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Cytotoxicity and genotoxicity of manganese in meristematic cells of *Glycine max* L. root

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Abstract. A crucial method for evaluating the potential harm to the genome caused by contaminants at levels exceeding the optimal threshold is the chromosomal plant assay. This paper reports on a study that examined the effects of varying concentrations of manganese (Mn) on the mitotic index (MI), cell kinetics index (CKI), and abnormality index (AI) in *Glycine max* L. root tip cells. Percentage of mitotic index, abnormality index, cell kinetics index, in root meristems of *Glycine max* L. at control and varying concentrations of Mn were evaluated. The findings showed that Mn doses that were utilized for seed treatment caused distinct differences in chromosomal activity of *Glycine max* L. root tip cells, with a decreased mitotic index and cell kinetics, and an increased abnormality index. Treatment was conducted at room temperature for 24 hours, 48 hours, and 72 hours at four different concentrations of Mn: CN (Control), 5µM, 10µM, 15µM, and 20µM. The control group was treated with distilled water. The findings demonstrated that Mn has cytotoxic and genotoxic effects on Glycine *max L.* root tip cells.

Keywords: Manganese (Mn), *Glycine max* L, mitotic index, abnormality index, cell kinetics.

INTRODUCTION

In many parts of the world, heavy metal contamination has an adverse effect on the biosphere, which is hazardous for environment. Metal contamination can come from a variety of sources, including mining, industry, agricultural chemicals, fuel, combustion byproducts, etc. (Siddiqui 2013; Siddiqui 2025a; Lee et al. 2024). Additionally, industrialization created industrial effluents that contained various pollutants, such as organic, inorganic, and radioactive trace elements, microbes, which might potentially contaminate soil (Siddiqui 2012; Siddiqui 2015; Chukwu et al. 2025). Heavy metals like Mn, Pb, Cd, Hg, and Ni are the most problematic worldwide pollutants. Industries release heavy metals and other pollutants into the environment, which are harmful to humans, animals, and plants. Although they are trace elements, these heavy metals are crucial to many physiological processes in living beings (Siddiqui et al. 2007; Siddiqui et al. 2009; Siddiqui et al. 2021; Hafeez et al. 2023; Espinola et al. 2025; Elik and Gül 2025). There is a sig-

nificant qualitative difference between natural environmental changes seen in the past and those seen in the present, especially when considering the current overextended anthropogenic activity (Siddiqui 2018; Siddiqui and Suliman 2021). These days, neither humans nor other superior organisms have developed genetic defenses against anthropogenic pollutants, such as chemicals released by industry, some of which are xenobiotics – things that have never existed in nature (Üstündağ et al. 2023; Siddiqui 2025 b; Vieira et al. 2025).

Although Mn is a chemical element that is necessary for proper nutrition, it can also be hazardous under some circumstances. Scientists are still trying to comprehend the various effects of its toxicity and deficiency on living things. However, Mn is unquestionably extremely hazardous at high concentrations, leading to several diseases dependent on the production of reactive oxygen species (ROS) (Ertürk et al. 2021; Vijaya Kumar et al. 2025; Rao et al. 2025; Xia et al. 2025). Additionally, Mn may build up inside cell, leading to cytotoxicity and eventual cell death. Following alterations in gene expression and enzyme activity, Mn causes intracellular changes such as lipid peroxidation, chromosomal disintegration, and DNA helix breakage (Siddiqui and Al rumman 2020 a and b; Perfileva and Krutovsky 2024; Aseef and Venkatkumar 2025). The pathophysiology and toxicity of various diseases, including atherosclerosis, diabetes, chronic inflammatory diseases, neurological disorders, and cardiovascular diseases, have been linked to long-term oxidative stress in humans (Dorman 2023). Lactate dehydrogenase and lipid peroxidation, two cytotoxic measures, show that Mn causes oxidative stress in a time and concentration-dependent way (Jomova et al. 2025; Sobańska et al. 2021). Our study used cytogenetic analysis in *Glycine max* L. as a plant indicator for the degree of heavy metal pollution in crops to identify the mutagenic effects of Mn.

METHODOLOGY

Procurement of chemicals

Mn is supplied by Sigma-Aldrich (MERCK). Local distributor Bayouni sells Sigma-Aldrich (MERK) products in Saudi Arabia. *Glycine max* L (Fabaceae) seeds with 2n = 40 chromosomes, cultivar JS 335, were collected from Indian Ministry of Agriculture.

