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EFFECT OF BIOLOGICAL AND CHEMICAL PREPARATIONS ON PEROXIDASE ACTIVITY IN LEAVES OF TOMATO PLANTS

© Y. Kolomiets, I. Grygoryuk, L. Butsenko

*In terms of treating tomato variety Chaika with chemical preparations with active substances of aluminum phosphate, 570 g/l + phosphorous acid 80 g/l and mankozeb in concentration of 640 g/kg, the maximum increase in peroxidase activity in leaves of plants was observed in 12 hours. In terms of use of biological preparations based on living cells *Bacillus subtilis* and *Azotobacter chroococcum* its activity was maximum in 24 hours and ranged from 77.7 to 112.7 $\text{un}\cdot\text{mg}^{-1}\cdot\text{s}^{-1}$*

Keywords: tomato, peroxidase activity, biological, preparations, pesticides, *Bacillus subtilis*, *Azotobacter chroococcum*

*За обробки рослин томата сорту Чайка хімічними препаратами з діючими речовинами фосфіт алюмінію, 570 г/л + фосфориста кислота, 80 г/л та манкоцебом в концентрації 640 г/кг максимальне підвищення активності пероксидази в листках рослин відбувалось на 12 год. За умов застосування біопрепаратів на основі живих клітин *Bacillus subtilis* і *Azotobacter chroococcum* її активність була максимальною на 24 год. й коливалась в межах 77,7 – 112,7 од. $\cdot\text{мг}^{-1}\cdot\text{с}^{-1}$*

Ключові слова: томат, активність пероксидази, біопрепарати, пестициди, *Bacillus subtilis*, *Azotobacter chroococcum*

1. Introduction

In tomato plants it can be found a number of bacterial diseases affecting various organs: stem centrum necrosis, bacterial leaf spotting, fruit mottling (spotting), fruit top rot, watery rot of stems and fruits, bacterial wilt (brown rot) of stems, root cancer.

To protect plants from infectious organisms they are used pesticides in agriculture. In the second edition of the official “List of pesticides and agrochemicals permitted for use in Ukraine for 2010”, there is no information on the drugs recommended for bacterial lesions of tomato plants [1]. According to the obtained data, fungicides, which include mankozeb or copper hydroxide, significantly inhibit the growth of bacterial agents and the development of cancer and bacterial black spotting [2].

Recently, in Ukraine it was renewed the interest in biological methods of plant protection, which is based on the use of microorganisms or their metabolic products to inhibit the development of pathogens. Biologicals based on live bacterial cultures are characterized by low toxicity and broad spectrum of activity against plants and pathogens. The use of combined biologicals that include properties of bio-fertilizers, fungicides, and insecticides, makes it possible to solve a large number of problems of biological protection of plants and improve the quality of final products (vegetables, fruits), as well as soil fertility [3].

Preparations of chemical, biological and natural origin cause induced systemic resistance against pathogens by stimulating defense reactions of the plant body [4]. They activate a complex network of signaling pathways that include regulatory molecule messengers (salicylic and jasmonic acids and their derivatives, indolyl-3-acetic acid, hydrogen peroxide, nitric oxide and other compounds), resulting in the synthesis of *de novo* PR-proteins pathogenesis-related (*pathogenesis-related proteins*, PR). PR-proteins implement various mechanisms to protect cells, the earliest of which is the intensive formation of reactive oxygen species (ROS) [5, 6].

2. Literature review

Under the ROS control, there are hypersensitivity and apoptosis reactions, due to which in the pathogenesis conditions it is formed the zone of dead plant cells, rich in antimicrobial compounds. ROS and products of oxidative modification of biomolecules, produced under their influence, act as second messengers for signal transduction to activate the expression of protective genes [7]. The resulting ROS affect the metabolism of plants and phytopathogens. However, in the resistant plants, oxidative processes offset by higher content of antioxidants.

