

Cytotoxic Effects of Green Synthesis Se/ZnO Nanoparticles on *Allium cepa* and their Potential to Inhibit Mycotoxin Synthesis

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Despite their dubious safety, nanoparticles (NPs) are beneficial in many areas, particularly in agriculture. Though a variety of commercial nano-fertilizers, pesticides, and insecticides are available, little is known about their potential detrimental effects on plant cells. A Se/ZnO nanoparticle complex was synthesized utilizing *Ficus nitida* fruit extract as both a reducing and stabilizing agent, yielding an eco-friendly product. The common food plant *Allium cepa* was treated with Se/ZnO NP suspension. Transmission electron microscopic analyses of the NPs were performed, including Dynamic Light Scattering (DLS), zeta potential, X-ray diffraction, and FTIR characterizations. This study examined the cytological impact and chromosomal patterns of *Allium cepa* root meristems after treatment by Se/ZnO-NPs. Results show that all applied concentrations of NPs decreased the mitotic index (MI). The many chromosomal defects that were caused by NPs included disrupted and sticky chromosomes. Aflatoxin levels (B1, B2, G1, G2) were quantified in vegetables inoculated with *Aspergillus flavus*. Tomatoes and potatoes showed the highest contamination, in contrast, garlic and beet exhibited minimal or undetectable levels, suggesting resistance. The effect of Se/ZnO-NPs (0 to 40 ppm) on *A. flavus* growth and aflatoxin production was evaluated. While 5 ppm stimulated growth, higher concentrations significantly reduced both biomass and aflatoxins. These findings suggest that Se/ZnO-NPs treatment as an effective strategy to suppress *A. flavus* and its toxin production in contaminated crops.

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INTRODUCTION

Nanoparticles (NPs) can change the physicochemical processes of different substances, which can have a significant biological impact on cells (Atallah and Yassin 2020). It is challenging to predict their beneficial or detrimental impacts and their modes of action in biological systems and the environment because of the diverse sizes and forms of these entities (Chen *et al.* 2020). The biological technique provides a relatively safe alternative to the other methods used to synthesize NPs, even though cytotoxicity, genotoxicity, and oxidative stress are the main ways that NPs cause harm. The synthesis of plant and microbial extracts has garnered significant attention as an eco-friendly and sustainable method for producing a range of NPs (Karimi and Mahdavi Shahri 2021). The development and application of nanotechnology have impacted multiple domains, involving drugs and food production. Understanding the advantages and disadvantages of these products is essential for ensuring their sustainable and optimal use (Selim *et al.* 2025a; Amin *et al.* 2024, 2025). Nonetheless, the heightened prevalence of Se or ZnO nanoparticles (NPs) in commercial items has elicited escalating public apprehension regarding their toxicological and environmental ramifications. Toxicological investigations conducted in the past decade have revealed that ZnO NPs pose possible health and environmental concerns, but there have been no studies related to Se/ZnO NPs and their effects on plant cells, especially the mitotic index and abnormalities of cell phases. The ZnO NPs exhibit significant toxicity to bacteria, freshwater microalgae, mice, and human cells (Hou *et al.* 2018). Abdelsalam *et al.* (2022) observed that 150 ppm of amino-Zn NPs after 24 h, appeared to decrease the mitotic index by 35.3% on *Triticum aestivum* L compared to the control, which was 88.0%. In a study conducted on *Lens culinaris* Medik., the mitotic index in root tip cells decreased at all concentrations of ZnO-NPs treatment (25 and 50 ppm) in comparison to the control. Especially, the mitotic index was at its lowest point at the maximum concentration (50 ppm) (Dogan 2024).

Vegetables are highly perishable commodities, and their high moisture content makes them particularly susceptible to fungal contamination during postharvest handling, transportation, and storage (El-Taher *et al.* 2012; Abdelghany and Bakri 2019; Al-Rajhi *et al.* 2023a). Fungal species such as *Penicillium*, *Aspergillus*, and *Fusarium* are commonly associated with vegetable spoilage, causing visible decay, softening, discoloration, and unpleasant odors (Abdelghany 2014). These fungi typically invade through wounds, cracks, or damaged tissues and thrive under humid and warm conditions. In addition to physical spoilage, their presence reduces the nutritional and commercial value of vegetables, leading to significant economic losses. Among the various fungi associated with vegetable spoilage, *Aspergillus flavus* is of particular concern due to its ability to produce aflatoxins, which are highly toxic and carcinogenic secondary metabolites. Aflatoxins, particularly B1, B2, G1, and G2, pose a major threat to food safety and public health (Hamed *et al.* 2016). They can contaminate a wide range of agricultural products, including nuts, grains, spices, and, under favorable conditions, vegetables. Poor storage conditions, mechanical damage, and high humidity increase the risk of aflatoxin contamination in vegetable crops, especially in warm climates (Abd El-Ghany 2006; Mariutti and Valente 2009; Abdel-Kader *et al.* 2025). The consumption of aflatoxin-contaminated vegetables may result in serious health issues such as liver damage, immune suppression, and, with chronic exposure, liver cancer (Nuhu *et al.* 2025). Regulatory authorities have set strict maximum limits for aflatoxins in food products to protect

consumers. Therefore, identifying fungal contaminants and monitoring aflatoxin levels in vegetables is crucial for ensuring food safety. Preventive strategies include improving postharvest handling, proper drying and storage, and the use of antifungal treatments or biocontrol agents to minimize fungal growth and toxin production (Abdel Ghany 2014; Al-Rajhi *et al.* 2022, 2023b). It is hypothesized that some metal ions can be released in a controlled manner *via* nanoparticles. Ion exchange with the solid material is made possible by the nanomaterial's extremely high surface area, which results from its extremely small size. Compared to liquid soil supplements, the solid structure of the nanoparticles makes them less prone to leaching into groundwater. This study succeeded in creating Se/ZnO NPs based on underutilized *Ficus nitida* fruit extract, and also aimed to examine the effects of Se/ZnO NPs on chromosomal aberrations, mitotic index of the economic plant *Allium cepa* L., as well as on fungal growth and its mycotoxins.

