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RESEARCH ARTICLE

# Exploring lithium toxicity in rice: An *in vitro* study on morphological and biochemical parameters

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## Abstract

Lithium (Li) is a known emerging contaminant and its effects on plant growth and metabolism are not fully understood. Most of the studies have been conducted using soil as the medium or by using hydroponics. The present study explores how different concentrations of lithium affect growth, chlorophyll level, phenolic compound accumulation and Peroxidase (POD) activity in *Oryza sativa* L. under *in vitro* conditions. Seeds were cultured on Woody Plant Medium (WPM) supplemented with lithium sulfate ($Li_2SO_4$) for 21 days. Chlorophyll a, b and total chlorophyll content were quantified and total phenolic content and POD enzyme activity were assessed to evaluate oxidative stress and metabolic changes. Morphological observations, including growth and symptoms of stress, were also recorded. Chlorophyll a, b and total chlorophyll content showed an initial increase at lower lithium concentrations (1-2 mg/L), followed by a decline at (3-10 mg/L). In contrast, total phenolic content exhibited a consistent, dose-dependent increase, indicating progressive activation of oxidative stress pathways. POD enzyme activity peaked at 5 mg/L but declined at higher concentrations, likely due to enzyme inhibition under intensified stress conditions. Morphologically, lithium-treated plants displayed stunted growth, with pronounced stress symptoms such as browning of roots and stems at enhanced lithium levels of 3-10 mg/L. Growth inhibition at elevated lithium levels (2-10 mg/L) was evidenced by a reduction in biomass.

**Keywords**: chlorophyll; *in vitro* culture; lithium toxicity; *Oryza sativa*; POD

## Introduction

Lithium (Li) has become increasingly prominent in the environment due to its widespread use in lithium-ion batteries, ceramics, pharmaceuticals and industrial lubricants (1). As a result, lithium is now recognized as an emerging environmental contaminant (2). Lithium can enter ecosystems through mining runoff, improper disposal of lithium-ion batteries and industrial effluents due to its accumulation in soil and water (3). Batteries are essential to the future of sustainable technology, yet their production and disposal carry significant environmental and human costs that must be responsibly addressed (4). Despite the growing presence, lithium remains largely unregulated in environmental policies, raising concerns about its long-term ecological and agricultural impacts (5). Lithium can bioaccumulate in aquatic organisms and plants (6), potentially entering the human food chain (7). Recent reviews suggest that the exposure of lithium can pose a health hazard to humans and ecosystem (1,8).

Lithium contamination in agricultural soils can adversely affect crop growth; it can increase oxidative stress, cause nutrient imbalance, affect photosynthesis by altering chlorophyll content and reduce growth. Most of these studies have been conducted using soil or hydroponics (7,9). To better understand lithium's phytotoxic effects, there is a growing need for research using plant tissue culture systems. *In vitro* culture techniques offer a controlled, sterile environment that eliminates soil and microbial variability, allowing for precise assessment of lithium's impact on plant metabolism. These systems enable detailed studies of oxidative stress markers, chlorophyll degradation, enzyme activity and morphological screening. The present study aims to investigate the effects of varying lithium concentrations on growth, POD activity, chlorophyll content (chlorophyll a, b and total) and phenolic accumulation in *in vitro* germinated rice seedlings.

## Materials and Methods

### *In vitro* seed culture

The MO1 variety rice seeds were collected from the local market, washed in running tap water for 30 min prior to inoculation. The seeds were then treated with 0.1 % (w/v) mercuric chloride solution for 8 min. During post-sterilization, the seeds were washed thrice using sterile water to remove the traces of mercuric chloride. Later, the sterilized seeds were inoculated into the media WPM (10), containing 2 mg/L BA and 1 mg/L IAA, with different concentrations of $Li_2SO_4$ (1 mg/L to 10 mg/L), gelled with 0.8 % (w/v) plant tissue culture-grade agar. Lithium salt was added to the media before steam sterilization. Media without any lithium source served as the control. The cultures were incubated under a photoperiod of

16 hr of light and 8 hr of dark. The temperature of the culture room was maintained at 25 ± 2 ºC.

