

Ecologically Friendly Formulations Based on Tebuconazole for Plant Protection and Their Biological Efficacy

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ABSTRACT

Currently, nanotechnologies are being actively introduced into agriculture, in particular in the field of creating new effective plant protection products. This is achieved through the development of nanosized controlled release systems, such as polymer nanoparticles, micelles, and so on using a wide variety of materials. In the present study, we applied original approach based on “green” mechanochemical technology to prepare new nanocomposites of pesticide Tebuconazole (TBC) for treating wheat seeds against pathogenic microflora (*B. sorokiniana*, *Fusarium spp.*, *Alternaria spp.*, *Penicillium spp.*). The size distribution of nanoparticles for three TBC formulations (microcapsules, microemulsions and nanosuspensions) was measured using dynamic light scattering technique. All formulations contained nanoparticles (10-300 nm) and we aimed to find the most suitable size for effective penetration into cell membranes. The narrowest size distribution (225±40 nm) was observed for nanosuspension based on Licorice Extract (LE). The microcapsules based on Na-CMC also contained micro-sized particles (1,500 nm), which are apparently aggregates of nanoparticles. The laboratory and field biological tests revealed a high activity of the developed formulations against all pathogenic microflora under study, with a low retardant effect. Nanosuspension is considered as the most “environmentally friendly preparation”, since it contains only natural LE as an adjunct. This formulation with a consumption rate of 0.25 Lt⁻¹ suppressed 100% *B. sorokiniana*, *Fusarium spp.* and *Penicillium spp.* infections, possibly due to the presence of natural saponin glycyrrhizic acid, which interacts with plant membranes and promotes better penetration of TBC into the grain.

Keywords: Delivery systems, Mechanochemistry, Nanopesticides, Pathogenic microflora

INTRODUCTION

Technology of plant protection against diseases and pests is one of the main factors affecting the yield and quality of wheat grain (Vlasenko *et al.*, 2020). Seed treatment plays the main role in the protection of grain crops from early-season diseases and pests, since it protects the seedling at the first stages of its development from diseases most effectively, economically and environmentally safe, and therefore, allows to get a dense and healthy

stem - the main and decisive factor of the planned yield (Xiao *et al.*, 2020). The seed treatment is one of the targeted and cost-effective measures to protect plants from diseases and pests. The environmental friendliness of this method of using chemical preparations is explained by the fact that fungicides are applied only where they are really needed, with subsequent decomposition before the tillering period of plants, and their residues are not contained in the grain (Pereira *et al.*, 2021).

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Modern science and industry are developing and producing an increasing number of pesticides to meet the needs of agriculture, since the use of chemicals is one of the most common ways to increase yields. Seed dressing by nano-preparations is an efficient process that can change seed metabolism and signaling pathways, affecting not only germination and seedling establishment but also the entire plant lifecycle. A feature of the current level of scientific research is the development of new nanoscale forms with better penetration of the active substance into plant tissues, with reduced consumption rates, use of biofungicides and natural biopolymers, as well as complexes with biological and chemical inducers (Scharma *et al.*, 2015).

Among the wide variety of fungicides, triazole derivatives occupy a special place, since they have a wide spectrum of fungicidal activity and regulate plant growth (Paranjape *et al.*, 2014). These include fungicides containing Tebuconazole (TBC), which are widely used in agricultural practice to protect several crops (wheat, barley, rapeseed, corn, rice, vineyards, etc.) from powdery mildew, rust, rot, leaf spots and other spot diseases. TBC quickly penetrates into plants through vegetative organs and roots, suppresses the synthesis of ergosterol, preventing the formation of cell membranes of pathogens and disrupts metabolic processes, causing their death. On the other hand, triazole fungicides in the treatment of grain seeds affect the growth and development of seedlings, reducing the length of the coleoptile, the first leaf and internodes, and also affects development of the root system, reducing the number of primary roots, etc. (Korsukova *et al.*, 2016; Hameed and Farooq, 2021).

Preparations based on triazoles differ not only in the quantity and composition of active substances, but also in the dosage form. The most common commercial TBC preparations are suspensions or emulsions that are used for spraying the vegetative organs of plants (Paranjape *et al.*, 2014).

Pesticides with targeted and controlled release of active ingredients embedded in biodegradable matrices or coated with biodegradable coatings should be considered relevant in the light of the development of environmentally friendly formulations. The use of such formulations can reduce the amount of chemicals added to the soil and ensure their sustainable and controlled delivery during the growing season, preventing the immediate release of active ingredients (Volova *et al.*, 2017).

