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INVESTIGATING THE IMPACT OF TiO_2 NANOPARTICLES ON BIOACTIVE COMPOUNDS IN SWEET PEPPER SEEDLINGS: A COMPARISON OF FOLIAR AND ROOT APPLICATION METHODS

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ABSTRACT

Engineered TiO_2 nanoparticles (TiO_2-NPs) are broadly produced and utilized in various consumer products. However, plant uptake of NPs may lead to disruptions in physiological and metabolic processes, particularly when the plant's defense mechanisms are overwhelmed. In this study, sweet pepper seedlings were exposed to TiO_2-NPs via foliar (2.5% suspension) and root (0.5% suspension) methods, with plants treated with distilled water serving as controls. Results showed that foliar application caused higher accumulation of Ti in leaves as compared to stems, while root exposure led to a higher increase of Ti content in stems than in leaves. Additionally, foliar application led to alterations in chemical composition of the plants, including changes in malondialdehyde (MDA), L-ascorbic acid, total phenolics content, carotenoids, in total antioxidant capacity (TAC) and antioxidant enzymes activity. Root exposure also affected enzyme activity and TAC, but also altered H_2O_2, MDA and glutathione content. Chlorophylls remained at stable level in the leaves of the seedlings. Overall, these studies provide important information on plant-nanoparticle interactions and the potential effects of different nanoparticle application strategies. These data indicate also that the specific nanoparticles, applied at a controlled manner, have potential to boost the plant metabolism and improve stress tolerance, which is an important factor affecting crops' quality and productivity.

Key words: absorption pathway, antioxidants, *Capsicum annuum* L., titanium

INTRODUCTION

Nanoparticles (NPs) possess distinct physicochemical properties, including differences in energy levels, electronic structures, and reactivity, compared to their larger-sized counterparts (fine particles, FP) of the same composition. As a result, the biological activity of NPs is expected to differ to a certain extent from that of larger particles [Shi et al. 2013]. The wide use of TiO_2 nanoparticles (TiO_2-NPs) in consumer

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and industrial products is associated with their high catalytic activity, resulting from their smaller size and large specific surface area compared to FP [Satti et al. 2022, Shi et al. 2013]. Nanoparticles have various applications in agriculture, including degradation of pesticides, accelerating seed germination and growth, improving fertilization efficiency, combating crop diseases and purifying water [Fu et al. 2020, Wang et al. 2016a]. A review by Lyu et al. [2017] highlighted the effects of TiO_2-NPs on plant performance, including stimulation of enzyme activity, increasing chlorophyll concentration and photosynthesis performance, promotion of nutrients uptake, enhancement of tolerance to stress stimuli, and improvement of yield and quality of the crops. For instance, Raliya et al. [2015] found an increase in the length of roots and shoot, root nodule numbers, root area, and an increase in the content of chlorophylls in the leaves of *Vigna radiata* due to application of TiO_2-NPs. Similarly, Lei et al. [2008] observed that TiO_2-NPs caused in spinach plants an elevation of Rubisco activity, photosynthetic rate, chlorophyll formation, and ultimately an improvement in yield. Harmful effects of NPs on plants resulted from physico-chemical properties of the applied nanoparticles, the concentration and amount of the delivered NPs, plant ontogeny stage, growth environment, method of treatment application (roots, leaves) and the plant species. When utilized at high concentrations, nanoparticles, such as TiO_2-NPs, often exhibit phytotoxicity [Rafique et al. 2018]. According to Hou et al. [2019] the overproduction of reactive oxygen species (ROS) and the peroxidation of cellular membranes are linked to the harmful effects of TiO_2-NPs on living beings, resulting in protein degradation and reduced capacity of ionic transport, and the attachment of NPs to cells and intracellular organelles through electrostatic forces which causes various types of damages. Despite these findings, predicting the specific impacts of NPs exposure on plant organisms remains a challenge.

The mechanisms by which nanoparticles enter and are transported within different plant organs, as well as the potential effects they may cause, are not well understood. In-depth research is needed to fully explore these phenomena [Khan et al. 2022]. In general, the penetration of NPs into the leaf can occur mainly through the cuticle or stomata [Larue et al. 2014a], but possible routes include hairs, ion channels, protein carriers, endocytosis, tissue damage caused by various factors, including NPs [Hong et al. 2021]. Two main paths stand out for penetration the cuticule by NPs. Lipophilic pathway facilitates the diffusion of nonpolar substances through the cuticle, whereas hydrophilic pathway allows for the transportation of polar solutes via polar aqueous pores [Eichert and Goldbach 2008, Popp et al. 2005]. Estimated effective size of NPs in this case ranged from about 0.6 nm to 4.8 nm. Some reports pointed to foliar uptake via cuticle of NPs of diameter higher than 5 nm [Lv et al. 2019]. The stomatal pathway allows for the penetration of larger nanoparticles (NPs) as the stomatal sizes are typically in the range 3–10 μm/ca. 25 μm (width/length), however, some studies showed that the stomatal pathway can vary greatly in NPs permeability [Eichert et al. 2008]. Eichert and Goldbach [2008] suggested that the stomatal pathway of NPs penetration is probably located on the surface of the stomatal pores and transport takes place in a diffusion medium. According to these authors, substances can move through pore with average radius even greater than 20 nm. Some reports suggest that NPs may lead that the plant will form larger pores in cuticles and cell walls, and cause other structural changes in cells, which facilitate the permeation and transport of larger NPs [Wang et al. 2016b]. It should also be noted that the uptake of NPs by leaves depends on such species traits as leaf morphology, cuticle thickness and permeability, stomata size and density of NPs [Lv et al. 2019, Schwab et al. 2016].

After absorption by the roots, nanoparticles probably migrate to various tissues of the above-ground plants' parts and vice versa. Reports have shown that NPs can be taken up by the plants via roots and transported to leaves when roots are exposed to NPs [Wu et al. 2020]. However, the concept of root application of NPs and their uptake by plants is still controversial due to contradictory reports. Factors that can influence NPs uptake from the soil include root morphology, rhizospheric conditions, root exudates or microbe-plant relationships, but the most important trait is the pores' sizes and membrane channels which vary due to plant species and plant ontogeny stage [Khan et al. 2022]. After reaching the root epidermis, NPs can be transported through the apoplastic pathway and the symplastic pathway [Khan et al. 2022, Avellan et al. 2017], with the apoplastic pathway being more likely. Larue

et al. [2012] in experiment on wheat exposed its roots to TiO_2-NPs and reported size-dependent NPs uptake. The upper diameter was 140 nm above which NPs were not absorbed by the roots, the lower threshold diameter was 36 nm above which NPs were accumulated in parenchyma of wheat root, not reaching the stele, and in consequence not reaching the shoot. As it was mentioned above, nanoparticles may lead to oxidative stress connected with overproduction of ROS, such as superoxide radical ($O_2^{\bullet -}$), perhydroxyl radical ($HO_2^{\bullet}$), H_2O_2, singlet oxygen ($^1O_2$), hydroxyl radical ($^{\bullet}OH$), and peroxy radical ($ROO^{\bullet}$) that are hazardous to cells leading sometimes to its death [Rico et al. 2015, Sharma et al. 2012]. The defense system include a variety of scavengers, including superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione reductase (GR), glutathione peroxidase (GPX), guaicol peroxidase (GPOX), catalase (CAT), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR) or glutathione-S-transferase (GST) enzymes and non-enzymatic metabolites (i.e. ascorbate, carotenoids, tocopherols, glutathione, phenolics and some others compounds), protects plants against oxidative stress damages [Gill and Tuteja 2010]. The activation of plant defense mechanisms may depend on the NPs treatment strategies (leaves or roots application) that affect rate of infiltration of nanoparticles into the plant and effective transport of NPs to individual tissues and cells [Cocozza et al. 2019].

Given the complex and varied effects of nanoparticles on plants, this study aimed to investigate the changes in the activity of the antioxidant system in sweet pepper plants following exposure to TiO_2-NPs. Specifically, the study aimed to assess changes in the content of bioactive compounds, including the production of H_2O_2 and malondialdehyde (MDA), and in the fresh weight and dry weight of the plants as affected by NPs. The study hypothesized that: 1) when TiO_2-NPs are applied to plants, it is expected to elevate the levels of H_2O_2 and MDA, as well as induce changes in the activity of antioxidant enzymes and in the accumulation of non-enzymatic antioxidants; 2) the biochemical changes depend on the route of application of the nanoparticles (supplying of TiO_2-NPs by foliar or root application); 3) these changes are specific to the leaves and stems; 4) applied NPs at given concentrations that are not lethal to the plants can play a key role in improving stress tolerance in plants and alleviating stress consequences.

MATERIAL AND METHODS

Nanoparticles

The titanium (IV) dioxide nanoparticles (TiO_2-NPs) were delivered by PlasmaChem GmbH (Berlin, Germany). TiO_2-NPs were in form of anatase, sold as 5 wt% aqueous colloidal suspensions. The physical properties of the NPs are as described by the manufacturer's data: particle size 4–8 nm (the size of the nanoparticles specified by the manufacturer was confirmed, it can be seen in Fig. 1), effective surface area was 140 $m^2\ g^{-1}$ and pores volume by N_2 absorption ca. 0.035 $cm^3\ g^{-1}$.

