EFFECTS OF MODERN SEED PROTECTANTS ON VIABILITY AND FUNCTIONAL ACTIVITY OF BIOLOGICAL AGENTS OF MICROBIAL PREPARATIONS

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The influence of seed protectants crop viability and functional activity of bacterial cells - biological agents microbial preparations. The possibility of the use of protectants compatible with biological products, bio-agents which exhibit resistance to the pesticide active ingredient.

Keywords: microbial drugs, viability of microorganisms, insecticides, fungicides.

It is known that the level of crop yields greatly depends on the phytosanitary status of crops. Thus, according to the FAO, the global agriculture from pests is lost at least a third of the crop, and during mass breeding crop dies almost completely. In Ukraine, the average loss of production of major crops from pests are 25 – 30%, including: wheat – 27%, corn – 29%, sugar beet – 27% [1]. Therefore, the use of plant protection products is an integral part of modern technologies of growing crops.

At the same time, the use of fungicides and insecticides for pre-treatment of seeds is not always reasonable due to their incompatibility with bacterial preparations of aimed on the improvement of soil fertility which use is becoming more common in crops growing practices [2]. At seed treatment its surface becomes an interaction area of bacteria with a chemical agent which toxicity can considerably reduce the effect of inoculation. Thus the current study was focused on the opportunities study of beneficial microorganisms introduction into the agrocenosis at use of modern pesticides for seeds treatment.

The purpose of given research was to determine the viability and functional activity of bacterial cells – biological agents of microbial preparations under the actions of protectants.

Materials and methods. The study was performed using the following bacteria: *Bradyrhizobium japonicum* M-8 (bio-agent of Rhizohumin); *Paenibacillus polymyxa* EF (bio-agent of Polimixobakteryn); *Achromobacter album* 1122 (bio-agent of Albobakteryn); *Azospirillum brasilense* 410 (bio-agent of Microhumin); fungicides: Alpha protectant, t.k.s. (*tebuconazole*, 60 g/l + *imazalil*, 100 g/l; UK), Alpha Epoxyl (*epoxiconazole*, 187 g/l + *thiophanate-methyl*, 310 g/l, UK), Absolute (*carbendazim*, 500 g/l, Ukraine), Alios (*triticonazole*, 300 g/l, Germany), Viale Trust (*tebuconazole*, 60 g/l + *thiabendazole*,

80 g/l, Russia), Viale TT (*tebuconazole* 60 g/l + *thiabendazole* 80 g/l, Russia), Vinets (*flutriafol*, 30 g/l + *thiabendazole*, 45 g/l, Ukraine), Gizmo 60 (*tebuconazole*, 60 g/l, Austria), Colfugo Super (*carbendazim*, 200 g/l, Ukraine), Corriolis (*triticonazole*, 200 g/l, Switzerland), Forsazh 500 Sc (*carbendazim*, 500 g/l, Ukraine), Funaben T 480 (*tiuram*, 332 g/l + *carbendazim*, 148 g/l, Poland); and insecticides: Celeste Top 312,5 Fs (*difenoconazole*, 25 g/l + *fludioxonil*, 25 g/l + *tiametoxam*, 262.5 g/l, 700 g/kg, Switzerland), Tabooo (*imidacloprid*, 500 g/l, Russia).

Protectants were sterilized with diethyl ether in 15 cm³ bottles with a cotton stopper. Sterility verification was performed by sowing aqueous suspension of protectants in culture medium. Qualitative responses of bacteria – bio-agents of microbial preparations on the fungicides action was determined using paper discs [3]. The resistance degree of bacterial strains to protectants was determined using Zibalskiy method [4]. The titer of bacterial cells in suspensions and liquid microbial preparations was determined by using Goryaeva camera [5]. Dissolving activity of phosphorus compounds by microorganisms was determined colorimetrically by the Erdei method [6].

Doses of microbial agents and protectants used for seeds treatment were selected in accordance to the label instructions.

