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Effect of Selenium Nanocomposites Based on Natural Polymer Matrices on the Biomass and Storage of Potato Tubers in a Field Experiment

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Abstract: The effect of chemically synthesized selenium (Se) nanocomposites (NCs) based on the natural polymer matrices arabinogalactan (AG), carrageenan (CAR), and starch (ST) on potato tuber traits, storage, and crop structure was studied in a field trial. Parental potato tubers were sprayed by Se/AG NC, Se/ST NC, and Se/CAR NC 14 days before planting in the field. The results showed that Se/AG NC and Se/CAR NC increased the number and weight of tubers in the first generation (F1) obtained from the plants grown from the treated tubers. It was found that Se/AG NC and Se/ST NC decreased the median weight of shoots after 230 days of storage of the F1 tubers, preventing their premature germination, and Se/AG NC decreased the number of rotten tubers. All three Se NCs significantly improved the storage by increasing the number of healthy scab-, dry-pitted-rot-, and wireworm-free tubers in the F1 after 230-day-long storage, except Se/CAR NC regarding dry pitted rot. Selenium/ST NC significantly increased the number of tubers, and Se/CAR NC their mass, and both decreased the number of rotten tubers in the second generation (F2). Selenium NCs affected crop structure in both generations.

Keywords: arabinogalactan; carrageenan; diene conjugates; field experiment; glutathione peroxidase; nanocomposites; phytopathogens; potato tubers; biomass; shoots; selenium; starch

1. Introduction

Global climate change leads to an active expansion of habitats for phytopathogenic microorganisms [1,2]. In response, the use of pesticides in global agriculture is increasing [3]. However, most pesticides, while being primarily effective against phytopathogens, could also be toxic and pollute the environment [4] by penetrating soil and groundwater and accumulating in plants [5]. They may have a negative impact on animals [6–9] and humans [10–12]. In this regard, it is extremely important to search for new effective and environmentally friendly agents against phytopathogens [13]. In this regard, various active bioagents of plant origin and biopesticides are being actively investigated now [14–17].

Biopesticides are natural compounds of biological origin that are used to control various agricultural pests that infect plants in forests, gardens, and agricultural lands [16]. They are different depending on the origin: microbial, produced with the help of genetically modified organisms and biochemical pesticides (pheromones, plant extracts, and oils, insect growth regulators). Basically, biopesticides are aimed at regulating the number of insects. Thus, as an alternative to chemical pesticides, it was proposed to use essential oils, which are a complex mixture of hydrocarbons with traces of compounds based on sulfur and nitrogen extracted from plants. Key components of horticultural oils include paraffin and olefin. They are recommended to treat cultivated plants to protect them from harmful insects [14]. In addition, insects and birds, extracts of various trees were used as an alternative to insecticides [15]. Plant terpenoid compounds, flavonoids, alkaloids, polyphenols, cyanogenic glucosides, quinones, amides, aldehydes, thiophenes, amino acids, and saccharides are proposed to be used as attractants, nematicides, fungicides, repellents, insecticides, and insect growth regulators for pest control [17].

Nanotechnologies are being actively introduced into various spheres of human economic activity [18–22]. In particular, there are encouraging developments in the field of agrochemistry [23–28]. Innovative nanopesticides are nanomaterials developed for plant protection and characterized by the following properties: minimizing losses during the application, increasing leaf coverage, increasing stability, and reducing the amount of consumption of active substances. Nanopesticide preparations can be divided into self-organizing systems, such as liposomes, dendrimers, metallic and bimetallic nanoparticles (NPs), and active encapsulating ingredients, such as nanoemulsion, polymeric nanoparticles, lipid, nanoparticles, and nanotubes [29]. Biopesticides can also be created on the basis of nanoparticles (NPs) [13,30], and we tested a few of them in this study. Nanobiopesticides are created by encapsulation of biomolecules extracted from plants, fungi, and bacteria, which have a prolonged action, or also with the help of numerous biomaterials synthesized by biogenic processes to obtain biocompatible and more effective biopesticides [31]. Biologically derived nanopesticides can be made using any element, including metals such as Ag, Cu, SiO_2, and ZnO, with a wide spectrum of pest control efficiency [32]. We have studied bionanopesticide selenium (Se) nanocomposites (NCs) based on natural polymer matrices and tested them in field conditions. Among them are Se NCs with different contents of Se NPs based on arabinogalactan (AG) [33–35], starch (ST) [36], and carrageenan (CAR) [37], as well as silver NCs based on humic substances of various compositions (shales, coals, and pelloids) [38,39], manganese NCs based on AG and CAR, and sulfur NCs based on ST [40]. It has been shown that these NCs reduce the viability of the phytopathogenic bacterium *Clavibacter sepedonicus* and the phytopathogenic fungus *Phytophthora cactorum* [35]. Some of them stimulated the growth and development of potatoes in vitro, germination of soybean and pea seeds [41], and increased biomass of potato tubers and quality of potato crop in field experiments. At the same time, the accumulation of NPs in plant tissues after their treatment with nanocomposites was not observed [33,34,37]. It is also important to note that the studied NCs did not inhibit the viability of soil microorganisms *Acinetobacter guillouiae*, *Rhodococcus erythropolis*, and *Pseudomonas oryzihabitans* [35]. Published laboratory data on the biological activity of NCs indicate the promise and relative safety of their use against phytopathogens for plant health control and improvement [33,34,37]. These experiments were carried out under in vitro conditions. However, for a comprehensive assessment of the effect of NCs on potatoes, it is necessary to conduct, in addition to laboratory experiments also, trials in natural conditions of potato vegetation and storage. This paper presents new data on the effect of Se NCs based on natural polymer matrices on the biomass, storage, and sprouting of potato tubers in the field in order to develop an effective and environmentally friendly growth stimulant for agricultural plants. Our main objective in this study was not so much to assess the effect of pre-planting treatment on the productivity and structure of potatoes, which would require multiple-year observations, but to study its immediate effect on the storage of the crop and its productivity in the next generation as a part of a systemic effect of nanocomposites. In our previous in vitro studies, we have shown that the NCs presented here have the properties of pesticides that kill pathogenic microflora but do not adversely affect the soil microflora and the plants themselves, which are treated with NCs [33–37,40,42]. In this study, we studied the effect of NC on the productivity, germination, and storage of uninfected potatoes in the field conditions. At the same time, some growth-stimulating effects were found.

2. Materials and Methods

2.1. Nanocomposites (NCs)

Arabinogalactan-based Se NC (Se/AG NC, 5.92% Se), starch-based Se NC (Se/ST NC, 1.46% Se), and kappa-carrageenan-based Se NC (Se/CAR NC, 3.67% Se), already detail studied in the laboratory improvement of potatoes [33–37], were used in the research. Since the NCs had different Se content due to the specific conditions for their synthesis, aqueous solutions of NCs were normalized to 0.000625% of Se. This concentration was selected in the previous experiments with potatoes [33–37].

