Influence of Biologically Active Substances on Key Indicators of the Conditions of Winter Wheat Ecocenosis

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Influence of biologically active substances on key indicators of the conditions of winter wheat ecocenosis

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Abstract. The article deals with the study of the effect of the application of the herbicide Granstar Gold 75, w.g. (water-soluble granulate) and plant growth regulator Regoplant on some ecological indicators of ecocenosis of winter wheat sowing (lipid peroxidation reactions in winter wheat plants by the activity of malonic dialdehyde content accumulation, enzymatic activity, total number of rhizosphere bacteria and anatomical structure of winter wheat leaves). The choice of research topic is due to the fact that currently obtaining high yields of winter wheat is closely connected to the widespread use of chemicals, in particular, herbicides, which by their nature are physiologically active substances that can affect both plants and soil microbiota. This, in turn, leads to the search for environmentally safe elements of technologies for growing winter wheat. One such element may be the use of herbicides together with plant growth regulators. The obtained experimental data testify to the protective ability of the growth regulator Regoplant against the winter wheat plants, as evidenced by a decrease in the activity of malonic dialdehyde accumulation, changes in the activity of the enzymatic defense system, decrease the number of epidermis cells and an increase in total rhizosphere bacteria in case of Regoplant use together with Granstar Gold 75, w.g. compared with the experiment variants, where the herbicide was applied without a growth regulator. That is, the use of growth regulator in a mixture with herbicide to some extent eliminates the toxic effect of xenobiotics, which has a positive effect on the state of ecobiosis of winter wheat sowing.

1 Introduction

The current stage of agriculture intensification is associated with the widespread use of various chemical compounds, which, along with increasing crop yields, significantly change the living conditions of both cultivated plants and soil biota. Therefore, research aimed at developing more environmentally friendly technologies for growing major crops are relevant and promising [1].

Also an urgent issue of modern agroecology is the development of scientifically sound criteria for assessing the environmental risks of herbicides in the technology of growing major cereals, in particular, winter wheat. After all, the peculiarity of agriculture in Ukraine is the extremely high variety of agri-environmental conditions, which cause certain environmental restrictions on the permissible range and conditions of application of herbicides. In the practice of the chemical method of winter wheat crops protection from weeds, a promising measure is the integrated use of herbicides and plant growth stimulants in tank mixtures. This method of using preparations in terms of environmental feasibility contributes to leveling the negative effects of herbicides on winter wheat plants and ecocenoses in general, which in turn has a positive effect on improving the ecological state of the environment [2].

However, the ingress of herbicide active ingredients into the plant organism can cause stress, which contributes to the formation of reactive oxygen intermediates (ROI) such as hydrogen peroxide, singlet oxygen etc., which are produced both by enzymatic oxidation and by redox reactions. Subsequently, the enhanced ROI formation activates free radical oxidative processes [3, 4].

To reduce the level of ROI, which are toxic to the plant organism, is the activation of lipid peroxidation reactions (LP) [5]. Activation of LP is one of the rapid and nonspecific reactions of living organism cells to the stress factors [6].

Also, the LP activation can cause structural-functional and physiological-biochemical disorders in cell membranes [7].

It is established that the process of ROI (reactive oxygen intermediate) formation is natural and also occurs under optimal conditions of plant growth and development, but under the influence of external factors, including xenobiotics, it is greatly enhanced, so to eliminate ROI (hydrogen peroxide, singlet oxygen, etc.)...
in plant cells activate antioxidant systems protection, including enzymatic ones [8].

Adaptation of the plant organism to stressful conditions due to the toxic effects of xenobiotics occurs with the help of components of the enzymatic defense system, including enzymes of oxidoreductase (catalase, peroxidase and polyphenol oxidase), because they play a significant role in plant protection reactions [9].

In general, the activity of lipid peroxidation (LP) and the activity of the enzymatic defense system components can be the environmental indicators of assessing the degree of xenobiotics influence.

Therefore, the LP activation is considered as a universal response of a living system to the action of stressors, in particular, herbicides. In the body of plants under normal conditions, the intensity of LP is maintained at a steady level, which is provided by a system of antioxidant protection, consisting of antioxidant enzymes and low molecular weight compounds.

