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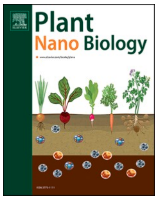

Larrea nitida extract-loaded nanodispersions as a novel bio-stimulant for tomato plants☆

Felipe Rocha [a], Laura Svetaz [b], Maximiliano Sortino [b], Valeria Alina Campos-Bermudez [a,*], Sebastián Pablo Rius [a,*]

[a] *Centro de Estudios Fotosintéticos y Bioquímicos (CEFOBI-CONICET), Universidad Nacional de Rosario, Suipacha 531, S2002LRK, Argentina*
[b] *Farmacognosia, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional del Rosario, Suipacha 531, Rosario CP 2000, Argentina*

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ABSTRACT

Larrea nitida Cav. (Zygophyllaceae) hydrophobic extract (LE) is rich in antimicrobial and antioxidant compounds. In our previous study, water-soluble nanodispersions without (PZ) and with LE (PZLE) were produced to facilitate their application in agriculture. The foliar treatment was carried out with water (control), PZ and PZLE thrice a week at concentrations of 33 and 100 $mg.L^{-1}$ on 1-week-old tomato seedlings (*Solanum lycopersicum* L. variety *perita platensis*). PZLE at a concentration of 100 $mg.L^{-1}$ significantly induced a growth promotion effect and decreased the activity of the antioxidant enzymes guaiacol peroxidase (GPx) and catalase (CAT), probably due to the presence of antioxidant compounds in PZLE. However, 5 days after infection with *Pseudomona syringae* pv. tomato, the activity of CAT increased by 73 % compared to the control, as a response to the stress caused by the infection. Additionally, plants treated with PZLE at 33 and 100 $mg.L^{-1}$ exhibited reductions in infection symptoms of 44 % and 76 %, respectively. As for both concentrations of PZ, no significant differences were found when compared to the control, suggesting a protective effect of PZLE. The photosynthetic parameters of the plants, such as Phi2 (φ_{PSII}), non-photochemical quenching (NPQ) and the relative chlorophyll in the leaves of these plants remained unaffected, suggesting no detrimental effects of PZLE on photosynthetic efficiency. PZLE demonstrates potential as a bio-stimulant for tomato plants, offering a complementary approach to traditional agricultural inputs.

1. Introduction

Bionanomaterials containing plant extract complexed with metallic materials for application in agriculture have been widely studied in recent years (Abdelkhalek and Al-Askar, 2020; Acharya et al., 2019; Singh et al., 2019). In fact, synthetic fertilizers can be an environmental issue, as well as the use of agrochemicals to treat pathogenicity in plants (Tudi et al., 2021). Nanomaterial-based bio-stimulants can be a promising alternative, due to the fact these materials can be produced at a relatively low cost, while mitigating environmental risks (Akhtar et al., 2022; Bairwa et al., 2023; Javed et al., 2022). However, the lack of well-established quality control for these products can be an issue, as well as the scarcity of studies regarding the safety of these materials (Nonget et al., 2022; Rai et al., 2023)

Even though the use of green synthesized nanoparticles has been evaluated in some studies with promising results in terms of mitigation of abiotic stress in plants (Alharbi et al., 2022; Mubashir et al., 2023; Pérez-Labrada et al., 2019), the foliar application of these materials as biostimulants to provide additional resistance against infection with phytopathogens is scarce in the literature. Since *Larrea nitida* extract (LE) contains antioxidant and antimicrobial compounds, such as polyphenols and organic acids, it is hypothesized that water-soluble LE-loaded nanodispersions can be a suitable bio-stimulant in contrast with the synthetic ones. In fact, polyphenol-rich extracts have been successfully used as a tool to promote a growth promotion effect and regulation of the immune system of plants by affecting the expression of genes related to biotic and abiotic stresses (Šamec et al., 2021; Singh et al., 2019). Moreover, the antioxidant properties of these compounds can neutralize some deleterious reactive species present in the tissue of plants, which can also play a role in the modulation of some antioxidant

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* Correspondence to: Universidad Nacional de Rosario, Suipacha 531, S2002LRK, Argentina.
E-mail addresses: campos@cefobi-conicet.gov.ar (V.A. Campos-Bermudez), rius@cefobi-conicet.gov.ar (S.P. Rius).

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enzymes, such as catalase (CAT) and guaiacol peroxidase (GPx) (Attia et al., 2023; Jang et al., 2020; Naqve et al., 2021). CAT is an enzyme that decomposes H_2O_2 into water and oxygen. Hydrogen peroxide is a reactive oxygen species produced during mitochondrial electron transport, photorespiratory oxidation, and oxidation of fatty acids (Gomes et al. 2022). GPx is an enzyme present in plants that is responsible for neutralizing free radicals by oxidation of electron donors, especially aromatic ones. For this purpose, H_2O_2 is used as an oxidant agent by the enzyme (Sharma et al. 2012). These enzymes play an important role in the defense mechanisms of plants (Ahmad et al. 2020; Erofeeva, 2015).

