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Research article

Impact of Green Synthesis of Silver Nanoparticles on Antioxidant Activity in Drought-Sensitive and Drought-Tolerant *Pimpinella anisum* L.

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ABSTRACT
The rapid advancement of nanotechnology has raised concerns about nanoparticles (NPs) potentially entering the environment, particularly their effects on plants, which are vital to ecosystems. This study explores the effects of green-synthesized silver nanoparticles (AgNPs) on three important antioxidant enzymes: catalase (CAT), superoxide dismutase (SOD), and guaiacol peroxidase (GPOx). We examined two varieties of anise (*Pimpinella anisum* L.), specifically the drought-sensitive (DS) and drought-tolerant (DT) varieties. The objective was to assess how these two types of anise respond to stress caused by silver nanoparticles. Anise plants were exposed to silver nanoparticles (AgNP) at 1, 5, and 10 mg/L concentrations for 21 and 28 days. The activity of catalase (CAT) did not show significant changes across all concentrations and remained inactive. Only a significant increase in CAT activity was observed in the plants treated with 5 mg/L AgNPs after 28 days. Superoxide dismutase (SOD) activity significantly increased in the treated plants exposed to 5 mg/L AgNPs after 21 days. For guaiacol peroxidase (GPOx), the treated plants showed significant increases at both 1 mg/L and 5 mg/L AgNPs after 21 days, while other results varied and lacked statistical significance. Overall, the increased enzyme activity in DT anise at low AgNP concentrations suggests a low level of toxicity and indicates that these plants are more resilient to AgNP stress. These findings highlight the complexity of AgNP effects on antioxidant activity in anise and underline the need for further research on the long-term impacts of AgNP exposure, particularly concerning the preservation of drought-tolerant anise in the face of global warming

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Introduction

Nanoparticles have a long history, with Michael Faraday describing the optical properties of metallic nanoparticles in 1857 [1]. While the global nanotechnology market had a market share of 79.14 billion US dollars in 2023, the market is expected to be worth 91.18 billion US dollars in 2024 [2]. More than 1,000 commercial products on the market currently contain nanoparticles (NPs). Common NPs used in household, industrial, and healthcare products include Au (gold), Ag (silver), ZnO (zinc oxide), CuO (copper oxide), TiO2 (titanium dioxide), Fe3O4/Fe2O3 (iron oxides), and CeO2 (cerium oxide)[3]. In addition, incorporating Ag, ZnO, TiO, and SiO (silicon dioxide) NPs into agrochemicals has great potential in nanotechnology-based smart agriculture [4]. With the expansion of nanotechnology applications in various sectors, the possibility of NPs entering the environment as waste-containing nanomaterials increases, necessitating research on plant responses to NPs [5]. Silver nanoparticles have gained attention for their unique properties and diverse applications, including medicine and environmental therapy [6]. These nanoparticles exhibit antimicrobial properties, which makes them effective against microorganisms and have potential in drug delivery systems [7]. The exploration and understanding of metallic and natural nanoparticles have contributed to various fields, including nanotechnology and the use of plants such as *Pimpinella anisum* for their valuable properties [8]. *Pimpinella anisum* L.(anise), a member of the Apiaceae family, is a versatile aromatic plant with numerous historical uses. Its dried fruits, known as anise seeds, are popular as flavouring agents in confectionery and liqueurs [9]. Anise has been traditionally used to treat digestive disorders and possesses potential antimicrobial, antioxidant, and anti-inflammatory effects [10]. It

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exhibits analgesic, antispasmodic, anticonvulsant, and potential antidepressant-like activities [11]. Chemical analysis reveals bioactive compounds like anethole with antimicrobial and antioxidant activity [12]. Anise seed extract has been shown to have cytotoxic effects on cancer cells, like the chemotherapy drug cisplatin, and may have potential applications in nanotechnology. Despite their essential applications, nanoparticles are still being debated regarding their beneficial effects on plants on the one hand and their harmful and toxic effects on the other [13]. AgNPs inhibited Ribulose-1,5-bisphosphate carboxylase/oxygenase activity (Rubisco) and the protective ability of PSII in the higher aquatic plant model *Spirodela polyrhiza* L. [14]. In addition to lower photosynthetic rates, growth inhibition induced by NPs has also been associated with increased oxidative stress [15] However, whether the arrest of photosynthesis or the induction of oxidative stress is the dominant effect of NPs is a matter of debate, as they go hand in hand Although the accumulation of NPs in chloroplasts and damage to the photosynthetic apparatus supports the former [16], the fact that to reach chloroplasts, they must cross the plasma membrane, where they can catalyze reactive oxygen species (ROS) via NADPH oxidases [17]. ROS production, membrane structure and function damage, and antioxidant enzymatic activity fluctuations across plant species have been documented as typical responses to NPs [18]. However, some studies also showed that the treatment plants and photosynthetic microorganisms with NPs increased phenols' production, which may function as antioxidants to suppress ROS [19]. Because of the toxicity associated with nanoparticles remains a concern in terms of their effect on cellular enzymes such as superoxide dismutase, catalase, and Guaiacol peroxidase, which is critical [20] because these enzymes are essential for cellular defense against oxidative stress [21]. They act as antioxidants, preventing the formation of harmful free radicals and reactive species in cells [22]. Several toxicological studies have been performed to measure their levels [22]. Examining the functions and mechanisms for analyzing the effects of AgNPs (Silver Nanoparticles) on the enzymes CAT (Catalase), SOD (Superoxide Dismutase), and GPOx (Glycol Peroxidase) [23]. Overall, CAT, SOD, and GPOx are vital enzymes in the plant's antioxidant defense system [24]. When studying the effects of AgNPs on these enzymes, it is essential to assess how their activity and expression may be altered, as any disruptions to their functions could have significant implications for the plant's overall health and stress response [25]. Detoxification mechanisms to eliminate the toxic effects of AgNPs may differ from plant to plant. In this case, it is not easy to about conclude how different detoxification pathways are activated in response to different AgNPs conditions in different plant species [26]. These different results vary depending on the size, shape, exposure concentration, and amount of aggregation of AgNPs. Therefore, the physical and chemical properties of AgNPs and their effects on plant morphology, physiology, and biochemistry vary greatly depending on the plant type or variety [27]. Considering that the most important factors affecting the penetration of NPs into cells in the literature review are Np type, amount, and plant type, studying the effect of NP is essential for scientific studies [28]. In addition to all this information, the nanoparticle synthesis method is crucial in scientific studies [29]. Chemical methods used for silver nanoparticle synthesis can often lead to toxic solvents, high energy consumption, and the production of harmful by-products. It is important to use environmentally friendly methods for synthesizing silver nanoparticles (AgNPs) [30]. In our study, we synthesized AgNPs that are expected to have a circular shape and dimensions ranging from 10 to 100 nm, utilizing an environmentally friendly green synthesis method [31]. In the synthesis, we used *S. sclaera* leaves [32]. Whose optimization we know well. Anise is a highly valuable medical ingredient plant, and silver nanoparticles have shown promising potential in many applications. The study's results can advance our understanding of the effects of AgNPs on plant growth and their potential applications in agriculture and medicine. Our objective is to synthesize silver nanoparticles (AgNPs) and apply the synthesized product to calluses, followed by an evaluation of the antioxidant activity of these calluses. This research will investigate, for the first time, the effect of AgNPs synthesized from *Salvia sclarea* on the toxicity of calluses in *P. anisum* L. The summary of history is illustrated in Figure 1.

