

## Salicylic Acid Triggers Adaptation Cadmium Cytogenetic Toxicity in Roots of *Nigella sativa* L

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CADMIUM (Cd) is a toxic heavy-metal pollutant in the environment, it is a nonessential element, which strongly inhibits plant growth and development, and causes plant death even at very low concentrations. Root tip cells of *Nigella sativa* were separately treated with different concentrations (5, 10, 25 and 50ppm) of cadmium for 3, 6, 12 and 24h and the results were recorded. The results showed that all concentrations of cadmium reduced the mitotic index and caused a disturbance in the frequencies of mitotic phases. The treatment with 50ppm of metal for 24h was the most effective in reducing the mitotic activity and inducing the highest percentage of mitotic abnormalities. The different types of abnormalities were irregularities, bridges, sickness at different phases, disturbed chromosomes or bi nucleated cells, forward and lagging chromosomes. Also, in this study three concentrations of SA (0.01, 0.1 and 0.2mM) for 6 and 12h were used to recover the cytotoxicity of the cadmium treatment (50ppm for 3 and 24h). This Post treatment with SA resulted in increasing MI and significant reduction of chromosomal abnormalities. These results illustrate the ameliorating effect of SA under stress conditions and reveal that SA is effective in alleviating the toxic effects of heavy metals at all applied concentrations.

**Keyword:** Cadmium (Cd), Heavy metals, Salicylic acid (SA), *Nigella sativa* (*N. sativa*), Mitotic activity (MI), Chromosomal aberrations(CAs).

### Introduction

Cadmium (Cd) is non-essential nutrient elements but toxic heavy metal for biology, especially at high concentrations in soil (Schutzendubel et al., 2001; He et al., 2011 and Rascio & Navari-Izzo, 2011). The cadmium (Cd) level in soil and aquatic environments is increasing with intensive anthropogenic activities, including industrial, agricultural and/or urban development (Li et al., 2012). Cd can be taken up by roots and accumulate at high concentrations in plant tissues (Irfan et al., 2014). This heavy metal, which most likely enters the cell through the existing mineral uptake machinery, also constitutes a serious threat to human health (Lin & Aarts, 2012). Increasing concentrations of Cd in soils represent a threat to plants because of its mobility and phytotoxicity (Wahid et al., 2010). Cadmium may limit plant growth and induce numerous physiological and

metabolic disturbances, both at the whole plant and cellular levels. It is well documented that Cd exposure, for example, can cause growth inhibition related to reduction of mitotic activity, induction of chromosome disorders and nuclear abnormalities in the apical meristems (Liu et al., 2003/2004; Unyayar et al., 2006; Zhang et al., 2009; Qin et al., 2010; Aslam et al., 2014 and Wang et al., 2014). Cd was found to decrease the mitotic index (MI) and induced chromosomal aberrations and micronucleus (MN) formation (El-Ghamery et al., 2001; Aslam et al., 2014 and Wang et al., 2014 and 2016).

In plants, cadmium is an easily absorbed and rapidly translocated heavy metal and causes strong toxicity even at relatively low concentrations (Das, et al 1997). Cadmium characteristically inhibits root growth and cell division in plants such as *Allium cepa*, *tradescantia*, *Vicia faba*

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DOI: 10.21608/ejbo.2018.3049.1159

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(Steinkellner et al., 1998), *Nicotiana tabacum* (Fojtova & Kovarik, 2000). Cd damages the nucleoli in cells of root tip (Nolan et al., 2003 and Wang et al., 2016) and inhibits the DNA repair mechanism (Rossman et al., 1992 and Wang et al., 2014). Numerous experimental studies have shown the genotoxicity of Cd salts (Seoane & Dulout, 2001). Cd induces clastogenic and genotoxic disturbance in plants (Seoane & Dulout, 2001) and inhibits root growth and cell division in different plants (Fojtova & Kovarik, 2000). The possible pathways of Cd-induced genotoxicity may involve the interaction of the metal with DNA and damage the DNA, either directly or indirectly (Valverde et al., 2001 and Bezrukova et al., 2016). At physiological level, Cd directly or indirectly induces reactive oxygen species (ROS) which affects the redox status of the cell and causes oxidative damage to proteins, lipids and other biomolecules (Stojs et al., 2000 and Schützendübel et al., 2001).

Salicylic acid (SA) is a compound that is chemically like aspirin. It functions as a phytohormone, an important factor in environmental stress tolerance in plants (Bosch et al., 2007). Salicylic acid (SA) is an essential component of plant resistance to pathogens and also plays an important role in mediating plant responses to some abiotic stresses (He et al., 2010). The application of SA may either be harmful or provide protection during abiotic stress, depending on the plant species, concentration and the mode of application (Horváth et al., 2007). The ameliorating effect of SA treatment on seed germination and seedling growth was also shown during  $Pb^{2+}$  or  $Hg^{2+}$  stress in rice (Mishra et al., 1997). Later, much more attention was attracted by the role of SA in Cd tolerance (Krantev et al., 2008 and Popova et al., 2009). SA produces an ameliorative protective effect in plants in response to abiotic stress, such as metal toxicity, heat, chilling, osmotic and salt stress (Borsani et al., 2001; Janda et al., 2001.; Singh & Usha, 2003.; Wang & Li, 2006; Gunes et al., 2007; He & Zhu, 2008; Szepesi et al., 2008; Ivanova et al., 2008; Popova et al., 2009 and Gondor et al., 2016) and drought (Bechtold et al., 2010). SA also elevates negative action of the stress-inducing factors (Horvath et al., 2007 and Hayat et al., 2010). The considerable interests have been focused on SA due to its ability to induce a protective measure on plant under stress factors (Sakhabutdinova et al., 2003). Exogenous SA application may be responsible

