EFFECTS OF BIOPESTICIDE CARBECOL, FUNECOL AND BIOFERTILIZER ECOLIT ON PHOTOSYNTHETIC PIGMENTS AND HYDROGEN PEROXIDE CONTENTS IN TOMATO (Solanum lycopersicum L.) PLANTS

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Abstract

The photosynthetic pigments and hydrogen peroxide content is a physiological marker to evaluate plant physiology and determine the response to application of different kinds of plant protection products. The aim of this study was to determine the response of leaf chlorophylls and hydrogen peroxide contents in tomato plants to application of biofungicide Carbecol alone or in combination with biofungicide Funecol and biofertilizer Ecolit. A greenhouse experiment was conducted out with tomato (Solanum lycopersicum L., cv Manusa) plants. Experimental results showed that plant treatments with Carbecol increased the contents of photosynthetic pigments in tomato plants, in particular chlorophyll a. The highest concentration of photosynthetic pigments was registered in treatment with integrated application of Carbecol, Funecol and Ecolit. The treatments with Carbecol and Ecolit. The treatments with Carbecol in photosynthetic pigments was observed in treatment with application of hydrogen peroxide in leaves. The highest level of this metabolite in leaves was observed in treatment with application of biofertilizer Ecolit. The integrate use of biofungicides and biofertilizer Ecolit increased tomato yield by 14%.

Key words: Carbecol, Funecol, Ecolit, photosynthetic pigments, hydrogen peroxide, tomato.

INTRODUCTION

Tomato (Solanum lycopersicum L.) is one of the major vegetables in many countries, as well as in the Republic of Moldova. This crop plays an important role in human nutrition and food security. It is well known that tomato is sensitive to infection by many phytopaphogens, especially by Phytophtora infestans. To control the late blight producers use a large range of fungicides (Egel et al., 2019). Some studies demonstrated that application of protection products has negative consequences on physiological state of crops as well as on yield quality. Therefore, it is necessary to identify the innovative ecological plant protection products with minimum impact on plants on one hand, and on the other hand they must be effective for the protection of crops against phytopaphogens (Bahramisharif & Rose, 2019). Nowadays, ecological plant protection products have become a relevant particularity of durable agriculture development for increasing productivity with good quality of vegetables. The application of chemical pesticides

photosynthetic diminishes the pigments, increases reactive oxygen species (ROS) in provokes plants. and imbalance of physiological constituents and metabolic processes (Cerný et al., 2018). Abd-El-Kareem et al. (2012) demonstrated that treatments of potato with potassium bicarbonates, essential plant oils and humic substances have a higher beneficial potential to reduce late blight disease accompanied with reducing yield losses. There is a body literature data which confirmed that biopesticides have an agronomic and economic importance with a large range of their functions to improve the plant resistance to some diseases, in particular to late blight of tomato and potato plants (Ngegba et al., 2022). The level of photosynthetic pigments concentrations determines the plant growth and productivity, the plant resilience to stress factors. The use of plant protection products has not only the capacity to control the diseases but they can alter a range of metabolites in leaves of plants. Likewise, the application of humic substances has ability to protect crops against phytopathogen attack and associated with

accumulation of chlorophylls in leaves (Jindo et al., 2020). Hydrogen peroxide (H₂O₂) has been shown to play a key role in a range of physiological processes including photosynthesis. senescence. stomatal movement, plant growth and development (Noctor & Foyer, 1998). However, the hydrogen peroxide at higher concentration has a toxic effect responsible for reducing plant tolerance to unfavorable conditions (Nazir et al., 2020). At the same time, the hydrogen peroxide is considered a strong antiseptic due to its toxic effects on some infections of crops. Analysis of the literature data revealed that most of the studies were carry out to assess only the capacity of ecological protection products of diseases control of crops (Ngegba et al., 2022). Unfortunately, there are a few investigations focused to determine their influence on changes of photosynthetic pigments and hydrogen peroxide (Mourad et al., 2017). To address this knowledge gap, this study was aimed to investigate the effects of biopesticide Carbecol applied alone or in combination with biopesticide Funecol and biofertilizer Ecolit on the contents of chlorophyll a, b, carotenoids and hydrogen peroxide in leaves of tomato plants. Also, we determined the influence of treatments on tomato fruit yield.

