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Mitigating effect of PGP-bacteria *Pseudomonas koreensis* IB4 on growth and biochemical parameters of wheat plants during their treatment with herbicides

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In this study strain *Pseudomonas koreensis* IB4, belonging to PGP-bacteria, with various useful properties such as nitrogen fixation, phytohormonal and fungicidal activity was studied. It was able to mitigate stress and significantly improve the condition of wheat plants against the background of stress caused by such herbicides as: "Chistalan" (commercial name) — based on 2,4-D (2-ethylhexyl ether) and dicamba (sodium salt); 2) "Nanomet" (commercial name) — based on metsulfuron methyl. This herbicides were chosen because of their wide prevalence and applicability. After treatment, the plants were evaluated according to several parameters — growth characteristics, the content of proline, malondialdehyde (MDA) and chlorophylls. In our experiments herbicides caused an increase in the amount of MDA in the cells, for "Chistalan" this parameter exceeded the control by 104% and by 73% for "Nanomet", there was also influence on other parameters. The strain *Pseudomonas koreensis* IB4 studied in this work, when combined with herbicide spraying, decreased the amount of MDA, normalized the amount of proline, chlorophylls and growth parameters. This paper shows that PGP bacteria are able to mitigate the negative effects of agrochemicals, herbicides in particular.

Keywords: *Pseudomonas koreensis*, herbicidal stress, PGP-bacteria, anti-stress activity, wheat

INTRODUCTION

The key place in economic well — being is given to food issues, namely, agriculture.One of the main problems of which are weeds, causing a decrease in yield up to 35 % (Oerke, 2006). Herbicides play an important role here, they allow to extend the productivity of agricultural crops, eliminating competition for nutrients, space and resources. However, there is also a negative impact. Being xenobiotics by their nature, herbicides can cause damage to the microbiota and biochemistry of soils (Cycon et al. 2013). In addition, their influence on the cultivated plants themselves is not without a trace. The use of chemicals causes stress in plants and shortening of the vegetation period (Kutuzova et al. 2006, Kumar, Singh, 2010, Su et al. 2018). Thus, there are two main problems: firstly, the long decomposition time of pesticides (Poddymkina, 2007); secondly, harmful effect on cultivated plants (Stetsov, 2015). The microbial decomposition of pesticides in soil is well

observed in scientific literature (Silva et al. 2007, Martins et al. 2011, Kanissery, Sims, 2011). But the influence of bacteria on the negative processes in plants, including the so - called "herbicidal stress", remains poorly understood. It is known that photosynthesis and the antioxidant system are vulnerable to abiotic stresses during the formation of plant biomass. There are only a few works on this topic in the literature. (Ahemad, Khan, 2010; Nahi et al. 2016; Bourahla et al. 2018; Chennappa et al. 2018, Kutilkin et al. 2018; Chetverikov et al. 2020). The interaction of flora and bacteria in the processes of hormonal regulation, nitrogen fixation, resistance to phytopathogens, nutrition, allows the former to successfully cope with negative factors. The association of PGP bacteria and plants, due to the complex nature of interaction, contributes to the overcoming of abiotic and biotic stresses by agricultural crops (Backer et al. 2018; Orozco-Mosqueda et al. 2018; Chetverikov et al. 2020; Khatoon et al. 2020). This paper investigates the anti-stress effect of the *Pseudomonas koreensis* IB4 strain of PGP bacteria on wheat seedlings against the background of pesticide stress. The purpose of this work is to evaluate the ability of *P. koreensis* IB4 to mitigate the negative effect caused by herbicides with various active substances: 1) "Chistalan" — based on 2,4-D (2-ethylhexyl ether) and Dicamba (sodium salt); 2) "Nanomet" — based on metsulfuron methyl. These herbicides were chosen because of their wide prevalence and applicability.

MATERIALS AND METHODS

The object of research was a museum strain *P. koreensis* IB4 deposited in the collection of microorganisms of the Ufa Institute of biology – Subdivision of the Ufa Federal Research Centre of the RAS. The strain has the properties of PGP microorganisms: production of phytohormones, fixation of atmospheric nitrogen, fungicidal activity (Rafikova et al. 2016), as well as resistance to herbicides.

