The toxic effect of aluminum on cell division and chromosome morphology of black seeds plant (*Nigella sativa* L.) in relation to seed germination and radicle growth

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Abstract: The effect of aluminum (Al^{3+}) was evaluated in black seeds plant (*Nigella sativa* L.). Different aluminum concentrations as well as different exposure times were applied to investigate cytogenetical alterations in black seeds plant (*Nigella sativa* L.) meristem cells. Different concentrations of Al^{3+} ($AlCl_3.6H_2O$) ranging from 200 to 5000 ppm were tested for different durations of 6, 12, 18, 24 and 36h. In this plant, following the treatments, germination percentage, radicle growth and mitotic index were declined by increasing the concentration and treatment duration. Radicle length was depressed but not blocked by the applied concentration. The treatments with the concentrations more than 3000 ppm for 36h were toxic and nearly inhibited cell division. The inhibition of mitotic activity induced by Al^{3+} is paralleled with a reduction in germination percentage and root growth. There was a gradual increase in metaphase frequency in all treatments with increasing concentration and treatment time. Increase metaphase frequency was accompanied by deceased frequency of the other mitotic division stages. The mitotic investigation of dividing cells in root tip cells of the studied plant revealed an abundance of dose-dependent of abnormalities for each treatment. Al^{3+} induced a number of abnormalities as dominant types. These types include irregular prophase, vacuolated nucleus, stickiness, disturbed meta- and anaphase, C-meta- and C-anaphases, laggards, chromosomal bridges. In addition, less frequent types were also recorded such as diagonal, fragment, unequal distribution and micronuclei. In conclusion, aluminum is significantly stressful in *Nigella sativa* L. culminating in morphological and cytological alterations.

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1. Introduction

Heavy metals are significant environmental pollutants and their toxicity is a problem of increasing significance for ecological, evolutionary, nutritional and environmental reasons (Nagajyoti *et al.*, 2010 and Jaishankar *et al.*, 2014). The increase of heavy metals potential in nature and industrial environment cause mutagenic and carcinogenic effects (Pandey and Upadhyay, 2010). Heavy metals toxicity in plants varies with plant species, specific metal, concentration, chemical form and soil composition and pH, as many heavy metals are considered to be essential for plant growth. Plants are stationary, and roots of a plant are the primary contact site for heavy metal ions. Aluminum, as a light metal, is sometimes counted as heavy metal in view of its toxicity (Blann and Ahmed 2014).

Aluminum element (Al) is the third most abundant metallic element found in the earth's crust after oxygen and silicon (Gupta *et al.*, 2013). Acid toxicity is mainly caused by a lack of essential nutrients in the soil and excessive toxic metals in the plant root zone. Of the toxic metals, aluminum is the most prevalent and most toxic. Aluminum is one of the most abundant minerals in the soil; comprising approximately 7% (Biao *et al.,* 2013). Saxena and Misra (2010) considered aluminum toxicity as a major inhibitor on limiting plant growth that affects large agricultural areas, which resulted in decrease crop yields principally in acid aluminum has no biological role and is a toxic non-essential metal to microorganisms (Olaniran *et al.*, 2013).

A large part of the literature dealing with effects of Al on plant growth refers to Al as a non-essential and toxic element. The best descriptive symptom of Al toxicity in plants as indicated by Foy (1974) appears as inhibition of root growth, which can only be explained by an inhibitory effect of Al on cell division. It is well known that roots are the primary target of aluminum phytotoxic (Doncheva *et al.*, 2005 and Li *et al.*, 2015), in which Al exposure causes inhibition of cell elongation and cell division, leading to root stunting accompanied by reducing water and nutrient uptake (Panda *et al.,* 2009). The initial and most dramatic symptom of Al toxicity is inhibition of root elongation or root growth (Barceló and Poschenrieder, 2002) where the Al inhibited root cell division after hours or even days of exposure, thus

reducing water and nutrient uptake, consequently resulting in poor plant growth and yield (Wang *et al*., 2006). The major consequences of Al exposure are the decrease of plant production and the inhibition of root growth. The root growth inhibition may be directly/indirectly responsible for the loss of plant production (Silva, 2012). Root elongation is a reliable parameter used in the bioassay and quantitative of heavy metals toxicity, because root growth is particularly sensitive to toxic metals (Sahi and Singh, 1996).

The works related to aluminum effect seed germination, root growth and cell division have been mainly undergone in some species like *Allium cepa* (Fiskesjö, 1988 and Qin *et al*., 2010), *Allium sativum* (Roy *et al.,* 1989), *Triticum aestivum* (Aniol, 1995), *Vicia faba* (Zhang, 1995), *Arabidopsis thaliana* (Richards *et al.,* 1998), *Sorghum bicolor* (Peixoto *et al.,* 2001), *Hordeum vulgare* (Pan *et al.,* 2001), *Oryza rufipogon* (Cao *et al*., 2011), *Helianthus annuus* (Chakravarty and Srivastava, 1992; Kumar and Srivastava, 2006 and Li *et al*., 2015)**,** *Glycine max* (Noya *et al*., 2014) and *Zea mays* (Liu and Jiang, 2001; Doncheva *et al*., 2005; Vardar *et al*., 2011 and Alvarez Bello *et al*., 2012). In this concern, Yi *et al*. (2010) demonstrated that aluminum chloride is a clear clastogenic/genotoxic and cytotoxic agent in *Vicia* root cells. No reports or limited information are available about the cytotoxicity effects of Al on root cell in black seeds plant (*Nigella sativa* L.). Therefore, the observation of the root-tip constitutes a rapid and sensitive method for environmental monitoring. El-Ghamery *et al.* (2002 and 2003) considered *Nigella sativa* L., a species of large an economical importance, as a very well characterized cytogenetically following its treatment with Zn^{2+} and Ni^{2+}. So, it can serve as useful biomarkers for detection of Al toxicity or as cytogenetic test could be used for the genotoxicity monitoring of aluminum compounds contamination. Thus, the present study aims to investigate, by cytogenetic tests, the effect of aluminum exposure to *Nigella sativa* L. and also to understand the toxic mechanisms of aluminum involved.