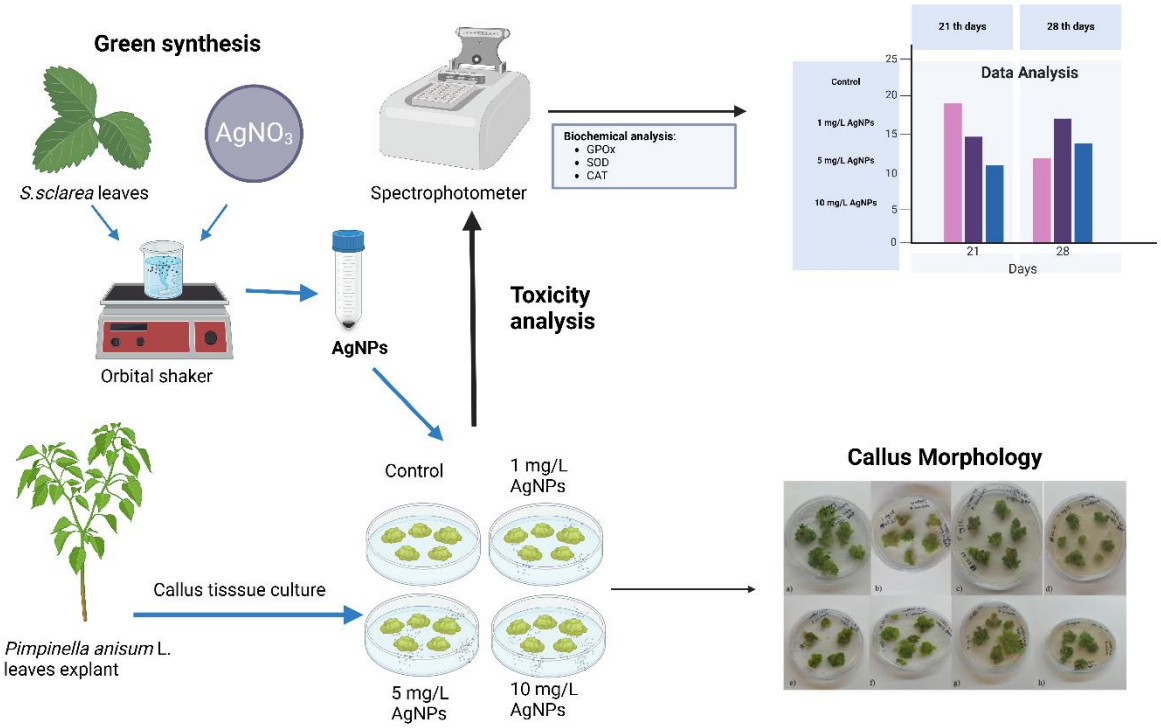

Fig.1 The flowchart summarizing the production of AgNPs through green synthesis, callus acquisition, and analysis is presented above. Created in BioRender. Ulusoy, E. (2025) https://BioRender.com/q83l859.

Material and Methods

Synthesis and Characterization of AgNPs.

Green Synthesis of AgNPs. Synthesis of Silver Nanoparticles (AgNPs) Using *Salvia* Leaf Extract: *Salvia* leaf extract was prepared by boiling 10 g of leaves in distilled water. (1 g:10ml) for 30 min and then filtering the mixture. The filtered extract was then added to 1M AgNO3 solution at a ratio of 1:9. The mixture was heated to 50˚ in a water bath and stirred continuously overnight for incubation. The solution was covered to prevent evaporation. The formation of AgNPs was indicated by the yellow to deep black of the solution. The obtained nanoparticles were precipitated by centrifugation at 10,000 rcf for 1 h. The preparation is illustrated in Figure 2.

Characterization of nanoparticles using scanning electron microscopy (SEM) and zeta potential measurements. The absorption spectra of the AgNP solution were recorded using a ThermoScientific-Genesys 180 UV-Vis spectrophotometer over a range of 300 to 600 nm, along with Zeta size measurements. Beginning with SEM, we deposited a colloidal silver nanoparticle solution onto a gold-coated substrate, allowing us to visualize particle morphology, size distribution, and surface features in detail (Malvern Zetasizer-NanoS, Cambridge, England). Concurrently, Zeta measurements and information on particle surface charge and stability were obtained by consulting the literature [33].

Pimpinella anisum Callus Production:

Seed Sterilization. The anise seeds used in this study were collected in 2022 from plants cultivated in the natural habitats near Kozagac village (37°05’84.0"N, 29°65’97.0” E, 15902 Cavdır, Burdur, Turkey). The seeds were provided by Durmuş EFE, a local breeder, who supplied two varieties: drought-sensitive (DS) and drought-tolerant (DT), the latter of which requires minimal irrigation for growth. Both DS and DT seeds were thoughtfully chosen to compare and understand a range of potential responses. *P. anisum* seeds were sterilized using a 25% bleach solution. A sterilization solution was prepared by mixing 50 ml of commercial bleach with 150 ml of distilled water. The seeds were subsequently submerged in the prepared bleach solution within the confines of a controlled laminar flow hood environment. Following this exposure, the seeds underwent a meticulous sterilization process through a sequence of three consecutive rinses utilizing sterile distilled water. Each rinse cycle was extended to a duration of 10 minutes, making sure that this rigorous rinsing protocol was executed meticulously [34].

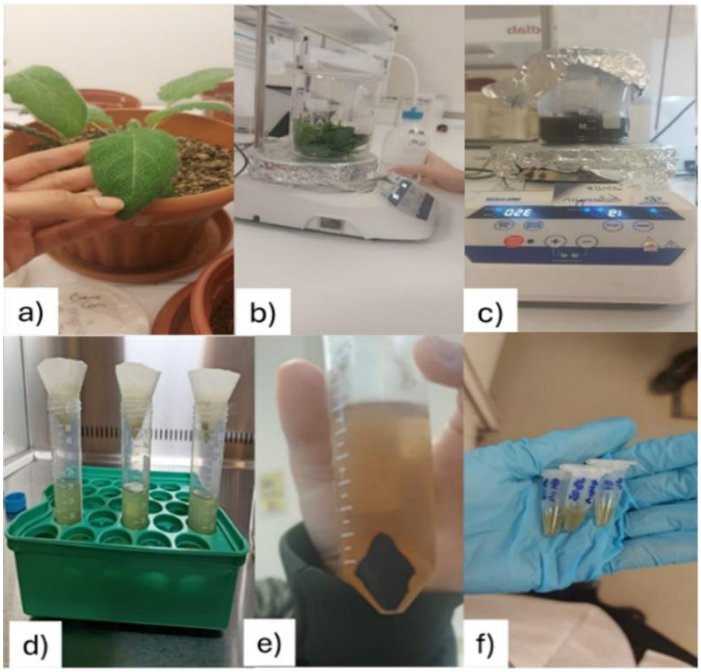

Fig. 2 a) *S. sclarea* grown in the Tissue Culture Lab at Yıldız Technical University (YTU). b) Preparation of *S. sclarea* leaves for extraction. c) AgNPs UV 400-467 nm d) Filtration of *S. sclarea* leaf extract. e) AgNPs after centrifugation. F) Eppendorf tube containing AgNPs.

***Callus formation*.** Fostering Natural Germination of *Anisum* Seeds. A growth hormone-free medium was prepared by dissolving 4.4 g of Murashige and Skoog (MS), 30 g of sucrose, and 7 g of agar in half the final volume of distilled water [35]. The pH of the solution was adjusted to 5,7. Following pH adjustment, the agar was added. The medium was autoclaved. The medium was poured into sterile Petri dishes within a laminar flow hood and allowed to cool until the point of seed placement—this hormone-free medium was composed without the addition of growth hormones. Seeds germinated in darkness were incubated in a medium at a constant temperature of 25±1°C, following a photoperiod of 16 hours of light and 8 hours of darkness.

***Leaf explant preparation and callus formation*.** Ripe anise leaves can be expected approximately 4 to 6 weeks after seed germination. In this study, ten leaf explants were taken, with the medium tested in three repetitions for each experimental group. A systematic approach was employed using a modified MS medium enriched with growth hormones to promote vigorous leaf growth in mature anise plants. For callus formation, leaf segments from aseptic anise seedlings that were 4 to 6 weeks old were utilized as explants. Explants measuring 1 cm square were placed in an MS medium containing 2 mg/L of 2,4-dichlorophenoxyacetic acid (2,4-D) hormone [36].