Plant material and treatment

For cytological examination, a homogeneous batch of *Glycine max* L (Fabaceae) seeds with 2n = 40 chromosomes, cultivar JS 335, were collected. *Glycine max* seeds were immersed in water overnight. The seeds were then treated with various concentrations of Mn (CN, 5µM, 10µM, 15µM, and 20µM) for 2 hours with CN serving as control with only distilled water. For root tip germination, the seeds were placed in a Petri dish lined with filter paper, covered, and incubated at 22-25 °C for 2-3 days. The root tips were properly cleaned with distilled water, then fixed in Carnoy's fixative (1 glacial acetic acid: 3 ethanol) for 24 hours and stored in 90% alcohol.

Glycine max L. test

For germination, 30 *Glycine max* seeds were put in Petri Plates with 3 mL of each of the test solutions of Mn. The seeds were subjected to treatments in a germination chamber (Quimis) with regulated temperatures of 23±3 °C every 96 hours. The size of the roots were measured using a digital calliper (Digmess), and the number of seeds that germinated were manually counted. The roots were subsequently gathered and preserved in Carnoy fixative. The microscope slides were prepared by Siddiqui et al. (2022 a), and they were stained for 1 hour and 30 minutes using Schiff's reagent based on Feulgen method. Ten slides containing *Glycine max*. root meristems were made for each treatment, and 500 cells were counted from each slide, for a total of 5000 cells per treatment. The slides were examined using an optical microscope that had a 400x magnification.

Analysis of cytotoxicity and genotoxicity

For cytotoxicity analysis, MI and CKI were evaluated. The indices were calculated using the formulas described below:

$$\text{Mitotic Index (MI)} = \frac{\text{No. of cells in cell division} \times 100}{\text{Total No. of counted cells}}$$

Formula for calculating cell kinetics index (CKI)

$$\text{Interphase index} = \frac{\text{No. of interphase cells} \times 100}{\text{Total No. of cells analyzed}}$$

$$\text{Prophase index} = \frac{\text{No. of prophase cells} \times 100}{\text{Total No. of cells analyzed}}$$

$$\text{Metaphase index} = \frac{\text{No. of metaphase cells} \times 100}{\text{Total No. of cells analyzed}}$$

$$\text{Anaphase index} = \frac{\text{No. of anaphase cells} \times 100}{\text{Total No. of cells analyzed}}$$

$$\text{Telophase index} = \frac{\text{No. of telophase cells} \times 100}{\text{Total No. of cells analyzed}}$$

For genotoxicity analysis, chromosomal abnormality index was evaluated. AI was calculated using the formulas described below:

$$\text{AI} = \frac{(\text{No. of cells with chromosomal alteration} \times 100)}{\text{Total No. of counted cells}}$$

Statistical analysis

A one-way ANOVA test was used to examine the significance of differences between variables using the GPIS 1.13 program (GRAPHPAD, California, USA). All results were presented as mean ± SE.

RESULT

Effect of Mn on MI in Glycine max root tips

The control samples had the highest MI values, which reached 1.52% after 24 h (Figure 1 A), 1.35% after 48 h (Figure1 B), and 72 h (Figure 1 C). After Mn treatment for 24 h, MI decreased significantly ($p<0.05$) dose-dependently from 10 µM (1.01±0.10), 15 µM (0.98±0.10), and 20 µM (0.97±0.09) concentrations, with no significant difference ($p>0.05$) observed at 5 µM (1.48±0.15) concentration. After 48 h, MI decreased significantly ($p<0.05$) dose-dependently from 10 µM (1.02±0.10), 15 µM (1.05±0.13), and 20µM (0.87±0.13) concentrations, with no significant difference ($p>0.05$) observed at 5µM (1.28±0.13). Similarly, on Mn treatment for 72 h, MI decreased significantly ($p<0.05$) dose-dependently from 10 µM (1.17±0.22), 15 µM (1.07±0.11), and 20 µM (0.70±0.08) concentrations, with no significant difference ($p>0.05$) observed at 5 µM (1.25±0.22) as compared to control.

Effect of Mn on Abnormality Index, in Glycine max root tips

In our study, we found that AI, such as anaphase bridges, dissociated chromosomes in metaphase and anaphase, chromosomal bridging, were caused by incorrect separation and retarded chromosomes in anaphase (Figure 3). These were the most common abnormalities after treating root meristematic cells of *Glycine max* with Mn.

