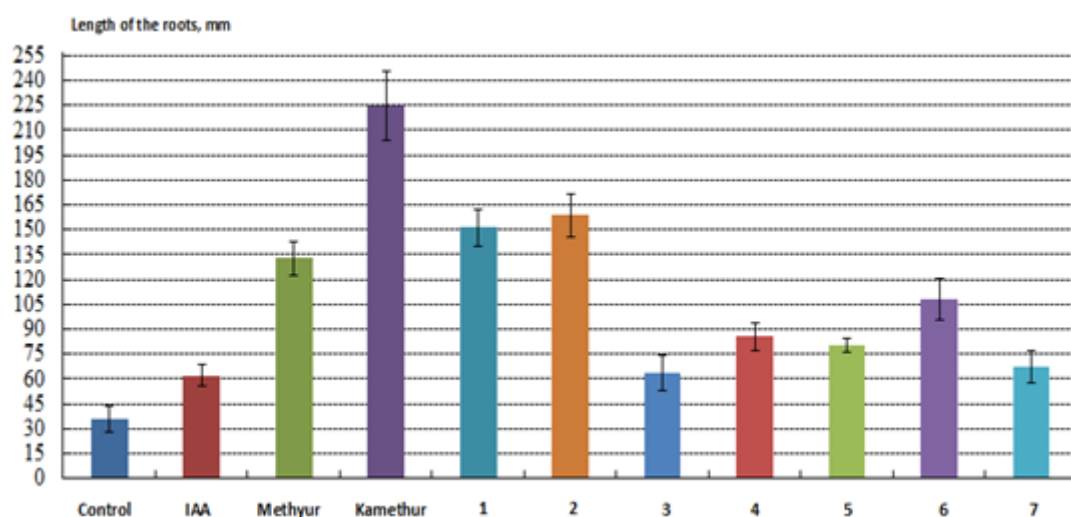
The lower regulatory effect on the length of the shoots (mm) was found in auxin IAA and synthetic compounds, pyrimidine derivatives № 6 and 7, under the influence of which these indicators increased: by 33.36% - under the influence of auxin IAA and by 40.39 - 44.44% - under the influence of compounds № 6 and 7, respectively, compared to the same indicator in the control maize plants (**Figure 2**).

Figure 2. Regulatory effect of auxin IAA, synthetic compounds Methyur, Kamethur and pyrimidine derivatives (№ 1-7) at a concentration of 10^{-6} M on the length of the shoots (mm) of 4-week-old maize (*Zea mays* L.) variety Twist grown under conditions of heat and drought stress, compared to control maize plants.



The highest regulatory effect on the length of the roots (mm) was found in synthetic compounds Methyur, Kamethur and pyrimidine derivatives № 1, 2 and 6, under the influence of which these indicators increased: by 271.96% - under the influence of Methyur, by 529.91% - under the influence of Kamethur, by 202.8–344.86% - under the influence of compounds № 1, 2 and 6, respectively, compared to the same indicator in the control maize plants (**Figure 3**).