for activation of defense genes (El-Tayeb et al., 2006) and it is supposed that exogenous SA as antioxidants may have wider application as means for the simultaneously increasing resistance not to one but to several stress-inducing factors. SA may be applied in agriculture for increasing the plant production quality. The majority using the SA as protective agent before the treatment plant with abiotic factor as recorded by many investigators (Umebese et al., 2013; Jyothsna & Murthy, 2016 and Abdul Halim & Phang, 2017). This work deals with the effect of cadmium chloride on the course of mitosis and on cell divisions in *Nigella sativa* (black seed) root tip meristem cells as well as with protective effect of SA on these processes.

### Materials and Methods

In this investigation *Nigella sativa* ( $2n=12$ ) plant used as test plant. This plant considered to be a good material for cytological studies as it has relatively low number of chromosomes of suitable sizes with good stain ability and a marker telocentric. The seeds were kept soaked for some time in distilled water followed by Sodium hypochloride solution for 2min. Thereafter the seeds were thoroughly washed with running water. Presoaked seeds were allowed to germinate.

#### To study the toxic effect of cadmium

Ten germinated seeds of *Nigella sativa* with radicle 2-3cm length, were treated with different concentrations of  $CdCl_2$  (5, 10, 25 and 50ppm) for 3, 6, 12 and 24h. Control germinated seeds were placed in distilled water.

#### To study the effect of salicylic acid

Ten germinated seeds of *Nigella sativa* with radicle 2-3cm length, were treated with different concentrations of salicylic acid (0.0, 0.01, and 0.2mM) for 6 and 12h. Control germinated seeds were placed in distilled water.

#### To study the recovering effects of cadmium by salicylic acid ( $C_7H_6O_3$ )

Root tip cells were treated with 50ppm concentration of cadmium for 3 and 24h soaked in different concentrations of salicylic acid (SA) (0.0, 0.01, and 0.2mM) for 6 and 12h.

#### Mitotic preparation

After each of the three treatments (1, 2 and 3), the roots were cut off and immediately fixed in glacial acetic acid: absolute ethyl alcohol (1:3 v/v)

for overnight (Qian, 2004). Root tips were stained by using the Feulgen squash technique (Darlington & La Cour, 1976). At least three slides for each treatment were examined to determine the mitotic index (MI), and the frequency of mitotic phases. Dividing cells in the same slides were analyzed for determination of the percentage of different types of abnormalities and their total percentages of abnormalities were also calculated.

#### Statistical analysis

Each treatment was made in three replicates. For statistical analysis, one-way ANOVA (Sigma Plot13.0 software) SPSS was used to determine significance at  $P < 0.05$  (Duncan, 1955).

#### Results

The effects of different concentrations of  $\text{CdCl}_2$  (5, 10, 25, and 50ppm) for different durations (3, 6, 12 and 24h) on mitotic index of *Nigella sativa* L. given in Table 1 and represented in Fig.1. In general, the mitotic index values reduced in the treated roots was a dose and time dependent

increased with increasing concentrations from 5 to 50ppm. It was also observed that, with the same concentration, mitotic index decreased with prolonging treatment period. Thus, after the treatment with 50ppm concentration at 24h the mitotic index reached to the lowest value of 4.75% compared the control value of 13.41%. Also, the results in Table 1 showed that the percentage of each mitotic phase in treated root tips of *N. sativa* L. was changed following the treatments with the  $\text{CdCl}_2$  and did not depend on concentration and time of treatment. The results in Table 2 and Fig.2 showed the percentages of total abnormal mitotic cells induced in treated root tips increased with increasing treatment times and concentrations and the total percentage of abnormalities at prophase stage was higher than those at the other mitotic stages. The total percentage of abnormalities induced at metaphase was higher than that in anaphase and telophase for all treatments. In the same manner, the total percentage of abnormalities induced at anaphase stage was higher than that present in telophase for all treatments.

**TABLE 1. Mitotic index and mitotic phases of *N. sativa* L. meristematic cells exposed to different concentrations of cadmium chloride after 3, 6, 12 and 24 hours.**