The control samples showed AI values of (0.0±0.0) at 24 h, 48 h, and 72 h. Following a 24 h Mn treatment, AI increased significantly ($p<0.05$) dose-dependently at 5 µM (0.39±0.059), 10 µM (0.40±0.049), 15 µM (0.46±0.050), and very significantly ($p<0.01$) at 20 µM (0.65±0.087) (Figure 2A). The AI increases significantly ($p<0.05$) from 5 µM (0.25±0.08), 10 µM (0.29±0.07), and very significantly ($p<0.05$) at 15 µM (0.42±0.06), and at 20 µM (0.53±0.07) dose-dependently following 48 h of Mn treatment (Figure 2B). AI increased significantly ($P<0.05$) dose-dependently from 5µM, which is (0.17±0.042), 10 µM (0.290.092), and 15 µM (0.410.093), and very significantly ($p<0.01$) at 20 µM (0.46±0.065) following 72 h of Mn treatment as compared to control (Figure 2C).

Effect of Mn on cell kinetics index in Glycine max root tips

Table 1 shows that Mn decreases CKI of *Glycine max* root tip cells in interphase, prophase, metaphase, anaphase, and telophase at 24 h, 48 h, and 72 h from 5 µM to 20 µM (Table 1). At 24 h of exposure to Mn, prophase (1.43±0.24), metaphase (0.83±0.06) and telophase (0.60±0.08) increased very significantly ($p<0.01$) in 5 µM. However, at 10 µM (0.15±0.02), there was a significant decrease ($p<0.05$) in telophase and at 15 µM (0.10±0.01 and 20 µM (0.13±0.01), there was a very significant decrease in telophase. Non-significant ($p>0.05$) decreases were reported in interphase and anaphase from 5 µM to 20 µM as compared to control.

After 48 h, in prophase, metaphase, anaphase, and telophase, very significant ($p<0.01$) increase was reported at 5 µM. In the case of interphase and prophase, non-significant ($p> 0.05$) decreases were reported from 5 µM to 20 µM. But in the case of anaphase and telophase, there was a significant decrease ($p<0.05$) at 10 µM to 20 µM as compared to control. After 72 h of exposure to Mn, interphase and telophase were very significantly ($p<0.01$) decreased from 5 µM to 20 µM. However, at anaphase at 20 µM, significant ($p<0.05$) decrease was reported. But in the case of prophase and metaphase, there was a non-significant decrease ($p> 0.05$) from 5 µM to 20 µM as compared to control.

Pearson correlation coefficients between MI, AI, and CKI on Mn treatments with different time intervals

The heat map of Pearson correlation coefficients displays the correlation among measured MI, AI, and CKI in Figure 4 (24 h), Figure 5 (48 h) and Figure 6 (72 h). The blue and red squares signify positive and nega-

