

Research Article

Murtaza Hasan*, Muhammad Sajjad, Ayesha Zafar, Riaz Hussain, Syed Ishtiaq Anjum, Muhammad Zia, Zahid Ihsan, and Xugang Shu*

Blueprinting morpho-anatomical episodes via green silver nanoparticles foliation

<https://doi.org/10.1515/gps-2022-0050>

received November 16, 2021; accepted April 06, 2022

Abstract: Traditional fertilization not only enhances the acidification of soil but also leads toward eutrophication. Here stimulatory and inhibitory effects of foliar fertilization of *Cordia* based silver nanoparticles (AgNPs) were studied on lettuce (*Lactuca sativa* L.) biomass accumulation, antioxidant activity, and morphological and anatomical modifications. The AgNPs were foliar supplied with a concentration of 25, 50, and 100 ppm along with control (deionized water) and negative control AgNO₃ for consecutive 25 days. The *L. sativa* fresh and dry biomass accumulation were significantly higher by 53%, leaf area by 25%, and leaf water holding capacity by 207% for foliar sprayed at 25 ppm concentration of AgNPs. The application of AgNPs and AgNO₃ had significantly shortened the shoot length (52%), while foliar spray of AgNPs promoted the root length (40%). Among different concentrations of AgNPs, the 50 ppm improved the thickness of stem epidermis (44%), hypodermis (130%), and cortex cell area (372%). For antioxidant studies, the 25 ppm of

AgNPs depicted the highest anti-oxidative activity for 2,2-diphenyl-1-picryl hydrazyl radical scavenging activity (75%), total antioxidant capacity (167%), total phenolic content (292%), and total reducing power (60%), while 50 ppm showed the maximum activity for total flavonoid content (243%) as compared to control. Thus, we may conclude that the AgNPs have both stimulatory and inhibitory effects on *L. sativa*. These effects were dependent on the concentration of the nanoparticles and have varied for different growth, antioxidants, and anatomical traits of the plant.

Keywords: *Cordia*, nanometals, *Lactuca sativa*, AgNPs, nano-fertilizer

1 Introduction

Excessive utilization of fertilizers for requirement of enhancing population for food has become the challenging task for researcher in the field of agriculture. The health index of soil alters by rapid use of artificial fertilizers which leads toward the loss of fertility of soil thereby decrease in the production rate [1,2]. The soil conditions in the agro-ecosystem have been changed by the anthropogenic activities, extensive cropping system, and intensive land use management. Furthermore, these human activities have also influenced the diversity of soil bacteria which ultimately leads toward the loss of soil fertility [3,4]. To combat this, nanotechnology, an emerging science, has been recently spreading all over the world with a fast rate of research and scientific development. The role of nanoparticles (NPs) has been studied extensively in different fields including agriculture [5]. Researchers have reported its beneficial effects, phytotoxicity, growth promoters, and their role as nano fertilizers contribution in plants [6]. Although the silver nanoparticles (AgNPs) application trend is increasing in the field of biotechnology, very few documented record is present on their method of preparation and concentration of dose [7,8]. Approximately, 25% of the total NPs used in the nanotechnology-based products are comprised of silver

* **Corresponding author: Murtaza Hasan**, Department of Chemistry, College of Chemistry and Chemical Engineering, Zhongkai University of Agriculture and Engineering, Guangzhou 510225, China; Department of Biotechnology, The Islamia University of Bahawalpur, Bahawalpur 63100, Pakistan, e-mail: murtaza@zhku.edu.pk

* **Corresponding author: Xugang Shu**, College of Chemistry and Chemical Engineering, Zhongkai University of Agriculture and Engineering, Guangzhou 510225, China, e-mail: xgshu@21cn.com

Muhammad Sajjad: Department of Biotechnology, The Islamia University of Bahawalpur, Bahawalpur 63100, Pakistan

Ayesha Zafar: Department of Biotechnology, The Islamia University of Bahawalpur, Bahawalpur 63100, Pakistan; Department of Biomedical Engineering, College of Future Technology, Peking University, Beijing 100871, China

Riaz Hussain, Syed Ishtiaq Anjum: Department of Zoology, Kohat University of Science and Technology, Kohat 26000, Pakistan

Muhammad Zia: Department of Biotechnology, Quaid-i-Azam University, Islamabad 45320, Pakistan

Zahid Ihsan: Cholistan Institute of Desert Studies (CIDS), The Islamia University of Bahawalpur, Bahawalpur 63100, Pakistan

owing to its antifungal, antibacterial, and antimicrobial utilizations [9–11]. In agriculture, the silver-based NPs are chiefly used in nano-pesticides to encounter plant diseases [12]. The main problem with AgNPs is their concentration and exposure time which determined the fate of crops.

Certain applications regarding the environmental issues involve the implementation of AgNPs. It has been observed that AgNPs are found to be very effective in the sunscreens, fiber industry, food industry, and certain agricultural and biomedical industries due to their powerful bioactivity [13,14]. Nano-agriculture is the implementation of nanotechnology in the field of crop and agriculture in which nano-sized particles are used to suppress the pests population and to enhance the production by focusing on the targeted farming [15]. Nanoparticles in the form of nanoemulsion and as nano-fertilizer increases the availability of micronutrients to the plants which enhances the direct implementation of required nutrients to the system of plants, improve their growth at seedling stage, and decreases the fertilizer waste [16,17]. But the rapid use of nano-fertilizers through foliar spray increases the nutrient bioavailability with fast response which enhanced the growth and production of plants [18,19]. As the reactivity of nano-fertilizers is very high, these fertilizers can penetrate in the cuticle and manage the controlled release and delivery of nutrients to the targeted part [20]. This foliar technique of nano-fertilizers greatly affects the quality, production, growth, abiotic stress alleviation, toxicity of heavy metals, and nutrient use efficiency of the crops [21]. Stampoulis *et al.* observed that Ag content in *Cucurbita pepo* shoots was approximately 5 times higher in plants exposed to $10\text{--}1,000\text{ m}\cdot\text{L}^{-1}$ AgNPs than those treated with bulk Ag powder at similar concentrations, due to higher levels of ion release from AgNPs [22]. Easy penetration of NPs into plants and specific properties of NPs can cause a strong reaction at various levels, including alterations in metabolic processes [23].

Due to the diversified properties of NPs, *i.e.*, composition, structure, size, and stability, nanoparticles affect the plants in both negative and positive way [24]. Positive effects are greatly studied in the crops production in which efficient NPs are synthesized for the bioactivity against pests, growth regulation, nutrition, and soil conditioners [25]. Silver nanoparticles greatly influenced the production and efficiency of certain hormones of plants such as auxins and cytokinins [26]. Negative effects of NPs are also widely observed and resulted in a decrease in growth, development, pigments quantity, and yield [27,28]. Exposure to AgNPs can lead to an oxidative stress in plant, for instance, higher concentrations of AgNPs produced excessive quantity of reactive oxygen species

(ROS) including superoxide radicals and H_2O_2 that created oxidative stress in *Brassica rapa* seedlings [29,30]. Different concentrations of NPs, morphology, size, and time duration have influenced the cell and plant morphological and physiological response in various plants. There are many benefits of green synthesized AgNPs in different disciplines such as medical, food, health care centers [31,32], environmental science, cosmetics, and many other industries [33]. However, agriculture is very special and need unique characteristics of AgNPs for controlling pesticides, fungicides, and insecticides [34,35]. Exposure to higher concentration of NPs generally results in over production of ROS and consequently cytotoxicity, while exposure to lower concentration of NPs usually stimulates nontoxic modulation of redox signaling which favors plants against different environmental stresses. For these reasons, the current study was designed to investigate the impact of green synthesized AgNPs on morphological and physiological parameters of lettuce' (*Lactuca sativa* L.). Lettuce is a well-known annual herb belonging to Asteraceae family, which is used commonly in most parts of the world as leaf vegetable. Therefore, the study was used to observe the changes in concentration of NPs with respect to metabolite marker in response to AgNPs. The growth parameters, biomass accumulation, and leaf contents were also measured. The antioxidant activity, phenolic content, and anatomical traits of leaf, stem, and root of *L. sativa* against silver nitrate and AgNPs were determined. Furthermore, a comparison of effect of different concentrations as well as on possible alterations in the plant response from stimulatory to inhibitory have been observed as well.

