UDC [632.3+632.952]:66-965.61:661.16

DOI: 10.15372/CSD20180304

Nanopesticides Based on Supramolecular Complexes of Tebuconazole for Cereal Seed Treatment

E. S. METELEVA[1], V. I. EVSEENKO[1], O. I. TEPLYAKOVA[2], S. S. KHALIKOV[3], N. E. POLYAKOV[4], I. E. APANASENKO[4], A. V. DUSHKIN[1], N. G. VLASENKO[2]

[1]*Institute of Solid State Chemistry and Mechanochemistry, Siberian Branch, Russian Academy of Sciences, Novosibirsk, Russia*

E-mail: dushkin@solid.nsk.su

[2]*Siberian Research Institute of Agriculture and Chemicalization of Agriculture SFSCA RAS, Siberian Branch, Russian Academy of Sciences,*
Novosibirsk Region, Krasnoobsk, Russia

[3]*Nesmeyanov Institute of Organoelement Compounds, Russian Academy of Sciences, Moscow, Russia*

[4]*Voevodsky Institute of Chemical Kinetics and Combustion, Siberian Branch, Russian Academy of Sciences, Novosibirsk, Russia*

(Received April 20, 2018)

Abstract

To develop innovative integrated seed protectants, the mechanochemical synthesis of supramolecular complexes of tebuconazole (TBC) has been applied. Seed protectants formulations with improved environmental properties were obtained using the polysaccharide arabinogalactan, glycyrrhizic acid, and its disodium salt, and also dry liquorice root extract as compounds that formed supramolecular systems composed of TBC species. The high efficiency of the resulting compounds in regard to pathogenic microflora in the treatment of spring wheat and barley seeds, in the suppression of the development of root rot, and also the lack of the retardant effect and a positive impact on the productivity of these crops was demonstrated under laboratory and field conditions. Furthermore, the compounds allow twice the reduction of TBC consumption rate.

Keywords: tebuconazole, mechanochemistry, polysaccharides, saponins, host-guest complexes, supramolecular systems, fungicidal compounds, biological efficiency, productivity

INTRODUCTION

The basis of the modern range of chemical plant protection in grain production of Russia is nitrogen-containing systemic fungicides, such as tebuconazole, triticonazole, difenocanozole, cyproconazol, difenocanozole, *etc.* [1]. Their preparative forms are permanently being improved [2, 3]. The indicated triazole derivatives may have an effect on biochemical and physiological functions of plants causing both retardand and stimulating effects [4]. For example, tebuconazole-containing preparations are able to increase the abscisic acid content in wheat plants, increasing their adaptation to low temperatures, which is especially important for the Siberian region [5]. On the other side, the retardant effect of triazoles, which increases under unfavourable conditions of plant growth and development, may result in a reduction in the field germination of cereals by 25–30 % [6]. In this regard, in plant protection, there is developed an area on the one side, related to toxicity neutralization of fungicides for gramineous plants, and on the other one – to the strengthening of their biological effectiveness against pathogens. Nanotechnological principles for the development of new formulations should play an important, if not decisive, role in the development of these areas.

As described previously, it is possible to improve the phytosanitary effect in the fight against pathogens of common root rot of thiabendazole – tebuconazole preparations using biogenic iron hydroxide nanoparticles enhancing their fungicidal properties [7]. Biogenic ferrihydrite nanoparticles doped with aluminium or cobalt enhance the effect of seed treatment with fungicide, and undoped ferrihydrite nanoparticles reduce [8]. The introduction of iron hydroxide nanoparticles statistically significantly reduces the inhibiting effect of fungicides in respect to sprouts, which appears in increasing the germinating ability of seeds, sprout dry mass, the length of the overground part and a root system, and also the total length of sprouts [9].

In this regard, the elaboration of polyfunctional seed protectants on the basis of development of supramolecular systems, *i. e.* carriers of protectant molecules, intermolecular formations of the guest-host type, where structure-forming substances are high-molecular-mass natural compounds or their self-associates, should be considered promising and relevant. Herewith, in view of the thermal stability of the used components, the lack of suitable solvents simultaneously dissolving all compounds that are ingredients of the systems, and also the insufficient chemical stability of the components in traditional liquid-phase technology processes, the use of the solid-phase technique for the preparation of solid dispersed systems (SD) of constituent elements that form the sought supramolecular systems ensuring high solubility on dissolving, adsorption of the active materials, and the total increase in their biological activity is promising [10]. Herewith, the process of mechanochemical treatment of a mixture of solids is most often carried out in ball mills and, if necessary, can be repeatedly scaled up [11, 12].

In our opinion, promising auxiliary substances include vegetable polysaccharides [13] that, in addition to the formation of intermolecular complexes, possess useful functions of moisture containment and acceleration of the beginning of physiological biochemical processes leading to the activation of seed embryo growth, and in combination with biopreparations – increased germination capacity and resistance of sprouts to phytopathogens [14]. In our view, other promising auxiliary compounds are glycyrrhizic acid and its salts [15] in significant amounts (in 24 %) contained in roots of Ural liquorice widely grown in the western part of Russia [16, 17]. The roots and rhizomes of liquorice, apart from traces of essential oil, vitamins, proteins, bitter (up to 4 %) and resinous (3–4 %) substances, lipids (about 4 %), polysaccharides (4–6) % pectin and starch), monosaccharides and disaccharides (in 20 %) contain more significant from a pharmacological point of view flavonoids (3–4 %) and triterpene saponins i.e. glycyrrhizic acid (about 20 %). Along with the polysaccharide arabinogalactan, the reserves of these plant substances in Russia are almost unlimited.

In fact, the area of development of supramolecular systems containing biologically active fungicide compounds is close to the scientific field of generating the so-called delivery systems of drugs widely used in pharmaceutical chemistry under the term Drug Delivery Systems. Part of a team of authors has been carried out research in this area for a number of years. There have been acquired results that allow repeatedly reducing drug dosages with the preservation of their action, and also decreasing their toxic effects [18, 19]. The physiological mechanism of these effects lies in enhanced water solubility, membrane adsorption, and pharmacological parameters [20].

We used the same approach to develop nanofungicide preparations based on supramolecular systems formed by vegetable organic matter, i.e. natural saponin, such as glycyrrhizin, and polysaccharide arabinogalactan and comprising molecules of trichlorobenzoic acid (TBA) as the active substance [21, 22].

The main purpose of the present research was profound physicochemical and biophysical investigations of intermolecular interactions of substances entering systems in solid phases and aqueous solutions by current structural and spectral methods, capacity towards membrane permeability over artificial membranes, and conducting biophysical experiments to assess the penetration of the active substance into the internal volume of grain, followed by correlation with the results of biological studies on the compositions on cereals. At the initial stage of works, the purpose was to reach understanding mechanisms of changes of the biological action of the developed systems. In the first part of the research, we used pure commercially produced substances with a sufficiently high cost and in dosages often different from those used in agriculture, which allowed avoiding ambiguity in the interpretation of experimental data and, ultimately justifying the criteria for generating systems with improved characteristics. However, at the final stage of experiments, we used an inexpensive auxiliary substance, *i.e.* liquorice root extract (LE) that was produced in the Siberian region and contained poly- and monosaccharides and glycyrrhizic acid as the main components. The indicated economic criteria of accessibility and cost also include quite valuable properties that appear in the quality of the stimulating agent for plant growth processes [23], and also the antiviral activity of its components [16, 17].

EXPERIMENTAL

Physical and chemical research

Components of the developed protectants were as follows: tebuconazole (TBA, Shenzhen Sunrising Industry Co., Ltd., China), basic substance content of >98.0; glycyrrhizic acid (GA) (Shaanxi Pioneer Biotech Co., Ltd, China), >98.14 %; its disodium salt (Na_2GA) (Shaanx Pioneer Biotech Co., Ltd, China), >91.14 %; the polysaccharide arabinogalactan (AG) with a molecular mass of ~15 kDa isolated from *Larix sibirica* Ledeb. and *Larix gmelinii* Rupr. larch wood (Ametis JSC, Russia), >97 %; dry extract of Ural liquorice root *Glycyrrhiza uralensis* Fisch. (Viterra Co., Ltd., Russia), GA mass fraction of 20–25 mass %. The combined mechanochemical treatment of TBA and in mass ratios of TBA/auxiliary substance of 1 : 5; 1 : 10 and 1 : 20, were carried out under the conditions described earlier [14]. The treatment was carried out for 12 h taking samples every 2 h.