EXPERIMENTAL

Extraction of *Ficus nitida* Fruits

The fruits of *Ficus nitida* (common name: curtain fig) were obtained from the Street garden at Sakaka city, Aljouf Region (Saudi Arabia). *Ficus* fruits contain many active compounds, such as phenolic compounds, fatty acids, and sugars. Such compounds may act as stabilizers, capping agents, or reducing agents, thereby facilitating the preparation of NPs. The most common bioactive substances present in many species are phenolic acids, which include caffeic, chlorogenic, and gallic acids (dos Anjos Cruz *et al.*, 2022).

The fruits were crushed after being cleaned with water that was distilled and let to air dry for two weeks. 5 g of air-dried powder and 100 mL of distilled water were combined, and the combination was sonicated for two hours at 50 °C in a water bath to create the fruit aqueous extract. The filtrate was kept in a refrigerator at 4 °C after the extract was filtered through filter paper (Whatman No. 1).

Synthesis of Selenium/ Zinc Oxide Nanoparticles based on *Ficus nitida* Fruit Extract

A magnetic stirrer was used to mix 30 mL of the aqueous fruit extract in a 250 mL flask to produce Se/ZnO nanoparticles. The flask was then filled with ten milliliters of sodium selenite (1 mM) and zinc nitrate (1 mM). The mixture was agitated for 48 h at 250 °C. The reaction mixture's color change indicated the formation of Se/ZnO NPs. The nanospheres were precipitated using centrifugation for 20 minutes at 4 °C and 14,000 rpm. The pellets were thoroughly cleaned in deionized water before freeze-drying (Selim *et al.* 2025b).

Characterization of Se/ZnO NPs

Utilizing an X-ray diffractometer (D8-Advance; Bruker, Germany), the microstructure of the nanomaterial was evaluated. The Scherrer equation, $D = 0.9 \lambda / \beta \cos \theta$, was used to measure the particle size of NPs. Then the d -spacing ($d_{hkl} = \lambda / (2 \sin \theta)$) values (Table 1). A transmission electron microscope (HR-TEM, JEOL-JEM-2100) was used to assess the generated NPs' size and shape. Using Fourier transform infrared spectroscopy, the functional groups connected to the produced NPs was analyzed. A Malvern Zetasizer

2000 (Nano-ZS; Malvern Instruments, Malvern, UK) with dynamic light scattering (DLS) was used to measure the mean size and zeta potential of Se/ZnO NPs suspended in deionized water at 25 °C.

Cytological Studies

Different concentrations of Se/ZnO NPs (5, 10, and 20 ppm) were produced as solutions in deionized water. The vitality of the seedlings was assessed after 6, 12, and 24 hours of the seeds sprouting in a small bottle containing varying concentrations of Se/ZnO NPs. Following treatment, the seeds were removed from the treatment device and rinsed with deionized water. To facilitate cytological processing, the tops of the roots were carefully cut. The root tips of the research plants were used for cytological processing after careful extraction. Using freshly made Carnoy's fixative (3:1 v/v absolute alcohol and glacial acetic acid, respectively), the root tips of both treated and untreated plants were fixed. The tips ranged in length from 1 to 2 cm. Feulgen squash was used to create cytological slides (Darlington and La Cour 1976). To confirm and count the chromosomal anomalies, the slides were examined under a microscope and photographed using an XSZ-N 107 research microscope equipped with a Premiere MA88-900 digital camera.

Fungal Isolation and Identification

To remove outside contaminants without compromising internal fungal structures, small pieces of spoiled vegetables (potato, garlic, beet, tomato, eggplant, onion, carrot, cucumber, broccoli, and pepper) were removed from areas that were obviously decomposed and surface-sterilized for 2 min using 1% sodium hypochlorite. After sterilizing, these segments were placed on potato dextrose agar (PDA) plates and incubated at 30°C to encourage the growth of fungi. The same incubation conditions were used to subculture newly formed fungal colonies in order to guarantee culture purity. Morphological and microscopic criteria were used to identify the dominant isolated fungus. The identification process followed the taxonomic keys and descriptions provided by Samson *et al.* (1995).

Industrial Infection of Vegetables with *Aspergillus flavus* for Mycotoxin Production

A toxigenic strain of *A. flavus* (previously isolated and confirmed for aflatoxin production) was cultured on potato dextrose agar (PDA) plates and incubated at 28 ± 2 °C for 7 days. *Conidia* were harvested by adding sterile 0.1% of polysorbate 80 (Tween 80) solution to the plate and gently scraping the surface. The spore suspension was filtered through sterile muslin cloth and adjusted to a concentration of 1×10^6 spores/mL using a hemocytometer. Ten types of fresh vegetables, such as potato, garlic, beet, tomato, eggplant, onion, carrot, cucumber, broccoli, and pepper were selected based on their economic relevance and susceptibility to fungal contamination. Vegetables were washed under running tap water, surface sterilized with 1% sodium hypochlorite for 2 min, and rinsed three times with sterile distilled water. Each vegetable was wounded at 2 to 3 sites (approx. 5 mm depth) using a sterile scalpel. A 50 µL aliquot of the *A. flavus* spore suspension was pipetted into each wound site. Control samples were treated with sterile polysorbate 80 solution without spores. Inoculated vegetables were placed individually in sterile plastic containers lined with moist filter paper to maintain high humidity ($\geq 90\%$). Incubation was conducted at 28 ± 2 °C for 15 days under dark conditions to mimic storage

environments conducive to aflatoxin production (Dijksterhuis and Houbroken 2025). After the incubation period, visibly infected tissue from each vegetable was excised, homogenized, and subjected to mycotoxin extraction. Aflatoxins were extracted using a mixture of methanol: water (80:20, v/v). Quantification of aflatoxins (B1, B2, G1, and G2) was performed using an ELISA kit. The aflatoxin concentrations were expressed in µg/kg of fresh weight.

