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Research Article

Selenium nanoparticles mitigate lead-induced genotoxicity and phytotoxicity in *Pisum sativum* by modulating *LOX1* and *JAR1* gene expression

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Abstract

This study investigated the effects of lead nitrate ($Pb(NO_3)_2$) at 90, 180, and 270 mg L^{-1} on pea (*Pisum sativum* L.) and evaluated the potential of selenium nanoparticles (SeNPs) at 10, 30, and 60 mg L^{-1} to alleviate lead-induced genotoxicity. We analyzed seedling and vegetative growth, DNA damage using the comet assay, and the expression profiles of the stress-related genes *LOX1* and *JAR1*. $Pb(NO_3)_2$ significantly inhibited pea growth, leading to severe reductions in shoot and root lengths, biomass, and number of leaves. Conversely, SeNPs alone enhanced these growth parameters and effectively mitigated lead-induced phytotoxicity. The 60 mg L^{-1} SeNP treatment, especially when combined with lead, demonstrated the most pronounced improvement in shoot and root development. Comet assay analysis in leaf cells revealed a concentration-dependent increase in DNA damage following $Pb(NO_3)_2$ exposure. The highest genotoxicity, characterized by increased percentages of tailed DNA, tail lengths, and tail moments, was consistently observed with the 270 mg L^{-1} $Pb(NO_3)_2$ treatment, alone or combined with 10 mg L^{-1} SeNP. In contrast, the 60 mg L^{-1} SeNPs+90 mg L^{-1} $Pb(NO_3)_2$ treatment resulted in the lowest DNA damage among lead-exposed groups. Furthermore, 270 mg L^{-1} $Pb(NO_3)_2$ significantly upregulated *LOX1* and *JAR1* expression. The sole application of 60 mg L^{-1} SeNPs substantially reduced *LOX1* expression below control levels. Notably, the 60 mg L^{-1} SeNP+270 mg L^{-1} $Pb(NO_3)_2$ combined treatment significantly downregulated the expression of both genes compared to the 270 mg L^{-1} $Pb(NO_3)_2$ treatment alone. These findings suggest that SeNPs can modulate lead-induced stress and mitigate genotoxicity in *P. sativum* by potentially influencing the *LOX1* and *JAR1* signaling pathways.

Keywords: comet assay; cytogenetic; gene expression; genotoxicity; lead; nanoparticles; qPCR; selenium

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Introduction

Lead (Pb) contamination represents a significant global environmental challenge, posing substantial threats to both ecosystem health and agricultural productivity (Ali *et al.*, 2024).. Its pervasive presence in soil and water leads to widespread plant uptake, triggering detrimental effects at physiological, biochemical, and genetic levels (Gupta *et al.*, 2024). *Pisum sativum* L. (garden pea) serves as a vital legume crop and a valuable model organism for investigating heavy metal stress impacts due to its economic importance and well-characterized genome (Galal *et al.*, 2021). Lead exposure consistently induces genotoxicity in plants, manifesting as DNA damage, and altered gene expression, consequently impairing growth, development, and yield (Nagaraju *et al.*, 2022).

In the pursuit of effective strategies to mitigate lead toxicity in plants, nanotechnology has emerged as a promising field. Selenium nanoparticles (SeNPs) have garnered considerable attention due to their unique physicochemical properties and demonstrated potential in alleviating heavy metal stress (Ahmad *et al.*, 2024). Selenium, an essential micronutrient at low concentrations, exhibits antioxidant properties and plays a crucial role in plant defense mechanisms. At the nanoscale, the increased surface area and reactivity of selenium can amplify its protective effects against lead-induced damage (Ghassemi Toussi *et al.*, 2025).

The comet assay, also known as single-cell gel electrophoresis, is a sensitive and widely used technique for assessing DNA damage in individual cells. This assay is particularly valuable in environmental toxicology studies, including the evaluation of the genotoxic effects of heavy metals (Collins *et al.*, 2023). Heavy metals, such as lead, mercury, cadmium, and arsenic. The comet assay provides a rapid and reliable method for detecting and quantifying this DNA damage, making it an essential tool in understanding the mechanisms of heavy metal toxicity and their potential risks to human and environmental health (Ji *et al.*, 2022).

Plants respond to diverse environmental stresses through complex molecular mechanisms that involve specific gene regulation. Among these, *LOX1* (Lipoxygenase 1) and *JAR1* (Jasmonate Resistant 1) play crucial roles in stress signaling (Li *et al.*, 2022; Singh *et al.*, 2022). *LOX1* initiates the jasmonic acid (JA) biosynthesis pathway by catalyzing the oxygenation of polyunsaturated fatty acids. Subsequently, *JAR1* functions by conjugating JA to isoleucine, thereby forming the bioactive hormone JA-Ile. The coordinated expression of these genes is essential for regulating plant defenses and developmental processes in response to various stressors, including heavy metals (Samanta and Roychoudhury, 2025).

LOX1 and *JAR1* were specifically chosen as they represent two critical, sequential steps in the biosynthesis and activation of the Jasmonate (JA) signaling pathway, a central regulator of plant defense against diverse abiotic stresses, including drought, salinity, and heavy metal toxicity (Raza *et al.*, 2021). *LOX1* is essential for the initial synthesis of JA, while *JAR1* catalyzes its final activation into the JA-Ile conjugate (Ding *et al.*, 2016). By focusing on these two key genes, this study aims to bridge the critical knowledge gap concerning the mechanistic involvement of the JA pathway in heavy metal detoxification and tolerance. This targeted assessment provides an evaluation of their potential as genetic markers for developing metal-tolerant crop varieties.

Therefore, this study evaluated the effectiveness of selenium nanoparticles (SeNPs) in counteracting lead (Pb(NO3)2)-induced genotoxicity in *P. sativum*. Specifically, we analyzed phenotypic parameters, comet assay results, and alterations in the expression of the key stress-response genes, *LOX1* and *JAR1*, following lead exposure and SeNPs treatment. This comprehensive approach aims to elucidate the protective mechanisms conferred by selenium nanoparticles against lead-induced genotoxicity in *P. sativum*. The findings are anticipated to offer valuable insights into the potential application of nanotechnology in developing sustainable strategies for mitigating heavy metal contamination in agricultural systems, thereby enhancing crop productivity and environmental safety in lead-polluted areas.

