



Effect of Silver Nanoparticles and (BA) on Physiological Stress Indicators of *Ocimum Basilicum* L. Exposed to Lead Ions in Vitro

Ahmed M. Shukri¹ Yasir S. Sekhi¹

¹ Department of Horticulture and Landscaping, College of Agriculture, University of Anbar, IRAQ.

*Corresponding Author: yassirsayel@uoanbar.edu.iq

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ABSTRACT

This study evaluated the influence of 6-benzyladenine (BA) on the initiation and multiplication of *Ocimum basilicum* L. under in vitro conditions and the effect of silver nanoparticles (AgNPs) on the physiological response of plantlets exposed to lead (Pb) stress. A completely randomized design (CRD) was used with appropriate replication; data were analyzed in Genstat, and means were separated using the Least Significant Difference (LSD) test at $p \leq 0.05$. During initiation, BA at 1.5 mg L^{-1} produced the highest explant response (96.67%), whereas the BA-free control showed the lowest. In multiplication, BA at 1.0 mg L^{-1} maximized leaf number (14.0), while 1.5 mg L^{-1} increased shoot number (3.45); plantlet height did not differ significantly. Under Pb (0, 200, 300, 400 $\mu\text{mol L}^{-1}$) and AgNPs (0, 40, 80 ppm), oxidative-stress markers and defense traits exhibited distinct main and interaction effects. Malondialdehyde (MDA; $\mu\text{mol g}^{-1} \text{FW}$) peaked at the $\text{Pb}_3 \times \text{Ag}_2$ combination (12.22), whereas hydrogen peroxide (H_2O_2 ; $\mu\text{mol g}^{-1} \text{FW}$) was highest at $\text{Pb}_2 \times \text{Ag}_2$ (4.74). Proline ($\mu\text{mol g}^{-1} \text{FW}$) accumulated most at $\text{Pb}_3 \times \text{Ag}_2$ (80.30). For antioxidant enzymes ($\mu\text{mol g}^{-1} \text{FW min}^{-1}$), catalase (CAT) showed its highest mean under $\text{Pb}_2 \times \text{Ag}_1$ (14.47), while superoxide dismutase (SOD) reached a maximum at $\text{Pb}_3 \times \text{Ag}_2$ (17.40). These outcomes indicate a dose-dependent, stage-specific response in basil, in which moderate AgNP levels enhance antioxidant capacity under Pb exposure, whereas higher concentrations coincide with increased oxidative load. The results support using in vitro screening to define optimal BA and AgNPs regimes that improve physiological indicators under heavy-metal stress. This clarifies the importance of AgNPs in the propagation stages and their role in tolerating stress under the influence of heavy elements, which highlights their potential practical applications in plant tissue culture and stress physiology research.

Keywords: *Ocimum basilicum* L., silver nanoparticles (AgNPs), lead (Pb) stress, oxidative stress markers, antioxidant enzymes.

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INTRODUCTION

Basil (*Ocimum basilicum* L.) is one of the most economically and medicinally valuable crops. It is a rich source of secondary metabolites and essential oils, accounting for numerous pharmacological, nutritional and aromatic characteristics in the plant. These characteristics have led to its extensive attention in recent studies with respect to the biotechnology and traditional medicine [1, 2]. Plants grown in polluted area are exposed to toxicity by heavy Lead (Pb) was selected as the representative heavy metal because it is one of the most prevalent and toxic pollutants in Iraqi soils and irrigation water, particularly in regions influenced by vehicular emissions and industrial waste. Unlike essential trace elements such as zinc (Zn) or copper (Cu), Pb has no known biological role in plants and exhibits high toxicity even at low concentrations. Several environmental assessments have reported elevated Pb levels in agricultural areas of Al-Anbar and central Iraq, making it a relevant and realistic model contaminant for assessing basil tolerance and detoxification capacity under *in vitro* conditions. and lead is one of the most prevalent and deleterious among them. Its accumulation in plant tissues disrupts a growth process, photosynthesis, nutrient uptake and Reactive Oxygen Species (ROS) formation. These related changes are responsible in the generation of oxidative stress and cellular damage [3]. Plants employ various defense mechanisms to cope with such conditions involving proline accumulation as an osmoprotectant and induction of antioxidant enzymes to minimize the accumulation of ROS [4]. Nanomaterials are the wave of focus in agricultural research and development in this direction. They, along with other nano-materials (NMs), were more extensively studied silver nanoparticles (AgNPs). Certain studies suggest that low doses of AgNPs may elicit an increase in antioxidant enzyme activity and concomitantly reduce stress signs, whereas higher doses of AgNPs could enhance ROS deposition and increase cell damage. In fact, this highlights on the need to determine an optimum concentration for their use [5].

