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Phytotoxicity and Genotoxicity Evaluations of Chromium Hexavalent (Cr^{VI}) on *Allium cepa* and *Nigella sativa* Root Cells

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AS ONE of the leading global causes of environmental contamination, heavy metals hurt agriculture and human health through the food chain. Using the mitotic index (MI), chromosomal aberrations (CAs), and DNA damage, the cytotoxic and genotoxic effects of $Cr^{(VI)}$ on the root tips of *Allium cepa* and *Nigella sativa* were assessed. Six $Cr^{(VI)}$ concentrations (50, 100, 400, 700, 1000, and 2000ppm) were administered for 6, 12, 18, and 24h. The germination percentages of *A. cepa* and *N. sativa* seeds decreased at 400ppm for both plants, without germination detected in *N. sativa* L. at or above 700 ppm. The MI in the treated group was significantly lower than the control cells after exposure to the various concentrations of $Cr^{(VI)}$ for different exposure times. The reduction of MI in *N. sativa* root tips was more pronounced than that of *A. cepa*. The largest abnormal percentages were identified in *N. sativa* after 24h of exposure to 100ppm $Cr^{(VI)}$, whereas *A. cepa* showed abnormal percentages after 24h of exposure to 400ppm $Cr^{(VI)}$. In all mitotic phases, distinct aberrations in the division of root tip cells were observed in both plant species. A few examples of these aberrations were stickiness, C-mitosis, anaphase–telophase bridges, disturbed laggards, and micronuclei. The comet test, which show the single-strand breaks in DNA, was used to determine how dangerous is $Cr^{(VI)}$ to DNA in *A. cepa* root cells. DNA damage was significantly obvious in the comet experiment than in the control at all concentrations. Evidence from both *A. cepa* and *N. sativa* root meristemic cells suggested that $Cr^{(VI)}$ is cytotoxic and genotoxic and induces DNA damage in a dose-dependent manner.

Keywords: Chromium, Comet assay, Cytotoxicity, Mitotic index, Seed germination.

Introduction

In addition for being a significant contributor to environmental degradation worldwide, heavy metal pollution directly impacts agriculture and subsequently the human health. Chromium (Cr) is the seventh most common "heavy metal" element in the earth's crust (Sadler, 1995). Several valence states of Cr exist; however, the trivalent (Cr^{III}) and hexavalent (Cr^{VI}) forms are the most stable and shared in the earth's crust (Kimbrough et al., 1999). $Cr^{(VI)}$ is a documented human carcinogen that has been classified as a Group A carcinogen (Winberry, 1998). The widespread pollution of water and soil with Cr resulting from various industrial Applications or natural human-driven processes is a significant environmental concern. Chromium is used in various processes, including leather tanning, mining, painting, petroleum refining, textile manufacturing, fungicides, and wood preservation (Mishra, 2016). Chromate ($-CrO_4$)$^{-2}$ and dichromate ($-Cr_2O_7$)$^{-2}$ anions are formed when $Cr^{(VI)}$ combines with oxygen; they are both very soluble, highly mobile in soil/water systems, and often more poisonous than $Cr^{(III)}$ (Shanker et al., 2009). As a result of its propensity to generate reactive oxygen species (ROS) in biological systems, $Cr^{(VI)}$ is phytotoxic, cytotoxic, and genotoxic element (Shanker et al., 2005; Nickens et al., 2010; Malherbe et al., 2011; Oliveira, 2012; Eleftheriou et al., 2012, 2013). The United Nations Environment Program strongly recommends utilizing assays with vascular plants to investigate genotoxic chemicals. In this context, it has been demonstrated that plants are

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promising tools because toxins generate equivalent chromosome aberrations in plant cells and animal cells in culture (Ditika & Anila, 2013). Plant bioassays have been used to help determining the metal and xenobiotic phytotoxicity (Di Salvatore et al., 2008). The *A. cepa* L. plant bioassay can determine the harmful effects of heavy metals, wastewater, complex mixtures, and nanoparticles by measuring chromosome aberrations and micronuclei, the mitotic index, root growth, DNA damage, and cell death (Leme & Marin-Morales, 2009; Chakraborty et al., 2010; Panda et al., 2011). Over the past decade, the cyto-genotoxic potential of surface water and effluents has been evaluated using the *A. cepa* (common onion) bioassay, which is a quick, cheap, and sensitive method of toxicity testing (Leme & Marin-Morales, 2009; Masood & Malik, 2013). Ditika & Anila (2013) revealed that the sensitivity of the *A. cepa* root to chemicals is likely due to its significant number of metacentric chromosomes and the large size of its chromosomes. Furthermore, *N. sativa* L. is chosen because of its cytological originality (Saha & Datta, 2002) and its yield of a commercially valuable spice which has enormous therapeutic value (Datta et al., 2012).

The ability of onion (*A. cepa*) to absorb Cr has made it useful in evaluating abnormalities in chromosomes that lead to DNA damage and mitotic cycle disturbances (Zayed et al., 1998). Tedesco & Laughinghouse (2012) recognized that DNA damage, chromosomal alterations, and perturbations in the mitotic cycle could all be evaluated in *A. cepa*. The *A. cepa* test has been emphasized as an indication of human health and a means of diagnosing toxicity and genotoxicity (El-Shahaby et al., 2002).

Rapid and low-cost implementations are essential in agricultural regions contaminated with heavy metals such as chromium. The ability of a seed to germinate in a medium containing Cr would show tolerance to this metal, because germination is the first physiological function that is altered by Cr (Akinci & Akinci, 2010).

Multiple sources agreed that a seedling's earliest developmental stages of seedlings are critical for deciding whether it will be affected by heavy metals such as chromium (Pandey et al., 2008; Kundu et al., 2018). Amin et al. (2019) analyzed the toxicity, tolerance, and accumulation of $Cr^{(VI)}$ in six biofuel plant species using soils with eight different Cr concentrations. Those researchers found that the germination rates and other growth metrics dropped significantly when seeds were subjected to extreme Cr stress. Previous researches demonstrated that increasing quantities of chromium negatively affected plant germination, growth, and yield (Lakshmi & Sundaramoorthy, 2003; Purohit et al., 2003; Chidambaram et al., 2006; Sundaramoorthy et al., 2006; Youssef, 2020). Because of its speed and sensitivity, the comet test is gaining importance in the evaluation of the DNA-damaging by environmental contaminants. The measurement of comet tails is crucial because they represent free DNA fragments and indicate damage to particular cells (Klaude et al., 1996; Hafez & Fouad, 2020).