The AG from Siberian larch, *Larix sibirica* Ledeb. (Wood Chemistry Ltd., Irkutsk, Russia) was purified by passing it through a polyamide column [43] and used for synthesis of the Se/AG NC. The CAR (potassium-sodium salt of sulfated anhydro-polysaccharide) of WR-78 type (CP Kelco ApS, Lille Skensved, Denmark) was used for synthesis of the Se/CAR NC. Starch from potato as a water-soluble reagent (Sigma-Aldrich, Saint Louis, MO, USA) was used for synthesis of the Se/ST NC. Selenium dioxide (99.8%, Sigma-Aldrich, Saint Louis, MO, USA) as a Se precursor and L-ascorbic acid as a reductant (99.0%, Sigma-Aldrich, Saint Louis, MO, USA) were used for synthesis of the Se/AG and Se/CAR NCs. Available sodium bis(2-phenylethyl)diselenophosphinate [44] as a selenium precursor, and hydrogen peroxide water solution (30%, Sigma-Aldrich, Saint Louis, MO, USA) as an oxidant were used for synthesis of the Se/ST NC. The synthesis and characterization of NCs were described in detail in [35]. All resulting NCs were well-soluble in water.

2.1.1. Se/AG NC

Arabinogalactan (Wood Chemistry Ltd., Irkutsk, Russia) (4.0 g) was dissolved in water (30 mL) at room temperature with stirring on a magnetic stirrer for 1 h. Powders of Se dioxide (99.8%, Sigma-Aldrich, Saint Louis, MO, USA) (1.0 g) and L-ascorbic acid (99.0%, Sigma-Aldrich, Saint Louis, MO, USA) (0.4 g) were successively added to the resulting solution. The reaction mixture was stirred for 30 min at room temperature. Then, the reaction mixture was poured into 150 mL of ethanol, and the formed Se/AG NC precipitates were filtered, washed on the filter with ethanol, and dried in air to constant weight.

2.1.2. Se/CAR NC

Carrageenan (CP Kelco ApS, Lille Skensved, Denmark) (5.0 g) was dissolved in water (350 mL) at room temperature under stirring on a magnetic stirrer for 12 h until complete dissolution. The solution of Se dioxide (99.8%, Sigma-Aldrich, Saint Louis, MO, USA) (0.375 g) in water (5 mL) and solution of L-ascorbic acid (99.0%, Sigma-Aldrich, Saint Louis, MO, USA) (0.227 g) in water (5 mL) were successively added. The reaction mixture was stirred for 24 h at room temperature. Then, the reaction mixture was poured into 1500 mL of ethanol, and the resulting Se/CAR NC precipitate was filtered, washed on a filter with ethanol, and dried in air to constant weight.

2.1.3. Se/ST NC

Starch (Sigma-Aldrich, Saint Louis, MO, USA) (2.0 g) was dissolved in water (250 mL) at room temperature under stirring on a magnetic stirrer. Then, the temperature was raised for 10 min until the resulting mixture boiled, cooled to 40 °C, sodium bis(2-phenylethyl)diselenophosphinate [44] powder (0.3 g) was added with stirring, and the mixture was held at 40 °C for 3 h. Then, concentrated aqueous hydrogen peroxide (Sigma-Aldrich, Saint Louis, MO, USA) (30%, 10 mL) was added, and the reaction mixture was additionally held at the same temperature for 1 h. Then, the reaction mixture was poured into 1000 mL of ethanol, and the resulting Se/ST NC precipitate was filtered, washed on a filter with ethanol, and dried in air to constant weight.

2.2. Potato Material and Experiments

In our earlier studies of effects of Se/AG, Se/CAR, and Se/ST NCs on potatoes in vitro, we concluded that the Se nanoparticles that composed Se NCs had main effect and showed that their biopolymer matrices (polysaccharides) did not have a negative effect on the viability of both potato plants and their phytopathogens, while the Se NCs significantly suppressed phytopathogens [36,37,42]. Therefore, labor-intensive field experiments in this study were carried out using only Se NCs without separate analysis of their polysaccharides without Se.

Potato tubers *Solanum tuberosum* L., variety "Gala", early maturing, high-yielding were used in the work. This variety belongs to the table varieties of German selection, which are characterized by high plasticity, and easily adapt to various soil compositions and climates. The choice of this variety was also due to the fact that it is not well-resistant to fungal and bacterial infectious diseases. This property facilitates detection of the NC effects on susceptibility of tubers to diseases. The scheme of the experiment is presented in Figure 1.

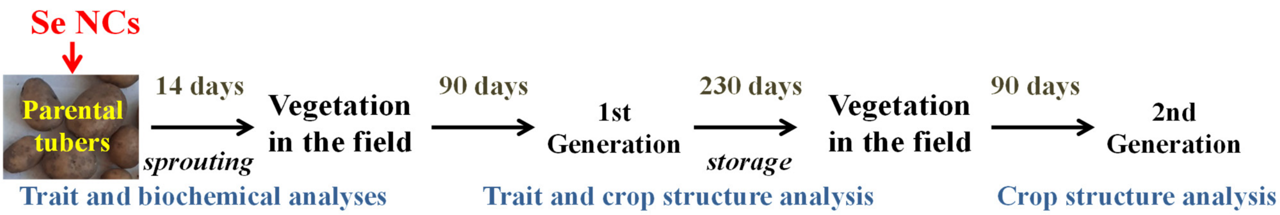

Figure 1. Scheme of the experiment.

Potato tubers were treated in April 2020 by spraying with aqueous solutions of Se NCs based on natural polymer matrices. Control tubers were sprayed with water. The amount of sprayed nanocomposite was 0.105 mg/L for Se/AG NC, 0.428 mg/L for Se/ST NC, and 0.170 mg/L for Se/CAR NC, which corresponds to the Se concentration of 6.25 µg/mL in the final solution. Potato tubers were sprayed from all sides with a spray gun (0.4 L/ton). They were sprouted in dark for 14 days. Then, in half of the tubers, the length and weight of their shoots and roots, as well as biochemical parameters, were analyzed. The rest of the tubers were planted in the experimental field site of the Siberian Institute of Plant Physiology and Biochemistry (Irkutsk, Russia). There were 30 tubers in each treatment variant and control.

The experiment was set up on gray forest soil, typical for the southern part of Eastern Siberia, with the following agrochemical parameters: humus content—7–8% (C); total nitrogen—0.13%; mobile phosphorus (P_2O_5)—159 mg/kg; exchangeable potassium (K_2O)—139 mg/kg; pH KCl = 5.6. In general, in terms of fertility, the soil was characterized as slightly acidic, rather humus-rich. The soil was loam and clay, highly cultivated with humidity level of 10% and density 1.39 g/cm^3. Agrochemical analyzes of soil and plants were performed according to [45]. All experiments were carried out on a natural infectious background in three fields with three plots (repeats) per field with randomized plot design. Plot area was 100 m^2 (20 m $\times$ 5 m). Common for this region, agrotechniques of crop cultivation were used: the cultivation sites were periodically weeded and hilled without additional irrigation and fertilization in the natural conditions of a sharply continental climate of Eastern Siberia (Irkutsk, Russia). The duration of both first and second vegetation was 90 days during the growing seasons. The summer of 2020 on the territory of the experimental site in Irkutsk, Irkutsk Region, Russia, where the experiment was conducted, was warm and long. The period from June to mid-July was dry, then frequent rains were noted until the end of August. In 2020, summer, similar to spring, began 25–30 days earlier than normal climatic terms. This was the earliest start of summer in the previous

60 years. From the beginning of the second decade of June, abnormally hot dry weather was established in the region and remained almost until mid-July. A long-term deficit of precipitation and an elevated temperature regime led to the development of an atmospheric-soil drought in the steppe zone of the southern, central, and southern Upper Lena regions. Precipitation fell unevenly, with 75% of precipitation from the monthly norm falling on 15–16 July. The average temperatures in July, the warmest month, ranged from +15 to +20 °C. The maximum temperatures exceeded +30 °C.