Also, when using herbicides, there is a danger of exposure of this type of xenobiotics on non-target objects, which are soil microorganisms. Herbicide studies have shown that xenobiotics and other biologically active substances can change the number of soil microbiota. In turn, microorganisms, due to their physiological properties, are quite sensitive to various environmental factors and anthropogenic influences and can be used in the diagnosis of environmental assessment of crop growing technologies [10, 11].

Thus, the studies by H.A. Valid Ibrahim [12] have shown that the application of the herbicide Stomp (5.0 l/ha) in sunflower and maize areas caused a decrease in the micromycetes number in the rhizosphere of plants, along with the use of Frontier in the rates of 1.7 and 1.5 l/ha contributed to the growth of their number.

The use of herbicides in winter wheat crops revealed a toxic effect of Lentipur, which inhibited the development of bacteria and actinomycetes in the crop rhizosphere by 1.5 and 1.3 times, respectively, and micromycetes – 1.8 times [13], while on maize areas the herbicide Bazys 75 at the rate of 20 g/ha did not have a pronounced negative effect on the number of major microbiota groups [14].

Other studies have shown that tank mixtures of herbicides with plant growth regulators contribute to its growth, which has a positive effect on the ecocenosis of crops by activating the rate of xenobiotics detoxification [15].

In general, most works covering the effect of xenobiotics on the soil microbiota contain data on both the inhibitory and stimulating effect of the latter on microorganisms and the course of microbiological processes in the soil [16, 17].

That is, today there is no single opinion about the impact of herbicide application on the soil microbiota and its number [18].

Also one of ecological indicators of an organism condition of winter wheat plants can be anatomic and morphological structure of epidermis of its leaves. Anatomical signs of a plant organism during its development under the influence of ecological factors, including anthropogenic character, constantly change, plants, the condition of which can characterize the depth of different factors influence on the plant organism.

The epidermis of plant leaves is a multifunctional tissue, the size and condition of the cells of which are able to characterize the water balance of the plant, the intensity of the processes of carbon dioxide absorption, which in turn has an impact on plant productivity. Histological features of primary integumentary cells depend on many biotic and environmental factors [19].

It is known from the practice of growing crops that the introduction of biologically active substances, including herbicides and plant growth regulators, significantly affects the formation of the structure of the epidermis of plant leaves [20].

Thus, the use of herbicides in crops of spring barley, winter wheat and other increases the number of epidermal cells of leaves by 5–30% and the formation of the number of stomata per unit of the leaf surface area compared to control [21].

It is known that the anatomical and morphological structure of a plant leaf significantly depends on changes in environmental factors, especially anthropogenic, which are herbicides [22].

Therefore, the anatomical and morphological structure of the vegetative organs of plants, in particular the leaves, is evidence of the plasticity of the plant organism [23].

In this regard, the change in the structure of the leaves epidermis is an indicator that characterizes the ecological condition and adaptation of the plant organism to environmental factors [24].

Modern toxicants such as herbicides have a systemic effect, which is manifested immediately by application of the toxicant on the leaves. From the leaf surface through the epithelial and cuticular formations of the active substances of products penetrate into the cells of the columnar and spongy parenchyma of the leaf mesophyll and from there - to other tissues and organs of plants.

Due to the change in the balance of endogenous phytohormones due to the influence of the active substances of herbicides, disturbances in the course of growth processes in the plant organism can occur, causing morphological and anatomical changes in tissues and organs. Growth regulators of plants of exogenous origin also have a significant impact on the anatomical structure of plant tissues and organs, in particular due to the growth of mitotic activity of meristematic tissues [25, 26].

Based on the above, one of the tasks of our research was to study the change in lipid peroxidation reactions, enzymatic activity, the number of rhizosphere microbiota and the anatomical structure of winter wheat leaves as environmental indicators of the herbicide
Granstar Gold 75, w.g. and plant growth regulator Regoplant on the ecocenosis of the winter wheat sowing.

2 Material and Methods

Research on this topic was conducted in the field and laboratory conditions of the Department of Biology of Uman National University of Horticulture on the plantings of soft winter wheat Lazurna (originator – Institute of Plant Physiology and Genetics of the National Academy of Sciences of Ukraine) during 2018–2020. The herbicide Granstar Gold 75 v.g. (manufacturer – American company DuPont, active substance – Tribenuron-methyl, 750 g/kg) in the rates of 15, 20, 25 and 30 g/ha and the growth regulator Regoplant (50 ml/ha) (manufacturer – CJSC «Vysokyi Vrozhai») before the emergence of the flag leaf of the crop.

