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**ANTIVIRAL ACTIVITY OF THE SYNTHETIC COMPOUND 4,6-DIPHENIL [1,3,5]
TRIAZINE-2-THIONE AGAINST ZYMV (ZUCCHINI YELLOW MOSAIC VIRUS)
DISEASE IN CUCUMIS SATIVUS L. PLANTS**

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A new biological compound containing several functional groups and having antioxidant properties – 4,6-Diphenil[1,3,5]triazine-2-thione - has been synthesized. The seedlings of cucumber plants were infected with ZYMV (Zucchini yellow mosaic virus) virus and the effect of the virus on the plants was confirmed by serological methods. The resistance of healthy plant seedlings treated with the synthetic biologically active substance at the initial stage of the vegetation period was established to be higher compared with that of the seedlings treated after being infected with a virus. The optimal concentration (0.05%) of the synthetic biologically active substance, which ensures normal metabolism in plant cells during pathogen infection, was determined.

Keywords: *Cucumis sativus L., synthetic antioxidant substance, malondialdehyde, peroxidase, superoxide dismutase*

Introduction

Currently, more than 1.500 types of viruses are known to infect plants [1]. Some viruses have a wide range of host-plants. Viral infections cause enormous damage to agriculture. These phytopathogens sometimes negatively affect the quality indices of the product, thereby reducing productivity by 80-100%. In this regard, the protection of plants from viruses is one of the current problems. In agricultural practice, more attention is paid to the development of virus-resistant plant varieties. Although chemical treatment of plants is widely used in the world today, great attention is paid to the creation of virus-free plants using genetic engineering methods. In this regard, the synthesis of new inhibitors of antioxidants, enzymes, isoenzymes, and the development of new methods is very urgent. Intensive research is currently underway to find new natural and synthetic antioxidants, and the number of these substances is constantly growing [2, 3]. Several small-molecule steroids and dipeptides have been found to have antioxidant properties. These substances interact directly with active radicals to reduce hydroperoxides and variable valency metals. The research conducted at the Institute of Chemistry of Additives named after Academician A.Guliyev of ANAS [4] revealed that when functional groups having antioxidant

properties were included in one molecule, their antioxidant effects were enhanced and in many cases, intramolecular synergism was observed.

The main purpose of the research was to synthesize a new functional derivative of synthetic antioxidants composed of triazines, which contain various functional groups with a range of properties and to establish the effect of this compound on the activity and isoenzyme content of some enzymatic components in the antioxidant defense system involved in the plant adaptation against pathogens.

Materials and methods

Synthesis of the antiviral substance:

After adding 1.7 g of 33.5% aqueous solution of ammonia to the mixture containing 0.15 mol of thiocarbamide, 0.2 mol of benzaldehyde and 20 ml of isopropyl alcohol dropwise, the mixture was stirred at 40°C for 4 hours. After the reaction, the mixture was kept at room temperature for 24 hours. The precipitated white crystals of 4,6-Diphenil[1,3,5]triazine-2-thione were filtered and crystallized in ethyl alcohol after washing with dichloride methane.

The research object and cultivation of the plant material: The “Absheron” variety of

the cucumber plant (*Cucumis sativus* L.) was used as the study object. Plant seeds were grown in artificial climate chambers, at a photoperiod of 8h/16h, at $25 \pm 1^\circ\text{C}$ and 60-70% relative humidity. A part of two-week-old seedlings was infected artificially with ZYMV. Another part of seedlings was treated using the synthetic substance with 2 various concentrations (0.05% and 0.1%) before and after infection. Thus, 8 variants were chosen for the experiment: 1 – Control – healthy plants; 2 – Plants treated with 50mM synthetic substance; 3 – Plants treated with 100mM synthetic substance; 4 – ZYMV-infected plants; 5 – Plants infected with ZYMV before the treatment with 50mM synthetic substance; 6 – Plants infected with ZYMV before the treatment with 100mM synthetic substance; 7 – Plants infected with ZYMV after the treatment with 50mM synthetic substance; 8 – Plants infected with ZYMV virus after treatment with 100mM synthetic substance.