After the end of the growing season, the weight and number of tubers obtained from experimental plants, as well as the structure of the crop and the number of rotten tubers, were analyzed. In order to determine the yield structure, the tubers were ranked according to the Russian state food potato specifications (GOST 33996-2016 and 7176-2017) of their weight into the following categories: (1) large tubers weighing more than 150 g; (2) commercial tubers weighing 85–150 g; (3) seed tubers—50–80 g; (4) small tubers—less than 50 g.

After the analysis, in autumn 2020, the tubers obtained from the field experiment were placed in mesh bags and stored in containers in a vegetable store at an air temperature of +4 °C; and a humidity of 75%. After 230 days of storage, the quality of the tubers was assessed visually and by the following nine traits: (1) total tuber weight (g), (2) median tuber weight (g), (3) median number of shoots per tuber, (4) median length of shoots (cm), (5) median shoot weight (g), (6) tubers affected by scab (%), (7) tubers affected by dry pitted rot (%), (8) green tubers (%) and (9) tubers damaged by wireworm (%). Further, in May 2021, these tubers were planted in the field without any treatments. After 90 days of vegetation, the crop structure, weight, and number of tubers were evaluated again in the second generation (F2). Numbers of tubers and shoots studied in each generation are presented in Table 1.

Table 1. Numbers of tubers and shoots (in brackets) studied for each trait in the parental tubers, and in the first and second generations obtained from tubers treated with selenium (Se) nanocomposites (Se/AG, Se/ST and Se/CAR NCs) and in the control (C).

Trait	Parental Tubers				First Generation				Second Generation			
	C	Se/AG	Se/ST	Se/CAR	C	Se/AG	Se/ST	Se/CAR	C	Se/AG	Se/ST	Se/CAR
Mean length and weight of shoots measured after 14 days following treatment and in control	20 (84)	20 (87)	20 (96)	20 (66)	10	10	10	10				
Content of LPO and DC	9	9	9	9								
Number of planted tubers	10	10	10	10	20	20	20	20				
Mean number and biomass of tubers per plant					64	112	70	122				
Crop structure and number of rotten tubers					64	112	70	122	334	232	419	406
Analysis of sprouting and health condition after 230 days of storage					64	112	70	122				

Note: DC—diene conjugates; LPO—lipid peroxidation; Se/AG—arabinogalactan-based Se nanocomposites (NCs); Se/ST—starch-based Se NCs; Se/CAR—kappa-carrageenan-based Se NCs.

2.3. Biochemical Analysis

As indicators of stress load on plants, the content of lipid peroxidation (LPO) and diene conjugates (DC) primary products were measured in the tissues of potato tuber shoots using a method with hexane and isopropanol described in [46,47]. Activity of glutathione peroxidase (EC 1.11.1.9, GPx) in shoots was assessed by change in the content of glutathione in samples before and after incubation with 5,5′-dithiobis-2-nitrobenzoic acid in the color reaction with this substrate [48].

Statistical data processing was carried out using the SigmaPlot v.12.5 program (SYSTAT Software, Chicago, IL, USA). The data obtained after treatment were statistically compared with controls using the nonparametric Mann–Whitney U test, Kruskal–Wallis test, and Fisher's exact test.

3. Results

3.1. Biochemical Analysis

After spraying with aqueous solutions of Se NCs, potato tubers were sprouted for 14 days, and then the length and weight of potato shoots (Table 2) and the level of DC (Figure 2A) and GPX (Figure 2B) were measured. It was found that Se/AG and Se/ST NCs had no significant effect on the length and weight of shoots, and a significant effect was observed only after treatment by Se/CAR NC (Table 2).

Table 2. Mean length and weight of potato shoots † (±SE) measured in the parental tubers after 14 days of sprouting following treatment with selenium (Se) nanocomposites (NCs) and in the control.

Shoot Trait	Control (84)	Se/AG NC (87)	Se/ST NC (96)	Se/CAR NC (66)
length, cm	2.04 ± 0.10	2.20 ± 0.11	1.90 ± 0.08	3.12 ± 0.15 *
weight, g	0.27 ± 0.02	0.32 ± 0.02	0.26 ± 0.02	0.46 ± 0.04 *

Note: Se/AG—arabinogalactan-based Se nanocomposites (NCs); Se/ST—starch-based Se NCs; Se/CAR—kappa-carrageenan-based Se NCs. † Total numbers of shoots measured in 20 tubers per each treatment and control are presented in brackets; * significant differences from control, $p < 0.05$ based on Mann–Whitney *U* test.

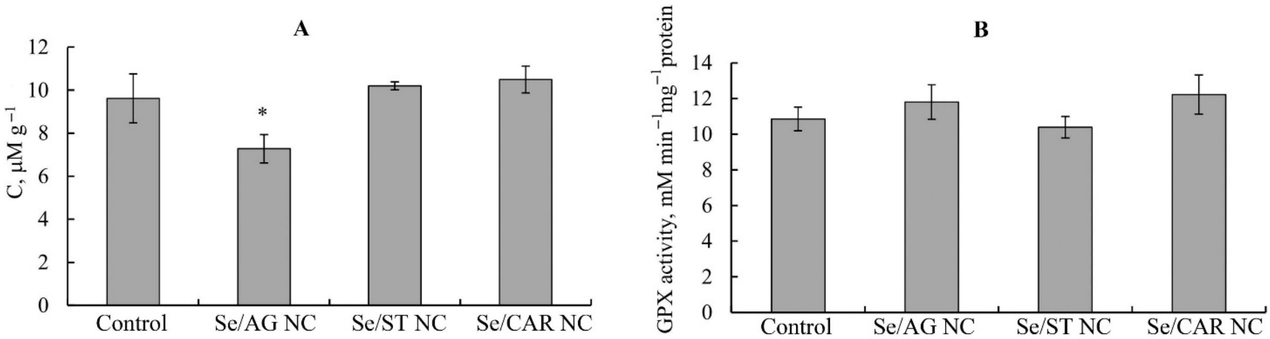

Figure 2. Effect of selenium (Se) nanocomposites (NCs) on the content of diene conjugates (DC) (**A**) and on glutathione peroxidase (GPX) activity (**B**) measured in the parental potato tuber shoot tissues after 14 days of sprouting following treatment and in the control (mean value of μM per g of wet weight); the vertical bars indicate the standard error (±SE); * Significant difference from control, $p < 0.05$ based on Mann–Whitney U test of shoots in 30 tubers per each treatment and control. Se/AG NC, Se/ST NC and Se/CAR NC are arabinogalactan-, starch- and kappa-carrageenan-based Se nanocomposites.

The effect on the level of DC in the tissues of potato shoots was noted only for Se/AG NC, with a significant decrease in DC compared to the control (Figure 2A). The activity of the GPX enzyme in the tissues of potato shoots did not change under the influence of Se NCs (Figure 2B).

3.2. Field Experiment (1st Generation)

The potato tubers remaining after biochemical analysis of DC and LPO were sprouted and planted in the field to determine the effect of Se NCs on the biomass of tubers in the field. Se/AG NC had the maximum stimulating effect on all studied parameters (Figure 3).

