International Journal of Agricultural Research

ISSN 1816-4897

Academic Journals Inc.

www.academicjournals.com

International Journal of Agricultural Research 7 (8): 376-387, 2012
ISSN 1816-4897 / DOI: 10.3923/ijar.2012.376.387
© 2012 Academic Journals Inc.

Protective Effects of Some Antioxidant Metals against Chromosomal Damage Induced by Cadmium in *Vicia faba* plants

Zeinab M. El-Ashry and Fawzia I. Mohamed
Department of Genetics and Cytology, National Research Centre, Dokki, Cairo, Egypt

Corresponding Author: Zeinab M. El-Ashry, Department of Genetics and Cytology, National Research Centre, Dokki, Cairo, Egypt

ABSTRACT

Cadmium toxicity and the chemoprotective effects of calcium (Ca^{2+}), zinc (Zn^{2+}) and selenium (Se^{4+}) at different concentrations (10^{-6}-10^{-4} M) for 24 h. are studied on the root growth, mitotic index, chromosomal aberrations and micro-nuclei of *Vicia faba* plants. The three concentrations of Cd^{2+} (10^{-5}-10^{-3} M) induced inhibition of root-growth. The percentage of root-growth reached to 10.82, 6.03 and 4.01%, respectively. After treatment with all Cd^{+2} concentrations together with (10^{-6}-10^{-4} M) of Ca^{2+}, All concentrations of Ca^{2+} improved the percentage of root-growth. The effects of Se^{4+} and Zn^{2+} had less obvious than Ca^{2+}. Three metals have a protective role against the genotoxicity of Cd^{2+} on root-growth especially at low concentration. A highly significant decrease in the percentage of MI appeared after treatment with the highest concentration of Cd^{2+}(10^{-3} M) which reached 4.01% but after treatment with Cd^{+2} together with 10^{-6} M of Ca^{2+}, Se^{4+} and Zn^{2+} reached to 11.30, 9.70 and 9.10%, respectively. Cd^{2+} induced a wide rang of mitotic abnormalities. All the tested concentrations of Cd^{2+}, caused statistically significant percentage of chromosome aberrations. The percentage reached 25.50, 32.60 and 48.51% after treatment with 10^{-5}, 10^{-4} and 10^{-3} M Cd^{2+}, respectively compared with 0.81% for the control. After treatment Cd^{+2} concentrations (10^{-5}-10^{-3} M) together with 10^{-6}-10^{-4} M of Ca^{2+}, Zn^{2+} and Se^{+4}, respectively, the frequency of chromosomal aberration and micro-nuclei decreased. These treatments had strong antagonism effects. The degree of antimutagenic effects of Ca^{2+}, Zn^{2+} and Se^{4+} against Cd^{2+} was related to their concentration. The present study cleared that the protective effects of Ca^{2+}, Zn^{2+} and Se^{4+} against Cd^{2+}. The degree of this protective effects was Ca^{2+}>Se^{4+}>Zn^{2+}.

Key words: Antagonism, calcium, selenium, zinc, micro-nuclei, *Vicia faba*

INTRODUCTION

Heavy metals are ubiquitous in nature and represent one of the major groups of environmental pollutants (Nriagu, 1990; Ernst, 1998). Cadmium (Cd) is the fifth most toxic metal to vertebrates and the fourth most toxic metal to vascular plants. Cadmium (Cd) cause high decrease in plant growth (Chen and Kao, 1995; Ouzounidou *et al*., 1997; Ali *et al*., 2001), photosynthetic characteristics (Ali *et al*., 2001; Zengin and Munzuroglu, 2005), mineral nutrition (Kim *et al*., 2003), enzyme activity (Schutzendubel *et al*., 2001; Scebba *et al*., 2006).

At low concentration of cadmium (Cd) may adversely affect the plant reproduction by inhibiting pollen germination and tube growth (Xiong and Peng, 2001).

Increased industrial usage of Cd^{2+} has caused an increase in Cd^{2+} production and constant rate in contamination of soil and water (Amin, 2001; Hsu and Kao, 2007). Continuous use of phosphate fertilizers lead to significant increase in the Cd^{2+} contents of many agricultural soils (Alloway, 1995). Cadmium (Cd^{2+}) toxicity have been carried out on different plants. It was found to inhibit seed germination and root growth (Chen and Kao, 1995), decrease the mitotic index of root cells (Zhang and Xiao, 1998) and produce the chlorophilic mutations (Reddy and Vaidyanath, 1978). Cadmium causes mitotic irregularities comprises c-mitoses, anaphase bridges, breaks, stickiness, lagging, vagrant chromosomes and micronuclei in different plants as *Hordeum vulgare*, *Vicia faba* and *Lens culinaris* (Gomez-Arroyo *et al*., 1989; Li and Zheng, 1992; Zhang and Yang, 1994; Kiran and Sahin, 2006; Siddiqui *et al*., 2009) damaged the nucleolar structural (Zhang, 1997) and reduced the fidelity of DNA and R NA synthesis (Enger *et al*., 1979). Also, there are reports on Cd^{2+} poisoning of soil (Xu, 1990; Amin, 2001).

It is evident that certain chemicals posses properties that directly or indirectly reduce or eliminate the mutagenic activity of other chemicals. This means that the search for mutagenic inhibitors may be useful for discovering antimutagenic agents.

In recent years, there are many reports indicating that vitamins improved and prevent the chemical toxicities of metal on biological activities (Anderson *et al*., 1994; El-Ashry, 2003; Schrauzer, 2008; Taspinar *et al*., 2011).

The purpose of the present investigation was to study the effects of some metal (Ca^{2+}, Se^{4+} and Zn^{2+}) with different concentration against Cd^{2+} induced genotoxic effects on root growth and cell division in *Vicia faba* plants in order to minimize the chromosomal aberrations.

MATERIALS AND METHODS

The experiment was carried out at the Genetic and Cytology Department, National Research Center, Cairo, Egypt, 2011, 2012.

