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The Response of Rapeseed (*Brassica napus* L.) Seedlings to Silver and Gold Nanoparticles

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Abstract: With the advancement of nanotechnology and the increasing utilization of nanoparticles (NPs), their production and release into the environment are on the rise. Consequently, it is crucial to continuously monitor the toxicity of nanoparticles for humans, animals, and plants, as well as their impact on the environment. This is particularly significant in relation to human health and food production, given the escalating use of nanomaterials in agriculture and horticulture. The aim of the study was to investigate the response of rapeseed seedlings to silver nanoparticles (AgNPs) and gold nanoparticles (AuNPs) over different periods of exposure. This research analyzed the impact of these nanoparticles on the biochemical response of rapeseed seedlings after 7, 14, and 21 days of growth in their presence. This study assessed the activity of guaiacol peroxidase (GPOX), pyrogallol peroxidase (PPOX), superoxide dismutase (SOD), and free protein content, as well as the interactions between key elements responsible for oxidative stress and the antioxidant response. The findings demonstrated a significant effect of AgNPs and AuNPs on stimulating the response of rapeseed seedlings, with the activity of PPOX, GPOX, and SOD being dependent on the exposure time and the type and dose of nanoparticles used. Enzyme activity increased with the length of exposure time, while the content of free protein decreased over the weeks. The most intense reaction of seedlings was observed in the case of GPOX, with the lowest activity observed in PPOX and SOD. High effects of the nanoparticle type and rate were also observed in the correlation matrix. This study suggests that a comprehensive analysis of plant reactions to nanoparticles could have a significant impact on the proper and effective use of nanoparticles in agriculture and horticulture. This could lead to the environmentally friendly production of high-quality plant material.

Keywords: silver nanoparticles; gold nanoparticles; guaiacol peroxidase; pyrogallol peroxidase; superoxide dismutase; free protein; antioxidant activity

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1. Introduction

With the growing population and the ever-increasing demand for food, as well as taking into account climate change and soil and food contamination with harmful compounds from fertilizers and pesticides, modern agriculture and horticulture must implement innovative cultivation methods. In intensively developing urban and suburban areas, soil used for agricultural crops is particularly exposed to contamination and pollution, so the production of healthy, uncontaminated food safe for human health within urban agriculture is an important problem. Changes and improvements in food production are aimed at increasing efficiency, and also at producing food that is safe for the consumer's health. In order to adapt to these requirements, the latest technologies and products manufactured based on the achievements of scientists in recent years are used. One of the technologies that have become present in modern agriculture and horticulture is the introduction of

materials based on nanotechnology. With the emergence of nanotechnology in the agricultural sector, efficient and more environmentally friendly nanopreparations are available, such as nanoemulsions, nanopolymers, nanopesticides, nanoherbicides, and nanofertilizers produced for sustainable agriculture [1,2].

Nanotechnology deals with the design and creation of materials in which at least one of the dimensions is between 1 and 100 nm [3]. These materials are characterized primarily by high reactivity resulting from the increased ratio of specific surface to volume, which causes the nanoparticles to have different physicochemical properties that can change the reactivity and thus their biological activity [4,5]. The properties of nanoparticles and their reactivity are influenced by the core composition, shape, surface properties, stability, and method of their production [6–8]. Due to their unique properties, these materials can be used in almost all branches of industry [9–13] and above all, in medicine and pharmacy, in elements of drug delivery systems and tissue imaging [14].

In agricultural and horticultural practices, nanomaterials are used to reduce nutrient losses, which positively affects the size and quality of crops, and to reduce the number of plant protection products. Nanotechnologies offer opportunities for solving agricultural and environmental problems in order to enhance crop productivity, to improve the soil health, and to reduce the costs of plant production, as well as offer possibilities for using nanomaterials to improve food safety [15].

The reactions of plants to the presence of nanoparticles in the environment are very complex and depend on the properties of the nanoparticles such as their shape or size, and also on the plant's genotype, development stage, and cultivation conditions [12]. Tools that enable the study of these relationships are projects carried out in a system of physically controlled conditions with precisely defined substrate components, such as in vitro plant cultures. Most of this information and known applications concern silver nanoparticles (AgNPs) and gold nanoparticles (AuNPs), mainly due to their antibacterial and antifungal properties [16–18]. However, there are also reports on nanoparticles of carbon, copper, aluminum, zinc, iron, silicon, titanium, and others [16,19–21].

AgNPs are known for their strong antibacterial, antifungal, and antiviral properties, and hence are used in a range of products such as clothing, cosmetics, pharmaceuticals, and medical devices [22,23]. Also, AuNPs have distinct properties such as good conductivity, chemical stability, and resistance to bacterial infection, and are used as an antimicrobial agent [24]. These characteristics make them suitable for applications in electronics, catalysis, and sensing technologies [25]. Their uses also extend into the medical field, where they are used in imaging, therapy, and drug delivery [26–28]. AgNPs and AuNPs exhibit a phenomenon called "Surface Plasmon Resonance", where they absorb and scatter light with extraordinary efficiency [29,30]. This property has led to their use in a variety of light-based technologies, such as photovoltaics, optoelectronics, and molecular diagnostics [25]. Metal nanoparticles can also affect the physiological and biochemical processes of plants. AgNPs content in the environment can affect seed germination processes, the growth of shoots and roots, and through interactions with proteins, enzymes and carbohydrates, it can also affect changes in the amount of biomass [31–36]. Moreover, AgNPs act as an ethylene inhibitor and activate antioxidants, influencing the chlorophyll content [37–39]. When examining the impact of AuNPs on physiological processes and the growth and development of plants, including agricultural ones, attention should be paid to the various effects depending on the size and shape of the particles and their concentrations. Therefore, very diverse effects on seed germination, plant growth, photosynthetic intensity, and the activation of reactive oxygen species (ROS) scavenging enzymes have been noted [40]. Some researchers have described the positive impact of AuNPs on the seed germination process [41–43], increases in surface area or the number of leaves, elongation growth of plants, chlorophyll content, sugar content, which translates into the quality yields [42,43], or a significant impact on the regulation of the antioxidant system [44]. Another effect studied with AuNPs is genotoxicity, which may be caused by direct interaction with DNA or through the formation of ROS and induction of oxidative stress [45,46]. The overproduction of ROS

or their ineffective removal can lead to very serious problems, disorders in cell functioning, lipid peroxidation, damage to carbohydrates and proteins, and damage in DNA [47–49]. The actions of ROS on proteins can cause specific modifications to amino acid residues, the fragmentation of the polypeptide chain, the formation of cross-links and aggregates, and alterations in charge [50]. Although the overproduction of ROS is harmful, if they are present in cells in low concentrations, they affect the proper functioning of the cell. They are produced by natural processes used for the regulation of physiological processes and play important roles as signal functions [51]. An increase in ROS has substantial implications on the activity of antioxidant enzymes in plants under nanoparticles stress [52–54]. Plants have developed mechanisms to prevent oxidative stress, employing enzymes and other compounds that inhibit or neutralize free radicals. The balance between the synthesis of ROS and their detoxification is controlled by the cellular antioxidant system, which consists of enzymes such as superoxide dismutase (SOD), peroxidase (POX), catalase (CAT), ascorbic acid peroxidase (AAP), and glutathione reductase (GR) and compounds such as ascorbic acid (AA), cysteine (Cys), glutathione (GSH), tocopherol, carotenoids, hydroquinones, and polyamines [55].

To study the reactions of plants to the presence of nanoparticles in the environment under in vitro culture conditions, we used rapeseed seedlings. Rapeseed cultivars, due to an even and rapid seed germination period, are often used in in vitro cultures. Disinfected, sterile seedlings may be obtained within a week of culture. Furthermore, rapeseed, as one of the most important crops globally, with its oil serving as a key ingredient in various industries, including food, biodiesel, and industrial applications, was chosen for the experiment. As a major source of vegetable oil, rapeseed contributes to the global edible oil production and plays a vital role in meeting the growing demand for functional, healthier, and sustainable oil alternatives mainly due to its high content (approx. 90%) of 18-carbon unsaturated acids and many bioactive compounds such as antioxidant compounds [56,57].

Extensive research has been conducted by the team over many years to investigate the impact of AgNPs and AuNPs on the growth and health of rapeseed. The goal of the research is to develop a technology for the use of these nanoparticles in agriculture. The research has allowed the team to specify the doses of nanoparticles, their impact on the growth of rapeseed seedlings, oxidative stress, and other related markers. In a previous study, the team presented the changing morphological, physiological, and biochemical parameters of plants under the influence of lengths of exposure to two concentrations of AgNPs and AuNPs. The data available on this subject are still very limited. The morphological parameters of rapeseed seedlings, such as the length and weight of shoots and roots, and the content of photosynthetic pigments (chlorophyll a, b, and total), carotenoids, anthocyanins, phenolics, free sugars, and H_2O_2 were determined. In a recent study, the team further examined the impact of the length of exposure of rapeseed seedlings to AgNPs and AuNPs on the activity of antioxidant enzymes such as SOD, GPOX, PPOX, and free protein content and their interactions with photosynthetic pigments (chlorophyll a, b, and total), carotenoids, anthocyanins, and H_2O_2 (data from our previous study [31]). The research was conducted in in vitro culture conditions on synthetic media, examining the reaction of rapeseed seedlings after 7, 14, and 21 days of exposure to nanoparticles.

The findings contribute to a better understanding of the role of the length of plant exposure, which is a crucial element influencing the interaction between metal nanoparticles and plants. Despite numerous reports on the effects of metal nanoparticles on plant development and physiological processes, further extensive research is needed to understand the role of other factors on the NP/plant interaction and the potential of AgNPs and AuNPs in inducing plant responses to oxidative stress. This research is crucial in the context of using nanotechnology to develop and support global, sustainable agriculture.

2. Materials and Methods

2.1. Plant Material

Seeds of spring rapeseed 'Feliks' from Strzelce Plant Breeding Ltd. (Strzelce, Poland) were used.

2.2. In Vitro Cultures of Rapeseed Plants

The micropropagation method described by Tomaszewska-Sowa et al. [31,58] was used in the experiment. The sterile seeds were inoculated on MS Basal medium [59] (Sigma Aldrich, Saint Louis, MO, USA) with 10 seeds per jar. The experiment took place in a growth chamber with a temperature of 24 ± 1 °C and a 16-h photoperiod under OSRAM L36W/77 Fluora lamps (OSRAM, Munich, Germany). The photosynthetic photon flux density was set to 40 $\mu mol \cdot m^{-2} \cdot s^{-1}$, providing the necessary light source for growth.