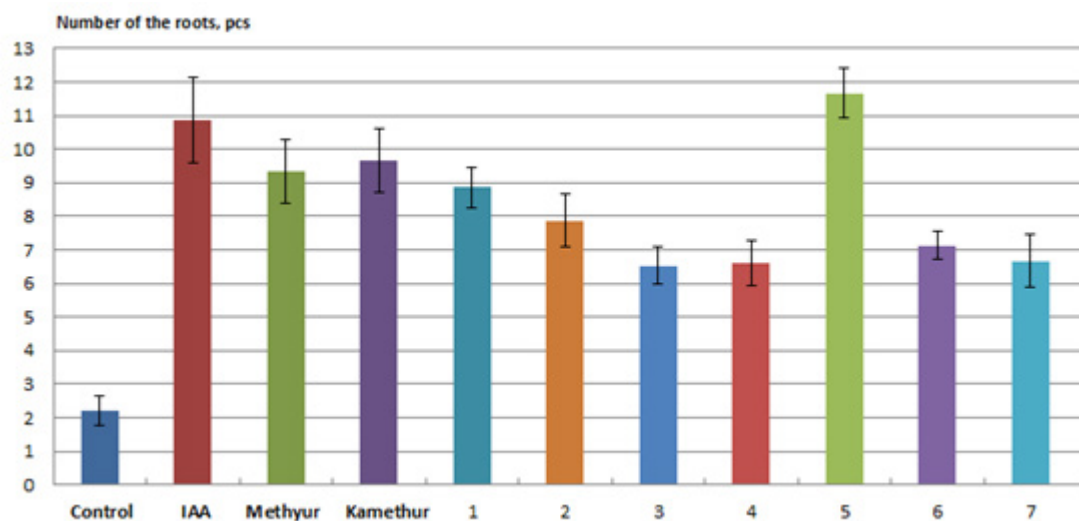
Figure 3. Regulatory effect of auxin IAA, synthetic compounds Methyur, Kamethur and pyrimidine derivatives (№ 1-7) at a concentration of 10^{-6} M on the length of the roots (mm) of 4-week-old maize (*Zea mays* L.) variety Twist grown under conditions of heat and drought stress, compared to control maize plants.



The lower regulatory effect on the length of the roots (mm) was found in auxin IAA and synthetic compounds, pyrimidine derivatives № 3, 4, 5 and 7, under the influence of which these indicators increased: by 73.83% - under the influence of auxin IAA and by 77.57–139.25% - under the influence of compounds № 6 and 7, respectively, compared to the same indicator in the control maize plants (**Figure 3**).

The highest regulatory effect on the number of the roots (pcs) was found in auxin IAA, synthetic compounds Methyur, Kamethur and pyrimidine derivatives № 1, 2, 5 and 6, under the influence of which these indicators increased: by 393.94% - under the influence of auxin IAA, by 324.24% - under the influence of Methyur, by 339.39% - under the influence of Kamethur, by 224.24–430.3% - under the influence of compounds № 1, 2, 5 and 6, respectively, compared to the same indicator in the control maize plants (**Figure 4**).

Figure 4. Regulatory effect of auxin IAA, synthetic compounds Methyur, Kamethur and pyrimidine derivatives (№ 1-7) at a concentration of 10^{-6} M on the number of the roots (pcs) of 4-week-old maize (*Zea mays* L.) variety Twist grown under conditions of heat and drought stress, compared to control maize plants.