Pathogens can cause great losses in agriculture as they can significantly impair the quality and yield of crop production, as well as restriction to exports (Savary et al., 2019). The phytobacteria *Pseudomonas syringae* pv. tomato is a pathogen that infects tomato plants (*S. lycopersicum* L.) causing the bacterial speck, which is characterized by dark and brown lesions on the leaves, fruits, and stem. This disease, which is favored by the combination of low temperatures and high-moisture level conditions, is of concern in tomato crops, as it causes severe defoliation and underdevelopment of fruits (Wei and Collmer, 2018).

Therefore, this study aims to address these gaps by evaluating the efficacy of water-soluble LE-loaded nanoparticles as a bio-stimulant, focusing on their potential to enhance resistance against phytopathogens and as a growth promoter agent. Specifically, the impact of these nanoparticles on the infection resistance against the phytobacteria *P. syringae*, as well as on some antioxidant enzymes related to biotic and abiotic stresses in tomato plants (*S. lycopersicum* L. variety *perita platensis*) were evaluated in this work. The solid dispersion technique was used in our previous study (Rocha et al., 2023) to produce nanoparticles containing LE with polyethylene glycol (PEG) and zinc acetate with increased solubility in water to facilitate their use in agriculture.

2. Materials and methods

2.1. Materials

Blank (PZ) and LE-loaded nanoparticles (PZLE) were produced and characterized by Rocha et al. (2023). PZ are nanodispersions produced with PEG and zinc acetate in equal proportions (w/w), whereas PZLE contains 9.1 % (w/w) of LE complexed with a mixture of equal proportions of PEG and zinc acetate (w/w). Importantly, it is reported that the antimicrobial properties typical of LE were maintained after its transformation into nanoparticles.

Tris buffer and buffer potassium phosphate (PE) pH 7.2 (Merck, Germany), 10 % H_2O_2 and guaiacol (Cicarelli, Argentina) were used to evaluate the enzymatic activity of CAT and GPx. Hydrochloric acid (HCl) 0.1 M and sodium hydroxide (NaOH) 0.1 M (Cicarelli, Argentina) were used to adjust the pH of the solutions.

P. syringae pv. *tomato* DC3000 strain was obtained from the sample collection of the Center of Photosynthetic and Biochemical Studies (CEFOBI) at the Universidad Nacional de Rosario. 100 mL of LB medium was prepared by adding 1 g of triptein, 0.5 g of sodium chloride and 0.5 g of yeast extract (Cicarelli, Argentina) to 100 mL of distilled water. Rifampin (PhytoTech, USA) were diluted in Dimethyl sulfoxide (DMSO, Merck, Germany), and kanamycin (Sigma-Aldrich, USA) was diluted in distilled water. Both antibiotics were added to the LB medium to grow *P. syringae* pv. tomato culture. Silwet L-77 (Loveland Industries, Inc., Greeley, CO) was used as a surfactant for the following experiments.

2.2. Greenhouse conditions

Tomato plants (*S. lycopersicum* L. variety *perita platensis*) were grown in greenhouse conditions with supplemental visible lighting to 100 $\mu mol.m^{-2}.s^{-1}$, with a cycle of 16 hours of light and 8 hours of dark a day at a temperature of approximately 24 ºC. The plants were cultivated in soil with pH ranging from 5 to 5.8, humidity of approximately 60 %, nitrate (250–450 ppm), phosphate (30–100 ppm), and potassium (200 – 300 ppm), with ashes content varying from 15 % to 20 %. All treatments were watered with the same amount of water every 48 hours.

2.3. Foliar treatment

The solutions containing 33 and 100 $mg.L^{-1}$ of PZ (PZ 33 and PZ 100) and PZLE (PZLE 33 and PZLE 100), respectively, were ultrasonicated in an ultrasound (Elma, Transsonic TI-H-10, Germany) with a frequency of 25 kHz for 20 min at room temperature (25 ºC), and then homogenized under magnetic stirring for 15 min. 1-week-old tomato seedlings were foliar treated thrice a week with a spray containing water (control), PZ, and PZLE solutions with the addition of the surfactant Silwet 0.005 % (v/v) for all treatments, based on the methodology proposed by Ghani et al. (2022). Each seedling was treated with approximately 0.33 mL of the solution containing the nanoparticles.

2.4. Growth promotion effect and photosynthetic parameters

The growth promotion and photosynthetic parameters were evaluated following the methodology described by Wang et al. (2018) and Azam et al. (2022), with some modifications. To evaluate the effects of the application of the nanoparticles on growth promotion of tomato plants, 3-week-old plants ($n = 6$ for each treatment) were weighed immediately after harvest for determination of the shoot fresh weight. After that, the plants were dried in an oven at 80 ºC for 2 days for determination of the shoot dry weight.

