Journal of Agricultural Science and Technology (2025) Vol. 27 (1): 179-188

Factors Affecting the Processes of Penetration of Protectants into Tissue of Seeds and Increasing the Yield of Crops

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ABSTRACT

In our previous studies, we prepared by mechanical treatment and tested several formulations of plant protection products based on Tebuconazole (TBC) with different delivery systems. As a result of those studies, polysaccharides showed high efficiency in increasing the solubility and the effectiveness of products based on these polymers. An important task in developing an effective seed treatment is to increase the efficiency of adhesion and penetration. However, the question arises as to which factor is more important for plant protection or which factor plays the main role in the activity of the protectants: the amount of dressing agent on the surface of the seed or the amount penetrated into the grain? This question remained unanswered in previous experiments and the purpose of this study was to find an answer to this question. For this purpose, protectants of various compositions based on TBC and polysaccharides were prepared and spring wheat seeds were treated with them. At the same time, these seeds were divided into two variants: seeds treated with protectant (TBC on the surface that managed to penetrate inside the seeds) and seeds whose surface was washed by water and, so, seeds were without sorbed Tebuconazole (TBC only inside the seeds). Thus, seeds were prepared that contained only the Adsorbed tebuconazole (SPrA) and seeds containing the drug that Penetrated into the grain (SPrP). These two types of seeds were used in biological tests and the results obtained were compared. An analysis of seed germination and infection with the main pathogens showed that SPrA seeds had an advantage over SPrP seeds both in germination and in protection from diseases. Further research in this direction will help to understand the effect of the penetration of drugs into plants on the possibility of increasing their efficiency and yield of grain crops.

Keywords: Biological efficacy, Mechanical treatment, Polysaccharides, Seed dressing, Tebuconazole.

[DOI: 10.22034/JAST.27.1.179]

INTRODUCTION

Modern agriculture cannot be imagined without pesticides to protect plants from diseases and pests. At the same time, the chemical pesticides cause irreparable harm to the environment. One of the prospective approaches for their use is protectants for plant seed dressing. A protective coating could also contain essential nutrients for germinating plants. It allows crops to grow even in unproductive soils (Sharma *et al.,* 2015). Another approach, which could improve the effectiveness of protectants, is the use of nanotechnologies, in particular, nanocarriers based on different delivery systems (Pereira *et al.,* 2021). This approach is actively used in the medical chemistry, but the application of nanotechnology in agricultural sector is relatively new and in the early stages of development (Yadav and

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Yadav, 2016). Currently, scientists developed a new area of nanotechnology, which could revolutionize agriculture nano-pesticides: nanosized controlled release systems. According to the recent studies, nano-pesticides have a wide range of benefits, including increased stability and sustainability of yields of crops to pathogens, pests, and weeds (An *et al.,* 2022).

Seed dressing is one of the targeted and cost-effective measures to protect plants from diseases and pests. In the process of treatment, pesticides are applied to the seeds to destroy not only external, but also internal infections of plant origin, protect both seeds and seedlings in the field from soil-dwelling phytopathogens and various pests (Vlasenko *et al.,* 2020). 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. In all developed countries, dressing is considered as a legally mandatory phytosanitary measure (Afzal *et al.,* 2020).

In our opinion, two important factors will affect the effectiveness of the protectants for seeds dressing: adhesion of the drug to the surface of the seeds and the degree of its penetration into the seeds through plant membranes. Unfortunately, there is no data on the differentiation of these two factors on the biological activity of protectants. The important task in the development of effective product for seed dressing is the increase of the adhesion and penetration efficacy (Sharma *et al.,* 2015). In the present study, an attempt was made to determine the contribution of each of the factors: improved adhesion of the active substance to the surface, improved penetration of the active substance into the seed. Understanding the significance of each of these factors is a big step towards the rational development and use of plant protection products.

