A comparative study for the cytological effect of ZnO nanoparticles and ZnO bulk on *Allium cepa* L.

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Abstract: In this study we compared the cytological effect and chromosomal behavior of root meristems of *Allium cepa*, induced by ZnO bulk and ZnO nanoparticles (NP). Four different concentrations of ZnO bulk and ZnO nanoparticles (2.5, 5, 10 and 20 ppm) were used to treat the root tips at different durations (2, 4, and 6 hours). The influence of these treatments on germination percentage and radicle length as well as mitotic index and chromosomal aberration was investigated. Our results indicate that all applied concentrations of ZnO bulk and ZnO nanoparticles caused a reduction in seed germination percent and radicle growth, as well as in mitotic index (MI) of *Allium cepa* seeds as compared to control. These reductions were accompanied by increase in concentration and/or duration of treatment. The total percentage of chromosomal aberrations were variable, with the change in concentrations and duration of treatment. Both bulk ZnO molecules and ZnO nanoparticles, induced different types of chromosomal abnormalities such as micro-nuclei, disturbed chromosomes, chromosomal stickiness, laggards, bridges, chromosomal fragmentation and diagonal. These abnormalities indicates true clastogenic possibility of ZnO bulk and ZnO nanoparticles.

[A.A. El-Ghamery, E. A. Abdel-Azeem, M.A. El-Kholy, O. A. Gamal El-Dein. **A comparative study for the cytological effect of ZnO nanoparticles and ZnO bulk on *Allium cepa* L.** *Nat Sci* 2016;14(2):87-96]. ISSN 1545-0740 (print); ISSN 2375-7167 (online). http://www.sciencepub.net/nature. 12. doi:10.7537/marsnsj140216.12.

Keywords: ZnO bulk (non-nano); ZnO nanoparticles (NPs); Zinc, *Allium cepa* radicle meristems; mitosis; chromosomal aberrations; mitotic index (MI); seed germination.

1. Introduction

Nanotechnology field is the future of all scientific applications due to the new physical and chemical properties that the nano scale materials have, compared to its bulk materials of origin. In the past decade ZnO nanoparticles (ZnO NPs) usage in the consumer's daily products has increased. Therefore Zn^{2+} deposition in the environment is increasing and there's no doubt that there's a global concern about toxicological and environmental effects of ZnO NPs. Recently, toxicological studies were carried out on zinc oxide nanoparticles especially in the last ten years showed that ZnO NPs have potential health as well as environmental hazard effect [1]. ZnO NPs can cause viral toxicity to bacteria, *Daphnia magna* (freshwater microalga), mice, and even human cells [2], [3] and [4].

The effect of zinc on M-phase and G_1 of the plant cell cycle in the synchronous TBY-2 tobacco cell suspension, was studied [5]. This study demonstrated that toxic but sublethal concentrations of Zn^{+2} can transvers the cell cycle of TBY-2 tobacco. It has also showed that the higher concentrations of Zn^{+2} does not result in substantial cell death, but it does have differential effects on the cell cycle; M-phase lengthens and G1 shortens.

In another study, the cytological effects of Zn^{2+} on the germination and root growth of *Nigella sativa* L. and *Triticum aestivum* L. was investigated, when treated with zinc sulfate[6]. The treatments reduced the germination percentage of *N. sativa* seeds and *T. aestivum* grains and inhibited the root growth of both plants. Treatments also caused different chromosomal aberrations such as chromosomal stickiness, chromosome breaks and chromosomal bridges at ana-telophases. So they concluded that, these abnormalities indicate true clastogenic potential of Zn^{2+}.

On the other hand, an investigation of the cytogenetic and genotoxic effects of zinc oxide nanoparticles on root cells of *Allium cepa* was done by [7]. Results of this study showed high percentage of micro nucleated cells in interphase cells of *Allium cepa* root tip cells treated with ZnO NPs, compared to control roots. This was explained due to the possible mechanism for higher intrinsic toxicity of ZnO NPs to *A. cepa* could be the release of reactive oxygen species which could convert fatty acid to toxic lipid peroxides, destroying biological membranes.

Phytotoxic and genotoxic effects of ZnO nanoparticles on garlic (*Allium sativum* L.) was investigated (Shaymurat, *et al*. 2011). This investigation showed that ZnO NPs caused concentration-dependent inhibition of root length, mitosis index decrease and induced several kinds of mitotic aberrations, like chromosome stickiness, bridges, breakages and laggings. This investigation provided new information for the possible genotoxic effects of ZnO NPs on plants.

In a recent study, the effect of zinc oxide nanoparticles on cytology and seed germination in onion was studied [9]. Results of this investigation showed decrease in mitotic index and increase in chromosomal abnormalities were observed in higher treatments of zinc oxide nanoparticles. Germination indices showed increased values in lower concentrations; however these decreased significantly at higher concentrations.

2. Materials and methods

Allium cepa L. was selected as test plant, due to its high profile as an environmental genetic indicator [10] and [11]. *Allium cepa* L. seeds (Giza-20) was purchased from The Agricultural Research Center, Cairo University, Giza, Egypt.