Materials and methods

Plant materials and experiment layout

Seeds of *P. sativum* L. (cv. 'Master B') were used for controlled pot experiment. The study comprised sixteen treatments, including control and fifteen treatments of lead nitrate and selenium nanoparticles applied individually or in combinations in different concentrations as presented in Table 1. The applied treatments were arranged in Completely Randomized Design (CRD) with three replicates. Each replicate consisted of a plastic pot filled with 5 kg of soil sown with eight seeds. The trial was conducted at the Faculty of Agriculture, Zagazig University, Egypt (30°35' N, 31°30' E) during the winter of 2025. Lead nitrate was applied to soil at concentrations of 0, 90, 180, and 270 mg L^{-1}. Selenium nanoparticles were exogenously applied at 0, 10, 30, and 60 mg L^{-1}. The corresponding SeNP doses were applied onto leaves at 2 and 5 weeks post-germination and on buds at 6 weeks.

Table 1. Experiment applied treatments using lead nitrate ($Pb(NO_3)_2$) and selenium nanoparticle (SeNPs) in pea plants across growth stages

Treatments	Seeds and soil treatments $Pb(NO_3)_2$	Leaves treatments (SeNPs)		Floral buds treatments (SeNPs)
		Two weeks after germination	Five weeks after germination	Six weeks after germination
Control	-	-	-	-
T1	-	SeNPs (10 mg L^{-1})	SeNPs (10 mg L^{-1})	SeNPs (10 mg L^{-1})
T2	-	SeNPs (30 mg L^{-1})	SeNPs (30 mg L^{-1})	SeNPs (30 mg L^{-1})
T3	-	SeNPs (60 mg L^{-1})	SeNPs (60 mg L^{-1})	SeNPs (60 mg L^{-1})
T4	$Pb(NO_3)_2$ (90 mg L^{-1})	-	-	-
T5	$Pb(NO_3)_2$ (180 mg L^{-1})	-	-	-
T6	$Pb(NO_3)_2$ (270 mg L^{-1})	-	-	-
T7	$Pb(NO_3)_2$ (90 mg L^{-1})	SeNPs (10 mg L^{-1})	SeNPs (10 mg L^{-1})	SeNPs (10 mg L^{-1})
T8	$Pb(NO_3)_2$ (180 mg L^{-1})	SeNPs (10 mg L^{-1})	SeNPs (10 mg L^{-1})	SeNPs (10 mg L^{-1})
T9	$Pb(NO_3)_2$ (270 mg L^{-1})	SeNPs (10 mg L^{-1})	SeNPs (10 mg L^{-1})	SeNPs (10 mg L^{-1})
T10	$Pb(NO_3)_2$ (90 mg L^{-1})	SeNPs (30 mg L^{-1})	SeNPs (30 mg L^{-1})	SeNPs (30 mg L^{-1})
T11	$Pb(NO_3)_2$ (180 mg L^{-1})	SeNPs (30 mg L-1)	SeNPs (30 mg L^{-1})	SeNPs (30 mg L^{-1})
T12	$Pb(NO_3)_2$ (270 mg L^{-1})	SeNPs (30 mg L^{-1})	SeNPs (30 mg L^{-1})	SeNPs (30 mg L^{-1})
T13	$Pb(NO_3)_2$ (90 mg L^{-1})	SeNPs (60 mg L^{-1})	SeNPs (60 mg L^{-1})	SeNPs (60 mg L^{-1})
T14	$Pb(NO_3)_2$ (180 mg L^{-1})	SeNPs (60 mg L^{-1})	SeNPs (60 mg L^{-1})	SeNPs (60 mg L^{-1})
T15	$Pb(NO_3)_2$ (270 mg L^{-1})	SeNPs (60 mg L^{-1})	SeNPs (60 mg L^{-1})	SeNPs (60 mg L^{-1})

Selenium nanoparticles (SeNPs) synthesis

Selenium nanoparticles (SeNPs) were biogenically synthesized with minor modifications to the protocol described by (Alam *et al.*, 2019). Briefly, 900 mL of a freshly prepared 10 mM aqueous solution of sodium selenite (Na_2SeO_3) was reacted with 100 mL of a diluted methanol extract of *Gossypium barbadense* leaves (prepared by diluting a 3 mg stock extract in 100 mL distilled water). The reaction was carried out at 60 °C for 12 hours under continuous stirring at 1000 rpm using a hot plate with a magnetic stirrer. Following the reaction, the synthesized SeNPs were isolated and purified through centrifugation at 8000 rpm for 20 minutes. The resulting nanoparticle pellet was then redispersed in acetone. Finally, the purified SeNPs were air-dried and stored at 4 °C for subsequent analysis.

Characterization of selenium nanoparticles

The synthesized selenium nanoparticles (SeNPs) underwent initial pH determination via digital potentiometry (Eutech Cyberscan pH 300). Subsequently, their optical properties were characterized by UV-

Vis spectrophotometry (Rigol Ultra-3660) across the 200–800 nm spectral range. Morphological assessment, size analysis, and surface topography of the SeNPs were conducted using Field Emission Scanning Electron Microscopy (FESEM). For FESEM, SeNP suspensions were sonicated for 15 minutes at ambient temperature. A droplet of the dispersed sample was deposited onto a glass substrate, air-dried, sputter-coated with gold, and imaged using a Zeiss Evo-MA 10 FESEM (Germany). High-Resolution Transmission Electron Microscopy (HR-TEM) was utilized for detailed nanostructural elucidation. For HR-TEM, selenium nanopowder was dispersed in ethanol via sonication, deposited onto a copper grid, and air-dried prior to analysis with a JEOL-2100 HR-TEM.

Recorded seedling and growth parameters

Samples from each pot were collected at 25 days after sowing (seedling stage), and 45 days after sowing (vegetative growth stage) to determine root and shoot length (cm), root and shoot fresh weight (g), root and shoot dry weight (g), and number of leaves.

Comet assay for assessment of plant nuclear DNA damage

Nuclei were isolated from individual leaf samples by gently slicing leaves into a fringe within a Petri dish containing 200 µL of ice-cold 400 mM Tris-HCl buffer (pH 7.5) under yellow light, a method optimized for minimizing control cell DNA damage. Resulting nuclear suspensions were mixed 1:1 with 1% low melting point (LMP) agarose in phosphate-buffered saline (PBS) at 40 °C, layered onto slides pre-coated with 1% normal melting point (NMP) agarose, and solidified on ice. Following removal of the coverslip, a 0.5% LMP agarose overlay was applied and solidified. Single cell gel electrophoresis (SCGE) slides were then incubated with mutagen solutions for 2 hours at 26 °C, rinsed in cold distilled water, and subjected to electrophoresis in 300 mM NaOH, 1 mM EDTA (pH >13) at 16 V and 300 mA for 30 minutes at 4 °C, conditions previously established for optimal control cell integrity and linear comet induction. Gels were neutralized with 400 mM Tris-HCl (pH 7.5), stained with 20 µg/mL ethidium bromide, and analyzed using fluorescence microscopy (546 nm excitation, 590 nm barrier filters) and a computerized image analysis system (Komet 3.1) to quantify DNA damage via tail DNA percentage and tail moment, with 50 cells analyzed per slide (Juchimiuk *et al.*, 2006).