Visual diagnosis of plant viruses: Infected cucumber samples revealed by the visual diagnostics were tested using serological methods [5]. To this end, the leaf samples were tested with virus-specific immunostrips and analyzed for appropriate specific antibodies (IgG) preplaced on a tablet prepared for IFA. In plant samples with positive results, the virus concentration was determined spectrophotometrically based on optical density (Stat Fax Microplate, Awareness Technology, USA).

Quantification of malondialdehyde: The content of MDA, a product of lipid peroxidation in plants, was determined spectrophotometrically by reaction with thiobarbituric acid at 532 and 600 nm [6].

Obtaining the enzyme extract: To obtain the enzyme extract, 0.5 g of leaf samples are crushed in liquid nitrogen and homogenized in 100 mM Na-phosphate (pH 7.8) buffer containing 1 mM EDTA, 2 mM FMSF, 1% PVP. The homogenate was centrifuged at 4°C , 15,000 g, for 20 minutes. The obtained supernatant was used for the analysis of antioxidant enzymes.

The activity and isoenzyme content of superoxide dismutase (SOD, EC 1.15.1.1):

The enzyme activity was determined spectrophotometrically at 450 nm using the SOD Assay Kit (Sigma Aldrich). The isoenzyme content of the SOD was determined in 10% PAAG according to the [9]. After electrophoretic separation the activity staining for SOD isozymes was performed as reported by [7].

The activity and isoenzyme content of guaiacol peroxidase (GPO, EC 1.11.1.7):

The enzyme activity was determined spectrophotometrically at 470 nm [8]. The isoenzyme content of the GPO was determined in 7% (PAAG according to the [9]. Detection of guaiacol peroxidase on electrophoretic gel was performed as reported by [10].

Statistical analysis: Statistical analysis was performed in 3 biological replications using the computer program Excel 2016.

Results and discussions

The effect of ZYMV on plant seedlings was observed phenotypically 10, 14, and 18 days after infection. The characteristic symptoms of the virus such as yellowing, the formation of mosaics of various degrees, and twisting were detected in the leaves. The weakening of viral symptoms occurred in plant samples treated with synthetic antiviral agents. Immunostrip and DAS-ELISA methods confirmed the presence of ZYMV in the samples tested 14 days after infection. According to the phenotypic observations, the treatment of plant seeds using the synthetic biologically active substance before sowing reduced the germination percentage of seeds, the plant stem diameter, and the leaf surface area.

A change in the content of malondialdehyde (MDA), a product of lipid peroxidation, is one of the main indicators of plant stress [11]. In our experiments, the MDA content in healthy plants was 3.7 mM/g wet biomass (Figure 1). In virus-infected samples, this parameter increased 1.5-fold reaching 5.5 mM/g fresh biomass.

