

Article

Effect of Silver Nanoparticles on Growth of Wheat: Is It Stage-Specific or Not?

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Abstract

Experimental studies published to date on the effects of silver nanoparticles (AgNPs) on plants have yielded highly contradictory results: reported outcomes range from growth inhibition to stimulation. The objective of this research was to test the hypothesis that the ontogenetic stage at the time of exposure to AgNPs is a key determinant of both the qualitative profile and quantitative magnitude of plant responses. For this purpose, laboratory seed priming and small-plot field experiments with wheat plants (*Triticum aestivum* L.) treated with stabilized dispersions of AgNPs at 1–100 mg·L^{−1} were conducted. It was shown that seed priming with low concentrations of AgNPs (1–5 mg·L^{−1}) did not affect wheat seedling growth, whereas dispersions at ≥25 mg·L^{−1} suppressed development. In agreement, antioxidant enzyme activities (POD, CAT, PPO) increased at 1–5 mg·L^{−1} and decreased at 100 mg·L^{−1}. By contrast, foliar treatments of field-grown wheat increased plant population density, plant height, spike structure metrics, and grain yield. The optimal regimen—three foliar applications at 5 mg·L^{−1}—increased grain yield by 12.1% from 5.89 t·ha^{−1} to 6.60 t·ha^{−1}. At low doses of AgNPs, activities of peroxidase, catalase, and polyphenol oxidase in seedlings tissues increased, indicating activation of nonspecific defense mechanisms; at higher concentrations, activities of these enzymes decreased, indicating antioxidant system exhaustion and dysfunction. The findings demonstrate dose- and stage-dependent effects and corroborate the central role of the developmental stage of wheat in determining responses to AgNPs, indicating opportunities to optimize stage-aware, low-dose application regimes to enhance productivity while minimizing phytotoxic risk.

Keywords: antioxidant enzymes; foliar application; nanoprimering; silver nanoparticles (AgNPs); sustainable agriculture; wheat; plant growth stimulation; grain yield



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1. Introduction

Silver nanoparticles (AgNPs) are ultrafine particles of metallic silver with a size of 1–100 nm in diameter [1,2]. Owing to their very high specific surface area (up to $100 \text{ m}^2 \cdot \text{g}^{-1}$), AgNPs exhibit a set of distinctive physicochemical, optical, and biological properties that are not inherent in the bulk form of silver [3–5]. Their antimicrobial activity against pathogenic bacteria [6,7], fungi [8,9], and viruses [10] has been extensively studied, leading to widespread use of AgNPs in medical and veterinary products for treating infected skin wounds, managing inflammatory diseases, preventing microbial complications, and as coatings for medical devices [11,12]. Furthermore, these antimicrobial properties have driven incorporation of AgNPs into a broad range of consumer products, including cosmetics and cleaning agents, disinfectants, packaging materials, and water-purification filters [13–16].

In recent years, the effects of AgNPs on plants—particularly on growth, development, and crop productivity—have been intensively investigated [17,18]. Numerous studies have shown that AgNPs can penetrate plant tissues via both roots and leaves, and subsequently translocate through the vascular system, and exert complex effects on multiple physiological processes [19,20]. Alterations in the photosynthetic apparatus have been observed, including changes in chlorophyll content and chlorophyll fluorescence, as well as in the abundance and activity of proteins involved in the formation of photosystems, which may lead to either enhancement or suppression of photosynthetic activity [21,22]. Another important component of the plant response to AgNPs is a shift in the pro-/antioxidant balance due to overproduction of reactive oxygen species (ROS), induced by AgNPs, which stimulates the activities of redox homeostasis enzymes [23,24]. Proteomic studies indicate changes in the regulation of proteins of primary metabolism, including enzymes involved in respiration, and the biosynthesis of nucleic acids, proteins, and lipids, as well as proteins associated with polypeptide folding and cell wall biogenesis [21,25]. Furthermore, stress-responsive proteins are activated, including chaperonins and chitinases implicated in pathogen defense and the regulation of cell wall structure [26]. Particular attention has been given to changes in hormonal regulation: AgNPs affect the pathways associated with auxin, abscisic acid (ABA), and ethylene, which control root and shoot growth and development, as well as fruit ripening processes [27].

The described molecular and physiological alterations at the cellular level manifest at the whole-plant scale as changes in growth parameters. Nevertheless, the published experimental data are highly contradictory. Some studies report growth-promoting effects of AgNPs—seen as faster germination, greater root and shoot length, enhanced tillering, and accelerated leaf-area development—ultimately resulting in higher biomass and yield [28–30]. Other studies, by contrast, describe suppression of these processes and slower plant growth and development [31,32]. This divergence has been attributed to multiple factors. The applied dose of AgNPs is considered the most important factor. At relatively low concentrations, plants often display a stimulatory response characterized by activation of the antioxidant system, enhancement of photosynthetic activity, and induction of stress-protective proteins [33,34]. Such changes can increase plant tolerance to adverse conditions and promote growth [35]. However, exceeding a biologically optimal concentration range leads to accumulation of ROS, disruption of hormonal regulation, membrane damage, and inhibition of key metabolic enzymes, resulting in growth retardation and reduced productivity [36–38]. The physicochemical characteristics of the NPs themselves, including size, shape, and stabilizer type, which determine the colloidal stability of dispersions, bioavailability, and the rate of release of cytotoxic silver ions Ag^+ , are also influential [26,39]. In addition, the experimental conditions are of significant importance. For example, in soil systems, the bioavailability of AgNPs may be reduced by their immobilization on

porous solid surfaces and by interactions with soil organic matter, whereas in laboratory hydroponic assays plants are exposed to AgNPs directly [40,41]. In addition to the factors outlined above, based on the analysis of extensive experimental evidence, we hypothesized that the plant developmental stage at which exposure to AgNPs occurs plays a key role [42]. We proposed that plant responses to AgNPs may differ qualitatively depending on the maturity of physiological systems and the degree of development of the antioxidant defense system, thereby explaining the opposing effects observed.

Within the agenda of sustainable agriculture, technologies that deliver agronomic gains at minimal material input and environmental cost are of particular interest [43]. The divergent outcomes reported for nanosilver therefore underscore the need to define application regimens that are both effective and safe. Determining whether plant ontogeny modulates responsiveness to nanosilver is essential for designing stage-aware, low-dose regimens that maximize agronomic benefit while minimizing inputs and environmental risk. Thus, the objective of this study was to experimentally evaluate the effects of stabilized dispersions of AgNPs on wheat at distinct developmental stages by assessing changes in growth metrics and yield components. In addition, to explain the observed responses, activities of antioxidant enzymes were measured in parallel with changes in plant morphological parameters.