Extraction of total RNA

Total RNA was extracted from leaf tissue using the GeneJET Plant RNA Purification Mini Kit (Thermo Fisher Scientific, K0801), incorporating an on-column DNase I digestion step with the RNase-Free DNase Set (Qiagen, 79254) to eliminate genomic DNA contamination. RNA quality and quantity were assessed using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific) via UV-Vis spectrophotometry, and RNA integrity was confirmed by gel electrophoresis.

Quantitative real-time PCR analysis of gene expression

One-step RT-qPCR was performed using the LightCycler 480 system (Roche) with a 25 µL reaction volume containing 3 µL of RNA template, 0.5 µL each of forward and reverse primers (20 pmol), 12.5 µL of 2x QuantiTect SYBR Green PCR Master Mix, and 8.25 µL of nuclease-free water. The amplification protocol consisted of reverse transcription at 50 °C for 30 minutes, followed by initial denaturation at 94 °C for 15 minutes. Forty amplification cycles were performed, each with denaturation at 94 °C for 15 seconds, annealing at 60 °C for 30 seconds (with fluorescence detection), and extension at 72 °C for 40 seconds. A dissociation curve analysis was conducted with denaturation at 94 °C for 1 minute, annealing at 60 °C for 1 minute, and a final denaturation at 94 °C for 1 minute to confirm specific product amplification. The primers for the genes were as follows: *LOX1* forward, 5'-TGACACTGTTCAAAAAGACATTGA-3' and reverse, 5'-GTGACCCTTTTCGACAGCTT-3'; *JAR1* forward, 5'-GAAAAGGGGGTGTGATGCTA-3' and

reverse, 5'-GCATTCTTGTATCCCGGATTT-3'; and *Actin* forward, 5'-CCAAATCATGTTTGAGGCTTTTAA-3' and reverse, 5'-GTGAAAGAACGGCCTGAATAGC-3'. Cycle threshold (Ct) values were determined using the Stratagene MX3005P software. Relative gene expression was calculated using the ΔΔCt method, as described by (Yuan *et al.*, 2006), with normalization against reference genes to account for variations in RNA input. Dissociation curves were compared across samples to exclude non-specific amplification ($2^{-\Delta\Delta ct}$).

Whereas ΔΔCt = ΔCt reference – ΔCt target

Δ*Ct target* = *Ct control* – *Ct treatment* and Δ*Ct reference* = *Ct control- Ct treatment*

Statistical analysis

Data were analyzed using one-way ANOVA, and means were separated using the Least Significant Differences (LSD) test at a significance of $P < 0.01$. Data are expressed as mean ± standard deviation (SD).

Results

Seedling performance

The effects of lead nitrate ($Pb(NO_3)_2$) and selenium nanoparticles (SeNPs) on *P. sativum* were evaluated at the seedling stage. The measurements of shoot and root length, biomass accumulation, and leaf development revealed distinct responses to individual or combined treatments during this critical stage. Lead nitrate ($Pb(NO_3)_2$) caused significant reductions in seedling growth parameters of *P. sativum* (Table 2). The highest Pb concentration (T6, 270 mg L^{-1}) induced the most severe inhibition with reduced shoot length (12.17 cm) and root length (12.67 cm) which were significantly lower than the non-stressed control (22.0 cm and 18.67 cm, respectively). The applied SeNPs-only (T1-T3) significantly enhanced shoot and root growth and surpassed the untreated control. Furthermore, the combined SeNPs with $Pb(NO_3)_2$ treatments displayed significant mitigation of Pb toxicity, particularly with higher concentrations of SeNPs (T13-T15) compared to Pb-alone treatments.

Table 2. Effects of lead nitrate ($Pb(NO_3)_2$) and selenium nanoparticles (SeNPs) on seedling parameters of *P. sativum* under different treatment combinations

Treatment	Shoot length (cm)	Root length (cm)	Shoot fresh weight (g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)	Number of leaves
Control	22.00 ± 1.00 de	18.67 ± 1.53 cd	1.38 ± 0.069 cd	1.38 ± 0.042 bcd	0.340 ± 0.044 abc	0.208 ± 0.014 d-g	4.33 ± 0.58 abc
T1	20.50 ± 0.87 e	19.00 ± 2.00 bcd	1.45 ± 0.080 bcd	1.43 ± 0.112 bc	0.363 ± 0.084 ab	0.306 ± 0.026 bc	4.67 ± 0.58 ab
T2	22.33 ± 0.58 cde	17.33 ± 1.53 def	1.64 ± 0.091 ab	1.77 ± 0.057 a	0.252 ± 0.035 bcd	0.165 ± 0.028 fg	5.00 ± 1.00 a
T3	25.33 ± 0.58 ab	21.00 ± 0.87 ab	1.75 ± 0.184 a	1.80 ± 0.064 a	0.353 ± 0.066 ab	0.282 ± 0.037 cd	4.67 ± 0.58 ab
T4	16.33 ± 0.58 fg	13.33 ± 1.53 hi	0.57 ± 0.070 g	1.05 ± 0.021 g	0.163 ± 0.033 d	0.384 ± 0.038 ab	3.67 ± 0.58 bcd
T5	14.17 ± 0.76 gh	12.33 ± 0.58 i	0.56 ± 0.088 g	0.48 ± 0.031 h	0.168 ± 0.032 d	0.299 ± 0.014 bc	3.00 ± 0.01 d
T6	12.17 ± 0.76 h	12.67 ± 0.58 i	0.40 ± 0.058 g	0.30 ± 0.009 i	0.172 ± 0.011 d	0.188 ± 0.028 efg	3.00 ± 0.01 d
T7	17.00 ± 1.00 f	16.33 ± 0.58 efg	1.19 ± 0.082 de	1.20 ± 0.007 efg	0.258 ± 0.024 bcd	0.267 ± 0.027 cde	4.00 ± 0.01 a-d
T8	16.50 ± 0.50 fg	15.33 ± 0.58 fgh	1.11 ± 0.121 ef	1.13 ± 0.002 fg	0.177 ± 0.018 cd	0.238 ± 0.006 c-g	3.33 ± 0.58 cd
T9	15.17 ± 0.76 fg	14.50 ± 0.50 ghi	1.02 ± 0.086 ef	1.12 ± 0.012 fg	0.262 ± 0.061 a-d	0.160 ± 0.038 fg	3.00 ± 0.01 d
T10	21.00 ± 1.73 e	19.67 ± 1.15 bc	1.54 ± 0.098 abc	1.34 ± 0.02 cde	0.278 ± 0.023 a-d	0.282 ± 0.060 cd	4.00 ± 0.01 a-d
T11	25.00 ± 1.00 ab	19.00 ± 1.00 bcd	1.24 ± 0.194 de	1.27 ± 0.054 def	0.362 ± 0.054 ab	0.243 ± 0.029 c-f	3.67 ± 0.58 bcd
T12	23.67 ± 1.53 bcd	18.33 ± 0.58 cde	0.85 ± 0.066 f	1.25 ± 0.061 def	0.302 ± 0.021 a-d	0.187 ± 0.041 efg	3.00 ± 0.01 d
T13	27.33 ± 1.15 a	22.50 ± 0.50 a	1.65 ± 0.136 ab	1.51 ± 0.032 b	0.345 ± 0.039 ab	0.469 ± 0.043 a	4.33 ± 0.58 abc
T14	25.67 ± 1.53 ab	18.67 ± 0.58 cd	1.37 ± 0.173 cd	1.39 ± 0.063 bcd	0.339 ± 0.086 abc	0.152 ± 0.049 g	4.00 ± 0.01 a-d
T15	24.67 ± 1.53 bc	17.50 ± 0.50 c-f	1.37 ± 0.132 cd	1.34 ± 0.120 cde	0.424 ± 0.024 a	0.208 ± 0.051 d-g	4.00 ± 0.01 a-d
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Data are expressed as means ± standard deviation (SD). Means with different letters are significantly different according to the Least Significant Differences Test ($P < 0.01$)