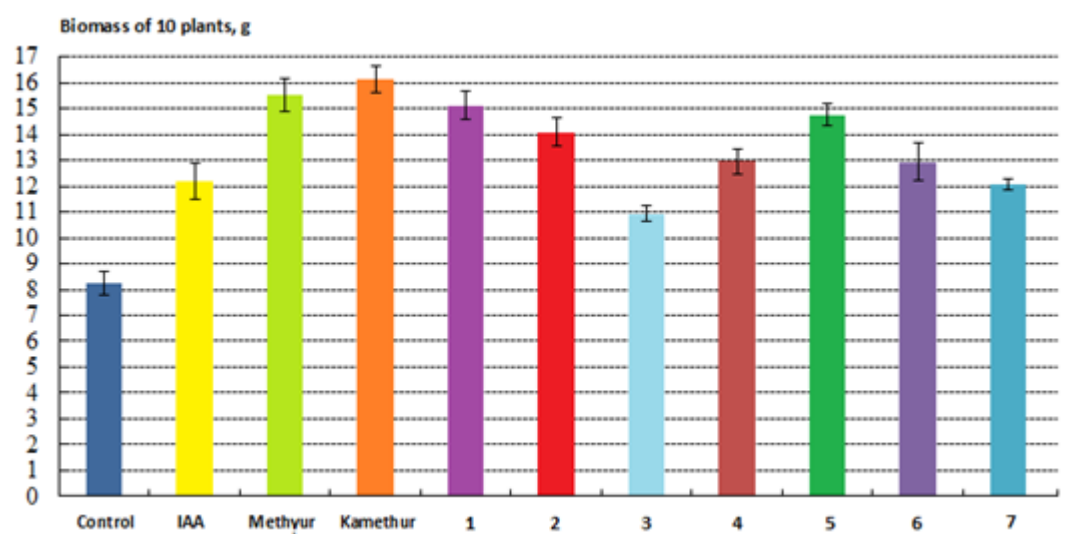


The lower regulatory effect on the number of the roots (pcs) was found in synthetic compounds, pyrimidine derivatives № 3, 4 and 7, under the influence of which these indicators increased by 196.97–203.03%, respectively, compared to the same indicator in the control maize plants (**Figure 4**).

The highest regulatory effect on the biomass of 10 plants (g) was found in auxin IAA, synthetic compounds Methyur, Kamethur and pyrimidine derivatives № 1, 2, 4, 5, 6 and 7, under the influence of which these indicators increased: by 48.15% - under the influence of auxin IAA, by 88.69% - under the influence of Methyur, by 95.91% - under the influence of Kamethur, by 46.61–83.83% - under the influence of compounds № 1, 2, 4, 5, 6 and 7, respectively, compared to the same indicator in the control maize plants (**Figure 5**).

The lower regulatory effect on the biomass of 10 plants (g) was found in pyrimidine derivative № 3, under the influence of which these indicators increased by 33.07%, compared to the same indicator in the control maize plants (**Figure 5**).

Figure 5. Regulatory effect of auxin IAA, synthetic compounds Methyur, Kamethur and pyrimidine derivatives (№ 1-7) at a concentration of 10^{-6} M on the biomass of 10 plants (g) of 4-week-old maize (*Zea mays* L.) variety Twist grown under conditions of heat and drought stress, compared to control maize plants.



The obtained data indicate that the synthetic compounds Methyur, Kamethur and pyrimidine derivatives № 1, 2, 5 and 6 showed the highest regulatory effect on the maize growth parameters, while the synthetic compounds, pyrimidine derivatives № 3, 4 and 7 showed a lower regulatory effect on the maize growth parameters compared to the same indicators in the

control maize plants. The regulatory effect of the synthetic compounds Methyur, Kamethur and pyrimidine derivatives № 1, 2, 5 and 6 was similar or higher than the effect of the plant hormone auxin IAA.

Obviously, this fact is due to the auxin-like and cytokinin-like effects of synthetic compounds on the processes of division, elongation and differentiation of plant cells, which are the basis for the growth and development of meristems of roots and shoots of maize in the vegetative phase, as well as on plant protection from damage caused by heat and drought stress, and the prevention of plant wilting and death [9 – 15, 18 – 20].

Study of the regulatory effect of pyrimidine derivatives on photosynthesis in maize under conditions of drought and heat stress

A comparative analysis of the regulatory effect of auxin IAA (1H-indol-3-yl)acetic acid and synthetic compounds, derivatives of 6-methyl-2-mercapto-4-hydroxypyrimidine sodium and potassium salts (Methyur and Kamethur) and pyrimidine (compounds № 1-7), applied at a concentration of 10^{-6} M, on the content of photosynthetic pigments: chlorophylls a and b, carotenoids (mg/g FW) in maize leaves, grown in the laboratory during the vegetative phase under conditions of heat and drought stress, was carried out.

