



Effect of chlorpyrifos and lindane in root cells of *Allium fistulosum*: Genotoxicity, cytotoxicity, and oxidative stress

Eunice Danilú Couoh-Puga¹ · María Concepción Gómez-Maldonado¹ · Karla Susana Escalante-Herrera² · Erick Antonio Osorio-Mendoza³ · Gissela Moreno-Ortiz¹ · Elsa Noreña-Barroso¹ · Julian L. Wissner¹ · Gabriela Rodríguez-Fuentes¹

Accepted: 23 June 2025 / Published online: 22 July 2025
© The Author(s) 2025

Abstract

Object This study aimed to determine the effects of chlorpyrifos and lindane on the roots of Cambray onion (*Allium fistulosum*).

Methods We assessed growth, the mitotic index (MI), the development of nuclear abnormalities (NA), the expression of POLD1 and CDC2 genes (key components of DNA replication, repair, and cell cycle progression), and oxidative stress. Onion roots were first grown for 24 h in distilled water; then, they were exposed for 72 h to three concentrations of chlorpyrifos (0.1, 1, and 10 µg/L) and lindane (100, 300, and 500 µg/L).

Results The results indicated significant differences from the controls, showing lower MI and higher NA in treatments with either pesticide. Lindane exposure led to increased expression of POLD1 and CDC2, while chlorpyrifos exposure resulted in increased expression of CDC2 but not POLD1. The findings of oxidative stress biomarkers indicate significant differences between the controls and the roots treated with either pesticide, accompanied by increases in antioxidant enzyme activities and lipid peroxidation.

Conclusion The combination of traditional microscopy-based results in the *Allium* sp. test, along with the evaluation of oxidative stress biomarkers and the expression of genes involved in DNA duplication and repair, indicated that exposure to chlorpyrifos and lindane, pesticides commonly found in Yucatan groundwater, is cytotoxic, genotoxic, and induces oxidative stress. In the case of chlorpyrifos, the concentrations tested in this study are lower than the limits allowed for drinking water in Mexican regulations. They are close to levels detected in groundwater.

Keywords Chlorpyrifos · Lindane · Antioxidant enzymes · POLD1 · CDC2, *Allium fistulosum*

Introduction

Pesticides are chemicals used to combat insects, weeds, fungi, bacteria, and other organisms. These substances are typically designed to disrupt the physiological functions of targeted organisms, leading to dysfunction and reduced vitality [1]. Pesticide residues can significantly contribute to environmental contamination, affecting air, water, and soil. This situation threatens the coexistence of plant and animal communities [2]. Organophosphate (OPPs) and organochlorine pesticides (OCPs) are the primary insecticides used in tropical crops [3]. Lindane is an organochlorine pesticide known for its notable toxicity, environmental persistence, bioaccumulation, and long-range transport; these characteristics complicate the assessment of this compound's effects on the environment

✉ Gabriela Rodríguez-Fuentes
grf@unam.mx

¹ Unidad de Química en Sisal, Facultad de Química, Universidad Nacional Autónoma de México, Puerto de Abrigo S/N, Sisal, Yucatan 97355, México

² Unidad Multidisciplinaria de Docencia E Investigación, Facultad de Ciencias, Universidad Nacional Autónoma de México, Puerto de Abrigo S/N, Sisal, Yucatan, Mexico

³ Facultad de Ciencias, Universidad Nacional Autónoma de México, Av. Universidad 3000, Ciudad Universitaria, Coyoacan, 04510 Mexico City, Mexico

and human health [4, 4, 5]. OCPs cause severe disruptions in the endocrine system and result in significant adverse health effects, including carcinogenic and non-carcinogenic damage to living beings [6–8]. Chlorpyrifos, on the other hand, is an OPP that inhibits acetylcholinesterase (AChE) in animals and plants [9]. Chlorpyrifos is considered a low-persistence pesticide in organisms and the environment. However, its adverse effects include genotoxicity, immunotoxicity, cytotoxicity, oxidative stress, neurotoxicity, and mutagenicity. Its use and regulation are ambiguous, and there is ongoing debate about the issue [10–12]. OCPs are listed as priority xenobiotics under the Stockholm Convention. OCPs persist in water bodies due to runoff from adjacent fields and roads and can travel considerable distances from emission sources. In this context, bioassays are valuable methods for identifying and evaluating the hazards of chemicals and their mixtures on the environment, enabling the detection of toxic and genotoxic impacts on biota. Plants serve as biological models, providing an economical and reliable alternative in toxicological studies to assess negative responses to pesticides [13, 14]. The *Allium sp.* test (AT) is a valuable tool for this purpose [15–17]. The AT enables the identification of genotoxicity, which results in morphological changes in chromosomes and cytotoxicity in response to environmental stressors that affect mitosis and cytokinesis.

The Mexican norm for drinking water, NOM-127-SSA1-2021 [18], has set a maximum lindane contaminant level (MCL) of 2.0 µg/L; however, this concentration exceeds the limit established by international legislation. For example, the Environmental Protection Agency established a permissible limit of 0.2 µg/L [19]. Australian legislation states that typical lindane values have been occasionally reported in drinking water at concentrations of less than 1 µg/L [20]. Regarding chlorpyrifos, Mexican legislation and the World Health Organization [21] recommend a tolerable quantity of chlorpyrifos in drinking water of 30 µg/L; however, other international legislation recommends less than 10 µg/L [19].

Environmental water concentrations of lindane in Yucatan wells have been reported as high as 8.5 µg/L during the north wind season [22], while other studies reported concentrations of 0.14 and 0.17 µg/L for chlorpyrifos and lindane, respectively [23].

The present study assessed the potential cytotoxic and genotoxic effects of two commonly found pesticides in Yucatan groundwater: chlorpyrifos (0.1, 1.0, and 10 µg/L) and lindane (100, 300, and 500 µg/L), using Cambray onion (*Allium fistulosum*) as a biological model. We evaluated root growth, mitotic index (MI), nuclear abnormalities (NA), gene expression of CDC2 and POLD1, and oxidative stress biomarkers. These biomarkers provide valuable information for environmental monitoring programs, as they have been

used to measure organism responses to pesticide exposure in the aquatic environment [24].

Materials and methods

Experimental design

Cambray onion (*A. fistulosum*) bulbs were purchased from a local market in Merida, Mexico. Similar-sized (20–30 mm) and healthy bulbs were obtained and washed under running tap water. All dry scales and roots were carefully removed with a sharp knife to avoid damaging the root primordia. The onion bulbs were then placed in plastic cups (60 mL capacity) with their basal ends submerged in distilled water to promote root growth in a dark room at 25 ± 1 °C for 48 h. Subsequently, the experimental design included two controls (one with distilled water and the other with 0.01% ethanol), along with concentrations of 0.1, 1.0, and 10 µg/L of chlorpyrifos and 100, 300, and 500 µg/L of lindane, for 72 h under a 12/12-h photoperiod. Each treatment was tested in triplicate.