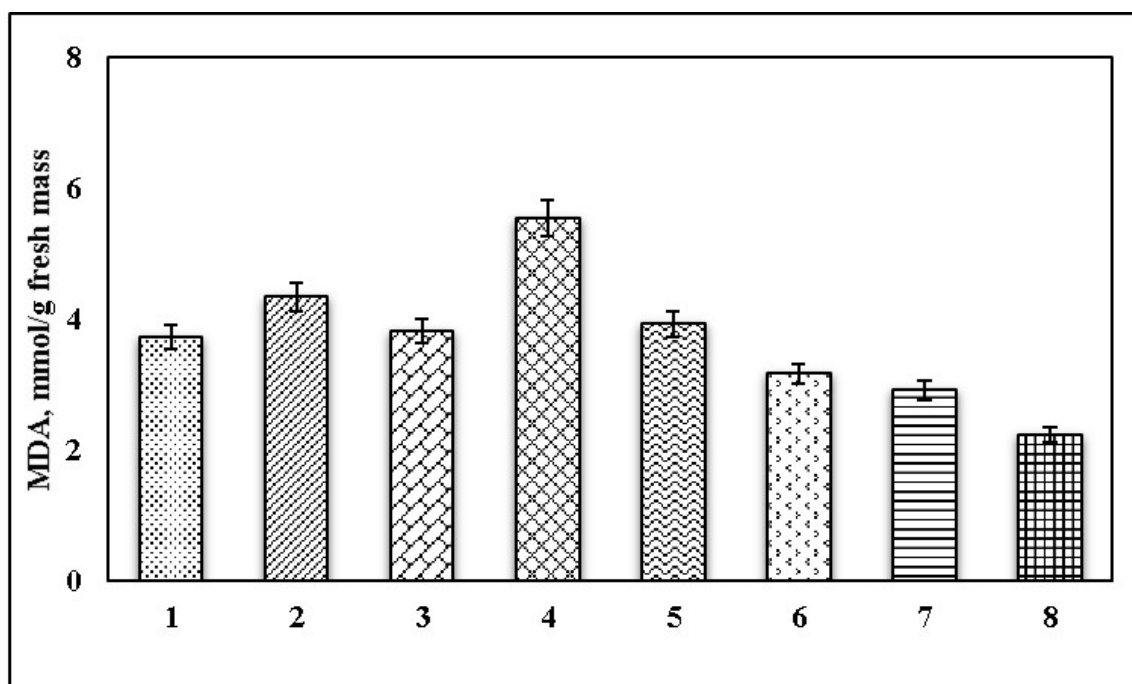


Fig. 1. MDA content in the roots of cucumber plants (*Cucumis sativus* L.)

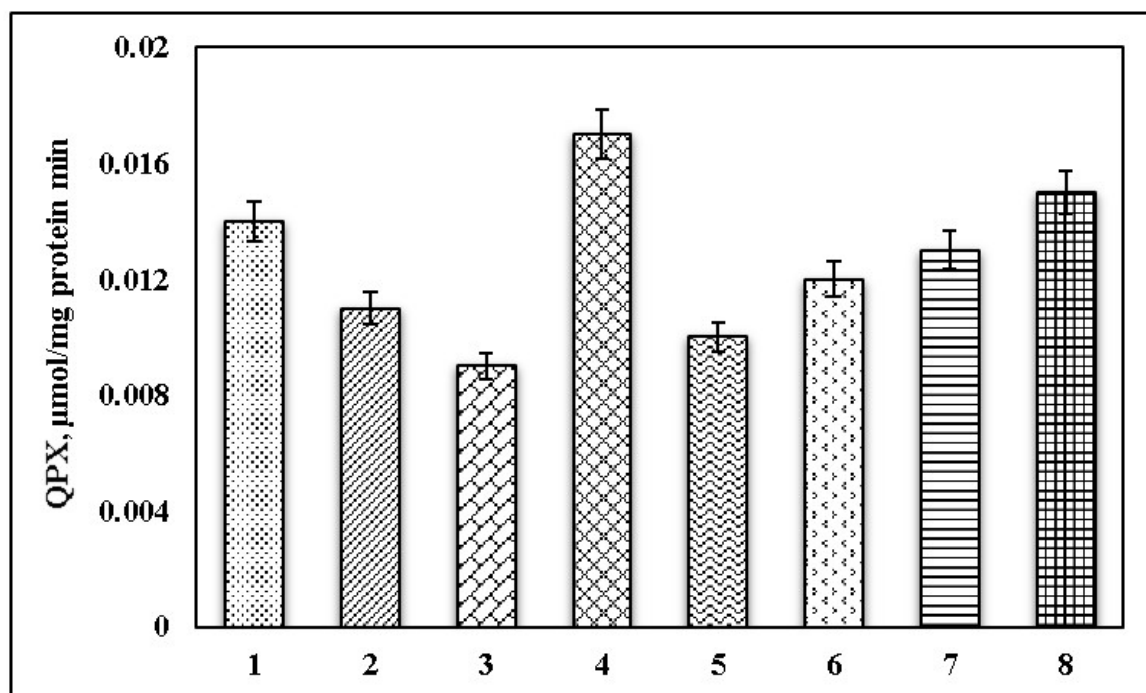


Fig. 2. The guaiacol peroxidase activity in the roots of cucumber plants (*Cucumis sativus* L.)