2. Material and Methods

2.1. Seed Germination Test

Healthy and uniform size seeds of black seeds plant (*Nigella sativa* L.) obtained from Sods Research Station in Bani Swif, Egypt and aluminum chloride ($AlCl_3$. $6H_2O$) provided or supplied from LOBA chemi 107, Wodehouse Road, Mubai 40005, India. The aluminum substance is dissolved in redistilled water and the applied concentrations were 200, 400, 800, 1000, 3000 and 5000 ppm after a preliminary test which showed that concentrations higher than 5000 ppm applied for 36 h exerted toxic effect on cells. To evaluate the rate of seed germination of black seeds plant (*Nigella sativa* L.) under the stress of Al^{3+}, seeds of the plant presoaked in distilled water for 6h, were placed directly in different concentrations of Al^{3+}, ranging from 200 to 5000 ppm for different treatment times (6, 12, 18, 24 and 36h). For each treatment, a triplicate of 30 seeds was used. Seeds were surface sterilized with 10% sodium hypochlorite for ten minutes then washed with sterile distilled water and placed on sterile filter paper in the Petri dishes. Fresh test solutions were added to the Petri dishes and the plates were placed in the dark for different treatment times at 24 ± 1°C to facilitate linear growth. The treated seeds (30 for each treatment) were washed carefully with distilled water then transferred to Petri-dishes containing filter paper moistened with distilled water and allowed to germinate at room temperature 24 ± 1°C for 5-7 days. Control seeds were treated with distilled water. Radicle length 5 mm was considered as germinated. The germinated seeds were counted and the percentages were calculated.

2.2. Radicle Growth Investigation

To study the effect of Al^{3+} on radicle growth, the seeds were germinated in distilled water till appearance the radicle. Thirty of the germinated seeds were immersed in suitable amount of each tested concentrations of 200, 400, 800, 1000, 3000 and 5000 ppm of aluminum chloride ($Al\ Cl_3$) for the different times of 6, 12, 18, 24 and 36 hours. Similarly, 25 germinated seeds were soaked in distilled water for the same period was run with each treatment as the control. Following the treatments, the treated seedlings together with each control samples were kept in the dark at 23-25^{o}C, in order to minimize the fluctuation in the rate of cell division (Evans *et al*., 1957). At the end of each treatment time, the length of the radicle was measured. The relative change of radicle length was calculated as a percentage of the variance from the control or expressed as percent of controls.

2.3. Cytological Examination

Ten germinated seeds, with radicle 2-3 cm long, were treated with different concentrations for different times. Control germinated seeds were placed in distilled water. After each treatment, the roots were cut off and immediately fixed in glacial acetic acid: absolute ethyl alcohol (1:3 v/v) for overnight. The root tips were stained by using the Feulgen squash technique. 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.

2.4. Statistical Analysis

Each treatment was made in three replicates. For statistical analysis, one-way ANOVA (Sigma Plot 13.0 software) SPSS was used to determine significance at $p < 0.05$ and at $P < 0.01$.

3. Results

Results of effects of Al^{3+} on the percentage of seed germination of the tested plant are represented in Fig. (1-A). The frequency of seed germination, as shown in figure (1-A), was found to be affected by $AlCl_3$ at different applied concentrations. The inhibition effect increased with increasing concentration of $AlCl_3$. A significant reduction in total seed germination percentage was observed starting at the $AlCl_3$ concentration of 200 ppm compared with the control values. The highest concentration (5000 ppm) caused a reduction of about 54% in percentage of germinated seeds compared with the control. Moreover, results revealed that the percentage of germination was over than 80 % at the concentrations of 200-800 but about 50%-80% at concentrations of 1000-5000, which indicate that $AlCl_3$ is toxic at higher concentrations.

To evaluate the Al^{3+} toxicity on the radicle elongation, black seeds plant (*Nigella sativa* L.) roots were exposed to different concentrations of $AlCl_3$ (200, 400, 800, 1000, 3000 and 5000 ppm) and the average root lengths were measured up to 120h.The effects of Al^{3+} on radicle growth of *Nigella sativa* L.varied with the Al^{3+} concentration and treatment time. There was a significant inhibition of radicle growth in seedlings treated with 200 ppm and 5000 ppm of Al (Fig. 1-B) during the whole course of treatment. Versus the control there was hardly any root growth with 5000 ppm of Al. Whereas there was no toxic effect At 200 ppm of Al. Radicle elongation as T/ C ratio were reduced to 50 % in 800 ppm, 14 % in 3000 ppm, 7 % in 5000 ppm, compare to the control length value (100 %) after 36 h period (Fig. 1-B). Although the radicle growths were not significantly affected up to 24 h, they started decreasing and exhibited a stationary phase up to 36 h. The results revealed the intensive inhibition occurred in 5000 ppm of $AlCl_3$. In general, the root morphology was nearly normal for all the treatments without showing an obvious difference in its appearance of color and rigidity.

Results of the microscopic evaluation of the effects of Al^{3+} on mitotic cells are presented in (Fig. 1-c) and Table 1. Root tips of black seeds plant exposed to different concentrations of Al^{3+} showed that compared to control, mitotic division inhibition was concentration dependent at all concentrations used in this study for each treatment time. The mitotic index (MI) showed significant differences in comparison with the control for all Al^{3+} treatments. Maximum reduction in MI was observed with the treatment of 5000 ppm for 36h, where the MI was 0.34 lower than the value seen in the control. There were no dividing cells at the 5000 ppm concentrations of Al with some stages of the mitotic division.

Effect of Al^{3+} on the different mitotic phases is given in Table (1). For each treatment time, the increase or decrease in the frequency of each stage was negatively correlated to the increase of the concentration compared to the control value. For 6 and 12h, the increase of the frequencies of metaphase and anaphase was on the expanse of the frequencies of the other stages. For 18h, the increase of the frequencies of prophase and metaphase was only recorded for almost all concentrations of Al, In contrast for 24h, the increase of the frequencies of prophase and telophase was on the expanse of the frequencies of the other stages. For 36h, the increase of the frequencies of metaphase was only recorded on the expanse of the frequencies of the other stages.

Results in Table (1) also, show the frequency of abnormalities in each mitotic phase and the total percentages of abnormalities induced in root tip cells of tested plant. With respect to chromosomal abnormalities, all treatments showed significant differences compared with the control. The percentage of aberrant cells at all the tested concentrations of Al increased as concentration of Al increased, the number of dividing cells aberrant cells increased as the mitotic indices decreased Table (1). The total frequency of abnormal dividing cells is clearly a dose and treatment time dependent. For each treatment time (6, 12, 18, 24 and 36 hours), increasing the concentration from 200 ppm to 5000 ppm gradually increased the frequency of total abnormal cells with all treatment times. The frequency of the total abnormal dividing cells following 6 hours treatment ranged from 63.22% to 84.62 % and from 78.69% to 100% for 12 hours treatment compared to their respective control values of 2.22% and 3.08, respectively. Also, the frequency of the abnormal dividing cells was ranged from 80.39% to 100 % for 18 hours treatment compared to its respective control value of 2.50. At the 24 and 36 hour treatments, the all tested concentrations of Al Cl_3 induced 100% of abnormalities.