Effect of Bimetallic Se/ZnO NPs on Growth and Mycotoxin Production by *Aspergillus flavus*

The following experimental protocol was used to assess the impact of bimetallic Se/ZnO NPs on the growth and production of aflatoxin by *A. flavus*: A toxigenic strain of *A. flavus* was cultivated and kept on PDA slants at 4°C. Se/ZnO NPs were suspended in sterile distilled water and sonicated to ensure even dispersion. Five concentrations (0, 5, 10, 20, and 40 ppm) were prepared and aseptically added to 100 mL of sterilized PDB in 250 mL Erlenmeyer flasks. Each flask was inoculated with 1 mL of a freshly made spore suspension from *A. flavus* (1×10^6 spores/mL). The flasks were incubated at 28 °C under stationary conditions for 10 days. After incubation, the fungal biomass was harvested by filtration through pre-weighed Whatman No. 1 filter paper, washed with sterile distilled water, and dried at 60 °C to constant weight. The biomass was expressed as mg/100 mL of culture medium (Al-Rajhi *et al.* 2022). The culture filtrates were collected and subjected to liquid-liquid extraction for aflatoxin determination.

Mycotoxins Assay

Mycotoxin analysis was conducted using a microtiter plate enzyme-linked immunosorbent assay (ELISA) with an automated Chem-well reader and commercial test kits for *Aspergillus* and *Fusarium* mycotoxins (R-Biopharm). The assay targeted aflatoxins B1, B2, G1, G2, and zearalenone, following the manufacturer's protocol (Al-Rajhi *et al.* 2024). For sample preparation, 10 mL of homogenized fungal broth was mixed with 20 mL of 70% methanol and stirred for 10 min using a magnetic stirrer. The mixture was then filtered through Whatman No. 1 filter paper. A volume of 5 mL of the filtrate was diluted with 15 mL of distilled water and 0.25 mL of polysorbate 20, followed by 2 min of mixing using a magnetic stirrer. For the ELISA procedure, 50 µL of each standard toxin solution (5, 10, 20, 45 ppb) and 50 µL of the prepared sample extract were added to individual wells of the microtiter plate. Plates were incubated at room temperature. After incubation, the wells were emptied and washed three times with 250 µL of PBS-polysorbate buffer (pH 7.2). Subsequently, 50 µL of enzyme conjugate and 50 µL of the chromogenic substrate tetramethylbenzidine (TMB) were added to each well and incubated in the dark at room temperature for 30 min (Hamed *et al.* 2016). The reaction was stopped by adding 100 µL of 1 M H₂SO₄, and absorbance was measured at 450 nm using the ELISA reader.

Analytical Statistics

Minitab 18 was utilized for statistical computations at the probability level of 0.05. Analyses of variance, one-way ANOVA, and post hoc Tukey's test were employed to examine quantitative data with a parametric distribution.

RESULTS AND DISCUSSION

Characterization of Se/ZnO NPs

A complex of Se/ZnO NPs was created using *Ficus nitida* fruit extract as a reducing and stabilizing agent, resulting in an environmentally friendly product. Zinc oxide NPs were combined with selenium (Se) to yield Se/ZnO NPs. The inclusion of Se may improve electrical properties, surface characteristics, and overall efficiency (Song *et al.* 2022). The nanoparticle manufacturing process demonstrated a notable color change to a dark brown hue. This visually confirmed the formation of Se/ZnO NPs (Mirzaei *et al.* 2021).

TEM and DLS analysis (Figs. 1A, 1C) are critical for assessing the shape, dimensions, distribution, and surface area of the synthesized Se/ZnO NPs. The TEM images obtained demonstrate that the NPs had semispherical and irregular shapes (Fig. 1A). The particle size was determined to be 48.8 nm, as measured by DLS analysis (Fig. 1C). The FTIR spectrum (Fig. 1B) reveals the vibrations and expanding peaks associated with functional groups that share in NPs formation. In the NPs solution, eight significant peaks were observed at 3415, 2919, 1602, 1413, 1348, 1118, 615, and 509 cm^{-1} . The presence of various functional groups in fruit extract, such as hydroxyl, carbonyl group, facilitated the reduction, capping, and stabilization of Se/ZnO NPs (Nguyen *et al.* 2023). The vibrations caused by stretching of O-H (alcohols and phenols) are located at 3415 cm^{-1} (Demir *et al.* 2018). The stretching vibration of the carboxyl group C=O accounts for the absorbance band at 1710 cm^{-1} . The ZnO bond was recognized as the origin of a significant vibration band at 615 cm^{-1} (El-Badri *et al.* 2021). At 509 cm^{-1} , the presence of selenium metal was observed (Jyoti *et al.* 2016). Figure 1D illustrates the investigation of the zeta potential of the synthesized Se/ZnO NPs at the synthesis pH of 7.2. The synthesized NPs exhibited a consistently negative surface zeta potential at the pH level analyzed in this study. The zeta potential of the preparation at a neutral pH of 7.2 was measured at -33.0 mV, as shown in Fig. 1D.