2 Materials and methods

2.1 Chemicals and equipment

Silver nitrate, *Cordia myxa* plant, coconut peat medium, plastic pots, 90% absolute alcohol, safranine, dimethyl sulfoxide DMSO, hydroponic apparatus, magnetic stirrer, UV-Vis spectroscopy, centrifuge machine, seedling tray, and Eppendorf tubes.

2.2 *Cordia myxa* based synthesis of AgNPs

The AgNPs were synthesized using the *Cordia myxa* branches extract as previously reported [9,31]. First, 1 M aqueous stock solution of AgNO_3 was prepared and mixed with *C. myxa* branches extract in a 1:1 ratio. The whole

mixture was heated and stirred with a magnetic stirrer (100 rpm) at 70°C for 30–50 min. Continuous visual observation of mixtures was recorded by changing the color change. The bio reduction of Ag ions in the aqueous solution was periodically monitored by UV-Vis spectroscopy. When the reaction was completed, end product was collected via centrifuge (30 min at 7,000 rpm). The supernatant was stored in Falcon tubes and kept at 4°C.

2.3 Foliar application of AGNPs on *L. sativa*

The seeds of *L. sativa* were purchased from market and hand sown in a seedling tray having coconut peat medium, porous peat mainly consisting of holocellulose and lignin content. As the root system developed, the seedlings started absorbing water. The seeds starting germination after 5–7 days. As the seeds attained maximum germination, sprinkling of water spray continued everyday as per requirement. All the seedlings were tagged with specific concentration experimental group and kept in net pot which were placed in hydroponic environment. Plastic containers were filled with 100 mL of Hoagland's solution. Lettuce seedlings were labelled with five groups, i.e., control group without AgNPs, 25, 50, and 100 ppm NPs, and silver nitrate as negative group. The experiment was carried out in triplets with each group having 8–10 plants. 12 mL of AgNPs solution were supplied in different concentrations every day for 25 days along with control having distilled water and negative control with silver nitrate solution (0.01 M). All the groups were placed in an open atmosphere and maintained on 14 h light/10 h dark cycle at ~22°C. The foliar application of all concentrations in the experimental group were continuous till the harvesting for analysis of morpho-anatomical parameter.

2.4 Plant morphological studies

Lettuce seedling morphological characteristics were recorded as in our previous reports by slight modification for height of plant (cm), dry weight accumulation (g), fresh weight accumulation (g), shoot length (cm), and root length (cm), leaf area, leaf frequency per plant by following a standard agronomic procedure.

2.5 Anatomical characterization of *L. sativa*

Anatomical studies were performed for leaf, stem, and root separately. The sectioning of the *L. sativa* parts

was carried after complete dehydration process containing double stains to prepare permanent slides. Then, microscope was used to observe these permanent slides. Different fast green solutions and certain staining solutions were prepared which had the concentrations of about 90% absolute alcohol, 70% alcohol, 50% alcohol, 30% alcohol, and 70% alcohol along with safranin. The photographs were captured by a digital camera, which was 24 mega pixels, mounted on the microscope. Furthermore, the anatomical characterization of root, stem, and leaves included: thickness of lamina (μm), thickness of palisade cells (μm), thickness of cuticle (μm), thickness of spongy cells (μm), thickness of mid rib (μm), thickness of abaxial epidermis (μm), thickness of adaxial epidermis (μm), thickness of bundle sheath (μm), and number of vascular bundles. Cell area of leaf tissues were measured for the area of palisade cells (μm^2), bundle sheath cells (μm^2), abaxial cells (μm^2), adaxial cells (μm^2), and spongy cells (μm^2). Root anatomical characterization included: thickness of pericycle (μm), thickness of cortex (μm), thickness of epidermis at 10 \times (μm), thickness of endodermis (μm), thickness of hypodermis (μm), and number of vascular bundles. Cell area of root tissues were measured for the hypodermis cells (μm^2), cortical cells (μm^2), epidermis cells (μm^2), and endodermis cells (μm^2). Stem anatomical characterization included: thickness of hypodermis (μm), thickness of epidermis (μm), thickness of endodermis (μm), thickness of cortex (μm), thickness of pith (μm), diameter of stem, and number of vascular bundles. Cell area of stem tissues were measured for the hypodermal cells (μm^2), endodermal cells (μm^2), epidermal cells (μm^2), and cortex cells (μm^2).

2.6 Determination of *L. sativa* antioxidant activity

The anti-oxidant potential of lettuce seedling in response to different concentrations of *cordia*-stabilized AgNPs were determined. Dimethyl sulfoxide (DMSO) ($10\text{ mg}\cdot\text{mL}^{-1}$) was used to prepare suspension in Eppendorf tubes. Every sample was kept at room temperature for 48 h and then centrifuged at 12,000 rpm for 15 min. The supernatant obtained from each group was used to determine the activities of different antioxidants. 1,1-di-phenyl-2-picryl-hydrazyl (DPPH) assay was used to analyze the antioxidants. The capability of sample to absorb DPPH ion was analyzed by the following formula:

$$\text{DPPH scavenging effect} = (\text{Control OD} - \text{Sample OD} / \text{Control OD}) \times 100 \quad (1)$$

Percent inhibition of the test sample

$$= \% \text{ Scavenging activity} = (1 - Ab_s/Ab_c) \times 100 \quad (2)$$

where Ab_s represents the absorbance of DPPH solution with sample and Ab_c represents the absorbance of negative control (having solvents and reagents only). The ability to reduce other elements was determined by the methods carried out by previously reported method [36,37]. To determine the total antioxidant capacity, phosphomolybdenum method, already reported in ref. [38], was adopted. Methanol (0.3 mL) was used as a blank in place of plant extract. The total antioxidant capacity was expressed as number of grams equivalent of ascorbic acid. Folin-Denis reagent method was used to calculate the total phenolic content (TPC) [39]. Gallic acid or trihydroxy benzoic acid was taken as standard and DMSO was used as blank and they were run in parallel. The obtained TPC was analyzed as microgram trihydroxy benzoic acid equivalent per mg FW ($\mu\text{g GAE per mg FW}$). AlCl_3 colorimetric method was used to estimate the total flavonoid content (TFC) of crude extract as used by previous researcher [40]. Absorbance was calculated by using microtiter plate at 415 nm after 15 min of incubation.

2.7 Statistical analysis

The effect of different concentrations of cordia-based stabilized AgNPs significance testing was analyzed by using the least significant difference (LSD) test; differences were determined to be statistically significant when p -value < 0.05 . The results were represented as a mean value \pm standard deviation.

3 Results

3.1 Lettuce growth studies

The foliar application of AgNPs with 25, 50, and 100 ppm NPs concentration and their impact on morphology, physiological, and anatomical changes and their antioxidant response in the lettuce plant are shown in Figure 1. In our previous reports, the AgNPs were applied hydroponically and their influence was observed. In the current study, the foliar application of AgNPs was applied for 25 days to