2. Materials and Methods

2.1. Materials

The following reagents were used for the synthesis of silver nanoparticles: silver nitrate (AgNO_3 , $\geq 99\%$, Sigma-Aldrich, St. Louis, MO, USA); sodium borohydride (NaBH_4 , $\geq 99\%$, Sigma-Aldrich, St. Louis, MO, USA); sodium laureth sulfate ($\geq 99\%$, RusChem LLC, Moscow, Russia); and sodium amphopolycarboxyglycinate (30 wt% aqueous solution, AkzoNobel, Amsterdam, The Netherlands). Distilled water was used in all experiments where needed.

2.2. Preparation of Dispersions of AgNPs

APCG was selected as a colloidal stabilizer because, as shown in our previous works, APCG-capped dispersions of AgNPs exhibit high colloidal stability and, consequently, strong biological activity [44,45]. SLES was included as a reference because sulfate-based anionic surfactants are well-studied, commercially available stabilizers for dispersions of AgNPs [44,46,47]. Notably, at the working pH both APCG- and SLES-capped AgNPs carry a negative ζ -potential, enabling comparison of biological activity while minimizing the influence of surface charge.

Aqueous dispersions of silver nanoparticles stabilized with sodium laureth sulfate (SLES) or sodium amphopolycarboxyglycinate (APCG) were prepared by chemical reduction of silver nitrate with sodium borohydride. The structural formulas of the stabilizers are shown in Figure 1.

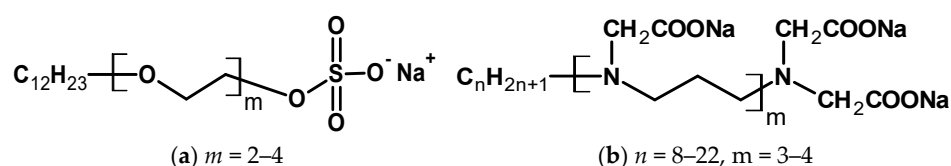


Figure 1. Structural formulas of the stabilizers used for colloidal stabilization of dispersions of AgNPs: (a) SLES; (b) APCG.

For all preparations, the minimum stabilizer-to-silver mass ratio that ensured high colloidal stability of the dispersions was used. For the preparation of 1 L of a dispersion of AgNPs with a silver concentration of $200 \text{ mg} \cdot \text{L}^{-1}$, 200 g of an aqueous solution containing

0.3149 g of AgNO_3 was added dropwise to 600 g of an aqueous stabilizer solution containing 1.6000 g of either SLES or APCG under vigorous stirring (500 rpm). After 15 min of stirring, 200 g of a solution containing 0.1408 g of NaBH_4 was added dropwise. The release of hydrogen and a color change in the reaction mixture from colorless to yellow–brown indicated the formation of AgNPs. The mixture was stirred for 2 h until visible hydrogen evolution ceased. The resulting dispersions were characterized by transmission electron microscopy (TEM), selected area electron diffraction (SAED), UV–Vis spectroscopy, and dynamic light scattering (DLS).

2.3. UV–Vis Spectrophotometry

UV–Vis absorption spectra were recorded on a UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan) in the range of 330–700 nm using quartz cuvettes (optical path length 10 mm).

2.4. TEM and SAED

TEM micrographs and microdiffraction patterns were obtained using a JEOL JEM-2100 (JEOL, Tokyo, Japan) operated at 200 kV. Samples were prepared by applying 1–2 μL of dispersion ($100 \text{ mg} \cdot \text{L}^{-1}$) on carbon grids, followed by air drying.

2.5. DLS

Hydrodynamic diameters and zeta potential (ζ -potential) of the obtained nanoparticles were determined using a Zetasizer Nano ZS (Malvern Instruments, Malvern, UK). Measurements were performed in quartz cuvettes (10 mm optical path; scattering angle 90°) at a concentration of AgNPs of $50 \text{ mg} \cdot \text{L}^{-1}$. The data were processed in DynaLS v2.0 software using a monomodal Gaussian distribution model.

2.6. Effect of Priming with Dispersions of AgNPs on Seed Germination

Wheat seeds (*Triticum aestivum* L.) were soaked in sterile distilled water for 12 h at room temperature. After soaking, the seeds were placed in 9 cm Petri dishes on moistened filter paper, 10 seeds per dish, with four replicates per treatment. Test solutions consisted of dispersions of AgNPs stabilized with SLES or APCG at 1, 5, 25, and $100 \text{ mg} \cdot \text{L}^{-1}$, as well as solutions of the stabilizers at concentrations those used in the corresponding dispersions. Five milliliters of the corresponding dispersion or solution were added to each dish; control dishes received 5 mL of sterile distilled water. Dishes were covered with aluminum foil to exclude light and incubated in darkness at 25°C for 48 h. The dishes were then transferred to a climate chamber (Sanyo MLR-351H, SANYO Electric Co., Ltd., Osaka, Japan) set to $25 \pm 0.5^\circ\text{C}$, 60% relative humidity, a 12 h light/12 h dark photoperiod, and 1200 lx illumination (cool fluorescent lamps). On day 7, the following parameters were assessed: root and shoot length (digital caliper, 0.1 mm resolution) and total fresh biomass of seedlings (determined gravimetrically after blotting excess moisture with filter paper).

2.7. Field Trials

The field study was conducted on winter wheat (cv. Skipetr, *Triticum aestivum* L.). Plants exhibit a semi-prostrate growth habit and short to medium height. The spike is white, cylindrical, loose to medium in density, and of medium length. Plant height is 79–96 cm; lodging resistance is high. The growing period is 297–338 days, with enhanced winter hardiness.

Field trials were conducted in 2024 in the Ostrogozhsky District, Voronezh Oblast, Russia. The experimental site was located at 134–159 m a.s.l. on slopes with gradients of 6.23 – 15.15° . Soils were typical Chernozems with 4.35% humus in the arable layer, pH 5.5, available phosphorus (as P_2O_5) 130–144 mg kg^{-1} , and exchangeable potassium (as K_2O) 172–181 mg kg^{-1} . During the winter of 2023–2024, adverse weather conditions resulted

in 18% winterkill in the control plots. Sowing took place on 11 September 2023. Each experimental plot measured 60 m²; the trial was conducted with three replicates. The preceding crop was clean (black) fallow.