Both shoot and root biomass (fresh and dry weight) were severely reduced by $Pb(NO_3)_2$ treatments (T4-T6) compared to the non-stressed control. Among these treatments, T6 exhibited the lowest values of shoot fresh weight (0.40 g), root fresh weight (0.30 g), shoot dry weight (0.172 g) and root dry weight (0.188 g). On the other hand, SeNPs-only treatments in different concentrations (T1-T3) enhanced biomass accumulation compared to the untreated control. Among these treatments, T3 demonstrated significantly higher fresh and dry shoot and root weights. The combined application of 60 mg L^{-1} SeNPs with $Pb(NO_3)_2$ at the applied three concentrations (T13-T15) significantly mitigated the Pb-induced biomass reduction, with T13 exhibited the most improvement producing biomass parameters. The number of leaves reflected similar trends, with the lowest counts under Pb-only treatments (T5-T6). Selenium application in Pb-stressed plants (T13-T15) resulted in a significant increase in leaf number.

Vegetative growth performance

The results of vegetative growth stage demonstrated the effects of $Pb(NO_3)_2$ and SeNPs on *P. sativum* development (Table 3). In Pb-only treatments (T4-T6), vegetative shoot and root length were significantly reduced, with T6 exhibited the lowest values of shoot length (22.33 cm) and root length (19.50 cm), which were significantly lower than the untreated control (shoot length 34.67 cm and root length 26.83 cm). SeNP-only treatment in the applied thre concentrations (T1-T3) exhibited improved vegetative growth, with T3 produced the highest shoot length (38.67 cm) and root length (28.33 cm), significantly greater than untreated control plants. Shoot and root fresh weights followed similar patterns, with Pb-only treatments caused significant reductions in particular T6 (shoot fresh weight 0.57 g; root fresh weight 1.25 g). Whereas SeNP-only treatments, especially T3, exhibited significant improvement compared to untreated plants. Moreover, combined treatments enhanced these parameters, with T13 surpassed Pb-only plants. The lowest number of leaves was assigned for Pb-only treatments in particular T6 (6.67 leaves) and increased in combined treatments, with T13, and T14 displayed the highest counts (10.67 and 10.33 leaves) compared to stressed control (Pb-only plants).

Table 3. Effects of lead nitrate ($Pb(NO_3)_2$) and selenium nanoparticles (SeNPs) on vegetative growth parameters of *P. sativum* under different treatment combinations

Treatment	Shoot length (cm)	Root length (cm)	Shoot fresh weight (g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)	Number of leaves
Control	34.67 ± 0.50 de	26.83 ± 0.76 cde	1.59 ± 0.14 e	1.36 ± 0.06 f	0.544 ± 0.07 c	0.417 ± 0.08 a-d	9.33 ± 0.58 abc
T1	36.67 ± 1.53 bc	26.33 ± 0.58 de	1.59 ± 0.23 e	1.37 ± 0.17 f	0.679 ± 0.10 ab	0.439 ± 0.10 a-d	10.00 ± 10 ab
T2	37.00 ± 1.04 abc	26.67 ± 0.58 cde	2.39 ± 0.17 bc	1.80 ± 0.17 e	0.584 ± 0.03 bc	0.549 ± 0.10 a	10.67 ± 0.58 a
T3	38.67 ± 0.50 a	28.33 ± 0.76 b	2.83 ± 0.12 a	1.93 ± 0.07 de	0.749 ± 0.09 a	0.446 ± 0.12 abc	7.67 ± 0.58 de
T4	26.67 ± 0.50 h	22.67 ± 0.58 hi	0.92 ± 0.12 f	1.30 ± 0.05 f	0.403 ± 0.06 d	0.233 ± 0.08 fgh	7.67 ± 0.58 de
T5	24.67 ± 0.87 i	21.33 ± 0.58 ij	0.60 ± 0.05 f	1.25 ± 0.05 f	0.399 ± 0.06 d	0.156 ± 0.02 gh	6.00 ± 1.00 f
T6	22.33 ± 0.58 j	19.50 ± 0.50 k	0.57 ± 0.20 f	1.25 ± 0.06 f	0.277 ± 0.02 e	0.113 ± 0.01 h	6.67 ± 0.58 ef
T7	27.00 ± 1.00 h	23.83 ± 0.29 gh	2.33 ± 0.32 c	2.80 ± 0.21 a	0.518 ± 0.04 c	0.476 ± 0.06 ab	8.33 ± 0.58 cd
T8	26.67 ± 0.58 h	20.50 ± 0.50 jk	2.40 ± 0.16 bc	2.18 ± 0.06 d	0.394 ± 0.02 d	0.364 ± 0.04 b-f	8.67 ± 0.58 bcd
T9	25.33 ± 0.58 hi	19.67 ± 0.58 k	1.80 ± 0.20 de	1.85 ± 0.10 e	0.327 ± 0.01 de	0.228 ± 0.02 fgh	7.67 ± 0.58 de
T10	33.00 ± 0.76 ef	27.50 ± 0.50 bcd	2.18 ± 0.16 cd	1.82 ± 0.17 e	0.588 ± 0.03 bc	0.397 ± 0.06 a-e	9.67 ± 0.58 abc
T11	32.67 ± 0.58 fg	26.83 ± 1.04 cde	2.32 ± 0.34 c	2.18 ± 0.20 cd	0.548 ± 0.02 c	0.243 ± 0.03 e-h	8.67 ± 0.58 bcd
T12	31.00 ± 1.00 g	24.83 ± 0.29 fg	2.34 ± 0.09 c	2.74 ± 0.11 a	0.532 ± 0.02 c	0.245 ± 0.03 e-h	8.33 ± 0.58 cd
T13	37.33 ± 0.58 ab	29.83 ± 0.58 a	2.99 ± 0.13 a	2.68 ± 0.07 ab	0.622 ± 0.03 bc	0.540 ± 0.11 a	10.67 ± 0.58 a
T14	35.33 ± 0.58 cd	27.83 ± 0.76 bc	2.84 ± 0.09 a	2.45 ± 0.08 bc	0.571 ± 0.07 bc	0.296 ± 0.06 c-g	10.33 ± 0.58 a
T15	34.00 ± 1.00 def	25.83 ± 0.29 ef	2.77 ± 0.14 ab	2.65 ± 0.12 ab	0.386 ± 0.02 de	0.283 ± 0.06 d-g	9.33 ± 0.58 abc
***P*-value**	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Data are expressed as means ± standard deviation (SD). Means with different letters are significantly different according to the Least Significant Differences Test ($P < 0.01$)