2.3. Nanoparticles

AgNPs and AuNPs obtained from Nanoparticles Innovation NPIN s.c. (Łódź, Poland) were used in the study. The nanocolloids were synthesized using the seed-mediated growth method, following the procedures outlined by Domeradzka-Gajda et al. [60] and Pudlarz et al. [61], as per the manufacturer's information. The final metal concentration after synthesis was maintained at 100 ppm.

Scanning transmission electron microscopy (Nova Nano SEM 450, FEI, accelerating voltage 30 kV) was employed for size and size-distribution analysis, revealing the obtained sizes of 20 ± 3 nm for AgNPs and 20 ± 2 nm for AuNPs. Dynamic Light Scattering (Nano ZS Zetasizer system, Malvern Instruments, Worcestershire, UK) was also employed to measure the hydrodynamic sizes, which were determined to be 23 ± 4 nm for AgNPs and 24 ± 5 nm for AuNPs.

2.4. Treatment of In Vitro Plants with AgNPs and AuNPs

AgNPs and AuNPs were applied onto the medium surface. Nanoparticles were diluted to concentrations of 50 and 100 ppm and applied in an amount of 1 mL per culture jar with a sterile pipette tip that was gently mixed to cover the surfaces of all seeds. Seeds that germinated on the MS medium without the application of AgNPs or AuNPs were used as the control. Concentrations were selected based on our previous research [31] and also on the literature data presented by numerous authors who considered these concentrations the most representative for the effects of nanoparticles on plants. For each experimental treatment, four repetitions were used. Biochemical analyses of rapeseed seedlings were performed for each experimental variant 7, 14, and 21 days after inoculating the seeds on the MS medium and starting the germination process (Figure 1).

Figure 1. In vitro cultures of rapeseed plants on MS Basal medium 21 days after inoculation.

2.5. Free Protein Content and Enzymes Assessments

2.5.1. Free Protein Content Assessment

The protein concentration was determined using the method of Bradford [62]. Bovine albumin at a concentration ranging from 0 to 50 µg was used as a standard for the calibration curve.

2.5.2. Extraction of Enzymes

Enzymes were extracted in a cold, 50 mM sodium phosphate buffer, pH 7.0, with an addition of 1 M NaCl. In total, 250 mg of fresh tissue was homogenized in an ice-cold mortar and pestle with 2 mL of extract buffer. After homogenization, the extract was centrifuged in 15,000× *g*, 4 °C for 30 min. After that, the supernatant was collected in a new tub, and the pellet was discarded.

2.5.3. Guaiacol and Pyrogallol Peroxidase Assessments

GPOX and PPOX have been assessed with the adapted methods described by Zahir et al. [63] and Chance and Maehly [64] in a 96-well microplate format. The reaction mixture contained 10 µL of enzyme extract, 10 µL of guaiacol or pyrogallol, and 250 µL of reaction buffer (phosphate buffer, 50 mM, pH 7.0) per well. The reaction began after the addition of 10 µL of H_2O_2 solution. Immediately, the absorbance (470 nm for guaiacol and 430 nm for pyrogallol) was measured for 2 min. For the blank, the reaction contained 260 µL of phosphate buffer instead of 10 µL of enzyme extract. One unit of peroxidase activity is the amount of enzyme oxidizing 1 nmol substrate in min^{-1}.

The spectrophotometric analysis of the extracts was carried out using a UV-VIS BioPhotometer (Eppendorf, Hamburg, Germany).

2.5.4. Superoxide Dismutase Assessment

SOD activity was measured with the use of the method used by Zahir et al. [63] in a 96-well microplate format. For the SOD activity assay, 2.5 µL enzyme extract (per well of a microplate), 250.5 µL of the reaction solution, and 25 µL H_2O were used. For light control, 253 µL reaction solution and 25 µL H_2O were added. Then, the microplate was put under a 30 W UV lamp at a distance of 20 cm from the light source. Every 5 min., the microplate was taken under the UV lamp, and the absorbance was measured (560 nm). After 30 min. of exposure, the reaction had stopped. The activity of SOD was expressed as the 50% photoinhibition as described by McCord and Fridovich [65].

2.6. Statistical Analysis

The data obtained from the conducted experiments underwent statistical analysis using MS Excel and R Core Team (version 4.0.4) with the R Studio overlay. When the data did not follow a normal distribution, normalization was performed until a normal distribution was achieved and confirmed using the Shapiro–Wilk test. The choice of normalization method depended on the skewness of the data, with either square root or log10 transformations applied.

For the analysis of variance and determination of homogeneous groups, a two-way analysis of variance was conducted. The Tukey post hoc test was used with a significance level (alpha) set to 0.05. Libraries agricolae, rstatix, tidyverse, and moments were utilized for this analysis.

To assess the presence of correlations, a linear r Pearson correlation analysis was performed at a significance level of 0.05. Obtained data (GPOX, PPOX, SOD) were also correlated with the content of hydrogen peroxide, chlorophyll a and b, carotenoids, and anthocyanins (data presented by Tomaszewska-Sowa et al. 2022 [31]). The results were presented in the form of a correlation matrix, which displayed the relationships between the examined features.

3. Results

3.1. Effect of Nanoparticles on the Free Protein Content

The content of free protein in plant tissues showed no significant differences during the initial two weeks of the experiment, as indicated in Table 1. Interestingly, the highest content of free protein in the first week was observed in plant tissues treated with 50 ppm of AuNPs (18.45 ± 1.71 mg/gFW). No substantial variations were observed in the second week. However, during the third week, a noticeable decrease in the content of free protein was observed across all tested plant variants. Despite this rapid decline, there were no significant differences observed among the tested combinations.

Table 1. Influence of nanoparticle type and concentration on the free protein content 7, 14, and 21 days after rapeseed treatment.

Treatment Time (Days)	Nanoparticles	Free Protein (mg/gFW)
7	Control	16.82 ± 0.80 ab
	AgNPs 50 ppm	15.78 ± 0.60 abc
	AgNPs 100 ppm	17.03 ± 0.39 ab
	AuNPs 50 ppm	18.45 ± 1.71 a
	AuNPs 100 ppm	17.91 ± 1.56 ab
14	Control	11.90 ± 1.08 cd
	AgNPs 50 ppm	13.54 ± 1.34 bc
	AgNPs 100 ppm	15.79 ± 0.73 abc
	AuNPs 50 ppm	16.91 ± 0.83 ab
	AuNPs 100 ppm	16.26 ± 0.30 abc
21	Control	4.26 ± 0.31 e
	AgNPs 50 ppm	5.51 ± 0.57 e
	AgNPs 100 ppm	4.07 ± 0.51 e
	AuNPs 50 ppm	7.91 ± 0.91 de
	AuNPs 100 ppm	7.16 ± 0.88 e

Values are presented as means ± standard deviation; the same lowercase letters indicate non-statistically significant differences according to Tukey's test ($p \leq 0.05$). Abbreviations: AgNPs—silver nanoparticles, AuNPs—gold nanoparticles.

Furthermore, the analysis revealed significant effects of the time factor ($p = 0.0000$) and the testing variant/combination factor ($p = 0.0001$). However, no significant interaction was observed between the studied factors ($p = 0.3125$).

3.2. Effect of Nanoparticles on the Guaiacol Peroxidase (GPOX) Activity

The analysis of GPOX activity, performed using two-way ANOVA and the Tukey HSD test ($p = 0.05$, agricolae R library), revealed variations in the enzyme activity across different experimental conditions, including total activity (per gram of fresh weight) and specific activity in tissues (per 1 mg of protein) (Table 2).

During the first week of the experiment, following exposure to nanoparticles, high activity was observed in plant tissues treated with AuNPs, specifically AuNPs 50 ppm (916.69 ± 527.76 U/gFM) and AuNPs 100 ppm (1034.64 ± 151.85 U/gFM). The lowest activity was observed in plants treated with AgNPs 50 ppm (432.14 ± 295.18 U/gFM) and control plants (553.23 ± 249.02 U/gFM). In the second week of exposure, plants treated with AgNPs 50 ppm exhibited the highest activity (1556.92 ± 677.10 U/gFM), while control plants (327.05 ± 106.25 U/gFM) and AuNPs 50 ppm (412.82 ± 26.20 U/gFM) showed the lowest activity. In the third week, plants treated with AuNPs 50 ppm displayed the highest activity (1220.90 ± 294.45 U/gFM), while those treated with AgNPs 50 ppm had the lowest activity (61.02 ± 16.67 U/gFM).

Table 2. Influence of nanoparticle type and concentration on the guaiacol peroxidase (GPOX) activity in rapeseed 7, 14, and 21 days after treatment.

Treatment Time (Days)	Nanoparticles	GPOX U/gFW	GPOX U/mg
7	Control	553.23 ± 249.02 ab	38.53 ± 18.66 abc
	AgNPs 50 ppm	432.14 ± 295.18 abc	30.22 ± 20.56 c
	AgNPs 100 ppm	763.80 ± 324.83 ab	48.78 ± 19.50 abc
	AuNPs 50 ppm	916.69 ± 527.76 ab	58.70 ± 33.72 abc
	AuNPs 100 ppm	1034.64 ± 151.85 a	72.02 ± 12.29 abc
14	Control	327.05 ± 106.25 abc	31.06 ± 10.51 bc
	AgNPs 50 ppm	1556.92 ± 677.10 a	113.79 ± 41.46 abc
	AgNPs 100 ppm	766.24 ± 402.14 ab	49.98 ± 24.97 abc
	AuNPs 50 ppm	412.82 ± 26.20 ab	25.92 ± 2.27 bc
	AuNPs 100 ppm	703.98 ± 123.30 ab	45.75 ± 7.72 abc
21	Control	552.58 ± 113.32 ab	190.40 ± 46.23 ab
	AgNPs 50 ppm	61.02 ± 16.67 c	16.11 ± 6.54 c
	AgNPs 100 ppm	139.45 ± 48.30 bc	50.00 ± 16.26 abc
	AuNPs 50 ppm	1220.90 ± 294.45 a	241.53 ± 98.85 a
	AuNPs 100 ppm	524.87 ± 159.34 ab	117.73 ± 54.37 abc

Values are presented as means ± standard deviation; the same lowercase letters indicate non-statistically significant differences according to Tukey's test ($p \leq 0.05$). Abbreviations: AgNPs—silver nanoparticles, AuNPs—gold nanoparticles.

