

The effect of biopreparations on soybean germination ability and contamination with seed infection

Dina Kurilova*

V.S. Pustovoit All-Russian Research Institute of Oil Crops, Filatova street, 17, 350038 Krasnodar, Russian Federation

Abstract. We studied the application efficiency of biopreparations for soybean seed treatment against seed infection. We established the positive effect of biopreparations application on the germination of soybean seeds. In the laboratory conditions, we observed the best protective effect against a complex of pathogens of seed infection in variants with the treatment of seeds with the laboratory samples of biopreparations 14-3 *Pseudomonas chlororaphis*, LC and Xk-1 *Chaetomium olivacium*, WP, as well as with biopreparations Trichoderma Veride 471, WP and Fitosporin-M, WP. In the field conditions, we observed an increase in plant density in relation to control by more than 10.0 % in 14-3 *P. chlororaphis*, LC and Fitolavin, SC, Fitosporin-M and Trichoderma Veride 471, WP. Also, these biopreparations (except Fitolavin, SC) showed a good growth-stimulating effect.

1 Introduction

The most part of the total number of soybean diseases are seed-borne. In Russia, the most harmful among the complex of pathogens affecting soybean seeds are cotyledonous bacteriosis and fusarium of seeds and seedlings, which reduce field germination that causes a strong thinness of sowings [1]. The diseases pathogens enter the soil with seeds, accumulate in it and subsequently serve as a source of infection even when healthy seeds are sown [2].

In 2018-2019, we carried out a phytoexamination of soybean seeds (21 samples) and according to it the laboratory germination ranged from 64.5 to 97.5 %. The reason for the decrease in germination was the infection with bacterioses (pathogens are bacteria from the genera *Pseudomonas* Migula, *Ervinia* Winslow et al. emend. Hauben et al., *Xanthomonas* Dowson). fusarium (pathogens are fungi of the genus *Fusarium* Link.) also affected seeds, but to a lesser degree and it is often manifested itself together with bacteriosis. We observed alternaria blight (pathogens are *Alternaria* Ness) affection in all years of research, but not in every sample. Cercospora blight (pathogen is *Cercospora* Fresen.) appeared only on the seeds of the 2018 harvest. Moreover, in all the years of research, the infective matter of mold fungi of the genera *Penicillium* Link. (up to 4.5 %), *Aspergillus* Ness. (up to 2.1 %),

* Corresponding author: charel@yandex.ru

and *Mucor* Fresen. (up to 22.1 %) was present on the seed coat of soybean seeds, but they had no effect on germination ability [3].

The presowing treatment with chemical disinfectants is the main method of seed disinfection. However, despite the importance of chemicals in disease control, there is a problem of resistance of phytopathogens to fungicides. As a result, higher dosages are used and new chemical compounds are introduced that cause contamination of food and the environment with toxic substances, as well as increase the cost of food production [4]. In this regard, currently, there is an increased interest in environmentally sound and relatively safe biopreparations, which are becoming a valid alternative to chemical pesticides.

The strategy of modern biological plant protection includes the use of biotic substances (antibiotics and substances of plant origin) and the application of biopreparations based on live cultures of microorganisms.

One of the trends in biocontrol is the development and application of biopreparations based on antagonist bacteria that can synthesize a wide range of antibiotics and other metabolites active against bacteria and fungi that cause plant diseases in agriculture [5-8]. Bacteria of the genus *Bacillus* and *Pseudomonas* produce various compounds that not only participate in the pathogens biocontrol, but also stimulate plant growth (PGPP), which makes them potential candidates for most agricultural and biotechnological applications [9-11]. *Trichoderma* spp. has the greatest potential among fungi-antagonists, the strains of which are widely used all over the world as biocontrol agents and plant biostimulants on many agricultural crops. Some *Trichoderma* strains are capable of stimulating plant growth and defense responses [12-14], including those in soybean [15]. Fungi of the genus *Chaetomium* are also known for their antagonistic activity against soil and seed pathogens [16-18].

