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Research Article

Plant growth promoting Rhizobacteria strain role in protecting crops sensitive to sulfonylurea herbicides from stress

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Abstract: The residues of metsulfuron-methyl in the soil can be a negative factor for the growth of susceptible crops to this herbicide. There are many successful examples of the use of bacteria to increase crop yields and protect plants against stress factors. The purpose of this work was to study the possibility of reducing the phytotoxic effect of metsulfuron-methyl in the soil on sugar beet using plant growth-promoting bacteria. Under greenhouse conditions, sugar beet seeds and bacteria were simultaneously placed in soil previously contaminated with methsulfuron-methyl. The weight of plants, leaf area, amount of proline, malondialdehyde, and flavonoids were measured. Suppression of the growth of young plants and oxidative damage caused by herbicides have been recorded. When sugar beet interacted with bacteria, *Pseudomonas protegens* DA1.2, oxidative stress caused by herbicide was mitigated, and the mass of plants increased. Treatment with bacteria against the background of herbicidal stress affected the dynamics of the content of flavonoids and proline, which play a role in the anti-stress reactions of plants.

Keywords: sugar beet, herbicidal stress, phytotoxicity, metsulfuron-methyl, *Pseudomonas protegens*, PGPR

Introduction

The sulfonylurea class of herbicides is widely used in agriculture due to their low danger to humans and animals (including beneficial insects), slow migration along the soil profile, and low application rates. Often, grain crops are treated with cheap sulfonylurea herbicides with a long period of persistence in the soil (Chkanikov *et al*., 2019).

The decomposition rate of persistent herbicides can vary significantly depending on weather conditions, agricultural practices, and other factors, and there are no simple methods for monitoring them in the soil. The hydrolysis half-life of the most slowly degradable substances from the sulfonylurea class can be more than 500 days in unfavorable soil-climatic conditions (Sarmah and Sabadie, 2002). All this makes it difficult to predict the residual amount of metsulfuron-methyl and chlorsulfuron at the time of subsequent sowing crops. The consequence is the appearance of stress symptoms in plants or even partial death of crops sensitive to these herbicides in the year

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following the use of herbicides (Kaur and Brar, 2014; Mehdizadeh and Abadan, 2018). Sugar beet is very sensitive to metsulfuron-methyl and is used to test the bioactivity of sulfonylureas (Szmigielski *et al.*, 2018).

Sustainable agriculture implies the inclusion of environmental factors in practice. For example, it was obtaining benefits from the relationship of plants with microorganisms. There are many successful examples of the use of bacteria to increase crop yields and protect against stress factors (Singh, 2018). A new development approach uses bacteria to reduce herbicidal stress in cultivated plants. In the field experiments, an improvement in the growth of wheat and legumes under the action of bacteria was observed against the background of the use of herbicides of different classes (Ahemad and Khan, 2010; Bourahla *et al*., 2018; Chetverikov *et al*., 2021). *Burkholderia cepacia* strain PSBB1 mitigated the toxicity of glyphosate and enhanced the size, dry matter, symbiosis, seed attributes, and nutritional contents of chickpeas (Shahid and Khan, 2018). Further, *B. cepacia* declined the levels of catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX), glutathione peroxidase (GPX), and malondialdehyde (MDA) contents at 4332 µg/kg soil glyphosate. Inthama *et al*. (2021) screened soil bacteria that could degrade paraquat and, at the same time, promote plant growth. The cowpea plants grown in paraquat-contaminated soil with *Bacillus aryabhattai* showed longer root and shoot lengths. Another study (Motamedi *et al*., 2022) was conducted to evaluate the effect of native plant growth-promoting bacteria isolated from the *Medicago sativa* rhizosphere, including *Serratia rubidaea, Pseudomonas putida, Synorhizobium meliloti*, on *M. sativa* and soil microbiota in the presence and absence of imazethapyr herbicide. Bacterial inoculation, in most cases, increased microbial population, plant biomass, and antioxidant activities.

The purpose of our work was to study the possibility of reducing the phytotoxic effect of metsulfuron-methyl residues in the soil on sugar beet seedlings using plant growth-promoting bacteria.

