Vol. 18(29), pp. 783-796, September, 2019
DOI: 10.5897/AJB2019.16918
Article Number: D39771B61816
ISSN: 1684-5315

http://www.academicjournals.org/AJB

ACADEMIC JOURNALS
expand your knowledge

African Journal of Biotechnology

Full Length Research Paper

Cytogenetic and molecular assessment of some nanoparticles using *Allium sativum* assay

Mona S. Al-Ahmadi

Department of Biology, College of Science, University of Imam Abdulrahman Bin Faisal, P. O. Box 1982, Dammam 31441, Kingdom of Saudi Arabia.

Received 15 July, 2019; Accepted 26 August, 2019

One of the primary objectives in agriculture is providing high-quality crops to consumers. Multiple techniques and methods are utilized to achieve this objective, including nanotechnology that depends on the use of very small materials, which will help in decreasing the amounts usually used with similar effects. Nanomaterials are used as fertilizers and also as component of nano-pesticides for plants. Despite their benefits, however, studies have noted their potential for cytotoxicity and genotoxicity. In this study, five nanoparticles (NPs) were tested to assess their effects on plants. The chromosomal aberration assay was used. The results showed that some NPs decreased the mitotic index (MI) significantly, which indicates the NPs' potential cytotoxicity. In addition, different NPs' treatments caused different types of chromosomal abnormalities e.g., chromosomes stickiness and disturbance of the metaphase and anaphase, lagging chromosomes, bridges, disturbed poles, micronuclei, s-metaphase, s-telophase, c- metaphase and bi-nucleus cells. All treatments had significant effects at p≤005. Treatments with NPs concentrations for 24 h affected the DNA content, AlO_2 and Fe_3O_4 NPs' increased the DNA content, while CeO_2, TiO_2 and Ag NPs' decreased it. High concentrations of the tested NPs decreased the DNA content. The study results showed that CeO_2 was the most harmful NP compared to the control and other NPs. Some types of chromosome abnormalities such as lagging chromosomes, bridge, and micronuclei indicate potential genotoxicity for these NPs. Despite of the positive effects, they also had negative side effects such as decreasing the MI and increasing the occurrence of different types of chromosomal abnormalities.

Key words: Cytotoxicity, genotoxicity, nanoparticles, mitotic chromosomes abnormalities.

INTRODUCTION

Nanotechnology has been used in many fields. In agriculture, one of these technologies involves the use of different elements in nano sizes, which can give satisfactory results using a low amount of the element compared with its natural size. Nanomaterials are used in various applications such as plants protection, nutrition and of farm practices management due to their small size, high surface-to-volume ratio, and unique optical properties (Ghormade et al., 2011).

Nanoparticles (NPs) interact with plants, causing many

E-mail: nsadeghi@sina.tums.ac.ir. Tel: +98-21-66954713. Fax: +098-21-66461178.

Table 1. Nanoparticles Concentrations and their description.

Nanoparticles	Description	Concentration %		Reference
		Low	High	
AlO_2 NPs	Form nano powder particle size <50 nm (TEM); surface area >40 m^2/g (BET); mp 2040°C (lit.)	20 mg	40 mg	Lee et al. (2010)
Fe_3O_4 NPs	Form nano powder particle size 50-100 nm (SEM); surface area >60 m^2/g; mp 1538°C (lit.)	0.025 g	5.9 g	Sheykhbaglou et al. (2010)
CeO_2 NPs	Form nano powder particle size <100 nm; mp >400°C	0.012 g	0.024 g	Ma et al. (2013)
TiO_2 NPs	Form, nano powder primary particle size 21 nm (TEM); surface area 35-65 m^2/g (BET); mp 1850°C >350°C (lit.)	10 mg	20 mg	Song et al. (2012)
Ag NPs	Form nanoparticles contains sodium citrate as stabilizer concentration 0.02 mg/ml in aqueous buffer particle size 10 nm (TEM); density 0.997 g/mL at 25°C	0.0005 mg	0.001 mg	Salama (2012)

morphological and physiological changes depending on their properties (Khodakovskaya et al., 2012). Chen and von Mikecz (2005) demonstrated that some NPs can enter cell nuclei and may directly affect the structure and function of the DNA genome. The efficacy of NPs depends on their concentrations, and these concentrations differ from plant to plant (Siddiqui et al., 2015). Also, NPs can have positive and negative impacts in higher plants (edible plants) and on their consumers in the food chain (Rico et al., 2011).

The minute size of NPs, smaller than cells and cellular organelles, allows them to penetrate those basic biological structures, disrupting their normal function (Buzea et al., 2007). Zheng et al., (2005) concluded that the up-take efficiency and effects of NPs on growth and metabolic function vary among plants. The concentrations of NPs affect processes like germination and plant growth. Babu et al., (2008) also suggested that the NPs' size gives them free entry inside cells, where they can interfere in normal cell function. Landsiedel et al., (2009), Kovacic and Somanathan, 2010 and Siddiqui et al., (2015) suggested that the ability of NPs to penetrate cells easily allows them to affect the intercellular organelles and nucleic acids. NPs characteristics such as their small size, their shape and their large surface-area-to-mass ratio, and their propensity to cross cell barriers and their interaction with intercellular contribute to potential cellular and genetic toxicity caused by the induction of oxidative stress. Hunt et al., (2013) assessed the effects of nano silver on *Caenorhabditis elegans* by measuring the 8-OH guanine levels and found that the silver induced oxidative damage in DNA. A similar result was found by Çekiç et al., (2017) in tomato plants. Cobalt oxide NPs were investigated by Faisal et al., (2016) to assess their effect on eggplant DNA. The results indicated that cobalt oxide NPs induced DNA strand breaks and apoptosis. Also, NPs cause chromosomal aberrations as several researchers have discussed in their study of these effects in higher plants (Kumari et al., 2009; Ghosh et al., 2010; Landa et al., 2012; Mukherjee et al., 2016; Debnath et al., 2018).

Higher plants are recognized as being excellent indicators of the cytogenetic and mutagenic effects of environmental chemicals. The study of these plants is also useful for detecting environmental mutagens indoors and outdoors. These plants are highly reliable bioassays for monitoring and testing for genotoxins because of their high sensitivity (Grant, 1999).

In this study, five NPs were tested to estimate their cytotoxicity and genotoxicity using a chromosomal aberration assay and to determine their effect on the DNA content of *Allium sativum*.

MATERIALS AND METHODS

Tested materials

Table 1 shows NPs and different concentrations chosen depending on previous studies that found out that treatment with these concentrations had a positive effect on root length, yield and quality, biomass, and plant growth without serious harm on plants.

Sample preparations

A. sativum, common name (garlic) 2n = 16, gained from local markets were used as testing material. The loose outer scales and

old roots were scraped and suspended in small beaker with distilled water.

Treatments

A. sativum were suspended in a small beaker (50 ml) with distilled water to encourage the root tips to grow until they reached 0.5 to 1 cm in length; they were then transferred to another beaker containing freshly prepared solutions of tested NPs, Aluminium oxide, Ferric oxide, Cerium oxide, Titanium oxide and Silver. Low concentrations (20 mg, 0.025 g, 0.012 g, 10 mg, 0.0005 mg) and high concentrations (40 mg, 5.9 g, 0.024 g, 20 mg, 0.001 mg) sequentially, and left for different periods of time (6, 16, and 24 h). One bulb of garlic was used for each treatment. The negative control was root tips treated with distilled water only, used as a qualified sample to compare for the effects of tested materials.

Slides preparation

The treated roots tips and negative control (untreated) were detached, fixed in freshly prepared 3:1 (v/v) ethanol alcohol: glacial acetic acid for 24 h. The root tips of *A. sativum* were hydrolyzed in 1N HCL at 60°C for 8 min. The root tips were then washed with distilled water several times and stained with 1% acetocarmin. Five temporary slides were prepared using the squash technique. Two root tips on each slide were examined for the effects of NPs on the mitotic index (MI). The same slides were analyzed for the types and frequencies of chromosomal abnormalities produced by the examined NPs.

DNA studies

Fisher bioreagents Sure-prep RNA/DNA/Protein purification kit was used to extract the genetic material from plant tissues following the instruction of HiPuraTM product. The plant DNA isolation was done using the CTAB method. The concentration of the isolated DNA was measured by Scan drop (Analytik Jena) device.

Scoring of slides and data analysis

Studying slides

The slides were viewed under light microscope (Phenix P H 50 DB047VU) using the 40X objective lens immersion. The demonstrative slides for each physical aberration were photo-graphed using Phenix micro Image analyzer Software 2008 EnV2, 2.

Mitotic index

On one slide for each treatment, a total of 2000 cells, were scored. The mitotic index (MI) was expressed as the number of dividing cells per total cells scored, as per the following equation:

Mitotic index (MI) = (Total number of dividing cell/Total number of cell examined) × 100

Cytotoxicity

The mitotic index of the treated cells was compared with that of the negative control sample.