The resulting systems were analysed by HPLC for the content of the active substance (TBA), its water solubility from the resulting compounds, gel-penetrating chromatography, dynamic ^1H NMR spectroscopy ЯМР (^1H NMR spectra in D_2O solutions were taken using Bruker BioSpin AG NMR AVHD-500X spectrometer), granulometric, powder X-ray phase analysis and thermal analysis according to methods described in [21]. The optimum time of mechanochemical treatment was selected by the criteria of the maximum water solubility of TBA, provided that its content in the resulting system was not less than 98 mass % from initial.

In addition, the degree of absorption of TBA into the internal volume of spent grain and the rate of its transmembrane transfer over model membranes were found.

The TBA concentration in a solution was measured by HPLC using Agilent 1200 chromatograph with Zorbax Eclipse XDB-C18 column 4.6 · 50 mm, (column temperature of +30 °C, diode-matrix detector). The acetonitrile–water (1:1) system, a flow rate of 1 mL/min, a sample volume of 5 μL, detection at a wavelength of 220 nm were used as an eluent. The TBA concentrations were determined in reference to its specially prepared solution in ethanol.

The TBA content was determined according to dilution of the resulting systems in ethanol. Afterwards, these solutions were analysed.

Phase solubility investigation was carried out according to the method described by Higuchi and Connors (Higuchi and Connors. Samples of mechanically treated systems with the maximum solubility of TBA were placed into a flat-bottom flask; 10 mL of distilled water was added, and stirred at +30 °C for 1 h. After reaching equilibrium, the system was centrifuged at 12000 r/min for 10 min followed by filtration of the resulting supernates using a paper filter. The TBA concentration in the filtrate was determined by HPLC according to the overall technique described in [22]. The phase solubility diagram was plotted using the total molar concentration of complexing agents as the X-axis and the total molar concentration of TBA as the Y-axis.

The measurement of trans-membrane permeability over artificial membranes was made by the PAMPA method that was used to predict the permeability of bioactive compounds through cell membranes [24]. To carry out analysis, there were used special 12-position Transwell cells with a 12 mm diameter polycarbonate membrane, 0.4 μm pore size of and an area of 1.12 cm^2 (Corning Incorporated, art. 3401) by the method described in [25]. The donor cell contained the investigated sample of the test material (0.002 g per TBA) in 0.5 mL of distilled water, the "acceptor" cell – 1.5 mL of water. The assembly of these cells was incubated in an orbital shaker at + 37 °C. At certain intervals, the selection of 1 mL of the solution from the acceptor cell was carried out with its replacement by an equal amount of water. The TBC concentration in the selected solutions was measured by the HPLC method according to the previously indicated procedure.

To determine TBA penetration into the internal seed volume, the grain of wheat (Omskaya 36 grade) was treated with an aqueous suspension of the resulting systems, and also with TBA per 0.03 g of the active substance (TBA) per 100 g of seeds. Mixing the suspension with the seed was carried out in VM-1 roller drum for 1 h (with a drive speed of 342 r/min) without loading grinding pearls. The processed grain was sprouted for 3 days on moistened filter paper at +25 °C. Then the grain, together with the sprouts, was dried to a constant mass in a vacuum cabinet at +60 °C, there was washout of TBC that did not penetrate into the grain volume (grain together with sprouts) and remained on its surface with several portions of ethanol until the disappearance of the peak of TBC in HPLC chromatograms. After that, the seed was dried in a vacuum oven at +60 °C for 24 h and milled in an impact mill (coffee-grinder). Afterwards, a known amount of ethanol was added to the sample of the powder, the mixture was stirred for 20 min and filtered through a paper filter. The TBA concentration in the resulting solution was determined by the HPLC method and the mass of TBA contained in 100 g of dry grain was computed. The moisture content of the initial grain, determined by mass loss during drying to a constant mass (+60 °C in vacuum) was 9 mass %.

Biological tests

To assess protectant efficacy, there were carried out laboratory and field tests. Biotests of the TBA system with AG were carried out for Novosibirskaya 29 wheat, TBA systems with GA and Na_2GA – for Omskaya 36 wheat and Acha spring barley, Acha, TBA, and liquorice extract (LE) – Omskaya 36 Wheat. To determine the level of improvement of the seed material by compositions, the seeds were placed on moistened filter paper [26]. Laboratory experiments included several variants, each of which had a blank experiment (without fungicide treatment). In the first variant, the seeds were treated with TBA/AG 1/5 with a flow rate of 0.3 kg/t of seeds. In the second variant, the seeds were treated with TBA/Na_2GA 1/5 in a dose of 0.3 and 0.1 kg/t (TBA/Na_2GA and 0.3 TBA/Na_2GA 0.1, respectively), and also TBA/GA 1/5 in a dose of 0.3 and 0.1 kg/t (TBA/Na_2GA 0.3 и TBA/Na_2GA 0.1, correspondingly). The TBA flow rate was 0.017 kg/t. The third variant, in addition to the test experiment, included the processing of the systems TBA/LR 1/10 in a dose of 0.5 kg/t.

The Bayer fungicide-protectant Raxil KS (TBA, 60 g/L) with a consumption rate of 0.5 L/t of seeds was used as a chemical standard in both experiments.

The treatment with preparations was carried out 24 h prior to trial establishment. The solutions were prepared in an amount of water consumption used under production conditions (10 L/t).

The first field experiment was laid out according to the following pattern: 1) monitoring (seeds not treated with fungicide): 2) standard (prior to sowing the seeds were treated with the drug Karsil in a dose of 0.5 L/t); 3) the processing of TBA/AG/surfactant systems in a dose of 0.3 kg/t. The scheme of the second experiment included the following variants: 1) monitoring (seeds not treated with fungicide): 2) standard (prior to sowing, the seeds were treated with the drug Raxil in a dose of 0.5 L/t); 3) prior to sowing, the seeds were treated with the system BA/Na_2GA 1/5 in a dose of 0.3 and 0.1 kg/t (TBA/Na_2GA and 0.3 TBQ/Na_2GA 0.1, respectively). In the third experiment, the following variants were available: 1) monitoring; 2) standard (prior to sowing, seeds were treated with Raxil in a dose of

0.5 L/t); 3) seed treatment before sowing using TBA/ES 1/10 in a dose of 0.5 kg/t.

The experiment was laid according to the vapour precursor. Experiment repeatability is fourfold. The area of the plot is 20–25 m^2 and the placement is systematic. Mordanting was performed with moisture of 10 L/t of seeds. Harvesting was carried out by direct combining. Yield was adjusted to standard moisture content and purity according to GOST 1386.5–93 and 1386-2–81. Accounting of stand density, the productive tilling capacity of plants and selection of bundles to analyse the structure of productivity was carried out directly before harvesting [27]. The development and occurrence of common root rot were taken into account according to the method [28], the presence of growth-regulating effects – in phases 3 and 5 leaves.

Mathematical data processing of field and laboratory experiments was carried out using the software packages Snedecor [29] and Statistica 6.0.

RESULTS AND DISCUSSION

Justification of optimum conditions for preparation of protectant systems

According to the totality of characteristics of maximum solubility and stability enhancement of TBA, there were selected the best compositions and conditions of their preparation for further physicochemical and biological research:

– TBA/AG mass ratio of 1/10, mechanochemical treatment time of 6 h, increased the water solubility of TBA is 3 times;

– TBA/Na_2GA 1/5, 6 h, 2.9 times;

– TBA/GA 1/5, 4 h, 34 times;

– TBA/ES 1/10, 24 h, 15.2 times.

