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Research Article

Screening of pesticides for the control of *Pseudomonas savastanoi* pv. *glycinea* and *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* on soybean

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Abstract: In Russia, soybean *Glycine max* cultivation covers over 3 million hectares, but the low average yield leaves the potential of this crop in Russia still unattainable. Soybean diseases are among the main obstacles to increasing soybean yields. Chemical control remains an important tool for managing bacterial diseases, as most soybean cultivars are susceptible, but the list of recommended pesticides is very short. We screened 17 commercial pesticides with reported antibacterial activity as potential agents to combat bacterial blight and bacterial tan spot/wilt of soybeans. Several methods were used to carry out the assay: in vitro screening and determination of the minimal bactericidal concentrations, analysis of phytotoxicity, and testing biological efficiency on seeds and leaves after artificial inoculation with pathogens. The results presented here show that all tested pesticides possessed a significant antibacterial effect in vitro. Still, some plant protection agents (Kocide 2000, Serebromedin, Kasumin, and Phytoplasmin) had a phytotoxic impact on germinating seeds and on green plants, at least in the early vegetation stage, when applied at the rates recommended by the manufacturers. The results showed that for seed treatment to control bacterial blight *Pseudomonas savastanoi* pv. *glycinea*, pesticide Thiram (TMTD) was the most effective (39% efficacy compared to control), while for the disease on vegetating plants, variants treated with Physan 20 and Daimondaisen showed the highest efficacy (54.5% and 45.5%, respectively). For bacterial tan spot/wilt, *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* on germinating seeds, the highest efficacy was shown with the Ridomil Gold treatment (66.2% efficiency by AUPDC), and on vegetating plants, with Diamondasen (reduction of disease severity by 64.4%). Our results allow us to recommend these pesticides for controlling two primary bacterial diseases of soybean.

Keywords: *Pseudomonas savastanoi*, *Curtobacterium flaccumfaciens*, Pesticides, Soybean, Bacterial diseases, Phytotoxicity, Chemical control

Introduction

Soybean *Glycine max* (L.) Merr. is a legume crop plant cultivated for at least 8000 years (Lee *et al.*, 2011). World soybean production in 2020 reached 353 million tons, of which 235 million were produced in Brazil and the USA (World Food and Agriculture, 2020). The average yield

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of soybeans across the world for 2016-2020 was 2.79 t/ha (Langemeier *et al.,* 2021). In Russia in 2021, soybean cultivation covered 3,071 thousand hectares, and the gross harvest was 4.76 million tons, with an average yield of 1.59 t/ha (The Ministry of Agriculture, 2021), leaving Russia among the lowest-yielding countries for this crop. Thus, the potential of this crop in Russia remains unattainable. Soybean diseases are among the main obstacles to increasing soybean yields (Hartman, 2015). Among them, bacterial diseases pose a serious threat to soybean cultivation, among which bacterial blight caus. agent *Pseudomonas savastanoi* pv. *glycinea* (Zhang *et al.,* 2018) is the most common in the Russian Federation (Tarakanov *et al.* 2022a) (hereinafter–Psg), and bacterial tan spot/wilt caus. agent *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* (Huang *et al.,* 2009) (hereinafter–Cff) was placed second in economic importance (Tarakanov *et al.,* 2022b). Bacterial diseases of soybean can reduce crop yields by up to 40% (Jagtap *et al*., 2012; Huang *et al.,* 2007).

A combination of agrotechnical methods and semi-resistant cultivars is insufficient to solve the problem, and the search for chemical and biological agents to combat bacterial pathogens is essential. The success of bacterial plant pathogen control depends on selecting effective agrochemicals/pesticides to combat them. Given the vast number of fungicides used in agriculture, the range of registered pesticides with antibacterial effects is very limited in Russia. Moreover, due to the rapid development of genetic resistance of phytopathogenic bacteria to different active ingredients, it is important to expand the range of pesticides that can be used to in rotation on soybeans to control bacterial pathogens.

Plant pathogenic bacteria have short generation times, allowing rapid development of high-density populations. The bacterial pathogens spread and disease development usually depend on the presence of free water on the plant surface. Bacterial disease problems are best managed prophylactically before symptoms appear. This is consistent with soybean production strategies that emphasize seed treatment with little or no post-planting disease control.

Most pesticides used in agriculture have a wide range of targets. On the one hand, it enables their application for controlling a wide range of pathogens. On the other hand, it determines their potential influence on non-target organisms and can enhance the accumulation of pesticide tolerance within pathogenic populations. Therefore, the work aimed to screen for pesticides, both in vitro and in planta, capable of controlling the development of bacterial pathogens of soybeans.