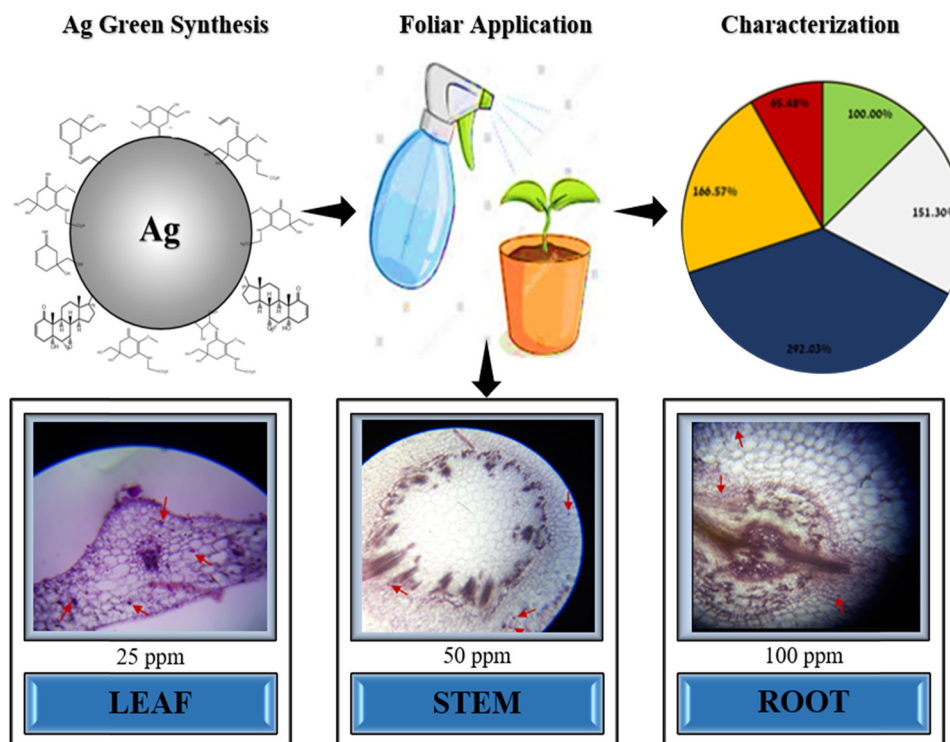


Figure 1: Illustration of foliar spray of silver nanoparticles on *L. sativa* and their morpho-anatomical changes.

investigate the morpho-anatomical and antioxidant responses. Initially, the plant height and number of leaves were higher in the control group.

There was a decrease of 30% in the number of leaves per plant and 36% in plant height in the negative control. The application of AgNPs progressively declined the above studied growth traits. The response of lettuce was concentration dependent as mean values of plant height and number of leaves per plant were gradually decreased with an increase in AgNPs concentration (25, 50, and 100 ppm). On contrary to plant height, lettuce fresh and dry biomass accumulation were significantly higher for foliar sprayed AgNPs over control (Figure 2a). The low concentration of AgNPs had accumulated almost 53% higher fresh and dry weight as compared to control.

The AgNO₃ proved fatal and lowered plant dry biomass accumulation by 83%. With an increase in AgNPs concentration, the lettuce biomass accumulation capacity

gradually decreased but still remained higher than both positive and negative controls. Shoot length of the lettuce displayed a similar trend as that of plant height (Figure 2a). The effect of AgNPs was antagonistic for shoot length to root length. The application of AgNPs and AgNO₃ has significantly shortened shoot length (52%), while foliar spray of AgNPs promoted root length (40%). It is concluded that the application of AgNPs negatively affected leaf emergence, shoot length, and plant height, while it promoted plant capacity for biomass accumulation and roots elongation. The foliar application of AgNPs has significantly improved leaf area and water content as compared to control (Figure 2a). The application of AgNO₃ exhibited a damaging effect on both these traits. The lowest concentration (25 ppm) of AgNPs displayed an astonishing improvement in leaf water holding capacity (207%) with a nominal increase in leaf area (25%). The response of leaf area and water content were significantly altered with the change in concentrations of AgNPs.

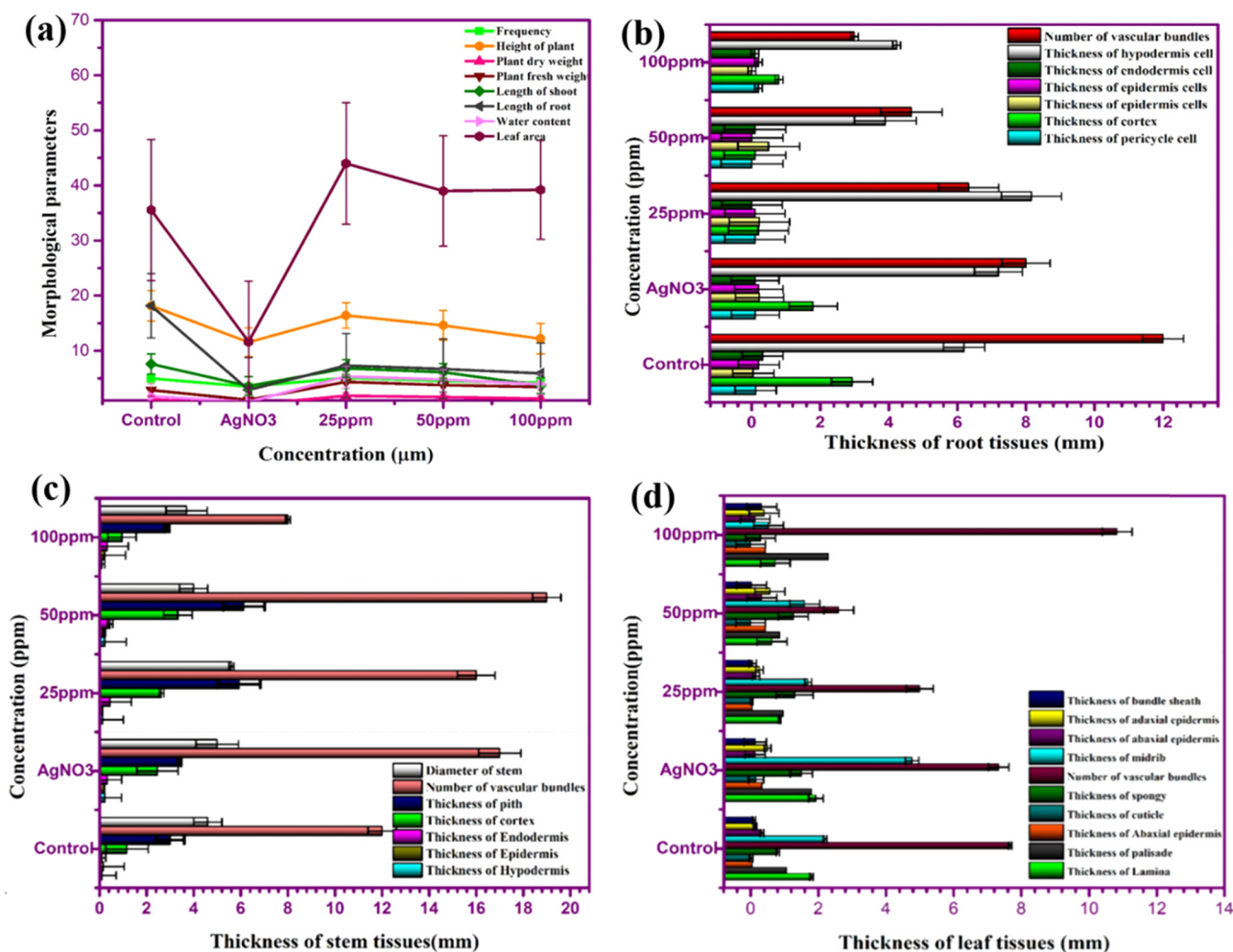


Figure 2: Foliar application of silver nanoparticles with 25, 50, and 100 ppm on *L. sativa* morpho-anatomical changes (a), thickness of root tissue (b), thickness of stem tissue (c), and thickness of leaf tissue (d).

3.2 Morpho-anatomical characterization of lettuce

The data of thickness of root diameter and number of vascular bundles are presented in Figure 2b. The application of AgNO₃ and AgNPs has significantly declined the number of vascular bundles and root diameter, while 25 ppm of AgNPs marginally improved root diameter. The application of all concentrations of AgNPs severely dropped the root epidermis and hypodermis thickness (Figure 2b). On the contrary, the application of AgNO₃ had improved thickness of epidermis by 101% but it did not affect the thickness of endodermis. The application of 100 ppm of AgNPs improved the thickness of pericycle by 64%. The application of AgNPs displayed a severe negative effect on all root anatomical characteristics except for the thickness of pericycle and diameter of root.

