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ARTICLE

Effect of Selenium, Copper and Manganese Nanocomposites in Polysaccharide Matrices on the Content of Photosynthetic Pigments in Potato Leaf Tissues

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ABSTRACT: The article presents the study of the effect of nanocomposites (NCs) based on selenium (Se), copper (Cu), and manganese (Mn) nanoparticles (NPs) embedded in a matrix of natural polysaccharides—arabinogalactan (AG), carrageenan (CAR), and starch (ST)—on the content of chlorophylls (Chls) and carotenoids in potato tissues *in vitro*. Potatoes were grown for 28 days on Murashige-Skoog (MS) medium with the addition of a NC, then pigments were isolated from leaf tissues, and their content was determined spectrophotometrically. Both a stimulating effect and an inhibitory effect of different NCs on the pigment content were found. Se and Cu NCs in the AG matrices (Se/AG and Cu/AG NCs) increased the Chl content both in the leaf tissues of healthy potato plants and plants infected with the phytopathogenic bacterium *Clavibacter sepedonicus* (*Cms*). Mn NCs increased the content of carotenoids and Chls in potatoes infected with *Cms*. A decrease in the pigment content was detected when growing potatoes on a medium with the addition of Cu NC based on a ST matrix (Cu/ST NC), which is probably due to the high content of NPs in this NC (20%). It was concluded that NCs based on an AG matrix are capable of exerting a stimulating effect on the content of potato pigments, which can be used to increase the yield of this crop.

KEYWORDS: *Clavibacter sepedonicus*; copper; manganese; nanocomposites; nanoparticles; potato; selenium

1 Introduction

In recent years, global climate change and the rapid advancement of agricultural technology have fueled growing interest in new materials and technologies for agricultural production. Crop yield losses can reach 40%, and the long-term use of pesticides does not fully address the problem, causing pollution, reduced soil fertility, the emergence of resistant pathogens, reduced biodiversity, and harm to humans and animals [1]. Consequently, there is an urgent need for innovative and efficient agricultural technologies

to address global food production and security challenges. Nanomaterials (NM), which are currently being actively studied, serve as such advanced agents [2–4]. They possess solubility, high reactivity, electrical and magnetic properties, and high biological activity. Artificial NMs find application in various technical fields [5,6], medicine, pharmacology [7,8], food industry [9], and cosmetics [10]. In agriculture, the use of such substances is promising in various aspects of veterinary medicine [11], as feed additives for livestock [12], and in the field of agrochemistry as new highly effective pesticides [13–15], plant health agents against phytopathogens [16], and agents for increasing plant resistance to stress [17].

It is known that, when nanoparticles (NPs) are applied to plants, they may affect, in addition to the antioxidant system, photosynthetic processes, including the content of photosynthetic pigments [18–20]. The composition of photosynthetic pigments in the leaves of higher plants varies widely depending on the species, life form, ontogenetic stages, and environmental conditions of vegetation [21]. Chlorophyll (Chl) and carotenoids are the main photosynthetic pigments that contribute to the efficient absorption of light by plants [22]. The chlorophyll (Chl) family plays a pivotal role in light harvesting by absorbing light across distinct regions of the spectrum, thereby enabling photosynthetic organisms to adapt to diverse environmental conditions under both long-term acclimation and short-term light fluctuations. Carotenoids perform multiple essential functions in photosynthesis, contributing to light absorption and serving as key antioxidants that mitigate photodamage and photoinhibition [23].

In our previous studies, we investigated nanocomposites (NCs), defined as metal or non-metal NPs embedded within natural polymer matrices [24]. To evaluate their potential for enhancing plant health, we examined the biological activity of NCs based on selenium (Se) [25,26], manganese (Mn) [27], and copper (Cu) [28] NPs incorporated via chemical synthesis into natural polymer matrices—carrageenan (CAR), arabinogalactan (AG), and starch (ST). It was demonstrated that Se-based NCs [25] and Cu-based NCs [28] at a concentration of 0.000625%, as well as Mn-based NCs [27] at 0.00625%, significantly reduced the viability of the phytopathogenic bacteria *Clavibacter sepedonicus* (Cms), the causative agent of potato ring rot, and *Pectobacterium carotovorum*, which infects a broad spectrum of crops ranging from vegetables to woody plants [29,30].

Furthermore, at antimicrobial concentrations, these NCs stimulated the growth and development of potato plants *in vitro*, both healthy and *Cms*-infected [26–28]. The effects of NC treatment were evaluated using *in vitro* biometric parameters and biochemical indicators of the antioxidant defense system, including reactive oxygen species (ROS) levels, antioxidant enzyme activities, and lipid peroxidation intensity [26–28].

In addition to these studies, including assessments of NC safety for plant systems, it is essential to investigate their influence on photosynthetic pigment content. Accordingly, the present study examines the effects of NCs and their precursor compounds on the levels of photosynthetic pigments (Chl *a*, Chl *b*, and carotenoids) in potato leaf tissues.

2 Materials and Methods

2.1 Synthesis of Nanocomposites (NCs)

For NC synthesis, arabinogalactan (AG) was isolated from the polysaccharide fraction of Siberian larch (*Larix sibirica* Ledeb.) (OOO Wood Chemistry, Irkutsk, Russia). The obtained AG was subsequently purified to remove residual impurities and flavonoids by chromatography on a polyamide column. The NCs were synthesized at room temperature according to the methods described below.