The microencapsulated form of TBC was obtained by the oil-in-water emulsion method and the kinetics of its release in water as well as the biological efficiency of these compounds in the fight against wheat rust in spring wheat has been studied (Asrar *et al.*, 2004). The release of tebuconazole from the matrix microparticles was found to be controlled by diffusion.

Preparations based on TBC is obtained by its mechanochemical modification with polysaccharides (Khalikov *et al.*, 2019) in the form of Solid Dispersions (SD). With higher water solubility, it showed high fungicidal activity against root rot pathogens (*Helminthosporium* spp., *Fusarium* spp.) and other fungal species (in particular, *Penizillium* spp.) at lower consumption rates of TBC (10-30 g t⁻¹) in comparison with the preparations used in practice. At the same time, SDs were not only more economically profitable, but also less toxic. Continuing these studies, SDs of TBC with arabinogalactan (from larch wood *Larix sibirica* and *Larix gmelinii*) and saponin (*Sapindus trifoliates*) were obtained, which allowed preparations with improved physicochemical, technological and biological parameters.

Suspension formulations of TBC (Vlasenko *et al.*, 2020) were obtained without using additional components, such as surfactants, emulsifiers, stabilizers, etc. These preparations showed a synergy of biological properties, manifested in accelerating the growth of plants and the length of their root system, reducing the development of seed diseases, as well as the

prospects for using such preparation forms of TBC.

In order to reduce the amount of active ingredient without reducing its bioavailability, TBC nanoemulsions were developed using a low-energy method (Díaz-Blancas *et al.*, 2016). The reduced surface tension of the systems under study may be useful for agricultural applications. An analysis showed that, over the past few years, triazoles have become promising candidates for use in functional nanomaterials.

Nano-materials functionalized with 1,2,4- and 1,2,3-triazole are promising for use not only in medicine (drug delivery), but also in agriculture (Hameed and Farooq, 2021). At the same time, research and development of new formulations of triazoles remains relevant in order to increase their effectiveness and safety.

The purpose of this research was to develop the technology of alternative preparation forms in the form of nanosuspensions, microcapsules and microemulsions based on TBC and to study the impact of these forms on the phytosanitary condition of seeds and the growth and development of spring wheat seedlings formed from the grains processed by them.

MATERIALS AND METHODS

Tebuconazole (TBC)-[(RS)-1p-chlorophenyl-4,4-dimethyl-3-(1H-1,2,4-triazol-1-yl-methyl) pentan-3-yl] is a systemic fungicide used for seed treatment of grain crops. It dissolves well in organic solvents, poorly soluble in water (Paranjape *et al.*, 2014). Production of Jiangsu Huifeng Bio Agriculture Co., Ltd. (China), main substance content $\geq 97.84\%$.

Sodium salt of Carboxymethyl Cellulose (Na-CMC) of the CEKOL 700 brand from CP Kelco, Finland.

Licorice Extract (LE) is a dry fine powder of light to dark brown color with a content of 25% Glycyrrhizic acid (GA) (Chauhan *et*

al., 2018). Production of "Visterra", Altai, Russia.

Sodium Dioctyl Sulfosuccinate (SDS) with a basic substance content of 96% (Acras Organics, New Jersey, USA) is a fine, hygroscopic powder for using in the food and chemical industry as a detergent (Vlasenko *et al.*, 2020).

Mechanochemical Preparation of TBC Formulations

The microencapsulated forms (further called microcapsules) of TBC were obtained by the following way: one g of TBC was dissolved in 10.5 g of dimethylformamide (DMF) and the resulting solution was dispersed into a flask from a suspension obtained from 3 g of Na-CMC, 20 g of ethyl acetate and 1 g of surfactant (Tween 80) while stirring on a rotary mixer at a speed of 300 rpm during 0.5 hour. Subsequently, 15.6 g of methanol and 10 g of distilled water were poured into the resulting system. Dispersion was continued at 350 rpm for 1 hour. The precipitated crystals were filtered off and dried in a desiccator over anhydrous calcium sulfate under reduced pressure. After drying for 24 hours, 3.1 g (yield 78%) of microcapsules were obtained in the form of a white powder of the composition TBC: Na-CMC= 1: 3.