In the previous studies we tested several compositions of plant protection products with water soluble delivery systems, polysaccharide Arabinogalactan (AG) and saponin Glycyrrhizin (GA) prepared by innovative "green" mechanochemical technology. As a result of these studies, both AG and GA showed high efficiency in increasing solubility and permeability of some lipophilic fungicides (tebuconazole, imazalil, prochloraz) (Khalikov *et al.,* 2015; 2023). Previously, when studying the processes of fungicides penetration through cell membranes, which mainly consisted of phospholipids forming bilayer structure, it was noted that any changes in its structure and dynamics could significantly affect the properties and functions of the cell membrane and associated proteins (Matsuoka *et al.,* 2015).

The properties of delivery systems based on Glycyrrhizic Acid (GA) were already studied in the models significant to medicinal chemistry (Selyutina *et al.,* 2017-a). Due to its amphiphilic properties, GA is capable to form micelles in water solution and "host-guest" complexes with hydrophobic molecule of tebuconazole (Vlasenko *et al.,* 2019). Given the physicochemical characteristics of GA and its disodium salt (Na_2GA), which forms solutions with a lower viscosity in contrast to GA, a synergistic effect could be expected from the use of Na_2GA as a drug delivery system (Selyutina *et al.,* 2020).

It was found that GA was able to interact with the cell membrane, affecting its properties, such as elasticity and permeability. GA penetrates into phospholipid bilayer and forms self-associates inside it, which lead also to changes in lipids mobility (Selyutina *et al.,* 2016).

Arabinogalactan is a natural polysaccharide consisting of arabinose and galactose fragments (Chauhan *et al.,* 2018) and demonstrates strong binding with different lipophilic biologically active molecules that results in a significant increase in their solubility (Selyutina *et al.,* 2020). In contrast to GA, AG is located on the surface of the lipid bilayer and does not penetrate inside it. However, AG is also able to affect lipid mobility and enhance

[DOI: 10.22034/JAST.27.1.179]

membrane permeability (Selyutina *et al.,* 2017). Therefore, we tried to use these promising properties of GA and AG in the development of the nano-pesticides (Khalikov *et al.,* 2015; 2023). An increase in the penetration of pesticides into seeds in the presence of both delivery vehicles was detected, but the greatest effect was observed in the presence of AG. Obtained results show that the effect of polysaccharides and oligosaccharides on the penetration efficacy of nanopesticides under the presowing seed treatment might be associated with the solubility enhancement and affinity of the delivery systems to the surface of seeds, as well as due to modification of cell membranes by poly- and oligosaccharides (Selyutina *et al.,* 2017-b). However, it is still unclear which factor is determinant in the increase of nano-pesticide efficiency: solubility enhancement improved adhesion to the seed surface or a better penetration of the pesticide into the seed.

In order to provide physicochemical and biological studies, the authors (Meteleva *et al.,* 2018) obtained Solid Dispersions (SD) of TBC with AG, GA, Na_2GA and Licorice Extract (LE), as substances that form supramolecular systems when the corresponding SD is dissolved in water. According to the results of these studies, it was found that the obtained SDs form supramolecular systems, which allow the transport of TBC through artificial and plant membranes. An attempt was made to establish the correlation of "biological activity of the protectants and the degree of penetration of TBC". The expected result of biological activity was the sum of TBC on the surface of the seed and the drug that penetrated inside.

A continuation of these studies is the work of Vlasenko *et al.* (2019) on reducing the TBC norm by obtaining its SDs with GA, Na_2GA, and LE. In this study, the consumption rates of TBC were reduced by 1.7- 4.5 times, while maintaining biological efficiency. In field experiments, during pre-sowing seed treatment, wheat growth and development increased its standing density, productive bushiness, and yield by 0.52–0.69 t/ ha. In this work, the biological effect was also the sum of the action of TBC on the surface of the treated seeds and TBC that penetrated into the seed (Selyutina *et al.,* 2020).