2.1.1 Selenium-Containing NCs

AG (1.0 g) and sodium bis(2-phenylethyl) diselenophosphinate (0.136 g) were dissolved in 50 mL distilled water under magnetic stirring. The solution was incubated at 35–40°C for 3 h, followed by the addition of 30% H_2O_2. The reaction mixture was precipitated into a fourfold excess of acetone (or ethanol) to isolate the $Se^{(0)}$-containing NC. The precipitate was washed with the same solvent, filtered, and air-dried. The yield was 97% (calculated relative to Se in the precursor), with a final Se content of 3.4%.

The resulting NC was obtained as a water-soluble orange-red powder. X-ray diffraction (D8 ADVANCE, Bruker Corporation, Billerica, Massachusetts, USA) indicated an X-ray amorphous structure. The formation of amorphous $Se^{(0)}$ NPs was confirmed by a characteristic absorption band at ~310 nm and the absence of crystalline reflections in the diffraction pattern. Transmission electron microscopy (TEM) revealed predominantly spherical NPs with sizes ranging from 20 to 65 nm (average ~25 nm), uniformly distributed within the AG matrix [26].

2.1.2 Manganese-Containing NCs

$Mn(OH)_2$/AG NC was synthesized by dissolving AG (2 g; Mw 18 kDa) in 5 mL distilled water, followed by the addition of $MnSO_4{\cdot}5H_2O$ (0.4 g) dissolved in 3 mL water. Subsequently, NH_4OH (0.1 mL) and hydrazine (0.2 mL) were added under continuous magnetic stirring. The reaction mixture was stirred for 3 h at room temperature, then precipitated with excess ethanol. The precipitate was washed with ethanol and dried to yield 1.68 g of $Mn(OH)_2$/AG NC.

The NPs are presumed to form as hydrated manganese hydroxide species ($Mn(OH)_2{\cdot}nH_2O$), stabilized within the AG matrix via interactions with oxygen-containing functional groups, primarily hydroxyl moieties. TEM revealed electron-dense, predominantly spherical nanoparticles with diameters of 3–6 nm. The nanoparticles exhibited minimal aggregation and were uniformly dispersed throughout the polymer matrix, maintaining spatial separation within the bulk material. The resulting NCs were found to be paramagnetic particles [27].

To synthesize $Mn(OH)_2$/CAR NC, kappa-carrageenan (3 g, 1100 kDa) were stirred in H_2O (150 mL) and heated at 50°C until the mixture become homogeneous. $MnSO_4{\cdot}5H_2O$ (0.69 g) in H_2O (3 mL) and NH_4OH (0.3 mL) were then added. After 24 h, the reaction product was precipitated in alcohol. Thorough washing of the precipitate with alcohol yielded 2.4 g of $Mn(OH)_2$/CAR NC. The resulting NC was a fine brown or light-brown powder, the color intensity of which depended on the mass content of Mn in the sample. The average Mn mass in $Mn(OH)_2$/AG and $Mn(OH)_2$/CAR NCs was 5.2 and 20.3 wt%, respectively [27].

2.1.3 Copper-Containing NCs

To synthesize Cu_2O/AG NC, an aqueous solution of $CuCl_2{\cdot}2H_2O$ (0.09 g in 2 mL H_2O) was added dropwise to a solution of AG (1 g) in 6 mL distilled water under vigorous stirring. The mixture was heated to 320 K (47°C) and maintained for 30 min. Subsequently, 5 mL of an aqueous solution containing $NaBH_4$ (0.08 g) and NaOH (0.003 g) was added as a reducing system. The reaction was continued for 3 h under vigorous stirring. The mixture was filtered, and the product was isolated from the filtrate by double reprecipitation with ethanol to remove low-molecular-weight impurities, followed by vacuum drying. The Cu content, determined by energy-dispersive X-ray (EDX) microanalysis, was 7.5% [28].

To synthesize Cu_2O/ST NC, $CuCl_2{\cdot}2H_2O$ (0.8 g in 8 mL H_2O) was added to a solution of ST (potato starch, grade Extra, 3 g) in 25 mL water under vigorous stirring. The reaction mixture was maintained at 50°C for 30 min, followed by the addition of 15 mL of an aqueous $NaBH_4$ (0.7 g) and NaOH (0.03 g) solution.

After stirring for 3 h, the mixture was filtered. The product was purified by double reprecipitation from ethanol and dried under vacuum. The copper content determined by EDX analysis was 20.3%.

Both NCs were obtained as polymer-stabilized Cu_2O NPs dispersed within the respective polysaccharide matrices. The NC solutions, in which the Cu content in the final concentration was 0.000625%, were used for the experiments [28].

2.2 Potato Treatment with NCs

Potato plants of Lukyanovsky variety were used for the *in vitro* experiment. This variety was provided by the A.G. Lorkh Federal Potato Research Centre (Kraskovo Village, Lyubertsy City, Moscow Region, Russia). It is a mid-early table variety resistant to the golden potato cyst nematode and moderately resistant to late blight. This variety was selected because it is considered susceptible to *Cms* [31], so all physiological reactions are more pronounced in this variety. Micropropagation of test-tube plants was accomplished by cuttings. Control plants were grown on standard Murashige-Skoog (MS) medium. NCs were added as components of the MS medium microsalts, where $MnSO_4{\cdot}4H_2O$ was replaced by $Mn(OH)_2$/AG NC, and $CuSO_4{\cdot}4H_2O$ was replaced by Cu_2O/AG NC. Se NC was added to the MS medium. All NCs were added to the MS medium at a final NP concentration of 0.000625%. The experimental variants are presented in Table 1.