For the photosynthetic parameters, 8 leaves of 3-week-old plants were used to determine some photosynthetic parameters, such as Phi2 (φ_{PSII}), non-photochemical quenching (NPQ), and the relative chlorophyll. The parameters were determined with a MultispeQ device v2.0 and the measurements were uploaded to the PhotosynQ platform (http://www.photosynq.org).

The evaluation of growth promotion effect and photosynthetic parameters were determined in quadruplicate and the results were expressed in percentage (%) relative to the control with water with Silwet 0.005 % (v/v).

2.5. Evaluation of the symptoms after infection with P. syringae pv. tomato

The in vitro infection with the phytobacteria *P. syringae* pv. tomato and evaluation of the progression of the symptoms were carried out according to the methodology proposed by Makovitzki et al. (2007), with some modifications. The third and fourth leaves of four 3-week-old tomato plants of each treatment (control, PZ 33, PZ 100, PZLE 33, and PZLE 100) were detached, sterilized for 1 minute by immersion in ethanol 70 % and washed with sterile distilled water. After that, the leaves were infected with 5 µL of the culture added with Silwet 0.005 % (v/v), to facilitate the penetration of the bacteria into the leaf, while placed in humidified glass recipients. Then, the glass recipients were sealed and placed in greenhouse conditions, while the symptoms were evaluated for 4 days. *P. syringae* culture was grown in LB medium added with 0.1 % rifampin (50 $mg.mL^{-1}$) and 0.1 % kanamycin (50 $mg.mL^{-1}$) (v/v) until reaching an optical density of 2.0 ($OD_{630} = 2.0$) at 28 ºC and constant agitation of 180 rpm.

After the infection, the symptoms were evaluated for 4 days both visually and with the free software ImageJ (http://imagej.nih.gov/ij), as proposed in this work carried out by Agostini et al. (2021). The leaf area damage was calculated for all the treatments and expressed in percentage (%) 4 days after infection in plants of each treatment.

2.6. *Effects of the foliar treatment on the modulation of the enzymatic activity of CAT and GPx*

The third and fourth leaves of four 3-week-old tomato plants of each treatment were detached and the fresh tissue (200 mg) was immersed in 600 µL of Tris-HCl 0.1 M (pH 6.8) and ground in a pre-chilled pestle and mortar placed on an ice bath. After that, the samples were centrifuged at 12,000 g for 20 minutes at 4 °C, and the supernatant was collected for the evaluation of the enzymatic activity (Sadati et al., 2022).

For the evaluation of the modulation of CAT activity, the analyses were carried out according to the methodology proposed by Rocha et al. (2018), with some modifications. 2.5 µL of leaf extract and 2.5 µL of H_2O_2 10 % were added to 295 µL of buffer Tris-HCl (pH 6.8), and the activity of the enzyme was evaluated by determining the decomposition of H_2O_2 at 240 nm in an UV-Vis spectrophotometer (JASCO, Japan) for 3 minutes. The enzymatic activity was calculated using a molar extinction coefficient for H_2O_2 of 39.4 $M^{-1}.cm^{-1}$.

The activity of GPx was evaluated according to the methodology proposed by Erofeeva (2015), with some modifications. 0.2 % guaiacol (v/v) was added to PE for the enzymatic analysis. After that, 2.5 µL of the leaf extract and 2.5 µL of H_2O_2 10 % were added to 295 µL of the mixture guaiacol:PE, and the activity of the enzyme was measured by measuring the oxidation of guaiacol at 470 nm in a UV-Vis spectrophotometer (JASCO, Japan) for 3 minutes. A molar extinction coefficient of 26.6 $M^{-1}.cm^{-1}$ was used to calculate the activity of the enzyme.

The modulation of the activity of CAT and GPx was evaluated in 3-week-old plants before and 5 days after infection with *P. syringae* pv. tomato. The amount of protein in the samples was determined according to the methodology proposed by Bradford (1976), and the enzymatic activity was determined in $\Delta E.min^{-1}.mg^{-1}$of protein, where ΔE is the absorbance variation. The experiments were carried out in quadruplicate and expressed in percentage (%) relative to the control with water added with the surfactant Silwet 0.005 % (v/v).

2.7. *Statistical analysis*

All the experiments were performed in triplicate. ANOVA one-way using $p < 0.05$ was done with the software R version 4.2.0 (v. 4.2.0; R Core Team, 2022). When appropriate, the significant differences between the means of the treatments were determined by Tukey's test at a level of significance of 5 %.

3. Results

3.1. *Growth promotion effect and photosynthetic parameters*

Plants treated with PZLE 100 presented higher shoot fresh and dry weights (53 and 44 % higher), respectively, compared to the control and other treatments ($p < 0.05$) (Figs. 1 and 2). For the plants treated with PZ 100, the shoot fresh weight was significantly decreased (26 %) compared to the control.