The analysis of variance demonstrated the significance of both the week and nanoparticle dose factors for the examined feature. The significance level for the time factor was $p = 0.0123$, and for the combination of factors, it was $p = 0.0038$. Furthermore, a significant interaction between the studied factors was observed at a significance level of $p = 0.0000$. In terms of specific activity, less variability was observed compared to general activity. During the first week, plants exposed to AuNPs 100 ppm (72.02 ± 12.29 U/mg) displayed the highest activity, while AgNPs 50 ppm (30.22 ± 20.56 U/mg) exhibited the lowest activity. In the second week, the lowest activity was observed in plants exposed to AuNPs 50 ppm (25.92 ± 2.27 U/mg) and control plants (31.06 ± 10.51 U/mg), while AgNPs 50 ppm (113.79 ± 41.46 U/mg) exhibited the highest activity. In the third week, plants exposed to AuNPs 50 ppm (241.53 ± 98.85 U/mg) and control plants (190.40 ± 46.23 U/mg) displayed the highest activity, while the lowest activity was observed in plant tissues exposed to AgNPs 50 ppm (16.11 ± 6.54 U/mg).

Regarding specific GPOX activity, significance was observed for the time factor ($p = 0.0144$) and the combination of factors ($p = 0.0500$). Furthermore, the interaction between the tested factors was found to be significant at a level of $p = 0.0003$.

3.3. Effect of Nanoparticles on the Pyrogallol Peroxidase (PPOX) Activity

The analysis of peroxidase activity measured using pyrogallol as a substrate exhibited similar trends in enzyme activity compared to GPOX, both in terms of total activity and specific activity (Table 3). However, there was no significant effect observed for the time factor (time of exposure) on total activity ($p = 0.5643$), or for the combination/testing variant ($p = 0.2224$). Nevertheless, a significant interaction between the factors was observed at a level of $p = 0.0019$. Regarding the specific activity of PPOX, no significant effect of the testing variant/combination factor was observed ($p = 0.6897$). However, the duration of exposure/time factor was found to be significant ($p = 0.000$). Additionally, there was a significant interaction observed between the factors at a level of $p = 0.0314$.

Table 3. Influence of nanoparticle type and concentration on the pyrogallol peroxidase activity (PPOX) in seedlings 7, 14, and 21 days after rapeseed treatment.

Treatment Time (Days)	Nanoparticles	PPOX U/gFW	PPOX U/mg
7	Control	1627.33 ± 483.90 ab	111.18 ± 37.50 de
	AgNPs 50 ppm	1401.21 ± 267.77 ab	99.96 ± 18.16 de
	AgNPs 100 ppm	1981.58 ± 294.05 ab	130.08 ± 20.00 a–e
	AuNPs 50 ppm	2300.20 ± 1097.37 ab	147.35 ± 70.13 cde
	AuNPs 100 ppm	2195.55 ± 271.86 a	153.06 ± 24.44 a–e
14	Control	1840.08 ± 169.87 ab	166.41 ± 13.94 a–e
	AgNPs 50 ppm	3285.83 ± 1306.83 a	296.07 ± 151.11 a–e
	AgNPs 100 ppm	1720.24 ± 402.91 ab	118.00 ± 34.20 cde
	AuNPs 50 ppm	1487.45 ± 194.04 ab	94.86 ± 17.14 e
	AuNPs 100 ppm	1711.74 ± 172.24 ab	112.20 ± 12.20 b–e
21	Control	1813.16 ± 324.26 ab	582.58 ± 54.27 a
	AgNPs 50 ppm	1027.94 ± 26.38 b	260.23 ± 42.56 a–d
	AgNPs 100 ppm	1361.74 ± 61.03 ab	513.61 ± 96.08 ab
	AuNPs 50 ppm	2978.14 ± 450.67 a	526.92 ± 82.62 ab
	AuNPs 100 ppm	2043.12 ± 399.56 ab	424.46 ± 121.04 abc

Values are presented as means ± standard deviation; the same lowercase letters indicate non-statistically significant differences according to Tukey's test ($p \leq 0.05$). Abbreviations: AgNPs—silver nanoparticles, AuNPs—gold nanoparticles.

3.4. Effect of Nanoparticles on the Superoxide Dismutase (SOD) Activity

The analysis of SOD activity, in terms of total activity, observed in tissues, exhibited moderate variation throughout the experiment (Table 4). In the first week of exposure, the lowest SOD activity was observed in plant tissues exposed to AgNPs 100 ppm, measuring 950.50 ± 16.22 U/gFM. The activity observed in the other experimental variants was at a similar level. Similarly, in the second week of exposure, plant tissues displayed comparable activity levels to those observed in the first week. The highest SOD activity was found in plants exposed to AuNPs 50 ppm, measuring 1345.71 ± 39.86 U/gFM. In the third week of plant exposure, the highest SOD activity in tissues was observed in the AuNPs 100 ppm variant, reaching 1394.09 ± 53.03 U/gFM. Simultaneously, the lowest statistically significant activity value was observed in the AgNPs 100 ppm variant (924.46 ± 87.16 U/gFM).

Table 4. Influence of nanoparticle type and concentration on the superoxide dismutase (SOD) activity in seedlings 7, 14, and 21 days after rapeseed treatment.

Treatment Time (Days)	Nanoparticles	SOD U/gFW	SOD U/mg
7	Control	1112.72 ± 64.78 a–d	198.99 ± 10.33 a–d
	AgNPs 50 ppm	1004.31 ± 27.81 bcd	189.76 ± 12.47 a–d
	AgNPs 100 ppm	950.50 ± 16.22 cd	182.30 ± 6.88 bcd
	AuNPs 50 ppm	1210.05 ± 93.17 a–d	227.64 ± 82.34 a–d
	AuNPs 100 ppm	1168.62 ± 36.16 a–d	137.58 ± 22.07 d
14	Control	1045.78 ± 43.80 a–d	260.89 ± 21.06 a–d
	AgNPs 50 ppm	1228.17 ± 95.10 a–d	342.36 ± 27.28 ab
	AgNPs 100 ppm	1255.37 ± 49.68 a–d	330.95 ± 41.88 abc
	AuNPs 50 ppm	1345.71 ± 39.86 ab	356.83 ± 56.87 a
	AuNPs 100 ppm	1293.21 ± 30.89 abc	288.37 ± 35.63 a–d
21	Control	1113.20 ± 65.42 a–d	171.18 ± 18.35 bcd
	AgNPs 50 ppm	1010.52 ± 115.91 bcd	178.56 ± 20.86 bcd
	AgNPs 100 ppm	924.46 ± 87.16 d	122.64 ± 14.10 d
	AuNPs 50 ppm	1304.79 ± 136.31 abc	163.96 ± 21.17 cd
	AuNPs 100 ppm	1394.09 ± 53.03 a	207.07 ± 27.49 a–d

Values are presented as means ± standard deviation; the same lowercase letters indicate non-statistically significant differences according to Tukey's test ($p \leq 0.05$). Abbreviations: AgNPs—silver nanoparticles, AuNPs—gold nanoparticles.

The analysis of variance demonstrated the significance of the time/week factor at a level of $p = 0.0104$, as well as the combination/study variant factor at a level of $p = 0.0000$. Additionally, the interaction between the studied factors was found to be significant at a level of $p = 0.0597$. On the other hand, the specific activity of SOD during the experiment showed low variability. In the first week, the lowest activity was observed in plant tissues treated with 100 ppm AuNPs (137.58 ± 22.07 U/mg). A decrease in SOD activity in plant tissues was observed in the third week, with the AgNPs 100 ppm variant displaying the lowest activity level (122.64 ± 14.10 U/mg) throughout the entire experiment. The analysis of variance revealed the significance of the time factor at a level of $p = 0.0000$, while the combination/experimental variant factor was not significant ($p = 0.4950$). Furthermore, the interaction between the two studied factors was not statistically significant ($p = 0.3580$).

3.5. Linear r Pearson Correlation Analysis

The correlation analysis revealed substantial interdependencies in the production of the analyzed elements when influenced by AgNPs and AuNPs. For the control plants, a predominantly positive correlation was observed between the content of chlorophylls (total, a, and b) and carotenoids (Figure 2). Additionally, the total and specific activities of GPOX and PPOX were positively correlated, with the highest correlation noticed for the specific activities of these enzymes. Furthermore, a strong positive correlation was found between the total and specific activities of GPOX.

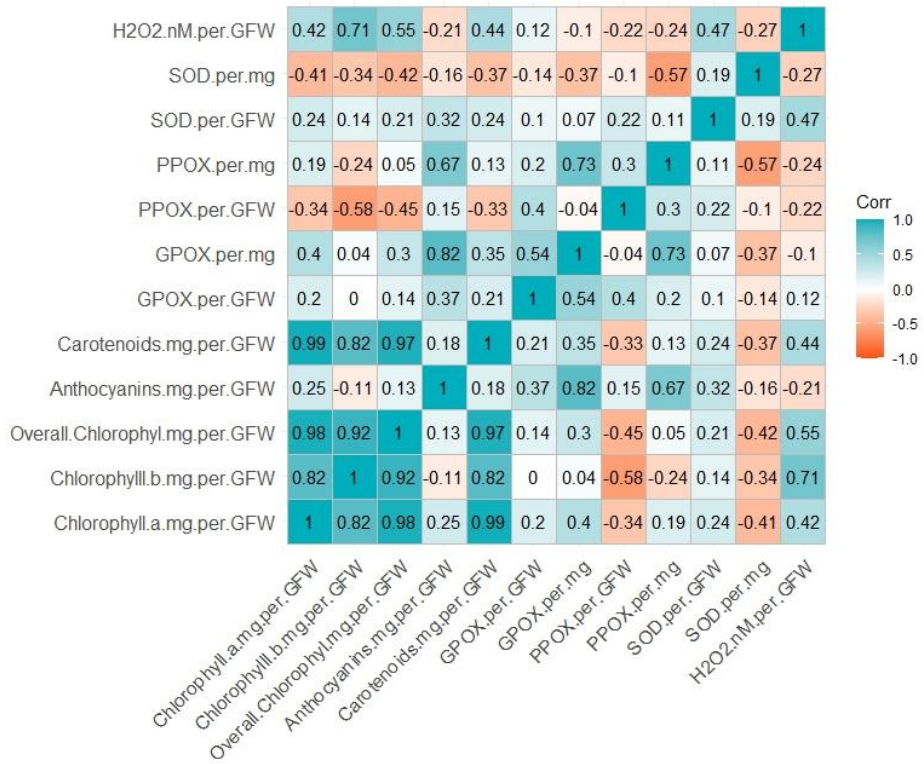

Figure 2. Pearson's r correlation matrix between examined features—Control.