Plant materials: Seed of *Vicia faba* L. (var. Giza 3) was used in the present study. The seeds were kindly supplied by the Legume Crops Research Section, Agricultural Research Center, Giza, Egypt.

Experimental agents: Cadmium sulphate ($CdSO_4$), Calcium chloride ($CaCl_2$), Sodium selenite (Na_2SeO_3) and Zinc sulphate ($ZnSO_4$) were purchased from Foluka company. The chemicals solutions were prepared by dissolving in distilled water at room temperature (23±1°C). The concentrations used for Cd^{2+} were from (10^{-5}-10^{-3} M). The doses of $CaCl_2$, Na_2SeO_3 and $ZnSO_4$ were from (10^{-6}-10^{-4} M), respectively.

Effect on early growth stages: Seeds of *Vicia faba* were soaked in tap water for 24 h. Then seeds germinated in rolls of filter paper moistened with tap water. When the roots reached 1.5-3.0 cm in length, they were divided and treated in 5 groups as shown in Table 1. The roots were immersed in different concentrations of experimental agents ($CdSO_4$, $CaCl_2$, Na_2SeO_3, $ZnSO_4$) for 24 h. then the number of survived plants was recorded and the lengths of the shoot and the root were estimated after 6 days.

Three replicates were conducted for each treatment and 15 seeds were used for each replicate.

Cytological studies: When the roots of *Vicia faba* germinated seeds reached 2-3 cm in length, they were divided and treated in 5 groups as shown in Table 1. The roots of all treatments were cut

Table 1: Treatment groups of Cd^{2+}, Ca^{2+}, Zn^{2+} , Se^{4+} and abbreviations used

Plant groups	Treatments for 24 h	Abbreviated in the text
I	Distilled water	Control (C)
II	Cadmium concentration	Cd^{2+}
		A = (10^{-5} Cd)
		B = (10^{-4} Cd)
		C = (10^{-3} Cd)
III	Cadmium+calcium concentration	Cd^{2}++Ca^{2+}
	1 = 10^{-6} Ca^{2+}	A+1, A+2, A+3
	2 = 10^{-5} Ca^{2+}	B+1, B+2, B+3
	3 = 10^{-4} Ca^{2+}	C+1, C+2, C+3
IV	Cadmium+zinc concentration	Cd^{2+}+ Zn^{2+}
	4 = 10^{-6} Zn^{2+}	A+4, A+5, A+6
	5 = 10^{-5} Zn^{2+}	B+4, B+5, B+6
	6 = 10^{-4} Zn^{2+}	C+4, C+5, C+6
V	Cadmium+selenium concentration	Cd^{2+}+ Se^{4+}
	7 = 10^{-6} Se^{4+}	A+7, A+8, A+9
	8 = 10^{-5} Se^{4+}	B+7, B+8, B+9
	9 = 10^{-4} Se^{4+}	C+7, C+8, C+9

and fixed in 3:1 absolute ethyl alcohol and glacial acetic acid (v/v) for 24 h. The roots were hydrolyzed in 1 N HCl at 60°C for 10 min, then stained and squash with Feulgen squash technique (Sharma and Sharma, 1980).

Statically analysis: All cytological data were statically analyzed using t-test. Score was taken from 9 roots (3 roots/ replicate). The percentage of shoot and root lengths, Mitotic Index (MI) and total number of chromosomal aberrations was estimated. The types of aberrations as (disturbed chromosome) was estimated:

$$MI = \frac{\text{No. of dividing cells}}{\text{No. of counted cells}} \times 100$$

$$\text{Chromosomal aberrations (\%)} = \frac{\text{No. of total aberrations cells}}{\text{No. of counted cells}} \times 100$$

$$\text{Chromosome stickiness (\%)} = \frac{\text{No. of chromosome stickiness}}{\text{No. of total aberrations cells}} \times 100$$

RESULTS AND DISCUSSION

Effect on early growth stages

Effect of cadmium: Table 2-4 showed that, Cd^{2+} had obvious toxic effects on the root-growth of *Vicia faba* seeds.

A significant decrease effect of root-growth was noticed for all the treatment of Cd^{2+} concentrations after 24 h. The degree of inhibition showed a positive relation with the concentrations of Cd^{2+}. The percentage of root-growth reached 10.82% with concentration 10^{-5} M

Table 2: Percentage of root-growth, mitotic index, total and different types of abnormalities after treatment of *Vicia faba* with different concentrations of calcium together with cadmium concentrations for 24 h

				Different types of mitotic abnormalities (%)					
Treatment (M)	Root-growth (%)	Total mitotic index (%)	Abnormal mitoses (%)	Disturbed	Sticky	Bridge	Lagging	Fragment	Micro-nuclei
Control	18.13	-	0.92±0.67	-	-	-	-	-	-
A: 10^{-5} Cd^{2+}	10.82	7.80±0.87**	26.50±0.71**	32.77	20.51	17.18	11.90	10.44	7.20
A+1	15.82	15.80±0.66**	14.20±0.61**	31.05	26.24	13.69	14.52	10.40	4.10
A+2	13.01	13.33±0.87**	17.60±0.77**	33.81	24.03	18.97	10.94	8.90	4.60
A+3	11.21	10.20±0.74**	19.30±0.81**	24.52	30.07	16.29	13.14	13.00	5.90
B: 10^{-4} Cd^{2+}	6.03	5.90±0.77**	32.60±0.72**	29.07	34.88	7.81	13.95	5.79	8.50
B+1	12.12	14.01±0.69**	18.20±0.78**	26.44	40.23	9.19	12.64	7.30	4.20
B+2	11.01	11.20±0.76**	21.30±0.77**	22.81	33.33	9.52	19.35	9.29	5.70
B+3	10.10	10.33±0.08**	23.40±0.82**	31.25	31.00	9.38	18.74	3.13	6.50
C: 10^{-3} Cd^{2+}	4.11	4.01±0.78**	48.51±0.86**	24.07	39.52	16.04	3.14	8.03	9.20
C+1	11.11	11.30±0.75**	24.66±0.77**	26.91	32.37	14.15	10.35	9.62	6.60
C+2	10.20	9.60±0.82**	20.33±0.81**	34.29	27.14	12.04	9.02	8.67	8.84
C+3	9.81	8.33±0.78**	29.60±0.67**	33.520	34.07	16.04	7.23	3.14	6.00