Application of AgNPs to *P. anisum L.* Callus Cultures

According to Jebor (2016), we transferred to different plant growth medium the callus formed over a period of 4 to 6 weeks, cutting it into pieces weighing up to 250 mg. These pieces were placed into a plant growth medium, specifically prepared with control and silver nanoparticles (AgNPs) groups at concentrations of 0, 1, 5, and 10 mg in MS medium containing 2 mg/L of BAP (Benzyl aminopurine) and 2 mg/L of 2,4-D. Our study will evaluate the zero-concentration group as the control. The calli will be incubated in a climate cabinet (SANYO MLR-351H) at a temperature of 25 ± 2°C for 16 hours under a photoperiod provided by white fluorescent light at an intensity of 50 µmol/m²/s. Reactive oxygen species (ROS) analyses of the calli will be conducted 21 and 28 days after the initiation of the culture.

Antioxidant enzyme analyses

SOD, GPOx, and CAT analyses, for which the methods are detailed below, were performed on the 21st and 28 th days and examined whether they caused oxidative stress in Calli. In this process, 1 g of callus tissue is homogenized at +4°C using 2 ml of extraction solution containing 0.1 mM

EDTA, 1% PVP, 0.5% Triton X-, and 100-mM PBS at pH 7.8. After homogenization, the mixture was centrifuged at +4°C at 18,000 g for 20 min, and the supernatant was collected. This supernatant will be used to measure antioxidant enzyme activity [37].

Catalase (CAT) activity. A 1.5 ml measurement buffer of 200 mM PBS at pH 7 and 71 mM H_2O_2 was incubated at 30˚ for 3 minutes. After the incubation, the measurement buffer was transferred to a quartz cuvette, and 37.5 µl of supernatant was added. Spectrophotometric measurements were conducted kinetically at 240 nm for 2 minutes. The catalase activity was calculated using the formula Δ Abs (240 nm) / (minute x mg protein) and expressed in units per mg of protein (U/mg protein) [38].

Superoxide dismutase (SOD) activity. Two µl of supernatant and twenty µl of riboflavin (0.2 mM) were added to 2 ml of buffer (100 mM PBS pH7, 2 M Na_2CO_3, 0.5 M EDTA, 300 mM L-Methionine, 7.5 mM NBT and the samples are exposed to 15 W white, fluorescent light until a visible colour change occurs. Following the colour change, the absorbance of the samples at 560 nm was measured spectrophotometrically. SOD enzyme activity was determined using a formula that considers the change in absorbance over time and protein content and expressed as U/mg protein [39].

Guaiacol peroxidase (GPOx) activity. A measurement buffer of 100 mM PBS at pH 5.8, 5 mM H_2O_2, and 15 mM Guaiacol was prepared. Then, ten µl of supernatant was introduced into this buffer and was placed in 1.5 ml quartz tubes. The spectrophotometric analysis (IMPLEN Nanophotometer-P330, Germany) is conducted kinetically at 470 nm for 120 seconds. GPOx activity is quantified using the formula Δ Abs (470 nm) / (minutes x mg protein) and expressed as U/mg protein [40].

Results

Characterization of AgNPs

AgNPs synthesized from *S. sclaera* leaf extract were measured by zeta particle size, potential, and multiple distribution index analysis. The particle size dispersion is shown in Figure 3. The size distribution of the synthesized NPs varied between 13,54-342 nm, with an average size of ~95 nm. The zeta potential was measured as (mV): -10,7. Additionally, UV-Vis measurements indicated wavelength peaks in the range of 400 nm on average.

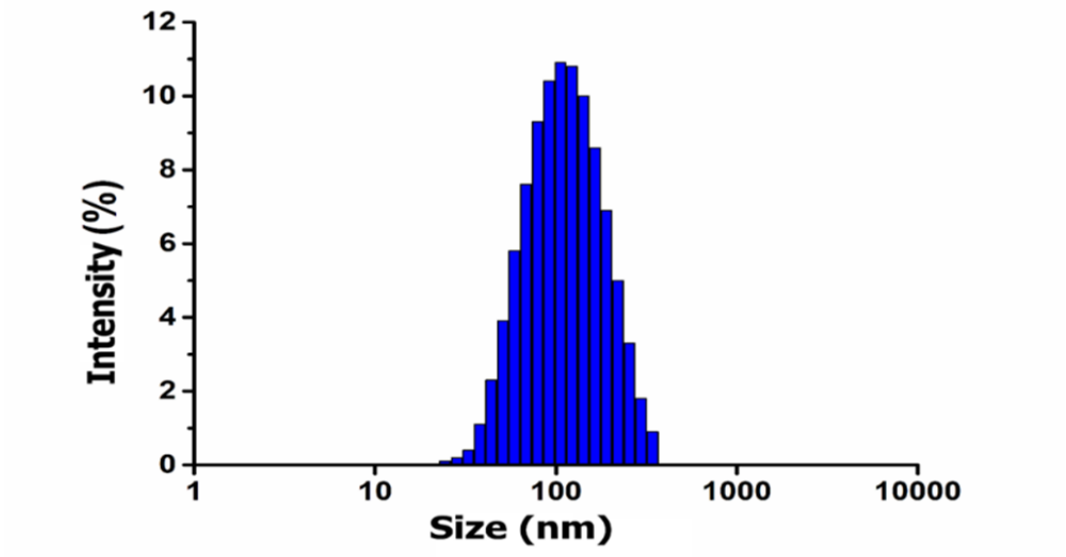

Fig.3 Intensity-based size was measured by Zeta Sizer; here is the distribution of the synthesized silver nanoparticles.

SEM images Figure 4 indicated that the NPs displayed a spherical morphology. The SEM findings aligned with the size measurements obtained using dynamic light scattering, confirming that the particles exhibited a uniform size distribution.

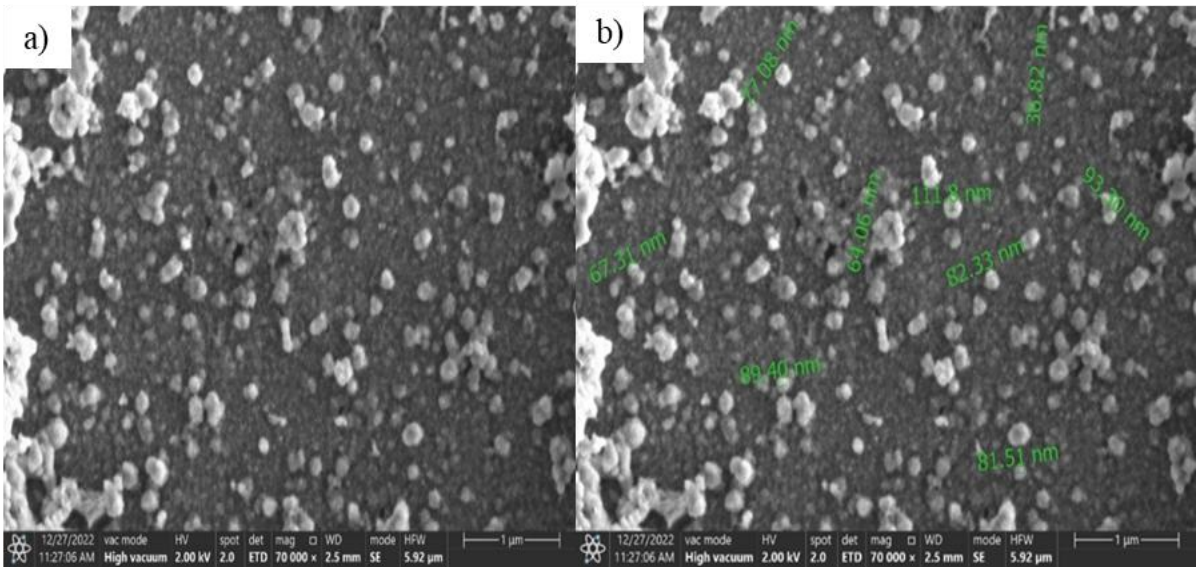

Fig.4 High-resolution SEM image showing spherical structure with a detailed focus on size, shape, and surface features for further analysis. a) High-resolution SEM image (70000x) acquired under high vacuum (2 kV). This focus on size, shape, and surface features allows for further analysis. b) This complements the high-resolution view of Figure 4a and captures the broader distribution and potential cluster formation within the synthesized AgNPs.