Materials and Methods

Bacterial strains

The chosen pesticides were tested against pure cultures of bacterial pathogens *Psg* (strain CFBP 2214) and *Cff* (strain CFBP 3418). The bacteria were stored at-80 °C in 15% glycerol in the Plant Protection Department collection at the Russian State Agrarian University. Previous studies have shown that these strains are genetically similar to Russian populations of Psg and Cff (Tarakanov *et al.* 2022a; Tarakanov *et al.* 2023).

Pesticides

For the study, pesticides registered in Russia with previously reported antibacterial activity against other bacteria were selected from the State catalog (2023). Table 1 shows the general characteristics of the pesticides. Application rates of disease control agents that are not on the approved list of pesticides and agrochemicals allowed for use in the territory of the Russian Federation were taken from relevant literature sources. The application rate for each pesticide was calculated as the average of the maximum and minimum rates, as recommended by the manufacturer.

Determination of minimum bactericidal concentrations (MBC)

The MBC values of the bactericides were estimated in vitro using a standard serial dilution method according to (Balouiri *et al*., 2016) and (Tarakanov *et al*., 2022c). To do this, serial dilutions of each pesticide were carried out in standard sterile 96-well plates (Corning Co., USA). The bacteria in liquid LB medium were added and cultured in a shaker incubator at 28 °C for 24 h. After cultivation, each dilution was spread onto LB agar Petri plates, and the number of colonies was counted after 4 days of incubation at 28 °C.

Table 1 Pesticides used in this study.

No.	Commercial name of the pesticide	Active substance (s)	Code of a.i. in FRAC	Content a.i., %	Crop	Rate of application	Working solution	Manufacturer (country)
Liquid formulations								
1	Phytoplasmin, SL	Macrolide tylosin complex	48	20	Tomato	Seed treatment, 3.5 l/t	300-600 l/ha	Pharmbiomed (Russ. Fed.)
2	Phytolavin, SL	Complex of strepto-tricin antibiotics (BA-120000 AU/ml)	25	3.2	Wheat	Seed treatment, 1.75 l/t	10 l/t	
						Spraying, 1.75 l/ha	300 l/ha	
3	TMTD, WSC	Thiram	M 03	40	Soybean	Seed treatment, 7 l/t	10 l/t	Avgust (Russ. Fed.)
4	Heraclion, SC	Azoxystrobin + Tebuconazole + Thiram	11 + 3 + M 03	1.5 + 2.5 + 40	Soybean	Seed treatment, 1.1 l/t	6-8 l/t	Shchelkovo Agrochem (Russ. Fed.)
5	Nanocolloid 2, SL	Colloidal silver nanoparticles	M	-	Cabbage	Spraying, 9.0 l/ha [Orynbayev *et al.*, 2019]	300 l/ha	Koncern Nanoindustriya (Russ. Fed.)
6	Zerox, SL	Colloidal silver	M	0.3	Cabbage	Spraying, 9 l/ha [Orynbayev *et al.*, 2019]	300 l/ha	Nanobiotekh (Russ. Fed.)
7	Physan 20, SL	Quaternary ammonium compounds	-	20	Tomato	Spraying, 1.4 l/ha [Baysal *et al.*, 2015]	300 l/ha	Maril Products Inc., (USA)
8	Bravo, SC	Chlorothalonil	M 05	50	Tomato, Onion	Spraying, 3 l/ha	400 l/ha	Syngenta Russia (Russ. Fed.)
						Seed treatment, 7.5 kg/t [kharbanda *et al.*, 1979]	10 l/t	
9	Strekar, SC	Phytobacteriomycin (streptotricin antibiotics complex)	25	7	Tomato, Cereal Crops	Spraying, 1.75 l/ha	300-400 l/ha	Pharmbiomed (Russ. Fed.)
10	Kasumin, SL	Kasugamycin	24	2	Rice	Spraying, 1.37 l/ha	300 l/ha	Hokko Chemical Industry Ko., Ltd (Япония)
11	Serebromedin, SL	Silver and copper nanoparticles	M	-	Vegetable Peas	Spraying, 12 l/ha	300 l/ha	Ekoforvard (Russ. Fed.)
						Soaking the seeds for 10 minutes	-	
Dry formulations								
12	Polyram, WDG	Methiram (polycarbocin)	M 03	70	Potato	Spraying, 2 kg/ha	300-600 l/ha	BASF (Germany)
13	Diamondisen, WP	Mancozeb	M 03	80	Tomato	Spraying, 1.6 kg/ha	200-600 l/ha	Corteva (USA)
						Seed treatment, 3 kg/t [Basave *et al.*,2020]	10 l/t	
14	Rovral, WP	Iprodione	2	50	Sunflower	Seed treatment, 4 kg/t	10 l/t	FMC Chemical (Бельгия)
15	Ridomil Gold, WDG	Copper oxychloride + Mephenoxam	M 01 + 4	14.2 + 2	Tomato, Potato	Spraying, 2.5 kg/ha	300-500 l/ha	Syngenta Russia (Russ. Fed.)
						Seed treatment, 2 kg/t [martins *et al.*, 2015]	10 kg/t	
16	Kocide 2000, WDG	Copper hydroxide	M 01	35	Potato	Spraying, 1.75 kg/ha	200-400 l/ha	Dupont Science and Technology (USA)
						Soaking the seeds for 10 minutes [Estefani *et al.*, 2007]	-	
17	Efatol, WP	Aluminum Phosphoethyl	P 07	80	Rapeseed	Spraying, 1.2 kg/ha	200-400 l/ha	Himagromarketing. RU (Russ. Fed.)
18	Curzate, WP	Copper chloroxide + Cymoxanil	M 01 + 27	68.9 + 4.2	Tomato	Spraying, 2.25 kg/ha	400-600 l/ha	Dupont Science and Technology (USA)