The effect of the synthetic antioxidant on the plant reduced the intensity of stress, as a result, the MDA content in the cells decreased and approached the control. Similar results were obtained in experiments with maize plants exposed to salt stress. High concentrations (200 mM) of NaCl and Na₂SO₄ were found to increase the fresh and dry weight of maize plants slightly, to decrease membrane permeability of roots, and increase the content of MDA and SOD activity in leaves and roots [12].

Peroxidases, which are common in nature and have a wide range of effects, can be considered as a marker of plant tolerance to stress. They control the regulation of the amount of H₂O₂ and organic peroxides in the cell. An increase in the activity of guaiacol peroxidase was observed depending on the degree of infection in the roots of diseased plants compared to healthy plants (Figure 2, Example 4). Thus, the guaiacol peroxidase content in the ZYMV-infected plant sample increased 1.2-fold compared to the control variant. The increase in the activity of guaiacol peroxidase under the influence of a pathogenic virus in plants can be attributed to an increase in the amount of phenolic compounds that are the substrate of this enzyme during stress. The experiments performed with potato plants showed that the peroxidase amount increased not only in leaves but also in roots [13]. The researchers detected also a correlation between chlorosis degree induced by the virus and amounts of peroxidases.

When the plant was treated using the synthetic substance before and after infection, the activity of guaiacol peroxidase was lower than that in the infected plants. Thus, the activity of the enzyme in the 5th, 6th, 7th, and 8th samples treated with the synthetic physiological substance was 1.7, 1.4, 1.3, and 1.1-fold lower than that in the infected sample. As can be seen, the synthetic substance reducing the effect of the virus on the plant has a significant effect on the activity of the enzyme.

The electrophoretic analysis revealed that the activity of guaiacol peroxidase (GPO) increased in infected plants compared to healthy plants. In all variants, four isoforms of GPO were detected. At 0.05% concentration, the synthetic antioxidant substance increased the enzyme activity (Figure 3, Example 5), whereas, at 0.1% concentration, this compound significantly inhibited the GPO activity (Figure 3, Example 8).

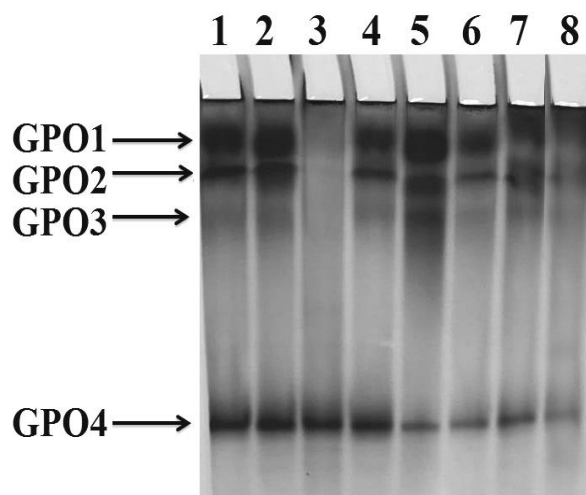


Fig. 3. Electrophoregram of the isoenzyme content of guaiacol peroxidase in the roots of cucumber plants (*Cucumis sativus* L.)

According to the results of the spectrophotometric analysis, the activity of SOD, one of the main enzymes of the antioxidant defense system, increased about 1.13-fold in ZYMV-infected plants compared to healthy plants (Figure 4). However, in the plant treated with the synthetic antioxidant, the activity of superoxide dismutase decreased approaching the control variant.

Based on the results of the electrophoretic analysis, 5 isoforms of superoxide dismutase exist in both control variants and plants treated with the biologically active substance (Figure 5, samples 1, 2, 3).