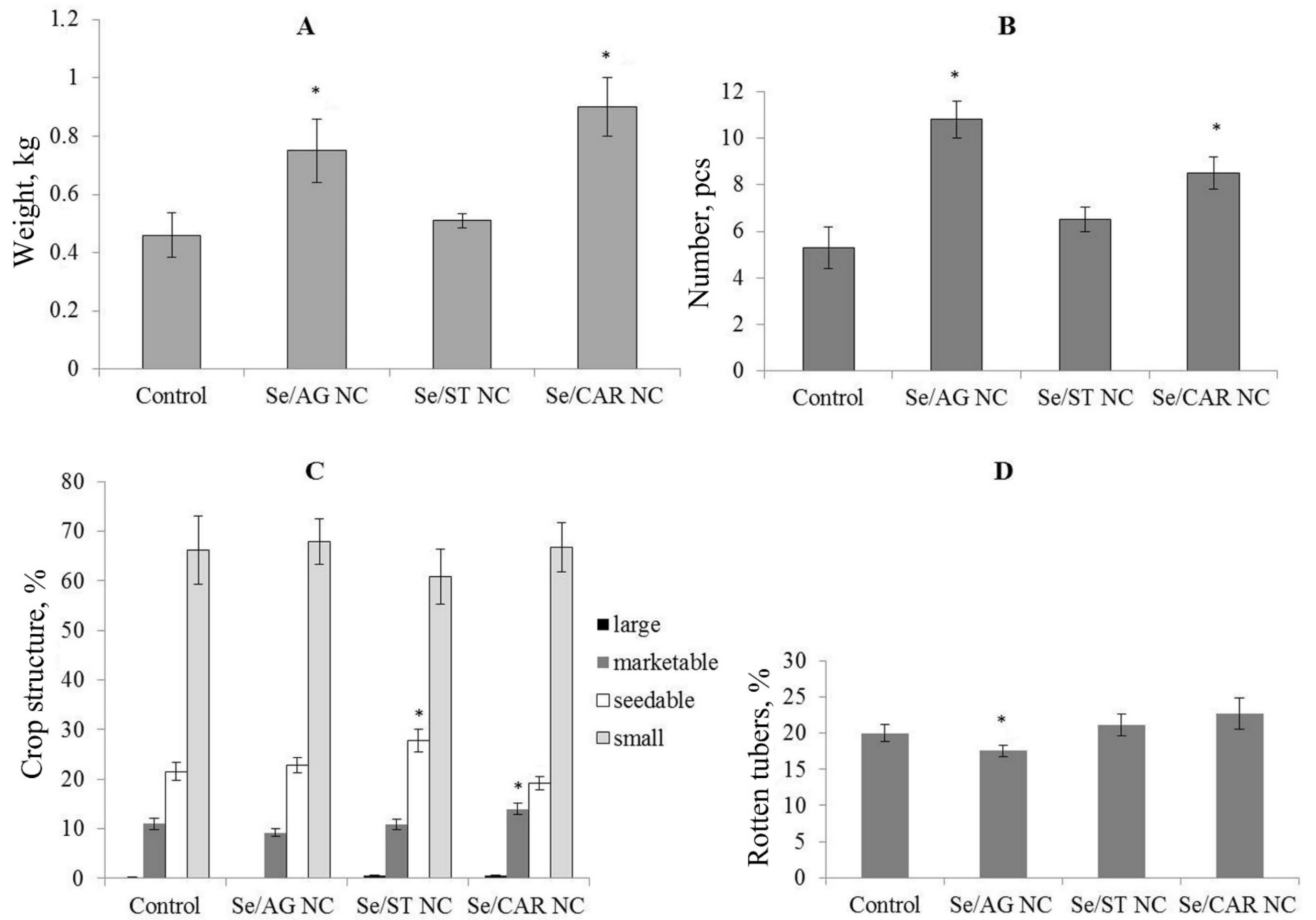

Figure 3. Effect of treatment of potato tubers with selenium (Se) nanocomposites (NCs) on the weight of tubers (**A**) obtained from the first-generation plants grown from treated tubers, their number; (**B**), crop structure—proportion of large, marketable, seedable and small tubers; (**C**) percentage of rotten tubers; (**D**) in the first generation; * Significant differences from control, $p < 0.05$ based on Mann–Whitney *U* test. Se/AG NC, Se/ST NC and Se/CAR NC are arabinogalactan-, starch- and kappa-carrageenan-based Se nanocomposites.

Compared to the control, it significantly stimulated an increase in the mean tuber weight and its number per plant (Figure 3A,B). Analysis of the crop structure did not reveal any effect of Se/AG NC on the studied parameters. At the same time, a decrease in the number of decayed tubers in the yield obtained from plants grown from tubers treated with Se/AG NC was found (Figure 3D). Selenium/ST NC did not have a pronounced effect on the biomass of tubers. It did not affect the mean mass of tubers (Figure 3A) and their number in one plant (Figure 3B). However, the treatment with Se/ST NC increased the number of seed tubers in the crop structure compared to the control (Figure 3C).

Selenium/CAR NC, compared with the control, significantly stimulated an increase in the mean tuber mass in the hole (Figure 3A) but did not affect the number of tubers per plant (Figure 3B) and the crop structure (Figure 3D). Thus, the field experiment demonstrated the stimulating effect of Se/AG and Se/CAR NCs on the biomass of tubers.

3.3. Tuber Storage Analysis

After 230 days of storage of the F1 tubers obtained from the treated and untreated (control) tubers, their quality was assessed visually (Figure 4) and for the nine traits (Table 3).