There were also high positive correlations between the GPOX and PPOX specific activities and anthocyanins. Hydrogen peroxide content and chlorophyll content, especially chlorophyll b, the total activity of SOD, and carotenoids were positively correlated as well. In contrast, the specific activity of SOD and total activity of PPOX displayed a negative correlation with the chlorophylls and the specific activity of GPOX, particularly, a high-level negative correlation with the specific activity of PPOX.

In plants treated with 50 ppm AgNPs, a positive correlation was predominantly observed for the content of chlorophylls and carotenoids, and for the total and specific activities of PPOX, GPOX and SOD (Figure 3). Furthermore, a strong positive correlation

was found between the total and specific activities of GPOX. Additionally, such a positive correlation was found between the total and specific activities of GPOX, PPOX, and SOD enzymes as well as between carotenoids, chlorophylls (total and a), and the specific activity of PPOX. In the opposite direction, a negative correlation was found between chlorophylls, carotenoids, anthocyanins, and GPOX activities. Moreover, a negative correlation was noted between the specific activity of SOD and anthocyanins as well as between hydrogen peroxide and GPOX and SOD activities.

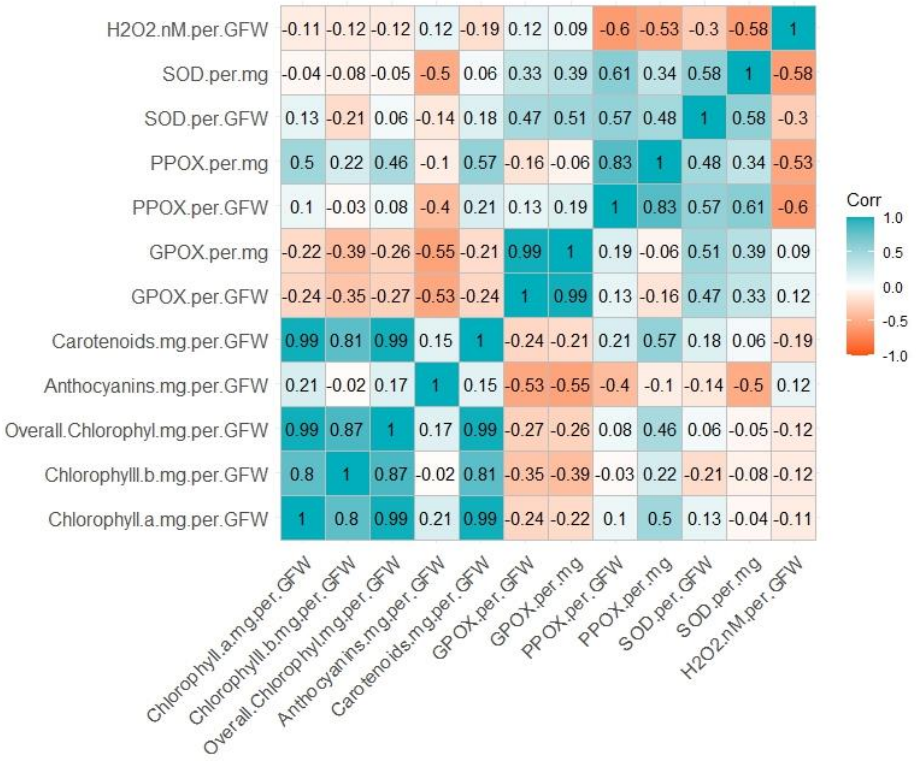

Figure 3. Pearson's r correlation matrix between examined features—AgNPs 50 ppm.

A higher rate of AgNPs, 100 ppm, affected the correlation matrix of analyzed elements differently compared to that with a lower rate except, especially, for a positive correlation of the content of chlorophylls and carotenoids (Figure 4). A high negative correlation was observed between hydrogen peroxide and chlorophylls, carotenoids, and SOD activity (total and specific). Furthermore, such a correlation was also found between the specific activities of PPOX and SOD. Moreover, anthocyanins were positively correlated with other analyzed elements in most of the cases, especially with GPOX activities.

In the case of plants exposed to 50 ppm AuNPs, a positive correlation was predominantly observed for the content of chlorophylls, carotenoids, and the specific activity of SOD and also for the total and specific activities of PPOX and GPOX (Figure 5). In the opposite direction, a higher negative correlation was found between the specific activity of GPOX and anthocyanins and total activity of SOD. Moreover, a negative correlation was noted between hydrogen peroxide and the total activities of GPOX and PPOX.

Plants treated with a higher dose of AuNPs 100 ppm showed a positive correlation in most of the instances, particularly between chlorophylls, carotenoids, and the specific activity of PPOX and total activity of SOD (Figure 6). The total and specific SOD activities, as well as the correlation between the total and specific PPOX activities and between carotenoids and the specific activity of PPOX and total activity of SOD, also demonstrated a positive correlation. In the opposite direction, a negative correlation was observed between the total activity of GPOX and chlorophylls, carotenoids, as well as between hydroxyl peroxide and chlorophylls, carotenoids, and the total and specific activities of SOD.

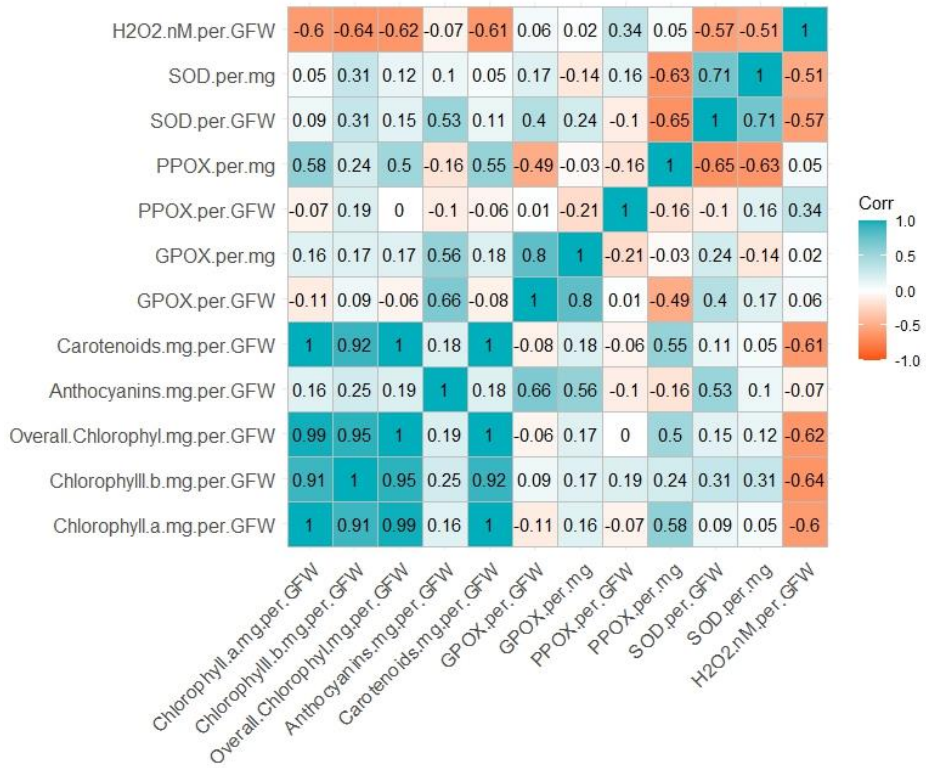

Figure 4. Pearson's r correlation matrix between examined features—AgNPs 100 ppm.

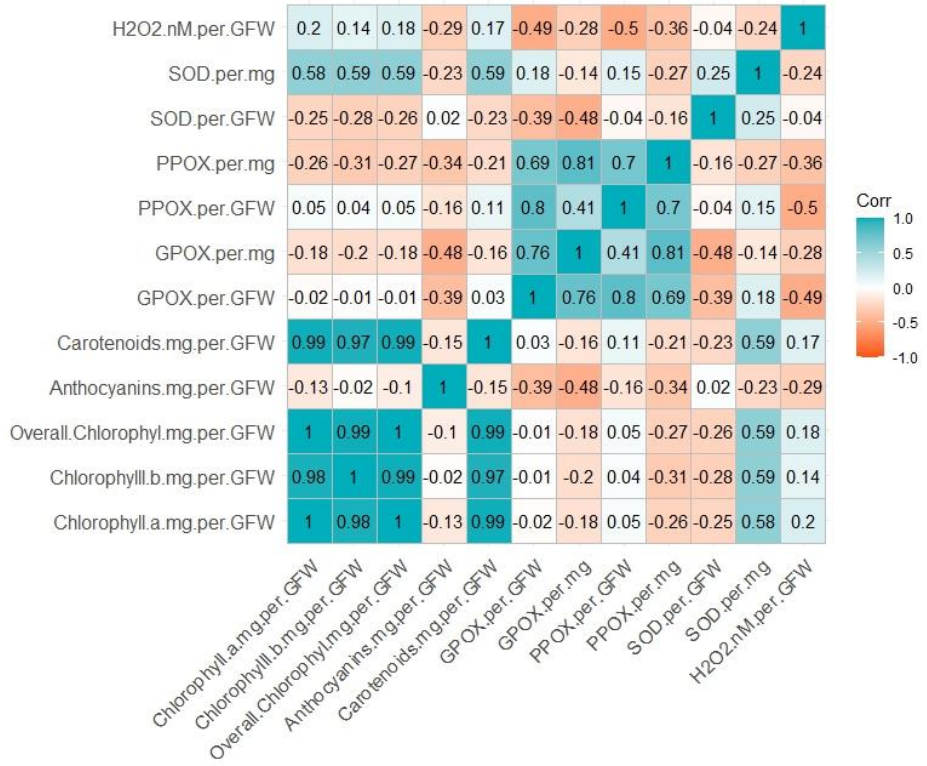

Figure 5. Pearson's r correlation matrix between examined features—AuNPs 50 ppm.