With the opening of the signal and the protective role of ROS, attention is paid to oxidoreductase, regulat-

ing their levels in the cell. Among them, of particular interest are peroxidases, the activity of which correlates with the development of plant resistance against biological and abiotic stresses [8, 9]. Enzyme activation is the most important link of signaling system, the function of which is transferring and multiplication of elicitor signal, culminating with expression of protective genes and protein biosynthesis, which determine the response of plants to infection and the impact of elicitors. Being constitutionally required, peroxidase as multifunctional enzyme is involved in photosynthesis redox reactions, respiration processes, protein metabolism and plant growth process regulations, allowing it to respond quickly to infection with phyto-pathogens. Its substrates are phyto-hormones (abscisic and gibberellin acids, auxins), causing the regulation of physiologically active substances in plant tissues [10]. Peroxidase utilizes ROS in the polymerization of phenolic compounds to form lignin. Directly involved in the synthesis of lignin, which limits the supply of nutrients to the pathogen in the zone of its penetration into the tissues of plants, are anionic peroxidase [11].

However, the biochemical processes occurring in plants under the influence of plant protection products, are still poorly understood. Any preparations caused rearrangement of metabolism in the plant cell. However, the regulation paths may be different. Ascertainment of these questions is necessary for the correct use of chemical and biological agents with an aim to the regulation of plant growth and development, increasing their resistance to adverse conditions, producing a high yield, improve the quality of agricultural products and the preservation of favorable ecological situation. Thus, the present study was carried out to determine the induction of peroxidase activity in tomato plants in response to the application of chemical and biological preparations.

3. Aim and research problems

Aim – determination of redox enzyme peroxidase activity in the leaves of tomato plants in terms of treating with chemical and biological preparations.

Research problems: identify the optimal pH for the molecular forms of peroxidase, the influence of biological and chemical preparations on peroxidase activity, the antibacterial activity of biological preparations against the agents of bacterial cancer *C. michiganensis* subsp. *michiganensis* and bacterial black spotting *X. vesicatoria*.

4. Materials and methods

For research it was taken Chaika tomato variety, which, according to the data, in culture in vitro showed resistance to bacterial cancer and bacterial mottle, and bacterial black spot [12]. Plants of Chaika tomato varieties were grown in the scientific research field “Horticultural Garden” of National University of Life and Environmental Sciences of Ukraine. They were used chemical pesticides with active ingredients, including Fital (aluminum phosphite, 570 g/l + phosphorous acid 80 g/l); Metaksyl (metalaxyl, 80 g/kg + mankotseb, 640 g/kg) and Kuprosat (threemain copper sulfate, 345 g/l). Biologicals were used in concentrations recommended by the

manufacturer, including Phytohelp containing a concentrated blend of natural bacteria *Bacillus subtilis* (4×10^9 CFU/cm³), micro- and macroelements, biologically active products of microorganisms (BTU-center, Ukraine) [13], Fitotsyd – living cells and spores of natural endophyte bacteria *Bacillus subtilis* (1×10^9 – 4×10^9 CFU/cm³), their active metabolites (BTU center, Ukraine) [10], Azotofit – cells of natural nitrogen-gathering bacteria *Azotobacter chroococcum* (1×10^9 CFU/cm³), macro- and microelements, BAP of bacteria (BTU center, Ukraine) [13].

Peroxidase enzyme activity in leaves of tomato plants was measured using spectrophotometric method according to the optical density of the reaction products formed by oxidation of benzidine per second for 120 s at wavelength 590 nm. before treatment, in 1, 6, 12 and 24 hours after spraying with preparations. Tissue sample weight of 200–300 mg was ground in cold porcelain mortar with cold pestle in 2 ml of acetate buffer (pH 4,7; 5,0; 5,5). The resulting homogenates was centrifuged for 5 minutes at 12 000 g. To store the samples were placed in a refrigerator at 4° C. The reaction mixture contained 150 mcl of 0,2 M Na-acetate buffer (pH 4,7; 5,0; 5,5), 150 mcl of 0,01 % solution of muriatic benzidine of 50 mcl extract, 200 mcl of 0,3 % hydrogen peroxide, and 200 mcl of distilled water. The control cuvette contained 150 mcl of 0,2 M Na-acetate buffer (pH 4,7; 5,0; 5,5), 200 mcl of 0.01 % solution of muriatic benzidine, 50 mcl of extract, and 400 mcl of distilled water [14].