MATERIALS AND METHODS

A greenhouse experiment was conducted to examine the potential effects of Carbecol alone or in combination with Ecolit and Funecol on photosynthetic pigments, hydrogen peroxide contents in leaves and productivity of tomato (cv Manusa). The experiment was performed out at the Institute of Genetics, Physiology and Plant Protection of the Republic of Moldova. The soil used for experiment was carbonated chernozem. The experiment included 6 treatments. The biorational products Carbecol and Funecol have the potential to control late blight of tomato. Individual and combined applications of the Carbecol. Ecolit and Funecol have been done at the following stages of the plant development: 1-st at 7 days after transplanting; 2-nd at the intensive growing stage; the 3-rd at flowering stage and the 4-th at the fruits development stage. Carbecol was applied taking in consideration the dose of 6 kg/ha. Biofertilizer Ecolit was applied at dose 3 L/ha and fungicide Funecol at concentration 0.4%. After the last treatment the leaves were collected from each variant in three replicates. Photosynthetic leaf pigments chlorophyll *a* and b and carotenoids were quantified by the method of Lichtenthaler & Wellburn (1987) through a spectrophotometer at 663 nm, at 644 nm and at 470 nm. Hvdrogen peroxide concentration in leaves was determined Velikova according to et al. (2000).Absorbance was measured using a UV-Vis spectrophotometer at 390 nm. Hydrogen peroxide contents are expressed as umol of H_2O_2 g⁻¹ fresh weight. At physiological maturity, tomato fruits from each variant were harvested and weighed separately to determine fruit yield. The factorial treatments were distributed in three replicates according to the Complete Randomly Block Design. The data were analyzed by using STATISTIC 7 program. All the results were the means \pm SE of three replicates.

RESULTS AND DISCUSSIONS

Evaluation of chlorophyll a and b, and carotenoids contents is a biochemical marker to determine the plant responses to treatments with agrochemicals used in integrated crops protection. Likewise, the chlorophyll content is a tolerance parameter to unfavorable factors of plants (Hosseinzadeh et al., 2016). In general, we visually observed that tomato plants grown under treatments of ecological substances were greener compared to untreated plants (control). Mean data for photosynthetic pigments concentrations shows variable responses. Experimental results of this study revealed that concentrations of pigments in tomato leaves were related to treatments. The effects of treatments with Carbecol alone or in combination with biofertilizer Ecolit and

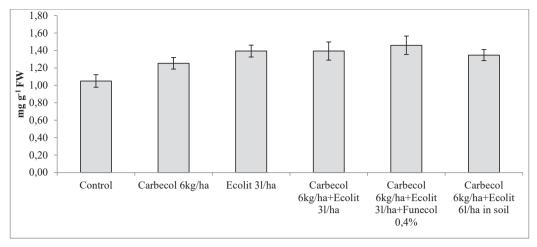


Figure 1. Effects of treatments of Carbecol alone or in combination with Ecolit and Funecol on the changes of the chlorophyll *a* content of *Solanum lycopersicum* L. plants.All the data are the means of three replicates (n = 3); vertical bars show standard errors (± SE)

Funecol on chlorophyll a and b contents are shown in Figures 1 and 2, respectively. It is necessary to note that in the variants with application of biopesticide tomato plants were less infected by Phytophtora infestans pathogen compared to control one (data are not shown). Experimental results shown that mean chlorophyll *a* contents ranged from 1.05 to 1.46 mg g/FW in depending on treatments. As we expected. the lowest concentration of chlorophyll *a* was observed in control plants.

Our present findings are in line with the findings of other researchers (Egel et al., 2019). However, the most increase of chlorophyll a concentration (by 39%) was registered in treatment with integrated application of Carbecol, Ecolit and Funecol (variant 6). The chlorophyll b contents varied among different treatments, the differences between the treatments and control variant were significant (Figure 2).

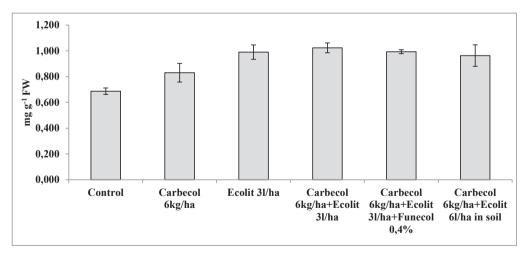


Figure 2. Effects of treatments of Carbecol alone or in combination with Ecolit and Funecol on the changes of the chlorophyll b content of *Solanum lycopersicum* L. plants. All the data are the means of three replicates (n = 3); vertical bars show standard errors (± SE)

