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MULTIFUNCTIONAL BIOLOGICS WHICH COMBINE MICROBIAL ANTI-FUNGAL STRAINS WITH CHITOSAN IMPROVE SOFT WHEAT (*Triticum aestivum* L.) YIELD AND GRAIN QUALITY

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Abstract

The developing of effective and high-tech preparations for microbiological plant protection is a crucial problem of agricultural biotechnology. In this paper, we revealed differences in the crop structure, grain introscopic characteristics and resistance of soft wheat plants to root rot when using novel multifunctional bioactive preparations. Our objective was to investigate effects of developed bioactive compositions based on microbial antagonists of plant pathogenic and chitosan complexes on spring soft wheat (*Triticum aestivum* L.) variety Leningradskaya 6. Plant protection against root rot, productivity and grain quality assessed by the methods of microfocus X-ray radiography and gas-discharge visualization were estimated in two-year field tests (Leningrad Province, 2016-2017). The weather conditions during growing season of 2016 were more favorable for wheat plants compared to 2017 due to a slight temperature fluctuation and a significant amount of precipitation. The number of spikelets per spike, flag and pre-flag leaf area, the weight of spike and vegetative parts were the indicators for wheat productivity, germination energy, seedling length, dynamics of plant growth phase and height, the number, length and weight of roots. According to significant positive influence of the studied compositions on yield structure, the biopreparations rank as follows: in 2016 — Vitaplan, Zh (OOO AgroBioTekhnologiya, Russia) > Vitaplan, Zh + Chitosan II (test preparation containing 50 and 100 kDa chitosans with the addition of 0.1 % vanillin, FSBSI VIZR) > Gamair, SP (OOO AgroBioTekhnologiya, Russia) > Chitosan I (test preparation containing 50 and 100 kDa chitosans with the addition of 0.05 % salicylic acid, FSBSI VIZR) > Chitosan II; in 2017 — Vitaplan, Zh > Vitaplan, SP > Vitaplan, Zh + Chitosan II > Gamair, SP > Chitosan II. In 2016, a combined use of Vitaplan, Zh and Chitosan II changed significantly not only the plant vegetative part weight, but also the spike weight, while separate use of Chitosan II significantly increased the vegetative biomass only. In 2017, the same combination of the biologicals made the flag leaf 86.84 % larger and the root weight 83.33 % higher compared to the control. In 2016, Chitosan I led to reliable 19.0 % increase (t = 3,0; $p < 0.05$) in potential grain yield compared to the control, however, there were no significant differences for Vitaplan, Zh, Chitosan II and their combination Vitaplan, Zh + Chitosan II. On the contrary, in 2017 Vitaplan, Zh + Chitosan II caused the maximum reliable (t = 7.2; $p < 0.05$) increase in yield (by 82.6 %). Vitaplan, Zh and Vitaplan, Zh + Chitosan II possess maximum efficiency against Helminthosporium root rot. Due to Vitaplan, Zh + Chitosan II, in 2016 root rot disease frequency was 80 % lower compared to the control, and in 2017 no symptoms were observed which may be due to less favorable weather conditions for root rot disease in 2017 compared to 2016. According to our findings, the potential grain yield in wheat correlates significantly and positively with grain X-radiographic projection area, integrated grain brightness and total intensity of the gas-discharge fluorescence. Chitosan I, Chitosan II and Vitaplan, F + Chitosan II have the greatest impact on grain structure and quality parameters assessed by X-ray and gas-discharge visualization. Perhaps the effectiveness of the studied drugs depended on weather conditions, but was generally positive in terms of the main assessed indicators. Thus, our data convincingly indicate the effectiveness of multifunctional biologics which combine microbial antagonists of fungal plant pathogens with chitosan, an activator of plant diseases resistance, to protect wheat against root rot, to increase grain yield with better quality.

Keywords: *Triticum aestivum* L., spring soft wheat, biological preparations, chitosan composition, yield structure, root rot, grain quality, microsofus X-ray, gas discharge visualization

Spring wheat is the main food crop and an important item of Russian export. Obtainment of high stable yield of quality spring wheat grain is only possible subject to a number of measures that include the use of general soil-protective technologies and methods of enhancement of soil fertility, correct crop rotation with sufficient saturation of bare fallows optimal for the conditions and objectives of variety cultivation as well as compliance with agrotechnical requirements meeting biological peculiarities of the crop variety. Currently, however, the potential productivity of crops is frequently achieved only by a third, which supports the necessity to improve cultivation technologies [1, 2[.

Obviously, optimization of conditions for growing and development of plants throughout all ontogenesis stages is one of the most important objectives in crop production. Its achievement is to the large extent connected with development of production technologies and application of environmentally-friendly multifunctional preparative forms capable of effectively reducing the spread and development of dangerous diseases and improving the disease resistance of plants as well as stimulating their growth and development.

Creation of effectiveness and high-technology preparative forms for microbiological protection of plants is the key issue of agricultural biotechnology. Such forms include such biological preparations registered in Russia as Vitaplan, Alirin-B and Gamair which are manufactured in the form of wettable powders, suspension concentrates, tablet and liquid forms (joint development of OOO AgroBioTekhnologiya, Moscow, and All-Russian Institute of Plant Protection) [3, 4]. Biological preparations have demonstrated high effectiveness in control of diseases of principal agricultural crops, promoted the increase in yield and quality of plant products. In addition, in some instances it was found that introduced strains of antagonist microbes considerably affect the variety composition of a complex of soil-inhabiting plant pathogenic fungi and suppressive properites of soils in agrocoenosis [5].

