ISSN 0147-6874, Moscow University Soil Science Bulletin, 2021, Vol. 76, No. 2, pp. 78–83. © Allerton Press, Inc., 2021.
Russian Text © The Author(s), 2021, published in Vestnik Moskovskogo Universiteta, Seriya 17: Pochvovedenie, 2021, No. 2, pp. 38–44.

ECOLOGY

Assessment of the Possibility of the Development of a Highly Effective Universal Stimulant for Presowing Treatment of Seeds of Grain Crops

I. V. Gorepekin[a],* and G. N. Fedotov[a],**

[a] *Department of Soil Science, Moscow State University, Moscow, Russia*

**e-mail: decembrist96@yandex.ru*

***e-mail: gennadiy.fedotov@gmail.com*

Received December 16, 2020; revised December 17, 2020; accepted January 20, 2021

Abstract—We have studied the possibility of the creation of a highly effective universal stimulating preparation for presowing treatment of seeds of various grain crops on the basis of a sorption-stimulating preparation (SSP), consisting of calcium bentonite (CB), humate (H), brewery yeast autolysate (BYA), and gibberellin (Gibb). The use of some nonionic surfactants as the preparation components results in the disintegration of bentonite aggregates into individual montmorillonite particles and in an increase in the active surface of the sorption complex. This enables a 1.5-time rise in the SSP efficiency by increasing the gibberellin concentration. As a result, we have obtained a five-component substance (SSP-5), including CB, H, BYA, Gibb, and PEG. Its efficiency for spring wheat of the cultivar Liza on soddy-podzolic soil is 56%. The introduction of other biologically active substances into the preparation in order to compensate their possible lack in seeds shows that they stimulate some seeds, but the general effect on other ones is reduced. It may be assumed that the creation of a universal preparation, which would be highly effective for seeds of all crops, is not possible. Gibberellin stimulates seed development much more often than other phytohormones, so we tested the SSP-5 effect on seeds of different crops and cultivars. It is revealed that this preparation is effective for seed stimulation in most cases, but its efficiency significantly varies.

Keywords: allelotoxicity, seed stimulation, presowing seed treatment, humates, bentonites, gibberellins, 6-benzylaminopurine, brassinolide, paraaminobenzoic acid, 3-indolylacetic acid, phytohormone balance in seeds

DOI: 10.3103/S0147687421020022

INTRODUCTION

Stimulating presowing treatment of seeds is well known, and various preparations are recommended for it [6, 12, 14, 15]. It is supposed that the small effect and nonreproducibility of seeds when using biologically active substances (BAS) are related to the depressing effect of soil allelotoxins [1, 3–5, 7–10, 13, 20–27]. The protection of seeds from the inhibitory effect of allelotoxins by sorption preparations based on calcium bentonite (CB) and humate (H) results in a significant increase in the use of some preparations containing stimulating substances: plant growth hormones, substrates of respiratory metabolism, and broad-spectrum stimulants [17, 20].

Calcium bentonite and humate in the preparation form a bentonite–humate complex, which fixes soil allelotoxins and reduces their input to seeds. Brewery yeast autolysate (BYA) prevents the fixation of stimulating BAS of soil on the sorption bentonite–humate complex. It has been shown that the presence of these substances and the individual application of various stimulating components (plant growth hormones, substrates of respiratory metabolism, and broad-spectrum stimulants) into the preparation significantly increase the stimulating effect of most BAS at the initial stage of plant development from seeds. A significant stimulating effect (from 20 to 36%) is seen, when such sorption-stimulating preparations (SSP) are applied [17, 20]. In particular, it has been found that the introduction of a nonionic surfactant—Polysorbate-20—into SSP accelerates the development of plants at early stages [20].

However, it should be pointed out that the preparations have been tested for small number of cultivars of spring wheat on soddy-podzolic soil. This does not enable us to conclude that they will stimulate the development of seeds of all cultivars of grain crops.

The aim of this work is to evaluate the possibilities of creating a highly effective universal stimulating preparation to accelerate the germination of seeds of various grain crops after their presowing treatment and the development of plants from them.