The microemulsion form of TBC was carried out according to the following way: organic phase, prepared from 20 g of TBC and a mixture of 20 g of DMF, 20 g of cyclohexanone and 15 g of surfactant (Tween 80), was poured into a three-necked reaction flask (volume 1 L) and heated at $T=40^{\circ}\text{C}$ for 0.15 hour. Then, with stirring at a speed of 100 rpm, an aqueous phase of 12 g of propylene glycol and 22 g of distilled water was added to the organic phase, and the mixture was kept for 0.5 hour. The resulting preparation was a transparent slightly yellowish liquid weighing 96.6 g (yield 97.6%) with a TBC content of 18.3%.

The nanosuspension form of TBC was obtained according to method described by



Vlasenko *et al.* (2020). The mixture from 20 g of TBC, 76 g of LE was processed mechanically for 0.5 hour to obtain a homogeneous mixture. To this mixture, 4 g of the SDS was added and the machining was continued for 0.5 h. The drum was charged with 48 g of distilled water and a suspension was prepared with machining for 1 hour. The resulting suspension was separated from the metal balls through a sieve (cell size 0.5-1.0 mm) and received 95 g (95% yield) of a flowable TBC suspension (13.5% TBC).

Determination of Particle Sizes in Dispersions of Microcapsules, Microemulsions and Nanosuspensions

Dynamic Light Scattering (DLS) technology was used to estimate the average particle size and Polydispersity Index (PDI) of all compositions by Photocor Complex Instrument (Photocor, Moscow) at 25°C. The compositions were dispersed in distilled water before measurement. The results were obtained by measuring three times and taking the average value.

Laboratory Test of TBC Formulations

Three types of experiments were made with the use of seeds calibrated by size (weight of 1000 grains was 39.8 g) of spring soft wheat Novosibirskaya 31. All experiments included 9 options: 1- Control; without seed treatment by fungicides; 2 and 3- Seed treatment by commercial fungicide Raxil, consumption rate 0.5 and 0.25 L t⁻¹ (corresponding contents of TBC were 60 g and 30 g); 4 and 5- Seed treatment by tebuconazole microcapsules based on Na-CMC, 1: 3. 25%, consumption rate 0.5 and 0.25 kg t⁻¹ (corresponding contents of TBC were 125 g and 62.5 g); 6 and 7- Seed treatment with TBC nanosuspension based on LE and surfactants, 13.5%, consumption rate 0.5 and 0.25 L t⁻¹ (corresponding contents of TBC were 67.5 g and 33.8 g); 8 and 9- Seed treatment by microemulsion TBC, 18.3%, consumption rate 0.5 and 0.25 L t⁻¹ (corresponding contents of TBC were

91.5 g and 45.8 g). The seeds were treated with moisture (10.0 L t⁻¹). After treatment, the seeds were kept in plastic containers for 6 days, after which they were used in the planned experiments (Vlasenko *et al.*, 2020).

Experiment No. 1, studied the phytosanitary state of the seeds treated with TBC, the damage to the formed seedlings, their growth and development. Conditions: caryopses were placed in rolls of filter paper, germination was carried out for 10 days (5 days, T= 26°C, thermostat; 5 days-room conditions, natural light, T= 20°C, constant humidity). At the end of the experiment, the contamination of seeds, the number of normally formed seedlings, their number with affected roots and root zone were determined. The growth parameters taken into account included height of the sprout, number of roots, length of the main root, and the biomass of the seedlings.

In experiment No. 2, the presence and strength of the growth-regulating effects of TBC preparative forms used as a fungicide-dressing agent were determined. Conditions: wet chamber method. The caryopses were placed on a wet bed [filter paper, 4 Layers= bed for germination of caryopses - moistened Filter Paper (FP-substrate) or in plastic cups (n= 40)], T= 20 ° C, natural light. At night, the seed cups were covered with plastic caps. The dynamics of germination energy (after 1, 2, 3 days), germination (after 7 days) were determined. Growth indices (total length of roots of 1 seedling, height of a seedling) were recorded after 3 and 7 days (number of roots of 1 seedling; height of a sprout; total biomass of roots of 1 seedling, 1 root, and a sprout).

In experiment No. 3, the effect of TBC preparative forms on the growth and development of seedlings of spring soft wheat, formed from the caryopses treated by them on a soil substrate, was studied. Conditions: The caryopses were grown on a soil substrate (leached chernozem, 500 g/plastic cup/40 caryopses), covered with 1-layer gauze, T= +20°C natural light, controlled soil moisture. At night, the cups with seeds, as in experiment No. 2, were

covered with plastic caps. The growth and development of seedlings were monitored during the first 7 days. The germination energy, germination capacity, sprout height, and seedling biomass were determined and the presence of symptoms of damage to the root and root parts was noted.