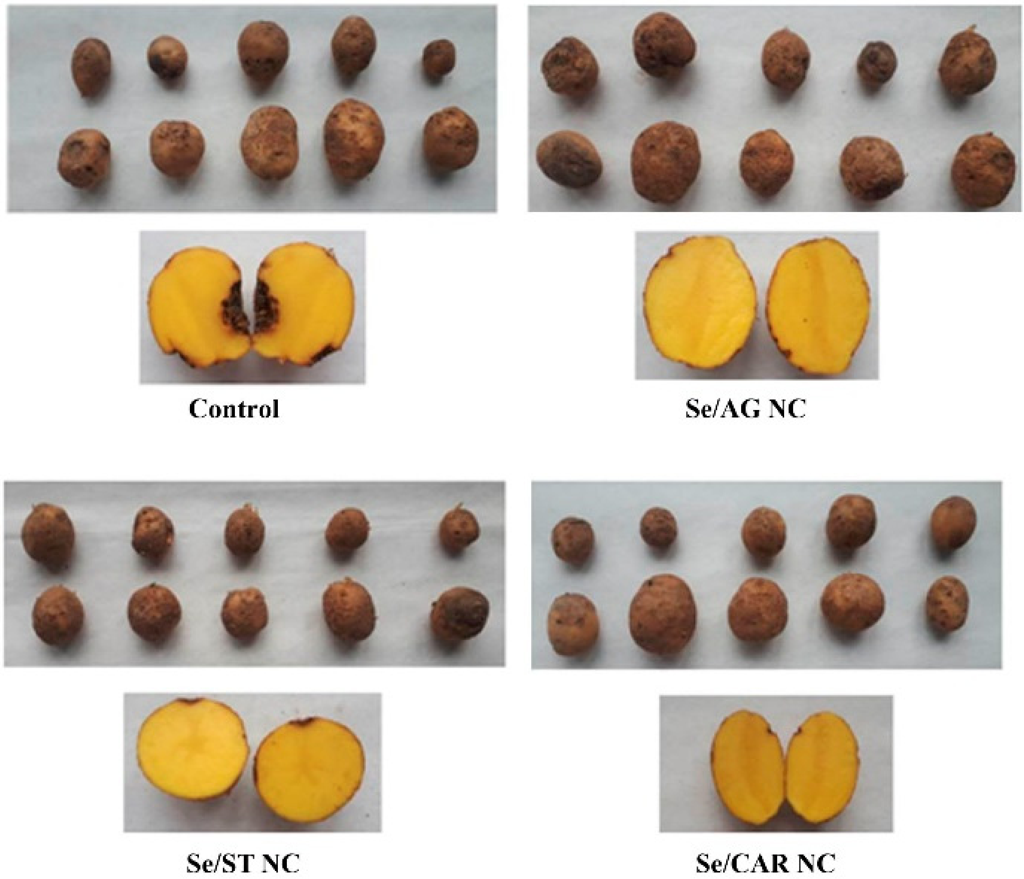

Figure 4. Potato tubers in the first generation obtained from plants grown from tubers treated with selenium (Se) nanocomposites (NCs) and untreated tubers (control) after 230 days of storage. Se/AG NC, Se/ST NC and Se/CAR NC are arabinogalactan-, starch- and kappa-carrageenan-based Se nanocomposites.

Table 3. Analysis of tuber traits in the first generation obtained from plants grown from tubers treated with selenium (Se) nanocomposites (NCs) after 230 days of storage.

Trait		Control	Se/AG NC	Se/ST NC	Se/CAR NC
Total tuber weight, g (based on the total number of plants studied in the brackets)	1st repeat	3990 (90)	8180 (112)	4735 (70)	8885 (122)
	2nd repeat	17,765 (412)	12,499 (296)	17,463 (378)	14,914 (325)
Median tuber weight, g		54.0 [29.5; 85.5]	42.0 [22.5; 68.3] *	41.5 [24.1; 69.8]	56.0 [32.0; 90.0]
Median number of shoots per tuber		3 [3; 4]	3 [2; 4]	3 [2; 4]	3 [2; 4]
Median length of shoots, cm		1.4 [0.8; 2.3]	1.6 [0.2; 1.9]	1.4 [0.7;1.9]	1.4 [0.7;2.1]
Median shoot weight, g		0.16 [0.07; 0.27]	0.13 [0.06; 0.19] *	0.13 [0.04; 0.22] *	0.13 [0.05; 0.22]

Note: Se/AG—arabinogalactan-based Se nanocomposites (NCs); Se/ST—starch-based Se NCs; Se/CAR—kappa-carrageenan-based Se NCs. The range of values is presented in square brackets as the interquartile values between the 25th and 75th percentile; * significant differences from control at $p \leq 0.05$ based on Kruskal–Wallis test. The numbers in round brackets present total number of tubers measured.

When visually inspecting the tubers, it was found that there were many tubers affected by diseases (scab, dry pitted rot) and wireworm in the control. There was no internal damage to tubers obtained from plants grown from tubers treated with Se NCs compared to controls (Figure 4). After the storage period, there was no shrinkage of tubers, and no difference was observed in the total mass of tubers in potatoes obtained from plants grown from treated tubers compared to the control (Table 3). Treatment by Se/AG NC decreased the median weight of tubers (Table 3, Figure 5). The median number and length of shoots were not affected by Se NCs, but Se/AG and Se/ST NCs decreased the median weight of shoots (Table 3, Figure 5).

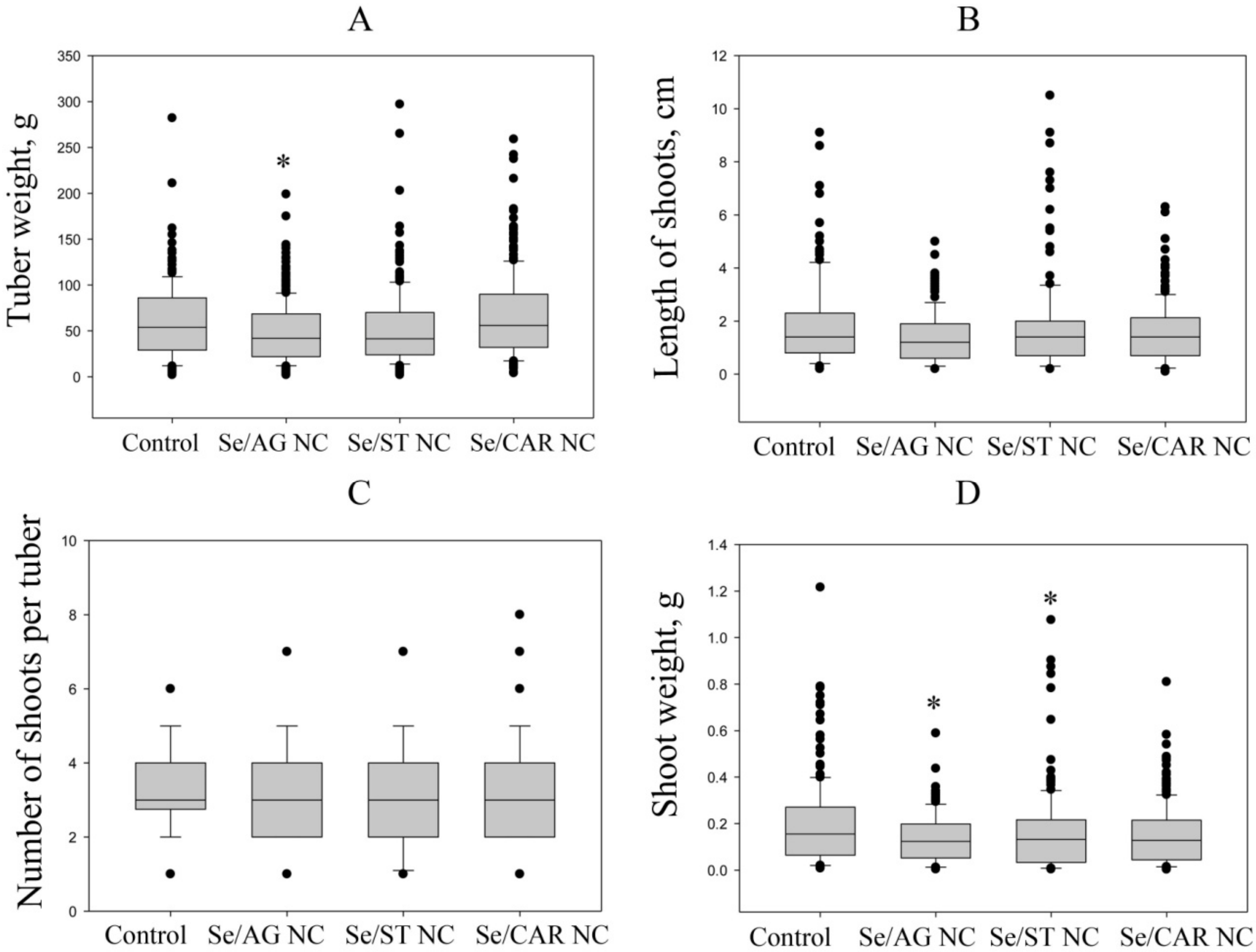

Figure 5. Box-plots of tuber traits (**A**) tuber wight, (**B**) length of shoots, (**C**) number of shoots per tuber, (**D**) shoot wight in the first generation obtained from plants grown from tubers treated with selenium (Se) nanocomposites (NCs) after 230 days of storage; * Significant differences from control at $p \leq 0.05$ based on Kruskal–Wallis test. Se/AG NC, Se/ST NC and Se/CAR NC are arabinogalactan-, starch- and kappa-carrageenan-based Se nanocomposites.