Two concentrations of TiO_2-NPs were prepared: 2.5% for foliar application and 0.5% for root appli-

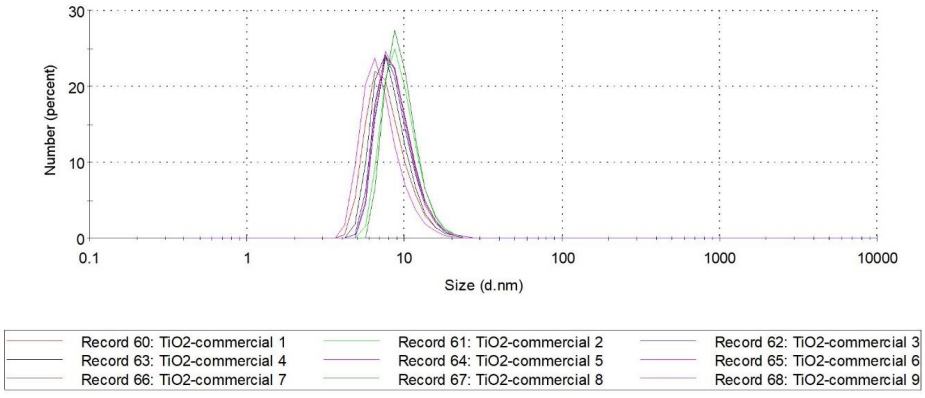


Fig. 1. Measuring the size distribution of TiO_2 nanoparticles, according to the number of particles

cation by adding deionized water to stock suspension. Mentioned concentrations for application strategies resulted from the observed phytotoxic effect of TiO_2 nanoparticles applied at a concentration of 2.5% to the roots (preliminary experiment), which caused hypocotyl and epicotyl browning, plant wilting and death within 2 days. This led to additional testing, which showed a concentration of 0.5% above which visible signs of toxicity occurred with NPs application to the roots. However, no chlorotic or necrotic changes were observed on the leaves after foliar spraying with a 2.5% TiO_2 suspension, this concentration was selected after analyzing data obtained from a previous study [Jurkow et al. 2020].

Plant culture, exposure, and sampling

Sweet pepper (*Capsicum annuum* L.) cultivar Chouca F_1 (Clause Vegetable Seeds, Hazera, Warsaw, Poland) was used in the experiment. Seedlings were bought from Krasoń – A Group of Vegetable Seedling Producers (Piaski, Poland, 51°21'0.24"N, 19°40'7.55"E). The seeds were sown in the spring on April 20. Sweet pepper was grown in multicell black trays (150 cells, pyramid shape each cell with a volume of 30 mL) filled with peat substrate. Two experiments were performed at the same time, consisting of foliar and root application of TiO_2-NPs to the plants, both leaves and stems were analysed. The scheme of the sampled plant part and abbreviated naming of the experimental treatments is presented in the Figure 2.

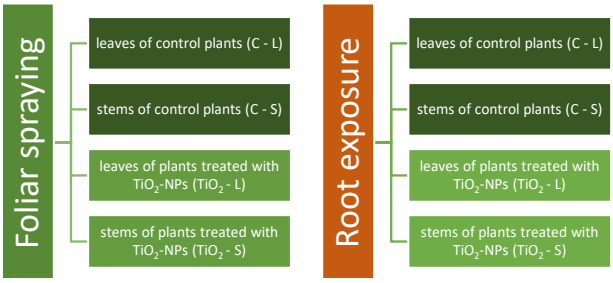


Fig. 2. Scheme of the experiments

Before the foliar treatment with nanoparticles, on May 26, the plants were positioned on a rolling table located in a compartment in a Venlo-type greenhouse (50°5'3.22"N, 19°57'2.21"E) and received irrigation through flooding of the entire table. Tap water was used each time for irrigation the plants. No additional fertilizers were applied to the plants. Growth conditions were as follow: natural light and CO_2 level, day length ca. 16 h, average temperature was set as 23/19°C (day/night), relative humidity ca. 75%, shading curtains were deployed automatically depending on the intensity of external irradiation (30% below 300 W m^{-2} to 90% at over 800 W m^{-2}). Experiment started on May 28 when seedlings had ca. 6–7 leaves. To ensure particle disaggregation before the start of the experiment, the nanoparticle suspensions were ultrasonicated for 10 minutes prior to application. The plants were then separated into two groups. For the first group, sweet pepper leaves were sprayed with a 2.5% TiO_2-NPs suspension using a hand sprayer equipped with a mist nozzle, each multicell tray was provided with 150 mL of the suspension (approximately 1 mL per plant); deionized water was used to control plants at the same time (Fig. 3A). Experimental treatment consisted of 300 seedlings, 100 plants in one repetition. The plants were supplied with water without wetting the leaves, by flooding the production table to 3/4 of the height of the cells. For the experiment with root delivery of nanoparticles, 120 plants were randomly selected from seedling trays, whose roots were washed out from the peat substrate (Fig. 3B). Of these plants, 60 seedlings were placed in plastic test tubes in 25 mL of 0.5% TiO_2-NPs suspension, another 60 plants in deionized water of the same volume. In this study, one repetition consisted of 20 plants. The TiO_2-NPs suspension was subjected to ultrasonication for 10 minutes before use, and the environmental conditions were kept consistent with those of the foliar experiment.

Sampling was performed after a 5-day exposure period for both the foliar and root exposure experiments. The leaves and stems of the sweet pepper plants were carefully harvested. Plant samples were first washed using tap water, followed by a rinse with deionized water. The fresh plant material was used to determine the average fresh weight of the plant, content of dry weight, L-ascorbic acid, glutathione, and photosynthetic pigments. A segment of the plant samples was frozen with liquid nitrogen and next kept at –40°C, allowing for future assessment of outstanding biochemical parameters.

Fig. 3. Illustration of the experiments with sweet pepper seedlings: A) foliar-treated plants, B) part of the plants whose roots were immersed in NPs suspension/deionized water. White arrows point treatments with nanoparticles, while yellow arrows – control plants

Fresh and dry weight determination

Fifteen sweet pepper seedlings from 300 plants constituting a treatment were taken randomly for determining fresh weight (FW) of the shoot. It was done with a Ohaus PA214CM/1 balance (OHAUS Europe GmbH, Nänikon, Switzerland) and the results expressed in grams per plant. Determination of dry weight was performed by the dryer method [Pijanowski et al. 1964]. The samples were transferred into the oven (Binder ED 23, BINDER GmbH, Tuttlingen, Germany) at 65°C up to a constant weight was reached. The results were presented as grams of dry weight (DW) in 100 g of fresh weight.

Total Ti concentration

The plant material, consisting of chopped sweet pepper leaves or stems, was dried in an oven at 65°C until a constant weight was achieved and then ground into a fine powder by Pulverisette 14 mill (Fritsch GmbH, Idar-Oberstein, Germany) fitted with a 0.5 mm mesh sieve. To determine the Ti content, the following procedure was employed: 0.2 g of plant samples were placed in 50 mL TFM vessels and mineralized with 10 mL of HNO_3 (65%, Merck no. 100443.2500) using an MDS-2000 microwave digestion system (CEM Corporation, Matthews, NC, USA). The samples underwent a 10-minute mineralization process to reach 200°C, followed by an additional 5 minutes at this temperature. Once cooled, the samples were transferred to 25 mL volumetric flasks using redistilled water. These prepared samples were diluted 10 times before Ti content determinations commenced using inductively coupled plasma mass spectrometry (ICP-MS/MS) with a triple quadruple spectrometer (iCAP TQ ICP-MS Thermo Fisher Scientific, Bremen, Germany). The measurement mode of 48Ti | 48Ti.14N4.1H10 (S-TQ-NH3) [Vincent 2017] was utilized.

Hydrogen peroxide and malondialdehyde quantification

To determine the hydrogen peroxide (H_2O_2) content, 2 g of fresh plant samples were ground and homogenized with 10.0 mL of 0.1% trichloroacetic acid (TCA) in an ice bath at 4°C. The resulting homogenate was centrifuged at 13,968 × g for 10 minutes. Next, 0.5 mL of K-phosphate buffer (pH 7.6) and 1 mL of 1 M KI (potassium iodide) were added to 0.5 mL of the supernatant. The control sample comprised 0.5 mL of 0.1% trichloroacetic acid (TCA), 0.5 mL of K-phosphate buffer (pH 7.6), and 1 mL of 1 M KI. All samples were mixed and left in darkness for 1 hour. Absorbance values were recorded using a UV-VIS Helios Beta spectrophotometer (Thermo Fisher Scientific Inc., Waltham, USA) at 390 nm. A calibration curve of H_2O_2 was employed to calculate hydrogen peroxide content, which was subsequently expressed as µmol H_2O_2 per 1 g FW.

Malondialdehyde content was assessed following the method outlined by Dhindsa and Matowe [1981]. Plant samples (1.0 g) were combined with 10 mL

of 0.1% trichloroacetic acid at 4°C and centrifuged (13 968 × g, 10 min, 4°C). Subsequently, 0.5 mL of the extracts were mixed with 0.5 mL of phosphate buffer (pH 7.6) and 1 mL of 0.5% thiobarbituric acid (TBA), previously dissolved in 20% TCA. The control sample consisted of 0.5 mL TCA, 0.5 mL phosphate buffer (pH 7.6), and 1 mL of 0.5% TBA dissolved in 20% TCA. All mixtures were incubated in a hot water bath at 95°C for 30 minutes before cooling to room temperature. Absorbance was measured first at 532 nm (MDA-TBA complex peak) and then at 600 nm (nonspecific absorption) using a UV-VIS Helios Beta spectrophotometer. A molar absorption coefficient (ε) of 155 mM^{-1} cm^{-1} was applied to compute MDA content, ultimately expressed as μmol g^{-1} FW.