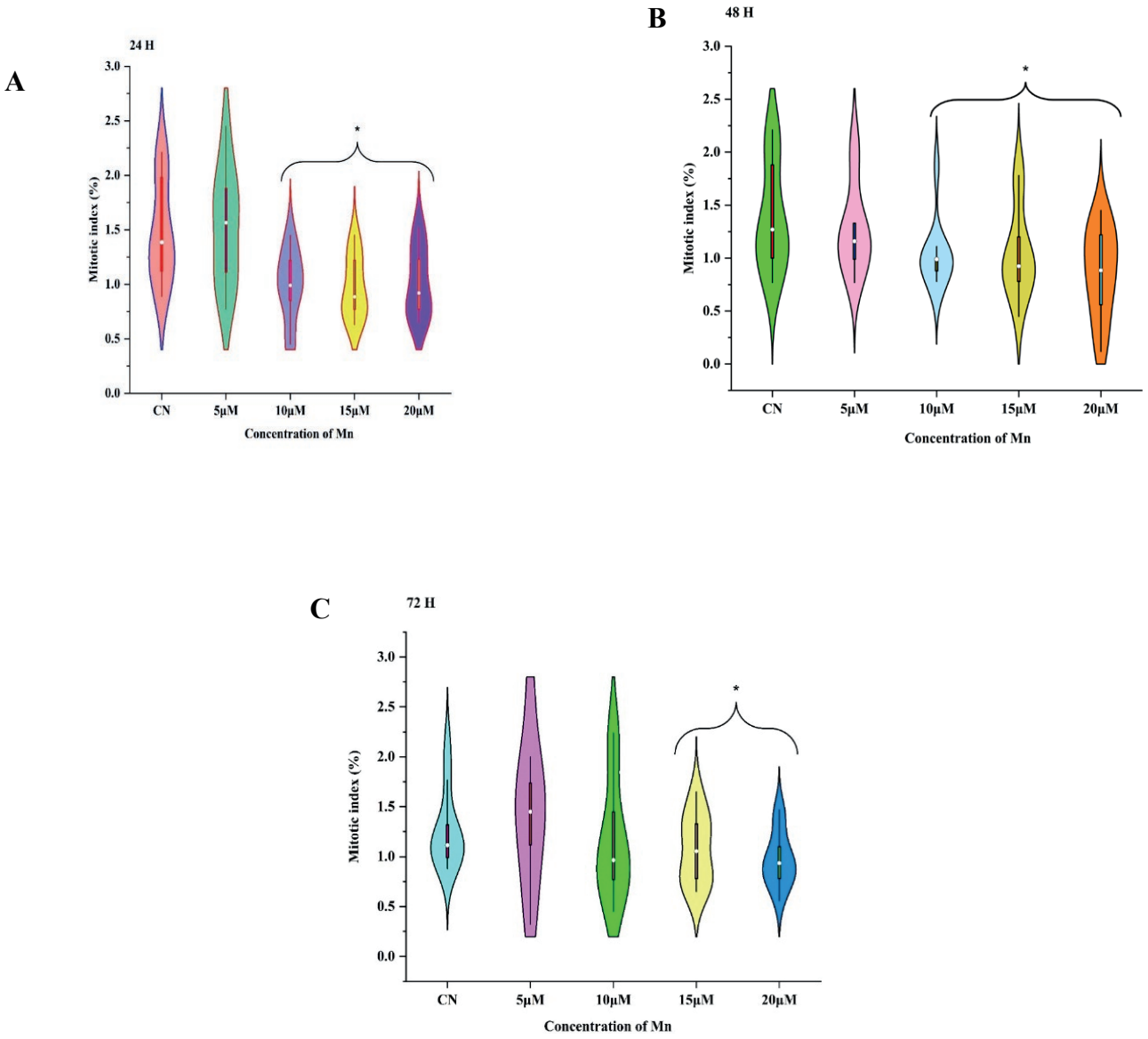

Figure 1. Effect of Mn on MI in *Glycine max* root tips at 24 h (A), 48 h (B), and 72 h (C).

tive correlations, respectively. Negative correlations were reported between MI and AI (-0.020) after treatment of Mn for 24 h. A negative correlation was noticed in MI and interphase of CKI (-0.02). On the other hand, a strong positive correlation was noticed between MI and CKI (prophase (0.51), metaphase (0.31), anaphase (0.56), and telophase (0.26) (Figure 4). However, positive correlations were noticed between AI and different phases of CKI, interphase (0.22), metaphase (0.24), anaphase (0.04), and telophase (0.29) and a negative correlation in prophase (-0.05), after treatment of Mn for 24 h.

Negative correlations were reported between MI and AI (-0.07) after treatment of Mn for 48 h. On the other hand, a positive correlation was noticed between MI and phases of CKI (Interphase (0.07), prophase (0.15), metaphase (0.01), anaphase (0.22) and telophase (0.40) (Figure 5). However, negative correlations were reported between AI and different phases of CKI: interphase (-0.20), prophase (-0.32), metaphase (-0.16), anaphase (-0.24), and telophase (-0.26) after treatment of Mn for 48 h (Figure 5). These two parameters are negatively correlated with each other.

A negative correlation was reported between MI and AI (-0.05) after treatment of Mn for 72 h. On the other hand, negative and positive correlations were noticed between MI and phases of CKI: interphase (-0.14), pro-

