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RESEARCH PAPER

Phytotoxic Nature of Different Concentrations of Zinc Oxide Nanoparticles on *Allium Cepa L.* Root Meristem Cells

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ABSTRACT

This paper conducted to study the effect of different levels of zinc nanoparticles at concentrations (0,20, 40, 80, 100) µg/ml on the meristematic cells of plant roots *Allium cepa*, were used at different periods24, 42 and72 hour by estimating their effect on root length, root morphology, cell morphology, chromosomal abnormalities, mitotic index, Mitotic inhibition (Minh), micronucleus and percentage of abnormal cells in different phases of cell division and comparing them with control by hydroponics of *A. cepa* . The results showed the cytogenetic and genotoxic effects of ZnO NPs on the meristematic cells of *A. cepa* roots at high concentrations, as it had a negative effect on growth, as it was shown that the elongation of roots was severely inhibited at the highest concentration of zinc oxide NPs compared to the control (untreated *A. cepa* roots), significant effects of zinc nanoparticles were also observed on the chromosomal aberration and mitotic index with increasing concentration by decreasing of divided cell rates ,chromosomal aberration were also observed in divided cells of onion roots, which exposed to different concentrations at different period ,the higher frequent type of this chromosomal aberrations which observed are stickiness , disturbed, micronucleus and binucleated, no chromosomal aberration was observed in the control (untreated onion root tips) as for the percentage of abnormal cells in different stages of mitosis, it increased with the increase of ZnO NPs concentration, while division stages in root tips were clearly normal in control. The bio-uptake of ZnO NPs was the cause of reactive oxygen species (ROS) generation, which in turn was probably the cause of genotoxicity and the DNA aberrations observed in this study.

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INTRODUCTION

Nanotechnology is one of the very new technologies when compared to other technologies, as it uses nanomaterials with sizes ranging from (1-100 nanometers) and these materials have shown positive effects in wide areas of life sciences [1]. The attractiveness of nanoparticles lies in the fact that they can be engineered to work in a way that natural materials cannot do, The high surface area of nanomaterials per unit volume, as well as their improved chemical reactivity [2], which made it used in new applications as there are many applications for particles, including protecting and producing crops, diagnosing and following up on diseases, reducing the time for nanomedicine to reach the circulatory system , Food processing and storage, treating drinking water, treating air pollution, cosmetics, paint, and others, which will

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be reflected on the daily life of the individual [3].

Published research related to living organisms has shown that in many cases, nanomaterials may pose increased health and environmental risks when compared to regular forms of the same materials nanomaterials can be travel to ecosystems through different pathways and thus cause toxicity to living organisms which affects the biodiversity and abundance of ecosystems [4].

Several studies have focused on the behavior of nanomaterials in natural and artificial water samples [5] among those nanomaterials is zinc nanoparticles (ZnO NPs) which have attracted a lot of attention because they have different physical and chemical properties than their normal state. ZnO NPs is one of the most widely used nanoparticles in industries, because it is a chemically stable substance that has good transparency and UV blocking properties, these properties make zinc oxide nanoparticles have many applications, including medical, pharmaceutical and food applications, zinc oxide nanocrystals have also been used to create invisible sunlight that blocks ultraviolet light [6]. However, the increased use of ZnO NPs in many consumer products increased the possibility of environmental pollution by these factors and their damage to living systems [7].

Nanotechnology has been applied to some living organisms, including plants, which are an essential component of all ecosystems, where plants play an important role in the fate and transport of nanoparticles in the environment through uptake and bioaccumulation [8].

The effect of nanomaterials on a plant varies depending on the type of plant, as well as the chemical composition, concentration, size, and other important chemical and physical properties of NPs. There are several studies that dealt with both the stimulating and inhibitory effects of NPs on plant growth at different stages of growth that can be absorbed by plant roots and transported to shoots through vascular systems depending on the composition, shape, size, and internal anatomy [9], there are many studies that dealt with the effect of nanoparticles on plants, a number of researchers analyzed the mutagenic and genotoxic activity of various nanoparticles on different types of plants, including the study of [10] on *Vicia faba,* and a study [11] on *Arabidopsis thaliana*, in addition to a study [12] on *Zea mays, A. cepa* L was a share of those studies, including a study by [13-15]and others. *A. cepa* L belongs to the family Amaryllidaceae [16] which is one of the higher plants used in genetic tests and is an effective genetic parameter for environmental monitoring [13].

The Apex root system of *A. cepa* was used to evaluate the genetic toxicity of many environmental pollutants, It depends on this system because of some important advantages, including the ease of working with it in the laboratory and in the field in terms of speed of growth and the formation of a large number of roots in a short time, It is one of the simple and economical plants, has simple genetic systems and has a fast germination rate[17] , In addition to the regular size of *A. cepa* chromosomes, which are characterized by their large size and small number (2=16) as validated by the United Nations Environment Program, the World Health Organization and the US Environmental Protection Agency[18].

Studies have dealt with the toxic effect of nanoparticles on plants, especially with regard to their mechanisms, absorption and passage within the food chain, when studying the effect of ZnO NPs on *Arabidopsis* [19] indicated that the toxicity of nanoparticles is stronger than solutions containing the same concentration of ordinary zinc. As for studying[15] dealt with the effects of ZnO nanoparticles on the morphology of *Allium sativum* L, which showed the complete growth inhibitory effect when exposing ZnO_2 nanoparticles to *Allium sativum* at a concentration of 50 µg/ml. Various cellular effects have been observed by[20] when studying the genotoxicity of silver nanoparticles in *Allium cepa*, he noticed a decrease in the mitotic index, which reached (27.62%) compared to the control (60.30%) with an increase in the concentration of silver nanoparticles. While [14] used high concentrations of titanium dioxide nanoparticles to measure its toxic effect on *Allium cepa*, as the plant was treated with four different concentrations of TiO_2 NP (12.5, 25, 50, 100) mg/ml, the study showed a decrease in the mitotic index to 21 compared to the control 69.

Because the industrial production and commercial applications of ZnO NPs nanoparticles have increased significantly in recent times, which has increased the possibility of environmental pollution by these factors and their consequent harmful effects on living systems, so this study was designed to evaluate the genotoxic damage of ZnO NPs when exposed to different concentrations of

ZnO NPs. Zinc nanoparticles on mitotic activity and chromosomal behavior in meristematic cells of roots *A. cepa* L when absorbed and then the oxidative stress it generates.

MATERIALS AND METHODS

Characteristics of zinc nanocomposites used in the study

Zinc nanocomposites were purchased from the local market ready in the form of nano-zinc oxide and belonged to one of the approved American companies, Sky spring nanomaterial's Inc. In the form of a white to light yellow powder with a purity of 99% and a size of (10-30) nanometers. Fig. 1.

Examination of nanocomposites using atomic force microscopy (AFM)

The zinc nanoparticles were examined by atomic force microscopy (AFM) for the purpose of identifying and mapping surfaces with nanoscale dimensions**.**

Examination of nanocomposites using X-ray diffraction analysis

X-ray diffraction (XRD) analysis of zinc oxide was carried out using a (Shimadzu X-Ray Diffractometer XRD 6000) in the service laboratory of Ibn Al-Haytham College of Education for Pure Sciences / University of Baghdad, in order to know the crystalline structure of the solids from by projecting the X-ray spectrum of the material to be examined.

Examination of nanocomposites using Scanning electron microscope (SEM)

The structural characteristics of nanoparticles in terms of shape and size were determined by scanning electron microscopy (SEM).

Preparation of nano–solutions

The solutions of the studied nano-elements (zinc) were prepared from zinc oxide nanocomposites, the NPs were suspended directly in deionized water (DI water), after that, it was mixed using an ultrasonic homogenizer (Haesler, Germany) for 15 min.[21] to prepare four suspensions of NPs with different concentrations (20, 40, 60, and 100) µg/ml.

Tested plants: A. cepa L.