Analysis of antioxidant enzymes activity

The activity of peroxidases was assessed using the procedure where 2 g of samples were crushed in the 4°C with 10 mL of 50 mM potassium phosphate buffer adjusted on pH 7.0 and containing 1 mM EDTA (ethylene diamine tetra-acetic acid), 1% PVP (polyvinyl pyrrolidone), and 1 mM PMSF (phenyl methane sulfonyl fluoride). The mixture was centrifuged at 13 968 × g for 15 min at 4°C, and the supernatant was utilized as the enzyme extract. The ascorbate peroxidase (APX) assay was performed following the method by Nakano and Asada [1981]. APX activity was determined in samples containing 1.85 mL of 50 mM potassium phosphate buffer (pH 7.0), 0.5 mL of 0.5 mM ascorbate (AsA), 0.5 mL of 0.1 mM hydrogen peroxide, and 0.15 mL of the supernatant. The AsA oxidation, dependent on H_2O_2, was monitored by the decrease in absorbance at 290 nm (Helios Beta spectrophotometer), using ε = 2.8 mM^{-1} cm^{-1} to calculate the enzyme activity, expressed as μmol AsA min^{-1} g^{-1} FW. Guaiacol peroxidase (GPOX) activity was assessed based on method of Zhang et al. [2005], using guaiacol as a substrate. The reaction sample included 1.4 mL of 50 mM phosphate buffer (pH 7.0), 0.2 mL of 4% guaiacol, and 1 mL of 1% H_2O_2. Enzyme reactions were initiated by adding 0.4 mL of the extract to the reaction mixture. Sample absorbance was measured with a UV-VIS Helios Beta spectrophotometer at 470 nm, and ε = 26.6 mM^{-1} cm^{-1} was applied to determine GPOX activity, which was subsequently expressed as μmol tetraguaiacol min^{-1} g^{-1} FW.

Catalase (CAT) activity was assessed using the following method [Aebi 1984]: 2 g of plant samples were blended with 10 mL of 50 mM potassium phosphate buffer (pH 7.0). Blending was made in an ice bath (4°C). The mixture was centrifuged at 13 968 × g for 15 minutes at 4°C, and the resulting supernatant was utilized to measure CAT activity. The reaction medium included 1.8 mL of 50 mM phosphate buffer (pH 7.0) and 1 mL of 1% H_2O_2. Adding 0.2 mL of the extract to the reaction mixture initiated the enzymatic reaction. Absorbance was determined at 240 nm (UV-VIS Helios Beta spectrophotometer) against a control sample (2.0 mL of 50 mM phosphate buffer (pH 7.0) with 1 mL of 1% H_2O_2). CAT activity was expressed as μmol H_2O_2 min^{-1} g^{-1} FW.

Determination of ascorbate, glutathione and total phenolics

The iodometric technique was employed to assess the L-ascorbic acid (AsA) content, as described by Ikewuchi and Ikewuchi [2011]. Plant sample (2.5 g) was homogenized with 10 mL of the acidity regulator, which was 1% oxalic acid, and, after 30 min, the 5 mL of filtered extract including 1 mL of 1% starch was titrated with a solution of iodine in potassium iodide. If there is ascorbic acid in the solution, it reacts with iodine on an ongoing basis and the starch has no color. Iodine, after all the ascorbic acid was oxidized, formed a blue complex with starch and marked the end of the titration. The AsA content was calculated considering the amount of iodine in potassium iodide solution used for the titration.

The method by Guri [1983], with slight modifications, was utilized to measure glutathione (GSH, reduced form). In this approach, fresh leaves (2 g) were chopped and then blended in an ice mortar (4°C) with 10.0 mL of 0.5 mM EDTA dissolved in 3% TCA. The extract was centrifuged at 13 968 × g for 10 minutes at a temperature of 4°C. Following that, 5 mL of K-phosphate buffer (pH 7.0) was added to 2 mL of supernatant to adjust the pH to approximately 7.0. This mixture (2.0 mL) was transferred to a tube where an additional 1 mL of the same K-phosphate buffer was added. Subsequently, 0.1 mL of Ellman's reagent (5,5-dithiobis-2-nitrobenzoic acid) was introduced to the sample. The GSH content was determined by reading the absorbance at 412 nm using a UV-VIS Helios Beta spectrophotometer, against a control sample prepared similarly, but with 1.1 mL of K-phosphate buffer and without Ellman's reagent.

The concentration of phenolic compounds was assessed using the Folin-Ciocâlteu method, as described by Djeridane et al. [2006]. The plant samples (2.0 g) were chopped and homogenized with 10 mL of 80% methanol, followed by centrifugation for 10 minutes at 4°C at 3492 × g. Then, 2 mL of 2% sodium carbonate was added to the supernatant (0.1 mL). After another 2 minutes, Folin-Ciocâlteu's reagent (0.1 mL), diluted with deionized water (1:1 v/v), was added to each sample. All samples were incubated for 45 minutes at approximately 22°C in a dark room. The absorbance was measured at 750 nm by the UV-VIS Helios Beta device. The total phenolics (TP) content was expressed as gallic acid equivalents (GAE) per 1 g of fresh weight (FW).

Total antioxidant activity

The total antioxidant capacity (TAC) was assessed using a method outlined by Molyneux [2004], which employs the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical. A 2 g sample of chopped plant material was homogenized with 10 mL of 80% methanol and then centrifuged at 3,492 × g for 10 minutes at 4°C. To the supernatant (0.1 mL), 4.9 mL of 0.1 mM DPPH dissolved in 80% methanol was added. The reaction mixture was thoroughly mixed and allowed to rest for 15 minutes at room temperature in the dark. The absorbance was recorded at 517 nm using the UV-VIS Helios Beta spectrophotometer. A standard calibration curve was prepared by plotting the Trolox concentration against the percentage of DPPH scavenged. The antioxidant activity was expressed as 1 mg of Trolox per 1 g of FW.

Photosynthetic pigment measurement

The content of photosynthetic pigments: chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*) and carotenoids (Car) was determined according to Lichtenthaler and Wellburn [1983]. Fresh leaves (0.1 g) were homogenized with 25 mL of 80% (v/v) acetone, magnesium carbonate (3 mg, $MgCO_3$) was used as a stabilizer. The samples were tightly covered and incubated for 0.5 h in the dark room. After this time, the obtained suspension was filtered (paper No. 978774513, POCH S.A., Gliwice, Poland). Absorption was measured by UV-VIS spectrophotometer at 646, 663, and 470 nm to determination of the Chl *a*, Chl *b*, and for analyse of total Car concentration. The final contents of these pigments were obtained from the equations published by Lichtenthaler and Wellburn [1983]. The ratio Chl *a*/*b* and ratio Car/Chls was also presented.

Statistical analyses

Statistica 13.3 (TIBCO Software Inc., Palo Alto, CA, USA) was used for analysis of the results. Treatments data were compared using one-way ANOVA, and next by Tukey's HSD test when data were statistically different ($P \leq 0.05$). Standard deviations (SD) were calculated on all data sets. Differences in fresh weight were checked by using t test at $P \leq 0.05$. Heatmaps were created also in Statistica 13.3 to study disparity and similarity patterns in gathered data for sweet pepper plants. Data were standardized before creating heatmaps.

RESULTS

The treatment by TiO_2-NPs led to a significant increase in DW content of the sweet pepper leaves for both foliar and root treatments, by 11.3% (from 12.10 g 100 g^{-1} FW for C–L to 13.47 g 100 g^{-1} FW for TiO_2-L) and 22.1% (from 11.37 g 100 g^{-1} FW for C–L to 13.88 g 100 g^{-1} FW for TiO_2-L), respectively (Fig. 4). Foliar spraying with TiO_2-NPs also increased the DW content in the stems of the seedlings by 5.1% (from 7.08 g 100 g^{-1} FW for C–S to 7.44 g 100 g^{-1} FW for TiO_2–S). However, this increase was not observed in stems of the plants whose roots were immersed in a suspension of nanoparticles. In general, higher content of DW was found in the leaves than in the stems of the seedlings.

Foliar and root application of TiO_2-NPs did not affect shoot FW of the sweet pepper seedlings (Fig. 5). In the case of foliar spraying, shoots of control seedlings weighed 4.79 g/plant, on average, while plants treated with TiO_2 – 4.34 g/plant, difference was not significant ($P = 0.153$). When roots of the plants were exposed to TiO_2-NPs, weight of control plant was 4.59 g, on average, while for the plants treated with nanoparticles – 4.73 g/plant. In this case there was also no differences between means ($P = 0.669$). The shoot weight of the seedlings treated with TiO_2-NPs via foliar spraying fluctuated for control from 3.01 to 6.45 g/plant (median was 4.92 g/plant) and for plants sprayed with TiO_2-NPs from 3.24 to 5.45 g/plant (median was 4.49 g/plant). With root application, the weight of control plants'

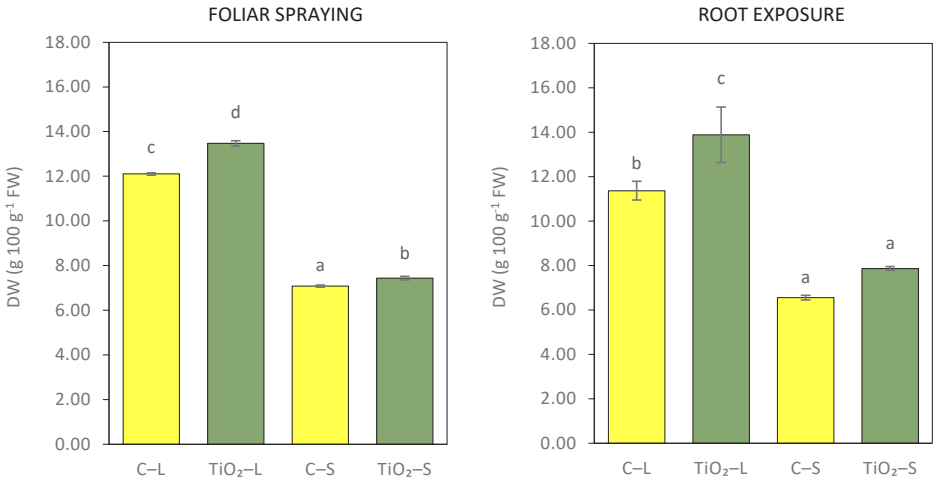


Fig. 4. Changes in the content of dry weight (DW) in sweet pepper seedlings leaves (L) and stems (S) caused by the treatments with TiO_2 nanoparticles. Distilled water served as a control (C). Values are means (n = 3) ± standard deviation. Means with the same letters were not significantly different according to Tukey's HSD test ($P \leq 0.05$)