Genotoxicity test

Chromosomal aberration per dose of each NP was examined; the percentage of cells with aberrations of each dose for each NP was scored and compared with that of the negative control as per the following equation:

Chromosomal aberration frequency (CF) = Total number of abnormal cell/Total number of normal cell

Statistical analysis

A two-way analysis of variance was used for determining the significance of difference at $p \leq 0.05$ (SPSS 16.0 for Windows statistical package).

RESULTS

Mitotic index (MI)

The NPs effects on the MI of A. sativum root tip cells

Table 2 and Figures 1 and 2 show the treatment results; it appears that the AlO_2 NPs 20 and 40 mg concentrations decreased the MI after treatment for 24 h. This treatment was insignificant at $p \geq 0.05$, while treatment with a low concentration of Fe_3O_4 for 24 h was significant at $p \leq 0.05$. Also, CeO_2 NPs decreased the MI after treatment with a low concentration for 6 h and high concentration for 24 h. This result was insignificant at $p \geq 0.05$. The TiO_2 NPs treatment with a low concentration and a high concentration for 24 h decreased the MI. This result was significant at $p \leq 0.05$. Treatment with a high concentration of Ag NPs for 16 h decreased the MI. This result was significant at $P \leq 0.05$. Some treatments with a low concentration of NPs increased the MI. The AlO_2 low concentration for 16 h, high and low concentrations of Fe_3O_4, also high and low concentrations of CeO_2 for 16 h, and a low concentration of Ag for 6 h increased the MI compared to the control. These results were insignificant $p \geq 0.05$.

Chromosomal aberrations (CA)

Examining the cytological aberrations in plants is an excellent way to detect genetic hazards that environmental substances may pose (Grant, 1978).

Table 3a-b and figures 3, 4, and 5 show the types of abnormalities found in the mitotic chromosomes of *A. sativum* root tip cells after treatment with different concentrations of NPs.

All tested materials affected the chromosomes and increased chromosomal aberrations compared to the control, and the results were significant at $p \leq 0.05$. The most harmful concentrations were AlO_2 NPs after treatment with a high concentration of 40 mg for 6 and 16 h (0.2) compared to the control (0.04), Fe_3O_4 NPs after

Table 2. Effects of different concentration of some nano-particles for different periods of time on mitotic index and chromosomal aberrations frequency.

Material	Concentration (%)	Time of duration (h)	No. of total cells	Mutant cells	Mitotic index	CA
Distilled Water	Distilled Water	6	2139	6	8	0.04
	Distilled Water	16	2054	5	7	36
	Distilled Water	24	2037	3	9	2
ALO_2 NPs	20 mg	6	2182	14	8	0.1
	20 mg	16	2126	18	8	0.12
	20 mg	24	2067	21	8.8	0.12
	40 mg	6	2305	30	8	0.2
	40 mg	16	2160	22	7	0.2
	40 mg	24	2201	17	7	0.1
Fe_3O_4 NPs	0.025g	6	2254	16	6	0.1
	0.025g	16	2178	21	7	0.1
	0.025g	24	2060	5	5	0.1
	0.05g	6	2122	27	7	0.2
	0.05g	16	2135	14	8	0.1
	0.05g	24	2125	18	6	0.1
CeO_2 NPs	0.012 g	6	2021	16	7	0.1
	0.012 g	16	2144	13	8	0.1
	0.012 g	24	2054	14	8	0.1
	0.024 g	6	2022	17	9	0.1
	0.024 g	16	2087	19	8	0.1
	0.024 g	24	2068	16	7	0.1
TiO_2 NPs	10 mg	6	2189	14	7	0.1
	10 mg	16	2317	17	7	0.1
	10 mg	24	2191	17	6	0.1
	20 mg	6	2120	11	7	0.1
	20 mg	16	2214	13	7	0.1
	20 mg	24	2262	13	7	0.1
Ag NPs	0.0005 mg	6	2019	18	10	0.1
	0.0005 mg	16	2089	17	7	0.1
	0.0005 mg	24	2076	16	8	0.1
	0.001 mg	6	2237	12	7	0.1
	0.001 mg	16	2054	5	5	0.1
	0.001 mg	24	2244	32	8	0.2

treatment with a high concentration of 0.05 g for 6 h (0.2) and Ag NPs (0.2) after treatment with a high concentration 0.001 mg for 24 h compared to the control (0.02).

The types of chromosomal abnormalities scored after treatment with different NPs were chromosomal disturbance and stickiness during metaphase and anaphase, sticky telophase, chromosomes bridges during the anaphase and telophase, micronuclei, lagging chromosomes, star metaphase and star telophase, bi-nucleus cells, and disturbed poles during anaphase. Specific types of chromosome aberrations were scored after treatment with some NPs and they were C-metaphase, lagging chromosome in the telophase stage micronuclei, bi-nucleus cells, after treatment with AlO_2 and Fe_3O_4 NPs, furthermore AlO_2 NPs caused the formation of abnormal anaphase poles. Treatment with CeO_2 NPs caused the formation of Star-metaphase, ring chromosome, C-metaphase and telophase bridge. The types of the chromosomal abnormalities scored after treatment with TiO_2 NPs were S-metaphase, lagging chromosome during anaphase stage, S-telophase,

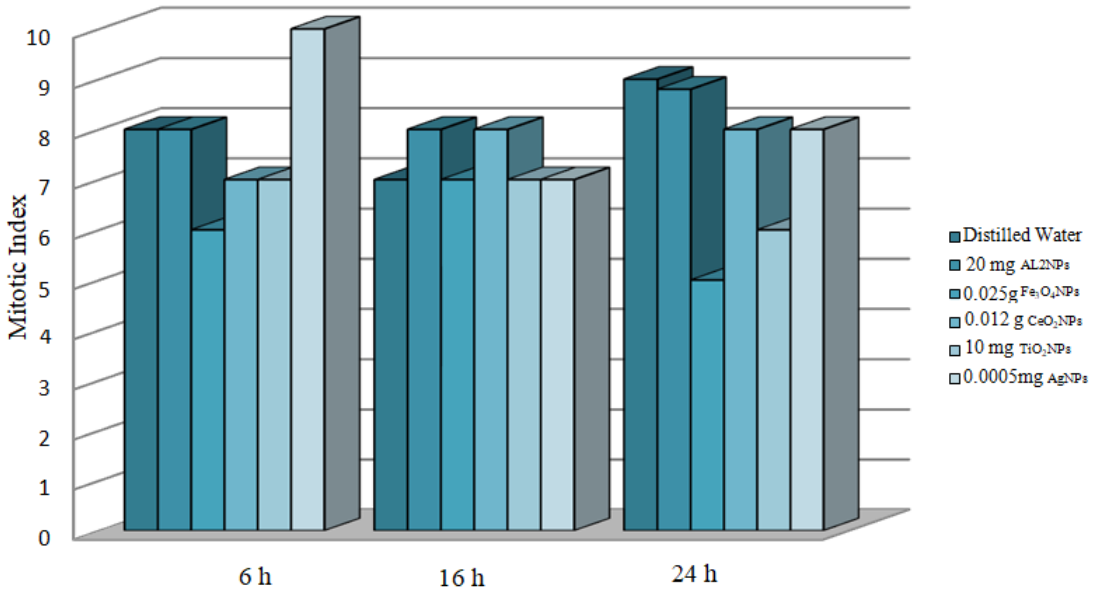

Figure 1. Effects of low concentrations of some nano-particles for different periods of time on mitotic index of *Allium sativum.*

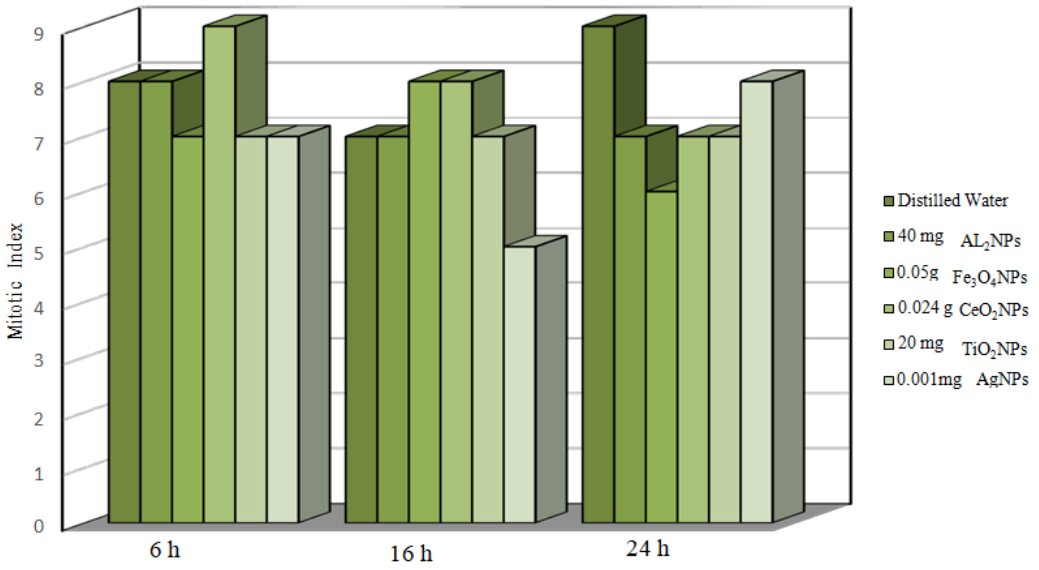

Figure 2. Effects of high concentrations of some nano-particles for different periods of time on mitotic index of *Allium sativum.*

bi-nucleus cells and micronuclei. Ag NPs produced lagging chromosome, S- anaphase, abnormal pole of anaphase stage, chromosomes bridges and bi-nucleus cells. Some types of chromosome abnormalities indicated the potential genotoxicity of tested NPs, e.g., micronuclei, lagging chromosomes, and the chromosome bridges during anaphase and telophase.