Physical and chemical research

Solid phases of protectant compositions. All X-ray diffraction patterns of TBA mixtures and auxiliary compounds contain characteristics reflections of the crystalline phase of TBA. Their intensity decreases resulting from mechanochemical treatment, however, the presence of the residual crystalline phase is apparent. In thermograms of DSC of mixtures of TBA and auxiliary compounds, there are characteristic endothermic melting peaks of the crystalline phase of TBA. Their areas decrease after mechanochemical treatment by 3–10 times, but there is also the residual crystal phase. The thermograms and X-ray diffraction patterns of auxiliary compounds, there are no apparent thermal effects of phase transitions, which indicates their amorphous state and the lack of the crystalline structure.

According to SEM data, during mechanochemical treatment, crystalline TBA and arabinogalactan (GA and Na_2GA) particles are decomposed. Polydisperse powders mainly consisting of the irregularly shaped particles (5–20 μm in size) and their aggregates are generated.

Solutions of protectant compositions. Figure 1 gives the data on the granulometric composition of suspensions of TBA and its systems. It can be seen that the TBA substance (see Fig. 1, *a*) is a polydisperse powder with particle sizes from 1 to 262 μm. The size distribution is monomodal with a maximum of 60 μm. Herewith, 80 % of the sample mass is concentrated in the particle size range of 20–135 μm.

Resulting from mechanochemical treatment (MT) with auxiliary compounds, particle distribution becomes bimodal and there are approximate maxima near 3–5 and 50–60 μm. It is most likely that the large fraction is initial unmilled TBA particles. Apparently, they are precisely the “remnants” of the crystalline phase of TBA, apparent in X-ray diffraction patterns of XPA and DSC thermograms. The origin of the fine fraction needs to be discussed, as with simple grinding of solid particles by mechanical action under these conditions, the granulometric image would have to look different: there would be a gradual increase in the quantity of small fractions and a shift in the maximum distribution towards small sizes. As a whole, the distribution would be “broadened” and shifted towards particles decreasing. This phenomenon proceeds during the mechanical treatment of the TBA substance without complexing additives [30]. The picture observed by us attests to a different mechanism of appearance of small fractions. In our view, micron and submicron particles are formed as a result of the water suspension formation cycle: solid composition > supersaturated aqueous solution > precipitation. Herewith, a supersaturated solution is generated due to the dissolution of TBA as intermolecular complexes formed in solid phases under mechanochemical

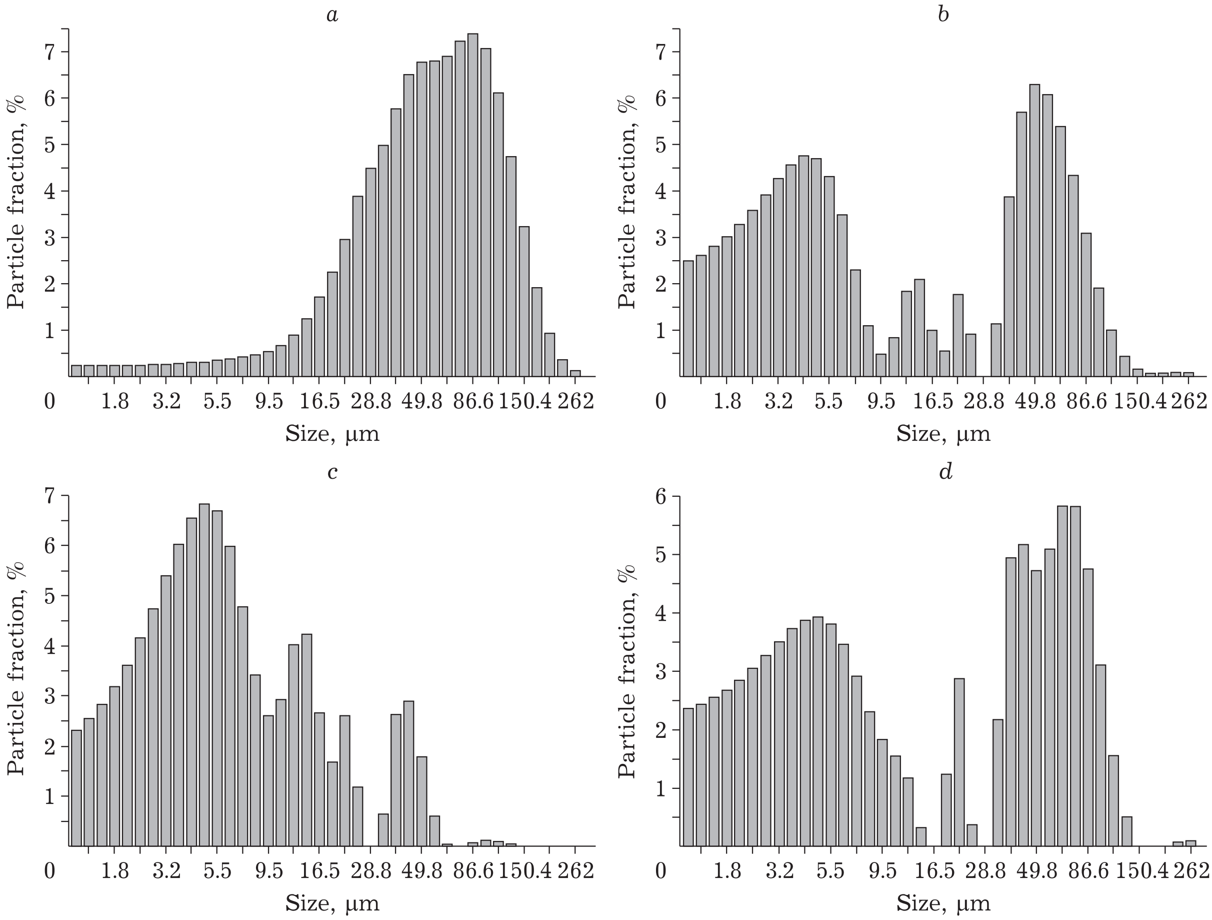

Fig. 1. Granulometric composition of: *a* – suspension of TBA substance; *b* – precipitate of dispersion TBA/AG 1/10, m/t, 6 h; *c* – suspension of system TBA/GA 1/5, m/t, 4 h; *d* – precipitate of dispersion TBA/Na_2GA 1/5, m/t, 6 h.

action and dissolution of its amorphous phase. Note that the mass fraction of these small fractions is 50–70 %, which is close to our assessment of the crystallinity degree loss from the data of X-ray structural analysis and thermal analysis. It is also worth noting that the fine fraction facilitates an increase in the biological activity of substances due to accelerated dissolution and high solubility, and this indicates the advantage of our mechanochemical method for producing these complexes.

To determine the stoichiometry and thermodynamic parameters of mechanochemically produced complexes at +30 °C, phase diagrams were examined. In the case of GA and its salt, they are AL-type diagrams (Fig. 2) and assume the formation of a complex with a molecular ratio of TBA/Na_2GA (GA) micelle of 1/1:

TBA + GA micelle ↔ [TBA/GA micelle]

Thus, in the solutions under study, Na_2GA (GA) micelles are sort of a single molecule with a fixed structure. Given very narrow molecular mass distribution of micelles in the explored concentration range, this assumption may be justified. The equilibrium constant (K_c) of this system was computed according to the formula

$$K_c = \text{Slope}/S_0(1 - \text{Slope}) \quad (1)$$

While the chemical potential ΔG was determined as

$$\Delta G = -RT\ln K_c \quad (2)$$

Considering the low solubility of TBA in water (0.097 mM), instead of computed values of S_0 acquired from solubility diagrams by the least squares method and having significant error values, a physicochemical and experimentally determined equilibrium solubility of TBA of 0.097 mmol/L was adopted. Table 1 gives the resulting thermodynamic parameters: K_c is the stability constant of supramolecular complexes and ΔG is the chemical potential of their formation. In case of AG, the acquired phase diagrams are of a more com-