Over the past years, new-generation preparations have been developed, e.g. the disease-resistance inductors based on chitosan, the results of application of which are widely discussed [6-8]. Chitosan is a natural polymer with β-D-glucosamine and N-acetyl-β-D-glucosamine units [9] obtained through deacetylation of chitin of crustaceans, insects, and fungi [10-13]. Positive effects of chitosan on plant growth and development were confirmed [14]. Treatment of wheat leaves with chitosan resulted in increase in concentration of phenolic acids, particularly ferulic acid. Chitosan stimulates generation of precursors of lignin (p-coumaric, ferulic, sinapic acids), and synthesis of phenolic acids possessing antimicrobial properties, i.e. benzoic, p-coumaric, caffeic, protocatechuic, chlorogenic, ferulic and gallus acids [15, 16]. Wheat plants treated with chitosan and exposed to drought stress, as compared to control set, demonstrated considerable increase in growth, germination rate, grain moist content, length and activity of roots, as well as changes in physiological indicators (i.e. activity of superoxide dismutase, peroxidase, catalase, malondialdehyde and chlorophyll contents) [17]. Treatment with chitosan, through increasing the chlorophyll content in wheat leaves, caused a 13.6% yield increase as compared to control group [17].

In the recent years, the research is carried out in Russia to create the compositions based on immobilization of antagonist microbes *Bacillus subtilis* M-22 and *Trichoderma viride* T-36 in chitin-chitosan carriers for effective protection of vegetable crops from Fusarium infection and nematodes [18, 19]. High protective effect (up to 70%) of such complex biopreparations is caused by combination of properties of antagonist microbe with the ability of chitosan to enhance, in conjunction with biologically active substances, the mechanisms of natural resistance of plants to pathogens. It also demonstrates the synergic effect of composition components.

Seeds of required quality are the condition the high wheat yield [20]. In this regard, there is a number of standard tests regulated by ISTA (International Seed Testing Association) and of promising seed quality control tests based on imaging technologies. The method of microfocus X-ray radiography is for many years used both in Russia [21-22] and abroad (23-26). It is used to detect various structural seed defects (stress cracks, enzyme-mycotic attrition, internal germination, latent pest colonization, Sunn pest contamination, physical damage and defectiveness of grain kernel, blind-seed disease). Computer microtomography allows researchers to obtain a 3D image of the internal caryopsis structure [27] and visualize some structural defects [28].

Over the past 10 years, the data was obtained regarding the possibility to use the method of terahertz imaging to determine the seed variety purity [29], seed quality [30] and ultra-early forecasting of laboratory germination rate of seeds [31]. Seed imaging in terahertz range enables detection of changes occurring during germination just 6 hours after seed soaking [31).

The presented work for the first time demonstrates the effectiveness of multifunctional preparations based on microorganism strains, the antagonists of infection agents, and activators of plant disease resistance, the chitosan compounds, for increasing the yield and protection of spring soft wheat from diseases. The results define the differences in the wheat yield structure and resistance to root rot upon application of multifunctional preparations and identify their impact on introscopic characteristics of the grain.

The purpose of our study is to justify the feasibility of use of multifunctional preparations based on antagonists of infection agents and chitosan compounds for spring soft wheat protection from root rot and increase of grain yield, as well as to evaluate the quality of grain through microfocus X-ray radiography and gas-discharge imaging.

Techniques. The experiments were run on spring soft wheat plants (*Triticum aestivum* L.) variety Leningradskaya 6 (k-6490; provided by the department of genetic wheat resources of Vavilov All-Russian Institute of Plant Genetic Resources, VIR) in 2016-2017 (VIR experimental field). The seeding was performed on May 7 on a 1.0 m^2 plot through row cropping with 15-cm row spacing and spacing in the row of 1-2 cm (400 seeds/m^2). Depth of seeding 5-6 cm.

In 2016 experiment pattern provided for the following scenarios: no treatment (control); Gamair, SP (OOO AgroBioTekhnologiya, Russia) as a standard; Vitaplan, Zh (OOO AgroBioTekhnologiya, Russia); chitosan complex Chitosan I (experimental sample, All-Russian Institute of Plant Protection, FSBSI VIZR); Vitaplan + Chitosan II complex. Gamair, SP is a fungicide based on *Bacillus subtilis* M-22 strain (wetting powder, viable cell titer defined in CFU/g). Powder (5 g) was dissolved in 10 l of water and used to treat 1 t of seeds by semidry method. Vitaplan, Zh is a culture liquid of *B. subtilis* VKM B-2604D and *B. subtilis* VKM B-2605D strains (1:1 ratio, live cells and *B. subtilis* spores titer of 10^{10} CFU/ml). The seeds (50 g) were soaked in 100 ml of culture liquid for 1 hour. Chitosan I complex contains 100 kDa and 50 kDa chitosans (1:1 in weight parts), a mix of succinic and glutamic acid (organic acids) at the ratio of chitosan:organic acids 1:1 and 0.05 % salicylic acid. Chitosan II complex includes 100 kDa and 50 kDa chitosans (1:1), a mix of succinic and glutamic acid (organic acids) of chitosan:organic acids 1:1 and 0.1% of vanillin. 50 kDa and 100 kDa chitosans were obtained by us through oxidative breakdown of 150 kDa chitosan (85% deacetylation) with sodium nitrite in acidic conditions (Bioprogress, Russia). The seeds were treated with the both complexes by semidry method, 80 g per 10 l of water per 1 t of seeds. When treating with Vitaplan, Zh + Chitosan II complex, Chitosan II was added to culture liquid of Vitaplan Zh biopreparation until the Chitosan II concentration reached 0.1% (50 g of seeds were soaked in 100 ml of culture liquid for 1 hour). Vegetating plant in 2016 were treated on June 24, July 9 and 19. The standard, Gamair, SP, was used as 10 g of preparation per 300 l of water. Vitaplan, Zh was dissolved in water to one-tenth, liquid consumption was 100 ml/m^2. When spraying the plants with aqueous solutions of Chitosan I and Chitosan II preparations, the concentration (0.1%) was measured by the main component (chitosan; liquid consumption was 100 ml/m^2). In a scenario that included Chitosan II complex, indoleacetic acid (0.0015%) was added as the main plant growth hormone instead of vanillin. When using Vitaplan, Zh + Chitosan II complex, culture liquid with the titer of 10^{10} CFU/ml was water-dissolved to one-tenth; liquid consumption was 100 ml/m^2.