Concentrations of lindane and chlorpyrifos used in the bioassays were verified through solid-phase microextraction and gas chromatography-mass spectrometry (SPME/GC-MS), following the method previously described by our group [25]. A 10 mL sample aliquot was placed in a 20 mL SPME vial (Supelco, USA) with a PTFE/silicone septum (SU860101, Supelco). SPME was conducted via direct immersion for 20 min at 50 °C with a 65 µm PDMS/DVB SPME fiber (57,310-U, Supelco), while magnetic stirring was maintained at 700–900 rpm. After extraction, both pesticides were quantified using an Agilent Technologies 7890B Series Gas Chromatography System equipped with a 5977B mass detector and a J&W HP-5MS capillary column (30 m × 0.25 mm and 0.25 µm film thickness). The inlet temperature was set at 250 °C, and samples were injected in splitless mode with a desorption time of 10 min. The oven temperature program began at 50 °C for 1 min, followed by three different ramps: 1) 15 °C/min to 180 °C (hold time 5 min), 2) 2.5 °C/min to 200 °C (hold time 2 min), 3) 30 °C/min to 290 °C (hold time 2.3 min). The carrier gas used was helium (ultra-pure grade) with a flow rate of 0.8 mL/min. Mass spectra (m/z 50–600) were recorded at a rate of five scans per second at 70 eV, with the interface and ion source temperatures set at 290 and 230 °C, respectively. The MS ionization mode utilized was electronic ionization (EI). Mass spectrometric analysis for quantitative determinations was performed using selected ion monitoring (SIM Mode) of two characteristic fragment ions (m/z) for each analyte (181 and 183 for lindane; 199 and 97 for chlorpyrifos). Calibration solutions were employed to quantify lindane and chlorpyrifos in the samples.

Three roots were measured using a caliper at the beginning of the exposure, after 24 h of growth in distilled water, and at the end of the exposure period. The roots were collected separately for analysis. Three roots were preserved in 70% ethanol at room temperature for further cytological studies. After 72 h of exposure, four roots were fixed in RNA later and stored at -80°C for molecular analysis, while four roots were also kept at -80°C for biochemical analysis.

Mitotic index and nuclear abnormalities

Mitotic activity in the onion root tips was studied using the squash technique [26]. The roots were prepared for microscopic examination with aceto-carmine (Sigma, USA) to stain the chromosomes [27, 28]. The stained and macerated milky root tips were placed on glass slides, covered with coverslips, and observed under a microscope, starting with lower magnifications. Cell counts were conducted for both dividing and non-dividing cells. The number of dividing cells out of 1000 cells was counted and recorded. Similarly, the number of abnormal cells was also counted and noted. The mitotic index (MI) was calculated by determining the number of cells in metaphase, anaphase, or telophase out of 1000 cells and expressed as a percentage. The nuclear abnormalities (NA) were documented as the number of cells displaying micronuclei, buds, kidney-shaped condensed nuclei, multinucleation, or nuclear buds in 1000 cells during interphase relative to the number of cells in interphase. Micrographs of the cells were captured with a Velab VE-M5LCD camera attached to a standard light microscope.

Gene expression

The RNA was extracted from roots using the Gene Elute™ Mammalian Total RNA Miniprep Kit (Sigma, USA) following the user manual for gene expression analysis. The quality and quantity of the isolated RNA were assessed with a NanoDrop One (Thermo Scientific, USA) at 260 nm and subsequently stored at -80°C . For RT-qPCR, 250 ng of total RNA was used to synthesize cDNA with the iScript® Reverse Transcription Supermix Kit (Biorad, USA). Quantitative analysis of CDC2 and POLD1 expression in onion

was conducted with gene-specific primers (Table 1), previously utilized [29, 30], using iTaq Universal SYBR Green Supermix according to the user manual on the CFX96 Real-Time System (Biorad, USA). For qPCR, 1 μL of cDNA was applied with iTaq SYBR Green Mix (Biorad, USA). For POLD1, an initial denaturation took place at 95°C for 3 min, followed by 37 cycles of 40 s at 95°C , 60 s at 65°C , and 40 s at 72°C , concluding with a melting curve from 95°C for 50 s to 55°C for 20 s. For the CDC2 gene, the qPCR was performed with an initial denaturation at 95°C for 3 min, followed by 37 cycles of 40 s at 95°C , 60 s at 58°C , and 40 s at 72°C , finishing with a melting curve from 95°C for 50 s to 55°C for 20 s. B-actin was used as a house-keeping gene, utilizing the informed Bayesian method for gene expression analysis. The optimal annealing temperature was verified for each set of primers by gradient PCR. Before qPCR, all amplicons were purified and sequenced to confirm primer specificity, and efficiency curves were run to validate the method.

Oxidative stress biomarkers

Onion roots were homogenized with 0.05 M Tris Buffer at pH 7.4 in a 1:100 (w/v) ratio for each sampling treatment, using a Potter–Elvehjem homogenizer. After homogenization, 200 μL of the homogenate was taken from each sample and centrifuged at 12,298 RCF for 5 min at 4°C .

The supernatant was collected and stored at -80°C . The measurement of catalase (CAT) was conducted according to the method previously developed [31, 32]. The assay quantified the hydrogen peroxide reduction rate at 405 nm over 10 min, with measurements taken every 15 s as the reaction proceeded in the presence of ammonium molybdate. Glutathione-S-transferase (GST) levels were measured using a Sigma Aldrich Assay Kit CS04 [33], with CDNB at 100 mM as the substrate for spectrophotometric measurement at 340 nm every 15 s for 5 min. Superoxide dismutase (SOD) activity was assessed using the Sigma Aldrich Assay Kit 19,160. The total glutathione (GSH) concentration was determined with the Sigma Aldrich Glutathione Assay Kit CS0260. Protein carbonylation (PO) quantification was based on the method previously described [34], while lipid

Table 1 Primer sequences used for qRT-PCR to assess genotoxic effects on *Allium fistulosum* roots

Gene	Sequence (5' → 3')
B-actin (Housekeeping)	F: ATATCCAGCCTCTTGTGTGCGA
R: TAGGGGCATCATCACCAGCAAA	
POLD1	F: AGACGACTCGCTGTGTATTGCT
R: CCAGTAACTCGTGCCATCTCCA	
CDC2	F: TTATGGGTACCCCAAACGAA
R: TTGTCCCAAGATCCCTGAAG	

peroxidation (LPO) quantification was performed by the FOX method utilizing the Sigma Aldrich PeroxiDetect Kit. The proteins in the supernatant were analyzed using the Bradford method [35] with bovine serum albumin (BSA) as a standard to standardize all enzyme activities in U mg^{-1} protein.

