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Study of selected crop plants responses in the pesticide – biostimulator – pathogen system

PhD dissertation

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Abstract

Crops play a key role in ensuring the food security of society. In the group of cereals, wheat (*Triticum aestivum*) is a crop of key importance in the human economy, which is used primarily for consumption purposes. Among leafy vegetables, lettuce (*Lactuca sativa*) is a widely cultivated alimentary plant, which is a rich source of fiber, minerals and vitamins. However, pressure of agrophages (pathogens, weeds and pests) adversely affects plant health and makes it difficult to ensure stable and high – quality crops. In order to protect crop plants against fungal pathogens, fungicides are used, which, despite their beneficial role in plant protection, are abiotic stress factors and can affect changes in the metabolic profile of crop plants. A common fungal pathogen in the cultivation of lettuce is *Botrytis cinerea*, which is the etiological agent of gray mold, while fungi of the genus *Fusarium* (including *Fusarium culmorum*) are frequent in the cultivation of wheat and are responsible e.g. for fusariosis of the ears. Moreover, the presence of pathogens constitutes biotic stress and negatively affects plants, leading to deterioration of crop quality and metabolic changes. In recent years, there has been a growing interest in biostimulators, i.e. environmentally friendly compounds that can improve the growth and condition of plants, especially in unfavorable climatic and soil conditions. However, there are few scientific reports presenting multidimensional studies of the response of crop plants in the pesticide – biostimulator – pathogen system at the level of plant metabolites.

The main goal of this PhD dissertation was a comprehensive analysis of the responses of two crop plants, commonly used for consumption purposes, from the group of leafy vegetables (*Lactuca sativa*) and cereals (*Triticum aestivum*) to the impact of pesticides, biostimulators and pathogenic fungi (*Botrytis cinerea* and *Fusarium culmorum*). Moreover, the collateral goal was to determine the role of organic and inorganic biostimulators in mitigating abiotic stress induced by pesticides and biotic stress caused by the presence of pathogenic fungi, as well as the impact of biostimulators and pathogens on the dynamics of pesticides dissipation in plants.

The above goals have been achieved through comprehensive scientific studies and statistical analyses. The research for the PhD thesis included experiments under controlled conditions and involved a modern analytical workshop, taking into account spectrophotometric

techniques as well as liquid and gas chromatography coupled with tandem mass spectrometry (LC – GC – MS / MS).

In the research for this PhD thesis, a different response of monocotyledonous and dicotyledonous plants to abiotic and biotic stress was obtained.

It was determined that the separate application of two fungicides: aniline (fluazinam) with quinone (dithianon) or guanidine (dodine) in lettuce resulted in increased biosynthesis of carotenoids, protein and phenolic compounds, and a reduction of carbohydrate concentration compared to the application of single fungicides. The fungicides used: fluazinam, dithianon and dodine induced the activity of antioxidant enzymes (catalase, NADH – dependent peroxidase and superoxide dismutase), non – enzymatic antioxidants (glutathione and ascorbate) and increased the antioxidant potential expressed by the activity of the DPPH radical. However, no higher activity of oxidative stress markers was demonstrated, except for ascorbate, in alleviating abiotic stress caused by the separate use of two fungicides (dithianon + fluazinam or dodine + fluazinam) compared to single fungicides (fluazinam, dithianon, dodine). The presence of the second active substance in lettuce increased the half – life (DT_{50}) of fluazinam and shortened the dissipation time of dithianon and dodine.

The fungicide azoxystrobin was introduced into the next tested system and lettuce plants were inoculated with the pathogenic fungus *B. cinerea*. It was indicated that the combined effect of abiotic stress, caused by the use of fungicides (azoxystrobin or fluazinam) and biotic stress (*B. cinerea*), intensified the decrease of chlorophylls and carotenoids concentration and the increase of carbohydrates, phenolic compounds and protein level compared to non – inoculated plants treated with fungicides. It was determined that the combined abiotic and biotic stress induces the activity of antioxidant enzymes (especially catalase and NADH – dependent peroxidase) and the concentration of ascorbate, compared to non – inoculated plants exposed to abiotic stress. Different levels of the analyzed metabolites were determined depending on the concentration of fungicides during their degradation (dynamics of dissipation). Moreover, it was determined that the pathogenic fungus *B. cinerea* affects the dynamics of fungicide decay, shortening the half – life of azoxystrobin and prolonging the decay of fluazinam.