In 2017 experiment included five scenarios: no treatment (control); Vitaplan, SP as a standard, 10 g of preparation per 300 l of water; Vitaplan, Zh; Chitosan II chitosan complex; Vitaplan, Zh + Chitosan II complex. Wheat seeds prior to seeding were treated and vegetative plants were sprayed according to the pattern applied in 2016.

During wheat tillering, the number, length and weight of roots (primary radicle root, radicle and coleoptile roots) were measured. The number and length of nodal roots were also defined. In each scenario, each 20 plants were evaluated twice. Wheat ontogenesis phases were registered by Eucarpia (EC) scale (Zadoks scale).

In studying the yield structure, the data of productive and overall tilling capacity, plant height, ear length, number of spikelets per ear, ear weight were evaluated. Weigh of vegetating parts of plants, area of flag and pre-flag leaves were measured in accordance with methodological guidelines [32].

Potential wheat yield Y_p (t/ha) was measured by productive tilling capacity and number of plants per 1 m^2: $Y_p = M_E T_P P_D \times 10000$, where M_E is the grain weight per ear of a single plant, t; T_P is a productive tilling capacity of a sample (the number of stems with ears per a single plant); P_D is plant density (the number of plants per 1 m^2).

The plant affection by root rot was defined in field conditions during wheat tillering phase (on July 15, 2016) by the generally accepted scale: 0 – epicotyl unaffected, 1 – isolated stains on epicotyl, 2 – major lesions, 3 – severe lesions, the plant died. In each experiment scenario, each 20 plants were evaluated twice.

Development of root rot was estimated by average weighted extent of plant affection R_e [33]:

$$Re = \frac{\Sigma(ab)100}{AK},$$

where *a* is the number of plants with similar symptoms, *b* is the corresponding score, A is a number of plants studied (healthy and diseased), K is the maximum scale score.

In 2016, a laboratory experiment was also held to define the grain germination energy (%) upon treatment with the aforesaid biological preparations and chitosan compounds (control group remained untreated) (commenced on May 30). In each experiment version, Petri dishes were used to analyze 100 grains (June 1), the length of seedlings was measured 1 day after transferring to moist chamber (June 2) and on the next day (June 3).

Microfocus X-ray radiography and gas-discharge visualization (GDV) methods were applied to evaluate introscopic characteristics of the grain. X-ray radiography of wheat grains was performed with a serial mobile X-ray unit PRDU-02 (ZAO ELTEKH-Med, Russia), ×3,0 zoom coefficient. Analysis of digital X -ray images of wheat grains was carried out with Agrus-Bio software (OOO ArgusSoft, Russia). On an X-ray projection of caryopsis, the area (cm^2), perimeter (cm), length (cm), width (cm), circularity (relative units), elongation (relative units), irregularity (relative units), average brightness (brightness units), standard brightness deviation (brightness units), optical density (relative units) and integrated optical density (relative units) were measured. Gas-discharge visualization (electrophotography with registration and quantitation of characteristics of corona effect emerging upon seeds exposure to high-energy electromagnetic field) was carried out on a serial GRV-Kamera apparatus equipped with analytical software GRV-Nauchnaya Laboratoriya (OOO Biotekhprogress, Russia). The following parameters of digital gas-discharge images of grain were analyzed: luminescence area (pixels), total luminescence intensity (relative units), form factor (relative units), average isoline radius (pixels), normalized standard deviation of isoline radius (pixels), isoline length (pixels), isoline-measured entropy (relative units), isoline-measured fractality (relative units).

Statistical analysis was carried out with SPSS 21.0, Statistica 6.0, MS Excel 2016 software [34]. In calculations, the methods of parametric statistics (based on mean *M* and standard error of mean ±SEM, 95 % confidence intervals and Student's *t*-test) and multivariate statistics (cluster and factor analysis) were used.

Results. Weather conditions in 2016 in Leningrad Province were characterized by higher average monthly temperature (the standard was exceeded by 3.4 °C in May, and the excess was within 1°C in June through August; the precipitation in May was 64 % of the standard, however, during the summer months it exceeded the standard considerably (137% in June, 191% in July, 227% in August). In May-July 2017, reduction in average monthly temperature was within 2.5 °C vs. the standard; precipitation in May reached only 29%, which is considerably below the standard; in summer, that indicator increased and reached 115% of the standard in June, 115% in July and 175% in August. Thus, vegetation period of 2016 was characterized by more favorable weather conditions for plant growth, given insignificant temperature fluctuations and considerable amount of precipitation.