The concentration of the pesticide that provided a 99.9% reduction in bacterial colonies was taken as the MBC value. Three repetitions of the experiment were done. The specificity of the bactericidal effect was calculated as the ratio of the higher MBC to the lower MBC.

Pesticides phytotoxicity

Pesticide phytotoxicity for seed treatment was tested by the "over paper" following protocol developed by ISTA (ISTA, 1999). Fifty seeds per treatment were used in three independent replications of the experiment. To assay the phytotoxicity of pesticides on vegetative plants, soybean was grown in a peat-perlite mix. At the R1 growth stage, seedlings were transplanted from trays into 1 L plastic pots and sprayed with the same pesticide suspension as previously used, with water as the control. Phytotoxicity was assessed according to the scale of Sathiyamurthi *et al*. (2017) 72 h after treatment. Three plants per pesticide were treated, and the experiments were repeated three times.

Seeds inoculation

The previously described method (Tarakanov *et al*. 2022b) was used with minor changes. The bacteria were grown from a fresh inoculum (early log phase) on King B and YDC agar for Psg and Cff, respectively, for 72 h at 19 °C. Biomass collected by spatula was transferred to a sterile test tube with 5 ml of sterile distilled water, and adjusted to 10^8 CFU/ml (OD_{600}≈0.2) using "Nanodrop One" spectrophotometer (ThermoFisher Scientific, USA). Before inoculation, healthy soybean seeds were washed twice, laid out on wet paper towels to swell for 3-4 h, and then transferred to a vacuum chamber. For 100 g of seeds, the volume of bacterial suspension added was 190 ml. Inoculum was applied at a pressure of-10^5 Pa to completely cover the seeds. After 10 min of exposition in a vacuum, the seeds were removed and left on dry paper towels for 24 h under the hood.

Inoculation of vegetative plants

Inoculation by Psg and Cff of vegetative soybean parts (stage of the first triple leaves) was done using the same suspension of bacteria prepared the same way as for seed inoculation, with airbrush 1113 AirControl airbrush (JAS, Ningbo, China), applying 5-7 ml per plant (on all leaves).

Cultivation of plants and treatment with pesticides

For all experiments, soybean seeds of the cultivar "Kasatka" (Federal Scientific Agroengineering Center VIM, Russia) were used, tested for health and quality in accordance with State Standard (GOST) R 52325-2005. For soybean seeds, the pesticides were applied at the recommended rate with a working solution of 10 l/t, by adding the pesticide suspension to the seeds in a plastic test tube and stirring with a vortex centrifuge. The control was treated with sterile water. The pesticide-treated seeds were sown into 40-cell plastic trays (volume of 120 cm3/cell; Agroflorapak, Russia) filled with a peat-perlite mixture (Veltorf, Russia). The experiment was carried out in a complete randomization design, with three independent repetitions and three trays per variant. The pesticide treatment of vegetative plants was carried out at the rates described by manufacturers or by literary sources. 24 h after inoculation, the plants were treated with 5 ml of pesticide suspension per plant using a hand-spray gun, in a triple-replicate design (4 plants per treatment). Negative controls included plants without pesticide application but treated with equivalent amounts of water and inoculated by Psg or Cff.

After the treatments, the plants were kept in a greenhouse at 28/22 °C (day/night) under natural sunlight (about 14 h of daylight and 10 h of night) and watered as needed.

Symptoms assaying

In the seed treatment experiment, symptoms were assessed at 9, 13, 17, and 20 days after sowing. In seeds infected with Psg, the incidence of the disease as described before (Tarakanov *et. al.* 2022c), taken as a percentage of plants with symptoms to the total number of plants (by the formula: P (%) = (n*100): N; where P–incidence rate; n–amount of plants with symptoms of the disease; N–total amount of plants in repetition). The damage for the seeds inoculated with Cff was assessed using a previously published scale (0–no symptoms;

1–wilting on one of yjhe primary leaves; 2–wilting on both of primary leaves; 3–wilting on first ternary leaves; 4–death of seedling after the development of first leaves, 5–no seedlings or complete withering and loss of turgor in adult plants) (Hsieh *et al.*, 2003). AUPDC (Area Under Progress Disease Curve) was calculated according to the method of Tarakanov *et al*. (2023) using the above scale in MS Excel 2007.

