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Research Article

Impact of nickel toxicity on mitosis and chromosomal behaviour in germinating *Pisum sativum* L.

Jayshri D Chaudhari

Department of Botany, Sir P T Sarvajanik College of Science, Athwalines, Surat, 395001, India.

Correspondence
Jayshri D Chaudhari
jayaganvit@gmail.com

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Environmental contamination by heavy metals like nickel (Ni) adversely affects plant growth by interfering with essential cellular processes. The present study examines nickel's cytotoxic and genotoxic effects on *Pisum sativum* L., an important leguminous crop. Germinating seeds of *Pisum sativum* were treated with varying concentrations of nickel chloride (75 ppm, 100 ppm, and 125 ppm). Root tips were subjected to cytological analysis using standard squash techniques to assess the mitotic index and chromosomal behaviour. Nickel treatment significantly reduced the mitotic index and induced a range of chromosomal abnormalities, including stickiness, bridges, laggards, and disturbed metaphases and anaphases. These effects were more pronounced at higher concentrations. The study demonstrates that nickel toxicity disrupts normal mitotic processes and induces chromosomal aberrations in *Pisum sativum*, suggesting potential risks to crop development and genetic stability in nickel-contaminated soils.

Keywords: *nickel chloride, chromosomal behavior, stickiness, bridges, laggards*

Introduction

Heavy metal contamination, particularly by nickel (Ni), is a growing concern in agricultural systems due to its detrimental effects on plant health and productivity. Although Ni functions as a micronutrient essential for enzymes like urease and hydrogenase (Brown et al., 1987), its elevated presence in soil can disrupt numerous physiological, biochemical, and cytological processes in plants (Yavaş et al., 2022). High concentrations of Ni impair photosynthesis, water uptake, nitrogen metabolism, and hormonal balance, ultimately reducing plant growth and vigor (Yu et al., 2024; Bhat et al., 2023). *Pisum sativum* L. (pea), a leguminous plant of significant agronomic and nutritional value, is cultivated extensively and has long served as a classical model in genetics and plant biology due to its well-characterized genome and ease of growth (Parihar et al., 2023). Its sensitivity to environmental pollutants, heavy metals, makes it ideal for evaluating metal stress's cytotoxic and genotoxic effects (Chaudhari, 2023). Ni contamination in soils, mainly resulting from industrial discharge, mining, and overuse of chemical fertilizers, has been linked to several cytological anomalies. In *P. sativum* and other plant species, Ni stress is associated with reduced mitotic index, abnormal spindle formation, chromatin condensation, micronuclei, c-mitosis, chromosome bridges, and laggards in root meristem cells (Sharma & Dubey, 2005; Dmitrieva et al., 2006; Lal, 2010; Patra et al., 2004; Manna et al., 2023). These mitotic irregularities are considered strong indicators of Ni-induced genotoxicity. Recent investigations have further highlighted the structural and molecular consequences of Ni exposure. For instance, Chaudhari (2023) observed considerable anatomical deformation in the roots and stems of *Pisum sativum* seedlings exposed to Ni, while Yu et al. (2024) demonstrated oxidative damage and disturbed micronutrient homeostasis in tomato. Similarly, Bhat et al. (2023) showed that Ni stress in maize could be mitigated through exogenous application of nitric oxide donors, indicating potential intervention strategies. Moreover, in *Allium cepa*, Ni has been shown to induce DNA fragmentation and chromosomal aberrations, effects that can be counteracted with natural antioxidants such as pomegranate seed extract (Yılmaz et al., 2023). Hormonal regulators like gibberellic acid have also demonstrated protective effects against Ni-

induced cytotoxicity in soybean by modulating the glyoxalase system and ROS detoxification pathways (Bhat et al., 2023). Understanding these cytological responses is crucial for assessing nickel's genotoxic risks in food crops and exploring tolerance mechanisms and informing phytoremediation strategies. This study aims to investigate the cytological impact of Ni on mitotic activity and chromosomal behavior in *Pisum sativum* root meristems, particularly under conditions relevant to agricultural soils in Gujarat, where the crop is widely cultivated.

Materials and Methods

The germination procedure was conducted using Petri dishes. The seeds were surface-sterilized with hydrogen peroxide (H_2O_2) to prevent surface bacterial and fungal contamination. Nickel chloride ($NiCl_2$) was used to prepare different concentrations of ppm solutions in pure distilled water, which also served as the control in the study. In each Petri dish, 10 seeds were placed on cotton and treated with 40 mL of the prepared solution. This treatment was applied once at the beginning of the germination process.