Comet assay of treated pea seedling leaf cells

Comet assay analysis of leaf cells revealed significant variations in DNA damage across samples (Figure 1). Images A and B displayed minimal DNA damage, characterized by intact DNA concentrated in the comet head. In contrast, images C through P showed a progressive increase in DNA damage, evidenced by the elongation and increased intensity of the comet tail. Notably, images F-P exhibited the most substantial DNA fragmentation.

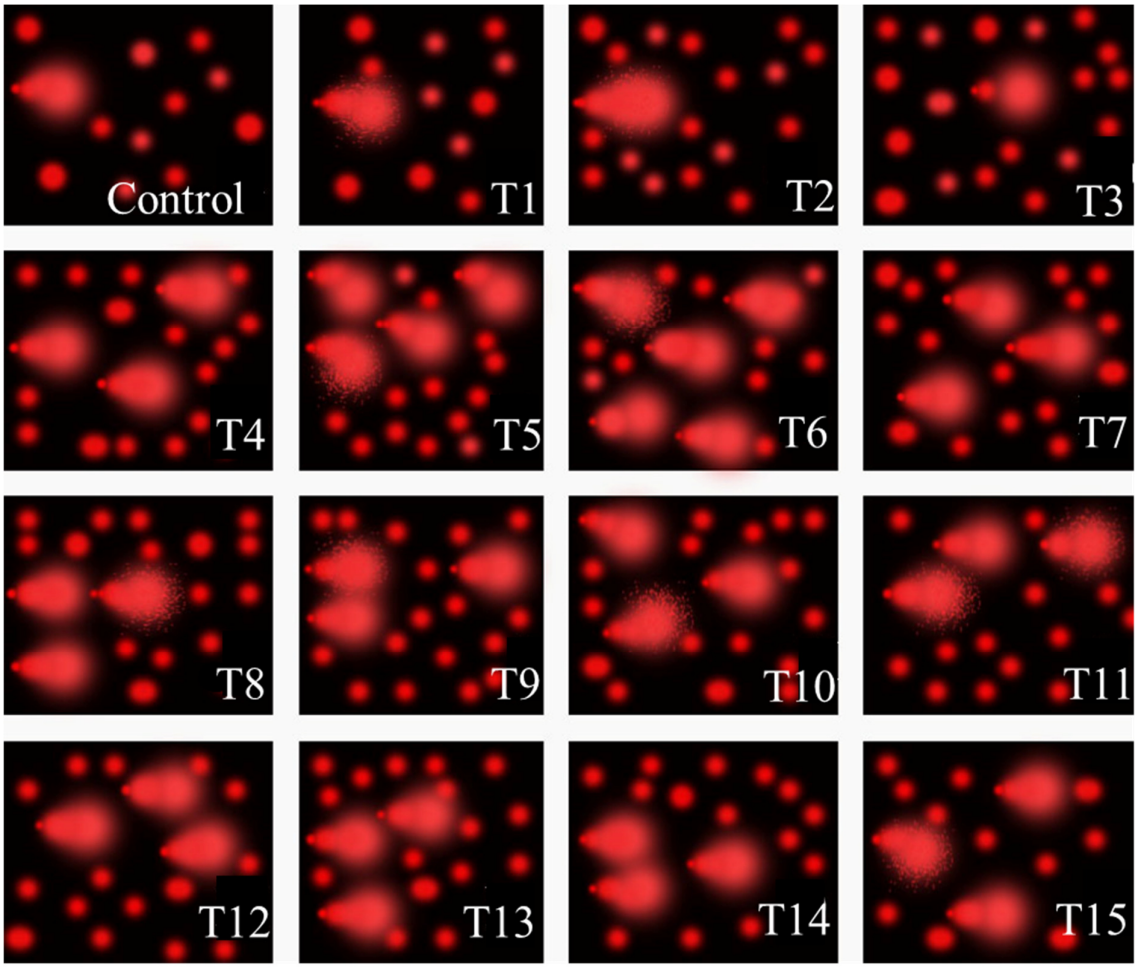


Figure 1. Comet assay showing DNA damage in *P. sativum* leaf cells. Images depict the induction of DNA damage in 15 treatments (T1-T15) and a control, as visualized by the Comet assay

Figure 2A illustrates the percentage of tailed and untailed DNA across various treatments (T1-T15) and a control. The control and treatments T1-T3 exhibited predominantly untailed DNA, ranging from 97% to 98%, with only 2-3% tailed DNA. In contrast, treatments T4-T15 showed a notable increase in tailed DNA, ranging from 5% to 19%, and a corresponding decrease in untailed DNA (81-95%). Notably, T6 displayed the highest percentage of tailed DNA (19%), while T13 exhibited the lowest (5%) among treatments with increased tailed DNA.