In experiments with vegetative plants, the number of diseased plants and the number of diseased leaves per plant were counted at 12 days post-inoculation, and the diseased area on leaves was measured using the LeafDoctor program for Android (Pethybridge *et al.*, 2015).

Statistical analysis of data

The data obtained in the experiments were statistically analysed using ANOVA/ MANOVA, the Duncan multiple-range test, Spearman's Rank-Order Correlation, and factor analysis with the Statistica v.10 program (StatSoft, TIBCO, Palo Alto, CA, USA). The data obtained as percentages were converted to arcsines before the statistical analysis.

Results

Antibacterial activity in vitro

Fourteen pesticides were included in the in vitro assay; Serebromedin, Kasumin, Kocide 2000, and Phytoplasmin were not used in this analysis because they caused phytotoxicity in germinating seeds or leaves. All tested pesticides showed a statistically significant antibacterial effect against the reference strains Psg and Cff; however, they differed significantly in their MBC values (Table 2). Strekar and Nanocolloid 2 showed the lowest efficiency (high average MBC = 3.5 and 2.5, respectively), whereas Diamodisen, Polyram, and Curzate/Phizan 20 were the most bacteriocidal (average MBC = 0.002, 0.005, and 0.09, respectively).

Table 2 Minimum bactericidal concentration (MBC) of tested pesticides against reference strains of Psg (CFBP 2214) and Cff (CFBP 3418).

№	Pesticide	Psg (CFBP 2214)[1, 2]	Cff (CFBP 3418)[1, 2]	Specificity of bactericidal effect[3]
1	TMTD	0.78 h	0.39 g	2.00
2	Physan 20	0.15 e	0.05 b	3.00
3	Phytolavin	0.78 h	0.78 i	1.00
4	Strekar	5.00 m	2.00 k	2.50
5	Curzate	0.049 bc	0.15 d	3.06
6	Zerox	1.50 j	0.50 h	3.00
7	Nanocolloid 2	2.50 l	2.50 l	1.00
8	Bravo	0.50 c	2.50 e	5.00
9	Heraclion	0.31 f	0.10 c	3.10
10	Diamondisen	0.003 a	0.001 a	3.00
11	Ridomil Gold	0.10 d	0.10 c	1.00
12	Rovral	1.25 i	0.40 g	3.13
13	Efatol	0.31 f	0.30 f	1.03
14	Polyram	0.001 a	0.01 a	10.00
	SD	0.006	0.003	-

1. Concentration of the working solution, % by active ingredient.
2. The values in the lines marked with different letters indicate a significant difference when using the Duncan criterion ($p = 0.05$).
3. The specificity of the bactericidal effect was calculated as the ratio of the higher MBC to the lower MBC.

Non-parametric correlation between the reaction of Psg and Cff to the tested pesticide was high and reliable (Spearman Rank Order Correlations = 0.87, Gamma Correlations = 0.72, and Kendall Tau Correlations = 0.71, all are significant at $p < 0.05$). Despite the fact, Factor Analysis (data not shown) revealed three main factors responsible for 59.2%, 28.7%, and 8,9% of the data variation, and grouped treatments into four classes; 1) first class with significant main effect of 1st Factor (Curzate; Efatol; Ridomil Gold; Phizan 20; Heraclion; Diamondisen; and TMTD), 2) second Class with main effect of 2nd Factor (Phytolavin and Nanocolloid 2), 3) third Class–with main 3rd Factor (Strekar; Zerox; and Rovral), and 4) fourth Class–with negative effect of 4th Factor (Polyram). The difference in MBC between the two pathogens ranged from 1 (equal) to 10-fold. The greatest difference between MBC of pesticide against Psg and Cff was found for Polyram (0.001 vs. 0.01).

Assessment of the phytotoxicity of pesticides

Reductions in germination rate and root length estimated the phytotoxicity of the 18 tested pesticides on seedlings. The results are shown in Figures 1A-1B. According to the data, both the minimum seedling root length and germination rate were observed in variants treated with Kocide 2000 and Serebromedin. Still, the difference was statistically significant only after Kocide 2000 treatment. Under foliar application, phytotoxicity, expressed as leaf chlorosis, was observed after treatments with Serebromedin, Kasumin, and Phytoplasmin (Fig. 1C, 2).