Table summarizes the effects of biological preparations and chitosan complexes on spring soft wheat productivity indicators and potential yield. In 2016 (under increased average monthly temperatures and considerable excess of precipitation during summer month) in a scenario where the seeds were treated with Gamair, SP, the germination energy was 25% higher than in control group, and reached the maximum value of 96.5%. Significant ($p < 0.05$) increase in the length of the sprout occurred in the scenarios with Gamair, SP and Vitaplan, Zh, by 73.7% and 69.5%, respectively.

Leningradskaya 6 variety spring soft wheat (*Triticum aestivum* L.) productivity upon application of multifunctional preparations based on antagonist microorganisms and chitosan compounds (*M*±SEM, St. Petersburg—Pushkin, a test field, 2016)

Experiment scenario	E_g, %	$L_{s.}$, mm	P, score	h, cm	$N_{r.}$, pcs.	$L_{r.}$, mm	M_r, h	N_{nr}, pcs.	L_{nr}, mm	N_{se}, pcs.	$S_{fl.}$, cm^2	S_{pfl}, cm^2	M_e, g	M_{vp}, g
Control (вода)	71.4	9.6±2.0	62.5±1.6	81.3±3.8	4.4±0.6	61.0±5.4	0.3±0.1	6.5±0.7	56.4±4.1	12.8±0.6	7.1±0.4	7.6±0.5	0.4±0.1	2.0±0.2
Gamair, SP	96.5	16.6±1.9*	64.5±0.6	86.6±3.0	6.4±0.4*	73.6±4.7	0.3±0.05	5.9±0.7	41.2±4.7*	13.5±0.6	7.9±0.6	9.2±0.4*	0.5±0.1	2.4±0.2
Vitaplan, Zh	86.8	16.2±1.9*	67.8±1.1*	90.1±2.5*	6.5±0.5*	76.8±4.8*	0.3±0.04	7.0±0.5	54.6±3.9	14.2±0.5	6.4±0.6	8.4±0.8	0.5±0.03	2.3±0.1
Vitaplan, Zh + Chitozan II	74.4	19.6±2.0	69.4±0.8*	98.6±2.8	5.9±0.5*	62.1±3.8	0.4±0.02	7.4±0.7	48.0±4.8	13.9±0.6	8.1±0.4*	7.6±0.5	0.6±0.03*	2.5±0.2*
Chitosan I	82.4	9.1±1.6	67.9±0.9*	87.9±2.6	6.2±0.5*	69.1±3.4	0.3±0.05	6.9±0.7	53.3±3.9	14.3±0.5	7.5±0.7	8.0±0.5	0.6±0.1	2.5±0.3
Chitosan II	79.8	8.9±1.2	60.7±2.2	83.6±3.6	4.6±0.5	70.4±5.0	0.4±0.1	7.7±0.7	66.1±5.1	12.2±0.8	7.9±0.7	6.6±1.1	0.6±0.1	2.7±0.3

N o t e. $E_{g.}$ — grain germination energy, $L_{s.}$ — sprout length, P — plant phase, h — plant height, $N_{r.}$ — number of roots, $L_{r.}$ — root length, $M_{r.}$ — root weight, N_{nr} — number of nodal roots, L_{nr} — nodal root length, N_{se} — number of spikelets per ear, S_{fl} — flag leaf area, S_{pfl} — pre-flag leaf area, M_e — ear weight, M_{vp} — vegetation part weight.
* Differences with control group are statistically significant at $p < 0.05$.

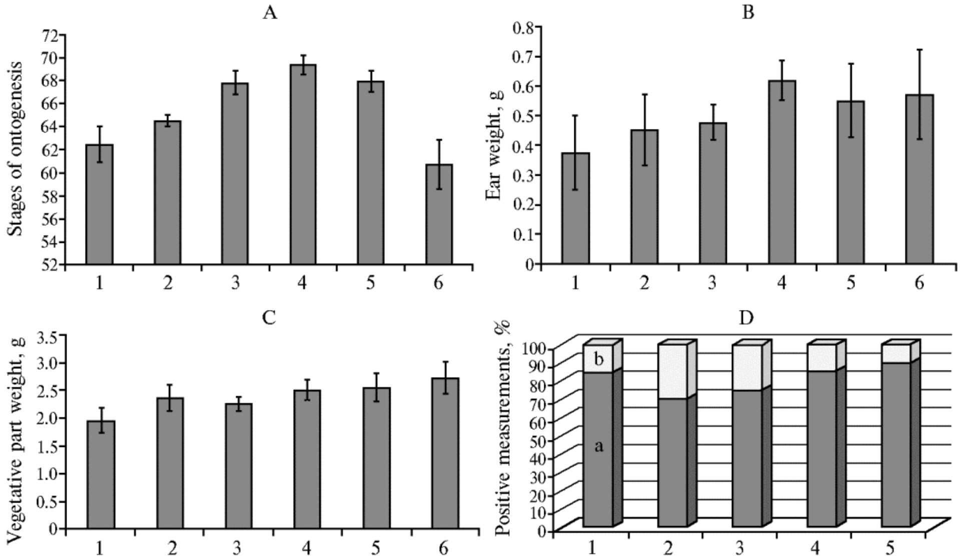

Fig. 1. Leningradskaya 6 variety spring soft wheat (*Triticum aestivum* L.) productivity indicators upon application of multifunctional preparations based on antagonist microorganisms and chitosan compounds: A —Eucarpia (EC)-scale ontogenesis phase, B — ear weight, C — vegetation part weight, D — complex of indicators (a — number of positive changes, b — number of significant positive changes); 1 — control (water), 2 — Gamair, SP, 3 — Vitaplan, Zh, 4 — Vitaplan, Zh + Chitosan II, 5 — Chitosan I, 6 — Chitosan II (St. Petersburg—Pushkin, a test field, 2016).