Statistical data processing were performed using Statistica 7.0 and Excel 13 software.

RESULTS AND DISCUSSION

Determination of Particle Sizes in Dispersions of Microcapsules, Microemulsions and Nanosuspensions

Three formulations of TBC in the form of microcapsules: TBC:Na-CMC (1:3); microemulsions (18.3% of TBC) and nanosuspensions (13.5% of TBC) easily form working dispersions for seed dressing, had good adhesion to the seeds surface and penetrating ability into wheat grains.

To establish the particle size distribution in dilute aqueous dispersions of the preparative formulations forms obtained in this work, the Dynamic Light Scattering (DLS) method was used. The size

distributions of the particles in three formulations are shown in Figure 1.

As can be seen in Figure 1, particle size in aqueous dispersion of most of the compositions corresponds to the nanometer range (10-300 nm), which is most suitable for effective penetration into cell membranes. The narrowest monodisperse size distribution (225 ± 40 nm) was observed for nanosuspension particles based on LE, which were measured by DLS. It can be assumed that these are the micelles of Glycyrrhizic Acid (GA), the content of which in the extract is about 25% (Selyutina and Polyakov, 2019). Similar data on the sizes of GA micelles were obtained by other authors (Yang *et al.*, 2015; Cai *et al.*, 2019). The smallest particles were observed for microemulsions, which demonstrate bimodal size distribution (16 ± 8 and 120 ± 90 nm). Bimodal distribution was observed in three different experiments, the typical is shown in Figure 1. The possibility of the application of DLS in the study of bimodal size distributions is described by Kato *et al.* (2016). The dispersion of the inclusion complexes of TBC with Na-CMC

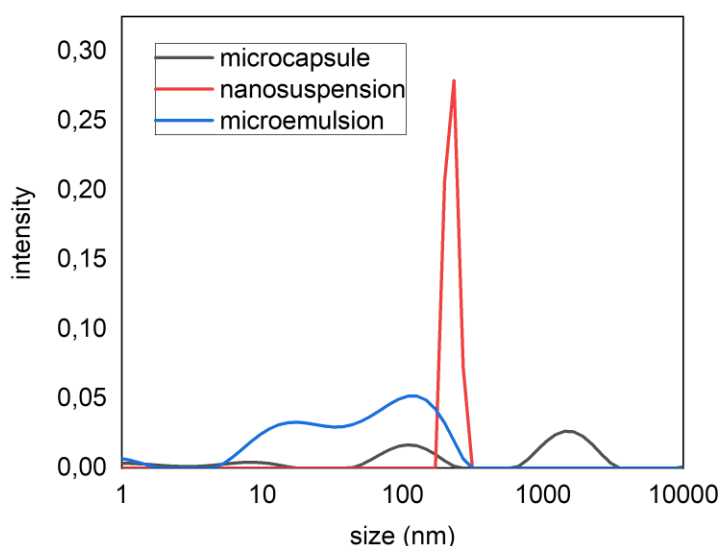


Figure 1. Dynamic light scattering measurement of particle size in water dispersions of microcapsules, microemulsions and nanosuspensions.



(microcapsules) also had bimodal size distribution (115 ± 56 and 1510 ± 590 nm). We assumed that large micro-sized particles around 1,500 nm were apparently aggregates of nanoparticles.

Laboratory Test of TBC Formulations

Study of the effect of seed treatment by three formulations of tebuconazole on the phytosanitary state of caryopses, growth and development of spring wheat seedlings.

The carried out phytoexamination showed a high fungicidal activity of the studied formulations of TBC (Table 1).

Use of these preparations as seed dressing agents led to a decrease in the complex contamination by 1.8-5.5 times (seed contamination in the control reached 84.9%). The maximum phytosanitary effect was obtained in the variant of using the nanosuspension of TBC with a consumption rate of 0.5 L t^{-1} . This preparation suppressed 100% helminthosporium-fusarium infection and 68.6-35.6% - alternaria, possibly due to the presence of natural saponin Glycyrrhizic Acid (GA) in the nanosuspensions, which

interact with plant membranes and promote better penetration of TBC into the plant cell (Selyutina et al., 2014, 2016, 2017, 2020). Placing seeds in rolls of filter paper showed a high biological efficiency of the tested forms of TBC against the main pathogen of common root rot *B. sorokiniana*, which in 4 out of 6 variants (nanosuspension TBC, 0.5 and 0.25; microcapsules TBC, 0.25, microemulsion TBC, 0.25) reached 100%, in 2 (microemulsion TBC, 0.5 and microcapsules TBC, 0.5) –80.5 and 64.8%. A commercial fungicide (Raxil, 0.5 and 0.25 L t^{-1}) inhibited *B. sorokiniana* by 100%. *Fusarium spp.* were not completely controlled by both Raxil, 0.5 and 0.25 L t^{-1} (Biological efficiency= 19.1 and 77.8%), and two studied TBC preparative forms - microcapsules based on Na-CMC with a flow rate of 0, 25 kg t^{-1} and microemulsion 0.5 L t^{-1} (81.9 and 62.7%).