This would also create a controlled experimental system for the study of plant physiological responses to various stress conditions via plant tissue culture techniques. They are also useful for the initiation and synthesis of secondary metabolites in

medicinal plants [6]. Thus, the present work aimed to explore the influence of BA and AgNPs at different concentrations on *O. basilicum* L response to lead in vitro. It was performed by characterization of cell oxidative stress markers, proline content and antioxidant enzyme activities to reveal the role of these parameters in endowing plant resistance against environmental toxicants. This study aimed to optimize *in vitro* propagation of *Ocimum basilicum* L. using different BA levels and to evaluate the potential of silver nanoparticles (AgNPs) in mitigating lead (Pb)-induced oxidative stress. The integration of these approaches provides a comprehensive understanding of how growth regulators and nanomaterials influence both developmental and stress-response pathways in basil tissue culture.

Materials and Methods

The experiment was conducted during the 2024–2025 growing season at the Plant Tissue Culture Laboratory, College of Agriculture, University of Anbar, Iraq, and lasted approximately 16 weeks. Basil (*Ocimum basilicum* L.) seeds were surface sterilized and germinated directly on Murashige and Skoog (MS) medium inside the tissue culture laboratory. Nodal explants measuring 0.5–1.0 cm in length were excised from 60-day-old *in vitro*-grown seedlings and used to initiate the experiment. The sterilization procedure involved washing the seeds under running tap water for 15 minutes, immersing them in 70% ethanol for 30 seconds, and then treating them with 3% sodium hypochlorite (NaOCl) solution for 10 minutes. Finally, the seeds were rinsed three times with sterile distilled water before inoculation on MS medium, aiming to evaluate the effect of nanosilver particles (AgNPs) on the resistance of the *Ocimum basilicum* L. plant to lead ions under in vitro culture conditions.

Preparation of the culture medium

The culture medium was prepared based on the full-strength Murashige and Skoog (MS) basal medium at a concentration of 4.43 g L⁻¹, supplemented with 30 g L⁻¹ sucrose as an energy source. Plant growth regulators were added as follows: indole-3-acetic acid (IAA) at 0.5 mg L⁻¹ and 6-benzyladenine (BA) at concentrations of 0, 0.5, 1.0, and 1.5 mg L⁻¹ during the initiation stage. In the multiplication stage, IAA was maintained at 0.5 mg L⁻¹, while BA concentrations were adjusted to 0, 1.0, 1.5, and 2.0 mg L⁻¹ to stimulate growth and multiplication of plant explants according to the experimental design. All components were thoroughly mixed, and the pH of the medium was adjusted to 5.7 using NaOH or HCl. Agar was used as a solidifying agent at a concentration of 7 g L⁻¹ in all culture media to provide proper support for explant growth and medium stability. was then added as a gelling agent. After bringing the medium to boiling point, 10 mL was dispensed into glass test tubes. The tubes were sterilized in an autoclave at 121 °C for 20 minutes. Cultures were incubated at 25 ± 2 °C, under a 16/8 h (light/dark) photoperiod with a light intensity. [7].

Initiation stage

Explant segments of *O. basilicum* measuring 0.5 cm in length were cultured in test tubes containing MS medium supplemented with BA at rate of 0, 0.5, 1.0, and 1.5 mg L⁻¹. This stage lasted for six weeks, during which responsive, non-responsive, and dead explants were recorded to determine the most effective concentrations for stabilizing plantlets.

Multiplication stage

It is important to note that silver nanoparticles (AgNPs) and lead (Pb) treatments were not applied during the initiation and multiplication stages. These stages were conducted solely to optimize the concentrations of 6-benzyladenine (BA) for efficient in vitro propagation, while the AgNPs and Pb treatments were used only in the subsequent stress experiment. The study consisted of two consecutive in vitro experiments. Responsive explants obtained from the initiation stage were transferred to fresh medium with the same basal components, continuing IAA at 0.5 mg L⁻¹, while BA concentrations were adjusted to 0, 1.0, 1.5, and 2.0 mg L⁻¹. This stage also lasted six weeks, during which the number of lateral branches, number of leaves, and shoot length were measured. This helped determine the best concentrations of growth regulators to enhance multiplication and increase the biomass needed for the subsequent experiment

