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ENHANCEMENT OF WHEAT VIRUS-RESISTANCE AT APPLICATION OF THE SE NANOPARTICLES CITRATES AND CONSORTIUM OF SOIL MICROORGANISMS

SUMMARY

The influence of pre-sowing seed treatments of selenium nanocitrates (SeNPs) and application of soil formed microorganism consortium (biological preparation (BP) Extrakon) on wheat plants infected with wheat streak mosaic virus (WSMV) were investigated in greenhouse and fields conditions in 2018-2019 (on territories of Zabolotny Institute of Microbiology and Virology of NASU).

The pre-sowing seed treatments of 1% selenium nanocitrates (SeNPs) with application of BP Extrakon initiating the growth of juvenile wheat in laboratory experiments were found.

In the field experiment on variants of WSMV-infected plants, two weeks after inoculation (tillering stage), we observed the appearance of characteristic symptoms of yellow mosaic with intermittent strokes on the leaves. In the boot phase, a lag in the growth of WSMV-infected plants compared with intact plants was already clearly visible, which was confirmed by the ELISA test.

It was shown, increase of the quantum efficiency of PS II (F_v/F_p) of WSMV-infected plants at pre-sowing treatment of 1% SeNPs and application of microbial consortium (BP Extrakon) compared with virus-infected plants without treatment.

In plant tissue of all experimental variants increase activity of superoxide dismutase was shown. It was shown more significant increase of the SOD-activity on the variant of WSMV- infected plants with application of BP Extrakon

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and SeNPs. Estimation of 1000-grain weight and grain productivity confirmed the effectiveness of the combined use of BP Extrakon with pre-sowing treatment of SeNPs on both intact and WSMV-infected plants. Thus, mixed application both SeNPs pre-sowing treatment and adding in soil of soil forming microbial consortium has a bio protective effect on wheat plants enhancing the resistance of plants cells to viral damage.

Keywords: Wheat, WSMV, Se nanoparticles, consortium of soil microorganisms, chlorophyll a fluorescence induction

INTRODUCTION

The wheat streak mosaic virus (WSMV) causes wheat mosaic, a disease of cereals and grasses, which threatens incomplete harvest wheat worldwide. Infected plants lag behind in growth and have a yellow mosaic with intermittent strokes on the leaves. It is known that WSMV affects winter and spring wheat (*Triticum aestivum* L.) and depending on environmental conditions and cultivation technology, the loss of wheat yield from viral damage can reach 60% or more (Hadi *et al.*, 2011; Singh *et al.* 2018). In general, studies of the effects of plant damage by pathogens have focused on the effects of nutrient availability, on plant susceptibility to infections and disease transmission rates (Dordas, 2008; Butsenko *et al.*, 2018; Dankevych *et al.* 2018). This effect depended on the interaction between the phytopathogen and the host, as well as on the type of fertilization of plants. Investigations of the effect of adding macronutrients on virus replication have shown that the effects vary depending on the nutrients and the pathological system, with the addition of nitrogen and phosphorus usually increases the titer of the virus (Pennazio and Roggero, 1997; Miller *et al.*, 2015).

In addition, a modern direction in crop production is the use of nanotechnology (Prasad *et al.*, 2017; Liu and Lal, 2015; Ljubičić, N. *et al.*, 2020), in particular to reduce the percentage of infection by phytopathogenic microorganisms, including viruses – in resource-saving technologies. Promising agents for the control of phytopathogenic microorganisms can be selenium nanoparticles (SeNPs), which are used in biomedicine and agriculture due to their high biological activity, including antioxidant and antimicrobial characteristics, and highness of biocompatibility and biosafety too (Wadhvani *et al.*, 2016).

It should be noted that with the current deterioration of soils at the global level it is extremely important to use microbiological technologies for its reproduction and in eco-technologies for growing plants (Kumar *et al.*, 2015). It is known that agronomically useful soil microorganisms are used in crop production as bio stimulants, bio fertilizers and bio protectors, due to complex mechanisms of plant-microbial interaction that improve plant growth, development and stability (Nihorimbere *et al.*, 2011).

Therefore, the aim of our work was to study the role of pre-sowing seed treatment with selenium nanoparticle citrates together with using soil-forming microbial consortium in enhancing virus resistance and productivity formation in conditions of WSMV infection.

