Egypt. J. Soil Sci. **Vol. 65**, No. **3**, pp: 1345 - 1364 (2025)

Egyptian Journal of Soil Science
http://ejss.journals.ekb.eg/

Lithium Toxicity Alleviation in *Vicia faba* Plants Using Zinc Oxide Nanoparticles and Biochar

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BY ACTING as nano-fertilizers, nanoparticles (NPs) have recently emerged as promising agents for plants to alleviate abiotic stressors in addition to biochar (BC). Specifically, the effect of Nanoparticle zinc oxide (ZnO-NPs) or BC on plant responses to lithium (Li) stress has not been studied, as Li is one of the emerging contaminants. Combining ZnO-NPs and BC is a promising approach to sustainable agriculture and environmental health. Thus, this study was performed through an aquatic experiment to investigate the effect of Li toxicity on the morphological parameters, enzyme activity, metal accumulations, and cytogenic toxicity parameters of *Vicia faba* plants, as well as to study the positive or negative effect of ZnO-NPs and BC on Li toxicity. Fifty mg L^{-1} of Li was used to present the toxicity of Li on plants, while 2% biochar and 25 and 100 mg L^{-1} of ZnO-NPs were applied to examine the alleviation effect on Li toxicity. The addition of Li led to a decrease in the root length (13.3 cm) and the number of leaves (5.7) compared to the control (18.5 cm and 8.3, respectively). Li led to flocculated effects of catalase, total soluble peroxidase, and polyphenol oxidase activities. Li or higher concentration of ZnO-NPs caused higher accumulation of both metals in plants. The bioaccumulation factor was also very high in Li or Zn treatments, and the treatment behavior was markedly varied. Li toxicity decreased the mitotic index (1.42%) compared to the control (8.45%) and increased the abnormalities from 0% to 11.33%. ZnO-NPs showed unexpected results, especially at 100 mg L^{-1}. Biochar enhanced most of the investigated parameters; however, the enhancement was greater when BC was combined with 25 mg l^{-1} ZnO-NPs, indicating that combining BC and a low rate of ZnO-NPs can be a promising solution to alleviate lithium toxicity in the environment. However, more studies are required to address the optimal concentration of BC and ZnO-NPs to eliminate Li from the environment.
Keywords: Lithium toxicity; Emerging contaminants; Zinc oxide; Nanoparticles; Biochar; *Vicia faba*.

1. Introduction

Increasing food productivity by 60% to fulfill the needs of a world population of 10 billion people by 2050 is one of the most pressing concerns of the current decade. Nevertheless, the agriculture sector in many parts of the globe faces many environmental challenges, including biotic and abiotic pressures. These pressures are exacerbated by climate change at a time when expanding agricultural areas and meeting food population demand are not at all possible. Plant germination, growth, and agricultural yield are significantly influenced by abiotic factors such as severe temperature, drought or flood, salt, and toxic metals (Faizan et al., 2021a; Hemathilake and Gunathilake, 2022; EL-Fakharany et al., 2023). Nanotechnology is currently utilized in numerous applications, with nanomaterials (NMs) playing an increasingly significant role in medicine, optics, physics, electronics, basic and applied sciences, and bio-nanotechnology (Faizan et al., 2021a and b, Song et al., 2024a). Because of its positive effects, ZnO-NPs have become one of the most utilized metal oxide NPs in bio-applications; it is commonly used in agriculture to boost plant production while reducing adverse effects resulting from heavy metals (HMs) stress on plants (Faizan et al., 2021a and b).

Many studies have shown that using ZnO-NPs as a foliar spray is the best way to avoid plant micronutrient deficits (Faizan et al., 2021c). Researchers have revealed that using ZnO-NPs has successfully alleviated the toxicity of some metals such as Cd toxicity in a variety of crop plants, including Zea mays and Triticum

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Received: 27/05/2025; Accepted: 09/07/2025
DOI: 10.21608/EJSS.2025.389839.2194

aestivum, by enhancing their growth and yield (Faizan et al., 2021c); and toxicity of Cu on Solanum lycopersicum by increasing photosynthetic and antioxidants enzyme activities, plant growth, cell viability, and proline that improved the resistance of plants to Cu stress (Faizan et al., 2021a). Also, ZnO-NPs reduced the As concentration in soybean shoots and roots, enhanced their length, photosynthesis, transpiration rates, and enzyme activities, and improved growth factors (Ahmed et al., 2020).

In addition to using NPs in HMs remediation, many researchers proved that BC also as a carbonaceous, porous, highly stable, and eco-friendly material to effective improvement of soil quality, retards the toxicity of HMs, and decrease the mobility/bioavailability of HMs in contaminated soil, thus reducing their plant uptake and enhancing plant growth and productivity, (Amirahmadi et al., 2020, Shi et al., 2020, Mancy and Sheta, 2021, Murtaza et al., 2021, El-Nahas et al., 2022, Shiade et al., 2023, Zahra et al., 2025), as well adsorbing HMs from aqueous solution (Obar et al., 2024). Many studies stated that using BC with NPs can alleviate the adverse effects of heavy metal pollution (Ali et al., 2019; Seleiman et al., 2020). The BC application will regulate antioxidant development in response to stress, and affect the cell permeability, metal adsorption, and uptake in the plants by interfering with the electrical characteristics of the enzymes and membrane activity. Furthermore, metal contamination management is related to soluble protein and nucleic acid development because of the elevated activity of ATPase. Also, adding BC to polluted soils eliminates lipid peroxidation by reducing reactive oxygen species (ROS) generation under metal stress. Furthermore, BC detoxifies contaminants from plants by promoting their metabolic and enzymatic activities (Haider et al., 2022; Eissa et al., 2025). Thus, BC can support sustainable, eco-friendly agricultural practices and tackle contamination (Song et al., 2024b).

Lithium (Li) is the first metal in the alkali metals group (IA) that naturally exists in its elemental state. Li has been applied in many industrial applications, including batteries, detectors, ceramics, humectants, and nuclear reactors, indicating a significant contribution to the global economy (Tanveer et al., 2019; Shen et al., 2020). Over the last decade, the increased desire for higher charge density batteries has caused a substantial global Li output, which makes Li one of the most essential metals in society, with an increasing demand for many technological applications (Tkatcheva et al., 2015; Kamran and Park, 2022). Zhang et al. (2024) reported 85000 tons of Li in 2018 worldwide (double the consumption of 2016), and a 160000 tons projection in 2025. Thus, it becomes an emergent contaminant that should be considered. Hence, Li's large-scale industry has led to Li pollution in agriculture (Tanveer et al., 2019; Shen et al., 2020). Lithium has low toxicity; however, plants may easily absorb Li (i.e., it is a highly mobile element) from soil polluted by anthropogenic sources, making it a potential source of risk for organisms and the environment due to this high mobility and higher accumulation (Hayyat et al., 2020; Elbehiry et al., 2020). Tanveer et al. (2019) reported that Li is highly harmful to plants and inhibits their development significantly. Plant physiology and biochemistry are also altered, and plant development is reduced because of oxidative damage to photosynthesis and DNA. Li also induces necrosis and degrades chlorophyll content, which might be attributed to increased catalytic activity of chlorophyllase, red chlorophyll catabolite reductase, pheophorbide oxygenase, and Mg-dechelatase, all of which are involved in chlorophyll bleaching (Tanveer et al., 2019).

The faba bean (*Vicia faba, L.*) is a major leguminous crop that is produced extensively in Egypt and the Mediterranean region (Fayed et al., 2021; Mansour et al., 2021; Alnefaie et al., 2023). The green or dried seeds of beans are consumed by humans and animals (Fayed et al., 2021). In addition to starch, cellulose, minerals, and vitamin C, this crop is a significant source of protein, making up around 25–35% of its composition (Fayed et al., 2021; Alnefaie et al., 2023). It also contributes significantly to crop rotation, improves soil fertility, and increases soil N fixation like other legumes (Fayed et al., 2021).

By reviewing the published literature, no studies have reduced lithium pollution and mitigated its adverse effects on plants by BC or NPs. As well, till recently, generally, Li toxicity has gained little attention in research (Elbehiry et al., 2021). Thus, this work aims to investigate the ZnO-NPs effect on Li toxicity and the growth indicators of Vicia faba plants, as well as investigate the impact of BC individually or combined with ZnO-NPs on mitigating Li toxicity and improving plant growth indicators.

2. Materials and Methods

2.1 Seeds

Beans (*Vicia faba*) seeds (Sakha 1) were obtained from the Agricultural Research Center (ARC), Sakha, Kafr-Elshiekh, Egypt. The seeds were washed and immersed in a sterile solution for a day before cultivation.

2.2. Treatment preparation and application rates

2.2.1. Biochar preparation

The dates nuclei were collected locally, cleaned thoroughly with tap water and multiple times with DI water, dried for 24 hrs (at 105° C), and pyrolyzed into BC in a porcelain airtight jar under a restricted oxygen environment in a furnace (ZBX1, China) at 500 ° C for 4 hours. Biochar was added at 2% concentration alone or mixed with Li and ZnO-NPs. The properties of BC were mentioned in Elbasiouny et al. (2023).