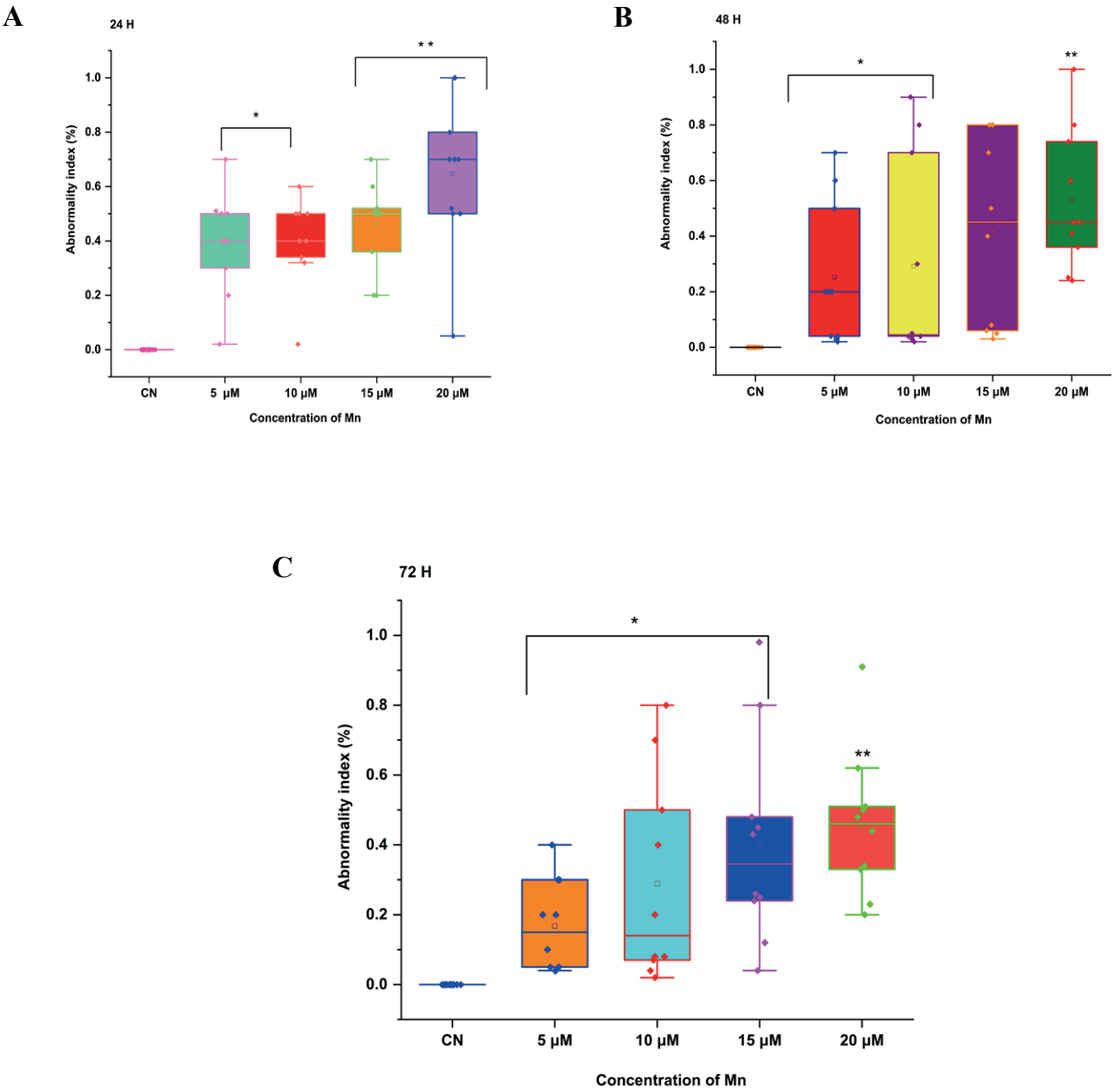

Figure 2. Effect of Mn on AI in *Glycine max* root tips at 24 h (A), 48 h (B), and 72 h (C).

phase (-0.15), metaphase (0.09), anaphase (-0.15) and telophase (0.06) (Figure 6). A positive correlation was reported between AI and interphase (0.17) of CKI, and negative correlations were noticed in prophase (-0.07), metaphase (-0.39), anaphase (-0.28), and telophase (-0.27) after treatment of Mn for 72 h (Figure 6).

DISCUSSION

On the basis of above data, it is obvious that Mn, a heavy metal, can cause cytotoxic and genotoxic effects on root tip cells of *Glycine max*. Findings indicate that when treatment dosages increase, the frequency of MI, CKI decreases and AI rises. It is practically obvious from this decrease in MI and increase in AI that Mn has a clastogenic impact on chromosomes at DNA level. How-