All preparations (in both consumption rates) effectively protected the root system- biological efficacy of using microcapsules was 83.3 and 100%, nanosuspensions- 100 and 92.5%; microemulsion- 94.9 and 95.2% and this phytosanitary effect was not inferior to that obtained from seed treatment with

Table 1. Efficiency of seed treatment with tebuconazole formulations, germination in filter paper rolls.

Treatments	Seed infestation, %					In normally formed seedlings affected (%)		
	<i>B. sorokiniana</i>	<i>Fusarium</i> spp.	<i>Alternaria</i> spp.	<i>Penicillium</i> spp.	Bacteriosis	total	Root part of sprouts	Roots
Control	26.7	9.4	48.8	0	0	84.9	44.4	68.1
Raxil, 0.5	0	7.6	32.5	0	0	40.1	27.3	4.2
Raxil, 0.25	0	2.0	53.1	0	0	55.1	21.5	9.8
Microcapsules TBC, 0.5	9.4	0	28.7	0	0	38.1	27.1	11.4
Microcapsules TBC, 0.25	0	1.7	45.6	0	0	47.2	22.6	0
Nanosuspension TBC, 0.5	0	0	15.3	0	0	15.3	0	0
Nanosuspension TBC, 0.25	0	0	31.0	0	0	31.0	12.1	5.1
Microemulsion TBC, 0.5	5.2	3.5	15.5	0	0	24.2	5.2	3.5
Microemulsion TBC, 0.25	0	0	40.0	0	0	40.0	21.7	3.3

Raxil- 93.8 and 85.6%. The highest level of protection of the root part of seedlings was provided by seed treatment with nanosuspension and microemulsion with a consumption rate of 0.5 L t^{-1} . In the first case, the biological efficiency was 100%, in the second- 88.3%. For the presence of LE and SDS as surfactants that improve the penetration of TBC through plant membranes. For microemulsions, this can be explained by the presence of solvents (DMF, propylene glycol) in their composition, which increase the solubility of TBC and improve its passage through cell membranes (Zhang *et al.*, 2020). Reducing the rate of consumption of nanosuspensions reduced the protection effect to 72.7%, and microemulsions- to 51.1%. Treatment of seeds with a reduced consumption rate of a commercial fungicide did not increase the frequency of occurrence of seedlings with an affected root zone (Biological efficiency= 38.5 and 51.6%).

In the experimental variants, the number of normally formed seedlings increased up to 21-23% after treating the seeds with the microemulsion. In the variants of the microcapsules and the nanosuspensions, the development of seedlings determined the consumption rate. The decreasing of these rates increased the number of normally developed ones from 15.2 to 21.4% (microcapsules) and from 8 to 16.3% (nanosuspension). In addition to the manifestation of the fungicidal properties of TBC, it was found that all forms of TBC affected the growth and development of the plant, positive effect in the formation of roots and the accumulation of biomass, while a negative effect on the height of the sprout and the length of the roots. In wheat seedlings treated with microcapsules, the number of roots increased by 7.0 and 10.4% for two consumption rates; with nanosuspension - by 10.2 and 10.6%; and with microemulsion – by 10.4 and 10.3%. The best stimulating effect was provided by the reduced consumption rates of TBC because of its retardant effect. However, all protected seedlings accumulated more

biomass than in the control (by 17 and 69% - when using microcapsules; by 47 and 50% - nanosuspensions; by 72 and 75% - microemulsions. When using Raxil the indicator increased by 40 and 64%.