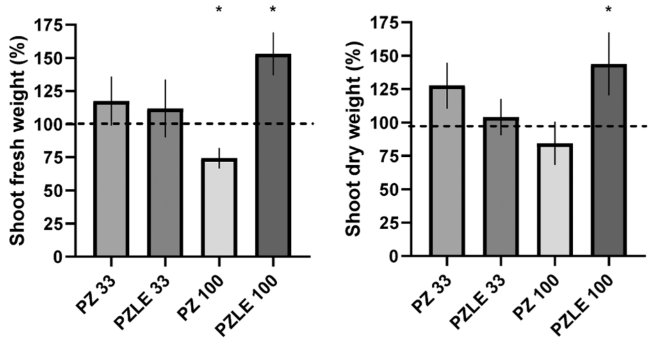

Fig. 1. – Shoot fresh and dry weight of 3-week-old tomato plants (%) treated with PZ and PZLE at concentrations of 33 and 100 $mg.L^{-1}$ relative to the control with water. Values are mean ± SD (n = 6 per group). Significance was determined by one-way analysis of variance (ANOVA) followed by Tukey's test. *$p < 0.05$.

The photosynthetic parameters, such as Phi2 (φ_{PSII}), non-photochemical quenching (NPQ), and the relative chlorophyll were not affected by treatment with the nanoparticles when compared to the control. φ_{PSII} is related to the photosystem II electron transport efficiency, which measures the incoming light that is used for photosynthesis. NPQ reflects the amount of incoming light that is not used for photosynthetic processes, in order to dissipate the excess of energy, thus avoiding damage to the plant (Bussotti et al. 2020; Jang et al. 2020).

The results are in accordance with data available in the literature. In this study carried out by Wang et al. (2018), one moistening treatment of 3-week-old tomato seedlings (*Solanum lycopersicum* L.) with chemically synthesized ZnO NPs up to a concentration of 200 $mg.L^{-1}$ in water did not affect the photosynthetic parameters nor the relative chlorophyll compared to plants treated only with water (Fig. 3).

3.2. *Effects of the foliar treatment on the modulation of the enzymatic activity of CAT and GPx*

Before infection with the pathogen *P. syringae* pv. tomato, the plants treated with blank nanodispersions (PZ 33 and 100) presented a significant increase in the activity of both GPx and CAT, in contrast with a significant decrease observed for PZLE 100 when compared to the control (Fig. 4).

The enzymatic activity of CAT 5 days after infection with *P. syringae* pv. tomato significantly decreased for PZLE 33, following the same behavior observed for PZ 33, where a decreasing trend is also observed when compared to the control.

Regarding the activity of GPx after infection, except for PZ 100, no significant differences were found for the other treatments when compared to the control. The increase in the activity of this enzyme can be attributed to increased ROS in the plant tissues because of the treatment with PZ at higher concentrations, in addition to the oxidative stress caused by the pathogenicity (Al-Qurainy, 2021; Xie et al., 2019).

3.3. *Evaluation of the symptoms after infection P. syringae pv. tomato*

It is possible to observe a significant protective effect of the foliar treatment PZLE at both concentrations (33 and 100 $mg.L^{-1}$) according to the progression of the symptoms on leaves of tomato plants 4 days after infection with the phytobacteria *P. syringae* pv. tomato (Figs. 5 and 6), especially when compared to PZ and the control, meaning that the presence of LE in the nanoparticles played a role as a bio-stimulant in these plants.

4. Discussion

PZ and PZLE nanoparticles used in this study were produced and characterized in our previous study (Rocha et al., 2023). The characterization of both PZ and PZLE shows a significant presence of zinc oxide nanoparticles (ZnO NPs), PEG, and LE, meaning that both PZ and PZLE can act as analogs to ZnO NPs.

The effects of the nanoparticles on the growth promotion of tomato plants can be explained by the presence of LE, which is an extract rich in antioxidant and phenolic compounds (Moreno et al., 2020a, 2020b). In addition to that, the increased solubility in water and a higher surface area of contact, PZLE 100 provided a significant improvement regarding growth parameters. Similar results regarding the use of plant extracts as a growth promotion agent have been reported in the literature. For example, Singh et al. (2019) also observed this increase in the shoot and root lengths of wheat seedlings treated with chemically and green synthesized ZnO NPs with *Aloe barbadensis Mill* extract at a concentration of 0–500 $mg.L^{-1}$. According to the authors, the green synthesized