The soil of the experiment was the low-humic heavy loam podzolized chernozem on loess. Its arable layer contains 3.5 % of humus, 88 and 132 mg/kg of phosphorus and potassium mobile compounds (by Chirikov method) respectively, 103 mg/kg of nitrogen easily hydrolyzed compounds (by Cornfield method), 6.2 of pH, 2.26 smol/kg of soil hydrolytic acidity [27].

The experiment was laid down by a systematic method with sequential placement of variants in four repetitions. A detailed scheme of the experiment is shown in figure 1.

The herbicide and the growth regulator were applied with the DS-3WF-3 battery-powered knapsack sprayer at the rate of 200 l/ha of the working mixture. The rates of herbicide application on the experimental plots were calculated based on the hectare rates of their application, taking into account the area of the plot and the rate of liquid consumption. The total area of one experimental plot was 40 m² and the accounting area – 20 m².

The intensity of the LP reactions in winter wheat leaves was determined by the method of malonic dialdehyde accumulation in the modification of V.V. Rogozhyn [28]. The activity of catalase, peroxidase and polyphenoloxidase in winter wheat leaves was determined by the methods described by V.V. Rogozhyn [28].

The number of microorganisms in the ecocenosis of winter wheat plantings in the crop flowering stage. The soil samples from winter wheat rhizosphere were taken in accordance with the generally accepted methods [29]. The total number of rhizosphere bacteria are used as a supply source mainly by the organic forms of nitrogen (ammonification group) – by sowing a soil suspension of appropriate dilutions on agar medium MPA (meat-and-peptone agar) [30]. The number of microorganisms was expressed in the colony forming units (CFU) and thousands of cells in 1 g of completely dry soil.

The anatomical structure of winter wheat leaves was studied according to the Hrytsaienko’s method [31].

3 Results and Discussion

As indicated above, the introduction of xenobiotics, which are the anthropogenic toxicants, causes the activation of lipid peroxidation reactions in response to increasing levels of ROI.

Studies conducted during 2018–2020 found that the intensity of LP reactions, which was monitored by the level of malonic dialdehyde accumulation in winter wheat plants, was different and depended on the rate of xenobiotic use, both individually and in combination with the growth regulator Regoplant (fig. 1).
MDA/g of raw substance, compared with the herbicide application without Regoplant.

Such indicators of LP reactions obtained with the combined use of drugs may indicate a positive environmental effect of the growth regulator on the winter wheat ecocenosis, because the slowdown in the accumulation of malonic dialdehyde indicates a decrease in the toxic effect of the herbicide on winter wheat plants.

The study of the intensity of LP reactions on the fifth day after application of the herbicide and plant growth regulator showed that it increased compared to the previous period, but there was a similar between the LP reaction and the application rates of Granstar Gold 75, w.g. both separately and in conjunction with Regoplant (fig. 2).

**Fig 2.** LP reactions in winter wheat plants under the application of the herbicide Granstar Gold 75, w.g. and growth regulator Regoplant (fifth day after application), the average for 2018–2020 (LSD05=5.2).

1. Without preparation (control); 2. Regoplant 50 ml/ha; 3. Granstar Gold 75, w.g., 15 g/ha; 4. Granstar Gold 75, w.g., 20 g/ha; 5. Granstar Gold 75, w.g., 25 g/ha; 6. Granstar Gold 75, w.g., 30 g/ha; 7. Granstar Gold 75, w.g., 15 g/ha + Regoplant 50 ml/ha; 8. Granstar Gold 75, w.g., 20 g/ha + Regoplant 50 ml/ha; 9. Granstar Gold 75, w.g., 25 g/ha + Regoplant 50 ml/ha; 10. Granstar Gold 75, w.g., 30 g/ha + Regoplant 50 ml/ha.

Along with the study of the effect of the herbicide and plant growth regulator on the LP reaction to the malonic dialdehyde accumulation, the change in the activity of key enzymes of the oxidoreductase class (catalase, peroxidase and polyphenoloxidase) was also studied. These components of the enzymatic protection system are part of the formation of plant resistance to stressors, which in our case are herbicides.