Selenium NCs had an ambiguous but rather positive effect on the infectious status of tubers (Table 4). The results showed abundant scab damage to tubers, which is typical for the region where the tests were carried out. From 50 to 92% of control tubers were affected by scab. Selenium/AG NC reduced the number of tubers affected by scab. Treatments with Se/ST NC and Se/CAR NCs reduced the number of tubers affected by this infection only in the first plot, but in the remaining plots, the number of tubers affected by scab remained high. Selenium/AG and Se/ST NCs also reduced the number of tubers affected by dry pitted rot in the first plot. Potato treatment with Se/CAR NC did not affect dry pitted rot at all (Table 4). The number of tubers affected by the wireworm decreased under the treatments with all three NCs. Green tubers were noted only in control samples (Table 4).

Table 4. Number of tubers in the visual analysis of healthy traits in the first generation tubers obtained from plants grown from tubers treated with selenium (Se) nanocomposites (NCs) after 230 days of storage.

Tuber Trait	Effect	Se/AG NC	Control	*p* *	Se/ST NC	Control	*p* *	Se/CAR NC	Control	*p* *
Plot 1										
scab	affected unaffected	0 106	59 5	0.0000	35 36	59 5	0.0000	34 23	59 5	0.0000
dry pitted rot	affected unaffected	10 96	17 46	0.0042	6 65	17 46	0.0057	12 45	17 46	0.5244
wireworm	affected unaffected	0 96	62 1	0.0000	0 71	62 1	0.0000	0 57	62 1	0.0000
color	green normal	6 90	0 63	0.0819	0 71	0 63	1.0000	0 57	0 63	1.0000
Plot 2										
scab	affected unaffected	80 35	32 32	0.0106	76 14	32 32	0.0000	80 21	32 32	0.0001
dry pitted rot	affected unaffected	11 104	10 54	0.2355	63 27	10 54	0.0000	10 91	10 54	0.3297
wireworm	affected unaffected	0 115	22 42	0.0000	0 90	22 42	0.0000	0 101	22 42	0.0000
color	green normal	0 115	0 64	1.0000	0 90	0 64	1.0000	0 101	0 64	1.0000
Plot 3										
scab	affected unaffected	103 12	37 15	0.0055	65 12	37 15	0.0805	86 29	37 15	0.7049
dry pitted rot	affected unaffected	80 35	5 47	0.0000	46 31	5 47	0.0000	17 98	5 47	0.4625
wireworm	affected unaffected	0 115	35 17	0.0000	0 77	35 17	0.0000	0 115	35 17	0.0000
color	green normal	0 115	0 52	1.0000	0 77	0 52	1.0000	25 90	0 52	0.0000

Note: Se/AG—arabinogalactan-based Se nanocomposites (NCs); Se/ST—starch-based Se NCs; Se/CAR—kappa-carrageenan-based Se NCs. * Two-sided *p*-values for difference between NC treatment and control for the studied traits based on the Fisher's exact test.

Thus, the analysis carried out after the storage period did not reveal any negative effect of treatments by Se NCs on the storage quality of tubers. Moreover, Se NCs suppressed the development of infectious diseases in tubers during storage.

3.4. Field Experiment (2nd Generation)

In order to evaluate the effect of the Se NC treatments on the biomass of tubers in the second generation, the tubers of the first generation obtained from plants grown from tubers treated with Se NCs and untreated tubers (control) were planted in the soil after 230 days of storage to obtain the second-generation crop. There was a slight but not statistically significant increase in the median weight of tubers after treatment by Se/CAR NC compared with the control (Figure 6A; Table 5). The number of tubers increased only under treatment by Se/ST NC (Figure 6B). No significant effect of Se NCs was found on the yield structure, except Se/AG NC, which increased the number of small tubers compared to the control (Figure 6C; Table 5). A statistically significant decrease of 20% in rotten tubers was observed in the treatments by Se/ST and Se/CAR NCs compared to the control (Figure 6D). Thus, despite the low biomass of tubers in the second generation, a positive effect of Se NCs on some studied traits was found, especially for Se/ST NC, which stimulated an increase in the weight of tubers and also reduced the number of rotten potatoes.