Application of AgNPs to *P. anisum L.* Callus Cultures

All morphological and physiological characteristics were observed to be affected by AgNPs stress, with a more pronounced effect on the transformation of callus color from green to yellowish as the amount of AgNPs increased.

Changes in AgNPs concentration had a noticeable impact on the morphological attributes. As the concentration of AgNPs increased, a more prominent effect was observed, particularly in the DT variety, which is much bigger and greener in colour than DS. DS and DT comparison of different concentrations of AgNPs in 21st days. Figure 5 indicated the callus formations.

Antioxidant enzyme activation

In our experiment, we studied the toxicity of AgNPs at three different concentrations (1 mg/L, 5 mg/L, and 10 mg/L) on Anis plants. Next, we evaluated the activation of antioxidant enzymes, including CAT, SOD, and GPOx, 21 and 28 days after AgNPs were applied to the plant. It is worth noting that the results showed distinct trends in enzyme activity.

***CAT Activation results*.** The CAT enzyme activity results in both DS and DT conditions after 21 and 28 days displayed inconsistencies, lacking coherent trends or statistical significance across all concentrations, as shown in Figure 6. However, the 5 mg/L AgNPs concentration after 28 days in DS conditions showed a substantial increase, reflecting a noteworthy **p, suggesting a statistically significant change at that concentration and time point (Table 1&2).

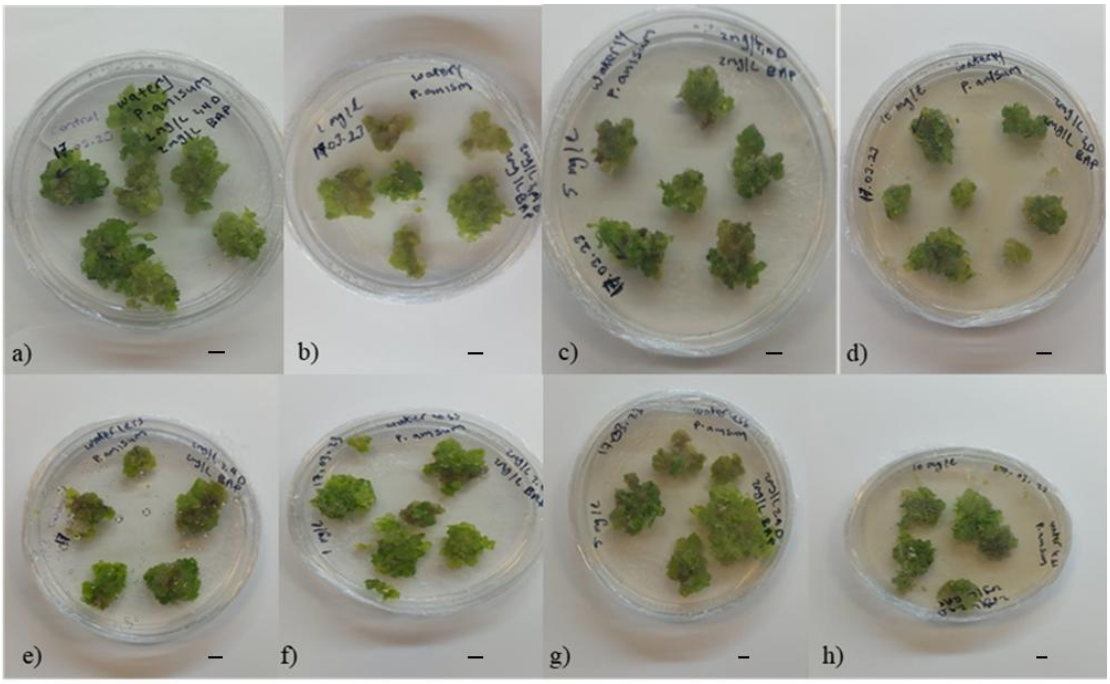

Fig.5 The images represent the progressive transformation of callus color from green to yellowish, reductions in size, and textural changes. 21st day, The DS (top row) and DT (bottom row) are shown for comparison. (a) DS control (b) DS 1mg/L AgNPs (c) DS 5mg/L AgNPs (d) DS 10mg/L AgNPs (e) DT control. (f) DT1mg/L AgNPs (g) DT 5mg/L AgNPs (h)DT 10 mg/L AgNPs. The scale bar was calculated and added as 1 cm based on the size of each image.

Table 1 Compared to the control group, Catalase (CAT) activities in response to AgNPs treatment in **DS** *P. anisum* plants. Change and significance levels in the distribution of AgNPs according to days **P <0.01 and ns (no significant difference).

	21 days		**28 days**	
1 mg/L AgNPs	51.98 %	ns	22.77 %	ns
5 mg/L AgNPs	33.36 %	ns	83.76 %	**
10 mg/L AgNPs	28.32 %	ns	40.71 %	ns

Table 2 Catalase (CAT) activities in response to AgNPs treatment in **DT** *P. anisum* plants in comparison to the control group. Change and significance levels in the distribution of AgNPs are determined by the days. ns (indicates no significant difference).

	21 days		**28 days**	
1 mg/L AgNPs	158.37%	ns	74.36%	ns
5 mg/L AgNPs	50.00 %	ns	57.00 %	ns
10 mg/L AgNPs	49.00 %	ns	40.51 %	ns

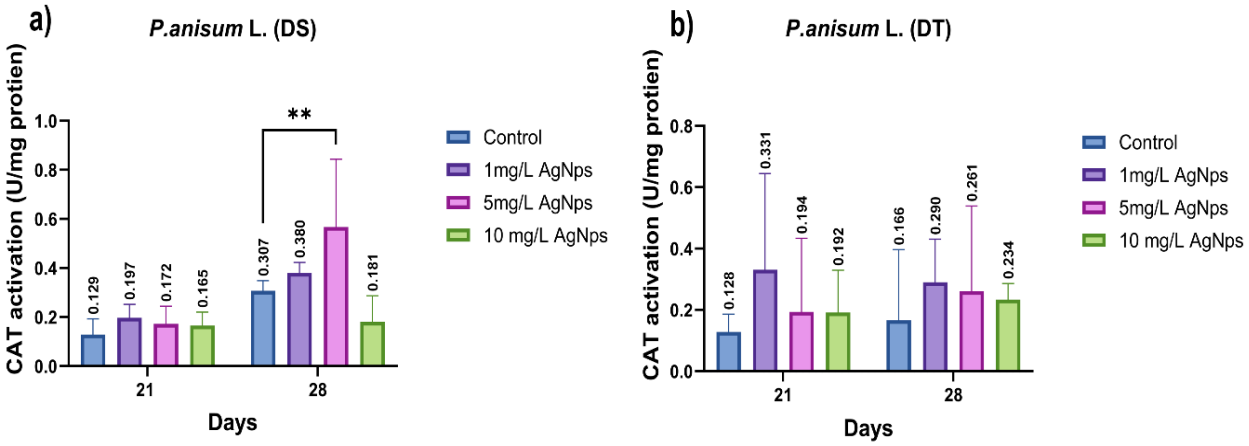

Fig.6 a) CAT enzyme activity graph on the 21st and 28th day applied to control and 1, 5 and 10 mg/L AgNPs Drought-sensitive (DS) anise. ** indicates P < 0.01. b) In DT conditions, the CAT enzyme activity did not produce statistically significant findings.