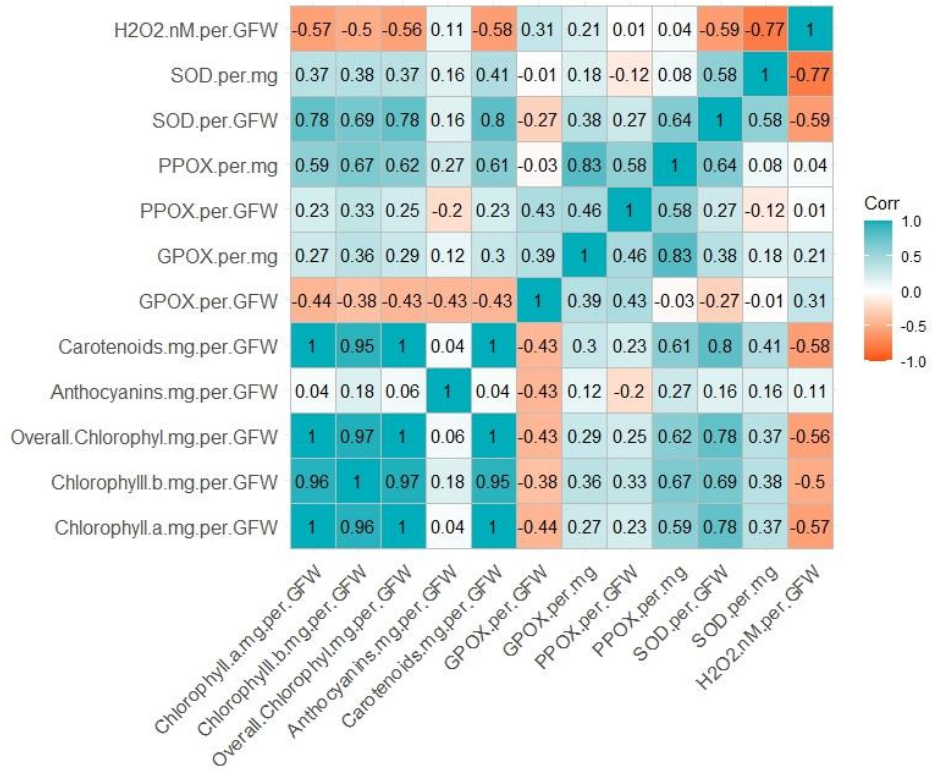

Figure 6. Pearson's r correlation matrix between examined features—AuNPs 100 ppm.

4. Discussion

Due to AgNPs' and AuNPs' high antimicrobial properties, they have multiple applications in the field of agriculture. Depending on the plant species, size, and the concentration of nanoparticles, both positive and negative effects have been reported [46]. Used as protective soaking or sprays, they are very effective in controlling various plant pathogens [66,67]. This could happen through the generation of reactive oxygen species (ROS) that damage the cellular components of the pathogens, disrupting their multiplication and plants growth [68]. They also lead to better germination rates and healthier, more robust plants when used to treat seeds before planting, enhancing plant growth and yield [69]. Despite the benefits, AgNPs or other stressors [70] may also cause oxidative stress in plants by producing excessive ROS, which can lead to cellular damage if the plants' antioxidant defenses are unable to neutralize them [71]. Some research indicates that exposure to AgNPs may impact gene expression in plants, turning certain genes on or off, it also affects the disruption transport of phytohormones [20,72,73].

The measurement of antioxidant enzyme activity can potentially serve as an effective indicator of the toxic impacts of environmental factors on plants. In our study, we explored the impact of nanoparticle exposure duration, type, and dosage on the activity of GPOX, PPOX, and SOD in rapeseed seedlings. Nevertheless, we did not observe a clear ascending or descending trend. During the first week, enzyme activity remained relatively consistent with that of the control group. Yet, in the second week, we noted the highest activity of GPOX for rapeseed seedlings exposed to AgNPs, particularly at a concentration of 50 ppm. Conversely, during the third week of the experiment, AuNPs exhibited the highest influence on GPOX activity, notably at the same concentration of 50 ppm. Research by Sharma et al. [71] showed that metal ions can alter enzyme activity in plant tissues, corroborating our findings of variations in GPOX activity following exposure to AuNPs and AgNPs. The observed elevated GPOX activity in plants treated with AuNPs is in line with studies like Manaf et al.'s [74], where increased peroxidase activity was seen under stress conditions. Our findings of time-dependent enzyme activity align with Slesak et al. [75], who showed different kinetics in plant stress responses, including enzyme activity

variations. The significant interaction between the studied factors—time and nanoparticle dose—resonates with the findings from Alharby et al. [76], who reported the influence of multiple factors on enzymatic activity. Our results also found less variability in specific activity compared to total activity, similar to observations of changes in enzyme activity levels made by Radić et al. [77]. Contrarily, some studies like Kao's [78] reported a decreased enzyme activity under heavy metal stress, contradicting our findings of increased GPOX activity with nanoparticle treatment. The fluctuating levels of GPOX activity observed in our study could be seen as inconsistent when compared to the research of Smirnoff [79], who reported a more uniform stress response of plants. The significant time-dependent effect in our study is at odds with findings from studies like Missaoui et al.'s [80], where CAT activity was found to be relatively constant over time. Our finding that nanoparticle dose significantly affects enzyme activity contradicts the findings of Movafeghi [81], who reported that enzyme activity can remain unaltered with varying exposure levels.

We observed similar trends in the peroxidase activity using pyrogallol as a substrate and in GPOX activity. This observation suggests a possible similar response mechanism to exposure conditions. The presence of nanoparticles can lead to an upregulation in PPOX activity as part of the plant's response to stress. However, the response of PPOX activity to nanoparticle treatment can be variable. For instance, Lei et al. [82] found an increase in PPOX activity in spinach plants treated with titanium nanoparticles, suggesting a stress response. Our research has shown that nanoparticles can impact PPOX activity differently depending on the duration of exposure. Jurkow et al. [83] observed some variations in PPOX activity over time, indicating a potential exhaustion of antioxidant defense systems in prolonged nanoparticle exposure. Different nanoparticles or metal ions also seem to affect PPOX activity in varied ways. While some nanoparticles like copper and zinc oxide have been found to significantly increase PPOX activity [84], others like silver nanoparticles and ions have been observed to decrease it [85].

The activity of SOD can be significantly influenced by the treatment of plants with nanoparticles. SOD is an important antioxidant enzyme in plants that helps scavenge reactive oxygen species (ROS), including superoxide radicals. Nanoparticles, due to their small size and large surface area, can easily interact with biological systems and potentially cause oxidative stress. In response to such stress, plants often exhibit altered activities in their antioxidant enzymes, including SOD, as part of their defense mechanisms [52]. The literature data have shown that different types of nanoparticles can elicit varying responses in SOD activity. Vannini et al. [86] also noted an increase in SOD activity in *Eruca sativa* cell cultures treated with AgNPs. This heightened SOD activity is a key part of the plant's defense mechanism against nanoparticle-induced stress. Barbasz and others [85] found no effect of AgNPs on SOD activity and opposite results for peroxidases in two tested wheat varieties. Conversely, exposure to titanium dioxide nanoparticles caused a decrease in SOD activity in wheat seedlings, possibly due to the excessive ROS production surpassing the detoxifying capacity of SOD [45]. The concentration of nanoparticles and the duration of exposure can also influence SOD activity. Tripathi et al. [87] observed an increase in SOD activity in higher concentrations demonstrating a dose-dependent response. In a study by Szymańska et al. [88], *Arabidopsis thaliana* plants exposed to TiO_2NPs showed an increase in SOD activity, indicative of an antioxidant response. However, the reaction was less pronounced than in plants exposed to other types of nanoparticles, suggesting that AuNPs might be less stressful for the plants. Moreover, the duration of nanoparticle exposure can modulate the response of SOD activity. An initial increase in SOD activity might be followed by a decline over time, potentially due to the exhaustion of the antioxidant defense system in prolonged stress conditions [89]. Nonetheless, the effects of AgNPs and AuNPs on SOD activity may vary considerably, influenced significantly by numerous factors such as the plant species and its growth stage. By studying the effects and optimizing the use of nanoparticles in agriculture, their potential can be fully exploited so that they can be a modern tool in agriculture and contribute to the development of environmentally friendly and efficient agricultural practices [90].

A study conducted by Barbasz and colleagues [85] examined the effects of AgNPs and ions on the activity of antioxidative enzymes in the callus cells of two varieties of wheat. The results showed that there was no effect of silver on the SOD activity, whereas CAT activity was significantly decreased. The changes in the activity of peroxidases in both varieties were opposite. From the other side, Iqbal et al. [91] undertook a study focused on understanding the impact of AgNPs on various physiological, biochemical, and antioxidant parameters of wheat under heat stress conditions. They discovered that the introduction of AgNPs resulted in protective effects on the plant tissues under stress. Specifically, wheat plants treated with AgNPs demonstrated a significant augmentation in dry matter content, coupled with an increase in antioxidant defense under heat stress conditions. Findings from Gunjan and colleagues [92] also imply that the enzymatic complex, comprised of APX, GPOX, and glutathione reductase (GR), may play a role in a plant's defense mechanism against the oxidative stress triggered by nanometals in *Brassica juncea*. Authors investigated the effects of AuNPs on the antioxidative enzyme activity in *B. juncea* seedlings. They found that the activities of those enzymes increased in response to elevated AuNPs concentrations (200 ppm). These activities were notably higher than those of CAT. In a study by Sharma et al. [93], there was also increased GPOX activity observed in *B. juncea* seedlings exposed to AgNPs. Similar results were obtained by Tripathi and colleagues in their research [87]. They observed that AgNPs, when used at high concentrations, significantly stimulated the activity of both SOD and APX. However, they also found that these nanoparticles inhibited the activity of GR and dehydroascorbate reductase (DHAR) in *Pisum sativum* seedlings.

Tripathi et al. [87] also found increased ascorbate peroxidase activity in pea seedlings upon AgNPs exposure, suggesting an activated antioxidant response to nanoparticle-induced stress. The impact of AuNPs on peroxidases activity is less understood. However, a study by Sharma et al. [93] showed that AgNPs can stimulate the production of hydrogen peroxide in *B. juncea* seedlings, leading to an increase in GPOX activity. This suggests that even though AuNPs are generally considered less toxic than AgNPs, they can still induce oxidative stress responses in plants, including the activation of GPOX.