Materials and Methods

Bacterial strain

The bacterium strain *Pseudomonas protegens* DA1.2 was isolated by the authors from the rhizosphere of Trifolium repens L. growing on the anthropogenic soil of the Republic of Bashkortostan (Russian Federation) and deposited in the All-Russian Collection of Microorganisms as VCM B-3542D. It retains viability in the presence of metsulfuron-methyl (0.1% by weight), synthesizes indolyl acetic acid (0.87 mg/L) - a plant growth stimulator (Table 1), fixes atmospheric nitrogen, and shows antagonism to phytopathogenic fungi. Its anti-stress influence and positive effect on wheat yield were established in the field conditions of the Southern Urals (Chetverikov *et al.*, 2021). Bacteria were cultivated in King B medium (King *et al.*, 1954) in an orbital shaker (160 rpm) at a temperature of 28 °C for 72 h.

Table 1 Properties of *Pseudomonas protegens* DA1.2 (Chetverikov *et al*., 2021).

Properties	Values
Similarity of the 16S rRNA sequence (1410 bp), GenBank MT267792	*P. protegens* CHA0(T), 100%
Nitrogenase activity, nmol C_2H_4/(h·ml)	21.30 ± 0.22
Indoleacetic acid production, ng/ml	870 ± 44
Solubilization of phosphates	+
Antagonism:	
Alternaria alternate, A. solani, Bipolaris sorokiniana, Botrytis cirnea, Fusarium culmorum, F. gibbosum, F. graminearum, F. solani, F. oxysporum, Rhizoctonia solani	+

Design of a laboratory experiment

In a laboratory experiment, the soil taken from the arable layer of Chernozem Haplic was treated with the herbicide Nanomet (active ingredient - metsulfuron-methyl, 600 g/kg) at concentrations of 0.1 mg/kg and 0.5 mg/kg of soil, which corresponded to the recommended dose of the herbicide and the excess of this dose.

Sugar beet *Beta vulgaris* L. subsp. *vulgaris* variety Cascade 3 F1 was grown on a light site in 0.5 l vessels filled with soil previously

contaminated with the herbicide of various exposure durations (3; 90; 180; 270, and 360 days). Photon flux density was 190 µmol/(m^2·s), photoperiod was 14-h, the temperature was 24-26 °C, and soil moisture was 60-80% of the total moisture capacity. Half of the plants were treated with the culture of *P. protegens* DA 1.2, diluted with water to a titer of $2 \cdot 10^6$ cells/ml. Each variant of the experiment was repeated in five vessels.

Growth parameters

Growth and weight parameters of shoots were measured on the 10th day after spraying with bacteria. For each variant of the experiment, 30 plants were used. Shoots were weighed on analytical scales HR-250AZG (AND, Japan). The total area of cotyledon and true leaves was determined using the ImageJ program (National Institutes of Health. MA, USA, imagej.net, Accessed 30th May 2022).

Biochemical parameters

The amount of proline in the leaves was measured using ninhydrin (Bates *et al.* 1973), calibration was carried out using L-proline (Sigma, United States). Malondialdehyde (MDA) in the leaves was assessed based on its reaction with thiobarbituric acid (Costa *et al.*, 2002). It was chosen as a marker of oxidative stress. All measurements were carried out on fresh material.

The flavonoids in the leaves were measured using the DUALEX SCIENTIFIC + device (FORCE-A, France) according to the manufacturer's recommendations.

Statistical analysis

The data was analyzed in Statistica (Statsoft) software (version 10). The significance of differences between the averages was assessed by ANOVA followed by Duncan's test ($p \leq 0.05$).

Results

Growth parameters

In the control group of plants (on untreated herbicide soil), the values of all indicators varied slightly throughout the experiment. In the variant treated with the liquid culture of *P. protegens* DA1.2 increased weight by 27.6% (Fig. 1), and the total leaf area increased by 16.7% was observed (Fig. 2), compared with the control.