The seedlings were harvested after 21 days of growth and kept in an oven at 30 °C for 48 hr for measuring the biomass as dry wt. Biochemical analysis were performed on *in vitro*-grown seedlings after 21 days of incubation. Any observable morphological changes in the *in vitro* germinated rice seedlings were also recorded.

### Chlorophyll and total soluble phenolics

Chlorophyll a, b and total chlorophyll were quantified using the acetone extraction method as described previously (11). The amount of phenolics was expressed as catechol equivalents. Phenolic content was estimated using alcohol extracts of the seedlings, following the Folin-Ciocalteu assay protocol (12).

### Estimation of POD activity

Changes in POD activity were measured using 500 mg of fresh weight tissue. Enzyme extraction was carried out using 0.167 M potassium phosphate buffer at pH 7.88 (13).

### Statistical analysis

The experiments were done in three replicates. The biochemical values were expressed as mean ± standard error. Comparison of means were performed by using Duncan's multiple range test using SPSS version 30.

## Results

Significant morphological changes were evident in rice seedlings exposed to lithium concentrations ranging from 4-10 mg/L. These alterations prominently affected root and leaf growth and appearance when compared to controls. A marked reduction in overall plant growth was observed, particularly in seedlings treated with lithium concentration range of 4-10 mg/L, indicative of lithium-induced growth inhibition (Fig. 1).

Seedlings subjected to lithium exposure at higher concentrations (4-10 mg/L) displayed pronounced browning at the stem base and root regions (Fig. 1C-H). Moreover, leaf yellowing (chlorosis) and extensive root necrosis became apparent, especially at the highest lithium concentration (10 mg/L) (Fig. 1H). Even though germination rate was 100 % in all the treatments (Supplementary Fig. 1, 2), lithium exposure exerted a noticeable qualitative impact on germinated seeds, leading to visible symptoms such as reduced vigor and signs of physiological stress such as discoloration of leaves and tissue browning (Supplementary Fig. 3, 4).

The growth and biochemical data are summarized in Table 1. The experiment demonstrated that higher concentrations of lithium reduced biomass compared to the control group. The influence of lithium on biomass accumulation in rice seedlings revealed a clear dose-dependent inhibitory effect. As lithium concentrations increased from 1-10 mg/L, there was a progressive decline in dry biomass, with the highest biomass recorded in the control group (26.4 mg) and the lowest at 10 mg/L (15.47 mg). The differences in the biomass accumulation were not significant during the concentration range of 5-9 mg/L (Table 1).

Interestingly, chlorophyll content exhibited a biphasic response to lithium treatment. At lower concentrations (1-3 mg/L), chlorophyll a, b and total chlorophyll levels increased significantly, peaking at 1 mg/L with a total chlorophyll content of 1135.66 µg/g fresh weight, substantially higher than the control. A very low levels of chlorophyll was observed at 10 mg/L, where visible yellowing of leaves was noted. The biochemical stress response was further evidenced by the accumulation of phenolic compounds and the activity of POD. Total phenolic content increased steadily with rising lithium concentrations, from 32.95 µg/g in the control to 179.1 µg/g at 10 mg/L, highlighting a robust oxidative stress response. On the contrary, POD activity results do not exhibit a constant decrease or increase with increasing concentration of Li. Compared to the control (0.96 ΔA475/min/mg fresh wt), POD activity increased at lower to moderate lithium concentrations, peaking at 5 mg/L (1.26 ΔA475/min/mg fresh


**Fig. 1.** Growth and morphological changes in *in vitro* cultures of rice under varying lithium concentrations.

A= Control; B, C, D, E, F, G and H indicates lithium concentrations of 4, 5, 6, 7, 8, 9 and 10 mg/L, Note the yellowing of leaves at 10 mg/L

wt). However, beyond this concentration, the activity showed a slight decline, dropping to 1.05 at 10 mg/L. The POD activity of the treatments exhibited a significant difference when compared to controls(Table 1).