Table 1. The stimulation effect of the preparation composition used for presowing treatment of spring wheat seeds on the development of seedlings

Series	The composition of the stimulating preparation and the concentration of components	Effect, %
1	CB (40 g/L), H (10 g/L), BYA (12 g/L) + P-20 (120 mg/L)	+17 ± 2
	CB (40 g/L), H (10 g/L), BYA (12 g/L) + Gibb (100 mg/L)	+23 ± 3
	CB (40 g/L), Г (10 g/L), BYA (12 g/L) + Gibb (100 mg/L) + P-20 (120 mg/L)	+31 ± 4
	CB (40 g/L), H (10 g/L), BYA (12 g/L) + Gibb (100 mg/L) + P-60 (120 mg/L)	+11 ± 2
	CB (40 g/L), H (10 g/L), BYA (12 g/L) + Gibb (100 mg/L) + P-80 (120 mg/L)	+20 ± 3
2	CB (40 g/L), H (10 g/L), BYA (12 g/L) + Gibb (100 mg/L) + PEG-400 (120 mg/L)	+32 ± 4
	CB (40 g/L), H (10 g/L), BYA (12 g/L) + Gibb (100 mg/L) + PEG-1000 (120 mg/L)	+32 ± 4
	CB (40 g/L), H (10 g/L), BYA (12 g/L) + Gibb (100 mg/L) + PEG-4000 (120 mg/L)	+32 ± 4
	CB (40 g/L), H (10 g/L), BYA (12 g/L) + Gibb (100 mg/L) + PEG-20 000 (120 mg/L)	+32 ± 4
3	CB (40 g/L), H (10 g/L), BYA (12 g/L) + Gibb (100 mg/L) + PEG-400 (200 mg/L)	+34 ± 4
	CB (40 g/L), H (10 g/L), BYA (12 g/L) + Gibb (100 mg/L) + PEG-400 (300 mg/L)	+36 ± 4

MATERIALS AND METHODS

We studied seeds of grain crops recognized for the Nonchernozemic Zone: spring wheat (*Triticum*) of the varieties Liza, Zlata, Ester, Agata, and Lyubava; spring barley (*Hordeum*) of the varieties Zlatoyar, El'f, Yaromir, Moskovskii-86, and Vladimir; winter rye (*Secale*) of the varieties Tat'yana, Moskovskaya-12, and Moskovskaya-15; and winter wheat (*Triticum*) of the varieties Moskovskaya-56 and Nemchinovskaya-17.

The seeds were germinated on agro-soddy deep-podzolic light-loamy soil formed on fluvioglacial (ancient lacustrine) sediments underlain by carbonateless loess-like (mantle) loam (floodplain of the Yakhroma River, Moscow oblast) from a depth of 92 cm. After the sampling, soil was stored under conditions that provided the preservation of its moisture [16].

Potassium humate made of brown coal (Agrotekhnologii Scientific–Innovative Center, Russia) and calcium bentonite (OST 18-49-71, Russia) were used to protect seeds from soil allelotoxins. Five biologically active substances added to the sorption preparations included: 12 g/L of brewery yeast autolysate (Biotekh Plus, Russia); 100–400 mg/L of gibberellin (Gibb) (China), 6-benzylaminopurine (6-BAP); brassinolide (Brass), containing 0.1% of brassinosteroids; and paraaminobenzoic (PABA) and 3-indolylacetic (3-IAA) acids as broad-spectrum stimulants. Polysorbates (P-20, P-60, and P-80) of the concentration 120 mg/L and polyethylene glycol (PEG) (Merck) with the molecular weight of 400, 1000, 4000, and 20000 at the concentration of 120–300 mg/L were used as nonionic surfactants. The treatment was performed by a semi-dry method at the rate of 40 L of the solution per one ton of seeds. We also studied the change in the integral length of seedlings of 7.5 g of seeds (~200 seeds) to increase the reproducibility of the obtained data. This parameter was determined by the express method based on the linear correlation between the bulk volume of germinated seeds in water and the length of their seedlings [18].

The experiments were performed in six replications, and the results were then statistically processed. Since we used 1000–1200 seeds in one experiment, the error related to their different quality was minimized to 7%.

Electronic-microscopic studies were performed with the use of a JEOL-6060A six-raster electronic microscope (JEOL, Japan) to determine the distribution pattern of bentonite particles on the surface of seeds during their treatment by the preparation.

