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Pre-sowing treatment of Soybean seeds against seed infection

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ABSTRACT

Currently, a promising area in plant protection is the use of stimulants and biofungicides to stimulate seed germination, plant growth, and development, to increase resistance to diseases, and to improve the quality and quantity of the products obtained. In the laboratory conditions, effective protective and stimulating compositions for soybean seed treatment have been developed that suppress fungal and bacterial infection, improve the sowing qualities of the seeds and the growth of the seedlings, and prevent seed mold. The research was aimed at assessing the effectiveness of the protective and stimulating compositions in the treatment of soybean seeds against microflora of the seeds and their effect on the sowing properties. The results of the studies have shown that the treatment of soybean seeds with stimulants in combination with a treater significantly improves their sowing qualities, suppresses fungal and bacterial infections, and intensifies the growth of plants and the root system. A process chart of integrated soybean protection from harmful organisms for the Agropark Ontustik LLC has been developed and tested, including seeds treatment with the protective and stimulating compositions against seed infection. For the transfer and adaptation of foreign plant protection products in the production conditions, the effectiveness of soybean seed treatment with the protective and stimulating compositions chosen based on laboratory experiments has been assessed. The experiments were performed on the crops of the Agropark Ontustik LLC.

Key words: *Phytoexpertise, Soybean, Seeds, Infection, Treater, Stimulant, Effectiveness.*

Introduction

Among the crops pathogenic microflora, seed infection takes a special place. It is well known that seeds spread more than 60 % of all crop pathogens. The microflora found on the seeds can be saprotrophic (penicillia, aspergilli, mucor, alternaria, etc.) and pathogenic (smut, helminthosporiosis, fusariumblight, septoria blight, etc.) (Govorov *et al.,* 2018; Sagitov *et al.,* 2014; Ram *et al.,* 2018). The pathogenic complex of the seeds includes dozens of fungi and bacteria species. Practice shows that currently the quality of seeded seeds of crops is worsening, and infestation with a complex of pathogenic and saprophytic microflora is growing. This entire complex of the pathogenic and saprophytic microflora causes enzyme-mycotic depletion of the seeds, which affects their germination rate, germination vigor, and plant development during the vegetation season. Upon seed germination, they accumulate in the rhizosphere of the root system and cause root and foot rot (Ismailova *et al.,* 2018; Lebedintseva and Tyuterov, 1994; Begunov *et al.,* 2003; Kamthane and Rakh 2013).

Seeds mass infestation with pathogenic fungi species often reduces the germination vigor and the germination rate. The injuriousness largely depends on the depth of mycelium localization and the number of infected seeds.

The seeding of infected seeds results in disease transition to growing plants and thus creates and maintains the foci of infection in the field. Seed infestation with microflora occurs at various times — during the vegetation season, during harvesting, especially in the conditions of high humidity, during threshing or afterharvesting grain conditioning, during storage due to violations of the conditions, and upon placing seeds with high humidity for storage (Sagitov *et al.,* 2014; Hima *et al.,* 2016; Sonakar, Jesu Dasu, Singh, Maurya and Maurya, 2014; Li Ming, *et al.,* 2007; GanYunbo and Stulenineke, 2002).

In the case of poor-quality pre-sowing treatment, improper choice of seed treater, seed infection will start spreading intensively upon seed germination, and will pose a danger to plants that will not be able to provide full-fledged harvest. Besides, many pathogens produce mycotoxins that affect the physiological processes in plants, inhibit the seedling and root growth, and can remain present in the end product. This not only reduces the yield but also worsens the quality of the product.

In this regard, the choice of the treater should be based on the results of the preliminary phytoexpertise for identifying the species composition of the seeds microflora. This will allow choosing the right preparation, given its action, and will ensure the effectiveness of the measure.

The soybean seedsphytoexpertise showed strong infestation with the pathogenic microflora, which creates a dangerous infectious background for the manifestation of diseases – root rot, fusarium blight, alternariosis, and bacterioses, as well as seed mold and rot. By the degree of seeds infestation, the choice of highly effective fungicides with a wide range of fungicidal and bactericidal properties is needed (Agaev *et al.,* 2009; Belyea 2004).