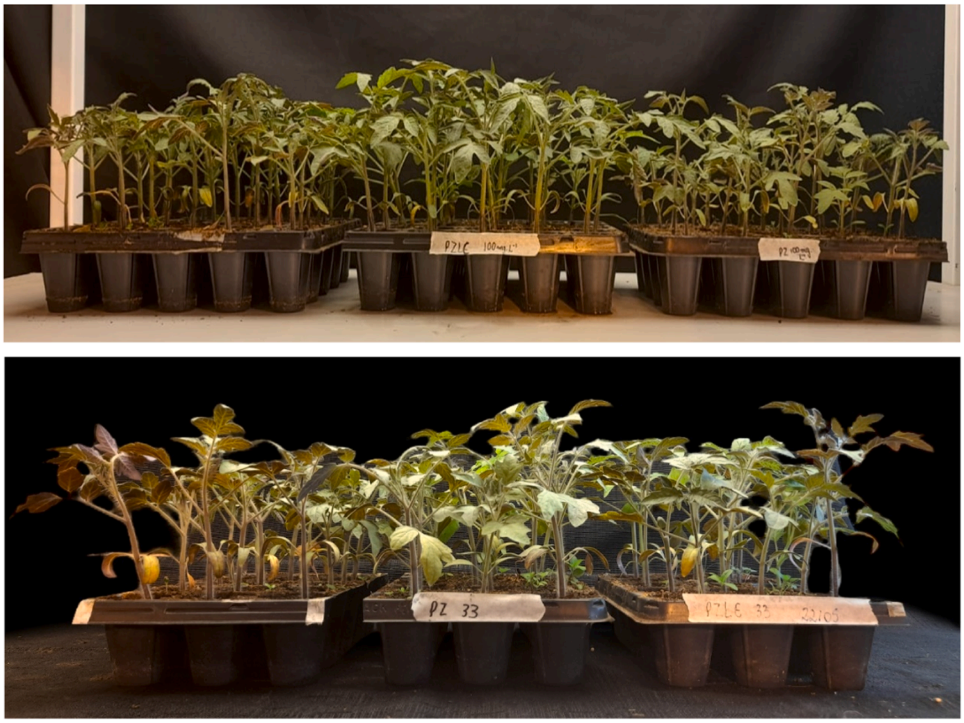

Fig. 2. – 3-week-old tomato plants treated with the control with water, PZ 33, and PZLE 33 from left to right, respectively (top), and 3-week-old tomato plants treated with the control with water, PZLE 100 and PZ 100 from left to right, respectively (bottom).

nanoparticles presented a better performance regarding the growth promotion effect on these plants at a concentration of 62 $mg.L^{-1}$. Similarly, Jang et al. (2020) reported a growth promotion effect on germinated seedlings cultivated in soil with different plant extracts (soybean leaf, soybean stem, Chinese chive, onion, and tomato). These plants presented 16–36 % higher fresh shoot weight compared to the control. The results indicate that the presence of polyphenols and the antioxidant activity of the extract present in the nanoparticles can play a significant role in the growth promotion of plants even at low concentrations. On the other hand, plants treated with PZ 100 presented a decrease in the fresh and dry shoot weight. This behavior can be explained by the fact that ZnO NPs at higher concentrations can contribute to an increase of reactive oxygen species (ROS) in plants, playing a role in their oxidative stress. This increase in ROS can be attributed to the fraction of bioavailable zinc that can accumulate in the plant tissue, causing toxicity, which might have affected the growth promotion of these plants (Krishnamurthy and Rathinasabapathi, 2013; Stanton et al. 2019). When foliar applied to plants, ZnO NPs can interact with the cuticle, where environmental factors like pH and temperature influence the release of zinc ions. Studies suggest that an acidic or neutral pH significantly contributes to release zinc ions, allowing them to diffuse into the plant tissues. The cuticle's structure, along with the stomatal pores, can also influence how much of the zinc is absorbed, in the sense that if the release rate is too high, it may lead to phytotoxicity, especially in sensitive plant species (Strekalovskaya et al., 2024; Wang et al. 2023). In this study carried out by Javed et al. (2022), the authors observed the same behavior for mung bean seeds treated with chemical and biologically synthesized ZnO NPs for seeds primed with a solution containing 5 $mg.mL^{-1}$ of NPs. According to the authors, biologically synthesized ZnO NPs with plant extract presented a better performance when compared to the control and chemically synthesized ZnO NPs in terms of growth promotion. Importantly, the authors reported that chemically synthesized ZnO NPs negatively affected the growth of these plants. Even though PEG can act as an osmotic agent when applied to plants, studies in the literature show that at low concentrations, this polymer is not sufficient to cause toxicity. In fact, the threshold concentration of PEG for simulating drought stress is usually around 1–5 % (w/v) at a low dose (Ayaz et al. 2015; Zhang and Shi, 2018; Hernández-Pérez et al., 2021). Taking into consideration that the nanoparticles were diluted to a final concentration of 33 and 100 $mg.mL^{-1}$, the concentration of PEG is less than 0.05 % (w/v), which strongly suggests that the oxidative stress was mainly caused by the accumulation of zinc ions.