1: 10^{-6} Ca, 2: 10^{-5} Ca, 3: 10^{-4} Ca, **Significant at $p<0.01$

Table 3: Percentage of root-growth, mitotic index, total and different types of abnormalities after treatment of *Vicia faba* with different concentrations of selenium together with cadmium concentrations for 24 h

				Different types of mitotic abnormalities (%)					
Treatment (M)	Root-growth (%)	Total mitotic index (%)	Abnormal mitoses (%)	Disturbed	Sticky	Bridge	Lagging	Fragment	Micro-nuclei
Control	18.13	18.90±0.68	0.81±0.67**	-	-	-	-	-	-
A: 10^{-5} Cd^{2+}	10.82	7.80±0.87**	25.50±0.78**	32.77	20.51	17.18	11.9	10.44	7.2
A+ 4	13.62	13.80±0.91**	16.70±0.67**	28.01	34.78	11.95	15.21	4.65	5.4
A+ 5	12.10	10.70±0.86**	19.30±0.77**	31.25	31.00	9.38	18.44	3.13	6.8
A+ 6	10.33	10.20±0.74**	19.30±0.81**	31.25	36.21	9.39	10.53	4.13	8.5
B: 10^{-4} Cd^{2+}	6.03	5.90±0.77**	32.60±0.72**	29.07	34.88	7.81	13.95	5.79	8.5
B+ 4	12.33	12.20±0.73**	21.20±0.77**	30.11	32.22	12.88	13.18	6.26	5.35
B+ 5	11.75	10.10±0.86**	22.30±0.79**	33.81	24.03	15.52	10.94	8.90	6.8
B+ 6	9.55	9.69±0.79**	24.90±0.82**	23.29	30.07	16.04	12.50	10.00	8.1
C: 10^{-3} Cd^{2+}	4.11	4.01±0.78**	48.51±0.86**	24.07	39.52	16.04	3.14	8.03	9.2
C+ 4	11.39	9.70±0.88**	26.90±0.82**	24.39	33.43	16.67	16.67	4.64	4.2
C+5	10.10	8.50±0.79**	28.40±0.91**	28.55	31.63	15.78	13.00	5.64	5.4
C+6	9.93	0.86±7.80**	31.02±0.77**	26.02	32.65	16.10	11.66	6.77	6.8

4: 10^{-6} Se, 5: 10^{-5} Se, 6: 10^{-4} Se, **Significant at $p<0.01$

Cd^{2+} but with the concentration (10^{-4} and 10^{-3} M Cd^{2+}), reached to 6.03 and 4.01%, respectively compared to 18.13% for the control. Also, at high concentration some roots showed a twisted appearance and become yellow and stubbly. Cd^{2+} concentrations were found to have no effect on shoot growth.

The results clear that the inhibition of root-growth on *Vicia faba* root- tip meristems after treated with different concentrations of Cd^{2+}. These results are agreement with the effect of Cd^{2+} on other plants (Ouzounidou *et al*., 1997; Ali *et al*., 2001). Chen and Kao (1995), showed that Cd^{2+} had toxic effects on the root growth of rice seedling. The reduced germination and inhibition of root and body development on *Sorghum bicolor* L. exposed to different Cadmium concentrations were reported by Pandit and Prasannakumar (1999).

Table 4: Percentage of root-growth, mitotic index, total and different types of abnormalities after treatment of *Vicia faba* with different concentrations of zinc together with cadmium concentrations for 24 h

				Different types of mitotic abnormalities (%)					
Treatment (M)	Root-growth (%)	Total mitotic index (%)	Abnormal mitoses (%)	Disturbed	Sticky Bridge	Lagging	Fragment	Micro-nuclei	
Control	18.13	18.90±0.68**	0.81± 0.67	----	---	----	-----	----	----
A: 10^{-5} Cd^{2+}	10.82	7.80±0.87**	25.50±0.78**	32.77	20.51	17.18	11.9	10.44	7.2
A+ 7	12.85	11.88±0.79**	18.60±0.91**	28.01	34.78	11.95	15.21	4.65	5.4
A+ 8	11.39	9.70±0.97**	20.50±0.78**	40.1	23.8	13.19	13	2.1	7.82
A+ 9	10.01	8.60±0.82**	18.60±0.88**	42	20.95	14.49	13.3	2.9	6.36
B: 10^{-4} Cd^{2+}	6.03	5.90±0.77**	32.60± 0.72**	29.07	34.88	7.81	13.95	5.79	8.5
B+ 7	11.75	10.61±0.91**	22.30±0.73**	27.61	38.99	13.22	12.04	3.43	4.71
B+8	10.35	8.70± 0.82**	24.90±0.81**	20.9	30.4	14.8	11.7	5.9	6.8
B+9	8.77	7.60± 0.67**	28.80±0.92**	36.1	24	14.01	12.6	6.9	6.4
C: 10^{-3} Cd^{2+}	4.11	4.01± 0.78**	48.51±0.86**	24.07	33.52	16.04	3.14	8.03	9.2
C+7	11.00	9.10± 0.68**	28.40±0.79**	32.54	30.99	12	13.06	6.04	5.37
C+8	10.01	7.80± 0.88**	31.50±0.89**	27.61	31.16	15	13.1	6.02	7.11
C+ 9	8.15	7.10±0.92**	36.20±0.78**	20.13	42.2	10.2	10.2	9.24	8.11

7: 10^{-6} Zn, 8: 10^{-5} Zn, 9: 10^{-4} Zn, **Significant at $p<0.01$

Effect of Ca^{2+}, Se^{4+} and Zn^{2+}: All concentrations of Ca^{2+} (10^{-6}-10^{-4} M) caused a high significant increase in the percentage of root-growth. The maximum percentage reached 15.82, 12.12,11.11 at concentrations 10^{-6}-10^{-4} M Ca^{2+} together with 10^{-5}-10^{-3} M Cd^{2+} compared to Cd^{2+} without Ca^{2+} which are 10.82 , 6.03, 4.11 (Table 2). These results are agreement with the addition of $CaCl_2$ to cadmium-stressed common bean plants improved the stem fresh weight, root length, number of flowers and number of pods per plant (Suzuki, 2005; Ismail, 2008).