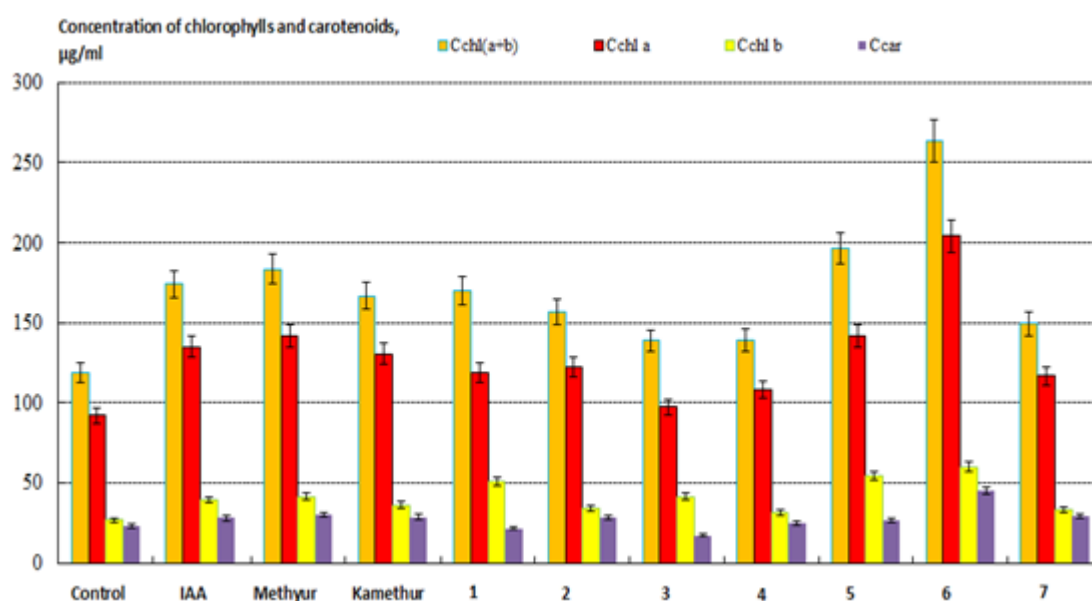
The conducted studies showed that synthetic compounds, derivatives of 6-methyl-2-mercapto-4-hydroxypyrimidine sodium and potassium salts (Methyur and Kamethur) and pyrimidine derivatives (compounds № 1-7), applied at a concentration of 10^{-6} M, exhibit a regulatory effect similar or higher than the effect of auxin IAA on the content of

chlorophylls and carotenoids in maize leaves during the vegetative phase under conditions of heat and drought stress (Figure 6).

The highest regulatory effect on the content of chlorophylls and carotenoids in maize leaves was found in auxin IAA, derivatives of 6-methyl-2-mercapto-4-hydroxypyrimidine sodium and potassium salts (Methyur and Kamethur) and pyrimidine derivatives № 1, 2, 5, 6, and 7 (Figure 6).

The content of chlorophyll a increased: by 46.61% - under the influence of IAA, by 54.37% - under the influence of Methyur, by 41.82% - under the influence of Kamethur, by 26.8–121.7% - under the influence of pyrimidine derivatives № 1, 2, 5, 6 and 7, compared to the same indicator in the control maize plants (Figure 6). The content of chlorophyll b increased: by 46.19% - under the influence of IAA, by 54.45% - under the influence of Methyur, by 35.36% - under the influence of Kamethur, by 23.18–124.18% - under the influence of pyrimidine derivatives № 1, 2, 5, 6 and 7, compared to the same indicator in the control maize plants (Figure 6). The content of chlorophylls a+b increased: by 46.52% - under the influence of IAA, by 54.4% - under the influence of Methyur, by 40.36% - under the influence of Kamethur, by 25.99–122.26% - under the influence of pyrimidine derivatives № 1, 2, 5, 6 and 7, compared to the same indicator in the control maize plants (Figure 6). The content of carotenoids increased: by 22.22% - under the influence of IAA, by 31.43% - under the influence of Methyur, by 25.62% - under the influence of Kamethur, by 15.84–97.05% - under the influence of pyrimidine derivatives № 1, 2, 5, 6 and 7, compared to the same indicator in the control maize plants (Figure 6).