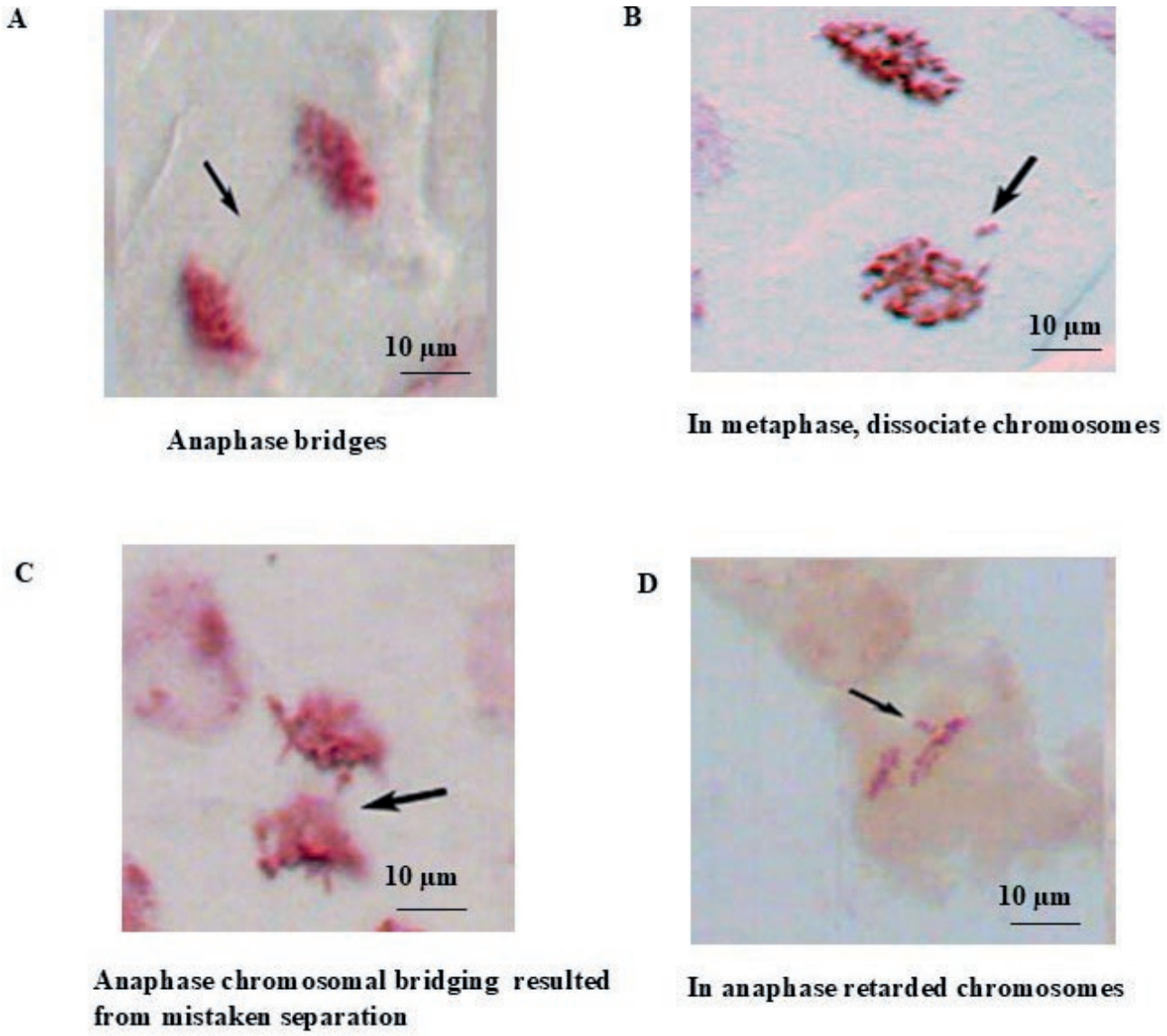

Figure 3. Chromosomal abnormality in Mn-treated *Glycine max* root tips. A. Anaphase bridges B. In metaphase, dissociate chromosomes C. Anaphase chromosomal bridging resulted from mistaken separation D. In anaphase retarded chromosomes. Bar. 10 µm.

ever, excessive and negligent use of these heavy metals and agrochemicals often leads to their selective accumulation on agricultural fields topsoil layers (Mauser et al. 2025; Shahwar and Ansari 2022; Aslam and Aslam et al. 2021; Sarkar et al. 2022), which eventually reduces soil fertility. The rapid emergence of pathogen resistance to these agrochemicals lowers the effectiveness of pesticides and causes more harmful side effects. One of these consequences is the problem of eliminating insects that are beneficial to environment from the ecosystem (Fairoj et al. 2024; Yu et al. 2025).

MI is a crucial indicator for determining heavy metal's potential for genotoxicity. Exposure to pesticides, heavy metals, and other chemical pollutants was linked to a decrease in MI in many plant species (Siddiqui and Al-Rumman, 2022a, b, and c; Siddiqui 2025 b). Chromatin abnormalities brought on by metal-DNA interaction or cell cycle delays result in decreased MI (Siddiqui 2023; Siddiqui 2024 a). Spindle-related chromosomal abnormality during cell division is also a result of primary mechanism of action of pesticides and heavy metals in mitosis (Firbas and Amon 2014). In this work, Mn demonstrated cytotoxicity by lowering MI dose-dependently. This implies that Mn causes mito-depression in *Glycine max*. Numerous investigations have demonstrated that differences in mitotic cycle's duration may be

Table 1. CKI in *Glycine max*, treated with different concentrations of Mn for 24 h, 48 h, and 72 h.