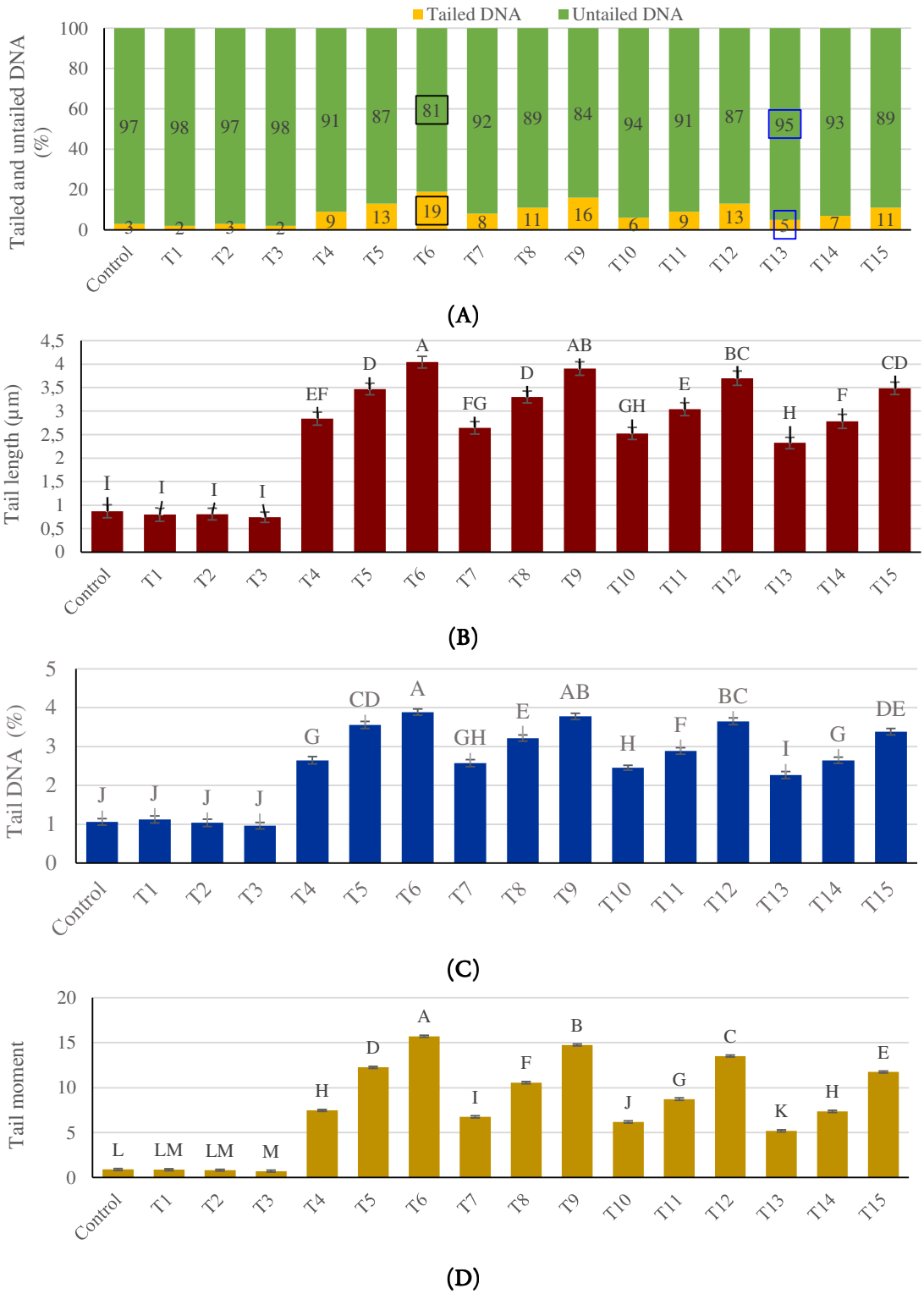


Figure 2. The effects of individual and combined treatments of selenium nanoparticles (SeNPs) and lead nitrate ($Pb(NO_3)_2$) on *P. sativum* leaf nuclei were assessed by Comet assay: (A) tailed and untailed DNA percentages, (B) tail length, (C) tail DNA percentage, and (D) tail moment values
Columns sharing the same letter not significantly different from each other ($p<0.05$)

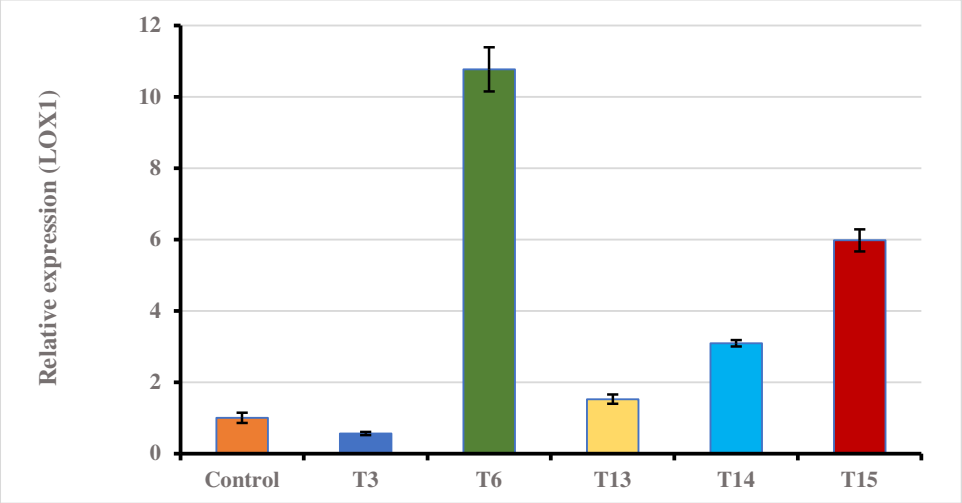
Figure 2B depicts the DNA tail length (µm) for the same treatments and control. The control and T1-T3 showed minimal tail lengths, all below 1 µm, indicating negligible DNA damage. Beginning with T4, a substantial increase in tail length was observed, ranging from 2.32 µm to 4.04 µm. Treatments T6 and T9 exhibited the highest tail lengths (approximately 4.04 µm and 3.90 µm, respectively), while T13 showed the lowest among treatments with significant damage (approximately 2.32 µm).

Figure 2C presents the percentage of DNA in the tail. The control and T1-T3 displayed low percentages, all below 1.5%, indicating minimal DNA damage. Starting with T4, a significant increase in tail DNA percentage was observed, ranging from 2.26% to 3.88%. Treatments T6 and T9 exhibited the highest percentages (approximately 3.88% and 3.77%, respectively), while T13 showed the lowest among treatments with substantial damage (approximately 2.26%).