Treatments were applied by foliar spraying to field-grown wheat stands. Dispersions of AgNPs were applied at 1–25 mg·L^{−1}, with concentrations chosen based on economic feasibility. A working spray volume of 200 L ha^{−1} was used, with either two or three applications at the following stages: first—tillering (Zadoks 25–29); second—stem elongation (flag leaf emergence, Zadoks 37–39); third—heading (Zadoks 55–59). Treatments were performed in the evening under wind speeds ≤ 5 m s^{−1} and in the absence of precipitation. Six treatment regimens were tested:

1. Control—background fertilization NPK 40:60:40 kg ha^{−1} (as N, P₂O₅, and K₂O);
2. NPK 40:60:40 + two foliar applications at tillering and stem elongation of an APCG solution at 200 mg·L^{−1};
3. NPK 40:60:40 + two foliar applications at tillering and stem elongation of a dispersion of AgNPs at 1 mg·L^{−1};
4. NPK 40:60:40 + two foliar applications at tillering and stem elongation of a dispersion of AgNPs at 5 mg·L^{−1};
5. NPK 40:60:40 + three foliar applications at tillering, stem elongation, and heading of a dispersion of AgNPs at 5 mg·L^{−1};
6. NPK 40:60:40 + two foliar applications at tillering and stem elongation of a dispersion of AgNPs at 25 mg·L^{−1}.

Weeds were controlled uniformly across all plots using the standard herbicide program of the farm, with applications pre-emergence and at tillering (Zadoks 20–29).

Plant population density was recorded manually at the tillering, stem elongation, heading, and milk stages (Zadoks 73–77). Plant height, and number of productive stems were recorded manually at the milk stage, following [48]. After harvest, the number of spikelets per spike and grains per spike, grain weight per spike, thousand-kernel weight (TKW), and grain yield were determined.

2.8. Antioxidant Enzyme Activity

To determine antioxidant enzyme activity, stem tissue from wheat seedlings was collected on day 7 after germination in Petri dishes, and tissue from field-grown plants was sampled at late heading (Zadoks 59). Samples were immediately frozen in liquid nitrogen for subsequent analyses. For extract preparation, 200 mg of stem or leaf tissue was homogenized in 2 mL of ice-cold phosphate buffer (pH 6.8–7.2) containing 1 wt% polyvinylpyrrolidone (PVP). The homogenate was centrifuged at 12,000× *g* for 15 min at 4 °C, and the resulting supernatant was used for the enzyme assays.

2.8.1. Peroxidase (POD) Activity

POD activity was determined spectrophotometrically by the guaiacol method, monitoring the increase in absorbance at 470 nm every 15 s over 3 min [49]. The reaction mixture contained 1.7 mL of 50 mM phosphate buffer (pH 7.0), 200 µL of 0.1 M guaiacol, 100 µL of 0.15% H₂O₂, and 100 µL of enzyme extract.

2.8.2. Catalase (CAT) Activity

CAT activity was determined spectrophotometrically according to Aebi [50] by recording the decrease in absorbance at 240 nm due to H₂O₂ decomposition every 10 s over 1 min. The reaction mixture contained 1.9 mL of 10 mM H₂O₂ prepared in 50 mM phosphate buffer (pH 7.0) and 100 µL of enzyme extract.

2.8.3. Polyphenol Oxidase (PPO) Activity

PPO activity was determined by the catechol method [51], recording the increase in absorbance at 420 nm every 10 s over 3 min. The reaction mixture contained 1.8 mL of 50 mM phosphate buffer (pH 6.8), 100 μ L of 0.33 M catechol, and 100 μ L of enzyme extract.

2.9. Statistical Analysis

Data from in vitro experiments are presented as mean $\pm$ standard deviation (SD) from three independent biological replicates ($n = 3$). Statistical significance was determined using one-way ANOVA followed by Tukey's Honestly Significant Difference (HSD) test ($p < 0.05$) implemented in Python 3.11.4 SciPy and statsmodels libraries (Supplementary Materials, Figure S1). For field trials, LSD for morphological traits, grain yield components, and grain yield were calculated at $p < 0.05$ according to Dospekhov, using Fisher's F-test [52].

3. Results

3.1. Preparation and Colloidal Characteristics of Dispersions of AgNPs

The minimum concentrations of SLES and APCG required to ensure colloidal stability of dispersions of AgNPs were determined by comparing the light absorbance of the dispersions at $\lambda = 420$ nm. The most stable dispersions, whether stabilized with SLES or with APCG, were obtained at a silver/stabilizer mass ratio of 1:8. The resulting dispersions of AgNPs were characterized by TEM and DLS.

According to TEM, APCG-stabilized AgNPs had a smaller mean diameter (10.4 ± 4.9 nm) than that of SLES-stabilized AgNPs (21.9 ± 9.1 nm) (Figure 2; Table 1). Moreover, particles in the AgNPs–SLES dispersion were frequently multiparticle aggregates of heterogeneous morphology, indicative of undesirable coagulation. Notably, the DLS-derived diameters of the obtained AgNPs exceeded those measured by TEM, which was expected, as DLS measures the hydrodynamic diameter, which includes the adsorbed surfactant layer and the hydration shell surrounding the NPs.