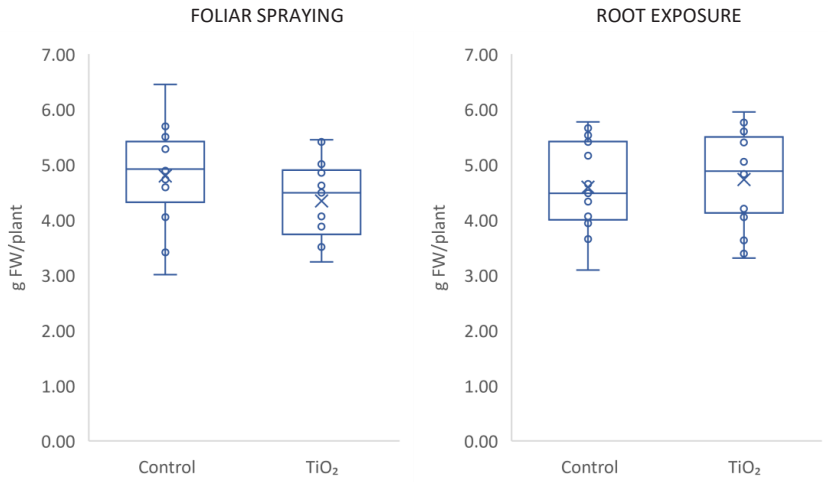


Fig. 5. Distribution of data in quartiles with the distinction of the means (×) and internal points representing shoot fresh weight (FW) of sweet pepper seedlings treated with TiO_2 nanoparticles. Distilled water served as a control (C). Whiskers indicate variability outside the upper and lower quartiles, lines inside the box showed medians

shoots ranged from 3.09 to 5.77 g/plant (median was 4.48 g/plant), while for plants treated with nanoparticles the minimum weight was 3.31 g/plant and the maximum 5.95 g/plant with median at 4.88 g/plant.

Sweet pepper seedlings showed higher concentrations of Ti in the leaves and in the stems, regardless of the treatment strategies which included leaves or roots exposure (Fig. 6). When TiO_2-NPs were applied via foliar spraying, the amount of Ti increased in the leaves and in the stems of the seedlings in comparison to control (by 8697%, from 1.32 to 115.70 mg kg^{-1} DW and by 4103%, from 1.85 to 77.77 mg kg^{-1} DW, respectively for C–L and C–S), however, concentration of this element was lower in the stems than in the leaves, difference reached 32.8%. TiO_2-NPs applied to roots caused considerable increase in Ti concentration in the seedlings' stems (by 557% in comparison to control C–S, from 3.35 to 22.00 mg kg^{-1} DW)

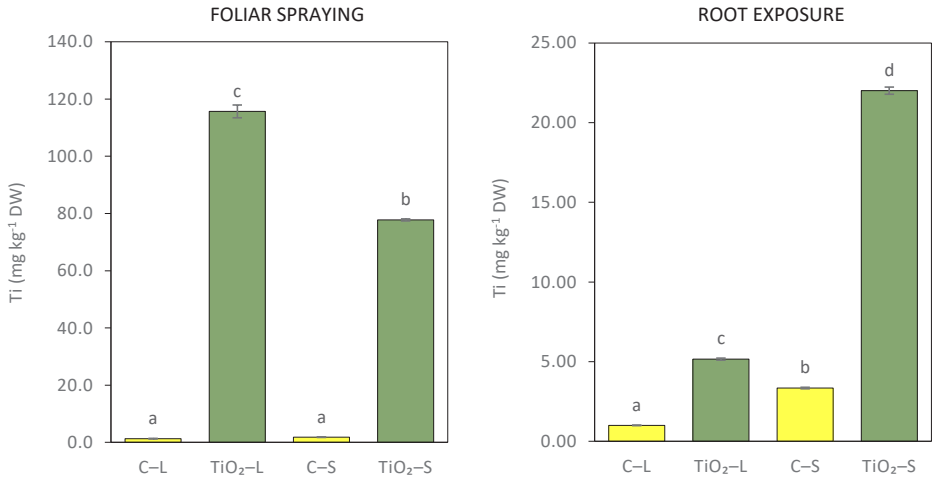


Fig. 6. Changes in the content of titanium (Ti) in sweet pepper seedlings leaves (L) and stems (S) caused by the treatments with TiO_2 nanoparticles. Distilled water served as a control (C). Values are means (n = 3) ± standard deviation. Means with the same letters were not significantly different according to Tukey's HSD test ($P \leq 0.05$)

and leaves (by 416% than in C–L plants, from 1.00 to 5.16 mg kg^{-1} DW), higher Ti content was found in the stems than in the leaves (by 327%).

Foliar spraying of seedlings with TiO_2-NPs did not affect content of H_2O_2 in their leaves and stems (Fig. 7A). However, roots treatment caused notable increase in H_2O_2 concentration in the leaves (by 20.7% in comparison to control C–L, from 1.32 to 1.60 µmol g^{-1} FW), but not in the stems of sweet pepper plants (Fig. 7B). More H_2O_2 was determined in the leaves of plants than in their stems. Additionally, the content of MDA in leaves was found to be higher than in stems of the plants that were subjected to foliar spraying of TiO_2-NPs (Fig. 7C). Furthermore, foliar spraying of TiO_2-NPs caused a markedly increase in MDA level in the plants by 20.7% when compared to the control (C–L) (from 7.73 to 9.33 µmol g^{-1} FW). When the plant roots were placed in the TiO_2-NPs suspension, there was a decrease in the MDA content in the stems when compared to the control seedlings (by 68.0%, from 13.42 to 4.30 µmol g^{-1} FW) (Fig. 7D). However, a different direction of changes was observed for the leaves, but this was not statistically significant.

Exposure of sweet pepper seedlings to TiO_2-NPs via foliar spraying resulted in statistically significant alterations in the activity of APX, GPOX, and CAT enzymes in the leaves, but not in the stems (Fig. 8). The foliar treatment with nanoparticles led to an increase in activity of GPOX and CAT in the leaves of seedlings (by 26.8%, from 2.245 to 2.848 µmol tetraguaiacol min^{-1} g^{-1} FW and by 38.7%, from 16.245 to 22.529 µmol H_2O_2 min^{-1} g^{-1} FW, respectively, as compared to the control group, Fig. 8C and 8E), but APX activity decreased (by 49.5% when compared to the control group, from 0.060 to 0.030 µmol AsA min^{-1} g^{-1} FW, Fig. 8A). Generally, for this treatment strategy, the activity of APX and CAT was lower in the stems than in the leaves, while higher activity of GPOX was noted in the stems. When the roots of sweet pepper plants were exposed to TiO_2-NPs, there was an increase in GPOX and CAT activity in the stems (Fig. 8D and 8F), by 10.8% (from 5.311 to 5.882 µmol tetraguaiacol min^{-1} g^{-1} FW) and 116.1% (from 3.563 to 7.701 µmol H_2O_2 min^{-1} g^{-1} FW), respectively, when compared to C–S. Additionally, activity of GPOX increased in the leaves of the plants whose roots were subjected to TiO_2-NPs (by 81.4% when compared to the C–L, from 1.321 to 2.396 µmol tetraguaiacol min^{-1} g^{-1} FW). However, activity of APX was reduced in the stems of the plants due to root treatment with TiO_2-NPs, by 60.1%, from 0.019 to 0.008 µmol AsA min^{-1} g^{-1} FW (Fig. 8B). When the roots were exposed to TiO_2-NPs, plants showed lower activity of APX and CAT in the stems than in the leaves, while lower activity of GPOX was noted in the leaves

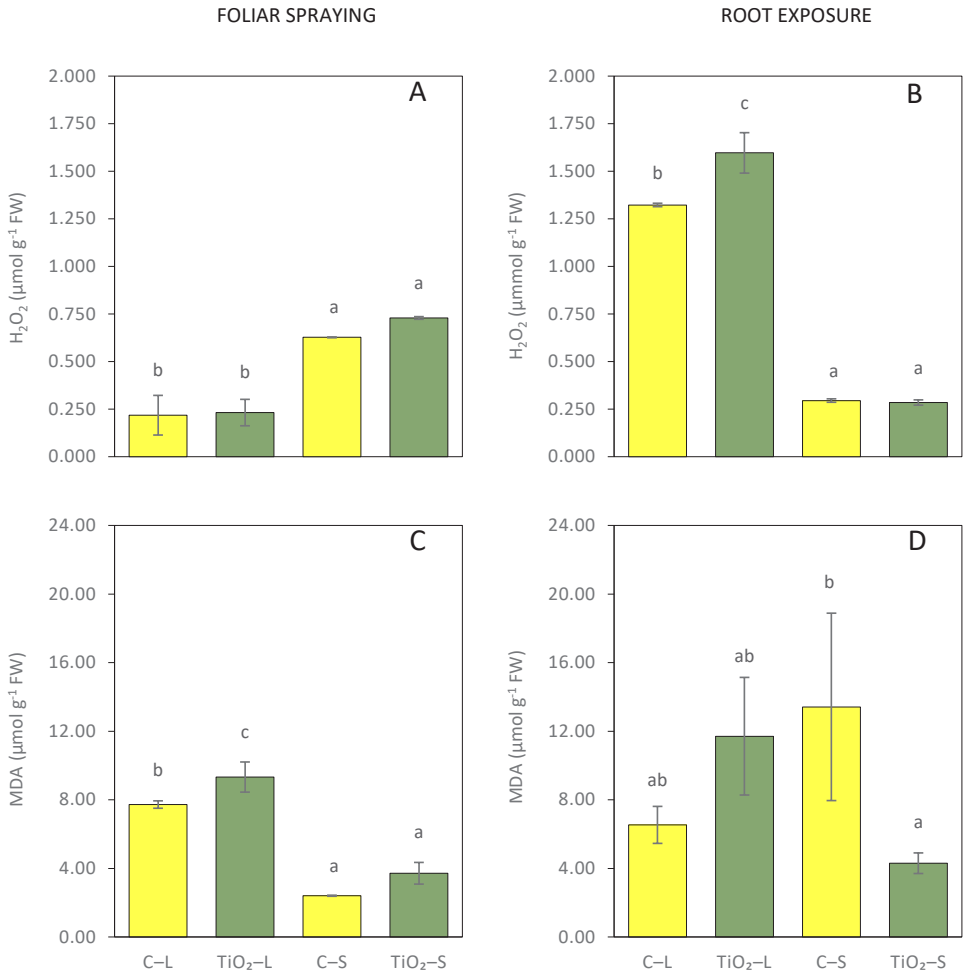


Fig. 7. Changes in the content of hydrogen peroxide – H_2O_2 (A, B) and malondialdehyde – MDA (C, D), respectively, in sweet pepper seedlings leaves (L) and stems (S) caused by the treatments with TiO_2 nanoparticles. Distilled water served as a control (C). Values are means (n = 3) ± standard deviation. Means with the same letters were not significantly different according to Tukey's HSD test ($P \leq 0.05$)