Mean chlorophyll **b** contents ranged from 0.687 to 1.023 mg g/FW in depending on treatments. Hence, this implies that the application of ecological products was effective on the chlorophvll contents of the plants. Experimental data confirmed that chlorophyll **b** content increased with various treatments; moreover, the significant increase was recorded for Carbecol + Ecolit treatment. The lowest level of chlorophyll b was recorded in the control treatment. We suppose that integrated use of ecological products with biofertilizer stimulates the uptake of nutrients, in particular magnesium. This nutrient participates in the chlorophyll synthesis. At the same time combined treatments of biopesticide and biofertilizer Ecolit significantly reduced the attack by Phytophtora infestans. Hence, in this research the chlorophyll b content exhibited pronounced responses to application of biorational plant protection products. We may conclude that application of ecological protection products taken in this study could affect beneficially the photosynthetic pigments status of tomato plants. Chlorophylls are sensitive to oxidation and carotenoids play a significant role in the protection of chlorophylls against damage by different adverse factors. In addition, carotenoids are more stable pigments than chlorophylls (Loggini et al., 1999).

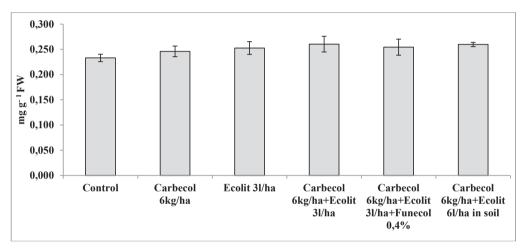
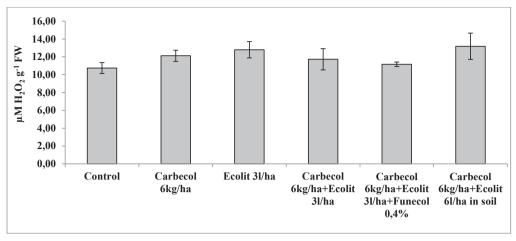


Figure 3. Effects of treatments of Carbecol alone or in combination with Ecolit and Funecol on the changes of carotenoids content of *Solanum lycopersicum* L. plants. All the data are the means of three replicates (n = 3); vertical bars show standard errors (± SE)

The analysis of carotenoids content in the leaves showed less responses to treatments than chlorophylls one. However, it was observed a moderate increase of carotenoids under different treatments in comparison with control plants (Figure 3). The highest (0.260 mg g/FW) and lowest (0.233 mg g/FW) concentrations of carotenoids were observed in the treatments with integrated use of all products and control variant, respectively. Therefore, the contents of carotenoids were light increased in leaves of tomato plants in response to application of plant protection ecological products. As we mentioned in the INTRODUCTION the hydrogen peroxide has a significant role in the physiological activity of the plants. As a rule

this metabolite is produced predominantly in photosynthesis processes of the and photorespiration (Noctor & Foyer, 1998). To our knowledge, this research for the first time has examined the effects of treatments of plant protection products on changes of hydrogen peroxide content in tomato plants. The data of its concentration in leaves of tomato plants is illustrated in Figure 4. The experimental results revealed that the hydrogen peroxide contents in leaves were higher under treatments than their respective untreated plants. The accumulation of hydrogen peroxide in leaves changed under treatments with Carbecol alone or under combined application with biofertilizer Ecolit and biofungicide Funecol.



 $\label{eq:Figure 4. Hydrogen peroxide (H_2O_2, \mu mol \ g^{-1} \ FW) \ content \ in \ leaves \ of \ tomato \ exposed \ to \ different \ treatments. \\ Bars \ represent \ the \ means \ of \ three \ replicates \ \pm \ SE$

Experimental results find out that the separate use of these products increased the concentration of hydrogen peroxide by 12.8% and 19.1%, respectively. As a whole, mean value of hydrogen peroxide contents were higher in treatment with integrated foliage application of the Carbecol and Ecolit applied in the soil (by 23.2%) over the control plants. However, it was observed that integrated application of Carbecol, Ecolit and Funecol insignificantly reduced its content in leaves (variant 5), but the value was higher than in control variant (10.74 micromoli H_2O_2/g FW). Our results are in concordance with the studies of Sidiqqui and coworkers (2009) in treatment of plants with extracts of compost tea. While treatments improved the biochemical parameters of plants in this study we also determined their influence on fruit yield of tomato. The data of tomato fruit yield is presented in Figure 5.

