Biological Preparations with Different Mechanisms of Action for Protecting Potato against Fungal Diseases

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Abstract—Mycological analysis throughout the vegetation period of potato (*Solanum tuberosum*) made it possible to study in detail the structure of the micromycete community, to determine typical dominant (frequency, more than 60%), typical common (frequency, 30 to 60%), typical rare (frequency, 10 to 30%), and casual (frequency, less than 10%) species and to estimate changes in the microorganism community caused by plant protection preparations with different mechanisms of action. It was shown that, as a result of occurrence of resistant forms, synthetic preparations against fungal pathogens of potato (such as TMTD, Ridomil gold MC, and Cupricol) were only slightly more effective than biological preparations (Trichodermin and AgroChit), with the former considerably changing the natural saprophytic mycological community. An increase in the soil pool of *Trichoderma harzianum* as a result of application of a biological preparation based on this antagonistic fungus correlated with its effectiveness against the soil pathogen *Fusarium* sp., which causes root rot. A chitosan-based elicitor preparation more effectively suppressed the development of early (*Alternaria* sp. and *Macrosporium* sp.) blighting of leaves and had a weaker effect on soil microflora.

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For a long-term strategy of effective and ecologically substantiated agronomy, it is necessary to assess the state of agrobiocenosis, which experiences various changes due to pesticides. The selection of protectants against phytopathogens is one of the key factors of the phytosanitary state of planting and productivity of crops.

Synthetic fungicides adversely affect the complex of microorganisms that, together with the root (rhizosphere) and aboveground (phyllosphere) systems of plants, forms a complex ecological niche and can effectively compete with phytopathogens. This eventually has a detrimental effect on plants themselves. This consequence of chemicalization of agriculture results from disregarding the processes of self-regulation in biocenoses, as a result of which the integrity of the existing agroecosystems is disturbed.

As an alternative to synthetic preparations, biological preparations based on microorganisms antagonistic to the pathogens of cultivated plants are finding an ever broader distribution in agriculture. However, the effect of the vast majority of such biological preparations is based on direct biological control, proceeding from the principle of that certain representatives of microflora dominate in the environment [1, 2]. Characteristic representatives of such agents exerting direct biological control are microscopic fungi from the genus *Trichoderma*. These fungi produce a set of antiphytopathogenic factors, the key ones of which are hydrolases (chitinases and glucanases); this ability underlies the biological protection of plants from pathogens [3, 4].

Another, indirect principle of plant protection implies the induction of nonspecific resistance, which is based on enhancement of personal protective mechanisms of plants using elicitors-natural biologically active compounds [5], among which chitin and its derivative chitosan occupy a special place [6, 7]. The results of application of chitin and chitosan to immunize plants against fungal infections indicate that these biopolymers have a considerable positive effect [8]. It was reported that chitosan doe not have a direct effect on microorganisms in vivo, which principally distinguishes it from other commonly used protectants against phytopathogens. However, the effect of elicitor preparations on the structure of the microorganism community in a real agrocenosis has not yet been elucidated.

When selecting the means of plant protection, the effectiveness of direct suppression of phytopathogens is brought to the forefront, whereas little attention has been given to occurring rearrangements in species composition and the proportion of representatives of soil microflora, which serves as an informative index of the strength of anthropogenic impact caused to agrophytocenosis. Changes in the structure of the complex of microorganisms are reflected in the spatial frequency of species occurrence, which corresponds to the position of species in the complexes and makes it possible to assess the scale of disturbance and change in biocoenotic equilibrium in the microorganism community caused by plant protectants.

The goal of this study was to estimate how preparations with different mechanisms of action affect the structure of the microorganism community in the agrocenosis—the key parameter reflecting the state of plantings and productivity of crops.

MATERIALS AND METHODS

Field tests of preparations were performed at Lenin OPKh, Tatarstan, with the potato (*Solanum tuberosum*) cultivar Lugovskoi. The size of each experimental plot was 1 hectare. Soil on plots was light-gray heavy loam (pH of salt extract, 5.5–6.0; humus content, 2.5%). Spring winter was a forecrop for potato.