2.2.2. Nano-Zinc Oxide Preparation

ZnO Nanopowder was synthesized by the sol-gel process as follows: Zinc acetate [$Zn(CH_3CO_2)_2.2H_2O$] and NaOH were used as a precursor material and the solvent, respectively. Zinc acetate was dissolved in NaOH at a molar ratio 1:85. After stirring, the solution was refluxed at 70-75℃ for 4 hours. Then, filtration was done with Whitman filter paper. During the filtration, the solution was washed with ethanol many times to avoid impurities. After filtration, the filtered sample was heated at 90 °C in the oven for 2 hours. The heated sample was ground. Transmission Electron Microscopy (TEM) and X-ray diffraction (XRD) were performed to determine the size of ZnO-NPs (Amutha & Dhanalakshmi, 2017).

2.2.3. Lithium and zinc oxide nanoparticle solutions preparation

Lithium solution was prepared as 50 mg L^{-1}, while zinc oxide-NPs (ZnO-NPs) solution was prepared as 25 and 100 mg L^{-1} and dispersed well in water before each application.

2.3. Experiment design and treatments

Twelve treatments were prepared as in Table 1. The plants were grown in 15 by 15 cm tubs with small holes approximately 1.5 cm wide. In addition to the treatment, a water tap was placed at a fixed level below the holes in each tub. Thirty faba beans were placed above the holes (to allow the roots to grow without drowning the plants). Growth continued for 45 days, and the water was weakly supplied to reach the same level as the starting point of the experiment. At the end of the experiment, each plant was carefully removed from the growth box without breaking the roots, as dense entanglement was observed between the roots in the growth basin. It was also observed that the roots of plants treated with BC were black. The plants were washed well with tap water, then washed several times with distilled water, and then morphological measurements (plant height, root and shoot length, root hairs, and leaf parameters) were taken. Some whole plants were kept dry to measure the elements in them, and some other plants were kept in the fridge to measure enzymatic activity and cytogenetic effects.

Table 1. Treatments in the experiment.

Treatments	Description
T1	Control (Tap water without any additions)
T2	25 mg L^{-1} ZnO-NPs
T3	100 mg L^{-1} ZnO-NPs
T4	50 mg L^{-1} Li
T5	25 mg L^{-1} ZnO-NPs +50 mg L^{-1} Li
T6	100 mg L^{-1} ZnO-NPs +50 mg L^{-1} Li
T7	BC (2%)
T8	BC+ 25 mg L^{-1} ZnO-NPs
T9	BC+100 mg L^{-1} ZnO-NPs
T10	BC+50 mg L^{-1} Li
T11	BC+ 25 mg L^{-1} ZnO-NPs +50 mg L^{-1} Li
T12	BC+ 100 mg L^{-1} ZnO-NPs +50 mg L^{-1} Li

3. Plant analyses

3.1 Germination% = the ratio of germinated seeds number and whole seeds number * 100 Eq. 1

3.2. Morphological traits

On a fresh plant sample, the morphological features of fine roots and root and shoot lengths were immediately assessed (after washing). Fresh plants were weighed, then oven-dried for 72 hrs. (at 60 °C). Maximum leaf width (W) and length (L) were measured to calculate leaf area according to the proposed model suggested by Peksen et al. (2007).

3.3. Enzyme activities in plants

0.5 g of plant leaves were mixed in 3 ml of 50 mM TRIS buffer (pH 7.8) containing 1 mM EDTA-2Na and 7.5% polyvinylpyrrolidone (at 0-4˚C). The mixture was centrifuged (at 4˚C) for 20 min, at 12,000 rpm and the activities of total soluble enzymes were determined in the supernatant spectrophotometrically (UV-160A spectrophotometer, Shimadzu, Japan) at 25˚C (Hafez, 2010). The catalase activity (CAT) was assessed (Aebi, 1984), as well as peroxidase (POX) (Hammerschmidt et al, 1982), and polyphenol oxidase (PPO) (Boeckx et al., 2025) at the absorbance of 240, 470, and 495 nm.

3.4. Metals in plants

Ash dried (at 450 °C for 4 hours) plant material (1g) was used for extracting metals (Zn and Li) with 20% HCl (Jones et a., 1991), then metals in plants were detected using atomic absorption spectrometry (GAS; GBC Avanta E; Victoria; Australia).

3.5. Bioaccumulation factor

The BAF is the ratio between the metal concentration in the organism (CO) and its concentration at steady state in the water (CW) as in Eq. 2

$$BCF = CO / CW \qquad \text{Eq. 2} \quad \text{(Agarwal et al., 2022)}$$

3.6. Cytological evaluation

The root seedling tips (1.5-2 cm) were preserved for 24 hrs. in a solution of Carnoy's fixative (1 glacial CH_3COOH: 3 C_2H_5OH absolute) and then stored in 70% C_2H_5OH at 4°C until cytological analysis. For cytological preparations, 2% aceto-carmine stain was used (Darlington, 1976). At least 3000 investigated cells (1000 cells/replicate) were examined under a light microscope to determine the mitotic index, number, and abnormality types.

Abnormal cells percent and mitotic index (MI) were calculated as follows:

Miotic index (MI) = (whole dividing cells/ whole cells (dividing and non-dividing)) x 100 Eq. 3

Abnormal cells% % = (whole abnormal cells / whole dividing cells) x 100 Eq. 4 (Darlington, 1976).

3.7. Statistical analyses

The experiment was completely randomly designed. The data were statistically analyzed to show the difference between the treatment means using One-Way ANOVA at a significance level of $P \leq 0.05$ by SPSS 20 software (IBM SPSS Statistics Software, IBM, Armonk, New York). A Duncan's test was used to compare the treatment means, and the data were presented as a mean and standard deviation (SD).

4. Results

4.1. Characterization of ZnO-NPs

The TEM image for ZnO-NPs shows the particle size in Figure 1. The average particle size of ZnO-NPs ranged from 20 to 25nm. The XRD pattern of the ZnO-NPs shows an apparent broadening of the XRD peak lines, indicating that the prepared particles are in the nanoscale range, as shown in Figure 2, consistent with the TEM image. The diffraction peaks located at 31.8, 34.4, 36.2, 47.5, 56.5, 62.8, 66.3, 67.9, 69.1, 72.5, and 76.90 were identified as identical to those of pure zinc oxide.

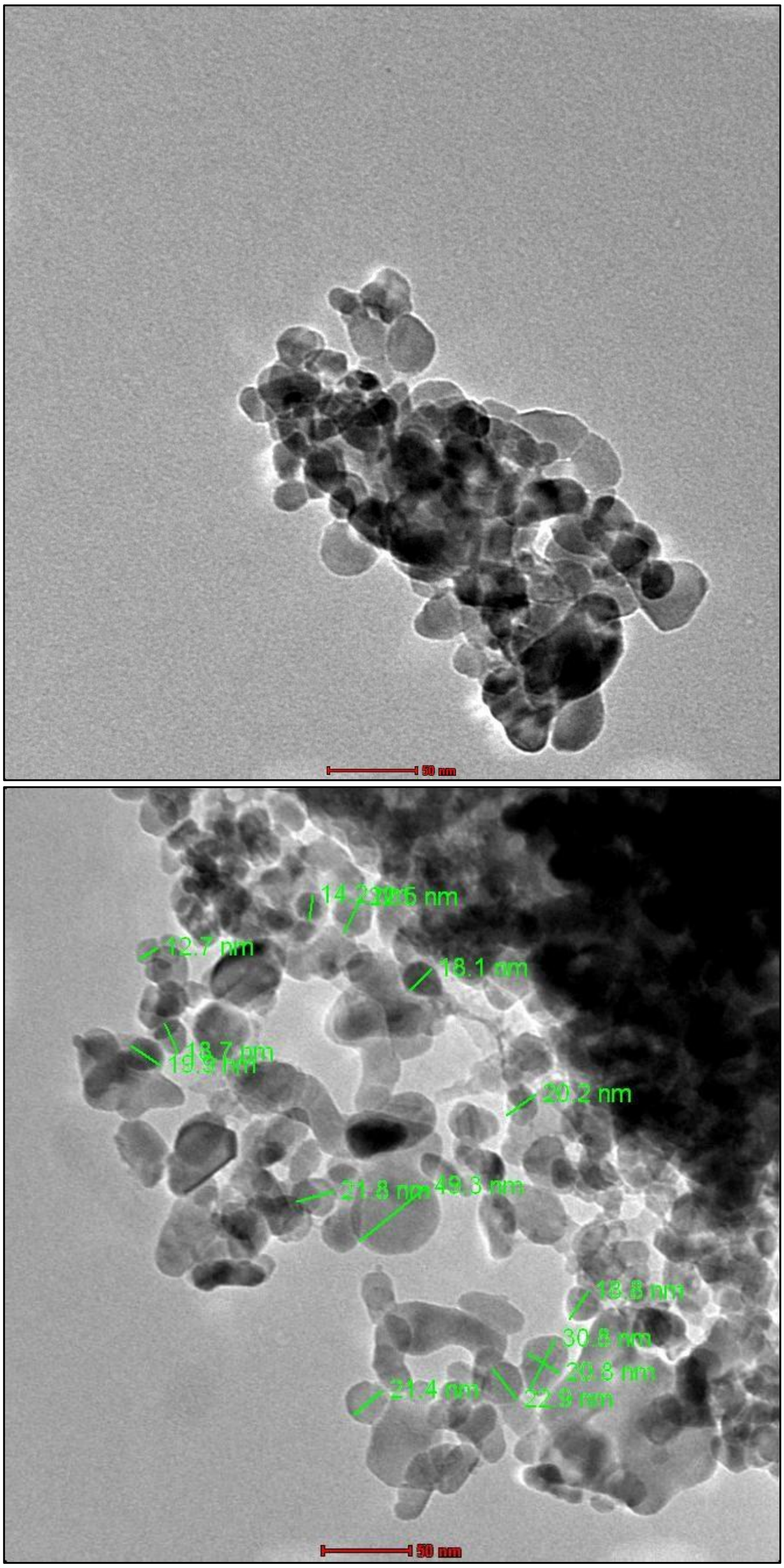

Fig. 1. TEM images for ZnO-NPs.