This trend suggests that lithium, particularly at higher concentrations, exerts a toxic effect on plant growth, likely by interfering with essential physiological processes. Even at the lowest tested concentration (1 mg/L), a slight reduction in biomass was observed, indicating that rice seedlings are sensitive to lithium exposure.

## Discussion

The current results suggest a stimulatory effect of trace lithium levels on photosynthetic pigment synthesis, possibly due to a mild stress-induced enhancement of metabolic activity. Higher concentrations of lithium were able to reduce chlorophyll content in *in vitro* grown seedlings. Yellowing of leaves observed at 10 mg/L of lithium was correlated with very low amount of chlorophyll accumulation, indicating chlorosis. Thus, a biphasic response was exhibited. Studies indicate that in some plants lithium at lower concentrations enhance chlorophyll content, but at higher levels it is inhibited. Decrease in photosynthetic pigment accumulation was reported in *Apocynum pictum* (14), *Brassica carinata* (15) and *Zea mays* (16). Such a reduction can led to oxidative stress (16). A hormesis effect of lithium exposure to chlorophyll content was noted earlier in *Chromochloris zofingiensis*, an algae (17). Similar hormesis effect of lithium was also found in the present experiment.

Similar to the current experimental results, lithium affecting quality root growth was also reported in *Brassica carinata* (15). It is well established that POD activity increase in plants is a clear indicator of pollution stress (18). The increased POD activity exhibited by the cultured plants on exposure to lithium certainly resulted in enhanced phenol accumulation. It is suggested that POD enzymes may facilitate the oxidative polymerization of phenols, thereby enhancing the plant's antioxidant capacity and structural integrity (19). These biochemical changes are part of a broader adaptive mechanism that helps plants mitigate damage and maintain physiological balance (20). Phenolics are known antioxidants and their accumulation suggests an adaptive mechanism to counteract lithium-induced oxidative damage. In contrast, POD activity remained relatively stable between 1-5 mg/L, with no statistically significant differences, implying that this enzyme may not be the primary antioxidant defence at lower lithium exposures. In an earlier study, it was reported that lithium exposure induces morphological and biochemical changes such as damage of shoot tip, discolouration of stem, reduced root growth and necrotic lesions along with enhanced phenolic accumulation in *in vitro* seedlings of *Vigna radiata* (21).

Lithium exposure to the plants has been experimented using soil as the medium and hydroponics, while some of the experiments used foliar application method (7). The experiment using a tissue culture *in vitro* system is more convenient, easy to regulate control parameters and provides a reliable result. Using a plant tissue culture system provides a controlled and uniform environment, eliminating variability associated with soil conditions and allowing precise analysis of growth and stress responses (22). This system also enables the direct assessment of specific treatments, such as lithium exposure, on plant physiology without interference from microbial or soil-derived factors.

## Conclusion

The current experiment proved the feasibility of adopting *in vitro* culture as a tool for accessing the effects of next generation pollutants like lithium on plants. The study demonstrates that lithium exerts a concentration-dependent effect on phenolic accumulation indicating the biochemical stress. At lower concentrations lithium was beneficial for improving chlorophyll content. Higher lithium levels (≥4 mg/L) induced significant morphological deformities, reduced growth, chlorosis and biochemical stress responses. Phenolic accumulation pattern revealed a sustained oxidative stress response, while POD activity peaked at moderate concentrations (5 mg/L), potentially reflecting an upper threshold for effective enzymatic defence before toxicity suppresses enzyme function. The use of *in vitro* culture systems of plants in this study proved advantageous for experimentally evaluating lithium-induced physiological and biochemical changes. These findings not only contribute to the understanding of lithium phytotoxicity but also reinforce the potential of plant tissue culture as a sensitive platform for early screening of environmental contaminants. Further investigations on the molecular mechanisms underlying lithium stress will be essential for developing strategies to mitigate its impact on agricultural productivity and ecosystem health.