Regarding the photosynthetic parameters Phi2 (ΦPSII), non-photochemical quenching (NPQ), and relative chlorophyll content remained unchanged across all treatments, indicating that PZ and PZLE do not adversely affect photosynthesis.

In terms of modulation of enzymatic activity, the increased activity of the antioxidant enzymes for the plants treated with PZ 33, PZLE 33 and PZ 100 can be related to the presence of ROS, further contributing to the increased activity of these antioxidant enzymes. As for the plants treated with PZLE 100, it was possible to observe a significant decrease in the activity of these enzymes, which can be related to the presence of antioxidant compounds in LE in higher concentrations, further contributing to the neutralization of ROS (He et al., 2017). In this study carried out by Ahmad et al. (2020), 39-day-old soybean plants stressed with arsenic were foliar treated with ZnO NPs at concentrations of 50 and 100 $mg.L^{-1}$ every alternate day for 2 weeks. These plants had the activities of CAT further enhanced by the treatment with the nanoparticles. Ghani et al. (2022) reported increased activity of CAT and GPx compared to the control of 34-day-old cucumber seedlings (*Cucumis sativus* L.) treated with a foliar treatment of ZnO NPs at a concentration of 25 and 100 $mg.L^{-1}$ thrice a week for 2 consecutive weeks. Importantly, ZnO NPs at higher concentrations can be used to alleviate the stress caused by the presence of osmotic agents in plants by regulating the activity of some antioxidant enzymes (Ahmed et al. 2023; Sadati et al. 2022), which might explain the difference in the activity of GPx between PZ 33 and PZ 100 ($p < 0.05$). Even though the accumulation of zinc in high concentrations can contribute to an increase in ROS in plant tissues, ZnO NPs have been widely used to mitigate biotic and abiotic stresses by regulating the expressions of some genes related to the defense system in plants (Daniel et al. 2023; Hezaveh et al. 2019). In fact, zinc is considered a very important micronutrient in plants as it is a

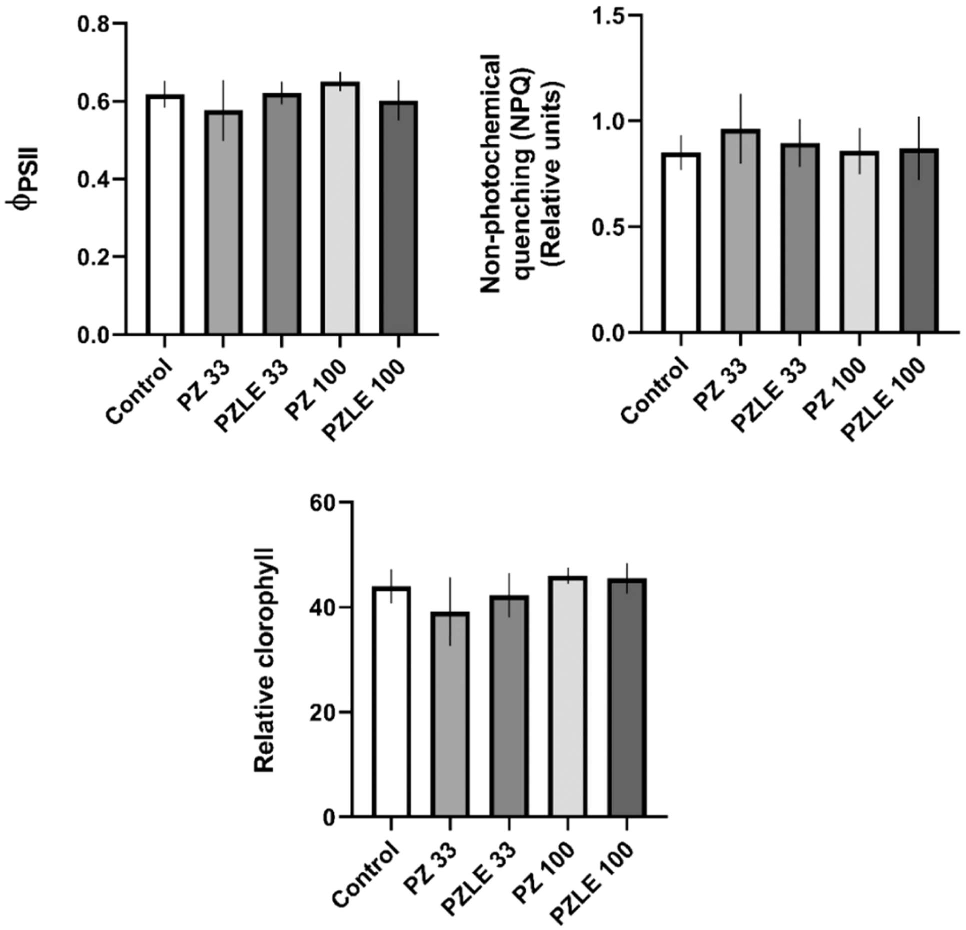

Fig. 3. – φ_2, NPQ, and relative chlorophyll of 3-week-old plants treated with water (control), PZ and PZLE (33 and 100 $mg.L^{-1}$). Values are mean ± SD (n = 8 per group).