The correlation study's findings provide valuable insights into the complex interplay of various elements and activities in the presence of AgNPs and AuNPs, shedding light on their potential impact on plant physiology and biochemistry. We observed large differences in the activity of oxidative stress markers under the influence of the tested doses of silver and gold nanoparticles. In plants not exposed to nanoparticles, the presence of H_2O_2 is positively correlated with the content of chlorophyll, carotenoids, and specific SOD activity. However, there is no clear correlation with PPOX, GPOX, and anthocyanin compounds. The presence of AgNPs at the dose of 50 ppm significantly modifies the seedlings' response. It causes a decrease in SOD and PPOX activity. In turn, at a higher dose of 100 ppm, the activity of SOD, carotenoids, and the chlorophyll content decrease. An identical situation occurs in the case of a higher dose of AuNPs. This may make plants more susceptible to damages caused by the presence of H_2O_2. In turn, the presence of AuNPs at a lower dose causes only a decrease in the activity of peroxidases at an average level. In other cases, there is no correlation of H_2O_2 with the other tested markers.

5. Conclusions

Although there are many reports about the impact of nanoparticles, it is difficult to find information on the impact of the length of exposure to AgNPs and AuNPs on the physiological and biochemical processes occurring in plants. Therefore, we examined plant responses, which varied depending on the duration of nanoparticle exposure. Our research suggests a significant impact of AgNP and AuNP in stimulating the response of rapeseed seedlings, as indicated especially by the activity of GPOX and to a lesser extent PPOX and SOD. We found that the exposure time of rapeseed seedlings to nanoparticles is a very important factor influencing the content of total protein and the activity of the tested enzymes. Enzyme activity tends to increases with the length of exposure time, while the content of free protein decreases over the weeks. The type and dose of nanoparticles used

also affect the analyzed markers to quite a high extent. Typically, a greater influence of AuNPs was observed in the first and third week, while AgNPs seemed to have a more potent effect in the second week. All these studies will further the understanding of complex plant responses to nanoparticle stress (length of exposure and concentration). The possibilities of applications of nanomaterials offer great potential, but the long-term effects of their use in agriculture on the environment and human health are not yet fully known. It is necessary to develop safe and sustainable practices for the use of nanomaterials in agriculture as an element of innovative technologies combining novel, non-chemical means of plant production and protection [94–97].

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References

1. Fraceto, L.F.; Grillo, R.; de Medeiros, G.A.; Scognamiglio, V.; Rea, G.; Bartolucci, C. Nanotechnology in agriculture: Which innovation potential does it have? *Front. Environ. Sci.* **2016**, *4*, 20. [CrossRef]
2. Mittal, D.; Kaur, G.; Singh, P.; Yadav, K.; Ali, S.A. Nanoparticle-Based Sustainable Agriculture and Food Science: Recent Advances and Future Outlook. *Front. Nanotechnol.* **2020**, *2*, 579954. [CrossRef]
3. Jeevanandam, J.; Barhoum, A.; Chan, Y.S.; Dufresne, A.; Danquah, M.K. Review on nanoparticles and nanostructured materials: History, sources, toxicity and regulations. *Beilstein J. Nanotechnol.* **2018**, *9*, 1050–1074. [CrossRef] [PubMed]
4. Radad, K.; Al-Shraim, M.; Moldzio, R.; Rausch, W.D. Recent advances in benefits and hazards of engineered nanoparticles. *Environ. Toxicol. Pharmacol.* **2012**, *34*, 661–672. [CrossRef] [PubMed]
5. Khodakovskaya, M.V.; de Silva, K.; Biris, A.S.; Dervishi, E.; Villagarcia, H. Carbon nanotubes induce growth enhancement of tobacco cells. *ACS Nano* **2012**, *6*, 2128–2135. [CrossRef] [PubMed]
6. Lovecká, P.; Macůrková, A.; Záruba, K.; Hubáček, T.; Siegel, J.; Valentová, O. Genomic Damage Induced in *Nicotiana tabacum* L. Plants by Colloidal Solution with Silver and Gold Nanoparticles. *Plants* **2021**, *10*, 1260. [CrossRef]
7. Rastogi, A.; Zivcak, M.; Sytar, O.; Kalaji, H.M.; He, X.; Mbarki, S.; Brestic, M. Impact of Metal and Metal Oxide Nanoparticles on Plant: A Critical Review. *Front. Chem.* **2017**, *5*, 78. [CrossRef] [PubMed]
8. Wang, P.; Lombi, E.; Zjao, F.J.; Kopittke, P.M. Nanotechnology: A new opportunity in plant sciences. *Trends Plant Sci.* **2016**, *21*, 699–712. [CrossRef]
9. Leso, V.; Fontana, L.; Iavicoli, I. Biomedical nanotechnology: Occupational views. *Nano Today* **2019**, *24*, 10–14. [CrossRef]
10. Stefan, L.; Monchaud, D. Applications of guanine quartets in nanotechnology and chemical biology. *Nat. Rev. Chem.* **2019**, *3*, 650–668. [CrossRef]
11. Li, W.Q.; Qing, T.; Li, C.C.; Li, F.; Ge, F.; Fei, J.J.; Peijnenburg, W.J. Integration ofsubcellular partitioning and chemical forms to understand silver nanoparticles toxicity to lettuce (*Lactuca sativa* L.) under different exposure pathways. *Chemosphere* **2020**, *258*, 127349. [CrossRef] [PubMed]
12. Yan, A.; Chen, Z. Impacts of Silver Nanoparticles on Plants: A Focus on the Phytotoxicity and Underlying Mechanism. *Int. J. Mol. Sci.* **2019**, *20*, 1003. [CrossRef] [PubMed]
13. Yaqoob, A.A.; Parveen, T.; Umar, K.; Mohamad, M.N. Role of nanomaterials in the treatment of wastewater: A review. *Water* **2020**, *12*, 495. [CrossRef]
14. Sim, S.; Wong, N.K. Nanotechnology and its use in imaging and drug delivery (Review). *Biomed. Rep.* **2021**, *14*, 42. [CrossRef] [PubMed]
15. Usman, M.; Farooq, M.; Wakeel, A.; Nawaz, A.; Cheema, S.A.; Rehman, H.; Ashraf, I.; Sanaullah, M. Nanotechnology in agriculture: Current status, challenges and future opportunities. *Sci. Total. Environ.* **2020**, *721*, 137778. [CrossRef] [PubMed]

16. Alvarez Perez, S.; Tapia, M.A.M.; González Vega, M.E.; Ardisana, E.F.; Chávez Medina, J.A.; Flores Zamora, G.L.; Bustamante, D.V. Nanotechnology and Plant Tissue Culture. In *Plant Nanobionics*; Prasad, R., Ed.; Nanotechnology in the Life Sciences; Springer: Cham, Switzerland, 2019; pp. 333–370.
17. Moradpour, M.; Aziz, M.A.; Abdullah, S.N.A. Establishment of in vitro culture of rubber (*Hevea brasiliensis*) from field-derived explants: Effective role of silver nanoparticles in reducing contamination and browning. *J. Nanomed. Nanotechnol.* **2016**, *7*, 375. [CrossRef]
18. Parzymies, M.; Pudelska, K.; Poniewozik, M. The use of nano-silver for disinfection of *Pennisetum alopecuroides* plant material for tissue culture. *Acta Sci. Pol. Hortorum Cultus* **2019**, *18*, 128–135. [CrossRef]
19. Hatami, M. Toxicity assessment of multi-walled carbon nanotubes on *Cucurbita pepo* L. under well-watered and water-stressed conditions. *Ecotoxicol. Environ. Saf.* **2017**, *142*, 274–283. [CrossRef]
20. Cvjetko, P.; Milošic, A.; Domijan, A.M.; Vinkovic Vrcek, I.; Tolic, S.; Peharec Štefanic, P.; Letofsky-Papst, I.; Tkalec, M.; Balen, B. Toxicity of silver ions and differently coated silver nanoparticles in *Allium cepa* roots. *Ecotoxicol. Environ. Saf.* **2017**, *137*, 18–28. [CrossRef]
21. Timoteo de Oliveira, C.; Paiva, R.; Reis, M.V.; Claro, P.I.C.; Ferraz, L.M.; Marconcini, J.M.; de Oliveira, J.E. In vitro growth of *Physalis peruviana* L. affected by silver nanoparticles. *3 Biotech* **2019**, *9*, 145. [CrossRef]
22. Kokura, S.; Handa, O.; Takagi, T.; Ishikawa, T.; Naito, Y.; Yoshikawa, T. Silver nanoparticles as a safe preservative for use in cosmetics. *Nanomedicine* **2010**, *6*, 570–574. [CrossRef] [PubMed]
23. Marambio-Jones, C.; Hoek, E.M. A review of the antibacterial effects of silver nanomaterials and potential implications for human health and the environment. *J. Nanopart. Res.* **2010**, *12*, 1531–1551. [CrossRef]
24. Hammami, I.; Alabdallah, N.M. Gold nanoparticles: Synthesis properties and applications. *J. King Saud Univ.-Sci.* **2021**, *33*, 101560. [CrossRef]
25. Das, M.; Shim, K.H.; An, S.S.A.; Yi, D. Review on gold nanoparticles and their applications. *J. Toxiol. Env.* **2012**, *3*, 193–205. [CrossRef]
26. Panyala, N.R.; Pena-Mendez, E.M.; Havel, J. Gold and nano-gold in medicine: Overview, toxicology and perspectives. *J. Appl. Biomed.* **2009**, *7*, 75–91. [CrossRef]
27. Ahmed, J.; Gultekinoglu, M.; Edirsinghe, M. Bacterial cellulose micro-nano fibres for wound healing applications. *Biotechnol. Adv.* **2020**, *41*, 107549. [CrossRef] [PubMed]
28. Matharu, R.K.; Ciric, L.; Ren, G.; Edirsinghe, M. Comparative study of the antimicrobial effects of tungsten nanoparticles and tungsten nanocomposite fibres on hospital acquired bacterial and viral pathogens. *J. Nanomater.* **2020**, *10*, 1017. [CrossRef] [PubMed]
29. Chou Chau, Y.F.; Ming, T.Y.; Chou Chao, C.T.; Thotagamuge, R.; Kooh, M.R.R.; Huang, H.J.; Lim, C.M.; Chiang, H.-P. Significantly enhanced coupling effect and gap plasmon resonance in a MIM-cavity based sensing structure. *Sci. Rep.* **2021**, *11*, 18515. [CrossRef]
30. Chau, Y.; Yang, T.; Lee, W. Coupling technique for efficient interfacing between silica waveguides and planar photonic crystal circuits. *Appl. Opt.* **2004**, *43*, 6656–6663.
31. Tomaszewska-Sowa, M.; Lisiecki, K.; Pańka, D. Response of Rapeseed (*Brassica napus* L.) to Silver and Gold Nanoparticles as a Function of Concentration and Length of Exposure. *Agronomy* **2022**, *12*, 2885. [CrossRef]
32. Stampoulis, D.; Sinha, S.K.; White, J.C. Assay-dependent phytotoxicity of nanoparticles to plants. *Environ. Sci. Technol.* **2009**, *43*, 9473–9479. [CrossRef] [PubMed]
33. Qian, H.; Peng, X.; Han, X.; Ren, J.; Sun, L.; Fu, Z. Comparison of the toxicity of silver nanoparticles and silver ions on the growth of terrestrial plant model *Arabidopsis thaliana*. *J. Environ. Sci.* **2013**, *25*, 1947–1956. [CrossRef] [PubMed]
34. Ma, X.; Geisler-Lee, J.; Deng, Y.; Kolmakov, A. Interactions between engineered nanoparticles (ENPs) and plants: Phytotoxicity, uptake and accumulation. *Sci. Total Environ.* **2010**, *408*, 3053–3061. [CrossRef] [PubMed]
35. Nair, P.M.G.; Chung, I.M. Physiological and molecular level effects of silver nanoparticles exposure in rice (*Oryza sativa* L.) seedlings. *Chemosphere* **2014**, *112*, 105–113. [CrossRef] [PubMed]
36. Siddiqi, K.S.; Husen, A. Plant response to silver nanoparticles: A critical review. *Crit. Rev. Biotechnol.* **2021**, *42*, 973–990. [CrossRef]
37. Jiang, H.S.; Li, M.; Chang, F.Y.; Li, W.; Yin, L.Y. Physiological analysis of silver nanoparticles and $AgNO_3$ toxicity to *Spirodela polyrhiza*. *Environ. Toxicol. Chem.* **2012**, *31*, 1880–1886. [CrossRef]
38. Falco, W.F.; Queiroz, A.M.; Fernandes, J.; Botero, E.R.; Falcao, E.A.; Guimaraes, F.E.G.; M'Peko, J.C.; Oliveira, S.L.; Colbeck, I.; Caires, A.R.L. Interaction between chlorophyll and silver nanoparticles: A close analysis of chlorophyll fluorescence quenching. *J. Photochem. Photobiol. A Chem.* **2015**, *299*, 203–209. [CrossRef]
39. Noori, A.; Ngo, A.; Gutierrez, P.; Theberge, S.; White, J.C. Silver nanoparticle detection and accumulation in tomato (*Lycopersicon esculentum*). *J. Nanopart. Res.* **2020**, *22*, 131. [CrossRef]
40. Ferrari, E.; Barbero, F.; Busquets-Fité, M.; Franz-Wachtel, M.; Köhler, H.-R.; Puntes, V.; Kemmerling, B. Growth-Promoting Gold Nanoparticles Decrease Stress Responses in Arabidopsis Seedlings. *Nanomaterials* **2021**, *11*, 3161. [CrossRef]
41. Savithramma, N.; Ankanna, S.; Bhumi, G. Effect of nanoparticles on seed germination and seedling growth of *Boswellia ovalifoliolata*—An endemic and endangered medicinal tree taxon. *Nanovis* **2012**, *2*, 61–68.
42. Arora, S.; Sharma, P.; Kumar, S.; Nayan, R.; Khanna, P.K.; Zaidi, M.G.H. Gold-nanoparticle induced enhancement in growth and seed yield of *Brassica juncea*. *Plant Growth Regul.* **2012**, *66*, 303–310. [CrossRef]