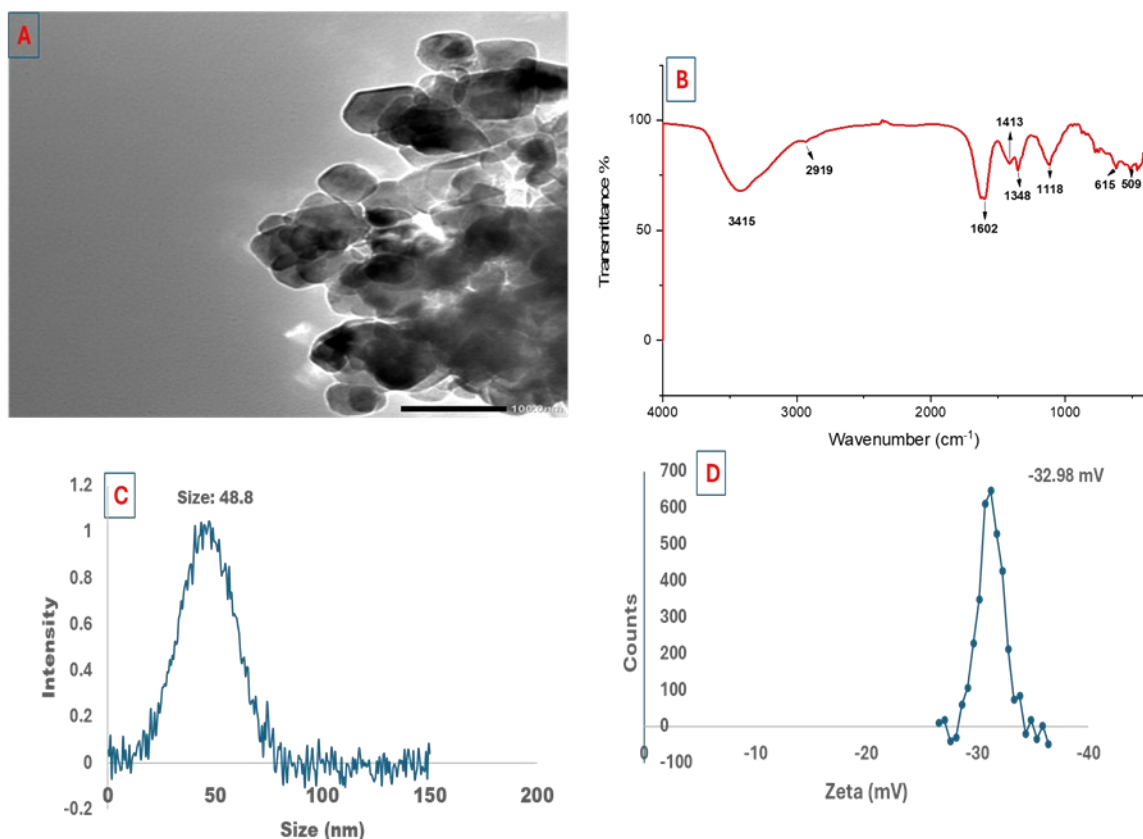


Fig. 1. TEM image (A), FTIR (B), DLS analysis (C), and Zeta potential (D) of Se/ZnO NPs

XRD Analysis

One useful method for determining the crystal structure of NPs is the XRD spectrum. The XRD diffraction spectra of the produced Se/ZnO NPs are shown in Fig. 2, where a clear and noticeable Bragg reflection was present.

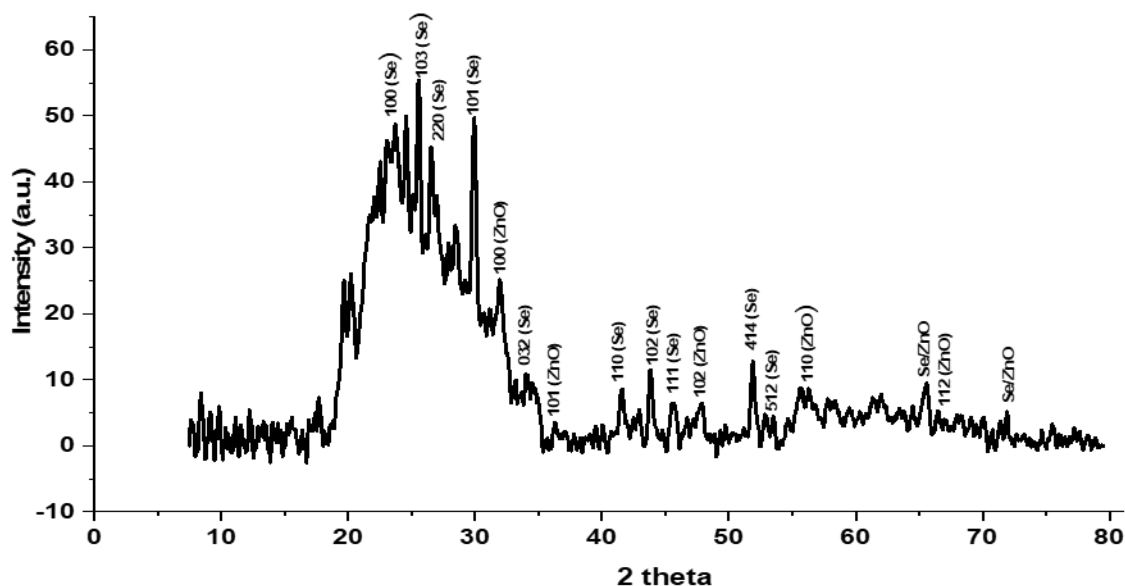


Fig. 2. XRD analysis of the Se/ZnO NPs

Table 1. XRD Parameters for Se/ZnO NPs

2θ (°)	FWHM (β)	Size (nm)	d -spacing
23.46	5.77	1.405	1.93
25.61	6.79	1.199	1.78
27.19	7.22	1.131	1.68
29.93	1.32	6.229	1.54
31.97	0.99	8.336	1.45
33.56	1.13	7.339	1.39
36.69	1.32	6.340	1.28
41.58	1.36	6.437	1.16
43.84	0.37	22.96	1.11
45.63	0.46	18.52	1.07
47.59	1.07	8.071	1.04
51.88	0.87	10.15	0.97
53.54	0.65	13.68	0.95
56.43	7.35	1.225	0.92
68.32	0.87	11.03	0.82
Average	0.62	8.27	1.27