Biological efficacy of control bacterial pathogens in soybean plants

The primary pathogen population on seeds, calculated by titrating solutions of homogenized seeds on King B and YD media, was ≈ 103 CFU per seed for both pathogens. Pesticides tested *in vitro* were ranked according to the MBC index, and three with the lowest MBC for both pathogens were selected for further study: Diamondisen, Polyram, and Phyzan 20. From the list of pesticides applied for seed treatment, some were ranked by MBC, and three of the best were selected for further testing: TMTD, Diamondisen, and Ridomil Gold.

Biological effectiveness against Psg. Bacterial blight infection on soybean seeds was reduced only by TMTD (Thiram). It suppressed disease development at the 21st day after inoculation by 39% compared with the control variant (Fig. 3A). However, the difference in disease severity across all protection variants was statistically insignificant. It can be explained by the non-systemic activity of TMTD and the survival of pathogens located under the seed coat (Murthy *et al.*, 1976).

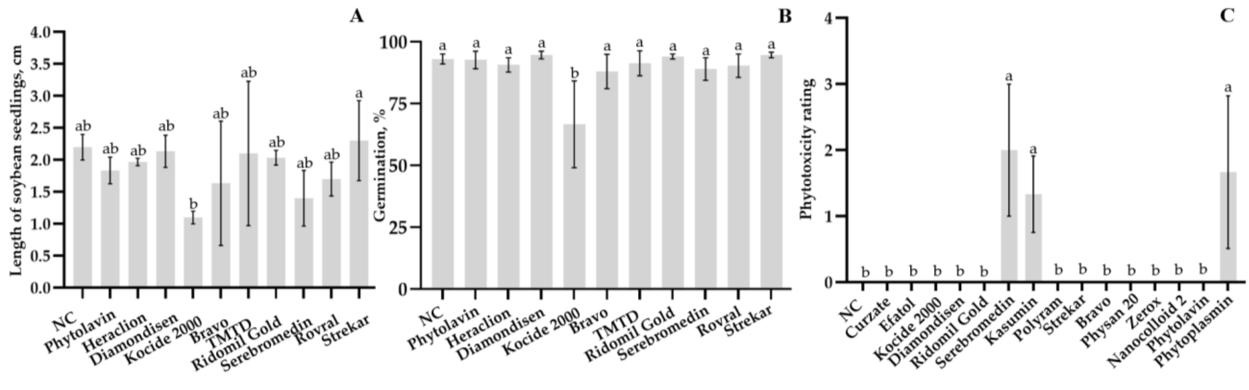

Figure 1 Soybean seedlings length (A), seed germination rate (B), average score of phytotoxity (C) after treatment by the pesticides. NC-negative control (treatment by water).

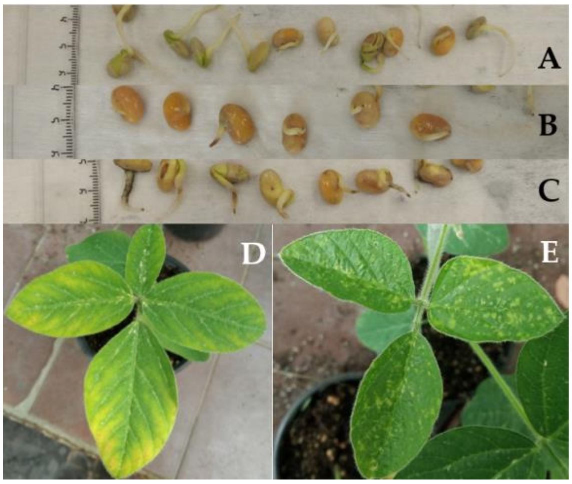

Figure 2 Symptoms of toxic effect of the pesticides applied for seeds treatment (A-C) and green soybean plants (D-E). A–Strekar (no toxic effect), B–Kocide 2000; C-D–Serebromedin; E–Phytoplasmin.

Biological effectiveness against Cff. AUDPC values for tan spot/wilt in soybean across all treatments were lower than those of the control variant at the 95% significance level. The lowest values of the disease development were obtained for TMTD and Ridomil Gold (AUDPC values are lower by 43.0% and 66.2%, respectively, than the control) (Fig. 3B).

Control of vegetative plant infection

Biological effectiveness against Psg. Three pesticides—Diamondisen, Polyram, and Phizan 20—were evaluated. All pesticides reduced the severity of bacterial blight (Fig. 4A, 5A-B). Phizan 20 was the most effective, with a 54.5% reduction in disease symptoms compared to the control, followed by Diamondisen (45.5%) and Polyram (36.4%). However, the incidence of infection on plants was reduced significantly only after Diamondisen treatment (65.9% decrease) (Fig. 4A).