Plants of soft spring wheat (*Triticum aestivum L.*) of the Kinelskaya Jubilejnaya variety were grown in a phytotron chamber in liter vessels filled with a mixture of sand and black earth soil in a ratio of 1:9, at a photon flux density of 190 micromol$\cdot m^{-2}\cdot s^{-1}$, a 14-hour photoperiod and in the temperature range 24-26 °C. Soil moisture was maintained at the level of 60 - 80% of the total moisture capacity.

Herbicidal stress was induced by products: 1) based on 2,4-D (2-ethylhexyl ether) and dicamba (sodium salt) — "Chistalan" (LLC "AHK-AGRO", Russia); 2) on the basis of a representative of sulfonylureas of metsulfuron-methyl — "Nanomet" (LLC "Pesticides.ru", Russia). These substances selectively act against broadleaf weeds growing among the crops of spring barley, spring and winter wheat.

Wheat plants were treated by spraying on the seventh day after the emergence of seedlings with the following compositions (based on one vessel): 1) 0.9 µl of “Chistalan”; 2) 13 µg of “Nanomet”;3) $5*10^7$ CFU of strain IB4. Herbicide amounts were determined based on working concentrations, taking into account the application rates according to the manufacturer's recommendations. The bacterial suspension was introduced both separately and together with the herbicide. Vessels with control plants were not exposed.

After three days after the treatment of plants, the content of chlorophyll, proline and malondialdehyde (MDA) in the leaves was determined.

Growth and weight parameters of shoots and roots were determined on the 14th day after spraying. For each variant of the experiment, 30 plants were grown.

The chlorophyll content was determined spectro photometrically after extraction with 96% ethanol (Vernon, 1960) and expressed in mg/g of dry weight.

The proline content was measured using a ninhydrin reagent, as described earlier (Bates et al. 1973), according to a calibration curve build on L-proline (Sigma, USA) of known concentrations, and expressed in µg/g of dry weight.

The MDA content was quantified by the Costa method (Costa et al. 2002) and expressed in µmol/g of dry mass.

Fresh material was used to measure the dry mass of roots and shoots, the length of shoots, proline, chlorophyll and MDA in the leaves.

The data were expressed as averages calculated using MS Excel software. The reliability of the differences between the averages was analyzed using the t-test.

RESULTS AND DISCUSSION

In this work, the ability of the PGP microorganism (*P. koreensis* IB4 *)* to mitigate the negative effects of herbicides was studied. Evaluation of this fact in relation to the strain of *P. koreensis* IB4 was carried out under controlled conditions of a laboratory experiment.

Thus, after treatment with "Chistalan", a significant decrease in the mass of shoots and

roots was observed, by 36% and 42%, respectively (Fig. 1). The length of the shoot at the same time decreased slightly, by 7%. "Nanomet" had a similar effect, but to a lesser extent, the weight reduction did not exceed 24% with the same slight decrease in the length of the shoot. Such a different influence of the tested agrochemicals is explained by belonging to different groups of their active substances.

With sole bacterial treatment, there was no significant change in the green part of wheat plants (the increase was equal to 3%). However, there was a decrease in root weight by 18%. This phenomenon is explained by the fact that the impact of bacteria does not necessarily cause an unambiguous increase in both the mass of roots and the mass of shoots. In the Riggs study, the treatment of maize with bacterial suspension did not lead to an increase in the above-mentioned characteristics, but at the same time a higher yield was achieved (Riggset al, 2001). For the "Nanomet + IB4" experiment variant, the values were at the control level. In the "Chistalan + IB4" variant, these values were lower than the control, but significantly higher than when treating wheat plants with only the "Chistalan" herbicide by 33% for the root mass and by 23% for the mass of shoots. Which indicates a decrease in the toxicity of herbicides when combined with *P. koreensis* IB4.