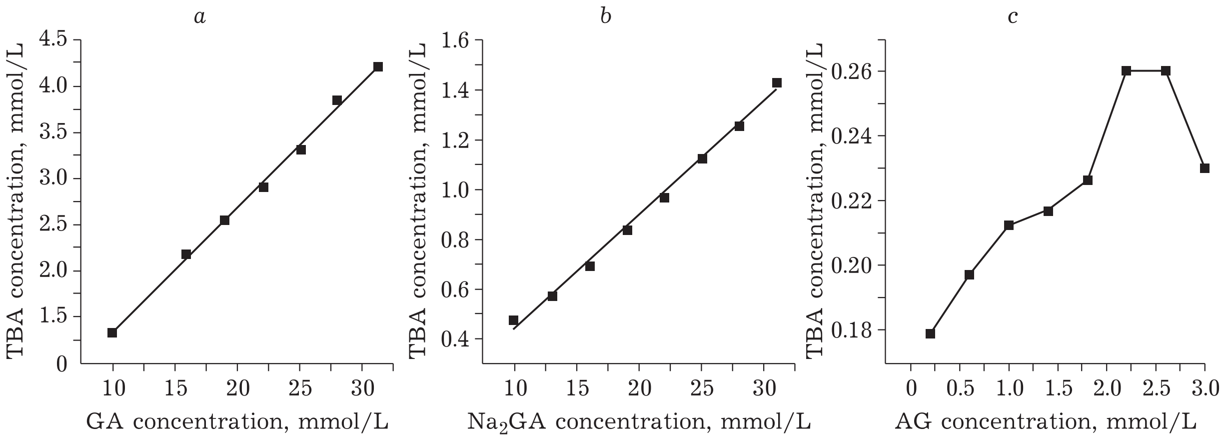

Fig. 2. Phase diagrams of solubility of complex TBA/GA 1/5 (*a*), TBA/Na_2GA 1/5 (*b*), TBA/AG 1/10 (*c*) in aqueous solutions at 30 °C. GA concentration, mmol/L TBA concentration, mmol/L.

plex form, probably due to changed complexation stoichiometry with different solution concentrations; it does not seem to be possible to compute K_c and ΔG parameters.

For additional proof of the formation of intermolecular complexes of TBA with molecules of auxiliary compounds, this work used the method of dynamic ^{1}H NMR spectroscopy. It is known that the spin-spin T_2 relaxation times are very sensitive to the intermolecular interaction and the diffusion mobility of molecules [31]. This is due to changes in the time of rotational reorientation of molecules in the complex or in contact with TBA molecules in GA micelles due to slowing down diffusion. Typical values of T_2 for molecules in solutions are 0.5–5 s, and in combination with AG or GA – 0.05–0.2 s [32].

In general, in the experiment, there may be observed the bi- or monoexponential kinetics of the echo signal decay depending on the rate of exchange between the complex and the solution.

The work measured relaxation times of aromatic protons of TBA and its systems, such as TBA/AG 1/10, TBA/GA 1/10, and TBA/Na_2GA 1/10 obtained by the mechanochemical method in an aqueous solution with a concentration of TBA of 1 mM. Figure 3 gives NMR signal decay kinetics and relaxation times of TBA protons in a free state and as a complex with AG and GA salt. The relaxation time of TBA in the GA solution was even shorter, less than 10 ms, indicating the full inclusion of TBA in micelles. All measured kinetics are described by monoexponential dependence, which indicates that there is a fast exchange of guest molecules between the complex and the solution. As can be seen from Fig. 3, the decay kinetics of the TBA proton echo signal is significantly shortened in solutions of the studied systems compared to a pure aqueous solution of TBA. This proves the formation of intermolecular complexes in case of systems with AG or the inclusion of TBA molecules in micelles formed by the GA or its sodium salt.

In addition, an aqueous solution of LE was analysed using the NMR method. Figure 4 gives the ^{1}H NMR spectrum of an aqueous solution of dry extract after centrifugation and insoluble precipitate removal. The GA content in extract (25 %) was determined by comparison of this spectrum with that of a solution of pure GA. Apart from, GA signals, the main components of the NMR spectrum of LE are those of polysaccharides and oligosaccharides (signals in the range of 3.2–4.2 ppm). As demonstrated by the measurement of T_2 relaxation time of GA protons in extract solution (45 ms) GA is present in the aggregated (micellar) state.

Further, complexation of TBA with glycyrrhizic acid in liquorice root extract was explored during aqueous dissolution of a mechanochemically activated mixture of TBA and

TABLE 1

Thermodynamic parameters of complexation

Complex	K_c, mol/L	ΔG, kJ/mol
TBA/GA 1/5	1611±38	−18.60±0.06
TBA/Na_2GA 1/5	492±14	−15.62±0.07

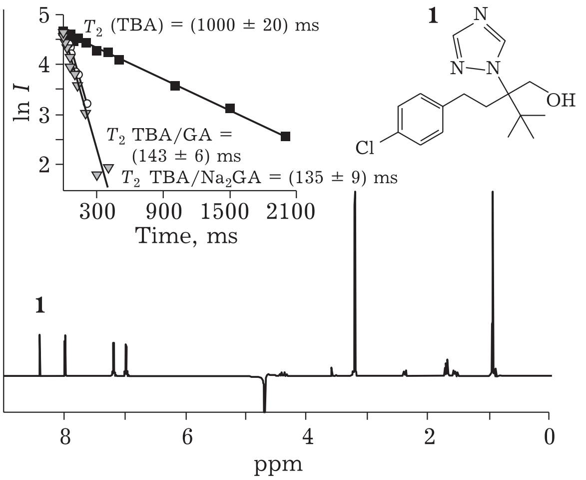

Fig. 3. ^{1}H NMR spectrum of TBA in a 20 % aqueous methanol solution. The digit **1** indicates protons, for which relaxation times T_2 were measured; in the inset, decay kinetics of echo signal and relaxation times of ^{1}H protons of TBA in D_2O solution for pure TBA and its complexes (1 : 10) with arabinogalactan and GA disodium salt are indicated.

extract powder in a ratio of 1 : 5 and 1 : 10. Associates formation was proven by the reduced time of the spin-spin relaxation of TBA protons. The acquired values of the relaxation time are typical for the molecules included in glycyrrhizic acid micelles and are in line with the relaxation time of the GA molecule itself in the micelle.

To investigate the structure of aqueous solutions of the systems, the gel-filtration chromatography technique was used according to the procedure described in the experimental section. In all cases, chromatograms of aqueous solutions of TBA systems with GA and Na_2GA contained peaks of high molecular mass formations of micelles with a mass of 60–100 kDa (herewith, the MM of GA is 837 Da).

There were no peaks of a low molecular mass compound (TBA), which, in addition to the data regarding increasing the solubility of TBA attests to the inclusion of TBA molecules into micelles. We have earlier demonstrated this physicochemical mechanism of water solubility increase on the example of drugs [12]. In case of arabinogalactan-containing systems, there was a single peak in gel chromatograms, with a calculated molecular mass of 14.5–15.0 kDa, belonging to the macromolecule of the specified polysaccharide, which indicated the lack of its mechano-destruction and probably attests to the binding of TBA molecules with AG macromolecules to an intermolecular complex.