Figure 6. Regulatory effect of auxin IAA, synthetic compounds Methyur, Kamethur and pyrimidine derivatives (№ 1-7) at a concentration of 10^{-6} M on the content of chlorophyll a, chlorophyll b, and carotenoids ($\mu\text{g/ml}$) in 4-week-old maize (*Zea mays* L.) variety Twist grown under conditions of heat and drought stress, compared to control maize plants.



The lower regulatory effect on the content of chlorophylls and carotenoids in maize leaves was found in pyrimidine derivatives № 3 and 4, under the influence of which the content of chlorophyll a increased by 5.83–17.42%, the content of chlorophyll b increased by 16.51–54.35%, the content of chlorophylls a+b increased by 16.76–17.22%, the content of carotenoids increased by 8.43%, compared to the same indicator in the control maize plants (**Figure 6**).

The obtained data indicate that the synthetic compounds Methyur, Kamethur and pyrimidine derivatives № 1, 2, 5, 6 and 7 showed the highest regulatory effect on the content of chlorophylls and carotenoids in maize leaves, while the synthetic compounds, pyrimidine derivatives № 3 and 4 showed a lower regulatory effect on the content of chlorophylls and carotenoids in maize leaves compared to the same indicators in the control maize plants. The regulatory effect of the synthetic compounds Methyur, Kamethur and pyrimidine derivatives № 1, 2, 5, 6 and 7 was similar or higher than the regulatory effect of the plant hormone auxin IAA.

Apparently, the regulatory effect of synthetic compounds is associated with their cytokinin-like effect on enhancing the biosynthesis of chlorophylls and carotenoids in maize leaves, which play an important role in plant productivity, and preventing leaf senescence and degradation of chlorophylls

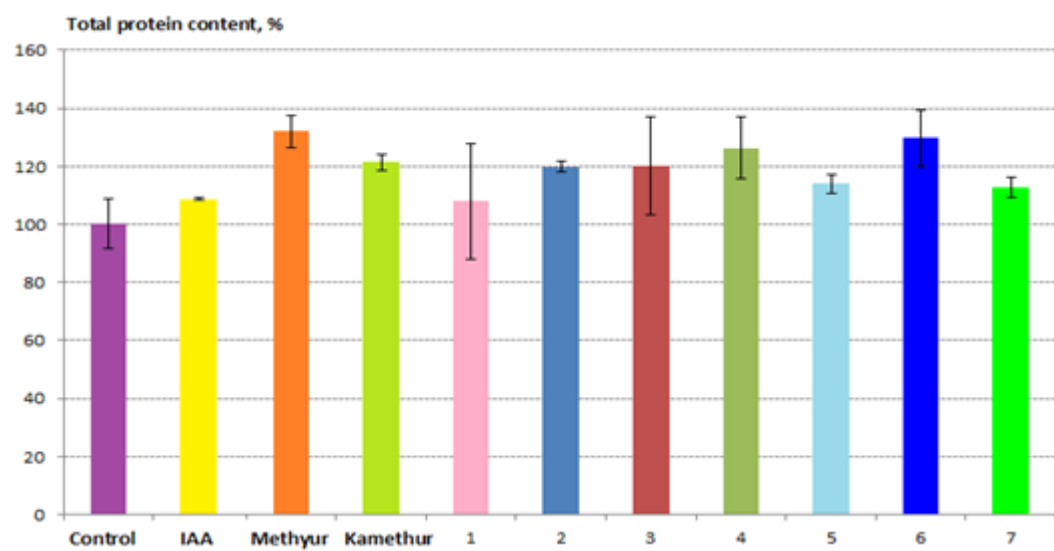
and carotenoids under conditions of heat and drought stress [49 - 52].

Study of the regulatory effect of pyrimidine derivatives on the content of total soluble protein in maize under conditions of heat and drought stress

A comparative analysis of the regulatory effect of auxin IAA (1H-indol-3-yl)acetic acid and synthetic compounds, derivatives of 6-methyl-2-mercapto-4-hydroxypyrimidine sodium and potassium salts (Methyur and Kamethur) and pyrimidine (compounds № 1-7), applied at a concentration of 10^{-6} M, on the content of total soluble protein (g/100 g FW) in maize leaves, grown in the laboratory during the vegetative phase under conditions of heat and drought stress, was carried out.