In the current study, the red local onion variety was used (the stage of small bulbs resulting from planting seeds and which are grown to produce large bulbs). The bulbs of onions *A.cepa* L were obtained from the local market, dry bulbs roots

Fig. 1. Nano-ZnO material from sky spring nanomaterial's Inc.

were removed from the base with a sharp blade before using them in the experiment to obtain fresh meristematic tissues, onions weighing 20-25 g were grown in a cylindrical glass flask without NPs with renewable water supply every 24 hour.

A.cepa L. treatment with zinc nanoparticles

Onions were used as a biosystem to study the genetic effects of zinc nanoparticles ,well-grown bulbs of homogeneous root length were transferred to glass flasks containing different concentrations of (20, 40, 60 and 100 µg/ml) of zinc nanoparticles, and the intensity of the effect was compared with the control solution free of nanocomposites, then incubated at 25±2°C and light system 12:12 hour lighting: dark: optimum conditions , The samples were left for 72 hour, taking into account the exchange of solutions for each treatment with freshly prepared solutions every 24 hours. After the exposure time, the best onion follicles in terms of root length growth were selected. The root length of all onion follicles was measured in the presence and absence of nanomaterials at different intervals using the measuring tape, after taking the roots lengths, the average was calculated for each concentration, and the length of the control samples was calculated. Three replicates were made for each concentration, and the standard methods described in [22] were followed.

Cytological analysis

In order to determine the effect of ZnO NPs in meristematic cells of *A.cepa* roots, slides of the growing apex were prepared in the group treated with zinc nanoparticles and the control. The apical root tips of 2 mm were collected from each of the follicles and then fixed in 1:3 acetic acid – ethanol, the root tips were then hydrolysed in 1N HCl at 60 C° for 5 min and then washed three times with distilled water with an interval of 2 min, then dry the excess water with filter paper, root tips were squashed on a microscopic slide and stained with acetocarmine for 15 minutes. After staining, the slide cover was placed and lightly pressed with the thumb in order to get rid of the excess dye, slides were and examined directly by inverted microscope (Optika, Italy) using an oil lens at 100X, magnification, approximately 1000 cells a dividing and non-dividing cell were examined for each slide and from different locations in the slide, and duplicate slides were recorded for all samples selected in each category [21,22].

Data treatment

In order to determine the effect of ZnO NPs in onion apical cells different stages of mitosis and aberrant chromosomes were counted to determine mitotic index, Phase index (PI), Mitotic inhibition, Percentage of abnormal cells and micronucleus index (MN) [13,23].

Mitotic index

Mitotic index was calculated:

Mitotic index= (number of dividing cells / total number of dividing and no. dividing cells) *100.

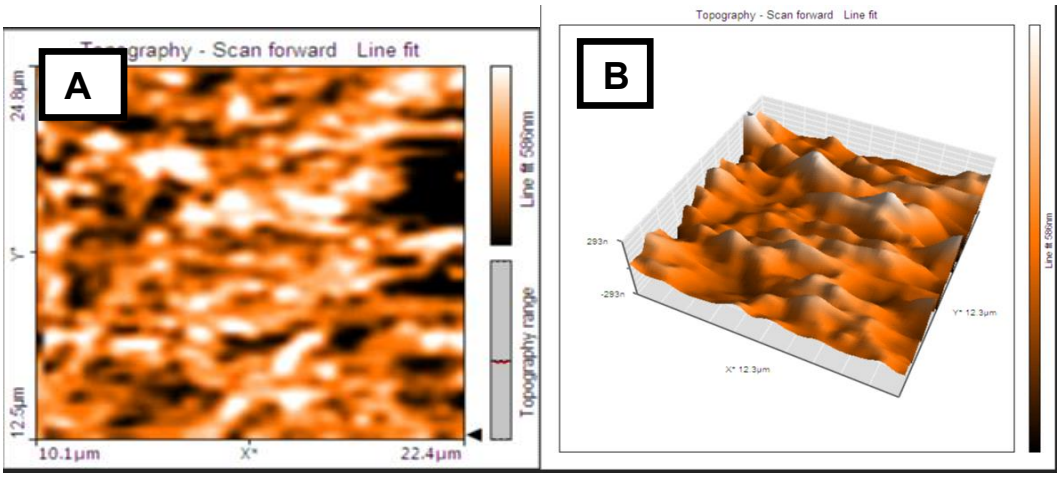

Fig. 2. Atomic force microscope (AFM) image for the zinc oxide (ZnONPs) nanoparticles, A: two-dimensional image (topography image); B, three-dimension image.

according to the following equation:

$$\text{Mitotic index(MI)} = \frac{T_{DC}}{T_C} \times 100 \quad (1)$$

Mitotic inhibition (Minh)

Mitotic inhibition: was also calculated:

Mitotic inhibition (Minh)= (number of non-dividing cells in exposed - number of non-dividing cells in control groups/ dividing cells in the control group) *100. according to the following equation:

$$\text{Minh} = \frac{\text{NDC} - \text{NDCc}}{\text{DCc}} \times 100 \quad (2)$$

Phase index (PI)

Phase index (PI) was also calculated:

Phase Index = (Total no. of cells observed/ Total no. of dividing cells) *100. according to the following equation:

$$\text{Phase index(PI)} = \frac{T_C}{T_{DC}} \times 100 \quad (3)$$

Percentage of abnormal cells

The percentage of abnormal cells was calculated: The percentage of abnormal cells= (total no. of abnormal cells/ total no. of dividing cells**)** *100. according to the following equation:

$$\text{Total percentage of abnormal cells} = \frac{T_{abn}}{T_{DC}} \times 100$$

where T_{DC} = total no. of dividing cells; T_C = total no. cells observed, T_{abn} = total no. of abnormal cells, (NDC) number of non-dividing cells in exposed and control groups (NDC_C), dividing cells in the control group (DC_C) [13,14].

Bio-adsorption of zinc nanoparticles:

A. cepa nanocomposite-treated root tips were washed with distilled water and then dried at 60 °C for 24 h. Then the dried roots were crushed using a sterile pestle, then the sample was digested with concentrated nitric acid and the dissolved fraction was filtered through a filter paper with a diameter of 0.45 µm. Then the concentration

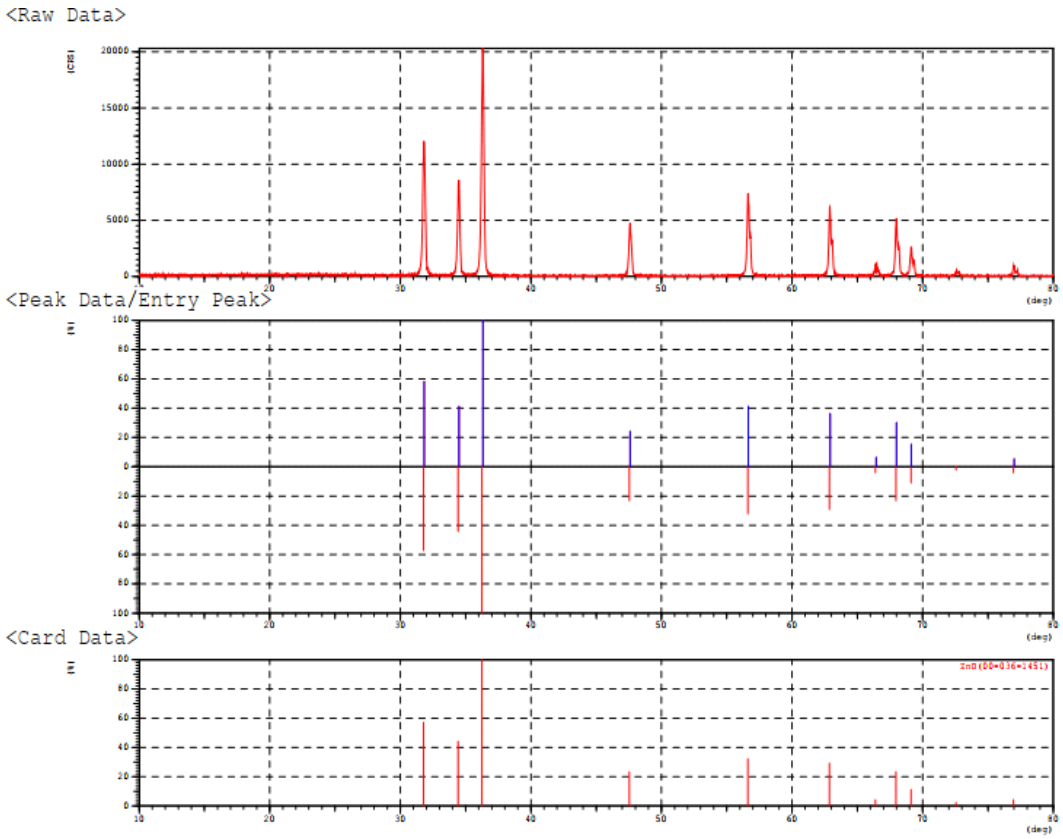

Fig. 3. X-ray diffraction (XRD) spectrum of zinc oxide nanoparticles.