MATERIAL AND METHODS

In laboratory, field experiments and experiments in the greenhouse used: 1% solution of citrates of nanoparticles Se – SeNPs (Se content: 100 g/l) and soil-forming microbial consortium (biological product (BP) "Extrakon" (Ukraine)). BP Extrakon consists of a consortium of soil cellulolytic and heterotrophic microorganisms inoculated into a peat-like substrate, which are in a functionally active state and are closely connected by trophic bonds: (*Sporocytophaga mixococcoides*, *Sorangium cellulosum*, *Cellvibrio mixtus*, *Trichoderma viride*, *Pseudomonas fluorescens*, *P. putida*, *Bacillus subtilis*, *B. sphaericus*, *B. megaterium*, *B. pumilus*).

In fields of conditions, plants of wheat *Triticum aestivum* L. variety Pecheryanka were grown on an experimental plot of the D.K. Zabolotny Institute of Microbiology and Virology of NASU in 2018-2019. The area of the experimental plot is 50 m², the soil is sod-podzolic. Repetition in the experiment four times. N₉₀P₉₀K₉₀ (ammonium nitrate, granular superphosphate, and potassium chloride) was applied to the soil before sowing.

The scheme of experiments: 1 – control (intact plants); 2 – WSMV-infected plants; 3 – pre-sowing treatment (p.-s. treatment) of 1% SeNPs solution; 4 – addition in soil of BP Extrakon + p.-s. treatment of 1% SeNPs solution; 5 – p.-s. treatment of 1% SeNPs solution + WSMV; 6 – BP Extrakon + p.-s. treatment of 1% SeNPs solution + WSMV.

The scheme of experiments in the greenhouse: 1 – control (intact plants); 2 – WSMV-infected plants; 3 – BP Extrakon; 4 – BP Extrakon + WSMV-infected; 5 – p.-s. treatment of 1% SeNPs solution + BP Extrakon; 6 – p.-s. treatment of 1% of SeNPs solution + BP Extrakon + WSMV.

In laboratory experiments 1: in Petri dishes decomposed of seed grains of 50 pcs, in three replications. The scheme of experiments: 1 – control (intact plants); 2 – p.-s. treatment of 1% of SeNPs solution.

After 7 days, the effect of this measure on the growth of the roots of juvenile plants in comparison with intact plants was determined.

In a laboratory experiment 2, using the roll method evaluated the effect of p.-s. treatment of 1% SeNPs solution together with BP Extrakon application. To do this, 50 seeds were laid out on rectangular filter paper, size 75x15 cm, it was tightly twisted and immersed in filtered water. The scheme of laboratory experiments 2: 1 – control (intact plants); 2 – p.-s. treatment of 1% of SeNPs solution; 3 – p.-s. treatment of 1% SeNPs solution + BP Extrakon.

In field experiments and in the greenhouse, inoculation of wheat plants with freshly prepared virus-containing material was carried out in stage of the second pair of true leaves, by the method of mechanical inoculation of leaves with preliminary dusting with carborundum. Isolation of viral material was performed by homogenization of freshly cut leaves of diseased plants with clear symptoms of WSMV with the addition of 0.1 M phosphate buffer pH 7.0. The plant homogenate was filtered through a nylon sieve and used to mechanically infect the plants. Infection of plants was carried out using a glass spatula or

fingers in disposable gloves soaked in inoculum. Excess inoculum was washed off with water (Wetzel, 1984). Antigen detection was performed by enzyme-linked immunosorbent assay (ELISA) using diagnostic sera for WSMV (Bradwell *et al.*, 1990; Gnutova, 1993). Solid-phase ELISA (sandwich variant) was performed using commercial test systems to WSMV "Loewe" (Germany). The reaction results were recorded on a reader of Termo Labsystems Opsis MR (USA) with Dynex Revelation Quicklink software at wavelengths of 405/630 nm. Values that exceeded the negative control three times were considered reliable (Crowther, 1995).

Studies of the activity of the antioxidant enzyme superoxide dismutase (SOD) were performed in leaf tissue on the stage of heading – flowering. The activity of SOD was determined by the ability of the enzyme to inhibit the photochemical reduction of nitro blue tetrazolium (Beyer and Fridovich, 1987). The portion of plant material weighing 150–200 mg was ground in a cooled mortar in 300–400 μ l of extraction buffer. The resulting homogenate was centrifuged at 12,000 g for 5 minutes. For each sample made two identical tubes with the reaction mixture and extract. One test tube was placed in the dark, the other – in the light. In addition, control tubes were prepared without enzymatic extraction – to calculate the maximum formation of formazan. The reaction was started by adding 20 μ l of 0.025% riboflavin to all tubes, which were stirred rapidly.