Retardant Effect in Different Formulations of Tebuconazole

All formulations of TBC had an ambiguous effect on the dynamics of germination of caryopses and, a day later, a decrease in the germination of grains was observed compared to the control in all experiments, namely: Raxil reduced the germination of grains by 12.5 and 37.5%, microcapsules by 5.8 and 8.5%, nanosuspensions by 25.0 and 12.5%, microemulsions at 35.0 and 47.4%. After 3 days, the number of normally formed seedlings exceeded the control in the variants of using microcapsules - by 7.5 and 10%, while the nanosuspension and microemulsion have not inhibited germination only at low consumption rate of 0.25 L t^{-1} with a decrease in the consumption rate. Over a 7-day period, the microemulsion and nanosuspension with a consumption rate of 0.5 L t^{-1} had the greatest inhibitory effect, which was previously explained by the influence of solvents and surfactants included in the microemulsion and nanosuspension of TBC (Zhang *et al.*, 2020).

The retardant effect revealed in the first experiment manifested itself even more strongly when studying the direction of the growth-regulating effect of TBC formulations on the development of the host plant at the earliest stage of organogenesis. The germination of treated caryopses in a humid chamber at $T= 20^{\circ}\text{C}$ was significantly inhibited. Both reduced root formation and growth of embryonic roots and sprout formation were noted.

According to the control, when using microcapsules, the number of roots decreased by 5.7% and 4.1%; nanosuspensions- by 19.4 and 17.6%. The weakening of the inhibitory effect (by 1.4 times) with a decrease in the rate of TBC was noticeably manifested when the seeds were treated with nanosuspension.



Microcapsules based on Na-CMC inhibited root growth of three-day-old seedlings weaker (by 10.2 and 9.2%, respectively, consumption rates), nanosuspension and microemulsion - stronger (by 44.7 and 35.6%; 48.6 and 31.6%). With a decrease in their consumption rates, the retardant effect weakened by 1.0, 1.25, and 1.53 times. A similar orientation was traced in the sprout height indicator. It differed slightly from the control when using microcapsules, and significantly - when treating seeds with nanosuspension in both consumption rates (less by 47.0 and 29.7%) and microemulsion at a consumption rate of 0.5 L t⁻¹ - for 50.3%.

Only when using microcapsules based on Na-CMC, the development of 7-day-old shoots was not inhibited. The rest of the studied formulations inhibited the development of sprouts not only in relatively pure control (by 31 and 21.4% - nanosuspension; by 24.8 and 10.0% - microemulsion), but also by the Raxil (by 16.8 and 9.3% - nanosuspension; at 19.3 and 7.6% - microemulsion). The accumulated biomass significantly exceeded the control only during germination of caryopses treated with microcapsules based on Na-CMC: when they were used at a consumption rate of 0.5 L t⁻¹, the mass of sprouts increased (by 10.7%), at 0.25 L t⁻¹ - by 18%. In other variants, with the exception of nanosuspension, which had a

negative effect on the accumulation of seedling mass, the aboveground mass was formed at the level of pure control, and the underground mass was lower than that.

Growth and Development of Seedlings of Spring Soft wheat

When the phosphate buffer-substrate was changed to soil, the intensity of seedling development varied according to the variants of the experiment, and was inferior to that in the control on both dates of counting (Figure 2).

The height of three-day-old sprouts decreased by 34.9 and 18.8% when using microcapsules, by 30.9 and 39.5% - nanosuspensions, by 51.4% and 30.0% - microemulsions, by 31.1 and 13.9% - Raxil; seven-day, respectively, by 24.5 and 13.7%; 34.4 and 34.8%; 44.0 and 15.3%; 23.9 and 18.5%. After 7 days, the biomass of seedlings in the control was 18.5±1.44 mg; when using Raxil- 18.08±0.86 and 20.5±0.5 mg; microcapsules- 18.18±1.99 and 24.45±1.66 mg; nanosuspension- 19.83±3.74 and 14.0±0.71 mg; microemulsion- 17.13±0.43 and 18.25±0.85 mg.

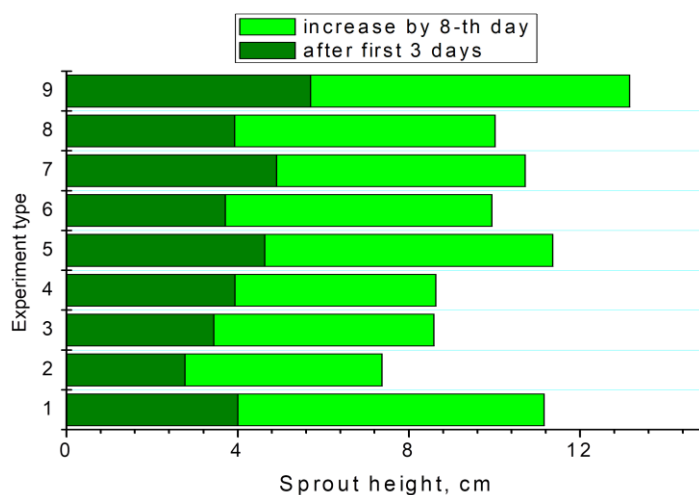


Figure 2. Influence of TBC formulations on the germination of spring wheat caryopses on a soil substrate: (1) Control; (2 and 3) Raxil (0.5 and 0.25 L t⁻¹); (4 and 5) Microcapsules (0.5 and 0.25 L t⁻¹); (6 and 7) Nanosuspension (0.5 and 0.25 L t⁻¹), (8 and 9) Microemulsion (0.5 and 0.25 L t⁻¹).