43. Gopinath, K.; Gowri, S.; Karthika, V.; Arumugam, A. Green synthesis of gold nanoparticles from fruit extract of *Terminalia arjuna*, for the enhanced seed germination activity of *Gloriosa superba*. *J. Nanostruct. Chem.* **2014**, *4*, 115. [CrossRef]
44. Kumar, V.; Guleria, P.; Kumar, V.; Yadav, S.K. Gold nanoparticle exposure induces growth and yield enhancement in *Arabidopsis thaliana*. *Sci. Total Environ.* **2013**, *461*, 462–468. [CrossRef]
45. Ghosh, M.; Ghosh, I.; Godderis, L.; Hoet, P.; Mukherjee, A. Genotoxicity of engineered nanoparticles in higher plants. *Mutat. Res. Toxicol. Environ. Mutagen.* **2019**, *842*, 132–145. [CrossRef] [PubMed]
46. Burketová, L.; Martinec, J.; Siegel, J.; Macůrková, A.; Maryška, L.; Valentová, O. Noble metal nanoparticles in agriculture: Impacts on plants, associated microorganisms, and biotechnological practices. *Biotechnol. Adv.* **2022**, *58*, 107929. [CrossRef] [PubMed]
47. Møller, I.M.; Jensen, P.E.; Hansson, A. Oxidative modifications to cellular components in plants. *Annu. Rev. Plant Biol.* **2007**, *58*, 459–481. [CrossRef] [PubMed]
48. Huang, H.; Ullah, F.; Zhou, D.X.; Yi, M.; Zhao, Y. Mechanisms of ROS regulation of plant development and stress responses. *Front. Plant. Sci.* **2019**, *10*, 800. [CrossRef]
49. Wang, S.; Wu, X.M.; Liu, C.H.; Shang, J.Y.; Gao, F.; Guo, H.S. *Verticillium dahliae* chromatin remodeling facilitates the DNA damage repair in response to plant ROS stress. *PLoS Pathog.* **2020**, *16*, e1008481. [CrossRef]
50. Romero-Puertas, M.C.; Palma, J.M.; Gomez, M.; Del Rio, L.A.; Sandalio, L.M. Cadmium causes oxidative modification of proteins in pea plants. *Plant Cell Environ.* **2002**, *25*, 677–686. [CrossRef]
51. Zhang, L.; Wu, M.; Teng, Y.; Jia, S.; Yu, D.; Wei, T.; Chen, C.; Song, W. Overexpression of the Glutathione Peroxidase 5 (RcGPOX5) Gene From *Rhodiola crenulata* Increases Drought Tolerance in *Salvia miltiorrhiza*. *Front. Plant Sci.* **2018**, *9*, 1950. [CrossRef]
52. Faisal, M.; Saquib, Q.; Alatar, A.A.; Al-Khedhairy, A.A.; Hegazy, A.K.; Musarrat, J. Phytotoxic hazards of NiO-nanoparticles in tomato: A study on mechanism of cell death. *J. Hazard. Mater.* **2013**, *250*, 318–332. [CrossRef] [PubMed]
53. Jiang, H.S.; Qiu, X.N.; Li, G.B.; Li, W.; Yin, L.Y. Silver nanoparticles induced accumulation of reactive oxygen species and alteration of antioxidant systems in the aquatic plant *Spirodela polyrhiza*. *Environ. Toxicol. Chem.* **2014**, *33*, 1398–1405. [CrossRef] [PubMed]
54. da Costa, M.V.J.; Sharma, P.K. Effect of copper oxide nanoparticles on growth, morphology, photosynthesis, and antioxidant response in *Oryza sativa*. *Photosynthetica* **2016**, *54*, 110–119. [CrossRef]
55. Stroiński, A. Some physiological and biochemical aspects of plant resistance to cadmium effect. I. Antioxidative system. *Acta Physiol. Plant.* **1999**, *21*, 175–188. [CrossRef]
56. Orsavova, J.; Misurcova, L.; Ambrozova, J.V.; Vicha, R.; Mlcek, J. Fatty acids composition of vegetable oils and its contribution to dietary energy intake and dependence of cardiovascular mortality on dietary intake of fatty acids. *Int. J. Mol. Sci.* **2015**, *16*, 12871–12890. [CrossRef]
57. Wang, Y.; Meng, G.; Chen, S.; Chen, Y.; Jiang, J.; Wang, Y.-P. Correlation analysis of phenolic contents andantioxidation in yellow- and black-seeded *Brassica napus*. *Molecules* **2018**, *23*, 1815. [CrossRef]
58. Tomaszewska-Sowa, M.; Siwik-Ziomek, A.; Figas, A.; Bocian, K. Assessment of metal nanoparticle-induced morphological and physiological changes in in vitro cultures of rapeseed (*Brassica napus* L.). *EJPAU* **2018**, *21*, 10. [CrossRef]
59. Murashige, T.; Skoog, F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* **1962**, *15*, 473–497. [CrossRef]
60. Domeradzka-Gajda, Y.K.; Nocuń, M.; Roszak, J.; Janasik, B.; Quarles, C.D., Jr.; Wąsowicz, W.; Grobelny, J.; Tomaszewska, E.; Celichowski, G.; Ranoszek-Soliwoda, K.; et al. A study on the in vitro percutaneous absorption of silver nanoparticles in combination with aluminum chloride, methyl paraben or di-n-butyl phthalate. *Toxicol. Lett.* **2017**, *272*, 38–48. [CrossRef]
61. Pudlarz, A.; Czechowska, E.; Ranoszek-Soliwoda, K.; Tomaszewska, E.; Celichowski, G.; Grobelny, J.; Szemraj, J. Immobilization of Recombinant Human Catalase on Gold and Silver Nanoparticles. *Appl. Biochem. Biotechnol.* **2018**, *185*, 717–735. [CrossRef]
62. Bradford, M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248–254. [CrossRef]
63. Zahir, S.; Zhang, F.; Chen, J.; Zhu, S. Determination of Oxidative Stress and Antioxidant Enzyme Activity for Physiological Phenotyping During Heavy Metal Exposure. *Methods Mol. Biol.* **2021**, *2326*, 241–249. [CrossRef]
64. Chance, B.; Maehly, A.C. Assay of catalases and peroxidases. *Meth. Enzymol.* **1955**, *2*, 764–775. [CrossRef]
65. McCord, J.M.; Fridovich, I. Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). *J. Biol. Chem.* **1969**, *244*, 6049–6055. [CrossRef]
66. Jo, Y.K.; Kim, B.H.; Jung, G. Antifungal activity of silver ions and nanoparticles on phytopathogenic fungi. *Plant Dis.* **2009**, *93*, 1037–1043. [CrossRef]
67. Mishra, S.; Singh, B.R.; Singh, A.; Keswani, C.; Naqvi, A.H.; Singh, H.B. Biofabricated silver nanoparticles act as a strong fungicide against *Bipolaris sorokiniana* causing spot blotch disease in wheat. *PLoS ONE* **2014**, *9*, e97881. [CrossRef]
68. Thuesombat, P.; Hannongbua, S.; Akasit, S.; Chadchawan, S. Effect of silver nanoparticles on rice (*Oryza sativa* L. cv. KDML 105) seed germination and seedling growth. *Ecotoxicol. Environ. Saf.* **2014**, *104*, 302–309. [CrossRef]
69. Mahakham, W.; Sarmah, A.K.; Maensiri, S.; Theerakulpisut, P. Nanopriming technology for enhancing germination and starch metabolism of aged rice seeds using phytosynthesized silver nanoparticles. *Sci. Rep.* **2017**, *7*. [CrossRef]
70. Xalxo, R.; Yadu, B.; Chandrakar, V.; Kumar, M.; Chandra, J.; Sahu, K. Silver Nanoparticles and Their Morpho-Physiological Responses on Plants. In *Plant Responses to Nanomaterials, Recent Interventions, and Physiological and Biochemical Responses*; Singh, V.P., Singh, S., Tripathi, D.K., Prasad, S.M., Chauhan, D.K., Eds.; Springer Nature: Cham, Switzerland, 2021; pp. 183–216. [CrossRef]