Conc.	FI	FP	FM	FA	FT
24 h					
CN	10.19 ± 0.56	0.71±0.08	0.20 ± 0.03	0.28±0.06	0.33±0.03
5 µM	10.30 ± 0.63	1.43±0.24**	0.83 ± 0.06**	0.45±0.07	0.60±0.08**
10 µM	9.10 ± 0.73	0.49±0.14	0.25 ± 0.07	0.16±0.02	0.15±.020*
15 µM	5.50 ± 0.17	0.55±0.17	0.15 ± 0.02	0.12±0.02	0.10±0.01**
20 µM	3.70 ± 0.12	0.37±0.13	0.15 ± 0.02	0.12±0.01	0.13±0.01**
48 h					
CN	10.20±0.56	0.73±0.07	0.17±0.03	0.28±0.06	0.31±0.05
5 µM	10.29±0.63	1.06±0.16	0.56±0.08**	0.32±0.06	0.35±0.04
10 µM	9.13±0.65	0.42±0.128	0.15±0.018	0.11±0.01*	0.14±0.02**
15 µM	8.32±0.53	0.39±0.12	0.15±0.010	0.13±0.01*	0.12±0.11**
20 µM	8.32±0.53	0.38±0.12	0.16±0.017	0.12±0.01*	0.12±0.01**
72 h					
CN	10.28±0.08	0.85±0.07	0.79±0.05	0.49±0.08	0.74± 0.05
5 µM	9.49±0.71**	0.37±0.74	0.45±0.06	0.21±0.03	0.24±0.04**
10 µM	8.24±0.04**	0.69±0.26	0.141±0.02	0.31±0.13	0.31±0.14**
15 µM	8.78±0.43**	0.79±0.26	0.15±0.03	0.41±0.13	0.41±0.14**
20 µM	7.80±0.84**	0.39±0.13	1.23±0.12	0.11±0.01*	0.11±0.03**

Where CN = control, FI (Frequency of interphase), FP (Frequency of prophase), FM (Frequency of metaphase), FA (Frequency of anaphase), and FT (Frequency of telophase).

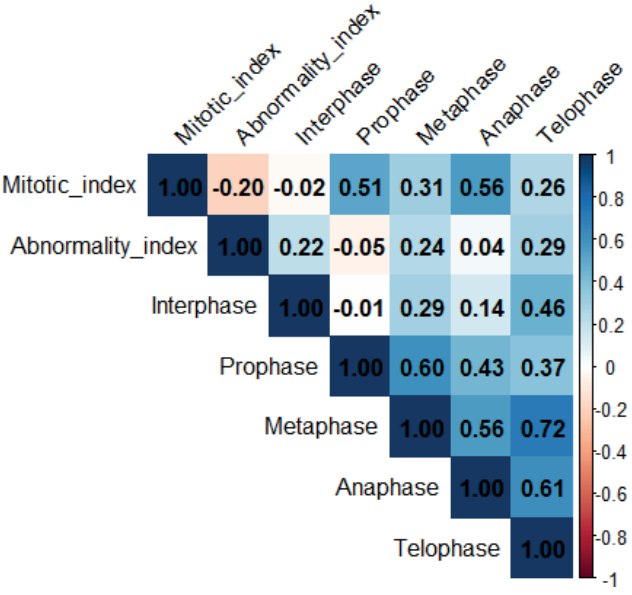

Figure 4. Pearson's correlation coefficients were calculated to assess the relationships among different variables (MI, AI, and CKI) under Mn treatments for 24 hours.