We used the fungicidal preparation Trichodermin and the elicitor preparation AgroChit. Trichodermin, obtained from culture liquid and spores of Trichoderma harzianum F-432 from the collection of the Department of Microbiology, Kazan State University, was manufactured by the Republican Station for Plant Protection (Kazan). It was used once for the preplanting treatment of tubers (1.5 l/ton) and twice during the vegetation period (1.5 l/hectare). AgroChit, obtained from lowmolecular-weight chitosan, was developed at the Bioengineering Center, Russian Academy of Sciences, and manufactured at ZAO BioChit (Moscow). It was also used once for the treatment of tubers (1.5 l/ton) and twice during vegetation (1.5 l/hectare). The chemical preparation TMTD (ZAO August, Moscow) was used as a reference agent for the preplanting treatment of tubers (0.5 kg/ton); the systemic fungicide Ridomil gold MC (OOO Singenta, Moscow) and the contact fungicide Cupricol (KNITI-VT, Kazan) were used as reference agents for the treatment during the vegetation period (2.5 and 5 kg/hectare, respectively). Intact plants served as a control.

Soil samples were taken in spring before planting potato (to determine the initial state of the microbiological community), after planting (to reveal changes in the microbiocenosis and to detect soil infection), and during intensive development of diseases on potato vines (to estimate the dynamics of accumulation of inoculum of certain representatives of microflora in soil) [9].

The structure of the complex and the frequency of the occurrence of soil micromycetes were determined by multiple dilution of aqueous suspension of soil samples followed by inoculation of solid nutrient media (potato–glucose agar, potato–sucrose agar, and wort agar). The same solid media were used for subsequent mycological tests. Samples were incubated in light at room temperature for 5 days. Micromycetes were identified and counted by direct microscopy. With respect to the frequency of occurrence of microorganisms, typical dominant (frequency, more than 60%), typical frequent (frequency, 30–60%), typical rare (frequency, 10–30%), and casual (frequency, less then 10%) species were distinguished.

The rate of development of diseases on potato vines was determined by thrice counting the percentage of infected green parts of plants during vegetation period: after the appearance of primary infection foci, during mass-scale development of diseases, and before harvesting. The biological effectiveness of preparations was estimated by visual examination and direct microscopy of potato leaves. The structure of the micromycete community of potato phyllosphere and rhizosphere was determined using mycological analysis on solid nutrient media [5]. The distribution of potato tuber diseases was assessed by analyzing medium-size tubers after harvesting.

RESULTS AND DISCUSSION

Mycocenosis of soil. Mycological analysis of soil samples from the experimental field on nutrient media revealed the presence of microscopic fungi belonging to 17 genera. The greatest diversity of micromycetes was observed in the soil samples taken before planting potato and in the control (16 and 14 genera, respectively; Table 1). In all variants, soil samples contained pathogens from the genera Rhizoctonia (Rhizoctonia blight of potato tubers and powdery scab of potato vines), Geotrichum (rubbery tuber rot), Oospora (skin spot), and Phytium (root and tuber rot). Fungi of the genus Fusarium (tracheomycosis of potato, root and tuber rot) were classified in the group of typical rare and typical common species. A considerable decrease in the frequency of occurrence of representatives of this genus was only observed in the variant with Trichodermin; in this variant, marked saturation of the mycocenosis with Trichoderma was observed.

The treatment of tubers and then plants with fungicides and biological preparations during vegetation period decreased the population density of *Phytophthora* sp. (the causal agent of potato late blight) and *Sclerotinia* sp. (the causal agent of white rot of stems).

At all stages of analysis, soil samples abounded in saprophytic micromycetes of the genera *Penicillium* and *Mucor*. Some representatives of these genera can suppress the germination of seedlings, thereby increasing the possibility of young plants becoming infected. The representatives of these genera were classified with typical common and typical dominant species, with the manner of treatment having little effect on their dominant position.