of Zno NPs inside the roots was determined using a spectrophotometer. Zn+2 ions leaked from nanoparticles were also evaluated. The concentrations of 20, 40, 80 and 100 µg/ml. Zno NPs were incubated under laboratory conditions for 4 hours and the nanoparticles were separated using a series of filtration using 0.1 mm diameter filter papers. The minute Zn^{+2} ions were estimated using a spectrophotometer. [14].

Statistical Analysis

The statistical analysis was done using SPSS, the variation between four concentrations of ZnO nanoparticles and the control (untreated A. cepa roots) among hours was done by one-way analysis of variance (ANOVA) and least significant differences (LSD) at $p<0.05$.

RESULTS AND DISCUSSION

Physicochemical characterization of ZnO NPs

Examination of nanoparticles using an atomic force microscopy (AFM)

The size range and surface morphology of zinc oxide nanoparticles (ZnO) were assessed by atomic force microscopy (AFM). The two- and three-dimensional geography of ZnO NPs was presented in Fig. 2. The type of each atom and its location were identified on the 3-D diagram of the topography of the material's surface at the atomic level. Direct observation of the image exposed spherical shapes of zinc nanoparticles. Fig. 2A. (Topography image) shows the 2-D image of zinc nanoparticles showing molecular clusters, while Fig. 2B shows the 3-D image of a section of zinc nanoparticle surface, where the height of the molecular cluster is about 293 nm. The average particle sizes were in the order of 29 nm.

Examination of nanoparticles using X-Ray diffraction (XRD) analysis

X-ray diffraction (XRD) analysis of zinc oxide nanoparticles, which is one of the basic methods used by chemists to examine the chemical and physical composition of unknown materials, was performed. (XRD) analysis is used to measure the crystallinity of particles when their crystallinity is sometimes not ideal, as well as to reveal the nature of material particles, this study showed that zinc oxide nanoparticles possess a crystalline nature, which agreed with the study [25] when they examined zinc oxide nanoparticles (XRD) analysis, which showed the crystalline nature of the zinc oxide nanoparticles. Our results also showed the bio-crystallization on the surface of the nano-zinc oxide, as the strong and narrow the diffraction peaks indicate that the crystal size was very small, diffraction peaks indicate that the product has good crystalline structure and there were three prominent peaks, and the strongest peaks were 3, 1, and 2 at 36.3°, 31.8 ° and 34.4 °, respectively

Table 1. X-ray diffraction (XRD) analysis of zinc oxide nanoparticles: Peak Data List

no.	2Theta (deg)	I/I1	d (A)	FWHM(deg)	Intensity (Counts)	Integrated Int (Counts)
1	31.8	2.80867	58	0.19230	1299	14121
2	34.4	2.59823	41	0.18400	926	9638
3	36.3	2.47143	100	0.18340	2258	23813
4	47.6	1.90864	24	0.18300	550	5681
5	56.6	1.62334	41	0.16780	916	8910
6	62.9	1.47608	36	0.17340	804	8098
7	66.4	1.40615	6	0.18010	131	1337
8	68	1.37748	30	0.17820	674	6788
9	69.1	1.35762	15	0.16720	338	3280
10	77	1.23729	5	0.16630	124	1131

(Table 1, Fig. 3). The diffraction plots also show the presence of other low intensity reflections opposite (31.8°,34.4°,36.3°,47.6°,56.6°,62.9°,66.4 °,68°,69.1°,77°) Table 1. All the diffraction peaks of the nanoparticles in this study are consistent with the crystal structure of ZnO NPs in the study [26].

Examination of ZnO NPs nanocomposites using scanning electron microscopy (SEM)

The SEM image of ZnO NPs was high resolution, as shown in Fig. 4 which shows that the particles were spherical in shape to hexagonal shape, this is in agreement with the study of [13] when studying

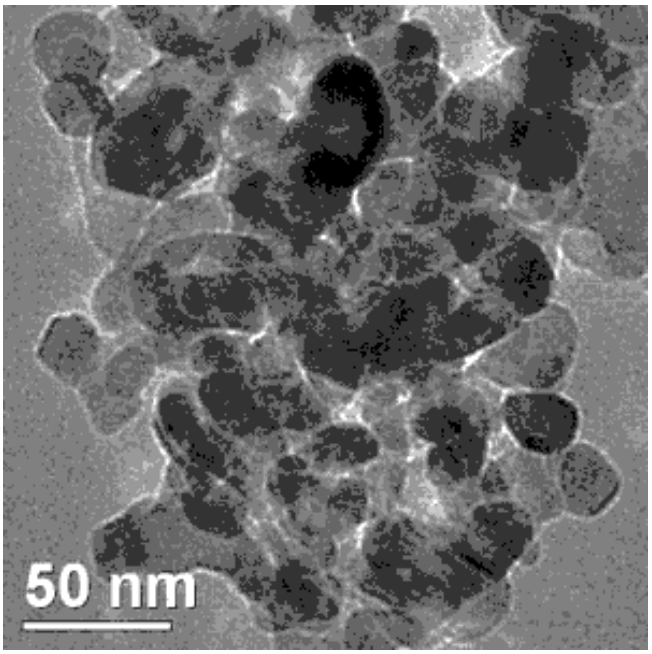

Fig. 4. Scanning electron microscope (SEM) images of zinc oxide nanoparticles

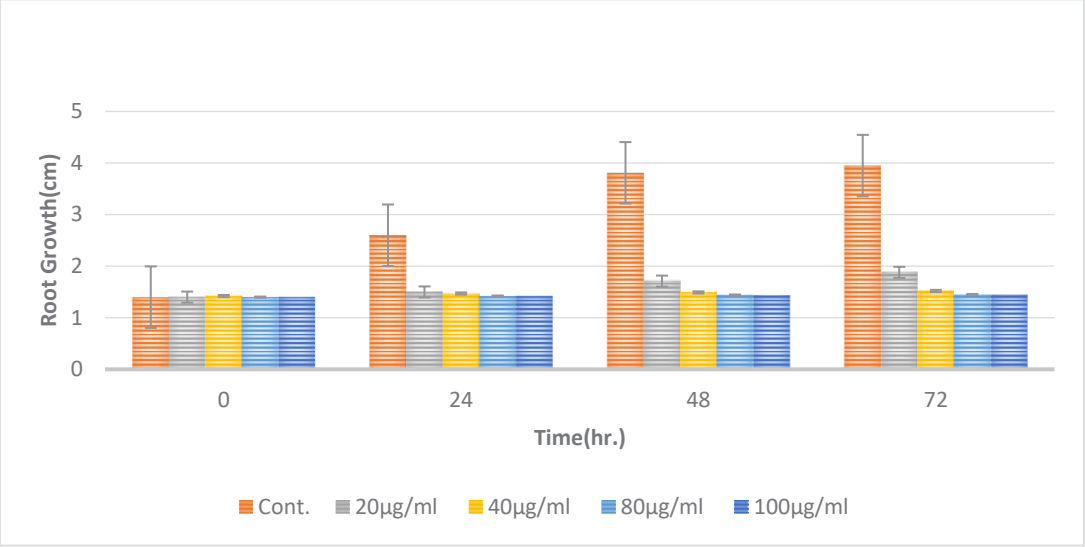

Fig. 5. Relative Mean root length among different concentrations of ZnO nanoparticles.

zinc nanoparticles.