SOD Activation results. SOD activity in DS after 21 days indicated a significant increase with a 48.42% increase in 1 mg/L AgNPs and an increase in 5 mg/L AgNPs, although 10 mg/L AgNPs did not statistically significant results, as shown in Figure 7. However, after 28 days, all concentrations showed a decrease in SOD activity in DS. On the contrary, the SOD activity in DT showed a decrease, particularly evident with a decrease of 1 mg/L AgNPs and a significant decrease of -57.60% in 10 mg/L AgNPs. However, it showed an increase of 42.26% in the condition of 5 mg/L AgNPs after 21 days. After 28 days, a similar decrease pattern emerged within 1 mg/L AgNPs and 10 mg/L AgNPs, with a significant increase of 322.88% in 5 mg/L AgNPs. At both 21 and 28 days, SOD enzyme activity in DS conditions exhibited compelling results. These results highlight the robust impact of AgNPs on SOD activity over time in DS conditions. In contrast, the 10 mg/L AgNPs concentration demonstrated a p, suggesting a lack of statistical significance in this case (Table 3). In DT conditions, SOD enzyme activity displayed highly noteworthy results both after 21 and 28 days of exposure to AgNPs. For all three concentrations (1 mg/L, 5 mg/L, and 10 mg/L AgNPs), the ****P were consistently found to be, at both time points. This indicates a strong and consistent level of statistical significance, emphasizing the substantial impact of AgNPs on SOD activity under the conditions (Table 3&4).

Table 3. Superoxide dismutase (SOD) activities were examined in response to AgNPs treatment in (DS) *P. anisum* plants compared to the control group. The change and significance levels in the distribution of AgNPs were analyzed over several days. The statistical significance was indicated as follows: ****P < 0.0001, ***P < 0.001, and ns (no significant difference).

	21 days		**28 days**	
1 mg/L AgNPs	48.42 %	**** ↑	- 37.96 %	**** ↓
5 mg/L AgNPs	21.01 %	**** ↑	- 81.13 %	**** ↓
10 mg/L AgNPs	-0.78 %	ns ↓	- 78.49 %	**** ↓

Table 4. Superoxide Dismutase (SOD) activities significantly increased in DT *P. anisum* plants treated with AgNPs compared to the control group, with a high level of statistical significance (****P <0.0001) observed after both 21 and 28 days of treatment.

	21 days		**28 days**	
1 mg/L AgNPs	- 38.14 %	****	- 42.42 %	****
5 mg/L AgNPs	42.26 %	****	322.88 %	****
10 mg/L AgNPs	- 57.60 %	****	- 63.58 %	****

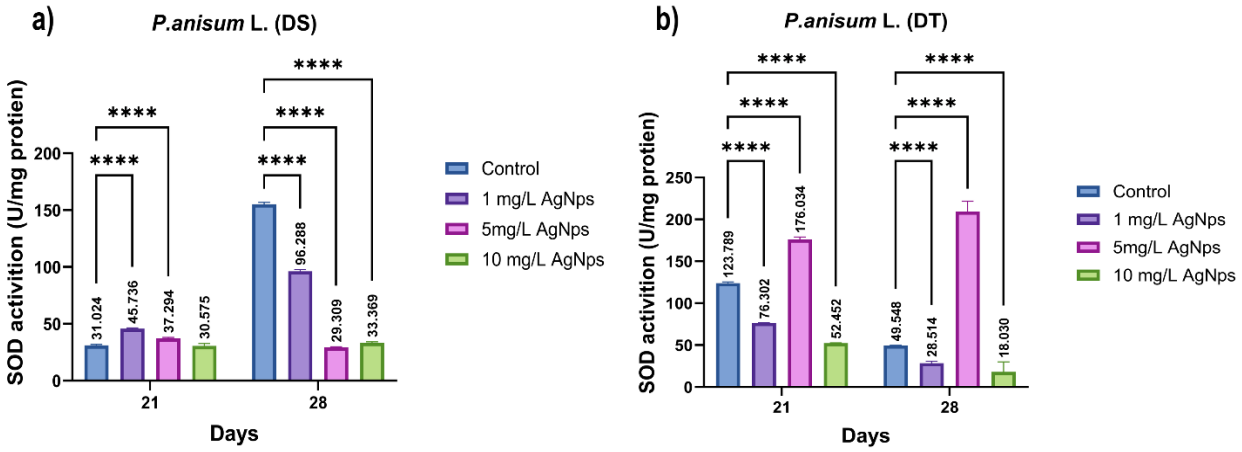

Fig.7 a) SOD enzyme activity graph on 21 and 28 days applied to control and 1, 5, and 10 mg/L AgNPs DS anise. **** indicates P <0.0001. b), SOD enzyme activity graph on 21 and 28 days applied to control and 1, 5, and 10 mg/L AgNPs DT anise. **** indicates P <0.0001.

GPOx Activation results. Turning to GPOx activity in DS condition after 21 days, it showed an impressive increase of 5 mg/L AgNPs, although the results were less interpretable for 1 mg/L and 10 mg/L AgNPs. After 28 days, a significant increase of was recorded for 1 mg/L AgNPs, but the results remained elusive for 5 mg/L and 10 mg/L AgNPs. On the contrary, in DT conditions, after 21 days, the enzyme response appeared inconsistent, with no sense in the case of 10 mg/L AgNPs and a striking increase of 1 mg/L AgNPs, an increase in 5 mg/L. AgNPs. After 28 days, a significant increase was seen in the case of 5 mg/L AgNPs, with an increase of 160.00%, and 10 mg/L AgNPs, which showed an increase of 150.43%, while 1 mg/L AgNPs continued to give results without statistical significance.

In DS conditions, GPOx enzyme activity exhibited varying results after 21 and 28 days of AgNPs exposure. After 21 days, for the 1 mg/L AgNPs concentration, the p indicated no statistical significance, while the 5 mg/L AgNPs concentration displayed a p suggesting a significant impact. However, the 10 mg/L AgNPs concentration had a p of indicating no statistical significance. After 28 days, the 1 mg/L AgNP concentration showed a p of indicating a significant impact, while the 5 mg/L and 10 mg/L AgNP concentrations had ps of signifying no statistical significance, as shown in Figure 8. In DT conditions, GPOx enzyme activity exhibited diverse outcomes following 21 and 28 days of AgNPs exposures. After 21 days, the 1 mg/L AgNPs concentration displayed a p, indicating a highly significant impact, while the 5 mg/L AgNPs concentration showed a p, denoting a significant effect. In contrast, the 10 mg/L AgNPs concentration yielded a p marked as 'ns' (non-significant). After 28 days, the 1 mg/L AgNPs concentration had a p labelled as 'ns,' implying no statistical significance, whereas the 5 mg/L AgNPs concentration presented a p a significant effect. Similarly, the 10 mg/L AgNPs concentration had a p representing a significant impact (Table 5&6).

Table 5. Guaiacol peroxidase (GPOx) activities in DS *P. anisum* plants in response to AgNPs treatment compared to the control group, showing changes and significance levels over time. Significance levels are denoted as **P < 0.01** for significant differences, and 'ns' indicates no significant difference.

	21 days		**28 days**	
1 mg/L AgNPs	22.55 %	ns ↑	93.01 %	** ↑
5 mg/L AgNPs	98.12 %	** ↑	26.00 %	ns ↑
10 mg/L AgNPs	35.5 %	ns ↓	25.57 %	ns ↑

Table 6. Guaiacol peroxidase (GPOx) activities in response to AgNPs treatment in DT *P. anisum* plants compared to the control group. The table presents changes in GPOx activity and significance levels of AgNPs distribution over different time points. Significance levels are indicated as P < 0.01 for significant differences and 'ns' for no significant difference.