Treatments		Counted cells	Mitotic index (MI±S.E)	Mitotic phases %			
Time (h)	Conc. (ppm)			Prophase	Metaphase	Anaphase	Telophase
3	Control	1680	13.39 ± 0.3	48.88	22.23	15.55	13.34
	5	1668	13.19 ± 0.06	49.54	23.64	14.09	12.73
	10	1660	12.65 ± 0.04	50.95	24.28	13.34	11.43
	25	1645	12.15 ± 0.08*	51.50	24.50	13.00	11.00
	50	1635	11.31 ± 0.02*	54.05	25.40	11.90	8.65
6	Control	1695	13.57 ± 0.5	49.56	23.92	15.22	11.30
	5	1678	12.81 ± 0.03	51.17	24.65	13.95	10.23
	10	1650	11.82 ± 0.02*	53.33	25.64	11.28	9.75
	25	1625	10.09 ± 0.05*	57.93	27.44	9.15	5.48
	50	1610	9.19 ± 0.01*	59.46	27.70	8.78	4.06
12	Control	1675	13.25 ± 0.01	50.45	21.62	14.86	13.06
	5	1655	11.84 ± 0.02	53.57	22.96	12.75	10.72
	10	1630	10.12 ± 0.05*	56.36	24.25	10.30	9.09
	25	1615	9.10 ± 0.03*	57.82	25.85	8.85	7.48
		1600	6.56 ± 0.05*	61.90	28.57	5.73	3.80
24	Control	1670	13.41 ± 0.06	51.35	21.87	14.28	12.50
	5	1650	10.30 ± 0.01*	55.29	23.53	11.76	9.42
	10	1620	8.02 ± 0.04*	57.70	26.92	8.46	6.92
	25	1590	6.28 ± 0.05**	60.00	28.00	7.00	5.00
	50	1580	4.75 ± 0.03**	64.00	29.33	4.00	2.67

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

**TABLE 2. Percentages of abnormal cells in different mitotic stages and total abnormalities after treating *Nigella sativa* L root tip cells with different concentrations of CdCl<sub>2</sub> for different treatment times**

Treatments	Time (h)	Conc. (ppm)	Total abnormal % (X ± S.E)	Abnormal mitotic phases %							% of aberrations type													
				Prophase	Metaphase	Anaphase	Telophase	Irreg.	St.	Brid.	Lagg.	For.	Dis.	c-met.	Bi.	Micro.								
		Control	0.88 ± 0.15	0.88	0	0	0	0.88	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
		5	4.54 ± 0.12	2.27	1.36	0.91	0	2.27	0	0.45	0.46	0	0.91	0.45	0	0	0	0	0	0	0	0	0	
3		10	10.47 ± 0.02*	4.76	2.38	2.38	0.95	4.67	1.44	0.48	1.42	0.48	0.94	0.48	0.47	0	0	0	0	0	0	0	0	0
		25	18.00 ± 0.03*	8.00	4.50	3.50	2.00	6.00	5.50	1.00	2.00	0.50	1.50	1.00	0.50	0.14	0	0	0	0	0	0	0	0
		50	24.32 ± 0.05*	10.81	5.40	5.40	2.71	8.10	8.11	2.16	1.62	0.54	2.16	1.08	0.55	0.13	0	0	0	0	0	0	0	0
		Control	1.30 ± 0.16	1.30	0	0	0	1.30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6		5	6.97 ± 0.22	2.33	2.32	2.32	0	2.33	1.38	0.92	0.92	0	0.92	0.46	0	0.06	0	0	0	0	0	0	0	0
		10	16.92 ± 0.05*	5.13	5.13	4.10	2.56	4.10	6.14	2.05	1.02	0	2.05	1.02	0.13	0	0	0	0	0	0	0	0	0
		25	25.60 ± 0.03*	10.97	6.09	4.88	3.66	9.14	8.54	2.44	1.83	0.61	2.44	0.61	1.22	0.13	0	0	0	0	0	0	0	0
		50	33.78 ± 0.05**	16.90	8.10	6.75	2.03	13.5	12.20	3.39	1.35	0	3.39	0	0	0	0	0	0	0	0	0	0	0
		Control	1.80 ± 0.14	0.90	0.90	0	0	0.90	0	0	0	0	0.90	0	0	0	0	0	0	0	0	0	0	0
		5	10.20 ± 0.05*	4.08	2.04	2.04	2.04	4.08	2.04	1.02	1.02	0	1.02	0	1.02	0	0	0	0	0	0	0	0	0
12		10	21.21 ± 0.02*	9.09	6.06	3.03	3.03	5.45	9.68	1.21	1.81	0.62	1.82	0	0.62	0	0	0	0	0	0	0	0	0
		25	40.81 ± 0.03**	17.00	13.61	6.80	3.40	10.2	19.04	2.04	4.76	0	3.40	0	1.36	0	0	0	0	0	0	0	0	0
		50	57.14 ± 0.04**	28.57	19.04	5.73	3.80	16.2	28.57	2.86	0	0	9.52	0	0	0.13	0	0	0	0	0	0	0	0
		Control	1.78 ± 0.06	1.78	0	0	0	1.78	0	0	0	0	1.78	0	0	0	0	0	0	0	0	0	0	0
		5	20.58 ± 0.03**	5.88	5.88	5.88	2.94	4.12	9.40	2.35	2.34	0	1.17	1.17	0	0.13	0	0	0	0	0	0	0	0
24		10	43.85 ± 0.01**	15.39	13.08	8.46	6.92	8.46	18.47	3.07	5.38	0	3.85	1.53	3.07	0	0	0	0	0	0	0	0	0
		25	55.00 ± 0.03**	28.00	15.00	7.00	5.00	16.0	32.00	2.00	0	0	5.00	0	0	0	0	0	0	0	0	0	0	0
		50	72.00 ± 0.04**	46.66	18.67	4.00	2.67	26.6	36.00	0	0	0	9.34	0	0	0	0	0	0	0	0	0	0	0

S.E., Standard error; \* Significant at 5% level (P≤0.05); \*\*Significant at 1% level (P≤0.01).