Al^{3+} treatments induced a considerable range of chromosomal abnormalities in all stages of mitosis in root tip cells of black seeds plant (*Nigella sativa* L.) plant (Table 1). From the results, at 6 hours treatment the percentages of prophase were more than that at metaphase stage for almost all treatments. Also at the treatment for 6 hours, telophase stage was the less

affected by Al Cl_3 treatment than the other stages than at metaphase stage for almost all treatments. No abnormal cells were observed at ana-telophases for the 5000 ppm treatment with 6h treatment time. At 12 hours, the treatment with 5000 ppm applied for 12 hours, the total percentages of abnormalities induced at metaphase (54.55%) were higher than that induced at anaphase stage (27.27%) and telophase stage (18.18%). No abnormalities were recorded at prophase stages. At 18 hours treatments the percentage of abnormalities at prophase stage was higher than those at other stages for each applied concentration. The percentages of abnormalities were in the following sequence: prophase > metaphase > telophase > anaphase. At 24 hours treatments, the percentages of abnormal cells at metaphase stage were more than at whereas the percentages anaphase stage were more other stages for all concentrations except the 5000 ppm. The percentages of abnormalities were in the following sequence: metaphase > prophase > telophase > anaphase. No abnormalities were recorded at metaphase and anaphase stages for the treatments with the 5000 ppm of Al Cl_3. At 36 hours treatments, only one abnormal cell was recorded at prophase and telophase stages for the treatments with the 5000 ppm of Al Cl_3. The percentages of abnormal cells at prophase stage were more than at other stages for almost the all concentrations in contrast to percentages of abnormal cells at telophase stage. The percentages of abnormal cells at metaphase stage were more than at anaphase stages for almost the all concentrations.

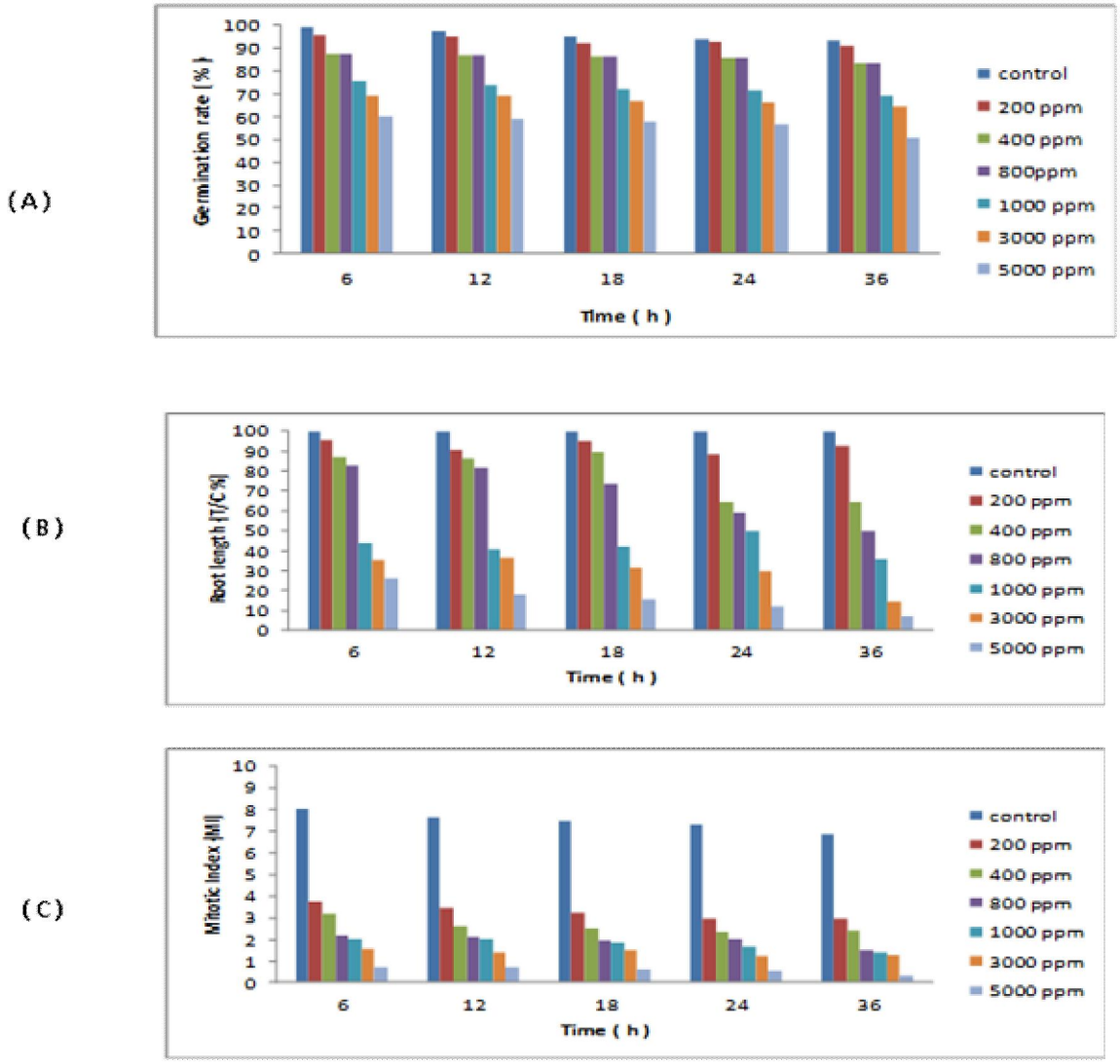

Figure 1. Effect of different concentrations of ($AlCl_3$) on germination percentage **(A)** root length as T/C % **(B)** and mitotic index (MI) **(C)** in *Nigella sativa*

Table 1. Frequency of mitotic phases and the percentage of total abnormal mitotic phases after treatment of *Nigella sativa* root tips with different concentrations of ($AlCl_3$) for 6, 12, 18, 24 and 36 hours

