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Influence of growth regulators on decrease of Fungicides Xenobiotic effect in winter wheat crops

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ABSTRACT

The conducted study has shown that growth regulators have a significant effect on the yield of winter wheat, both when used separately and in combination with fungicides. Processing crops with growth regulators increased the yield of winter wheat from 0.29 to 0.56 t/ha. The introduction of growth regulators in the tillering stage of winter wheat turned out to be more preferable. The yield increase from growth regulators in this phase compared with the phase of output into the tube was 5%. The combined use of growth regulators Silk and Immunocytophyte with the fungicide Colosal Pro significantly reduced the level of malondialdehyde (MDA) in comparison with the control variant. This indicates the anti-stress effect of the drugs, as well as the rapid transition from the beginning of the signal stress stage to the adaptation stage to the stress factor. The use of fungicides had a significant stressful effect on the growth and development of the root system of winter wheat. When using Immunocytophyte, the greatest decrease in the adverse effect of fungicides on winter wheat plants was observed. The combined use of Immunocytophyte with Kolosal Pro increased the daily growth of the roots of the culture by 40% compared with the option where only one fungicide was used.

Key words: *Winter wheat, Plant growth regulator, Fungicide, Lipid peroxidation, Root mass.*

Introduction

In world agriculture, the crop yield losses from diseases caused by phytopathogens of different groups start from 10%, and with the occurrence of epiphytotics this value reaches up to 80% and more, depending on the crop. The history has witnessed a fair amount of examples when the development of destructive diseases led to the total harvest failure, which provoked the greatest famine and people's death (Levkina, 1976) cites the classical example of rice helminthosporiosis development that provoked famine in Bengal (India) in 1924, which claimed two million people.

The strength and mortality of rice helminthosporiosis can be estimated as equal to phytophtora epiphytotics in Ireland in 1845 (Gorlenko, 1976). The issue of plants protection from diseases is becoming more acute due to the expansion of the pathogens spectrum, non-compliance with agrotechnical measures, and a high proportion of grain crops in the structure of the sown area. Application of fungicides for cropping capacity improvement is one of the main concepts in the modern plant protection system (Treikale, Afanaseva and Pugacheva 2011).

To date, the list of pesticides and agrochemicals includes more than 120 items, recommended for fighting destructive diseases. Unfortunately, fungicides, as well as other chemicals used in agricultural

industry, have not only a positive effect in reducing the development and spread of phytopathogens but also an adverse effect on the cultivated culture, which has been confirmed by a number of authors (Vikhreva, 2010). Negative effects may occur in the form of viability decrease, slow growth and development of the crop, weakening of metabolic processes, visible burns and stains (Tyukina *et al.,* 2013).

Materials and Methods

In order to study the effect of combined application of growth regulators and fungicides on the winter wheat yield in 2009-2011 the three-factor field experiment was conducted on the experimental field of Mordovskii agricultural research. The accounting area of three orders' plot equaled 30 m^2.

On the plots of the first order the periods of fungicides and growth regulators applications were studied in the following way: I – during the tillering stage (in spring, with the renewal of spring vegetation of plants) (Zadox scale from 21 to 29), II – during the shooting stage (Zadox scale from 30 to 39). On the plots of the second order the fungicides effect was estimated in the following way: 1 – without fungicides (control), 2 – Colosal Pro (rate of application – propiconazole + tebuconazole 300 + 200g/L) with consumption rate of 0.4 l/ha, 3 – Benorad (benomyl 500 g/kg) with consumption rate of 0.6 kg/ha. On the plots of the third order the growth regulators effects were studied in the following way: 1 – water (control), 2 – Albit (poly-beta-hydroxybutyric acid 6.2 g/kg + magnesium sulphate 29.8 + potassium phosphate 91.1 + potassium nitrate 91.2 + carbamide 181.5 g/kg) 30 g/ha; 3 – Silk (100 g/L triterpenic acid) 30 mL/ha; 4 – Immunocytophyte (20 g/kg arachidonic acid ethyl) 1 tablet/ha.

The plot soil is leached medium-humic medium-power black soil with heavy loams. The content of active forms of potassium and phosphor is average for grain crops. Wheat of Moskovskaya 39 variety was grown for the experiment. Working fluid solution was prepared in the amount of 300 l/ha.