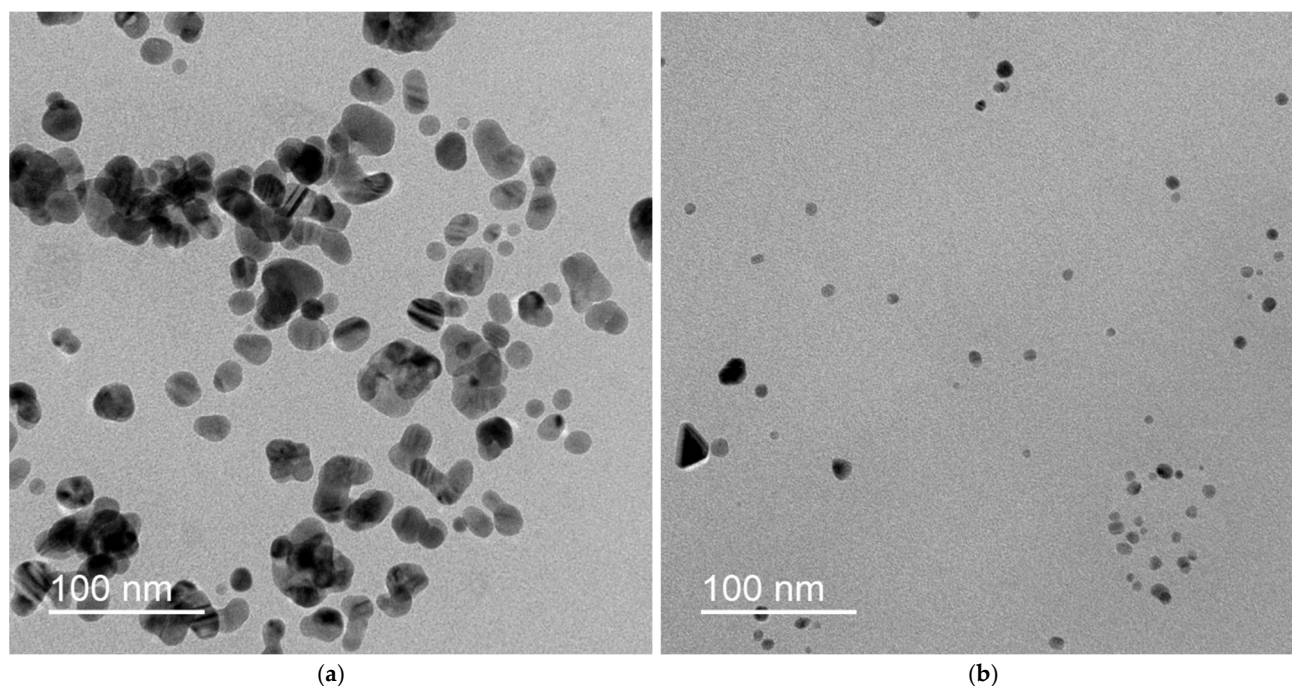


Figure 2. TEM images of silver nanoparticles stabilized with (a) SLES; (b) APCG.

Table 1. Colloidal characteristics of dispersions of AgNPs; d_{TEM} —mean particle diameter determined by TEM; d_{DLS} —mean hydrodynamic diameter determined by DLS; ζ —zeta potential (electrokinetic potential).

Stabilizer	d_{TEM} , nm	d_{DLS} , nm	ζ , mV
SLES	21.9 ± 9.1	60.2 ± 8.8	−28.2
APCG	10.4 ± 4.9	27.2 ± 5.3	−52.6

To confirm the crystalline phase of silver, SAED in TEM was used (Figure 3). Interplanar d-spacings were obtained from the diameters of the first four diffraction rings. The measured values for the AgNPs–APCG sample (2.35, 2.02, 1.43, 1.22 Å) were in agreement with the standard values for metallic Ag (2.36, 2.04, 1.44, 1.23 Å), confirming the crystalline phase of metallic silver [53].

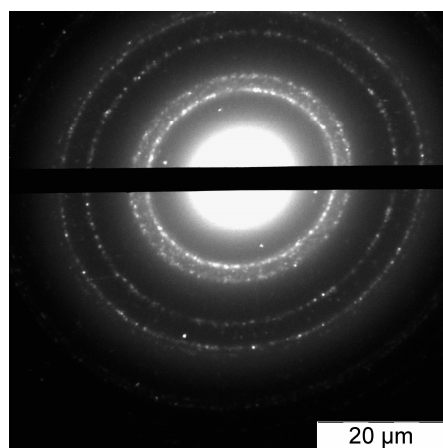


Figure 3. Electron microdiffraction pattern of APCG-stabilized AgNPs.

The stability of dispersions of AgNPs stabilized with SLES (AgNPs–SLES) and with APCG (AgNPs–APCG) was evaluated by UV–Vis spectroscopy at a silver concentration of $10 \text{ mg} \cdot \text{L}^{-1}$, 24 h after preparation and again after 2 months (Figure 4). The results confirmed the partial aggregation of AgNPs–SLES dispersion as its localized surface plasmon resonance (LSPR) decreased in intensity and showed a slight red shift two months after synthesis.

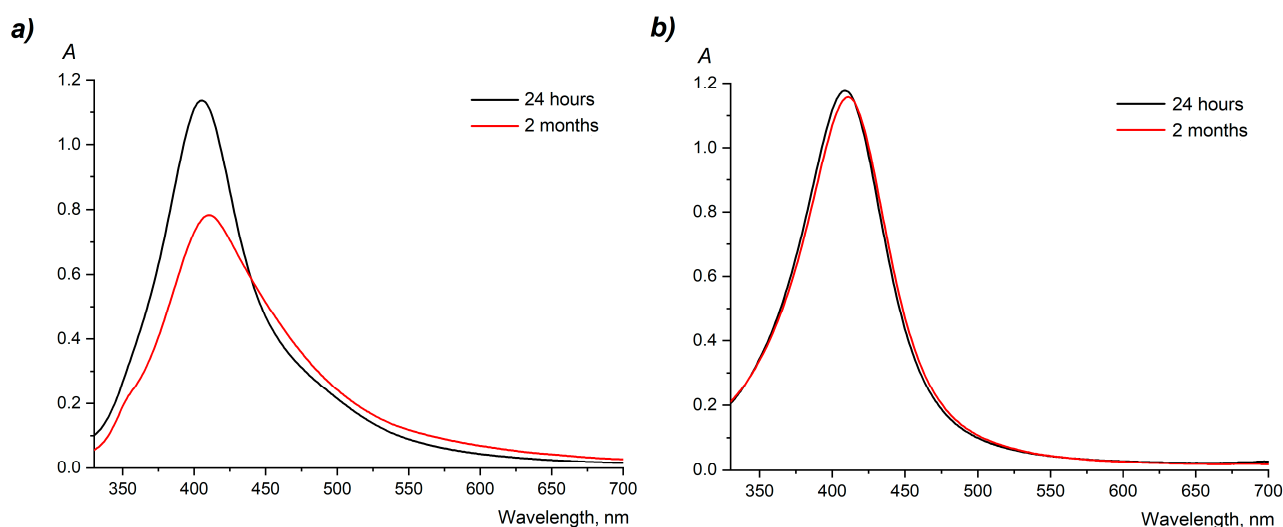


Figure 4. UV–Vis absorption spectra of dispersions of AgNPs stabilized with (a) SLES and (b) APCG. “A” refers to “absorption”.