	21 days		**28 days**	
1 mg/L AgNPs	186.24%	*** ↑	11.65 %	ns ↑
5 mg/L AgNPs	109.53%	* ↑	160.00 %	** ↑
10 mg/L AgNPs	-37.40 %	ns ↓	150.43 %	* ↑

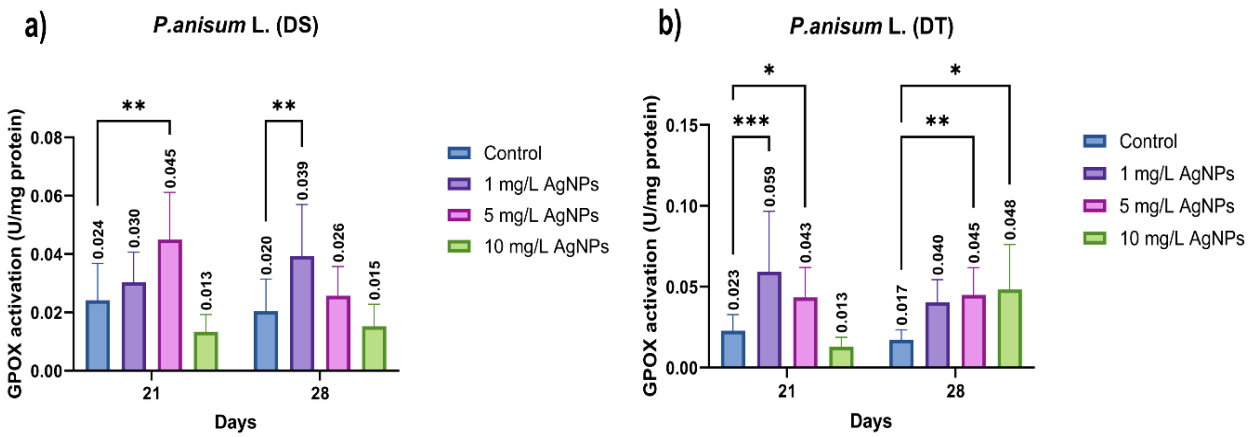

Fig.8 a), GPOx enzyme activity graph on 21 and 28 days applied to control and 1, 5 and 10 mg/L AgNPs DS anise. **** indicates ** indicates P < 0.01. b), GPOx enzyme activity graph on 21 and 28 days applied to control and 1, 5 and 10 mg/L AgNPs DT anise. *** indicates P < 0.001, ** indicates P < 0.01 and * indicates P < 0.05.

Discussion and Conclusion

The goal of this study is to analyse the antioxidant activity of different enzymes, such as CAT, SOD, and GPOx when exposed to AgNPs in two anise types: DT and DS. In 2018, [41] found that the CAT activity was consistent across all the tested AgNPs concentrations in the *Lemna minor*. Also, the study of [42] found

that the effects of AgNPs on CAT activity were not significant in *Allium cepa* roots. Another study by [43]noted that AgNPs had a lesser impact on *Brassica* seedlings as they reduced the accumulation of AgNPs and improved the activities of CAT. However, other studies like [44]. They recorded that AgNPs at 10 mg/L significantly increased the maximum level of CAT activity in plantlets. They also noted that the activity increased. Our results support these findings, indicating that the treatment of AgNPs at varying concentrations did not significantly alter the levels of the CAT enzyme. CAT enzyme activity results after 21 and 28 days, in both DS and DT conditions, displayed inconsistencies, lacking coherent trends or statistical significance across all concentrations.

Continuing with the SOD result, in a study conducted by [44], researchers observed that plants treated with 20 mg/L AgNPs experienced a 17.6% reduction in their SOD activity. Optimal SOD values were found at 2 mg/L for both Ag ion and Ag NP treatments. On the other hand, the highest SOD levels were observed in the treated Ag ions at 10 mg/L. The researchers observed that concentrations of antioxidant enzymes at this level significantly increased SOD enzyme activity. The decrease in SOD activity exhibited at higher concentrations was also notable. In a study conducted by [45], researchers observed a significant increase in SOD activity levels upon exposure to 10 mg/L AgNP. Also, a study conducted by researchers [46] revealed that SOD activity levels were higher in all low AgNPs treatments. This is consistent with the results of our study; we observed that enzyme activity significantly increased in the DT after 21 at 5 mg/L AgNPs days of AgNPs exposure. This is in line with the results of another study [47], which showed that SOD activity levels were higher in tomato plants when exposed to 5 mg/L AgNPs. Also, a study [48] confirmed that the SOD activity was higher when compared to the activity of the control group when the AgNPs concentrations were 2 and 5 mg/dm3. But when AgNPs concentrations were 10, 20, and 50 mg/dm3, the SOD activity decreased. The results of these two studies highlight the complex relationship between plant enzymes and AgNPs. These results support our hypothesis that the increase in SOD activity is due to exposure to different AgNPs concentrations and environmental conditions. The most striking increase in activity, a 322.88% increase, was observed in the DT SOD enzyme after 21 days at 5 mg/L AgNPs, indicating higher enzyme performance under these conditions. Conversely, the most significant decrease, a −63.58% reduction, in DT SOD enzyme was observed after 21 days at 10 mg/L AgNPs, indicating a significant reduction in enzyme functionality at this concentration. These results underscore the complex interaction between AgNPs and plant enzymes, highlighting their potential impact on plant antioxidant defence mechanisms. Similarly, in the investigation of Guaiacol Peroxidase (GPOx) activity in response to silver nanoparticles (AgNPs), multiple studies have contributed to our understanding of the concentration-dependent effects of AgNPs on this antioxidant enzyme. The study of [49] observed a reduction in GPOx activity with AgNPs treatment at 2 mM, indicating an inhibitory effect at this concentration. As the concentration increased to 6 mM, GPOx activity showed a gradual decrease, suggesting a dose-dependent response. This concentration-dependent modulation implies that higher doses of silver nanoparticles may down-regulate the enzyme's functionality, potentially impacting the plant's antioxidant defence mechanism. Moreover, it explored the impact of different AgNPs concentrations on GPOx activity, showing altered GPOx activity in radish plants treated with 50 mg/L and 100 mg/L of AgNPs [50]. The results demonstrated that varying concentrations of AgNPs could influence GPOx activity, highlighting a nuanced response to different nanoparticle concentrations. Another study contributed to this understanding by reporting continuous increases in GPOx activity in *B. juncea* seedlings with increasing concentrations of Ag-NPs from 25 ppm to 400 ppm. This observation aligns with the notion that GPOx activity can be positively influenced by higher concentrations of silver nanoparticles [51]. In our specific investigation, we explored the concentration-dependent effects of AgNPs on GPOx activity in both DS and DT conditions. In the DS condition after the 21st day, GPOx activity displayed a notable increase at 5 mg/L AgNPs, while results for 1 mg/L and 10 mg/L AgNPs were less interpretable. After the 28th day, a significant increase was recorded for 1 mg/L AgNPs, but the results remained elusive for 5 mg/L and 10 mg/L AgNPs. In DT conditions after the 21st day, the enzyme response appeared inconsistent, with no clear trend for 10 mg/L AgNPs, a significant increase for 1 mg/L AgNPs, and an increase for 5 mg/L AgNPs. After the 28th day, a significant increase was observed for 5 mg/L AgNPs (160.00%), and 10 mg/L AgNPs showed an increase of 150.43%, while 1 mg/L AgNPs continued to yield results without statistical significance. These detailed findings underscore the complexity of the relationship between AgNPs concentrations and GPOx activity, providing valuable insights into the nuanced responses of antioxidant enzymes under different exposure conditions.

In conclusion, under DT conditions, there was an increase in the working capacity of plant enzyme activities, with a significant rise at 10 mg/L on the 28th day. These results collectively emphasize the concentration-dependent and complex impact of AgNPs on GPOx enzyme activity in plant systems. According to the results obtained, antioxidant enzyme activity works actively in 1 and 5 mg/L AgNPs concentrations in both

groups, but at the highest dose of 10 mg/L AgNPs concentration reduced the working capacity of the plant's defense mechanism. As a result, it was observed that plant tolerance of 1 mg/L AgNPs dose was high in both groups. However, despite the increase in toxic values at 1 and 5 mg/L, it was observed that the tolerance capacity increased more in the DT condition compared to the DS after the 21st day. While toxic values were evaluated to be relatively high at 10 mg/L, it was observed that it seriously limited the ROS enzyme working capacity of the plant.

Abbreviations

NPs: Nanoparticles; *P. anisum: Pimpinella anisum*; *S. sclarea*: *Salvia sclarea*; AgNPs: silver nanoparticles; ROS: Reactive Oxygen Species; CAT: Catalase enzyme; SOD: Superoxide Dismutase enzyme; GPOx: Guaiacol Peroxidase enzyme; DS: drought-sensitive; DT: drought-tolerant type.

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Data Availability statement

The author confirms that the data supporting this study are cited in the article.

Compliance with ethical standards

Conflict of interest / Çıkar çatışması

The author declare no conflict of interest.