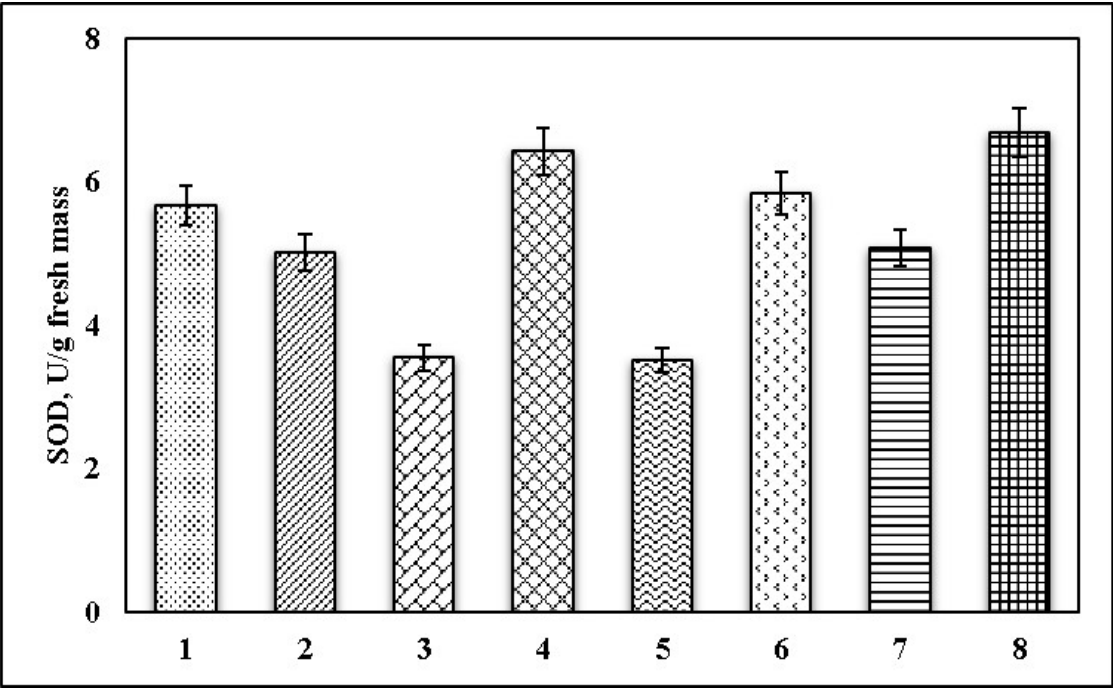


Fig. 4. The superoxide dismutase activity in the roots of cucumber plants (*Cucumis sativus* L.). In the virus-infected sample, the synthesis of a new isoform (SOD 4) due to the influence of the pathogen suggests a key role of this isoform in the antioxidant defense system of the plants

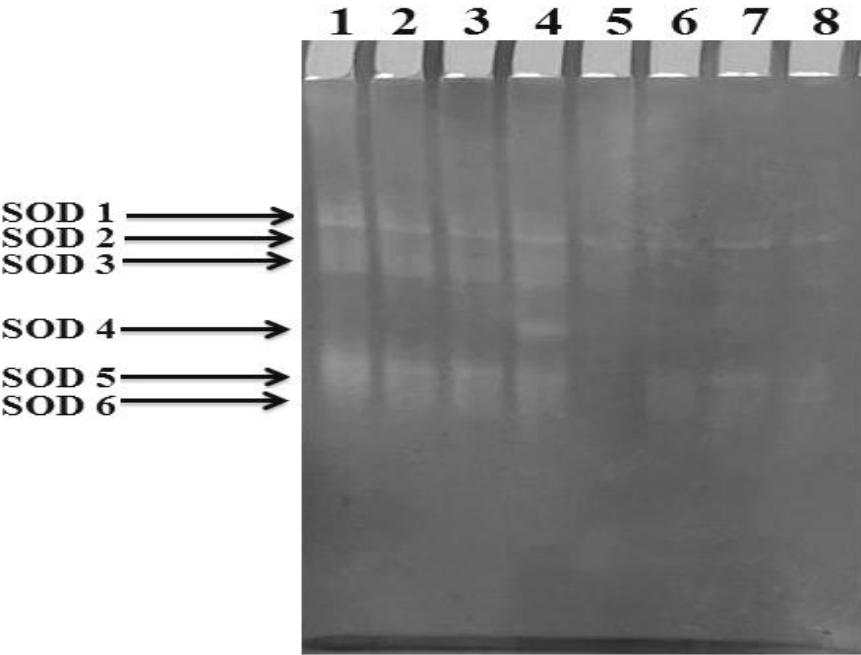


Fig.5. Electrophoregram of the isoenzyme content of superoxide dismutase in the roots of cucumber plants (*Cucumis sativus* L.)