The conducted studies showed that synthetic compounds, derivatives of 6-methyl-2-mercapto-4-hydroxypyrimidine sodium and potassium salts (Methyur and Kamethur) and pyrimidine derivatives (compounds № 1-7), applied at a concentration of 10^{-6} M, exhibit a regulatory effect higher than the effect of auxin IAA on the content of total soluble protein in maize leaves during the vegetative phase under heat and drought conditions (**Figure 7**).

Figure 7. Regulatory effect of auxin IAA, synthetic compounds Methyur, Kamethur and pyrimidine derivatives (№ 1-7) at a concentration of 10^{-6} M on the content of total soluble protein (%) in 4-week-old maize (*Zea mays* L.) variety Twist grown under conditions of heat and drought stress, compared to control maize plants.



The highest regulatory effect on the content of total soluble protein in maize leaves was found in derivatives of 6-methyl-2-mercapto-4-hydroxypyrimidine sodium and potassium salts (Methyur and Kamethur) and pyrimidine derivatives № 2-7 (Figure 7). The content of total soluble protein in maize leaves increased: by 31.96% - under the influence of Methyur, by 21.39% - under the influence of Kamethur, by 12.73–29.75% - under the influence of pyrimidine derivatives № 2-7, compared to the same indicator in the control maize plants (**Figure 7**).

The lower regulatory effect on the content of total soluble protein in maize leaves was found in auxin IAA and pyrimidine derivative №1, under the influence of which the content of total soluble protein increased by: 8.66% - under the influence of IAA and by

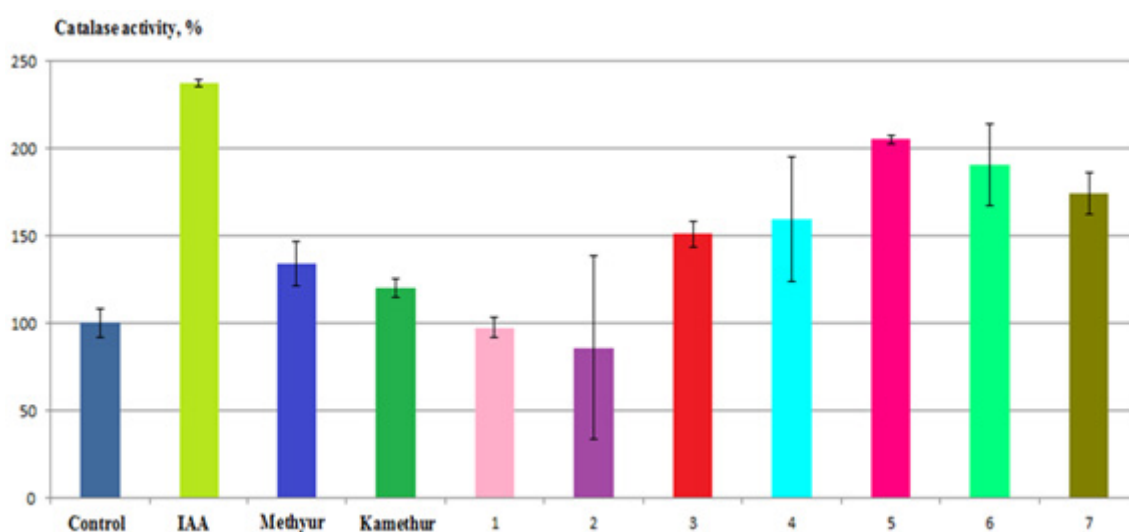
7.89% - under the influence of pyrimidine derivative №1, compared to the same indicator in the control maize plants (**Figure 7**). The obtained data indicate that the synthetic compounds Methyur, Kamethur and pyrimidine derivatives № 2-7 showed the highest regulatory effect on the content of total soluble protein in maize leaves, while the synthetic compound, pyrimidine derivative № 1 showed a lower regulatory effect on the content of total soluble protein in maize leaves compared to the same indicators in the control maize plants. The regulatory effect of the synthetic compounds Methyur, Kamethur and pyrimidine derivatives № 2-7 was higher than the regulatory effect of the plant hormone auxin IAA. Apparently, the regulatory effect of synthetic compounds is associated with their auxin-like and cytokinin-like effects on enhancing the biosynthesis of antioxidant enzymes and regulatory stress-associated proteins in maize leaves, which play an important role in plant growth, metabolism, and plant protection against heat and drought stress [19 – 31].