Effectiveness in relation to Cff. The results of the experiments show that all pesticides reduced the incidence and prevalence of bacterial tan spot and soybean withering. For disease development, the most effective treatment was Diamondisen (reducing disease severity by 64.4% compared to the control), followed by Phyzan 20 (45.4%). Polyram did not differ significantly from the negative control (Fig. 4A, 5C-D). However, based on disease incidence, Physan 20 was the most effective (a 77.8% decrease).

Discussion

Plant disease control is an essential technique for soybean cultivation. To combat bacterial diseases of soybean in Russia, the range of permitted pesticides is insufficient, and it is necessary to expand the list to enable scientifically effective control of bacterial pathogens. In this work, a screening of pesticides capable of controlling the development of bacterial pathogens of soybeans was carried out.

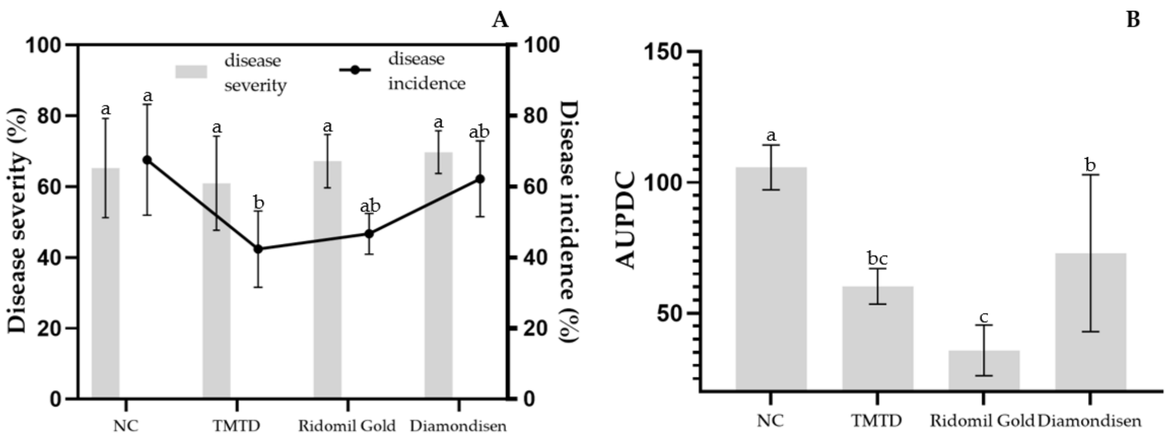

Figure 3 Severity and incidence of A) the bacterial blight (Psg) and AUDPC (%) of tan spot/wilt (Cff) (B) for on infected plants 21 days after inoculation.

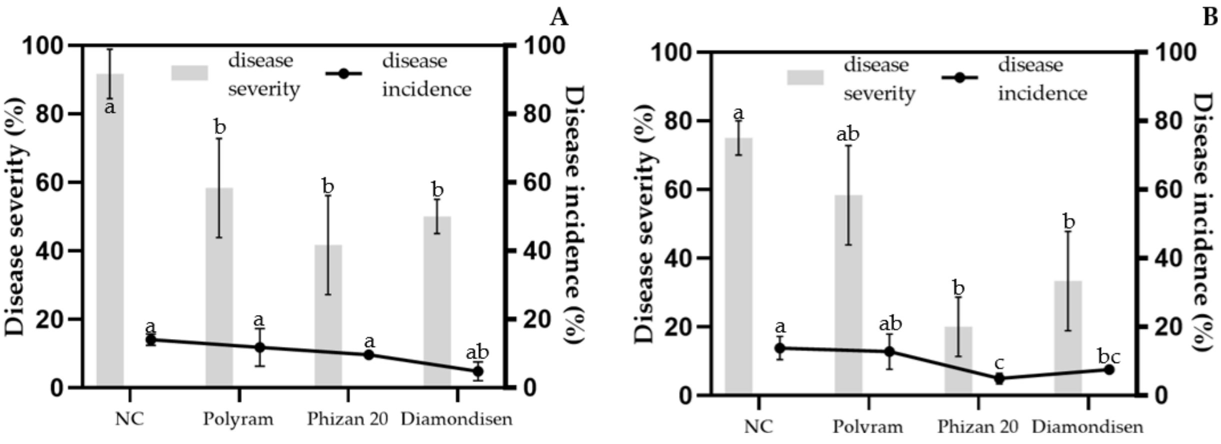

Figure 4 Severity and incidence for Psg (A) and Cff (B) at 12th day after green plants inoculation.