This study compared the reactions of *A. cepa* and *N. sativa* to heavy metal stress by measuring the effects of chromium at six concentrations over a variety of treatment intervals (from 6 to 24h) on seed germination and plant growth, chromosomal frequency and architecture, and DNA damage.

Materials and Methods

A. cepa seeds were received from the Onion Research Department of the Field Crops Research Institute at the Agricultural Research Center in Giza, Egypt. The Medicinal and Aromatic Plants Research Center (Giza, Egypt) provided seeds of *N. sativa* plants. Potassium dichromate ($K_2Cr_2O_7$) was acquired from Advent Chembio PVT. LTD. TM (CAS No. 7778-50-9) MW 294.185) as a source of hexavalent chromium $Cr^{(VI)}$.

Preparation of hexavalent chromium concentrations, seed germination and root length measurements

The effect of various concentrations of hexavalent chromium (50, 100, 400, 700, 1000, and 2000ppm) on the germination rate of onion (*A. cepa*) and black seed (*N. sativa*) was examined. The seeds of the two plants were selected, soaked in a sodium hypochlorite solution (20% V/V) for 15min, washed many times with deionized water, and placed directly in distilled water for 2h. For each treatment, 30 seeds were utilized in triplicate. The treated seeds were rinsed adequately with distilled water before being placed in Petri dishes containing a wet filter paper with solution of various concentrations of $Cr^{(VI)}$, ranging from 50 to 2000ppm. Germination time was detected 5 and 9 days after incubation for onions and black seed plants, respectively. The seeds were deemed germinated when the radicle protruded from the seed coat by 2mm. The germination percentage was

calculated using the following formula: G percent= (number of germinated seeds / total number of planted seeds) ×100 (Scott et al., 1984). After 10 days for onions and 14 days for black seeds, the radicle length was measured after each treatment period. The relative change in root length was determined as a percentage of the variation from the control or reported as a percentage of the control.

Cytological investigations

In deionized water, $Cr^{(VI)}$ solutions at various concentrations (50, 100, 400, 700, 1000, and 2000ppm) were prepared as follows: 0.1414, 0.2828, 1.131, 1.980, 2.8282, and 5.657g/L, respectively, from $K_2Cr_2O_7$ powder. After the sprouting seeds were placed in a tiny bottle with various concentrations of $Cr^{(VI)}$ for varying amounts of time (6, 12, 18 and 24h), the viability of the seedlings was determined. The seeds were taken from the treatment apparatuses and washed with deionized water after treatment. Carefully cut root tips were employed for cytological processing. The root tips of the studied plants were meticulously removed and utilized for cytological processing. Briefly, the root tips (1–2cm in length) from untreated and treated plants were fixed in freshly prepared Carnoy's fixative (3:1 v/v absolute alcohol and glacial acetic acid, respectively). Cytological slide preparations were made using the Feulgen squash technique (Darlington & La Cour, 1976). The slides were examined microscopically for checking and counting the chromosomal aberrations, and photography using an XSZ-N 107 research microscope equipped with a Premiere MA88-900 digital camera.

Single-cell electrophoresis (comet assay)

Onion (*A. cepa*) and black seed plant (*N. sativa*) seeds that had germinated were taken out of their respective treatment solutions and washed well under running water before being used in the alkaline comet test (Patnaik et al., 2011). First clean slides were coated with 50µL of 1% standard melting point agarose (type I, Sigma-Aldrich, USA) diluted in distilled water, and allowed to dry at room temperature for an overnight, then labeled. After root cutting out, the roots were frozen in a 60-mm Petri dish, and then sprayed with 100–200µL of a cold Tris-HCl buffer pH 7.4. After slicing the roots using a fresh razor blade, the nuclei were extracted in the same buffer and carefully transferred by a pipette into a micro centrifuge tube at 4°C. First, the afterward nuclear suspension was mixed with an equivalent volume (1:1) of 1.5 % LMPA dissolved in Tris-HCl buffer at 37 °C to create a 0.75% low-melting point-agarose (LMPA, type VII, Sigma-Aldrich, USA) nuclear suspension. A 20mm ´ 40mm coverslip was used to apply 80µL of the prepared nuclear suspension to the slides at 37°C. The coverslip was removed carefully after the agarose had been set for 5min on the cold metal plate. Another layer of 0.75% (80µL) LMPA in Tris-HCl buffer was placed on top of the nuclear layer and allowed to gel. The slides containing the agarose-embedded nuclei were submerged in an alkaline solution (300mM NaOH and 1mM EDTA) in a horizontal electrophoresis unit for 10min after the coverslips were removed. In the same alkaline buffer at 4°C, 0.75V cm^{-1} and 300mA, electrophoresis was run out for 15min. Afterward the slides were washed in distilled water and then neutralized in 0.4M Tris buffer (pH 7.4). After that rinsing the slides for 5 min in distilled water, then 200µL of ethidium bromide solution (2g mL^{-1}) was used to stain the nuclei on each slide plate. A fluorescence attachment was used (excitation filter, 515–560nm ;and barrier filter, 560nm) for an Olympus BX51 microscope, a Cohu camera, and the Kinetic KometTM Imaging Software 5.5 (AndorTM Technology, www.andor.com) was used to analyze comets.

Ten roots on two slides were used to compare the treatments. At least 50 comets were ranked on each slide and comet photographs were acquired at a magnification of 1009. Among the many parameters used by researchers to evaluate comets is the so-called Olive tail moment (OTM), which is calculated by taking the difference between the comet's head and tail intensity centroids (centers of gravity) along the X axis and the percentage of DNA in the tail. Based on this trait, the differences in DNA distribution inside the tail can be detected. As a result, one micrometer (OTM) is considered an absolute parameter (Kumaravel et al., 2009). The entire process of analyzing the comet was carried out under yellow or low-level lighting.