constituent of many proteins and enzymes, being essential in the regulation of these elements and, thus, in many metabolic reactions (Saleem et al. 2022). Importantly, CAT and GPx are important enzymes that play a role against biotic (i.e., pathogenicity) and abiotic stress (i.e., radiation, lack of nutrients, salinity, and temperature disturbance). These enzymes neutralize deleterious reactive species that can induce reactions, affecting some processes in plants, such as homeostasis and ion transportation in plant cells, leading to the accumulation of reactive species that can impair cell organelles, membranes, and functions (Sofo et al. 2015, Yemelyanov et al. 2023).

In terms of biotic stress resistance, PZLE demonstrated notable efficacy against *P. syringae* pv. tomato. Plants treated with PZLE at 33 and 100 $mg.L^{-1}$ exhibited a reduction of 44 and 76 %, respectively, in the infection symptoms 5 days post-infection compared to the control. Regarding the activity of GPx after infection, it was possible to observe a significant decrease in GPx activity for PZLE 33, following the same trend observed for PZ 33 when compared to the control. This behavior can be explained by the lower concentration of these particles, which consequently contributes to producing less ROS. Moreover, polyphenols and antioxidant compounds present in LE might have been absorbed by the plants through the stomata or the cuticle present on the surface of the leaves, further contributing to the neutralization of some reactive species produced during the infection (Wang et al. 2023). On the other hand, the activity of CAT was significantly increased for the plants treated with PZ and PZLE at a concentration of 100 $mg.L^{-1}$. Importantly, the presence of LE in the nanodispersions further enhanced the activity of CAT when compared to PZ 100 ($p < 0.05$). Sadati et al. (2022) reported that plants foliar treated once with 1 $mg.L^{-1}$ of ZnO NPs and submitted to drought stress had the catalase gene CAT1 significantly up-regulated. The results suggest that the combination of the treatment with PZ and PZLE at higher concentrations with the infection with *P. syringae* might have played a role in the expression of CAT to mitigate the oxidative stress caused by the infection. Similar results regarding the infection resistance against phytopathogens in plants as a result of foliar application with nanoparticles are reported in the literature. In this study carried out by Elmer et al. (2021), the foliar treatment with chemically synthesized ZnO NPs (500 $mg.L^{-1}$) did not suppress fusarium wilt development in Chrysanthemum plants, in contrast with a significant effect observed for the plants treated with of copper oxide nanoparticles at the same concentration. The improved resistance against *P. syringae* observed with PZLE treatments suggest that LE may enhance plant immune responses, as no difference were seen with PZ. In fact, in this work carried out by Aina et al. (2022), the authors reported that phenolic compounds from seaweed, for example, present great potential to be used as bio-stimulants in plants as they promote resilience against various stress conditions.

Data in the literature regarding the effects of foliar treatment with green synthesized nanoparticles on antioxidant enzymes after induced pathogenicity in plants is scarce in the literature. However, similar results regarding the modulation of the activity of some antioxidant enzymes of plants submitted to conditions of stress, such as drought and the presence of toxic compounds, have been recently reported in the literature as a consequence of the treatment with ZnO NPs. According to these studies, the increase in the activity of some antioxidant enzymes in treated plants under conditions of stress is indicative of an attempt to mitigate these effects, as these enzymes are responsible for neutralizing