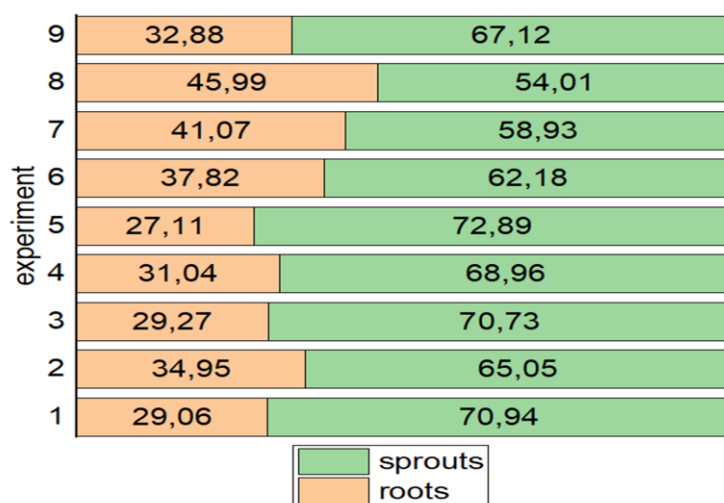


Figure 3. The ratio of biomass (air-dry,%) sprout: roots in wheat seedlings, grown from seeds treated by formulations of tebuconazole, the substrate is chernozem leached, after 7 days: (1) Control, without seed treatment with TBC; (2 and 3) Raxil, 0.5 and 0.25 L t⁻¹; (4 and 5) Microcapsules based on NaCMC, 0.5 and 0.25 kg t⁻¹; (6 and 7) Nanosuspension, 0.5 and 0.25 L t⁻¹; (8 and 9) Microemulsion, 0.5 and 0.25 L t⁻¹.

The aerial part developed more intensively during germination of seeds treated with TBC microcapsules based on Na-CMC, 0.25 kg t⁻¹, which is comparable to the growth rate observed in the variant with a reduced consumption rate of a commercial fungicide (Figure 3).

The biomass of sprouts protected by microcapsules at a reduced consumption rate reached the highest value in the experiment-17.81±1.52 mg, which is higher than the indicator in the control and in the variant with Raxil, 0.25 in 1.3 and 1.2 times. The root system was not inhibited during germination of caryopses treated with microemulsion with a consumption rate of 0.5 L t⁻¹, as indicated by a significantly higher value (1.5 times) than in the pure control root biomass of 1 seedling. On the soil substrate, all spring wheat seeds treated with TBC formulations had germination energy and germination capacity at the standart level. These indicators reached a maximum (100%) in the case of using both rates of consumption of microemulsion, microcapsules based on NaCMC and nanosuspension with a reduced rate of consumption. Symptoms of common root rot lesions were not found in all variants.

CONCLUSIONS

To prepare new formulations of tebuconazole for treating wheat seeds against pathogenic microflora (*B. sorokiniana*, *Fusarium spp.*, *Alternaria spp.*, *Penicillium spp.*), in the present study, we applied original approach based on “green” mechanochemical technology (Vlasenko *et al.*, 2020). The particles size distributions for three TBC formulations: Microcapsules (I), microemulsions (II) and nanosuspensions (III) were measured using dynamic light scattering technique. It was demonstrated that all formulations contained nanoparticles (10-300 nm), which is the most suitable size for effective penetration into cell membranes. The narrowest monodisperse size distribution (225±40 nm) was observed for nanosuspension III based on LE. We assume that these are the micelles of GA, the content of which in the LE is about 25% (Selyutina and Polyakov, 2019). Similar data on the sizes of GA micelles were obtained by other authors (Yang *et al.*, 2015; Cai *et al.*, 2019). The smallest particles were observed for microemulsions, which demonstrate bimodal size distribution (16±8 and 120±90 nm). The



dispersion of the inclusion complexes of TBC with Na-CMC (microcapsules) also has bimodal size distribution (115 ± 56 nm and $1,510\pm 590$). We can assume that large micro-sized particles around 1,500 nm are apparently aggregates of nanoparticles.