Effects of zinc nanocomposites on average root length of A. cepa

Mean root lengths were recorded for all the four treatment samples in each concentration group of 20, 40,80 and 100 µg/ml of ZnO NPs nanoparticles**.** In general, it was clear that zinc nanocomposites inhibited the average length of onion roots compared with the control treatment (distilled water), and the increase in the four concentrations led to an increase in the inhibition of the average root length. Through the results that were reached and shown in Fig. 5 which shows the effect of zinc nanoparticles on the length of the onion root, these results recorded a decrease in root lengths 72 hours after treatment of ZnO NPs nanoparticles that amounted to (1.88, 1.52, 1.45,1.44) cm, at concentrations 20, 40, 80, 100, µg/ml respectively compared to the control 3.95 cm, the decrease in root length upon exposure to high concentrations of ZnO NPs is due to the toxicological mechanism of zinc oxide NPs, which is closely related to the chemical composition, structure, particle size and surface area of NPs [27] ,In addition to the concentration of ZnO nanoparticles, this was confirmed by the study of [15] on *Allium sativum* L. when exposed to ZnO NPs. as there was a complete inhibition of *Allium sativum* root growth when treated with a

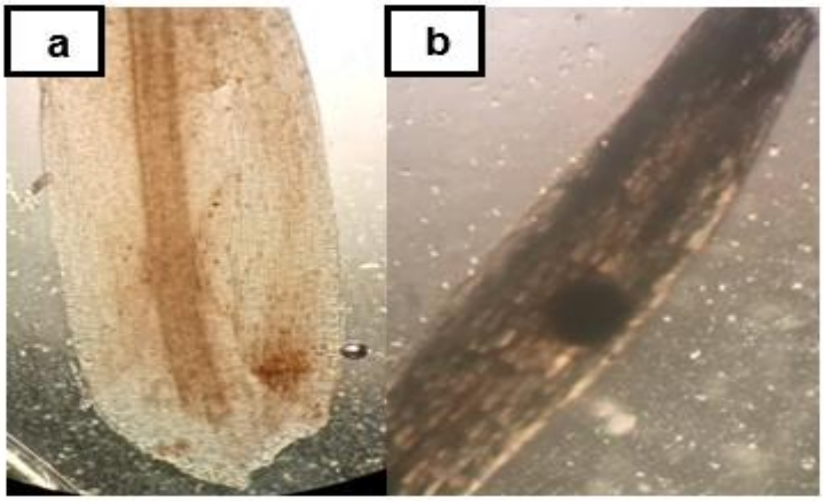

Fig. 6. Adsorption and morphological phenotype of A. cepa roots: without NPs (a), with zinc oxide NPs (b).

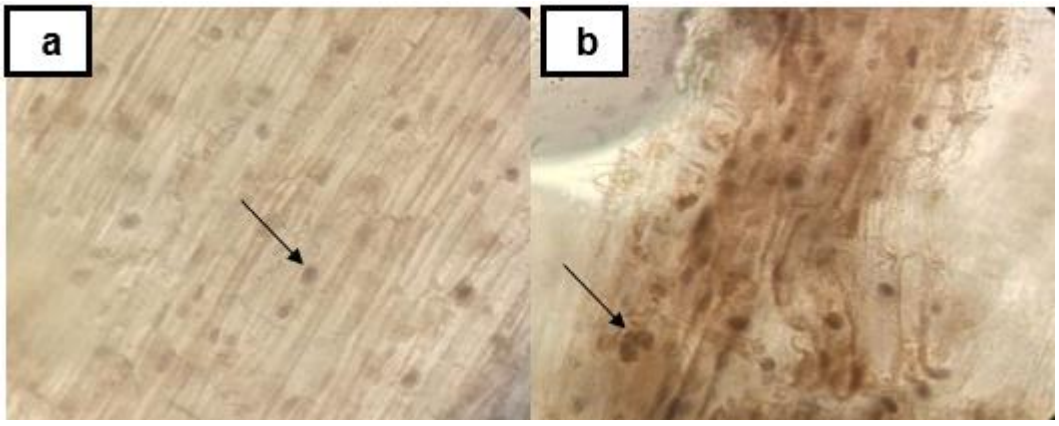

Fig. 7. Microscopic cellular phenotype of *A. cepa* roots: without NPs (control) (a), with zinc oxide NPs (b).

concentration of 50 mg/l and the result obtained in this study is consistent with the findings of the study [28] on onion plants when exposed to nano-zinc oxide, the root lengths were reduced to 1.50, 1.25, and 1.24 cm at concentrations 5, 10, and 20 µg/ml, respectively, compared to the control (4.75). The study on onion plants when exposed to Cuo Nanoparticles stated that the average root lengths of all samples treated with nanoparticles were the lowest lengths 1.8 cm, reaching at the highest concentration 0.1 g /100 ml compared to the control 3.78.[29] reported that the percentage of germination and root length of *Raphanus sativum* upon exposure to TiO_2 nanoparticles (n-TiO2) was significantly reduced when co-exposure to n-TiO2 and $CdCl_2$ at the highest concentration (250 ,1000) mg/L respectively compared to control ($p < 0.05$). The effect of zinc oxide nanoparticles (NPs) on the root system of *A. cepa* was examined by light microscopy, The root surface of the control was free of nanoparticle adhesion (Fig. 6A), however, the treatment of the plant with zinc oxide (NPs) led to damage in the morphological shape as a result of the effect of nanoparticles on the root systems (Fig. 6.B). The cellular phenotype of untreated *A. cepa* roots was also examined with nano-zinc oxide, and the normal direction of cleavage and cellular network was observed in untreated samples (Fig. 7A), While, cells were morphologically damaged in the treated samples (Fig. 7B). Zinc oxide nanoparticles are able to penetrate the roots of *A. cepa* and influence root elongation, metabolism, and genetic material, The effect of nanoparticles on the morphology of onion roots is in agreement with the study [28] on the evaluation of the hazardous and toxic nature of zinc oxide nanoparticles using *Allium cepa.*

Cytological analysis

A. cepa samples were analyzed after treatments using Zno nanoparticles at concentrations of (20, 40, 80, 100) µg/ml, as mentioned in the Materials and Methods section.

Influence of Zno NPs on Number of dividing cell and mitotic index of Allium cepa

The number of dividing cells (DC) was calculated per 1000 cells in the taken concentrations and there was a decreasing trend with increasing concentration between all treatment groups. The results showed that the maximum values for the number of dividing cells reached 471 per 1000 cells at the lowest concentration (20) µg/ml, while (DC) was it reached 375 cells at the highest concentration of 100 µg/ml compared to the control samples (622) Table 2.