Zinc oxide nanoparticles powder was purchased from Nanostructured & Amorphous Materials, Inc. Houston, TX, USA. The non nano ZnO was purchased from a local chemicals store. The physical characteristics and SEM picture of the ZnO nanoparticles according to the manufacturer's data are: purity, 99.5%, size 20 nm (SEM). **Figure.1**

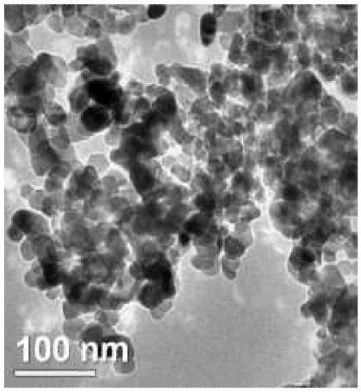

Fig.1 Scanning Electron Microscope for ZnO NPs.

Fig.2 Ultrasonic vibrator

ZnO bulk and ZnO engineered nanoparticles (50 mg/L) was suspended directly in 1000 ml of distilled water to prepare 50 ppm of both materials, then it was dispersed using ultrasonic vibrator (Eumax, ultrasonicator,UD50SH-2L, 50/60Hz) Figure 2. For 30 minutes [12].

Four concentrations were diluted to prepare treatment concentrations (2.5, 5, 10, 20ppm).

$$\text{Mitotic index (MI)} = \frac{\text{Total divided cells}}{\text{Total counted cells}} \times 100$$

2.1. Germination percentage

Seeds of *Allium cepa* were soaked in distilled water for 2 hours, then it was dipped in different concentrations of ZnO bulk and ZnO NPs suspension (2.5 ppm, 5ppm, 10ppm, and 20ppm) for 2, 4, 6hours. After treatment three replicates of each treatment and control seeds, were placed in petridishes with filter paper soaked with distilled water, each replicate has 25seed which were allowed to grow in room temperature for 72 hours. Germinated seeds of each treatment and germination percentage were scored.

2.2. Radicle length

The seeds of *Allium cepa* L. were soaked in distilled water for 2 hours and then were allowed to grow on a petridish until the root radical starts to grow then seedlings are treated with four different concentrations of ZnO bulk and ZnO NP (2.5ppm, 5ppm, 10ppm and 20ppm) for different durations(2 hours, 4 hours and 6 hours). After treatment duration, seeds were washed with distilled water and then were allowed to grow in distilled water for 72 hours at room temperature (23°C±) with the control seeds which grown in distilled water. Then the radicle length of each seed was measured.

T/C ratio was calculated as the following;

$$T/C = \frac{\text{Radicle length of treated seeds}}{\text{Radicle length of control seeds}} \times 100$$

2.3. Cytological observation

For cytological observation, radicle of (2.5cm-3cm length) was treated with four different concentrations (2.5ppm, 5ppm, 10ppm and 20ppm) of zinc oxide bulk and zinc oxide nanoparticles for three different durations (2hours, 4hours and 6hours). After each treatment, germinated seeds were fixed immediately by Carnoy's fixative solution. A freshly prepared mixture of absolute ethyl alcohol and glacial acetic acid (3: 1 v/v) for 24 hours and then transferred into 70% ethyl alcohol in the refrigerator for mitotic study.

Mitotic study was carried out by following Feulgen squash technique [13]. Treated seeds with radicle were hydrolyzed in (1N HCl) at 60° C for 5-6 min and then stained with Feulgen stain for 45-60min. Stained root tips was cut and squashed on slides by

45% glacial acetic acid. Four replicates for each treatment were prepared and a control replicates for each of the treatment duration was prepared. Slides were examined by (WETZLAR leitz, Germany) light microscopy using (40X) objective lens and (15x) eye lens, to record mitotic phases and chromosomal abnormalities.

All mitotic phases and chromosomal abnormality of each phase was recorded to calculate mitotic index, phase index and total abnormality types and percentage of each type to analyze the effect of ZnO bulk and ZnO NPs as the following:

$$\text{Phase index (PI)} = \frac{\text{Phase counted cells}}{\text{Total divided cells}} \times 100$$

$$\text{Total ab. cells \%} = \frac{\text{Total no. of abnormal cells}}{\text{Total divided cells}} \times 100$$

$$\text{Chromoscmal aberration \%} = \frac{\text{Aberation type}}{\text{Total divided cells}} \times 100$$

Statistical analyses were carried out by the procedure of, one way ANOVA descriptive analysis using PASW statistics. Data were expressed as mean ± standard error (SE) which was driven by four replicates. The statistical significance of the difference between values(mitotic index and total abnormalities) of treated samples and control samples was evaluated by means of the *t*-test at two levels of significance; $p \leq 0.05$ (significant*) and $p \leq 0.01$ (highly significant **).

3. Results

3. 1. Seed Germination

The influence of Zinc oxide (bulk & NPs) on *Allium cepa* seeds germination% is represented in **Fig. 3.** and **Fig. 4.** In seeds treated with ZnO bulk all applied concentrations caused inhibition in germination% for all durations of treatment compared with control value. From our results, we conclude that the inhibitory effects of ZnO NPs was more than ZnO bulk in decreasing germination%.