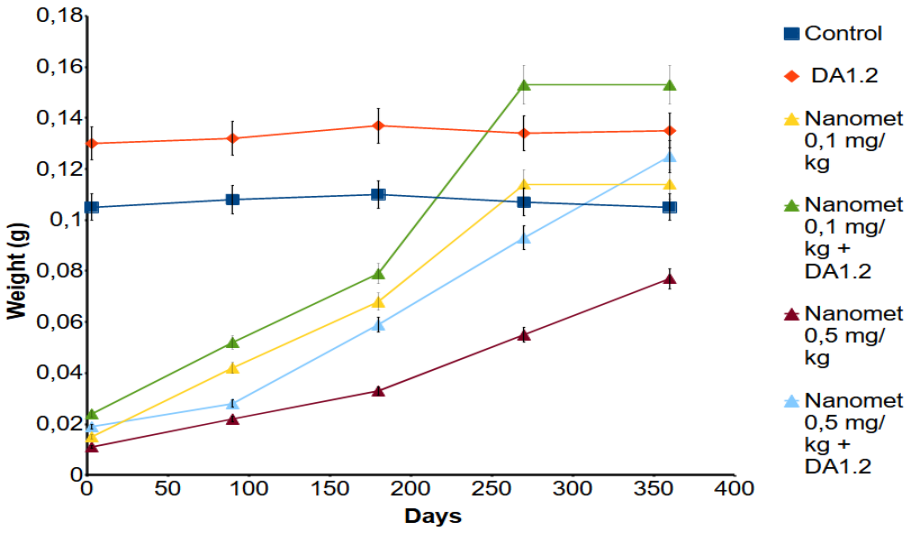

Figure 1 Dynamics of the average weight of shoot of ten-day sugar beet plants depending on the use of bacteria and the time since soil was treated with herbicide. Control - absence of any treatments; Nanomet - herbicide treatment with Nanomet at the written dose; DA1.2 - treatment with bacterial culture of *Pseudomonas protegens* DA1.2. A standard error is drawn for each value.

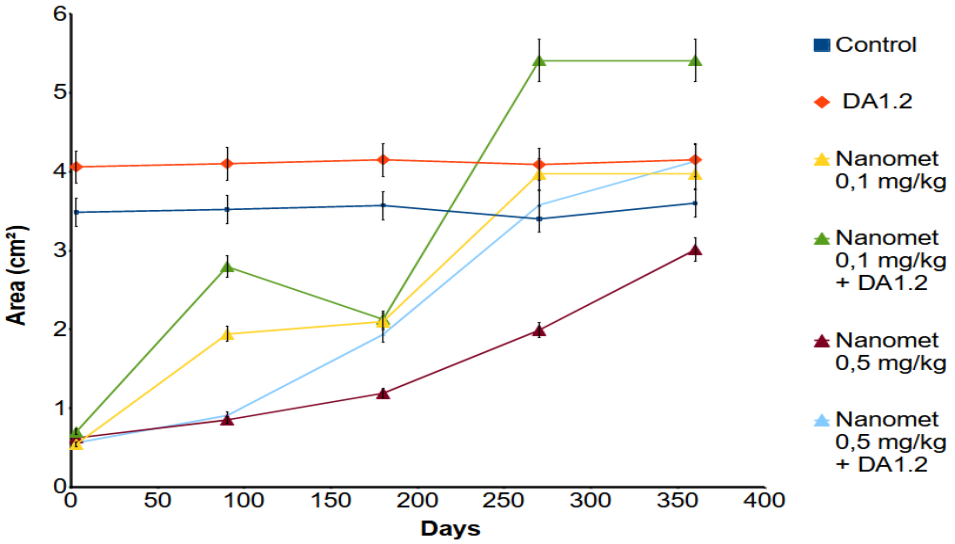

Figure 2 Dynamics of the average leaf area of ten-day sugar beet plants depending on the use of bacteria and the time since soil was treated with herbicide. Control - absence of any treatments; Nanomet - herbicide treatment with Nanomet at the written dose; DA1.2 - treatment with bacterial culture of *Pseudomonas protegens* DA1.2. A standard error is drawn for each value.