Treatment		No. of counted cells	No. of dividing cells	MI±SE	Mitotic phase (%)				No of abnormal cells	Abnormal mitotic phase (%)				Total abno cells%
Time (h)	Conc. Ppm				Prophase	metaphase	anaphase	Telophase		Prophase	metaphase	anaphase	Telophase	
6	Control	1685	135	8.01±0	26.66	25.18	23.96	25.18	3	2.22	0	0	0	2.22
	200	2336	87	3.72±0**	24.13	32.18	22.98	20.68	55	9.20	25.29	16.09	12.64	63.22
	400	1676	53	3.16±0**	26.41	26.41	32.07	15.09	35	16.98	15.09	24.53	9.43	66.04
	800	1650	36	2.18±0**	27.77	16.66	33.33	22.22	25	13.89	11.11	33.33	11.11	69.44
	1000	1676	34	2.02±0**	11.76	11.76	52.94	23.52	27	11.76	11.76	44.12	11.76	79.41
	3000	1790	28	1.56±0**	17.85	39.28	25	17,85	23	14.29	28.57	28.57	10.71	82.14
	5000	1735	13	0.74±0**	23.07	76.92	0	0	11	23.08	61.54	0	0	84.62
12	Control	1699	130	7.65±0.57	27.69	24.61	23.07	24.61	4	2.31	0.77	0	0	3.08
	200	1750	61	3.48±0.33**	18.03	24.59	27.86	29.50	48	13.11	21.31	19.67	24.59	78.69
	400	1738	46	2.64±0**	21.73	32.60	21.73	23.91	37	19.57	28.26	17.39	15.22	80.43
	800	1993	42	2.10±0**	30.95	21.42	35.71	11.90	35	21.43	11.90	35.71	14.29	83.33
	1000	1402	28	1.99±0**	7.14	3.57	82.14	7.14	25	7.14	7.14	71.43	3.57	89.29
	3000	1480	21	1.41±0**	28.57	28.57	23.80	19.04	19	19.05	23.81	23.81	23.81	90.48
	5000	1566	11	0.70±0**	0	81.81	0	18.18	11	0	54.55	27.27	18.18	100
18	Control	1608	120	7.46±0	30.83	25.83	19.16	24.16	3	2.50	0	0	0	2.50
	200	1566	51	3.25±0**	62.74	9.80	11.76	15.68	41	49.02	11.76	0.00	19.61	80.39
	400	1440	36	2.50±0.33**	45.9	30.55	0	22.22	30	41.67	27.78	0.00	13.89	83.33
	800	1316	26	1.97±0**	42.30	19.23	11.53	26.92	24	42.31	23.08	7.69	19.23	92.31
	1000	1026	19	1.85±0**	26.31	52.63	5.26	15.78	19	15.79	57.89	5.26	15.79	100
	3000	1530	23	1.50±0.3**	30.43	52.17	4.34	13.04	23	30.43	56.52	4.35	8.70	100
	5000	1130	7	0.60±0**	42.8	57.14	0	0	7	42.86	57.14	0	0	100
24	Control	1500	110	7.33±0	29.09	27.27	27.27	16.36	1	0.91	0	0	0	0.91
	200	1310	39	2.97±0**	30.76	33.33	10.25	25.64	39	30.77	35.90	15.38	17.95	100
	400	1306	31	2.37±0**	25.80	32.25	19.35	22.58	31	25.81	35.48	22.58	16.13	100
	800	1203	24	1.99±0**	58.33	25.00	0	16.66	24	58.33	20.83	0.00	20.83	100
	1000	1246	21	1.68±0**	28.57	52.38	0	19.04	21	23.81	52.38	4.76	19.05	100
	3000	1306	16	1.22±0**	25.00	37.50	18.75	18.75	16	25	37.50	18.75	18.75	100
	5000	1813	7	0.56±0 **	42.85	0	14.28	42.85	7	71.43	0.00	0.00	28.57	100
36	Control	1936	133	6.86±0.33	27.07	18.80	24.06	30.07	2	1.5	0	0	0	1.5
	200	1986	29	2.94±0**	31.03	24.13	13.79	31.03	29	27.58	20.68	17.24	34.48	100
	400	1006	24	2.38±0.3**	25.00	41.66	16.66	16.66	24	37.50	29.16	12.50	20.83	100
	800	1000	15	1.50±0**	20.00	26.66	26.66	26.66	15	26.66	20.00	20.00	33.33	100
	1000	1013	14	1.38±0**	28.57	28.57	16.28	28.57	14	35.71	21.42	14.28	28.57	100
	3000	1096	14	1.27±0**	28.57	28.57	28.57	14.28	14	50	21.42	14.28	14.28	100
	5000	576	2	0..34±0**	50.00	0	0	50.00	2	50	0	0	50	0

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

Frequencies of the different types of abnormalities induced in root tip cells of *Nigella sativa* following the treatment with aluminum chloride are given in Table (2). At prophase stage: three types of chromosomal abnormalities were recorded such as irregular prophase, vacuolated prophase and sticky. The irregular prophase was the most common abnormal type induced by almost all treatments (25 of the 30 different treatments). The other two types (vacuolated nucleus and sticky prophase) were recorded in 15 and 13 of the 30 different treatments for the first and the second types, respectively. At metaphase stage: four types were recorded in the following order: sticky metaphase, C-metaphase, disturbed metaphase and fragment chromosome at metaphase. The last type was the lowest common of abnormality at this stage whereas stickiness was the most frequent type. In addition, C-anaphase type was recorded in a few cells. At anaphase stage: cells with abnormal anaphase according to their frequencies were in the following sequence: stickiness, bridges, forwarded chromosome(s), diagonal (depolarized), disturbed anaphase and star anaphase were observed in some treatments but with low frequency. The cells with sticky chromosome were the most frequent type whereas the forwarded chromosome and star chromosomes were the less frequent types. At telophase stage: only the sticky chromosomes were recorded in all abnormal cells. In this investigation, there is no correlation between the frequency of each observed type of abnormalities and the increasing of the concentration for each treatment time. Moreover, a little number of cells was observed with a micronucleus at interphase stage.