3.3 Anatomy of stem tissue

Stem anatomical studies reported thickness of endodermis and hypodermis of about 0.1 μm; however, the thickness of epidermis was around 0.16 μm (Figure 2c). The foliar application of AgNO₃ improved thickness of epidermis and hypodermis by about 25–130%, while the thickness of endodermis remained unchanged. The application of AgNPs displayed a variable response to the thickness of stem dermis layers. Their response varied significantly depending upon the concentration of the AgNPs. For example, the 25–50 ppm concentration of AgNPs improved thickness of endodermis by about 30% and 100 ppm concentration of AgNPs reduced it by about 40%. The medium dose of AgNPs incremented the thickness of hypodermis by 130% and its highest dose brought no change in thickness of hypodermis as compared to the control. The observed thickness of epidermis of the lettuce plants, which were treated with AgNPs concentration of 25 and 100 ppm, showed 19% decrease, while the plants treated with 50 ppm concentration showed 44% increase (Figure 2c).

Figure 2c displays the effect of AgNO₃ and AgNPs on stem diameter, cortex thickness, pith thickness, and number of vascular bundles. The application of AgNO₃ positively improved all these traits. The application of AgNPs also promoted these traits except for stem diameter that significantly declined (18%) at all concentrations. The application of 50 ppm of AgNPs promoted the production of the number of vascular bundles by 58%. The observed thickness of pith of the stem, which were treated with 25 and

50 ppm concentrations, were 5.93 and 6.13 μm, as compared to the control group (3 μm), these showed 97% and 104% upsurge in pith thickness, respectively. Similarly, 25 and 50 ppm concentrations of AgNPs showed 126% and 187% increase in cortex thickness, respectively. The 100 ppm dose of AgNPs proved toxic and significantly declined in all anatomical characteristics of the stem (Figure 2c).

3.4 Anatomy of leaf tissue

Figure 2d presents the data for leaf thickness parameters. All studied leaf tissues displayed a variable response to foliar application of AgNPs in different concentrations. The lower concentration of AgNPs showed a significant improvement in cuticle thickness, while the plants which were implemented by the 50 and 100 ppm concentrations completely lost their cuticle. The application of AgNO₃ improved the thickness of midrib and lamina, while the AgNPs gradually decreased the thickness of these traits. Silver nitrate improved the adaxial epidermis thickness by 284% and decreased the abaxial epidermis thickness by 60% as compared to the control. The AgNPs concentration of 25, 50, and 100 ppm showed 100%, 338%, and 207% decrease in adaxial epidermis thickness and 51%, 0%, and 60% decrease in abaxial epidermis thickness, respectively. The application of AgNO₃ and 100 ppm AgNPs improved the palisade thickness by 69% and 117%, respectively as compared to the control (Figure 2d). The low and medium levels of AgNPs slightly declined the thickness of palisade cells. On the contrary, the 25–50 ppm AgNPs positively improved the thickness of spongy cells, while 100 ppm concentration sharply declined it. The foliar spray of AgNO₃ had the thickness of spongy cells of about 1 μm that was 65% higher than control. Except for 50 ppm concentration of AgNPs that improved thickness of bundle sheath cells by 230%, the remaining treatments of silver had a non-significant consequence on bundle sheath cells.

3.5 Cell area plant vegetative part

Cell area of root tissues (epidermis, hypodermis, endodermis, and cortex) is presented in Figure 3a. Control group had the area of cortex cells of about 0.31 μm². It was studied that cortex cell area of negative control group, i.e., AgNO₃ was 0.48 μm² which showed about 55% increase as compared to the control. Similarly, AgNO₃ positively affected

root epidermis and hypodermis cell area. The application of AgNPs at a concentration of 50 ppm favored the endodermis cell area.

The effect of AgNO_3 and all concentrations of AgNPs were non-significant for the area of epidermis, endodermis, and hypodermis of stem except for 100 ppm that displayed a severe decline in hypodermis area (Figure 3b). The application of AgNO_3 produced the maximum cortex cell area that was 460% higher as compared to the control. Among the AgNPs, the 50 ppm concentration improved the cortex cell area by 372% as compared to control. Thus, it is concluded that stem cortex area is an important trait to study the effect of silver on lettuce plant. Area of adaxial and abaxial epidermis depicted almost similar trend against the foliar application of both AgNO_3 and AgNPs (Figure 3c). The application of 25–50 ppm AgNPs decreased the area of adaxial and abaxial epidermis by 29–56%, while the foliar spray of 100 ppm improved these traits by 129–433%, respectively. The application of AgNO_3 stretched the area of adaxial and abaxial epidermis by 400–1,500% approximately. The application of AgNO_3 significantly enhanced the area of spongy cell by 384% and palisade by 370%, while the application of AgNPs significantly declined the area of spongy cell and palisade cell. The concentration of AgNPs had an inverse relationship with the area of spongy cell and palisade cell. All levels of AgNPs displayed negative effect on both the areas of spongy cell and palisade cell. The negative effect of AgNPs was more severe on the area of spongy cell as compared to the area of palisade cell. The effect of AgNO_3 and AgNPs were non-significant to the area of bundle sheath cells; however, it decreased slightly with the application of AgNPs.

3.6 Foliar application of AgNPs and silver nitrate on lettuce

Foliar application and the uptake of AgNPs in root, stem, and leaves were performed using anatomical microscopic studies. These microscopic studies exposed the uptake and retention of the AgNPs via leaf route and their anatomical changes in cells of root, stem, and leaves. The presence of AgNPs in the root, stem, and leaves are marked by red arrows in Figure 4. The foliar application of 25 ppm AgNPs showed maximum absorption in the leaf tissue as compared to stem and root (Figure 4a). Similarly, the influence of 50 ppm was also observed in the vegetative part of lettuce via foliar application but the leaf shows maximum uptake of NPs because of direct exposure and having stomata. Significant changes in cell morphology, structure, and physiology have been observed in root, stem, and leaves section (Figure 4b). In case of Figure 4c, NPs-induced cell death via apoptosis was observed based on the anatomy results. Significant changes in the anatomy of all parts of plant were observed.

3.7 Anti-oxidative response of lettuce in foliar application of AgNPs and AgNO_3

The anti-oxidative response of lettuce was measured for DPPH activity, total antioxidant capacity (TAC), TPCTPC, TFC, and total reducing power (TRP). Control group had DPPH of $28.2 \mu\text{g}\cdot\text{mg}^{-1}$ and TAC of $18.3 \mu\text{g}\cdot\text{mg}^{-1}$ and observations showed an increase of 34% in DPPH inhibition (Figure 5a) and 59% in TAC for foliar application of AgNO_3 as compared to the control (Figure 5b). The

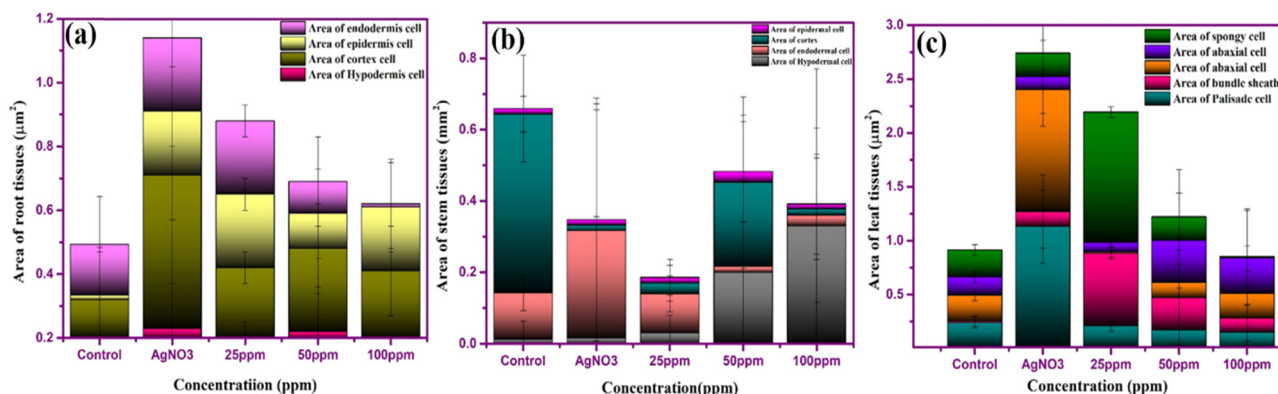


Figure 3: Effect of different concentrations of Ag NPs and AgNO_3 (1 M) on area of endodermis, epidermis, cortex, and hypodermis cell: (a) root tissue, (b) stem tissue, and (c) area of spongy cell, abaxial, bundle sheath, and palisade cell in leaf.