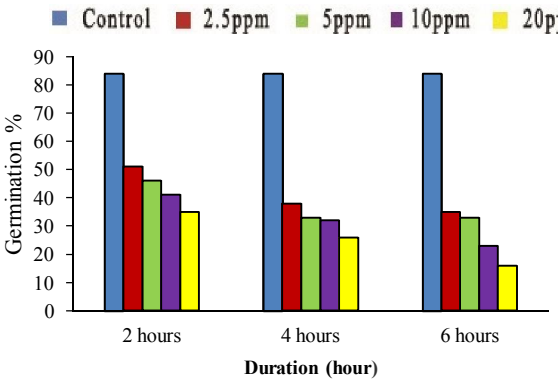

Fig.3 Effect of ZnO bulk on *Allium cepa* germination %

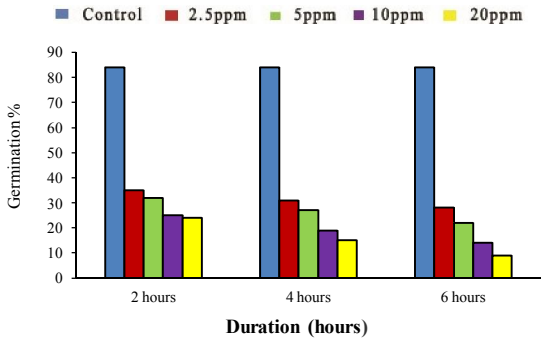

Fig. 4. Effect of ZnO NPs. on *Allium cepa* germination %

3.2. Radicle length

The effects of ZnO bulk and ZnO NPs on radicle growth of *Allium cepa* seeds is shown in **Fig.5**. and **Fig.6**. Comparing the radicle length of each treatment and the

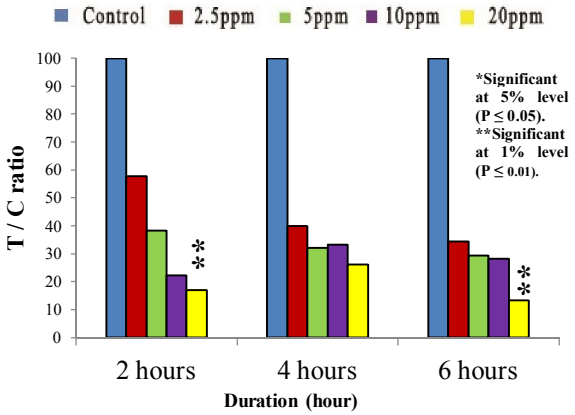

Fig.5. Effect of ZnO NPs. on *Allium cepa* radicle length

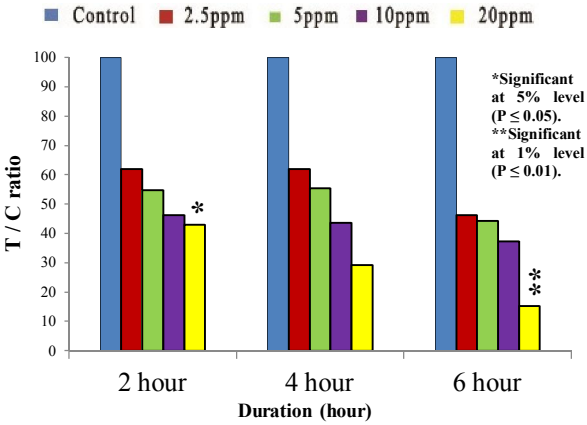

Fig.6. Effect of ZnO bulk on *Allium cepa* radicle length

control (T/C ratio). In seeds treated with ZnO bulk all applied concentrations caused inhibition in radicle growth for all durations of treatment compared to control value. These inhibition increases with increasing the concentration for each treatment time. The lowest value of radicle growth is 15.18% and was observed at 20 ppm for 6hour Similar in seeds treated with ZnO NPs showed decrease in radicle growth with all treatments. From our results we concluded that, the inhibitory effect of ZnO NP was higher than ZnO bulk in reducing radicle growth.

3.3. Mitotic study

The effect of ZnO bulk and ZnO NPs on the mitotic index (MI) is shown in **Fig.7** and **Fig.8**. According to these results both ZnO bulk and ZnO NP have a mitodepressive effect on *Allium cepa* root tip cells, but