The effects of Se^{4+} and Zn^{2+} had less effects obvious than Ca^{2+}. The high percentage of root-growth with 10^{-6} M Se^{4+} and Zn^{2+} reached to 13.62 and 12.85%, respectively after treatment together with 10^{-5} M Cd^{2+} (Table 3, 4). This result showed that Ca^{2+} had more antagonistic effects on root-growth than Se^{4+} and Zn^{2+} but these three metals have a protective role against the genotoxicity of Cd^{2+} on root-growth especially at low concentration.

Cakmak (2000) reported that, zinc is known to have a stabilizing and protective effect on the biomembranes against Cd induced oxidative and peroxidative damage, loss of plasma membrane integrity.

Effect on mitotic division

Effect of Cd^{2+}: The ability of different concentrations of Cd^{2+} affected the mitotic activity, total percentage of abnormalities and the types of abnormalities shown in Tables 2-4. From these results, it could be concluded that, a significant decrease in mitotic index appeared after treatment with all concentrations of Cd^{2+} (10^{-5}-10^{-3} M). A highly significant decrease in the percentage of MI appeared after treatment with the concentration of Cd^{2+}(10^{-5}-10^{-3} M) which reached 7.80, 5.90 and 4.01%, respectively as compared to the control 18.90%, the mitotic index decreased progressively with increased Cd^{2+} concentration. The reduction of Mitotic Index (MI) was significantly decreased upon Cd^{2+} treatment and was found to be directly proportional to the concentration of Cd^{2+} used. There were highly significant differences between these concentrations. Mitotic index reflects the frequency of cell division and rat of root-growth. The reduction of mitotic index could be produced

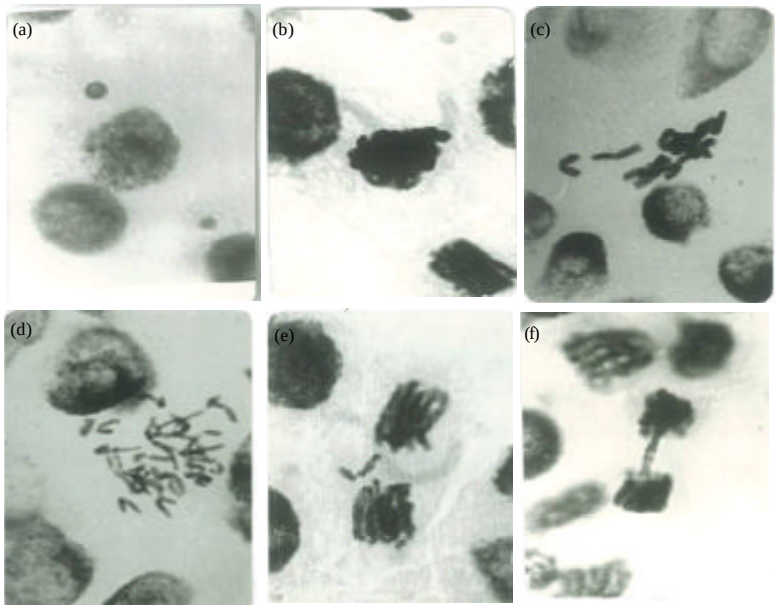

Fig. 1(a-f): Chromosomal abnormalities induced in root-tip cells of *Vicia faba* following after treatment with Cd^{2+} and Cd^{2+} with Ca^{2+}, Se^{4+} or Zn^{2+} (a) Interphase with micro-nuclei after treated with (10^{-3}) Cd^{2+}, (b) Metaphase sticky after treated with 10^{-4} Cd^{2+} with 10^{-6} Ca^{2+}, (c) Metaphase with two lagging after treated with 10^{-5} Cd^{2+} with 10^{-6} Se^{4+}, (d) Disturbed anaphase after treated with 10^{-4} Cd^{2+} with 10^{-4} Zn^{2+}, (e) Telophase with lagging and (f) Telophase with bridge after treated with 10^{-5} Cd^{2+} with 10^{-5} Ze^{2+}

either by prolonging the time of mitotic cycle as a whole, or through the permanent inhibition of mitosis of some cells (Borboa and de la Torre, 1996).

There is evidence that Cd^{2+} much more easily taken up by plants than other heavy metals (Stoeppler, 1991), thus, cadmium at higher concentrations has serious inhibitory and toxic effects on cell division (Greger *et al.*, 1991). Earlier workers showed that Cd^{2+} is an extremely toxic element, and it could reduce the MI and induce chromosomal aberrations (Brown and Martin, 1981). Similar effects have been noted in the present study, Li *et al.* (1992) reported that Cd^{2+} concentrations reduced the MI and it had genotoxic effects at the chromosomal level in *Allium cepa* roots. The same results were obtained by George (2000) and Muneer *et al.* (2011).

Treatment of *Vicia faba* with different concentrations of Cd^{2+} for 24 h induced a wide rang of mitotic abnormalities. The percentage of chromosome aberrations increased with increasing the concentration of Cd^{2+}. All the tested concentrations of Cd^{2+}, caused statically significant percentage of chromosome aberrations. Such percentage reached 25.50, 32.60 and 48.51% after treatment with 10^{-5}, 10^{-4} and 10^{-3} M Cd^{2+}, respectively compared with 0.81% for the control (Table 2-4). These results indicated that the concentrations of Cd^{2+} (10^{-5}-10^{-3} M) are the more damage the chromosomes suffer.