Thus, the aim of our research was to evaluate the effectiveness of soybean seeds treatment with biopreparations based on bacterial and fungal strains in the laboratory conditions against the main pathogens that reduce germination.

2 Materials and methods

The objects of research were the soybean variety Slavia (breeding of V.S. Pustovoit All-Russian Research of Oil Crops), commercial biopreparations Fitosporin-M, WP (> 2 billion CFU/g) based on the strain 26 D *Bacillus subtilis*, *Trichoderma* Veride 471 (> billion spores/g) and Fitolavin, SC (BA-120000 removal unit/ml, 32 g/l) – phytoantibiotic, an antibiotic of the streptotri group, as well as the laboratory samples of biopreparations based on the strains 14-3 *Pseudomonas chlororaphis*, LC (3.5×10^{10} CFU/g), Xk-1 *Chaetomium olivacium*, LC (1.3×10^8 CFU/g) and Xk-1 *Ch. olivacium*, WP (1.0×10^9 CFU/g), developed in the laboratory of biotechnology of V.S. Pustovoit All-Russian Research Institute of Oil Crops [19, 20]. We chose the chemical pesticide TMTD, WSC (thiram 400 g/l), recommended for use the Russian Federation on soybean against seed mold, Ascochyta blight, Fusarium and bacteriosis, as a standard.

In order to determine the effect of biopreparations and the laboratory samples of biopreparations on the seeds and seedlings of soybean, we placed the treated seeds for germination in sterile rolls of filter paper moistened with sterile tap water in a moist chamber for 7 days at a temperature of + 25 °C. We determined the germination ability by the total number of normally germinated seeds according to GOST 12038-84. We classified the seeds with soft decomposed endosperm, blackened or rotten germs, seedlings with dead (partially or completely) roots, cotyledons, budlets, hypocotyl, epicotyl as unfruitful. We classified hard and imbibed non-germinated seeds with no signs of affection by any diseases as non-germinated. The length and weight of the root and seedling were the

parameters for analyzing the effect on soybean seedlings. We calculated the plant density at the stage of full germination on an area of 1 m² in course of 4 replications.

3 Research results

Bacteriosis and fusarium affection was the main reason for the decrease in seed germination. All tested biopreparations showed a protective effect. Due to the decrease in seed infection, the number of normally germinated seeds was higher in the variants than in the control – by 5.0-19.0 %. The maximum germination was in the variants with 14-3 *P. chlororaphis* (91.0 %) and Fitosporin-M (90.0 %). Also, we observed a high percentage of germinated seeds in the variants with Xk-1 *Ch. Olivacium* (86.0-87.0 %) and standard TMTD (85.5 %) (Table 1).

Table 1. The effect of biopreparations on the laboratory germination and pathogenic microflora of soybean seeds (V.S. Pustovoit All-Russian Research Institute of Oil Crops, 2020)

Variant	Application rate, l/t, kg/t	Laboratory germination, %	Biological effectiveness, %			Plant density,	
			bacteriosis	fusarium	mold fungi*	pcs/m ²	% to control
Control (without treatment)	-	72.0	25.0**	7.5**	8.5**	30.2	-
TMTD, WSC (standard)	6.0	85.5	54.0	73.3	100	34.6	14.6
Fitosporin-M, WP	2.0	90.0	64.0	20.0	11.8	33.8	11.9
Fitolavin, SC	2.0	76.0	12.0	6.7	0	34.8	15.2
Trichoderma Veride 471, WP	2.5	81.0	48.0	80.0	94.1	33.4	10.0
14-3 <i>P. chlororaphis</i> , LC	3.0	91.0	64.0	86.7	76.5	35.0	15.9
Xk-1 <i>Ch. olivacium</i> , LC	3.0	86.0	44.0	33.3	29.4	31.5	4.3
Xk-1 <i>Ch. olivacium</i> , WP	0.05	87.0	48.0	73.3	76.5	32.6	7.9
HCP ₀₅						3.75	-

* fungi of the genera *Penicillium* Link. and *Aspergillus* Ness.;