Tubes to determine the maximum formation of formazan, placed in a dark place. The remaining tubes were installed under two fluorescent lamps. The reaction lasted 15 minutes. At the end of the time, the reaction was stopped by turning off the light. The optical density is measured at a wavelength of 560 nm. The enzyme activity was calculated by determining the percentage inhibition per min. The increase in absorbance without the enzyme extract was taken as 100%. Fifty percent of inhibition was taken as equivalent to 1 unit of SOD activity. To calculate the activity of SOD per gram of dry weight (units/g dry wt) (Giannopolitis and Ries, 1977; Chakrabarty *et al.*, 2009).

The photochemical activity of the leaves was determined by the biophysical method of chlorophyll a fluorescence induction (ICF) using a portable device "Florotest" (Ukraine).

The device is equipped with a liquid crystal display (128×64 pixels) and a remote optoelectronic sensor with a wavelength of 470 ± 15 nm, the area of the irradiation spot not less than 15 mm² and illumination within it not less than 2.4 W/m². The spectral range of fluorescence intensity measurements is in the range from 670 to 800 nm. The data measured by the device was transferred to a PC via the USB port of the computer using the software "Floratest", which comes with the device and allows you to display this data in tabular or graphical form. Dark adaptation of leaves before measurements was in range: 6-20 minutes. Replications measurements on each variant – threefold. The evaluation of the influence of experimental factors was performed by changes in the value of the quantum efficiency parameter PS II: F_v/F_p ($F_v = F_p - F_0$), where F_0 – the

minimum fluorescence; F_p – maximum (peak) fluorescence, F_v – variable fluorescence (Sharma *et al.*, 2015; Lichtenthaler *et al.*, 2007; Huliaieva, *et al.* 2018).

Accounting for grain productivity in the experimental plots was done at the end of the growing season at full grain maturity. Statistical processing of the obtained results was performed using computer programs MS Excel.

RESULTS AND DISCUSSION

In the laboratory experiment, it was found that pre-sowing treatment of wheat seeds with 1% solution of SeNPs increased root length from 4.16 cm in the control to 7.99 cm, and therefore 1.92 times. It should be noted, that pre-sowing treatment of 1% SeNPs and application of BP Extrakon several-fold enhanced the growth-stimulating effect of pre-sowing treatment of 1% SeNPs by showing a synergistic effect. Increase of 16.5% of juvenile plants leaf weight at pre-sowing treatment of 1% SeNPs and adding of Extrakon was shown.

In the field experiment on variants of virus-infected plants, two weeks after inoculation, we observed the appearance of characteristic symptoms: yellow mosaic with intermittent strokes on the leaves (tillering phase) (Fig. 1 A). In the boot phase, the lag in the growth of infected compared to intact plants was already clearly visible (Fig. 1 B)

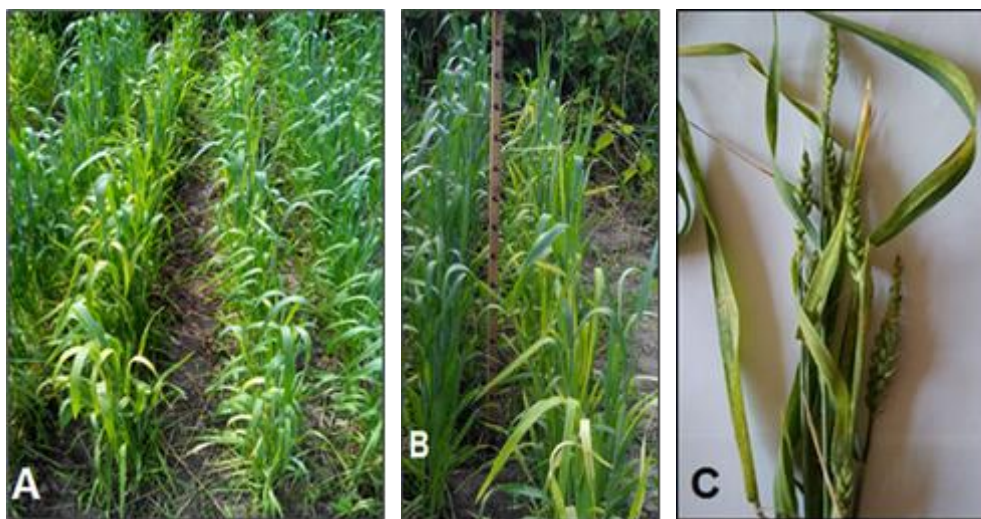


Fig. 1. The WSMV-infected wheat plants in different growth stage: the tillering phase (A) and the boot phase (B), the milk-wax ripeness phase (C).