The observation of the winter wheat root system development depending on the growth regulators and fungicides was conducted in laboratory conditions on a roll culture. The seeds of the studied culture were soaked for 6 hours in the tested concentrations of growth regulators and fungicides; control variant was placed in water. After soaking, the seeds were washed with distilled water and placed in a roll culture in the amount of 100 pcs. Per roll. The rolls were made of filter paper with polyethylene padding that lowered water evaporation, facilitated the measurement of the root system and plants care.

Rolls were submerged into the Knop culture solution (g/L: $Ca(NO_3)_2$ – 1; KH_2PO_4 – 0,25; $MgSO_4$ – 0,25; KCl – 0,125). As a source of Ferrum, 5 mL of $FeSO_4$ solution and citric acid were added to 1 liter of the finished salt solution. To prepare the solution 1.5 g of ferric sulfate was dissolved in 150-200 mL of water; 1.7 g of citric acid was dissolved in another container in the same amount of water. Then both solutions were mixed together and brought up to the volume of 500 mL. The culture solution was changed on the 3rd, 7th and 10th day from the establishing day.

Two weeks later, the winter wheat seedlings roots development was evaluated. The daily growth of the roots, the volume, the total and the working surface of the roots were determined by the method of (Tretiakov *et al.,* 2005).

Results and Discussion

Analyzing three-year research it can be noted the studied preparations both separately and combined significantly affected yield of winter wheat (Table 1).

The diversity of weather conditions made it possible to identify the level of stress effect of fungicides and the effect of growth regulators on its decrease. The results indicate that the use of growth regulators significantly increased the yield of winter wheat from 0.29 to 0.56 t/ha.

When comparing the periods of their application, the results showed that it is more preferable to use this group of preparations in the shooting stage, especially for Silk and Immunocytophyte, as the increase was about 5% if compared with application in the tillering stage.

The active material of Immunocytophyte preparation is arachidonic acid ethyl, which helps to mobilize plants immunity, in particular, splitting and transfer of reserve substances into plant cells in an easily accessible form occurs in plant cells since all immune responses are associated with active energy consumption. Presence of available energy sources enhances growth processes, thus Immunocytophyte acts as a stimulant. Terpene compounds in the composition of Silk preparation, in

addition to the fungistatic effect, are not pathogenic, help to activate the isoprenoid pathway of the metabolites synthesis, stabilizing the hormones balance.

Application of fungicides in average over three years increased the yield of the cultivated crop by 4 to 15%. It should be noted that these figures were significantly affected by results of 2010 when the application of fungicides without phytopathogene complex reduced the winter wheat yield. The most preferable period for their application turned to be shooting stage. Application of Benorad added 0.5 t/ha to the yield and Colosal Pro added 0.2 t/ha if compared with preparation treatment during the tillering stage.

Speaking about the effectiveness of the combined application of growth regulators and fungicides it should be noted that, on average over three years, application of Silk and Immunocytophyte with Colosal Pro during the shooting stage was the most effective. In these variants, the yield increase ranged from 10 to 12% if compared with the variants where only fungicide was applied. The application of Benorad in combination with Silk and Immunocytophyte in the shooting stage phase slightly underperformed in effectiveness to variants with the application of Colosal Pro.

Our studies have shown that the application of growth regulating preparations Silk and Immunocytophyte contributed to increasing the yield of winter wheat. In addition, the combined application of these preparations with fungicides Colosal Pro and Benorad leveled their stressful effect on winter wheat plants and contributed to increasing its yield.

One of the important stress response of the plant is the activation of lipid peroxidation (LPO). Reactive oxygen species (ROI) play a significant role in

Table 1. Effect of growth regulators and fungicides on the winter wheat yield in average for three years