Statistical analysis

Data were analyzed using a multivariate statistical approach. Permutational MANOVA (PERMANOVA) and Principal Coordinate Analysis (PCO) were performed using Primer v7.0 + PERMANOVA add-on. The data were transformed using the function $\log_{10}(x + 1)$ and were normalized. The resemblance among samples was calculated using the Euclidean Distance [36]. PERMANOVA was conducted using unrestricted permutation of raw data (9999 permutations) to generate a pseudo-F [37]. Pairwise tests were performed using a Monte Carlo test.

Gene expressions were analyzed and quantified using "informed" Bayesian methods, with b-actin serving as the housekeeping gene, following the procedure previously reported [38]. The analysis was conducted using the MCMC.qPCR package of the R software (version 4.2.2). In the qPCR model, qPCR data are represented as counts (i.e., the transcript count). Therefore, a generalized linear model with Poisson-lognormal errors was fitted. Once the counts were obtained, $\log_2$ abundance was applied to compare the expression levels of target genes across various samples or experimental conditions.

Results and discussion

In this study, we evaluated the effects of chlorpyrifos and lindane pesticides on *Allium fistulosum*. The meristematic regions of the onion roots were exposed to control (C, distilled water), control solvent (CS, ethanol at 0.01% in distilled water), and concentrations of 0.1, 1.0, and 10 $\mu\text{g/L}$ of chlorpyrifos, along with 100, 300, and 500 $\mu\text{g/L}$ of lindane. (Table 2) presents the concentrations measured by MEFS/GC–MS of lindane and chlorpyrifos in the solutions used for the bioassays.

The PCO analysis of the effects of lindane and chlorpyrifos on growth, MI, and NA is presented in Fig. 1A. For lindane, 76.87% of the total variation is explained by the first two axes. PCO1 was highly correlated with MI and NA, while PCO2 was correlated with root growth. It is evident that MI is higher in controls, and the number of NA increased in roots treated with lindane (Table 3). Growth was similar across all treatments. PERMANOVA indicated significant differences between treatments ($P = 0.0008$, Pseudo-F = 3.4338); pair-wise tests with a Monte Carlo

Table 2 Measured pesticide concentrations in the solutions employed to perform the bioassays using MSPE and GC–MS

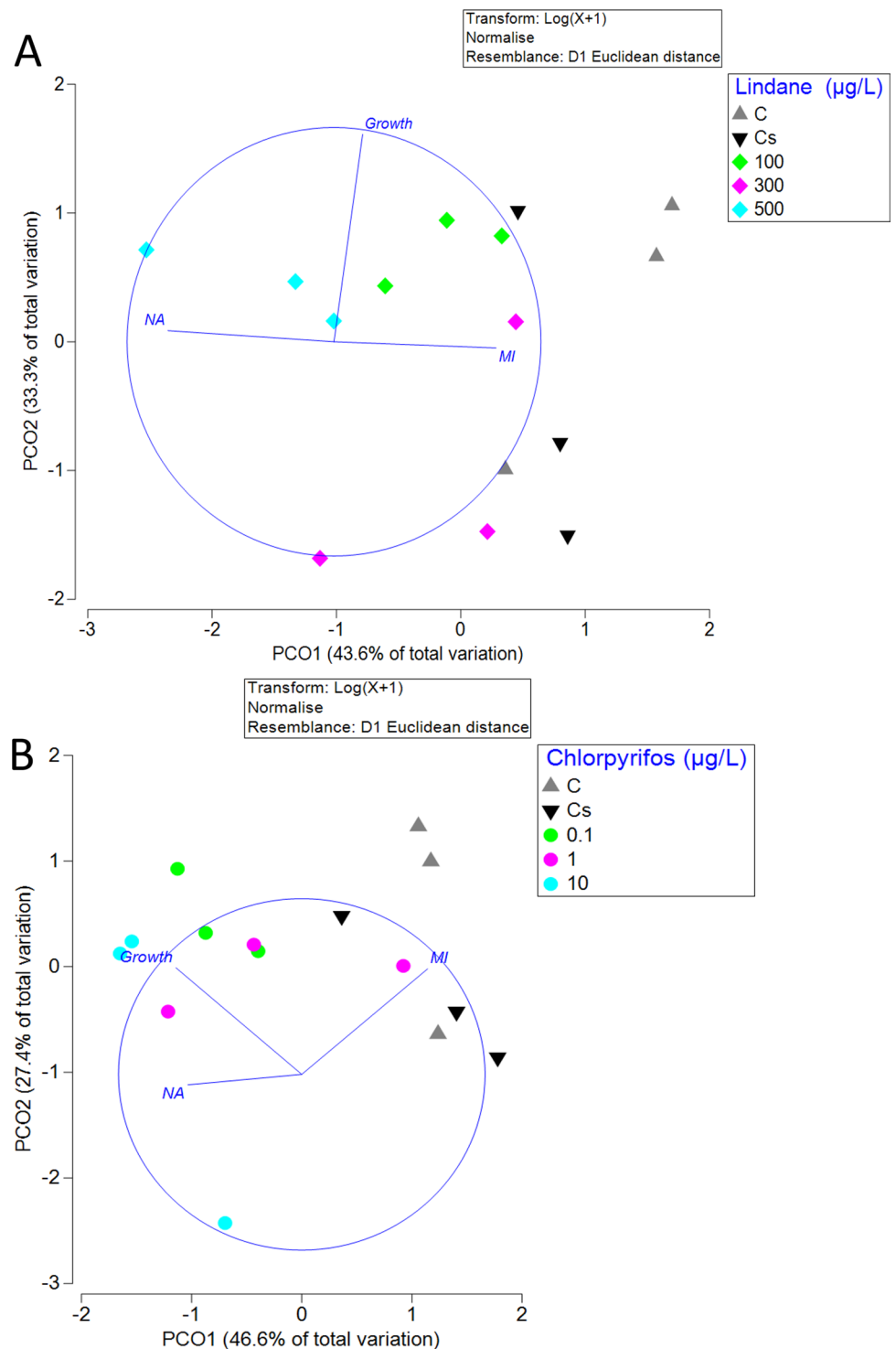
Chlorpyrifos nominal	Measured $\mu\text{g/L}$	Lindane nominal	Measured $\mu\text{g/L}$
Control	ND	Control	ND
Control solvent	ND	Control solvent	ND
0.1 $\mu\text{g/L}$	0.10 ± 0.01	100 $\mu\text{g/L}$	114.9 ± 10.9
1.0 $\mu\text{g/L}$	0.87 ± 0.13	300 $\mu\text{g/L}$	254.6 ± 3.5
10 $\mu\text{g/L}$	9.86 ± 1.01	500 $\mu\text{g/L}$	460.1 ± 6.7

*ND = Not detected

test indicated significant differences between controls and treatments at 100 $\mu\text{g/L}$ ($P(\text{MC}) = 0.0429$) and 500 $\mu\text{g/L}$ ($P(\text{MC}) = 0.0122$) of lindane. In roots treated with chlorpyrifos (Fig. 1B), the total variation is explained by the first two axes at 73.99%. PCO1 correlates similarly with the three measured variables, and PCO2 correlates with MI and growth. As observed in lindane exposure, MI is higher in controls than in treatments with chlorpyrifos, and the number of NA is higher in roots treated with the pesticide than in controls (Table 3). Growth was similar across all treatments. PERMANOVA indicated significant differences between treatments ($P = 0.0017$, Pseudo-F = 3.397). Pair-wise tests with Monte Carlo indicated significant differences between controls and roots treated with 0.1 and 10 $\mu\text{g/L}$ of chlorpyrifos ($P(\text{MC}) = 0.0228$ and 0.033, respectively). Therefore, these results demonstrate differences between controls and treatments with both pesticides.