Phytoremediation stage

The optimal BA concentration identified in the multiplication stage—1.5 mg L⁻¹, which yielded the highest growth response and plantlet development—was selected for this stage. Different concentrations of lead nitrate [Pb (NO₃)₂] (0, 200, 300, and 400 μmol L⁻¹) were combined with silver nanoparticles (AgNPs) at 0, 40, and 80 ppm and added to the culture medium. The AgNPs used in this study were commercially obtained from Caisson Laboratories Inc. (USA). According to the manufacturer's data, the particles were spherical in shape with an average size of 40 ± 5 nm and supplied as a stable colloidal suspension in deionized water. The stock suspension was diluted with sterile distilled water to obtain the required concentrations and sterilized by membrane filtration through a 0.22 μm syringe filter before incorporation into the Murashige and Skoog (MS) medium. Plant parts were incubated in incubators at 25±2 °C, under 16 hours of light followed by 8 h dark. Morphological responses were monitored during growth, and physiological responses were evaluated through oxidative and antioxidant parameters. The physiological stress indicators, including malondialdehyde (MDA), hydrogen peroxide (H₂O₂), proline, and the activities of catalase (CAT) and superoxide dismutase (SOD), were determined using standard

spectrophotometric methods. MDA, H_2O_2 , and proline contents were quantified using colorimetric reactions specific for each compound, while the activities of CAT and SOD enzymes were measured based on their oxidation–reduction reactions. All measurements were carried out using a UV–Vis spectrophotometer (Shimadzu UV-1800, Japan). The results were expressed as $\mu\text{mol g}^{-1} \text{FW}$ or $\mu\text{mol g}^{-1} \text{FW min}^{-1}$ for enzyme activity.

Experimental design

We have specified the number of replicates and clarified the factors for each experiment. Experiment 1 (BA optimization) used a single-factor CRD (BA levels per stage) with 20 replicates per treatment. Experiment 2 (stress tolerance) used a 3 (AgNPs: 0, 40, 80 ppm) $\times$ 4 (Pb: 0, 200, 300, 400 μM) factorial CRD with 20 replicates per combination. Experimental unit = one explant per culture vessel; vessels were randomized. ANOVA models (one-way for BA; two-way for AgNPs $\times$ Pb) and p-level/LSD are reported in the manuscript tables. Data were analyzed using analysis of variance (ANOVA), and treatment means were compared using the Least Significant Difference (LSD) test at $p \leq 0.05$ [8].

The studied characteristics included morphological parameters such as the number of shoots, number of leaves, and plantlet height during the initiation and multiplication stages. In the phytoremediation stage, physiological and biochemical indicators were measured, including malondialdehyde (MDA), hydrogen peroxide (H_2O_2), proline content, and the enzymatic activities of catalase (CAT) and superoxide dismutase (SOD).

Results and Discussion:

Initiation Stage

Percentage of Dead Parts (%)

The percentage of dead parts (%) was determined by visually assessing each plantlet at the end of the culture period. The number of necrotic or dead explants was divided by the total number of cultured explants and multiplied by 100, according to the following formula:

$$\text{Dead parts (\%)} = (\text{Number of dead explants} / \text{Total explants}) \times 100.$$

The percentage of dead, non-responsive, and responsive explants was recorded at the end of the six-week initiation stage to evaluate the efficiency of BA concentrations

$$\text{Percentage of Response to Dead Parts} = \frac{\text{Number of Dead Plant Parts}}{\text{Total Number of Planted Parts}} \times 100$$

$$\text{Percentage of Live Plant Parts Not Responding to Growth (\%)} = \frac{\text{Number of Live Plants Not Responding}}{\text{Total Number of Planted Parts}} \times 100$$

$$\text{Percentage of Plants Responding to Growth \%} = \frac{\text{Number of Plants Responding to Growth}}{\text{Total Number of Planted Parts}} \times 100$$

The results of Table (1) showed that the highest response rate was The difference in optimal BA concentration between the initiation (1.5 mg L^{-1}) and multiplication (1.0 mg L^{-1})

stages is attributed to the distinct physiological requirements of basil tissues during *in vitro* development. A relatively higher BA level (1.5 mg L^{-1}) is needed during initiation to activate dormant meristems and promote bud induction, whereas a lower concentration (1.0 mg L^{-1}) during multiplication maintains balanced cell division and elongation without inducing hyperhydricity or callus formation. This variation reflects the shift in hormonal sensitivity as the explants progress from morphogenic induction to organized shoot proliferation. 96.67% at BA 1.5 mg L^{-1} . This difference may be attributed to the distinct physiological needs of explants at each stage, where higher BA levels (1.5 mg L^{-1}) stimulate meristem activation during initiation, while lower levels (1.0 mg L^{-1}) maintain hormonal balance and promote stable shoot proliferation during multiplication while the lowest rate was 80% at BA's absence. BA.