Variant			Seed yield, t/ha	Seed yield change					
Treatment period	Fungicide	Growth regulator		In relation to absolute control		From fungicides		From regulators	
				t	%	t	%	t	%
Tillering	Control	Control	2,92	–	–	–	–	–	–
		Albit	3,21	0.29	9	–	–	0.29	9
		Silk	3,30	0.39	11	–	–	0.39	11
		Immunocytophyte	3,44	0.53	15	–	–	0.53	15
	Colosal Pro	Control	3,24	0.33	9	0.33	11	–	–
		Albit	3,53	0.62	18	0.32	10	0.29	9
		Silk	3.63	0.72	21	0.33	10	0.39	12
		Immunocytophyte	3.70	0.78	23	0.25	7	0.45	14
	Benorad	Control	3.05	0.14	4	0.14	5	–	–
		Albit	3.45	0.53	15	0.24	7	0.40	13
		Silk	3.57	0.65	19	0.27	8	0.52	17
		Immunocytophyte	3.66	0.75	22	0.22	6	0.61	20
Shooting	Control	Control	2.92	–	–	–	–	–	–
		Albit	3.19	0.27	8	–	–	0.27	9
		Silk	3.37	0.46	13	–	–	0.46	16
		Immunocytophyte	3.48	0.56	16	–	–	0.56	19
	Colosal Pro	Control	3.44	0.53	15	0.53	18	–	–
		Albit	3.67	0.75	22	0.48	15	0.22	6
		Silk	3.76	0.85	25	0.39	12	0.32	9
		Immunocytophyte	3.86	0.94	27	0.38	11	0.42	12
	Benorad	Control	3.35	0.43	13	0.43	15	–	–
		Albit	3.45	0.53	15	0.26	8	0.10	3
		Silk	3.54	0.63	18	0.17	5	0.19	6
		Immunocytophyte	3.67	0.75	22	0.19	5	0.32	10
SSD_{05} *	–	0.60	–	0.34	–	0.08	–		

Note: *SSD – Smallest significant difference.

the triggering of programmed cell death in response to various infections, abiotic effects, etc., which are accompanied by a sharp increase in the oxidative status of the plant with simultaneous induction of the pathogen dependent proteins synthesis. Thus, ROI appearing during the infection trigger defense mechanisms of the plant body and at the same time can cause programmed cell death (Vanyushin, 2001). Lipid peroxidation was defined by accumulation in the decomposition product tissues, i.e. malondialdehyde (MDA). Studied factors significantly affected MDA concentration.

Analysis of multi-year average data allowed establishing a number of patterns of preparations action on lipid peroxidation (Table 2).

Effect of preparation applied during the tillering stage by the defining moment significantly decreased. MDA level on plots with Colosal Pro applied during tillering stage exceeded control variant by 9%. On plots, with Immunocytophyte application, the figures were 9% lower than in control. Arachidonic acid ethyl acts as an active elicitor in triggering nonspecific immunity, one of the fast reactions to contamination is a strengthening of the oxidative process in the cell.

At the same time, according to Selye (1979) stress theory, active synthesis processes are happening in the body during adaptation, including the processes aimed at detoxifying active radicals, which are oxidation products. This can explain a significant decrease of MDA content after applying Immonocytophyte combined with Colosal Pro both in variants with treating during tillering and shooting.

The sharp increase of MDA content by 80% was noted in the variant with Colosal Pro and by 39% after Benorad application if compared with control on plots with fungicides application during shooting stage, which shows a significant physiological effect of fungicides on wheat plants.

Triazoles effect on pathogens is associated with styrols synthesis inhibition, in particular of ergosterol, but in wheat plants triazoles also have effect sites. According to Yurina and Ditchenko (2009) propiconazole affects the work of both passive and active ion transportation system of plant cell plasma membrane increasing permeability for Na+ ions. Affecting the biocatalysts of isoprenoid route, triazoles inhibit synthesis of terpene series, reduce the content of lipochromes and gibberellin and increase the content of abscisic acid (Rademacher *et al.*, 1987; Taton *et al.*, 1988; Sorokin *et al.*, 2009).

Thus, the physiological effect of triazoles on the plant is ambiguous. It also should be noted, that in field conditions the plants are not isolated from a wide range of stress factors, in this aspect relatively short-term inhibition effect of fungicides is compensated by the decrease of disease intensity and the increase of resistance.

It should be noted, that combined application of Colossal Pro with growth regulators Silk and Immunocytophyte contributed to the significant decrease of MDA content, lower than in control variants. These points out the significant anti-stress effect of preparations, a fast transition from anxiety phase, i.e. the primary stress stage to the adaptation stage. Shakirova (2001) states in her work, those weak stressors are able to induce restoring processes enhancing, which leads to forming of long nonhost resistance; physiological stress, inducing

Table 2. Effect of growth regulators and fungicides on MDA content, 10^{-2} μm/g of leaves wet mass in average over 3 years