The XRD's sharp peaks demonstrated the crystalline nature of the NPs generated. According to the hkl miller indices, the peaks at 23.4°, 25.6°, 27.2°, 29.9°, 33.5°, 41.5°, 43.8°, 45.6°, 51.8°, and 53.5° respectively correspond to crystal planes 100, 103, 220, 101, 032, 110, 102, 111, 414, and 512 crystalline Se (COD9008579, 9008581, and JCPDS card No. 06-362) (El-Batal *et al.* 2023) is indicated by these peaks. Another group of 2θ was visible in the XRD spectrum in Fig. 2 at 31.9°, 36.6°, 47.5°, 56.4°, and 68.3°. Accordingly, these angles corresponded to the Miller indices of 100, 101, 102, 110, and 112. Crystalline ZnO (COD 2107059, 1011259, and JCPDS 5-0664) is represented by these peaks. Se/ZnO NPs (average = 8.27 nm) had particle sizes that were slightly smaller than the TEM measurement, according to the Scherer equation. The amorphous peaks were

seen in the XRD pattern. According to Indhira *et al.* (2023), Se-ZnO NPs must be heated to roughly 400 °C to achieve a crystalline form. It can degrade the bioactive metabolites in the specimen and stop biological activity at this temperature point. This implies that the NPs are crystalline and that *Ficus nitida* fruit extract is a stabilizing agent. XRD parameters for Se/ZnO NPs are shown in Table 1.

Genotoxic Effects of Se/ZnO NPs

The potential phytotoxic and genotoxic impacts of Se/ZnO Nanocomposite on garlic (Tables 2 and 3, and Figs. 3 and 4) were examined. The Se/ZnO NPs inhibited mitosis index in a concentration-dependent manner and created several mitotic abnormalities, including chromosomal stickiness, irregular prophase, disturbed metaphase, C-metaphase, and bridges. Higher concentrations of ZnO NPs were found to exacerbate chromosomal aberrations and reduce the mitotic index (Raskar and Laware, 2014). Table 2 illustrates how Se/ZnO NPs affect the mitotic index (MI). According to the findings, the Se/ZnO NPs caused the root tip cells of *Allium cepa* to undergo mitodepression. Nevertheless, in comparison to lower concentrations and control cells, larger concentrations of nanoparticles had a noticeably bigger effect on the MI value. The reduction observed was substantial across all treatments for NPs, with a particularly remarkable significance noted in the treatment involving 20 ppm Se/ZnO NP over a 24-h duration, where the MI was measured at 1.80 ± 0.096 , in comparison to the control group's MI of 5.11 ± 0.044 .

Zinc ions are responsible for the reduction in the rate of mitotic division because they impede the transition of cells from the S (DNA synthesis) phase to the M (mitosis) phase. Exposure to ZnO has the effect of preventing many cells from progressing to prophase and interfering with the mitotic cycle. The decrease in the MI value can be used to gauge the degree of cytotoxicity (Ramesh *et al.* 2014). The findings indicated an elevated frequency of prophase and metaphase stages at the expense of other mitotic phases in Se/ZnO NPs. The accumulation observed during these stages suggests that zinc ions play a significant role in modulating the sequence of mitotic division, effectively diminishing the number of cells that proceed to mitotic division by obstructing the process after prophase. Table 3 presents the percentages of chromosomal abnormalities attributed to NPs. The percentage of abnormalities reached 100% following treatment with 20 ppm for 24-h. The abnormal percentage was 42.4% following treatment with 5 ppm for 6-h, in contrast to 16.5% observed in control cells. Table 3 indicates that NPs elicited various types of mitotic abnormal cells in the root tip of *Allium cepa* seeds. Several of these types are illustrated in Fig. 3. The proportion of these types varied with the concentration and duration of treatment. These categories encompassed sticky at various stages, irregular prophase, and C-C-metaphase. During prophase, a high frequency of irregular prophase and sticky prophase was observed. The frequency of irregular prophase was elevated during shorter treatment durations, whereas sticky prophase was more prevalent at the longest treatment duration. The irregular formation of prophase is influenced by the effects of Se and Zn ions on the individualization of chromatin threads into normal. ZnO NPs' genotoxicity and biochemical effects on *Vicia faba*, *Nicotiana tabacum*, and *Allium cepa* plants were assessed by Ghosh *et al.* (2016). According to their findings, *Allium cepa* cells' root meristems displayed higher chromosome abnormalities, cell-cycle, micronucleus production, and membrane integrity loss.