DNA content

Table 4, 5 and figures 6 and 7 show the effect of different concentrations of NPs (AlO_2, Fe_3O_4, CeO_2, TiO_2, Ag) on DNA content after 24 h.

All the tested NPs affected the DNA content. Specifically, the content decreased after treatment with

Table 3. Types of chromosomal aberrations scored after treatment with different concentrations of Nano-particles for different periods of time on root tip cells of *Allium sativum*.

Tested material		Control			ALO_2 NPs						Fe_3O_4 NPs					
					20 mg			40 mg			0.025 mg			0.05 mg		
Type of CA		**6**	**16**	**24**	**6**	**16**	**24**	**6**	**16**	**24**	**6**	**16**	**24**	**6**	**16**	**24**
Sticky	Metaphase	-	0.007	0.006	0.01	0.02	0.02	0.04	0.09	0.04	0.034	0.06	0.009	0.007	0.02	0.01
Disturb	Metaphase	-	0.03	-	-	0.04	0.03	0.05	0.007	0.03	-	-	0.03	0.04	0.03	0.0008
Lagging	Metaphase	-	-	-	-	-	-	-	-	-	-	-	-	0.007	-	-
Fragments	Metaphase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0008
c-metaphase	Metaphase	-	-	-	-	-	-	-	-	0.006	0.007	-	-	-	-	-
Sticky	Anaphase	-	-	-	-	0.006	-	-	0.007	0.006	-	0.013	-	-	-	-
Disturb	Anaphase	-	-	-	0.01	0.006	-	-	-	-	0.014	-	-	-	0.006	0.02
S. anaphase	Anaphase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lagging	Anaphase	-	-	-	-	-	-	-	-	-	-	-	-	0.007	-	-
Bridge	Anaphase	0.04	-	0.01	0.045	0.01	0.04	0.05	0.05	0.02	0.014	0.04	0.009	0.06	0.002	0.05
Sticky disturb	Telophase	-	-	-	-	-	-	-	-	-	0.02	0.013	-	-	-	-
Lagging bridge	Telophase	-	-	-	-	-	0.005	-	-	-	-	0.006	-	-	0.006	0.0008
Dis polar	Telophase	-	-	-	-	0.006	-	-	-	-	-	-	-	-	-	-
Fragment	Telophase	-	-	-	-	-	0.005	-	-	-	-	-	-	-	-	-
Bi-nucleate		-	-	-	0.01	0.006	-	0.017	-	-	0.007	-	-	-	-	-
Micronuclei		-	-	-	-	-	-	0.005	-	0.006	0.014	0.006	-	-	0.006	0.02
%		0.04	0.036	0.02	0.08	0.12	0.12	0.2	0.15	0.11	0.1	0.1	0.05	0.18	0.08	0.1

low concentrations of Ag, TiO_2 and CeO_2 NPs (50.65, 55.32 and 97.63 ng/µl, respectively), and the results were significant at $P \leq 0.05$. AlO_2 NPs and Fe_3O_4 NPs increased the DNA concentration (391.34 and 234.07 ng/µl, respectively) and these results were significant at $P \leq 0.05$ compared to the control (144.73 ng/µl). Treatments with high concentrations affected the DNA content. The NPs Fe_3O_4, Ag and CeO_2 (130.37l, 124.65, 119.33 ng/µl, respectively) decreased the DNA concentration, and these results were significant at $p \leq 0.05$; the results showed that CeO_2 NPs were the most harmful and that TiO_2 NPs were the least harmful followed by AlO_2 compared to the control (144.73 ng/µl).

DISCUSSION

Mitotic index (MI)

The NPs treatments reduced the MI. The decrease of MI might have resulted from the effect of the NPs during S-phase which inhibited the DNA synthesis. The decrease might also be due to the activation of enzymes by decreasing or inhibiting the enzymes, particularly the enzymes that involved in DNA replication or cell division (Sudhakar et al., 2001).

AlO_2 NPs caused decreased MI. This effect may be due to the blockage at GI stage, which disturbs the DNA synthesis (Mohandas and Grant, 1972). A similar result was found by Rajeshwari et al., (2015).

The effect of Fe_3O_4 NPs on cells was as reported by Alarifi et al., (2014), that is, the cell

Table 4. Types of chromosomal aberrations scored after treatment with different concentrations of Nano- particles for different periods of time on root tip cells of *Allium sativum*

Tested material		Control			ALO_2 NPs						Fe_3O_4 NPs						Ag NPs					
					20 mg			40 mg			0.025 mg			0.05 mg			0.0005 mg-l			0.001 mgl-1		
Type of CA		6	16	24	6	16	24	6	16	24	6	16	24	6	16	24	6	16	24	6	16	24
Sticky		-	0.007	0.006	0.03	0.01	0.05	-	0.02	0.04	0.03	0.03	0.04			0.01	0.02	0.03	0.02	0.007	0.009	0.06
Disturb		-	0.03	-	0.03	0.006	-	0.02	0.02	0.03	0.007	0.01	0.02	0.04	0.03	0.007	0.005	0.03	0.03	0.03	0.03	0.06
Lagging	Metaphase	-	-	-	-	-	-	0.006	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Fragments		-	-	-	-	0.006	-	-	-	0.006	-	-	-	-	-	-	-	-	-	-	-	-
c-metaphase		-	-	-	-	0.006	-	-	0.006	0.006	-	0.006	-	-	0.006	-	-	-	-	-	-	-
Sticky		-	-	-	-	-	0.01	0.006	0.006	-	-			-	0.007	0.005	-					
Disturb		-	-	-	0.007	0.006	0.006	0.006	-	-	0.007	-	0.007	0.02	0.006	0.01	-	0.01	-	0.03	-	0.01
S. anaphase		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.006	-	-	0.006
Polar	Anaphase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.006	-	-	-
Lagging		-	-	-	-	-	-	0.01	0.006	-	0.007	0.006	0.007	-	-	-	-	-	0.006	-	0.009	-
Bridge		0.04	-	0.01	0.04	0.04	0.03	-	0.06	0.03	0.05	0.03	0.05	0.007	0.03	0.05	0.04	0.05	0.02	0.04	-	0.05
Sticky		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.01	-	-	0.006
bridge		-	-	-	-	-	-	0.006	-	-	-	-	-	-	-	-	-	-		-	-	-
S. anaphase	Telophase	-	-	-	-	-	-	-	-	-	-	0.006	-	-	-	-	-	-		-	-	-
Bi-nucleate		-	-	-	-	-	-	-	-	-	-	0.02	-	-	-	-	-	-	0.01	-	-	-
Micronuclei		-	-	-	-	-	-	-	-	-	0.007	-	-	0.007	-	-	-	-	-	-	-	-
%		0.04	0.036	0.02	0.1	0.07	0.09	0.1	0.1	0.1	0.1	0.1	0.12	0.08	0.08	0.09	0.09	0.11	0.1	0.08	0.05	0.2

death mediated by the reactive oxygen species (ROS) triggered mitochondrial pathway as evidenced by the cleavage of caspase-3 activity and caused an imbalance between the production and degradation of ROS and induced oxidative stress. NPs may change the production of ROS and affect antioxidation defense and so induce oxidative stress (Srinivas et al., 2011). More explanations of iron oxide reaction were reported by Zhongwen et al., (2012), that the cytotoxicity ability of iron oxide, iron oxide trapped in acidic lysosomes of the cell, and they catalyze decomposition of H_2O_2 to produce hydroxyl radicals through peroxidase-similar activity.

The cytotoxicity of CeO_2 may be due to the oxidative stress (Jezek and HIavata, 2005). Park et al., (2008) found that CeO_2 caused cytotoxicity because of the introduction of ROS, and that the free radical species produced by CeO_2 NPs significantly reduce the levels of cellular antioxidants. Also, Sendra et al., (2016) suggested that the toxicity of CeO_2 NPs may be due to their photocatalytic properties. Similar results were demonstrated by Liman et al., (2019).

The TiO_2 NPs decreased the MI compared to the control. Pakrashi et al. (2014) found that TiO_2 NPs increased ROS and that this was the main contribution to the toxic effects. Castiglione et al., (2011) produced similar results in a study of the effect of TiO_2 NPs on *Vicia faba* and *Zea mays*, while Klien and Godnic (2012) in a study of the effect of TiO_2 NPs on rodents.