The types of mitotic abnormalities noticed in the present study were, disturbed chromosomes, Sticky, laggard, bridges, fragments and micro-nuclei. These results are in agreement with those obtained by George (2002) and Muneer *et al.* (2011). The most common types of mitotic abnormalities were disturbed metaphase and disturbed anaphase after treated with 10^{-4} Cd^{2+}

together with 10^{-4} Zn^{2+} (Fig. 1d). The percentage of disturbed chromosome reached to 32.77% with 10^{-5} M Cd^{2+}. The disturbed chromosomes were noted in all treatment of Cd^{2+}. It is produced as a result of spindle disturbed (Abd-El-Salam *et al*., 1993; Amer and Aly, 2001).

Stickiness (clumped chromosomes) was noticed in the different mitotic stages. It was the dominant types in metaphase especially after treatment with the high concentration of Cd^{2+} (10^{-4}-10^{-5} M). The percentage of stickiness reached to 39.52% with Cd^{2+} (10^{-3} M). Metaphase sticky after treated with 10^{-4} Cd^{2+} with 10^{-6} Ca^{2+} (Fig.1b). Stickiness may result from physical adhesion involving mainly the proteinaceous matrix of chromatin material (Patil and Bhat, 1992; El-Ghamery *et al*., 2003).

Bridges represented one of the common types of abnormalities induced by Cd^{2+}. The percentage of bridges reached to 16.04% with Cd^{2+} (10^{-5} M). The formation of bridges can be interpreted on the bases of the general stickiness of chromosomes and subsequent failure of anaphase separation (Abraham and Koshy, 1979) or may be the result of chromosome breakage and reunion (El-Kodary *et al*., 1990). The presence of both stickiness and bridges in our results support these conclusions. Telophase with bridge after treated with 10^{-5} Cd^{2+} with 10^{-5} Ze^{2+} (Fig. 1f).

Fragments and lagging chromosomes were noticed in the different mitotic stages (Table 2-4, Fig. 1c, f). The percentage of lagging reached to 13.95% with 10^{-4} Cd^{2+} also, percentage of fragment chromosomes are 10.44% with10^{-5} Cd^{2+}. Metaphase with two lagging after treated with 10^{-5} Cd^{2+} together with 10^{-6} Se^{4+} (Fig. 1c) and telophase with lagging after treated with 10^{-5} Cd^{2+} together with 10^{-5} Ze^{2+} (Fig. 1f). The induced of lagging chromosomes could be attributed to the spindle apparatus (Pandey and Upadhyay, 2010). A considerable percentage of micro- nuclei were observed in the different mitotic stages. Micro-nuclei can be originated from fragments or from laggard, Interphase with micro-nuclei after treated with 10^{-3} M Cd^{2+} (Fig. 1a) which reached to 9.20%. The occurrence of bridges, fragments and laggards may lead to loss of genetic materials and the formation of micro-nuclei (Gustavino *et al*., 1987). The induction of these aberrations has been found to be an active the mutagenicity of Cd^{2+} (Fiskesjo, 1985; Liu *et al*., 1992).

Effect of Ca^{2+}, Se^{4+} and Zn^{2+}: In the protective effect experiments, the present data indicated that Ca^{2+}, Se^{4+} and Ze^{2+} had obviously protective effects against Cd^{2+} induced inhibition of cell division, chromosomal aberration and micro-nuclei formation (Table 2, 4).

It is evident from the present results that, treatment Cd^{2+} concentrations (10^{-5}-10^{-3} M) together with (10^{-6}-10^{-4} M) Ca^{2+} showed strong improvement of MI compared with same concentrations without Ca^{2+}. The 10^{-5} M Cd^{2+} together with 10^{-6} M Ca^{2+} concentration showed significant increase in MI, the percentage reached 15.80% compared with 7.80% without Ca^{2+} (Table 2). On the other hand, the depression of MI was still observed but intensity of reduction was much less in the concentrations (10^{-5}, 10^{-4} M) Cd^{2+} together with (10^{-6}-10^{-4}) Ca^{2+} when compared to the same concentration without Ca^{2+}. Also, these results showed that Ca^{2+} minimized the chromosomal aberration induced by Cd^{2+} toxicity. It demonstrated that Ca reduced the uptake of Cd and caused a modest reduction in Cd toxicity (Gipps and Coller, 1982; El-Enany, 1995).

Calcium plays an important role in regulation of cellular physiological function and metabolism in plants (Hepler and Wayne, 1985). The accumulation of Cd^{2+} in cells may arouse the metabolism abnormality of Ca^{2+} (Chal and Zhu, 1983). Since Ca^{2+} and Cd^{2+} have similar binding sites, Ca^{2+} can substitute or replace Cd^{2+} and activate the activity of Ca-ATPase when the concentration of Ca^{2+} in cells increase, thus reducing the toxic effects of Cd^{2+} on cells and decrease the number of abnormal dividing cells (Wang, 1992; Taspinar *et al*., 2011).

One reason for Ca ion alleviation of Cd toxicity is the displacement of cell-surface toxic cations by Ca. Since plasma membrane surface has usually negative charge, high levels of Ca would reduce cell-surface negativity and alleviate the harmfulness of cationic toxicants (Kinraide, 1998). The other proposed mechanism is the uptake of Cd through calcium channels (Perfus-Barbeoch *et al.*, 2002). Due to high concentrations of Ca around Ca channels, there would be a competition between Cd and Ca ions influx (Suzuki, 2005; Shahrtash *et al.*, 2011).

In the present study, the different concentration of Selenium (10^{-6}-10^{-4} M) cause increase in MI for all concentration of Cd^{2+} especially the treatment with 10^{-5} M of Cd^{2+} together with 10^{-6} M of Se^{4+}. Table 3 clears the percentage of MI which reached to 11.88% as compared with the same concentration without Se^{4+} (7.80%). On the other hand, the percentage of MI was increased. This improved may due to the protective role played by Se^{4+}.