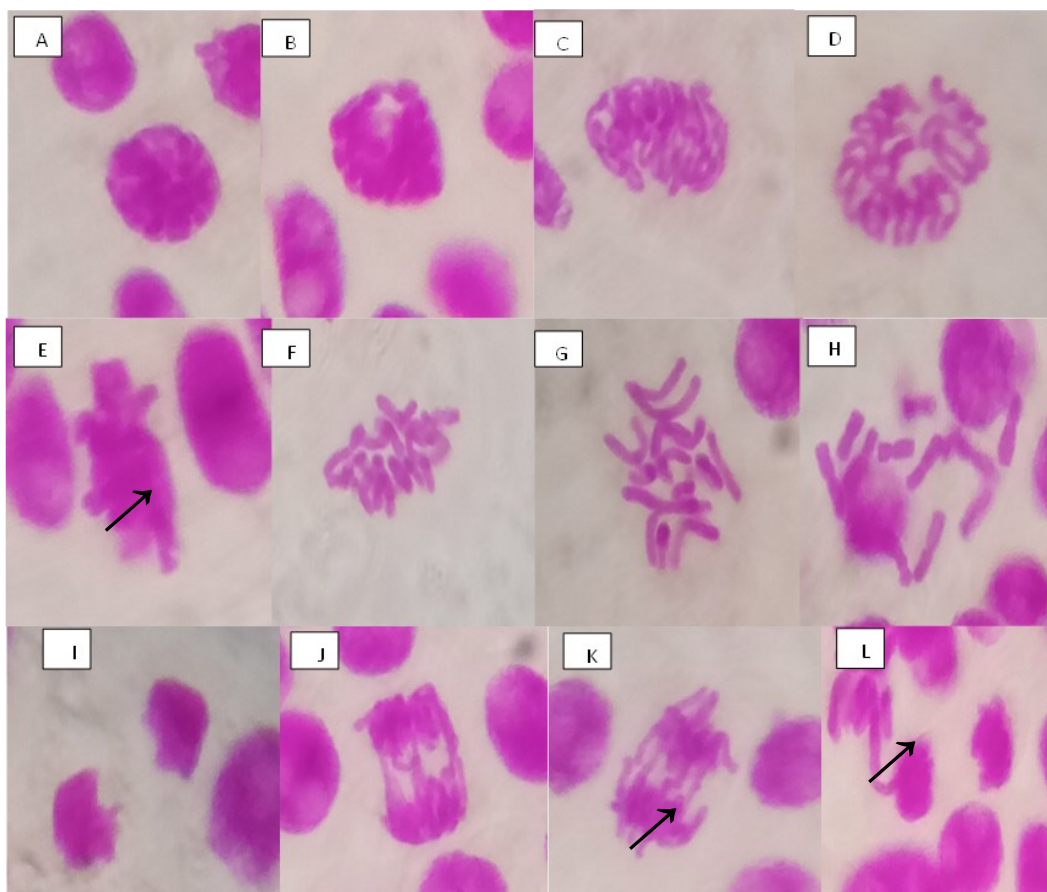


Fig. 3. Different types of chromosomal abnormalities in *Allium cepa* response to different times and concentrations of biogenic bimetallic Se/ZnO NPs: (A) Sticky prophase, (B) sticky vacuolated nucleus at prophase, (C, D) irregular prophase, (E) sticky metaphase, (F) disturbed metaphase, (G, H) C- metaphase, (I) Sticky anaphase, (J) sticky anaphase with multi-bridges, (K) sticky anaphase with multi-bridges and forward chromosomes, and (L) sticky telophase with forward chromosome.

Table 2. Types and Percentages of Mitotic Abnormal and Total Abnormal Cells in *Allium cepa* Root Tips Treated with Se/ZnO NPs at Different Times

Treatments		Counted Cells	Divided Cells	Mitotic Index (MI $\pm$ S.E)	Mitotic Phases (%)			
Conc. (ppm)	Time (h)				Prophase	Metaphase	Anaphase	Telophase
Control		5345	273	5.11 $\pm$ 0.044	50	28.88	5.56	15.55
5ppm	6	5599	257	4.59 $\pm$ 0.103	46.15	38.46	0.00	15.38
	12	5089	192	3.77 $\pm$ 0.085	38.98	40.68	3.39	16.95
	24	5215	148	2.84 $\pm$ 0.022	43.14	39.22	3.92	13.73
10 ppm	6	5486	205	3.74 $\pm$ 0.118	37.14	30.00	14.29	18.57
	12	5275	150	2.84 $\pm$ 0.135	39.22	23.53	15.69	21.57
	24	5417	135	2.49 $\pm$ 0.105	33.33	26.19	19.05	21.43
20 ppm	6	5693	156	2.74 $\pm$ 0.063	28.85	40.38	17.31	13.46
	12	5401	114	2.11 $\pm$ 0.108	30.30	30.30	18.18	21.21
	24	5228	94	1.80 $\pm$ 0.096	31.03	27.59	20.69	20.69

Table 3. Types and Percentages of Mitotic Abnormal and Total Abnormal Cells in *Allium cepa* Root Tips Treated with Se/ZnO NPs at Different Times and Concentrations

Treatments		Divided Cells	Total Abnormal Cells	Ab. (%)	Abnormal Mitotic Phases (%)			
Conc. (ppm)	Time (h)				Prophase	Metaphase	Anaphase	Telophase
Control		273	45	16.48 $\pm$ 0.81	6.59	5.13	1.83	2.93
5 ppm	6	257	109	42.41 $\pm$ 0.91	16.34	15.56	2.33	8.17
	12	192	110	57.29 $\pm$ 0.53	20.31	19.79	2.60	14.58
	24	148	108	72.97 $\pm$ 1.05	23.65	31.08	4.05	14.19
10 ppm	6	205	141	68.78 $\pm$ 0.97	17.56	22.93	14.63	13.66
	12	150	123	82.00 $\pm$ 0.98	28.67	21.33	15.33	16.67
	24	135	125	92.59 $\pm$ 0.50	26.67	25.19	19.26	21.48
20 ppm	6	156	138	88.46 $\pm$ 1.50	23.08	35.90	17.31	12.18
	12	114	110	96.49 $\pm$ 0.83	30.70	27.19	17.54	21.05
	24	94	94	100 $\pm$ 0.00	31.91	27.66	20.21	20.21

Types of Aflatoxins - Aflatoxins B1, B2, G1, and G2-detected in Various Vegetables