Foliar spraying with TiO_2-NPs led to an increase in AsA and TP contents in the leaves and TP in stems of sweet pepper (Fig. 9A and 9C), differences reached 17.4% (from 53.99 to 63.38 mg 100 g^{-1} FW), 5.5% (from 1.90 to 2.01 mg GAE g^{-1} FW) and 8.4% (from 0.84 to 0.91 mg GAE g^{-1} FW), respectively, in relation to control plants (C–L and C–S), while GSH content remained unchanged (Fig. 9E). When leaves of the plants were sprayed with TiO_2-NPs suspension, concentrations of AsA, TP and GSH were generally lower in the stems than in the leaves. There were no changes in L-ascorbic acid and total phenolics contents in the leaves and in the stems of the plants which roots were exposed to TiO_2-NPs (Fig. 9B and 9D). Lower contents of these organic compounds were observed in the stems than in the leaves. Root exposure to TiO_2-NPs induced an increase in GSH concentration in the seedlings' leaves, by 47.0%, from 176.79 to 259.84 µg g^{-1} FW, when compared to control seedlings (C–L, Fig. 9F). Application of nanoparticles to the roots did not affect level of GSH in the stems of sweet pepper. At the same time, leaves of seedlings treated with TiO_2-NPs (via roots) had higher level of glutathione than the stems of these plants.

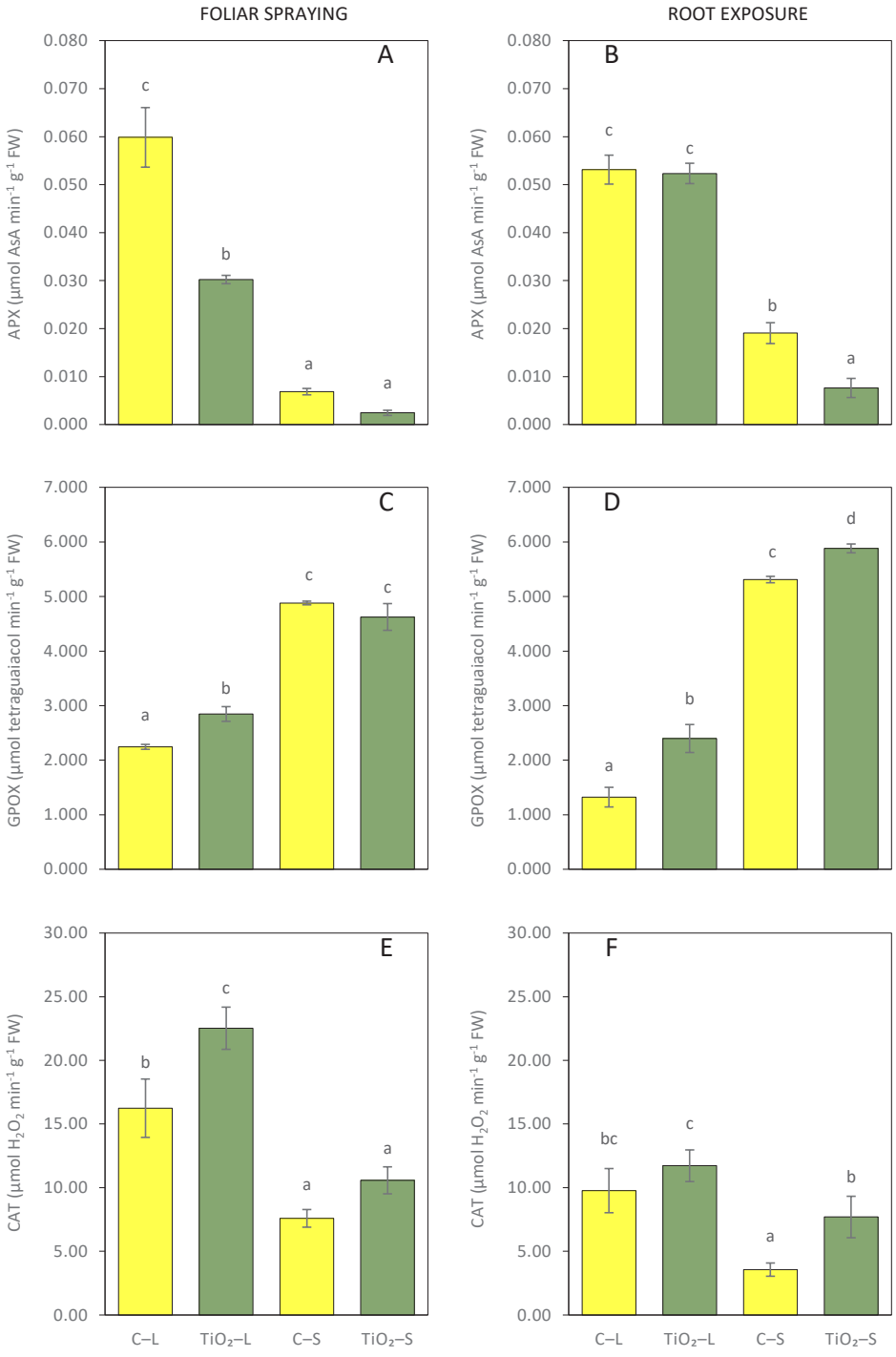


Fig. 8. Changes in the activity of ascorbic peroxidase – APX (A, B), guaiacol peroxidase – GPOX (C, D) and catalase – CAT (E, F), respectively, in sweet pepper seedlings leaves (L) and stems (S) caused by the treatments with TiO_2 nanoparticles. Distilled water served as a control (C). Values are means (n = 3) ± standard deviation. Means with the same letters were not significantly different according to Tukey's HSD test ($P \leq 0.05$)

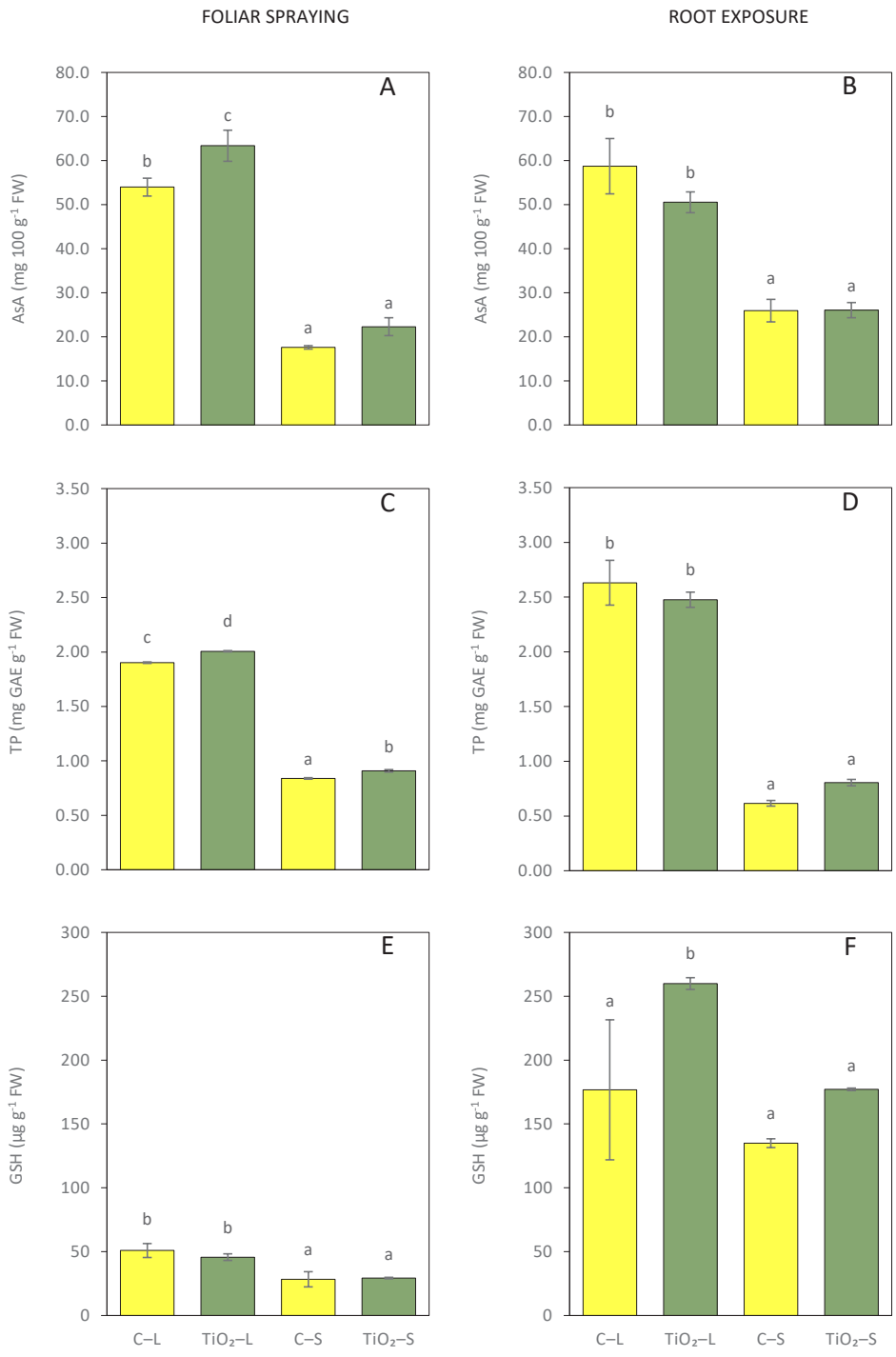


Fig. 9. Changes in the content of ascorbic acid – AsA (A, B), total phenolics – TP (C, D) and glutathione – GSH (E, F), respectively, in sweet pepper seedlings leaves (L) and stems (S) caused by the treatments with TiO_2 nanoparticles. Distilled water served as a control (C). Values are means (n = 3) ± standard deviation. Means with the same letters were not significantly different according to Tukey's HSD test ($P \leq 0.05$)