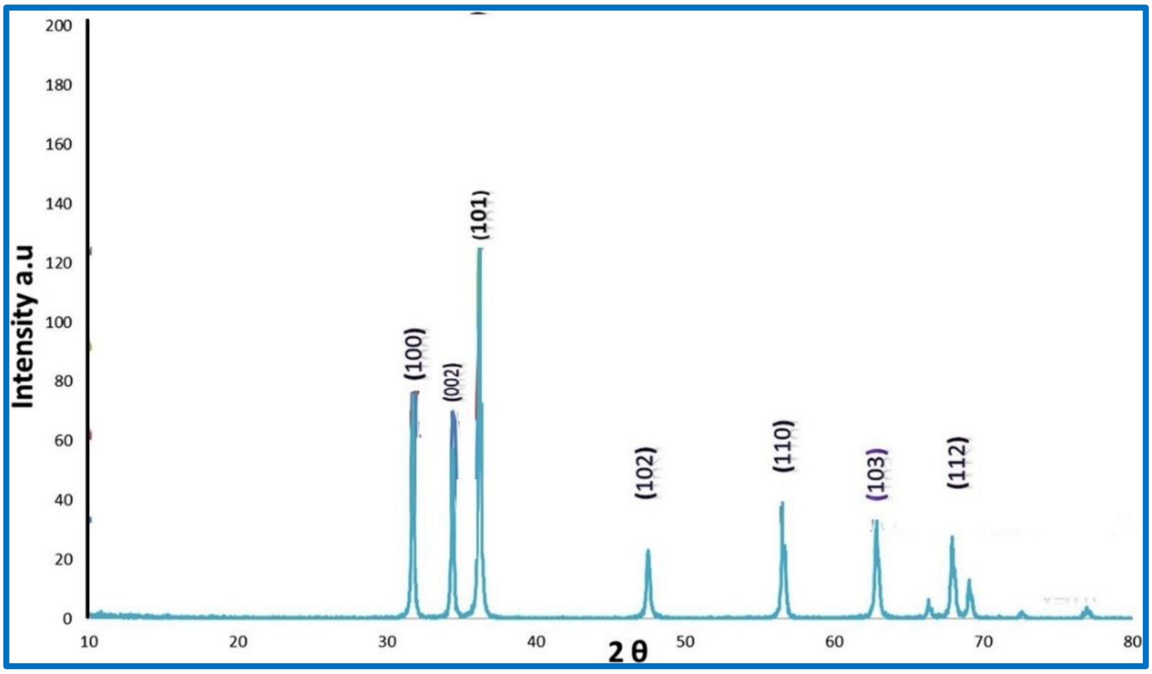

Fig. 2. XRD patterns of present ZnO-NPs.

4.2. Germination percentages

The germination percentage across treatments varied considerably, as shown in Figure 3. The Li treatment (T4) recorded the lowest germination percentage (58.6%) compared to the control (T1) (the highest value) (83.3%), implying that Li exposure negatively impacted the seeds' germination, and suggesting that Li toxicity interferes with the physiological processes necessary for adequate germination. Additionally, the results showed that both ZnO-NPs negatively impacted seed germination compared to the control; however, when combined with Li, the germination percentage improved compared to either Li or ZnO-NPs alone. Furthermore, BC contributed to a higher germination rate; BC + a low concentration of ZnO-NPs improved the germination rate, which was the best and close to the control, suggesting that BC may alleviate these adverse effects and enhance germination performance, especially when combined with a low concentration of ZnO-NPs.

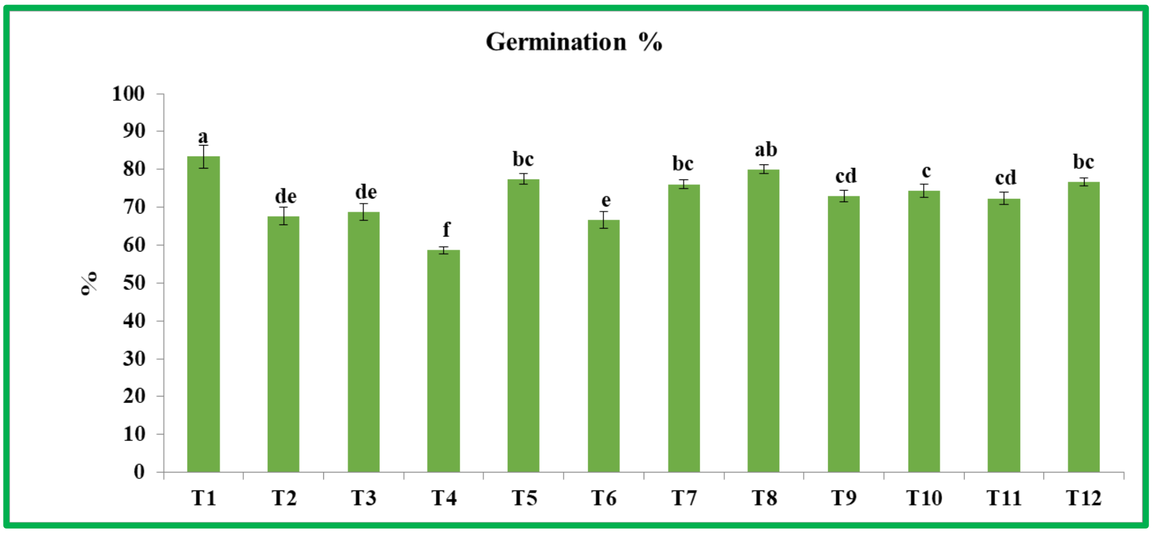

Fig. 3. Germination Percentage of Vicia Faba as affected by the investigated treatments.

T1: Control; T2: 25 mg l^{-1} ZnO-NPs; T3: 100 mg l^{-1} ZnO-NPs; T4: 50 mg l^{-1} Li; T5: 25 mg l^{-1} ZnO-NPs +50 mg l^{-1} Li; T6: 100 mg l^{-1} ZnO-NPs +50 mg l^{-1} Li; T7: BC; T8: BC+ 25 mg l^{-1} ZnO-NPs; T9: BC+100 mg l^{-1} ZnO-NPs; T10: BC+50 mg l^{-1} Li; T11: BC+ 25 mg l^{-1} ZnO-NPs +50 mg l^{-1} Li; T12: BC+ 100 mg l^{-1} ZnO-NPs +50 mg l^{-1} Li

4.3. Plant morphology

Plants grown under different treatments exhibited pronounced variations in the values of morphological growth parameters compared to the control (Tables 2 and 3). Root length in T2 and T3 (ZnO-NPs: 25 and 100 mg l^{-1}), T4 (Li), or T5 (25 mg l^{-1} ZnO-NPs + Li) was decreased significantly compared to control, while the root length in T6 (100 mg l^{-1} ZnO-NPs +50 mg l^{-1} Li) was significantly increased (20.7 cm) compared to control (18.5 cm). Following the BC application, root length increased substantially compared to the control. The highest value of root length in BC treatments was recorded in T11 (29.7 cm), while the lowest value was recorded in T10 (19.3 cm), indicating improvement in root length resulting from BC applications. The variation in shoot length values was apparent in all treatments, and some treatments significantly decreased or increased insignificantly compared to the control, as shown in Table 2. Ansari et al. (2024) confirmed that the dose-dependent reduction in seedling and shoot growth follows NPs application. T12 was the exception; its value increased significantly than the control (28 cm) and was recorded 42 cm, indicating the mitigation effect of combining BC and ZnO-NPs. However, T12 recorded the lowest number of root hairs. The root hairs recorded the highest significant value in T9 (61 hairs), then T7 (BC) (57.3). The number of leaves was lower than the control (8.3) in all treatments except in T2 and T3.

Table 2. Morphological growth parameters of faba bean at the end of the experiment (mean ± standard deviation).