In present study, to assess the possible positive as well as negative influence of Zno NPs, we focused on mitosis in *A. cepa.*

The mitotic index (MI%) was studied to determine the rate of cell division, zinc nanoparticles showed cytotoxicity through the

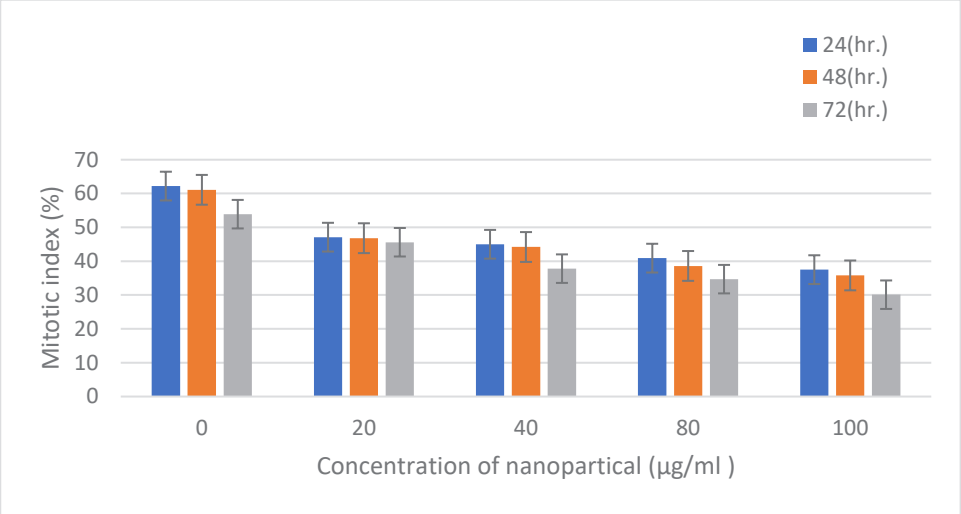

Fig. 8. effect different concentrations of ZnO nanoparticles on the mitotic index (MI%). In root -tip meristem of *A. cepa*.

decrease in the mitotic index depending on the dose. The mitotic index (MI%) decreased for samples treated with concentrations (20, 40, 80 and 100) µg/ml ZnO NPs. All concentrations of Zinc nanoparticles decreased MI in *A. cepa* root meristematic cells for 24–72 h. Fig. 8. The maximum decrease in the mitotic index of *A. cepa* roots exposed with ZnO NPs at a concentration of 100 µg/ml after exposure period 24,48,72 hours reached (37.5, 35.8, 30.1) respectively compared to the control treatment 62.2, 61.1, 53.9 respectively. Fig. 8. It is clear from the results of this study that ZnO NPs led to a significant ($p<0.05$) decrease in the MI% compared to the control treatment and for all the concentrations used and within the time periods, the relationship was inverse between the concentration and mitotic index as an increase in the mitotic index was observed as the concentration of Zinc nanoparticles increased. These results are in agreement with previous studies of ZnO NPs that showed the decrease in cell division index as a result of exposure to nanoparticles, which is due to changes in the duration of the mitotic cycle due to the slower development of cells from the S-phase (synthesis DNA) to the M-phase (mitosis) of cell cycle, the decrease in the mitotic index represents a decrease in the number of dividing cells and this

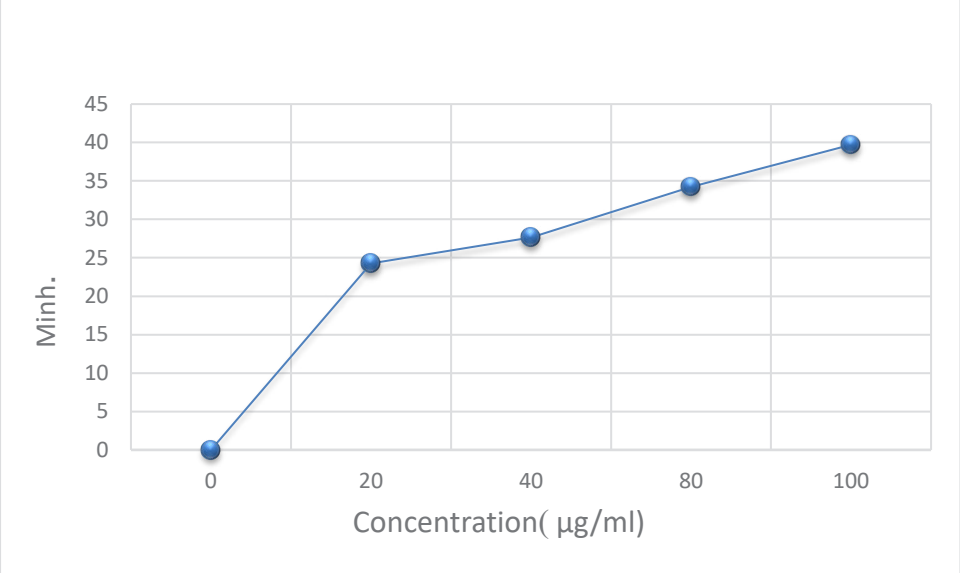

Fig. 9. Mitotic inhibition among different concentration of ZnO nanoparticles

Table 2. Mitotic inhibition with the Standard Error (S.E) among different concentrations of ZnO nanoparticles. Total cells analyses, 1000.

Con. (µg/ml)	Number of dividing cells	Non-dividing cells	Mitotic inhibition ± S.E
0	622	378	-
20	471	529	24.28±5.19
40	450	550	27.65±3.46
80	409	591	34.24±4.04
100	375	625	39.71±4.61

leads to a decrease in growth where nanoparticles penetrate the plant systems and interfere with the internal components of the cells, thus weakening the stages of cell division. The nanoparticles interfere with the normal development of division, thus preventing a number of cells from entering the prophase and ending the mitotic cycle during the prophase, thus inhibiting the synthesis of DNA and protein. [30]. The current study agreed with [13] when studying zinc nanoparticles and their effect on cell division in *Allium cepa*. Mitotic index decreased to 30.1 compared to 62.3 control. The current study also agreed with study [31] which demonstrated that Ag NPs lead to a decrease in the mitotic index of *A. cepa* plant, as confirmed by [14] that the decrease in mitotic index is dose-dependent, as the mitotic index decreased from 69 to 21 in the root of *A. cepa* when treated TiO_2 NPs.

Influence of Zno NPs on Mitotic inhibition (Minh) of A. cepa

Mitotic inhibition (Minh): was calculated for all the treatment samples, Minh was found to be increasing with the treatment concentration as maximum was found in 100 µg/ml (39.71) and least was found to be in 20 µg/ml (24.28). Table 2 shows the relative mitotic inhibition in four exposure groups of nanoparticles. Increasing trend in mitotic inhibition was found to be (20 <40< 80<100) µg/ml samples (Fig. 9). The increase in the percentage of inhibition with increasing concentration is due to the role of nanoparticles, which are characterized by their ultra-small size

Table 3. Mitotic Index in percentage (MI %) with the Standard Error (SE) for different exposure concentrations of ZnO NPs as Phase Index in percentage (after 24 hour of incubation)

Concentration(µg/ml)	Mitotic phases (%) ± S.E				MI% ± S.E
	Prophase%	Metaphase%	Anaphase%	Telophase%	
0	60.3±4.67	1.9±0.11	0.9±0.11	0.7±0.11	62.43±4.76
20	48.3±4.09	0.9±0.11	0.7±0.11	0.5±0.11	47.6±3.96
40	40.7±3.57	0.3±0.05	0.3±0.05	0.3±0.05	44.9±3.66
80	34.3±2.94	0.1±0.01	0.1±0.02	0.2±0.05	41.03±2.88
100	29.4±2.48	0.1±0.01	0.1±0.02	0.2±0.05	37.7±2.65

Table 4. Mitotic Index in percentage (MI %) with the Standard Error (S.E) for different exposure concentrations of ZnO NPs as Phase Index in percentage (after 48 hour of incubation)

Concentration(µg/m)	Mitotic phases (%)± S.E				MI% ± S.E
	Prophase%	Metaphase%	Anaphase%	Telophase%	
0	65.4±4.96	2.1±0.11	0.8±0.05	0.8±0.05	61.1±4.96
20	54.3±4.38	1.2±0.11	0.6±0.11	0.6±0.11	46.8±4.38
40	52.7±3.57	0.4±0.05	0.4±0.05	0.2±0.05	44.2±3.57
80	44.3±3.00	0.1±0.03	0.1±0.005	0±0	38.6±3.00
100	39.4±2.48	0.1±0.02	0.1±0.01	0.1±0.03	35.8±2.48

(<100 nm), which helps them to interact easily with the cell surface and then penetrate into the cytosol and thus affect the cell. [32].