It is established that on the average for the years of research by the application of herbicide in the rates of 15, 20, 25 and 30 g/ha without plant growth regulator catalase activity increased compared to the control version of the experiment by 1.28; 1.35; 1.41 and 1.31 times; peroxidase activity – 1.19; 1.25; 1.28 and 1.22 times and polyphenol oxidase activity – 1.09; 1.15; 1.23 and 1.25 times in accordance with the standards of Granstar Gold 75, w.g. (fig. 3).

1. Without preparation (control); 2. Regoplant 50 ml/ha; 3. Granstar Gold 75, w.g., 15 g/ha; 4. Granstar Gold 75, w.g., 20 g/ha; 5. Granstar Gold 75, w.g., 25 g/ha; 6. Granstar Gold 75, w.g., 30 g/ha; 7. Granstar Gold 75, w.g., 15 g/ha + Regoplant 50 ml/ha; 8. Granstar Gold 75, w.g., 20 g/ha + Regoplant 50 ml/ha; 9. Granstar Gold 75, w.g., 25 g/ha + Regoplant 50 ml/ha; 10. Granstar Gold 75, w.g., 30 g/ha + Regoplant 50 ml/ha.

With the joint application of Granstar Gold 75, w.g. and Regoplant there is an increase in the activity of the studied enzymes compared with the use of herbicide without plant growth regulator.

In particular, for making 15, 20, 25 and 30 g/ha Granstar Gold 75, w.g. in combination with Regoplant, catalase activity against control increased by 1.37; 1.41; 1.46 and 1.38 times; peroxidase activity in 1.24; 1.32; 1.36 and 1.22 times and polyphenoloxidase activity in 1.18; 1.21; 1.24 and 1.29 times according to the rates of herbicide application.

Study of the soil microbiota of the rhizosphere of winter wheat in the flowering stage revealed that such an indicator of the state of ecocenosis of crops as the total number of rhizosphere bacteria has a certain tendency to change under the action of the herbicide Granstar Gold 75, w.g. introduced both separately and in conjunction with the growth regulator Regoplant (fig. 4).

It was found that in case of 50 g/ha Regoplant application, the total number of rhizosphere bacteria compared to the control version of the experiment on average over the years of research increased by 230 thousand CFU. In case of the Granstar Gold 75 w.g. herbicide application in the rates of 15, 20, 25 and 30 g/ha there was a certain inhibition of the rhizosphere...
bacteria development compared to the action of Regoplant, but their number exceeded the control variant by 180, 120, 50 and 10 thousand CFU, respectively.

Increase in the total number of rhizosphere bacteria under the action of Granstar Gold 75, w.g. while increasing the rate of xenobiotics use indicates the toxic effect of the chemical on the ecocenosis of winter wheat sowings.

With the joint application of herbicide and plant growth regulator, there was an increase in the total number of rhizosphere bacteria both against control and against the experiment variants with the use of Granstar Gold 75, w.g. without Regoplant, which indicates a positive effect of plant growth regulator on the ecocenosis of winter wheat plantings.

In particular, under the action of 15, 20, 25 and 30 g/ha of herbicide in a mixture with Regoplant, the total number of rhizosphere bacteria compared to the control variant increased by 220, 170, 120 and 80 thousand CFU, respectively. Compared with the use of herbicide without growth regulator, there was an increase in the number of rhizosphere bacteria by 40, 50, 70 and 70 thousand CFU in accordance with the rates of xenobiotics.

It was established that the studied preparations also had an effect on the anatomical structure of winter wheat leaves.

In particular, under the action of 15, 20, 25 and 30 g/ha of Granstar Gold 75, w.g. there is an increase in the number of epidermal cells from 134 to 178 pcs/mm². When the herbicide is co-administered with a growth regulator, there is a slight decrease in the number of epidermal cells compared to a single application of Granstar Gold 75, w.g., however, with increasing rate of the preparation in the mixture, the number of cells also to increase from 115 to 161 pcs/mm² (fig. 5).