Following this initial treatment, tap water was used daily to maintain moisture. Daily observations were made for fungal growth and other signs of contamination in the Petri dishes. Plants were used to establish the sublethal (LC20), lethal (LC50) and super-lethal (LC80) concentrations for $NiCl_2$ shown in Table 1. Chromosomal behaviour was studied after seed germination.

Table 1. The details for $NiCl_2$ treatments in the *Pisum sativum* L.

Plant	Percentage Mortality	$NiCl_2$ concentration
***Pisum sativum* L.**	20% (Sub lethal)	75ppm (LC20)
	50% (Lethal)	100ppm (LC50)
	80% (Super lethal)	125ppm (LC80)

Chromosomal studies

Root tip excision and slide preparation

The root tips were treated with $NiCl_2$, and the controls were preserved in a 3:1methanol-acetic acid solution. Because the rate of mitosis was higher during this time, the root tip was fixed between 9:30 and 10:30 am. The staining method used was that of Darlington & La Cour (1976) and Conger & Fairchild (1953). For the current investigation, aceto orcine staining and aceto orcine – aceto carmine staining were determined to be appropriate. For initial observations, a temporary squash preparation of the material was utilized, and DPX was used to make the preparations permanent. After screening hundreds of cells in each group, the mitotic index was calculated. Per hundreds of cells, the percentage of abnormal cells was scored.

Calculation of mitotic index (in percentage) and percentage of aberrant cells

The slides of the root tips of the control and treated plants at the third, fifth, and seventh days of growth were examined in order to calculate the mitotic index. A total of 100 cells were seen on the slide. The number of cells in the prophase, metaphase, anaphase, and telophase stages was determined. Using the following formulas, the mitotic index and the proportion of aberrant cells were determined.

$$\text{Mitotic index (\%)} = \frac{\text{No. of dividing cells}}{\text{Total no. of cells studied}} \times 100$$

$$\text{Percentage of aberrant cells} = \frac{\text{No. of aberrant cells}}{\text{No. of dividing cells}} \times 100$$

Photography

The photos of the chromosomes of *Pisum sativum* were shot using a Sony T-10 digital camera and an X5 zoom lens. The pictures were uploaded to an IBM ThinkPad laptop, and then prints were made on an HP-4288 printer. With a Carl-Zeis photomicroscope with planophotochromatic objectives and Kodak 100 ASA-35mm colour film, photomicrographs were taken. Green, yellow, or daylight filters were applied.

Results

Cytological observations

Pisum sativum L. (2n = 14) exhibited normal mitotic division in the control group. All phases- prophase, metaphase, late metaphase, anaphase, late anaphase, and telophase were clearly distinguishable without any cytological aberrations (Figure 1 A–F).

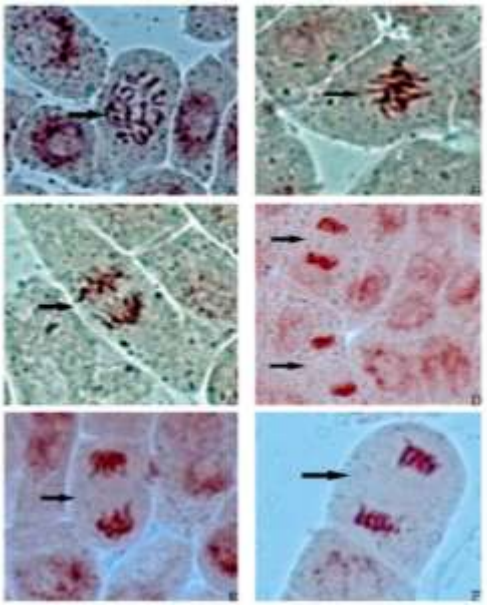


Figure 1. Pattern of chromosomal segregation at normal conditions (A-F)

In contrast, plants treated with 75 ppm $NiCl_2$ showed significant mitotic abnormalities. The most frequently observed anomalies included sticky chromosomes during metaphase (Figure 2 A–C), chromosome bridges during late metaphase (Figure 2 D), and vagrant chromosomes accompanied by chromosomal stickiness (Figure 2 E–F). Chromosome bridges persisted through metaphase and telophase in several cells.