3.2. Effect of Seed Priming with Dispersions of AgNPs on Wheat Seedlings Growth

Photographs of Petri dishes with wheat seedlings exposed to dispersions of AgNPs stabilized with SLES and APCG at various concentrations are shown in Figure 5.

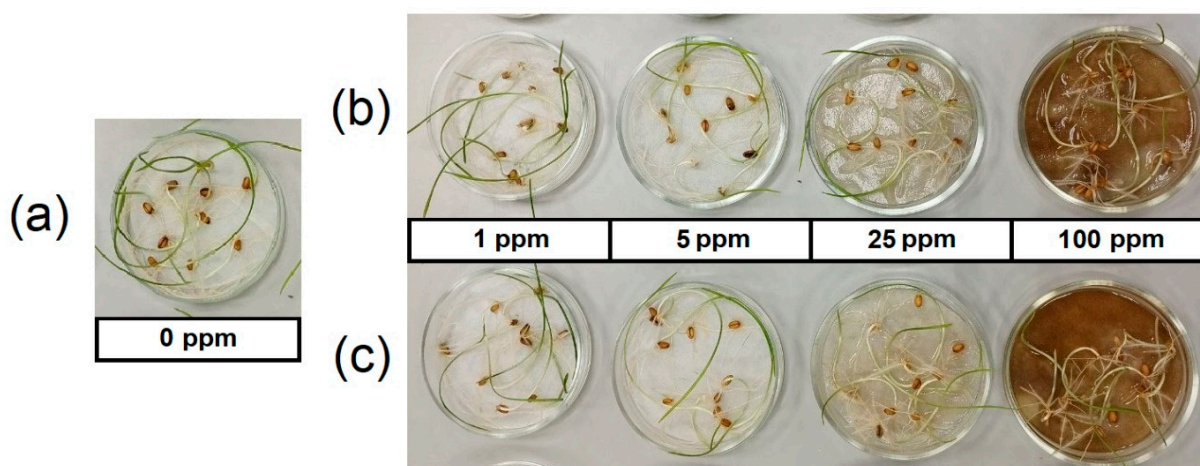


Figure 5. Photographs of Petri dishes with wheat seedlings exposed to AgNPs: (a) control; (b) AgNPs–SLES dispersion; (c) AgNPs–APCG dispersion.

The effect of exposure to silver nanoparticles on plant seedlings was assessed on the 7th day of the experiment by measuring root length, shoot length, and seedling mass as growth parameters. The results are presented in Figure 6. Low concentrations (1–5 mg·L^{−1}) of dispersions AgNPs produced little to no effect on wheat seedling growth metrics. An exception was observed for APCG-stabilized dispersions, which at low concentrations elicited modest increases in seedling stem length and total fresh seedling mass. At higher concentrations (≥25 mg·L^{−1}), exposure to stabilized dispersions of AgNPs caused marked reductions in root length and seedling mass. The APCG stabilizer itself exhibited pronounced phytotoxicity toward wheat seedlings as well.

3.3. Field Trials

The results of field trials demonstrated a pronounced positive effect of treatments with dispersions of AgNPs on plant population density, grain yield and yield components of winter wheat (cv. Skipetr) (Tables 2 and 3). Thus, in treatments receiving dispersions of AgNPs (treatments 3–6), plant population density increased at the tillering, stem elongation, heading, and milk stages. The highest plant population density was observed in treatment 5 (three applications of a 5 mg·L^{−1} dispersion of AgNPs), reaching 380 plants·m^{−2} at the milk stage compared with 352 plants·m^{−2} in the control.

Analysis of spike-structure metrics at harvest (Table 3) indicates that three applications of a dispersion of AgNPs at 5 mg·L^{−1} (treatment 5) increased the average number of spikelets per spike from 19.0 to 21.0 and grains per spike from 29.0 to 31.2 compared with the control. These increases were accompanied by a rise in grain weight per spike to 1.46 g, exceeding the control value of 1.24 g. In addition, mean plant height increased, in agreement with previous reports [54,55]. When the application concentration of AgNPs was either increased or decreased, or when the number of applications was reduced (treatments 3, 4, and 6), the magnitude of the positive effect declined: values remained above the control, gains in spike-structure metrics and grain weight per spike were smaller (grains per spike 30.6–31.0; grain weight per spike 1.31–1.44 g).