Statistical analysis

The mitotic index (MI) was computed by scoring dividing cells from a population of 2000 –3000 non-dividing (interphase) cells. Statistical analysis was performed on the outcomes. The statistical significance of variations in the mitotic index and chromosomal abnormality frequency between treatments and controls was examined. Each treatment was performed in triplicate. The significance levels of $P< 0.05$and $P< 0.01$ were

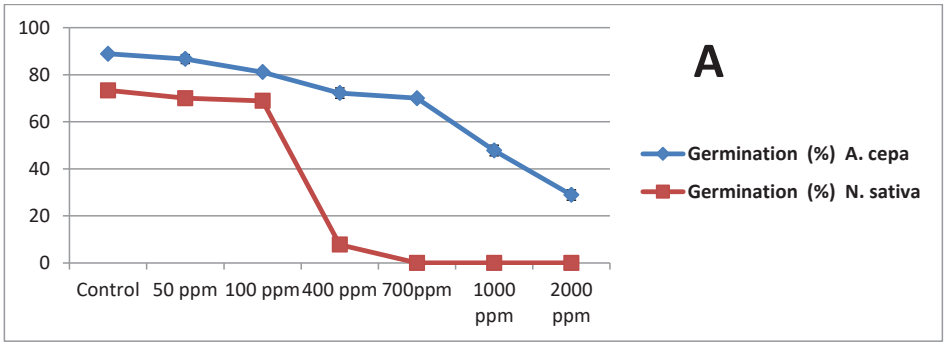
determined using one-way ANOVA (Sigma Plot 13.0 software) and SPSS for statistical analysis.

Results

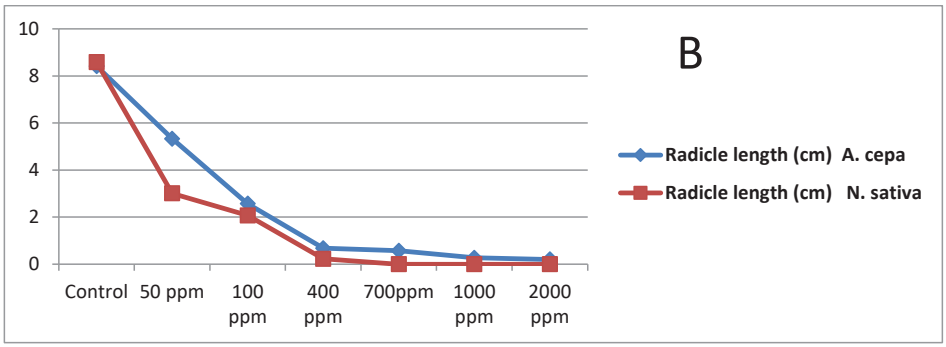
Effect of hexavalent chromium on seed germination and root length

As shown in Fig. 1a, more significant amounts of chromium inhibited the germination of specific plants compared with the control. All concentrations had comparable effects on the germination of *A. cepa* and *N. sativa*, with a considerable decrease in germination beginning at 400ppm. *N. sativa* performed poorly in response to increasing chromium concentrations, with no germination observed at 700, 1000, and 2000 ppm (Fig. 1a and c). There were significant differences in the germination percentage (GP. %) in *A. cepa* (Fig. 1a and c) at all levels of Cr, with reductions of 72.22 %, 70 %, 47.78 %, and 20.89 % for concentrations of 400, 700, 1000, and 2000 ppm, respectively.

A: Germination percentage (%)



B: Radicle Length (cm)



C: The morphological characteristics of the treated plants at all concentrations

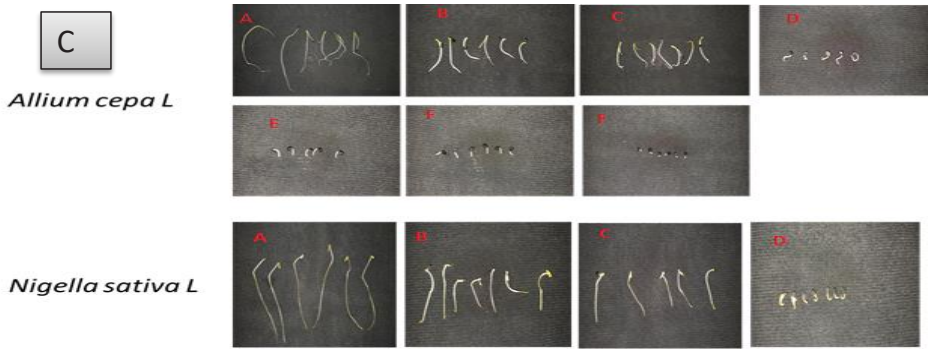


Fig. 1. Effect of different concentrations of hexavalent chromium $Cr^{(VI)}$ on *Allium cepa* and *Nigella sativa:* (A) germination percentage (percent), (B) radicle length (cm), and (C) morphological characteristics of the treated plants at all concentrations (where a, b, c and d in the black boxes were the control, 50ppm, 100ppm, and 400ppm, respectively, and e, f, and g were 700, 1000, and 2000ppm, respectively).

In addition, all *A. cepa* and *N. sativa* seeds were directly treated with $Cr^{(VI)}$ solutions for 5 and 9 days, respectively. However, the resulting seedlings developed differently based on Cr concentration (Fig. 1b). When exposed to Cr concentrations as low as 400ppm, the transition zone between the root and cotyledon became clearly defined in *A. cepa*. The cotyledon partially emerged from the seed coat and grew at medium and high concentrations, whereas the roots did not grow. The average root length measurements used to check the correlation between the root growth and concentration (Fig. 1b). According to these values (Fig. 1b), harmful effects were observed between 400 and 2000ppm. Above 50ppm, a modest development of radicles was detected in the tested plants. Treating two plants with 100ppm or more of Cr dramatically reduced the development of radicles. *N. sativa* showed no radicle emergence over 400ppm.

At Cr concentrations of 400, 700, 1000, and 2000ppm, the average root length of *A. cepa* was significantly impacted by 0.68 ± 0.04, 0.57 ± 0.03, 0.27 ± 0.02, and 0.2 ± 0.00cm, respectively. Similarly, increases in chromium at 50, 100, and 400ppm in *N. sativa* significantly reduced root length relative to the control plants by 3.01 ± 0.01, 2.07 ± 0.05, and 0.23 ± 0.01cm, respectively. All plants failed to develop radicles beyond 400 ppm, demonstrating that greater levels of chromium severely inhibited root germination in *A. cepa* and *N. sativa*.