Thus, soil in an agrocenosis may be regarded a potential source of infection. Before planting, it contains the complete set of potato-specific pathogens, the majority of which remained in soil and were not exposed to the preparations used. In addition, the soil samples contained a large number of saprophytic fungi that attenuated the resistance of seedlings to diseases.

	Frequency of occurrence, %									
Microorganism	V	Control		Reference preparations		AgroChit		Trichodermin		
	v	VI	VIII	VI	VIII	VI	VIII	VI	VIII	
Fusarium	36.7	23.3	30.0	23.3	26.7	26.7	20.0	20.3	6.6	
Rhizoctonia	20.0	26.6	30.0	20.0	23.3	20.0	20.0	20.0	20.0	
Phytophthora	16.0	16.0	16.0	-	6.6	3.3	3.3	_	-	
Alternaria, Macrosporium	13.3	10.0	3.3	10.0	_	_	3.3	10.0	_	
Geotrichum	13.3	16.7	13.3	13.3	13.3	13.3	13.3	13.3	23.3	
Oospora	13.3	10.0	23.3	13.3	16.0	10.0	10.0	16.7	26.7	
Phytium	13.3	16.7	16.7	6.7	3.3	13.3	13.3	3.3	3.3	
Sclerotinia	16.6	3.3	6.7	3.3	-	3.3	_	-	_	
Cladosporium	3.3	3.3	3.3	-	-	3.3	_	3.3	-	
Aspergillus	3.3	3.3	20.0	-	20.0	_	3.3	-	10.0	
Penicillium	60.0	53.0	33.0	53.0	46.7	40.0	30.0	30.0	30.0	
Mortierella	6.6	_	3.3	3.3	-	-	_	_	-	
Halobyscus	6.7	6.7	_	-	-	3.3	_	-	_	
Mucor	100.0	60.0	56.7	60.0	60.0	56.7	56.7	60.0	60.0	
Humicola	_	3.3	9.9	-	_	_	_	-	_	
Mycogone	6.7	_	_	-	_	3.3	_	—	_	
Trichoderma	3.3	_	_	-	10.0	_	_	36.7	36.7	

Table 1. Mycological analysis of soil*

* V, May (soil before planting); VI, June; VIII, August.

Dynamics of infections		Control	Reference preparations	AgroChit	Trichodermin	
Late blight	VI	1	1.0	1.0	1.0	1.0
		2	0.1	0.05	0.05	0.05
	VII	1	12.4	8.0	8.0	9.0
		2	3.5	0.6	0.6	0.7
	VIII	1	15.0	12.0	10.0	10.0
		2	8.0	2.1	2.5	2.6
Early blight	VI	1	50	40	40	45
		2	1.1	0.5	0.3	0.3
	VII	1	90	80	80	90
		2	9.1	1.8	1.1	1.0
	VIII	1	100	100	100	100
		2	12.0	4.8	5.5	4.5

Table 2. Distribution (1) and development (2) of diseases on leaves, %*

* VI, June; VII, July; VIII, August.

Distribution and development of plant diseases (visual observations). The first observation in June showed no significant influence of the tested preparations on the distribution and rate of development of early (caused by *Alternaria* and *Macrosporium*) and late (caused by *Phytophthora*) blights on potato vines compared to the control and reference samples (Table 2). Later, in June, we noticed that AgroChit and Trichodermin had a protective effect, which was expressed as a marked decrease in the rate of development of the potato disease caused by *Alternaria*, as well as distribution and development of the potato blight caused by *Phytophthora*. The degree of protection ensured by the biological preparations was close to that characteristic

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of the reference preparations. The same tendency was observed during the third examination. Note the widespread occurrence of the early potato blight caused by *Alternaria*, which was detected on all plants in all experimental variants.

Thus, biological preparations were nearly as effective as the reference preparations and suppressed the development of potato diseases induced by *Phytophthora* and *Alternaria* compared to the control.

Structure of the complex of microorganisms inhabiting the phyllosphere. The microorganisms inhabiting the phyllosphere of potato were somewhat less diverse than the soil microflora; however, during vegetation it underwent greater changes depending on the preparations used.