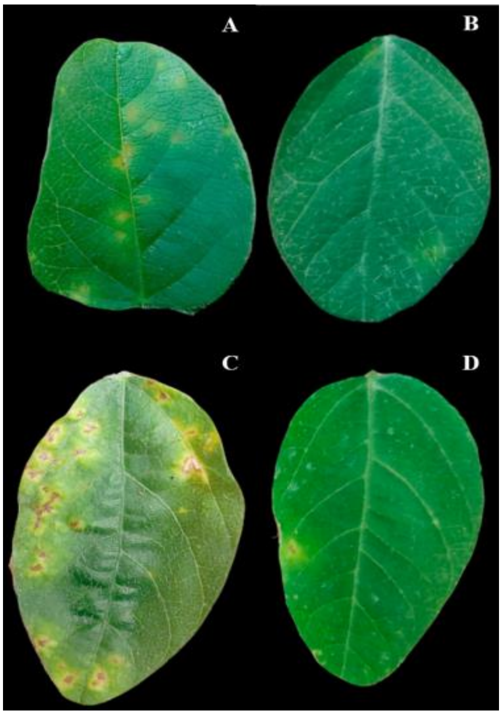

Figure 5 Symptoms caused by Psg (A, B) and Cff (C, D) of soybean plant treated by different pesticides: A–control (water), B–Kocide 2000, C–control (water); D–Diamondisen.

Currently, five pesticides are used in Russia to control bacterial pathogens of soybean: thiram-based compounds–for seed treatment (Tirada, TMTD, Heraklion)-and Vitaplan (Bacillus subtilis) and Benorad (a.i. benomil)–for plant protection during vegetation (State catalog, 2023). The antibacterial effect of tetra-methyl-thiuram-disulfide (Thiram) is well known (Dimond *et al.*, 1943; Kaars *et al.*, 1959; Akerström *et al.*, 1962). However, published studies on the bactericidal activity of Thiram indicate variable sensitivity among bacteria, particularly among gram-negative species (Long *et al.*, 2017). Benomyl is a benzimidazole fungicide that has been effectively used on crops and ornamental plants for many years with noticeable bactericidal effect (Goto *et al.*, 1994). Benomyl is metabolized to carbendazim. Thiram, benomyl, and their derivatives induce oxidative stress by generating oxygen, hydroxyl, and hydrogen peroxide free radicals, leading to membrane damage and bacterial cell death (Cui *et al.*, 2019).

In this work, eighteen chemical and biological plant protection agents registered for crop treatment in the Russian Federation (State catalog, 2023) were tested for phytotoxicity to soybean plants and for biological efficacy *in vitro/in planta* assays against two of the most harmful bacterial pathogens of soybean in Russia-*Pseudomonas savastanoi* pv. *glycinea* and *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* (Tarakanov *et al.*, 2022a, Tarakanov *et al.*, 2022b). Among the tested pesticides, Phytolavin, Strekar, Phytoplasmin belonged to the group containing complexes of streptomycin and tylosin (macrolide antibiotics), Kasumin–the antibiotic kasugomycin, TMTD, and Heraklion–tetramethylthiuram disulfide (Thiram), Nanocolloid 2, Zerox, Serebromedin, Curzate, and Kocide, 2000–contained colloidal silver and copper. Physan 20 had quaternary ammonium compounds as the main active ingredient, Bravo–Chlorothalonil, Polyram–Polycarbocin, Diamondisen–Mancozeb, Rovral –Iprodione, Efatol-Phosphoethyl Aluminum, and the preparation Ridomil Gold was a mixture of copper-containing active ingredient and metalaxyl. The results presented here show that all pesticides had different antibacterial effects in vitro. Still, some plant protection agents (Kocide 2000, Serebromedin, Kasumin, and Phytoplasmin) had a phytotoxic effect on germinating seeds and on green plants, at least in the early vegetation stage, when applied at the manufacturers' recommended rates. Similar results on the phytotoxicity of these pesticides have been reported before on other plants: for kasugamycin-on apple (Adaskaveg *et al.*, 2011), for copper hydrochloride (Kocide 2000 and others)–on many crops (Arunakumara *et al.*, 2013), and a macrolide tylosin complex (Phytoplasmin)–on cabbage (Dzhalilov *et al.*,2014). Other pesticides did not show significant phytotoxic effects regardless of the application method. Pesticides with obvious phytotoxicity (Kocide 2000 and Serebromedin–for seed treatment, and Phytoplasmin and Kasumin–for plant treatment) were excluded from further experiments.

Three pesticides normally applied for seed treatment (TMTD, Diamondisen, and Ridomil Gold) and three for vegetative plant treatment (Diamondisen, Polyram, and Phizan 20) were selected for further testing based on the lowest MBCs and minimal phytotoxic effects. They showed good efficiency when applied to artificially infected seeds and green plants, treating both tested pathogens–Psg and Cff. Among fungicides, Polyram was most effective, while Thiram was the least effective in combination studies with both Psg and Cff. The results obtained here were consistent with those previously reported by other authors (Jamdagni *et al*., 2018; Panáček *et al*., 2016), who performed MIC assays with geometric dilutions of antibiotics against several tested bacteria. They reported strong synergistic effects of Ag in combination with an array of antibiotics against pathogenic bacteria, even multidrug-resistant ones. Hence, the combination of the tested agents may have enhanced antimicrobial efficacy.