Effect of $Cr^{(VI)}$ on mitotic index

Cytological markers, such as the chromosomal abnormalities percentage, mitotic index (MI), and chromosomal aberrations (CAs), were used to evaluate the cytotoxicity and genotoxicity of Cr on onion and black seed plants. The investigation of the effect of various doses (50, 100, 400, 700, 1000, and 2000ppm) with four different durations of exposure to Cr revealed a dose–time-dependent decrease in the mitotic index in the treated group compared with the control cells ($P < 0.05$ and $P < 0.01$) (Tables 1, 2).

The highest onion and black seed MI values were recorded at 6h after 50ppm Cr treatment (6.35 ± 0.196 and 5.35 ± 0.19), whereas the lowest MI values were found at 24h after 2000ppm Cr treatment (1.07 ± 0.042 and 1.09 ± 0.04, respectively) (Tables 1, 2 and Fig. 2a, b). As the exposure duration or Cr concentration increased, the proportion of abnormal cells increased. The percentage of chromosomal abnormalities was reported to be more significant at all examined concentrations. The highest number of chromosome abnormalities in *A. cepa* occurred at Cr concentrations of 2000ppm with continuous exposure and at lower Cr concentrations with an extended exposure time of 24h. (Fig. 3a). When the exposure duration to *N. sativa* reached 18 h, the percentage of defective chromosomes reached the maximum of 100 % (Fig. 3b). *A. cepa* and *N. sativa* showed the lowest abnormality percentage at 50ppm for 6h (28.42%± 1.02 and 81.43 % ± 0.60%, respectively) as compared with the control (18, 58 ± 0.93, and 16.86 ± 0.48 %, respectively) (Fig. 3a and b).

Effect of $Cr^{(VI)}$ on chromosomal aberrations

Cells with bridge, forward, diagonal, vacuolated, lagging, micronucleus, fragmented, and sticky chromosomes were the most prevalent forms of aberrant cells when both plants were subjected to different concentrations of Cr. The analysis of the total number of CAs revealed that, stickiness was the most frequent aberration, with a high occurrence in cell division stages measured as 49.43 % and 56.04 % in *A. cepa* and *N. sativa*, respectively, followed by c-mitosis with ratio 13.15% and 22.08% CAs (Fig. 3c). These aberrations (disturbed and irregular) were grouped and measured as 16.06% and 10.64% for *A. cepa* and *N. sativa*, respectively. Bridge, forward, diagonal, vacuolated, lagging, and fragmented chromosomes were pooled together and labeled "others" (Fig. 3c). In *A.cepa and N.sativa*, they were attributed the ratio 21.16 % and 11.04%, micronucleus was found in *A. cepa* and *N. sativa* with ratio 0.21% and 0.20 % respectively (Fig. 3c). Types of abnormalities are illustrated in Fig. 4.

Effect of $Cr^{(VI)}$ on DNA damage

The DNA damage was caused by different Cr concentrations after 24 and 18h of treatment in root meristem cells of *A. cepa* and *N. sativa*, respectively, is reported in Table 3 and Fig. 5 as tail DNA percentage (percent of comet tail DNA) and tail length. The tail moment (TM) was assessed as a damage parameter. Root tip cells exposed to Cr concentrations for 24 and 18h showed a comet tail compared with control cells (Fig. 5), which indicates the instability of the nuclear DNA. Whereas the length of the comet tail increased as the Cr concentration and exposure time increased. At 50ppm Cr, DNA damage was severe, and at 2000ppm, the damage was extremely worse.

TABLE 1. Mitotic index and mitotic phase percentage in *Allium cepa* meristematic cells treated with various doses of hexavalent chromium $Cr^{(VI)}$

Treatments		Counted cells	Divided cells	Mitotic index (MI ± S.E)	Mitotic phases (%)			
Time (h)	Conc. (ppm)				Prophase	Metaphase	Anaphase	Telophase
6	Control	5745	323	5.62 ± 0.038	31.50	28.43	16.38	23.69
	50	2985	190	6.37 ± 0.196	45.46	22.70	13.55	18.29
	100	3216	200	6.22 ± 0.163	23.28	38.92	16.48	21.32
	400	3532	210	5.95 ± 0.392	36.28	30.86	17.74	15.12
	700	3683	204	5.54 ± 0.239	33.19	20.09	16.95	29.76
	1000	3511	181	5.16 ± 0.038	36.07	37.79	10.96	15.18
	2000	5612	165	2.94 ± 0.167**	30.55	26.18	17.37	25.90
12	50	4327	243	5.62 ± 0.162	40.97	18.60	15.28	25.15
	100	2977	144	4.84 ± 0.291	30.30	26.08	23.38	20.24
	400	4033	164	4.07 ± 0.039**	32.05	33.76	20.25	13.94
	700	3922	150	3.82 ± 0.174**	37.11	21.55	10.28	31.06
	1000	4483	150	3.35 ± 0.227**	27.12	53.23	9.67	9.98
	2000	5197	116	2.23 ± 0.081**	27.26	47.63	14.01	11.11
18	50	2712	141	5.20 ± 0.193	48.80	26.94	9.21	15.05
	100	4305	184	4.27 ± 0.160**	28.45	19.48	13.12	38.95
	400	3318	133	4.01 ± 0.388**	35.98	35.61	9.51	18.90
	700	3828	140	3.66 ± 0.318**	31.92	40.26	15.97	11.85
	1000	4319	84	1.94 ± 0.153**	22.11	51.87	12.34	13.68
	2000	5328	87	1.63 ± 0.226**	22.72	56.12*	10.23	10.93
24	50	2558	98	3.83 ± 0.176**	47.26	27.13	10.06	15.55
	100	3431	91	2.65 ± 0.175**	45.07	17.21	11.82	25.90
	400	3898	96	2.46 ± 0.181**	34.75	43.45	8.72	13.08
	700	2038	50	2.45 ± 0.198**	43.74	14.35	17.68	24.23
	1000	2916	39	1.34 ± 0.030**	17.86	26.32	24.21	31.61
	2000	5540	59	1.06 ± 0.042**	30.92	34.22	16.51	18.36