In all variants, *Phytophthora*, the causal agents of early and brown blight (*Alternaria* sp. and *Cladosporium* sp., respectively), as well as the pathogens that cause tracheomycosis and vascular disease in potato (*Fusarium* sp. and *Verticillium* sp., respectively), were present on potato leaves, with the representatives of the genera *Fusarium* and *Alternaria* being the most abundant (Table 3).

Similarly to the reference variant, the treatment with AgroChit and Trichodermin decreased the population density of fungi of the genus Alternaria in July to a certain level, which was then restored in August to a level that exceeded the initial one in June. Although the dynamics of distribution of the causal agents of early and brown blight of potato leaves were similar in both variants, the decrease in the population density of pathogens in the case of Alternaria was apparently due to the inhibitory effect of preparations, whereas in the case of *Cladosporium* sp., it was a consequence of the natural process of fungus development. The pathogen that caused late blight appeared in a marked amount only in July and almost did not progress further in August. All the preparations tested caused a twofold decrease in the frequency of occurrence of Phytophthora compared to the control. However, they had different effects on Fusarium sp. Similarly to the reference variant, AgroChit suppressed development of winter rot in potato during intensive spraying in June and July; however, after the termination of treatments in August, the abundance of this micromycete reached the control level. Conversely, the effect of Trichodermin was the same as in the case of soil infection: the population density of Fusarium was not restored. Furthermore, its population density decreased at the end of vegetation period; as a result, it became a typical rare, rather than a typical common, species. Similarly to the previous variant, in the case of the Trichoderma-based preparation, an increase in the population density of this antagonist on the above-ground parts of potato plants was observed.

Saprophytic micromycetes were dominated by the representatives of the genera *Mucor* and *Penicillium* due to their large pool in soil before planting potato.

However, it should be remembered that all representatives of micromycetes form a single biocoenotic complex, and even minor components of communities (*Acremonium* sp. and some others) can potentiate the effect of pathogens under certain ecological conditions.

Mycological analysis of tubers. Thirteen micromycete genera, seven of which were classified as dangerous pathogens (specifically, *Phytophthora, Fusarium, Phytium, Oospora, Geotrichium, Rhizoctonia,* and *Alternaria*), were isolated from untreated potato tubers (Table 4). A considerable proportion of phytopathogenic isolates was represented by *Phytophthora* sp., *Rhizoctonia* sp., and *Fusarium* sp., which belong to the group of typical rare and typical common species. The representatives of the genera *Phytium* and *Oospora* did not occur as frequently in the complex.

We also isolated saprophytic microscopic fungi from the tubers intended for planting, which were dominated by representatives of the genera *Penicillium* and *Mucor*, which might be due to their high sporulating activity and high antagonistic potential.

In the control variant, the degree of infection of the new-crop tubers with the phytopathogenic micromycetes increased and was approximately 1.5 to 2 times greater than that of the tubers used for planting.

Preplanting treatment of tubers and subsequent spraying of plants during vegetation with synthetic preparations decreased the population density of *Phytophthora* and *Fusarium* on the new-crop tubers; the frequency of occurrence of the saprophytes from the genera *Mucor* and *Penicillium* decreased as well. On the other hand, the frequency of occurrence of the causal agents of skin spot, rubbery rot, as well as *Rhizoctonia* and *Fusarium* blights on the tubers of the reference variant increased, which may be indicative of the occurrence of fungal forms resistant to these preparations.

In most cases, biological preparations improved the phytosanitary state of new-crop tubers. However, these preparations did not suppress the growth and development of the pathogens that caused bare patch and skin spot, although AgroChit slightly suppressed the development of *Fusarium* blight. However, Trichodermin tended to decrease the frequency of fungi of the genus *Fusarium*.

Potato productivity and crop quality. It is known that phytosanitary conditions during vegetation largely determine the crop quality. For this reason, the treatment of the tubers intended for planting and subsequent treatment of plants with AgroChit and Trichodermin improved the quality of new-crop tubers.