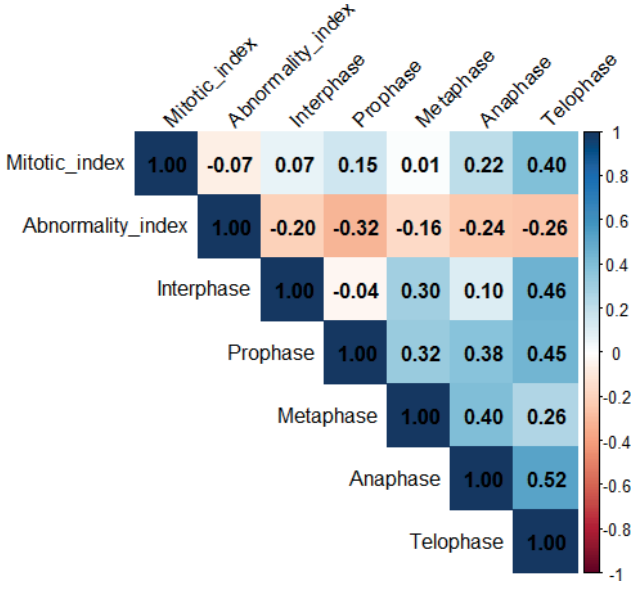

Figure 5. Pearson's correlation coefficients were calculated to assess the relationships among different variables (MI, AI, and CKI) under Mn treatments for 48 h.

the cause of decline in cell activity. A longer S phase has been associated by certain researchers with mitotic inhibition (Siddiqui 2024 ; Periakaruppan et al. 2023; Qian 2024). According to Das et al. (2023), heavy metals can enter DNA through nuclear pores or when the nuclear membrane splits into cells undergoing mitosis.

Aneugenic, tubergenic, and clastogenic effects are caused by the prevalence of these abnormalities (Sid-

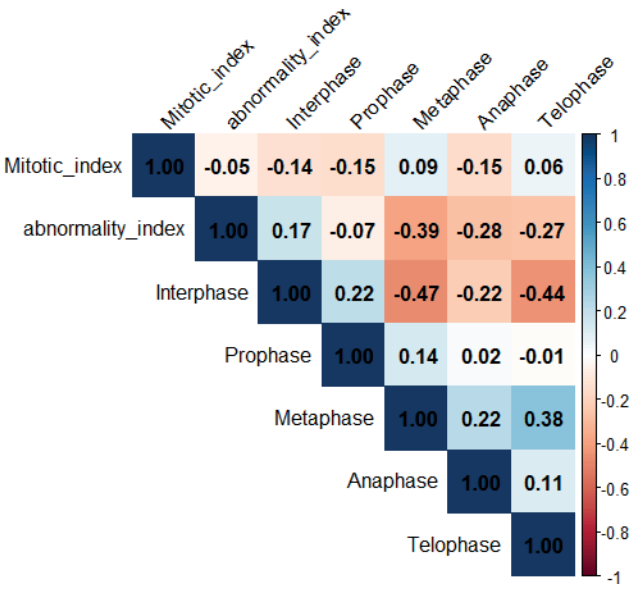

Figure 6. Pearson's correlation coefficients were calculated to assess the relationships among different variables (MI, AI, and CKI) under Mn treatments for 72 h.

diqui 2024 b; Siddiqui et al. 2012;–Bonciu et al. 2018; Bonciu et al. 2022). They are caused by spindle apparatus disorders, abnormal metaphase, telophase, anaphase, and bridges, as well as chromosomal breakage (fragments, micronuclei) (Hossain et al.2022; Faizan M, et al. 2022). The rising occurrence of AI has resulted from suppression of DNA synthesis during S-phase (Tumer et al., 2022). In the current study, different types of AI, such as anaphase bridges, dissociated chromosomes in metaphase and anaphase, chromosomal bridging was caused by incorrect separation and retarded chromosomes in anaphase were reported in *Glycine max* after treatment with Mn. Based on the results of proportions of distribution of mitotic phases, Mn reduced the percentage of interphase, prophase, metaphase, anaphase, and telophase in all concentrations dose-dependently.

The results align with those of (Liman et al. 2022; Munir et al. 2021; Yu et al. 2025). The percentage of telophase stage was reduced as compared to control. These results imply that a decrease in cell development during mitosis or the stoppage of one or more mitotic stages may be the cause of decline in telophase stages and, thereafter, MI (Ping et al. 2012; Siddiqui 2018 a and b). The results suggest that a reduction in cell development and the arrest of one or more mitotic phases may be the source of a drop in the proportion of prophase, metaphase, anaphase, and telophase at all doses, and therefore MI (Siddiqui 2014; Siddiqui 2016; Sarac et al. 2019).

CONCLUSION

The cytotoxic and genotoxic properties of Mn, which is commonly added to our agricultural fields due to environmental pollution, can cause mutations in *Glycine max*. The above studies suggest that natural resources pollution, industrial effluents, and agricultural practices for comforts should be curtailed, as incidence of heavy metal increases soil pollution, resulting in grave cytogenetic effects in plants and higher organisms. Thus, mutagenesis data obtained from plant tests are critical for genetic studies aimed at maintaining a stable ecosystem.

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