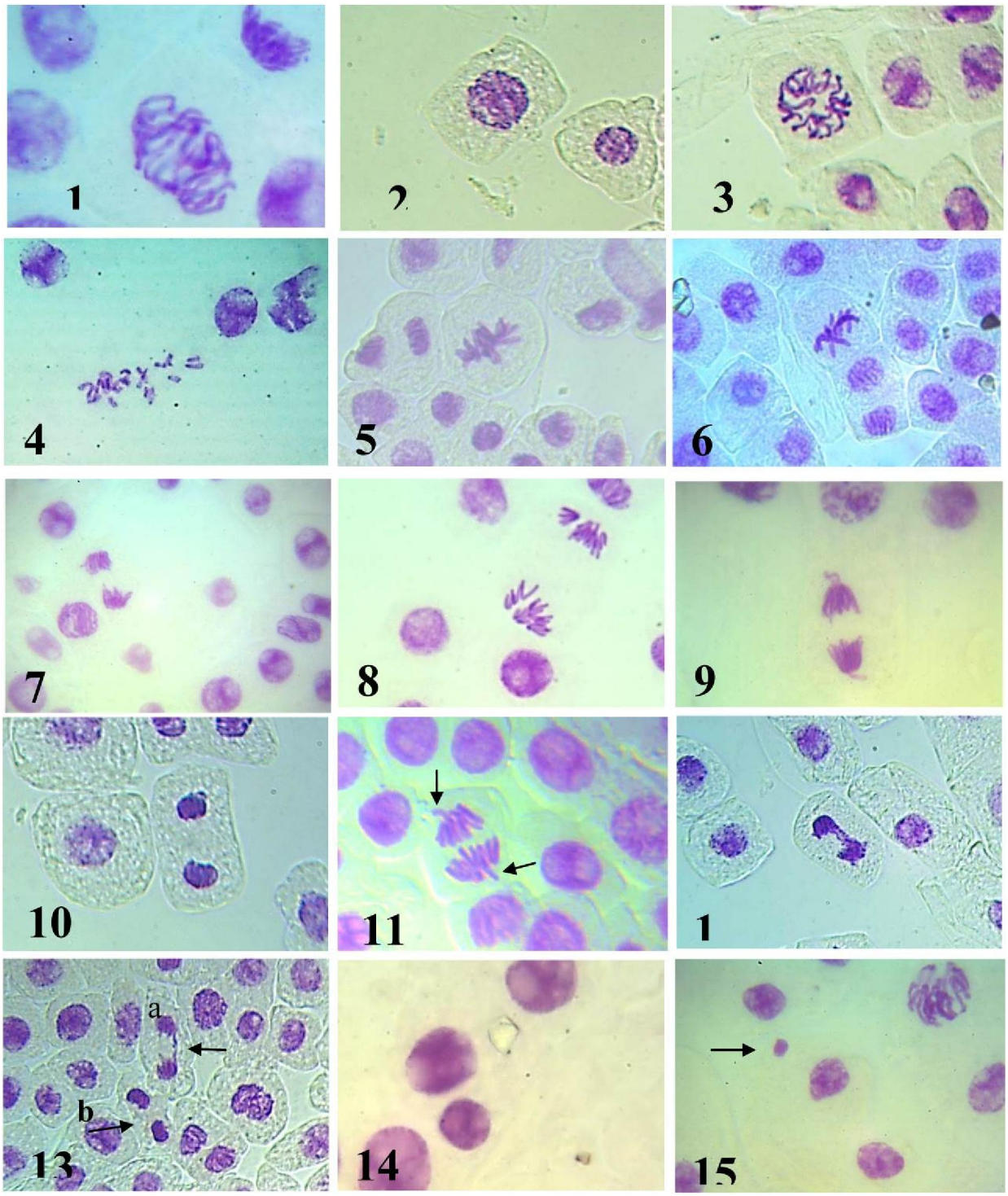

Figure 2. Some types of abnormalities induced in *Nigella sativa* root tip cells by different treatments of $AlCl_3$. (1) Irregular prophase. (2) Sticky prophase. (3) Vacuolated nucleus at prophase. (4) C-metaphase. (5) Sticky metaphase. (6) Fragment at metaphase. (7) Diagonal anaphase. (8) Tetra polar anaphase. (9) Sticky anaphase with forward chromosomes. (10) Sticky telophase. (11) Anaphase with forward chromosomes. (12) Multibridges at anaphase. (13-a) Single bridge at anaphase. (13-b) Sticky telophase. (14) Unequal sized nucleus. (15) Micronucleus.

Table 2. Types and percentages of mitotic abnormalities and the percentage of total abnormalities induced by different concentrations of ($AlCl_3$) in root tips of *Nigella sativa* after different treatment times

Treatment		Interphase	Prophase			Metaphase				Ana-Telophase						
Time (h)	Conc. (ppm)	Micro nuc.	Vac.	Irrgular	Sticky	C-met.	Dist.	Laging	sticky	sticky	s/forw.	Bridg.	Digon.	Dist.	Star	forward
		%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
6	Control	0	0	2.22	0	0	0	0	0	·	0	0	0	0	0	0
	200	·	0	6.90	2.30	8.05	4.60	4.60	8.05	14.94	0	1.15	6.90	2.30	2.30	2.30
	400	·	0	11.32	5.66	3.77	9.43	0	1.89	15.09	3.85	1.89	5.66	5.66	0	1.89
	800	·	0	13.89	0	0	5.56	0	5.56	16.67	8.33	11.11	0	5.56	0	2.78
	1000	·	0	11.76	0	0	0	0	11.76	17.55	0	5.88	8.82	14.71	8.82	0
	3000	·	3.57	10.71	0	7.14	3.57	3.57	14.29	21.42	7.40	3.57	0	0	0	7.14
	5000	·	0	23.08	0	0	0	0	61.54	·	0	0	0	0	0	0
12	Control	·	0	2.31	0	0	0	0	0.77	·	0	0	0	0	0	0
	200	·	0	13.11	0	9.84	0	0	11.48	34.43	0	0	9.84	0	0	0
	400	·	0	19.57	0	6.52	13.04	4.35	4.35	26.09	0	4.35	0	0	0	2.17
	800	·	0	21.43	0	2.38	2.38	0	7.14	16.67	0	4.76	16.67	4.76	0	7.14
	1000	·	0	7.14	0	3.57	0	0	3.57	17.86	0	17.86	14.29	7.14	17.86	0
	3000	·	0	4.76	14.29	0	0	0	23.81	47.62	0	0	0	0	0	0
	5000	·	0	0	0	18.18	36.36	0	0	45.45	0	0	0	0	0	0
18	Control	·	0	2.50	0	0	0	0	0	·	0	0	0	0	0	0
	200	0.5	0	29.41	9.80	5.88	5.88	0	0	19.61	0	0	0	0	0	0
	400	·	16.67	8.33	16.67	8.33	19.44	0	0	13.84	0	0	0	0	0	0
	800	·	0	30.77	11.54	23.08	0	0	0	26.92	0	0	0	0	0	0
	1000	·	5.26	5.26	5.26	15.79	10.53	10.53	21.05	21.05	0	0	0	0	0	0
	3000	·	13.04	17.39	0	21.74	17.39	4.35	13.04	13.05	0	0	0	0	0	0
	5000	·	28.57	14.29	0	28.57	0	14.29	14.29	·	0	0	0	0	0	0
24	Control	·	0	0.91	0	0	0	0	0		0	0	0	0	0	0
	200	·	10.26	20.51	0	17.95	0	7.69	10.26	25.64	0	5.13	2.56	0	0	0
	400	·	16.13	9.68	0	6.45	9.68	0	19.35	22.58	0	6.45	0	6.45	0	3.23
	800	·	20.83	12.50	25	12.50	0	0	8.33	20.83	0	0	0	0	0	0
	1000	·	9.52	14.29	0	4.76	28.57	0	19.05	19.05	0	0	0	0	0	0
	3000	·	25	0	0	12.50	0	0	25	25	0	0	12.50	0	0	0
	5000	·	0	42.86	28.57	0	0	0	0	28.57	0	0	0	0	0	0
36	Control	·	0	1.50	0	0	0	0	0	·	0	0	0	0	0	0
	200	·	6.90	13.79	6.90	3.45	6.90	0	10.34	44.82	0	3.45	0	3.45	0	0
	400	·	8.33	20.83	8.33	8.33	8.33	0	12.50	29.16	0	4.17	0	0	0	0
	800	·	6.67	20	0	0	0	0	20	46.66	0	0	0	6.67	0	0
	1000	·	28.57	0	7.14	0	0	0	21.43	35.71	0	0	7.14	0	0	0
	3000	·	28.57	0	21.43	0	0	0	21.43	14.29	0	0	14.29	0	0	0
	5000	·	0	0	50	0	0	0	0	50	0	0	0	0	0	0

