Biological Rationale for the Use of Polyfunctional Biological Products Based on Antagonist Microbes and Chitosan in Wheat Cultivation

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Abstract. The modern concept of resource-saving technology for the cultivation of agricultural crops, including wheat, is based on the use of growth-stimulating biologically active substances and beneficial microorganisms. Studies aimed at the development of polyfunctional compositions that reduce the harmfulness of pathogens and have a positive effect on the production process are especially promising. The paper provides data on the biological substantiation of the use of polyfunctional complexes based on B. subtilis strains and chitosan salicylate to protect wheat from diseases and increase yields. Polyfunctional complexes “Vitaplan, KZh + 0.1% chitosan salicylate” (submerged cultivation, composition) optimize the physiological state of wheat plants; significantly increase its productivity and disease resistance. It was shown that the combined use of Vitaplan, KZh and 0.1% chitosan salicylate increased wheat productivity and the biological efficiency of the polyfunctional complex against wheat yellow rust. The use of the complex did not have a significant effect on the indicators characterizing the growth of the vegetative mass of plants, but had a positive effect on the structure of wheat yield.

1. Introduction
The production of grain crops is the main branch of agriculture in the Russian Federation and plays a crucial role in ensuring the food security of the country. In addition, grain crops are necessary for the development of other sectors of the agro-industrial complex, and in particular, livestock and poultry farming. The productivity of cultivated plants significantly depends on both genetic and climatic factors, as well as on their combination. One of the ways to reduce the use of fertilizers without prejudice to plant nutrition is the use of biostimulants in crop production, which enhance the growth and development of plants, and increase their adaptive potential to cultivation conditions. The use of bioregulators is promising for optimizing the phytosanitary state of crops of various agricultural crops, including – to neutralize side effects after the use of chemical plant protection products.

Soil bacteria capable of stimulating plant growth and protecting them from diseases and abiotic factors mainly belong to the genera Acetobacter, Azospirillum, Azotobacter, Bacillus, Burkholderia,
Klebsiella, Pseudomonas, and Serratia. Treatment of plants with microbiological preparations, developed, in particular, on the basis of bacilli, can improve the absorption of available nutrients by plants and increase the effectiveness of protective measures [1]. Biological polysaccharides, including chitosan, can have a growth-stimulating effect on a plant, as well as increase their adaptive potential to natural and climatic factors [2]. In agriculture, chitosan is used mainly as an inducer of plant resistance to diseases, a biostimulant [3], and also to reduce the effects of abiotic stress in plants [4]. However, the exact metabolic response of plants to chitosan is still unclear; it may be associated with pleiotropic modulation of carbon and nitrogen metabolism [5].

The protective effect of biological products based on B. subtilis strains is based on the suppression of phytopathogenic microflora, in particular pathogens of root rot, as well as the ability to increase the systemic resistance of plants to diseases. The biological effectiveness of preparations based on the B. subtilis bacterium can be increased with enhancing the inducing activity of the antagonist microbe, since it has been experimentally shown that Bacillus strains induce systemic resistance to various bacterial and fungal plant pathogens [6].

One of the ways to enhance the inducing activity of a biological product is to include in its composition natural polysaccharides (chitin, chitosan and their oligomers), which are known to be powerful elicitors of plant immunity. At present, chitosan and preparations based on it are widely used as inducers of nonspecific resistance in plant protection against diseases; salicylic acid, as one of the main inducers of resistance, is often used to protect against phytopathogens [7].

Based on the idea that the biological activity of chitosan is determined with its ability to induce biochemical pathways leading to the activation of plant defense reactions and the formation of resistance to fungal, bacterial and viral diseases in them. It is assumed that chitosan and its derivatives will include (alone or in combination with bacterial cells) systemic resistance to phytopathogens.

2. Formulation of the problem
The aim of the study is to analyze the effectiveness of biological products and polyfunctional complexes based on the strains of Basillus subtilis and chitosan salicylate to increase productivity and protect wheat from pathogens of leaf diseases and root rot.

3. Materials and methods
Material for research – Leningradskaya 6, k-64900, a variety of spring soft wheat from the collection of the Department of Wheat Genetic Resources of the Federal State Budgetary Scientific Institution “Federal Research Center N.I. Vavilov All-Russian Institute of Plant Genetic Resources” (VIR). The objects of research are polyfunctional complexes and biological preparations created in the laboratory of microbiological plant protection of the Federal State Budgetary Scientific Institution VIZR.