Results and discussion. The effect of modern fungicides Gizmo 60 and Colfugo Super, Forsazh 500 Sc, used for treatment of barley seeds on the viability of *Azospirillum brasilense* 410 was studied using paper disks. Sterile fungicide suspensions with sterile water were used in the following ratio: gizmo 60 : water – 0.5:10; Super Colfugo : water – 3.0: 10; Forsazh 500 Sc : water – 1.2: 10. It was shown that neither of the studied fungicides had affected the growth of *Az. brasilense* 410.

Cell viability of *Az. brasilense* 410 under the influence of fungicides Gizmo 60, Colfugo Super, Forsazh 500 Sc was examined in the work solution consisted of Mikrohumин and protectants in the following ratio: Gizmo 60 : Mikrohumин – 0.5:10; Colfugo Super : Mikrohumин – 3.0:10, Forsazh 500 Sc : water – 1.2:10. On the next day the filter paper disk was moistened in the working mixture and placed on nutrient potato agar medium followed by the plates' examination on the presence or absence of bacteria biomass growth around the disc. It was established that examined protectants had no effect on the cell viability of *Az. brasilense* 410 grown in the in the working mixture used for seeds treatment.

The data obtained indicates the possibility of the simultaneous use of Mikrohumин and protectants Gizmo 60, Colfugo Super and Forsazh 500 Sc in spring barley growing technologies.

No adverse effects on cell growth of *Br. japonicum* M-8 were observed under the influence of Alpha protectant (no growth retardation zone was observed around the paper disk moistened with protectant) (Figure 1). Alpha Epoxyl on the other hand had shown a negative effect on cell growth of *Br. japonicum* M-8 – the 11 mm zone of growth retardation was formed around the paper disk impregnated with protectant (Fig. 2).

Figure 1. Effect of Alpha Protectant on the cell growth of *Br. japonicum* M-8 using paper disks (*paper disk is moistened in water suspension of water : Alpha Protectant – 10:0.5*)

Figure 2. Effect of Alpha Epoxyl on the cell growth of *Br. japonicum* M-8 using paper disks (*paper disk is moistened in water suspension of water : Alpha Epoxyl – 10:0.6*)

Cell viability of *Br. japonicum* M-8 in the working mixture under the influence of fungicide Alpha Epoxyl was not determined due to the negative effect it has on the growth of bacteria.

Effect of Alpha Protectant on cell viability of *Br. japonicum* M-8 was investigated in the working mixture of bacterial suspension and protectant in the ratio of 0.5:10. In 24 hours the filter paper disks moistened in working mixture were placed on a nutrient agar medium with pea broth. The presence or absence of the bacterial growth was used to determine the level of influence. It was revealed that Alpha Protectant does not adversely affect the cell viability of *Br. japonicum* M-8 in the working mixture used for treatment of soybean seeds (the growth of bacterial biomass was observed around the disc moistened in the working solution) (Fig. 3).

Figure 3. Effect of fungicide Alpha Protectant on the cell growth of *Br. japonicum* M-8 using paper disks (paper disk is moistened in water suspension of water : Alpha Protectant – 10:0.5)

The results obtained have showed that fungicide Alpha Protectant had no toxic effect on the growth and development of cells *Br. japonicum* M-8 – bio-agents of microbial preparation Rhizohumin, indicating the possibility of their use in soybean growing technology.

Bacteria *Br. japonicum* M-8 does not remain viable in the working mixture of fungicide Alpha Epoxyl used for treatment of soybean seeds, thus excluding their combined use.

The action of modern fungicide Funaben T 480 on viability of *Achr. album* 1122 cells was studied using the product suspension prepared in accordance to the recommendations of rapeseeds treatment. For this the sterilized fungicide and sterile water were used in the ratio of 2.5:10. It was established that fungicide Funaben T 480 had no toxic effect on cell growth of bacteria *Achr. album* 1122.