Ethical standards

The study is proper with ethical standards.

Authors' contributions

During the study Esma Ulusoy (E.U.) and Esraa Gaber (E.G.) designed the project, experiment, and acquired data. E. U. supervised the work, while both authors performed the experiments and statistical analysis.

References

1. K. L. Kelly, E. Coronado, L. L. Zhao, et G. C. Schatz, The optical properties of metal nanoparticles: The influence of size, shape, and dielectric environment », *Journal of Physical Chemistry B*, vol. 107, nº 3, p. 668-677, 2003. https://doi.org/10.1021/jp026731y
2. Nanotechnology Market Size, Share & Industry Analysis, By Type (Nanodevices and Nanosensors), By Industry (Electronics, Healthcare, Manufacturing, Energy & Power, Automotive, Aerospace & Defense, Food & Beverages, and Others), and Regional Forecast, 2024-2032. Source: https://www.fortunebusinessinsights.com/nanotechnology-market-108466
3. A. Mohajerani *et al.*, « Nanoparticles in construction materials and other applications, and implications of nanoparticle use », 1 octobre 2019, *MDPI AG*. https://doi.org/10.3390/ma12193052
4. S. Marella, A. R. Nirmal Kumar, et N. V. K. V. Prasad Tollamadugu, « Nanotechnology-based innovative technologies for high agricultural productivity: Opportunities, challenges, and future perspectives », dans *Recent Developments in Applied Microbiology and Biochemistry: Volume 2*, Elsevier, 2021, p. 211-220. doi: 10.1016/B978-0-12-821406-0.00019-9
5. M. Taran, M. Safaei, N. Karimi, et A. Almasi, « Benefits and application of nanotechnology in environmental science: an overview », *Biointerface Res Appl Chem*, vol. 11, nº 1, p. 7860-7870, janv. 2020, https://doi.org/10.33263/BRIAC111.78607870
6. L. Wei, J. Lu, H. Xu, A. Patel, Z. S. Chen, et G. Chen, « Silver nanoparticles: Synthesis, properties, and therapeutic applications », 1 mai 2015, *Elsevier Ltd*. DOI: 10.1016/j.drudis.2014.11.014
7. Z. Ferdous et A. Nemmar, « Health impact of silver nanoparticles: A review of the biodistribution and toxicity following various routes of exposure », 1 avril 2020, *MDPI AG*. https://doi.org/10.3390/ijms21072375
8. M. Usman *et al.*, « Nanotechnology in agriculture: Current status, challenges and future opportunities », 15 juin 2020, *Elsevier B.V.* DOI: 10.1016/j.scitotenv.2020.137778
9. W. Sun, M. H. Shahrajabian, et Q. Cheng, « Anise (Pimpinella anisum L .), a dominant spice and traditional medicinal herb for both food and medicinal purposes », *Cogent Biol*, vol. 5, nº 1, p. 1673688, janv. 2000, https://doi.org/10.1080/23312025.2019.1673688
10. K. Dzi *et al.*, « antioxidants Antioxidant Therapy in Inflammatory Bowel Diseases », 2021, https://doi.org/10.3390/antiox10030412
11. Asad, Mohd, et al. Traditional uses, phytochemistry and pharmacological activities of anisun (Pimpinella anisum l.): A comprehensive review. *Ann For Res*, 2022, 65.1: 5212-27. https://www.researchgate.net/publication/372935655
12. A. M. V. Ritter *et al.*, « Antihypernociceptive activity of anethole in experimental inflammatory pain », *Inflammopharmacology*, vol. 21, nº 2, p. 187-197, avr. 2013, DOI: 10.1007/s10787-012-0152-6

13. L. Zhao *et al.*, « Nano-Biotechnology in Agriculture: Use of Nanomaterials to Promote Plant GroDSh and Stress Tolerance », *J Agric Food Chem*, vol. 68, nº 7, p. 1935-1947, févr. 2020, https://doi.org/10.1021/acs.jafc.9b06615
14. H. S. Jiang *et al.*, « Silver nanoparticles induced reactive oxygen species via photosynthetic energy transport imbalance in an aquatic plant », *Nanotoxicology*, vol. 11, nº 2, p. 157-167, févr. 2017, https://doi.org/10.1080/17435390.2017.1278802
15. G. Marslin, C. J. Sheeba, et G. Franklin, « Nanoparticles alter secondary metabolism in plants via ROS burst », *Front Plant Sci*, vol. 8, mai 2017, https://doi.org/10.3389/fpls.2017.00832
16. A. Rasheed *et al.*, « The role of nanoparticles in plant biochemical, physiological, and molecular responses under drought stress: A review », 24 novembre 2022, *Frontiers Media S.A.* https://doi.org/10.3389/fpls.2022.976179
17. A. Sosan *et al.*, « Engineered silver nanoparticles are sensed at the plasma membrane and dramatically modify the physiology of Arabidopsis thaliana plants », *Plant Journal*, vol. 85, nº 2, p. 245-257, janv. 2016, https://doi.org/10.1111/tpj.13105
18. C. O. Dimkpa *et al.*, « CuO and ZnO nanoparticles: Phytotoxicity, metal speciation, and induction of oxidative stress in sand-grown wheat », *Journal of Nanoparticle Research*, vol. 14, nº 9, sept. 2012, DOI 10.1007/s11051-012-1125-9
19. M. Danish *et al.*, « Bacillus mojavensis, a Metal-Tolerant Plant GroDSh-Promoting Bacterium, Improves GroDSh, Photosynthetic Attributes, Gas Exchange Parameters, and Alkalo-Polyphenol Contents in Silver Nanoparticle (Ag-NP)-Treated Withania somnifera L. (Ashwagandha) », *ACS Omega*, vol. 7, nº 16, p. 13878-13893, avr. 2022, https://doi.org/10.1021/acsomega.2c00262
20. M. Thwala, N. Musee, L. Sikhwivhilu, et V. Wepener, « The oxidative toxicity of Ag and ZnO nanoparticles towards the aquatic plant Spirodela punctuta and the role of testing media parameters », *Environmental Sciences: Processes and Impacts*, vol. 15, nº 10, p. 1830-1843, oct. 2013, https://doi.org/10.1039/C3EM00235G
21. H. Sies, « Oxidative stress: A concept in redox biology and medicine », 1 avril 2015, *Elsevier B.V.* DOI: 10.1016/j.redox.2015.01.002
22. P. Sailaja Rao, S. Kalva, A. Yerramilli, et S. Mamidi, « Free Radicals and Tissue Damage: Role of Antioxidants », *Free Radicals and Antioxidants*, vol. 1, nº 4, p. 2-7, oct. 2011, https://doi.org/10.5530/ax.2011.4.2
23. A. Rastogi *et al.*, « Impact of metal and metal oxide nanoparticles on plant: A critical review », 2017, *Frontiers Media S.A.* https://doi.org/10.3389/fchem.2017.00078
24. K. Bela, E. Horváth, Á. Gallé, L. Szabados, I. Tari, et J. Csiszár, « Plant glutathione peroxidases: Emerging role of the antioxidant enzymes in plant development and stress responses », 5 mars 2015, *Urban und Fischer Verlag GmbH und Co. KG*. DOI: 10.1016/j.jplph.2014.12.014
25. M. A. Altaf *et al.*, « Melatonin Improves Drought Stress Tolerance of Tomato by Modulation Plant GroDSh, Root Architecture, Photosynthesis, and Antioxidant Defense System », *Antioxidants*, vol. 11, nº 2, févr. 2022, https://doi.org/10.3390/antiox11020309
26. J. Yang, W. Cao, et Y. Rui, « Interactions between nanoparticles and plants: Phytotoxicity and defense mechanisms », 2017, *Taylor and Francis Ltd.* https://doi.org/10.1080/17429145.2017.1310944
27. S. Kapoor, H. Sood, S. Saxena, et O. P. Chaurasia, « Green synthesis of silver nanoparticles using Rhodiola imbricata and Withania somnifera root extract and their potential catalytic, antioxidant, cytotoxic and groDSh-promoting activities », *Bioprocess Biosyst Eng*, vol. 45, nº 2, p. 365-380, févr. 2022, https://doi.org/10.1007/s00449-021-02666-9
28. D. K. Tripathi *et al.*, « An overview on manufactured nanoparticles in plants: Uptake, translocation, accumulation and phytotoxicity », *Plant Physiology and Biochemistry*, vol. 110, p. 2-12, janv. 2017, doi: 10.1016/j.plaphy.2016.07.030
29. P. G. Jamkhande, N. W. Ghule, A. H. Bamer, et M. G. Kalaskar, « Metal nanoparticles synthesis: An overview on methods of preparation, advantages and disadvantages, and applications », 1 octobre 2019, *Editions de Sante* https://doi.org/10.1016/j.jddst.2019.101174
30. A. Roy, O. Bulut, S. Some, A. K. Mandal, et M. D. Yilmaz, « Green synthesis of silver nanoparticles: Biomolecule-nanoparticle organizations targeting antimicrobial activity », 2019, *Royal Society of Chemistry*. https://doi.org/10.1039/C8RA08982E
31. A. A. Zahir *et al.*, « Green synthesis of silver and titanium dioxide nanoparticles using Euphorbia prostrata extract shows shift from apoptosis to G0/G1 arrest followed by necrotic cell death in Leishmania donovani », *Antimicrob Agents Chemother*, vol. 59, nº 8, p. 4782-4799, août 2015, DOI: 10.1128/AAC.00098-15
32. A. Balčiūnaitienė *et al.*, « Eucalyptus globulus and Salvia officinalis Extracts Mediated Green Synthesis of Silver Nanoparticles and Their Application as an Antioxidant and Antimicrobial Agent », *Plants*, vol. 11, nº 8, avr. 2022, https://doi.org/10.3390/plants11081085
33. T. J. I. Edison et M. G. Sethuraman, « Instant green synthesis of silver nanoparticles using Terminalia chebula fruit extract and evaluation of their catalytic activity on reduction of methylene blue », *Process Biochemistry*, vol. 47, nº 9, p. 1351-1357, sept. 2012, https://doi.org/10.1016/j.procbio.2012.04.025
34. B. E. Lindsey, L. Rivero, C. S. Calhoun, E. Grotewold, et J. Brkljacic, « Standardized method for high-throughput sterilization of Arabidopsis seeds », *Journal of Visualized Experiments*, vol. 2017, nº 128, oct. 2017, DOI: 10.3791/56587
35. K. Karimah, R. Yuniati, et W. Handayani, « In vitro culture from internodes of Melastoma malabathricum L. on Murashige and Skoog (1962) modified medium with thidiazuron and 1-naphthaleneacetic acid », dans *IOP Conference Series: Earth and Environmental Science*, Institute of Physics Publishing, avr. 2020. **DOI** 10.1088/1755-1315/481/1/012007
36. M. A. Jebor, A. R. Obayis, et K. T. Khashan, « Biochemical and Physiological Changes of Callus GroDSh and t-anethole Essential Oil Production from pimipinella anisum L. under Salinity Stress », 2016. https://www.researchgate.net/publication/330480877_Biochemical_and_Physiological_Changes_of_Callus_Growth_and_t-anethole_Essential_Oil_Production_from_pimipinella_anisum_L_under_Salinity_Stress
37. F. Yanik, B. A. Dalı, et B. Programı, « Marmara Üniversitesi Fen Bilimleri Enstitüsü Triticum Aestivum L. (Buğday)'Da Gümüş Nanopartikülleri İle Oluşturulmuş Programli Hücre Ölümünün Belirlenmesi », 2019. https://tez.yok.gov.tr/UlusalTezMerkezi/tezDetay.jsp?id=QPjMGVfoZQxOL1wb6V6TDg
38. Cho, Young-Wook; PARK, Eun-Hee; LIM, Chang-Jin. Glutathione S-transferase activities of S-type and L-type thioltransferases from Arabidopsis thaliana. *BMB Reports*, 2000, 33.2: 179-183. https://koreascience.kr/article/JAKO200010102464254.page