In next studies conducted in wheat plants, systems with biostimulators were tested. The studies indicated that inorganic biostimulators based on silicon and titanium compounds alleviated abiotic stress caused by spiroketalamine (spiroxamine) and triazole (cyproconazole, propiconazole, tebuconazole, triadimenol) fungicides at the level of protein, carbohydrates and phenolic compounds in the grain. It reflects the concentration increase of these compounds as a result of the application of biostimulators, compared to analogous treatments without their

use. The indicated effect of biostimulators is also expressed by an increase in the activity of antioxidant enzymes (catalase and NADH – dependent peroxidase). Titanium and silicon compounds improved phosphate nutrition by reducing the activity of acid phosphatases, the induction of which is characteristic in deficiency conditions of assimilable phosphorus forms. Biostimulators also reduced the contamination of wheat grain with mycotoxins. Based on multidimensional statistical analyzes expressed by principal component analysis and Pearson correlation coefficients, it was indicated that the concentration of amino acids such as phenylalanine, arginine, aspartic acid, isoleucine, tyrosine and glutamic acid is positively correlated with the activity of enzymes: catalase and NADH – dependent peroxidase, therefore they can be a building compounds for defense proteins against abiotic stress. Moreover, it was determined that the titanium and silicon biostimulators reduced the half – life (DT_{50}) of fungicides such as spiroxamine, tebuconazole and triadimenol, while titanium compounds affected the faster degradation of the fungicide propiconazole, which is crucial in optimizing the application time and grace period of pesticides.

Sulfonylurea herbicide (sulfosulfuron) was introduced into the next tested pesticide – biostimulator system in wheat and inoculation with the pathogenic fungus *F. culmorum* was carried out. The impact of the herbicide sulfosulfuron, spiroketalamine and triazole fungicides, and organic biostimulators (based on humic acids and nitrophenols) resulted in a different response at the level of some of the analyzed metabolites. The impact of biotic (*F. culmorum*) and abiotic (pesticides) stress combined with nitrophenols resulted in a reduced content of protein, carbohydrates and phenolic compounds in wheat grain, compared to the application of humic acids in inoculated plants exposed to pesticides. The impact of pesticides combined with humic acids reduced the activity of acid phosphatases to the greatest extent. It was also determined that the enzymes: catalase and NADH – dependent peroxidase conditioned the mitigation of abiotic stress caused by sulfonylurea herbicide and fungicides. However, only in the case of catalase, the induction of its activity in the pesticide – biostimulator – pathogen system was indicated. Moreover, biotic stress in wheat reduced the half – life of fungicides such as cyproconazole, spiroxamine and triadimenol. The impact of humic acids or nitrophenols in plants exposed to combined biotic and abiotic stress resulted in an increased shortening of the dissipation time of the herbicide sulfosulfuron and fungicides: propiconazole, cyproconazole, spiroxamine and tebuconazole, compared to the combined impact of organic biostimulators and pesticides in non – inoculated plants.

As a result of the research carried out under controlled conditions, various responses of crop plants in the pesticide – biostimulator – pathogen system were determined. The level of

metabolites may fluctuate periodically depending on the crop species, the type and concentration of the pesticide, the use of biostimulators, and the presence of pathogenic fungi. It was determined that oxidative stress markers are of key importance in alleviating stress caused by the use of pesticides, and their activity is related to the dynamics of pesticide dissipation. Organic/inorganic biostimulators caused the mitigation of abiotic/biotic stress in crop plants and increased the concentration of some of the desired metabolites. Moreover, pathogenic fungi and biostimulators can affect the dynamics of pesticide dissipation, which has a key impact on their grace period in crops intended for consumption. Statistical analyzes indicated correlations between some of the tested compounds in the experimental configurations used. The results of this PhD thesis shed new light on the responses of crop plants in the pesticide – biostimulator – pathogen system and can be implemented in agricultural practice.

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