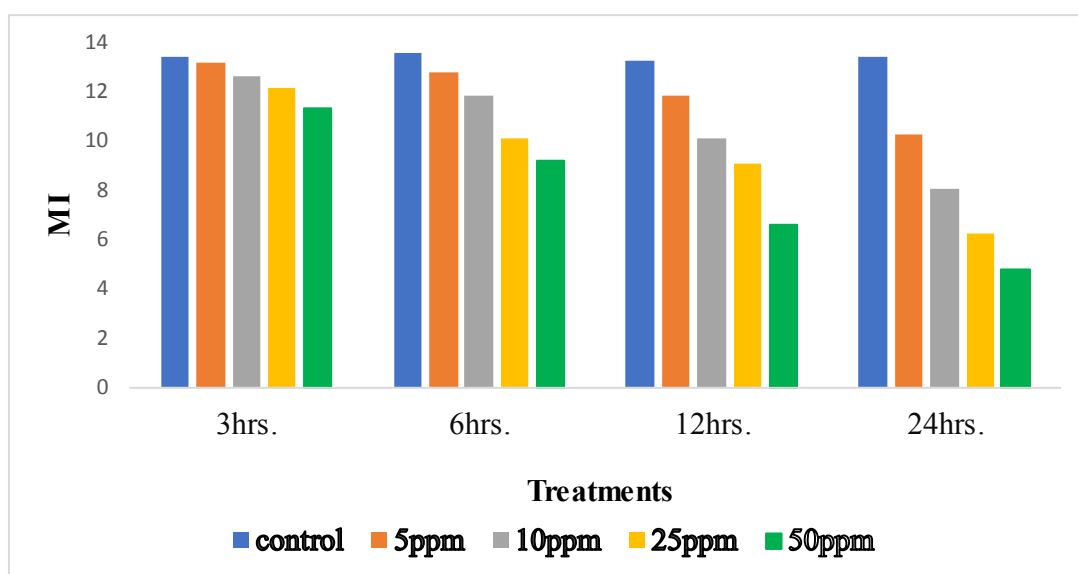


Fig. 1. Diagrammatic representation effect of different concentrations (5 to 50ppm) of  $\text{CdCl}_2$  on mitotic index of *Nigella sativa* L. seeds after four treatment periods (3, 6, 12 and 24h).