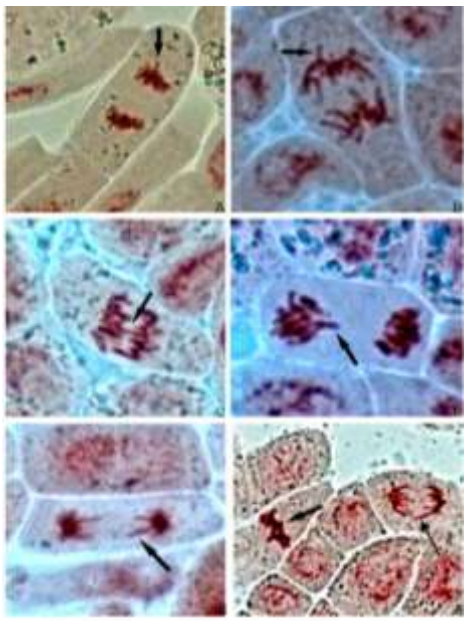


Figure 2. Pattern of chromosomal segregation at 75 ppm of $NiCl_2$ (A-F)

Exposure to 100 ppm $NiCl_2$ induced more severe mitotic disruptions. Chromosomal breaks and fragmentation were prominent at various mitotic stages (Figure 3 A–B), occasionally forming chromatin bridges between poles (Figure 3 C). Vagrant and laggard chromosomes were frequently recorded during telophase (Figure 3 D–E). Additionally, star-shaped chromosome configurations during anaphase and sticky metaphase were also observed (Figure 3 F).

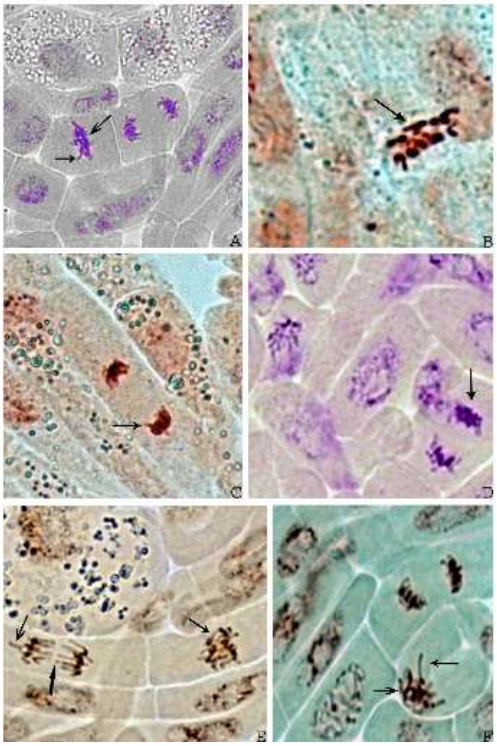


Figure 3. Pattern of chromosomal segregation at 100 ppm of $NiCl_2$ (A-F)

Further cytological damage at 100 ppm $NiCl_2$ included chromosomal bleaching during anaphase (Figure 4 A), disrupted anaphase with star configurations (Figure 4 D), and chromosomal projections directed toward the poles (Figure 4 B). Chromosomal fragmentation (Figure 4 B–D) and bridges connecting chromatid groups were documented during anaphase and telophase (Figure 4 C, E–F).

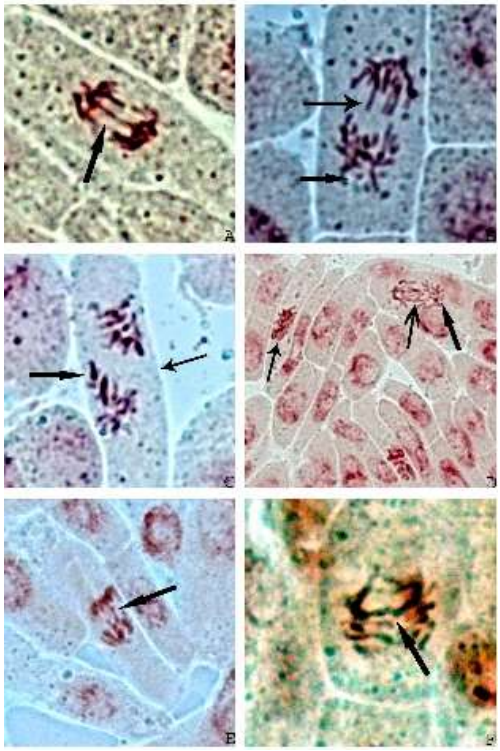


Figure 4. Pattern of chromosomal segregation at 100 ppm of $NiCl_2$ (A-F)

A notable cytological event under 100 ppm $NiCl_2$ was the inhibition of spindle fiber formation, suggestive of C-mitosis. Cells at this concentration frequently showed C-metaphase, chromosomal fragments, and laggard chromosomes during

anaphase (Figure 5 A–C). Nuclear deformation, including the presence of nuclear cavities, was evident (Figure 5 D), along with chromosomal stickiness, vagrant chromosomes, and ring chromosome formation (Figure 5 E–F).