Table (1). Effect of BA concentrations on the percentages of explants during the initiation stage

BA concentration (mg L^{-1})	Dead explants (%)	Viable non-responsive explants (%)	Responsive explants (%)
0	13.3	6.7	80.0
0.5	10.0	3.3	86.7
1.0	6.7	3.3	90.0
1.5	3.3	0.0	96.67

Effect of cytokinin (BA) on vegetative growth during the multiplication stage

Table (2) shows that the number of leaves reached 14.00 at 1.0 mg L^{-1} BA compared with 9.20 at 0.0. While the number of branches was 3.45 at 1.5 mg L^{-1} BA versus 0.95 at 0.0 mg L^{-1} , while plantlet height did not differ significantly. The results in Tables (1) and (2) indicate that supplementing the culture medium with cytokinin (BA) produced a significant increase in explant response during the initiation stage. The 1.5 mg L^{-1} BA treatment showed the highest response compared

with the BA-free control, confirming BA's central role in stimulating cell division and activating axillary bud formation. Jakovljević et al. (2) reported that tissue culture protocols using BA are effective for improving regeneration rates and encouraging bud formation in basil, in addition to supporting the production of secondary metabolites of economic value. The present data also show that BA at 1.0 mg L⁻¹ markedly increased leaf number, whereas the higher concentration (1.5 mg L⁻¹) was more effective at increasing the count of lateral branches. No significant differences were detected in shoot length among treatments, suggesting that BA's effects at this stage are focused primarily on shoot and leaf proliferation. These findings agree with [9], who found that BA-based micropropagation protocols in basil effectively enhanced multiplication and increased shoot number compared with other treatments.

Table (2). Effect of adding cytokinin (BA) on some morphological traits

BA concentration (mg L ⁻¹)	Number of leaves	Number of branches	shoot length
0	9.2	0.95	3.0
1.0	14.0	2.75	2.0
1.5	12.8	3.45	3.0
2.0	11.9	1.7	2.0
Mean BA	11.97	2.21	3.0
LSD 5%	2.437	0.4997	NS

Effect of lead and silver nanoparticles on physiological stress indicators Malondialdehyde (MDA)

Analysis of variance (Table 3) revealed significant differences in malondialdehyde (MDA) content in plantlets under the effects of silver nanoparticles (AgNPs) and lead (Pb). For AgNPs, treatment Ag₀ produced the highest mean (8.36 µmol g⁻¹ FW), whereas Ag₂ showed the lowest mean (5.04 µmol g⁻¹ FW). For Pb, treatment Pb₀ recorded the highest mean (8.66 µmol g⁻¹ FW), while Pb₃ had the lowest mean (5.42 µmol g⁻¹ FW). Regarding the interaction between Pb and AgNPs, the Pb₀×Ag₀ combination yielded the highest mean (12.22 µmol g⁻¹ FW), whereas Pb₃×Ag₂ resulted in the lowest mean (3.23 µmol g⁻¹ FW).

Table (3). Effect of adding lead (Pb) and silver nanoparticles (AgNPs) on plant MDA content

Pb (µmol L ⁻¹)	Ag ₀ (0 µmol g ⁻¹ FW)	Ag ₁ (40 µmol g ⁻¹ FW)	Ag ₂ (80 µmol g ⁻¹ FW)	Mean Pb
Pb ₀	5.47	6.2	6.78	6.15
Pb ₁	5.63	8.51	7.44	7.19
Pb ₂	3.23	6.04	6.99	5.42
Pb ₃	5.83	7.92	12.22	8.66
Mean Ag	5.04	7.71	8.36	
LSD 5%	Ag×Pb = 1.827		Ag = 0.914	Pb = 1.055

Hydrogen peroxide (H₂O₂)

Statistical analysis results in Table 4 indicated significant differences in (H₂O₂) in plantlets due to the effects of silver nanoparticles (AgNPs) and lead (Pb). For AgNPs, treatment Ag₀ produced the highest mean (3.21 µmol g⁻¹ FW), whereas Ag₂ recorded the lowest mean (1.35 µmol g⁻¹ FW). Regarding Pb, treatment Pb₀ achieved the highest mean (2.44 µmol g⁻¹ FW) compared with Pb₃ (1.58 µmol g⁻¹ FW). In terms of interaction effects, Pb₀×Ag₀ gave the maximum mean value (4.74 µmol g⁻¹ FW), while Pb₃×Ag₂ resulted in the minimum (1.00 µmol g⁻¹ FW).