The samples treated with Vitaplan, Zh preparation together with Chitosan II complex showed the highest growth rate by Eucarpia (EC) scale ontogenesis phases during the earing stage, significant increase of score by 11%, $t = 7.8$, $p < 0.05$ as compared to the control group. Maximum intensive plant development was ensured through treatment with Vitaplan, Zh and Chitosan I preparations (Fig. 1, A). Significant ($p < 0.05$) increase in plant height, by 10.8%, during the earing stage was observed for the scenario where the seeds were treated with Vitaplan, SP.

In all experiment scenarios that provided for the use of preparations, the number of roots from epicotyl (primary radicle root, radicle and coleoptile roots) has increased vs. the control group. The number of roots significantly increased upon application of Gamair, SP (by 44.7%, $t = 2.7$, $p < 0.05$), Vitaplan, Zh (by 46.3%, $t = 2.6$, $p < 0.05$), Vitaplan, Zh together with Chitosan II (by 32.3%, $t = 2.7$, $p < 0.05$), and Chitosan I (by 32.3%, $t = 2.3$, $p < 0.05$). Chitosan II chitin complex did not actually affect this indicator (see Table).

Virtually in all experiment scenarios (Vitaplan, Zh, Vitaplan, Zh + Chitosan II, Chitosan I), there was a significant ($p < 0.05$) increase in the number of wheat roots as compared to the control group. The preparations did not produce any significant effect on the number and length of nodal roots, number of spikelets per ear, area of flag and pre-flag leaves. At the same time, Vitaplan, Zh preparation when combined with Chitosan II complex has a considerable positive effect on wheat ear weight (a 65.2% increase vs. the control group, $t = 7.2$, $p < 0.05$) (see Fig., B), and also on the weight of green parts of the plant (by 28.4%, $t = 2.9$, $p < 0.05$) (see Fig., C). When Chitosan I was used, significant growth of only the weight of green parts occurred (by 39.8%, $p < 0.05$).

Figure 1 (D) shows the normalized stacked column chart reflecting variation in number of positive and statistically significant positive changes for 14 indicators of wheat productivity due to use of biopreparations and chitosan complexes as compared to the control. At first, the indicators with positive change in value of certain productivity indicators as compared to control values (as per scenarios) were selected. Then they were ranked using Student's *t*-test at p = 0.05, which gives a decrease in biological efficiency as follows: Vitaplan, Zh > Vitaplan, Zh + Chitosan II > Gamair, SP > Chitosan I > Chitosan II.

Cluster analysis (k-means method) [34] diveded all biological preparations and chitosan compounds into two groups of effectiveness based on changes of the average values of the set of indicators vs. control. The first group comprised Gamair, SP, Vitaplan, Zh, and Chitosan I, and Vitaplan, Zh + Chitosan II and Chitosan II formed the second group. Preparations of the second group, as compared to the first group, showed a more express effect on the root weight (by 12.7%, $t = 5.8$; $p < 0.05$), number of nodal roots (by 14.7%, $t = 6.7$; $p < 0.05$), length of nodal roots (by 13.0%, $t = 2.6$; $p < 0.05$), flag leaf area (by 10.4%, $t = 4.4$; $p < 0.05$), ear weight (by 27.1%, $t = 7.4$; $p < 0.05$), green part weight (by 11.7%, $t = 4.7$; $p < 0.05$). Use of Vitaplan, Zh + Chitosan II and Chitosan II resulted in insignificant drop in plant development (by 2.8% as per ontogenesis phase), significantly smaller number of roots and their length (by 25.7%, %; $t = 5.7$; $p < 0.05$ and by 11.3 %; $t = 4.4$; $p < 0.05$, respectively).

Principal factor analysis [34] using Varimax normalized axis rotation procedure (factor impacts in the procedure are normalized by dividing by square root of relevant dispersion) allowed evaluation of the interrelations between relative changes in wheat productivity indicators caused by biological preparations and chitosan compounds (Fig. 2, A). The highest effect on productivity was characteristic of Vitaplan, Zh + Chitosan II complex, while Chitosan II caused the slightest effect.

Chitosan I complex promoted a 19.0% increase in potential wheat yield ($t = 2.8$, $p < 0.05$) vs. the control (see Fig. 2, B). We have found no considerable differences in potential yield between scenarios for Vitaplan, Zh, Vitaplan, Zh + Chitosan II complex and Chitosan II. Upon Gamair, SP application, the potential wheat yield was 25.0% lower ($t = 3.5$, $p < 0.05$) compared to control.