In the contaminated soil with Nanomet herbicide at 0.1 mg/kg and 0.5 mg/kg (variants "Nanomet - 0.1 mg/kg" and "Nanomet - 0.5 mg/kg", respectively), the total leaf area and shoot fresh weight decreased significantly compared to the control. The weight and total area of beet leaves in the "Nanomet — 0.1 mg/kg" variant with the recommended dose of herbicide returned to the level of control indicators only after 270 days. The more polluted methsulfuron-methyl soil remained toxic even after one year.

In variants with combined treatment (herbicide and bacterial strain *P. protegens* DA1.2), an increase in leaf area and aboveground biomass of plants was observed. This was also observed with a five-fold excess of the herbicide dose in the variant "Nanomet - 0.5 mg/kg + *P. protegens* DA1.2" compared with "Nanomet - 0.5 mg/kg".

Biochemical parameters

There was no significant increase in MDA and proline levels in untreated soil and on the variant with sole *P. protegens* DA1.2 treatment (not listed in the chart). On the contrary, in soil contaminated with Nanomet herbicide at concentrations 0.1 mg/kg and 0.5 mg/kg (variants "Nanomet - 0.1 mg/kg" and "Nanomet - 0.5 mg/kg", respectively) a clear accumulation of proline and MDA (Fig. 3.) was observed. In "Nanomet - 0.5 mg/kg", the content of MDA on the third day was 182.4% higher than the control. An increased MDA (88% higher than the control) was observed even in the 12th month after the introduction of the herbicide into the soil. When planting sugar beet in "Nanomet - 0.1 mg/kg" on the 270th day after contamination, there were no abnormal proline and flavonoid levels compared to the control (Table 2). The detox trend was observed for "Nanomet - 0.5 mg/kg" too, but significantly less due to initially higher concentrations of contamination.

The treatment of samples containing metsulfuron-methyl by bacteria resulted in the amount of proline and MDA in beet leaves falling to the control indicators at earlier exposure periods. But even under the influence of bacteria, the concentration of MDA in sugar beet leaves normalized only if the amount of herbicide introduced into the soil at the beginning of the exposure was not excessive.