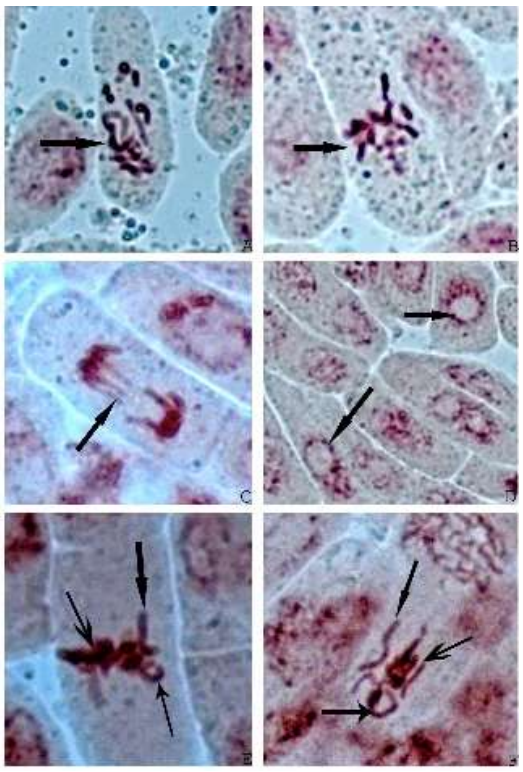


Figure 5. Pattern of chromosomal segregation at 100 ppm of $NiCl_2$ (A-F)

At 125 ppm $NiCl_2$, the severity of cytological abnormalities increased. C-mitosis was predominant, alongside ring chromosomes and chromosomal stickiness (Figure 6 A–C). Nuclear degeneration and nuclear cavities were common features (Figure 6 D). Low-magnification images revealed overall chromosomal abnormalities and changes in mitotic frequency (Figure 6 E).

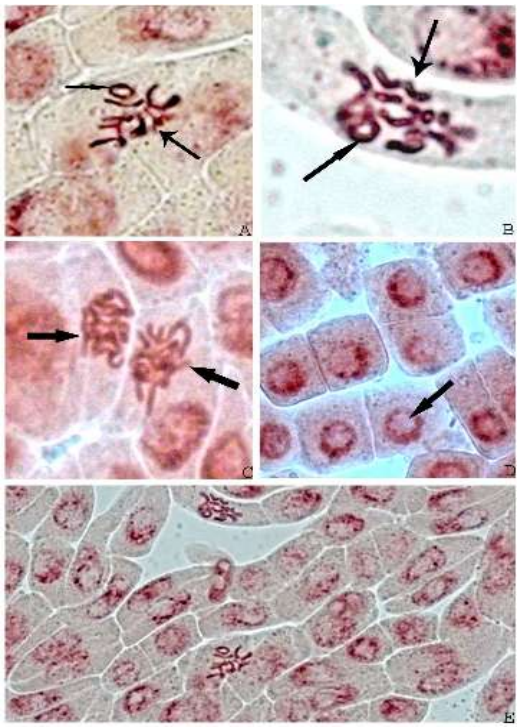


Figure 6. Pattern of chromosomal segregation at 125 ppm of $NiCl_2$ (A-E)

B. Mitotic index and frequency of aberrant cells

Table 2. Effect of $NiCl_2$ on the mitotic index and abnormal cells in *Pisum sativum* root tips on day 3

Treatment	Main Root MI (%)	Aberrant Cells (%)	Side Root MI (%)	Aberrant Cells (%)
Control	28.25 ± 2.11	2.50 ± 0.52	ND	ND
75 ppm $NiCl_2$	18.32 ± 0.45	15.25 ± 0.98	ND	ND
100 ppm $NiCl_2$	16.32 ± 2.36	17.32 ± 1.94	ND	ND
125 ppm $NiCl_2$	14.32 ± 1.82	19.98 ± 1.65	ND	ND

All the values are means ±S.D.
ND-Not developed during the study period.
NS-Not studied due to bursting and decay.