Currently, it is very relevant to introduce the technologies in which the reduction of the effects of adverse environmental factors, the enhancement of the adaptive ability of the plants, and the maximum use of the crops potential are achieved by the use of effective and environmentally friendly biological products, which, along with preserving the yield of the crops, contribute to reducing environmental pollution and agricultural products (Cunha and Filho, 2010; Shabaldas and Hoffman, 2007).

The high quality of the seeds is one of the main agronomic requirements that ensure high and stable yields of crops with other conditions being optimal.

Among legumes, soybean is high in essential nutrients. Its seeds contain up to 50 % protein and 20 % oil, as well as minerals and vitamins that allow using it both for food, fodder, and for technical purposes. It is also very important for increasing soil fertility. Nodule bacteria enrich the arable soil layer with organic nitrogen. However, soybean is a good precursor for many crops. The diversification of agriculture in the Republic determines an increase in soybean production by expanding the seeding areas, increasing the yield of this valuable crop, and reducing the crop losses from pests, especially from fungal and bacterial diseases.

Obtaining high and stable yields of soybean largely depends on the quality of the seed material. In agricultural practice, increasing attention is paid to the use of growth stimulants. Seed treatment with growth stimulants is currently a scientifically substantiated technique (Sagitov *et al.,* 2014; Agaev *et al.,* 2009). It allows achieving the maximum germination rate and germination vigor, reducing the environmental factors, and improving the quality and quantity of the products (Shabaldasand Hoffman 2007).

Therefore, many entrepreneurs involved in agricultural activities purchase highly efficient and qualitative growth stimulants for the crops cultivated. However, most of them are unable to effectively suppress seed infections. At the same time, seed treaters, while inhibiting seed infection in most cases, do not have a positive effect on the germination, the growth, and the development of the plants. To improve their effectiveness, their joint application is required. A combination of these two protection means will allow developing an effective method of seed treatment (Sagitov *et al.,* 2014; Agaev *et al.,* 2009).

Seed phytoexpertise allows predicting the agricultural plants' potential susceptibility to diseases and thereby gives a chance to preserve their yields and quality of the harvest. The purpose of phytoexpertise is to determine in the laboratory conditions the quantitative and qualitative composition of the pathogens transmitted with the seeds, as well as the seed germination rate, which may vary depending on the degree of infestation (Govorov *et al.,* 2018; Shabaldas and Hoffman, 2007).

Methods

The phytoexpertise of various soybean species was performed in the laboratory. With that, their sowing properties were assessed following the GOST for soybean — GOST 1275-2004 (2004). The germination vigor was assessed on day 3, laboratory germination rate — on day 7 by the number of germinating seeds. The seeding properties were determined in moist chambers. Fifty seeds were analyzed in four repetitions. The number of affected seeds and seedlings was accounted for; the species composition of the fungal and bacterial microflora was determined. The analyses were performed according to the guidelines of N. A. Naumova (1970) on two nutrient media: potato dextrose agar (PDA) and Czapek's agar (CZA). By the morphological properties of the fungi and bacteria colonies and their pure cultures, the fungal and bacterial microflora was determined. Also, the morphological properties of fungi were studied by morphological microscopy of sporulation and those of bacteria by checking the pathogenic properties.

Results and Discussion

For preparing the crops seeds, one should start with the mandatory phytopathological examination of the seeds, including the microbiological analysis of the composition of the fungal and bacterial phytopathogens. The research was aimed at assessing the effectiveness of the protective and stimulating compositions in the treatment of soybean seeds against microflora of the seeds and their effect on the sowing properties.

The results of phytoexpertise showed that the analyzed soybean seedswere infected with fungal and bacterial microflora, in particular, the fungi of genera *Penicillium* spp., *Alternaria* spp., *Fusarium* spp., and bacteria *Pseudomonas* spp., *Erwinia* spp.