Cell viability of *Achr. album* 1122 was investigated using the working mixture consisted of bacterial suspension and protectant in the ratio of 2.5:10. In 24 hours the filter paper disk moistened in working mixture was placed on nutrient agar medium MPA and in a few days the presence or absence of bacterial growth around the disc was determined. The studies have shown that fungicide Funaben T 480 had no effect on the viability of *Achr. album* 1122 used for treatment of rapeseed (the growth of bacterial biomass was observed around the disc) thus indicating the possibility of simultaneous use of *Achr. album* 1122 and Funaben T 480 in rape growing technologies.

The same methods of paper disks was used for investigation of the effect of modern fungicides: Absolute, Alios, Gizmo 60, Colfugo Super, Corriolis, Vial Trust, Vial TT, Vinets, Forsazh 500 Sc, Funaben T 480 and insecticides: Celeste Top 312,5 Fs, Taboo on the viability of *P. polymyxa* KB. The working suspensions were made of sterilized fungicide (used at label recommendations) and sterile water in the following ratio: Absolut:water – 2.0:10; Alios:water – 2.0: 10; Vinets:water – 1.8:10; Gizmo 60:water – 0.5:10; Colfugo Super:water – 3.0:10; Corriolis:water – 0.2:10; Vial Trust:water – 3.0:10; Vial TT:water – 0.15:10; Celeste Top 312,5 Fs:water – 2,0:10 Tabooo:water – 6,0:10; Forsazh 500 Sc:water –

1.2:10; Funaben T 480:water – 2.5:10. The experiments performed have showed that studied fungicides had no effect on growth of *P. polymyxa* KB.

Viability of *P. polymyxa* KB cells was studied using the working mixtures consisted of bacterial suspension and protectants in the following ratio: Absolut:Polymyxobacteryn – 2.0:10; Alios:Polymyxobacteryn – 2.0:10; Vinets:Polymyxobacteryn – 1.8: 10; Gizmo 60:Polymyxobacteryn – 0.5:10; Colfugo Super:Polymyxobacteryn – 3.0:10; Corriolis:Polymyxobacteryn – 0.2:10; Vial Trust:Polymyxobacteryn – 3.0:10; Vial TT:Polymyxobacteryn – 0.15:10; Celeste Top 312,5 Fs:Polymyxobacteryn – 2.0:10 Taboo:Polymyxobacteryn – 6.0: 10; Forsazh 500 Sc:Polymyxobacteryn – 1.2:10; Funaben T 480:Polymyxobacteryn – 2.5:10. After day exposure in the working mixture filter paper discs were placed on a potatoes nutrient agar medium. Plates were then checked for the presence or absence of bacterial growth around the disc. It was established that fungicides Absolute, Alios, Vinets, Gizmo 60, Colfugo Super, Corriolis, Vial Trust, Vial TT, Forsazh 500 Sc, Funaben T 480 and insecticides: Celeste Top 312,5 Fs, Taboo had no effect on the viability of *P. polymyxa* KB in the working mixture used for seeds treatment (bacterial growth around the discs moistened in the working mixture was observed).

Bacteria *P. polymyxa* KB remain their viability and functional activity in the working mixture of fungicides Absolute, Alios, Vial Trust, Vial TT, Vinets, Gizmo 60, Colfugo Super, Corriolis, Forsazh 500 Sc, Funaben T 480 and insecticide Celeste Top Fs 312.5, Taboo which indicates the possibility of their joint use and Polymyxobacteryn for seed treatment of sugar beet seeds, spring and winter wheat, barley, sunflower, corn, canola (Table. 1).

The results obtained may be projected on the other pesticides with the same active substances and their use in the crops growing technologies in combination with bacterial preparations.