Table 1: Experiments with potato plants *in vitro* using different types of NPs and NC matrix with and without *Cms* infection.

NP	NC Matrix	Without *Cms* Infection	With *Cms* Infection
–	–	Control (5)	*Cms* (5)
–	–	SeO_2 (3)	–
–	–	AG (3)	–
Se	AG	Se/AG NC (5)	Se/AG NC + *Cms* (5)
$Mn(OH)_2$	AG	Mn/AG NC (5)	Mn/AG NC + *Cms* (5)
	CAR	Mn/CAR NC (5)	Mn/CAR NC + *Cms* (5)
Cu_2O	AG	Cu/AG NC (5)	Cu/AG NC + *Cms* (5)
	ST	Cu/ST NC (5)	Cu/ST NC + *Cms* (5)
$Mn(OH)_2$ + Cu_2O	AG	Mn/AG NC + Cu/AG NC (3)	–

Note: Number of plants is indicating in the brackets; AG—arabinogalactan; CAR—carrageenan; ST—starch; NC—nanocomposite; NP—nanoparticle.

Two hours after adding the NC, 1 mL of the *Cms* suspension (titer = 1×10^9 CFU/mL) was added to the potato plant fragments using a sterile syringe, avoiding contact of the bacteria with the plants. The plants were grown under controlled conditions at 26°C and 5–6 Klux illumination for 28 days. Pigment content was then determined.

2.3 Determination of Photosynthetic Pigments

Pigments were extracted with 80% acetone and quantified using a Specord S 100 spectrophotometer (Analytik Jena, Jena, Germany). Chl and carotenoid contents were calculated using the Vernon [32] and Wettstein [33] formulas per unit fresh weight of leaf tissue. A sample of wet leaf tissue was homogenized in 10 mL of a mixture of petroleum ether and acetone, followed by pigment extraction with undiluted acetone. After combining the extracts, the mixture was brought to 25 mL with acetone, and the optical density of the resulting solution was measured at 644, 662 nm, and 440 nm (to determine Chl *a*, Chl *b*, and carotenoids, respectively).

2.4 Statistical Analysis

Microsoft Excel 2007 and SigmaPlot 12.5 programs were used for analysis and statistical processing of the results. The samples were tested for normality using the Shapiro-Wilk test [34]. The differences in pigment content between potato leaf tissue samples isolated from control plants *in vitro*, plants subjected to NCs and infected plants were estimated using the analysis of variance (ANOVA).

3 Results

3.1 Effect of Nanocomposites (NCs) on Photosynthetic Pigment Content in Potato Leaf Tissue

3.1.1 Selenium-Containing NCs

Examples of potato plants infested with *Cms* and treated with Se/AG NC are shown in Fig. 1. Leaves with pronounced lesions were observed in the *Cms*-infested plants. In the Se/AG NC treatment alone, the plants had a rich green color. In the combined Se/AG NC + *Cms* treatment, the color of the plants was less bright green than under the Se/AG NC treatment alone.

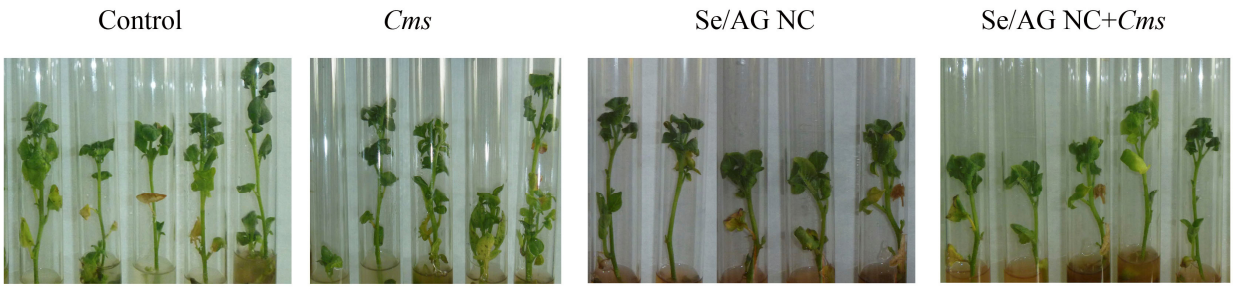

Figure 1: Examples of potato plants infested with *Cms* and treated with Se/AG NC.

It was found that a single treatment of healthy potato plants or those infected with the phytopathogenic *Cms* with Se oxide at a final Se concentration of 0.00625% resulted in a deviation of the Chl *a*/*b* ratio from approximately 1:3 in the control to 1:1.7 and 1:2, respectively, while the total Chl content increased in both cases (Table 2).