Previous studies have shown that onion root tip cells treated with pesticides can develop genotoxic effects, including cellular abnormalities such as lagging chromosomes and chromosome bridges, along with several injuries similar to those observed in our slides [39]. Despite these findings, root growth was not inhibited in any treatment (Table 3). However, the increased number of abnormal nuclei (NA) in pesticide-treated roots indicated that pesticide exposure triggered DNA damage. This is reflected in the expression levels of the POLD1 and CDC2 genes (Fig. 2), where significant differences in pesticide treatments compared to the control group ($P < 0.05$) were apparent. POLD1 gene expression increased primarily in response to lindane exposure, with all treatments showing significant differences relative to the control groups ($P < 0.05$, Fig. 2A). In contrast, POLD1 gene expression did not demonstrate significant differences following chlorpyrifos exposure (Fig. 2B). The POLD1 gene is involved in DNA replication and repair processes [40]. The regulation of POLD1 gene expression is complex and can be influenced by various factors, including environmental conditions and exposure to pollutants such as pesticides [41]. The increase in POLD1 gene expression indicates that plant cells initiate DNA repair mechanisms in response to

Fig. 1 Principal coordinate analysis (PCO) of root growth, Metaphase, Anaphase, and Telophase index (MI) and nuclear abnormalities (NA), in *Allium fistulosum* exposed to lindane (A) and chlorpyrifos (B)



damage caused by stressors [30]. This suggests that DNA damage affects cell mitosis and may lead to genomic instability and mutations [30, 42]. In this context, timely repair of DNA damage is crucial for maintaining genomic integrity, as DNA damage triggers the activation of DNA repair machinery, which delays or halts cell-cycle progression, allowing adequate time for damaged DNA to be repaired

before entering mitosis [43]. With regard to CDC2 gene expression, both chlorpyrifos and lindane exposure exhibited significant differences ($P < 0.05$), with increased expression in the treatment groups compared to the control groups (Fig. 2). CDC2 becomes activated in complexes with specific cyclins, managing entry into mitosis and ensuring the cell progresses to division only when it is ready. The CDC2

Table 3 Mitotic Index (MI), nuclear abnormalities (NA), and most cytological damage observed during exposures to lindane and chlorpyrifos in each pesticide treatment

LINDANE μg/L	MI %	NA %	Growth cm	Nuclear abnormalities
CONTROL	9.22 ± 1.36	0.34 ± 0.10	2.88 ± 0.92	NB, KSC
CS	6.78 ± 0.97	0.22 ± 0.13	2.35 ± 1.02	NB, KSC
100	6.64 ± 0.46	0.52 ± 0.32	3.2 ± 0.26	MN, BC, KSC
300	7.94 ± 2.22	1.06 ± 0.64	1.55 ± 1.15	MN, BC
500	6.51 ± 1.15	2.31 ± 0.40	2.88 ± 0.21	NEC, MN, BC
CHLORPYRIFOS μg/L	MI %	MI %	Growth cm	Nuclear abnormalities
CONTROL	9.22 ± 1.36	0.34 ± 0.10	2.88 ± 0.92	NB, KSC
CS	7.19 ± 0.72	0.26 ± 0.13	2.35 ± 1.02	NB, KSC
0.1	6.96 ± 0.85	1.99 ± 1.21	4.02 ± 0.43	BC, NB, KSC
1.0	7.55 ± 0.77	2.87 ± 2.37	2.80 ± 0.23	KSC, NEC, MN
10	4.46 ± 0.77	1.22 ± 0.78	2.14 ± 2.14	KSC, NEC, MN

* MI: Mitotic index as metaphase, Anaphase, and Telophase mean ±sd; NA: nuclear abnormalities mean ±sd; Growth mean±sd; sd: Standard deviation; NA present: KSC: kidney shape cell; MN: micronuclei; BC: binucleated cell; NB: nuclear budding; NEC: necrotic cells

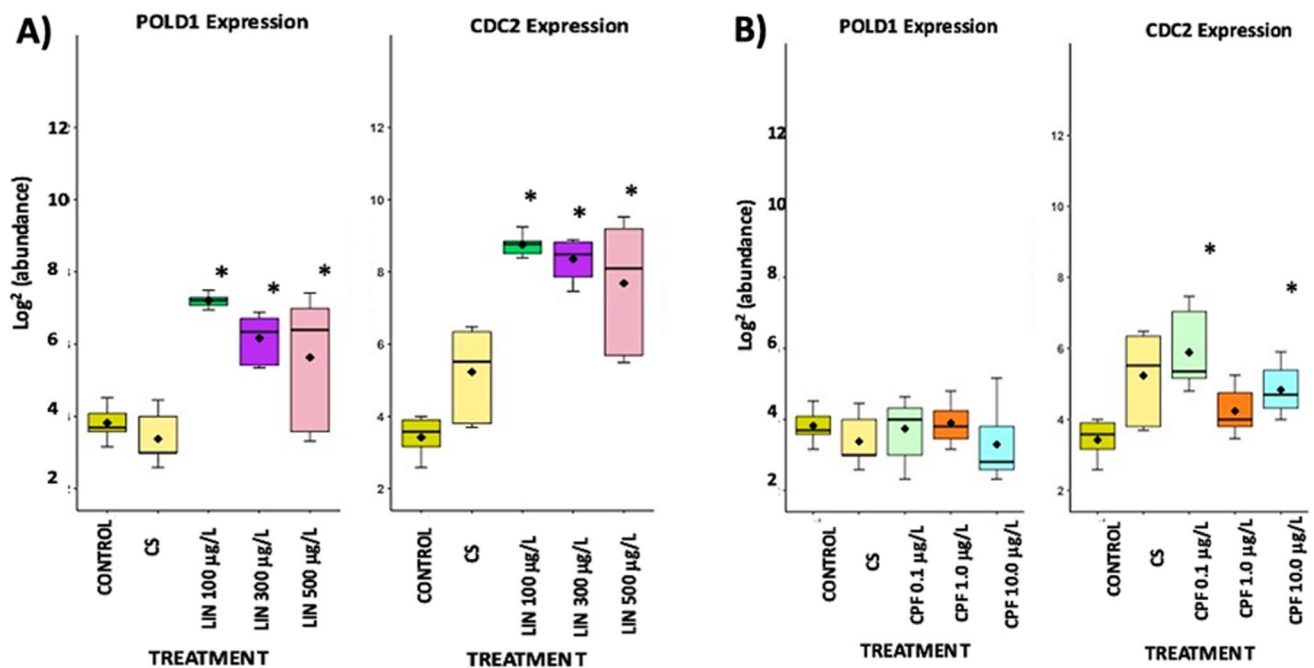


Fig. 2 POLD1, CDC2 gene expression in **A** Lindane (LIN) and **B** Chlorpyrifos (CPF) pesticide exposure. *The Bayesian method using an "informed" model using b-actin as a housekeeping gene indicates significant differences. ($P < 0.05$) with the control group