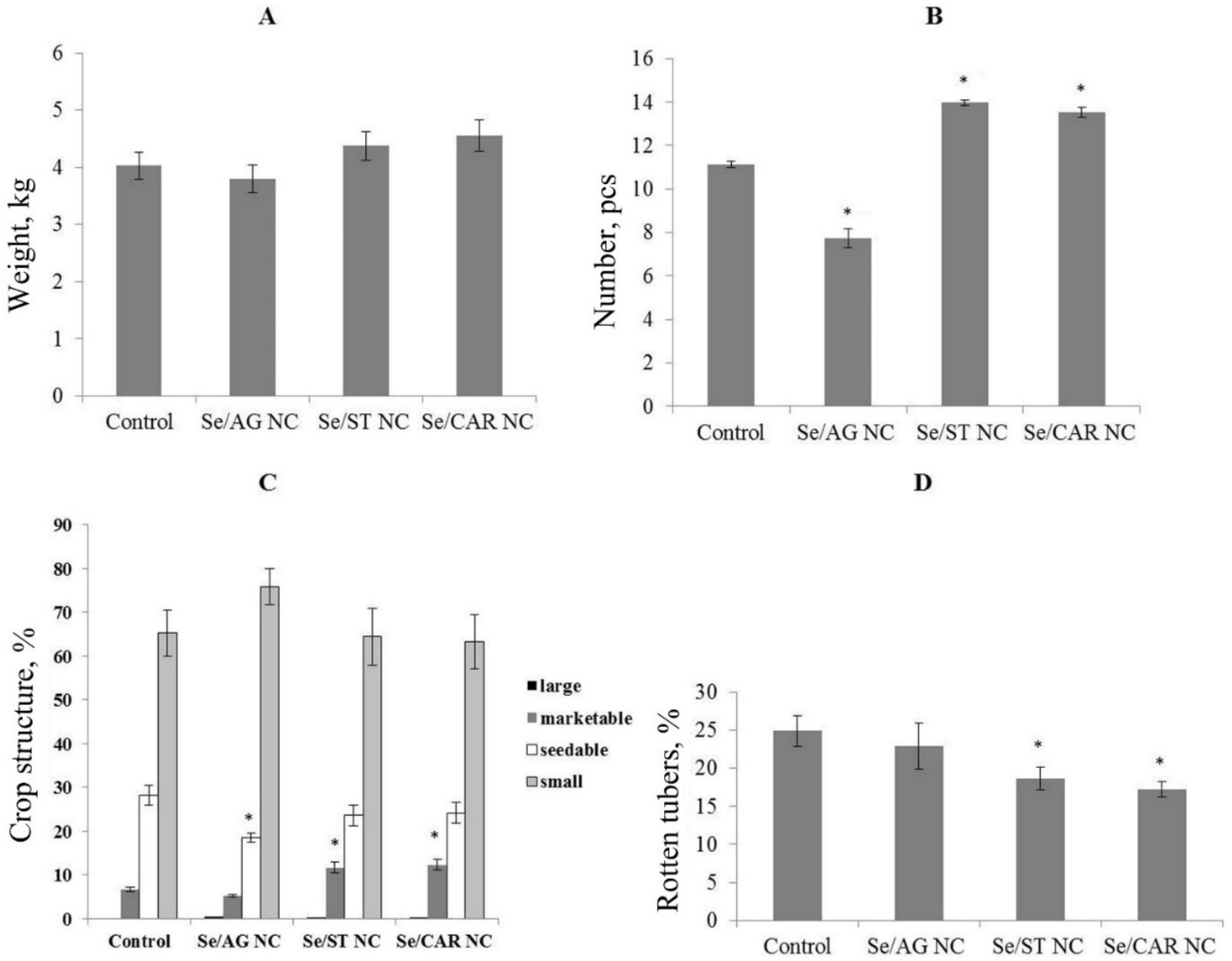

Figure 6. Effect of treatment of potato tubers with selenium (Se) nanocomposites (NCs) on the mean weight of tubers per plant obtained from plants in the second generation (**A**), their number (**B**), yield structure (**C**), and percentage of rotten tubers (**D**) in comparison with control; * Significant differences from control, $p < 0.05$ based on Mann–Whitney U test. Se/AG NC, Se/ST NC and Se/CAR NC are arabinogalactan-, starch- and kappa-carrageenan-based Se nanocomposites.

Table 5. Analysis of biomass of potato tubers in the second-generation tubers obtained from plants grown from parental tubers treated with selenium (Se) nanocomposites (NCs).

Trait	Control	Se/AG NC	Se/ST NC	Se/CAR NC
Total tuber weight, g (based on 20 plants)	13,462	8815	17,956	15,035
Total number of tubers	334	232	419	406
Median tuber weight, g	38 [20; 59]	37 [19; 50]	40 [21; 63]	41 [25; 64]

Note: Se/AG—arabinogalactan-based Se nanocomposites (NCs); Se/ST—starch-based Se NCs; Se/CAR—kappa-carrageenan-based Se NCs. The range of values is presented in square brackets as the interquartile values between the 25th and 75th percentile.

4. Discussion

Reactive oxygen species (ROS) are involved in the regulation of the most important physiological and biochemical cell processes both under and without stress [49–51]. Tuber dormancy and germination in potatoes can also be controlled by manipulating the content of ROS, especially the amount of hydrogen peroxide (H_2O_2), through the inhibition of catalase (CAT) activity. In contrast to the hormonal regulation of potato dormancy, little attention has been paid to the role of ROS. The participation of ROS such as superoxide anion radical (O_2), H_2O_2, and hydroxyl radicals (OH·) in the regulation of dormancy and germination has been demonstrated in several plant species, particularly in grapes [52]. The role of this process during potato germination is discussed in [53–55].

When the concentration of H_2O_2 is excessive, the mitochondria remain dormant, and germination decreases. Indeed, high concentrations of H_2O_2 are toxic to plants, activates LPO, and causes damage to the membrane wall and a decrease in membrane integrity [52]. Lipid peroxidation is a process in which free radicals (oxyl, peroxyl, hydroxyl) remove electrons from lipids and subsequently produce reactive intermediates that can enter into further reactions [56]. As a result of this process, primary products are formed, hydroperoxides, also called diene conjugates (DC), which are considered the primary product of LPO [57]. Therefore, as a biochemical marker of the effect of Se NCs on plant growth and development, we determined the content of primary LPO products, namely DC, in root and shoot tissues. Our results showed a decrease in the level of DC under the influence of Se/AG NC. The remaining Se NCs did not affect the level of DC (Figure 2A). This result indicates that the treatment of tubers with Se NCs does not cause a stress response in the tuber cells. The decrease in the level of DC under the influence of Se/AG NC may be associated with the antioxidant activity of Se NP. It was shown that green synthesized Se NPs (25–200 µg/mL) reduced the amount of a free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) by 61% [58]. Selenium NPs decorated with a different molecular weight of chitosan (1.5 kDa, 48 kDa, and 510 kDa) showed the strongest antioxidant activities. These NPs inactivated DPPH, ABTS+, and O_2- radicals [59]. The antioxidant activity of Se NCs has been proven repeatedly in animals, including the use of LPO products as an indicator. The antioxidant activity of Se NCs in animal tissues is mainly associated with the regulation of the activity of Se-containing enzymes that is the family of glutathione peroxidases (GPX) and thioredoxin reductase (TR), which detoxify a wide range of peroxides, such as H_2O_2, phospholipid hydroperoxides, fatty acid hydroperoxides, and thymine hydroperoxyl groups [60]. It has been shown in chickens that the addition of Se NP at doses of 0, 0.1, and 0.2 mg/kg to their diet increased the levels of GPX and superoxide dismutase (SOD) and reduced the level of the secondary LPO product, malondialdehyde (MDA) [61]. The antioxidant and hepatoprotective effect of Se NP was found in the study of MDA content, total antioxidant capacity, GPX, SOD, glutathione (GSH), and CAT activity in rat liver tissue [62].

The level of LPO in the body is controlled with the participation of antioxidant enzymes [51], including the important role of GPX, which is involved in the reduction of organic peroxides formed during LPO. It was previously shown that treatment of tubers with Se NCs leads to a significant increase in GPX activity in tomato tissues 50 days after treatment [63]. However, we did not reveal any changes in GPX activity in potatoes treated by any of the Se NCs tested in our experiments. Probably, stimulation of sprouting under the influence of Se/CAR NC is associated with other antioxidant enzymes. In addition, the results obtained on the content of DC and the levels of GPX activity indicate the absence of stress on potato tuber cells under the influence of NCs.

The data obtained in this study are summarized in Table 6. It was found that only treatment with Se/CAR NC had a stimulating effect on the length and weight of shoots sprouted from the treated parental tubers. Tuber sprouting is always accompanied by activation of oxidative processes [53].

Table 6. Summary data on the effect of selenium (Se) nanocomposites (NCs) on parental potato tuber traits and in the first and second generations in the field study.

Trait	Se/AG NC	Se/ST NC	Se/CAR NC
Parental tubers			
DC level	decreased (17%)	-	-
GPX activity	-	-	-
Shoot length	-	-	increased (53%)
Shoot weight	-	-	increased (70%)
First generation			
Crop structure	-	increased the number of seedable tubers (27%)	increased the number of marketable tubers (44%)
Mass of tubers	increased (59%)	-	increased (83%)
Number of tubers	increased (120%)	-	increased (80%)
Number of rotten tubers	decreased	-	-
Tuber weight after storage	decreased (23%)	-	-
Number of tubers affected by scab	decreased (90%)	decreased (7%)	decreased (4%)
Number of tubers affected by dry pitted rot	decreased (18%)	decreased (20%)	-
Number of tubers affected by wireworm	decreased (67%)	decreased (67%)	decreased (67%)
Shoot weight after storage	decreased (19%)	decreased (19%)	-
Second generation			
Crop structure	decreased the number of seedable tubers (32%)	increased the number of marketable tubers (140%)	increased the number of marketable tubers (160%)
Mass of tubers	-	-	increased (12%)
Number of tubers	-	increased (31%)	-
Number of rotten tubers	-	decreased (28%)	decreased (36%)

Note: DC—diene conjugates; GPX—glutathione peroxidase; Se/AG—arabinogalactan-based Se nanocomposites (NCs); Se/ST—starch-based Se NCs; Se/CAR—kappa-carrageenan-based Se NCs.