Table 3. Effect of $NiCl_2$ on *Pisum sativum* root tips on day 5

Treatment	Main Root MI (%)	Aberrant Cells (%)	Side Root MI (%)	Aberrant Cells (%)
Control	24.36 ± 1.09	2.69 ± 2.36	22.69 ± 1.32	3.26 ± 1.54
75 ppm $NiCl_2$	22.65 ± 1.37	38.36 ± 1.47	18.25 ± 1.87	20.36 ± 1.92
100 ppm $NiCl_2$	17.32 ± 1.56	45.36 ± 1.61	16.74 ± 0.21	28.94 ± 0.93
125 ppm $NiCl_2$	NS	NS	ND	ND

All the values are means ±S.D.
ND-Not developed during the study period.
NS-Not studied due to bursting and decay.

Table 4. Effect of $NiCl_2$ on *Pisum sativum* root tips on day 7

Treatment	Main Root MI (%)	Aberrant Cells (%)	Side Root MI (%)	Aberrant Cells (%)
Control	20.35 ± 0.36	3.26 ± 2.05	18.36 ± 0.41	3.03 ± 1.06
75 ppm $NiCl_2$	6.32 ± 2.36	40.54 ± 1.32	11.23 ± 0.52	14.36 ± 1.62
100 ppm $NiCl_2$	NS	NS	10.32 ± 0.47	22.36 ± 0.36
125 ppm $NiCl_2$	NS	NS	ND	ND

All the values are means ±S.D.
ND-Not developed during the study period.
NS-Not studied due to bursting and decay.

On day 3 (Table 2), the control group exhibited the highest MI (28.25 ± 2.11%), with minimal aberrant cells (2.50 ± 0.52%). In contrast, at 125 ppm, the MI dropped to 14.32 ± 1.82%, and abnormal cells rose to 19.98 ± 1.65%. By day 5 (Table 3), the MI in the control was 24.36 ± 1.09% (main root), while in 100 ppm and 125 ppm treatments, it reduced to 17.32 ± 1.56% and became undetectable in the main root due to tissue decay, respectively. Aberrant cell percentages increased to 45.36 ± 1.61% in the 100 ppm group. On day 7 (Table 4), MI further declined to 6.32 ± 2.36% in the main root at 75 ppm, while root tips at 100 ppm and 125 ppm showed signs of disintegration and were unfit for cytological study. However, growth was sustained through the emergence of lateral roots at these concentrations.

Discussion

The rate of cell division, duration of the mitotic cycle, and frequency of chromosomal aberrations induced in plants exposed to heavy metal-contaminated soils vary depending on the type and concentration of heavy metal ions, as well as the plant species or cultivar involved (Bessonova, 1991; Duan & Wan, 1995; Nyarai-Horvath et al., 1997; Sahi et al., 1998; Wang, 1999; Piechalak et al., 2002). In *Pisum sativum*, exposure to varying concentrations of nickel ions triggered diverse cytological abnormalities. A decline in the mitotic index suggests a mitodepressive effect, indicative of inhibited cell entry into mitosis. Nickel was observed to suppress cell division and cause chromosomal irregularities. Notably, an increase in nickel concentration corresponded to a marked decrease in the mitotic index. Lead nitrate, although a weak mutagen, exhibited a synergistic effect when combined with ionizing radiation due to its high toxicity (Dineva et al., 1993). In *Allium* species treated with lead compounds, prevalent chromosomal anomalies included anaphase bridges, chromatin stickiness, achromatic masses, diplochromosomes, centromeric splits, fragmentation, and chromosomal dissolution (Savić et al., 1989; Wierzbicka, 1989; Bhowmik, 2000). Rank and Nielsen (1998) demonstrated that genotoxicity is closely associated with industrial pollution levels and that the toxicity correlates with the concentration of heavy metals such as Pb, Ni, Cr, Zn, Cu, and Cd. Chang et al. (1997), using the *Allium cepa* assay, confirmed the genotoxic potential of lead-contaminated soils. Our findings reveal that nickel acts as a mitodepressive agent, inducing a variety of chromosomal aberrations in *Pisum sativum* root tip cells. Different concentrations of nickel led to specific