This effect is likely due to the development of compensatory metabolic processes under the influence of excessive Se in the plant growth medium, leading to an increase in total Chl content. The increase in the proportion of Chl *b* in the total Chl (*a* + *b*) is presumably due to the oxidation of Chl *a* caused by ROS generated by plant cells in response to the introduction of Se^{+4} ions. The introduction of Se/AG NC with a final Se concentration in the nutrient medium of 0.000625% was characterized mainly by maintaining the Chl *a*/*b* ratio at a level of 3:1, including in the infected plants. At the same time, the total Chl content in the leaves of infected plants was significantly reduced (by 3–5 times) compared to healthy plants, even after treatment with the NCs. In general, it should be noted that the treatment of plants with $Se^{(0)}$ NPs along is characterized by the absence of any negative impact on the Chl content and the ratio of its types in plant leaves, and the Chl levels were maintained at the level of control intact plants, which again confirms the safety of these NCs for potato plants.

Table 2: Content (mean ± S.D.) of Chl *a* and *b* and their ratio in the leaves of healthy and *Cms*-infected potato plants on the third day of incubation under the influence of Se/AG NC (6.4% Se) and its precursors SeO_2 and AG, and in the control.

Potato Plants	Chl	Control	SeO_2	AG	Se/AG NC
Healthy	Chl *a*, mg/g	1.23 ± 0.09	0.72 ± 0.02*	1.63 ± 0.03*	0.95 ± 0.10
	Chl *b*, mg/g	0.70 ± 0.05	0.26 ± 0.03*	0.49 ± 0.04	0.29 ± 0.12
	Chl (*a* + *b*), mg/g	1.94 ± 0.02	0.97 ± 0.02*	2.12 ± 0.03*	1.2 ± 0.08
	Chl *a*/Chl *b*	1.72	2.80	3.32	3.4
Infected with *Cms*	Chl *a*, mg/g	0.35 ± 0.06	0.26 ± 0.03*	0.30 ± 0.12	0.33 ± 0.08
	Chl *b*, mg/g	0.16 ± 0.03	0.09 ± 0.01*	0.10 ± 0.03*	0.12 ± 0.01*
	Chl (*a* + *b*), mg/g	0.51 ± 0.01	0.35 ± 0.04	0.40 ± 0.09	0.45 ± 0.03
	Chl *a*/Chl *b*	2.24	2.88	2.9	2.90

*$p \leq 0.01$ compared to control.

3.1.2 Copper-Containing NCs

Examples of potato plants infested with *Cms* and treated with Cu/AG and Cu/ST NCs are shown in Fig. 2. Treatment with Cu/AG NC resulted in an increase in the length and number of leaves on uninfected plants, as previously published [28]. The leaves were a deep green color. In the combined Cu/AG NC + *Cms* treatment, plants with partially dried leaves were observed, and some plants were light green. When plants were treated with Cu/ST NC, the leaves curled and turned yellow, and some leaves fell off in both uninfected and *Cms*-infected potatoes.

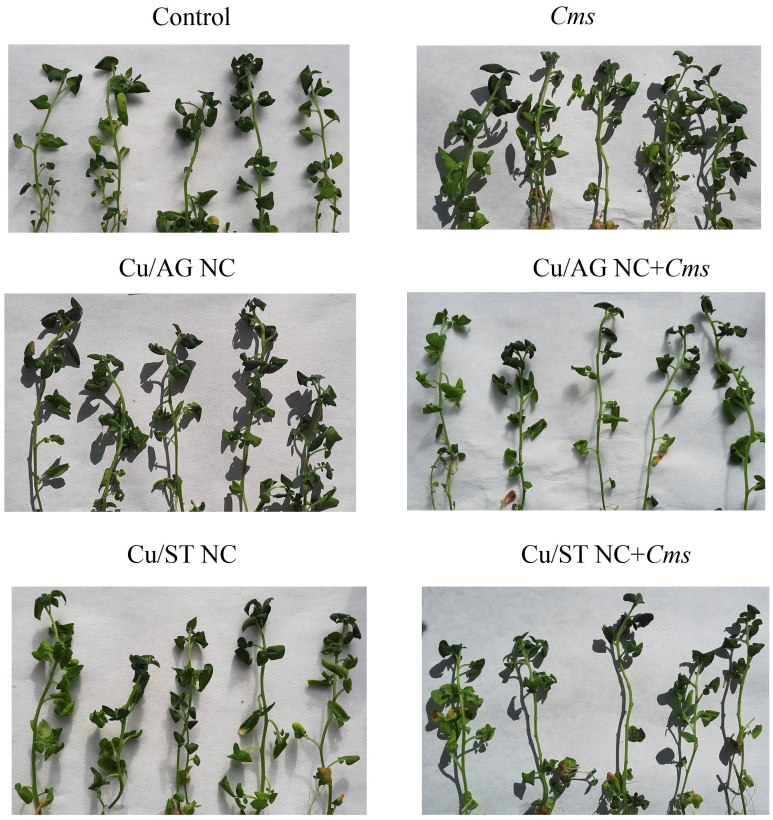

Figure 2: Examples of potato plants infested with *Cms* and treated with Cu/AG and Cu/ST NCs.

The results of *in vitro* potato incubation on nutrient media containing Cu NC are presented in Table 3.

Table 3: Effect of copper-containing NC on pigment concentration (mean ± S.D.) in potato leaf tissue *in vitro*.