In order to differentiate the biological activity caused by these two sources, i.e. on the seed and inside the seed, in the present work, we compared the biological effect of the so-called total amount of the protectants (on the surface of the seed plus inside the seed) and the activity of only the penetrated part into the seed of wheat. To perform this work, protectants based on TBC and plant metabolites (AG, GA, Na_2GA, and LE) were obtained by mechanochemistry at their mass ratio of 1:9.

MATERIALS AND METHODS

Materials

Tebuconazole (TBC)–(RS)-1p-chlorophenyl-4,4-dimethyl-3-(1H-1,2,4-triazol-1-yl-methyl) pentane-3-il- is a systemic fungicide for the treatment of grain seeds in the fight against phytopathogens transmitted with seeds, such as *Helminthosporium* spp., *Fusarium* spp., *Bipolaris sorokiniana, Penicillum* spp. It is also used for the treatment of vegetative plants of rapeseed and cereals as an integral component of the combined preparations. Solubility (25^{0}C) in water is 32.0 mg L^{-1} (Paranjape *et al.,* 2014). Produced by Shanshen Sunrising Industry Co., Ltd. (China), content of active ingredient ≥ 97.84%, powder.

Arabinogalactan (AG) is a polysaccharide consisting of arabinose and galactose units with molecular weight 15 kDa (97%, Ametis JSC, Russia). It is derived from Siberian larch *Larix sibirica* and differs from AG of other suppliers (Khalikov *et al.,* 2015).

Glycyrrhizic Acid (GA) – Triterpene glycoside from licorice root extract: Its chemical name is 20 β-Carboxy-11-oxo-30-

[DOI: 10.22034/JAST.27.1.179]

norolean-12-en-3β-yl-2-O-β-D-glucopyranuronosyl-alpha-D-glucopyranosiduronic acid (Selyutina *et al*., 2016). Production of Shaanxi Pioneer Biotech Co., Ltd, China; content of active ingredient ≥ 98.14%.

Disodium salt of Glycyrrhizic Acid (Na_2GA) (Selyutina *et al*., 2014): Production of Shaanxi Pioneer Biotech Co., Ltd, China- content of active ingredient ≥ 91.14%.

Licorice Extract (LE) is a dry fine powder of light to dark brown color with a content of 25% GA. Production of “Visterra", Altai Territory, Russia.

Methods

Mechanochemical Method for Obtaining Tebuconazole Composition

Dressing agents were obtained in the form of Solid Dispersions (SD) from TBC and polysaccharides (mass ratio of 1:9) by their joint mechanical processing in a roller mill LE-101. These SDs are finely dispersed powders that increase solubility in water and form supramolecular complexes in water with high biological efficiency (Khalikov*,* 2021).

Analysis of Solubility of Tebuconazole Composition

The solubility of TBC in various compositions was determined by HPLC on an Agilent 1100 liquid chromatograph (Germany) on a Hypersil HyPURITY Elite C18 analytical column (150×4.6 mm, 5 microns) in isocratic mode (Khalikov *et al.,* 2023). The temperature of the column thermostat was 30°C. Eluent-acetate buffer pH 3.4/acetonitrile in a ratio of 1:1. Eluent consumption – 1 mL min^{-1}. UV detection at a wavelength of 220 nm. Data on the measurement of the solubility of the studied solid dispersions are presented in Table 1.

Biological Tests of Tebuconazole Composition

Seed dressing of wheat seeds Novosibirsk 31 was carried out (Vlasenko *et al.,* 2020) as follows: wheat seeds (20.0 g) were placed in 150 mL round-bottomed glass flask. For seeds dressing, 5.0 g of 1% suspension of all compositions was used. The flask was rotated on the rotor (with speed of 100 rpm) for 15 minutes until the seeds completely poured out from the flask walls. After that, the seeds were placed in a Petri dish in the humid atmosphere of the desiccator for 3 days (with periodic spraying of the seeds with distilled water). Then, the seeds were dried in air at room temperature 24-25^{0}C for 3 days and divided into three parts:

A) 7.5 g of seeds for biological testing (to study the effect of TBC adsorbed on the surface of the seed and trapped inside the seed).