Ag NPs decreased the MI compared to the control. Patlolla et al., (2012) explained that the decrease in MI after treatment with different concentrations of Ag NPs might be due to a lower

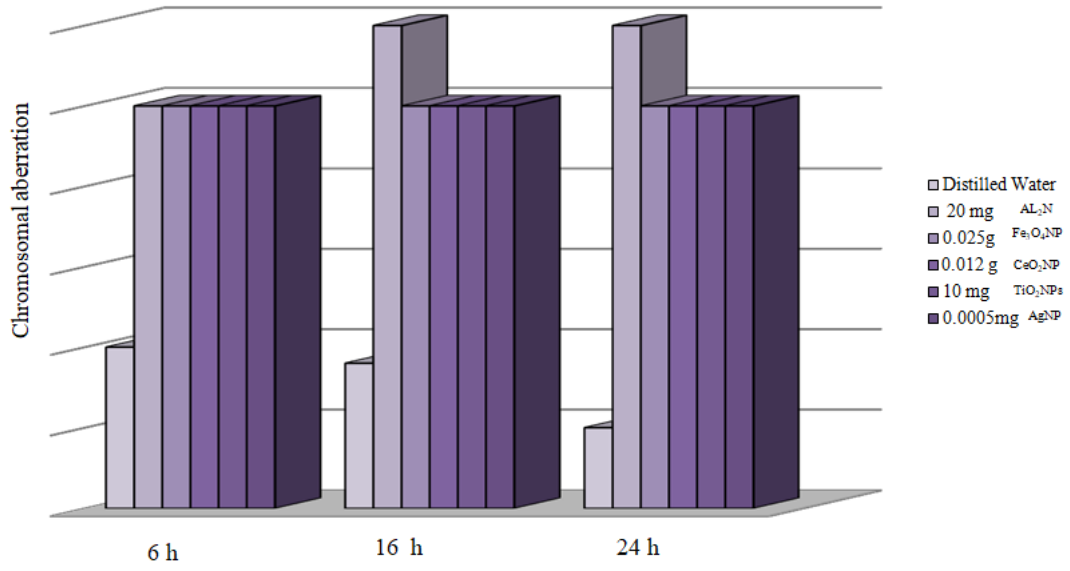

Figure 3. Effects of low concentrations of some nano-particles for different periods of time on chromosomal aberrations of *Allium sativum.*

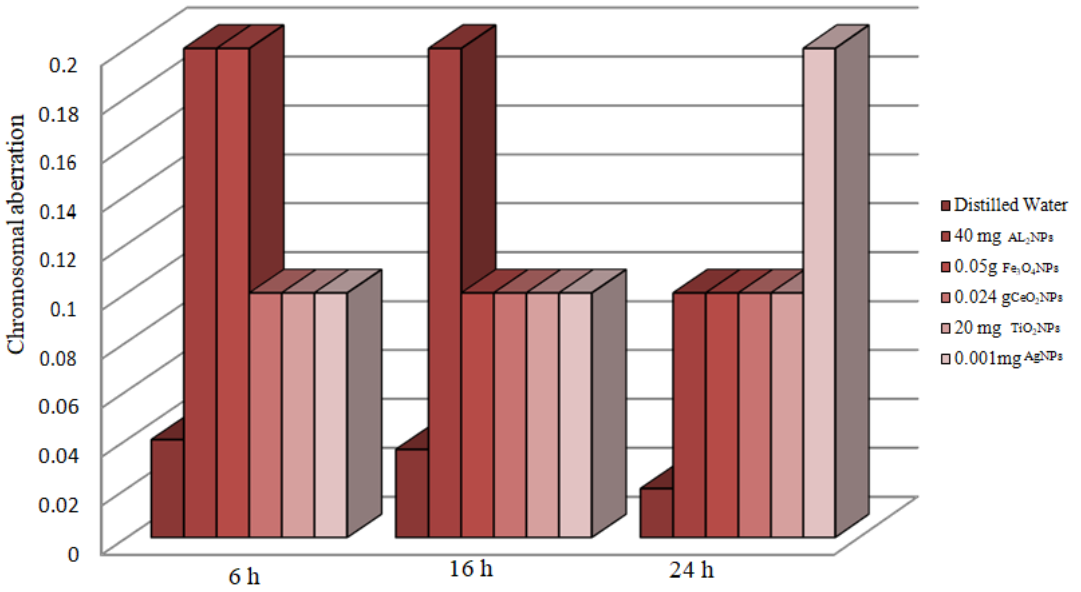

Figure 4. Effects of high concentrations of some nano-particles for different periods of time on chromosomal aberrations of *Allium sativum* root tip cells.

progression of cells from S-phase to M-phase of the cell cycle. Babu et al., (2008) suggested that Ag NPs might affect the DNA synthesis during the S-phase cell cycle, leading to mitodepressive effects and cytotoxicity. These NPs might also cause slower development of cells from the S-phase (DNA synthesis) to the M-phase (mitosis) of the cell cycle as a consequence of silver NPs exposure (Kumari et al., 2009). Similar results were found by Pulate et al. (2011).

Some treatments had no effect on the MI while others increased it. This variance might be due to the intrinsic plant detoxification mechanism of NPs when the plants are exposed to nanotoxicity. Free metal radicals, formed during oxidative stress, function as signaling molecules that later activate the ROS detoxification and antioxidant defense mechanisms in plants to deal

Table 5. Effects of low and high concentrations of different Nano-particles after 24 h of treatment.

Treatment	Control	ALO_2 NPs	Fe_3O_4 NPs	CeO_2 NPs	TiO_2 NPs	Ag NPs
Low CON.	Distilled Water 144.73 ng/µl	20 mg 391.34 ng/µl	0.025 mg 234.07 ng/µl	0.012 mg 97.63 ng/µl	10 mg 55.32 ng/µl	0.0005 mg 50.65 ng/µl
High CON.	Distilled Water 144.73 ng/µl	40 mg 136.2 ng/µl	0.05 g 130.37 ng/µl	0.024 g 119.33 ng/µl	20 mg 141.9 ng/µl	0.001 mg 124.65 ng/µl

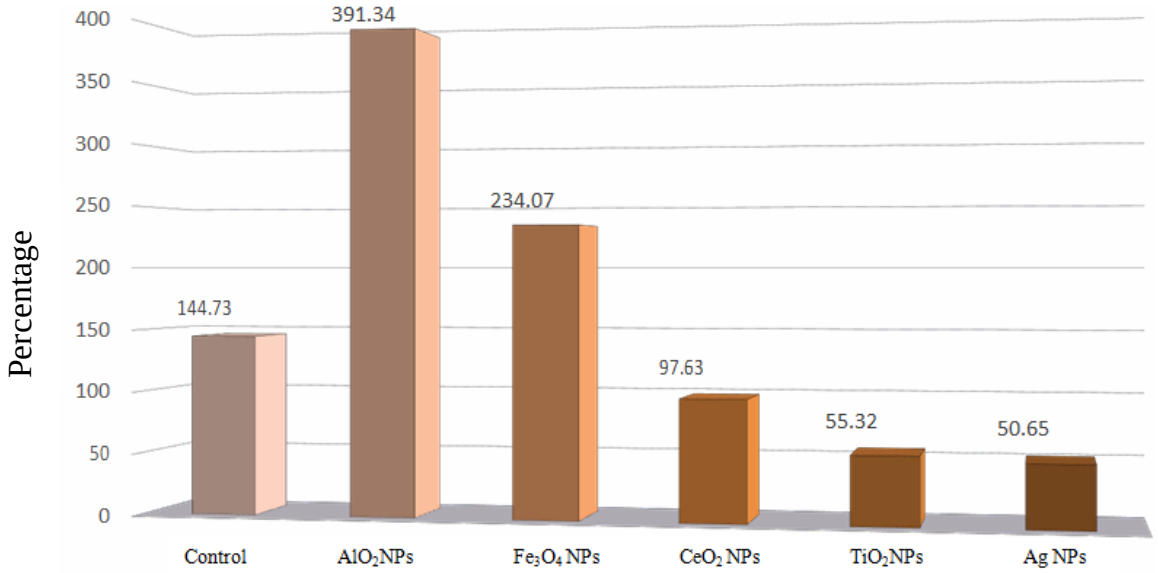

Figure 5. Effects of low concentrations of some nano-particles for 24 h on DNA content of *Allium sativum.*