The percentage of chromosomal aberrations was decreased. The decrease may due to the protective role played by Se^{4+}. The percentage of chromosomal aberrations with 10^{-3} M of Cd^{2+} are 48.51% become 26.90% after treated with 10^{-3} M of Cd^{2+} together with 10^{-6} M Se^{4+}.

The relation between Se^{4+} and Cd^{2+} has been studied in some organisms (Wang, 1992; Hassan *et al.*, 2006). Zhang and Xiao (1998) indicated that Se^{4+} decrease the MI and Micro-nuclei frequency in *Hordeum vulgare* induced by Cd^{2+}. The present data indicate that the antagonism of Se^{4+} against Cd^{2+} is less than Ca^{2+}.

Selenium is an essential trace element for human and animal and a component part of glutathione peroxidase (GSH-PX). Se^{4+} can act as antioxidant which can help to protect the living organism from damaging effects of free radicals (Gu and Zhang, 1993; Schrauzer, 2000; Nadiminty and Gao, 2009).

Selenium also clear the free radicals produced by Cd^{2+} which promotes DNA synthesis. When the concentration of Se^{4+} increased, the activity of GSH.Px was elevated while the mutagenic effects decreased (Valdiglesias *et al.*, 2010).

The present data also indicated that Zn^{2+} concentration (10^{-6}-10^{-4} M) minimized the genotoxicity of Cd^{2+}. The MI and abnormal cells showed improvement for all concentrations of Cd^{2+} especially the concentration 10^{-6} M Zn^{2+} together with 10^{-5} M Cd^{2+} could produce significant protective effect against the mitoinhibitory effect of Cd^{2+}. The percentage reached 11.88% as compared with the same concentration without Zn^{2+} 7.80% (Table 4). This protective mechanism may be due to the effect of Zn^{2+} on the defense system of organism and protection of cells against harmful factor (Rout and Das, 2003).

Zinc is a micro-nutrient, that is essential in the nutrition of all plants, is mainly combined with metalloenzymes or metallothioneins which regulates and control the activity of zinc enzyme and metabolism of nucleic acid (Cunnane, 1988; Chang *et al.*, 2006). Zn^{2+} is less toxic of all heavy metal although very high concentrations show cytotoxic effects (Chakravarty and Srivastava, 1992; Thirunavukkarasu *et al.*, 2008).

Niu (1995) proved that Zn may inhibit toxicity of Cd^{2+} in mouse cells. Wang (1992) suggested that the inhibition of Zn and Cd^{2+} toxicity resulted from the inhibition or competition of Zn in metallothioneins on Cd^{2+}. When the concentration of Zn^{2+} is higher than Cd^{2+} the replacement of Zn^{2+} by Cd^{2+} is obviously inhibited, thus the activity of Zinc enzyme will not decrease.

In conclusion, from our results it is clear that Ca^{2+}, Se^{4+} and Zn^{2+} plays an important role for improvement of root-growth, MI and abnormal mitosis.

Ca^{2+}, Se^{4+} and Zn^{2+} had obviously protective effects against Cd $^{2+}$induced inhibition of cell division and chromosomal aberrations. The antagonism of Ca^{2+} was strong than Se^{4+} and Zn^{2+} but

Se^{4+} is more than Zn^{2+} against Cd^{2+} toxicity was Ca^{2+}>Se^{4+}>Zn^{2+}. This, indicated that the administration of Ca^{2+}, Se^{4+} or Zn^{2+} were very much helpful in minimizing the mitoinhibition and the clastogenic effect induced by Cd^{2+}.

Finely the present results indicated that the administrations of antioxidant metals such as (Ca^{2+}, Se^{4+} and Zn^{2+}) was very much helpful in minimizing the mitoinhibition and genetic effect induced by chemo-pollutant.

REFERENCES

Abd-El-Salam, A.Z.E., H.Z. Hassan, F.M.I. Badawy and W.M. Abdel-Naby, 1993. The mutagenic potentialities of three pesticides on three biological systems. Egypt. J. Gent. Cytol., 22: 109-128.

Abraham, S. and M.P. Koshy, 1979. Mutagenic potential of green chillies. Cytologia, 44: 221-225.

Ali, G., P.S. Srivastava and M. Iqbal, 2001. Responses of *Bacopa monniera* cultures to cadmium toxicity. Bull. Environ. Contam. Toxico., 66: 342-349.

Alloway, B.J., 1995. Heavy Metals in Soils. 2nd Edn., Chapman and Hall, Glasgow, UK., Pages: 368.

Amer, S.M. and F.A. Aly, 2001. Genotoxic effect of 2,4-dichlorophenoxy acetic acid and its metabolite 2,4-dichlorophenol in mouse. Mut. Res., 494: 1-12.

Amin, A.W., 2001. Heavy metals content in maize as affected by sewage sludge application. ii-cytological effects. Egypt. J. Genet. Cytol., 29: 131-140.

Anderson, S., W. Sadinski, L. Shugart, P. Brussard and M. Depledge *et al*., 1994. Genetic and molecular ecotoxicology: A research frame work. Environ. Health Perspect., 102: 3-8.

Borboa, L. and C. de la Torre, 1996. The genotoxicity of Zn (II) and C(II) in *Allium cepa* root meristematic cells. New Phytol., 134: 481-486.

Brown, H. and M.H. Martin, 1981. Pretreeatment effects of cadmium on the root growth of *Holcus lanatus* L.. New Phytol., 89: 621-629.

Cakmak, I., 2000. Possible roles of zinc in protecting plant cells damage by reactive oxygen species. New Phytol., 146: 185-205.

Chakravarty, B. and J. Srivastava, 1992. Toxicity of some heavy metals *In vivo* and *In vitro Helianthus annuus*. Mutat. Res., 283: 287-294.

Chal, Z.F. and H.M. Zhu, 1983. Microelement. Peoples Medical Press, Beijing, China, pp: 186-202.