According to the results of phytoexpertise, one can say that the degree of seeds infestation with fungal and bacterial microflora can create a high infectious background for seed mold, root rot, fusarium blight, Alternaria blight, and bacterioses during the vegetation period, deteriorate the seeding properties of the seeds, and reduce the germination vigor of the plants. The level of the analyzed seeds infestation with the fungal and bacterial infection requires effective pre-sowing treatment with the preparations that have fungicidal and bactericidal properties, and with their combinations with stimulants that activate the physiological processes in plants.

The effectiveness of 26 protective and stimulating compositions on the soybean seedswas assessed in the laboratory conditions. With that, their effect on the seeding properties of the seeds (the germination vigor and the laboratory germination), on the growth vigor of the seedlings and the root system, on the fungal and bacterial infestation was assessed.

To develop the soybean protective and stimulating compositions, seed dressers (fungicides (TMTD w.s.c., Insure perform 12% s.c., Sertikor 050 s.c., Maxim 025 s.c.), insectofungicides (Celest Top 312.5 s.c., Prestige s.c.), insecticides (Kruiser 350 s.c., Actara 250 w.d.g., Tabu TMTD w.s.c.), and stimulants (Extrasol, potassium humophosphate, Phytolavin) were chosen based on the laboratory studies (Cunha and Filho, 2010). All preparations were tested in the recommended dosages.

Based on the studies, the most effective protective and stimulating compositions were selected, that had a positive effect on the seeding properties of the seeds (the germination vigor and the laboratory germination), on the growth vigor of the seedlings and the root system, which effectively inhibited fungal and bacterial microflora of the seeds, and the number of affected soybean seeds and seedlings.

During the phytoexpertise of the soybean seeds in the laboratory conditions, their seeding qualities (the germination vigor and the laboratory germination rate (Fig. 1), the number of affected seeds and seedlings, and the infestation rate with fungal and bacterial infections were established. The results of the experiments are shown in Tables 1, 2.

The results of checking the seeding properties of

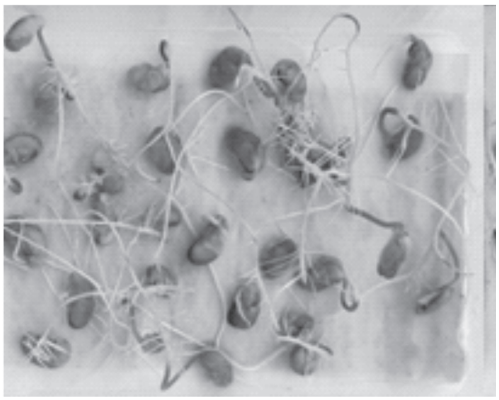


Fig. 1. Germination of the soybean seeds

the seeds of three soybean varieties (Table 1) also showed that they met the requirements (GOST 9669-61) and belonged to Class I. However, this revealed more thanthe half of affected seeds (mold and rot) at the level of 49 – 56%.

The phytopathological analyses of the soybean seeds also revealed fungal and bacterial microflora. The results of the studies are shown in Table 2 and Figures 2 and 3.

The results of the phytopathological analyses showed that all analyzed samples of soybean seedswere heavily infected by the fungal and bacterial microflora. The samples were dominated by the fungi of genera *Alternaria* and *Fusarium;* saprophytic species of the fungi of genera *Alternaria* and *Mucor* that caused seed mold were also found. The bacteria found were*Pseudomonas syringaepv. glycinea* causing burns, and *Pectobacteriumcaratovorum*– the pathogen of soft rot (Plant protection handbook, 2004). The infestation rate was 51 to 90%.