Study of the regulatory effect of pyrimidine derivatives on the catalase activity in maize under conditions of drought and heat stress

A comparative analysis of the regulatory effect of auxin IAA (1H-indol-3-yl)acetic acid and synthetic compounds, derivatives of 6-methyl-2-mercapto-4-hydroxypyrimidine sodium and potassium salts (Methyur and Kamethur) and pyrimidine (compounds № 1-7), applied at a concentration of 10^{-6} M, on catalase activity (mmol of decomposed H_2O_2 /min per 1 mg of protein) in maize leaves, grown in the laboratory during the vegetative phase under conditions of heat and drought stress, was carried out.

The conducted studies showed that synthetic compounds, derivatives of 6-methyl-2-mercapto-4-hydroxypyrimidine sodium and potassium salts (Methyur and Kamethur) and pyrimidine derivatives (compounds № 1-7), applied at a concentration of 10^{-6} M, exhibit a regulatory effect similar to or lower than the effect of auxin IAA on catalase activity in maize leaves during the vegetative phase under conditions of heat and drought stress (**Figure 8**).

Figure 8. Regulatory effect of auxin IAA, synthetic compounds Methyur, Kamethur and pyrimidine derivatives (№ 1-7) at a concentration of 10^{-6} M on catalase activity (%) in 4-week-old maize (*Zea mays* L.) variety Twist grown under conditions of heat and drought stress, compared to control maize plants.



The highest regulatory effect on catalase activity in maize leaves was found in auxin IAA, synthetic compounds, derivatives of 6-methyl-2-mercapto-4-hydroxypyrimidine sodium and potassium salts (Methyur and Kamethur) and pyrimidine derivatives № 3-7 (**Figure 8**). The catalase activity in maize leaves increased: by 137.25% - under the influence of auxin IAA, by 33.99% - under the influence of Methyur, by 19.95% - under the influence of Kamethur, by 50.98–104.9% - under the influence of pyrimidine derivatives № 3-7, compared to the same indicator in the control maize plants (**Figure 8**). At the same time, no statistically significant changes in catalase activity in maize leaves under the influence of synthetic compounds, pyrimidine derivatives № 1 and 2, were detected compared to the same indicator in control maize plants.

The obtained data indicate that the synthetic compounds Methyur, Kamethur and pyrimidine derivatives № 3-7 showed the highest regulatory effect on catalase activity in maize leaves, while the synthetic compounds, pyrimidine derivatives № 1 and 2 showed a lower regulatory effect on catalase activity in maize leaves compared to the same indicators in the control maize plants. The regulatory effect of the synthetic compounds Methyur, Kamethur and pyrimidine derivatives № 3-7 was similar to or lower than the effect of the plant hormone auxin IAA.

It is obvious that the regulatory effect of synthetic compounds is associated with their auxin-like and cytokinin-like effects on enhancing the activity of the catalase enzyme, which plays a key role in the adaptation of plant to oxidative stress caused by heat and drought stress [19 – 29].