Figure 5 gives the measurement results of the trans-membrane transport by RAMRA. It can be seen that the diffusion/transfer rate of TBA molecules significantly increases in case of its compositions with AG, Na_2GA, GA, and

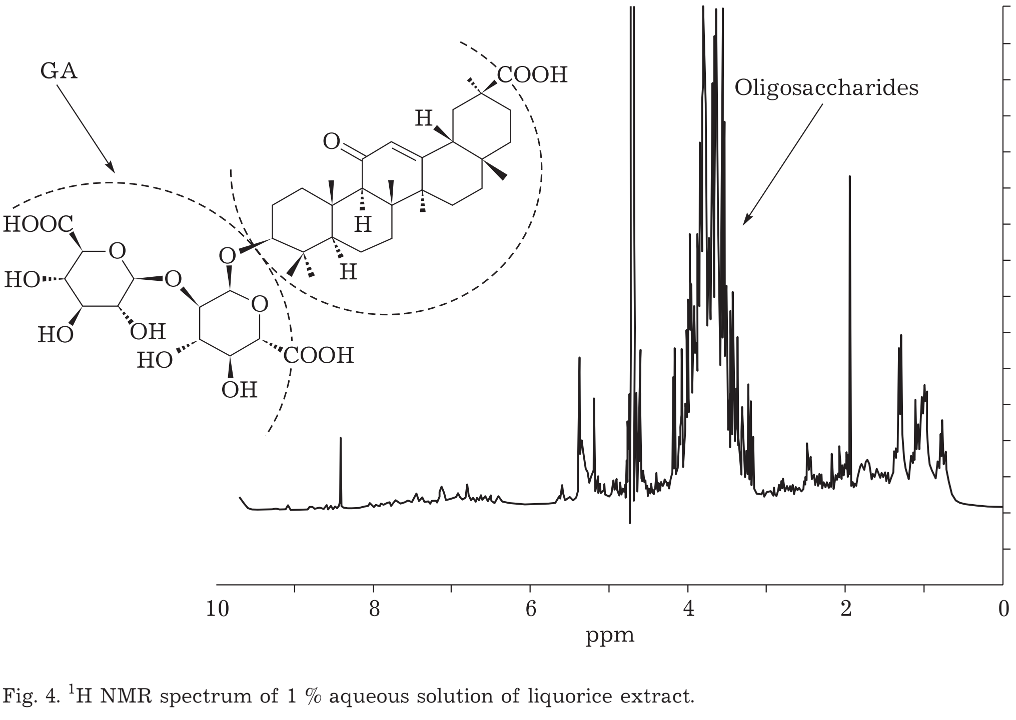

Fig. 4. ^{1}H NMR spectrum of 1 % aqueous solution of liquorice extract.

ES compared to the initial TBA substance. Herewith, the transfer from systems of TBA with AG and Na_2GA is higher than that from the TBA/GA system, which is found in the inverse ratio with an increase in the solubility from the above systems. In our view, this contradiction may be explained by the specifics of the interaction of the "excess" of the non-neutralized GA (auxiliary compound) with the artificial membrane material.

Table 2 gives research results of TBA adsorption transfer from the surface of the seed into its internal volume after treatment with suspensions of TBA and resulting systems, and also with the preparation Raxil that are taken in equal doses.

The reliability of the carried out measurements is proven by the cumulative data of the total number of the detected TBA and its actual agreement (within the accuracy of the experiment) with those introduced with seed treatment (see Table 2). Herewith, the penetration of TBA into the seed volume from the unmodified suspension of the TBA substance is ~2 times lower than for those from the resulting compositions of protectants and the reference preparation Raxil. These preparations demonstrate about the same absorption efficiency, which proves the correctness of the developed approach to generating innovative protectants based on supramolecular carriers of biologically active molecules, in our case, TBA.

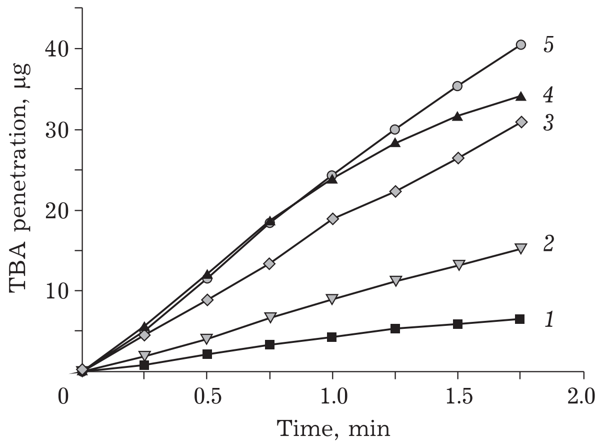

Fig. 5. Transfer dynamics of TBA from its systems through an artificial membrane, *i. e.* porous polycarbonate/hexadecan: *1* – initial TBA; *2* – TBA/GA 1/5, m/t; *3* – TBA/ES 1/10, m/t; *4* – TBA/Na_2GA 1/5, m/t; *5* – TBA/AG 1/10, m/t.

Biological tests

As demonstrated by the investigation of tebuconazole-containing systems over a naturally infected seed material, they are relatively active against pathogens of root rot of soft spring wheat and in the first place, against the main pathogen that is *Bipolaris sorokiniana* Shoem. Laboratory experiments showed that upon infestation of seeds of spring wheat Novosibirskaya 29 *B. sorokiniana* at a level of 20.9 %, the TBA/AG system inhibited the growth of pathogen by 100 %, the same as Raxil. The biological efficacy against fungi of the genus *Fusarium* in the system was 94.9 %, in commercial protestant – 79.8 %. Both preparations also suppressed the development of *Alternaria* spp. by 100 %.

In monitoring the dynamics of the germinating ability of seeds, the positive effect of using the drug TBA/AG was revealed at the earliest stages of growth: in 3 days, a greater number (16.3 %) of seedlings was recorded in this option. This value was higher than both monitoring (11.3 %), and an option with Raxil

TABLE 2

Adsorption of TBA molecules from the surface of germinated (3 days) grain into its internal volume

Composition	Treatment TBA, (mg) per 100 g of seeds calculated	Blank exp.* TBA, mg/100 g of seeds	TBA wash-off, mg/100 g of seeds	In paper, mg/100 g of seeds	In seeds, mg/100 g of seeds	Total TBA detected, mg/100 g of seeds
TBA	32	31	18	5	5	28
TBA/Na_2GA 1/5	31	31	15	4	10	29
TBA/GA 1/5	30	30	17	3	11	31
TBA AG 1/10	31	29	21	2	9	32
Raxil	30	28	14	2	13	29

* Wash-off from treated non-germinated grain.

(6.2 %) by 1.4 and 2.6 times. Upon completion of the experiment, an equally greater (95 %) number of plants than in the control (90 %) were formed in options TBA/AG and Raxil.

To improve process parameters (wettability, working suspension, adhesion to drug suspension to seed surface), *Sapindus trifoliatus* fruit containing saponins, *i.e.* natural surfactants, were included in the TBA/AG system [30]. During treatment of wheat seeds, TBA/AG/surfactant formed sprouts with better growth indicators than in the control (Fig. 6). Under its influence, there was increased the root length (14.08±0.11 cm against 9.58±0.84 cm in the control and 13.95±0.16 cm in a variant with Raxil) and air-dry biomass (10.57±0.63 g/plant *vs*. 8.53±1.71 in the control and 9.75±0.62 g/plant in a variant with Raxil). In a variant with treatment by TBA/AG/surfactant, sprout height (10.56±0.17 cm) did not differ from that in the version with Raxil (10.57±0.14 cm) and were higher than monitoring by 11 % (9.51±0.80 cm). Sprouts were formed more aligned than in monitoring. This is also proven by the biomass index of one sprout formed from corn seeds treated with TBA/AG/surfactant, the value of which varied (9.39–11.30 mg) to a much lesser extent than those from untreated (5.26–15.65 mg).

The high efficiency of seed treatment by TBA/AG/surfactant in limitation of the lesion of spring wheat plants by root rot was proven under field conditions. In the first steps of organogenesis, the drug had a healing effect on the primary roots, coleoptile, and the axilla of bottom sand leaves. Its efficacy in suppression of development and morbidity rate was high and reached 74.1 and 71.1 % (primary roots); 86.1 and 85.7 % (coleoptile), and 100 % (the axilla of bottom sand leaves). The efficiency of the drug TBA/AG/surfactant in limitation of development and prevalence of root rot was traced right up to the phase of milk ripeness of wheat. By this phase of development, the plants grown from the seeds treated by the system turned out to be taller (by 4.2 %), had a larger (by 8.9 %) biomass of the above-ground part and better (by 4.8 %) formed a shrub (Table 3). According to all indicators characterizing the spike structure, the experimental option was significantly different from monitoring. The process of forming the spike somewhat weaker depended on the use of the drug TBA/AG/surfactant; the length and the number of ears increased by 3.2 and 3.8 %. A similar growth (3.8 %) was also achieved according to the mass of 1000 seeds. The data analysis of number of grain content and grain productivity of the main spike revealed a significantly better effect of the drug TBA/AG/surfactant under field condition. The number of grains and their mass increased by 7.8 and 10.8 %

Fig. 6. Response of wheat plants on seed treatment: *a* – control; *b* – Raxil KS, 0.5 L/t; *c* – TBA/AG/surfactant, 1/5/1, 0.3 kg/t (laboratory experiment, method of coils, 2014).