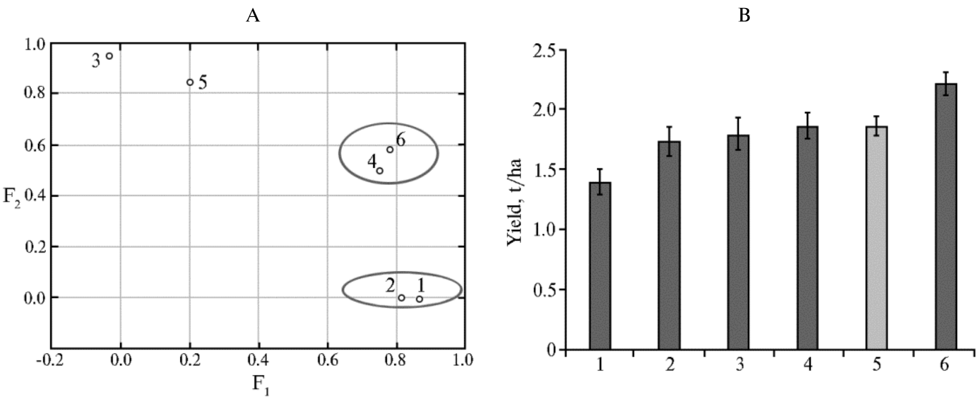

Fig. 2. Factor analysis (A) and potential yield (B) of Leningradskaya 6 variety spring soft wheat (*Triticum aestivum* L.) upon application of multifunctional preparations based on antagonist microorganisms and chitosan compounds: 1 — Gamair, SP, 2 — Vitaplan, Zh + Chitosan II, 3 — Chitosan II, 4 — Vitaplan, Zh, 5 — control (water), 6 — Chitosan I (St. Petersburg—Pushkin, a test field, 2016).

In 2017, at reduced average monthly temperature vs. the standard, insignificant precipitation (29% of May standard) and excess of precipitation during the summer month, maximum significant increase in yield (82.6 %, $p < 0.05$) occurred in Vitaplan, Zh + Chitosan II scenario (Fig. 3). As compared to 2016, in control group this indicator was significantly higher, by 77.1%. In Vitaplan, Zh and Vitaplan, Zh + Chitosan II scenarios, the yield has increased considerably, by 2.3 t/ha and 4.3 t/ha, respectively. Insignificant differences over years were observed for Chitosan II complex.

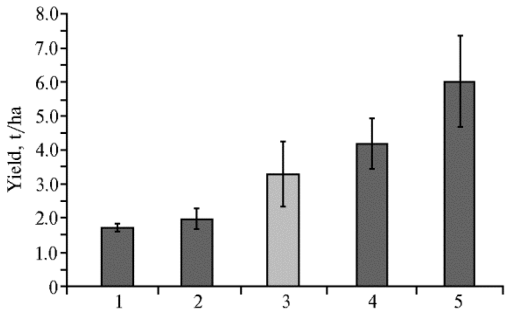

Fig. 3. Leningradskaya 6 variety spring soft wheat (*Triticum aestivum* L.) potential yield upon application of multifunctional preparations based on antagonist microorganisms and chitosan compounds: 1 — Vitaplan, SP, 2 — Chitosan II, 3 — control (water), 4 — Vitaplan, Zh, 5 — Vitaplan, Zh + Chitosan II (St. Petersburg—Pushkin, a test field, 2017).

In 2017, all preparations other than Vitaplan, Zh significantly and positively speeded up the plant development over phases (Vitaplan, Zh + Chitosan II by 25.04%, Chitosan II by 33.59%, Vitaplan, SP by 25.58%) and increased their height (Vitaplan, Zh + Chitosan II by 32.57%, Chitosan II by 45.22%, Vitaplan, SP by 49.44%) as compared to control (average increase by 10.0% and 16.2%, respectively). Vitaplan, Zh + Chitosan II complex increased the number of spikelets in an ear (by 7.66% vs. control), productive and overall tilling capacity (by 116.00% and 22.19%, respectiely). In this scenario, the plants also distinguished by larger flag leaf area (by 86.84%) and root weight (by 83.33%).

Figure 4 shows the number of positive (negative) and significantly positive (negative) changes in indicator values of wheat productivity caused by preparations as compared to the control group. By their biologic effectiveness, the preparations could be ranked as follows: Vitaplan, Zh > Vitaplan, SP > Vitaplan, Zh + Chitosan II > Chitosan II. Vitaplan, Zh + Chitosan II complex also caused the growth of the maximum number of wheat productivity indicators as compared to control group (90%, with 35% significant changes).

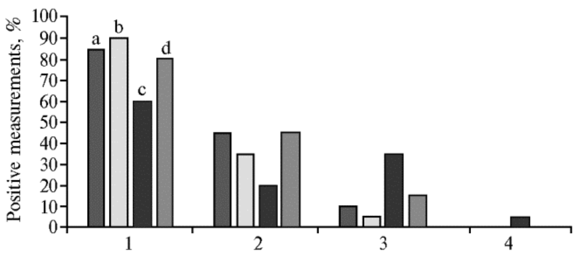

Fig. 4. Number of positive changes (1), **positive significant changes** (2), **negative changes** (3) **and negative significant changes** (4) **in values of productivity indicators compared to control in Leningradskaya 6 variety spring soft wheat (*Triticum aestivum* L.) upon application of multifunctional preparations based on antagonist microorganisms and chitosan compounds: a** — Vitaplan, Zh, b — Vitaplan, Zh + Chitosan II , c — Chitosan II , d — Vitaplan, SP (St. Petersburg—Pushkin, a test field, 2017).

Damage to plants caused by root rot was evaluated during the stem elongation phase. As the studies showed, the principal infection agent was *Bipolaris sorokiniana* (Sacc.) Shoem. Vitaplan, Zh and Vitaplan, Zh + Chitosan II complex demonstrated maximum effectiveness against Helminthosporium root rot. In 2016 Vitaplan, Zh + Chitosan II scenario provided the reduction in root rot occurrence by 80% as compared to the control group, and in 2017 no disease symptoms were found (Fig. 5).