A comprehensive analysis of the levels of four types of aflatoxins- Aflatoxin B1, B2, G1, and G2-detected in various vegetables artificially infected with *A. flavus* is shown in Table 4. Among the vegetables tested, tomatoes, potatoes, and peppers recorded the highest levels of aflatoxins across all types. Specifically, tomatoes showed the highest concentration of Aflatoxin B1 (4.50 $\pm$ 0.01 μ g/kg) and significant levels of B2, G1, and G2, suggesting that they are highly susceptible to contamination. Potatoes followed closely with 4.33 $\pm$ 0.06 μ g/kg of Aflatoxin B1 and high levels of B2 (1.95 $\pm$ 0.01 μ g/kg), G1 (1.10 $\pm$ 0.18 μ g/kg), and G2 (0.40 $\pm$ 0.04 μ g/kg). Peppers also exhibited substantial contamination, particularly with Aflatoxin B2 (1.76 $\pm$ 0.02 μ g/kg) and G2 (0.58 $\pm$ 0.04 μ g/kg). In contrast, garlic and beet exhibited no detectable levels of Aflatoxin G1 and G2 (0.00 $\pm$ 0.00 μ g/kg), which may indicate either resistance to *A. flavus* growth or its inability to produce them in those substrates. Garlic, in particular, had low levels of B1 and B2 (1.77 $\pm$ 0.06 and 0.31 $\pm$ 0.01 μ g/kg, respectively), while beet showed slightly higher but still moderate levels of B1 and B2. Onions also demonstrated relatively low aflatoxin levels, particularly for B1 (1.53 $\pm$ 0.18 μ g/kg), but detectable levels of G1 and G2 (0.58 $\pm$ 0.04

and 0.61 ± 0.02 $\mu\text{g/kg}$), suggesting selective susceptibility. Vegetables such as broccoli, eggplants, cucumbers, and carrots showed intermediate contamination levels, indicating that while not as heavily affected as tomatoes or potatoes, they are still vulnerable to mycotoxin accumulation under favorable conditions for fungal growth. The statistical differences among vegetable types were supported by the Least Significant Difference (LSD) values, which were 0.43, 0.22, 0.10, and 0.15 $\mu\text{g/kg}$ for Aflatoxins B1, B2, G1, and G2, respectively. These relatively low thresholds, particularly for G1 and G2, mean that even small variations in toxin levels are statistically meaningful. Generally, the data clearly showed that the susceptibility of vegetables to aflatoxin contamination by *A. flavus* varied significantly. Tomatoes and potatoes pose the highest risk, which is likely due to their high moisture content and soft tissue structure, which favor fungal colonization and toxin production. In contrast, garlic, beet, and to some extent onions appear to be more resistant, potentially due to their antimicrobial compounds or less favorable internal environments for fungal growth. These results underscore the importance of targeted postharvest handling, storage, and food safety monitoring, especially for the more vulnerable vegetables in the context of mycotoxin contamination.

Van de Perre *et al.* (2014) reported the presence of several fungi and different mycotoxins on various vegetables including tomatoes, soft red fruits, onions, and bell peppers. Capsicum and its derivative products are highly susceptible to contamination by mycotoxins, particularly during postharvest handling and storage under inadequate conditions. This vulnerability underscores the importance of developing effective antifungal strategies to minimize fungal growth and mycotoxin biosynthesis (Costa *et al.* 2019). Our results were agreeing with other studies, where the presence of *A. flavus* in fresh fruit of tomato has been reported (Segura-Palacios *et al.* 2021). While previous study, Maroutti (2009) informed the documentation of the fungi namely *A. flavus* and *A. parasiticus* besides the occurrence of aflatoxins in fresh tomato fruit as well as by-products including ketchup, pulp, paste, dried tomatoes preserved in oil and dehydrated tomatoes. Ali *et al.* (2024) revealed fungal contamination of onions by *Aspergillus niger*, *Fusarium* species, and *Rhizopus stolonifer*. Notably, *A. niger* isolated from the infected onion samples demonstrated the ability to produce ochratoxin A (Esuola and Ortega-Beltran 2025), highlighting its potential risk as a foodborne toxigenic pathogen.

Table 4. Mycotoxin Detection in Different Vegetables Infected with by *A. flavus*

Vegetables	Aflatoxin B1 ($\mu\text{g/kg}$)	Aflatoxin B2 ($\mu\text{g/kg}$)	Aflatoxin G1 ($\mu\text{g/kg}$)	Aflatoxin G2 ($\mu\text{g/kg}$)
Garlic	$1.77 \pm 0.06\text{d}$	$0.31 \pm 0.01\text{d}$	0.00 ± 0.0	0.00 ± 0.00
Broccoli	$2.40 \pm 0.04\text{c}$	$0.49 \pm 0.04\text{d}$	$0.29 \pm 0.01\text{e}$	$0.19 \pm 0.01\text{d}$
Peppers	$3.88 \pm 0.12\text{b}$	$1.76 \pm 0.02\text{ab}$	$1.47 \pm 0.24\text{a}$	$0.58 \pm 0.04\text{b}$
Eggplants	$2.65 \pm 0.04\text{c}$	$0.78 \pm 0.01\text{c}$	$0.56 \pm 0.02\text{cd}$	$0.31 \pm 0.02\text{c}$
Beet	$2.99 \pm 0.07\text{c}$	$0.71 \pm 0.04\text{c}$	$0.40 \pm 0.01\text{d}$	0.00 ± 0.00
Onions	$1.53 \pm 0.18\text{d}$	$0.87 \pm 0.03\text{c}$	$0.58 \pm 0.04\text{c}$	$0.61 \pm 0.02\text{b}$
Potatoes	$4.33 \pm 0.06\text{a}$	$1.95 \pm 0.01\text{a}$	$1.50 \pm 0.18\text{a}$	$0.89 \pm 0.05\text{a}$
Tomatoes	$4.50 \pm 0.01\text{a}$	$1.56 \pm 0.04\text{b}$	$0.78 \pm 0.07\text{b}$	$0.48 \pm 0.01\text{b}$
Cucumbers	$3.50 \pm 0.05\text{b}$	$0.88 \pm 0.01\text{c}$	$0.61 \pm 0.06\text{c}$	$0.23 \pm 0.03\text{c}$
Carrots	$2.66 \pm 0.01\text{c}$	$0.78 \pm 0.02\text{c}$	$0.66 \pm 0.01\text{c}$	$0.34 \pm 0.02\text{c}$
LSD	0.43	0.22	0.10	0.15