Calculation of peroxidase enzyme activity was provided by the formula [14]:

$$A = \frac{E(a \cdot \epsilon)}{c \cdot t},$$

where E is an extinction = 0,125;

a – the ratio of liquid taken for extract cooking, cm³/mg;

b – the degree of constant dilution of extract in the reaction mixture;

c – layer thickness, cm;

t – time, s.

The effect of biologics on bacteria was studied by method of diffusion in agar [15]. In Petrie dishes into holes made by sterile cork drill in the middle of frozen potato agar, with the help of sterile removable spouts it was added biological in the recommended by manufacturer concentrations. Then it was radially plated one-day old bacteria with titer 10^9 CFU/ml. The dishes were incubated for 48 hours at a temperature of 28 ± 1 °C. The antibacterial effect of the substance was set due to the diameter of the zone with no bacterial growth.

Statistical analysis of the results was performed using application software package STATISTICA v.6.0.

5. Results of the research

In the leaves of tomato plants of Chaika variety it was determined the optimal activity value of peroxidase loosely bound with cell wall. In particular, there was a shift of the pH optimum for peroxidase, caused by the advantage of enzyme forms synthesis in terms of treating with chemical and biological preparations (Fig. 1).

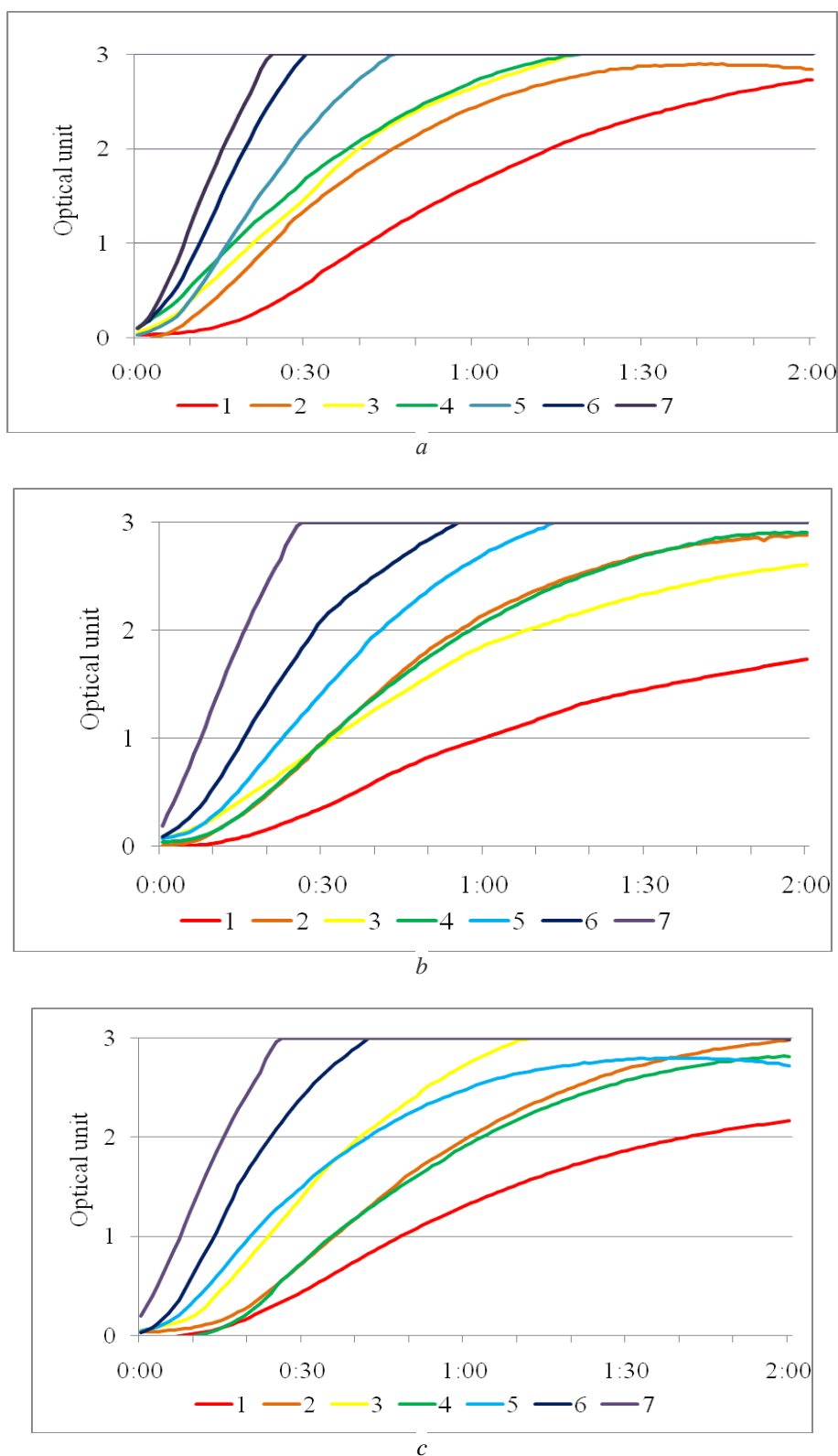


Fig. 1. Effect of pH Na-acetate buffer on peroxidase activity of anionic forms in the leaves of tomato plants of Chaika variety in terms of treating with preparations: 1 – kontrol, 2 – Fital, 3 – Kuproksat, 4 – Metaksyl, 5 – Azotofit, 6 – Phytohelp, 7 – Fitotsyd: *a* – pH 4,7; *b* – pH 5,0; *c* – pH 5,5

It was determined that plants treated with biological preparations are characterized by higher values of peroxidase activity $51,4-112,5 \text{ un.mg}^{-1}\cdot\text{s}^{-1}$ with pH=4,7 and pH=5,5, indicating the induction of synthesis of anionic and cationic-anionic forms. They were found two peaks of activity in leaves of tomato plants in terms of the actions of biological preparations Fitotsyd and Phytohelp