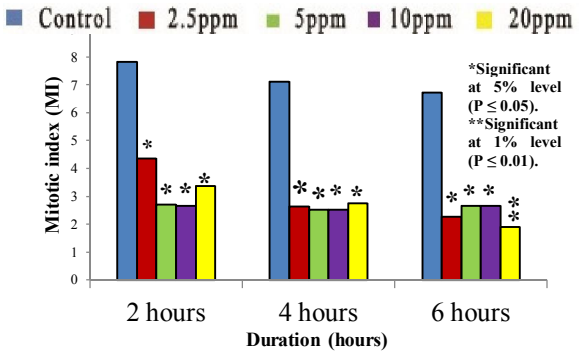

Fig.7. Effect of ZnO bulk on MI of *Allium cepa root tip cells*

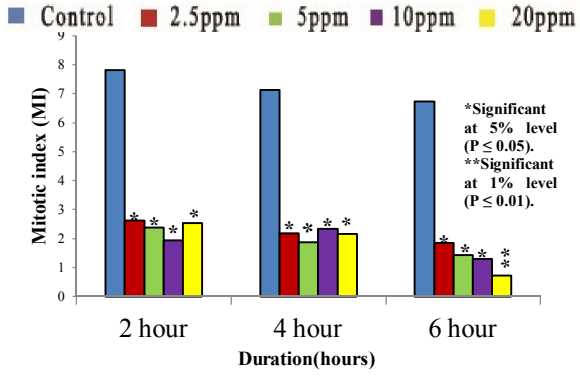

Fig.8. Effect of ZnO bulk on MI of *Allium cepa root tip cells*

ZnO NP has more effect on the mitotic index, compared to ZnO bulk and control cells. This reduction was statistically significant ($p \leq 0.05$) at all treatments for both, ZnO NP & ZnO bulk and was highly significant at the treatment with 20ppm ZnO NP for 6hours as MI was 0.72 ± 0.24 while the MI of ZnO bulk at the same treatment was 1.89 ± 0.016 when the control MI was 4.22 ± 0.89.

Percentages of chromosomal abnormalities caused by ZnO non-nano & ZnO NP are shown in **Table 1**. Abnormality percentage was 100% when treated with 10ppm for 2hours, 5ppm for 4hours and 10ppm, 20ppm for 6hours with ZnO bulk treatment. While ZnO NP chromosomal abnormality percentage was statistically high significant ($P \leq 0.01$) at 2hours and 4hours of treatments, and significant ($P \leq 0.05$) at 6hours. Abnormality Percentage was 100% when treated with 10ppm for 4hours and 2.5ppm, 5ppm, 10ppm and 20ppm for 6hours.

Meaning when treated with ZnO NPs, chromosomal aberrations was highest when treated for 6hours with all concentrations of treatments. In **Table 2** and **Table 3** are showing that, ZnO bulk and ZnO NP induced different types of mitotic abnormal cells in the root tip of *Allium cepa* seeds. Some of these types are represented in **Fig. 9** and **Fig. 10.** The percentage of these types are variable with the concentration and duration of treatment. These types included micro-nuclei at interphase, irregular prophase, disturbed- metaphase, sticky metaphase, sticky at different stages. Also other abnormalities were recorded, like lagging chromosome at metaphase, forward chromosome at anaphase and sticky anaphase with forward chromosome, but with less percentage than the previous ones.

4. Discussion

This investigation demonstrated that ZnO bulk and ZnO nanoparticles has a cytological influence on *Allium cepa* cells. Seed germination and root elongation is a rapid and widely used acute phytotoxicity test with several advantages: sensitivity, simplicity, low cost and suitability for unstable chemicals or samples [14]. Germination is normally known as a physiological process beginning with water imbibition by seeds and culminating in the emergence of the rootlet [15].

Table 1. Percentages of abnormal cells in difirrent mitotic stages and total abnormalities after treating *Allium cepa* root tip cells with different concenteraions of Zinc oxide bulk and Zinc oxide nanoparticles for different treatment times

Tretment		ZnO bulk					ZnO nanoparticles				
Time (hr)	Conc. (ppm)	Abnormal cells X ± S.E.%	Prophase %	Metaphase %	Anaphase %	Telophase %	Abnormal cells X ± S.E.%	Prophase %	Metaphase %	Anaphase %	Telophase %
2	Control	7.5 ± 0.23	3.8	1.3	2.5	0.0	7.5 ± 0.23	3.8	1.3	2.5	0.0
	2.5	97.3 ± 0.81**	58.1	21.6	14.9	2.7	73.8 ± 0.009**	28.6	23.8	21.4	0.0
	5	93.8 ± 1.34**	58.3	20.8	14.6	0.0	72 ± 0.009**	18.6	20.9	32.6	0.0
	10	100 ± 0.81**	38.2	26.5	29.4	5.9	73.8 ± 0.006**	23.8	19.0	31.0	2.4
	20	83.3 ± 3.38 *	23.3	23.3	21.7	15.0	84.6 ± 0.005**	18.4	28.9	39.5	0.0
4	Control	10.7 ± 3.13	1.3	2.7	6.7	0.0	10.7 ± 3.13	1.3	2.7	6.7	0.0
	2.5	90.6 ± 0.21**	41.5	24.5	24.5	0.0	97.8 ± 1.46**	54.3	15.2	26.1	2.2
	5	100 ± 0.94**	24.4	22.0	53.7	0.0	97 ± 1.40**	55.9	26.5	14.7	0.0
	10	95.7 ± 1.56**	53.2	21.3	21.3	0.0	97.7 ± 1.36**	50.0	25.0	22.7	0.0
	20	81.4 ± 0.47**	27.9	16.3	18.6	18.6	98 ± 1.01**	35.0	32.5	30.0	0.0
6	Control	11.1 ± 1.29	6.9	0	4.2	0.0	11.1 ± 1.29	6.9	0	4.2	0.0
	2.5	94.7 ± 0.53**	52.6	15.8	12.3	14.0	100 ± 0.00*	46.2	19.2	26.9	7.7
	5	97.6 ± 0.35**	46.5	23.3	16.3	11.6	100 ± 0.00*	53.8	23.1	19.2	3.8
	10	100 ± 0.28**	49.0	24.5	16.3	10.2	100 ± 0.00*	43.3	33.3	13.3	10.0
	20	100 ± 0.82**	24.0	36.0	28.0	12.0	100 ± 0.00	8.3	50.0	41.7	0.0

S.E.,standard error. *Significant at 5% level ($P \leq 0.05$). **Significant at 1% level ($P \leq 0.01$).