Table (4). Effect of adding lead (Pb) and silver nanoparticles (AgNPs) on plant H₂O₂ content

Pb (µmol g ⁻¹ FW)	Ag ₀ (0 µmol g ⁻¹ FW)	Ag ₁ (40 µmol g ⁻¹ FW)	Ag ₂ (80 µmol g ⁻¹ FW)	Mean Pb
Pb ₀	1.6	1.48	1.67	1.58
Pb ₁	1.42	2.04	2.98	2.15
Pb ₂	1.0	1.58	4.74	2.44
Pb ₃	1.38	1.57	3.46	2.14
Mean Ag	1.35	1.67	3.21	
LSD 5%	Ag×Pb = 0.9419		Ag = 0.4710	Pb = 0.5438

Proline

Table (5) shows significant differences in plantlet proline content under the influence of AgNP and Pb concentrations. Treatment Ag₀ recorded the highest mean (72.33 µmol g⁻¹ FW), while Ag₂ gave the lowest mean (61.28 µmol g⁻¹ FW). For Pb treatments, Pb₀ produced the highest mean (68.97 µmol g⁻¹ FW), whereas Pb₃ gave the lowest (63.77 µmol g⁻¹ FW).

FW). The interaction analysis revealed that Pb $\times$ Ag yielded the maximum mean (80.30 $\mu\text{mol g}^{-1}$ FW), while Pb $\times$ Ag resulted in the lowest value (59.63 $\mu\text{mol g}^{-1}$ FW).

Table (5). Effect of adding lead (Pb) and silver nanoparticles (AgNPs) on plant proline content

Pb ($\mu\text{mol L}^{-1}$ FW)	Ag0 (0 mg L^{-1} FW)	Ag1 (40 mg L^{-1} FW)	Ag2 (80 mg L^{-1} FW)	Mean Pb
Pb0	60.30	66.80	66.90	64.67
Pb1	59.63	61.33	70.33	63.77
Pb2	63.07	65.60	71.77	66.81
Pb3	62.10	64.50	80.30	68.97
Mean Ag	61.28	64.56	72.33	
LSD 5%	Ag $\times$ Pb = 4.453	Ag = 2.227	Pb = 2.571	

Catalase (CAT)

According to Table (6), catalase (CAT) activity showed significant variation in response to AgNP and Pb concentrations. Among the AgNP treatments, Ag recorded the highest mean (12.74 $\mu\text{mol g}^{-1}$), whereas Ag had the lowest mean (10.87 $\mu\text{mol g}^{-1}$). For Pb, treatment Pb gave the highest mean (13.29 $\mu\text{mol g}^{-1}$), while Pb produced the lowest (10.38 $\mu\text{mol g}^{-1}$). Although the interaction between Pb and AgNPs was not statistically significant, the Pb $\times$ Ag combination reached a relatively high value (14.47 $\mu\text{mol g}^{-1}$), whereas the Pb $\times$ Ag treatment showed the lowest mean (9.18 $\mu\text{mol g}^{-1}$).

Table (6). Effect of adding lead (Pb) and silver nanoparticles (AgNPs) on catalase (CAT) activity

Pb ($\mu\text{mol L}^{-1}$)	Ag0 (0 mg L^{-1})	Ag1 (40 mg L^{-1})	Ag2 (80 mg L^{-1})	Mean Pb
Pb0	9.18	10.94	11.02	10.38
Pb1	10.42	12.18	12.32	11.64
Pb2	12.64	14.47	12.77	13.29
Pb3	11.22	13.37	11.42	12.0
Mean Ag	10.87	12.74	11.88	
LSD 5%	Ag $\times$ Pb = NS	Ag = 1.104	Pb = 1.275	

Superoxide dismutase (SOD)

Result showed very highly significant differences on the activity of super oxide dismutase (SOD) enzyme by effect of nanosilver and lead concentrations (Table 7). Ag2 treatment showed a highest mean of 14.94 $\mu\text{mol g}^{-1}$, whereas Ag0 exhibited the lowest mean value of 12.40 $\mu\text{mol g}^{-1}$. The Pb3 treatment also outperformed others with an average of 14.55 micromoles/g, whereas the lowest average was at Pb0, at 12.46 micromoles/g. For the interactions, the Pb3 $\times$ Ag2 interaction gave the highest average, at 17.40 micromoles/g, while the Pb0 $\times$ Ag0 interaction recorded the lowest average, at 11.48 micromoles/g.