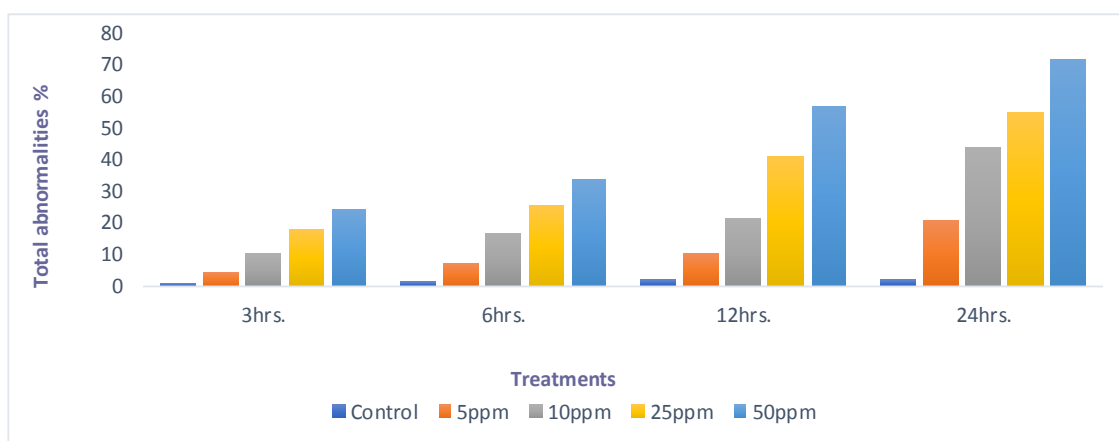


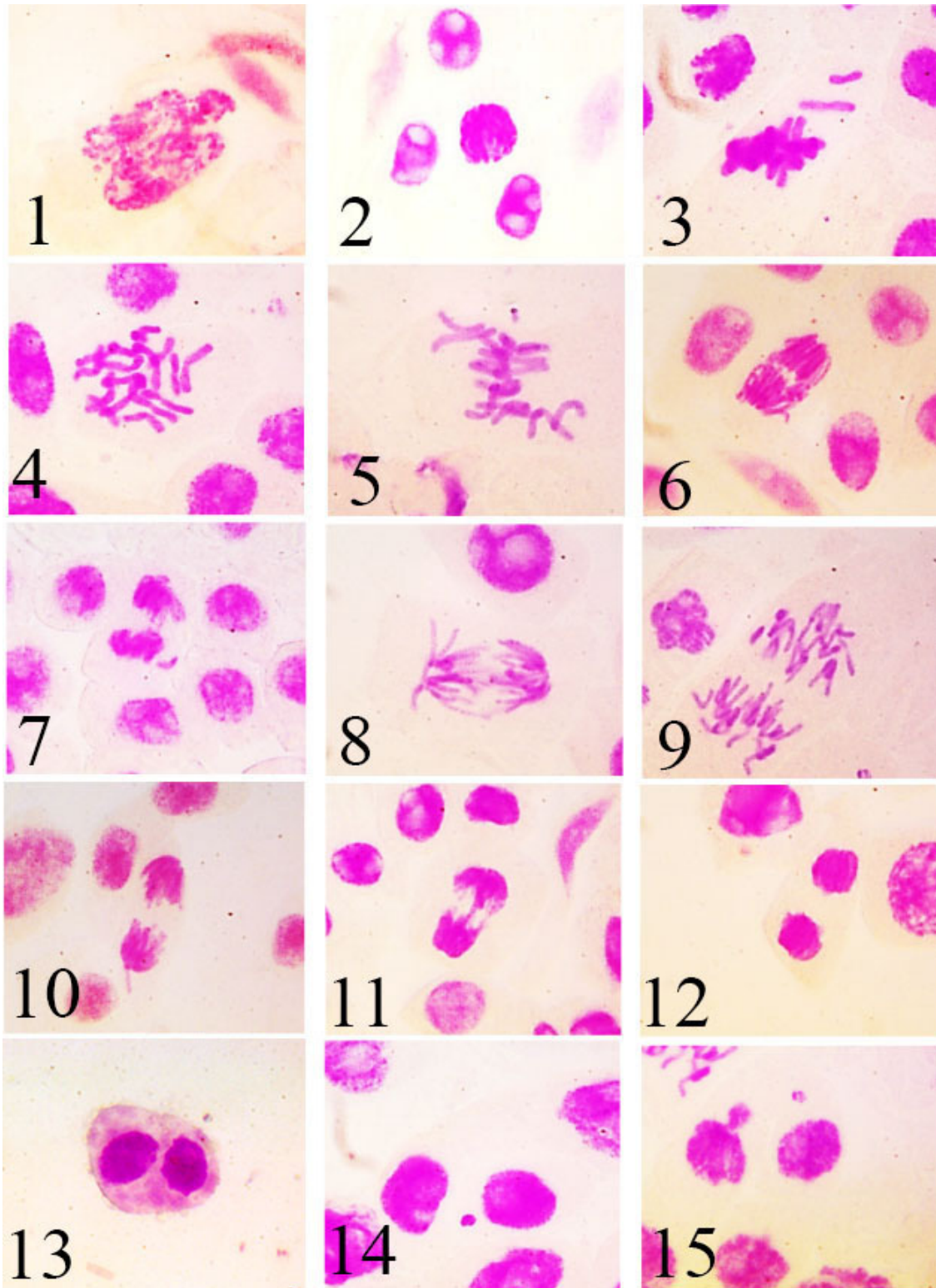
Fig. 2. Effect of different concentrations (5 to 50ppm) of  $\text{CdCl}_2$  on total abnormal cells of *Nigella sativa* seeds after four treatment periods (3, 6, 12 and 24h).

Cadmium induced irregular prophase, disturbed metaphases, c-metaphase, chromosome bridges, chromosome stickiness, laggards, forward chromosome, bi-nucleated cell and micronuclei, which are biomarkers of genotoxic events and chromosomal instability (Table 2 and Fig. 3 {1-15}).

In this study the results showed that, all concentrations of using SA caused an increased in MI in treated root tips compared with untreated root tip cells (control) for each period. This increase more clearly at the concentration (0.01mM), when MI recorded was 15.05 and 14.86 for 6 and 12h, respectively. Also, all applied concentrations of SA

caused elevate in total abnormal cells percentages and the concentration (0.1mM) was the lowest one and caused minimal increase in total abnormalities percentage in treated root tip cells. (Table 3).

Result presented in Table 4 clearly state that, using SA post-treatment caused a significant increase of MI at high concentrations (0.1-0.2mM) in all treated root tip cells. The improvement was more prevalent at higher concentrations of SA in root tips treated with  $\text{CdCl}_2$  for 24h. On contrast, the lower concentration of SA (0.01mM) for both duration of recovery caused a non-significant increase of MI in treated cells with  $\text{CdCl}_2$  for 3h, and for 24h.



**Fig. 3.** Chromosome and cellular aberrations observed in *N. sativa* L root meristem exposed to  $\text{CdCl}_2$ . (1) Irregular prophase, (2) Sticky prophase, (3) Un-oriented chromosomes, (4) C- metaphase, (5) Disturbed metaphase, (6) Bridges at anaphase, (7) Anaphase with chromatinal bridge and lagging chromosome, (8) Anaphase with multi bridge and forward chromosomes, (9) Disturbed anaphase, (10) Late anaphase, (11) Sticky anaphase with broken bridge, (12) Sticky telophase, (13) Bi nucleated cell, (14) Micronucleus and (15) Two cell with micronucleus.