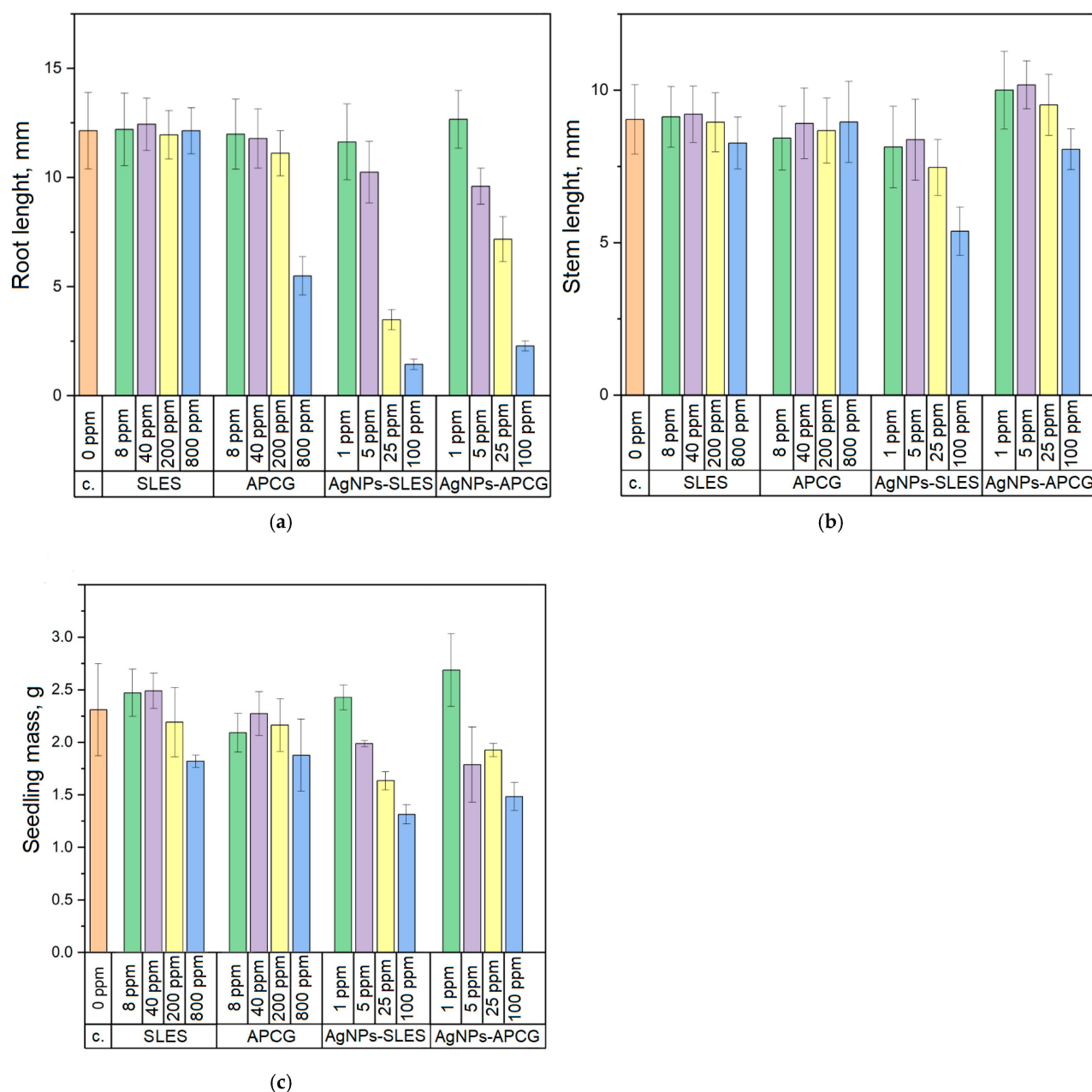


Figure 6. Growth parameters of wheat seedlings on day 7 of exposure to dispersions of silver nanoparticles: (a) root length; (b) shoot length; (c) seedling mass.

Table 2. Wheat plant population density (plants·m^{−2}) at key growth stages.

Treatment No.	Growth Stage			
	Tillering	Stem Elongation	Heading	Milk Stage
1	407	378	364	352
2	404	372	368	349
3	410	384	375	358
4	425	395	382	367
5	440	406	395	380
6	418	392	386	372
LSD _{0.05}	12	10	10	9

Table 3. Wheat plant height, grain yield components, and grain yield.

Treatment No.	Plant Height (mm)	Spikelets per Spike	Grains per Spike	Grain Weight per Spike (g·Spike ⁻¹)	Thousand-Kernel Weight (g)	Grain Yield (t·ha ⁻¹)
1	560	19.0	29.0	1.24	42.8	5.89
2	555	19.3	28.5	1.23	43.2	5.82
3	588	19.8	31.0	1.31	42.3	5.99
4	605	20.6	30.6	1.42	46.4	6.32
5	625	21.0	31.2	1.46	46.8	6.60
6	615	20.8	30.9	1.44	46.6	6.50
LSD _{0.05}	25	1.0	1.5	0.06	1.0	0.18

Cumulative improvements in morphological and spike-structure metrics resulted an increase in grain yield (Table 3). In the control, grain yield amounted to 5.89 t·ha⁻¹, whereas two applications of a dispersion of AgNPs at 5 mg·L⁻¹ (treatment 4) increased yield to 6.32 t·ha⁻¹; and three applications at 5 mg·L⁻¹ (treatment 5) increased it to 6.60 t·ha⁻¹. Thus, the yield gain in the optimal regime (treatment 5) was 0.71 t·ha⁻¹ (12.1%) higher relative to the control. Increasing the application concentration of dispersions of AgNPs did not confer additional yield benefits: treatment 6 resulted in 6.50 t·ha⁻¹, exceeding the control but remaining below the treatment regime of three applications of AgNPs dispersions at 5 mg·L⁻¹.

3.4. Antioxidant Enzyme Activity

Activities of antioxidant enzymes (POD, CAT, and PPO) were determined in stems of wheat seedlings and in leaves of field-grown plants exposed to dispersions of AgNPs. The results are presented in Tables 4 and 5.

Table 4. Activities of antioxidant enzymes in stems of wheat seedlings exposed to dispersions of AgNPs.

Dispersion	AgNPs Concentration (mg·L ⁻¹)	POD Activity (U·g ⁻¹ FW·s ⁻¹)	CAT Activity (μmol H ₂ O ₂ ·g ⁻¹ FW·min ⁻¹)	PPO Activity (U·g ⁻¹ FW·min ⁻¹)
-	0	189 ± 4	1119 ± 26	22.9 ± 1.2
	1	251 ± 4	1432 ± 40	28.1 ± 1.4
	5	270 ± 8	1600 ± 35	30.3 ± 1.3
	25	190 ± 7	1151 ± 29	23.1 ± 1.2
	100	45 ± 10	279 ± 19	6.6 ± 1.3
AgNPs-SLES	1	253 ± 7	1510 ± 31	29.5 ± 1.0
	5	285 ± 9	1685 ± 38	31.2 ± 1.4
	25	228 ± 8	1405 ± 33	26.3 ± 1.1
	100	81 ± 12	469 ± 30	9.4 ± 0.8

The obtained data indicate that changes in the activities of antioxidant enzymes in wheat following exposure to dispersions of AgNPs depend on the concentration of AgNPs, developmental stage, and treatment regimen. In wheat seedlings (Table 4), low concentrations (1–5 mg·L⁻¹) elicited increases in the activities of POD, CAT, and PPO. At 25 mg·L⁻¹, the activities of antioxidant enzymes remained above the control but declined relative to the maxima observed at 5 mg·L⁻¹ and at 100 mg·L⁻¹, the activities of all three enzymes decreased sharply (by approximately 3–4-fold relative to the control).

In field trials, two foliar applications of either an APCG solution at 200 mg·L⁻¹ or AgNPs-APCG at 1 mg·L⁻¹ (treatments 2 and 3, respectively) did not lead to significant changes in the activities of antioxidant enzymes compared with the control (Ta-

ble 5). However, increasing the concentration of dispersions of AgNPs to 5 and 25 mg·L⁻¹ (treatments 4–6) resulted in marked increases in the activities of POD, CAT, and PPO.