Table (7). Effect of adding lead (Pb) and silver nanoparticles (AgNPs) on superoxide dismutase (SOD)

Pb ($\mu\text{mol L}^{-1}$)	Ag0 (0 mg L^{-1})	Ag1 (40 mg L^{-1})	Ag2 (80 mg L^{-1})	Mean Pb
Pb0	11.48	11.83	14.07	12.46
Pb1	12.20	12.28	15.14	13.21
Pb2	12.96	15.25	13.13	13.78
Pb3	12.97	13.27	17.40	14.55
Mean Ag	12.40	13.15	14.94	
LSD 5%	Ag $\times$ Pb = 2.349	Ag = 1.175	Pb = 1.356	

Discussion

In response to the reviewer's comments, it is clarified that this study included two separate in vitro experiments. The first experiment aimed to optimize shoot initiation and multiplication using different concentrations of benzyladenine (BA), while the second evaluated the effects of silver nanoparticles (AgNPs) on basil plantlets exposed to lead (Pb) stress. The results of both were discussed together to link growth performance with stress tolerance rather than to suggest a direct interaction between BA and AgNPs. Moderate AgNP concentrations likely promoted oxidative balance by stimulating antioxidant enzyme activity (CAT and SOD) without inducing toxic oxidative damage. The lack of significant variation in leaf traits under Pb stress was due to these parameters being measured only during the BA experiment, while the Pb–AgNP stage focused mainly on biochemical stress indicators. The relatively short culture period and limited Pb mobility in the solid medium may also have minimized visible morphological differences. The results shown in Tables 3, 4, 5, 6, and 7 demonstrate a specific ethological and related physiological response, associated with high oxidative stress markers levels, induced by higher concentrations of Pb and AgNPs applied to basil plantlets. Both malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) levels were significantly higher at increased concentrations, representing lipid peroxidation and membrane destruction by the accumulation of Reactive Oxygen Species (ROS). These results are consistent with [3] and [10] who reported that as the amount of heavy metals accumulate in plant tissues, these oxidative stress indicators such as MDA, H_2O_2 rise. Similarly,

[11] identified Pb as a toxic element, and they concluded that this severely restricted vegetative growth via decreased cell division and photosynthesis in plantlets, on the other hand, proline accumulation was observed to be significantly high at higher concentration of Pb and AgNPs that may act as an osmolyte which regulate cellular osmotic potential and protect protein by acting as a secondary antioxidant. This finding is in agreement to the result of [1, 4, and 12], which considers proline as a major defense strategy in basil against environmental stresses [13], who also indicated that the K^+/Na^+ ratio was significant for plant tolerance under metal and salt stress. Although higher MDA and H_2O_2 levels generally indicate oxidative stress, the moderate increase observed under AgNP treatments may reflect an adaptive redox response that activates antioxidant enzymes (CAT and SOD), helping to restore oxidative balance rather than causing severe damage. With regard to antioxidant enzymes, activities of CAT and SOD were found to be significantly increased suggesting their important role as a first line of defense against ROS accumulation. CAT detoxicates one molecule of H_2O_2 to water and oxygen, while SOD promotes conversion of superoxide radicals to peroxides which are scavenged out. These data are also confirmed by [5] and [14] who noticed an increase in antioxidant enzyme activity on basil at certain AgNPs concentrations. Similarly, [15] suggested that the enhanced activity of CAT and SOD is a straightforward, pivotal plant defense response against increased ROS under metal stress, while [16] observed that antioxidant activity changes with the nature and degree of stress. Other works, such as [17] that genotype-specific regulations of ion uptake may explain differences in plantlet responses. [18] also proved that basil has strong physiological responses to environmental stress. Based on these results can be interpreted as indicating that at low doses, AgNPs primed the defense while repressing the oxidative load, while at high doses, they facilitated the stress. It therefore suggests that the AgNP levels should be regulated to maintain defense activation to cause minimal stress. This is consistent with the research by [19,20]. that stated that nanoparticles' concentrations in culture media demonstrate that nanomaterials could induce either a stimulatory or a non-stimulatory growth effect depending on their level in the culture media

Conclusion

The current study demonstrated that under lead stress, MDA and H_2O_2 contents escalated in plants while proline was accumulated as an antioxidant defense. AgNPs were shown to have a biphasic effect, that is, at the optimal concentrations they induced an increase in activities of CAT and SOD as defensive enzymes and reduced stress rather at high concentrations. Tissue culture proved to be a good tool for screening response of basil, and the results obtained here should aid in orienting further investigations towards formulating amelioration strategies, based on concentration of AgNP, in pollutant injury. The manuscript was revised to clearly separate the BA micropropagation phase from the Pb+AgNPs stress phase in both Materials and Methods and Results/Discussion. While BA and AgNPs were not tested as interacting factors, the best-performing plantlets from the BA phase were used as source material for the stress phase. We therefore discuss them in sequence (not as a single combined factor), and we added an orienting paragraph at the start of *Materials & Methods* plus a sentence in the *Conclusion* to reflect this two-stage design.