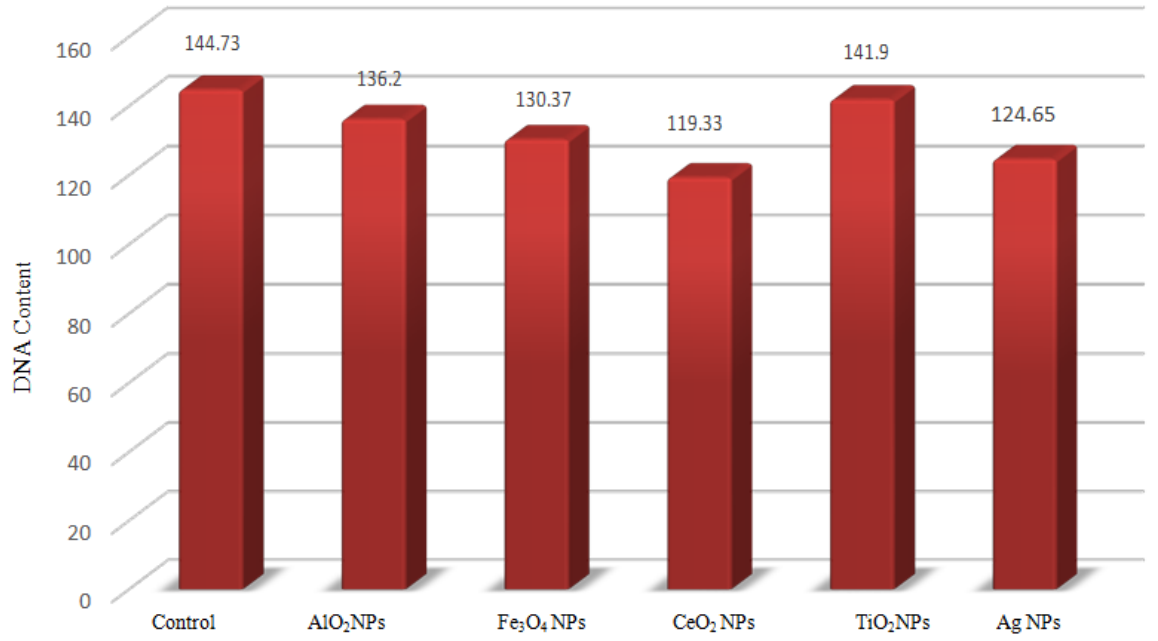

Figure 6. Effects of high concentrations of some nano-particles for 24 h on DNA content of *Allium sativum.*

with NPs toxicity (Zia-ur-Rehman et al., 2018).

Chromosomal aberrations (CA)

Treatments with different concentrations of NPs cause several types of chromosomal aberrations. Rajeshwari et al., (2015) found that AlO_2 NPs decreased the MI and increased the chromosomal aberration in root cells of *Allium cepa* due to the ROS generated by the interaction of AlO_2 NPs and root-tip cells.

The effects of Fe_3O_4 NPs were explained by Rajiv et al., (2015). They found that the metal-oxide NPs caused DNA damage and chromosomal aberrations due to the generation of ROS, which leads to cell death.

CeO_2 NPs also produce chromosomes abnormalities. In this respect, Benameur et al., (2015) demonstrated that chromosomal aberrations are consistent with cellular ROS production. Similar result was found by Liman et al., (2019).

Treatment of *A. sativum* with different concentrations of TiO_2 NPs for different time periods causes different types of chromosomal abnormalities; Ghosh et al., (2010) concluded that treatment with TiO_2 NPs caused chromosomal aberration due to the generation of superoxide radicals that sequentially resulted in lipid peroxidation in the cells. Trouiller et al., (2009) found that TiO_2 NPs are capable of causing oxidative bursts, resulting in DNA damage and the occurrence of micronuclei. Tavares et al. (2014) have the same effect of TiO_2 NPs in human lymphocytes.

Ag NPs also cause chromosomal abnormalities. Kumari et al., (2009) suggested that Ag NPs could penetrate plant system and may impair stages of cell division, causing chromosomal aberrations. Similar results were found by Pulate et al. (2011) and Patlolla et al. (2012).

The presence of disturbance, S-metaphase, S-anaphase, S-telophase, lagging chromosomes, abnormal anaphase poles, and sticky chromosomes of metaphase and telophase revealed that NPs affected spindle fibers. Several studies concluded that NPs cause chromosomal aberration by affecting the spindle fibers. These aberrations alter the direction of chromosomes during different stages of mitotic division. This may be due to the interaction of NPs with mitotic spindle apparatus, centrioles or their associated proteins leading to the loss or gain of chromosomes in daughter cells (Kuriyama and Sakai, 1974; Babu et al., 2008; Magdolenova et al., 2014).

The formation of chromosome stickiness involves the matrix of chromatin material which makes the chromosome stick or clump (Patil and Bhat, 1992). Klasterska et al., (1976) suggested that the stickiness of chromosomes arises due to the effect of NPs on nucleic acids, which causes polymerization and chromosomes stickiness. The formation of chromosomes bridges during anaphase and telophase may be due to chromosomal stickiness (EL-Khodar et al., 1990). Micronuclei being acentric fragments appear because of DNA breaks, especially during cell division, or because of laggards being excluded from the nucleus (Ma, 1982). These micronuclei could be owing to the inhibition of DNA synthesis at the S-phase (Kumari et al., 2009).

Grant (1978) reported that binucleate cells rise as a consequence of the inhibition of cell-plate formation. Huang et al., (2009) reported that due to the disruption of the mitotic checkpoint, PLKI protein function controls the mitosis process, including cytokinesis, when exposed to TiO_2 NPs.

DNA content

Different treatments of NPs affect the DNA content. Kwon et al., (2014) suggest that small NPs cross the cellular membranes more easily and this can increase the potential for DNA damage. Within cells, many NPs end up in the lysosomes but some also appear in the cytoplasm and other cellular organelles, e.g., the Golgi body, the mitochondria, and the nucleus (Yuliang et al., 2010). The molecular mechanisms of NPs mostly depend on their chemical properties. Auffan et al., (2009) concluded that chemically stable metallic NPs have no significant cellular toxicity, while NPs that can be oxidized, reduced, or dissolved are cytotoxic and genotoxic for cellular organisms. Mehrian and Lima (2016) and Brunner et al., (2006) suggested three mechanisms involved in NPs toxicity. The first is the toxic substance from soluble NPs released into exposed media. These substances could contribute to DNA damage by their involvement in ROS generation (Fenton-type reaction) (Kruszewski et al., 2011). The second mechanism is the ROS generated through surface interactions with the media. The third mechanism is the direct physical interaction of NPs with biological targets such as cell membranes or DNA (Brunner et al., 2006). NPs can also interact with the mitochondria and other cell components and disrupt their functions. The ROS that result from the transfer of electrons' energy to oxygen are highly reactive and potentially harmful to living organisms (Wu et al., 2014). Van Breusegern and Dat (2006) reported that ROS as a result of NP interaction will interact with almost all cellular components, producing protein change, lipid peroxidation, and DNA damage.

In this study, the treatment of *A. sativum* with AlO_2 showed that a low concentration increases DNA content and a high concentration decreases it. Sjorgen and Larsen (2017) suggested that Al_2 inhibits the cells' entrance into the S-phase during the cell cycle, which will affect DNA content by decreasing the content frequency. On the other hand, the S-phase cells entered the G2/M phase, leading to an increase of DNA content frequency. Similar results were found by Silva et al., (2000) and Jaskowiak et al., (2018). Wu et al. (2014) demonstrate that the reductive dissolution of iron oxide NPs induced a