Influence of Zno NPs on Phases index of Allium cepa

The results showed that the percentage of cells in different mitotic phases (prophase, metaphase, anaphase and telophase) decreased with the increase of ZnO NPs concentration when compared to the control (Tables 3-5). It is clear from the table that ZnO NPs caused a change in the prophase of onion root samples treated with ZnO NPs, and it was shown from the results that the prophase decreased significantly in the roots of onion plant exposed to all concentrations compared to the control treatment, and the highest decrease of the prophase was at the concentration 100, as it decreased to 22.1 within 72 hour of exposure Table 5. The highest value, metaphase, was recorded 1.2 after 48 h. of incubation of root treatment with nano-Zn containing 20 µg-mL when compared to the control (2.1) Table4., metaphase value also experienced a gradual decrease at 24,48, 72 h. up 0.1. As for the anaphase an of *A. cepa* root cells treated with ZnO NPs, it decreased significantly at concentration (100) µg/ml of ZnO NPs after 48,72 hours Tables 4 and 5. while the telophase decreased significantly at concentration (80) µg/ml at 42 ,72hour Tables 4 and 5. The reason may be due to a decrease anaphase and telophase to the retention of cells in the metaphase and then a decrease in the rate of cells that go into the anaphase and the telophase, The results of this study agree with [13]when studying zinc nanoparticles and their toxic effect on *Allium cepa.* the percentage of cells in different mitotic phases decreased with the increase in ZnO NPs concentration, as well as with [32] who studied the genotoxic effect of copper nanoparticles on *A. cepa*.

Effect of ZnO nanoparticles and cytotoxic modifications

Fig. 10 are shown A. cepa root of meristem cells exposed marked with acetocarmine reactions. Chromatin is differentially distributed in meristem cells in exposed A. cepa root as shown by arrows. Where the nuclei appear, abnormal shape have caused disruption of mitotic division, thus leading to cytotoxic effects. Our findings are in agreement with previous studies in which the exposure to ZnO nanoparticles to abnormal cell division during root elongation is associated [7].

Genotoxic parameters

Chromosomal aberration studies

Chromosomal aberration are changes in the structure of chromosomes resulting from breakage or exchange of a chromosome , it is evident from Table 6 that ZnO NPs it caused a significant increase in the incidence of chromosomal abnormalities at all concentrations used For each exposure period., it was also found that increasing the concentration of Zno NPs and the duration of exposure leads to an increase in the percentage of chromosomal abnormalities in onion root cells, this result is consistent with several studies that have found numerous chromosomal abnormalities in *A. cepa* roots exposed to nanoparticles as a study[19] on the effect of ZnO NPs on *Arabidopsis* and a study

Table 5. Mitotic Index in percentage (MI %) with the Standard Error (S.E) for different exposure concentrations of ZnO NPs as Phase Index in percentage (after 72 hour of incubation)

Concentration(µg/m)	Mitotic phases (%) ± S.E				MI% ± S.E
	Prophase%	Metaphase%	Anaphase%	Telophase%	
0	58.3±4.96	0.8±0.11	0.5±0.11	0.4±0.11	53.9±4.96
20	47.3±4.38	0.4±0.11	0.3±0.11	0.2±0.05	45.6±4.38
40	40.1±3.57	0.2±0.05	0.2±0.05	0.1±0.011	37.8±3.57
80	31.3±3.00	0.1±0.02	0.1±0.011	0±0	34.7±3.00
100	22.1±2.48	0.1±0.02	0±0	0.1±0.005	30.1±2.48

[14]of titanium dioxide nanoparticles on *Allium cepa*. The results showed that ZnoNPs have a clear effect on chromosomal behavior especially in metaphase and anaphase as compared to prophase and telophase. Different types of chromosomal aberrations were observed which included stickiness, fragment, micronucleus, binucleated, disturbed chromosome, no chromosomal aberration was observed in the control (untreated onion root tips). Table 6. shows the effect of ZnO NPs in the meristematic cells of the onion root of *A. cepa* on the results of chromosomal aberrations. There was an increase in chromosomal aberrations when the concentration was increased. The results showed that the chromosomal aberration of onion root for samples treated with (20, 40, 80, 100) mg/ml ZnO NPs were 17, 22, 28, 31, respectively compared to the control without any chromosomal aberration, a number of factors can contribute to the increase in chromosomal aberrations, the most important factor being the interference of chemicals during DNA repair [30]. The mechanism of nanotoxicity will be closely related to the chemical composition of nanoparticles, the size and surface area of nanoparticles, as nanoparticles can penetrate the plant system and then interfere with the internal cellular components, causing some changes such as changes in chromosomal aberrations, sister chromatid exchanges leading to obstruction in plant cell division [20].

The types of chromosomal abnormalities are c-mitosis, lagging and bridge, it does not appear at all the different concentrations or

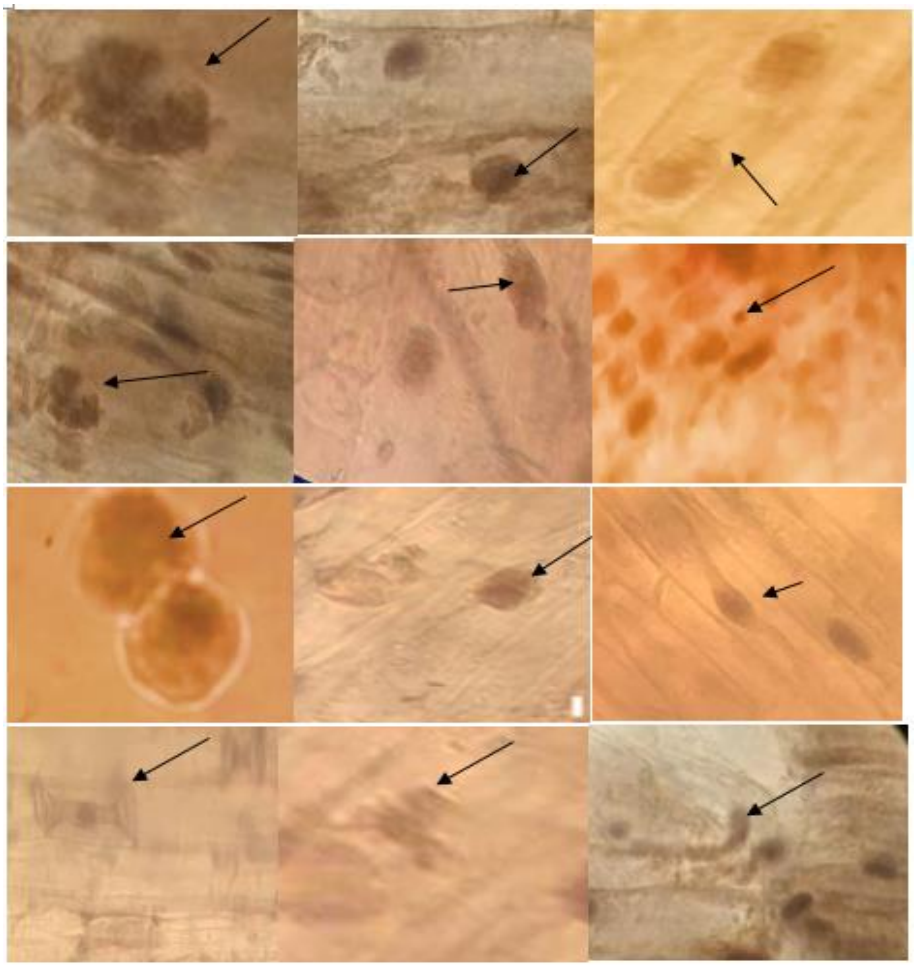

Fig. 10. Light microscopy images of *Allium cepa.* roots meristem stained with acetocarmine reactions: exposed. Arrows indicate DNA damage in cells of root meristem and high potential to interact with DNA.