References

- [1]. Azizah, N. S.; B. Irawan; J. Kusmoro; W. Safriansyah; K. Farabi; D. Oktavia ; M. Miranti, 2023. Sweet basil (*Ocimum basilicum* L.)—A review of its botany, phytochemistry, pharmacological activities, and biotechnological development. *Plants*, 12(24), 4148.
- [2]. Jakovljević, D.; M. Stanković; M. Warchoń; E. Skrzypek, 2022. Basil (*Ocimum* L.) cell and organ culture for the secondary metabolites production: a review. *Plant Cell, Tissue and Organ Culture (PCTOC)*, 149(1), 61–79.
- [3]. Georgiadou, E. C.; E. Kowalska; K. Patla; K. Kulbat; B. Smolińska; J. Leszczyńska; V. Fotopoulos, 2018. Influence of heavy metals (Ni, Cu, and Zn) on nitro-oxidative stress responses, proteome regulation and allergen production in basil (*Ocimum basilicum* L.) plants. *Frontiers in Plant Science*, 9, 862.
- [4]. Biswas, K.; S. Adhikari; A. Tarafdar; R. Kumar; S. Saha; P. Ghosh, 2020. Reactive oxygen species and antioxidant defence systems in plants: role and crosstalk under biotic stress. In: *Sustainable Agriculture in the Era of Climate Change*, 265–292. Springer, Cham.
- [5]. Shahraki, S. H.; T. Ahmadi; B. Jamali; M. Rahimi, 2024. The biochemical and growth-associated traits of basil (*Ocimum basilicum* L.) affected by silver nanoparticles and silver. *BMC Plant Biology*, 24(1), 92.
- [6]. Górski, F.; G. M. Gerotti; H. M. Magalhães, 2021. Relationship between auxins and cytokinins in the growth and organogenesis of *Ocimum basilicum* L. 'Grecco a Palla'. *Canadian Journal of Plant Science*, 101(5), 698–713. (Spelled "Gorski" in manuscript text.)
- [7]. Murashige, T.; F. Skoog, 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia Plantarum*, 15(3).
- [8]. Al-Rawi, K. M.; A. A. Khalaf-Allah, 2000. *Design and analysis of agricultural experiments*. Mosul University, Directorate of Books for Printing and Publishing, Iraq.
- [9]. Barberini, S.; C. Forti; M. Laura; R. Ciorba; C. Mascarello; A. Giovannini; ...; M. Savona, 2023. An optimized protocol for in vitro regeneration of *Ocimum basilicum* cv. FT Italiko. *Horticulturae*, 9(3), 407.

- [10]. Escoto, J., 2022. *Evaluation of plant growth regulators in the in vitro multiplication stage of basil Ocimum basilicum L.* Doctoral dissertation, Zamorano: Escuela Agrícola Panamericana.
- [11]. Lamhamdi, M. ; A. Bakrim; A. Aarab; R. Lafont; F. Sayah. 2011. Lead phytotoxicity on wheat (*Triticum aestivum* L.) seed germination and seedlings growth. *Comptes Rendus Biologies*. 334 (2): 118-126 <https://doi.org/10.1016/j.crv.2010.12.006>
- [12]. Pan, R.; Z. Zhang; Y. Li; S. Zhu; S. Anwar; J. Huang; ...; L. Yin, 2024. Stage-specific effects of silver nanoparticles on physiology during the early growth stages of rice. *Plants*, 13(23), 3454.
- [13]. Manikandan, R.; S. V. Sahi; P. Venkatachalam, 2015. Impact assessment of mercury accumulation and biochemical and molecular response of *Mentha arvensis*: a potential hyperaccumulator plant. *The Scientific World Journal*, 2015(1), 715217.
- [14]. Hussain, I.; N. B. Singh; A. Singh; H. Singh; S. C. Singh, 2016. Green synthesis of nanoparticles and its potential application. *Biotechnology Letters*, 38(4), 545–560.
- [15]. Rao, M. J.; M. Duan; C. Zhou; J. Jiao; P. Cheng; L. Yang; ...; B. Zheng, 2025. Antioxidant defense system in plants: reactive oxygen species production, signaling, and scavenging during abiotic stress-induced oxidative damage. *Horticulturae*, 11(5), 477.
- [16]. Kőszeghe, S.; C. Bereczki; A. Balog; K. Benedek, 2014. Comparing the effects of benzyladenine and meta-topolin on sweet basil (*Ocimum basilicum*) micropropagation. *Notulae Scientia Biologicae*, 6(4), 422–427.
- [17]. Ciriello, M.; L. Formisano; M. C. Kyriacou; P. Carillo; L. Scognamiglio; S. De Pascale; Y. Roupheal, 2022. Morpho-physiological and biochemical responses of hydroponically grown basil cultivars to salt stress. *Antioxidants*, 11(11), 2207.
- [18]. Dudai, N.; N. Nitzan; I. Gonda, 2020. *Ocimum basilicum* L. (Basil). In: *Medicinal, Aromatic and Stimulant Plants*, 377–405. Springer, Cham.
- [19]. Venkatachalam, P.; M. Jayaraj; R. Manikandan; N. Geetha; E. R. Rene; N. C. Sharma; S. V. Sahi, 2017. Zinc oxide nanoparticles (ZnONPs) alleviate heavy metal-induced toxicity in *Leucaena leucocephala* seedlings: a physiochemical analysis. *Plant Physiology and Biochemistry*, 110, 59–69.
- [20]. SekhI, Y., Kadhim, Z., & Hamad, A. (2022). Effect of Sodium Azide on some vegetative and biochemical properties of Strawberry under Polyethylene Glycol of Albion variety in vitro. *Annals of Agri-bio Research*, 28(1), 121-128.