Table 2. Types and percentages of mitotic abnormal cells and the percentage of total abnormal cells in *Allium cepa* root tips treated with ZnO Bulk at different times.

Treatments		Types and percentages of abnormal cells																			
		Interphase		Prophase			Metaphase				Anaphase								Telphase		
Time (hours)	Conc. (ppm)	Bi-Nuc %	Micro %	Irr. %	Stick %	Vac %	Distu %	Stick %	Lag %	C- %	Bri %	Distu %	C- %	Stick %	Dig %	Fw %	Lag %	Mult-Pol %	Lag %	Stick %	Dig %
2	Control	0	0	2.5	0	1.3	1.3	0	0	0	0.0	1.3	0	0.0	0	1.3	0.0	0.0	0.0	0.0	0.0
	2.5	0	1.4	54.1	1.4	2.7	16.2	2.7	1.4	1.4	2.8	0	2.3	4.2	1.4	1.4	1.4	1.4	1.4	0.0	1.3
	5	0	0.0	52.1	4.2	2.1	12.5	8.3	0.0	0.0	0.0	6.3	2.1	4.2	2.1	0.0	0.0	0.0	0.0	0.0	0.0
	10	0	2.9	26.5	8.8	2.9	14.7	11.8	0.0	0.0	5.8	2.9	2.9	12.0	2.9	0.0	2.9	0.0	5.9	0.0	0.0
	20	3.3	11.7	8.3	13.3	1.7	5.0	15.0	1.7	1.7	0.0	0.0	0.0	16.6	0.0	3.3	0.0	1.7	0.0	15.0	0.0
4	Control	0	0	1.3	0	0	1.3	0.0	1.3	0.0	0.0	1.3	0.0	0.0	0.0	5.3	0.0	0.0	0.0	0.0	0.0
	2.5	0	0	37.7	1.9	1.9	11.3	13.2	0	0	0.0	1.3	2.7	9.3	1.3	2.7	0.0	0.0	0.0	0.0	0.0
	5	0	0	14.6	4.9	4.9	7.3	9.8	0.0	4.9	2.4	0.0	0.0	46.4	2.4	0.0	0.0	2.4	0.0	0.0	0.0
	10	0	2.1	44.7	6.4	2.1	6.4	10.6	4.3	0.0	2.1	2.1	2.1	12.7	0.0	0.0	0.0	2.1	0.0	0.0	0.0
	20	7.0	18.6	11.6	9.3	7.0	9.3	7.0	0.0	0.0	2.3	0.0	0.0	16.3	0.0	0.0	0.0	0.0	0.0	18.6	0.0
6	Control	0.0	0	4.2	0	2.8	0	0.0	0.0	0.0	0.0	0.0	0.0	2.8	0.0	1.4	0.0	0.0	0.0	0.0	0.0
	2.5	7.0	15.8	10.5	38.6	3.5	3.5	8.8	0.0	3.5	1.8	0.0	1.8	3.6	0.0	3.5	1.8	0.0	0.0	14.0	0.0
	5	9.3	7.0	14.0	27.9	4.7	7.0	11.6	2.3	2.3	0.0	0.0	0.0	11.7	2.3	2.3	0.0	0.0	0.0	11.6	0.0
	10	2.0	28.6	16.3	30.6	2.0	14.3	8.2	2.0	0.0	2.1	0.0	0.0	14.2	0.0	0.0	0.0	0.0	0.0	10.2	0.0
	20	16.0	4.0	8.0	16.0	0.0	20.0	16.0	0.0	0.0	0.0	0.0	0.0	28.0	0.0	0.0	0.0	0.0	0.0	12.0	0.0

Table 3. Types and percentages of mitotic abnormal cells and the percentage of total abnormal cells in Allium cepa root tips treated with ZnO nanoparticles at different times.