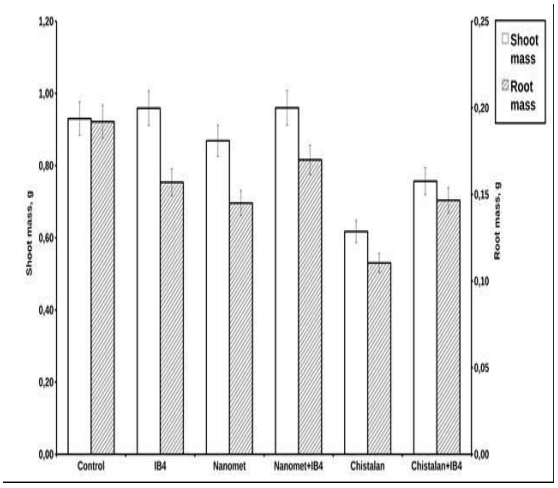

Figure 1: Effect of treatment on shoot and root mass.

When a plant is under stressful conditions, low-molecular metabolites accumulate in the cells. These markers allow to quantify the level of stress, some of them are MDA and proline. Thus, when exposed to the IB4 strain on plants, there was no accumulation of malondialdehyde (Fig. 2), and the proline content decreased by 34% at all (Fig. 3), i.e. when the growth characteristics changed, the plant was not under stressful conditions. When treated with auxin-like chemicals, the quantitative indicators of stress in plants change dramatically. When herbicides acces to wheat plants, enlarge in the activity of the enzyme peroxidase is observed in response to higher levels of free radicals and a decrease in protein concentration *(*Souahi et al. 2016*)*. MDA is one of the metabolites in lipid peroxidation and its accumulation in plants shows that they are under heavy oxidative stress. The high levels of MDA also shows an upturn in the number of free radicals (Carvalho et al. 2013). Thus, "Chistalan" heightened the level of MDA by 104%, "Nanomet" by 73%, which points out the occurrence of processes of strong oxidative stress in plant. When assessing the content of proline, it should be borne in mind that its concentration may reduce due to the suppression of protein biosynthesis (Fakhari et al. 2020) or vice versa may increase (Sharma, Dietz, 2006). The authors note the osmoprotective, antioxidant, signaling-regulatory and other functions of this amino acid (L. Szabados, A. Savouré, 2006; K. de Carvalho et al. 2013). An increase in the concentration of proline in wheat leaves was observed by researchers in response to contamination with metal ions, osmotic and temperature stresses (Yang et al. 2011.; Mwadzingeni et al. 2016). In our experiments, we observed a drop in proline concentration by 17% ("Chistalan") and 11% ("Nanomet"). In an experiment to mitigate the negative effects of the herbicides "Nanomet" and "Chistalan" with the strain IB4, when treated with the composition "herbicide + bacterium", the amount of MDA does not exceed the values in the control group of wheat plants for "Chistalan + IB4". For the variant "Nanomet + IB4", the amount of malondialdehyde was 19% lower than the control. Thus, there was a significant reduction in oxidative stress caused by exposure to herbicides in the presence of the strain *P. koreensis* IB4. A decrease in the level of MDA indicated a less damaging effect of reactive oxygen species in wheat leaves during inoculation of plants with *P. koreensis* IB4 bacteria, which contributed to maintaining their growth and indicated a favorable anti-stress effect of the bacterial strain studied in this work.

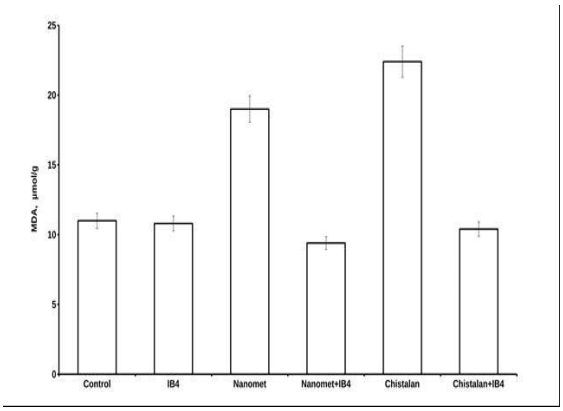

Figure 2: Effect of treatment om MDA content.