B) 7.5 g of seeds to find the amount of TBC adsorption on wheat seed.

C) 5.0 g of seeds to determine the degree of penetration of TBC into wheat seed.

Analysis of Penetration Process of Tebuconazole Composition

The amount of the TBC adsorption on the seed was determined as follows:

A sample of 7.5 g of treated seeds was immersed in a conical 50-mL flask and, after adding 10 mL of acetone, stirred on a magnetic stirrer for 10 minutes. Acetone filtrates were analyzed for the content of TBC, which is an indicator of the drug's adsorption on the seed (see Table 2). The remainder of wheat seeds were transferred to biological tests (to study the effect of TBC that penetrated into the seed).

The amount of TBC penetrated from various SDs was determined as follows:

A sample of 5.0 g of treated seeds was placed in a conical 25-mL flask and, after adding 10 mL of acetone, the system was stirred for 10 minutes. The seeds were separated by decanting the solvent and were

[DOI: 10.22034/JAST.27.1.179]

dried in air for 2 days at a temperature of 24-25°C. The dried seeds were crushed in a mortar and, after being loaded into a 50 mL conical flask, 10 mL of acetone was poured and the system was stirred on a magnetic stirrer for 1 hours. The acetone filtrate was analyzed for the content of TBC, which is an indicator of the penetration of TBC into the seed (see Table 2).

The biological effect was studied based on the development of spring wheat seedlings of Novosibirsk 31 variety. After treating their seeds with preparations in the form of SD based on TBC and polysaccharides (AG, GA, Na_2GA and LE), their infection with diseases was determined. In the laboratory experiment, the effect of drugs on the development of seed diseases (Vlasenko *et al.,* 2020) was evaluated on the length of the seedling and the length and number of roots using the "roll" method. As a control, untreated wheat seeds were used in comparison with treated seeds, and in the experiment with seeds washed from protectants, the control seed material was also washed with acetone. The results of biological investigations are presented in Tables 3 – 6.

Statistical Analysis

All experiments were repeated thrice and the resulted values were calculated as an arithmetic mean, and the error was calculated as mean value of least squares errors. The differences were considered statistically significant at $P< 0.01$ using a t-test (Dospekhov, 2012).

RESULTS AND DISCUSSION

Tebuconazole is the active substance of the most widely used fungicidal drugs for both seed and plant treatment during the growing season (Vlasenko *et al.,* 2020). Due to the poor solubility in water, various formulations of TBC have been developed, which, to some extent, improve its solubility and bioavailability. Despite this, the consumption rates of TBC in these preparations are quite high, for example, in the preparations of Raxil SC 60 or Raxil Ultra the TBC content is 60 and 120 g L^{-1}, respectively. Solid dispersions of TBC and polysaccharides obtained by machinery had 100% efficiency with a reduction in the flow rate of 10 – 15 g L^{-1}. These results were explained by a significant increase in the solubility of the obtained SDs (see Table 1).

To understand the difference in biological activity caused by the two sources (on the seed and inside the seed), the amount of TBC on the surface and inside the treated seeds were measured. The results are shown in Table 2.

Data analysis of Table 2 indicates that the sorption of TBC on the seed surface slightly depends on the adhesive properties of polysaccharides, and the amount of TBC on the surface is almost two times higher than inside the seed. Also, all compositions show effective penetration of TBC into the seed. This effect might be explained by the similar mechanism of influence of these carriers on the plant membranes and the earlier studied lipid membranes (Selyutina *et al.,* 2017a).

Table 1. Solubility[a] of tebuconazole compositions obtained after 3 hours mechanical treatment.