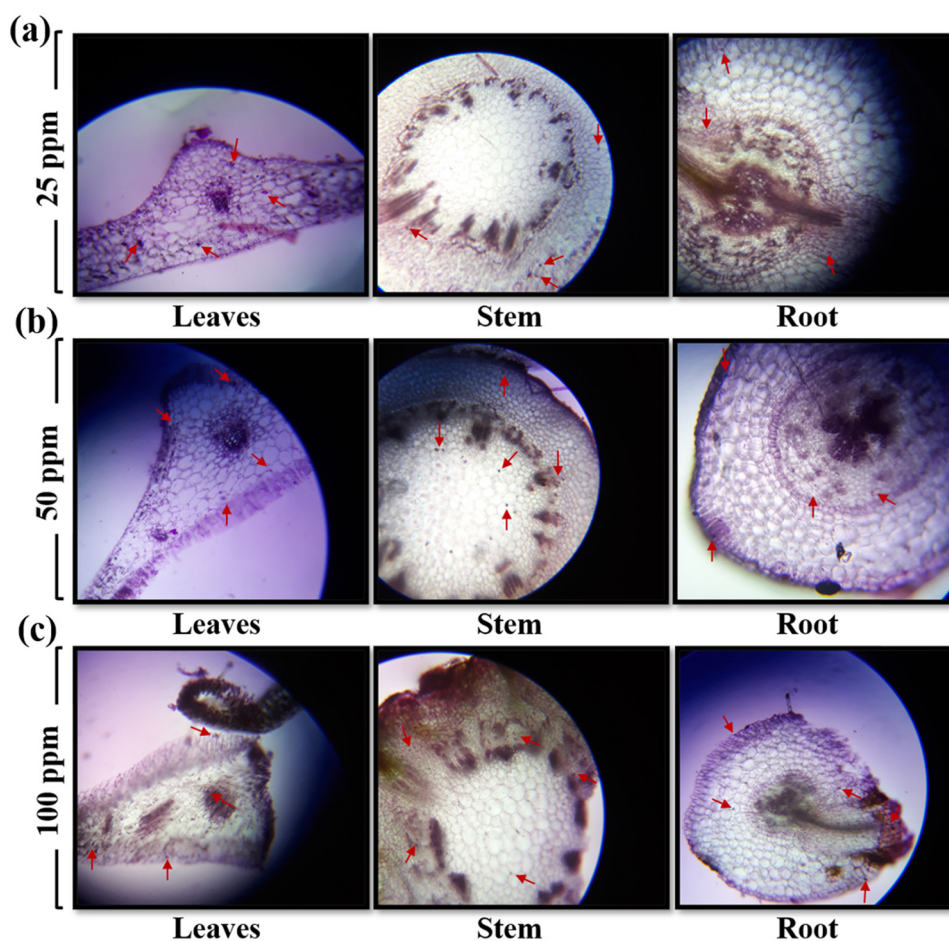


Figure 4: Morpho-anatomical analysis of *L. sativa* leaves, stem, and root: (a) 25 ppm, (b) 50 ppm, and (c) 100 ppm. The images were recorded under a light microscope (Nikon 104, Japan) at a resolution of 1,500× with the help of a 20 mega pixel digital camera.

observed DPPH and TAC inhibition of the plants, which were treated with 25, 50, and 100 ppm concentrations of AgNPs, were increased by approximately 75–167%, 61–120%, and 44–28%, respectively, as compared to the control.

The application of AgNPs and AgNO_3 significantly improved the activity of TPC, TFC, and TRP (Figure 5c–e). Among different concentrations of AgNPs, the 25 ppm depicted the highest anti-oxidative activity for TPC (292%) and TRP (60%) (Figure 5e), while 50 ppm showed the maximum activity for TFC (243%) as compared to control (Figure 5d). The anti-oxidative activity of *L. sativa* decreased considerably at 100 ppm concentration of AgNPs.

4 Discussion

Recently, the trend of foliar spray of NPs prepared by chemical and biological methods as nanofertilizers has

increased in the agricultural industry [41,42]. These NPs have enhanced the effectiveness of nanofertilizers and nanopesticides emulsion as compared to the traditional soil root application [43]. The effectiveness of foliar application helps plants to avoid nutrient from leaching, soil contamination, and economically feasible. Lettuce is a green fleshy vegetable having good source of essential nutrient and antioxidant molecules which has been used as soup, salads, and sandwiches throughout the world [44,45]. To increase the growth and production of lettuce, nanotechnological tools, and techniques have been effective for uptake of nutrients and ions by direct interaction with soil, air, and water [46]. The current study evaluated the response of *L. sativa* against the foliar exposure of AgNO_3 and AgNPs with various concentrations to investigate the optimal conditions as nanofertilizers or toxicants. Explicitly the effects induced by AgNPs were studied on *L. sativa* growth, biomass accumulation, leaf, stem, and root thickness, area, and anatomical traits. The effects were also studied on anti-oxidative

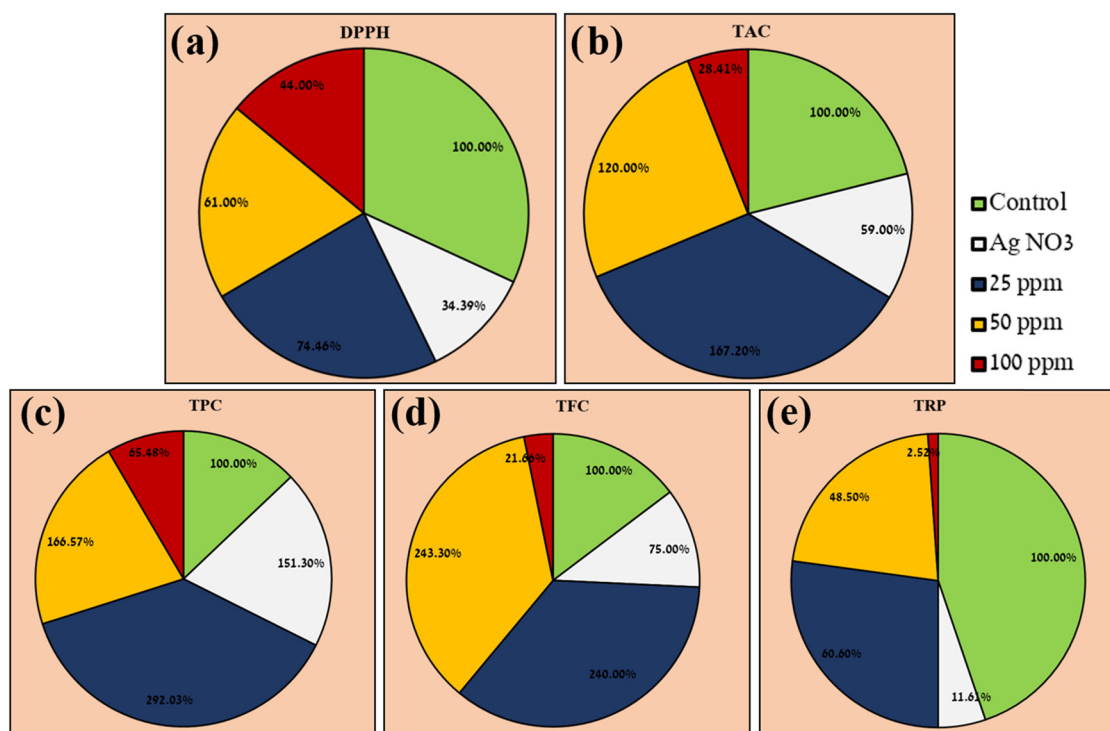


Figure 5: Effect of different concentrations of AgNPs and AgNO₃ (1 M) on: (a) free radical scavenging activity, (b) total antioxidant activity, (c) total phenolic content, (d) total flavonoid content, and (e) total reducing power levels in roots and leaves of lettuce plant.