S.E., standard error *Significant at $P < 0.05$ ** Significant at $P < 0.01$

TABLE 2. Mitotic index and mitotic phases proportion of *Nigella sativa* meristematic cells subjected to various doses of hexavalent chromium $Cr^{(VI)}$

Treatments		Counted cells	Divided cells	Mitotic index (MI ± S.E)	Mitotic phases (%)			
Time (h)	Conc. (ppm)				Prophase	Metaphase	Anaphase	Telo-phase
6	Control	5182	261	5.04 ± 0.092	45.89	25.65	8.36	20.09
	50	4435	237	5.34 ± 0.191	43.72	31.17	9.01	16.10
	100	5097	229	4.49 ± 0.211	38.85	27.98	12.21	20.95
	400	3532	155	4.39 ± 0.222	47.89	27.80	11.53	12.79
	700	4557	198	4.34 ± 0.142	33.37	30.22	24.53	11.88
	1000	4793	194	4.05 ± 0.112**	35.11	30.39	15.96	18.54
	2000	5481	195	3.56 ± 0.218**	31.39	31.93	15.33	21.35
12	50	5539	259	4.68 ± 0.227	35.64	37.80	14.28	12.29
	100	4922	210	4.27 ± 0.124	38.97	34.60	14.58	11.85
	400	5382	191	3.55 ± 0.139**	40.67	30.67	13.83	14.83
	700	5365	176	3.28 ± 0.092**	34.24	36.85	15.76	13.16
	1000	6093	162	2.66 ± 0.140**	29.86	55.97**	4.76	9.40
	2000	5645	142	2.52 ± 0.140**	50.32	22.78	12.75	14.14
18	50	5022	211	4.20 ± 0.191*	32.12	42.83	11.28	13.76
	100	4379	159	3.63 ± 0.107**	49.71	25.21	12.91	12.17
	400	6207	167	2.69 ± 0.188**	29.55	41.83	11.84	16.79
	700	6210	158	2.54 ± 0.150**	34.98	41.55	11.95	11.52
	1000	7395	179	2.42 ± 0.318**	30.64	43.28	19.43	6.66
	2000	4544	75	1.65 ± 0.178**	48.83	33.92	7.69	9.56
24	50	4847	189	3.90 ± 0.071**	31.33	46.76	9.17	12.74
	100	5758	179	3.11 ± 0.051**	30.95	32.42	17.45	19.18
	400	6988	155	2.22 ± 0.072**	28.27	49.31	13.93	8.49
	700	5789	113	1.95 ± 0.045**	29.55	39.31	20.71	10.44
	1000	5130	70	1.36 ± 0.177**	35.60	35.02	21.69	7.68
	2000	4192	46	1.10 ± 0.046**	51.71	29.50	15.29	3.51

S.E., sandard error *Significant at $P < 0.05$ ** Significant at $P < 0.01$

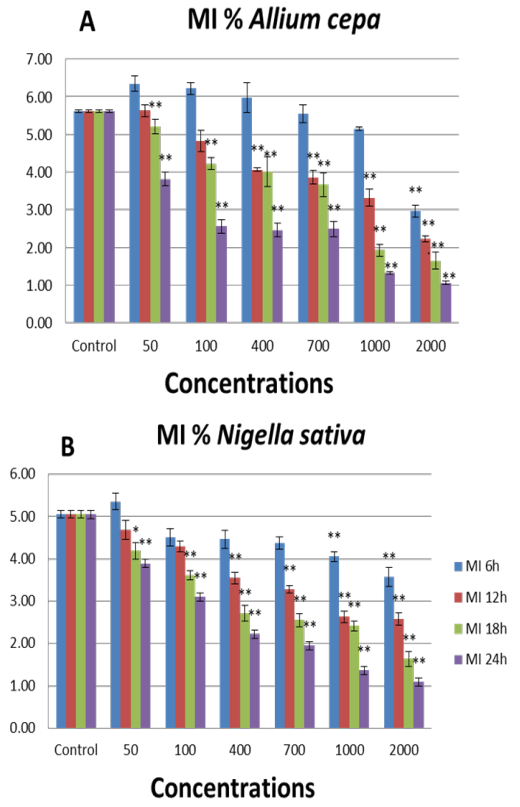
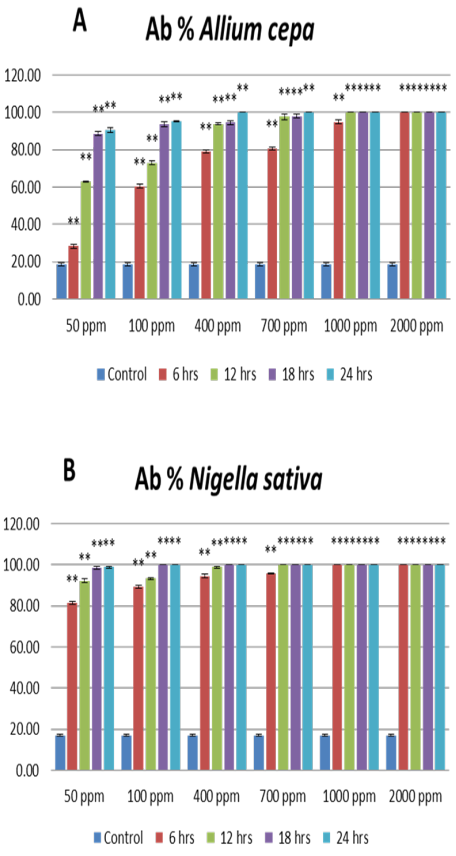


Fig. 2. Mitotic Index for (A) *A. cepa* and (B) *N. sativa*



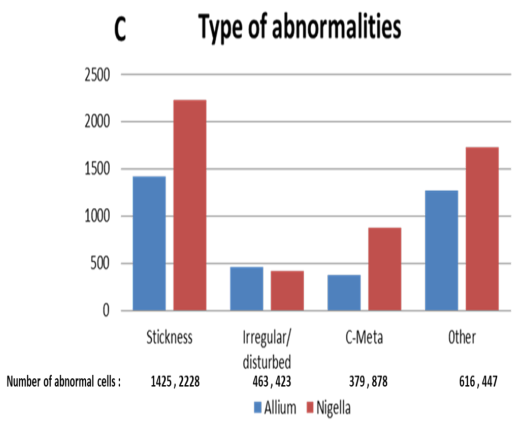


Fig. 3. Abnormalities caused by different concentrations of hexavalent chromium (Cr^{VI}) in *Allium cepa and Nigella sativa* Percentages : (A) Ab. % for *A. cepa*, (B) Ab. % for *N. sativa,* and (C) types of most abundant abnormalities , with Ab reffering to abnormalities.