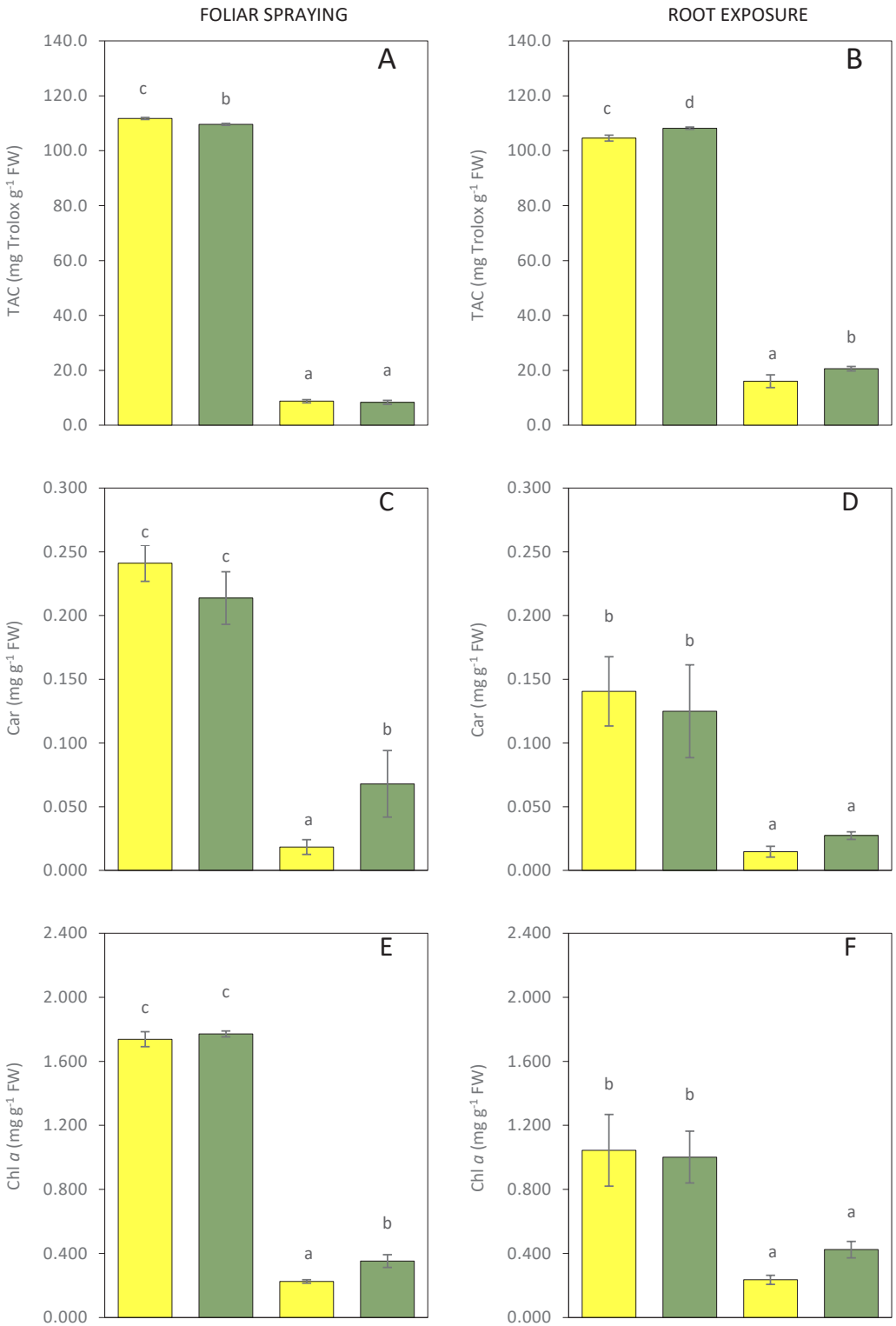
Sweet pepper seedlings subjected to foliar spraying with TiO_2-NPs showed lower TAC of the leaves, by ca. 2%, from 117.74 to 109.65 mg Trolox g^{-1} FW, in comparison to the control (C–L) (Fig. 10A). This was small change, but statistically significant. No effect of foliar spraying with nanoparticles was observed in the case of TAC of the stems. When TiO_2-NPs were applied to the roots of the seedlings, it caused significant increase of TAC of the leaves (by 3.5%, from 104.59 to 108.23 mg Trolox g^{-1} FW) and the stems (by 28.6%, from 16.03 to 20.60 mg Trolox g^{-1} FW) in comparison to control (C–L and C–S, respectively, Fig. 10B). In both treatment strategies (nanoparticles applied on the leaves or to the roots) lower antioxidant capacity was observed in the stems than in the leaves. Car and Chl *a* contents of sweet pepper plants increased significantly in their stems (from 0.018 to 0.068 mg g^{-1} FW and from 0.23 to 0.35 mg g^{-1} FW, respectively), but not in the leaves, upon exposure to TiO_2-NPs via foliar application, in relation to control C–S (Fig. 10C and 10E). The level of Chl *b* remained unchained (Fig. 10G). If plants' roots were exposed to TiO_2-NPs, there were no changes in Car, Chl *a* and Chl *b* concentrations in both leaves and stems of sweet pepper (Fig. 10D, 10F and 10H). In all cases, stems contained lower contents of these pigments than the leaves.

Foliar spraying of TiO_2-NPs increased Car/Chls and Chl *a*/*b* ratios in the stems of sweet pepper seedlings (Fig. 11A and 11C). These increases reached 170.6% (from 0.044 to 0.118) and 36.0% (from 1.124 to 1.529), respectively. No changes in these ratios were noted in the leaves. When TiO_2-NPs were applied to the roots, not any alterations were observed in the ratios of Car/Chls and Chl *a*/*b* in the leaves and in the stems of the seedlings also (Fig. 11B and 11D).

Figure 12 presents the heatmaps for the parameters of sweet pepper seedlings. Two heatmaps, referring to foliar application of nanoparticles and application of nanoparticles to the roots, were created. The color pattern ranges from dark green, which means lowest values to dark red presenting highest values, with transition values marked by gradually changing colors from dark green to dark red. Most of the parameters of plants treated with TiO_2-NPs via foliar spraying were higher in the leaves than in the stems. The only exception was GPOX activity and Car/Chls ratio being higher in the stems of such treated plants. Application of TiO_2 nanoparticles on the leaves significantly altered their chemical composition by increasing DW and TP content in the leaves and stems of the treated seedlings; MDA and AsA content together with GPOX and CAT activity in the leaves; Car and Chl *a* content as well as Car/Chls and Chl *a*/*b* ratio in the stems, but it decreased APX activity and TAC in the leaves. Concentration of H_2O_2, GSH and Chl *b* remained stable in both plant parts after spraying leaves with TiO_2-NPs. When nanoparticles were applied to the roots of the seedlings, it led to an increase in GPOX activity and TAC in both stems and leaves, CAT activity in stems or DW, GSH and H_2O_2 in leaves, but APX activity and MDA content decreased in the stems. Pigments (Car, Chl *a*, Chl *b*), AsA, TP content as well as Car/Chls and Chl *a*/*b* ratio remained unchanged. The content of titanium was elevated in the plants treated with TiO_2--NPs, when applied on the leaves, showing higher increase in the leaves than in the stems. On the other hand, in plants whose roots were placed in TiO_2-NPs suspension, more Ti was determined in the stems of the seedlings than in the leaves, in both cases values were higher in comparison to control stems and leaves (C–S and C–L, respectively).

DISCUSSION

In our study, the response of sweet pepper seedlings subjected to TiO_2-NPs were examined by evaluating changes in biochemical attributes. The nanoparticles were applied directly to the seedlings by spraying their leaves with a TiO_2-NPs suspension or by immersing the roots of the plants in a vial filled with the NPs suspension. In both cases, alterations in the biochemical composition of the leaves and stems of the plants were investigated. Our research showed that the nanoparticles led to significant changes in plant defense mechanisms consisting in changing the amount of non-enzymatic antioxidants and the activity of antioxidant enzymes in sweet pepper plants. This suggests that the plants suffered oxidative stress. Previous research on the phytotoxicity of metal and metal-based nanoparticles has shown that they can induce oxidative stress in various plant species [Sharma et al. 2019, Yang et al. 2017]. Factors that can influence this include the physico-chemical properties of the nanoparticles, the concentration and amount of the nanoparticles applied, and the plant species in question. The way in which the nanoparticles are taken up by the plants also


FOLIAR SPRAYING
ROOT EXPOSURE
A
B
C
D
E
F
TAC (mg Trolox g-1 FW)
Car (mg g-1 FW)
Chl a (mg g-1 FW)
140.0
120.0
100.0
80.0
60.0
40.0
20.0
0.0
0.300
0.250
0.200
0.150
0.100
0.050
0.000
2.400
2.000
1.600
1.200
0.800
0.400
0.000
a
b
c
d