activity and phenolic contents of *L. sativa* in response to biologically synthesized AgNPs and AgNO₃. The *L. sativa* response significantly varied depending upon the concentration of the NPs. The knowledge provided us relevant information for the evaluation of the positive effects on plants and potential risks of toxicity. The findings of this study displayed that AgNPs have negatively affected leaf emergence, shoot length, and plant height, while it promoted plant capacity for biomass accumulation and root length. Foliar application of AgNPs at 60 ppm concentration improved the fresh and dry weight accumulation of *Phaseolus vulgaris* by 27–30% [47] and *Zea mays* by 33–35%, while at 100 ppm it significantly declined the fresh and dry weight of both plants [48]. On the contrary, negative effects of AgNPs are also frequently reported in different plants. Application of AgNPs declined the growth and biomass accumulation of *Oryza sativa* and *Triticum aestivum* [49,50]. In the authors' opinion, the growth promotory potential of NPs depends on the concentration of AgNPs and it varies for each growth parameter. For example, plant height significantly decreased at 25 ppm concentration, while leaf water holding capacity considerably improved at this concentration. Although the results presented exhibited some variation in the anti-oxidative activity of *L. sativa* leaves treated with AgNPs at different concentrations. In general, the source of silver ions was

responsible for the increment of enzymatic activities in the treated plants, but the degree of this increase was dependent on the concentration of the applied NPs. The low and medium concentration of AgNPs reported the highest anti-oxidative activity (DPPH, TAC, TPC, TFC, and TRP). Similar kind of reports were reported earlier and showed that TiO₂ NPs enhanced the activity of superoxide dismutase, ascorbate peroxidase (APX), and glutathione peroxidase in *Spinacia oleracea* plant [51]. In another report, the APX activity improved in *Solanum tuberosum* plants by the application of AgNPs [52]. Upregulation of genes was documented in *A. thaliana* involved in the synthesis of glutathione when treated with the AgNPs [53]. To the best of our knowledge, the effect of AgNPs on DPPH, TAC, TPC, TFC, and TRP have not yet been presented in detail and the mechanisms of increase in the activity of these antioxidants observed in our experiment requires further in-depth research to explore the key physiochemical mechanisms regulating these antioxidants under foliar application of AgNPs. Application of higher concentrations of AgNPs enhanced the scavenging activity of DPPH by 37–44% as compared to control, while lower concentration of AgNPs did not produce any effect on DPPH [54]. Antioxidative activity of *Corchorus olitorius* increased in a concentration dependent manner for the AgNPs when mixed in the soil [55]. In another report,

DPPH free radical scavenging activity slightly improved at 20 ppm of AgNPs and tremendously enhanced at 50 ppm in *Echium amoenum*, which agrees with our findings [56]. Furthermore, one more researcher revealed that AgNPs and PtNPs at 40 ppm concentration have improved the total phenolic contents by 17–15% as compared to the control. Similarly, the AgNPs and PtNPs increased carotenoids content in *L. sativa* by 13–17% when applied with 20–40 ppm solutions [57]. Total phenolic contents were found maximum in *Triticum aestivum* seedlings that were treated with the 50 ppm concentration of AgNPs. Review of previous literature suggests that there is generally a positive correlation between the plant exposure to AgNPs and total phenolic contents. In our study, the same relationship was confirmed at the medium AgNP concentrations. Current study's results concluded that there is a need to investigate the nature of functional biomolecules which are responsible for the reduction process and play key role in the formation of NPs [58]. Furthermore, there is also a need to investigate the mechanism of stimulatory and inhibitory effect of AgNPs in model plants. This positive or negative effect will help to understand the all-molecular processes, signaling and action of AgNPs may open up new perspectives in the practical approach of scientist and new possibilities of creating beneficial combinations with other biologically active agents. Recently, green synthesized AgNPs were used as potential biomass feedstock for the sustainable production of biodiesel [59]. In another report, *Phoenix dactylifera* waste as bioreductant for effective dye degradation and antibacterial performance in wastewater treatment [60]. There is also a need to investigate the combinational use of AgNPs with other antimicrobial and antifungal agents for solving the problem of toxicity and avoid the possible risk of microbial resistance development.

5 Conclusion

The study evaluated the response of green synthesized AgNPs (25, 50, and 100 ppm) on lettuce growth, antioxidant activity, and morpho-anatomical changes in leaf, stem, and root cells. The response of lettuce plant depended on the applied concentration of the AgNPs. The lower concentrations of the NPs 25–50 ppm promoted different studied traits, while at 100 ppm it inhibited the functioning of the same traits. The higher dose of AgNPs proved fatal and resulted in phytotoxicity such as failure to develop cuticle cells, but in limited cases, the full dose of AgNPs (100 ppm) promoted the studied traits such as the thickness of pericycle

in root tissues. Finally, it can be concluded from this research that in-depth research is needed to explore the key physiochemical mechanisms regulating these morpho-anatomical changes in lettuce under foliar application of green synthesized AgNPs.

Acknowledgments: The authors acknowledge the financial support provided by The Islamia University of Bahawalpur, Pakistan, Higher Education Commission Pakistan and NRPU Project (9458), Guangdong Science and Technology Planning Project (2016A020210066), and Guangzhou Science and Technology Planning Project (201707010461).

Funding information: The Islamia University of Bahawalpur, Pakistan, Higher Education Commission Pakistan and NRPU Project (9458), Guangdong Science and Technology Planning Project (2016A020210066), and Guangzhou Science and Technology Planning Project (201707010461).

Author contributions: Murtaza Hasan: writing – original draft; Muhammad Sajjad: methodology and experimental design; Ayesha Zafar: writing – draft and review; Riaz Hussain: visualization, and software application; Syed Ishtiaq Anjum: designing of project; Muhammad Zia: methodology and draft of data; Zahid Ihsan: methodology and idea creating; Xugang Shu: resources of experimental work.

Conflict of interest: Authors state no conflict of interest.

References

- [1] Li J, Han G, Wang G, Liu X, Zhang Q, Chen Y, et al. Imbalanced nitrogen–phosphorus input alters soil organic carbon storage and mineralisation in a salt marsh. *Catena*. 2022;208:105720. doi: 10.1016/j.catena.2021.105720.
- [2] Chen X, Yan X, Wang M, Cai Y, Weng X, Su D, et al. Long-term excessive phosphorus fertilization alters soil phosphorus fractions in the acidic soil of pomelo orchards. *Soil Tillage Res.* 2022;215:105214. doi: 10.1016/j.still.2021.105214.
- [3] Sui X, Zhang R, Frey B, Yang L, Li MH, Ni H. Land use change effects on diversity of soil bacterial, acidobacterial and fungal communities in wetlands of the Sanjiang Plain, north-eastern China. *Sci Rep.* 2019;9(1):55063–4. doi: 10.1038/s41598-019-55063-4.
- [4] Yang T, Siddique KHM, Liu K. Cropping systems in agriculture and their impact on soil health – a review. *Glob Ecol Conserv.* 2020;23:e01118. doi: 10.1016/j.gecco.2020.e01118.
- [5] Hasan M, Rafique S, Zafar A, Loomba S, Khan R, Hassan SG, et al. Physiological and anti-oxidative response of biologically and chemically synthesized iron oxide: *Zea mays* a case study. *Heliyon*. 2020;5:e04595. doi: 10.1016/j.heliyon.2020.e04595.