Preparation	Solubility	
	Absolute (mg L^{-1})	Increase (Times)
TBC, initial	32.0	-
SD of composition TBC: AG (1:9)	185.6	5.8
SD of composition TBC: GA (1:9)	601.6	18.8
SD of composition TBC: Na_2GA (1:9)	144.0	4.5
SD of composition TBC: EL (1:9)	403.2	12.6

[a] Value of standard error±3%.

[DOI: 10.22034/JAST.27.1.179]

Table 2. Adsorption of TBC-based compositions on the seed and its penetration into the seed. (All experiments were repeated thrice and the resulting values were calculated as an arithmetic mean).

N	Composition	TBC[a] content (%)	
		On the grain (Adsorption)	Inside the grain (Penetration)
1	TBC : AG	61	39
2	TBC : GA	57	43
3	TBC : Na_2GA	71	29
4	TBC : EL	68	32

[a] Value of standard error±3%.

In the next step, we compared the biological effectiveness of total TBC adsorbed on the seed surface and entered into the seed (Treated Seeds= TS) with the biological effectiveness of the part of TBC penetrated into the grain (Seeds Treated and Washed= STW).

It was found that TS increased germination by 4–17%, and STW by 13–21% relative to the control variants (62 and 55%) (Table 3). The maximum increase in the percentage of germination in both cases was observed from the treatment of seeds with TBC with GA and TBC with EL: 14 and 17% in the first case, and 19 and 21% in the second. It should be noted that the percentage of germination of seeds when germinating in rolls is low, due to high humidity conditions.

Phytoanalysis of seeds sprouted in rolls showed the best results in reducing the infection rate of their main root rot pathogens (*Fusarium spp* and *Bipolaris sorokiniana*) from the protectants TBC: AG, TBC: GA and TBC: Na_2GA in, respectively, 3.3, 2.5, and 10 times. When analyzing the washed seeds, the best results were shown by the compositions TBC: AG, TBC: Na_2GA and TBC: EL, where the percentage of infection of seeds with these pathogens in relation to the control variant decreased by 8, 4, and 8 times, respectively. It should be also noted that the germination of washed seeds significantly reduces the level of damage to seedlings of *Alternaria spp*.

All of the studied preparations showed their growth-stimulating abilities to a greater or lesser extent by the 7th day of plant growth, increasing the root length by 0.8–4.1 cm (8.8–45.3%) relative to the control (9.05 cm) when germinating seeds were treated with protectants, and by 0.25–3.4 cm (2.2–29.3%) when protectants were washed off from the seed surface (in the control – 11.6 cm). Due to the retarding effect of tebuconazole on plant height, the length of

Table 3. Effect of treatment of spring wheat seeds with compositions of TBC on the percentage of germination and infection with the main pathogens.[a]

Composition	Seed germination rate (%)	Infection with diseases (%)			
		Fusarium spp.	*Bipolaris sorokiniana*	*Penicil-lium* spp.	*Alternaria* spp
Treated Seeds (TS)					
Control (Non-treated)	62	6	4	1	78
TBC : AG	66	3	0	1	30
TBC : GA	76	2	2	0	32
TBC : Na_2GA	73	1	0	0	33
TBC : EL	79	3	3	0	40
Seeds Treated and Washed (STW)					
Control (Only washed)	55	2	6	1	56
TBC : AG	68	1	0	1	8
TBC : GA	74	3	2	0	16
TBC : Na_2GA	68	0	2	0	15
TBC : EL	76	0	1	0	18

[a] All experiments were repeated thrice and the resulting values were calculated as an arithmetic mean.

[DOI: 10.22034/JAST.27.1.179]

sprouts in most variants was lower than the control (Table 4). The number of roots in the variants increased relative to the control (the number of roots in the control was 2.85 pcs.) by 10.5–28.1% for seeds with preparations on the surface of the seeds and by 5.5–6.8% for seeds from the surface of which the preparation was washed off (the number of roots in the control was 3.65 pcs.).