**TABLE 3. Effect of different concentration of salicylic acid on *N. sativa* L root tip cells for different durations.**

Treatments	Salicylic acid concentrations (mM)							
	6h				12h			
	Cont.	0.01	0.1	0.2	Cont.	0.01	0.1	0.2
Mitotic index	13.02	15.05*	14.11*	13.68	13.42	14.86*	14.63*	13.81*
Total abn. %	0.44	4.18*	2.67	3.18	0.50	6.25*	3.83	5.02*

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

**TABLE 4. Effect of different concentration of salicylic acid on treated cells of *N. sativa* L with cadmium chloride (50ppm) for different durations.**

Treatments	Concentrations	50ppm CdCl <sub>2</sub> for 3h		50ppm CdCl <sub>2</sub> for 24h	
		Mitotic index	Total abn. %	Mitotic index	Total abn. %
		Time (h)	Control	50 ppm CdCl <sub>2</sub> (A)	A+SA (0.01mM)
6	Control	13.02	0.44	13.42	0.50
	50 ppm CdCl <sub>2</sub> (A)	11.31	24.32	4.75	72.00
	A+SA (0.01mM)	11.40	21.22*	5.03	69.68
	A+SA (0.1mM)	13.28*	19.66**	7.33**	65.43**
	A+SA (0.2mM)	12.05*	20.45*	5.95**	67.05**
12	A+SA (0.01mM)	11.47	20.50*	6.11	68.88
	A+SA (0.1mM)	13.67**	16.80**	9.50**	59.85**
	A+SA (0.2mM)	12.25*	18.77**	7.25*	63.13**

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

Application of SA after CdCl<sub>2</sub> exposure resulted in the significant reduction of chromosomal abnormalities as shown in the Table 4. In treated cells with CdCl<sub>2</sub> for 3h, the recovery with SA for 6h, caused more than 10% decrease in CAs at 0.01 when the total abnormalities values were 21.22% compared with treated root tips of 24.32% but, the recovery with SA for 12h, caused more than 15% decrease in CAs at 0.01 when the total abnormalities values were 20.50%. In the same manner, the results showed greatest adaptive response to SA 0.1 and 0.2mM concentrations at which, SA caused more than 20% decrease in CAs in the root tip cells. Also, treated cells with CdCl<sub>2</sub> for 24h, the recovery with SA caused maximum decreased in CAs (more than 10%) at 0.1mM concentration of both duration of recovery.

## Discussion

Cadmium (Cd) is a strong environmental pollutant with high toxicity to animals and plants.

Positive results monitored in higher plant like the *N. sativa* indicate the presence of cytotoxic and/or genotoxic attributes of some heavy metals such as Cd. This can also be used to monitor ameliorative and protective effects of some other compounds such antioxidants.

In this study, the results revealed the different concentrations of cadmium applied for different periods caused a reduction in the MI values. This effect a time and a dose dependent. Similar effects of cadmium on the MI were reported in different plants such as in *Lactuca sativa*, *Eruca sativa* and *Coriaudrum sativum* (El-Ghamery et al., 2001), *Phaseolus vulgaris* (Çavusoğlu et al., 2009), in *Lens culinaris* (Kiran & Şahin, 2006), *Pisum sativum* (Fusconi et al., 2007), *Triticum aestivum* (Bezrukova et al., 2016), *Capsicum annum* (Aslam et al., 2014), *Vigna radiatae* (Munee et al., 2011), *Vigna unguiculata* (Thangaraja et al., 2013) *Vicia faba* (Li & Zheng, 1992; George, 2000; El-Ashry et al., 2012 and Arya & Mukherjea 2014),

in *Allium cepa* (Zou et al., 2012; Wang et al., 2014 and 2016 and Nataliya, 2015) and *Hordeum vulgare* (Shi et al., 2017). The decline of MI suggested that the Cd could prevent cells from going into cell division (Unyayar et al., 2006). In the same manner Seth et al. (2008) suggested that exposure to Cd prevented cells entering cell division phases, which then resulted in a decrease in MI. The reduction of MI in treated roots is probably due to disturbances in the cell cycle as well as chromatin function, which is induced by interactions between DNA and the heavy metal used (Çavusoğlu et al., 2009 and Wang et al., 2014). In this concern, Thangaraja et al. (2013) reported that the decrease in mitotic index is a result of cytotoxic effects.

The analysis for the number of cells in each phase of mitosis showed change in root treated with cadmium compared with untreated roots which causing accumulation cells at prophase and metaphase stages. In this respect, Bezrukova et al. (2016) reported that, decrease in cells at anaphase reflecting a reduction in MI and cadmium induces cell cycle checkpoints defects in the tested plant root tip cells.

The experimental findings of the present study showed that CdCl<sub>2</sub> can induce significant chromosomal abnormalities in the root meristem cells of *Nigella sativa* L as compared to control, indicates the genotoxic potential of heavy metal used. The results are in agreement with the results recorded in other plants by many investigators such as Li & Zheng (1992), George (2000), Kiran & Şahin (2006), Fusconi et al. (2007), Çavusoğlu et al. (2009), Munee et al. (2011), El-Ashry et al. (2012), Zou et al. (2012), Thangaraja et al. (2013), Aslam et al. (2014), Wang et al. (2014 and 2016), Nataliya (2015), Bezrukova et al. (2016) and Shi et al. (2017). They demonstrated the potential cytotoxic and genotoxic of cadmium in their tested plants meristematic cells exposed to different concentrations of cadmium. The aberrant mitotic stages might have been the outcome of spindle poisoning that cause chromosome disturbances during mitotic cell division (Zou et al., 2012). The total percentages of the abnormalities increased gradually with increase the heavy metal concentration and as the time of treatment prolonged. In general, the highest percentages of abnormalities induced by the CdCl<sub>2</sub> in all treatments were recorded in divided cells at prophase and metaphase stages in tested plant.