**Table 1.** Biochemical data of *in vitro* rice seedlings grown in presence of different concentrations of lithium salt

| Concentration of $Li_2SO_4$ (mg/L) | Biomass accumulation (mg dry wt) | Chlorophyll content ((µg/g fresh wt) | | | Phenol content (µg/g dry wt) | POD activity (ΔA 475nm/min/mg Fresh wt) |
|---|---|---|---|---|---|---|
| | | A | B | Total | | |
| Control | $26.40^{e} \pm 1.00$ | $649.31^{ef} \pm 0.84$ | $221.72^{f} \pm .51$ | $870.82^{g} \pm 1.8$ | $32.95^{a} \pm 0.09$ | $0.96^{a} \pm 0.04$ |
| 1 | $24.53^{de} \pm 1.67$ | $833.18^{g} \pm 1.42$ | $302.76^{h} \pm 1.18$ | $1135.66^{i} \pm 2.4$ | $46.06^{b} \pm 0.05$ | $1.18^{ef} \pm 0.07$ |
| 2 | $22.87^{cd} \pm 0.58$ | $688.76^{f} \pm 2.02$ | $256.48^{g} \pm 2.32$ | $945.00^{h} \pm 0.30$ | $62.06^{c} \pm 0.19$ | $1.22^{fg} \pm 0.03$ |
| 3 | $21.63^{c} \pm 0.82$ | $645.63^{ef} \pm 0.19$ | $218.5^{f} \pm 1.65$ | $863.93^{g} \pm 1.46$ | $73.52^{d} \pm 0.09$ | $1.20^{ef} \pm 0.02$ |
| 4 | $21.73^{c} \pm 0.71$ | $625.18^{e} \pm 0.19$ | $198.66^{e} \pm 1.65$ | $823.65^{f} \pm 1.46$ | $80.12^{e} \pm 0.71$ | $1.17^{def} \pm 0.02$ |
| 5 | $19.23^{b} \pm 0.09$ | $566.25^{d} \pm 0.84$ | $187.52^{de} \pm 1.51$ | $753.59^{e} \pm 1.82$ | $100.83^{f} \pm 3.00$ | $1.26^{g} \pm 0.01$ |
| 6 | $17.47^{ab} \pm 0.30$ | $552.17^{cd} \pm 0.72$ | $178.10^{d} \pm 1.31$ | $730.09^{de} \pm 2.04$ | $111.87^{g} \pm 0.36$ | $1.09^{bc} \pm 0.00$ |
| 7 | $18.30^{b} \pm 0.57$ | $517.16^{c} \pm 0.00$ | $187.35^{de} \pm 0.00$ | $704.34^{d} \pm 0.00$ | $130.32^{h} \pm 0.39$ | $1.16^{de} \pm 0.00$ |
| 8 | $18.00^{b} \pm 0.46$ | $517.15^{c} \pm 0.92$ | $157.23^{c} \pm 0.34$ | $674.22^{c} \pm 0.58$ | $151.90^{i} \pm 1.25$ | $1.08^{bc} \pm 0.00$ |
| 9 | $16.67^{ab} \pm 0.34$ | $383.17^{b} \pm 83.40$ | $129.92^{b} \pm 30.74$ | $512.96^{b} \pm 52.67$ | $157.75^{j} \pm 1.32$ | $1.13^{cd} \pm 0.01$ |
| 10 | $15.47^{a} \pm 0.15$ | $320.57^{a} \pm 0.92$ | $98.22^{a} \pm 0.34$ | $418.69^{a} \pm 0.58$ | $179.1^{k} \pm 0.73$ | $1.05^{b} \pm 0.00$ |

N=3; Data = Mean ± SE, Mean followed by same letters do not differ significantly by Duncan's multiple range test

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## Authors' contributions

DD carried out the *in vitro* studies, biochemical analysis and drafting of the manuscript. NS designed and supervised the research work and involved in drafting and finalizing of the manuscript.

## Compliance with ethical standards

**Conflict of interest:** Authors do not have any conflict of interests to declare.

**Ethical issues:** None

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