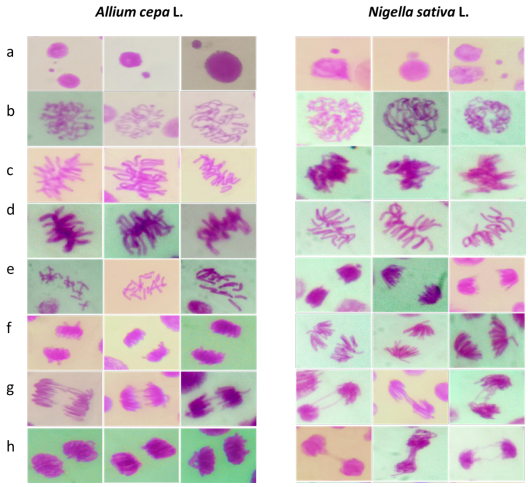


Fig. 4. Different types of chromosomal abnormalities in *Allium cepa* and *Nigella sativa:* (a) Micronucleus at interphase (b) Irregular prophase, (c) Disturbed metaphase in *A. cepa* and sticky metaphase in *N. sativa* , (d) Sticky metaphase in *A. cepa* and disturbed metaphase in *N. sativa,* (e) c-meta phase in *A. cepa* and sticky anaphase in *N. sativa,* (f) Sticky anaphase in *A. cepa* and anaphase with forward chromosomes in *N. sativa,* (g) Anaphase with forward and bridges in *A. cepa* and anaphase with bridges in *N. sativa* and (h) Sticky telophase with with forward chromosome in *A. cepa* and sticky telophase with bridges in *N. sativa*

TABLE 3. DNA damage in the nuclei of *Allium cepa* and *Nigella sativa* root meristems exposed to hexavalent Chromium ($Cr^{(VI)}$) was detected using the Comet method

	Concentration	Tails length (µm)	Tail DNA(%)	Tail moment
Allium cepa L.	Control	1.08 ± 0.09 ^{g}	1.25	1.35
	50ppm	2.82 ± 0.11 ^{f}	3.14	8.85
	100ppm	4.14 ± 0.15 ^{e}	4.17	17.26
	400ppm	5.90 ± 0.19 ^{d}	4.78	28.20
	700ppm	7.08 ± 0.32^{c}	6.05	42.83
	1000ppm	9.33 ± 0.40 ^{b}	8.12	75.76
	2000ppm	11.20 ± 0.47 ^{a}	9.39	105.17
Nigella sativa L.	Control	1.20 ± 0.10 ^{f}	1.39	1.67
	50ppm	3.05 ± 0.13 ^{e}	3.47	10.58
	100ppm	4.53 ± 0.18 ^{d}	4.36	19.75
	400ppm	6.62 ± 0.26 ^{c}	5.14	34.03
	700ppm	7.51 ± 0.48 ^{c}	6.38	47.91
	1000ppm	10.82 ± 0.57 ^{b}	9.03	97.70
	2000ppm	12.11 ± 0.60 ^{a}	10.28	124.49

* Data are presented as the mean ± standard error of mean (SEM). Values with different superscript letters [a (highest) to g (lowest) in the tail length column are significantly different at $P \leq 0.05$.

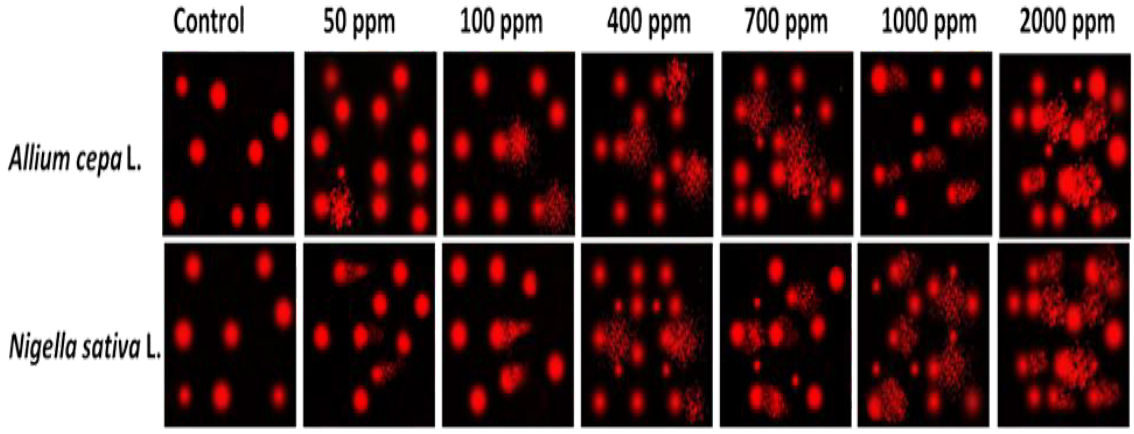


Fig. 5. Comet character in nuclei isolated from *Allium cepa* and *Nigella sativa* root meristems exposed to hexavalent Chromium (Cr^{VI}) at all concentrations

The ANOVA test revealed a substantial difference between the investigated values. In control samples of *A. cepa* and *N. sativa*, the measured TM was 1.35 and 1.67mm, whereas cells exposed to 2000ppm displayed TM value of 105.17 and 124.49mm, respectively. Multiple comparisons between the treatment and control groups revealed that variations in the mean values of the percentage of tail DNA were statistically significant at concentrations higher than 700ppm. The tail length and moment values varied significantly through all concentrations.