The field experiment was carried out in triplicate at the experimental field of the Pushkin laboratories of the VIR. The experiment scheme provided for the following options:
– Control (water);
– “Vitaplan, KZh” is a culture liquid, consisting of the strains of Basillus subtilis VKM B-2604D and B. subtilis VKM B-2605D in a ratio of 1: 1 (titer of living cells and spores / g B. subtilis - 1010 CFU / ml). For submerged cultivation of B. subtilis strains, a corn-molasses medium of optimized composition was used (corn extract – 30 g / L; molasses – 15 g / L (pH = 7.8). The culture liquid contains spores of producer strains, nutrient medium residues and metabolites released into the medium by microorganisms during fermentation;
– “Vitaplan, SP” (standard), is a biological product based on strains B. subtilis VKM B-2604D and B. subtilis VKM B-2605D. B. subtilis strain VKM-2604D is a producer of various antibiotics: polypeptide (bacteriocin group), polyene, and B. subtilis VKM B-2605D strain synthesizes a polypeptide close to bacillin and hexaene antibiotics, one of which belongs to the mediocidin subgroup;
– 0.1% chitosan salicylate (aqueous solution);
– polyfunctional complex: Vitaplan, KZh + 0.1% chitosan salicylate (chitosan salicylate was introduced into the nutrient medium for deep cultivation of strains);
polyfunctional complex Vitaplan, KZh + 0.1% chitosan salicylate (chitosan salicylate was introduced into the composition during the preparation of the final preparative form).

Seed treatment with biological products and polyfunctional complexes was carried out before sowing wheat, and during the growing season, the plants were sprayed four times during the tillering, tubing, heading and flowering phases.

To obtain a polyfunctional complex “Vitaplan, KZh + 0.1% chitosan salicylate (submerged cultivation)”, 0.1% chitosan salicylate was added to the initial corn-molasses medium. Then the flasks with the nutrient medium were sterilized, cooled, and inoculated with a 5-day culture of B. subtilis strains. The cultivation time is 3 days on an orbital shaker (180 rpm) at T = 27–28 °C. The composition “Vitaplan, KZh + 0.1% chitosan salicylate” was obtained with adding 0.1% chitosan salicylate to the culture liquid of the biological product Vitaplan, KZh.

Chitosan (60 kDa) was obtained with the method of oxidative destruction [8] from chitosan with a molecular weight of 150 kDa and a degree of deacetylation of 85% (“Bioprogress”, Russia). On its basis, chitosan salicylate was synthesized containing 25% ion-bound SA fragments. The formation of a salt between chitosan and SA was confirmed with the presence of characteristic bands from the СО₂ carboxylate group in the IR spectrum: 1552.92 cm⁻¹ and 1386.12 cm⁻¹. A broad strong band in the region of 3100–2600 cm⁻¹ contained stretching vibrations from the NH₃+ and OH functional groups.

Wheat productivity was studied by a set of indicators characterizing the growth, development and yield of a crop during the developmental phases of an embryonic shoot (3-leaf stage), heading-flowering and ripening [9]. Based on the data on productive tillering, the mass of grains of a spike of one plant, the biological productivity of wheat (g / plant) was calculated. With regard to the sown area, the yield was calculated in accordance with the expression:

\[ Y_p = 0.01 M_k K_p P_p \]

where \( Y_p \) is the yield (t / ha), \( M_k \) is the grain weight of an ear of one plant, g; \( K_p \) – productive tillering, \( P_p \) – seeding density per 1 m², calculated using data on field germination, plants / m².

Accounts of the development of pathogens of root rot and yellow rust were carried out both using generally accepted indicators (the intensity of the disease, the type of reaction) and calculated. The degree of development of wheat yellow rust was characterized with the length of the strip, the number of pustules in the strip, the total number of pustules per leaf, and the area of the pustules [10].

The experimental data processing algorithm included obtaining experimental data in the field, creating an electronic database in the IBM SPSS system, statistical analysis and generalization of the results of the experiment.

4. Results and discussion
As follows from the data in figure 1, the greatest effect on the yield of common wheat compared to the control was exerted with 0.1% chitosan salicylate (in the first replicate it increased by 39.9%, \( t = 3.3 \) and in the second, by 42.3%, \( t = 2.5 \)) and the polyfunctional complex: Vitaplan, KZh + 0.1 % chitosan salicylate (submerged cultivation) – in the third replication. The yield changed significantly from 1.8 t / ha (control) to 5t / ha (\( t = 13.7 \)) in the third replication. On average for replicates, the use of chitosan salicylate at a concentration of 0.1% led to an increase in wheat yield by 55.2% (\( t = 3.9 \)).

In the variant of the experiment with the use of 0.1% chitosan salicylate, a significant increase in \( P <0.05 \) of the maximum number of indicators of wheat productivity (10 out of 19) was revealed. The given indicators are the rate of plant development in the phase of ontogenesis – by 4%; plant heights – by 19%; the number of roots – by 40%; root mass – by 59%; total bushiness – by 70%; the mass of the vegetative part – by 17%; the number of spikelets per spike – by 10%; ear mass – by 12%; the number of grains in a spike – by 11%; grain mass of one spike – by 11%.
The combined use of the Vitaplan, KZh and 0.1% chitosan salicylate complex did not have a significant effect on the growth of general indicators characterizing the growth of the vegetative mass of plants, but significantly influenced the indicators of the structure of wheat yield. To the greatest extent, this tendency at $P < 0.05$ was revealed in the variant of the experiment “Vitaplan, KZh + 0.1% chitosan salicylate (composition)”, while an increase in the spike weight by 11% was noted; the number of spikelets per spike – by 6%; the number of grains in an ear - by 19%; the mass of grains of one spike - by 12%. It together determined the growth of potential yield when using the specified polyfunctional complex.