The present study reports a comparative analysis of the efficacy of commercial agricultural fungicides registered in Russia for soybean protection. In conclusion, we report effective agents with high antibacterial potential that can be further developed and optimized through combination and rotation for efficient agricultural use.

Conclusions

Thus, the study showed that the pesticide TMTD (a.i. thiram) at a consumption rate of 7.0 l/t has the greatest effectiveness in control of bacterial blight of soybean when treating seeds; on vegetative plants, the pesticide Diamondisen at a consumption rate of 1.6 kg/ha and Physan 20 (0.14 l/ha). Treatment with Ridomil Gold (2 kg/t) has the greatest effect on controlling bacterial tan spot/wilt on seeds and vegetative plants, while Diamondisen at 1.6 kg/ha has the greatest effect on controlling bacterial tan spot/wilt on seeds and vegetative plants. The pesticide Physan 20 (active ingredient: quaternary ammonium compounds) demonstrated the same high efficacy as well-known fungicides (e.g., mancozeb) used to combat diseases of vegetative plants. This fact demonstrates the high potential of this new class of pesticides for controlling bacterial plant diseases.

Conflicts of Interest

The authors declare no conflict of interest. The funders had no role in the design of the study, the collection, analysis, or interpretation of data, the writing of the manuscript, or the decision to publish the results.

Authors' Contributions

K. Troshin-Main Researcher (25%); R. Tarakanov-Subsidiary Researcher, Writer of the Introduction (20%); A. Ignatov-Writer of the

Discussion (20%); I. Jafarov-Data Analyzer (15%); F. Dzhalilov–Methodologist (20%).

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غربالگری آفت‌کش‌ها برای کنترل *Pseudomonas savastanoi* pv. *glycinea* و *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* روی سویا

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چکیده: در روسیه، کشت سویا *Glycine max* بیش از ۳ میلیون هکتار را پوشش می‌دهد، اما میانگین پایین عملکرد، پتانسیل این محصول را در روسیه هنوز دستنیافتنی می‌کند. بیماری‌های سویا ازجمله موانع اصلی افزایش عملکرد سویا هستند. کنترل شیمیایی همچنان ابزاری مهم برای مدیریت بیماری‌های باکتریایی است، زیرا اکثر ارقام سویا حساس هستند، اما فهرست آفت‌کش‌های توصیه شده بسیار کوتاه است. ما ۱۷ آفت‌کش تجاری با فعالیت ضدباکتریایی گزارش شده را به‌عنوان عوامل بالقوه برای مبارزه با سوختگی باکتریایی و لکه برنزه/پژمردگی باکتریایی سویا غربالگری کردیم. چندین روش برای انجام این سنجش استفاده شد: غربالگری آزمایشگاهی و تعیین حداقل غلظت باکتری‌کشی، تجزیه‌وتحلیل سمّیت گیاهی و آزمایش کارایی بیولوژیکی روی بذرها و برگ‌ها پس از تلقیح مصنوعی با عوامل بیماری‌زا. نتایج ارائه شده در اینجا نشان می‌دهد که همه آفت‌کش‌های آزمایش شده دارای اثر ضدباکتریایی قابل‌توجهی در شرایط آزمایشگاهی هستند. با این‌حال، برخی از عوامل محافظت از گیاه (Kocide 2000، Serebromedin، Kasumin و Phytoplasmin) حداقل در مرحله اولیه رشد، در صورت استفاده با مقادیر توصیه شده توسط تولیدکنندگان، تأثیر گیاه‌سوزی روی بذرهای درحال جوانه زدن و گیاهان سبز داشتند. نتایج نشان داد که برای تیمار بذر برای کنترل سوختگی باکتریایی *Pseudomonas savastanoi* pv. *glycinea*، آفت‌کش تیرام مؤثرترین بود. درحالی‌که برای این بیماری در گیاهان در حال رشد، گونه‌های تیمار شده بافیزان ۲۰ و دیاموندایسن بالاترین اثربخشی را نشان دادند. برای لکه برنزه/پژمردگی باکتریایی، *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* روی بذرهای در حال جوانه زدن، بالاترین اثربخشی با تیمار ریدومیل گلد با راندمان ۶۶٫۲٪ و روی گیاهان در حال رشد با دیاموندایسن نشان داده شد. براساس نتایج به‌دست آمده می‌توان آفت‌کش‌های فوق را برای کنترل دو بیماری باکتریایی اصلی سویا توصیه نمود.

واژگان کلیدی: *Pseudomonas savastanoi*، *Curtobacterium flaccumfaciens*، آفت‌کش‌ها، سویا، بیماری‌های باکتریایی، سمّیت گیاهی، کنترل شیمیایی