gene encodes a cyclin-dependent kinase essential for regulating cell cycle progression, particularly during the transition from interphase (G2 phase) to mitosis (M phase) [44, 45]. Therefore, we suggest that the decrease in mitotic index (MI) and the increase in NA under chlorpyrifos and lindane pesticide stress were mediated by DNA damage. Additionally, a coordinated and critical relationship was observed between the genes, with POLD1 highlighting its role in DNA replication and repair. Simultaneously, a coordinated and essential relationship between POLD1 and CDC2 governs cell

cycle progression, ensuring that cells only proceed to mitosis when DNA is accurately replicated and repaired. This coordination is vital for maintaining genomic integrity and cellular viability.

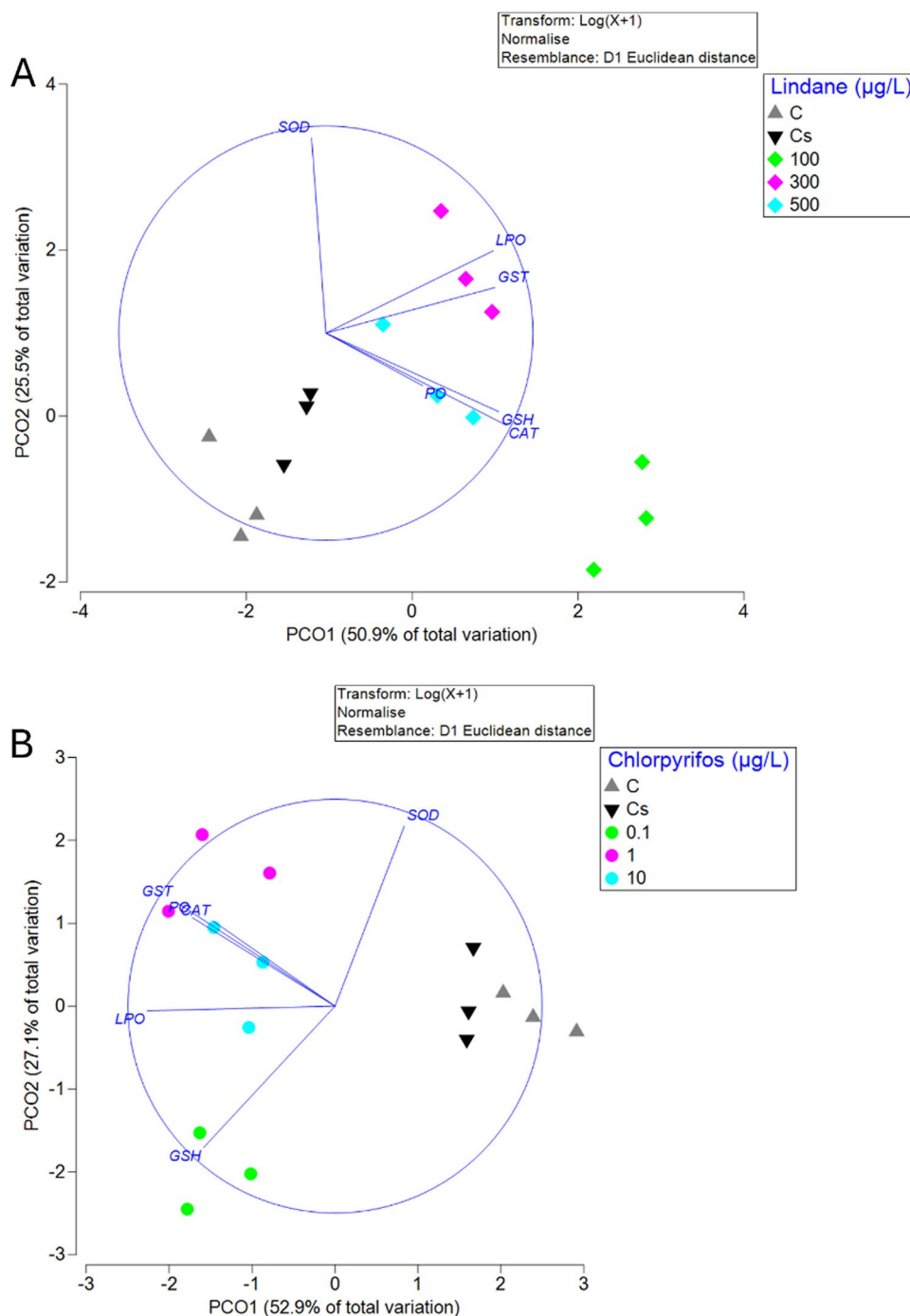
Additionally, incorporating more genes associated with toxicity into the *Allium* sp. test could enhance its utility as a bioindicator for environmental pollutants. Genes related to oxidative stress, apoptosis, and other cellular defense mechanisms, such as SOD2, GPX1, and CASP3, may provide insights into the broader cellular responses triggered by toxic

exposure. By examining these genetic expressions alongside POLD1 and CDC2, researchers can gain a more comprehensive understanding of the molecular pathways disrupted by stressors, thereby improving the predictive accuracy of cytotoxic and genotoxic effects across biological systems.

Regarding the PCO analysis applied to oxidative stress biomarkers in *A. fistulosum*, the total variation explained by the first two axes was 76.4% for lindane and 79.97% for chlorpyrifos (Fig. 3A and Fig. 3B). For lindane exposure, the variables that correlated with PCO1 were LPO (0.8081),

GST (0.818), GSH (0.825), and CAT (0.872). For the second principal coordinate, SOD (0.943) showed the highest correlation. The PERMANOVA indicated significant differences between treatments ($P=0.0008$, Pseudo- $F=3.42$). Pairwise tests with the Monte Carlo test revealed significant differences between the control groups and roots exposed to 100, 300, and 500 $\mu\text{g/L}$ ($P(\text{MC}) < 0.05$). The activity of SOD increased at 100, 300, and 500 $\mu\text{g/L}$. LPO and GST also increased in all treatments compared to controls. GST enzyme is involved in LPO repair [46]. CAT exhibits its

Fig. 3 Principal coordinate analysis (PCO) of oxidative stress biomarkers in *Allium fistulosum* exposed to **A** lindane and **B** chlorpyrifos. Response variables were included in the analysis: antioxidant enzymes activities (catalase, CAT; superoxide dismutase, SOD; glutathione-S-transferase, GST), total glutathione concentration (GSH), indicators of oxidative damage (protein carbonylation, PO; and lipid peroxidation, LPO)



highest activity at 100 µg/L, followed by a reduction at 300 and 500 µg/L. This behavior has been previously reported; at high concentrations of reactive oxygen species (ROS), CAT is inhibited due to excess substrate, indicating oxidative stress [47].