Experiment	Chl *a*, mg/g	Chl *b*, mg/g	Chl *a* + Chl *b*, mg/g	Carotenoids, mg/g
Control	0.80 ± 0.30	0.55 ± 0.08	1.35 ± 0.33	1.30 ± 0.24
Cms	0.53 ± 0.10	0.17 ± 0.06*	0.70 ± 0.16	1.22 ± 0.17
Cu/AG NC	1.00 ± 0.09	0.44 ± 0.01	1.44 ± 0.19	1.31 ± 0.03
Cu/AG NC + *Cms*	0.52 ± 0.03	0.37 ± 0.03	0.88 ± 0.06	1.41 ± 0.06
Cu/ST NC	0.24 ± 0.01**	0.37 ± 0.02	0.61 ± 0.03**	0.28 ± 0.01**
Cu/ST NC + *Cms*	0.19 ± 0.03**	0.26 ± 0.01	0.45 ± 0.01**	0.20 ± 0.01**

*$p \leq 0.05$ and **$p \leq 0.01$ compared to control.

Infection significantly reduced Chl *b* content. Cu/AG NC had no effect on potato pigment content. Cu/ST NC significantly reduced Chl *a* and carotenoids in both infected and uninfected plants. These data indicate that Cu/ST NC has a negative effect on the amount of photosynthetic pigments in potatoes. Therefore, Cu/AG NC was used in further experiments to study the combined effect of NCs on pigment content.

3.1.3 Manganese-Containing NCs

Examples of potato plants infested with *Cms* and treated with Mn/AG and Mn/CAR NCs are shown in Fig. 3. As in the previous series of experiments, potato plants infected with *Cms* had a light green color. Mn-containing NCs had no visual negative impact on uninfected plants, and even when potatoes were infected with *Cms*, they maintained the bright green color of the aboveground parts.

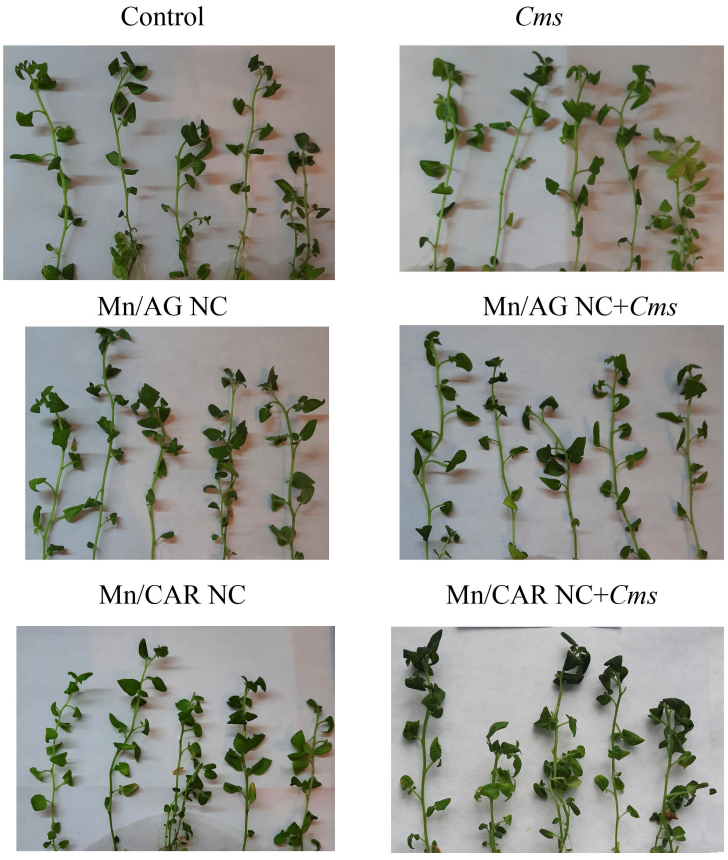

Figure 3: Examples of potato plants infested with *Cms* and treated with Mn/AG and Mn/CAR NCs.

The effect of Mn NCs *in vitro* on the pigment content in leaf tissues of both healthy potato plants and those infected with *Cms* is presented in Table 4. Infection reduced the content of all potato pigments. Mn/AG NC slightly reduced Chl *b* content in healthy plants. However, upon infection, this NC increased Chl *a* and carotenoids content. Mn/CAR NC reduced Chl *b* content. Therefore, Mn/AG NC was used in further experiments to study the combined effect of NCs on pigment content, as it increased pigment content under biotic stress.

Table 4: Effect of manganese-containing NC on pigment concentration (mean ± S.D.) in potato leaf tissue *in vitro*.

Experiment	Chl *a*, mg/g	Chl *b*, mg/g	Chl *a* + Chl *b*, mg/g	Carotenoids, mg/g
Control	1.28 ± 0.02	0.95 ± 0.02	2.23 ± 0.04	1.79 ± 0.05
Cms	0.40 ± 0.02**	0.29 ± 0.03**	0.69 ± 0.05**	1.04 ± 0.01**
Mn/AG NC	0.94 ± 0.12	0.78 ± 0.04*	1.72 ± 0.15*	1.71 ± 0.10
Mn/AG NC + *Cms*	1.69 ± 0.01**	0.87 ± 0.01	2.56 ± 0.03**	2.00 ± 0.01**
Mn/CAR NC	0.96 ± 0.20	0.62 ± 0.10*	1.58 ± 0.30	1.58 ± 0.11
Mn/CAR NC + *Cms*	1.54 ± 0.18	0.80 ± 0.06	2.35 ± 0.24	1.97 ± 0.11

*$p \leq 0.05$ and **$p \leq 0.01$ compared to control.