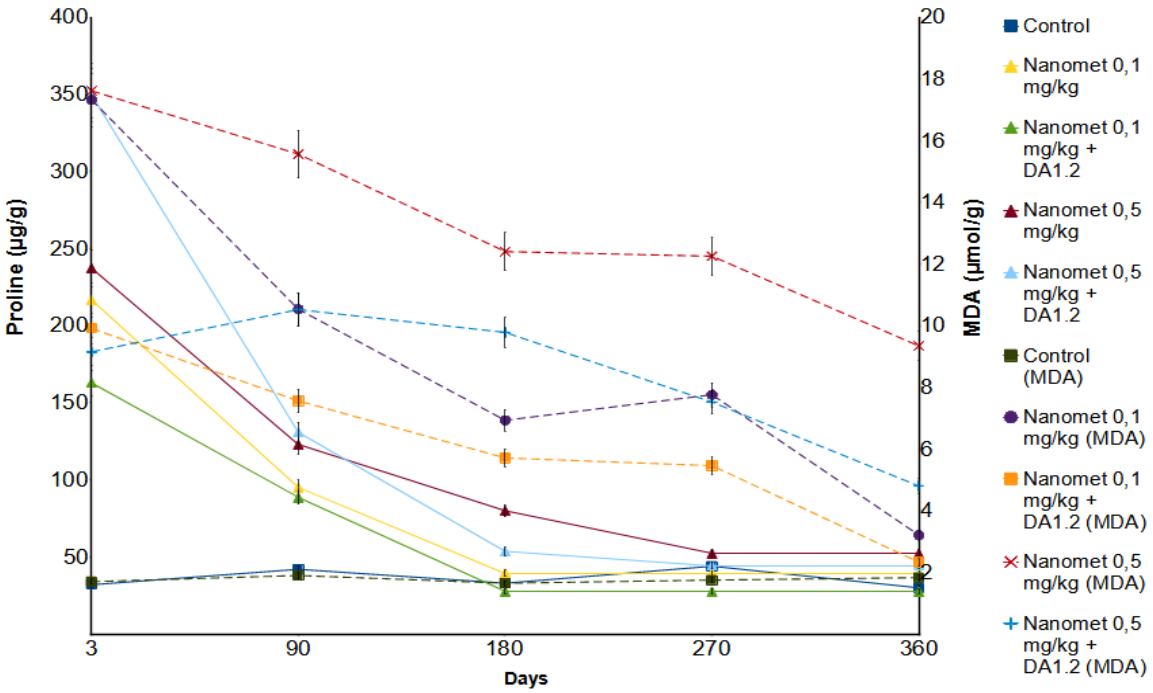

Figure 3 The amount of proline and malondialdehyde (MDA) in beet leaves depending on the use of bacteria and the time since soil was treated with herbicide. Control - absence of any treatments; Nanomet - herbicide treatment with Nanomet at the written dose; DA1.2 - treatment with bacterial culture of *Pseudomonas protegens* DA1.2. A standard error is drawn for each value. The concentration of proline is drawn with solid lines, and the concentration of MDA is drawn with dotted lines.

Table 2 The concentration of flavonoids in beet leaves depending on the time since soil was treated with bacterial culture of *Pseudomonas protegens* DA1.2 and Nanomet herbicide.

Days after treatment	Flavonoids content (conventional units)					
	Control	DA1.2	Nanomet (mg/kg)		Nanomet (mg/kg) + DA1.2	
			0.1	0.5	0.1	0.5
3	0.288 ± 0.020^{a}	0.422 ± 0.011^{b}	0.890 ± 0.096^{d}	0.822 ± 0.057^{d}	0.880 ± 0.058^{d}	0.705 ± 0.013^{c}
90	0.269 ± 0.026^{a}	0.387 ± 0.016^{b}	0.578 ± 0.065^{c}	0.723 ± 0.029^{d}	0.722 ± 0.054^{d}	0.82 ± 0.06^{d}
180	0,269 ± 0.007^{a}	0.389 ± 0.031^{bc}	0.340 ± 0.011^{b}	0.433 ± 0.011^{c}	0.364 ± 0.005^{b}	0.345 ± 0.027^{b}
270	0.277 ± 0.013^{a}	0.384 ± 0.020^{b}	0.288 ± 0.002^{a}	0.37 ± 0.012^{b}	0.293 ± 0.015^{a}	0.445 ± 0.028^{b}
360	0.274 ± 0.008^{a}	0.411 ± 0.024^{b}	0.280 ± 0.017^{a}	0.367 ± 0.022^{b}	0.268 ± 0.009^{a}	0.308 ± 0.033^{ab}

Average value ± standard error (n = 6). Means with the same letters in each row are not significantly different (Duncan's Multiple Range test, $p \leq 0.05$).

In the first days of the experiment and 90 days after its start, a high quantity of flavonoids was recorded in beet plants grown on herbicide-contaminated soil. This was more pronounced in plants sprayed with growth-stimulating bacteria. On the 90th day, the bacterial treatment further intensified their accumulation. In the future, the amount of flavonoids in beet leaves will decrease.

Discussion

In addition to growth characteristics, the levels of MDA, proline, and flavonoids were chosen as

stress indicators. This makes it possible to comprehensively assess the condition of plants and give numerical interpretations of the stress level caused by exposure to herbicides, namely the occurrence of oxidative stress. So, in the variant with only bacterial treatment, an increase in the weight and area of the leaves was not accompanied by the accumulation of stress molecules. On the contrary, it was observed that in the soil contaminated with Nanomet herbicide, the total leaf area and shoot fresh weight decreased significantly, indicating unfavorable conditions for the plant. In addition, there was a clear accumulation of proline and MDA. High levels of MDA indicate severe oxidative damage in beet cells, which may affect the yield and sugar content in the future.