Treatments		Types and percentages of abnormal cells																				
Time (hours)	Conc. (ppm)	Interphase	Prophase			Metaphase						Anaphase									Telophase	
		Micro-Nuc%	Vac %	Irr %	Stick %	Distu %	Stick %	Lag %	Fw %	C- %	Fr %	Bri %	Distu %	Dis-Fw %	Stick %	Dig %	Fw %	Lag %	Star %	Mult-Pol %	Stick %	Dig %
2	Control	0.0	1.3	2.5	0.0	1.3	0.0	0.0	0.0	0.0	0.0	0.0	1.3	0.0	0.0	0.0	1.3	0.0	0.0	0.0	0.0	0.0
	2.5	21.4	2.4	23.8	0.0	21.4	0.0	0.0	0.0	0.0	0.0	2.4	4.8	0.0	9.6	2.4	0.0	0.0	0.0	2.4	4.8	0.0
	5	23.3	0.0	16.3	2.3	11.6	7.0	0.0	2.3	0.0	0.0	2.3	2.3	2.3	13.9	2.3	4.7	2.3	0.0	0.0	2.3	0.0
	10	16.7	4.8	16.7	2.4	7.1	7.1	0.0	2.4	0.0	2.4	4.8	7.1	2.4	9.5	0.0	2.4	2.4	0.0	2.4	0.0	0.0
	20	13.2	5.3	10.5	2.6	11.1	7.9	0.0	5.3	0.0	2.6	7.9	7.9	2.6	13.1	0.0	2.6	2.6	0.0	2.6	0.0	0.0
4	Control	0.0	0.0	1.3	0.0	1.3	0.0	1.3	0.0	0.0	0.0	0.0	1.3	0.0	0.0	0.0	5.3	0.0	0.0	0.0	0.0	0.0
	2.5	0.0	0.0	52.2	2.2	10.9	0.0	0.0	0.0	4.3	0.0	2.2	2.2	2.2	17.4	0.0	2.2	0.0	0.0	0.0	2.2	0.0
	5	44.1	2.9	41.2	11.8	20.6	5.9	0.0	0.0	0.0	0.0	0.0	2.9	2.9	8.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	10	6.8	2.3	40.9	6.8	18.2	4.5	2.3	0.0	0.0	0.0	2.3	0.0	2.3	11.3	0.0	2.3	2.3	0.0	2.3	0.0	0.0
	20	5.0	2.5	30.0	5.0	30.0	2.5	0.0	0.0	0.0	0.0	0.0	7.5	2.5	20.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6	Control	0.0	2.8	4.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.8	0.0	1.4	0.0	0.0	0.0	0.0	0.0
	2.5	38.5	0.0	7.7	38.5	15.4	3.8	0.0	0.0	0.0	0.0	0.0	3.8	7.7	11.5	0.0	0.0	0.0	3.8	0.0	7.7	0.0
	5	57.7	3.8	3.8	46.2	19.2	3.8	0.0	0.0	0.0	0.0	0.0	3.8	3.8	7.7	0.0	0.0	0.0	3.8	0.0	3.8	0.0
	10	53.3	0.0	6.7	36.7	23.3	10.0	0.0	0.0	0.0	0.0	0.0	3.3	3.3	6.7	0.0	0.0	0.0	0.0	0.0	0.0	10.0
	20	183.3	0.0	8.3	0.0	41.7	8.3	0.0	0.0	0.0	0.0	0.0	16.7	0.0	25.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Treatments with ZnO bulk and ZnO NPs affected *Allium cepa* seeds germination percentage. It was highly significant at the highest concentration of ZnO NPs which showed the highest inhibitory effect compared to the same concentration of ZnO bulk. The inhibitory effect was less in ZnO bulk, than the ZnO NPs compared to control seeds. This result meets with previous studies[6], [16] and [9] in which germination % decreased with the increase of time and concentration of treatment. Radicle growth was also affected with the treatments of ZnO. Both treatments caused reduction in the radicle growth, as the reduction rate was significant at 4 hours of treatment which was higher in ZnO NPs than the ZnO bulk, but after 6hours of treatment the reduction was almost the same compared to control radicles, this result is in agreement with previous results [17], [8] and [18]. The reduction of germination rate and radicle growth might be due to the toxicity of Zn^{2+} induced chromosomal aberrations, as this aberrations could lead to mitotic arrest and cell death [19] and [6]. It might be also due to the toxicity of the metal ion through disturbance of the physiological processes by the fixation of the ions by the plant tissue [20].

Reduction in the rate of mitotic division can be attributed to the mitodepressive effect of ZnO bulk and ZnO NP. This decrease might be caused by zinc ions, which slows the progression of cells from S (DNA synthesis) phase to M (Mitosis) phase, preventing a number of cells from entering the prophase and blocking the mitosis cycle during interphase as a result of ZnO exposure [21]. It has been suggested that the cytotoxicity level can be determined by the decreased rate of the mitotic index [22].

In our study zinc ions induced an imbalance in frequency of abnormal mitotic phases in radicle meristems, and the degree of this imbalance is clearly dose-dependent [23], and type-dependent [24].

The results showed that prophase and metaphase stages frequency was increased on the expense of other mitotic stages in both bulk and nano zinc oxide. Accumulation in these stages indicates that zinc ions influence the sequence of mitotic division and reduced the number of cells entering mitotic division by blocking the process at the end of prophase [25]. It could also be because of interaction with the spindle which can cause an arrest of the cell division at metaphase [26]. Both ZnO bulk and ZnO NPs induced a number of chromosomal aberrations in all mitotic stages but the frequency of each type was variable.