In samples 5 and 6, the intensity of the enzyme isoforms, as well as their activity, decreased. However, in samples 7 and 8, these parameters remained at the control level. Studies by Hakmaoui and co-authors showed that the tobacco plant (*Nicotiana benthamiana*) responds to PMMoV (*Pepper mild mottle virus*) by altering the synthesis of various isoforms of the enzyme superoxide dismutase [14].

Thus, the results of the research showed that when plants were treated using the synthetic antioxidant in the early stages of the vegetation before being infected with the virus, their resistance to the pathogen was higher. A 0.05% concentration of the synthetic antiviral substance inhibited the activity of SOD while increasing the activity of GPO in virus-infected plants. No significant change in the isoenzyme content of both antioxidant enzymes was observed at 0.1% concentration of the synthetic antiviral substance.

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**ZYMV (ZUCCHINI YELLOW MOSAIC VIRUS) VIRUSU ILƏ YOLUXMUŞ CUCUMIS SATIVUS L.
BITKISINDƏ 4,6-DIFENIL[1,3,5]TRIAZIN-2-TIONUN MADDƏSİNİN ANTIVIRUS
FƏALLIĞININ TƏDQIQI**

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Tərkibində bir neçə funksional qrup olan yeni antioksidant xassəyə malik maddə - 4,6-difenil[1,3,5]triazin-2-tion sintez olunmuşdur. Xiyar (*Cucumis sativus* L.) bitkisinin cücərtiləri ZYMV (Zucchini yellow mosaic virus) virusu ilə yoluxdurulmuş, virusun bitkiyə təsiri seroloji metodlarla təsdiq edilmiş və müəyyən olunmuşdur ki, vegetasiyanın ilkin mərhələsində sağlam bitki cücərtilərini sintetik bioloji aktiv maddə ilə işlədikdə onun patogenə rezistentliyi virusla yoluxduqdan sonra maddə ilə işlənmiş bitkilərlə müqayisədə daha yüksək olur. Sintetik bioloji aktiv maddənin patogenlə yoluxma zamanı bitki hüceyrələrində normal metabolizmi təmin edən optimal qatılığı (0.05%) müəyyən olunmuşdur.

Açar sözlər: *Cucumis sativus* L., sintetik antioksidant maddə, malondialdehid, peroksidaza, superoksiddismutaza

**ИССЛЕДОВАНИЕ АНТИВИРУСНОЙ АКТИВНОСТИ СИНТЕТИЧЕСКОГО
ВЕЩЕСТВА 4,6-DIFENIL[1,3,5]TRIAZIN-2-TIONUN В РАСТЕНИИ CUCUMIS SATIVUS L.,
ПОРАЖЕННОМ ВИРУСОМ ZYMV (ZUCCHINI YELLOW MOSAIC VIRUS)**

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Синтезировано новое биологически активное вещество - 4,6-дифенил [1,3,5] триазин-2-тион, обладающее рядом антиоксидантных свойств. Антивирусная активность данного вещества изучалась на растениях *Cucumis sativus* L. Степень инфицированности определяли серологическими методами. Было выявлено, что при обработке здоровых проростков растений синтетическим биологически активным веществом на ранних стадиях вегетации их устойчивость к патогену была выше, чем у растений, обработанных после заражения вирусом. Определена оптимальная концентрация (0,05%) синтетического биологически активного вещества, обеспечивающая нормальный обмен веществ в клетках растений при инфицировании возбудителем.

Ключевые слова: *Cucumis sativus* L., синтетический антиоксидант, малоновый диальдегид, пероксидаза, супероксиддисмутаза