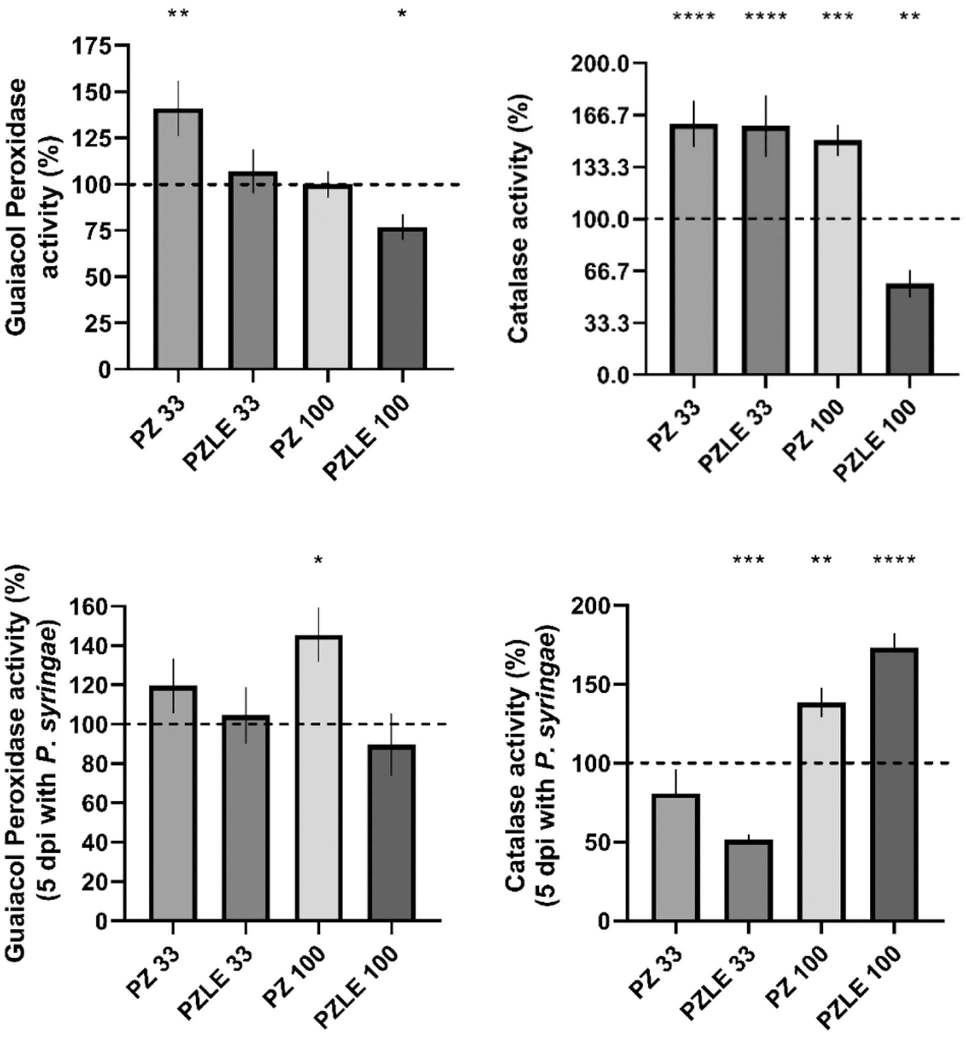

Fig. 4. – Enzymatic activity of GPx and CAT of leaf tissue of 3-week-old tomato plants treated with PZ and PZLE (33 and 100 $mg.L^{-1}$) compared to the control with water before (top) and 5 days after in vitro infection with *P. syringae* pv. tomato (bottom). Values are mean ± SD (n = 4 per group). Significance was determined by one-way analysis of variance (ANOVA) followed by Tukey's test. *p < 0.05; * *p < 0.01; * **p < 0.001; * ** *p < 0.0001.

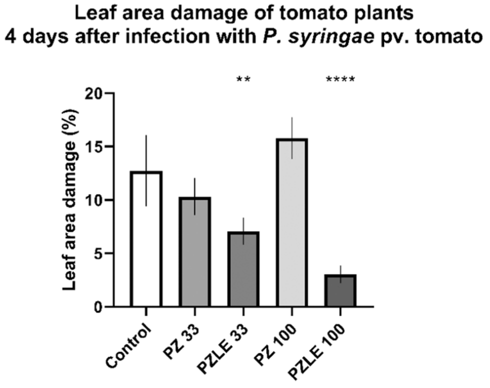

Fig. 5. – Leaf area damage 4 days after infection with *P. syringae* pv. tomato, expressed in percentage (%) relative to the total area of the leaf. Values are mean ± SD (n = 4 per group). Significance was determined by one-way analysis of variance (ANOVA) followed by Tukey's test. * *p < 0.01; * ** *p < 0.0001.

deleterious reactive species (Ahmad et al., 2020; Azim et al., 2022).

Fig. 6. Leaves of 3-week-old tomato plants treated with water (control), PZ 33, and PZLE 33 from left to right (top), and 3-week-old plants treated with water (control), PZ 100 and PZLE 100 from left to right (bottom), respectively, 4 days after infection with *P. syringae* pv. tomato.

5. Conclusions

The results suggest that the presence of LE in the nanodispersions contributed to the growth promotion of these plants, which can be also attributed to the increased surface area of contact of nanoparticles, assisting the bio-stimulation due to the presence of polyphenols and other antioxidant compounds.

PZLE significantly modulated the activity of some enzymes related to oxidative stress and present great potential to be applied in agriculture as a bio-stimulant due to the extra resistance provided against *P. syringae* pv. tomato, which is a pathogen of interest in tomato plants.

CRediT authorship contribution statement

Laura Svetaz: Resources, Methodology. **Maximiliano Sortino:** Resources, Methodology. **Valeria Alina Campos Bermudez:** Writing – review & editing, Resources, Project administration, Methodology, Conceptualization. **Sebastián Pablo Rius:** Writing – review & editing, Resources, Project administration, Methodology, Conceptualization. **Felipe Rocha:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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