Chang, K.L., T.C. Hung, B.S. Hsieh, Y.H. Chen, T.F. Chen and H.L. Cheng, 2006. Zinc at pharmacologic concentrations affects cytokine expression and Induces apoptosis of human peripheral blood mononuclear cells. Nutrition, 22: 465-474.

Chen, S.L. and C.H. Kao, 1995. Glutathione reduces the inhibition of rice seedling root growth caused by cadmium. Plant Growth Regul., 16: 249-252.

Cunnane, S.C., 1988. Zinc: Clinical and Biochemical Significance. CRC Press, Boca Raton, FL, USA., pp: 93-109.

El-Ashry, Z.M., 2003. Vitamin C antimutagenic effect against genotoxicity of cadmium chloride in *Vicia faba*. Egypt. J. Gent. Cytol., 32: 89-99.

El-Enany, A.E., 1995. Alleviation of cadmium toxicity on maize seedlings by calcium. Biol. Plantarum., 37: 93-99.

El-Ghamery, A.A., M.A. El-Kholy and M.A.A El-Yousser, 2003. Evaluation of cytological effects of Zn^{2+} in relation to germination and root growth of *Nigella sativa* L. and *Triticum aestivum* L. Mutat. Res., 537: 29-41.

El-Kodary, S., A. Habib and A. Haleim, 1990. Effect of the herbicide tribunal on root-mitosis of *Allium cepa*. Cytologia, 55: 209-215.

Enger, M.G., E.W. Cambell, R.L. Ratliff, R.A. Tobey, C.E. Hildebrand and R.J. Kissane, 1979. Cadmium-induced alterations in RNA metabolism in cultures of Chinese hamster cells sensitive to and resistant to the cytotoxic effects of cadmium. J. Toxicol. Environ. Health, 5: 711-728.

Ernst, W.H.O., 1998. Effects of Heavy Metals in Plants at the Cellular and Organismic Level Ecotoxicology. In: Bioaccumulation and Biological Effects of Chemicals, Gerrit, S. and M. Bernd (Ed.). Wiley and Spektrum Akademischer Verlag, Heidelberg, Berlin, pp: 587-620.

Fiskesjo, G., 1985. The Allium test as a standard in environmental monitoring. Hereditas, 102: 99-112.

George, N.M., 2000. Evaluation on mutagenic effects of the three heavy metals on *Vicia faba* plants. Cytologia, 65: 75-82.

George, N.M., 2002. Cytogenetic effect of some heavy metals on *Vicia faba*. Egypt. J. Genet. Cytol., 31: 37-46.

Gipps, J.F. and B.A.W. Coller, 1982. Effect of some nutrient cations on uptake of cadmium by *Chlorella pyrenoidosa*. Aust. J. Mar. Freshwater Res., 33: 979-987.

Gomez-Arroyo, S., J. Abarca-Hermandez and R. Vilialobos-Pietrini, 1989. Sister chromatid exchanges induced by cadmium in *Vicia faba*, Contam. Ambiment, 5: 71-82.

Greger, M., E. Brammer, S. Lindberg, G. Larsson and J. Idestam-Almquist, 1991. Uptake and physiological effects of cadmium in sugar beet (*Beta vulgaris*) related to mineral provision. J. Exp. Bot., 42: 729-737.

Gu, G.W. and H.W. Zhang, 1993. Trace elements and malignant tumour. Science and Technology, Shanghai, China, pp: 179-181.

Gustavino, B., E. Vitagliano, A. Sottili and M. Rizzoni, 1987. A comparison between short-tem evolution of micronuclei induced by X-ray and colchicine in root tips of *Vicia faba*. Mut. Res., 192: 109-119.

Hassan, N.H.A., M.A. Fahmy, A.A. Farghaly and E.S. Hassan, 2006. Antimutagenic effect of selenium and vitamins against the genotoxicity induced by cobalt chloride in mice. Cytologia, 71: 213-222.

Hepler, P.K. and R.O. Wayne, 1985. Calcium and plant development. Ann. Rev. Plant Physiol., 36: 397-439.

Hsu, Y.T. and C.H. Kao, 2007. Cadmium-induced oxidative damage in rice leaves is reduced by polyamines. Plant Soil, 291: 27-37.

Ismail, M.A., 2008. Involvement of Ca^{2+} in alleviation of Cd^{2+} toxicity in common bean (*Phaseolas vulgaris* L.) plants. Asian J. Biol. Sci., 1: 26-32.

Kim, C.G., J.N.B. Bell and S.A. Power, 2003. Effects of soil cadmium on *Pinus sylvestris* L. seedlings. Plant Soil, 257: 443-449.

Kinraide, T.B., 1998. Three mechanisms for the calcium alleviation of mineral toxicities. Plant Physiol., 118: 513-520.

Kiran, Y. and A. Sahin, 2006. The effect of cadmium on seed germination, root development and mitotic of root tip cells of Lentil (*Lens culinaris* Medik). World J. Agric. Sci., 2: 196-200.

Li, X.L. and G.R. Zheng, 1992. Study on mutagenic effects and antagonism of selenium and cadmium. Carcinog. Teratogen. Mutagen., 4: 19-21.

Li, Z., A. Hayashemoto and N. Murai, 1992. A sulfonylurea herbicide resistance gene from *Arabidopsis thaliana* as a new selectable marker for production of fertile transgenic rice plants. Plant Physiol., 100: 662-668.

Liu, D.H., W.S. Jiang and M.X. Li, 1992. Effects of cadmium on root growth and cell division of *Allium cepa*. Acta Sci. Circumtantiae, 12: 339-406.

Muneer, S., T.N. Qadri, Mahmooduzaffar and T.O. Siddiqi, 2011. Cytogenetic and biochemical Investigations to study the response of *Vigna radiate* to cadmium stress. Afr. J. Plant Sci., 5: 183-192.