71. Sharma, P.; Jha, A.B.; Dubey, R.S.; Pessarakli, M. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. *J. Bot.* **2012**, *2012*, 217037. [CrossRef]
72. Singh, N.; Manshian, B.; Jenkins, G.J.; Griffiths, S.M.; Williams, P.M.; Maffeis, T.G.; Wright, C.J.; Doak, S.H. NanoGenotoxicology: The DNA damaging potential of engineered nanomaterials. *Biomaterials* **2009**, *30*, 3891–3914. [CrossRef]
73. Yin, L.; Colman, B.P.; McGill, B.M.; Wright, J.P.; Bernhardt, E.S. Effects of silver nanoparticle exposure on germination and early growth of eleven wetland plants. *PLoS ONE* **2012**, *7*, e47674. [CrossRef]
74. Manaf, A.; Wang, X.; Tariq, F.; Jhanzab, H.M.; Bibi, Y.; Sher, A.; Razzaq, A.; Fiaz, S.; Tanveer, S.K.; Qayyum, A. Antioxidant Enzyme Activities Correlated with Growth Parameters of Wheat Sprayed with Silver and Gold Nanoparticle Suspensions. *Agronomy* **2021**, *11*, 1494. [CrossRef]
75. Slesak, I.; Libik, M.; Karpinska, B.; Karpinski, S.; Miszalski, Z. The role of hydrogen peroxide in regulation of plant metabolism and cellular signalling in response to environmental stresses. *Acta Biochim. Pol.* **2007**, *54*, 39–50. [CrossRef] [PubMed]
76. Alharby, H.F.; Metwali, E.M.R.; Fuller, M.P.; Aldhebiani, A.Y. Impact of application of zinc oxide nanoparticles on callus induction, plant regeneration, element content and antioxidant enzyme activity in tomato (*Solanum lycopersicum* Mill.) under salt stress. *Arch. Biol. Sci.* **2016**, *68*, 723–735. [CrossRef]
77. Radić, S.; Stipaničev, D.; Cvjetko, P.; Marijanović Rajčić, M.; Širac, S.; Pevalek-Kozlina, B.; Pavlica, M. Duckweed *Lemna minor* as a tool for testing toxicity and genotoxicity of surface waters. *Ecotoxicol. Environ. Safe* **2011**, *74*, 182–187. [CrossRef]
78. Kao, C.H. Role of glutathione in abiotic stress tolerance of rice plants. *J. Taiwan Agric. Res.* **2015**, *164*, 167–176.
79. Smirnoff, N. The role of active oxygen in the response of plants to water deficit and desiccation. *New Phytol.* **1993**, *125*, 27–58. [CrossRef]
80. Missaoui, T.; Smiri, M.; Chmingui, H.; Hafiane, A. Effects of nanosized titanium dioxide on the photosynthetic metabolism of fenugreek (*Trigonella foenum-graecum* L.). *Comptes. Rendus Biol.* **2017**, *340*, 499–511. [CrossRef]
81. Movafeghi, A.; Khataee, A.; Abedi, M.; Tarrahi, R.; Dadpour, M.; Vafaei, F. Effects of TiO2 nanoparticles on the aquatic plant *Spirodela polyrrhiza*: Evaluation of growth parameters, pigment contents and antioxidant enzyme activities. *J. Environ. Sci.* **2018**, *64*, 130–138. [CrossRef]
82. Lei, Z.; Mingyu, S.; Xiao, W.; Chao, L.; Chunxiang, Q.; Liang, C.; Hao, H.; Xiaoqing, L.; Fashui, H. Antioxidant stress is promoted by nano-anatase in spinach chloroplasts under UV-B radiation. *Biol. Trace Elem. Res.* **2008**, *121*, 69–79. [CrossRef]
83. Jurkow, R.; Pokluda, R.; Sękara, A.; Kalisz, A. Impact of foliar application of some metal nanoparticles on antioxidant system in oakleaf lettuce seedlings. *BMC Plant Biol.* **2020**, *20*, 290. [CrossRef] [PubMed]
84. Kim, S.; Lee, S.; Lee, I. Alteration of phytotoxicity and oxidant stress potential by metal oxide nanoparticles in *Cucumis sativus*. *Water Air Soil Pollut.* **2012**, *223*, 2799–2806. [CrossRef]
85. Barbasz, A.; Kreczmer, B.; Ocwieja, M. Effects of exposure of callus cells of two wheat varieties to silver nanoparticles and silver salt (AgNO3). *Acta Physiol. Plant.* **2016**, *38*, 76. [CrossRef]
86. Vannini, C.; Domingo, G.; Onelli, E.; Prinsi, B.; Marsoni, M.; Espen, L.; Bracale, M. Morphological and proteomic responses of *Eruca sativa* exposed to silver nanoparticles or silver nitrate. *PLoS ONE* **2013**, *8*, e68752. [CrossRef] [PubMed]
87. Tripathi, D.K.; Singh, S.; Singh, S.; Srivastava, P.K.; Singh, V.P.; Singh, S.; Prasad, S.M.; Singh, P.K.; Dubey, N.K.; Pandey, A.C.; et al. Nitric oxide alleviates silver nanoparticles(AgNps)-induced phytotoxicity in *Pisum sativum* seedlings. *Plant Physiol. Biochem.* **2017**, *110*, 167–177. [CrossRef] [PubMed]
88. Szymańska, R.; Kołodziej, K.; Ślesak, I.; Zimak-Piekarczyk, P.; Orzechowska, A.; Gabruk, M.; Żądło, A.; Habina, I.; Knap, W.; Burda, K.; et al. Titanium dioxide nanoparticles (100–1000 mg/l) can affect vitamin E response in Arabidopsis thaliana. *Environ. Pollut.* **2016**, *213*, 957–965. [CrossRef]
89. Shaw, A.; Ghosh, S.; Kalaji, M.; Bosa, K.; Brestic, M.; Zivcak, M.; Hossain, Z. Nano-CuO stress induced modulation of antioxidative defense and photosynthetic performance of Syrian barley (*Hordeum vulgare* L.). *Environ. Exp. Bot.* **2014**, *102*, 37–47. [CrossRef]
90. Parveen, A.; Sonkar, S.; Sarangi, P.K.; Singh, A.K.; Sahoo, U.K.; Gupta, R.; Prus, P.; Imbrea, F.; Șmuleac, L.; Pașcalău, R. Harnessing the Eco-Friendly Potential of *Asparagus racemosus* Leaf Extract Fabricated Ni/Ni(OH)$_2$ Nanoparticles for Sustainable Seed Germination and Seedling Growth of *Vigna radiata*. *Agronomy* **2023**, *13*, 2073. [CrossRef]
91. Iqbal, M.; Raja, N.I.; Mashwani, Z.-U.-R.; Hussain, M.; Ejaz, M.; Yasmeen, F. Effect of silver nanoparticles on growth of wheat under heat stress. *Iran. J. Sci. Technol. Trans. A Sci.* **2017**, *43*, 387–395. [CrossRef]
92. Gunjan, B.; Zaidi, M.G.H.; Sandeep, A. Impact of gold nanoparticles on physiological and biochemical characteristics of *Brasica juncea*. *J. Plant. Physiol. Biochem.* **2014**, *2*, 133. [CrossRef]
93. Sharma, P.; Bhatt, D.; Zaidi, M.G.H.; Saradhi, P.P.; Khanna, P.K.; Arora, S. Silver nanoparticle-mediated enhancement in growth and antioxidant status of *Brassica juncea*. *Appl. Biochem. Biotechnol.* **2012**, *167*, 2225–2233. [CrossRef] [PubMed]
94. Pedrini, S.; Merritt, D.J.; Stevens, J.; Dixon, K. Seed Coating: Science or Marketing Spin? *Trends Plant. Sci.* **2017**, *22*, 106–116. [CrossRef] [PubMed]
95. Decerle, J. Farmers and the "Farm to Fork" Strategy. In *Farm to Fork: Towards a Sustainable and Resilient Food System in Europe*; Ulmann, L., Mai Thanh, C., Eds.; The European Files/Les Dossiers Europeenes: Brussels, Belgium, 2020; Volume 64, p. 22.

96. Elshafie, H.S.; Camele, I. Applications of Absorbent Polymers for Sustainable Plant Protection and Crop Yield. *Sustainability* **2021**, *13*, 3253. [CrossRef]
97. Pańka, D.; Jeske, M.; Łukanowski, A.; Baturo-Cieśniewska, A.; Prus, P.; Maitah, M.; Maitah, K.; Malec, K.; Rymarz, D.; Muhire, J.d.D.; et al. Can Cold Plasma Be Used for Boosting Plant Growth and Plant Protection in Sustainable Plant Production? *Agronomy* **2022**, *12*, 841. [CrossRef]