exposure times that appear less rate, the most frequent abnormality was the appearance of stickiness, it may be stickiness the result of a defect in non-histone proteins that have a role in chromosomal regulation and are necessary for chromosome segregation, or stickiness may be the result of interlacing chromatin fibers between chromosomes [32]. The appearance of adherent chromosomes was the most frequent abnormality, or the reason may be due to the fact that the materials used are highly toxic, and this toxicity is usually irreversible and may lead to cell death [34]. As for the appearance micronucleus in meristematic cells, which are pieces of chromosomes indicates that the nanoparticles used in the test are a clastogenic substance that has its own castogenic /genotoxic effects resulting in damage to DNA or chromosomes [32]. Disturbed chromosome demonstrated that NPs induced oxidative stress that was manifested in terms of DNA degradation. As for bridges, may arise due to a break in the chromosome, and the break may be in the chromatids of the chromosome to itself and then reconnect properly from the sticky end [32]. As for lagging chromosomes and c-mitosis, it is caused by the disruption of the spindle fiber apparatus, which leads to a delay in the division of the centromere [35] as nanoparticles exert a mitotic effect through the production lagging chromosomes, which fail to attach to the spindle apparatus [14]. The result obtained in this study is in agreement with the study of [15] on *Allium sativum* L when exposed to ZnO NPs, which found several types of mitotic aberrations, including chromosome stickiness, laggings, breakages and bridges, disturbed chromosome, many other studies also revealed DNA damage on exposure to different NPs as by study [24] when studying copper nanoparticles and their effect on cell division in *Allium cepa*.

Percentage of aberrant cells

The percentage of abnormal cells was calculated, the highest percentage was at the highest

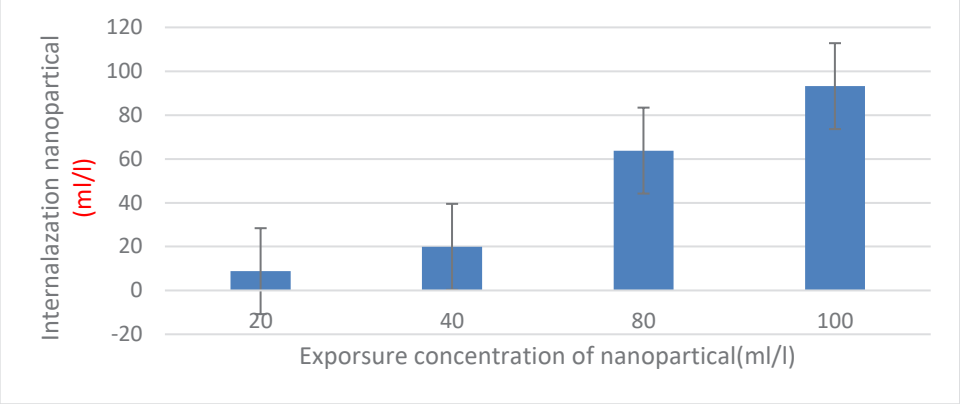

Fig. 11. Internalization of ZnO nanoparticlesl (µ/ml) into the root tips

Table 6. Genotoxicity parameters with the Standard Error (S.E) for different exposure concentrations of ZnO nanoparticles in *A. cepa* root, % of aberrant cells

Con. µg/ml	Stickiness± S.E	Bridge± S.E	c-mitosis ± S.E	Fragment± S.E	Laggards± S.E	Micronucleus ± S.E	Binucleated± S.E	Disturbed chromosom± S.E	Total Aberrations ± S.E	% of aberrant cells± S.E
0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
20	6±0.57	0±0	0±0	3±0.57	20±0	3±0.57	1±0.23	2±0.57	17±1.15	3.6±0.46
40	7±0.57	2±0.57	1±0.11	3±0.57	40±0	3±0.57	1±0.17	5±0.57	22±1.15	4.9±0.57
80	8±0.57	1±0.05	2±0.57	2±0.57	80±0	4±0.57	4±1.15	5±1.15	28±1.73	6.8±0.57
100	9±1.15	1±0.11	1±0.17	4±0.57	100±0	5±0.57	4±0.57	6±0.57	31±1.73	8.3±0.63

concentration of 100 to be 8.3%, while the lowest values were 3.6% in the lowest concentration 20 (Table 6).The reason for high abnormal cells at high concentration is due to that ZnO nanoparticles it has toxic properties as it increases with increasing concentration and small size of nanoparticles [36],thus the smaller particles induce cytotoxicity which leads to DNA damage through the oxidative action of nanoparticles by formation of reactive oxygen species (ROS)as well as deoxyribonucleic acid (DNA) degradation either by breaking strands or the removal of nuclides or a modification of the bases in the nuclides [37], the current study agreed with many studies that indicated damage to the DNA of the root of *A. cepa* upon exposure to various nanoparticles, which leads to deformation of plant cells [31,24].

Bio-adsorption of zinc nanoparticles

Analyzes showed a dose-dependent increased internalized of ZnO NPs nanoparticles into the root cells of *Allium cepa*. we noted that (8.8, 19.9, 63.8, 93.2) µ/ml of ZnO NPs were internalized to the roots upon exposure to20, 40, 80, 100 mg/ml concentrations respectively (Fig. 11). The involvement of soluble Zn^{2+} ions was ruled out due to the insoluble nature of the ZnO NPs which was substantiated by ionic analysis of the filtrate obtained after complete removal of suspended ZnO NPs. thus, the internalization of zince was confirmed to be in particulate form and not in ionic state, the uptake of metal ions by onion roots is one of the main causes of DNA damage leading to cell death due to elevated intracellular ROS levels. [38], this is in agreement with the study [13] when studying zinc nanoparticles and their harmful effect on DNA. and the study of [30] genotoxicity of silver nanoparticles in *Vicia faba*.

CONCLUSION

The use of ZnoNPs resulted in a significant inhibition in the average root length of *A. cepa*, Zno NPs can penetrate the plant system and possibly interfere with the intracellular components, causing cell damage and thus affecting the growth and cell division processes of the tested plant by affecting the elongation of *A. cepa* roots when treated with zinc oxide nanoparticles compared to the control. Our results show that ZnONPs are genotoxic in plant cells and exposure of *A. cepa* roots to ZnONPs causes cytotoxicity and genotoxicity due to its heavy accumulation in both cellular and chromosomal units, which indicates its dangerous phytotoxic nature at high concentrations, The decrease in MI, increase in chromosomal aberration, and Minh were observed to be dose dependent. There was an increase in chromosomal aberrations when increasing the concentration of zinc nanoparticles, and this effect was clear on chromosomal behavior, especially in metaphase and anaphase as compared to prophase and telophase, The most frequent abnormalities were, chromosomal, stickiness, fragment, micronucleus, binucleate, disturbed chromosome. Internalization of ZnO nanoparticles have been found to have detrimental effect on A. cepa leading to DNA damage.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

REFERENCES

1. Reza Mozafari M, Johnson C, Hatziantoniou S, Demetzos C. Nanoliposomes and Their Applications in Food Nanotechnology. J Liposome Res. 2008;18(4):309-327.
2. Saxena P, Harish. Nanoecotoxicological Reports of Engineered Metal Oxide Nanoparticles on Algae. Current Pollution Reports. 2018;4(2):128-142.
3. Rivero PJ, Urrutia A, Goicoechea J, Arregui FJ. Nanomaterials for Functional Textiles and Fibers. Nanoscale research letters. 2015;10(1):501-501.
4. Morales-Díaz AB, Ortega-Ortíz H, Juárez-Maldonado A, Cadenas-Pliego G, González-Morales S, Benavides-Mendoza A. Application of nanoelements in plant nutrition and its impact in ecosystems. Advances in Natural Sciences: Nanoscience and Nanotechnology. 2017;8(1):013001.
5. Peng Y-H, Tsai Y-C, Hsiung C-E, Lin Y-H, Shih Y-h. Influence of water chemistry on the environmental behaviors of commercial ZnO nanoparticles in various water and wastewater samples. J Hazard Mater. 2017;322:348-356.
6. Eixenberger JE, Anders CB, Hermann RJ, Brown RJ,