RESULTS AND DISCUSSION

Stimulating preparations are mainly used to accelerate seed germination and plant development at the initial stages. Therefore, the efficiency of this process is the key point. First, we have tried to enhance the effect of SSP. As it is pointed out above, the introduction of Polysorbate-20 into the sorption-stimulating preparation increases its efficiency. We have studied the effect of other nonionic surfactants (P-60, P-80, and PEG) introduced into SSP instead of Polysorbate-20.

Table 1 (series 1 and 2) clearly shows that not all nonionic surfactants increase the efficiency of SSP. Among the three studied substances, only the stimulating effect of polyethylene glycol is similar to that of Polysorbate-20. Its molecular weight does not affect the stimulation efficiency (series 2), while an increase in its concentration in the preparation solution to 300 mg/L (series 3) results in a greater effect, which is not the case for Polysorbate-20 [20].

The next stage is related to the study of the surface of seeds treated by SSP with and without Polysorbate-20. In the absence of Polysorbate-20, bentonite is located on the surface of grains in the form of aggregates of montmorillonite particles of about 10 μm microns in size (Fig. 1a). When Polysorbate-20 is applied, the

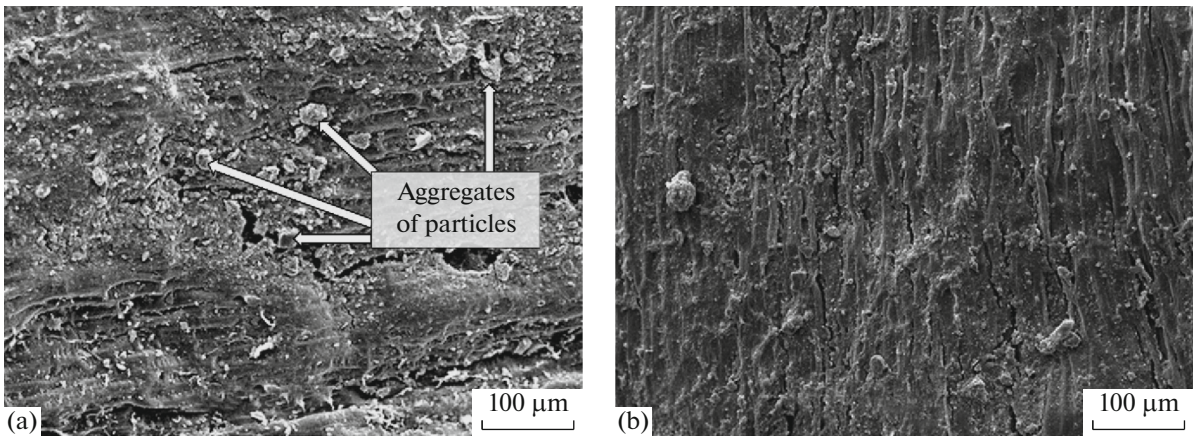

Fig. 1. The morphology of grain surface of the wheat variety Liza processed by sorption-stimulating preparation without (a) and with (b) Polysorbate-20.

aggregates practically disappear, because they are disintegrated into individual particles and evenly cover the grains (Fig. 1b). The decomposition of aggregates of montmorillonite particles results in the creation of a denser protective sorption layer, which more strongly prevents the penetration of allelotoxins into seeds. Thus, the introduction of nonionic surfactants into the preparation causes a decrease in the size of bentonite particles and improves the protection of the seed surface from penetration of allelotoxins from soil.

It should be taken into account that the decomposition of montmorillonite aggregates into individual particles may increase their active surface and hence the sorption capacity. As a result, a greater amount of gibberellin in SSP will be fixed on montmorillonite, and its concentration in the preparation suspension may become not optimal for seeds. We have studied the effect of increasing concentration of gibberellin in the preparation, containing bentonite (40 g/L), humate (10 g/L), BYA (12 g/L), and PEG (300 mg/L).

The data obtained (Fig. 2) confirm our assumption: the optimal concentration of gibberellin in the preparation increases from 100 to 300 mg/ L and causes a rise in the stimulation effect on seeds of spring wheat of the cultivar Liza from 36 to 56%. This highly effective preparation contains bentonite (40 g/L), humate (10 g/L), BYA (12 g/L), PEG (300 mg/L), and gibberellin (300 mg/L), and we term it SSP-5 (five-component sorption-stimulating preparation).