3.1.4 Combined NCs

Considering that Mn, like Cu, is an essential element for the successful functioning of the plant photosynthetic apparatus, it was interesting to test the combined effect of Mn- and Cu-containing NCs on the concentration of photosynthetic pigments in potato leaf tissue *in vitro*. Cu/AG NC was chosen as the Cu-containing NC in the combined treatment, because it was found that it did not have a negative effect on pigment content, unlike Cu/ST NC (Table 3). The obtained results are presented in Fig. 4. The results showed that Mn-containing NCs increased the carotenoids content in potato tissue. This effect was negated by combined treatment of plants by Mn-containing NCs together with Cu-containing NCs.

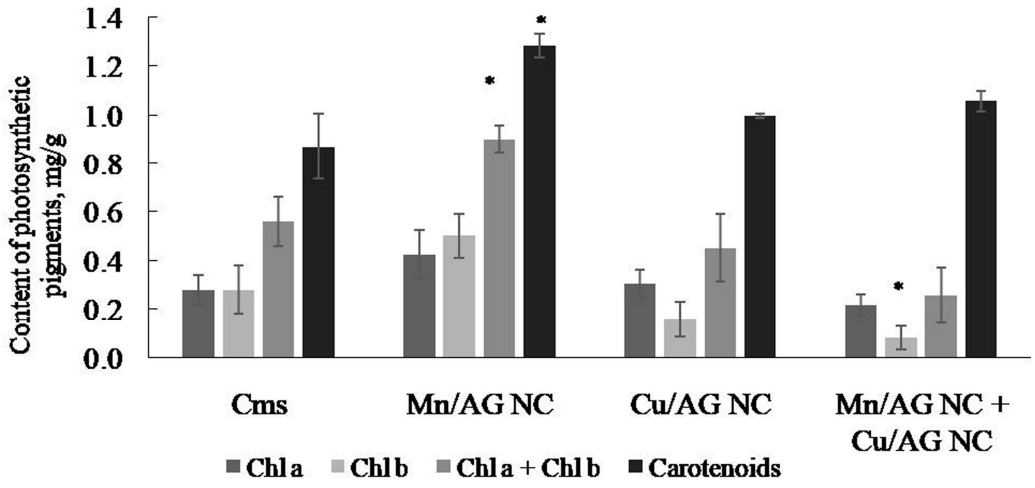

Figure 4: Effect of copper- and manganese-containing NC on the pigment content (mean ± S.D.) in potato leaf tissues *in vitro*; *$p \leq 0.01$ compared to control.

4 Discussion

Plants and photosynthetic bacteria convert solar radiation into chemical energy through photosynthesis, thereby sustaining primary productivity and supporting nearly all life on Earth. Photosynthetic pigments, principally Chls and carotenoids, are integral components of the photosynthetic apparatus, capturing light

energy to drive electron transport reactions [35]. Chl functions as the primary light-harvesting pigment, whereas carotenoids contribute both to light capture and to photoprotection by dissipating excess energy. Together, these pigments perform complementary roles that maintain photosynthetic efficiency and protect plant cells from oxidative damage [36].

Chl is the central pigment of the photosynthetic machinery, responsible for photon absorption and energy transfer to the reaction centers of photosystems [37]. Upon light absorption, Chl molecules undergo electronic excitation and transfer energy to the photosystems, thereby initiating photochemical reactions. In higher plants, Chl metabolism is tightly regulated through coordinated enzymatic steps and transcriptional control. Biosynthesis begins with glutamate, which is converted to 5-aminolevulinic acid and subsequently to protochlorophyllide (Pchlide) via a multistep pathway involving enzymes such as glutamyl-tRNA reductase and magnesium chelatase (subunit H) [35,38]. Precise regulation of Chl and its intermediates is critical, as Chl metabolism influences not only photosystem assembly but also processes including programmed cell death, the "stay-green" phenotype, and chloroplast–nucleus signaling [39]. Excessive accumulation of Pchlide, a potent photosensitizer, can promote reactive oxygen species (ROS) generation, leading to growth inhibition or accelerated cell death [40]. Chl degradation is induced during stress and senescence, and substantial progress has been made in elucidating its regulatory mechanisms [41,42]. Structurally, Chl a contains a methyl group that contributes to its photochemical properties and renders it more susceptible to photodegradation relative to other pigments, while Chl b broadens the absorption spectrum and enhances light-harvesting efficiency, thereby supporting biomass accumulation [43].

Carotenoids are isoprenoid pigments synthesized *de novo* in all photosynthetic organisms. In plants, they confer yellow, orange, and red coloration to leaves, flowers, fruits, and certain storage organs [44]. Functionally, carotenoids participate in photosynthesis, photoprotection, pigmentation, phytohormone biosynthesis, and intracellular signaling [45]. Synthesized in plastids, they are integral to light-harvesting complexes and play a critical role in protecting the photosynthetic apparatus from oxidative stress. In addition, carotenoids serve as precursors of apocarotenoids, including vitamin A derivatives and the phytohormones abscisic acid and strigolactones, which regulate plant development and stress responses [46,47].

Carotenoid biosynthesis originates from geranylgeranyl diphosphate and proceeds through a series of enzymatically regulated reactions leading to the formation of major carotenoids such as lutein, zeaxanthin, and β-carotene. Key enzymes include phytoene synthase, phytoene desaturase, and lycopene β-cyclase. This pathway is tightly controlled at the transcriptional level and responds to environmental and light signals, with red and blue wavelengths particularly effective in promoting carotenoid accumulation [48].