Treatments	Root length	Shoot length	No of root hairs	No of leaves
T1	$18.5^{d}\pm1.8$	$30.0^{bcd}\pm1.0$	$28.0^{e}\pm1.7$	$8.3^{bc}\pm1.5$
T2	$10.3^{f}\pm0.6$	$28.3^{cde}\pm2.1$	$51.7^{c}\pm2.9$	$10.3^{a}\pm0.6$
T3	$16.3^{d}\pm1.2$	$25.0^{efg}\pm2.0$	$29.7^{e}\pm1.5$	$9.7^{ab}\pm0.6$
T4	$13.3^{e}\pm1.2$	$29.0^{bcd}\pm1.0$	$29.7^{e}\pm2.9$	$7.7^{cd}\pm0.6$
T5	$9.8^{f}\pm1.0$	$30.7^{bcd}\pm1.2$	$28.3^{e}\pm0.6$	$5.7^{f}\pm0.6$
T6	$20.7^{bc}\pm0.6$	$32.7^{b}\pm2.5$	$51.3^{c}\pm3.2$	$5.7^{ef}\pm0.6$
T7	$21.0^{bc}\pm1.0$	$24.3^{fg}\pm2.1$	$57.3^{b}\pm2.3$	$6.3^{de}\pm0.6$
T8	$20.7^{bc}\pm0.6$	$30.7^{c}\pm1.2$	$38.7^{d}\pm2.3$	$5.7^{ef}\pm0.6$
T9	$22.0^{b}\pm2.6$	$27.0^{defg}\pm2.0$	$61.0^{a}\pm1.7$	$4.3^{f}\pm0.6$
T10	$19.3^{c}\pm2.3$	$23.3^{g}\pm2.1$	$21.0^{f}\pm2.0$	$6.7^{de}\pm1.2$
T11	$29.7^{a}\pm0.6$	$31.0^{bcd}\pm1.7$	$39.3^{d}\pm1.2$	$6.3^{de}\pm0.6$
T12	$20.7^{bc}\pm0.8$	$42.0^{a}\pm3.6$	$19.0^{f}\pm1.7$	$5.3^{ef}\pm1.2$

T1: Control; T2: 25 mg l^{-1} ZnO-NPs; T3: 100 mg l^{-1} ZnO-NPs; T4: 50 mg l^{-1} Li; T5: 25 mg l^{-1} ZnO-NPs +50 mg l^{-1} Li; T6: 100 mg l^{-1} ZnO-NPs +50 mg l^{-1} Li; T7: BC; T8: BC+ 25 mg l^{-1} ZnO-NPs; T9: BC+100 mg l^{-1} ZnO-NPs; T10: BC+50 mg l^{-1} Li; T11: BC+ 25 mg l^{-1} ZnO-NPs +50 mg l^{-1} Li; T12: BC+ 100 mg l^{-1} ZnO-NPs +50 mg l^{-1} Li

4.4. Enzyme activity in *Vicia faba*

The results in Figure 4 revealed that adding ZnO-NPs increased CAT activity in Vicia faba than in the control. Interestingly, the lower addition rate of ZnO-NPs had the most significant effect. When Li was added to the growing environment, it had a remarkable influence on the CAT enzyme, with the enzyme activity being higher than the values of CAT in the two addition rates of ZnO-NPs. When Li was mixed with ZnO-NPs (at 25 or 100 mg L^{-1}), the impact of Li on the CAT enzyme was significantly reduced. The CAT was increased in BC application and BC + ZnO-NPs, Li, or ZnO-NPs + Li compared to the control and other treatments, except for Li. The highest effect of BC was recorded in T10. On the other hand, the BC application mixed with ZnO-NPs + Li significantly decreased the CAT activity; however, the CAT values were higher than those of the control. Also, the ZnO-NPs application increased POX activity in *Vicia faba* compared to the control, with a higher effect in 100 mg l^{-1}. However, Li didn't affect POX activity, while when Li was mixed with ZnO-NPs, POX activity was increased markedly compared to the control, especially at the higher rate of ZnO-NPs. On the other hand, BC treatments were higher than the control, especially with a lower addition rate of ZnO-NPs + Li. Although the Li addition didn't remarkably affect POX, ZnO-NPs + Li treatments rose POX activity. Also, the BC addition to ZnO-NPs + Li increased the POX activity compared to the corresponding treatments without BC. Li addition alone or + 25 mg l^{-1} ZnO-NPs increased PPO activity in *Vicia faba* compared to the control, with a higher effect in Li treatment. However, 100 mg l^{-1} ZnO-NPs + Li decreased the PPO activity compared to the control. The BC remarkably declined the value of PPO compared with the corresponding treatments without BC. The highest effect with BC was recorded in T7, while the lowest was in T10. Thus, the BC or BC + 100 mg l^{-1} ZnO-NPs mitigated the adverse impact of Li compared with the control.

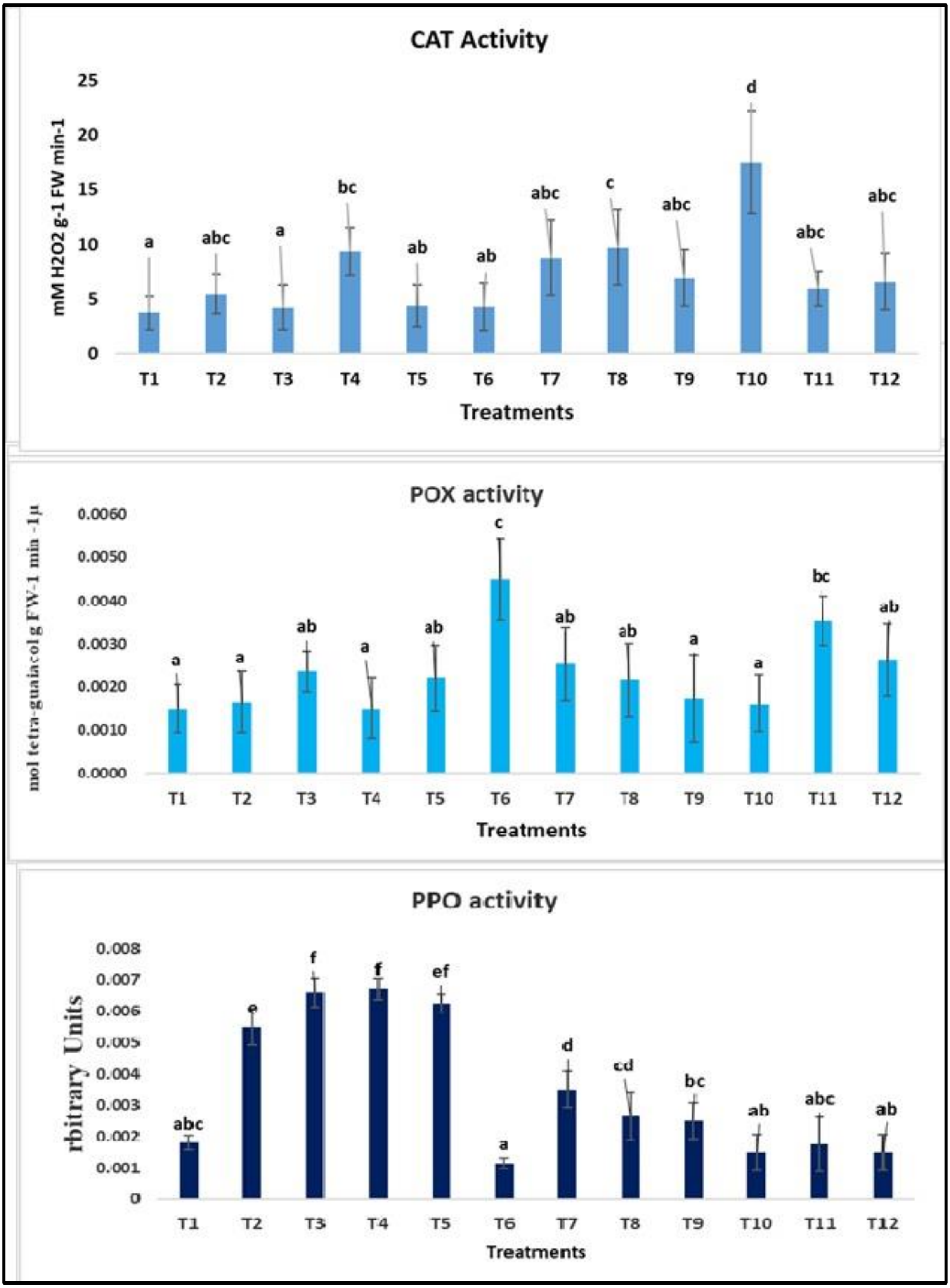

Fig. 4. Enzyme activities in *Vicia faba as affected by ZnO-NPs, Lithium, and Biochar*.

T1: Control; T2: 25 mg l^{-1} ZnO-NPs; T3: 100 mg l^{-1} ZnO-NPs; T4: 50 mg l^{-1} Li; T5: 25 mg l^{-1} ZnO-NPs +50 mg l^{-1} Li; T6: 100 mg l^{-1} ZnO-NPs +50 mg l^{-1} Li; T7: BC; T8: BC+ 25 mg l^{-1} ZnO-NPs; T9: BC+100 mg l^{-1} ZnO-NPs; T10: BC+50 mg l^{-1} Li; T11: BC+ 25 mg l^{-1} ZnO-NPs +50 mg l^{-1} Li; T12: BC+ 100 mg l^{-1} ZnO-NPs +50 mg l^{-1} Li**.** Bars on columns represent SD values.

4.5. Metal Concentrations in *Vicia Faba* Tissues

4.5.1. Li concentration Li

The concentration of Li in plant tissues was lower in ZnO-NPs treatments than in the control; however, the decrease was insignificant (Figure 5). The highest concentration of Li in plant tissues was in Li treatment (631.07 mg g^{-1}) as expected. According to Shahzad et al. (2016), Li threshold concentrations in plants are very variable. The moderate to severe toxic levels are 4–40 mg kg^{-1} of Li, which were recorded in citrus leaves. Thus, all Li treatments (Li, Li +Zn, and Li+ Zn+ BC) were higher than the severe limit, although the addition of BC significantly decreased Li concentration compared to Li treatment only. It was noticed that ZnO-NPs + Li decreased the Li concentration in plant tissues significantly compared to the Li treatment. The same trend was also noticed in all BC treatments, but not at the same rate as ZnO-NPs addition. However, the BC significantly decreased the Li concentration in all treatments, with a distinct decrease in BC + low rate of ZnO-NPs + Li.