For chlorpyrifos exposure, the variable which correlated with PCO1 was LPO (-0.9073); for PCO2, SOD was the variable that presented the highest correlation (0.8717) (Fig. 3B). The PERMANOVA showed significant differences between treatments (pseudo- $F=9.734$; $P=0.0001$). Pairwise tests using the Monte Carlo test revealed significant differences between control groups with chlorpyrifos treatment, particularly at higher exposure concentrations ($P<0.05$). Controls had lower concentrations of LPO and GSH and lower activities of SOD, CAT, and GST than treatments with chlorpyrifos.

Exposure to chlorpyrifos and lindane can induce oxidative stress, triggering specific responses from the antioxidant system. The overexpression of the POLD1 and CDC2 genes is involved in repair mechanisms and cellular replication, respectively. It is well known that plants utilize various processes at molecular, morphological, physiological, and biochemical levels to mitigate the adverse effects of stressors, such as pollutants [48]. When activated, the antioxidant system helps plants endure stressful conditions by reducing oxidative damage [49]. Plants possess a complex antioxidant system, which includes enzymes like CAT and SOD, to mitigate and repair damage caused by ROS [50]. For instance, SOD converts superoxide radicals into hydrogen peroxide, which is then broken down into water and oxygen by CAT; this process helps maintain cellular health, especially under environmental stress conditions, ensuring that plants can withstand and adapt to challenging environments [51]. Our results indicate that both chlorpyrifos and lindane treatments increase the activities of antioxidant enzymes. In this context, other studies have highlighted an increase in SOD activity in *Allium cepa* under stress generated by chlorpyrifos and mancozeb pesticides [52]. For LPO, higher values were observed in the chlorpyrifos and lindane treatments compared to the control groups; this variable was also most closely linked to NA. It is essential to note that lipid peroxidation has been reported as a physiological process occurring in both plants and animals; it is the primary molecular mechanism responsible for causing oxidative damage to cells, which can ultimately lead to cell death, rendering it an irreversible process [53]. Lipid peroxidation can disrupt redox homeostasis and contribute to disease development caused by stress in both plants and animals. The activation of this process can initiate a cascade of events, including the activation of inflammatory pathways and the release of pro-inflammatory cytokines [54].

The results of the current study indicate that the enzymatic system plays a crucial role in ROS defense during

the initial stages of pesticide stress. The findings illustrate the morphological changes in roots caused by varying concentrations of pesticides. At higher pesticide concentrations, *A. fistulosum* onion roots exhibit more pronounced toxic effects on cellular responses. Pesticide stress induces distinct responses in biomarkers utilized in this research, resulting in a positive correlation among them, which allows us to establish a connection between various levels of biological organization.

In conclusion, cytotoxic and genotoxic effects were observed following exposure to chlorpyrifos and lindane. Roots employed various defensive strategies under pesticide treatment, specific to the nature and mode of action of the pesticides. The pesticides increased nuclear abnormalities, indicating DNA damage and the accumulation of ROS, which triggered the activation of detoxification and antioxidant mechanisms. They suppressed cell cycle progression, decreased mitotic index (MI), and increased nuclear anomalies (NA) (Table 3). The mitotic-depressive activity could be attributed to cell cycle regulators, such as CDC2, and the delay in cell cycle timing. The occurrence of various chromosomal aberrations, including clastogenic aberrations, indicates DNA damage resulting from pesticide exposure.

Beyond the cytotoxic and genotoxic effects observed at the cellular level, these findings pose broader ecological implications. Pesticides like chlorpyrifos and lindane, when they leach into groundwater, can affect the health of aquatic systems.

Additionally, these pesticides pose significant risks to human health, particularly in regions that rely on groundwater for drinking. The levels of chlorpyrifos measured in this study, although near Mexican regulatory limits, underscore the potential dangers associated with long-term exposure. Prolonged contact with pesticide-tainted water has been connected to neurological disorders, developmental problems, and disruptions of the endocrine system in humans.

The use of traditional microscopy results in the *Allium* sp. test, along with the evaluation of oxidative stress biomarkers and the expression of genes involved in DNA duplication and repair, suggests that exposure to chlorpyrifos and lindane, pesticides commonly found in Yucatan groundwater, is cytotoxic, genotoxic, and leads to oxidative stress. For chlorpyrifos, the concentrations tested in this study are lower than those permitted for drinking water according to Mexican regulations and are comparable to values measured in groundwater. These findings underscore the urgent need for stricter regulations and sustainable agricultural practices to mitigate health and environmental consequences.

Acknowledgement This study was supported by a postdoctoral fellowship granted by UNAM's Dirección General de Asuntos del Personal Académico (DGAPA) to EDCP and JLW, along with grants UNAM-DGAPA-PAPIIT IN203321 and IN211724 awarded to GRF and ENB.

Author contributions Grant writing (Gabriela Rodríguez-Fuentes, Elsa Noreña Barroso), bioassay and sample processing (Eunice Danilú Couoh-Puga, María Concepción Gómez-Maldonado, Karla Susana Escalante-Herrera, Elsa Noreña Barroso, Erick Antonio Osorio-Mendoza, Gissela Moreno-Ortiz, Julian L Wissner) Gabriela Rodríguez-Fuentes), data analysis (Eunice Danilú Couoh-Puga, María, Erick Antonio Osorio-Mendoza, Gissela Moreno-Ortiz, Julian L Wissner), draft writing and editing (Eunice Danilú Couoh-Puga, Elsa Noreña Barroso, Gissela Moreno-Ortiz, Julian L Wissner, Gabriela Rodríguez-Fuentes).

Declarations

Conflict of interest The authors declare that the research was conducted without any commercial or financial relationships that could be interpreted as a potential conflict of interest.