تأثير جسيمات الفضة النانوية والبنزل أدنين في المؤشرات الفسيولوجية للإجهاد لنبات الريحان *Ocimum basilicum* L. المعرض لأيونات الرصاص خارج الجسم الحي

احمد محمود شكري¹ ياسر صايل صخي¹

جامعة الانبار، كلية الزراعة، قسم البستنة وهندسة الحدائق.

الخلاصة

تم تنفيذ هذه الدراسة في مختبر زراعة الانسجة النباتية - قسم البستنة وهندسة الحدائق/ كلية الزراعة/ جامعة الانبار، بهدف تقييم تأثير تراكيز من الساييتوكاينين (*BA*) في مرحلتي النشوء والتضاعف لنبات *Ocimum basilicum* L. تحت ظروف الزراعة المختبرية خارج الجسم الحي، اضافة الى دراسة دور الفضة النانوية (*AgNPs*) في استجابة النباتات لاجهاد ايونات الرصاص (*Pb*). اظهرت النتائج في مرحلة النشوء ان *BA* بتركيز 1.5 ملغم لتر⁻¹ اعطى اعلى نسبة استجابة بلغت 96.67%. وفي مرحلة التضاعف سجل *BA* بتركيز 1.0 ملغم لتر⁻¹ اعلى عدد اوراق بواقع 14.0 ورقة، بينما حقق التركيز 1.5 ملغم لتر⁻¹ اعلى عدد افرع بواقع 3.45 فرعاً، ولم يظهر فرق معنوي في ارتفاع النبات تحت تأثير الرصاص ومع وجود *AgNPs* (0 و 40 و 80 ppm) ارتفعت قيم المألون ثنائي الالديهايد (*MDA*) الى اعلى قيمة عند التداخل *Pb3×Ag2* بواقع 12.22 مايكرومول لتر⁻¹ ووزن طري، وسجل بيروكسيد الهيدروجين (*H₂ O₂*) اعلى قيمة عند التداخل *Pb2×Ag2* بلغت 4.74 مايكرومول لتر⁻¹ ووزن طري كما ارتفع البرولين الى اعلى قيمة عند التداخل *Pb3×Ag2* بواقع 80.30 مايكرومول لتر⁻¹ ووزن طري وفيما يخص الانزيمات الدفاعية فقد سجل الكاتاليز (*CAT*) اعلى نشاط عند التداخل *Pb2×Ag1* بواقع 14.47 مايكرومول لتر⁻¹ ووزن طري، بينما سجل السوبر اوكسيد ديسموتاز (*SOD*) اعلى قيمة عند التداخل *Pb3×Ag2* بواقع 17.40 مايكرومول لتر⁻¹ ووزن طري. وبشكل عام، يمكن القول ان *AgNPs* لم تكن عاملاً مثبطاً لمؤشرات الاكسدة، لكنها ساعدت على موازنة العلاقة بين انتاج *ROS* وتنشيط الاليات الدفاعية عند التراكيز المناسبة، مما يؤكد ان اعتماد التراكيز المثلى يحقق استجابة افضل ويرتبط بتحسين المؤشرات الفسيولوجية والصفات الانتاجية اللاحقة.

الكلمات المفتاحية: الريحان (*Ocimum basilicum* L.)، الفضة النانوية (*AgNPs*)، إجهاد الرصاص (*Pb*)، مؤشرات الإجهاد التأكسدي، الإنزيمات المضادة للأكسدة.