4. Discussion

Germination assay is especially effective to indicate phytotoxicity of soil contaminants and seed germination is considered the most sensitive parameter for evaluating the phytotoxicity of heavy metals (Valerio *et al*., 2007). Our results revealed that a significant reduction in total germination percentage was observed compared with control, starting at the Al concentration of 200 ppm. The highest concentration (5000 ppm) caused a reduction in total percentages of germinated seeds to a half value of the control. So, germination of the treated seeds was not completely inhibited. A similar result was observed where a reduction in the seed germination percentages has been observed under the action of aluminum for various varieties of barley (*Hordeum vulgare*) grains and the germination percentage was lower than the control value (Synzynys *et al*., 2013). Also, a decrease in the total percentage of germination was observed in the treatment of *Lactuca sativa* (Palmieri *et al*., 2014), *Zea mays* (Liu & Jiang, 2001 and Nasrin, 2013) and *Platycodon grandiflorum* (Zhu *et al*., 2010) with different concentrations of Al which demonstrates the toxicity of Al. These effects represent a serious hazard to plants because of the extreme importance of the root as an organ involved in obtaining nutrients and water. In contrast, the seed germination is not affected by Al whereas root and seedling developments are reduced following the treatment of white spruce (*Picea glauca*) seeds Nosko *et al.* (1988), wheat *Triticum aestivum* grains (Jamal *et al*., 2006) and *Nicotiana tabacum* seeds (Vandar *et al*., 2006). This metal has been reported not to inhibit germination found, however germination time was delayed, but

impaire the growth of new roots and seedling establishment (Nosko, *et al.,* 1988 and Rellén-Álvarez, *et al.,* 2006). The decrease in seed germination can be attributed to the accelerated breakdown of stored food material in seeds by the application of Al (Nasrin, 2013), which has no physiological activity (Lasat, 2002) in contrast to other essential mineral nutrients such as Ni and Zn.

Radicle elongation is the combination of cell division and cell elongation. Inhibition of radicle growth is considered to be primarily the result of inhibited cell elongation and expansion, prior to inhibiting cell division (Vardar *et al.*, 2007). The previous studies represent that root apex (root cap, meristem and elongation zone) is the first target of Al toxicity. Although several toxic effects of Al on plant growth have been proposed, the mechanism of Al toxicity is still to be clarified. For this reason, the present study demonstrates the effects of different Al concentrations on *Nigella sativa* root growth which has been suggested that radicle growth inhibition is the most evident symptom of Al toxicity. A Similar radicle growth inhibition was detected within different times and concentrations treatments in different plants such as *Allium cepa* (Liu *et al*., 1993b; De Campos & Viccini, 2003; Qin *et al*., 2010 and Achary *et al*., 2013), *Hordeum vulgare* (Pan *et al*., 2001 and Wang *et al.,* 2006), *Zea mays* (Liu and Jiang, 2001; Barceló & Poschenrieder, 2002; De Campos & Viccini, 2003 and Nasrin, 2013), *Vicia faba* (Liu *et al*., 1993c and Pan *et al*., 2001), *Triticum aestivum* (Jamal *et al*., 2001), *Triticum turgidum* (Frantzios *et al*., 2000), *Oryza sativa* (Mohanty *et al*., 2004, de Macêda *et al*., 2009 and Alvarez Bello *et al*., 2012), *Lactuca sativa* (Palmieri *et al*., 2014), *Glycine max* (Yu *et al*.2011 and Noya *et al.,* 2014), *Arachis hypoganea* (Huang *et al*., 2014), *Helianthus annuus* (Chakravanty & Sirvastava, 1992; Kumar and Srivastava, 2006 and Li *et al.,* 2015*), Platycodon grandiflorum* (Zhu *et al*., 2010) and *Nicotiana tabacum* (Vandar *et al*., 2006).