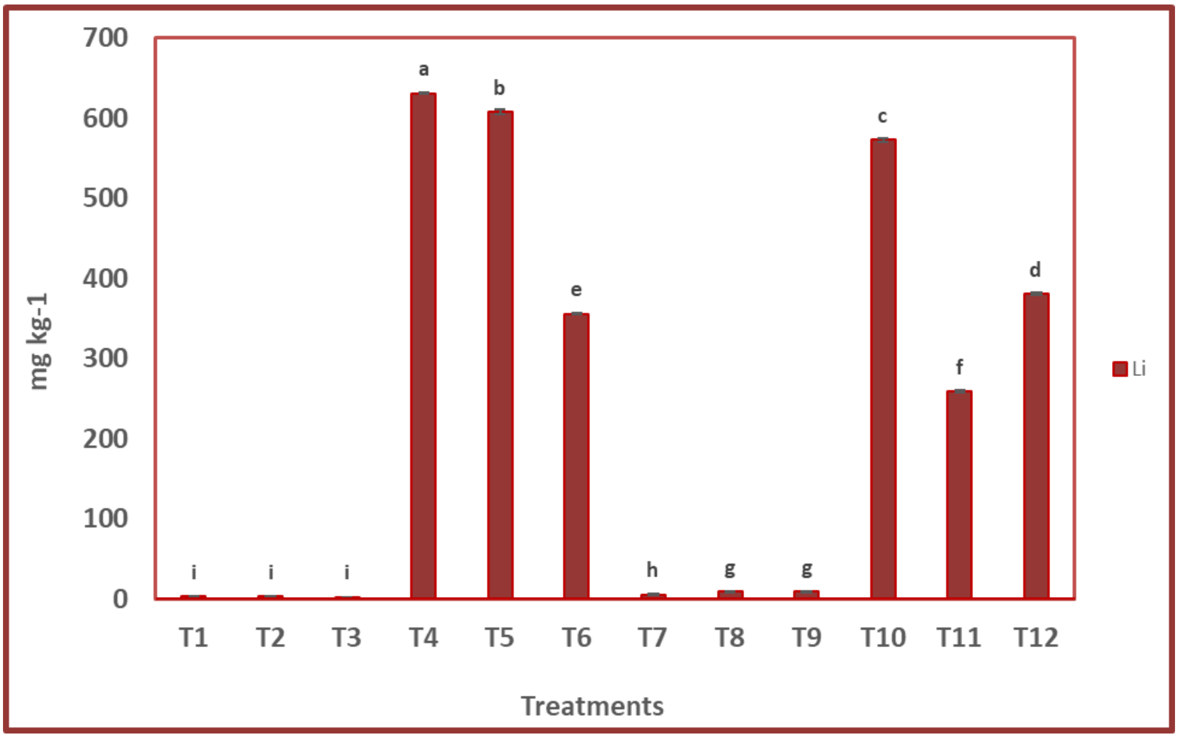

Fig. 5. Concentration of Li in *Vicia Faba* Tissues as affected by ZnO-NPs, Lithium, and Biochar.

T1: Control; T2: 25 mg l^{-1} ZnO-NPs; T3: 100 mg l^{-1} ZnO-NPs; T4: 50 mg l^{-1} Li; T5: 25 mg l^{-1} ZnO-NPs +50 mg l^{-1} Li; T6: 100 mg l^{-1} ZnO-NPs +50 mg l^{-1} Li; T7: BC; T8: BC+ 25 mg l^{-1} ZnO-NPs; T9: BC+100 mg l^{-1} ZnO-NPs; T10: BC+50 mg l^{-1} Li; T11: BC+ 25 mg l^{-1} ZnO-NPs +50 mg l^{-1} Li; T12: BC+ 100 mg l^{-1} ZnO-NPs +50 mg l^{-1} Li. Bars on columns represent SD values.

4.5.2. Zn concentration

The data in Figure 6 also showed increasing Zn concentrations in plant tissues compared to the control in all treatments except T4 and T7. The change in Zn concentration in T7 was insignificant compared to the control. However, it was markedly stated that the BC application decreased Zn concentration in plant tissues compared to their concentrations in ZnO-NPs or ZnO-NPs + Li treatments.

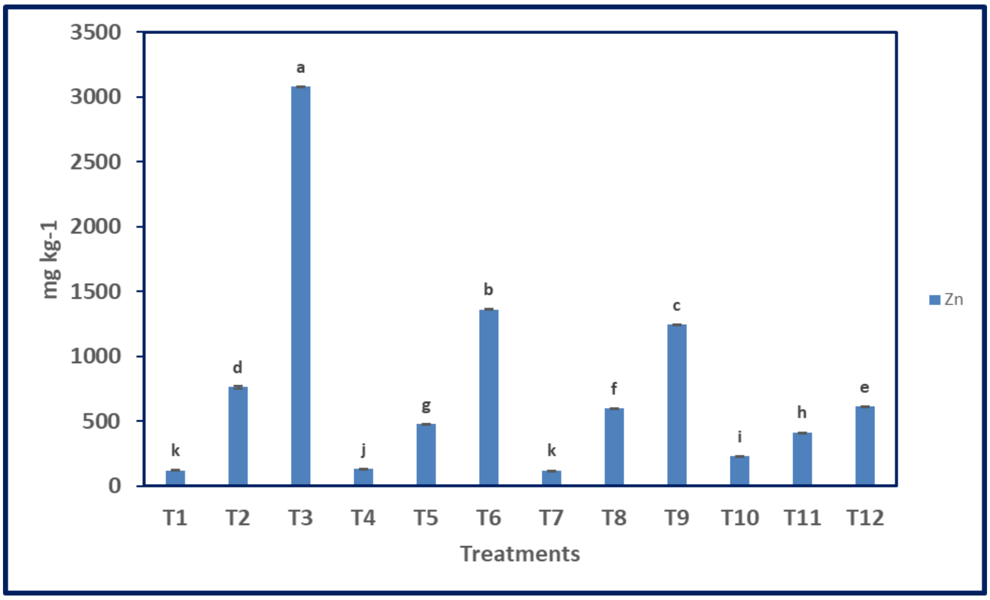

Fig. 6. Concentration of Zn in *Vicia Faba* Tissues as affected by ZnO-NPs, Lithium, and Biochar.

T1: Control; T2: 25 mg L^{-1} ZnO-NPs; T3: 100 mg L^{-1} ZnO-NPs; T4: 50 mg L^{-1} Li; T5: 25 mg L^{-1} ZnO-NPs +50 mg L^{-1} Li; T6: 100 mg L^{-1} ZnO-NPs +50 mg L^{-1} Li; T7: BC; T8: BC+ 25 mg L^{-1} ZnO-NPs; T9: BC+100 mg L^{-1} ZnO-NPs; T10: BC+50 mg L^{-1} Li; T11: BC+ 25 mg L^{-1} ZnO-NPs +50 mg L^{-1} Li; T12: BC+ 100 mg L^{-1} ZnO-NPs +50 mg L^{-1} Li. Bars on columns represent SD values.

4.6. Bioaccumulation of zinc and lithium in plant tissues

Bioaccumulation Factor (BAF) of Zn and Li in *Vicia faba*

This study determined the BAFs for Zn and Li in *Vicia faba*. As shown in Figure 7, BAF values in all treatments were higher than 1. *Vicia faba* exhibited markedly higher accumulation of Zn (30.8 in T3) than Li (12.6 in T4). In contrast, the BAF of Li was lower than that of Zn because the Li concentration in the plant was much lower than that of Zn. In our study, the BC application reduced the BCF of Zn and Li in *Vicia faba* plants, as Zn accumulation decreased by 35.8% and 55.8% in 25 and 100 mg l^{-1} of ZnO-NPs, respectively. Nonetheless, the BC reduced the BAF of Li by 5% compared to Li treatment only.

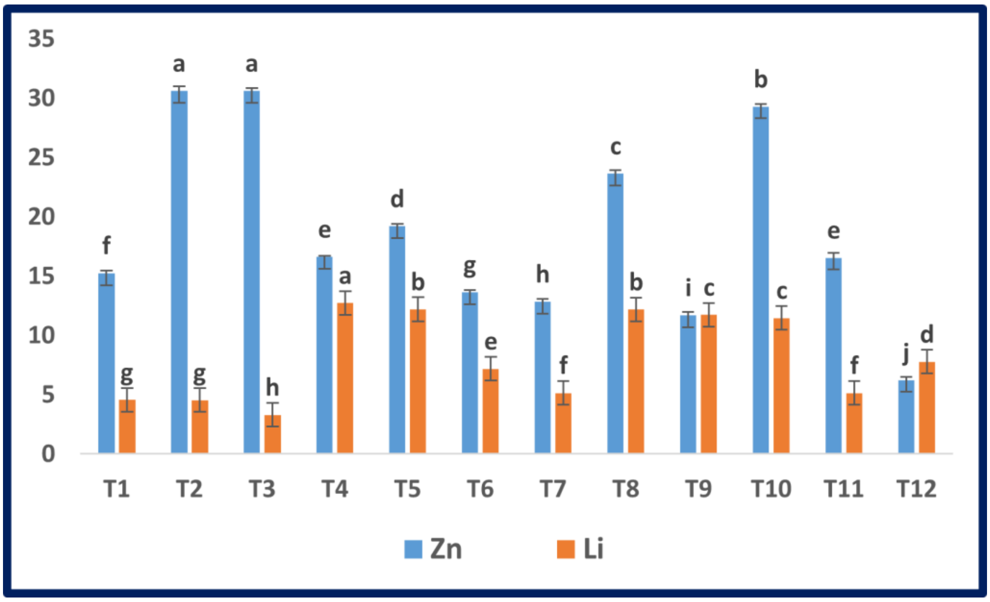

Fig. 7. Bioaccumulation factor of zinc and Li in Vicia faba.