The results in Table 5 demonstrate the impact of various doses (ranging from 0 to 40 ppm) of bimetallic Se/ZnO NPs on the growth and Aflatoxins (B1, B2, G1, and G2) production of *A. flavus*. *A. flavus* showed a baseline growth of 255 ± 2.12 mg and produced considerable amounts of all four aflatoxins, with Aflatoxin B1 at 6.54 ± 0.32 µg/kg, B2 at 3.68 ± 0.18 µg/kg, G1 at 2.42 ± 0.10 µg/kg, and G2 at 1.77 ± 0.01 µg/kg at 0 ppm (the control) of Se/ZnO NPs. Interestingly, when treated with a low concentration of 5 ppm Se/ZnO NPs, *A. flavus* growth significantly increased to 320 ± 3.23 mg. This unexpected stimulation may reflect a hormetic effect, where sublethal stress enhances biological activity, or it could result from trace element uptake stimulating fungal metabolism at low doses. However, with increasing nanoparticle concentrations, a gradual decline in *A. flavus* growth was evident. At 10 ppm, growth dropped to 278 ± 4.32 mg, and further reductions were seen at 20 ppm (223 ± 1.36 mg) and 40 ppm (168 ± 1.65 mg). This downward trend clearly suggests that higher doses of Se/ZnO NPs exert an inhibitory effect on *A. flavus* biomass, likely due to oxidative stress, cell wall damage, or Se/ZnO NPs interaction with vital cellular processes. Aflatoxin production followed a similar pattern. At 5 ppm, despite the increased *A. flavus* biomass, aflatoxin levels remained high, with AFB1 and B2 even exceeding control levels slightly. However, as Se/ZnO NPs concentrations increased, aflatoxin production decreased markedly. At 10 ppm, AFB1 dropped to 6.22 ± 0.25 µg/kg and continued decreasing to 2.66 ± 0.36 µg/kg at 20 ppm and 2.54 ± 0.26 µg/kg at 40 ppm. The same trend was observed with AFB2, AFG1, and AFG2, all of which were sharply reduced. Of particular note is Aflatoxin G2, which was completely undetectable at 40 ppm, suggesting a complete inhibition of its biosynthetic pathway under these conditions. The values of LSD provided—12.36 for *A. flavus* growth, 0.32 for AFB1, 0.54 for AFB2, 0.62 for AFG1, and 0.24 for AFG2—support the statistical significance of these changes. Previously, numerous investigations employed NPs of silver for controlling aflatoxins (Abdelghany *et al.* 2020) and other mycotoxins (Bakri *et al.* 2020; Al-Rajhi *et al.* 2022). According to Hassan *et al.* (2013), growth of fungi and its productivity of aflatoxins were suppressed *via* the addition of ZnO-NPs at 8 µg/mL, whereas the growth of ochratoxin A- and fumonisin B1-producing fungi, along with their mycotoxin production, was suppressed by incorporating 10 µg/mL of ZnO-NPs into the tested medium. Also, recent study demonstrated the ability of ZnO-CuO NPs to inhibit aflatoxin production in maize (Ngwenya *et al.* 2025).

Table 5. Effect of Se/ZnO NPs on Growth and Mycotoxin Production by *A. flavus*

Conc (ppm)	Growth mg/100 mL medium	Aflatoxin Quantity			
		Aflatoxin B1	Aflatoxin B2	Aflatoxin G1	Aflatoxin G2
0	255±2.12c	6.54±0.32b	3.68±0.18b	2.42±0.10b	1.77±0.01a
5	320±3.23a	7.59±0.15a	5.87±0.21a	3.92±0.06a	1.89±0.02a
10	278±4.32b	6.22±0.25b	3.12±0.08c	2.11±0.04b	1.58±0.06b
20	223±1.36d	2.66±0.36c	1.56±0.03d	0.86±0.09c	0.21±0.03c
40	168±1.65e	2.54±0.26c	1.12±0.04d	0.34±0.02c	0.0±0.00
LSD	12.36	0.32	0.54	0.62	0.24

CONCLUSIONS

1. Se/ZnO nanoparticles (NPs) exhibited a concentration-dependent dual effect on *Aspergillus flavus*, initially stimulating growth at very low levels but progressively suppressing both growth and mycotoxin production as the concentration increases.
2. The results highlight the potential of Se/ZnO NPs as an effective antifungal and anti-mycotoxigenic agent, particularly at higher concentrations (20 to 40 ppm).
3. Evidence was found of genetic disruption in *A. flavus* exposed to the Se/ZnO NPs, which raises concerns about possible unintended effects. Such risks, in addition to reports from previous studies on nanoparticle toxicity and environmental persistence, underline the importance of caution.
4. Therefore, while Se/ZnO NPs demonstrate promise for nanoparticle-based control strategies in food preservation and fungal contamination prevention, their potential risks must be carefully weighed. Further investigation is necessary to fully assess their safety, genetic impact, and compatibility with food and agricultural systems before large-scale application.

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