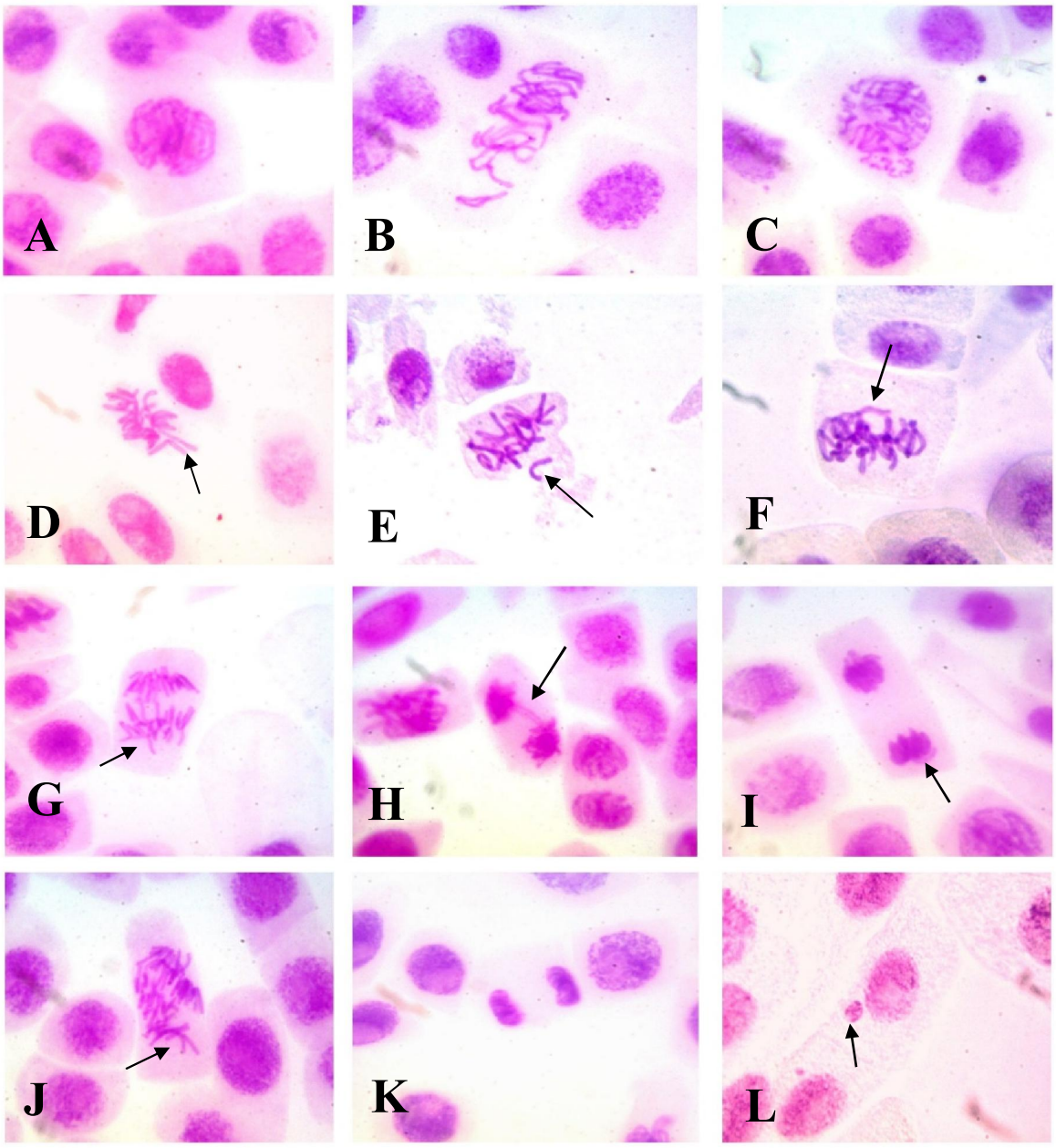

Fig.9. Mitotic abnormalities following ZnO bulk treatment in *Allium cepa* seeds. Magnification power 540x. **A.** sticky prophase; **B** irregular prophase; **C** irregular prophase; **D.** disturbed metaphase; **E.** disturbed metaphase with lagging chromosome **F.** disturbed metaphase; **G.** anaphase with forward chromosome and double bridge; **H.** Anaphase with double bridge; **I.** Sticky anaphase with forward chromosome **J.** Tetra polar anaphase **K.** sticky telophase **L.** micronucleus at interphase**.**