- [6] 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. doi: 10.3390/ijms20051003.
- [7] Fatima F, Hashim A, Anees S. Efficacy of nanoparticles as nanofertilizer production: a review. *Environ Sci Pollut Res.* 2021;28:1292–303. doi: 10.1007/s11356-020-11218-9.
- [8] Hasan M, Mahmood K, Mustafa G, Zafar A, Tariq T, Hassan SG, et al. Phytotoxic evaluation of phytosynthesized silver nanoparticles on lettuce. *Coatings.* 2021;11:225. doi: 10.3390/coatings11020225.
- [9] Zulfiqar H, Zafar A, Rasheed MN, Ali Z, Mehmood K, Mazher A, et al. Synthesis of silver nanoparticles using: *Fagonia cretica* and their antimicrobial activities. *Nanoscale Adv.* 2019;1:1707–13. doi: 10.1039/c8na00343b.
- [10] Hasan M, Zafar A, Shahzadi I, Luo F, Hassan SG, Tariq T, et al. Fractionation of biomolecules in *Withania coagulans* extract for bioreductive nanoparticle synthesis, antifungal and biofilm activity. *Molecules.* 2020;25:3478. doi: 10.3390/molecules25153478.
- [11] Hasan M, Altaf M, Zafar A, Hassan SG, Ali Z, Mustafa G, et al. Bioinspired synthesis of zinc oxide nano-flowers: a surface enhanced antibacterial and harvesting efficiency. *Mater Sci Eng C.* 2020;119:111280. doi: 10.1016/j.msec.2020.111280.
- [12] Mishra S, Singh HB. Biosynthesized silver nanoparticles as a nanoweapon against phytopathogens: exploring their scope and potential in agriculture. *Appl Microbiol Biotechnol.* 2015;99:1097–107. doi: 10.1007/s00253-014-6296-0.
- [13] Das CGA, Kumar VG, Dhas TH, Karthick V, Govindaraju K, Joselin M, et al. Antibacterial activity of silver nanoparticles (biosynthesis): a short review on recent advances. *Biocatal Agric Biotechnol.* 2020;27:101593. doi: 10.1016/j.bcab.2020.101593.
- [14] Vigneswari S, Amelia TSM, Hazwan MH, Mouriya GK, Bhubalan K, Amirul AAA, et al. Transformation of biowaste for medical applications: Incorporation of biologically derived silver nanoparticles as antimicrobial coating. *Antibiotics.* 2021;10:229. doi: 10.3390/antibiotics10030229.
- [15] Shahzad K, Manzoor F. Nanoformulations and their mode of action in insects: a review of biological interactions. *Drug Chem Toxicol.* 2021;44:1–11. doi: 10.1080/01480545.2018.1525393.
- [16] Upinder, Kumar, R. Nanotechnology: advancement for agricultural sustainability. In: Singh P, Singh R, Verma P, Bhadouria R, Kumar A, Kaushik M, editors. *Plant-microbes-engineered nanoparticles (PM-ENPs) nexus in agro-ecosystems.* Advances in Science, Technology & Innovation. Cham: Springer; 2021.
- [17] Mustafa G, Hasan M, Yamaguchi H, Hitachi K, Tsuchida K, Komatsu S. A comparative proteomic analysis of engineered and bio synthesized silver nanoparticles on soybean seedlings. *J Proteom.* 2020;224:103833. doi: 10.1016/j.jprot.2020.103833.
- [18] He J, Zhang L, He SY, Ryser ET, Li H, Zhang W. Stomata facilitates foliar sorption of silver nanoparticles by *Arabidopsis thaliana*. *Environ Pollut.* 2022;292:118448. doi: 10.1016/j.envpol.2021.118448.
- [19] Janmohammadi M, Amanzadeh T, Sabaghnia N, Dashti S. Impact of foliar application of nano micronutrient fertilizers and titanium dioxide nanoparticles on the growth and yield components of barley under supplemental irrigation. *Acta Agric Slovenica.* 2016;107:265–76. doi: 10.14720/aas.2016.107.2.01.
- [20] Hong J, Wang C, Wagner DC, Gardea-Torresdey JL, He F, Rico CM. Foliar application of nanoparticles: mechanisms of absorption, transfer, and multiple impacts. *Environ Sci Nano.* 2021;8(5):1196–210. doi: 10.1039/d0en01129k.
- [21] Salehi H, Chehregani A, Lucini L, Majd A, Gholami M. Morphological, proteomic and metabolomic insight into the effect of cerium dioxide nanoparticles to *Phaseolus vulgaris* L. under soil or foliar application. *Sci Total Environ.* 2018;617:1540–51. doi: 10.1016/j.scitotenv.2017.10.159.
- [22] Stampoulis D, Sinha SK, White JC. Assay-dependent phytotoxicity of nanoparticles to plants. *Environ Sci Technol.* 2009;24:9473–9. doi: 10.1021/es901695c.
- [23] Kumari R, Singh DP. Ameliorating effect of surfactants against silver nanoparticle toxicity in crop *Fagopyrum esculentum* L. *Environ Nanotechnol Monit Manag.* 2019;12:100254. doi: 10.1016/j.enmm.2019.100254.
- [24] Czyżowska A, Barbasz A. A review: zinc oxide nanoparticles—friends or enemies? *Int J Environ Health Res.* 2020;32:885–901. doi: 10.1080/09603123.2020.1805415.
- [25] Mahyoub JA. Bioactivity of two marine algae extracts and their synthesized silver nanoparticles as safe controls against *Musca domestica* housefly. *Entomol Res.* 2021;51:323–30. doi: 10.1111/1748-5967.12512.
- [26] Ismail G, Abou-Zeid H. The role of priming with biosynthesized silver nanoparticles in the response of *Triticum aestivum* L. to salt stress. *Egypt J Bot.* 2018;58:73–85. doi: 10.21608/ejbo.2017.1873.1128.
- [27] Jiráková K, Moskvín M, Urdziková LM, Rossner P, Elzeinova F, Chudichova M, et al. The negative effect of magnetic nanoparticles with ascorbic acid on peritoneal macrophages. *Neurochem Res.* 2020;45(1):159–70. doi: 10.1007/s11064-019-02790-9.
- [28] Goswami P, Yadav S, Mathur J. Positive and negative effects of nanoparticles on plants and their applications in agriculture. *Plant Sci Today.* 2019;6(2):232–42. doi: 10.14719/pst.2019.6.2.502.
- [29] Khan SA, Shahid S, Lee CS. Green synthesis of gold and silver nanoparticles using leaf extract of *clerodendrum inerme*; characterization, antimicrobial, and antioxidant activities. *Biomolecules.* 2020;10:835. doi: 10.3390/biom10060835.
- [30] Thiruvengadam M, Gurunathan S, Chung IM. Physiological, metabolic, and transcriptional effects of biologically-synthesized silver nanoparticles in turnip (*Brassica rapa* ssp. *rapa* L.). *Protoplasma.* 2015;252:1031–46. doi: 10.1007/s00709-014-0738-5.
- [31] Na-Phatthalung W, Keaonaborn D, Jaichuedee J, Keawchouy S, Sinyoung S, Musikavong C. Effect of silver nanoparticles and chlorine reaction time on the regulated and emerging disinfection by-products formation. *Environ Pollut.* 2022;292:118400. doi: 10.1016/j.envpol.2021.118400.
- [32] Naseer QA, Xue X, Wang X, Dang S, Din SU, Jamil J. Synthesis of silver nanoparticles using *Lactobacillus bulgaricus* and assessment of their antibacterial potential. *Braz J Biol.* 2022;82:232434. doi: 10.1590/1519-6984.232434.
- [33] Yonathan K, Mann R, Mahbub KR, Gunawan C. The impact of silver nanoparticles on microbial communities and antibiotic resistance determinants in the environment. *Environ Pollut.* 2022;293:118506. doi: 10.1016/j.envpol.2021.118506.
- [34] Kaziem AE, Yang L, Lin Y, Song Z, Xu H, Zhang Z. Efficiency of mesoporous silica/carboxymethyl β -glucan as a fungicide nano-delivery system for improving chlorothalonil bioactivity