T1: Control; T2: 25 mg L^{-1} ZnO-NPs; T3: 100 mg L^{-1} ZnO-NPs; T4: 50 mg L^{-1} Li; T5: 25 mg L^{-1} ZnO-NPs +50 mg L^{-1} Li; T6: 100 mg L^{-1} ZnO-NPs +50 mg L^{-1} Li; T7: BC; T8: BC+ 25 mg L^{-1} ZnO-NPs; T9: BC+100 mg L^{-1} ZnO-NPs; T10: BC+50 mg L^{-1} Li; T11: BC+ 25 mg L^{-1} ZnO-NPs +50 mg L^{-1} Li; T12: BC+ 100 mg L^{-1} ZnO-NPs +50 mg L^{-1} Li. Bars on columns represent SD values.

4.7. Cytogenetic analysis

4.7.1. Cytological effects on mitosis of root tips of *V. faba*

It has been shown from Table 3 that the treatments shifted the balance between phases; the highest prophase was recorded in T12 (81.89%) and T9 (75.56%), while the lowest was recorded in T11 (29.62%). Metaphase reached the highest peaks in T5 and T3 (30.55 and 30.43%, respectively), while it recorded the lowest in T4 (11.11%) and T7 (11.34%). Anaphase was mainly frequent in T2 (19.73%) and T5 (15.74%), whereas it was the lowest in T12 (2.8%). Telophase was the highest in T11 (50%), while it was less often in T4 (42.22%) and almost absent in T9 (0.56%). Table 4 shows the ZnO-NPs, Li, and BC influence on the mitotic index (%) and abnormalities (%) in *V. faba* root tips. ZnO-NPs modify the structure of biomolecules, including proteins and DNA, which affects the regular operation of plant cells and DNA (Pullagurala et al., 2018). Cytological evaluation revealed that the maximum value of the mitotic index was recorded in the control, BC, BC+25 mg $^{l-1}$ ZnO-NPs, BC+100 mg l^{-1} ZnO-NPs, and BC+100 mg $^{l-1}$ ZnO-NPs +50 mg $^{l-1}$ Li treatments, showing significant ($P < 0.05$) differences compared to other treatments. Thus, BC achieved better outcomes than ZnO-NPs.

Inducing abnormalities and decreasing mitotic index were markedly shown in 25 mg L^{-1} ZnO-NPs +50 mg L^{-1} Li, followed by 25, 100 mg L^{-1} ZnO-NPs, and 100 mg L^{-1} ZnO-NPs +50 mg L^{-1} Li, respectively. It was noticed that 25 mg L^{-1} ZnO-NPs recorded higher abnormalities than 100 mg L^{-1} ZnO-NPs and 100 mg L^{-1} ZnO-NPs mixed with 25 mg L^{-1} Li, and reduced the aberration compared to 100 mg L^{-1} ZnO-NPs. The most common mitotic abnormalities prompted by Zn and Li were stickiness, chromosomal disorders, bridges, and c-metaphase (Figure 8). The combination of BC with Zn and Li significantly reduced chromosomal aberration and undesirable side effects of Zn and Li; the best treatments are BC+100 mg L^{-1} ZnO-NPs, BC+25 mg L^{-1} ZnO-NPs +50 mg L^{-1} Li, and BC+100 mg L^{-1} ZnO-NPs +50 mg L^{-1} Li. The current study demonstrated that MI values increased differentially with various treatments of Zn, Li, and combinations with BC, with the highest value noted for the lower concentration of Zn combined with BC compared to other treatments (Table 4).

Table 3. The total investigated dividing and abnormal cell and mitotic phase (%) effects of different applied treatments.

Treatments	No. of examined cells	No. of dividing cells	No. of abnormal cells	Mitotic phase (%)			
				Prophase	Metaphase	Anaphase	Telophase
T1	3087	261	0	72.79	14.55	6.89	5.74
T2	3219	76	34	36.84	23.68	19.73	19.73
T3	3011	46	16	32.60	30.43	6.52	30.43
T4	3151	45	5	31.11	11.11	15.55	42.22
T5	3014	108	48	42.59	30.55	15.74	10.18
T6	3007	40	8	45	17.5	12.5	25
T7	3183	238	10	63.02	11.34	10.92	14.70
T8	3123	246	1	74.39	12.60	4.87	8.13
T9	2638	176	0	75.56	13.63	10.22	0.56
T10	3043	84	1	42.85	13.09	13.09	30.95
T11	3124	54	0	29.62	12.96	7.40	50
T12	3204	243	0	81.89	14.40	2.8	0.82

T1: Control; T2: 25 mg L^{-1} ZnO-NPs; T3: 100 mg L^{-1} ZnO-NPs; T4: 50 mg L^{-1} Li; T5: 25 mg L^{-1} ZnO-NPs +50 mg L^{-1} Li; T6: 100 mg L^{-1} ZnO-NPs +50 mg L^{-1} Li; T7: BC; T8: BC+ 25 mg L^{-1} ZnO-NPs; T9: BC+100 mg L^{-1} ZnO-NPs; T10: BC+50 mg L^{-1} Li; T11: BC+ 25 mg L^{-1} ZnO-NPs +50 mg L^{-1} Li; T12: BC+ 100 mg L^{-1} ZnO-NPs +50 mg L^{-1} Li. 0 means in detection.

Table 4. Mitotic index, types, and percent of V. faba root tip cells abnormalities in the applied treatments.

Treatments	Mitotic aberration (%)									Mitotic index (%)	Abnormalities (%)
	Stickiness	Disrupted	Multipolar anaphase	Fragments	Bridge	C-metaphase	Loss	Pole-to-pole metaphase	Laggard		
T1	0	0	0	0	0	0	0	0	0	8.45±2.20^{a}	0.00±0.00^{c}
T2	8.82	44.11	2.94	23.52	5.88	5.88	2.94	2.94	2.94	2.39±0.79^{b}	42.17±36.48^{ab}
T3	18.75	37.5	0	12.5	0	18.75	0	12.5	0	1.53±0.41^{b}	34.41±11.10^{ab}
T4	0	100	0	0	0	0	0	0	0	1.42±049^{b}	11.33±3.51^{c}
T5	8.33	54.16	2.08	20.83	6.25	2.08	4.16	2.08	-	3.53±1.72^{b}	48.16±13.77^{a}
T6	0	37.5	0	0	12.5	50	0	0	0	1.12±0.35^{b}	23.00±12.27^{bc}
T7	0	40	0	20	10	30	0	0	0	7.45±109^{a}	4.65±5.77^{c}
T8	0	0	0	0	0	100	0	0	0	7.88±0.46^{a}	0.39±0.67^{c}
T9	0	0	0	0	0	0	0	0	0	6.69±2.80^{a}	0.00±0.00^{c}
T10	0	0	0	0	100	0	0	0	0	2.75±0.59^{b}	1.33±2.30^{c}
T11	0	0	0	0	0	0	0	0	0	1.74±0.93^{b}	0.00±0.00^{c}
T12	0	0	0	0	0	0	0	0	0	7.64±2.16^{a}	0.00±0.00^{c}
sig										0.00	0.00

T1: Control; T2: 25 mg L^{-1} ZnO-NPs; T3: 100 mg L^{-1} ZnO-NPs; T4: 50 mg L^{-1} Li; T5: 25 mg L^{-1} ZnO-NPs +50 mg L^{-1} Li; T6: 100 mg L^{-1} ZnO-NPs +50 mg L^{-1} Li; T7: BC; T8: BC+ 25 mg L^{-1} ZnO-NPs; T9: BC+100 mg L^{-1} ZnO-NPs; T10: BC+50 mg L^{-1} Li; T11: BC+ 25 mg L^{-1} ZnO-NPs +50 mg L^{-1} Li; T12: BC+ 100 mg L^{-1} ZnO-NPs +50 mg L^{-1} Li.
0 means indetection

5. Discussion

The Findings showed an adverse effect on *Vicia Faba* germination induced by Li and showed variability by other treatments. Sarraf et al. (2024) reported that seed germination is hindered by metal(iod) stress, which can be attributed to reduced turgor pressure, particularly at elevated relative water content. Additionally, suppressing physiological and metabolic functions within the seed contributes to the obstruction of the germination process. Such variation in our treatments refers to Li, which hinders early plant development, potentially resulting in diminished growth and development if the stress persists. The ZnO-NPs application showed unanticipated findings compared to normal because many authors described its role in plant growth, such as Ďúranová et al. (2024), who reported that NPs application enhances seed quality, resulting in higher yields and greater resilience against biotic and abiotic stresses. This enhanced seed enzymes, consequently increasing germination rates. It also promotes the development of nano-pores in the shoot, improves water uptake, stimulates the production of ROS, activates antioxidant systems in seeds, and generates hydroxyl radicals, which play a crucial role in loosening cell walls, accelerating the hydrolysis of starch. They also noticed that ZnO-NPs positively correlated with physiological indicators in plants, possibly because of increased Zn bioavailability, which boosts plant physiological and metabolic processes. Zha et al. (2022) stated that ZnO-NPs are utilized as nano-fertilizers to improve Zn availability to plants and enhance seed germination and biomass.

Furthermore, it plays various critical roles in vital processes in plants such as respiration, photosynthesis, chlorophyll synthesis, protein metabolism, electron transport, and hormonal regulation. However, Pedruzzi et al. (2020) verified that the NPs' absorption, translocation, and bioaccumulation in plants are greatly impacted by plant species and NPs' characteristics such as size, shape, chemical composition, and stability, which may explain why ZnO-NPs suppress germination in Vicia Faba. Furthermore, much research on ZnO-NPs was conducted in soil; therefore, the effects on aquatic conditions may differ from those in this work.