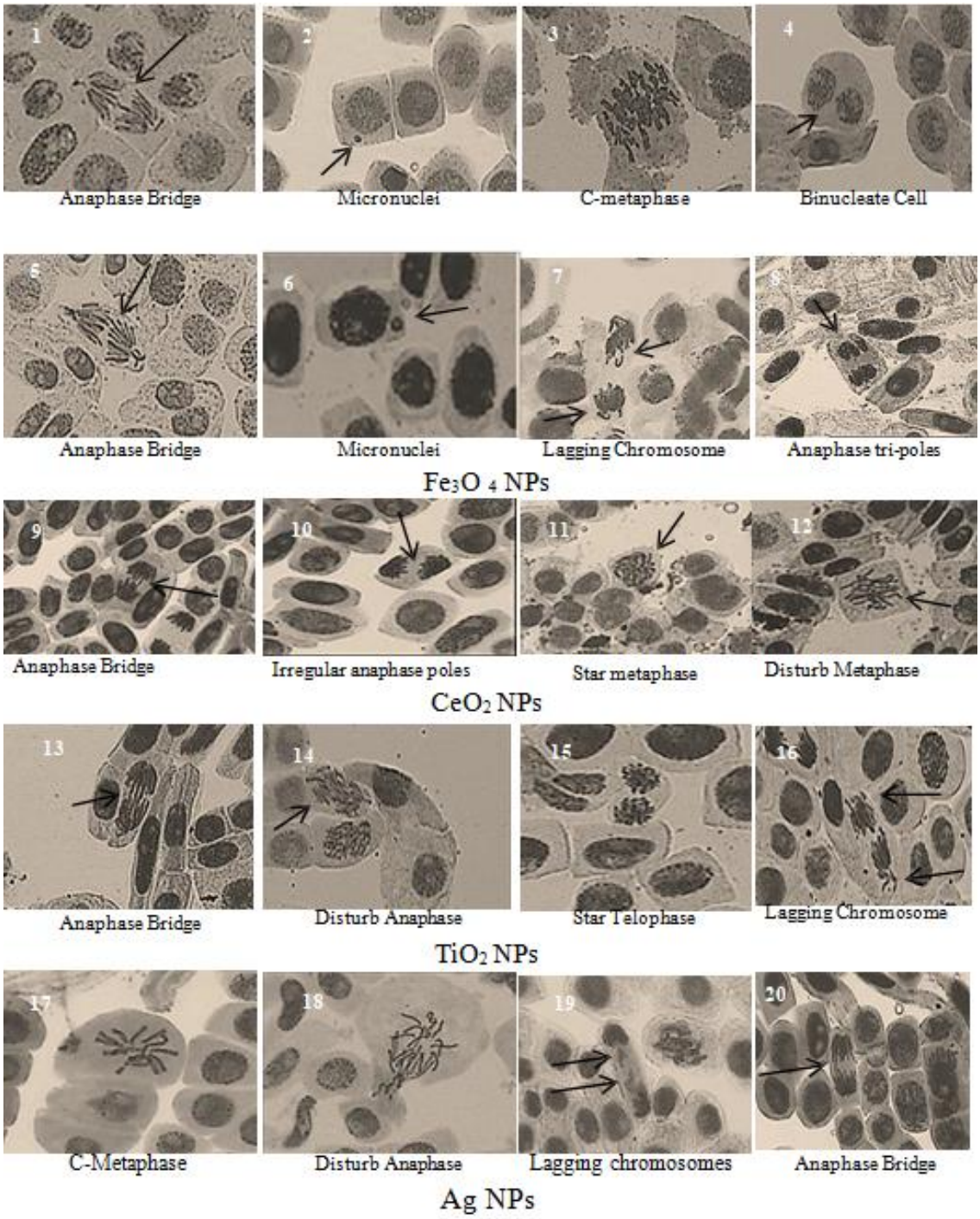

Figure 7. (1-4) Anaphase bridge, micronuclei, C-metaphase, binucleate cell. (5-8) Anaphase bridge, micronuclei, anaphase lagging chromosome, anaphase trip-polar. (9-12) Anaphase bridge, irregular anaphase polar, Star metaphase, disturb metaphase. (13-16) Anaphase bridge, disturb anaphase, Star telophase, anaphase lagging chromosome. (17-20) C-Metaphase, disturb anaphase, lagging chromosomes, anaphase bridge.

more homogeneous Fenton reaction, one that is more efficient in producing ROS. The availability of ROS inside the cell will affect cell components, and one of these components is DNA.

The CeO_2 NPs was the most harmful for DNA compared to other NPs and the control. This effect may be due to the instability of DNA caused by increasing oxidative stress, which leads to DNA damage that occurs due to the high presence of ROS (Mattiello et al., 2015). A high concentration of CeO_2 NPs effected DNA

content compared to the control but less than a low concentration, which has a greater impact on DNA content. This difference may be due to the superoxide dismutase (SOD) mimetic activity related to a high concentration of CeO_2 NPs, which causes the dismutation of superoxide anions into H_2O_2 (Mattiello et al., 2015). Vranová et al., (2002) suggested that the oxidative burst induced by the more harmful dose of CeO_2 NPs may be associated with the stimulation of cellular respiration that increases the signal requirement for energy. Mattiello et al., (2015) found that CeO_2 NPs affect the DNA by inducing visible modifications in the chromatin aggregation. A condensed chromatin is a part of the programmed cell death. A similar result was found by Liman et al., (2019) for the effect of CeO_2 NPs on the DNA content of *A. cepa*, by Benameur et al., (2015) for the effect on human dermal fibroblasts, and by Kumari et al., (2014) for the effect on Wistar rats. López-Moreno et al., (2010) found that CeO_2 NPs affect the integrity of DNA and genetic stability of soybean plants.

In this study, the TiO_2 NPs decreased the DNA content. As Rico et al., (2011) reported, this is due to the generation of superoxide radicals that cause lipid peroxidation in cells. Turkez and Geyikoglu (2007) reported that TiO_2 NPs could induce genotoxicity by inducing sister chromatid exchange and micronuclei in human white blood cells. Also, Ghosh et al., (2010) reported that the effect of TiO_2 NPs on DNA is due to the increased malondialdehyde (MDA) concentration that leads to lipid peroxidation, which leads to DNA damage. Pesnya (2013) concluded that TiO_2 NPs have a high potential to interact with DNA and cause primary DNA damage. The bio-uptake effect of TiO_2 NPs was explained by Pakrashi et al., (2014). They found a conjunction between the NPs uptake and the increase of ROS. An imbalance in intracellular ROS content caused by NPs exposure can induce DNA damages through oxidative stress owing to the oxidation of purine molecules (Afaq et al., 1998). Ghosh et al., (2012) found that treatment with TiO_2 NPs caused genotoxicity because of the generation of superoxide radicles. Schins and Knaapen (2007) suggested that the genotoxic effect of TiO_2 NPs might be due to oxidative stress and that the mechanism for this, as described by Donaldson et al., (1996) and Gilmour et al., (1997), is that TiO_2 NPs have hydroxyl radical activity. Similar results for TiO_2 NPs effect were found by Pakrashi et al., (2014).

Treatment of *A. sativum* with low and high concentrations of Ag NPs decreased the DNA content compared to the control. The Ag NPs induced toxicity due to their effect on ROS formation (Qian et al., 2013). Ma (1982) and Grant (1982) suggested that Ag NPs and their role in oxidative stress induced cellular death. Similar results were found by Sudhakar et al., (2001) and Babu et al., (2008). In higher plants, Saha and Gupta (2017) found that Ag NPs enter the plant cells and interfere with DNA repair, which leads to a blockage of DNA synthesis. Huijing et al., (2015) found that Ag NPs inhibit the new DNA synthesis in bacteria cells, which causes cell apoptosis.

This study showed that low concentrations of tested NPs had different effects on DNA. The Ag, TiO_2 and CeO_2 NPs decreased the DNA content, while AlO_2 and Fe_3O_4 NPs increased it. This difference may result from the ROS generation (Mcshan et al., 2014). Sharma et al. (2012) reported that ROS' destructive role depends on the equilibrium between ROS production and scavenging, that is, if a cell has developed a strong mechanism to control the ROS level by producing the enzymatic and non-enzymatic molecules needed to cope up with NPs-caused stress, it will decrease the effect of NPs on cell components including DNA.

The genotoxicity of NPs may result from their direct interaction with DNA or from indirect effects such as interacting with cells or tissues and releasing factors that cause harmful effects such as inflammation and oxidative stress (Singh et al., 2009; Magdolenova et al., 2014). Golbamaki et al., (2015) proposed that the genotoxic effects of NPs may be classified as primary genotoxicities or secondary genotoxicities. The second class may be due to the ROS generated during particle-induced inflammation, whereas the first class can be genotoxic without inflammation.

This study has revealed that different concentrations of the tested NPs affects the MI and that some treatments were significant at $p \leq 0.05$ particularly, Fe_3O_4 NPs after treatment with a low concentration for 24 h, TiO_2 NPs after treatment with low and high concentrations for 24 h, and Ag NPs after treatment with a high concentration for 16 h. This effect may be due to the free radicals generated by the interaction between NPs and cell components that raises the potential for cytotoxicity and decreases the MI. The tested NPs caused different types of chromosomal aberrations. Some of the scored types, e.g., micronuclei, lagging chromosomes, and chromosome bridges, indicated a genotoxic effect of NPs because these types of chromosome aberrations only occur if there is a direct effect on DNA. These NP effects may also be due to the time of interaction between the NPs and the cell cycle periods. It seems that NPs have greater effects during the S-phase of the cell cycle and wither this interaction starts during the beginning, middle, or end of the S-phase.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Afaq F, Abidi P, Matin R, Rahman Q (1998). Cytotoxicity, pro-oxidant effects and antioxidant depletion in rat lung alveolar macrophages exposed ultrafine titanium dioxide. Journal of Applied Toxicology 18(5):307-312.

Alarifi S, Ali D, ALkahtani S, Alhader MS (2014). Iron oxide nanoparticles induce oxidative stress, DNA damage, and caspase activation in the human breast cancer cell line. Biological Trace Element Research 159(1-3):416-424.

Auffan M, Rose J, Orsiere T, De Meo M, Thill A, Zeyons O, Proux O, Masion A, Chaurand P, Spalla O, Botta A, Wiesner MR, Bottero JY (2009). CeO2 nanoparticles induced DNA damage towards human dermal fibroblasts in vitro. Nanotoxicology 3(2):161-171.

Babu K, Deepa MA, Shankar SG, Rai S (2008). Effect of nano-Silver on Division and mitotic chromosomes: A prefatory Siren. Internet Journal of Nanotechnology 2(2):1-7.

Benameur L, Affuan M, Cassien M, Liu W, Culcasi M, Rahmouni H, Stocker P, Tassistro V,Bottero J, Rose J, Botta A, Pietri S (2015). DNA damage and oxidative stress induced by CeO2 nanoparticles in human dermal fibroblasts: Evidence of a clastogenic effect as a mechanism of genotoxicity. Nanotoxicology 9(6):696-705.