Different types of mitotic abnormalities were observed in *Nigella sativa* L root tip cells after treatments with CdCl<sub>2</sub>. The variation in the percentages of chromosomal aberration in this study was recorded and the total abnormalities previously rapidly by increasing the concentration (El-Ashry et al., 2012; Zou et al., 2012; Thangaraja et al., 2013; Wang et al., 2014 and 2016; Aslam et al., 2014; Nataliya, 2015; Bezrukova et al., 2016 and Shi et al., 2017). In *Nigella sativa* L these types were: Irregular prophase, stickiness at different phases, disturbed metaphase, C- metaphase, bridges, forward chromosome and lagging in addition to other types recorded in low frequency in some treatments such as bi-nucleated cells and micronucleus. Of these types of aberrations, stickiness, as a most frequent type, was recorded in the different mitotic phases in root tip cells of tested plant treated with CdCl<sub>2</sub>. This type of abnormality was recorded by many investigators following the treatment of different plants by cadmium (Zou et al., 2012; Wang et al., 2014 and 2016; Aslam et al., 2014 and Bezrukova et al., 2016).

In this study, in forms of irregular prophase (Fig. 3 {1}), where the DNA in chromatin threads may be despiraled and/or depolarized or the chromatin threads were not typically arranged, was a common type of abnormalities induced in treated root tip cells *N. sativa* L by heavy metal used and was recorded in a relatively high percentage in all treatments. The percentage of irregular prophase were higher than those in other types of stages. Formation of irregular prophase resulted from the its effect on the process of individualization of chromatin threads to normal chromosomes as stated by (Wang et al., 2014). The induction of sticky chromosomes (Fig. 3 {11 and 12}), indicates the toxic effect of cadmium on the organization of chromatin. This property is related to a disturbed balance of histones or other proteins that are responsible for controlling the proper structure of nuclear chromatin; generally, this imbalance leads irreversibly to cell death (El-Ghamery et al., 2003). Also, the induction of sticky chromosomes and laggard chromosomes (Fig. 3 {7}), are indicators of spindle poisoning (Onyemaobi et al., 2012). Bridges at anaphase (Fig. 3 {6 and 8}), can result from the terminal deletion or loss of telomeres. According to suggestion of El-Ghamery et al. (2003), chromosomal bridge could be a result of the failure of free anaphase separation, unequal translocation or inversion of chromosome segments.



The abnormal C-metaphases (Fig. 3 {4}) were formed because of the complete inactivation of division of the spindle (Fernandes et al., 2007). Consequently, arrest of cells in metaphase stage might be one of the causes of mitotic inhibition. C-metaphase induction could be due to inactivation of spindle followed by random scattering of chromosomes. The C-metaphase that we observed in treated meristems suggests that Cd<sub>2</sub> act on the mitotic spindle apparatus, probably interfering with the polymerization and depolymerization of microtubules (Seth et al., 2008).

Inhibition of cytokinesis following telophase was responsible for bi-nucleated cell formation visible in the next interphase of a new cell cycle (Fig. 3 {13}). Some authors suggested that phragmoplast inhibition at the early stage of telophase is the responsible disturbance for binucleated cell formation (Fiskesjö, 1997; Rank et al., 2002 and Majewska et al., 2003).

Micronuclei (Fig. 3 {14 and 15}) were observed after Cd treatments. The formation of micronucleus implies that CdCl<sub>2</sub> causes damage to DNA (Gabara et al., 1995 and Fusconi et al., 2007). Micronucleus can be derived from acentric fragments involving clastogenic activity, or from entire chromosomes involving aneugenic activity. This means that Cd is clastogens that induce chromosome breaks and/or aneugens explaining lagging chromosomes. This results in Similar with Fusconie et al. (2007) and Wang et al. (2014 and 2016). They recorded micronuclei formation following treatments their tested plants with cadmium.

Forward chromosome(s) (Fig. 3 {8}) have been described as a weak C-mitotic effects indicating risk of aneuploidy (Fiskesjö, 1985 and 1988). In this type, the arms of chromosomes pointed outward to the pole instead of centromere during the chromosome movement at ana-telophases.