Spearman's non-parametric correlation analysis of introscopic data obtained by radiography and gas-discharge visualization methods for the harvested grains has shown that potential yield of wheat Y_g positively and significantly ($p < 0.05$) correlates with the radiograph projection area $S_{p.}$ ($r = 0.9$), integrated brightness of grains $I_{g.\ int.}$ ($r = 0.8$) and total intensity of gas-discharge fluorescence $I_{gdf.\ total.}$ ($r = 0.8$). Dependencies among these indicators may be described by regression equations: $Y_g = 29.36 - 5.04S_{p.}^2 + 0.23S_{p.}^3$ ($r^2 = 0.8$); $Y_g = -10.46 + 0.000038I_{g.\ int.}^2 - 0.000000000029I_{gdf.\ total.}^3$ ($r^2 = 0.83$) and

$Y_g = -17.68 + 37.82\ I_{gdf.\ total.}^2 + 18.09 I_{gdf.\ total.}^3$ ($r^2 = 0.89$). Values of 1000-grain weight of M_{1000} positively correlate with integrated grain brightness $I_{g.\ int.}$: $M_{1000} = -1.99 + 0.000042 I_{g.\ int.}$ ($r^2 = 0.9$).

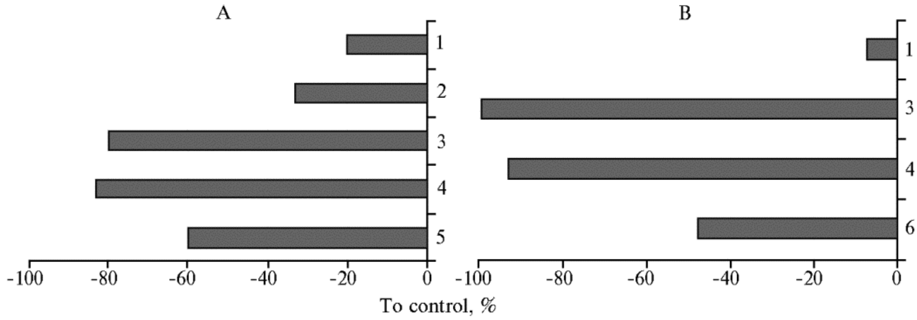

Fig. 5. Development of Helminthosporium root rot in Leningradskaya 6 vaiety spring soft wheat (*Triticum aestivum* L.) in 2016 (A) **and 2017** (B) **upon application of multifunctional preparations as compared to control:** 1 — Chitosan II, 2 — Chitosan I, 3 — Vitaplan, Zh + Chitosan II, 4 — Vitaplan, Zh, 5 — Gamair, SP, 6 — Vitaplan, SP (St. Petersburg—Pushkin, a test field).

According to microfocus X-ray radiography, in case of Chitosan I compound application wheat grains had better morphometric and densitometric parameters as compared to control group, i.e. considerably larger radiograph projection area (by 9.26%, $t = 2.5$), length (by 3.90%, $t = 2.5$) and width (by 5.84%, $t = 2.3$), increased perimeter (by 4.00%, $t = 2.4$) and average size (by 4.50%, $t = 2.7$), higher average fluorescence brightness (by 5.76%, $t = 3.5$) and significantly lower optical density (by 3.8%, $t = 3.5$). That is, treatment of wheat plants with Chisotan I compound resulted not only in increase of grain size but improved their endosperm density. Larger average brightness of radiographs was found in grains after use of Vitaplan, Zh + Chitosan II (by 6.00%, $t = 4.3$), Chitosan I compound (by 5.76%, $t = 3.5$) and Chitosan II (by 9.93%, $t = 5.4$) as compared to the control. The largest maximum brightness was observed in grains obtained in scenario with Chitosan I (6.4% increase, $t = 2.5$) and Chitosan II (10.2% increase, $t = 3.6$). Lower average radiograph brightness (by 7.6%, $t = 2.7$) vs. the control was discovered in grains obtained through use of Gamair, SP preparation. In the Chitosan II scenario, the grains had lower circle factor values (by 3.70%, $t = 3.0$), circularity (by 7.22%, $t = 2.9$) and larger elongation (by 4.90%, $t = 2.2$) vs. the control.

When Chitosan II was used, gas-discharge characteristics of wheat grains differed drastically from control group in form factor characterizing the irregularity of gas-discharge image and related to grain weight (23.1% higher, $t = 2.4$). Due to use of Chitosan I compound, the grains had larger isoline fractality values and lager gas-discharge isoline length as compared to control group (by 2.8%. $t = 3.8$ and by 20.9%, $t = 2.2$, respectively). These parameters are presumably also connected with size characteristics of grains. In Vitoplan, Zh + Chitosan II and Chitosan II scenarios, the total intensity of gas-discharge image of the grain was considerably less as compared to control (by 19.3%, $t = 4.1$ and by 15.9%, $t = 3.1$, respectively). Reduction in intensity of gas-discharge fluorescence is typical for the grains that have better growth indicators during germination.

Microfocus X-ray radiography and gas-discharge visualization demonstrated that, compared to control group, introscopic characteristics of grain were to the largest extent changed in Chitosan I, Vitaplan, Zh + Chitosan I and Chitosan II scenarios. Treatment of wheat with only Vitaplan, Zh biological preparation rendered no effect on introscopic characteristics of grains. With the use of Gamair, SP biological preparation (standard), introscopic characteristics of grains changed insignificantly.