The ELISA results showed that with the defeat of WSMV, the content of antigens on the 14th day after infection increased significantly – 5 times compared to the control (Fig. 2).

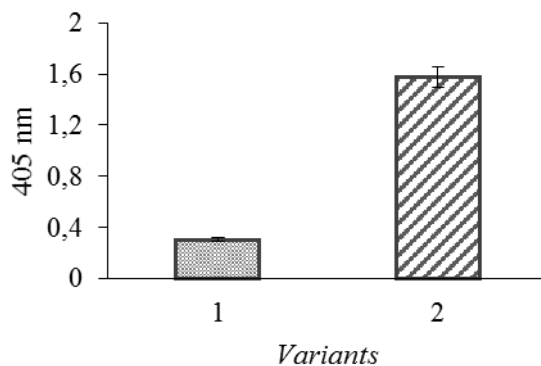


Fig. 2. The effect of artificial contamination of WSMV of spring wheat on the content of antigens, ELISA data 14 days: 1 – the intact plants (treatment with water, control 1); 2 – the WSMV-infected plants (treatment with water).

With ICF of method, we found decrease of quantum efficiency of PSII (F_v/F_p), which reflects decrease of quantity of photochemically active centers in PSII, in the leaves of virus-infected wheat plants (14 days after infection) (Fig. 3).

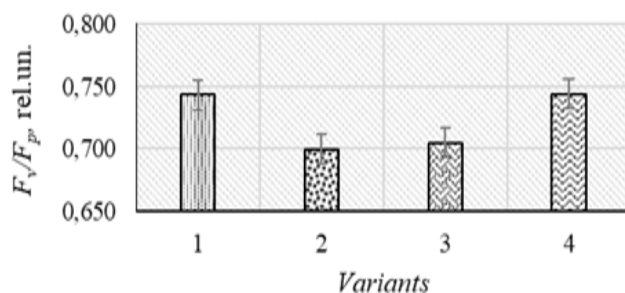


Fig. 3. The quantum efficiency of PSII of leaves of WSMV-infected wheat plant at pre-sowing treatment of 1% SeNPs and adding BP Extrakon, the tillering phase: 1 – Control (intact plants); 2 – WSMV-infected plants; 3 – WSMV + BP Extrakon; 4 – WSMV + p.s. treatment of 1% SeNPs + BP Extrakon (experiment in the greenhouse).

It is known that under the action of stresses of different nature, including the action of phytopathogenic microorganisms, in cells there is an accumulation of reactive oxygen species (ROS), in particular: superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), singlet oxygen (1O_2), etc. One of the main antioxidant enzymes involved in the regulation of ROS levels while maintaining resistance to adverse factors is superoxide dismutase (SOD), which catalyze the dismutation of superoxide radicals to molecular oxygen and hydrogen peroxide (Tyagi *et al.*, 2019; Tanase and Popa, 2014; Choudhury *et al.*, 2013).

Therefore, as a marker of resistance to the action of experimental measures, we determined the change in the activity of SOD in the tissues of the leaves. Evaluation of SOD activity (in the field experiment) in wheat leaves in the heading – flowering growth period showed its growth in all experimental variants in the following order: control (intact plants) > SeNPs + WSMV > BP Extrakon + SeNPs > SeNPs > WSMV > BP Extrakon + SeNP. Thus, the most significant increase in the activity of SOD, indicating an increase in plant resistance, was found in the variant Extrakon + SeNPs + WSMV (Fig. 4).