chromosomal changes at distinct mitotic stages, including chromosome fragments during telophase, disrupted anaphase with fragments, C-mitosis, chromosome bridges in late metaphase, vagrant chromosomes at telophase, sticky metaphase, and star-shaped chromosomes at anaphase. The mitotic index consistently declined with increased concentrations of nickel, in agreement with previous studies (Bessonova, 1991; Sengupta & Ghosh, 1993; Nyarai-Horvath et al., 1997; Sahi et al., 1998; Wang, 1999). Nickel-induced mitotic activity was found to depend on factors such as concentration, treatment duration, and root type (lateral or adventitious). Due to the high frequency of aberrant cells in the root tips, the impact of lead on microtubule organization in the meristematic tissues was also investigated. Disruptions were noted in interphase as a reduction or disarray of cortical microtubules, and during mitosis as spindle pole disorganization or absence (Strubinska et al., 2005). Rank and Nielsen (1998) further corroborated the correlation between industrial contamination, heavy metal concentration, and genotoxicity. Chang et al. (1997) reaffirmed these findings through the *Allium cepa* assay. Lead has also been reported to exhibit embryotoxic and gonadotoxic effects, whereas cadmium showed teratogenic effects (Ivanova et al., 2008). Deswal & Laura, 2018 reported alterations in prophase frequency in pea root tips following lead ion application. Nickel significantly suppressed mitotic activity in root meristems, potentially explaining its inhibitory effect on maize root growth, as cell elongation remained unaffected (L'Huillier et al., 1996). Nickel treatments did not induce abnormal prophase in *Pisum sativum* but led to considerable metaphase and anaphase–telophase abnormalities. These anomalies fluctuated without a consistent concentration-dependent pattern. At lower nickel concentrations, common mitotic abnormalities included stickiness, disturbed anaphase, laggards, C-metaphase, and chromosome bridges. Additionally, interphase cells exhibited micronuclei and multinucleation. Chromosomal breakage was evident in root tip cells, with higher frequencies of these aberrations recorded in certain pea seedling varieties compared to others (Deswal & Laura, 2018). The observed chromosomal aberrations, particularly at metaphase and anaphase–telophase stages, are in agreement with previous reports on the genotoxic effects of nickel (Deswal & Laura, 2018). Nickel significantly suppressed mitotic activity in root meristems, potentially explaining its inhibitory effect on maize root growth, as cell elongation remained unaffected (L'Huillier et al., 1996). Nickel treatments did not induce abnormal prophase in *Pisum sativum* but led to considerable metaphase and anaphase–telophase abnormalities. These anomalies fluctuated without a consistent concentration-dependent pattern. At lower nickel concentrations, common mitotic abnormalities included stickiness, disturbed anaphase, laggards, C-metaphase, and chromosome bridges. Additionally, interphase cells exhibited micronuclei and multinucleation. Chromosomal breakage was evident in root tip cells, with higher frequencies of these aberrations recorded in certain pea seedling varieties compared to others (Deswal & Laura, 2018). Recent studies have further confirmed that nickel induces oxidative stress, which interferes with spindle formation and chromosomal segregation, exacerbating cytological abnormalities during mitosis (Mustafa et al., 2023; Mahawar et al., 2023). Moreover, genotoxic assessments using Allium and Vicia systems support the observation that even sub-lethal nickel exposure can result in persistent mitotic spindle dysfunction and chromosomal disarray (Manna et al., 2022).

Conclusion

From this investigation, it was revealed that nickel was mitodepressive and induced various types of chromosomal aberrations in root tip cells of *Pisum sativum*. Different concentrations of nickel solution induced different types of chromosomal aberrations at various stages of cell division. Chromosome fragments at telophase disturb anaphase with fragment C- mitosis, Chromosome Bridge at late metaphase, vagrant chromosome at telophase, sticky metaphase, ring chromosome, star chromosomes at anaphase and sticky metaphase with vagrant chromosome etc. Applying various concentrations of nickel ions induces various abnormalities in *Pisum sativum.* In the present study, a decrease in the mitotic index level shows that experimental materials had mitodepressive effects, resulting in the inhibition of cells access to mitosis. It was found that cell division was inhibited by nickel. It caused chromosomal aberrations and normal cell division was affected. Mitotic index decreased with the increase of nickel concentration.

Author contributions

The author solely conceived and designed the study, conducted all experiments, collected and analyzed the data and prepared the manuscript

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Ethics approval: Not applicable.

AI tool declaration: The authors have not used AI and it's related to tools to write this manuscript.

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