The possibility to use SSP for the treatment of different varieties and crops is also important. The prospects of creation of such preparation may be evaluated by a variety (crop), the germination of seeds and the initial development stage of which are not stimulated by SSP-5. It is very efficient for the processing seeds of spring wheat of the variety Liza. However the data obtained clearly show that SSP-5 more slightly stimulates seeds of the varieties Lyubava and Zlata and practically does not stimulate seeds of the variety Agata (Table 2).

According to the theory of the limiting factor, seeds may only be stimulated by a substance necessary for their development, the concentration of which is not optimal in them. This is the case, if such substance cannot enter seeds from the soil in which they are sown. It is reasonable to assume that the content of BAS, which are necessary for seed germination and seedling development, differs in seeds of various crops and varieties (and probably grown under different conditions). In this regard, seeds of the cultivar, which is not stimulated by the described preparation, should have other limiting substances. If they are revealed, their deficit may be eliminated by their addition to the preparation.

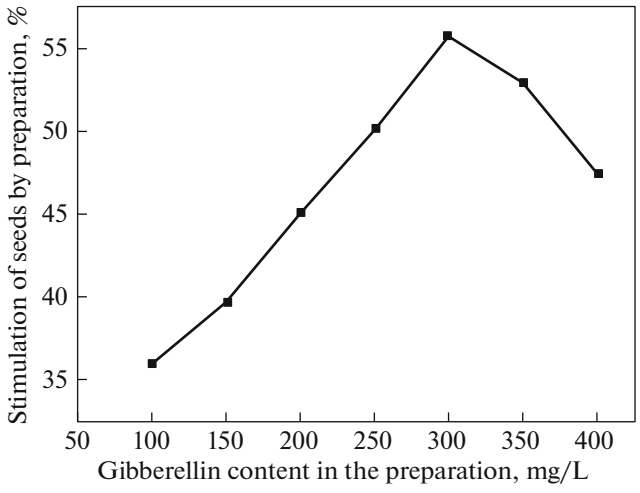

Fig. 2. The effect of increasing content of gibberellin in sorption-stimulating preparation, containing CB (40 g/L), H (10 g/L), BYA (12 g/L), and PEG-400 (300 mg/L), on the stimulation of spring wheat of the variety Liza at the early stage of development from seeds.

Table 2. Stimulation of the development of plants of spring wheat of different varieties from seeds at the early stages after their pre-sowing treatment by various SSP

Spring wheat variety	Increase in the total length of seedlings, %	
	CB (40 g/L), H (10 g/L), BYA (12 g/L), Gibb (0.3 g/L), PEG-400 (0.3 g/L) (SSP-5)	CB (40 g/L), H (10 g/L), BYA (12 g/L), Gibb (0.3 g/L), PEG-400 (0.3 g/L), 3-IAA (22 mg/L), 6-BAP (9.5 mg/L) (SSP-7)
Liza	56 ± 5	32 ± 3
Lyubava	32 ± 3	5 ± 2
Zlata	33 ± 4	19 ± 3
Agata	4 ± 2	34 ± 4

Brewery yeast autolysate, which is a component of the stimulating preparation, contains vitamins, amino acids, trace elements, etc. [2, 19]. Therefore the lack of these substances in seeds cannot limit the development of plants at the early stages. Hence, a special attention should be paid to plant growth hormones. It can be assumed that a preparation with balanced composition of plant hormones [11] will be effective for a larger number of crops and varieties.

Taking into account the above consideration, we have chosen seeds of the variety Agata of spring wheat for the development of an SSP with balanced plant hormones.

We have studied the effect of 6-benzylaminopurine, brassinolide, and paraaminobenzoic acid added to SSP-5, but all their tested concentrations do not exert a favorable impact on the variety Agata, though they are effective for the variety Liza. The application of 3-indolylacetic acid is also not very effective for the variety Liza, but its addition to SSP-5 applied on the variety Agata results in a significant increase in its stimulation (Fig. 3).

Concentrations of growth hormones in plants are balanced [11], and the disturbance of their ratio should result in slowing down plant development. Therefore, it is necessary to check the effect of brassinolide and 6-BAP added to the SSP with IAA on the stimulation of wheat seeds of the variety Agata. The addition of brassinolide does not cause an increase in the stimulation, and the introduction of 6-BAP into the SSP with IAA results in a slight increase in the effect (Fig. 4).