Table 5. Activities of antioxidant enzymes in leaves of mature wheat plants exposed to AgNPs-APCG dispersions.

Treatment No.	Peroxidase Activity (U·g ⁻¹ FW·s ⁻¹)	Catalase Activity (μmol H ₂ O ₂ ·g ⁻¹ FW·min ⁻¹)	Polyphenol Oxidase Activity (U·g ⁻¹ FW·min ⁻¹)
1	234 ± 16	1259 ± 43	21.2 ± 0.8
2	244 ± 19	1212 ± 43	21.3 ± 0.8
3	236 ± 18	1150 ± 26	20.3 ± 1.0
4	268 ± 14	1395 ± 35	23.8 ± 1.2
5	252 ± 12	1320 ± 31	22.6 ± 1.1
6	277 ± 10	1441 ± 41	24.6 ± 1.8

4. Discussion

4.1. Preparation and Colloidal Characteristics of Dispersions of AgNPs

According to TEM and SAED, the use of both APCG and SLES stabilizers yielded dispersions of nanometer-scale, zero-valent metallic silver (Figures 2 and 3). The absolute values of the negative ζ-potentials of the obtained AgNPs measured by DLS exceeded the commonly cited threshold value of 30 mV [56,57] indicating an electrostatic mechanism of colloidal stabilization (Table 1). This strongly negative ζ-potential arises from adsorption of anionic headgroups (carboxylate groups of APCG and sulfate groups of SLES), deprotonated at the working pH, onto Ag⁰. Together with the low ionic strength, this yields a large-magnitude negative ζ-potential and robust electrostatic stabilization of the dispersions of AgNPs.

APCG was a more effective stabilizer of AgNPs than SLES yielding smaller NPs with better colloidal stability. This is consistent with the more negative ζ-potential for APCG-stabilized AgNPs and can be rationalized by stronger, multipoint adsorption of carboxylate groups of APCG on the surface of NPs. Furthermore, the more negative surface potential, and superior temporal stability of the dispersion of AgNPs stabilized with APCG are expected to increase the biological activity of AgNPs in subsequent assays. However, in this work, the APCG-stabilized dispersion of AgNPs exhibited a slightly weaker inhibitory effect on wheat seedlings than the AgNPs–SLES dispersion (Figure 6). Although SLES showed lower intrinsic inhibitory activity than APCG, this discrepancy can stem from formulation-dependent differences in Ag⁺ release and bioavailability: SLES capping may accelerate Ag⁺ dissolution, enhance wetting and tissue penetration, and promote adhesion to root cell walls, whereas a thicker APCG corona can shield particle surfaces and reduce the effective dose at target sites. However, this question requires further investigation.

4.2. Effect of Seed Priming with Dispersions of AgNPs on Wheat Seedlings Growth

At low doses (1–5 mg·L⁻¹), AgNPs did not affect wheat seedling growth, whereas higher concentrations (≥25 mg·L⁻¹) produced adverse effects. Such inhibitory action of AgNPs is consistent with previously reported mechanisms whereby AgNPs at high concentrations induce oxidative stress leading to lipid peroxidation, protein and DNA damage, disrupt ion homeostasis (including Ca²⁺), thereby perturbing hormonal regulation (ethylene, ABA, auxin) and gene expression, impair the photosynthetic apparatus (chlorophyll loss and reduced photosynthetic efficiency), and affect other intracellular processes that govern root and shoot development [42].

In this work, to explain the observed changes in growth indicators of wheat seedlings, exposed to different concentrations of AgNPs, the activity of antioxidant enzymes in their

stem tissues was determined (Table 4). At low concentrations ($1\text{--}5\text{ mg}\cdot\text{L}^{-1}$) the activity of POD, CAT, and PPO increased, consistent with no growth inhibition and, in some cases (with dispersions of AgNPs stabilized with APCG), modest stimulation of stem elongation and fresh biomass. However, increasing the concentration of AgNPs to 25 and $100\text{ mg}\cdot\text{L}^{-1}$ resulted in the decline of antioxidant enzyme activity, suggesting a stronger perturbation of redox homeostasis to which seedlings could not mount a commensurate enzymatic response, which was accompanied by suppression of seedling growth and reduced biomass (Figure 6). This pattern is plausibly attributable to excessive ROS generation that exceeds the compensatory capacity of the antioxidant system, leading to damage within cellular compartments harboring these enzymes. Direct inactivation of enzymes by Ag^+ binding and/or immobilization on nanoparticle surfaces may also contribute. Further investigation is warranted to resolve the relative contributions of these mechanisms and to identify which systems in wheat seedlings are most sensitive to ROS. For example, the observed changes in growth parameters may reflect partial suppression of the photosynthetic apparatus and chlorophyll levels, which would limit carbon assimilation and thereby slow seedling growth. To address this issue, chlorophyll content could be measured in subsequent *in vitro* assays.

Notably, stem length was substantially less responsive than root length, in agreement with previous reports [58,59]. This pattern is plausibly explained by preferential accumulation of silver in roots relative to stems in *in vitro* seedling systems [59,60].

4.3. Field Trials

Under field conditions, foliar application of AgNPs–APCG dispersion generally improved plant population density at key growth stages plausibly attributable to improved plant survival due to enhanced tolerance to both high and low temperatures and other abiotic stresses induced by exposure to AgNPs (Table 2) [61]. Furthermore, grain yield, and yield component metrics were also improved (Table 3). The best results were obtained with repeated low-concentration applications (treatment No. 5—three applications at $5\text{ mg}\cdot\text{L}^{-1}$). However, if the concentration of AgNPs was increased, it resulted in a reduction in the magnitude of the positive effect. These results suggest the presence of a threshold application concentration for dispersions of AgNPs, above which stress responses attenuate the beneficial effect. Consistent with this interpretation, high doses of AgNPs have been reported to induce ROS production and thus disrupt redox homeostasis, leading to suppression of physiological functions, including the functioning of the photosynthetic apparatus and grain filling [36–38]. These results support the efficacy of repeated low-dose application of AgNPs dispersions, which may provide a sustained prolonged stimulatory effect on plant metabolism. It is also plausible that repeated applications of AgNPs enhances plant tolerance to abiotic stresses during key phenological stages, thereby contributing to higher yields under suboptimal weather conditions.