based on living cells of natural bacteria *Bacillus subtilis*. The optimal value for peroxidase activity for plant leaves in terms of treating with chemicals were characteristic with pH=4,7, which was accompanied by enhanced generation of anionic peroxidase. Thus, changing in activity of the molecular forms of peroxidase is likely to ensure the resistance of plants against complex of environmental factors

To analyze the intensity of synthesis of ROS, caused by chemical and biological preparations, and the study of adaptive properties of tomato plants of Chaika variety, it was studied the daily dynamics of

peroxidase activity (Table 1). During the experiment it was found that during the day peroxidase activity in leaves of plants remained at the initial level – 15,0–15,6 $\text{un.mg}^{-1}\cdot\text{s}^{-1}$.

Table 1

Change in activity of peroxidase enzyme ($\text{un.mg}^{-1}\cdot\text{s}^{-1}$) in the leaves of tomato plants of Chaika variety in terms of treating with preparations

Biologicals	before treatment	1 h	6 h	12 h	24 h
Kontrol	15,0±0,04	15,4±0,04	15,0±0,02	15,6±0,03	15,4±0,04
Fital	16,3±0,02	27,7±0,06	55,5±0,03	112,5±0,02	24,5±0,02
Kuproksat	19,3±0,05	36,0±0,02	7,62±0,02	31,5±0,05	13,5±0,06
Metaksyl	14,3±0,03	67,5±0,03	81,1±0,05	111,5±0,03	65,8±0,06
Azotofit	18,2±0,02	30,7±0,05	43,0±0,04	54,5±0,04	77,7±0,04
Phytohelp	16,2±0,02	24,0±0,04	32,6±0,02	41,5±0,02	94,7±0,03
Fitotsyd	17,6±0,04	23,2±0,02	23,2±0,02	80,5±0,04	112,7±0,03

Peroxidase activity in leaves of tomato plants treated with chemical preparations Fital and Metaksyl was maximal in 12 hours, while in 24 hours it took place its decrease by 41,0–78,2 %. In general, the impact of pesticides is found in diverse effects on metabolism in plants. They change the permeability of the cell membrane, the intensity of photosynthesis, respiration and activity of the related oxidation-reductive enzymes. The intensity and direction of these processes depends on the nature, norms, terms and forms of the preparation use and environmental conditions [11].