Analyzing the relationship between the chemical structure and physiological activity of synthetic compounds, pyrimidine derivatives № 1-7, we can conclude that their regulatory effect, similar to or superior to the effect of auxin IAA, was selective. Synthetic compounds, pyrimidine derivatives № 1, 2, 5, 6, and 7, showed the highest regulatory effect on maize growth parameters and on the content of photosynthetic pigments.

Apparently, the highest auxin-like and cytokinin-like regulatory effects of synthetic compounds, pyrimidine derivatives № 1, 2, 5, 6, and 7 on maize growth parameters and on the content of photosynthetic pigments is associated with the presence of substituents in the chemical structure of these compounds (Table 1): compound № 1 contains an ethylthio group in position 2, a hydroxyl group in position 4 and a methyl group in position 6; compound № 2 contains a propylthio group in position 2, a hydroxyl group in position 4 and a methyl group in position 6; compound № 5 is the sodium salt of 4-hydroxypyrimidine-2-thiolate; compound № 6 contains a methylthio group in position 2 and a hydroxyl group in position 4; compound № 7 contains a benzylthio group in position 2 and a hydroxyl group in position 4 (**Table 1**). At the same time, the decrease of auxin-like and cytokinin-like effects in synthetic compounds, pyrimidine derivatives № 3 and 4 on maize growth parameters and on the content of photosynthetic pigments in maize leaves can be explained by the presence of substituents in the chemical structures of these compounds: compound № 3 contains a benzylthio group in position 2, a hydroxyl group in position 4 and a methyl group in position 6; compound № 4 contains an isopropyl substituent in position 2, a hydroxyl group in position 4, and a methyl group in position 6 (**Table 1**).

It should also be noted that pyrimidine derivatives № 2, 3, 4, 5, 6, and 7 showed the highest regulatory effect on the content of total soluble protein in maize leaves, and pyrimidine derivatives № 3, 4, 5, 6, and 7 showed the highest regulatory effect on catalase activity in maize leaves. These important parameters play a protective role in the adaptation of maize to oxidative stress caused by heat and drought [19 – 29].

The results obtained in this work indicate the prospects for the practical application of synthetic compounds Methyur, Kamethur and all studied pyrimidine derivatives № 1-7, which improve the growth of shoots and the root system of maize, increase biomass, enhance the biosynthesis of chlorophyll, carotenoids, and proteins, increase the activity of the antioxidant enzyme catalase, and also prevent disruption of plant metabolism and reduction of growth and yield of maize

under conditions of heat and drought stress.

Similar results were obtained in our previously published works, which studied the regulatory effect of synthetic compounds, pyrimidine derivatives № 1–7 at concentrations of 10^{-6} M and 10^{-7} M, on the growth and photosynthesis of various agricultural crops, such as barley, chickpea, pea, and haricot bean [53 - 57]. It has been shown that the regulatory action of synthetic compounds, derivatives of pyrimidine, similar to the phytohormones auxins and cytokinins, is variety-specific and depends on their chemical structure and the concentration used. However, the protective effect of synthetic compounds, pyrimidine derivatives № 1–7 on plant adaptation to stress factors such as heat and drought has not been studied in our early studies.

CONCLUSION

A study was conducted on the regulatory effect of synthetic compounds, pyrimidine derivatives № 1–7, on the growth of maize in the vegetative phase under heat and drought stress conditions. The use of synthetic compounds, pyrimidine derivatives № 1–7, applied at a concentration of 10^{-6} M, improved the growth of shoots and roots of maize, and also increased the content of chlorophylls, carotenoids, total soluble protein and catalase activity in maize leaves, similar to the regulatory effect of auxin IAA, applied at the same concentration of 10^{-6} M. A correlation has been found between the chemical structure and the selectivity of the regulatory action of synthetic compounds, pyrimidine derivatives. Based on the obtained results, a conclusion was made about the growth-regulating and protective effect of the synthetic compounds, pyrimidine derivatives № 1–7, on the growth and development of maize against the background of heat and drought stress.

Statement Of Conflict Of Interest

The authors are declared that they have no conflict with this research article.

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