39. I. Cakmak et H. Marschner, « Magnesium Deficiency and High Light Intensity Enhance Activities of Superoxide Dismutase, Ascorbate Peroxidase, and Glutathione Reductase in Bean Leaves», 1992. doi: 10.1104/pp.98.4.1222
40. H. Birecka, K. A. Briber, et J. L. Catalfamo, « Comparative Studies on Tobacco Pith and Sweet Potato Root Isoperoxidases in Relation to Injury, Indoleacetic Acid, and Ethylene Effects », 1973. https://doi.org/10.1104/pp.52.1.43
41. S. P. P. Pereira *et al.*, « Phytotoxicity of silver nanoparticles to Lemna minor: Surface coating and exposure period-related effects », *Science of the Total Environment*, vol. 618, p. 1389-1399, mars 2018, DOI: 10.1016/j.scitotenv.2017.09.275
42. P. Cvjetko *et al.*, « Toxicity of silver ions and differently coated silver nanoparticles in Allium cepa roots », *Ecotoxicol Environ Saf*, vol. 137, p. 18-28, mars 2017, DOI: 10.1016/j.ecoenv.2016.11.009
43. K. Vishwakarma *et al.*, « Differential phytotoxic impact of plant mediated silver nanoparticles (AgNPs) and silver nitrate (AgNO3) on Brassica sp. », *Front Plant Sci*, vol. 8, oct. 2017, https://doi.org/10.3389/fpls.2017.01501
44. M. Bagherzadeh Homaee et A. A. Ehsanpour, « Silver nanoparticles and silver ions: Oxidative stress responses and toxicity in potato (Solanum tuberosum L) grown in vitro », *Hortic Environ Biotechnol*, vol. 57, nº 6, p. 544-553, déc. 2016, https://doi.org/10.1007/s13580-016-0083-z
45. X. Zou, P. Li, Q. Huang, et H. Zhang, « The different response mechanisms of Wolffia globosa: Light-induced silver nanoparticle toxicity », *Aquatic Toxicology*, vol. 176, p. 97-105, juill. 2016, DOI: 10.1016/j.aquatox.2016.04.019
46. H. S. Jiang, X. N. Qiu, G. B. Li, W. Li, et L. Y. Yin, « Silver nanoparticles induced accumulation of reactive oxygen species and alteration of antioxidant systems in the aquatic plant Spirodela polyrhiza », *Environ Toxicol Chem*, vol. 33, nº 6, p. 1398-1405, 2014, https://doi.org/10.1002/etc.2577
47. U. Song *et al.*, « Functional analyses of nanoparticle toxicity: A comparative study of the effects of TiO2 and Ag on tomatoes (Lycopersicon esculentum) », *Ecotoxicol Environ Saf*, vol. 93, p. 60-67, juill. 2013, DOI: 10.1016/j.ecoenv.2013.03.033
48. P. Jadczak, D. Kulpa, R. Drozd, W. Przewodowski, et A. Przewodowska, « Effect of aunps and AgNPs on the antioxidant system and antioxidant activity of lavender (Lavandula angustifolia mill.) from in vitro cultures », *Molecules*, vol. 25, nº 23, déc. 2020, https://doi.org/10.3390/molecules25235511
49. I. Khan *et al.*, « Physiological and biochemical responses of pearl millet (Pennisetum glaucum l.) seedlings exposed to silver nitrate (AgNO3) and silver nanoparticles (AgNPs) », *Int J Environ Res Public Health*, vol. 16, nº 13, juill. 2019, https://doi.org/10.3390/ijerph16132261
50. A. Tymoszuk, « Silver nanoparticles effects on in vitro germination, groDSh, and biochemical activity of tomato, radish, and kale seedlings », *Materials*, vol. 14, nº 18, sept. 2021, https://doi.org/10.3390/ma14185340
51. R. Jurkow, R. Pokluda, A. Sękara, et A. Kalisz, « Impact of foliar application of some metal nanoparticles on antioxidant system in oakleaf lettuce seedlings », *BMC Plant Biol*, vol. 20, nº 1, juin 2020, https://doi.org/10.1186/s12870-020-02490-5