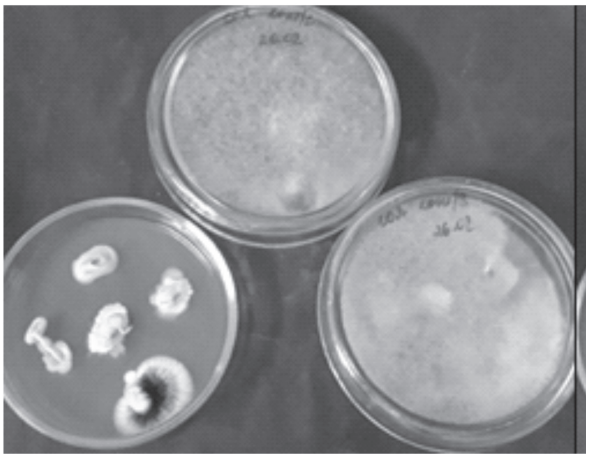


Fig. 2. The bacterial and fungal microflora on the soybean seeds (culture medium)

The results in Table 3 show that the treatment of the soybean seeds with the protective and stimulating compositions also significantly increases the germination vigor and the laboratory germination rate of seedlings. In the reference, the share of affected plants was 52 %. With that, the biological effectiveness of seed treatment in the variants was high and ranged between 92 and 96 %.

The laboratory experiments showed that the treatment of the soybean seedswith the protective and stimulating compositions suppressed fungal and bacterial infections in the seedsand improved the seeding properties and the seedling growth rate (Dzhaymurzina *et al.,* 2014; Dzhaymurzina *et al.,* 2015). The biological effectiveness of the protective and stimulating compositions in soybean against root rot in the branching phase was 82.5 – 73.2 %; before harvesting — 80 – 70.4%, and in the reference variant, this value was 68.0 – 66.4%, respectively (Table 4).

During the soybean-harvesting period, the yield data for the variants of the experiment and the biometric indicators were determined.

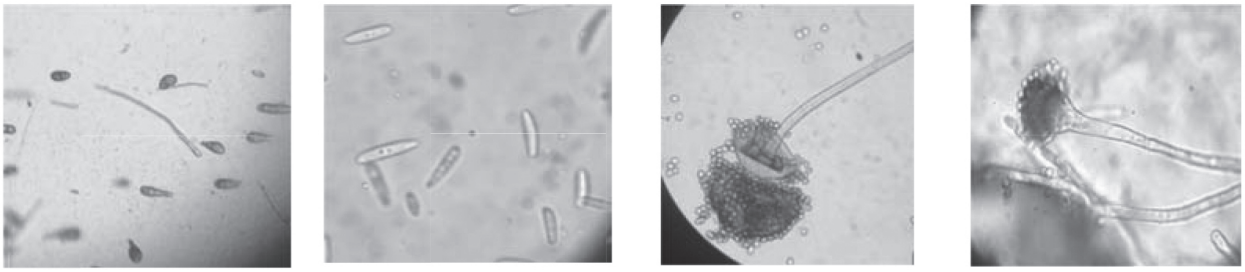
As one can see from Table 5, in all types of experiments, a positive effect of treatment with the protective and stimulating compositions on the biometric indicators of soybean such as plant height, the number of branches, and the number of beans and seeds per plantwas noted. The plant height in

Table 1. The seeding qualities of the soybean seeds (moist chamber)

Variety	Germination vigor, %		Laboratory germination,%	The number of affected seeds, %
	Accounting days			
	3	5	7	
Lastochka, superelita, 2018	90.0	98.0	98.5	51.0
Tourmaline, 2018	84.0	97.0	97.0	49.0
Luna, 2018	81	95	97	56.0

Table 2. Soybean seeds infestation by fungal and bacterial microflora (the nutrient medium)

No.	Variety	The number of affected seeds, %	Fungal microflora, %				Bacterial microflora, %
			Alternaria	*Fusarium*	*Mucor*	*Aspergillus*	
1	Lastochka, superelita, 2018	100	27	40	52	-	53
2	Tourmaline, 2018	100	4	-	97	41	51
3	Luna, 2018	100	72	30	38	10	90



conidia of fungus *Alternaria*macro- and microconidia of fungus *Fusarium* sporangia with spores of fungus *Mucor* spores of fungus *Aspergillus*

Fig. 3. The fungal microflora isolated from the soybean seeds

the variants treated with the protective and stimulating compositions was in the range between 101.5 and 113.3 cm, while in the reference variant, this value did not exceed 93.0 cm. The number of beans per plant in the treated variants was higher thanthat in the reference variant by 50.1 – 51.5 %, the number of seeds per plant also exceeded the parameters in the reference variant by 47.7% and 54.1%, respectively.