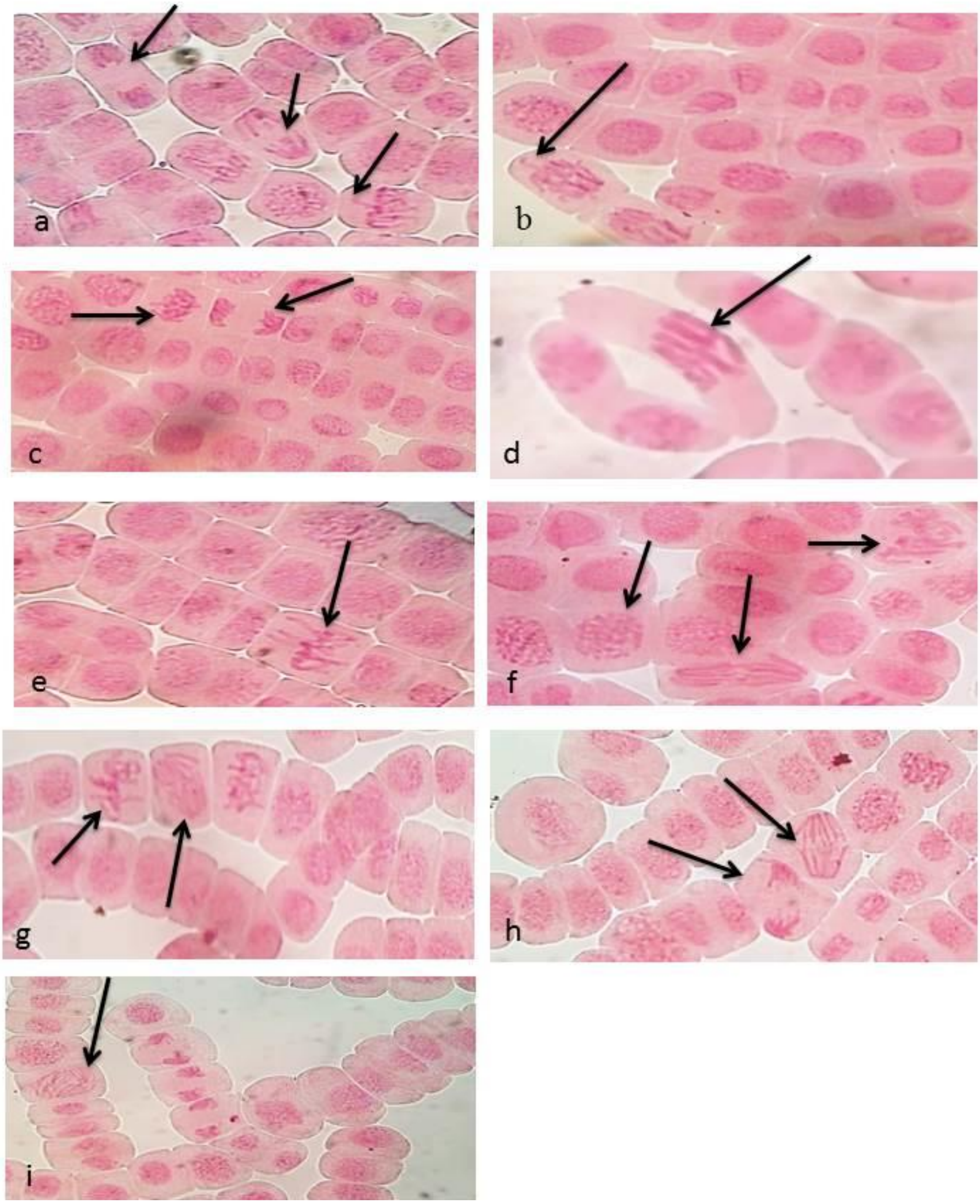

Fig. 8. Types of abnormalities observed in *V. faba* root tips cells under different treatments.

(a) Stickiness telophse, Anaphse with fragment, metaphase with Laggard chromosome, (b) Anaphse with Laggard chromosome, (c) Stickiness metaphase and telophase, (d) C-metaphase, (e) Pole-to-pole metaphase, (f) Stickiness prophase, Anaphase with bridge, C- metaphase, (g) Multipolar anaphase and disrupted metaphase, (h) Telophase with loss chromosome, Anaphase with bridge (i) Multipolar anaphase.

The Li adverse effect was mainly noticed in the morphological parameters, either alone or when mixed with the low addition of ZnO-NPs; nevertheless, the high addition of ZnO-NPs, BC, or the mix between both showed a positive effect compared to Li treatments. Shukla et al. (2023) noticed an enhancement in growth parameters when combining ZnO-NPs with BC to alleviate arsenic toxicity in rice fields. Shahzad et al. (2017) reported that some plants are sensitive to Li toxicity, while others are unaffected and show normal growth. Also, some Li has a positive effect on some plants (such as promoting the opening movement of folioles, faster maturation, and resistance to diseases with low concentration, ...) or an adverse effect on others (such as triggering chlorosis–like symptoms, necrotic spots, reducing assimilation organ area, reducing the water content of cells thus declining the growth….). They added that Li in the medium growth can affect several plant morphological processes. Tanveer et al. (2019) reported that the high toxicity of Li to plants led to a significant growth reduction, which can be interpreted by the decrease in root and shoot length in the Li treatment. Moreover, Tanveer et al. (2019) explained that Li-induced plant growth reduction can result from oxidative damage to the photosynthetic system and DNA.

Additionally, Li hinders the inositol cycle and Ca signaling in plants. Li also triggers necrosis and breakdown of chlorophyll concentration, which could result from the enhanced catalytic action of chlorophyllase, red chlorophyll catabolite reductase, pheophorbide oxygenase, and Mg-dechelatase, which are the main reasons for chlorophyll bleaching. On the other hand, the improved morphological growth parameters can be attributed to ZnO-NPs, BC, or, in some treatments, to both. Many authors, such as (Ali et al., 2019; Wu et al., 2020; Chen et al., 2024; Singh et al., 2024; Sharma et al., 2025), reported the alleviation of toxic elements by ZnO-NPs, which is considered an efficient and eco-friendly amendment. The effect of toxic trace elements on crops is based on their bioavailability, mainly at the root level. BC is one of the most effective substances that can reduce the availability of such metals to minimize their absorption by the root (Ali et al., 2019). It is confirmed that BC can also reduce the toxicity of toxic metals (Moradi et al., 2019; Jiang et al., 2022).

Furthermore, combining ZnO-NPs and BC revealed the elimination of the toxic metals' effects on plants (Ali et al., 2019; Seleiman et al., 2020). As suggested, ZnO-NPs helped reduce Li stress, leading to better plant growth. Combining BC with ZnO-NPs boosted plant development and likely lowered lithium levels in faba bean tissues. This combination enhanced the beans' resistance to Li stress by immobilizing Li by BC and reducing its toxicity with ZnO-NPs, as Shukla et al. (2023) conducted.

According to Pullagurala et al. (2018), the cellular processes of plants may likewise be impacted by ZnO-NPs in many ways. Stress enzymes like CAT have been demonstrated to increase after exposure to ZnO-NP. Afzal et al. (2023) stated that Li increased the activities of CAT and POX in quinoa, which is similar to our results. The BC or BC + ZnO-NPs showed a lower effect on PPO than the control. This notice was in line with Farhangi-Abriza and Torabian (2017), where they applied BC to mitigate the impact of abiotic stress (salinity) in bean seedlings. The application of BC decreased the PPO activity. High PPO activity under stress oxidizes and destroys hazardous chemicals, such as phenolic compounds, which are frequently observed to accumulate during stress (Farhangi-Abriza and Torabian, 2017).

Increased resistance to severe oxidative damage and alleviating stress have been linked to plants' high antioxidant content (Farhangi-Abriza and Torabian, 2017; Badawy et al., 2024). The NPs can act as enzymes and enhance the plants' tolerance because Zn is essential for plant life. ZnO-NPs were noticed to enhance tomato SOD and GPX-related genes under salinity. Additionally, titanium oxide and silicon oxide-NPs have been reported to increase the expression of antioxidant enzymes and the photosynthesis of plants under abiotic stress (Zia-ur-Rehman et al., 2023). Zha et al. (2022) conducted their experiment on the BC and ZnO-NPs effect on Cd and found an enhancement in the maize enzyme activity. They attributed this enhancement to the protection of the structure of plant membranes. This enhancement, which accords with our results, may be a mechanism of stress tolerance of maize towards Cd stress.

A lower concentration of ZnO-NPs associated with BC application has led to a lower concentration of Li than a higher application rate of ZnO-NPs. In this experiment, the Li addition rate was selected assuming that Li may accumulate in the environment in the future; however, if this happens, this rate will be extremely high and will cause many risks to food chains and humans. Based on Břendová et al. (2015), BC was a very effective regulator of the availability of Zn and Cd. It improves biomass growth and decreases the leaching of these elements. Biochar application decreased the mobility and translocation of HMs in the soil and plant parts. It also increased rapeseed's growth and biomass (Shahbaz *et al.,* 2018). This improvement might be attributed to the adsorption of toxic metals on the BC surface (Khan *et al.*, 2017; Arabyarmohammadi *et al.,* 2018) through some mechanisms such as precipitation, surface complexation, or ion exchange as reported by Bogusz *et al.* (2017). Moreover, this might result from BC's physical and chemical characteristics that offer new binding sites for HMs complexation and adsorption (Kamran *et al.,* 2020). According to Kamran *et al.* (2020), woodchip-derived BC has several C-, H-, and N-containing functional groups on the surface. The existence of C–O, C–H, N–H, OH, P–O, and Si–O on BC might increase the immobilization of HMs through complexation. Also, it is emphasized that BC has several CHN-bearing functional groups that can play an effective role in HMs stabilization (Bashir *et al.*, 2018). The mechanism involved in this improvement is also owing to BC's capability to increase plant resistance against metal stress by enhancing plant health and ROS scavenging (Ibrahim *et al.* 2018).