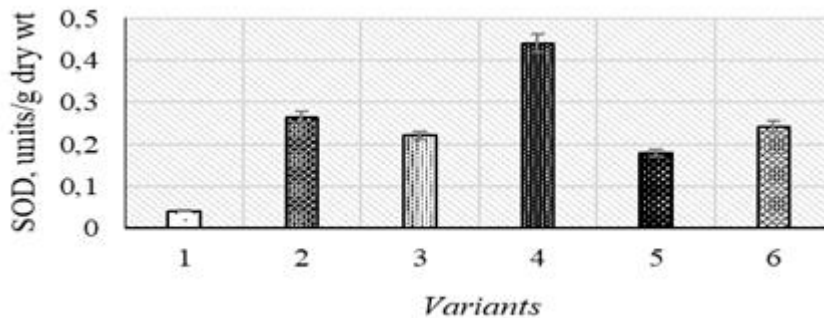


Fig. 4 The activity of SOD in the tissues of the leaves of WSMV-infected spring wheat under the influence of pre-sowing treatment of 1% SeNPs and adding of BP Extrakon (field experiment): 1– intact plants; 2 – WSMV- infected plants ; 3 – BP Extrakon + 1% SeNPs; 4 – BP Extrakon + 1% SeNPs + WSMV; 5 – 1% SeNPs + WSMV; 6 – 1% SeNPs.

Accordingly, in variants with WSMV-infected plants, a significant decrease in grain productivity and the thousand-kernel weight was observed compared to the control – by 29.7% (Table 1).

Table 1. The grain productivity of spring wheat at application of 1% SeNPs pre-sowing treatment, use of BP Extrakon and WSMV-infection

Variants	The thousand-kernel weight, g	The grain productivity, kg/10m ²
Control (intact plants)	34,8±1,39	3,17±0,12
WSMV-infected plants without treatment	21,4±0,86	2,23±0,11
1% SeNPs	34,2±1,37	3,12±0,15
1% SeNPs+ BP Extrakon	36,2±1,45	3,41±0,14
1% SeNPs+ WSMV	22,1±0,89	2,37±0,09
1% SeNPs+ BP Extrakon + WSMV	27,5±1,11	2,52±0,10

On variants with pre-sowing treatment with 1% solution of SeNPs and WSMV-infected plants was showed a tendency to increase grain productivity compared to WSMV-infected plants without treatment. The better effect on grain productivity was detected on variants of intact plants wheat with pre-sowing

treatment of 1% SeNPs solution with BP Extrakon application, where productivity increased by 7.6% relative to control. The application of pre-sowing treatment of 1% SeNPs and BP Extrakon on the variants with WSMV-infected plants allowed an increase of grain production by 13% compared with virus-infected plants without treatment, partially reducing losses from viral infection.

The effect of experimental treatment on the thousand-kernel weight had a similar tendency. Its effect it is possible be arranged in the following order: SeNPs + BP Extrakon > Control (intact plants) > SeNPs > SeNPs + BP Extrakon + WSMV > SeNPs + WSMV > WSMV-infected plants (see Table 1).

Thus, pre-sowing treatment of wheat seeds with selenium-nanocitrates with soil-forming microbial consortium application has not only bio stimulating, but also antioxidant and therefore – bio protective effect on plants – increasing the resistance of cells to viral damage.

CONCLUSIONS

Thus, the better effect in growth stimulating wheat plants at mixed application both 1% SeNPs and consortium of soilformed microorganisms (BP Extrakon) was found in laboratory conditions on the young plants.

In the field experiment on WSMV-infected wheat plants, two weeks after inoculation (in the tillering phase), we observed the appearance of characteristic symptoms: yellow mosaic with intermittent strokes on the leaves. In the boot phase, a lag in the growth of infected compared to intact plants was already clearly visible, which was confirmed by the ELISA test. It was shown, increase of the quantum efficiency of PS II (F_v/F_p) of WSMV-infected plants at mixed application both pre-sowing treatment of 1% SeNPs and microbial consortium (BP Extrakon) compared to virus-infected plants without treatment.

In plant tissue of all experimental variants increase activity of superoxide dismutase was shown. It was shown more significant increase of the SOD-activity on the variant of WSMV- infected plants at mixed application both application of BP Extrakon and SeNPs. Estimation of 1000-grain weight and grain productivity confirmed the effectiveness of the combined use of BP Extrakon with pre-sowing treatment of SeNPs on both intact and WSMV-infected plants.

Thus, mixed application both SeNPs pre-sowing treatment and adding in soil of soil-forming microbial consortium has not only bio stimulating, but also antioxidant and therefore – bio protective effect on wheat plants – increasing the resistance of cells to viral damage.

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