Ethical Approval Not applicable, a vegetable model was used in this study.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Pathak VM, Verma VK, Rawat BS, Kaur B, Babu N, Sharma A, Dewali S, Yadav M, Kumari R, Singh S, Mohapatra A, Pandey V, Rana N, Cunill JM (2022) Current status of pesticide effects on environment, human health and it's eco-friendly management as bioremediation: a comprehensive review. *Front Microbiol* 13:962619. <https://doi.org/10.3389/fmicb.2022.962619>
- Rajendran S (2003) Environment and health aspects of pesticide use in Indian agriculture. Proceedings of the third international conference on environment and health, Chennai, India, 15–17 December 2003 MJ Bunch, VM Suresh, T Vasantha Kumaran 353–73 Dep. Geogr., Univ. Madras, Chennai, Tamil Nadu, India/ Environ. Stud., York Univ. Toronto, ON, Canada.
- Singh P, Prasad SM (2018) Antioxidant enzyme responses to the oxidative stress due to chlorpyrifos, dimethoate and dieldrin stress in palak (*Spinacia oleracea* L.) and their toxicity alleviation by soil amendments in tropical croplands. *Sci Total Environ* 630:839–848. <https://doi.org/10.1016/j.scitotenv.2018.02.203>
- Velasco A, Hernández S, Ramírez M, Ortiz I (2014) Detection of residual organochlorine and organophosphorus pesticides in agricultural soil in Rio Verde region of San Luis Potosí, Mexico. *J Environ Sci Health B* 49(7):498–504. <https://doi.org/10.1080/03601234.2014.896670>
- UNEP/POPS/POPRC.17/INF/4 (2021) Persistent organic pollutants review committee seventeenth meeting geneva, 24–28 January* Item 4 (c) (i) of the provisional agenda** Technical work: consideration of chemicals proposed for listing in Annexes A, B and/or C to the Convention: chlorpyrifos
- U.S. EPA (1996) EPA/600/P-96/001F September 1996 PCBs: Cancer dose-response assessment and application to environmental mixtures. <http://www.epa.gov/epawaste/hazard/tsd/pcbs/pubs/pcb.pdf>
- Videla LA, Tapia G, Varela P, Cornejo P, Guerrero J, Israel Y (2004) Effects of acute gamma-hexachlorocyclohexane intoxication in relation to the redox regulation of nuclear factor-kappaB, cytokine gene expression, and liver injury in the rat. *Antioxidant Redox Signal* 6:471–480. <https://doi.org/10.1089/152308604322899530>
- Sumida K, Saito K, Oeda K, Yakabe Y, Otsuka M, Matsumoto H, Sekijima M, Nakayama K, Kawano Y, Shirai T (2007) A comparative study of gene expression profiles in rat liver after administration of alpha-hexachlorocyclohexane and lindane. *J Toxicol Sci* 32:261–288. <https://doi.org/10.2131/jts.32.261>
- U.S. EPA (1997) Office of prevention, pesticides and toxic substances, review of chlorpyrifos poisoning data, memorandum, 14 January.
- Ubaid-ur-Rahman H, Asghar W, Nazir W, Sandhu MA, Ahmed A, Khalid N (2020) A comprehensive review on chlorpyrifos toxicity with special reference to endocrine disruption: evidence of mechanisms, exposures and mitigation strategies. *Sci Total Environ*. <https://doi.org/10.1016/j.scitotenv.2020.142649>
- Wołjko E, Łozowicka B, Jabłońska-Trypuc A, Pietruszyńska M, Wydro U (2022) Chlorpyrifos occurrence and toxicological risk assessment: a review. *Int J Environ Res Public Health* 19(19):12209. <https://doi.org/10.3390/ijerph191912209>
- DuTeaux S, Koshlukova S E (2024) Chlorpyrifos, Editor(s): Philip Wexler, Encyclopedia of toxicology (Fourth Edition), Academic Press, Pages 1–13, ISBN 9780323854344, <https://doi.org/10.1016/B978-0-12-824315-2.00746-6>.
- Gogoi J, Karabi D, Dutta P (2021) Effect of preservatives and pesticides on mitotic index of *Allium cepa* roots: Biological model experiment for genotoxicity. *Poll Res* 40(3):777–781
- Camilo-Cotrim CF, Cardoso-Bailão EFL, Souza-Ondei L, Melo-Carneiro F, Madureira-Almeida L (2022) What can the *Allium cepa* test say about pesticide safety? A review. *Environ Sci Pollut Res Int* 29:48088–48104. <https://doi.org/10.1007/s11356-022-20695-z>
- Fiskesjö G (1985) The *Allium* test is a standard in environmental monitoring. *Hereditas* 102:99–112
- Fiskesjö G (1988) The *Allium* test — an alternative in environmental studies: the relative toxicity of metal ions. *Mutat Res* 197(2):243–260. [https://doi.org/10.1016/0027-5107\(88\)90096-6](https://doi.org/10.1016/0027-5107(88)90096-6)
- Bosio S, Laughinghouse HD IV (2012) Bioindicator of genotoxicity: the allium cepa test. INTECH. <https://doi.org/10.5772/31371>
- NORMA Oficial Mexicana NOM-127-SSA1–2021, Agua para uso y consumo humano. Límites permisibles de la calidad del agua. Norma Oficial Mexicana NOM-127-SSA1–2021 – Agua para uso y consumo humano. Límites permisibles de la calidad de agua
- U.S. EPA (2024). National primary drinking water regulations. United States Environmental Protection Agency. <https://www.epa.gov/ground-water-and-drinking-water/national-primary-drinking-water-regulations>
- NHMR, NRMCM (2011) Australian drinking water guidelines paper 6 national water quality management strategy. National Health and Medical Research Council, National Resource Management Ministerial Council, Commonwealth of Australia, Canberra.
- World Health Organization. (2021) A global overview of national regulations and standards for drinking-water quality.
- Giácoman-Vallejos G, Lizarraga-Castro I, Ponce-Caballero C, González-Sánchez A, Hernández-Núñez E (2018) Presence of DDT and lindane in a karstic groundwater aquifer in Yucatan.