Brunner TJ, Wick P, Manser P, Spohn P, Grass RN, Limbach LK, Bruinink A, Stark WJ (2006). In vitro cytotoxicity of oxide nanoparticles: Comparison to asbestos, silica, and the effect of particle solubility. Environmental Science & Technology 40(14):4374-4381.

Buzea C, Pacheco BI, Robbie K (2007). Nanomaterials and nanoparticles: Sources and toxicity. Biointerphases 2(4):17-72.

Castiglione MR, Giorgetti L, Geri C, Cermonini R (2011). The effects of nano-TiO2 on seed germination, development and mitosis of root tip cells of *Vicia narbonensis* L. and *Zea mays* L. Journal of Nanoparticle Research 13(6):2443-2449.

Çekiç FÖ, Ekinci S, Inal MS, Ünal D (2017). Silver nanoparticles induced genotoxicity and oxidative stress in tomato plants. Turkish Journal of Biology 41(5):700-707.

Chen M, von Mikecz A (2005). Formation on nucleoplasmic protein aggregates impairs nuclear function in response to SiO2 Nanoparticles. Experimental Cell Research 305(1):51-62.

Debnath P, Mondal A, Hajra A, Das C, Mondal K (2018). Cytogenetic effects of silver and gold nanoparticles on *Allium cepa* roots. Journal of Genetic Engineering and Biotechnology 16(2):519-526.

Donaldson K, Beswick PH, Gilmour PS (1996). Free radical activity associated with the surface of particles: a unifying factor in determining biological activity. Toxicology Letters 88(1-3):293-298.

EL-Khodar S, Habib A, Haliem A (1990). Effect of the herbicides tribunnil on root mitosis of *Allium cepa*. Cytologia 55:209-215.

Faisa M, Saquib Q, Alatar A, Han MAM (2016). Cobalt oxide nanoparticles aggravate DNA damage and cell death in eggplant via mitochondrial swelling and NO signaling pathway. Biolgical Research 49:20.

Ghormade V, Deshpande MV, Pakmikar KM (2011). Perspectives for nano-biotechnology enabled protection and nutrition of plants. Biotechnology Advances 29(6):792-803.

Ghosh M, Bandyopadhyay M, Mukherjee A (2010). Genotoxicity of titanium dioxide (TiO2) nanoparticles at two trophies levels: Plant and human lymphocytes. Chemosphere 81(10):1253-62

Ghosh M, Manivannan J, Silnha S, Chakraborty A, Mallic SK, Bandyopadhyay M, Mukherjee A (2012). *In vitro* and *in vivo* genotoxicity of silver nanoparticles. Mutation Research 749(1-2):60-69.

Gilmour P, Brown DM, Beswick PH, Benton E, MacNee W, Donaldson K (1997). Surface free radical activity of PM10 and ultrafine titanium dioxide: a unifying factor in their toxicity? The Annals of Occupational Hygiene 41(1):32-38.

Golbamaki N, Rasulev B, Cassano A, Robinson RIM, Benfenati E, Leszczynski J, Cronin MTD (2015). Genotoxicity of metal oxide nanomaterials: Review of recent data and discussion of possible mechanisms. Nanoscale 7(6):2154-98.

Grant WF (1978). Chromosome Aberrations in Plants as a Monitoring System. Environmental Health Perspectives 27:37-43

Grant WF (1982). Chromosome aberration assay in Allium. A report of the US Environmental Protection Agency Gene-Tox Program. Mutation Research 99(3):273-291.

Grant WF (1999). Higher plant assays for the detection of chromosomal aberrations and gene mutations—a brief historical background on their use for screening and monitoring environmental chemicals. Mutation Research /Fundamental and Molecular Mechanisms of Mutagenesis 426(2):107-112.

Huang S, Chueh PJ, Lin YW, Shih TS, Chuang SM (2009). Disturbed mitotic progression and genome segregation are involved in cell transformation mediated by nano-Tio2 long-term exposure. Toxicology and Applied Pharmacology 241(2):182-94.

Huijing B, Xiaoxu Y, Xhen X, Zhaoyang L, Dianjun W, Yunde L (2015). New Toxicity Mechanism of Nanoparticles. Promoting Apoptosis and Inhibiting Proliferation. PLoS One 10(3):e0122535.

Hunt PR, Marquis BJ, Tyner KM, Conklin S, Olejnik N, Nelson BC, Sprand RL (2013). Nano silver suppresses growth and induces oxidative damage to DNA in *Caenorhabditis elegans*. Journal of Applied Toxicology 33(10):1131-1142.

Jaskowiak J, Tkaczyk O, Slota M, Kwasniewska J, Szarejko L (2018). Analysis of Aluminum toxicity in *Hordeum vulgare* roots with an emphasis on DNA integrity and cell cycle. PLoS One 13(2):e0193156

Jezek P, Hlavata I (2005). Mitochondria in homeostasis of reactive oxygen species in cell, tissues and organism. The International Journal of Biochemistry and Cell Biology 37(12):2478-503.

Khodakovskaya MV, Silva K, Biris AS, Dervishi E, Villagarcia H (2012). Carbon Nanotubes Induce Growth Enhancement of Tobacco Cells. ACS Nano 6(3):2128-2135.

Klasterska I, Natarajan AT, Ramel C (1976). An interpretation of the origin of sub chromatid aberration and chromosome stickiness as a category of chromatid aberrations. Hereditas 83(2):153-162.

Klien K, Godnic J (2012). Genotoxicity of Metal Nanoparticles: Focus on *in vivo* Studies. Arh Hig Rada Toksikol 63(2):133-45.

Kovacic P, Somanathan R (2010). Biomechanisms of nanoparticles (toxicants, antioxidants and therapeutics): Electron transfer and reactive oxygen species. Journal of Nanoscience and Nanotechnology 10(12):7919-7930.

Kruszewski M, Brzoska K, brunborg G, Asare N, Dobrzynska M, Dusinska M, Marie Fjellsbo L, Georgantzopoulou A, Gromadzka-Ostrowska J, Gutleb AC, Lankoff A, Magdolenova Z, Pran ER, Rinna A, Instanes Ch Sandberg WJ, Schwarze PE, Maciej Stepkowski T, Wojewodzka M, Refsnes M (2011). Toxicity of silver nanomaterials in higher eukaryotes. Advances in Molecular Toxicology 5:179-218.

Kumari M, Kumari SI, Grover P (2014). Genotoxicity analysis of cerium oxide micro and nanoparticles in Wistar rats after 28 days of reported oral administration. Mutagenesis 29(6):467-479.

Kumari M, Mukherjee A, Chandrasekaran N (2009). Genotoxicity of Silver Nanoparticles on *Allium cepa*. Science of The Total Environment 407(19):5243-5246.

Kuriyama R, Sakai H (1974). Role of tubulin-SH group in polymerization to microtubules. Journal of Biochemistry 76(3):651-654.

Kwon JY, Koedrith P, Seo YR (2014). Current investigations into the genotoxicity of Zinc oxide and silica nanoparticles in mammalian models in vitro and in vivo. Carcinogenic/ genotoxic potential relevant mechanisms and biomarkers, artifacts, and limitations. International Journal of Nanomedicine 9(Suppl 2):271-86.

Landa P, Vankova R, Andrlova I, Hodek J, Marsik P, Storchova H, White JC, Vanek T (2012). Nanoparticle-specific changes in Arabidopsis thaliana gene expression after expouser to ZnO, TiO2, and gulleren soot. Journal of Hazardous Materials 241:55-62

Landsiedel R, Kapp MD, Schulz M, Wienc, K, Oesch F (2009). Genotoxicity investigations on nanomaterials: Methods, preparation and characterization of test material, potential artifacts and limitations- Many questions, some answers. Mutation Research 681(2-3):241-58.

Lee CW, Mahendra S, Zodrow K, Li DM, Tsai YC, Braam , Ivarez PJJ (2010). Developmental phytotoxicity of metal oxide nanoparticles to *Arabidopsis thaliana* L. Environmental Toxicology and Chemistry 29(3):669-675.

Liman R, Acikbas Y, Hakki I (2019). Cytotoxicity and genotoxicity of cerium oxide micro and nanoparticles by Allium and comet tests. Ecotoxicology and Environmental Safety 168:408-414.