Comparative tests of fungicides and biological preparations demonstrated certain differences in the efficiency of their effect (Table 5). It was found that the treatment of planting material or potato plantings with biological preparations decreased the degree of infection of new-crop tubers with *Phytophthora* and common potato scab, as well as *Fusarium* blights in the

		Frequency of occurrence, %										
Microorganism	Control			Reference preparations			AgroChit			Trichodermin		
	VI	VII	VIII	VI	VII	VIII	VI	VII	VIII	VI	VII	VIII
Fusarium	65.0	85.0	98.0	62.5	60.0	90.0	72.5	65.0	77.5	40.0	27.0	17.5
Phytophthora	_	22.5	20.0	-	12.5	12.5	-	10.0	10.0	_	7.5	10.0
Alternaria	62.5	67.5	95.0	55.0	37.5	70.0	57.5	27.5	75.0	50.0	42.5	82.0
Cladosporium	5.0	_	22.5	5.0	_	17.5	5.0	_	25.0	5.0	-	20.0
Aspergillus	_	7.5	_	-	12.5	-	2.5	2.5	-	5.0	7.5	_
Penicillium fellutanum	22.5	17.5	12.5	12.5	5.5	-	15.0	7.5	-	12.5	7.5	_
P. regulosum	10.0	15.0	20.0	7.5	10.0	17.0	7.5	12.5	22.5	5.0	10.0	17.5
Mucor	72.5	70.0	67.5	44.5	32.5	80.0	45.0	32.5	100.0	57.5	45.0	75.0
Trichoderma	7.5	10.0	7.5	7.5	7.5	_	12.5	10.0	-	12.5	45.0	25.0
Verticillium	2.5	5.0	-	-	2.5	_	-	2.5	-	_	-	2.5
Botrytis	_	5.0	10.0	-	2.5	5.0	-	2.5	7.5	_	-	_
Sthemphilium	_	_	10.0	-	_	7.5	-	-	10.0	_	-	5.0
Aureobasidium	-	10.0	17.5	-	5.0	12.5	-	7.5	15.0	-	2.5	5.0
Acremonium	7.5	10.0	10.0	-	_	-	2.5	_	-	2.5	-	_
Fusidium	7.5	5.0	5.0	5.0	_	—	2.5	_	-	2.5	_	_

Table 3. Mycological analysis of potato phyllosphere*

* VI, June; VII, July; VIII, August.

variant with Trichodermin. However, in these cases, the infection of tubers with *Rhizoctonia* blight increased compared to the reference variant. However, no damage resulting from the infection with bacterial blights was observed in tubers treated with the biological preparations, whereas the tubers of the control and reference variants were damaged.

Note that no traits of skin spots or rubbery rot were found during visual examination of new-crop tubers. However, mycological analysis on nutrient media revealed the presence of the causal agents of these diseases (*Oospora* sp. and *Geotrichum* sp.) on tubers in all experimental variants (Table 4). On the basis of the results obtained in this study, it can be predicted that skin spots or rubbery rot of potato will develop in the process of storing tubers.

Analysis of the biological efficiency of the biological preparations showed that Trichodermin and AgroChit can protect potato plantings from early and late blights and decrease the rate of development of diseases (Table 6), thereby improving crop quality and preservation.

Thus, all micromycete complexes, irrespective of the variant to which they belong, contained both saprophytic and phytopathogenic micromycetes, which together form a single biocoenotic complex. The complexes of micromycetes included both dangerous pathogens that cause potato diseases and minor components of communities from the genera *Acremonium*, *Nigrospora*, etc., which, under certain ecological conditions, can potentiate the effect of pathogens, with the planting material and the soil of agrophytocenosis serving as sources of infection.