In the variant of the experiment “Vitaplan, KZh + 0.1% chitosan salicylate (submerged cultivation)”, a significant increase was revealed only in the final indicator - the potential yield. The increase in yield in this variant of the experiment can be explained with an insignificant increase in almost all indicators characterizing the structure of wheat yield. The given indicators are spike length (by 3%), spike weight (by 9%), number of spikelets in a spike (by 0.1%); the number of grains per spike (by 4%); the mass of grains of one spike (by 9%); the mass of 1000 grains – by 4%.

In the variant of the experiment “Vitaplan, KZh” there was a significant increase at $P < 0.05$ of the weight of the spike in comparison with the control – by 29%; the mass of the vegetative part of plants – by 48%.

The least efficiency in relation to wheat productivity was shown with the biological product ”Vitaplan, SP”: the weight of a spike decreased – by 20%; the number of grains in a spike – by 14%; the mass of grains of one spike – by 22%; weight of 1000 grains – by 13%. In the indicated variant of the experiment, a tendency to decrease the yield of a single plant by 20% was revealed. However, it should be noted the positive effect of the biological product on the number of spikelets in a spike, which significantly increased by 8%.

The tendency for the greatest increase in productive tillering of wheat at $P <0.05$ compared to the control was determined in the variants of the experiment “0.1% chitosan salicylate” – by 63% and “Vitaplan, KZh + 0.1% chitosan salicylate (submerged cultivation)” – by 69%.

The greatest decrease in the intensity of development of wheat root rot, by 9%, was noted in the variant of the experiment using 0.1% chitosan salicylate. The root rot was caused with the fungus Bipolaris sorokiniana (Sacc.) Shoemaker.
During the period of phytosanitary monitoring, a strong infection of crops with the pathogen of yellow rust *Puccinia glumarum* Erikss & Henn was revealed (figure 2).

**Figure 2.** Uredopustules on wheat leaves cultivar Leningradskaya 6, k-64900 (a) and urediospores (b) of wheat yellow rust pathogen.

The greatest decrease in the intensity of development of wheat yellow rust at *P* < 0.05 by 40% was recorded in the variant of the experiment “Vitaplan, KZh + 0.1% chitosan salicylate (composition)” – figure 3. In the variants of the experiment “0.1% chitosan salicylate” and “Vitaplan, KZh + 0.1% chitosan salicylate (submerged cultivation)” the development of the disease decreased by 26% and 23%.

**Figure 3.** Influence of biological products and polyfunctional complexes on the intensity of development of yellow rust of wheat (R) in three replicates: control – 1; Vitaplan, KZh – 2; Vitaplan, SP – 3; 0.1% chitosan salicylate – 4; Vitaplan, KZh + 0.1% chitosan salicylate (submerged cultivation) – 5; Vitaplan, KZh + 0.1% chitosan salicylate (composition).

In the experiment variant “Vitaplan, KZh + 0.1% chitosan salicylate (composition)”, the number of yellow rust stripes decreased as much as possible at *P* < 0.05 – by 52%; the length of the stripes – by 57%; the number of pustules in the strip – by 76%.

In all variants of the experiment with the use of biological products and polyfunctional complexes based on chitosan, a decrease by more than 60–80% in the intensity of damage to wheat leaves by septoria was revealed (figure 4). The causative agent of the disease was the fungus *Stagonospora* (syn. *Septoria*) nodorum (Berk.) Castell. and Germano.
5. Conclusion
Polyfunctional complexes “Vitaplan, KZh + 0.1% chitosan salicylate” optimize the physiological state of wheat plants, significantly increase its productivity and disease resistance. The addition of 0.1% chitosan salicylate to the biological product “Vitaplan, KZh” increases the yield of wheat and increases the biological efficiency of the multifunctional complex against yellow rust of wheat. When plants were treated with 0.1% chitosan salicylate, the maximum yield of soft wheat was noted. In this variant of the experiment, an increase in 10 out of 19 indicators of productivity was revealed and a minimum development of root rot was noted. The combined use of Vitaplan, KZh and 0.1% chitosan salicylate did not have a significant effect on the growth of indicators characterizing the growth of the vegetative mass of plants, but had a positive effect on the structure of wheat yield. This tendency was especially clearly traced in the variant of the experiment “Vitaplan, KZh + 0.1% chitosan salicylate (composition)”. The results of the work can be used to develop a resource-saving technology for growing wheat and optimize the phytosanitary state of agroecosystems.

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