(monitoring 21.2 pcs and 0.789 g, respectively). The grain harvest in the experiment (1.62 t/ha) exceeded the control and reference variant.

As demonstrated by laboratory experiments, TBA systems with GA and Na_2GA are also relatively active against pathogens of root rot of spring soft wheat and ensure 100 % efficiency. Using inhibition level of fusarial infection for wheat seeds, the composition and system consumption rates were determined; TBA/Na_2GA 0.3 suppressed the pathogen completely, and TBA/Na_2GA 0.1 – by 70 % (seed contamination in the control of 6.4 %). During treatment of TBA/GA, both dosages ensured 100 % efficiency. In barley grains, TBA/GA did not suppress the growth of *Fusarium* spp. fungi in both consumption rates. Phytosanitary action *vs*. the composition and consumption rate of the systems was also observed with regard to *Alternaria* spp. fungi. Their development on wheat seeds successfully monitored by the complex TBA/Na_2GA 0.1 (biological efficiency 71.1 % and TBA/GA (biological efficiency of 71.8 and 74.4 %, in accordance with consumption rates of 0.1 and 0.3 kg/t). Barley seed treatment with TBA/Na_2GA in doses of 0.3 and 0.1 suppressed *Alternaria* spp. by 50.9 and 29.4 %, respectively. In case of treatment of barley grains with TBA/GA, the dependence on the dose consumption rate was not observed: *Alternaria* spp. it was controlled by 45.1 and 50.9 %.

Laboratory wheat seed germination was maximum (94.8 %; standard is 68.9 %) during seed treatment with TBA/Na_2GA 0.3. When using TBA/Na_2GA 0.1, it was 84.8 %; TBA/Na_2GA 0.3–83.3 % and TBA/GA 0.1 – 90.6 %, which was lower than in applying TBA/Na_2GA 0.3 (Fig. 7).

Plants that have a shorter main root but larger root biomass were formed from wheat kernels treated with the systems. There was observed a positive trend in all variants of using the systems. The maximum mass of the roots of one plant was acquired when processing wheat seeds with TBA/GA, 0.3 kg/t (26.7 % higher than in the control). Compared to the standard, where wheat sprout height reached 14.0 cm, the complex TBA/Na_2GA facilitated its increase by 20–22 %. The biomass of wheat sprouts was at a level of the net control and exceeded that of the standard (15.0 mg) by 1.2 times. There was a similar effect when using the complex TBA/GA only in a dose of 0.1 kg/t. Barley sprouts formed from kernels treated with TBA/Na_2GA 0.1 were higher by 27 %. The advantage of using the complex TBA/Na_2GA for barley is evidenced by the biomass indicator of sprouts that increased from 17.4 mg in the standard by 7.4 and 16 % in accordance with doses of 0.3 and 0.1 kg/t.

The efficiency of wheat seed treatment with the system TBA/Na_2GA 0.3 was 83.2 and 73.9 %, correspondingly, according to the development and prevalence of root rot and exceeded the standard, where the corresponding indicators were found at a level of 76.6 and 67.9 % (in the control, disease development index is 32.1 % and the disease prevalence is 83.3 %). With decreasing the consumption rate

TABLE 3

Effect of the drug TBA/AG/surfactant 1/5/1 (14 % of TBA) on productivity structure and yield of Novosibirskaya 29 spring wheat

Variants	Control	Raxil	TBA/AG/surfactant 1/5/1 (14 % of TBA)	$LSD_{0.5}$
Plant height, cm	61.9	63.5	64.6	0.50
Number of stems / plant, pcs	1.19	1.21	1.25	0.01
Spike length, cm	6.98	7.02	7.21	0.08
Number of spikelets in main spike, pcs	12.7	12.9	13.2	0.13
Number of grains therein, pcs	21.2	21.1	23.0	0.66
Mass of grain from main spike, g	0.79	0.78	0.89	0.01
Mass of 1000 grains, g	38.5	38.2	40.0	1.16
Productivity, t/ha	1.53	1.55	1.62	0.07

Note. Surfactant (*Sapindus trifoliatus* fruit) was added for better adhesion of suspensions of protectants to grain surface. In subsequent experiments, surfactant addition was not required, as glycyrrhizin derivatives have amphiphilic properties and act as surfactants themselves.

of TBA/Na_2GA, treatment efficiency decreased to 76.6 and 49.9 %, respectively. By the phase of stem extension, the phytosanitary effect in a variant with TBA/Na_2GA 0.3 weakened by 2.7 times, but this drug still better controlled the development of root rot in wheat crops.

The biological efficiency of TBA/Na_2GA in the three barley leaf phase in both consumption rates (49.6 and 51.1 %) was higher than that of the standard (37.9 %). The treatment effect was also observed during bush formation and the stem extension. A higher fungicidal effect for the barley was also observed when applying a higher consumption rate.

When using TBA/Na_2GA in doses of 0.3 and 0.1 kg/t, it was also demonstrated that under field conditions, the three leaf phase, the plant height of wheat (24.6 and 26.2 cm) was significantly higher than the pure control (23.0 cm) and Raxil (21.7 cm, $LSD_{0.5}$ = 0.17). In the five-leaf phase, there were significant differences in the variant of TBA/Na_2GA 0.1 (38.9 cm) with the control (37.9 cm), and also TBA/Na_2GA 0.1 and TBA/Na_2GA 0.3, kg/t (38.1 cm) with the standard (35.8 cm $LSD_{0.5}$ = 0.38). The height of control plants of barley in the three leaf phase (21.8 cm) and also treated with Raxil (21.2 cm), in relation to variants using the systems (22.5 and 23.5 cm, $LSD_{0.5}$ = 0.23) were significantly lower, which was also observed in the five-leaf phase of (in the control, 34.2 cm, in reference variant, 32.2 cm, and in variants with TBA/Na_2GA, 38.2 and 37.3 cm, $LSD_{0.5}$ = 0.35).

Seed material treatment had an effect on cereal planting density. By the phase of wax ripeness of wheat, there was a significantly higher (by 14.2 %) plant density in the variant with treatment by TBA/Na_2GA 0.1, but the productive plant stand increased by 30 % when using both consumption rates of this system. When sowing barley seeds treated with the systems, both indicators of seeding density significantly grew up: the number of plants/m^2 – by13.4–19.6 % (in the variant with Raxil – 10.6 %); productive stems – by 27.8–45.5 % (in the variant with Raxil – by 30 %).