Application period (factor A)	Growth regulator (factor C)	Fungicide (factor B)			Average on factor À	Average on factor Ñ
		Control	Colosal Pro	Benorad		
Tillering	Control	2.65	2.89	2.68	2.59	3.14
	Albit	2.60	2.60	2.63		2.64
	Silk	2.55	2.53	2.56		2.31
	Immunocytophyte	2.41	2.46	2.54		2.27
Shooting	Control	2.53	4.56	3.52	2.58	–
	Albit	2.30	3.24	2.47		
	Silk	2.00	1.99	2.21		
	Immunocytophyte	2.28	1.92	2.00		
	Average on factor Â	2.42	2.77	2.57	–	

Note: SSD_{05} of particular differences =0.18–0.34; SSD_{05} A=0.05–0.10 ; SSD_{05} B=0.06–0.12; SSD_{05} C=0.07–0.14

excessive metabolism activity, may enhance general adaptive mechanisms of plant body and contribute to its pre-adaptation to other possible stress factors.

In average for factor B the greatest level of MDA is 2.77*10^{-2} μm/g of leaves wet mass, was recorded on plots with Colosal Pro application, in control it was 2.42 × 10^{-2} μm/g. Among regulators Immunocytophyte shows the best effect, in average for factor C MDA level was 2.27×10^{-2} μm/g of leaves wet mass, in control is was – 3.14×10^{-2} μm/g.

The studies showed that fungicides stressfully effect development of wheat roots (Table 3). Application of Colosal Pro and Benorad reduces the daily root growth by 0.3 and 0.2 mm, reduces root system volume by 2.4 and 0.7 mm^3, total and working root surface by 75.47 and 37.15 mm^3, if compared with variants without fungicides treatment. The strongest decline in the studied parameters was recorded after applying Colosal Pro. A combined application of growth regulators with fungicides leveled the negative effect of the latter. The greatest decrease of negative effect of fungicides on winter wheat plants was recorded after application of Immunocytophyte. Application of this preparation with Colosal Pro increased daily growth of roots by 40%, root volume by 90%, total and working surface of roots by 41 and 40%, if compared with the variant where only fungicide was applied.

On the background of Benorad application, use of Immunocytophyte increased daily growth by 26%, root system volume by 16% and their total surface by 21% if compared with analogous variant without growth regulators.

Application of Silk for decreasing negative effect of fungicides was also highly effective. On the background of wheat grain treatment with Colosal Pro the daily growth of roots increased by 30%, volume by 71%, total and working surface by 32 and 91% if compared with the variant where only fungicide was used. On the variant with Benorad, application of Silk increased roots volume by 11%, total and working surface was increased by 13 and 39%.

Protective effect of Albit in decreasing negative effect of fungicides was somehow lower. Daily root growth at its use on the background of Colosal Pro application was higher by 17%, roots volume by 43%, total root surface increased by 14%, working surface increased by 6%. Use of Albit on the background of wheat grain treatment with Benorad significantly affected only root volume increase (11%).

Conclusion

Application of growth regulators promoted significant reduce of fungicides negative effect. Among the studied xenobiotics the most significant inhibitory action in winter wheat plants physiological processes was recorded in Colosal Pro, which belongs to triazoles class, with active material containing tebuconazole and propiconazole.

Table 3. Influence of growth regulators and fungicides on development of winter wheat root system

Variant		Daily root growth, mm/day	Root volume, ml^3/100 pcs.	Root surface, mm^2/100 pcs.	
Fungicide	Growth regulator			total	working
Control	Control	0.60	4.50	121.0	64.1
	Albit	0.59	4.46	119.3	65.4
	Silk	0.65	4.53	122.4	70.1
	Immunocytophyte	0.69	4.50	125.0	74.7
Colosal Pro	Control	0.30	2.10	45.7	26.9
	Albit	0.35	3.00	52.1	28.7
	Silk	0.39	3.60	60.4	51.5
	Immunocytophyte	0.42	4.00	64.4	37.6
Benorad	Control	0.39	3.80	74.4	49.0
	Albit	0.37	4.20	76.3	46.5
	Silk	0.39	4.20	84.3	30.0
	Immunocytophyte	0.49	4.40	90.0	50.0
SSD_{05} of particular differences		0.08	0.55		
	SSD_{05} À	0.04	0.28		
	SSD_{05} Â	Ff<Ft	0.32		
	SSD_{05} ÀÂ	0.08	0.56		

Growth regulators Immunocytophyte and Silk demonstrated most effective leveling of fungicides inhibitory action on growth and development of winter wheat.

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