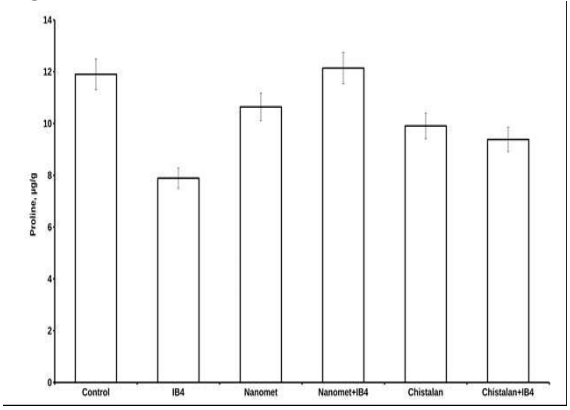

Figure 3: Effect of treatment on proline content.

Chlorophylls are an important plant system that allows light energy to be captured and involved into biochemical reactions. It is noted that the use of metsulfuron methyl augments the content of chlorophylls in wheat shoots (Singh et al, 2015). In our experiments, reduction in the content (Fig. 4) of chlorophylls in shoots was 7% for the "Nanomet" (the active substance is metsulfuron methyl) compared with the control. After treatment with "Chistalan", on the contrary, there was a decrease in chlorophylls by 7%. In the "Nanomet + IB4" and "Chistalan+IB4" experiments, the total content of chlorophyll was boosted by 11% and 7%, respectively, compared with herbicide treatment. That is, it has changed slightly. However, this parameter should be evaluated in conjunction with other markers. Avneet Kaur (Kaur, 2019) presents the results of both higher and lower level of chlorophylls depending on the dose of the herbicide, and therefore the degree of stress. According to the results of our experiment, with a slight change in the content of chlorophylls after spraying with herbicides, an increase in MDA and a decrease in the content of proline are observed (see the graph of chlorophylls, MDA and proline). When sprayed with a mixture of herbicide + bacterium, the concentration of MDA and proline declines. In other words, the reaction from the plant is complex.

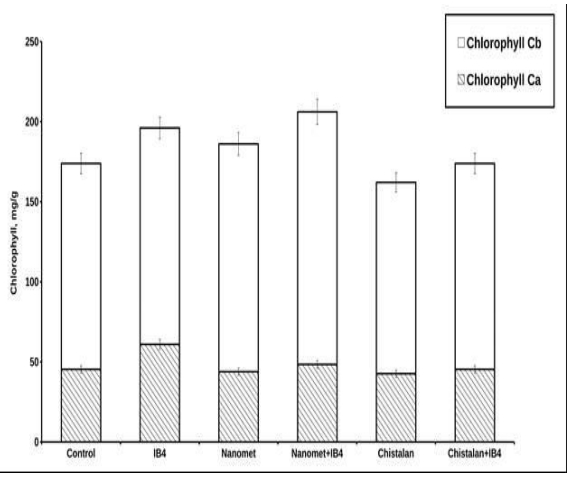

Figure 4: Effect of treatment on chlorophyll content (chlorophyll Ca and chlorophyll Cb).

CONCLUSION

When herbicides enter the plant body, changes occur at the level of biochemistry: the content of MDA increases, the content of proline changes. Which in turn leads to a shift in the concentration of chlorophylls and growth parameters. It is important to evaluate these phenomena in a complex. With an increase in the level of pigments responsible for photosynthesis and an increase in the mass of shoots and roots after treatment with herbicides, however, the plant may be under stress, as indicated by an increase in the content of MDA as a consequence of the accumulation of free radicals and a decrease in the concentration of proline due to a malfunction in the biosynthesis processes. *P. koreensis* IB4 bacteria, possessing a complex of useful properties such as nitrogen fixation, phytohormonal and fungicidal activity, are able to significantly improve the condition of wheat plants against the background of stress caused by herbicides.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

GH, SC, DC designed study, performed the statistical analysis, wrote and editing the manuscript. TR, ZS, NC were responsible for preparing the soil, sowing, monitoring of moisture content in the soil during the experiment, plant treatment with herbicides, with a composition based on culture fluid and their mixtures. AK, SS, DS, MT, AF analyzed the samples for the content of proline, MDA and chlorophylls. All authors read and approved the final version.

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