As mentioned above, due to transition to biological farming, the researchers lately pay special attention to development of alternative plant protection methods. There are several antagonistic microorganisms effective against the wide range of infecting agents, e.g. *Pseudomonas fluorescens* PCL1751 and *P. putida* PCL1760 [35], *Bacillus* spp. [5, 36, 37], as well as *Trichoderma* species [5, 38]. In general, they are expedient to be used in practice as a sound alternative to synthetic chemical fungicides. However, the effectiveness of biological preparations based on antagonistic microbes is often insufficient.

Another way to control diseases may be the enhancement of natural resistance of plants to pathogen. Compounds that launch own protective mechanisms in plants are called resistance inductors. Among them, special role is played by chitosan and its derivatives. Biological activity of chitosan is connected with its ability to induce protective plant immunity responses [39, 40]. Presence of chitosan in cell walls of some microorganisms, particularly plant pathogenic fungi, determines the most important property of this polymer, the pathogen-associated molecular pattern (PAMPs) that is recognized by plant pattern recognition receptors (PRR). This results in activation of a set of nonspecific plant protective responses (pattern-triggered immunity, PTI), including synthesis of phytoalexins, lignification of cell walls, deposition of callose, synthesis of PR proteins, generation of reactive oxygen species (ROS) and nitrogen (NO), etc. [41].

To enhance potential effect of microbe antagonists, many scientists research joint application of biological agent and resistance inductor. Rajkumar et al. [42] have demonstrated that chitin may stimulate the effectiveness of *Pseudomonas fluorescens* (SE21 and RD41 strains) in controlling pepper bare patch. Adding chitin improves the plant protection by stimulating the production of affined metabolites that promote antagonistic activity and/or stimulate plant protective properties. Co-treatment of vegetating pepper, cucumber, tomato plants with *Saccharomyces cerevisiae* conjointly and chitosan has reduced the development of mildew twice or more [43]. Niranjan et al. [44] reported the results of testing compositions containing two *Bacillus* strains and chitosan as a carrier, and established their capability of growth promoting and enhancing resistance of millet to mildew. The most effective method was based on combining the introduction of chitosan to soil along with treatment of seeds with antagonist microbe strains [45]. Thus, resistance inductors in combination with bioactive substances are very promising for future use of antagonistic microorganisms in controlling plant diseases, especially in greenhouses [46]. Of interest are the compositions of antagonist microbes, e.g. of *Bacillus* genus, with chitosan and its derivatives.

The main issue in assessing the effectiveness of plant treatment for the quality of seed and bread grain is quantitative objectification of stimulating activity. One of solutions may be the development and application of modern imaging facilities and information facilities for express diagnostics of latent grain heterogeneity. Germination parameters are closely related to morphometric indicators of seeds that can be defined through radiographic study, particularly, in lab tests larger seeds germinate earlier and better than smaller ones [47]. It was stated [48] that optical characteristics of radiographs are important for ensuring seed quality. Relative optical density parameter allows us to make conclusions regarding the density of internal seed tissues and hence their physiological quality [49].

It should be noted that the effectiveness of multifunctional preparation Vitaplan, Zh + Chitosan II in respect of potential wheat yield was determined to the largest extent by its effect on productive tilling capacity, which may be connected with the more developed root system (more roots and longer roots) and marked reduction in affection by Helminthosporium root rot when this preparation was applied, as compared to other scenarios we tested. In addition, the use of Vitaplan, Zh + Chitosan II complex demonstrated a more rapid plant passing through ontogenesis phases.

Thus, the research undertaken has convincingly demonstrated the prospects of multifunctional preparations combining useful traits of antagonistic microorganisms and chitosan, a plant disease-resistance activator, for protection of wheat from root rot, yield gain and improvement of grain quality. According to significant positive changes in productivity, the biopreparations rank as follows: in 2016 — Vitaplan, Zh > Vitaplan, Zh + Chitosan II > Gamair, SP > Chitosan I > Chitosan II; in 2017 — Vitaplan, Zh > Vitaplan, SP > Vitaplan, Zh + Chitosan II > Chitosan II. In 2016, a combination of Vitaplan, Zh and Chitosan II in the weather conditions more favorable for plant growth (higher temperature and precipitation), ensured a significant changes not only in the plant green part weight, but also in the spike weight, while separate use of Chitosan II significantly increased the green biomass only. In 2017 (at lower average monthly temperature and considerable amount of precipitation), in this scenario the plants distinguished by their flag leaf area and root weight (86.84 % and 83.33 % increase against control group). In 2016, Chitosan I led to reliable 19.0 % increase ($t = 3.0$; $p < 0.05$) in potential grain yield compared to the control, however, there were no significant differences for Vitaplan, Zh, Vitaplan, Zh + Chitosan II complex and Chitosan II. On the contrary, in 2017 Vitaplan, Zh + Chitosan II caused the maximum reliable ($t = 7.2$; $p < 0.05$) increase in yield, by 82.6 %. Vitaplan, Zh and Vitaplan, Zh + Chitosan II complex possess maximum efficiency against Helminthosporium root rot. In Vitaplan, Zh + Chitosan II scenario, in 2016 root rot occurance was 80 % lower compared to the control, and in 2017 no symptoms were observed, which may be due to less favorable weather conditions for root rot disease in 2017 compared to 2016 (particularly, lower average monthly temperatures and considerable amount of precipitation over the summer period). Potential grain yield in wheat correlates significantly and positively with grain X-radiographic projection area, integrated grain brightness and total intensity of the gas-discharge fluorescence. Chitosan I, Chitosan II and Vitaplan, F + Chitosan II have the greatest impact on grain structure and functional characteristics.

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