SA complete growth inducing properties are reported barley roots under Cd treatment (Matewally et al., 2003). The inhibition of growth under Cd treatment could be due to the inhibition of cell division and elongation rate of cells that mainly occurs by an irreversible inhibition proton pump responsible for the process (Liu et al., 2003/2004). In this investigation the results showed that, all concentrations of using SA

caused an increase in MI to a highest value of MI 15.05 which recorded by 0.01mM concentration for 6h compared with control value of 13.03. This observation contrasted with the results obtained by Mahfouz et al. (2014) which demonstrated a dose dependent decreased in MI of *Allium cepa* treated with different concentration of SA for different durations treatments. In this respect, He et al. (2010) reported that mitosis was one of the reasons for growth inhibition in the presence of Cd, and reduction in mitotic index of root tips is a concentration dependent. The pretreatment with SA increased the mitotic index. The increase of the mitotic index under Cd stress by SA pretreatment is probably related to shortening the phases of G<sub>2</sub> and prophase or facilitating the abnormal spindle formation (He et al., 2008). Also, increased the percentage of total abnormalities in treated root tips to the value of 6.25 compared with the value of control. The results obtained from the present investigation revealed that exogenous SA ameliorate the toxic effect of cadmium chloride. SA has been shown to alleviate the harmful effects of Cd in maize and pea plants (Krantev et al., 2008, Popova et al., 2009 and Drazic & Mihailovic, 2005). However, SA can also be a stress factor inducing oxidative stress when its concentration differs from the optimum level. The results obtained by Gondor et al. (2016) were demonstrated that the effect of exogenous SA depends greatly on its form, i.e., whether it is applied as an acid (SA) or a Na salt (NaSA). The mode of application may also be important, since different combinations may result in fundamentally different effects even at the same concentration.

At cytological level, SA post-treatment resulted in significant decrease in the percentage of cells with chromosomal abnormalities (CAs) from 24.32% to 19.66% for *N. sativa* L treated root tips with cadmium chloride (50ppm) for 3h and the period recovery 6h and from 72.00% to 59.85% for *N. sativa* treated root tips with CdCl<sub>2</sub> (50ppm) for 24h and the period recovery 12h which implies that SA conditioning conferred adaptive response to genotoxic stress of CdCl<sub>2</sub>. These results are in accordance with the earlier reports demonstrate that, SA-induced protective response to DNA damaging agents (Bautz & Freese, 1960 and Patra et al., 2005). Earlier results also suggested that SA treatment led to a decrease in oxidative injuries induced by Cd (Krantev et al., 2008; Popova et al., 2008 and Panda & Patra,

2007). The production of H<sub>2</sub>O<sub>2</sub> during abiotic stress serves as part of the signaling cascade, and increased levels of ROS might interact with SA. Responses to genotoxic stress include activation of distinct stress signaling pathways, delay of cell cycle progression, and induction of DNA repair (Albinsky et al., 1999). The application of SA provide protection during abiotic stress, depending on the plant species, the concentration and the mode of application (Horváth et al., 2007). In contrast, Mahfouz et al. (2014) reported several damages in *Vicia faba* after application high concentrations of SA.

### **Conclusion**

The experimental findings from the present study prove that SA is effective in inducing the adaptive response to cytotoxic and genotoxic stress since it significantly enhancement the MI values which decrease under cadmium treatments. Also, all applied concentrations of SA reduces the total percentage of abnormalities induced by the CdCl<sub>2</sub>. Efforts to understand the different mechanisms by which SA influence biological activities could help to better distinguish the advantages and disadvantages of its use and to clarify its possible protective role against heavy metals.

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(Received 28/ 2/2018;  
accepted 25/ 6/2018)

Zou, J., Yue, J., Jiang, W. and Liu, D. (2012) Effects of cadmium stress on root tip cells and

### تحفيز نبات حبة البركة للتأقلم مع السمية الوراثية للكادميوم بواسطة حمض الساليسيليك

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الكادميوم (Cd) هو عبارة عن مادة سامة من المعادن الثقيلة ملوثة للبيئة وهو عنصر غير أساسي، والذي يحول دون نمو النبات، ويسبب موت النبات حتى في تركيزات منخفضة للغاية. تم التعامل مع القمم النامية لجذور نبات حبة البركة بشكل منفصل مع تركيزات مختلفة (5، 10، 25 و 50 جزء في المليون) من الكادميوم لمدة 3 و 6 و 21 و 42 ساعة وسجلت النتائج. وأظهرت النتائج أن جميع تركيزات الكادميوم قللت معدل الإنقسام الميتوزى وتسببت في حدوث خلل في ترددات المراحل الأنقسامية. كانت المعاملة باستخدام 50 جزء من المليون من المعدن لمدة 42 ساعة هي الأكثر فعالية في الحد من النشاط الإنقسامى وإحداث أعلى نسبة من التشوهات الأنقسامية. كانت الأنواع المختلفة من التشوهات الناتجة من المعاملات تتمثل فى: كروموسومات غير تنظيمية، أو جسور، أو اللزوجة في المراحل المختلفة، أو كروموسومات مضطربة أو خلايا ثنائية النواة، أو كروموسومات متخلفة. أيضا، في هذه الدراسة تم استخدام ثلاثة تركيزات من حمض الساليسيليك (0.01، 0.1 و 0.2 ملم) لمدة 6 و 21 ساعة لعلاج السمية الخلوية الناتجة عن المعاملة بالكادميوم (50 جزء في المليون لمدة 3 و 42 ساعة). هذا العلاج بحمض الساليسيليك أسفر عن زيادة معدل الإنقسام الميتوزى وخفض كبير في تشوهات الكروموسومات. توضح هذه النتائج التأثير المحسن لحمض الساليسيليك في ظروف الإجهاد وتكشف أن حمض الساليسيليك فعال في التخفيف من التأثيرات السامة للمعادن الثقيلة في جميع التركيزات المطبقة.