On the 15th day of wheat plant growth in rolls, the indicators of biometrics of sprouts and roots and the number of roots retained the trends of the previous Measurement (Table 5): The main decrease in the length of sprouts compared to the control, an increase in the length of roots in the variants by 3.1–4.6 cm (41.4-60.5%), and an increase in the number of roots by 18.2–34.8% in the first method of using drugs, and an increase in their number by 8.2–14.2% in the second.

When studying the new protectants, a significant growth-stimulating effect was also observed on the air-dry biomass of sprouts and roots (Table 6). The level of growth of the aboveground biomass in the first case varied in the variants from 1.0 to 21.5% (the weight of sprouts in the control was 0.404 g) and in root – from 62.9% to 90.3% (the weight of roots: 0.197 g). When the preparations were washed off, the accumulation of biomass by sprouts was slightly more intense than the control

Table 4. Effect of treatment of spring wheat seeds with compositions of TBC on the biometrics of spring wheat seedlings, day 7.

Composition	**Biometrics**		
	Sprout length (cm)	Number of roots (pcs)	Root length (cm)
Treated Seeds (TS)			
Control	8.9±5.05	2.85±1.0	9.05±4.25
TBC : AG	7.2±2.4	3.65±1.0	11.35±2.3
TBC : GA	9.3±3.45	3.5±0.8	13.15±3.45
TBC : Na_2GA	8.15±3.45	3.55±1.0	10.85±4.0
TBC : EL	7.65±3.35	3.15±1.15	9.85±4.15
Seeds Treated and Washed (STW)			
Control	9.95±2.6	3.65±0.9	11.6±2.85
TBC : AG	10.2±2.0	3.9±0.85	15.0±2.0
TBC : GA	8.8±2.2	3.9±0.8	11.35±2.7
TBC : Na_2GA	9.3±2.25	3.85±0.85	11.85±2.85
TBC : EL	8.95±2.2	3.6±0.75	12.5±2.6

Table 5. Effect of TBC treatment of spring wheat seeds compositions on the biometrics of seedlings, after 15 days.

Composition	Biometrics		
	Sprout length, cm	Number of roots, pcs	Sprout length, cm
Treated Seeds (TS)			
Control	14.65±7.2	3.3±1.05	7.6±4.05
TBC : AG	11.15±3.5	4.4±0.9	11.4±2.3
TBC : GA	14.65±4.4	4.35±0.85	12.2±3.7
TBC : Na_2GA	11.75±5.0	4.45±1.0	11.0±3.6
TBC : EL	11.6±4.9	3.9±1.05	10.75±3.2
Seeds Treated and Washed (STW)			
Control	18.5±5.55	3.65±1.05	11.9±2.9
TBC : AG	15.4±3.75	4.05±0.8	13.9±2.35
TBC : GA	15.3±3.35	4.15±0.85	11.3±2.45
TBC : Na_2GA	15.9±3.9	4.17±0.8	11.9±2.6
TBC : EL	15.6±3.35	3.95±0.75	11.9±2.55

[DOI: 10.22034/JAST.27.1.179]

Table 6. Effect of treatment of spring wheat seeds with compositions of TBC on the air-dry biomass of sprouts and roots, g/50 plants.

Composition	Aboveground (sprouts)	Underground (roots)
Treated Seeds (TS)		
Control	0.404	0.197
TBC : AG	0.481	0.375
TBC : GA	0.491	0.361
TBC : Na_2GA	0.455	0.329
TBC : EL	0.408	0.321
Seeds Treated and Washed (STW)		
Control	0.459	0.384
TBC : AG	0.436	0.439
TBC : GA	0.470	0.370
TBC : Na_2GA	0.464	0.372
TBC : EL	0.459	0.368

indicator (0.459 g) only in the TBC: GA variants of compositions (by 2.4%) and TBC: Na_2GA (by 1.1%), and the root mass – when using TBC: AG, by 14.3% (in the control – 0.384 g).