Discussion

The toxicity of $Cr^{(VI)}$ differs depending on the type of plant and the source of the heavy metal, and there is a wide range of sensitivity or tolerance to $Cr^{(VI)}$ in the environment among different plant species (López-Luna et al., 2009). The results of this study proved that chromium is hazardous to seed germination at high concentrations. From the current publications revealed that $Cr^{(VI)}$ has previously been shown to reduce or inhibit the germination of plants depending on the Cr concentration and the plant genotype; for example, *Lycopersicum* L., *Triticum aestivum* L., *Hibiscus*

esculentus L., some essential pulses (such as gram , necessary pulses, *Lycopersicon esculentum* L., Green gram and Chickpea), *Brassica oleracea* L. *var*. *acephala* DC,as well as some crops from India, which was consistent with the present results, (Jun et al., 2009; López-Luna et al., 2009; Lakshmi & Sundaramoorthy, 2010; Ozdener et al., 2011; Datta et al., 2012; Amin et al., 2013; Singh & Sharma, 2017; Joshi et al., 2019; Hafiz & Ma, 2021). However, when exposed to 50µM $Cr^{(VI)}$, Panda (2007) discovered that the biomass and root length of *Oryza sativa* increased rapidly during the first 24h but gradually inhibited over the next 48h. Higher concentrations of $Cr^{(VI)}$ (2.0–3.2mM) had no inhibitory effect on the seed germination percentage in several pulse crops (López-Luna et al., 2016; Mathur et al., 2016). Several mechanisms, such as the effect of Cr on amylase activity and the subsequent transport of sugars to embryo axes, may have led to the negative effect of Cr on seed germination observed here, resulting in a low germination percentage. This was attributed to disruptions in respiration activity and the mobilization of significant seed reserves including starch, proteins, and lipids, which prevented normal seed imbibition. (Kumar et al., 2016; Steinbrecher & Leubner-Metzger, 2016; Medda & Mondal, 2017). Even though that protease activity increases in tandem with chromium treatment, it could be a factor in the reduced germination of chromium-treated seeds (Zied, 2001).

Contact Roots are the first organs in plants to come into contract with toxic materials, and they often accumulate more metals than do shoots (Shanker et al., 2004; Mangabeira et al., 2011), hence limiting growth and root development. Several investigations have shown that Cr(III, VI) is detrimental to root and shoot development and biomass. Under $Cr^{(III)}$ stress, Liu et al. (2008) observed a reduction in the root development of *A. cepa*. All plants failed to develop radicles beyond a Cr concentration of 400 ppm, demonstrating that greater levels of chromium severely inhibited root extension in *A. cepa* L. and *N. sativa* L. Similar findings were found in a photo-genotoxic assay of chromium (Cr) and arsenic (As) on *A. cepa* conducted by Gupta et al. (2018). In that research, root length significantly decreased with increasing Cr and As concentrations, suggesting a negative correlation between root length and Cr and As treatment. A hormetic dose-response analysis revealed that $Cr^{(VI)}$ accelerated root growth at low doses and retarded it at high levels (Calabrese & Blain, 2009; Belz et al., 2011). According to previous researches, the principal morphological impacts of heavy metals on roots include a reduction in root extension, root hair breakdown, and a reduction in root number (Mallick et al., 2010). The length of root and shoots of *Arachis hypogea* was altered by increasing $Cr^{(VI)}$ concentrations (Rajalakshmi et al., 2010). It has also been shown that the root growth of rice (*Oryza Sativa* L.) cultivars was inhibited to be a significantly greater extent than shoot growth (Rajalakshmi et al., 2010); this could be because chromium trapped in the vacuoles of the root cells, making it less toxic. This may be a natural way for the plant to deal with toxicity (Shanker et al., 2004).

Root meristemic cells can be examined for cytogenetic abnormalities under an optical microscope, thus allowing the speedy and accurate determination of the MI, chromosome breaks and aberrations, and micronuclei. This technique is invaluable in genotoxicity research (Prasad et al., 2012), spindle failure, and polyploidy and aneuploidy incidence (Lin & Aarts, 2012; Nalci et al., 2019). Moreover, the mitotic index shows how often cells divide, which is crucial in determining how fast roots grow and how well anti-mitotic agents work (Zou et al., 2006). The value of the MI in onion and black seed plants was at its beak 6h after treatment with a concentration of 50ppm of Cr, whereas it was at its lowest at 24h after treatment with a concentration of 2,000ppm of Cr. These findings were consistent with those of Eleftheriou et al. (2012) and Gupta et al. (2018), who discovered that $Cr^{(VI)}$ had a dose-and time-dependent detrimental effect on root growth rate and the MI during the cell division cycle in *A. cepa*. In addition, Hemachandra & Pathiratne (2015) found that the roots of *A. cepa* bulbs exposed to $Cr^{(VI)}$ exhibited the highest levels of growth inhibition, MI suppression, and nuclear abnormalities. According to the obtained results, the proportion of aberrant cells increased when the duration of exposure or Cr concentration increased. In this regard, Glińska et al. (2007) hypothesized that the suppression of MI in metal-exposed roots could stem from a disrupted cell cycle or chromatin disfunction resulting from the metal-DNA interaction. The mito-depressing effect of Cr on the roots of *A. cepa* and *N. sativa* reduces root length. This inhibition may result from a blockage of the G1 phase and suppression or inhibition of DNA synthesis at the S phase.

In contrast to other metals, chromium salts readily permeate the cell membrane. They are converted to trivalent type that directly react with DNA, resulting in the alteration of bases, single- and double-strand breaks, and different adducts such as Cr-DNA, DNA-Cr-DNA, and protein-Cr-DNA adducts. Cr causes genotoxicity by acting directly on DNA (Santos & Rodriguez, 2012).

Natural and environmental factors, such as radiation or chemicals, both contribute to the induction of chromosomal abnormalities (Russel, 2002). The effects of pollutants, such as heavy metals, on plants have been extensively studied, and the results of current investigations agree well with those of previous publications (Gupta et al., 2012; Hemachandra & Pathiratne, 2015). Increases in the proportion of aberrant root meristems suggest the genotoxicity of the tested chemicals (Ghosh et al., 2016). In the present study, a wide range of mitotic cell division defects in the two plant species analyzed here, with some concentrations approaching the maximum level of aberrations (100%). Increasing chromosomal abnormalities may result from a multitude of events. Chemical interference has the most significant impact during DNA repair. Sticky chromosomes, which are a hallmark of chromosome-harming effects, were among the most frequently detected abnormalities in the root tips of plants treated with Cr during metaphase, anaphase, and telophase. This finding directly contradicts those of Kumari et al. (2011), Ghosh et al. (2016), and Sun et al. (2019), who demonstrated that chromosomal stickiness was the most prevalent CA, supporting the significant DNA fragmentation observed in the comet assays conducted on *A. cepa* specimens. In addition, the enzyme system disrupted by the stickiness, that slowed down cell division (Mahakhode & Somkuwar, 2013). The high c-metaphase values indicate that Cr is aneugenic (Zou et al., 2006). The increased Cr content may cause oxidative damage, and abnormally high c-mitosis frequencies may indicate that the mitotic apparatus is partially blocked. Aneuploidy is more likely to be caused by the chromosome bridges formed when chromatids fuse together after being broken off from their homologous chromosomes (Leme & Marin-Morales, 2009).