López-Moreno M, Guadalupe R, José ÁH, Hiram C, Cristina E, José

RP, Jorge LG (2010). Evidence of the differential biotransformation and genotoxicity of ZnO and CeO2 nanoparticles on soybean (*Glycine max*) plants. Environmental Science and Technology 44(19):7315-7320.
Ma C, Chhikara S, Xing B, Musante C, White JC, Dhankher OP (2013). Physiological and molecular response of Arabidopsis thaliana L. to nanoparticle cerium and indium oxide exposure. ACS Sustainable Chemistry and Engineering 1(7):768-778.
Ma TH (1982). Vicia cytogenetic tests for environmental mutagen. A report of the US Environmental Protection Agency Gene-Tox Program. Mutation Research 99(3):257-271.
Magdolenova Z, Collins A, Kumar A, Dhawan A, Stone V, Dusinska M (2014). Mechanisms of genotoxicity. A review of in vitro and in vivo studies with engineered nanoparticles. Nanotoxicology 8(3):233-78.
Mattiello A, Filippi A, Poscic F, Musetti R, Salvtici MC, Giordano C, Vischi M, Bertolini A, Marchiol L (2015). Evidence of Phytotoxicity and Genotoxicity in *Horeum vulgare* L. Exposed to Ceo2 and TiO2 Nanoparticles. Forntiers in Plant Science 6:1043.
McShan D, Ray PC, Yu H (2014). Molecular toxicity mechanism of nano silver. Journal of Food and Drug Analysis 22(1):116-127.
Mehrian SK, De Lima R (2016). Nanoparticles cyto and genotoxicity in plants: Mechanisms and abnormalities. Environmental Nanotechnology, Monitoring and Management 6:184-193.
Mohandas T, Grant WF (1972). Cytogenetic effect of 2,4-D and amitol in relation to nuclear volume DNA content in some higher plants. Canadian Journal of Genetics and Cytology 14(4):773-783.
Mukherjee A, Peralta-Videa J, Gardea-Torresdey J (2016). Effects and Uptake of Nanoparticles in Plants. Engineered Nanoparticles and the Environment: Biophysicochemical Processes and Toxicity. https://doi.org/10.1002/9781119275855.ch20
Pakrashi S, Jerobin J, dalai S, Prathna TC (2014). In vivo Genotoxicity Assessment of Titanium Dioxide Nanoparticles by *Allium cepa* Root Tip Assay at High Exposure Concentration. PLoS One 9(2):e87789.
Park EJ, Choi J, Park YK, Park K (2008). Oxidative stress induced by cerium oxide nanoparticles in cultured BEAS-2B cells. Toxicology 245(1-2):90-100.
Patil BC, Bhat GI (1992). A comparative study of MH and EMS in the induction of chromosomal aberration on lateral root meristem in *Clitoria ternate* L. International Journal of Cytology 57(2):259-264.
Patlolla AK, Berry A, May L, Tchounwou PB (2012). Genotoxicity of silver nanoparticles in *Vicia faba*: a pilot study on the environmental monitoring of nanoparticles. International Journal of Environmental Research and Public Health 9(5):1649–1662.
Pesnya DS (2013). Cytogenetic effects of chitosan-capped silver nanoparticles in the *Allium cepa* test. International Journal of Cytology, Cytosystematics and Cytogenetics 66(3):275-281.
Pulate PV, Ghurde MU, Deshmukh VR (2011). Cytological effects of the biological and chemical silver-nanoparticles in *Allium cepa* L. International Journal of Innovations in Biological and Chemical Sciences 1:32–35.
Qian H, Peng X, Han X, Ren J, Zhengwei F (2013). Comparison of the toxicity of silver nanoparticles and silver ions on the growth of terrestrial plant model *Arabidopsis thaliana*. Journal of Environmental Sciences 25(9):1947-1955.
Rajeshwari A, Kavitha S, Sruthi Ann A, Deepak K, Anita M, Natarajan C, Amitava M (2015). Cytotoxicity of Aluminum oxide nanoparticles on *Allium cepa* root tip- effects of oxidative stress generation and bio uptake. Environmental Science and Pollution Research 22(14):11057-66.
Rajiv S, Erobin J, Saranya V, Nainawat M, Sharma M, Makwana P, Gayathri C, Bharath L, Singh M, Kumar M, Mukherjee M, Chandrasekaran N (2015). Comparative cytotoxicity and genotoxicity of cobalt (II, III) oxide, iron (III) oxide, silicon dioxide, and aluminum oxide nanoparticles on human lymphocytes in vitro. Human and Experimental Toxicology 35(2):170-183
Rico CM, Duarte S, Garden M, Peralta-Videa JR, Gardea-Torrcsdcy JL (2011). Interaction of nanoparticles with edible plants and their possible implications in the food chain. Journal of Agricultural and Food Chemistry 59(8):3485-3498.
Saha N, Gupta SD (2017). A Glimpase on Silver Nanoparticles Genotoxicity in Higher Plants. Global Journal of Nanomedicine 2(2):1-2.
Salam HMH (2012). Effects of silver nanoparticles in some crop plants, common bean (*Phaseolus vulgaris* L.) and corn (*Zea mays* L.). International Research Journal of Biotechnology 3(10):190-197.
Schins RP, Knaapen AM (2007). Genotoxicity of poorly soluble particles. Inhalation Toxicology 19(Suppl 1):189-198.
Sendra M, Moreno-Garrido G, Yeste P, Gatica JM, Blasco J (2016). Behaviour of CeO2 nanoparticles and bulk and their toxicity in freshwater and seawater microalgae. Universidad de Alicante pp. 99-101.
Sharma P, Jha AB, Dubey RS, Pessarakli M (2012). Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. Journal of Botany 217037:1-26.
Sheykhbaglou R, Sedghi M, Shishevan MT, Sharifi RS (2010). Effects of nano-iron oxide particles on agronomic traits of soybean. Notulae Scientia Biologicae 2(2):112-113.
Siddiqui MH, Mohamed HW, Mohammad F, Mutahhar YK (2015). Role of Nanoparticles in Plants. Nanotechnology and Plant Sciences 19-35
Silva JR, Smyth TJ, Moxley DF, Carter TE, Allen NS, Rufty TW (2000). Aluminum accumulating at nuclei of cell in the root tip. Fluorescence detection using lumogallion and confocal laser scanning microscopy. Plant Physiology 123(2):543-552.
Singh N, Manshian B, Jenkins GJ, Griffiths SM, Williams PM, Maffeis TG, Wright CJ, Doka SH (2009). The DNA damaging potential of engineered nanomaterials. Biomaterials 30(23-24):3891-3914.
Sjorgen CA, Larsen PB (2017). SUV2, which encode an ATR-related cell cycle checkpoint and putative plant ATRIP, is required for aluminum-dependant root growth inhibition in Arabidosis. Plant, Cell and Environment 40(9):1849-1860.
Song G, Gao Y, Wu H, Hou W, Zhang C, Ma H (2012). Physiological effect of anatase TiO2 nano-particles on *Lemna minor*. Environmental Toxicology and Chemistry 31(9):2147-2152.
Srinivas A, Rao PJ, Selam G, Murthy PB, Reddy PN (2011). Acute inhalation toxicity of cerium oxide nanoparticles in rats. Toxicology Letters 205(2):105-115.
Sudhakar R, Ninge Gowda KN, Govindappa V (2001). Mitotic Abnormalities Induced by Silk Dyeing Industry Effluents in the Cells of *Allium cepa*. Cytologia 66(3):235-239.
Tavares AM, Louro H, Antunes S, Quarre S, Simar S, De Temmerman PJ, Verleysen E, Mas J, Jense KA, Norppa H, Nesslany F, Silva MJ (2014). Genotoxicity evaluation on nanosized titanium dioxide, synthetic amorphous silica and multi-walled carbon nanotubes in human lymphocytes. Toxicology *in Vitro* 28(1):60-90.
Trouiller B, Reliene R, Westbrook A, Solaimani P, Schiestl RH (2009). Titanium Dioxide Nanoparticles induce DNA Damage and Genetic Instability in vivo in Mice. Cancer Research 69(22):8784-8789
Turkez H, Geyikoglu F (2007). An *in vivo* blood culture for evaluating the genotoxicity of titanium dioxide: the response of antioxidant enzymes. Toxicology and Industrial Health 23(1):19-23.
Van Breusegem F, Dat JF (2006). Reactive oxygen species in plant cell death. Plant Physiology 141:384-390.
Vranová E, Inzé D, Van Breusegem F(2002). Single transduction during oxidative stress. Journal of Experimental Botany 53(372):1227-1236.
Wu H, Yin J, Wamer WG, Zeng M (2014). Reactive oxygen species-related activities of nano-iron metal and nano-iron oxides. Journal of Food and Drug Analysis 22(1):86-94.
Yuliang Z, Bing W, Weiyue F, Chunli B (2010). Nanotoxicology: toxicological and biological activities of nanomaterials. In: Encyclopedia of Life support Systems (EOLSS): nanoscience and nanotechnologies. Paris: UNESCO-EOLSS Publisher. https://www.eolss.net/Sample-Chapters/C05/E6-152-35-00.pdf
Zheng L, Hong S, Lu C (2005). Effect of TiO2 on strength of naturally aged seeds and growth of spinach. Biological Trace Element Research 104(1):83-92.
Zhongwen Chen, Jun-Jie Yin, Yu-Ting Zhou, Zhang Y, Song L, Song M, Hu S, Gu N (2012). Dual enzyme-like activities of iron oxide nanoparticles and their implication for diminishing cytotoxicity. ACS Nano 6(5):4001-4012.
Zia-ur-Rehman M, Qayyum MF, Akmal F, Maqsood MA, Rizwan M, Waqar M, Azhar M (2018). Nanomaterials in Plants, Algae, and Microorganisms. Recent Progress of Nanotoxicology in Plants-Science Direct pp. 143-174.