Mycological analysis on nutrient media revealed a high population density of phytopathogens that were not detected during visual examination; this may be used for predicting respective diseases in future. We showed that the treatment with the synthetic preparations did not protect potato tubers from infection by common potato scab and that the sensitivity of fungi belonging to the genus Fusarium and certain other micromycete genera to these preparations decreased. These data led us to assume that the populations of pathogens contain resistant strains. This assumption accounts for the fact that the efficiency of the synthetic fungicides was comparable to the efficiency of the biological preparations. We also showed that the biological preparations AgroChit and Trichodermin, which were used for the treatment of potato tubers and plantings, had different effects on certain representatives of mycocenosis. AgroChit did not affect microorganisms directly; it only stimulated the development of nonspecific resistance in plants. It most strongly influenced the microflora of the phyllosphere and had a much weaker effect on soil microorganisms. AgroChit had a negligible effect on the frequency of occurrence of pathogens but decreased the rate of development of Alternaria and Phytophthora blights on leaves. Conversely, Trichodermin had a direct effect on the representatives of the micromycete community. It also affected the structure

				Frequen	cy of occurre	ence, %						
Microorganism	X 7	Control		Reference p	Reference preparations		AgroChit		dermin			
	V	VIII	IX	VIII	IX	VIII	IX	VIII	IX			
Fusarium. sambosinum	22.5	52.5	57.5	37.5	45.0	17.5	12.5	15.0	10.0			
F. gibbosum	17.5	30.0	30.0	20.0	30.0	10.0	10.5	7.5	5.0			
Rhizoctonia	32.5	50.0	72.5	17.5	30.0	40.0	52.5	37.5	47.5			
Phytophthora	27.5	15.0	12.5	12.5	5.0	5.0	2.5	_	2.5			
Alternaria	5.0	7.5	5.0	5.0	_	5.0	2.5	-	-			
Geotrichum	12.0	15.0	25.0	15.0	25.0	12.5	15.0	15.0	15.0			
Dospora	15.0	10.0	22.0	17.5	32.5	7.5	17.5	25.0	45.0			
Phytium	10.0	15.5	22.5	2.5	10.0	7.5	5.0	5.0	2.5			
Cladosporium	7.5	_	10.0	-	5.0	_	5.0	-	5.0			
Aspergillus	-	-	7.5	-	2.5	_	7.5	-	5.0			
Penicillium	87.5	85.0	40.0	57.5	45.0	75.0	32.5	85.0	32.5			
Mortierella	-	-	7.5	-	-	7.5	2.5	5.0	-			
Mucor	85.0	100.0	52.5	100.0	57.5	80.0	37.5	75.0	57.5			
Frichoderma	7.5	-	10.0	-	12.5	_	5.0	77.5	57.5			
Nigrospora	15.7	_	_	-	2.5	_	-	-	-			
Chaetomium	_	_	_	-	-	10.0	10.0	_	-			
Chephalosporium	_	-	_	-		_	7.5	_	2.5			

Table 4. Mycological analysis of potato tubers*

* V, May (tubers intended for planting); VIII, August; IX, September.

Disease	Planting material	Control	Reference preparations	AgroChit	Trichodermin
Phytophthora blight	8.3	11.4	6.8	3.8	3.5
Rhizoctonia blight	20	25	10	15	15
Common potato scab	20	30	50	15	25
Skin spots	4.2	_	-	_	_
Silver scab	2.1	1.6	1.6	_	1.8
Fusarium blight	4.2	11.4	9.8	5.8	3.6
Bacterial blights	4.2	6.6	3.2	-	-

Table 5. Distribution of diseases of tubers, %

Table 6. Potato yield and efficiency of preparations

	Yie	eld	Biological e	efficiency, %
Variant	hundred kilograms per hectare	percentage from control	with respect to <i>Alternaria</i> blight	with respect to <i>Phytophthora</i> blight
Control	89	100		
Reference preparations	110	123.6	71.0	73.9
AgroChit	120	134.8	72.4	70.2
Trichodermin	103	115.7	75.8	69.2

of soil microflora, having an especially strong effect on the micromycetes of the genus *Fusarium*. The presence of this antagonistic fungus in soil facilitates a decrease in the pool of this pathogen.

The treatment of potato tubers and plantings with the biological preparations decreased the degree of infection of new-crop tubers with phytopathogens, including *Fusarium* and *Phytophthora* blights and common potato scab.

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