The data obtained show that the effect on seeds may not only be increased at the 6-BAP concentration of about 10 mg/L, but also become sharply slighter, when the IAA concentration is beyond the optimal range. The important role of a balance between hormones is confirmed once again. In this connection, the combined effect of 6-BAP and IAA added to SSP-5 has been studied more comprehensively (Table 3).

The stimulating effect (31–34%) on spring wheat of the variety Agata is maximal, when 9.0–9.5 mg/L of 6-BAP and 22 mg/L of 3-IAA are added to SSP-5. Therefore, it is necessary to analyze this particular

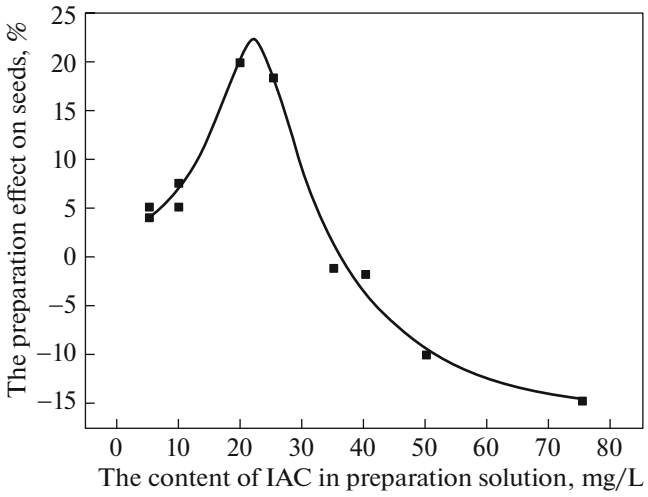

Fig. 3. The effect of IAA added to SSP-5 on the stimulation of seeds of spring wheat of the variety Agata at the early development stage.

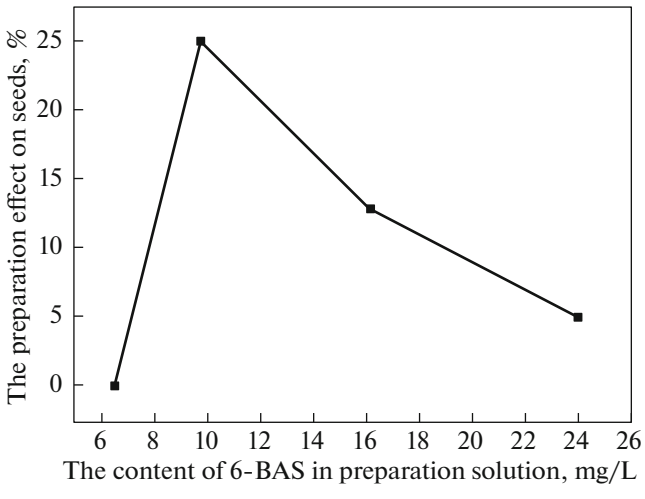

Fig. 4. The effect of the addition of 6-BAP to SSP-5 with IAA (22 mg/L) on the stimulation of spring wheat of the variety Agata at the early stage of development from seeds.

Table 3. The effect of 3-IAA and 6-BAP added to SSP-5 on the stimulation of spring wheat of the variety Agata at an early stage of the development from seeds

The content of 6-BAP in the preparation, mg/L	The content of 3-IAA in the preparation, mg/L	The stimulation value
11.0	25.0	0 ± 2
11.0	23.0	10 ± 2
9.0	23.0	14 ± 2
11.0	21.0	0 ± 2
9.0	21.0	14 ± 2
10.0	21.0	18 ± 3
9.0	22.0	31 ± 4
9.5	22.0	34 ± 4
10.0	22.0	18 ± 3
10.0	20.0	16 ± 3

composition of SSP as a stimulating preparation for the treatment of seeds of various grain crops. The first experiments on seeds of spring wheat (Table 2) have shown that the introduction of even small amounts of phytohormones (auxins and cytokinins) into the developed SSP results in a sharp decrease in the stimulating effect on seeds of spring wheat of the varieties Liza, Lyubava, and Zlata. It may be concluded that it is impossible to create a universal preparation to stimulate the development of seeds of any variety of grain crops. This is related to the fact that the content of phytohormones in seeds must be balanced, but may differ in various crops. The composition, which stimulates some varieties, does not exert a significant effect (at best) on others.