The herbicide application without a growth regulator at the rate of 15 g/ha increased the area of one epidermal cell by 6 μm² against the control variant, while under 15 and 20 g/ha of herbicide the cell area exceeded the control by 10 and 18 μm². The application of the maximum rate of 30 g/ha had a pronounced toxic effect on the body of winter wheat plants, which was manifested in a decrease in cell area compared to previous rates, and this figure was almost at the level of the control variant (fig. 6).
In case of joint use of Granstar Gold 75, w.g. and Regoplant, the protective effect of the growth regulator on the organism of winter wheat dew was observed, which was manifested in an increase in the area of epidermal cells compared to the variants of the experiment, where the herbicide was applied alone. The largest cell area was observed under the action of 25 g/ha of herbicide in a mixture with Regoplant – 30 μm² more than the control and 12 μm² more than the variant where Granstar Gold 75, w.g. made without a growth regulator.

When determining the volume of one cell, there was a tendency to reduce this indicator in proportion to the increase in the rate of herbicide application. In particular, under the action of 15, 20, 25 and 30 g/ha of product without Regoplant there was a decrease in the volume of one cell from 1520 to 1380 μm³, but these figures exceeded the control value.

With the joint use of Granstar Gold 75, w.g. and Regoplant there was a certain increase in the volume of one cell compared to the variants of the experiment, where the herbicide was used without a growth regulator, but the tendency to decrease the volume of one cell in proportion to the increase in herbicide application in the mixture was maintained. Thus, for making 15, 20, 25 and 30 g/ha of Granstar Gold 75, w.g. with the growth regulator, the volume of one cell varied from 1810 to 1670 μm³ according to the herbicide standards.

The tendency to decrease the number of epidermis cells of winter wheat leaves with a simultaneous increase in their surface, which can be traced in the variants of the experiment with the combined use of toxicant and plant growth regulator, indicates the formation of mesomorphic type of leaf blade. This type of leaf blade begins to form under more favorable growth conditions for the plant, so we can assume that the combined effect of the studied products has a positive effect on the winter wheat ecocenosis as a whole. In contrast, a slightly larger number of cells in the control variant (with a high level of weeding) and an increase in the rate of herbicide application with a decrease in the area and volume of one cell indicates the formation of xenomorphic leaf type, which develops under less favorable living conditions.

4 Conclusion

Therefore, the obtained experimental data indicate that the use of herbicide Granstar Gold 75, w.g. and plant growth regulator Regoplant has a certain influence on such indicators of ecological stability of ecobiosis of winter wheat plantings as the intensity of lipid peroxidation reactions in the plants, the activity of enzymes and the total number of rhizosphere bacteria in the soil.

The application of Granstar Gold 75, w.g. without plant growth regulator is characterized by a certain toxic effect on both winter wheat plants and soil microbiota, which is manifested in increased activity of accumulation of malonic dialdehyde (indicating the activation of the lipid peroxidation reactions), changes in the antioxidant enzymes activity and a certain decrease in the total number of rhizosphere bacteria. The degree of xenobiotic influence on the specified indicators of a condition of ecobiosis of winter wheat plantings has a tendency to strengthening together with the increase of the preparation use rate. So, in particular, on the third day after the application of Granstar Gold 75, w.g. in the rates of 15, 20, 25 and 30 g/ha without Regoplant, there was an increase in the intensity of LP reactions in winter wheat plants against control from 3.2 to 17.4 μMol MDA/g of raw substance, depending on the rate of xenobiotic application. The activity of the enzymatic defense system increased in 1.18–1.46 times, depending on the rate of herbicide and type of enzyme. The total number of rhizosphere bacteria under the action of the herbicide without a growth regulator decreased from 1680 to 1510 thousand CFU by increasing the rate of the drug from 15 to 30 g/ha.

The combined use of preparations indicates the protective effect of the growth regulator on winter wheat plants and soil microbiota, which is manifested in a certain slowdown in the reactions of lipid peroxidation and an increase in the number of rhizosphere bacteria. In particular, on the fifth day after the application, the accumulation of malonic dialdehyde, although exceeding the control variant, but against the variants of the experiment with the use of herbicide without Regoplant decreased by 1.7–7.1 μMol MDA/g of raw substance according to the herbicide rates in a mixture with plant growth regulator. Under the joint application of preparations by 40–70 thousand CFU, the number of rhizosphere bacteria increased compared to the use of herbicide without plant growth regulator. Also under the using of preparation was noted dependence to decrease the number of epidermis cells of winter wheat leaves with a simultaneous increase in their surface.

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