Carotenoids are catabolized by carotenoid cleavage dioxygenases, generating apocarotenoids such as abscisic acid and strigolactones [49]. These metabolites function as signaling molecules that regulate plant growth, development, and adaptive stress responses. The dynamic balance between carotenoid biosynthesis and degradation ensures adequate light harvesting and photoprotection while preventing excessive accumulation that could disrupt cellular homeostasis.

Beyond their role in pigmentation, carotenoids are essential for protecting the photosynthetic apparatus from photooxidative damage. They associate with Chls in photosystem I (PSI) and photosystem II (PSII) complexes and are embedded within the thylakoid membrane lipid bilayer, where they suppress uncontrolled energy transfer to molecular oxygen and limit ROS formation. Moreover, carotenoids function as accessory pigments by absorbing wavelengths outside the principal absorption range of Chls and transferring this energy to the reaction centers, thereby enhancing overall photosynthetic performance [36].

Chl *a*, the main photosynthetic pigment in plants, contains a methyl (-CH_3) group, whose key function is photon binding, and is more sensitive to photodegradation than other pigments [50]. Chl *b* plays an important role in improving light absorption efficiency and, thus, increasing energy and biomass production in plants [51]. In our experiments, Se NCs and Cu NCs increased Chl content. A similar effect was previously described in a study of strawberries (*Fragaria ananassa*) under greenhouse experimental conditions, where it was shown that the application of nanoselenium (at concentrations of 25, 50, 75, and 100 mg L^{-1}) and nanocopper (at concentrations of 50 and 100 mg L^{-1}) increased the Chl content and its fluorescence intensity [52]. Moreover, the increase in Chl *a* content under the treatments was more pronounced than that of Chl *b*. In addition, Se nanofertilizer increased Chl levels more than Cu NP-based nanofertilizer. The highest Chl values were recorded after the application of 100 mg L^{-1} Se nanofertilizer. CuO nanofertilizer had an obvious effect on the minimum and maximum fluorescence parameters (F_0 and F_m), with significantly higher values (513 and 1713) compared to the control and Se nanofertilizer [52].

Spraying the leaves of Moldavian dragonhead (*Dracocephalum moldavica* L.) with CuO NPs resulted in an increase in the photosynthetic pigments Chl *a* and Chl *b* by 77% and 123%, respectively [20]. When growing rice (*Oryza sativa* L.) under hydroponics, it was shown that the content of photosynthetic pigments initially increased and then decreased with increasing CuO NP concentrations (0, 10, 20, and 50 mg L^{-1}) [53]. Foliar treatment with 5 mg L^{-1} bimetallic NPs consisting of the micronutrients Cu and Fe increased the energy and efficiency of photosynthetic activity in rice *O. sativa* plants [54]. CuO and Fe_2O_3 NPs stimulated the growth of pomegranate *Punica granatum* L. variety Hegazy and provided resistance to the root-knot nematode *Meloidogyne javanica* [55]. An increase in the level of photosynthetic pigments, both chlorophylls and carotenoids, was observed. The authors believe that the effect of NPs partly depends on the increase in the production of photosynthetic pigments [55]. The Chl *a* content increased in sunflower seedlings after seed soaking in a Cu NP solution compared to the control, while the Chl *b* content decreased by 3–7% [56]. Bimetallic Cu-Se NPs synthesized using *Aspergillus niger* increased the resistance of eggplant *Solanum melongen* to the bacterial pathogen *Ralstonia solanacearum* and positively affected photosynthetic pigments, increasing the levels of chlorophyll and carotenoids [57]. In an experiment on wheat (*Triticum aestivum* L.) under greenhouse conditions, it was shown that photosynthetic pigments and gas exchange parameters were significantly increased when treated with Se NPs obtained by green synthesis, compared to the control [58].

Exogenous application of nanoselenium with glutathione (SeG) significantly enhanced the growth of cucumber *Cucumis sativus* plants and increased the content of photosynthetic pigments [59]. Se NPs (10 mg L^{-1}), chitosan (CS) (0.1%), and CS-Se NPs (at two concentrations of 5 and 10 mg L^{-1}) were shown to increase the content of photosynthetic pigments in leaf tissues of Moldovan lemon balm (*Dracocephalum moldavica* L.) [60]. Spraying the leaves of red bean (*Phaseolus vulgaris* L.) plants with Se NPs coated with gum arabic resulted in a significant increase in the content of photosynthetic pigments and the area of photosynthetic plant organs (cm^2 $plant^{-1}$) [61]. Under field conditions, spraying sweet wormwood (*Artemisia annua* L.) leaves with Se NPs resulted in a significant increase in photosynthetic pigments [62]. In common bean (*Phaseolus vulgaris*), CuO NPs were shown to improve plant vegetative growth and, in particular, increase the content of photosynthetic pigments: Chl *a* (2.96 mg/g), Chl *b* (1.93 mg/g), and total carotenoids (1.16 mg/g) [63]. Spraying tomatoes (*Solánum lycopérsicum*) with Se NPs promoted the accumulation of photosynthetic pigments [64]. Spraying lemon verbena (*Aloysia citrodora*) with Se NPs increased the content of photosynthetic pigments [65]. Se has been shown to have a beneficial effect on increasing photosynthetic pigments in *Cofféa arábica* leaf tissue [66]. In sesame (*Sesamum indicum* L.), Se NPs have been shown to increase Chl *a* and Chl *b* content [67]. Eco-friendly Se NPs synthesized