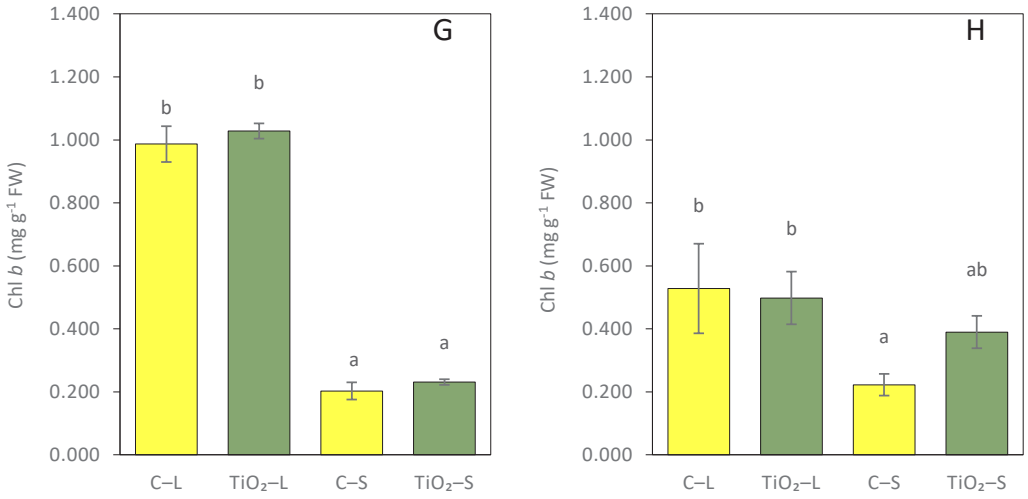


Fig. 10. Changes in the total antioxidant capacity – TAC (A, B) and in the content of carotenoids carotenoids – Car (C, D), chlorophyll *a* – Chl *a* (E, F) and chlorophyll *b* – Chl *b* (G, H), respectively, in sweet pepper seedlings leaves (L) and stems (S) caused by the treatments with TiO_2 nanoparticles. Distilled water served as a control (C). Values are means (n = 3) ± standard deviation. Means with the same letters were not significantly different according to Tukey's HSD test ($P \leq 0.05$)

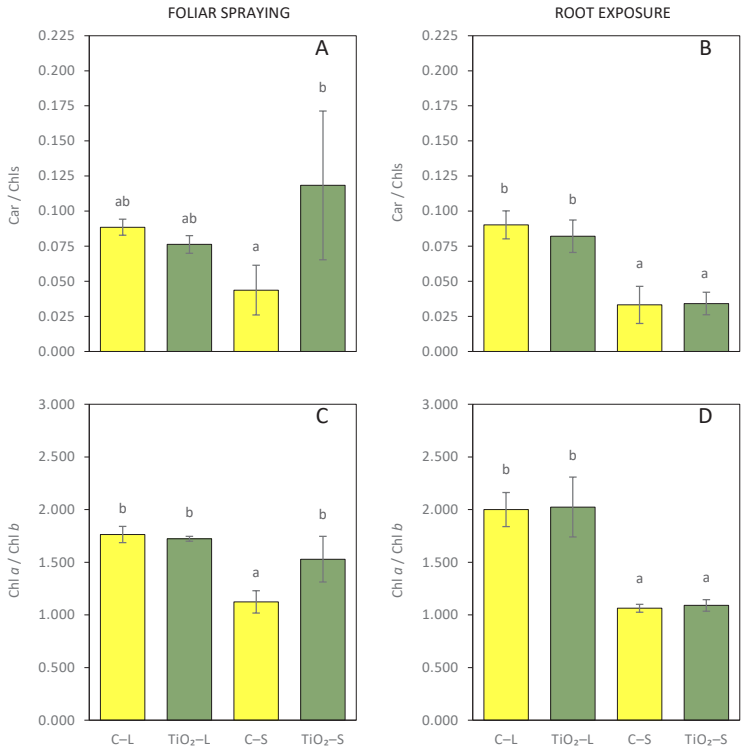


Fig. 11. Changes in the carotenoids/chlorophylls ratio – Car/Chls (A, B) and chlorophyll *a* to chlorophyll *b* ratio – Chl *a*/*b* (C, D), respectively in sweet pepper seedlings leaves (L) and stems (S) caused by the treatments with TiO_2 nanoparticles. Distilled water served as a control (C). Values are means (n = 3) ± standard deviation. Means with the same letters were not significantly different according to Tukey's HSD test ($P \leq 0.05$)

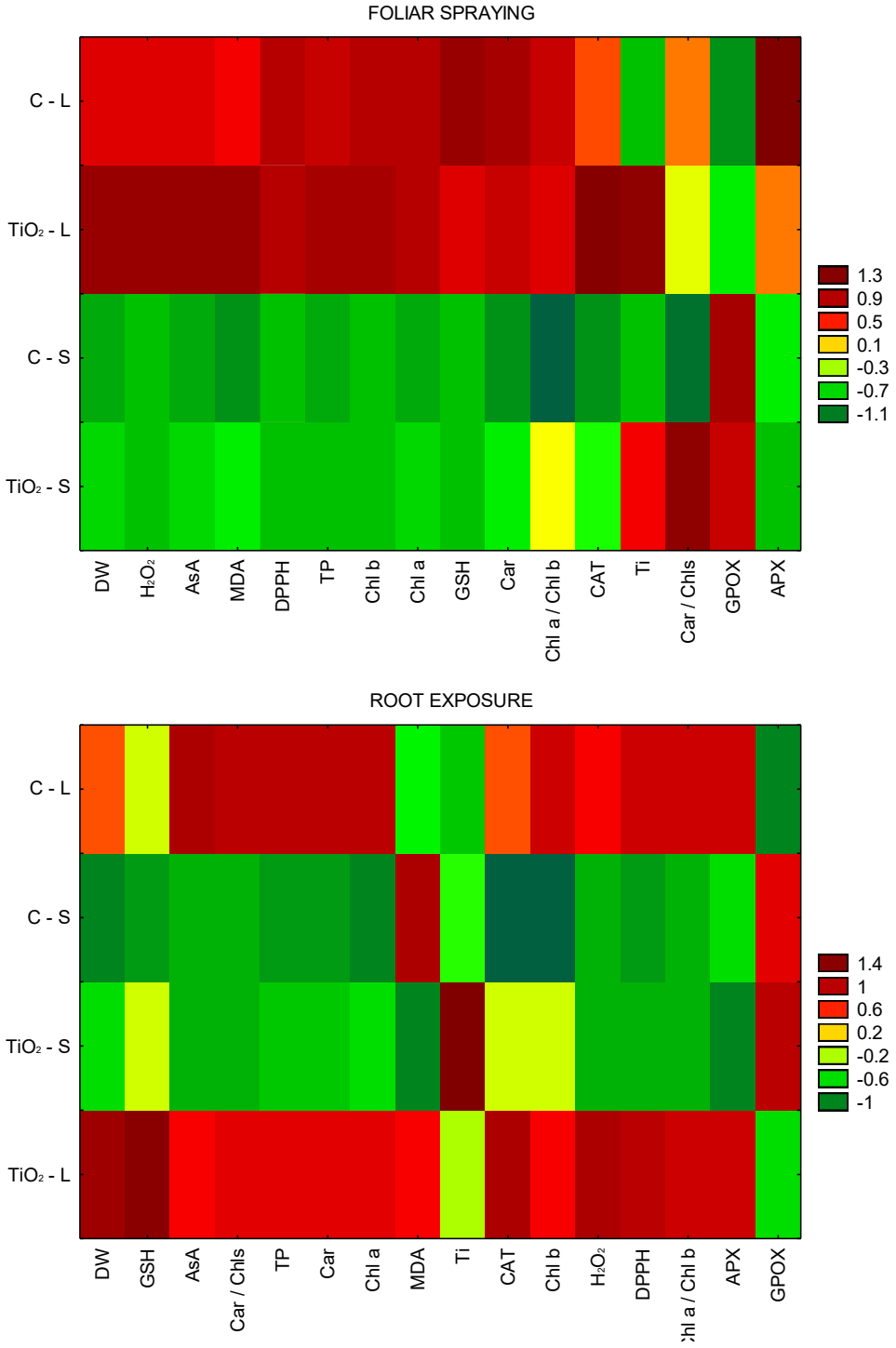


Fig. 12. Heatmaps of selected sweet pepper plants traits as depending on treatment strategies of nanoparticles application (on the leaves or to the roots). C – control plants treated with distilled water, TiO_2 – plants treated with TiO_2-NPs, L – leaves, S – stems

plays a role in determining the changes in the plants' chemical composition [Khan et al. 2022].

Overcoming the detrimental effects of oxidative stress by plants includes scavenging and deactivation of stress-induced and overproduced ROS [Sharma et al. 2019]. Plants initiate antioxidative defense system involves non-enzymatic components (including AsA, GSH, TP, tocopherols, Car) and antioxidants enzymes, like SOD, CAT, different peroxidases, and reductases [Das and Roychoudhury, 2014]. Changes in concentration or enzymatic activity of mentioned compounds as well as H_2O_2 and MDA contents were examined in the leaves and stems of pepper seedlings in present research.

In sweet pepper plants whose leaves were treated with TiO_2-NPs, MDA content notably increased in the leaves and slightly in the stems. Enhanced lipid peroxidation is associated with the imbalance between production and inactivation of ROS that is caused by various stresses (including those caused by metal-based NPs) [Yang et al. 2017], which means that the cell membranes of the leaves of sweet pepper plants were damage but not of the stems that can act as a translocation pathway or barrier between roots and leaves. Our findings are in similar line with those reported by Khan et al. [2019] in lentils after treatment with TiO_2--NPs, particularly at higher concentrations. However, our results revealed that H_2O_2 accumulation remained constant in treated and control sweet pepper plants, which suggests the involvement of other types of ROS than H_2O_2 in the generation of MDA. When nanoparticles were applied to the roots of seedlings, a slight increase in MDA content was observed in the leaves of treated plants, although this was not statistically significant. Conversely, MDA content in the stems of seedlings exposed to TiO_2-NPs decreased. Additionally, there was a rise in H_2O_2 concentration in the leaves of seedlings whose roots were subjected to TiO_2-NPs. This may be due to the fact that the amount of ROS transported from roots via stems or produced in stems of these plants was not sufficient to induce severe peroxidation of cell membrane lipids, as evidenced by small changes in MDA levels at the time of plant sampling. In contrast, seedlings exposed to NPs via roots, showed higher MDA formation in the stems.