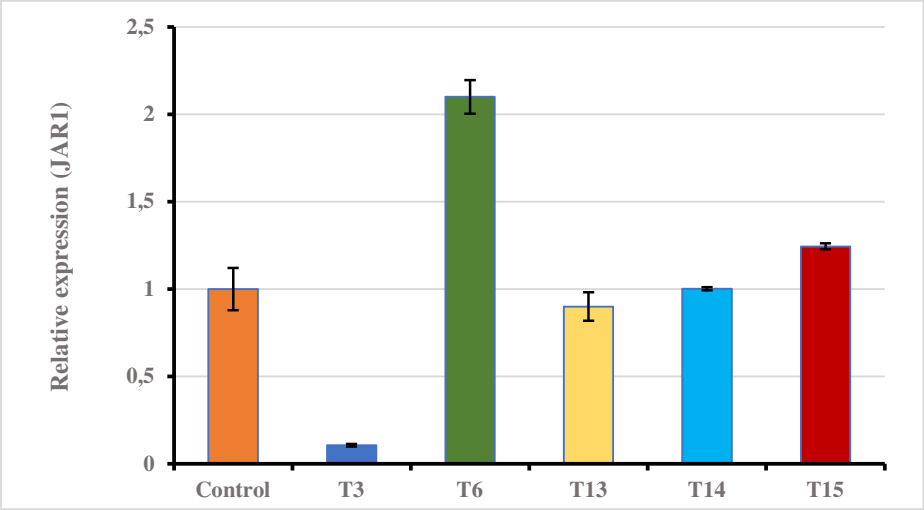
Figure 2D illustrates the DNA tail moment. The control and T1-T3 showed minimal tail moments, all below 1, indicating negligible DNA damage. Beginning with T4, a marked increase in tail moment was observed, ranging from 5.18 to 15.7. Treatments T6 and T9 exhibited the highest tail moments (approximately 15.7 and 14.74, respectively), while T13 showed the lowest among treatments with significant damage (approximately 5.18).

Effect of SeNPs and lead on LOX1 and JAR1 gene expression in pea seedlings

Figure 3A shows the relative expression of the *LOX1* gene in *P. sativum* seedlings under various treatments. Exposure to 270 mg L^{-1} $Pb(NO_3)_2$ significantly increased *LOX1* expression compared to the control. Conversely, 60 mg L^{-1} selenium nanoparticles (SeNPs) alone substantially reduced *LOX1* expression, even below control levels. Combined treatments of 60 mg L^{-1} SeNPs with increasing $Pb(NO_3)_2$ concentrations (90, 180, and 270 mg L^{-1}) showed a biphasic response: an initial decrease in *LOX1* expression at 90 mg L^{-1} $Pb(NO_3)_2$, followed by a progressive increase at 180 and 270 mg L^{-1} $Pb(NO_3)_2$. Notably, the combined treatment of 60 mg L^{-1} SeNPs + 270 mg L^{-1} $Pb(NO_3)_2$ resulted in a significant downregulation of *LOX1* compared to 270 mg L^{-1} $Pb(NO_3)_2$ alone. A similar expression pattern was observed for the *JAR1* gene (Figure 3B).



(A)



(B)

Figure 3. Effect of individual and combined SeNPs and $Pb(NO_3)_2$ on the relative expression of (A) *LOX1* and (B) *JAR1* Genes in *P. sativum* seedlings

T3: SeNPs (60 mg L^{-1}), T6: $Pb(NO_3)_2$ (270 mg L^{-1}), T13: $Pb(NO_3)_2$ (90 mg L^{-1}) + SeNPs (60 mg L^{-1}), T14: $Pb(NO_3)_2$ (180 mg L^{-1}) + SeNPs (60 mg L^{-1}), T15: $Pb(NO_3)_2$ (270 mg L^{-1}) + SeNPs (60 mg L^{-1})

Discussion

Lead contamination poses critical challenge to agricultural production due to its widespread presence and toxic effects on plant growth, development, and genetic integrity. In the present study, lead nitrate ($Pb(NO_3)_2$) treatments caused significant negative effects on *P. sativum* seedling establishment and growth performance as observed in all measured parameters, including shoot and root length, fresh and dry biomass, and number of leaves. Increasing levels of $Pb(NO_3)_2$ were associated with more considerable inhibition in seedling and vegetative growth parameters. The treated plants in high concentration in T6 (270 mg L^{-1} $Pb(NO_3)_2$) exhibited the most significant reduction in measured parameters compared to the non-stressed control. In this context, Elgharbawy *et al.* (2021), Gupta *et al.* (2024) and Rahman *et al.* (2024) elucidated that Pb exposure disrupts cell division and elongation, through oxidative damage to cellular structures and inhibition of physiological processes such as water and nutrient uptake. Moreover, Yuan *et al.* (2024); Zhang *et al.* (2024); Liu *et al.* (2025) depicted that Pb disrupts hormone balance, enzyme activity, and membrane function, ultimately stunting plant development. Otherwise, we found that selenium nanoparticle (SeNP) application demonstrated strong protective effects, particularly at higher doses. Plants in the SeNP-only treatments (T1–T3) exceeded untreated control values across all studied parameters. Furthermore, T13-T15 treatments in the presence of Pb stress produced greater shoot length, root length, fresh and dry mass, and leaf number than the Pb-only treatment. SeNP application mitigated the adverse impacts of Pb-induced phytotoxicity and enhanced plant resilience during early seedling establishment and growth stages. The current results indicated that combined SeNP and Pb treatments demonstrated that SeNPs are significantly associated to the mitigation of heavy metal toxicity during both seedling and vegetative growth stages, as reflected in all measured morphological and biomass parameters. As reported by Samynathan *et al.* (2023), Burmistrov *et al.* (2025) and Tsivileva (2025), the biostimulant role of bioavailable selenium at the nanoscale, which can promote cellular metabolism, photosynthesis, and antioxidant defense. Moreover, Zhu *et al.* (2022); Ran *et al.* (2024); Qin *et al.* (2025) deduced that SeNP enhance the antioxidant system, detoxification enzymes, stabilization of cell membranes, and reduces the translocation of Pb into photosynthetically active tissues.