- and reduce biotoxicity. *Chemosphere*. 2022;287:131902. doi: 10.1016/j.chemosphere.2021.131902.
- [35] Bapat MS, Singh H, Shukla SK, Singh PP, Vo DN, Yadav A, et al. Evaluating green silver nanoparticles as prospective biopesticides: An environmental standpoint. *Chemosphere*. 2022;286:131761. doi: 10.1016/j.chemosphere.2021.131761.
- [36] Zhao P, Guo Y, Zhang W, Chai H, Xing H, Xing M. Neurotoxicity induced by arsenic in *Gallus Gallus*: regulation of oxidative stress and heat shock protein response. *Chemosphere*. 2017;166:238–45. doi: 10.1016/j.chemosphere.2016.09.060.
- [37] Zafar A, Tariq T, Hasan M, Nazar M, Rasheed MN, Mehmood N, et al. Green-maturation of cobalt-oxide nano-sponges for reinforced bacterial apoptosis. *Colloids Interface Sci Commun*. 2021;45:100531. doi: 10.1016/j.colcom.2021.100531.
- [38] Luo F, Wang W, Chen M, Zheng Z, Zeng D, Hasan M, et al. Synthesis and efficacy of the *n*-carbamoyl-methionine copper on the growth performance, tissue mineralization, immunity, and enzymatic antioxidant capacity of Nile tilapia (*Oreochromis niloticus*). *ACS Omega*. 2020;35:22578–86. doi: 10.1021/acsomega.0c03220.
- [39] Kaur C, Kapoor HC. Anti-oxidant activity and total phenolic content of some Asian vegetables. *Int J Food Sci Technol*. 2002;37:153–61. doi: 10.1046/j.1365-2621.2002.00552.x.
- [40] Zhang M, Wang S, Sun L, Gun L, Lin Y, Shao J, et al. Ammonia induces changes in carbamoyl phosphate synthetase I and its regulation of glutamine synthesis and urea cycle in yellow catfish *Pelteobagrus fulvidraco*. *Fish Shellfish Immunol*. 2022;120:1–6. doi: 10.1016/j.fsi.2021.11.023.
- [41] Luo F, Fu Z, Wang M, Ke Z, Wang M, Wang W, et al. Growth performance, tissue mineralization, antioxidant activity and immune response of *Oreochromis niloticus* fed with conventional and gluconic acid zinc dietary supplements. *Aquacult Nutr*. 2021;27:897–907. doi: 10.1111/anu.13234.
- [42] Wasaya A. Improving growth and yield of Mungbean (*Vigna radiata* L.) through foliar application of silver and zinc nanoparticles. *Pure Appl Biol*. 2020;9:790–7. doi: 10.19045/bspab.2020.90085.
- [43] Shahid M, Dumat C, Khalid S, Rabbani F, Farooq AB, Amjad M, et al. Foliar uptake of arsenic nanoparticles by spinach: an assessment of physiological and human health risk implications. *Environ Sci Pollut Res*. 2019;26:20121–31. doi: 10.1007/s11356-018-3867-0.
- [44] Kim MJ, Moon Y, Tou JC, Mou B, Waterland NL. Nutritional value, bioactive compounds and health benefits of lettuce (*Lactuca sativa* L.). *J Food Compos Anal*. 2016;49:19–34. doi: 10.1016/j.jfca.2016.03.004.
- [45] Lei C, Engeseth NJ. Comparison of growth characteristics, functional qualities, and texture of hydroponically grown and soil-grown lettuce. *LWT*. 2021;150:790–7. doi: 10.1016/j.lwt.2021.111931.
- [46] Najafi S, Razavi SM, Khoshkam M, Asadi A. Effects of green synthesis of sulfur nanoparticles from *Cinnamomum zeylanicum* barks on physiological and biochemical factors of Lettuce (*Lactuca sativa*). *Physiol Mol Biol Plants*. 2020;26(5):1055–66. doi: 10.1007/s12298-020-00793-3.
- [47] Zea L, Salama HMH. Effects of silver nanoparticles in some crop plants, common bean (*Phaseolus vulgaris* L.) and corn. *Int Res J Biotechnol*. 2012;3:190–7.
- [48] Rajkumar T, Sapi A, Das G, Debnath T, Ansari AZ, Patra JK. Biosynthesis of silver nanoparticle using extract of *Zea mays* (corn flour) and investigation of its cytotoxicity effect and radical scavenging potential. *J Photochem Photobiol B Biol*. 2019;193:1–7. doi: 10.1016/j.jphotobiol.2019.01.008.
- [49] Yang Q, Shan W, Hu L, Zhao Y, Hou Y, Yin Y, et al. Uptake and transformation of silver nanoparticles and ions by rice plants revealed by dual stable isotope tracing. *Environ Sci Technol*. 2019;53:625–33. doi: 10.1021/acs.est.8b02471.
- [50] Iqbal M, Raja NI, Mishwani ZR, Wattoo FH, Hussain M, Ejaz M, et al. Assessment of AgNPs exposure on physiological and biochemical changes and antioxidative defence system in wheat (*Triticum aestivum* L) under heat stress. *IET Nanobiotechnol*. 2019;13:230–6. doi: 10.1049/iet-nbt.2018.5041.
- [51] Azmat R, Altaf I, Moin S. The reflection of the photocatalytic properties of TiO₂ nanoparticles on photosynthetic activity of *Spinacia oleracea* plants. *Pak J Bot*. 2020;52:1229–34. doi: 10.30848/PJB2020-4(2).
- [52] Bagherzadeh Homaee M, Ehsanpour AA. Physiological and biochemical responses of potato (*Solanum tuberosum*) to silver nanoparticles and silver nitrate treatments under *in vitro* conditions. *Ind J Plant Physiol*. 2015;20:353–9. doi: 10.1007/s40502-015-0188-x.
- [53] Ke M, Li Y, Qu Q, Ye Y, Peijnenburg WJGM, Zhang Z, et al. Offspring toxicity of silver nanoparticles to *Arabidopsis thaliana* flowering and floral development. *J Hazard Mater*. 2020;15:121975. doi: 10.1016/j.jhazmat.2019.121975.
- [54] Bawazeer S, Rauf A, Shah SUA, Shawky AM, Awthan YS, Bahattab OS, et al. Green synthesis of silver nanoparticles using *Tropaeolum majus*: phytochemical screening and anti-bacterial studies. *Green Process Synth*. 2021;10:85–94. doi: 10.1515/gps-2021-0003.
- [55] Azeez L, Lateef A, Wahab AA, Rufai MA, Salau AK, Ajai EO, et al. Phytomodulatory effects of silver nanoparticles on *Corchorus olitorius*: its antiphytopathogenic and hepatoprotective potentials. *Plant Physiol Biochem*. 2019;136:109–117. doi: 10.1016/j.plaphy.2018.12.006.
- [56] Abbasi F, Jamei R. Effects of silver nanoparticles and silver nitrate on antioxidant responses in *Echinium amoenum*. *Russ J Plant Physiol*. 2019;66:488–94. doi: 10.1134/S1021443719030026.
- [57] Mares-Briones F, Barragán-Mares O, López-Miranda JL, Esparza R, Rosas G. Bimetallic Ag@Pt core-shell nanoparticles and their catalytic activity by a green approach. *Mater Res Exp*. 2019;6:ab299c. doi: 10.1088/2053-1591/ab299c.
- [58] Hasan M, Zhongqiu T, Iqbal J, Umer A, Shiyong M, Rongji D, et al. Assessment of bioreducing and stabilizing potential of dragon's blood (*Dracaena cochinchinensis*, Lour. S. C. Chen) resin extract in synthesis of silver nanoparticles. *Nanosci Nanotechnol Lett*. 2013;5:780–4. doi: 10.1166/nnl.2013.1600.
- [59] Molnár Z, Bodai V, Szakacs G, Erdelyi B, Fograssy Z, Safran G, et al. Green synthesis of gold nanoparticles by thermophilic filamentous fungi. *Sci Rep*. 2018;8(1):8:3943. doi: 10.1038/s41598-018-22112-3.
- [60] Rambabu K, Bharath G, Banat F, Show PL. Green synthesis of zinc oxide nanoparticles using *Phoenix dactylifera* waste as bioreductant for effective dye degradation and antibacterial performance in wastewater treatment. *J Hazard Mater*. 2021;402:123560. doi: 10.1016/j.jhazmat.2020.123560.