Table 1. Effect of seed protectants on growth and viability of Paenibacillus polymyxa KB

Protectants [active ingredients, concentration]	Crop	Bacteria viability	
		On paper disks	In working suspension
Fungicides			
Absolute [carbendazim, 500 g/l]	Spring wheat	+	+
Alios [triticonazole, 300 g/l]	Corn	+	+
Vial Trust [tebuconazole, 60 g/l + thiabendazole, 80 g/l]	Winter wheat	+	+
Vial TT [tebuconazole 60 g/l + thiabendazole 80 g/l]	Winter wheat	+	+
Vinets [flutriafol, 30 g/l + thiabendazole, 45 g/l]	Cereals	+	+

Gizmo 60 [tebuconazole, 60 g/l]	Spring and winter wheat	+	+
Colfugo Super [carbendazim, 200 g/l]	Spring and winter wheat, sunflower	+	+
Corriolis [triticonazole, 200 g/l]	Winter wheat, corn	+	+
Forsazh 500 Sc [carbendazim, 500 g/l]	Winter wheat, sunflower	+	+
Funaben T 480 [tiuram, 332 g/l + carbendazim, 148 g/l]	Spring and winter wheat	+	+
Insecticides			
Celest Top 312,5 Fs [difenoconazole, 25 g/l + fludioxonil, 25 g/l + tiametoxam, 262,5 g/l]	Winter wheat	+	+
Taboo [imidacloprid, 500 g/l]	Sugar beets, Spring and winter wheat, rape	+	+

Note: “+” – no effect.

The success of the combined application of microbial preparations and protectants in crop growing technologies is guaranteed if fungicides and insecticides do not affect the functional activity of bacteria – bio-agents of microbial preparations. In this regard, we studied the activity of *P. polymyxa* KB and *Achr. album* 1122 to dissolve phosphates in liquid nutrient medium of Muromtsev under the influence of seed protectants.

It was established that *P. polymyxa* KB had retained its functional activity in medium with fungicides Absolute, Alios, Gizmo 60, Colfugo Super, Corriolis, Vial Trust, Vial TT, Vinets, Forsazh 500 Sc, Funaben T 480 and insecticides Celeste Top 312,5 Fs and Taboo. Phosphates dissolution by bacteria *P. polymyxa* KB in a liquid nutrient medium of Muromtsev with seed protectants had remained on the same level as in control without pesticides (Table 2).

Table 2. The rate of phosphates dissolution by *Paenibacillus polymyxa* KB bacteria in liquid nutrient medium under the influence of seed protectants

Variants	Amount of phosphorous, on P ₂ O ₅ basis, mg/100 ml*
<i>Paenibacillus polymyxa</i> KB, control	52,0
<i>Paenibacillus polymyxa</i> KB at combined cultivation with fungicides:	
Absolute	51,8
Alios	51,7
Vial Trust	51,4
Vial TT	51,3

Vinets	51,5
Gizmo 60	51,6
Colfugo Super	51,9
Corriolis	51,8
Forsazh 500 Sc	51,8
Funaben T 480	51,6
<i>Paenibacillus polymyxa</i> KB at combined cultivation with insecticides:	
Celest Top 312,5 Fs	51,3
Taboo	51,7

*) liquid nutrient medium of Muromtsev was used with $\text{Ca}_3(\text{PO}_4)_2 - 5 \text{ g/l}$ (229 mg P_2O_5 /100 ml)

Addition of fungicide Funaben T 480 into the liquid nutrient medium of Muromtsev had not affected the rates of phosphate dissolving by bacteria *Achr. album* 1122 which was 82.9 mg P_2O_5 / 100 ml while the control values were 83.5 mg P_2O_5 / 100 ml thus indicating that studied protectant had no toxic effect on the functional activity of bacteria *Achr album* 1122 (Table 3).

Table 3. The rate of phosphates dissolution by *Achromobacter album* 1122 bacteria in liquid nutrient medium under the influence of seed protectants

Variants	Amount of phosphorous, on P_2O_5 basis, mg/100 ml*
liquid nutrient medium of Muromtsev was used with $\text{Ca}_3(\text{PO}_4)_2 - 5 \text{ g/l}$ (229 mg P_2O_5 /100 ml)	
<i>Achromobacter album</i> 1122	83,5
<i>Achromobacter album</i> 1122 + Funaben T 480	82,9

Summarizing the performed studies it can be concluded that combined application of protectants and microbial preparations is possible in the crop growing technologies if bacteria – bio-agents of specific preparations are resistant to the active ingredients of pesticides.