The results of the field experiment demonstrated that treatment of tubers with Se/AG and Se/CAR NCs stimulated the biomass of tubers in the first generation by increasing both the mass and number of tubers. It is in agreement with our previous studies, which showed that Se/AG NCs stimulated the growth and development of potato plants as well as their root formation in vitro [34]. This effect may be due to the biological activity of not only Se NCs but also arabinogalactan, which stimulates the growth and development of plants [64]. Experiments carried out on plants in vitro showed a stimulating effect of Se/CAR NC on biometric traits and a decrease in the negative effect of potato infection with a phytopathogenic bacterium [37]. The different effects of Se NCs observed in our results can be associated with the nature of the matrix-different polysaccharides arabinogalactan [65], carrageenan [66], and starch [67], which have rich individual biological activity. Most likely, the specific polysaccharide shell of antimicrobial Se NCs, which is trophic for microorganisms, can be captured with varying degrees of preference by phytopathogens (a kind of "Trojan horse" principle implemented for targeted microbial delivery of Se NCs) potentially both outside the cell [68] and intracellularly [69]. In the case of the observed positive effect of Se/CAR NC on the length and weight of seedlings after treatment with parental tubers, the weight and number of tubers in the 1st generation and an increase in the weight of tubers in the second generation, the growth-stimulating effect of sulfated carrageenan polysaccharide may also take place [70,71]. However, these hypotheses require further detailed verification. Selenium/AG and Se/ST NCs reduced premature tuber sprouting during storage. All three Se NCs increased the number of healthy tubers in the first generation tubers after their storage for 230 days. This effect can be explained by the fungicidal [33] and antibacterial effects of Se NCs [33–37]. The present data showed an increase in plant resistance to infections under the influence of treatments with Se NCs.

In the second generation, Se/AG NC did not affect the biomass and number of the tubers per plant (Figure 6A,B). In addition, there was a significant decrease in the number of seed tubers in this treatment compared to the control (Figure 6C). Selenium/ST and Se/CAR NCs increased the total biomass of tubers, the number of tubers per plant, and the number of marketable tubers in the crop structure (Figure 6C; Table 4). Most of which were healthy, likely indicating the stimulation of induced potato resistance under the influence of Se/ST and Se/CAR NCs, which had a prolonged effect that lasted even in the second generation (Figure 6D). These transgenerational effects could be explained by stable epigenetic modifications caused by Se NCs and are worth to be studied further.

Over the past 5 years, many studies have been carried out to study the effect of Se NCs on the growth and development of plants, as well as their ability to resist stresses of various nature [72,73]. Most of the available literature data on the action of Se NCs indicated its positive effect on plants. For example, it has been shown that exogenous spraying by Se NCs increased the antioxidant potential of basil (*Ocimum basilicum* L.) [74] and enhanced the growth of tobacco (*Nicotiana tabacum* L.) [75] and peanut (*Arachis hypogaea* L.) [76]. The effect of selenium nanoparticles on seed germination was studied in *Hordeum vulgare* L. [77]. Nano-selenium dioxide increased the yield and intensity of plant growth and enhanced salt tolerance in *Phaseolus vulgaris* L. growing in a field experiment on saline soils. It was suggested that an increase in the growth of higher plants treated by Se NCs occurs due to an increase in the productivity of photosynthesis [78]. A change in the fatty acid profile of lipids in plant cells was also shown under the influence of Se NCs [77]. It has been shown that spraying pear, grape, and peach plants during vegetation with a solution containing Se NPs increased the photosynthesis rate [79]. In addition, it was found that Se NCs affect the activity of antioxidant enzymes in various plant organs, such as nitrate reductase in leaves and peroxidase in roots [80].

It was also shown that Se NCs could function as stimulators of plant development, improving their antioxidant defense system and, consequently, their ability to tolerate stress [75]. Se NCs affect cellular processes and, for example, regulate the activity of antioxidant enzymes and affect the photosynthetic apparatus. It was shown that Se NCs significantly reduced the content of heavy metals in rice grains grown on the polluted soil [81]. Spraying plants with a solution of Se NCs improved the growth and increased the yield of rice, radish, and corn, and accelerated the growth of lettuce plants. It was found that Se NCs not only enhance the resistance of tomato plants to salt [82] and biotic stress caused by a nematode (*Alternaria solani*) but also increase their yield [83]. The increased resistance of tomatoes to stress can be explained by the induction of some enzymes, such as SOD, ascorbate peroxidase, GP*X*, phenylalanine ammonia lyase in leaves, and GP*X* in fruits [84]. In addition, the content of chlorophylls *a* and *b* was increased in the leaves, and the amount of vitamin C, glutathione, phenols, and flavonoids increased in the fruits [85]. Salt stress tolerance and increased yields have been observed when strawberry plants were sprayed with *Fragaria ananassa* and treated with Se NCs [86]. The resulting effect was explained by a decrease in the level of LPO, an increase in the activity of antioxidant enzymes-SOD and peroxidase, as well as an increase in the content of proline in plant tissues [85,86]. In addition, an increase in the quality and nutritional properties of strawberries was noted due to an increase in the content of organic acids (for example, malic, citric, and succinic acids) and sugars (for example, glucose, fructose, and sucrose) in the berries of plants treated with Se NCs [87].

5. Conclusions

The problem of the growing global demand for food products leads to an increase in the use of pesticides. Most of them are aimed at regulating phytopathogenic fungi. At the same time, there are no pesticides effective against phytopathogenic bacteria. Selenium NCs in natural polymer matrices were studied by us in a number of earlier studies under in vitro conditions as new promising and environmentally safe agents for the recovery of plants from phytopathogens of not only fungal but also bacterial nature [33–40,42]. Promising data have been obtained on the bactericidal effect of Se NCs on the phytopathogenic bacterium *Clavibacter sepedonicus* and the phytopathogenic fungus *Phytophthora cactorum*. At the same time, these Se NCs did not have a negative effect on the vegetation of potato plants, did not accumulate in their tissues, and did not kill rhizospheric bacteria. As a continuation of these studies, the results of field studies of the treatment of potato tubers with Se NCs presented in this paper confirmed the observations obtained earlier in vitro that Se NCs do not adversely affect the viability of potatoes, and even some of them stimulate the formation of biomass. The treatment with Se/AG NCs was the most effective among used Se NCs for increasing the biomass of potato tubers in one growing season. At the same time, in the present study, some effects of Se NCs persisted even in the second generation. Selenium/CAR and Se/ST NCs promoted prolonged resistance to the tuber decay (rotting) and also increased biomass and the number of tubers, respectively, in the second generation. Treatment of seed tubers by Se NCs reduced the incidence of infectious diseases in potato tubers during their storage. These transgenerational effects could be explained by stable epigenetic modifications caused by Se NCs and are worth to be studied further. Thus, Se NCs in natural polymer matrices not only have an antimicrobial effect against phytopathogenic microorganisms but also have a healing effect on potatoes, presumably even transgenerational ones. However, we have to emphasize that obtained data are preliminary and need further verification in the multi-year studies.

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