via *Sternbergia candida* reduced salt stress in pepper (*Capsicum annuum* L.) plants by increasing the content of chlorophyll and carotenoids [68]. A similar stimulating effect of Se NPs on the amount of photosynthetic pigments was shown in wheat (*T. aestivum*) under salt stress in a field experiment [69]. Se NPs increased the content of photosynthetic pigments in the medicinal plant *Echium italicum* L. [70]. Spraying Se NPs on the leaves of broccoli (*Brassica oleracea* var. *italic*) mitigated the effects of drought by improving pigment stability and increasing carotenoid content [71]. In our experiments, Mn-containing NPs increased carotenoid content. In field experiments, the addition of CuO NPs to the soil significantly affected photosynthetic pigments (Chl *a*, Chl *b*, and carotenoids) in soybean (*Glycine max* L.). Carotenoid content was 2.5 times higher with nCuO-S treatment [43].

In our experiments, a decrease in pigments was also recorded under the influence of NPs. This effect was demonstrated by Cu/ST NC, as well as by combined Cu- and Mn-containing NCs. The high NP content of 20% in the NC apparently had a negative impact on plant cells, causing oxidative stress. Under the combined effect of the two NCs, Chl *b* was more sensitive than Chl *a* and carotenoids. Effect of studied NCs on potato photosynthetic pigments *in vitro* is briefly summarized in Table 5.

Table 5: Summary of the effect of NC on potato photosynthetic pigments *in vitro*.

Effect	Se NC	Cu NC	Mn NC	Cu/AG NC + Mn NC
Pigment content	Se/AG NC did not affect chlorophyll content in both healthy and infected plants.	• Cu_2O/AG NC increases chlorophyll and carotenoid content in healthy and infected plants. • Cu_2O/ST NC reduces pigment levels in healthy and infected plants.	Mn/AG NC causes an increase in the amount of chlorophylls and carotenoids during infection.	Decreased Chl *b* content

In the study presented here, we did not separately examine the possible effect of free Se, Cu, and Mn ions, which could hypothetically be formed during treatment with Se-, Cu-, and Mn-containing NCs, since the study presented here was preceded by a series of studies devoted to the biological effect of these metal-containing NCs (MCNCs) on potatoes *in vitro* [26–28]. We have previously published the results of several studies of the effect of these studied MCNCs on the biometric and biochemical characteristics of potato plants *in vitro* [26–28]. In particular, at the initial stage of the study, we compared the effect of Se NC with the effect of Se in ionic form. For example, as one of the controls for the study of the effect of Se NCs on the biometric and biochemical characteristics of potato plants *in vitro*, the effect of SeO_2 along was compared with the effect of Se NC [72]. It was found that SeO_2 had a negative effect on plant viability compared to the Se NC treatment. In another study, we also investigated the effect of sodium bis(2-phenylethyl) diselenophosphinate (BIS), a precursor of Se NC, on potato viability *in vitro* [26]. It was also found that when Se-containing NC and BIS were used at equal concentrations for plant treatment, BIS suppressed plant growth and development, while Se NC had a positive effect on all studied parameters.

Furthermore, in one of our earlier studies [73], we studied the content of free elemental Se from Se NC in potato tissues *in vitro* using energy dispersive X-ray microanalysis (EDXMA). It was found that the Se content in potato leaf tissues was 0.01–0.03% of air-dry weight, indicating insignificant Se penetration into potato tissues from Se NC and its insignificant accumulation in the plant body after Se NC treatment [73].

Similar studies of the effect of free Cu and Mn ions on potatoes *in vitro* were also carried out in early studies of Cu and Mn NCs and showed a positive and stronger effect of these metals in the composition of

NCs, and not in the form of free ions [27,28]. Therefore, we have every reason to believe that it is the studied NCs that: (1) reduce oxidative stress in plant tissues, which was reflected in a decrease in the amount of reactive oxygen species, modulation of the activity of antioxidant enzymes (catalase, peroxidase), a decrease in lipid peroxidation products—diene conjugates and malondialdehyde [26–28,72]; (2) activate proteins involved in pathogenesis, which is also confirmed by new data on an increase in the level of PR gene expression in potato tissues *in vitro* when they are treated with Se-, Cu-, and Mn-containing NCs, obtained by us recently and not yet published; (3) have an antibacterial effect on phytopathogenic bacteria, which we have previously shown in [74]; (4) have an effect on the hormonal status of plants (we are currently conducting research in this direction).

5 Conclusions

The results presented in this article demonstrate the absence of a negative effect of Se, Cu, and Mn NCs based on AG matrix on the content of photosynthetic pigments in potato leaf tissues *in vitro*. Se/AG and Cu/AG NCs increased the Chl content in both healthy plants and those infected with the pathogen *Cms*. Mn/AG NC increased the carotenoid content. Cu/ST NC decreased the amount of pigments in healthy plants and those infected with Cms. The combined effect of Mn/AG and Cu/ST NCs led to a decrease in Chl *b*. Thus, it can be concluded that NCs based on AG do not have a negative effect on the pigment system of plants, which is in agreement with our other studies, they can be considered as safe agents for plant enhancement and protection from phytopathogenic bacteria.

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