Although few micronuclei were discovered, this technique is crucial for determining the genotoxic and cytotoxic effects of mutagens and environmental contaminants. Acentric fragments or lagging chromosomes that cannot join with telophasic daughter nuclei are the most common causes of micronuclei. A loss of primary genes could lead to cell death via this process (Kirsch-Volders et al., 2011). Only a few micronuclei were observed here because of the short exposure time to Cr, which generally occurs after 48 to 72h of exposure, as described by Eleftheriou et al. (2012). Cr may cause chromosomal abnormalities in the metaphase and ana-telophase stages of mitosis. It has been shown through chromosomal anomalies that Cr can disrupt nucleic acids and cause chromosome breakage.

The comet test, which is a sensitive method for detecting DNA damage, was used to determine the genotoxicity of Cr in *A. cepa* root tip cells. This method permits the identification of DNA strand breakage in individual cells. At concentrations greater than 700ppm. Results revealed that statistically significant differences in the mean values of tail DNA percentage; however the tail length and moment values differed considerably at all concentrations. In a study using *A. cepa* bulbs, Patnaik et al. (2013) found that exposure to $Cr^{(VI)}$ at varying concentrations led to micronuclei, chromosomal abnormalities, and DNA damage in the plants' root cells, which agree with the present results. The comet assay showed that, compared with the controls, the measured amounts of Cr generated a substantial spectrum of DNA damage in the nuclei of *A. cepa* and *N. sativa* roots. Furthermore, permanent changes in DNA replication, repair, recombination, and transcription have been linked to $Cr^{(VI)}$ induced DNA damage or genotoxicity (Ueno et al., 1995; Casadevall et al., 1999; Rodriguez et al., 2011) because of the metal ion's propensity to generate ROS in plant or mammalian cells (Watanabe & Suzuki, 2002; Yadav & Sehrawat, 2011).

Conclusion

In this study, we investigate the responses of common onion (*A. cepa*) and black seed (*N. sativa*) to hexavalent chromium $Cr^{(VI)}$, which cause negative effects on plants. All applied concentrations of $Cr^{(VI)}$ for different treatment times cause a gradual decrease in the percent of germination and radicle lengths in *A. cepa* and *N. sativa*. Moreover, all concentrations of $Cr^{(VI)}$ applied for different treatment times cause both

cytotoxic and genotoxic effects on the tested plants by reducing the MI, causing an imbalance in the frequency of different mitotic phases, and inducing a wide range of chromosomal aberrations in root tip cells of *A. cepa* and *N. sativa.* There was a variation between the two plants in response to $Cr^{(VI)}$: the *N. sativa* responded to$Cr^{(VI)}$ more effectively than the *A. cepa.* Finally, $Cr^{(VI)}$ was found to cause severe DNA damage in *A. cepa* and *N. sativa* at all concentrations.

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تقييمات السمية النباتية والسمية الجينية للكروم سداسى التكافؤ على خلايا الجذور فى نباتى البصل وحبة البركة

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كواحد من الأسباب العالمية الرئيسية للتلوث البيئي، تضر المعادن الثقيلة بالزراعة وصحة الإنسان من خلال السلسلة الغذائية. باستخدام مؤشر معدل الانقسام (MI) ، والانحرافات الصبغية (CAs) ، وتلف الحمض النووي، تم تقييم التأثيرات السامة للخلايا والسمية الجينية للكروم سداسى التكافؤ على خلايا الجذور فى نباتى البصل وحبة البركة. تمت معاملة النباتات بستة تركيزات من الكروم السداسى (50 ، 100 ، 400 ، 700 ، 1000 ، 2000جزء في المليون) لمدة 6، 12، 18، 24 ساعة. انخفضت نسب إنبات بذور البصل وحبة البركة عند 400 جزء في المليون لكلا النباتين، مع ملاحظة عدم وجود انبات في نبات حبة البركة عند أو أعلى من 700 جزء في المليون. كان معدل الانقسام الميتوزى في المجموعة المعالجة أقل بكثير من خلايا الكنترول بعد التعرض لتركيزات مختلفة من الكروم سداسى التكافؤ لأوقات تعرض مختلفة. أيضا كان الانخفاض فى معدل الانقسام الميتوزى في خلايا القمم النامية لحبة البركة أكثر وضوحا منه فى نبات البصل. تم تحديد أكبر نسبة للشذوذ فى حبة البركة بعد 24 ساعة من التعرض لـ 100 جزء في المليون، بينما أظهر نبات البصل أكبر نسبة شذوذ بعد 24 ساعة من التعرض لـ 400 جزء في المليون. في كل مراحل الاقسام، لوحظ وجود شذوذ في الانقسام فى خلايا القمم النامية للجذور في كلا النباتين. بعض الأمثلة على أنواع الشذوذ هي اللزوجة، الطور الاستوائى الكولشيسينى، القناطر فى الطور الانفصالى والنهائى، الكروموسومات الشاردة المضطربة، والنويات الدقيقة. تم استخدام اختبار (Comet assay)، الذي يُظهر الانكسار أحادي الخيط في الحمض النووي، لتحديد مدى خطورة الكروم السداسى على الحمض النووي في خلايا الجذور للبصل. كان من الواضح تلف الحمض النووي بشكل كبير في تجربة المذنب مقارنةً بالكنترول في جميع التركيزات. تشير الدلائل المستمدة من كل من الخلايا المرستيمية لجذور البصل وحبة البركة إلى أن الكروم سداسى التكافؤ سام للخلايا وسام للجينات ويؤدي إلى تلف الحمض النووي بطريقة تعتمد على الجرعة.