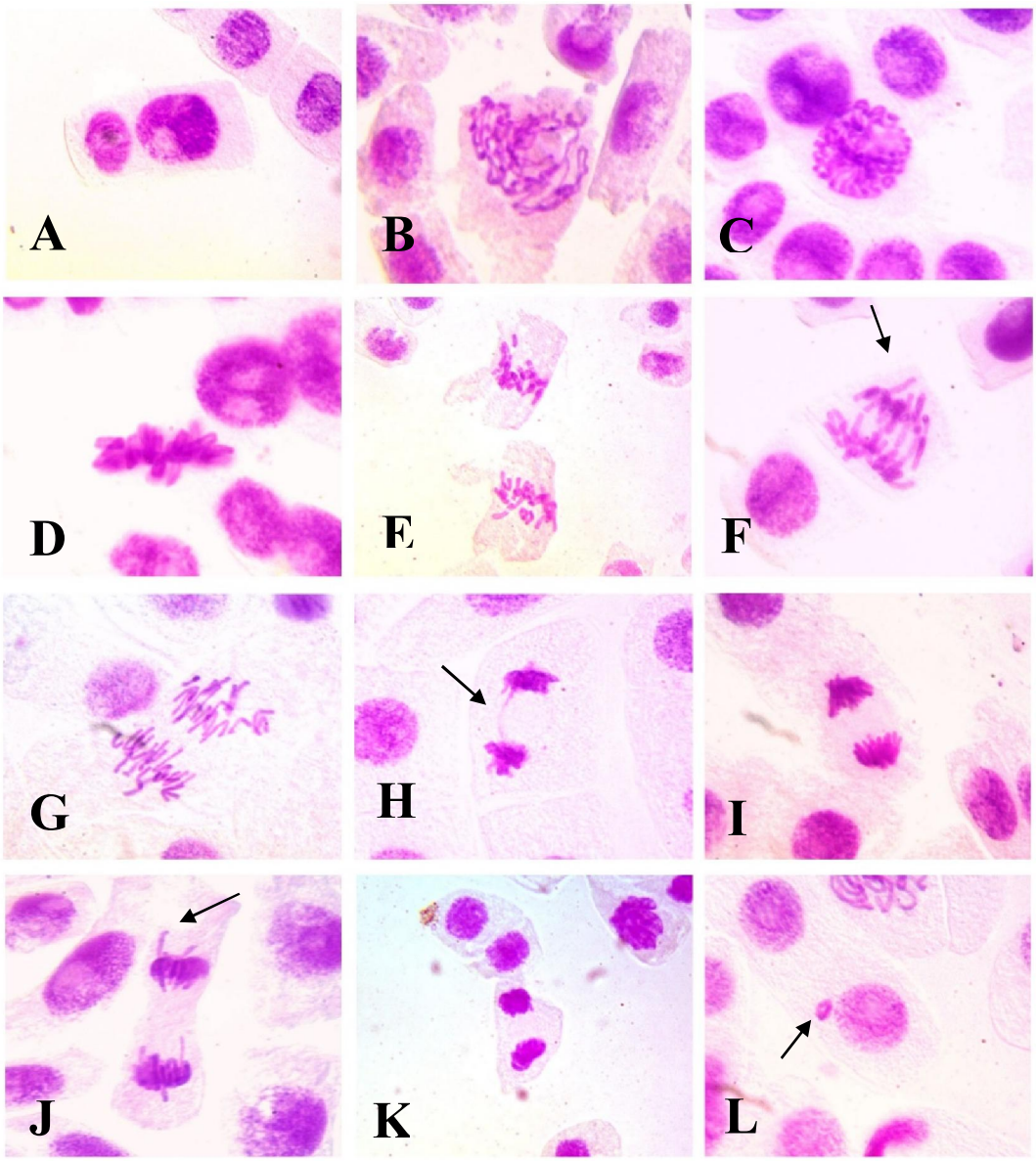

Fig.10. Mitotic abnormalities following ZnO NPs treatment in *Allium cepa* seeds. Magnification power 540x. **A**. binucleated cell at interphase **B.** Irregular prophase **C.** sticky prophase **D.** sticky metaphase **E.** disturbed metaphase **F.** anaphase with multiple bridges and forward chromosome. **G.** multipolar anaphase **H.** sticky anaphase with single bridge **I.** sticky anaphase **J.** telophase with forward chromosome **K.** sticky diagonal telophase **L.** micronucleus at interphase

Types of chromosomal aberrations observed during our study was almost similar for both treatments with ZnO, bulk & NPs. In prophase the presence of irregular prophase and sticky prophase, was observed with high frequency. The occurrence of irregular prophase was higher in the lower duration of treatment while the occurrence of sticky prophase at the longest treatment duration, was higher in frequency. Formation of irregular prophase results from the effect of Zn^{2+} on the process of individualization of chromatin threads to normal chromosomes [27]. Earlier studies [28] suggested that stickiness is a kind of physical adhesion including the protein matrix of chromatin material, while other study suggests that stickiness is due to depolymerization or degradation of the chromosomal DNA [29]. In our results observation of stickiness in different stages might be due to the polymerization effect of ZnO NPs on nucleic acid of the chromosome.

In metaphase the most prevailing types were disturbed metaphase and sticky metaphase. Presence of disturbed metaphase may suggest that Zn^{2+} caused partial disturbance in the spindle apparatus or partial suppression of spindle formation [30]. Sticky chromosomes in metaphase and anaphase was frequently observed at higher concentrations. This stickiness is also the explanation for observing sticky bridges at anaphase and telophase [31]. Lagging chromosomes, fragmentation and bridges were also recorded in a low frequency at metaphase and anaphase, which could be attributed to chromosome breaks and reunion of the broken ends [6]. This type of clastogenic or toxic effect of Zn^{2+} is irreversible [32].

In our observation, we recorded micro nucleus with treatment with ZnO bulk and with ZnO NPs, unlike other studies [7] and [9] which observed micronucleus in higher treatments with ZnO nanoparticles only, which was explained due to the effect of Reactive Oxygen Species that ZnO nanoparticles emerged in the treated cells, but our results proved otherwise.

In conclusion, our study showed that ZnO bulk and ZnO NPs has a toxic effect on *Allium cepa* seeds. The toxicity of the ZnO NPs didn't cause new types of chromosomal aberration different than ZnO bulk but showed higher percentage of aberration, compared to control cells. This means that the higher toxicity is only due to the nano scale particles which increased the reactivity of the ZnO, so carful usage of ZnO NPs is necessary due to its environmental hazard effect.

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2/17/2016