Gibberellin stimulates the development of seeds much more often than other phytohormones, so we have tested the composition of SSP, containing only gibberellin (SSP-5), which is the most effective for spring wheat of the variety Liza, on different cereal crops. The experiments have shown that the preparation of this composition remains effective in most cases, when used on soddy-podzolic soil, but the rate of the effect significantly varies (Table 4). For example, the stimulation is the highest for spring wheat of the varieties Liza (56%), Lyubava (32%), and Zlata (33%), as well as for spring barley of the varieties Moskovskii 86 (31%) and Vladimir (28%). At the same time, the stimulation of spring wheat of the varieties Agata and Ester is practically absent (4 and 3%, respectively), and spring barley of the variety El'f is slightly inhibited (–4%).

The data obtained testify to the need for laboratory experiments on seeds selected for sowing in order to test the efficiency of stimulants prior to their use in terrain conditions.

Table 4. The effect of presowing treatment of seeds of different crops and varieties by SSP-5 based on gibberellin on the germination stimulation and on the initial stage of plant development

Crop	Variety	Stimulation, %
Spring wheat	Liza	56 ± 5
	Lyubava	32 ± 3
	Zlata	33 ± 4
	Agata	4 ± 2
	Ester	3 ± 2
Spring barley	Zlatoyar	17 ± 2
	El'f	–4 ± 2
	Yaromir	25 ± 3
	Moskovskii-86	31 ± 4
	Vladimir	28 ± 3
Winter wheat	Moskovskaya-56	21 ± 2
	Nemchinovskaya-17	24 ± 3
Winter rye	Moskovskaya-15	11 ± 2
	Moskovskaya-12	15 ± 2
	Tat'yana	15 ± 2

CONCLUSIONS

Nonionic surfactants introduced into the sorption-stimulating preparation cause a rise in the active surface of montmorillonite particles and thus increase by 36–56% the preparation effect on seeds of spring wheat of the variety Liza processed and sown in soddy-podzolic soil.

Our experiments show that the need in a balance of the content of phytohormones in seeds is one of the main restrictions to create a universal stimulating preparation, which may be effectively used for various grain crops.

The elaborated sorption-stimulating preparation, including calcium bentonite, humate, BYA, gibberellins, and polyethylene glycol, preserves its efficiency when used for most grain crops.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests. The authors declare that they have no conflicts of interest.

Statement on the welfare of humans or animals. This article does not contain any studies involving animals performed by any of the authors.

REFERENCES

1. Berestetskii, O.A., Phytotoxines of soil microorganisms and their ecological role, in *Fitotoksicheskie svoistva pochvennykh mikroorganizmov* (Phytotoxic Properties of Soils Microorganisms), Leningrad, 1978.