By the milky stage, plants grown from seeds treated with TBA/Na_2GA turned out to be higher (by 10.3–11.0 % – wheat; by 13.0–15.6 % – barley), better formed a bush (by 1.1–1.3 times) than in the control (Table 4). In wheat and barley plants, the spike length significantly increased in all variants, where the seeds etched by the system were sown. The increased number of spikelets in the main spike for both cultures was acquired by during sowing the seeds treated with TBA/Na_2GA 0.3. Both consumption rates of TBA/Na_2GA facilitated a significant increase in the number of grains in the main spike of wheat and barley. The mass of grains in 1 plant of wheat was increased during seed treatment with both consumption rates, barley – in a variant of TBA/Na_2GA of 0.3. Both consumption rates of the system increased the mass of 1000 grains by 1.1 and 1.2 g (wheat) and 1.5 and 1.2 g (barley). Wheat and barley harvest in the experiment

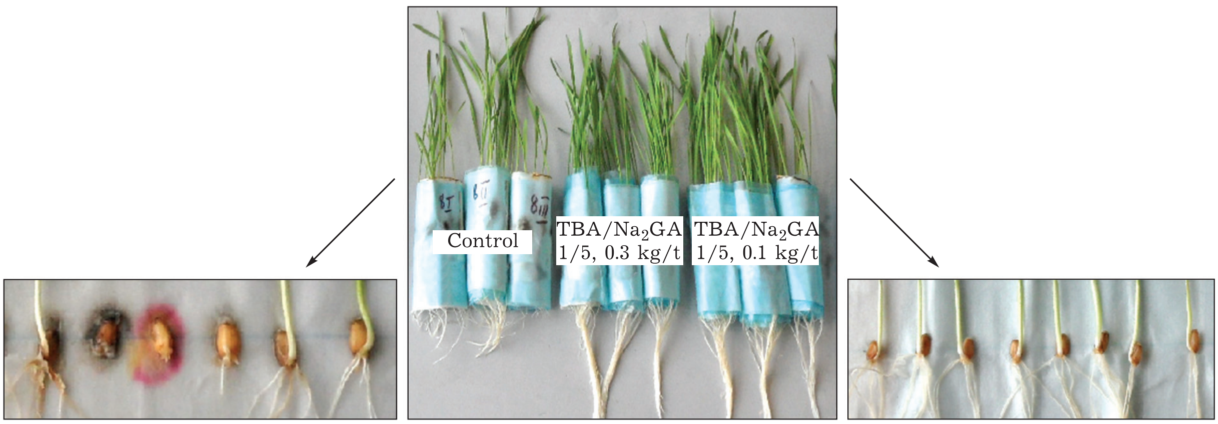

Fig. 7. Efficiency of seed treatment of spring wheat seeds with system TBA/Na_2GA 1/5 (laboratory experiment, method of rolls, 2016).

significantly exceeded the control by 0.2 and 0.14 t/ha (TBA/Na_2GA in a dose of 0.3 and 0.1 kg/t) and 0.19 and 0.14 t/ha; the use of Raxil increased the indicator by 0.16 and 0.07 t/ha.

Phytosanitary examination of the seed material treated with TBA/ES, 1/10 demonstrated the efficiency of the resulting complex to combat Helminthosporiose and Fusarium infection. Phytopathogenic fungi *B. sorokiniana* and *Fusarium* spp. (infection in control ~ 10 %) were suppressed by 100 %. It also inhibited the growth of mycelium and slowed down the conidiogenesis of fungi *Alternaria* spp. The energy of seed germination in the treatment with the developed system was 98 %, while in the control; it was 92 %, in the variant with Raxil – 88 %, and laboratory germination – 100, 97.5 and 87.5 %, respectively. Sprouts formed from seeds treated with TBA/ES had a more developed root system with an increased total root length of 43.7±0.65 cm (in the control – 37.9±0.81, in the variant with Raxil – 41.0±0.99 cm), the length of the main root – 12.5±0.19 (in the control – 10.9±0.21, in the variant with Raxil – 11.3±0.31 cm) and biomass – 7.75±0.48 mg (in the control – 6.67±0.24, in the variant with Raxil – 7.00±0.41 mg).

The high fungicidal effect under field conditions was also acquired in seed treatment with TBA/ES 1/10, where the index of root rot development in the primary root system decreased by 2.9 times compared to the control (the disease development index is 19.1 %) and 1.7 times compared to Raxil The damage of the secondary root system in using the developed system decreased by 73.3 % (in the control, the disease development index 11.6 %, in the variant with Raxil - 10.6 %). Seed treatment with TBA/ES 1/10 reliably protected the underground internode and the basal section of the stem; biological efficiency is 87.5 and 68.3 %.

In the 3–5 leaf phase, the height of plants grown from seeds treated with TBA/ES 1/10 was 10.9 and 3.6 % higher than that in the control (height – 22.1±0.27 and 22.1±0.27 cm). Herewith, in the 3-leaf phase, biomass accu-

TABLE 4

Effect of seed treatment with system TBA/Na_2GA (1/5) on productivity structure and yield of spring wheat and spring barley

Productivity indicator	Control	Raxil	TBA/Na_2GA 1/5		$HCP_{0.5}$
			0.3 kg/t	0.1 kg/t	
Omskaya 36 spring wheat					
Plant height, cm	86.7	94.8	96.3	95.7	1.71
Number of stems / plant, pcs	1.17	1.45	1.48	1.34	0.05
Spike length, cm	8.14	8.76	8.79	8.50	0.14
Number of spikelets in main spike, pcs	13.11	13.54	13.68	13.26	0.34
Number of grains therein, pcs	26.9	28.5	28.9	27.5	0.67
Mass of grain from main spike, g	0.99	1.06	1.06	0.98	0.03
Mass of grain from one plant, g	1.06	1.27	1.37	1.16	0.07
Mass of 1000 grains, g	33.7	34.4	34.8	34.9	0.04
Productivity, t/ha	2.45	2.61	2.65	2.59	0.53
Acha spring barley					
Plant height, cm	53.8	60.0	60.8	62.2	1.02
Number of stems / plant, pcs	1.34	1.58	1.72	1.43	0.08
Spike length, cm	7.7	8.0	8.5	8.3	0.46
Number of spikelets in main spike, pcs	10.6	11.1	11.5	10.6	0.57
Number of grains therein, pcs	18.8	20.3	21.2	19.9	0.98
Mass of grain from main spike, g	0.95	1.02	1.09	1.01	0.07
Mass of grain from one plant, g	1.21	1.47	1.61	1.29	0.10
Mass of 1000 grains, g	46.6	46.9	48.1	47.8	0.05
Productivity, t/ha	2.51	2.58	2.70	2.65	0.97

mulation by plants in the variant with TBA/ES 1/10 (83.7±4.06 mg) exceeded the control by 4.7 % and the standard by 15.9 %.

The resulting indicators of plant population density (603 pcs/m^2) it the beginning of vegetation indicate a slightly lower efficiency of the tebuconazole complex with ES than the fungicide Raxil (630 pcs/m^2, in the control, 540 pcs/m^2), but by the end of the growing season, the density of plants (499 pcs/m^2) in this option was higher than in the control (by 16.8 %) and in seed treatment with Raxil (by 6.2 %). In relation to the control variant (443 pcs/m^2), when using TBA/ES 1/10, the number of productive stems increased by 26.2 %, in relation to the standard – by 3.8 %.

Structure productivity analysis has proven the positive impact of seed treatment with TBA/ES 1/10 for almost all test characteristics (Table 5). The wheat grain harvest in the TBA/ES 1/10 variant was not inferior to the standard, and increased by 0.34 t/ha in relation to the control.

Thus, complexes of tebuconazole with arabinogalactan, glycyrrhizic acid sodium salt and liquorice root extract are efficient in improving corn seeds of spring soft wheat and spring barley from seed infection, reduce the development and prevalence of root rot in the first steps of organogenesis of cereal crops. They increase seed germination, biomass accumulation by plants, have a positive impact on grain productivity structure, and eventually guarantee grain yield increase.

CONCLUSION

Solid powder compositions of tebuconazole and a plant saponin (glycyrrhizin) and its derivatives have been obtained for the first time and characterized by various physicochemical methods.

Solubility parameters of tebuconazole from the resulting systems have been explored and the formation of supramolecular structures during their dissolving in water and the inclusion of tebuconazole molecules therein has been demonstrated.

Tebuconazole penetration from solutions of the resulting systems through artificial membranes and the seed coats has been assessed. According to the data set, the optimum composition and preparation conditions of systems have been substantiated.

Biological efficiency testing against root rot pathogens in treatment of spring wheat and spring barley seeds has been carried out. The high efficiency of the biological action of the developed systems has been demonstrated. This result correlates with the data of physical and chemical research of increasing the solubility and trans-membrane transfer of tebuconazole, which is facilitated by its inclusion into supramolecular delivery systems.