** the number of affected seeds in the control.

14-3 *P. chlororaphis* and Fitosporin-M (64.0 %) showed the maximum biological effectiveness against bacteriosis against the background of the control affection of 25.0 %, which exceeded the efficiency of the standard by 10.0 %. We also observed a protective effect in the variants with Trichoderma Veride 471 (48.0 %) and Xk-1 *Ch. olivacium* (44.0-48.0 %). 14-3 *P. chlororaphis* (86.7 %), Trichoderma Veride 471 (80.0 %) and Xk-1 *Ch. olivacium*, CII (73.3 %) showed the best results against fusarium, with the efficiency of the chemical standard TMTD of 73.3 %. The chemical standard TMTD showed 100 % effectiveness against the mold fungi of the genera *Penicillium* and *Aspergillus*. Among the biopreparations, Trichoderma Veride 471 (94.1 %), 14-3 *P. chlororaphis* and Xk-1 *Ch. olivacium*, CII (76.5 %) efficiently decreased their number.

The field tests of biopreparations have confirmed the data obtained in the laboratory. In all variants of the experiment, the plant density at the stage of full germination was 4.3-15.9 % higher than in the control. Seed treatment with Fitolavin, SC and 14-3, LC provided and increase in plant density by 15.2 and 15.9 %, respectively, compared with the control and exceeded the result of the chemical standard TMTD (Table 1).

In the laboratory experiment, in addition to the protective effect, we observed a positive effect of biopreparations on seedlings in the terms of stimulation of their growth. 14-3 was

the most effective in all parameters, providing, in comparison with the control, an increase in the root length (RL) by 13.7 %, the seedling (SL) by 8.4 %, the root weight (RW) by 25.0 %, the seedling weight (SW) by 11.9 %. Xk-1, WP also showed a good stimulating effect on the root system (RL – by 13.5 %, RW – by 18.8 %), however, the effect on the seedling was insignificant (SL – by 9.8 %, SW – by 1.2 %). Trichoderma Veride 471 had the maximum stimulating effect on the root length – by 29.0 %, while the root weight was at the level with the control. Fitosporin-M contributed to a significant increase in the seedling length and weight (by 24.4 and 6.0 %, respectively), while the root system of seedlings was less developed than in the control variant (Table 2).

Table 2. The effect of seed treatment with biopreparations and the laboratory samples of biopreparations based on the promising strains of antagonists on the soybean seedlings (V.S. Pustovoit All-Russian Research Institute of Oil Crops, 2020)

Variant	Root length		Root weight		Seedling length		Seedling weight	
	cm	% to control	g	% to control	cm	% to control	g	% to control
Control (without treatment)	11.20	-	0.16	-	5.50	-	0.84	-
TMTD, WSC (standard)	11.83	5.6	0.15	0	6.31	14.7	0.85	1.2
Fitosporin-M, WP	11.16	0	0.15	0	6.84	24.4	0.89	6.0
Fitolavin, SC	11.13	0	0.17	6.3	5.53	1.9	0.84	0
Trichoderma Veride 471, WP	14.45	29.0	0.16	0	5.90	7.3	0.83	0
14-3 <i>P. chlororaphis</i> , LC	12.73	13.7	0.20	25.0	5.96	8.4	0.94	11.9
Xk-1 <i>Ch. olivacium</i> , LC	12.19	8.8	0.17	6.3	5.67	3.1	0.81	0
Xk-1 <i>Ch. olivacium</i> , WP	12.71	13.5	0.19	18.8	6.04	9.8	0.85	1.2
HCP ₀₅	1.28	-	0.02	-	0.91	-	0.04	-

4 Conclusions

The laboratory sample of the biopreparation based on the strain 14-3 *P. chlororaphis*, LC had the best protective effect against the complex of pathogens of soybean seed infection and the maximum growth-stimulating effect on soybean seedlings. We also observed high efficiency in the variants with the treatment of seeds with the biopreparations Trichoderma Veride 471, WP, Fitosporin-M, WP and the laboratory sample of biopreparation based on the strain Xk-1 *Ch. olivacium*, WP.

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