Thus, the analysis of the biometric measurement data and the crop structure showed that the treatment of the soybean seedswith the protective and

Table 3. The effectiveness of the treatment of the soybean seeds with the protective and stimulating compositions

No.	Variants	Germination vigor, %	Laboratory germination, %	The intensity of seedlings development, %	The number of affected seeds and seedlings, %	Biological effectiveness, %
1	Reference	62	84	+	52	-
2	TMTD w.s.c. + Celest Top 312.5 s.c. + Extrasol	93	98	+++	2.0	96.0
3	TMTD w.s.c.+ Celest Top 312.5 s.c.+ potassium humophosphate	94	97	+++	2.0	96.0
4	TMTD w.s.c. + Celest Top 312.5 s.c. + Phytolavine	95	97	+++	4.3	92.0
5	TMTD w.s.c.+ Prestige s.c. + Extrasol	94	98	+++	3.0	94.0
6	TMTD w.s.c. + Prestige s.c. + potassium humophosphate	94	98	+++	2.0	96.0
7	TMTD w.s.c. + Prestige c.s. + Phytolavine	95	98	+++	5.0	92.0

Note: + low intensity, ++ medium intensity, +++ intensive development

Table 4. The biological effectiveness of the protective and stimulating compositions against soybean root rot, the Almaty region, Karasai district, Agropark Ontustik LLC (2019)

Variants	The rate of plant infestation with root rot		Biological effectiveness against root rot, %	
	In the branching phase	Before harvesting	In the branching phase	Before harvesting
TMTD w.s.c. + Celest Top 312.5 s.c. + Extrasol	1.7	2.5	82.5	80.0
TMTD w.s.c. + Celest Top 312.5 s.c. + potassium humophosphate	2.6	3.7	73.2	70.4
TMTD w.s.c. (reference)	3.1	4.2	68.0	66.4
Reference	9.7	12.5	-	-

Table 5. The economic effectiveness of the protective and stimulating compositions and their effect on the growth and development of soybean plants, the Almaty region, Karasai district, Agropark Ontustik LLC (2019)

Variant	Plant height	Number of branches	The number of beans per one plant	The number of seeds per one plant	The weight of 1,000 seeds, g
TMTD w.s.c. + Celest Top 312.5 s.c. + Extrasol	101.5	1.7	49.9	124.4	157.0
TMTD w.s.c. + Celest Top 312.5 s.c. + potassium humophosphate	113.3	1.8	56.9	141.5	168.5
TMTD w.s.c. (reference)	98.7	1.5	39.7	86.9	153.8
Reference	93.0	1.2	27.5	64.9	148.0
LSD_{05}	*6.4*				

stimulating compositions increased the soybean vegetative growth, the number of beans and seeds per plant, productivity, and the weight of 1,000 seeds.

Conclusion

Thus, the pre-sowing the treatment of the soybean seedswith the developed protective and stimulating compounds significantly improves their seeding properties and suppresses fungal and bacterial infections.

The analysis of the biometric measurement data and the crop structure shows that the treatment of the soybean seeds with the protective and stimulating compositions increases the soybean vegetative growth, the number of beans and seeds per plant, productivity, and the weight of 1,000 seeds.

As a result of using the developed integrated soybean protection system, an additional yield of 20 – 30 % has been also obtained.

To protect soybean crops from seed and soil infection, high-quality treatment of the seeds is needed before seeding. The preparation of seed material and choosing a proper treater provide the possibility to prevent the development of diseases in the field and to obtain good healthy seedlings.

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