The previously non-observable healing effect of tebuconazole systems/complexes with glycyrrhizic acid and its sodium salt, *i.e.* increased seed germination, biomass accumulation by plants, and productive haulm stand, spike grain productivity, and eventually enhanced grain harvest from 1 ha of bally crops of soft spring wheat and spring barley, has been found.

The fungicidal activity of tebuconazole in its systems with liquorice root extract, their healing effect and growth properties have been demonstrated. It is critical to note the prospects of the practical use of liquorice roots ex-

TABLE 5

Treatment effect by systems TBA/ES 1/10 on productivity structure of Omskaya 36 spring wheat

Productivity indicators	Control	Raxil	TBA/ES 1/10	LSD_{05}
Plant height, cm	102.5	105.1	103.6	0.98
Number of stems / plant, pcs	1.35	1.35	1.68	0.08
Spike length, cm	8.48	8.81	8.79	0.13
Number of spikelets in main spike, pcs	15.08	14.45	14.6	0.36
Number of grains therein, pcs	28.58	29.30	30.8	1.05
Mass of grain from main spike, g	1.30	1.33	1.40	0.05
Mass of grain from one plant, g	1.65	1.69	2.08	0.04
Mass of 1000 grains, g	36.2	37.6	39.2	0.07
Productivity, t/ha	2.45	2.71	2.79	0.20

tract in agriculture due to low costs, existing manufacture in the Siberian region, and unlimited raw materials base.

Based on the acquired biological data set, the developed systems are superior to the widely used commercial preparation Raxil and make it possible to reduce the dose consumption rate according to TBA up to ~2 times.

Acknowledgements

The investigation was carried out with the financial support of RFBR and Governments of the Novosibirsk region in the framework of the scientific project No. 17-43-540175 and within the state assignment to ISSCM SB RAS [project No. 0301-2018-0005].

REFERENCES

1 Abelentsev V. I., Achievements Sci. Technol. AIC, 2006, No. 9, P. 44–48.

2 Petrov D. V., Valitov R. R., Sapozhnikov Yu. E., Semenova G. E., Golovina I. G., Smolyanets R. I., Valitov R. B., Bashkir Chem. J., 2012, Vol. 19, No. 2, P. 21–24.

3 Karakotov S., Int. Agricult. J., 2015, No. 1, P. 9–13.

4 Korsukova A. V., Borovik O. A., Grabelnych O. I., Voinikov V. K., J. Stress Physiol. & Biochem., 2015, Vol. 11, No. 4, P. 118–127.

5 Korsukova A. V., Gornostai T. G., Grabeinych O. I., Dorofeev N. V., Pobezhimova T. P., Sokolova N. A., Dudareva L. V., Voinikov V. K., J. Stress Physiol. & Biochem., 2016, Vol. 12, No. 2, P. 72–79.

6 Abelentsev V. I., Protection and Quarantine of Plants, 2007, No. 3, P. 28–29.

7 Khizhnyak S. V., Muchkina E. Ya., Kuchkin A. G., Shevelev D. I., Samoylova V. A., Bull. KrasGAU, 2012, No. 5, P. 422–423.

8 Khizhnyak S. V. Shevelyov D. I., Samoylova V. A., Bull. KrasGAU, 2015, No. 10, P. 179–182.

9 Lankina E. P., Shevelev D. I., Khizhnyak S. V., Gurevich Yu. L., Bull. KrasGAU, 2011, No. 11, P. 129–133.

10 Dushkin A. V., Chem. Sustain. Dev., 2004, No. 3, P. 251–254. http://www.sibran.ru/en/journals/KhUR

11 Dushkin A. V., Mechanochemical synthesis of organic compounds and rapidly soluble materials: in High-energy Ball Milling. Mechanochemical Processing of Nanopowders, Oxford: Woodhead Publishing Limited, 2010, P. 249-273.

12 Dushkin A. V., Tolstikova T. G., Khvostov M. V., Tolstikov G. A., in Complex World of Polysacchraids, (D.N.Karunaratne, Ed.), Publisher: InTech, 2012, P. 573–602.

13 Dushkin A. V., Meteleva E. S., Tolstikova T. G., Khvostov M. V., Tolstikov G. A., Chem. Sustain. Dev., 2010 No. 6, P. 719–728. URL: http://www.sibran.ru/en/journals/KhUR

14 Burova Yu. A., Ibragimova S. A., Revin V. V., J. Ural Med. Acad. Sci., 2011, No. 4/1, P. 94.

15 Dushkin A. V., Meteleva E. S., Tolstikova T. G., Khvostov M. V., Dolgikh M. P., Tolstikov G. A., Chem. Sustain. Dev., 2010, No 4, P. 517–525. URL: http://www.sibran.ru/en/journals/KhUR

16 Tolstikov G. A., Baltina L. A., Shultz E. E., Pokrovsky A. G., Bioorg. Chem., 1997, Vol. 23, No. 9, P. 691–709.

17 Abramova G. A., Palagin M. V., Bull. PSEU, 2005, No. 1, P. 77–87.

18 Qihong Zhang, Polyakov N. E., Chistyachenko Y. S., Khvostov M. V., Frolova T. S., Tolstikova T. G., Dushkin A. V., Su Weike, Drug Delivery, 2018, Vol. 25, No. 1, P. 198–209.

19 Kong R., Zhu X., Meteleva E. S., Chistyachenko Yu. S., Suntsova L. P., Polyakov N. E., Khovstov M. V., Baev D. S., Tolstikova T. G., Yu J., Dushkin A. V., Su W., Int. J. Pharm., 2017, Vol. 534, P. 108–118.

20 Deep P. Patel, Bharat G., J. Current Pharm. Res., 2012, Vol. 9, No. 1, P. 1–5.

21 Dushkin A. V., Meteleva E. S., Khomichenko N. N., Vlasenko N. G., Teplyakova O. I., Khalikov S., Khalikov M. S., Adv. Current Nat. Sci., 2016, Vol. 11, No. 2, P. 296–300.

22 Pat. RU 2545797, 2017.

23 Vostokova V. A., Sukhenko L. T., Egorov M. A., Znaniye, 2017, No. 5–1(45), P. 11–16.

24 Kansy M., Senner F., Gubernator K., J. Med. Chem., 1998, Vol. 41, P. 1007–1010.

25 Mccallum M. M., High-throughput approaches for the evaluation of factors of influencing bioavailability of small molecules in pre-clinical drug development, Dissertations & Theses, Gradworks, 2013.

26 Perelly A., Gruhlke M., Slusarenko A. J., J. Plant Protection Res., 2013, Vol. 53, No. 4, P. 317–323.

27 Bagi F. F., Bodroža-Solarov M. I., Balaž F. F., Mastilović J. S., Stojšin V. B., Budakov D. B., Lazić S. D., Proc. Nat. Sci., Matica Srpska Novi Sad, 2011, No. 120, P. 119–126.

28 Teplyakov B. I., Protection and Quarantine of Plants, 2004, No. 7, P. 32–33.

29 Sorokin O. D., Applied Statistics on the Computer, 2nd ed., Novosibirsk, 2012. 282 p.

30 Khalikov S. S., Teplyakova O. I., Vlasenko N. G., Khalikov M. S., Evseyenko V. I., A.V. Dushkin V. I., Chem. Sustain. Dev., 2015, Vol. 23, No. 5, P. 591–599. URL: http://www.sibran.ru/en/journals/KhUR

31 Emsley J. W., Freeney J., Sutcliffe L. H., High Resolution Nuclear Magnetic Resonance Spectroscopy, Oxford: Pergamon Press, 1965.

32 Dushkin A. V., Meteleva E. S., Tolstikova T. G., Tolstikov G. A., Polyakov N. E., Medvedeva E. N., Neverova N.A. Bablin V. A., Izv. AN, Ser. Khim., 2008, No. 6, P. 1274–1282.