Therefore, our results indicate that SeNP foliar application can be a powerful strategy to sustain seedling establishment and growth of *P. sativum* in soils contaminated with toxic lead. The comet assay findings, showing minimal DNA damage in leaf cells treated with SeNPs and significant damage with $Pb(NO_3)_2$, align with recent research on the dual nature of these substances in plants. SeNPs are known to act as potent antioxidants, which helps to prevent oxidative stress and protect DNA. A study conducted by *Ikram et al. (2022)* shown that SeNPs can enhance a plant's antioxidant defense system by increasing the activity of enzymes like superoxide dismutase (SOD) and catalase (CAT), which scavenge reactive oxygen species (ROS) and, in turn, mitigate DNA damage. Conversely, Nagaraju *et al. (2022)* reported that $Pb(NO_3)_2$ is a well-established genotoxic agent that induces DNA damage. Lead exposure in plants leads to the overproduction of ROS, which can directly or indirectly attack DNA molecules, leading to breaks and other structural damage. This sharp contrast in results underscores the protective role of SeNPs and the damaging effect of lead.

Findings from different studies align with the presented results, emphasizing the genotoxic effects of lead and the protective role of selenium nanoparticles (SeNPs) in plant systems. Lead is a well-known environmental contaminant that readily accumulates in plant tissues. Even at low concentrations, this general protoplasmic poison can induce significant oxidative stress, leading to DNA damage (Carocci *et al.*, 2016; Jagota *et al.*, 2024). This is consistent with the significant DNA fragmentation observed in treatments containing $Pb(NO_3)_2$ (T4-T15), where the comet assay results, particularly the increased tail DNA percentage and tail length, directly indicate DNA strand breaks (Sharma *et al.*, 2025). The high levels of DNA damage in T6 (270 mg L^{-1} $Pb(NO_3)_2$ are comparable to other studies demonstrating dose-dependent increases in genotoxicity with heavy metal exposure in plants (Badr *et al.*, 2021). The most compelling point of discussion arises from the combination treatments, particularly T13 (90 mg L^{-1} $Pb(NO_3)_2$ + 60 mg L^{-1} SeNPs), which showed a significant reduction in DNA damage compared to treatments with lead alone. The protective effect of SeNPs against heavy metal toxicity is well-documented and is attributed to their ability to enhance the plant's antioxidant defense system, such as by increasing glutathione (GSH) levels, which helps to mitigate the ROS produced by lead stress that would otherwise cause DNA damage (Laslo *et al.*, 2022). Additionally, SeNPs can restrict the uptake and translocation of heavy metals from the roots to other parts of the plant, further reducing their toxic impact (Wang *et al.*, 2023). Our findings underscore the potential of SeNPs as a mitigating agent for heavy metal-induced genotoxicity in agriculture. The statistical analysis corroborating the significant differences in tail length further strengthens the validity of these findings, confirming that the observed variations in DNA damage are not due to random chance but are a consequence of the applied treatments.

The subsequent analysis of gene expression provides a potential association to the observed DNA damage. The upregulation of *LOX1* and *JAR1* genes by lead ($Pb(NO_3)_2$) suggests the activation of stress response pathways, potentially including those involved in DNA repair or signaling damage. The contrasting downregulation of these genes by selenium nanoparticles (SeNPs) alone indicates a potential protective role against lead toxicity. Numerous studies confirm that lead toxicity primarily stems from oxidative stress, which leads to DNA damage, lipid peroxidation, and alterations in gene expression (Balali-Mood *et al.*, 2021; Jagota *et al.*, 2024). This is consistent with the observed upregulation of genes like *LOX1* and *JAR1* in the presence of lead, as these genes are known to be involved in plant stress and defense pathways, particularly in the biosynthesis of jasmonates (JAs) (Chen *et al.*, 2021; Li *et al.*, 2022). The downregulation of these genes by SeNPs alone suggests their potential role in reducing basal cellular stress. The biphasic response observed with combined treatments of SeNPs and $Pb(NO_3)_2$, where low concentrations of $Pb(NO_3)_2$ show a mitigating effect of SeNPs while high concentrations overwhelm this protection, aligns with recent findings that show SeNPs protect against lead-induced toxicity by maintaining oxidant/antioxidant balance (El-fakharany *et al.*, 2022). However, at higher heavy metal concentrations, the protective capacity of the SeNPs is likely exceeded, allowing the toxic effects of lead to dominate and activate stress response genes. This modulation of gene expression by SeNPs at the molecular level, which reverses the lead-induced disruption of stress-related genes, provides a

compelling explanation for the reduction in overall cellular damage, which is reflected in improved histopathological and biochemical parameters.

Conclusions

The present study demonstrated that SeNPs effectively alleviate lead-induced phytotoxicity in *P. sativum* at the seedling and vegetative stages, as reflected by significant improvements in growth and biomass parameters. $Pb(NO_3)_2$ exposure alone caused significant reductions in shoot and root development, biomass accumulation, and leaf production, whereas SeNPs application, particularly at higher concentrations, significantly enhanced these parameters compared to the untreated control. The enhancement effect of SeNPs was consistent across developmental stages, indicating their potential as a protective against lead-induced phytotoxicity in plants. The comet assay provides a clear picture of the varying degrees of DNA damage induced by different treatments in leaf cells. The gene expression analysis of *LOX1* and *JAR1* suggests that SeNPs can influence the lead-induced stress response in pea seedlings. Future research could focus on directly linking the observed DNA damage levels with the expression of genes involved in DNA repair and stress response pathways to gain a more comprehensive understanding of the mechanisms underlying the differential genotoxic effects of the investigated treatments and the potential protective role of selenium nanoparticles. Although the study effectively measured DNA damage and the expression of two stress-related genes *LOX1* and *JAR1*, a more comprehensive understanding of the underlying protective mechanism of SeNPs would require analyzing a broader suite of genes involved in the stress response, Pb transport, and Se metabolism, and confirming the observed gene expression changes with corresponding protein-level data and physiological markers.

Authors' Contributions

Conceptualization: AAB, FMB, EA, AAH; Methodology: AAB, FMB, EA, AAH; Software: AAB, FMB, EA, FAS, DSA, ASJ, NMA, AAH; Validation: AAB, FMB, EA, FAS, DSA, ASJ, NMA, AAH; Formal analysis: AAB, FMB, EA, FAS, DSA, ASJ, NMA, AAH; Investigation: AAB, FMB, EA, AAH; Resources: AAB, FMB, EA, FAS, DSA, ASJ, NMA, AAH; Data curation: AAB, FMB, EA, FAS, DSA, ASJ, NMA, AAH; Writing original draft preparation: AAB, FMB, EA, FAS, DSA, ASJ, NMA, AAH; Writing review and editing: AAB, FMB, EA, FAS, DSA, ASJ, NMA, AAH; Supervision: AAB, FMB, EA, FAS, DSA, ASJ, NMA, AAH; Funding acquisition AAB, FMB, EA, FAS, DSA, ASJ, NMA, AAH.

All co-authors reviewed the final version and approved the manuscript before submission

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article

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