Preparations based on sulfonylureas have the same mechanism of action on plants sensitive to them, which consists in blocking the functions of the enzyme acetolactate synthase relative to the synthesis of essential aliphatic amino acids (Gerwick *et al*., 1993). Protein deficiency is accompanied by various physiological disorders. Several studies have shown that sulfonylureas cause oxidative stress in plants (Gar'kova *et al*., 2011; Souahi *et al*., 2016; Li *et al*., 2020).

The results of many previous studies indicate that the main way to survive when spraying herbicides with sulfonylureas is to possess a type of acetolactate synthase that is not inhibited by the herbicide. This path has been implemented in creating transgenic varieties (Fartyal *et al*., 2018; Guo *et al*., 2020), as well as in the natural selection of herbicide-resistant weeds (Deng *et al*., 2017). Measures not related to enzyme replacement are too weak to mitigate the phytotoxicity of large amounts of herbicide. The amount of herbicides in the soil decreases weeks or months after application and has less effect on synthesizingthe synthesis of essential amino acids in sensitive plants. This allows us to realize the stimulating potential of bacteria, which is confirmed by our data. The combination of herbicide and bacterial strain *P. protegens* DA1.2 led in parallel to an improvement in the growth parameters of beet plants and inhibition of the formation of MDA in the leaves. It indicates the mitigation of herbicidal stress in the presence of *P. protegens* DA1. 2.

The more pronounced resistance of sugar beet to herbicide after treatment with bacteria was not associated with lower susceptibility of the enzyme targeted. And we have not investigated the metabolism of metsulfuron-methyl by beet plants. There are articles proving the biodestruction of sulfonylureas of herbicides in soil (Łozowicka *et al*., 2021). The use of microorganisms is discussed with soil reclamation or mitigation of the residual effects of herbicides on following crops (Rainbird *et al*., 2018). Although the bacterium can decompose metsulfuron-methyl, we doubt that this is the main reason for the decrease in phytotoxicity since the time interval from the treatment with bacteria *P. protegens* DA1.2 to seed germination was too short for the initial biodegradation of the herbicide in the soil. Another possible reason for the stimulation of beet growth by bacteria against the background of herbicide may be better availability of nutrients for plants due to the vital activity of bacteria. This ability is characteristic of most plant growth-promoting bacteria, including *P. protegens* DA1.2. Since it manifests similarly in different soils and has been described many times (Singh, 2018), we have not explored it in detail.

Perhaps bacteria affect the manifestations of oxidative stress induced by the herbicide. Tétard-Jones and Edwards (2016) considered the paradigm that microbes could 'prime' resistance mechanisms in plants to enhance herbicide tolerance by inducing the host's stress responses to withstand the downstream toxicity caused by herbicides. In our study, changes in the dynamics of the amount of flavonoids and proline in beet leaves after spraying with microorganisms suggest that bacterial metabolites may affect the regulation of the stress response in plants. Proline is currently considered a multifunctional molecule in plants. Proteomic, genomic, and metabolomic studies have revealed that proline, produced under stressful conditions, can act as a compatible solute in osmotic adjustment, a free radical scavenger, a metal chelator, an activator of ROS detoxification pathways, a cell redox balancer, a cytosolic pH

buffer, a source of energy, a source of nitrogen and carbon, a stabilizer of subcellular structures and membranes including photosystem II, and can act as a signaling molecule (Soshinkova *et al*., 2013; Hossain *et al*., 2014; Siddique *et al*., 2018). In our study, proline synthesis played a significant role in the response of beet plants to herbicide exposure and was one of the mechanisms of plant cell protection. On the other hand, against the background of bacterial treatment, the accumulation of proline was not so large. Apparently, bacteria activate other plant defense mechanisms, so the importance of proline decreases.

The accumulation of flavonoids induced by abiotic stresses has been observed by other researchers (Fini *et al*., 2011; Agati *et al*., 2012). Flavonoids act as antioxidants and represent a secondary system of absorption of reactive oxygen in plants. For example, they can restore hydrogen peroxide coming from chloroplasts into vacuoles (Fini *et al*., 2011). Enhancing the synthesis of flavonoids by plants is considered a promising area of research (D'Amelia *et al*., 2018). We believe that the treatment with bacteria improved the protective mechanisms of plants when they were exposed to oxidative stress. The increased amount of flavonoids can be caused by the synthesis of indolyl acetic acid (IAA) by bacterial cells since polyphenolic compounds are regulators of auxin metabolism (Peer and Murphy, 2007). In response to the intake of IAA, the flavonoid level also increases.