In agreement with previous studies, we observed that all applied Al concentrations inhibited the radicle growth and the highest radicle growth inhibition was at 36 h with 5000 ppm of $AlCl_3$ of about of 93 % of the control value. In this concern, Barceló and Poschenrieder (2002) and De Campos & Viccini (2003) concluded that the most evident symptom of Al toxicity is root growth inhibition, which can be detected within 30 min (Ma *et al*., 2002 and De Campos & Viccini, 2003) to 2 hours (Kochian, 1995; Zheng and Yang, 2005; Doncheva *et al*., 2005; Arroyave *et al*., 2011). It has been suggested that inhibition of root growth induced by Al may result from disturbance of the cell divisions in the root meristematic zone (Doncheva *et al*., 2005). Also Liu *et al.* (1993a) concluded that the poisoning by Al^{3+} of the root tip cells of *Allium sativum* may result from the uptake and accumulation of Al and inhibition of Ca uptake, distribution of physiological activities of calmodulin (CaM) and the inhibition of some enzyme reactions. Al interactions with intracellular constituents, such as calmodulin, and DNA (Matsumoto *et al*., 1976 and Yamamoto *et al*., 2001). In this respect, retardation of root and seedling growth were either due to the toxicity of the metal ions through disturbance of the physiological processes and induction of chromosomal abnormalities (Synzynys *et al*., 2013) or affect root metabolism, which shows sensitivity to Al^{3+} toxicity by a reduction in lateral root size. This is due to reductions in both new cell formation and cell elongation in the extension region of the root (Aremu and Meshitsuka, 2005). In contrast Al at concentration as low as 5-10μM stimulated the root growth in *A. cepa* (Achary *et al*., 2013) and also within low concentration of 1.4 mmol/l in *Oryza rufipogon* (Cao *et al.,* 2011).

Previous investigations started to look at the cell cycle (de)regulation induced by Al, with some works focusing unbalances on mitosis phase and very few on other interphase phases (Silva, 2012). In our study, different mitotic phases in *Nigella sativa* following the treatment with Al showed a different response in deceasing or increasing in their frequencies than the control values. It is also provided evidence that the treatments had adverse effect on the normal progression of the mitotic cell cycle. It is clear that there was an increase in the percentage of dividing cells in metaphase with 3 treatment times and 2 treatment times in prophase whereas with one treatment time in anaphase and telophase. Wojciechowska and Kocik (1983) reported that an increase in the number of the divided in prophase stage was connected with the increase in telophase stage in root tip cells of *Vicia faba* treated by Al whereas Andrade *et al.* (2010) found that Al reduces the percentage of prophase and metaphase cells, reflecting a reduction in MI. Also, Doncheva *et al.* (2005) reported that Al-induced alterations in the number and the position of dividing cells in root tips. Prolonged exposures of treatments lead to Al interactions with the root cell division and the cytoskeleton (Silva, 2012). As well as, Al impaired the activity of the root meristem as indicated by reduction in its number of mitotic figures (de Macêdo *et al*., 2009). Our result may indicate that Al^{3+} blocks mitosis at the end of prophase and is considered as pro-metaphase or metaphase inhibitor. In this respect, Frantzios *et al.* (2000) concluded that the effect of Al on *Triticum turgidum* mitotic cells is greatly disturbed organization and function of the mitotic apparatus, as well as inhibition of cells from entering mitosis. Cell cycle analysis indicate that the number of cells in each

phase of mitosis changed in Al-exposed root tips, providing the indication of Al-induced abnormal progress through mitosis. Data in this study, demonstrate that Al induces cell cycle checkpoints defects in *Nigella sativa* root tips as reported by Yi *et al.* (2010) in *Vicia faba* root tip cells.

The inhibition of mitotic activity was almost time and concentration dependent. In this study, cell division decreased as exposure to Al increased for each treatment time, however, the mitotic activity in treated root tip cells of *Nigella sativa* never reached zero but reach to a minimum value of 0.34 ± 0. In contrast, the treatments of *Vicia faba* root tip cells with Al induced pycnosis formation which indicates complete inhibition of mitosis in root meristematic cells (Yi *et al*., 2010). Al-induced inhibition of cell division in root tip menistems as a primary effect of aluminium has been reported (Wojciechowska and Kocik, 1983). Decrease of mitotic activity was reported as a consequence of Al exposure in root tips of several species such as onion (Dovgalyuk *et al.,* 2001a; De Campos & Viccini, 2003 and Qin *et al*., 2010), wheat (Frantzios *et al*., 2001 and Rayburn *et al*., 2002), rice (Mohanty *et al*., 2004 and de Macêda *et al*., 2009 and Alvarez Bello *et al.,* 2012), maize (Llugany *et al*., 1995; Liu and Jiang, 2001; De Campos & Viccini, 2003 and Doncheva *et al*., 2005), barley (Budikova and Durcekova, 2004; Wang *et al*., 2006 and Synzynys *et al*., 2013), bean (Wojciechowska and Kocik, 1983; Marienfeld *et al.,* 2000; Pan *et al*., 2001; Liu *et al*., 1993c and Abdel Hamied *et al*., 2008), garlic (Liu *et al*., 1993a) and sunflower (Chakravanty & Sirvastava, 1992; Kumar and Srivastava, 2006 and Li *et al.,* 2015).

However, Doncheva *et al*. (2005) reported that inhibition of cell division (decrease of S-phase cells) in the proximal meristem after 5 min Al exposure and inhibition of root cell division in the apical meristem within 10 or 30 minutes. According to Posmyk *et al*. (2008), cell mitotic activity inhibition involves disturbances in the basic processes occurring during all the cell cycle phases. Moreover, the reduction of MI in treated roots is probably due to disturbances in the cell cycle as well as chromatin dysfunction, which is induced by interactions between DNA and the metal (Andrade *et al*., 2010). In this concern, Maksymiec (2007) considered cell cycle inhibition as the basis for root growth inhibition. In this respect, Roy *et al.* (1989) concluded that Al has a cytotoxic effect on root tip cell of *Allium sativum* during a time course study. Matsumoto *et al.* (1976) suggested that Al could bind to DNA of pea cell and inhibit its synthesis. When a great deal of Al^{3+} penetrates the cell, Al^{3+} ions associate directly with DNA and chromatin and the template activity of DNA for the RNA synthesis is reduced (Morimura and Matsumoto, 1978). Chromatin becomes more condensed and aggregated resulting in low transcriptional activity and this could be the cause of chromosomal damage and mitotic abnormalities (Zhang, 1995). Further evidences for the decrease in the MI could be either due to Al-induced DNA synthesis blockage (Mohanty *et al.,* 2004) or Al interacts with double-stranded DNA and influences its function (Wu *et al*., 2005).