Nadiminty, N. and A.C. Gao, 2009. Selenium and Androgen in Prostate Cancer. In: Androgen Action in Prostate Cancer, Tindall, D. and J. Mohler (Eds.). Springer Publishers, USA., pp: 755-780.

Niu, J.P., 1995. Joint toxic action of 29 pairs metal compounds on isolated rat hepatocytes. J. Environ. Health, 12: 56-58.

Nriagu, J.O., 1990. Global metal pollution poisoning the biosphere?. Environment, 32: 7-33.

Ouzounidou, G., M. Moustakas and E.P. Eleftheriou, 1997. Physiological and ultrastructural effects of cadmium on wheat (*Triticum aestivum* L.) leaves. Arch. Environ. Contam. Toxicol., 32: 154-160.

Pandey, R.M. and S.K. Upadhyay, 2010. Cytological effect of havy metals on root meristem cells of *Vicia faba* L. Toxicolo. Environ. Chem., 92: 89-96.

Pandit, B.R. and P.G. Prasannakumar, 1999. Effect of metals on Jowar (*Sorghum bicolor* L.) seedling growth, germination and absorption of elements. Pollut. Res., 18: 307-315.

Patil, B.C. and G.I. Bhat, 1992. A comparative study of MH and EMS in the induction of chromosomal aberrations on lateral root meristem in *Clitoria ternetea* L. Cytologia, 57: 259-264.

Perfus-Barbeoch, L., N. Leonhardt, A. Vavasseur and C. Forestier, 2002. Heavy metal toxicity: Cadmium permeates through calcium channels and disturbs the plant water status. Plant J., 32: 539-548.

Reddy, T. and K. Vaidyanath, 1978. Mutagenic potentiating and antimutgenic activity of certain metallic ions in the rice genetic system. Current Sci., 47: 513-515.

Rout, G.R. and P. Das, 2003. Effect of metal toxicity on plant growth and metabolism: I. Zinc. Agronomie, 23: 3-11.

Scebba, F., I. Arduini, L. Ercoli and L. Sebastiani, 2006. Cadmium effects on growth and antioxidant enzymes activities in *Miscanthus sinensis*. Biol. Plant., 50: 688-692.

Schrauzer, G.N., 2000. Anticarcinogenic effects of selenium. Cell Mol. Life Sci., 57: 1864-1873.

Schrauzer, G.N., 2008. Effect of selenium and low level of lead a mammary tumor development and growth in MMTV-infected female mice. Biol. Trace Elem. Res., 125: 268-275.

Schutzendubel, A., P. Schwanz, T. Teichmann, K. Gross and R. Langenfeld-Heyser, D.L. Godbold and A. Polle, 2001. Cadmium-induced changes in antioxidativ systems, hydrogen peroxide content and differentiation in Scots pine roots. Plant Physiol., 127: 887-898.

Shahrtash, M., S. Mohsenzadeh, H. Zare and H. Mohabatkar, 2011. Role of calcium in allevIation of cadmium toxicity in maize seedling. Elet. J. Environ. Agric. Food Chem., 10: 2404-2412.

Sharma, A. and K. Sharma, 1980. Chromosome Techniques: Theory and Practice. 3rd Edn., Butterworths, London, pp: 150-151.

Siddiqui, S., M.K. Meghvansi, M.A. Wani and F. Jabee, 2009. Evaluating cadmium toxicity in the root meristem of *Pisum sativum* L. Acta Physiol. Plantarum, 31: 531-536.

Stoeppler, M., 1991. Cadmium. In: Metals and their Compounds in the Environmental. Marian, E. (Ed.). VGH, Weinheim, Germany, pp: 803-851.

Suzuki, N., 2005. Alleviation by calcium of cadmium-induced root growth inhibition in *Arabidopsis* seedlings. Plant Biotechnol., 22: 19-25.

Taspinar, M.S., G. Agar, L. Alpsoy, N. Yildirim, S. Bozari and S. Sevsay, 2011. The protective role of zinc and calcium in *Vicia faba* seedlings subjected to cadmium stress. Toxicol. Ind. Health, 27: 73-80.

Thirunavukkarasu, C., K. Premkumar, A.K. Sheriff and D. Sakthisekaran, 2008. Sodium selenite enhances glutathione peroxidase activity and DNA strand break in hepatoma induced by N.nitrosodiethylamine and promoted by Phenobarbital. Mol. Cell. Biochem., 310: 129-139.

Valdiglesias, V., E. Pasaro, J. Mendez and B. Loffon, 2010. *In vitro* evaluation of selenium genotoxic, cytotoxic and protective effects: A review. Arch. Toxicol., 84: 337-351.

Wang, J., 1992. Trace Elements in Life Science. China Meteorological Press, Beijing, China, pp: 188-207.

Xiong, Z.T. and Y.H. Peng, 2001. Response of Pollen germination and tube like growth to cadmium either special reference to low concentration exposure. Ecotoxicol. Environ. Saf., 48: 51-55.

Xu, Q., 1990. The enrichment of cadmium and its leaching loss in soils of Beijing area with a view to the relationship between Zn and Cd. China J. Environ. Sci., 11: 17-21.

Zengin, F.K. and U.O. Munzuroglu, 2005. Effects of some heavy metals on content of chlorophyll, proline and some antioxidant chemicals in bean (*Phaseolus vulgaris* L.) seedlings. Acta Biol. Cracoviensia Series Bot., 47: 157-164.

Zhang, Y. and H. Xiao, 1998. Antagonistic effect of calcium, zinc and selenium against cadmium induced chromosomal aberrations and micronuclei in root cells of *Hordeum vulgare*. Mut. Res. Genet. Toxicol. Environ. Mutage., 420: 1-6.

Zhang, Y. and X. Yang, 1994. The toxic effects of cadmium on cell division and chromosomal morphology of *Hordeum vulgare*. Mut. Res., 312: 121-126.

Zhang, Y.X., 1997. Toxicity of heavey metals to hordeum vulgare. Acta Sci. Circumstantiae, 17: 199-205.