The responses of antioxidant enzyme activities correlated with improvements in spike-structure metrics and grain yield (Tables 3 and 5). Thus, dose-dependent increases in POD, CAT, and PPO are indicative of elevated levels of ROS in the cells of field-grown plants, within an adaptive signaling window. At low levels ROS function as signaling molecules: they regulate transcription via redox post-translational modifications and activate Ca^{2+} /MAPK cascades, intersecting with ABA, ethylene, salicylic and jasmonic acid pathways while upregulating antioxidant defenses [62,63]. Morphologically, ROS modulate stomatal conductance and photosynthetic acclimation and govern cell-wall remodeling and polar growth processes—including shoot and root elongation, lateral root formation and root-hair development [62,63]. Such controlled, moderate ROS increases could therefore underlie the improvements in morphometric traits, spike-structure metrics and grain yield

observed under low-dose, multi-application regimens. This interpretation accords with the concept of induced resistance, whereby controlled elicitation of defense pathways enhances stress tolerance and yield stability—an approach increasingly emphasized in sustainable crop protection [43].

Taken together, we suggest that the observed stage-dependent responses arise from differences in the maturation and robustness of physiological systems in wheat seedlings versus adult plants, including the development of antioxidant defenses. Furthermore, developmental changes in tissue barriers (e.g., thicker cuticles, altered apoplastic conductance) modulate AgNP uptake and translocation, yielding different local concentrations at target sites and, consequently, distinct physiological outcomes across growth stages.

These stage- and dose-dependent outcomes are consistent with our hypothesis that developmental stage is a primary moderator of plant responses to AgNPs, and accord with the majority of prior studies reporting inhibition of seedlings *in vitro* and stimulation of mature plants *in vivo* (in field conditions), as systematically summarized in our review [42]. To deepen understanding of stage-dependent mechanisms, additional experiments (e.g., chlorophyll and pigment content, antioxidant enzyme activities, Ag content in plant tissues, gene expression, etc.) can be conducted across different ontogenetic stages and application timings.

5. Conclusions

The effects of stabilized dispersions of silver nanoparticles on wheat plants at different stages of their ontogenesis were investigated in this study. It was found that the response to AgNPs depends strongly on the developmental stage. Thus, *in vitro* exposure of wheat seedlings resulted predominantly in neutral or negative outcomes, whereas foliar application to wheat plants under field conditions produced a pronounced positive effect. Specifically, low concentrations ($1\text{--}5\text{ mg}\cdot\text{L}^{-1}$) in seedling assays had little effect or, in case of APCG-stabilized dispersions, modestly stimulated growth, whereas $25\text{--}100\text{ mg}\cdot\text{L}^{-1}$ suppressed root length and biomass. In field trials, three foliar applications at $5\text{ mg}\cdot\text{L}^{-1}$ increased grain yield from 5.89 to $6.60\text{ t}\cdot\text{ha}^{-1}$ (+12.1%) and improved spike structure (spikelets per spike from 19.0 to 21.0 , respectively; grains per spike from 29.0 to 31.2 , respectively), while deviations from this regimen (changes in concentration or with fewer applications) yielded smaller gains.

The type of stabilizer also played an important role: APCG ensured high colloidal stability of the dispersions, resulting in smaller NPs ($10.4 \pm 4.9\text{ nm}$) and a more negative ζ -potential (-52.6 mV) than SLES ($21.9 \pm 9.1\text{ nm}$; -28.2 mV), features consistent with greater colloidal stability. Furthermore, at low concentrations, APCG-stabilized dispersions of AgNPs showed slight seedling stimulation relative to the control and SLES-stabilized dispersions. Thus, optimization of the concentration of AgNPs and the chemical nature of the stabilizer, together with the number and timing of applications, is critical for maximizing the growth-promoting potential of nanosilver in wheat.

The biological effects observed upon treatment of plants with dispersions of AgNPs were closely associated with changes in the activities of antioxidant enzymes, thereby shedding light on a plausible mechanism of action. Exposure to moderate doses of AgNPs (up to $5\text{ mg}\cdot\text{L}^{-1}$ in *in vitro* assays and up to $25\text{ mg}\cdot\text{L}^{-1}$ in field experiments) led to increased activities of POD, CAT, and PPO in both seedlings and mature plants, reflecting activation of nonspecific defense responses to moderate abiotic stress. This controlled activation of the antioxidant system likely underlies the enhanced tolerance of plants treated with dispersions of AgNPs to adverse biotic and abiotic factors and, consequently, contributes to growth stimulation. At sufficiently high doses ($\geq 25\text{ mg}\cdot\text{L}^{-1}$), by contrast, the compensatory

capacity of the enzymatic antioxidant system was exceeded: activities of antioxidant enzymes declined, and seedling growth was inhibited.

Taken together, the dose- and stage-dependent responses observed are consistent with induced stress resistance: appropriately low doses of AgNPs act as abiotic elicitors that pre-activate antioxidant and hormonal defense networks, improving tolerance to heat, drought, and pathogen pressure. In the context of sustainable agriculture, such elicitation can help maintain yield under variable conditions while reducing pesticide inputs. Thus, the practical significance of the present results is that they demonstrate that the appropriate use of stabilized dispersions of silver nanoparticles—with optimization of concentration and the application regimen—can constitute an effective approach to enhancing the stress tolerance and productivity of agricultural crops, whereas application under non-optimized conditions, by contrast, may lead to phytotoxic effects. These findings open broad prospects for incorporating dispersions of AgNPs into agricultural technologies as an innovative means of stimulating plant growth and increasing crop yield.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agronomy15112540/s1>, Figure S1. Effects of AgNPs-SLES and AgNPs-APCG on wheat seedling growth parameters after 7-day exposure.

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Abbreviations

The following abbreviations are used in this manuscript:

ABA	abscisic acid
AgNPs	silver nanoparticles
APCG	sodium amphopolycarboxyglycinate
CAT	catalase
DLS	dynamic light scattering
HSD	Honestly Significant Difference
LSD	least significant difference
MAPK	mitogen-activated protein kinases
POD	peroxidase
PPO	polyphenol oxidase
PVP	polyvinylpyrrolidone
ROS	reactive oxygen species

SAED	selected area electron diffraction
SD	standard deviation
SLES	sodium laureth sulfate
TEM	transmission electron microscopy
TKW	thousand-kernel weight
ζ-potential	electrokinetic potential (“zeta” potential)

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