- Mexico Groundwater Monitoring & Remediation 38(2):68–78. <https://doi.org/10.1111/gwmr.12267>
23. Cauich-Suárez L Y, Sánchez-Sánchez V E, Moreno-Ortiz G E, Noreña-Barroso E, Rodríguez-Fuentes G M (2024) Bioassays with *allium cepa* for the monitoring of toxicity in the groundwater of Yucatan, Mexico. Preprints 2024, 2024090818. <https://doi.org/10.20944/preprints202409.0818.v1>
 24. Sanchez-Hernandez J. (2007) Ecotoxicological perspectives of B-esterases in the assessment of pesticide contamination. In: Environmental Pollution. pp 1–45
 25. Rodríguez-Fuentes G, Rubio-Escalante FJ, Noreña-Barroso E, Escalante-Herrera KS, Schlenk D (2015) Impacts of oxidative stress on acetylcholinesterase transcription and activity in embryos of zebrafish (*Danio rerio*) following Chlorpyrifos exposure. Comp Biochem Physiol C Toxicol Pharmacol 172:19–25. <https://doi.org/10.1016/j.cbpc.2015.04.003>
 26. Singh HP, Batish DR, Kaur S, Setia N, Kohli RK (2005) Effects of 2-benzoxazolinone on the germination, early growth and morphogenetic response of mung bean (*Phaseolus aureus*). Ann Appl Biol 147(3):267–274. <https://doi.org/10.1111/j.1744-7348.2005.00031.x>
 27. Sharma CBSR (1983) Plant meristems as monitors of genetic toxicity of environmental chemicals. Curr Sci 52(1983):1000–1002
 28. Gupta S, Kumar A (2023) Chromosomes and the mitotic cell cycle phase in onion roots. Intern Med J 1(5):224–228
 29. Maity S, Chatterjee A, Guchhait R, De S, Pramanick K (2019) Cytogenotoxic potential of a hazardous material, polystyrene microparticles on *Allium cepa* L. J Hazard Mater. <https://doi.org/10.1016/j.jhazmat.2019.121560>
 30. Lyu G, Li D, Li S, Ning C, Qin R (2020) Genotoxic effects and proteomic analysis on *Allium cepa* var. *agrogarum* L. root cells under Pb stress. Ecotoxicology. <https://doi.org/10.1007/s10646-020-02236-x>
 31. Góth L (1991) A simple method for determination of serum catalase activity and revision of reference range. Clin Chim Acta 196:143–151. [https://doi.org/10.1016/0009-8981\(91\)90067-M](https://doi.org/10.1016/0009-8981(91)90067-M)
 32. Hadwan MH, Abed HN (2016) Data supporting the spectrophotometric method for the estimation of catalase activity. Data Brief 6:194–199. <https://doi.org/10.1016/j.dib.2015.12.012>
 33. Habig WH, Jakoby WB (1981) Glutathione S-transferases (rat and human). Detoxication and drug metabolism: conjugation and related systems. Methods Enzymol 77:218–231. [https://doi.org/10.1016/s0076-6879\(81\)77029-0](https://doi.org/10.1016/s0076-6879(81)77029-0)
 34. Mesquita CS, Oliveira R, Bento F, Geraldo D, Rodrigues JV, Marcos JC (2014) Simplified 2,4-dinitrophenylhydrazine spectrophotometric assay for quantification of carbonyls in oxidized proteins. Anal Biochem 458:69–71. <https://doi.org/10.1016/j.ab.2014.04.034>
 35. Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254. [https://doi.org/10.1016/0003-2697\(76\)90527-3](https://doi.org/10.1016/0003-2697(76)90527-3)
 36. Legendre P (2019) Numerical ecology. Encyclopedia of ecology. <https://doi.org/10.1016/B978-0-12-409548-9.10595-0>
 37. Anderson MJ (2001) A new method for non-parametric multivariate analysis of variance. Austral Ecol 26:32–46. <https://doi.org/10.1111/j.1442-9993.2001.01070.pp.x>
 38. Matz M V (2015) Bayesian analysis of qRT-PCR data using MCMC. *qpcr package: a tutorial*.
 39. Asita O A, Makhalemele R (2009) Genotoxic effects of dithane, malathion, and garden ripcord on onion root tip cells. Afr J Food Agric Nutr Dev 9(5).
 40. Gaudet P, Livstone MS, Lewis SE, Thomas PD (2011) Phylogenetic-based propagation of functional annotations within the gene ontology consortium. Brief Bioinform 12:449–462
 41. Nicolas E, Golemis EA, Arora S (2016) POLD1: central mediator of DNA replication and repair, and implication in cancer and other pathologies. Gene 590(1):128–141. <https://doi.org/10.1016/j.gene.2016.06.031>
 42. Rodriguez E, Azevedo R, Fernandes P, Santos CAO (2011) Cr (VI) induces DNA damage, cell cycle arrest and polyploidization: a flow cytometric and comet assay study in *Pisum sativum*. Chem Res Toxicol 24:1040–1047
 43. Endo M, Nakayama S, Umeda-Hara C, Ohtsuki N, Saika H, Umeda M, Toki S (2011) CDKB2 is involved in mitosis and the DNA damage response in rice. Plant J 69(6):967–977. <https://doi.org/10.1111/j.1365-3113x.2011.04847.x>
 44. Doerner PW (1994) Cell cycle regulation in plants. Plant Physiol 106(3):823–827
 45. Hemery A, Engler JdeA, Bergounioux C, Van Montagu M, Engler G, Inzé D, Ferreira P (1995) Dominant negative mutants of the Cdc2 kinase uncouple cell division from iterative plant development. EMBO J. <https://doi.org/10.1002/j.1460-2075.1995.tb00064.x>
 46. Sharma R, Yang Y, Sharma A, Awasthi S, Awasthi YC (2004) Antioxidant role of glutathione S-transferases: protection against oxidant toxicity and regulation of stress-mediated apoptosis. Antioxid Redox Signal 6(2):289–300. <https://doi.org/10.1089/152308604322899350>
 47. Sepasi Tehrani H, Moosavi Movahedi AA (2018) Catalase and its mysteries. Prog Biophys Mol Biol 140:5–12. <https://doi.org/10.1016/j.pbiomolbio.2018.03.001>
 48. Akbar MU, Aqeel M, Shah MS, Jeelani G, Iqbal N, Latif A, Elnour RO, Hashem M, Alzoubi OM, Habeeb T, Qasim M, Noman A (2023) Molecular regulation of antioxidants and secondary metabolites act in conjunction to defend plants against pathogenic infection. S Afr J Bot 161:247–257
 49. Ahmad P, Ahanger MA, Alyemeni MN, Wijaya L, Alam P (2017) Exogenous application of nitric oxide modulates osmolyte metabolism, antioxidants, enzymes of ascorbate-glutathione cycle and promotes growth under cadmium stress in tomato. Protoplasma 255(1):79–93. <https://doi.org/10.1007/s00709-017-1132-x>
 50. Kapoor D, Singh S, Kumar V, Romero R, Prasad R, Singh J (2019) Antioxidant enzymes regulation in plants in reference to reactive oxygen species (ROS) and reactive nitrogen species (RNS). Plant Gene. <https://doi.org/10.1016/j.plgene.2019.100182>
 51. Sharma P, Jha AB, Dubey RS, Pessarakli M (2012) Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. J Botany Article ID 217037:26. <https://doi.org/10.1155/2012/217037>
 52. Fatma F, Verma S, Kamal A, Srivastava A (2017) Phytotoxicity of pesticides mancozeb and chlorpyrifos: correlation with the antioxidative defence system in *Allium cepa*. Physiol Mol Biol Plants 24(1):115–123. <https://doi.org/10.1007/s12298-017-0490-3>
 53. Repetto M, Semprine J, Boveris A (2012) Lipid peroxidation: chemical mechanism, biological implications and analytical determination. INTECH. <https://doi.org/10.5772/45943>
 54. Chakraborty N, Mitra R, Dasgupta D, Ganguly R, Acharya K, Minkina T, Popova V, Churyukina E, Keswani C (2024) Unraveling lipid peroxidation-mediated regulation of redox homeostasis for sustaining plant health. Plant Physiol Biochem 206:108272. <https://doi.org/10.1016/j.plaphy.2023.108272>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.