Generally, all treatments exceeded the toxicity limit of Zn (99 mg kg^{-1}) of (WHO/FAO, 2007). It was also noticed that the increase in Zn was higher with the rise in the addition rate of ZnO-NPs, while Zn concentration was also lower in ZnO-NPs + Li treatments. Increasing Zn concentration in plant tissues except in T4 and T7 indicates that both BC and Li reduced the availability of Zn and thus its concentration in plant tissues which have two sides; positive if the Zn concentration was higher, therefore BC will reduce its toxicity which can result from accumulation; or negative which means that the high Li concentration will inhibit uptake of some vital elements to plant. However, it was also apparent that the BC addition to Li caused an increasing concentration of Zn compared to its corresponding Li treatment, implying that BC inhibits the Li effect on Zn concentration. Shukla et al. (2023) found a higher concentration of Zn when combining ZnO-NPs with BC to alleviate arsenic toxicity in rice crop fields. They explained that ZnO-NPs and ZnO-NPs + BC regulated plant growth by providing adequate nutrition, likely because of improved antioxidant activity and photosynthetic performance, since Zn is a component of many enzymes and acts as an antioxidant.

The BAF quantifies the ability of a plant to accumulate elements from the solution into its tissues. BAF, higher than 1, indicates that bioaccumulation happened, while a BAF less than 1 indicates no bioaccumulation (Proc et al., 2021). The elevated BAF of Zn implies that *Vicia faba* can effectively uptake Zn from the growing medium. Zinc is a vital micronutrient for plants. Zn plays a considerable role in the structure and function of many enzymes and proteins. Due to Zn's importance and toxicity for biological systems at specific concentrations, it significantly plays a role in plant metabolism and biochemistry. It is lacking or hazardous beyond the optimal range. It plays a role in plants' various cellular and physiological processes, enhancing their growth, development, and yield (Saleem et al., 2022). Although Li doesn't seem to be an essential element for plants, various plants can absorb a considerable concentration of Li (Shahzad et al., 2016). However, the BAF values of Li treatments show lower uptake and accumulation of Li in *Vicia faba*. The BAF results underlined a difference in the BAF of Zn and Li elements (as essential and nonessential elements) in *Vicia faba*. The BAF reveals the level of metal bioaccumulation in plants compared to the growth environment, which can improve the evaluation of the safety of consuming crops cultivated in a contaminated environment. An increased BAF value indicates the potential metal accumulation in the plant at a high rate, which could be unsafe for human health if consumed (Aziz et al., 2023). Generally, although Li has shown high toxicity (as well as Zn) in its treatment and high bioaccumulation factor, BC showed a high capacity to decrease it to a high extent (although it didn't reduce it to the safe limit), making it promising. However, more research is necessary to use the optimal amount of BC, which can effectively interact with Li concentration in the environment (soil or water). In this study, we used a Li concentration higher than the established toxic limit to simulate its effect when accumulated in the atmosphere; however, this gives an alarm that Li should be removed from the environment if it continues to be inappropriately and unsustainably eliminated.

The results showed that different treatments arrested the cell cycle at different mitotic stages. Some treatments stop it early (at prophase stage), others at metaphase, and some let it go on to the last stages. This pattern can help identify the cellular targets or mechanisms affected by each treatment. It can be concluded that Li clearly affects the telophase stage, which could mean that the cell didn't leave mitosis quickly or that there is a buildup due to problems with cytokinesis. Sarraf et al. (2024) found that BC improves plant performance when exposed to metal(loid) stress. It significantly modifies the activity of ROS-scavenging enzymes and develops an efficient electron transfer route to reduce the adverse effects of ROS on plants. Faizan et al. (2021a) stated that Zn releases from ZnO-NPs is also essential for cell division. On the other hand, exposing the root tips of *V. faba* to Zn and Li increased the percentage of chromosomal aberrations. Ansari et al. (2024) stated that the mitotic index can be reduced after the application of NPs to a critical point based on the dose. Kumar et al. (2021) reported that the negative or positive effects of using ZnO-NPs in agriculture depend on their type, dose, treatment method, developmental stage, and genotype of the plant species, and environmental conditions. They added that high concentrations of ZnO-NPs have been noticed to induce a wide range of toxicity, such as growth/yield inhibition, cytotoxicity, physiological aberrations, genotoxicity, and oxidative stress. In this study, the types and percentages of chromosomal aberrations varied among treatments. According to Hu et al. (2017), the mitotic index is a reliable measure of cytotoxicity.

Our findings are in line with those of Cavuşoğlu et al. (2009), that Zn metal ions significantly affected the viability of *P. vulgaris* root tip cells and that parameters like germination percent, root length, and weight gain are biomonitoring indicators for these effects. Furthermore, earlier experiments indicated that the MI values in the root tips of *A. cepa* and *V. faba* are reduced due to the increasing Zn or ZnO-NPs concentrations and the duration of exposure (Kumari et al. 2011; Taranath et al. 2015; Ghosh et al. 2016). As reported by Mahfouz (2019), the cytotoxic effect of ZnO-NPs, as revealed by a reduction in mitotic and meiotic divisions and the occurrence of chromosomal aberrations such as chromosome breaks, was verified by their impact on DNA profiles in the *Vicia faba*. Stickiness and several chromosomal aberrations (such as chromosomal breakage and bridges, ring chromosomes, and laggard chromosomes) were noted by Youssef and Elamawi (2020). This may be due to the binding of ZnO-NPs with DNA and proteins, which leads to negative changes in their physico-chemical characteristics, chromatin condensation within the nucleus, or reating inter- and intra-chromatid crosslinks. This recommendation is supported by the strong binding affinity of ZnO-NPs to DNA (Das et al. 2018), the formation of ZnO-NPs complexes with the ring N atom or NH site in nucleobases of DNA (Saha and Sarkar 2014), and the interaction and creation of a bioconjugate between protein and ZnO NPs (Bhunia et al., 2013). Moreover, cytological investigations revealed that lithium chloride can induce mitotic changes ranging from a decrease in mitotic activity to numerous mitotic abnormalities. The manifestation of these changes varied according to the Zn concentration. The abnormalities produced included chromosome stickiness, lagging chromosomes during metaphase and telophase, diagonal anaphase, chromosome bridges, and micronuclei in interphase cells.

MI values also decreased (Hammad et al., 2001). On the contrary, it was supposed that applying BC and HMs together effectively diminished chromosomal aberration and the adverse effects of Zn and Li. Li et al. (2016) mentioned that straw BC and hardwood BC significantly reduced rice plants' Pb and Cd levels. Thus, integrating BC and ZnO-NPs in agricultural practices presents a sustainable, practical method for tackling key issues like soil health, crop resilience, and environmental effects, while conserving natural resources and reducing the ecological footprint of agricultural activities. Combining BC and ZnO-NPs can enhance crop productivity, decrease dependence on synthetic inputs, and facilitate the shift toward more sustainable farming practices (Mazhar et al., 2024).

6. Conclusion

This experiment was conducted to determine the effect of lithium, as an emerging contaminant in the environment, on some parameters *in V. faba* plant. Biochar and ZnO-NPs were used to mitigate the expected adverse effects of lithium on the bean plant. The results demonstrated that lithium negatively affected the germination rate, some morphological traits, and the enzyme activities of the bean plant. There was also a significant accumulation of lithium in the plant, and lithium showed the highest rate of cytogenetic abnormalities and the lowest rate of mitotic index in *V.faba*. The results showed that using ZnO-NPs at two addition ratios to alleviate the negative effects of lithium showed adverse results in some parameters at the higher rate, especially. Using biochar alone or mixed with ZnO-NPs at two addition ratios showed some improvement in the above parameters, with a marked improvement in biochar + low rate of ZnO-NPs. Therefore, the results of this experiment indicate the potential of using biochar with ZnO-NPs to address toxic levels of lithium in the environment. However, further studies are needed on different growth media, including soil, for a better understanding of the impact of lithium on plants and for studying the addition rates and mechanisms of such amendments to mitigate the effects of increased lithium in the environment.

List of abbreviations:
BC: Biochar
Zinc: Zn
ZnO-NPs: Zinc oxide nanoparticles
Li: Lithium
BAF: Bioaccumulation Factor

Declarations

Ethics approval and consent to participate

Consent for publication: The article contains no such material that may be unlawful, defamatory, or which would, if published, in any way whatsoever, violate the terms and conditions as laid down in the agreement.

Availability of data and material: Not applicable.

Competing interests: The authors declare that there is no conflict of interest in the publication.

Funding: Not applicable.

Authors' contributions: Authors HE, MD, AZ, AAM, SFMD, AMS, HME, FE, write the original draft and HE, MD, AZ, FE edit and finalize the manuscript. All authors read and agree for submission of manuscript to the journal.

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