Reddy KM, Punnoose A, et al. Rapid Dissolution of ZnO Nanoparticles Induced by Biological Buffers Significantly Impacts Cytotoxicity. Chem Res Toxicol. 2017;30(8):1641-1651.
7. Ghodake G, Seo YD, Lee DS. Hazardous phytotoxic nature of cobalt and zinc oxide nanoparticles assessed using Allium cepa. J Hazard Mater. 2011;186(1):952-955.
8. Monica RC, Cremonini R. Nanoparticles and higher plants. Caryologia. 2009;62(2):161-165.
9. Ma X, Geisler-Lee J, Deng Y, Kolmakov A. Corrigendum to "Interactions between engineered nanoparticles (ENPs) and plants: Phytotoxicity, uptake and accumulation" [Sci. Total Environ. 408 (2010) 3053-3061]. Sci Total Environ. 2014;481:635.
10. Alhammad BA, Abdel-Aziz HMM, Seleiman MF, Tourky SMN. How Can Biological and Chemical Silver Nanoparticles Positively Impact Physio-Chemical and Chloroplast Ultrastructural Characteristics of Vicia faba Seedlings? Plants (Basel, Switzerland). 2023;12(13):2509.
11. Nair PMG, Chung IM. Impact of copper oxide nanoparticles exposure on Arabidopsis thaliana growth, root system development, root lignificaion, and molecular level changes. Environmental Science and Pollution Research. 2014;21(22):12709-12722.
12. Tulasi SL, Swamy AVVS, Pavani P, Subhashini V. Green synthesis of zinc oxide nanoparticles using schrebera swietenioides roxb., aqueous leaf extract and investigation of its effect on seed germination and plant growth on pigeon pea (cajanus cajan linn.). International Journal of Applied Pharmaceutics. 2022:193-199.
13. Kumari M, Khan SS, Pakrashi S, Mukherjee A, Chandrasekaran N. Cytogenetic and genotoxic effects of zinc oxide nanoparticles on root cells of Allium cepa. J Hazard Mater. 2011;190(1-3):613-621.
14. Pakrashi S, Jain N, Dalai S, Jayakumar J, Chandrasekaran PT, Raichur AM, et al. In vivo genotoxicity assessment of titanium dioxide nanoparticles by Allium cepa root tip assay at high exposure concentrations. PLoS One. 2014;9(2):e87789-e87789.
15. Shaymurat T, Gu J, Xu C, Yang Z, Zhao Q, Liu Y, et al. Phytotoxic and genotoxic effects of ZnO nanoparticles on garlic (Allium sativum L.): A morphological study. Nanotoxicology. 2011;6(3):241-248.
16. Deniz İG, Genç İ, Sarı D. Morphological and molecular data reveal a new species of Allium (Amaryllidaceae) from SW Anatolia, Turkey. Phytotaxa. 2015;212(4):283.
17. Gosavi VC, Daspute AA, Patil A, Gangurde A, Wagh SG, Sherkhane A, et al. Synthesis of green nanobiofertilizer using silver nanoparticles of Allium cepa extract Short title: Green nanofertilizer from Allium cepa. International Journal of Chemical Studies. 2020;8(4):1690-1694.
18. Küçük D, Liman R. Cytogenetic and genotoxic effects of 2-chlorophenol on Allium cepa L. root meristem cells. Environmental Science and Pollution Research. 2018;25(36):36117-36123.
19. Lee CW, Mahendra S, Zodrow K, Li D, Tsai YC, Braam J, et al. Developmental phytotoxicity of metal oxide nanoparticles to Arabidopsis thaliana. Environmental Toxicology and Chemistry. 2009;29(3):669-675.
20. Kumari M, Mukherjee A, Chandrasekaran N. Genotoxicity of silver nanoparticles in Allium cepa. Sci Total Environ. 2009;407(19):5243-5246.
21. A. Alwathnani H. Antibacterial Activity of Aqueous Extracts of Artemisia species against some Pathogenic Bacteria. Biosciences, Biotechnology Research Asia. 2017;14(2):621-624.
22. Fiskesjö G. Allium test for screening chemicals; evaluation of cytological parameters. Plants for Environmental Studies: CRC Press; 1997. p. 307-333.
23. Bakare AA, Adeyemi AO, Adeyemi A, Alabi OA, Osibanjo O. Cytogenotoxic effects of electronic waste leachate in Allium cepa. Caryologia. 2012;65(2):94-100.
24. Singh Z, Singh I. CTAB Surfactant Assisted and High pH Nano-Formulations of CuO Nanoparticles Pose Greater Cytotoxic and Genotoxic Effects. Sci Rep. 2019;9(1):5880-5880.
25. Djearamane S, Lim YM, Wong LS, Lee PF. Cellular accumulation and cytotoxic effects of zinc oxide nanoparticles in microalga Haematococcus pluvialis. PeerJ. 2019;7:e7582-e7582.
26. Ramesh M, Anbuvannan M, Viruthagiri G. Green synthesis of ZnO nanoparticles using Solanum nigrum leaf extract and their antibacterial activity. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2015;136:864-870.
27. Stampoulis D, Sinha SK, White JC. Assay-Dependent Phytotoxicity of Nanoparticles to Plants. Environmental Science & Technology. 2009;43(24):9473-9479.
28. Cortez Caillahua M, Cuadro Oria SM. Green Synthesis of Zinc Oxide Nanoparticles using extract of onion (Allium cepa) peel [Síntesis verde de nanopartículas de óxido de zinc utilizando extracto de cáscara de cebolla (Allium cepa)]. Journal of Nanotechnology. 2024;6(1):1-6.
29. Manesh RR, Grassi G, Bergami E, Marques-Santos LF, Faleri C, Liberatori G, et al. Co-exposure to titanium dioxide nanoparticles does not affect cadmium toxicity in radish seeds (Raphanus sativus). Ecotoxicology and Environmental Safety. 2018;148:359-366.
30. Patlolla AK, Berry A, May L, Tchounwou PB. Genotoxicity of silver nanoparticles in Vicia faba: a pilot study on the environmental monitoring of nanoparticles. International journal of environmental research and public health. 2012;9(5):1649-1662.
31. Becaro AA, Siqueira MC, Puti FC, de Moura MR, Correa DS, Marconcini JM, et al. Cytotoxic and genotoxic effects of silver nanoparticle/carboxymethyl cellulose on Allium cepa. Environmental Monitoring and Assessment. 2017;189(7).
32. Nagaonkar D, Shende S, Rai M. Biosynthesis of copper nanoparticles and its effect on actively dividing cells of mitosis in Allium cepa. Biotechnology Progress. 2015;31(2):557-565.
33. Verma A, Stellacci F. Effect of Surface Properties on Nanoparticle–Cell Interactions. Small. 2009;6(1):12-21.
34. Liu D, Jiang W, Li M. Effects of trivalent and hexavalent chromium on root growth and cell division of Allium cepa. Hereditas. 2008;117(1):23-29.
35. Türkoğlu Ş. Genotoxicity of five food preservatives tested on root tips of Allium cepa L. Mutation Research/Genetic Toxicology and Environmental Mutagenesis. 2007;626(1-2):4-14.
36. Xia Q, Li H, Liu Y, Zhang S, Feng Q, Xiao K. The effect of particle size on the genotoxicity of gold nanoparticles. Journal of Biomedical Materials Research Part A. 2016;105(3):710-719.
37. Cooke MS, Evans MD, Dizdaroglu M, Lunec J. Oxidative DNA damage: mechanisms, mutation, and disease. The FASEB Journal. 2003;17(10):1195-1214.
38. Xin F, Peng-Yu Q, Ke-Feng L, Chen-Yu S, Hui-Ping R, Xue-Hui S, et al. Author response for "A cleaved adhesin DNA vaccine targeting dendritic cell against Porphyromonas gingivalis –induced periodontal disease". Wiley; 2024.