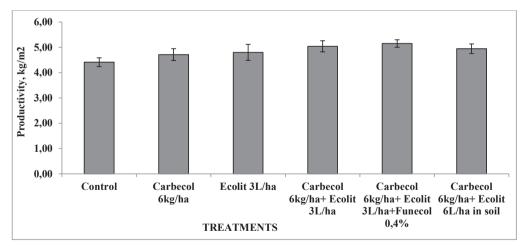


Figure 5. Effect of Carbecol alone or in combination with Ecolit and Funecol on the fruits productivity of tomato plants. Error bars represent LSD at $p \le 0.05$ of a mean pooled from three replications

As we supposed, the application of Carbecol alone or in combination with biofertilizer Ecolit and fungicide Funecol had positive influence on plant productivity (Figure 5). The highest value of the yield was obtained in treatment with integrated application of Carbecol, Ecolit and Funecol. In this treatment the yield increased by 14% compare to untreated plants (control). As we expected the lowest yield of tomato fruits were registered in control variant. Probably, the negative effect of higher infection of untreated plants by pathogen *Phytophtora infestans* could be attributed the adverse influence of late blight disease on plants physiology on the one hand and lesser supply of nutrients on the other hand because the biofertilizer was not applied in control variant, and consequently decreased yield of tomato.

CONCLUSIONS

Experimental results demonstrated that under treatments with Carbecol applied alone or in combination with biofertilizer Ecolit and biofungicide Funecol increased photosynthetic pigments in tomato plants.

The application of biofertilizer Ecolit alone increases the carotenoids contents in plants.

The highest concentration of hydrogen peroxide was obtained under the application of ecological fungicide Carbecol and biofertilizer Ecolit.

The integrated treatments with Carbecol, Ecolit and Funecol improved photosynthetic pigments status of plants and increased fruits yield of tomato. A field study in this regard should be conducted to further verify these results.

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REFERENCES

- Abd-El-Kareem, F., Fatten, M., & Abd-El Latif. (2012). Using bicarbonates for controlling late blight disease of potato plants under field conditions. *Life Science Journal*, 9(4), 2080–2085.
- Cerný, M., Hab'anov'a, H., Berka, M., Luklov'a, M., & Brzobohatý, B. (2018). Hydrogen peroxide: its role in plant biology and crosstalk with signaling networks. *International Journal of Molecular Sciences*, 19. 2812.

- Bahramisharif, A., & Rose, L.E. (2019). Efficacy of biological agents and compost on growth and resistance of tomatoes to late blight. *Planta*, 249. 799–813.
- Egel, D., Hoagland, L., Davis, J., Marchino, C., & Bloomquist, M. (2019). Efficacy of organic disease control products on common foliar diseases of tomato in field and greenhouse trials. *Crop Protection*, 122. 90–97.
- Hosseinzadeh, S. R., Amiri, H., & Ismaili A. (2016). Effect of vermicompost fertilizer on photosynthetic characteristics of chickpea (*Cicer arietinum* L.) under drought stress. *Photosynthetica*, 54. 87–92.
- Jindo, K., Olivares, F.L, Malcher, D.J.P., Sánchez-Monedero, M.A., Kempenaar, C., & Canellas, L.P. (2020). From Lab to Field: Role of humic substances under open-field and greenhouse conditions as biostimulant and biocontrol agent. *Frontiers in Plant Sciences*, 11. 426–434.
- Lichtenthaler, H.K., & Wellburn, A.R. (1983). Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochemistry Society Transactions*, 11. 591–592.
- Loggini, B.A., Brugnoli, S.E., & Navari-Izzo, F. (1999). Antioxidative defense system, pigment composition, and photosynthetic efficiency in two wheat cultivars subjected to drought. *Plant Physiology*, 119. 1091– 1099.
- Mourad, B., Baha, E., & Mokhtar, B. (2017). The impact of a hexaconazole fungicide on agronomic, biochemical parameters and yield components of green beans *Phaseolus vulgaris* cv. Djedida. *International Journal of Advanced Engineering and Management*, 2(6), 146–152.
- Nazir, F., Fariduddin, Q., & Khan, T.A. (2020). Hydrogen peroxide as a signaling molecule in plants and its crosstalk with other plant growth regulators under heavy metal stress. *Chemosphere*, 25. 126486.
- Ngegba, P.M., Cui, G., Khalid, M.Z., & Zhong, G. (2022). Use of botanical pesticides in agriculture as an alternative to synthetic pesticides. *Agriculture*, 12. 600. https://doi.org/10.3390/.
- Noctor, G., & Foyer, C.H. (1998). Ascorbate and glutathione: keeping active oxygen under control. *Annual Review of Plant Physiology and Plant Molecular Biology*, 49. 249–279.
- Siddiqui, Y., Meon, S., Ismail, R., & Rahmani, M. (2009). Bio-potential of compost tea from agro-waste to suppress *Choanephora cucurbitarum* L. the causal pathogen of wet rot of okra. *Biological Control*, 49(1), 38–44.
- Velikova, V., Yordanov, I., & Edreva, A. (2000). Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective role of exogenous polyamines. *Plant Science*, 151(1), 59– 66.