The results of laboratory test revealed a high biological activity of the developed formulations (I-III) against all pathogenic microflora under study, with a low retardant effect. In particular, composite III with a consumption rate of 0.25 L t^{-1} suppressed 100% *B. sorokiniana*, *Fusarium spp.* and *Penicillium spp.* infections, possibly due to the presence of GA in the nanosuspensions, which interact with plant membranes and promote better penetration of TBC into the grain. Also, it was demonstrated that new formulations of tebuconazole, in comparison with the previously obtained SD and commercially available Raxil, have a number of advantages both in the technology of preparation and dressing, as well as in biological effectiveness. All TBC formulations showed excellent activity against pathogenic microflora and low inhibitory effect.

Special attention should be paid to nanosuspension III, which is the most “ecologically friendly” green formulations based on natural LE. Its high activity and low retardant effect can be explained by the presence of the growth-stimulating effect of GA, as shown in our previous studies. These results open up prospects for the use of LE as components of various formulations of nanopesticides from the viewpoint of the design of environmentally friendly nano-preparations with a reduced consumption rate. We hope that our results may have implications for the use of nanopesticides in wheat processing, helping to improve their use while reducing harmful effects.

ACKNOWLEDGEMENTS

This work was supported by the Ministry of Science and Higher Education of the Russian Federation

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فرمولاسیونی سازگار با محیط زیست و مبتنی بر تبوکونازول برای حفاظت از گیاهان و اثر بخشی بیولوژیکی آنها

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چکیده

در حال حاضر، فناوری‌های نانو به گونه ای فعال به کشاورزی معرفی و وارد می شوند، به ویژه در زمینه تولید محصولات جدید موثر در حفاظت از گیاهان. این امر از طریق توسعه سامانه‌های رهاسازی و کنترل شده در



ابعاد نانو، مانند نانوذرات پلیمری، میسل‌ها و غیره، با استفاده از طیف گسترده‌ای از مواد به دست می‌آید. در این پژوهش، برای تهیه نانوکامپوزیت‌های جدید آفت‌کش تبوکونازول (TBC) برای تیمار بذر گندم در برابر میکرو فلور بیماری‌زا) شامل *B. sorokiniana*، *Fusarium spp.*، *Alternaria spp.*، *Penicillium spp.*) از رویکردی اصیل مبتنی بر فناوری "سبز" مکانیکی-شیمیایی (mechanochemical) استفاده شد. توزیع اندازه ذرات-نانو برای سه فرمولاسیون TBC (میکروکپسول‌ها، نانوسوسپانسیون‌ها، و میکروامولسیون) با استفاده از روش پراکندگی نور پویا (dynamic light scattering technique) اندازه‌گیری شد. همه فرمولاسیون‌ها حاوی نانوذرات (۱۰-۳۰۰ نانومتر) بودند و هدف ما یافتن مناسب‌ترین اندازه برای نفوذ مؤثر به غشای سلولی بود. باریکترین توزیع اندازه (۴۰ ± ۲۲۵ نانومتر) برای نانوسوسپانسیون بر اساس عصاره شیرین بیان (LE) مشاهده شد. میکروکپسول‌های مبتنی بر Na-CMC همچنین حاوی ذرات با اندازه میکرو (۱۵۰۰ نانومتر) بودند که ظاهراً دانه‌هایی بودند از نانوذرات (به-هم چسبیده). آزمون‌های بیولوژیکی آزمایشگاهی و صحرائی، فعالیت بالای فرمولاسیون‌های توسعه‌یافته را در برابر همه میکرو فلورهای بیماری‌زای مورد مطالعه نشان داد که بازدارندگی کمی داشتند. نانوسوسپانسیون به عنوان "سازگار ترین ماده با محیط زیست" در نظر گرفته می‌شود، زیرا فقط حاوی LE طبیعی به عنوان یک مکمل است. این فرمولاسیون با میزان مصرف ۰.۲۵ Lt توانست عفونت *B. sorokiniana*، *Fusarium spp.* و *Penicillium spp.* را ۱۰۰٪ کنترل کند. علت آن احتمالاً وجود ساپونین طبیعی اسید گلیسیریزیک (natural saponin glycyrrhizic acid) بود که با غشاهای گیاهی برهمکنش دارد و باعث نفوذ بهتر TBC به دانه می‌شود.