Aluminum produced a number of mitotic and chromosomal abnormalities in root tip cells of *Nigella sativa* which varied in their frequencies in the different mitotic stages. Similar to the finding of Roy *et al*. (1989) and Dovgalvuk *et al.* (2001a &b), the total abnormalities induced in present study increased with increasing the concentrations and treatment time. In this concern, Grant (1994) stated that mitotic aberrations are generally regarded as the results of an action on the spindle apparatus, whereas chromosomal aberrations indicate effects on chromosome directly or indirectly and the induction of these types provide a variable genetic assay for screening environmental pollution. The induction of abnormalities following the treatment of different plants with Al was previously recorded such as barley (Zhang, 1995; Patra *et al*., 2000; Pan *et al*., 2001 and Synzynys *et al*., 2013), wheat (Rayburn & Wetzel, 2002 and Rayburn *et al*., 2002), onion (Liu *et al*., 1993b; De Campos & Viccini, 2003; Qin *et al*., 2010; Achary & Panda, 2010), bean ((Wojciechowska and Kocik, 1983; Liu *et al*., 1993c; Abdel Hamied *et al*., 2008; Zhang *et al*., 2009 and Yi *et al*., 2010), garlic (liu *et al*., 1993a), rice (Mohanty *et al*., 2004 and de Macêda *et al*., 2009), sunflower (Chakravanty & Sirvastava, 1992; Kumar and Srivastava, 2006 and Li *et al.,* 2015) and lettuce (Palmieri *et al*., 2014). Mohanty *et al*. (2004) reported that cytological alterations seem to be due to the phytotoxic properties of Al. Presumably, the dissociation of the metabolic salt $AlCl_3$ altered the ionic environment of the cell, which might have led to a physiological change in nucleoprotein or denaturation of protein reflected as chromosomal aberrations.

Of these types of aberrations, stickiness, as a most frequent type, was recorded in the different mitotic phases in root tip cells of *Nigella sativa* treated with aluminum chloride. This type was recorded following treatment of different plants with Al (Patra *et al*., 2000; Rayburn *et al*., 2002; De Campos & Viccini, 2003; Mohanty *et al*., 2004; Kumar and Srivastava, 2006; Zhang *et al*., 2014 and Li *et al*., 2015). Chromosome stickiness consisted of pro- metaphase sticky chromosomes and ana-telophases sticky bridges. The induction of this type reflected the toxicity of aluminum which is most likely irreversible, and probably led to cell death (Pan *et al*. 2001 and Zhang *et al*., 2014). Stickiness has

been attributed to either the effect of aluminum on the denaturing activity of Al on nuclear proteins such as DNA topoisomerase II, which might also interfere with chromosome segregation (Panda and Panda, 2002), or binding with nucleic acids, mainly DNA, causes serious changes in their physic-chemical properties (El-Ghamery *et al*. 2002 & 2003 and Synzynys *et al*., 2013), or chromatin condensation of the nucleus (Zhang, 1995). Moreover, sticky chromosomes can cause loss of genetic material. Subsequently, the cell-division process occurs irregularly, with some chromosomes not adhering to the assembled chromosomal complex and being lost during the cell cycle.

The observation of C-metaphase as the second frequent type of abnormalities at metaphase and some cells C-anaphase at anaphase stage indicates either the action of the aluminum on the spindle fibers which involved in the processes of chromosome movement through regulation and control of de-polymerization and polymerization of the microtubules and preventing the continuation of the mitotic cycle (Li and Sun, 1991) or the spindle apparatus was only impaired or it is one of the consequences of inactivation of spindle apparatus connected with the delay in the division of centromere. As a result of this disorder, the cell cycle is interrupted in metaphase and the chromosomes are seen scattered and condensed with very well-defined centromeres (Fiskesjö, 1985). These dose-independent changes were observed at all concentrations. Both sticky chromosomes and C-metaphase cells are abnormalities that characterize the aneugenic action of the aluminum (Leme and Marin-Morales, 2009). The induction of some cells with disturbed metaphase chromosomes may suggest that aluminum likes other heavy metals causing partial disturbance in the spindle apparatus or partial suppression of spindle formation (El-Ghamery *et al*., 2003). Consequently, a few cells with multipolar were also observed at ana-teolphase referred to the effect of aluminum by causing splitting of the spindle-fibers into more than two directions (Dovgalyuk *et al.,* 2001a) or partial suppression of spindle formation (El-Ghamery *et al*., 2003).

At higher concentration, the occurrence of strongly stained nuclei throughout this study, characterized as condensed nuclei, is also an alteration of the normal structure of the nuclei. Nuclear condensation is a feature that is associated with the process of programmed cell death (Andrade *et al.* 2010).

In similar to the results of De Campos and Viccini (2003) and Li *et al.* (2015), a great increase in the occurrence of bridges in maize and sunflower, respectively, has been observed. We have observed not only anaphases with simple bridge, but also, with two, three and multiple bridges. An increase of anaphases with more than one bridge was detected more frequently in higher aluminum concentrations. The decrease of anaphase with single bridge was probably due to the increase of the other anaphase bridge type. One hypothesis to explain the increase in the number of bridges with or without fragments per abnormal anaphase can be related to disturbances caused by aluminum on spindle and DNA organization (Frantzios, *et al.* 2000). Chromosome bridges may have occurred due to the chromosomal stickiness and subsequent failure of free anaphase separation or may be attributed to an unequal translocation or inversion of chromosome segment. In the present investigation, a noticed few cells with fragments in the root tips is probably formed by acentric chromosome and also as a result of inversion. Fragmentation might have arisen due to stickiness of chromosomes and consequent failure of separation of chromatids to poles (El-Ghamery *et al*., 2003). Bridges lead to chromosome breakage and formation of acentric chromosome fragments which – free-floating in the cytoplasm - can be enveloped by membranes and produce micronuclei where a many cells were recorded with a micronucleus. The induction of micronuclei indicates that some mitotic cells can enter into mitotic (M) phase with DNA damage. In this study, micronuclei, which probably, arose from both clastogenic and aneugenic actions of the aluminum as suggested by Dovgalyuk *et al.* (2001b). The majority of micronuclei observed may have derived from a single chromosome that was lost from the whole set of chromosomes in the cell (De Campos and Viccini, 2003 and EL-Ghamery *et al*., 2003).

5. Conclusion

In conclusion, Mohanty *et al.* (2004) reported that the occurrence of different types of chromosomal aberrations, reduction in the amount of nuclear DNA and persistence of the phytotoxic effect at the post-treatment stage with aluminum on rice plant. Whereas our results demonstrate that aluminum chloride is a clear clastogenic/genotoxic and cytotoxic agent in *Nigella sativa* root cells. Also, the data about root growth and germination have provided new indicator for Al toxicity and tolerance where the higher concentrations applied did not stop the germination and kill the root. On the other hand, further studies for mechanism of the induction of mitotic aberrations (sticky chromosomes and micronuclei) by Al can allow new insights into the mutagenic effect of Al toxicity on plant nuclear material and the adaptation mechanism of plant to Al toxic environment.

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