Thus, when sugar beet interacted with bacteria *P. protegens* DA1.2, herbicidal stress was mitigated, the level of oxidative stress was reduced, and the weight of the plant and the leaf area increased, which provided the basis for increasing yield and resistance to adverse factors.

Conclusions

It was shown that after 12 months, the toxicity of the herbicide based on metsulfuron-methyl for sugar beet plants persisted, as evidenced by an increased level of MDA. It is obvious that if the recommended application doses are exceeded, the soil remains unsuitable for growing sugar beet for more than a year. To mitigate herbicidal stress on soils where herbicides based on metsulfuron-methyl were previously used, a strain of bacteria *Pseudomonas protegens* DA1.2 is recommended. It was shown that this strain reduces the phytotoxic effect of metsulfuron-methyl at the biochemical and macro levels and increases the resistance of sugar beet to negative factors.

Statement of Conflicting Interests

The Authors (Hkudaygulov G., Chetverikova D., Bakaeva M., Kenjieva A., Chetverikov S.) state that there is no conflict of interest. The study did not pursue a commercial interest in using Nanomet products for research. It was used due to its popularity in the region and accessibility.

Authors' Contributions

Chetverikov Sergey Pavlovich designed the experiments, analyzed and interpreted the results obtained during the experiment, and revised the paper. Hkudaygulov Gaisar Garaevich performed the calculation of leaf area and determination of plant growth characteristics. Bakaeva Margarita Dmitrievna and Chetverikova Darya Vladimirovna carried out the treatment of plants and soil and determined the biochemical parameters of plants. Kenjieva Aliya Abduljalilovna performed bacterial strain cultivation, titer determination (CFU), construction of graphs, tables, and writing of an introduction. Bakaeva Margarita Dmitrievna wrote the paper.

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نقش سویه‌هایی از باکتری‌های ریزوبیومی محرک رشد گیاه در محافظت از گیاهان حساس به تنش علفکش‌های سولفونیل اوره

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چکیده: بقایای متسولفورون-متیل در خاک می‌تواند عامل منفی برای رشد گیاهان زراعی حساس به این علفکش باشد. نمونه‌های موفق زیادی از استفاده از باکتری‌ها برای افزایش عملکرد محصول و محافظت از گیاهان در برابر عوامل استرس‌زا وجود دارد. هدف از این کار بررسی امکان کاهش اثر سمّیت گیاهی متسولفورون-متیل در خاک بر چغندرقند با استفاده از باکتری‌های محرک رشد گیاه بود. در شرایط گلخانه‌ای، بذور چغندرقند و باکتری‌ها به‌طور هم‌زمان در خاکی که قبلاً با متسولفورون-متیل آلوده شده بود قرار گرفتند. وزن بوته‌ها، سطح برگ، مقدار پرولین، مالون دی آلدئید و فلاونوئیدها اندازه‌گیری شدند. در این آزمایش، سرکوب رشد بوته‌های جوان و آسیب اکسیداتیو ناشی از علفکش‌ها ثبت شد. تیمار چغندرقند با باکتری‌های سودوموناس پروتوژن DA1.2 سبب کاهش استرس اکسیداتیو ناشی از علفکش و افزایش وزن بوته‌ها شد. تیمار با باکتری به‌منظور کاهش تنش علفکشی بر پویایی محتوای فلاونوئیدها و پرولین تأثیر می‌گذارد که در واکنش‌های ضداسترس گیاهان نقش دارند.

واژگان کلیدی: چغندرقند، تنش علفکش، سمّیت گیاهی، متسولفورون-متیل، سودوموناس پروتوژن، باکتری‌های ریزوبیومی محرک رشد گیاه (PGPR)