2. Goryaev, M.I., Hydrolysis herbal raw materials and biosynthesis, in *Trudy In-ta khimicheskikh nauk AN KazSSR* (Scientific Works of Institute for Chemical Sciences of Kazakh Soviet Socialist Republic Academy of Sciences), Alma-Ata, 1970, vol. 27.
3. Grodzinskii, A.M., *Allelopatiya rastenii i pochvoutomlenie* (Plants Allelopathy and Soil Fatigue), Kiev, 1991.
4. Grodzinskii, A.M., Bogdan, G.P., Golovko, E.A., et al., *Allelopaticheskoe pochvoutomlenie* (Allelopathical Soil Fatigue), Kiev, 1979.
5. Konoshina, S.N., How different ways for soil utilization influence onto its allelopathical activity, *Cand. Sci. (Agric.) Dissertation,* Orel, 2000.
6. Kravets, A.V., Bobrovskaya, D.L., Kasimova, L.V., and Zotikova, A.P., Pre-seeding treatment of spring wheat seeds by humic product extracted from bog, *Vestn. Altaisk. Gos. Agrar. Univ.,* 2011, no. 4(78).
7. Krasil'nikov, N.A., *Mikroorganizmy pochvy i vysshie rasteniya* (Soil Microorganisms and Higher Plants), Moscow, 1958.
8. Lobkov, V.T., *Ispol'zovanie pochvenno-biologicheskogo faktora v zemledelii* (Soil-Biological Factor Application in Agriculture), Orel, 2017.
9. Lobkov, V.T., Ecological foundations of Soil Fatigue in field agrocenosises of Russian Central Black Earth Region, *Extended Abstract of Doctoral Sci. (Agric.) Dissertation,* Kursk, 1994.
10. Mirchink, T.G., *Pochvennaya mikologiya* (Soil Mycology), Moscow, 1988.
11. Muromtsev, G.S., Chkanikov, D.I., Kulaeva, O.N., and Gamburg, K.Z., *Osnovy khimicheskoi regulyatsii rosta i produktivnosti rastenii* (Foundations of Chemical Regulation of Plants Growth and Productivity), Moscow, 1987.
12. Nikolaeva, M.G., Razumova, M.V., and Gladkova, V.N., *Spravochnik po prorashchivaniyu pokoyashchikhsya semyan* (Dormant Seed Sprouting. Handbook), Leningrad, 1985.
13. Rudakov, V.O. and Rudakov, O.L., Nature of soil phytotoxicosises and plant protecting problem, *Agro XXI,* 2009, nos. 1–3.
14. Ryabchinskaya, T.A., Kharchenko, G.L., Sarantseva, N.A., et al., Poly-functional effect of Albit agent under presowing processing of spring wheat seeds, *Agrokhimiya,* 2009, no. 10.
15. Sechnyak, L.K., Kindruk, N.A., Slyusarenko, O.K., et al., *Ekologiya semyan pshenitsy* (Wheat Seeds Ecology), Moscow, 1983.
16. Fedotov, G.N., Gorepekin, I.V., Lysak, L.V., and Potapov, D.I., Soil allelotoxicity and creation of sorption-stimulating preparation to accelerate plant development from spring wheat seeds at early stages, *Eurasian Soil Sci.,* 2020, vol. 53, no. 9, pp. 1302–1311.
17. Fedotov, G.N., Shalaev, V.S., Batyrev, Yu.P., and Gorepekin, I.V., Soil allelotoxicity and the way to increase application efficiency of gibberellins application for presowing processing, *Lesn. Vestn.,* 2019, vol. 23, no. 6.
18. Fedotov, G.N., Shoba, S.A., Fedotova, M.F., and Gorepekin, I.V., The impact of soil allelotoxicity on germination of grain seeds, *Eurasian Soil Sci.,* 2019, vol. 52, no. 4, pp. 448–455.
19. Chichina, T.V., The way to develop protein ingredients technology on the base of residual brewery yeast by using cold treatment, *Cand. Sci. (Eng.) Dissertation,* St. Petersburg, 2014.
20. Shoba, S.A., Gorepekin, I.V., Fedotov, G.N., and Gracheva, T.A., Plant growth hormones increase the stimulation efficiency of seedlings development for spring wheat seeds upon pre-sowing treatment, *Dokl. Biol. Sci.,* 2020, vol. 493, no. 1, pp. 128–132.
21. Shoba, S.A., Salimgareeva, O.A., Gorepekin, I.V., et al., Stimulation of seed germination by humic substances: on the nature of the phenomenon, *Dokl. Biol. Sci.,* 2019, vol. 487, no. 1, pp. 105–108.
22. *Allelopathy. A Physiological Process with Ecological Implications,* Reigosa, M.J., Ed., New York: Springer-Verlag, 2006.
23. Cheng, F. and Cheng, Z., Research progress on the use of plant allelopathy in agriculture and the physiological and ecological mechanisms of allelopathy, *Front. Plant Sci.,* 2015, vol. 6, art. 1020.
24. Ghulam, J., Shaukat, M., Arshad, N.C., et al., Allelochemicals: sources, toxicity and microbial transformation in soil—a review, *Ann. Microbiol.,* 2008, vol. 58, no. 3, pp. 351–357.
25. McCalla, T.M. and Haskins, F.A., Phytotoxic substances from soil microorganisms and crop residues, *Bacteriol. Rev.,* 1964, vol. 28, no. 2, pp. 181–207.
26. Rice, E.L., *Allelopathy,* New York, 1984.
27. Weiner, J., Plant allelochemical interference or soil chemical ecology? *Perspect. Plant Ecol. Evol. Syst.,* 2001, vol. 4, no. 1.

Translated by I. Bel'chenko