CONCLUSIONS

For effective treatment of wheat seeds, solid dispersions of tebuconazole with plant metabolites were prepared, forming supramolecular complexes when dissolved in water. These compositions increased solubility and effectiveness while reducing the dose of TBC by 10 times or more. These preparations in water formed stable working solutions, convenient for the process of treating soft spring wheat seeds Novosibirsk 31. Studies conducted using spring wheat seeds treated with TBC compositions showed its high (from 29 to 43%) penetrating ability into the seeds. This value was maximum in the case of the composition with GA. Comparison of the biological effectiveness of the treated seeds with the treated-and-washed seeds did not reveal differences in their effect on pathogens of common root rot. Approximately the same results were observed when comparing the growth processes of spring wheat seedlings. Such results can be explained by the fact that, during the initial treatment of seeds, the foci of diseases on the seeds are already suppressed and further washing of the drug residues from surface of the seeds no longer affects the biological results.

ACKNOWLEDGEMENTS

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

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[DOI: 10.22034/JAST.27.1.179]

عوامل موثر بر فرآیندهای نفوذ مواد محافظ به درون دانه بذر و افزایش عملکرد محصول

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چکیده

در پژوهش‌های قبلی، ما تیمارهایی مکانیکی را آماده کردیم و چندین فرمول از محصولات حفاظتی گیاهی (plant protection products) مبتنی بر تبوکونازول (TBC) را با سامانه‌های تحویل (delivery systems) مختلف آزمایش کردیم. در آن بررسی‌ها، پلی ساکاریدها کارایی بالایی درافزایش حلالیت و اثربخشی محصولات مبتنی بر این پلیمرها نشان دادند. یک کار مهم در تهیه یک تیمار بذر موثر، افزایش کارایی درچسبندگی به سطح بذر و نفوذ آن به درون ماده است. با این حال، این پرسش مطرح می شود که کدام ماده عامل برای حفظ گیاه اهمیت بیشتری دارد یا کدام ماده در فعالیت محافظ ها نقش اصلی را ایفا می کند: مقدار ماده پاشیده شده در سطح دانه یا میزان نفوذ ماده به داخل دانه؟ این سوال در آزمایش های قبلی بی پاسخ مانده بود ولی هدف از این پژوهش یافتن پاسخی برای این پرسش بود. برای این منظور، محافظ‌هایی از ترکیبات مختلف بر پایه TBC و پلی‌ساکارید تهیه و دانه‌های گندم بهاره با آن‌ها تیمار شدند. همزمان، این دانه ها به دو نوع تقسیم شدند: دانه های تیمار شده با محافظ (TBC بر روی سطح بذرکه توانسته به داخل بذرها نفوذ کند) و بذرهایی که سطح آنها با آب شسته شده و بنابراین دانه ها بدون تبوکونازول جذب شده بودند (TBC فقط در داخل بذر). بنابراین، بذرهایی تهیه شدند که فقط حاوی تبوکونازول جذب سطحی شده (SPrA) و دانه های حاوی ماده نفوذ کرده به دانه (SPrP) بودند. این دو نوع بذر در آزمایش‌های بیولوژیکی مورد استفاده قرار گرفتند و نتایج به دست آمده با هم مقایسه شدند. تجزیه و تحلیل جوانه زنی بذر و آلودگی با پاتوژن های اصلی نشان داد که بذر SPrA نسبت به بذر SPrP هم در جوانه زنی و هم در محافظت در برابر بیماری ها برتری داشت. تحقیقات بیشتر در این راستا، به درک تأثیر نفوذ مواد (داروها) به درون گیاهان و امکان افزایش کارایی و عملکرد محصولات غلات کمک می کند.

[DOI: 10.22034/JAST.27.1.179]