In response to the treating with biological preparations we have identified a gradual increase in activity of peroxidase in 1, 6, 12 and 24 hours. In the leaves of plants treated with *Bacillus subtilis*, the enzyme activity increased and was maximal in 24 hours 94,7–112,7 $\text{un.mg}^{-1}\cdot\text{s}^{-1}$. According to [16], endophytic bacteria *Bacillus subtilis* protect plants from pathogens, produce physiologically active substances and form induced systemic resistance.

Phytohelp and Phytocide showed high antibacterial activity against the agents of bacterial cancer *C. michiganensis* subsp. *michiganensis* and bacterial black spotting *X. vesicatoria*, and the no growth zone diameter ranged from 73 to 80 mm. Bacilli antagonistic activity against the phytopathogens associates with the synthesis of antibiotics, toxins, volatile organic compounds, phytohormones, and other exometabolites of different chemical nature. A significant place in manifestation of antagonism takes a lytic enzyme complex of bacteria of the *Bacillus* genus, which is able to destroy certain connections in the structure of cell walls peptidoglycan of gram-positive and gram-negative bacteria.

In terms of treating with Azotofit, based on *Azotobacter chroococcum*, peroxidase activity increased up to 77,7 $\text{un.mg}^{-1}\cdot\text{s}^{-1}$. In our study we examined the antibacterial activity of biologicals Azotofit based on nitrogen-fixing bacteria (*plant growth-promoting rhizobacteria* – PGPR-bacteria). Biological Azotofit, based on cells of nitrogen-fixing bacteria *Azotobacter chroococcum*, showed high antibacterial activity against the agent of bacterial cancer *C. michiganensis* subsp. *michiganensis*, no growth zone diameter was 78±2,0 mm. This preparation was middle-active against the agent of bacterial black spotting *X. vesicatoria* and showed no activity against the agent of bacterial speck of tomato

P. syringae pv. *tomato*. PGPR-bacteria positively affect plant growth and development due to the ability to fix atmospheric molecular nitrogen, to synthesize substances of hormonal nature, improve water and mineral nutrition of plants, and inhibit the development of pathogens, due to the synthesis of substances of bactericidal and fungicidal action.

Thus, a higher tendency of peroxidase activity in leaves of plants treated with biological preparations based on living cells *Bacillus subtilis* and *Azotobacter chroococcum*, confirm the increase in activity of non-specific defense reactions.

6. Conclusions

In terms of treating Chaika tomato variety with chemical and biological preparations, it was identified increased activity of peroxidase, which varied depending on the active ingredient of the preparation. The studied preparations with active substances aluminum phosphate, 570 g/l + phosphorous acid, 80 g/l, and mankotseb in a concentration of 640 g/kg have caused the maximal peroxidase activity in leaves of plants in 12 hours, which amounted up to 111,5–112,5 $\text{un.mg}^{-1}\cdot\text{s}^{-1}$, while in 24 hours – its reduction by 41,0–78,2 %. In terms of use biological preparations based on living cells *Bacillus subtilis* and *Azotobacter chroococcum*, peroxidase activity was maximal in 24 hours and ranged from 77,7–112,7 $\text{un.mg}^{-1}\cdot\text{s}^{-1}$. The increase in activity of peroxidase in leaves under the influence of chemical and biological preparations confirms the effectiveness of their use for growth and activation of tomato plant immunity.

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*Рекомендовано до публікації д-р біол. наук, професор, академік НААН України Федоренко В. П.
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Kolomiets Yulia, PhD, associate professor, Department of environmental biotechnology and biodiversity, National University of Life and Environmental Sciences of Ukraine, Heroyiv Oborony str., 15, Kyiv, Ukraine, 03041
E-mail: julyja@i.ua

Grigoryuk Ivan, Doctor of Biological Sciences, professor, corresponding member of NAS of Ukraine, Department of physiology, biochemistry plants and bioenergetics, National University of Life and Environmental Sciences of Ukraine, Heroyiv Oborony str., 15, Kyiv, Ukraine, 03041
E-mail: julyja@i.ua

Butsenko Lyudmila, PhD, associate professor, Department of plant pathogenic bacteria, D. K. Zabolotny Institute of Microbiology and Virology of the NASU, Zabolotnogo str., 154, Kyiv, Ukraine, 03143
E-mail: julyja@i.ua