Synthesis and accumulation of phenolics is induced by abiotic stress caused by nanoparticles [Chung et al. 2018]. In our previous study [Jurkow et al. 2020], the foliar application of SiO_2-NPs and TiO_2-NPs (all tested concentrations) and CeO_2-NPs and Fe_2O_3-NPs (the highest used concentrations) increased the total phenolics of oakleaf lettuce. Similarly, Ghorbanpour [2015] observed that TiO_2-NPs elevated the concentration of these compounds in common sage. In the present study, foliar spraying of the plants with TiO_2-NPs increased TP content in both leaves and stems of the seedlings. These results proved phenolics as effective components of antioxidant defense system, acting as chelators and scavenger of molecular species of active oxygen, protecting biological systems against the harmful effects of oxidative stress [Michalak 2006]. On the other hand, when nanoparticles were applied to the roots of sweet pepper seedlings, there were no changes in the TP content of the leaves and stems, which suggests induction of their synthesis in the tissues directly absorbing NPs. Moreover, the action of other antioxidants like GPOX and CAT as well as GSH took place in this case. This theory is supported by the higher total antioxidant capacity found in both leaves and stems of plants subjected to TiO_2-NPs via the roots.

It is known that GSH is oxidized by ROS and acts as a part of the antioxidant mechanisms that prevent excessive oxidation of cellular components [Noctor et al. 2012]. Ascorbic acid (AsA) is a universal non-enzymatic antioxidant that has the potential to scavenge ROS and modulate several functions in plants, especially under stress [Akram et al. 2017]. AsA is a reducing metabolite, neutralizing ROS and reducing molecules oxidized by ROS, being a significant antioxidant involved together with glutathione in the AsA-GSH pathway [Bilska et al. 2019, Yang et al., 2017]. In our previous study [Jurkow et al. 2020], we described an increase in the content of GSH in oakleaf lettuce subjected to various nanoparticles compared to the control. In the case of AsA, when TiO_2-NPs were applied, AsA concentration increased significantly, there was also an increase observed in some treatments with SiO_2-NPs and Fe_2O_3-NPs. A significant increase in GSH was also observed by Larue et al. [2014b] for lettuce exposed to TiO_2-NPs, while higher level of ascorbic acid in wheat treated with TiO_2-NPs was noted by Silva et al. [2019]. In our present study, an elevated level of GSH content in the leaves of sweet pepper supplied with TiO_2-NPs through the roots was observed.

However, the impact of TiO_2-NPs on plants after leaf spraying was not visible, with no changes in GSH level in the leaves and stems. Therefore, we assumed that GSH plays a crucial role in the tissues directly absorbing NPs. In the plants exposed to TiO_2-NPs via foliar application, a significantly higher concentration of AsA in the leaves of treated plants was observed when compared to the control. Although AsA belongs to a group of small molecules, it seems to play a crucial role in leaves as we did not observe any increase in AsA content in the stems of the plants. Although there are some evidences for inter-tissues transport of AsA [Zheng et al. 2022], it still requires more research to confirm such assumptions. Observed increase in the plants' leaves sprayed with TiO_2-NPs was correlated with a similar content of H_2O_2 in treated and control plants, pointing to the action of AsA in deactivating H_2O_2. In the plants subjected to nanoparticles via roots, AsA content remained unchanged, whilst GSH and H_2O_2 contents increased. This suggests that plants accumulated GSH to regenerate AsA that is a substrate in AsA-GSH cycle, used for detoxification of H_2O_2 in plants [Shan et al. 2018, Chumyam et al. 2017], but in this case detoxification has not yet occurred. These dependencies indicate that we observed different steps of Foyer-Halliwell-Asada pathway in the plants subjected to different strategies of TiO_2-NPs application (foliar or root route).

Carotenoids detoxify various ROS, they are also able to quench triplet chlorophylls being the major source of $^1O_2$ in plant leaves [Ramel et al. 2012]. Present study showed an increase in carotenoids in the stems of plants when TiO_2 nanoparticles were applied on their leaves. This observation was not confirmed in our previous research on oakleaf lettuce, where all tested concentrations of TiO_2-NPs showed a significant increase in the carotenoids content compared to the control [Jurkow et al. 2020]. In the literature there are data showing both the increase in the concentration of carotenoids after the application of TiO_2-NPs [Mohammadi et al. 2014] and the lack of their effective impact on the biosynthesis of these compounds [Larue et al. 2014b].

Several enzymes sustain redox homeostasis in plants under stress conditions by directly capturing specific ROS and ROS by-products [Gupta et al. 2018]. Among these enzymes, APX is one of the major peroxidases in plants with a role relying on reducing H_2O_2. APX works in conjunction with AsA as an electron donor and as a part of the AsA-GSH pathway [Ishikawa and Shigeoka 2008]. In our study, we observed a decrease in APX activity in the leaves of the plants sprayed with TiO_2-NPs and in the stems of the plants exposed to TiO_2-NPs via roots. This is similar to the data published by Servin et al. [2013] for cucumber treated with TiO_2-NPs, whose leaves were also characterized by lower activity of APX at concentrations of 500 mg L^{-1} and 700 mg L^{-1}, while meaningless difference in APX activity was found for lower concentrations than in control. In our experiment, the changes in APX activity seem to be related to CAT activity. It is known that CAT and APX are very important components of the antioxidant machinery that regulate H_2O_2 content [Sing et al. 2019]. Our study revealed higher activity of CAT in the leaves of foliar-sprayed plants and in the stems of the plants whose roots were placed in a TiO_2-NPs suspension. Servin et al. [2013] found that TiO_2-NPs can induce CAT activity in cucumber treated with nanoparticles concentration ranging from 250 to 750 mg L^{-1}. Lei et al. [2008] noted an increase in CAT activity in the chloroplasts of spinach plants subjected to TiO_2-NPs. However, in our previous study on oakleaf lettuce, we did not find a significant effect of TiO_2-NPs on CAT [Jurkow et al. 2020] but activity of GPOX increased in the plants sprayed with all tested concentrations of Fe_2O_3-NPs and with higher applied concentrations of TiO_2-NPs and SiO_2--NPs [Jurkow et al. 2020]. In the present study, GPOX activity also increased in sweet pepper plants due to TiO_2-NPs treatment. GPOX acts as an oxidizer of aromatic electron donors like guaiacol or pyragallol at the expense of H_2O_2. GPOX can therefore be a quencher of reactive intermediate forms of O_2 and peroxy radicals formed in plants under stress [Sharma et al. 2012, Vangronsveld and Clijsters 1994]. Our results proved the important role of GPOX in detoxification of reactive oxygen species – its activity increases in the leaves (foliar spraying of the plants) and in the leaves and stems (TiO_2-NPs via root exposure). The results of Lei et al. [2008] also showed that nano-anatase (TiO_2--NPs) treatment increased GPOX activity in spinach. Treatment of sweet pepper seedlings with TiO_2-NPs via roots did not cause any changes in chlorophyll pigments, while foliar spraying with nanoparticles elevated

the level of Chl *a*, but only in the stems of the plants. Mustafa et al. [2021] described an elevated content of Chl *a* and Chl *b* in wheat plants exposed to TiO_2-NPs at a concentration of 40 mg L^{-1}, but higher concentrations (60 and 80 mg L^{-1}) had a detrimental effect on chlorophylls, decreasing their content. The results suggest that the concentration of TiO_2-NPs was not high enough to cause the destruction of chlorophyll in sweet pepper plants. Data on fresh weight (no differences between treated plants and control) and dry weight (an increase under TiO_2-NPs treatment connected rather with slight dehydration) also pointed to a small to medium impact of the applied TiO_2-NPs.

As expected, treatment of the plants with TiO_2-NPs led to elevation in total Ti content in the leaves and in the stems when compared to the control. An increase in Ti content in plants subjected to TiO_2-NPs was also described by Jurkow et al. [2020] for oakleaf lettuce, and by Larue et al. [2014b] for lettuce. There was a very clear difference between the tested plant parts in the accumulation of titanium depending on the route of nanoparticles application. Foliar-sprayed plants showed the highest Ti concentration in the leaves, next in the stems, whereas a higher level of Ti in plants placed in NPs suspension was found in the stems, and next in the leaves of plants.

CONCLUSIONS

Our results indicated that the concentration of TiO_2--NPs was adequate to affect the metabolic pathways and antioxidant system of sweet pepper seedlings. This was manifested by changes in concentrations of non-enzymatic antioxidants and alterations in antioxidant enzymes activities. However, it did not cause visible phytotoxic effects on the plants, as evidenced by no significant effects of TiO_2-NPs on fresh weight and no decisive influence on photosynthetic pigments content. This work also provides new information about the specific metabolic response in different plant parts (leaves and stems) to TiO_2 nanoparticles applied via leaves and roots. Results of our study showed that nanoparticles applied to the plants caused oxidative stress and activation of antioxidant mechanisms. Because the stress stimuli were not lethal, thus repair and defense processes in the plant have been stimulated. Plants potentially become more tolerant of various stresses (cross-tolerance) that can occur in later stages of ontogenesis, which promotes harmonious growth and development, leading to higher productivity. Commercial fertilizers containing bulk Ti are used for improving crop production. It is very possible that they will be replaced by nanofertilizers containing this element. For that reason, we need to intensify investigations on the mechanisms underlying the beneficial or phytotoxic effects of TiO_2-NPs on the plants.

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CONFLICT OF INTEREST

No conflict of interest declared.

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DATA AVAILABILITY

The data supporting the findings of this study are available from the corresponding author, upon request.

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