Comparative genotoxicity of heavy metals in root meristems of *Cuminum cyminum* L.

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ABSTRACT: Effects of heavy metals are very extreme on biological organisms. Pollution causes accumulation of metals in soil which adversely affects the yield attributes of the crop plants. *Cuminum cyminum* L., (Cumin) is a major spice growing crop of India. The present study has been focused to scrutinize the cytogenetic effects of Chromium (Cr), Cadmium (Cd) and Lead (Pb) on root meristems of *Cuminum cyminum* L. During experiment, germinated seeds of cumin were exposed to four different concentrations viz., 50ppm, 100ppm, 150ppm and 200ppm of metallic salts of Chromium (Cr₂O₃), Cadmium (CdCl₂) and Lead [Pb(NO₃)₂], respectively. The AMI% (Active Mitotic Index) was observed to show a sharp decline whereas TAB% (Total abnormality percentage) was elevated with increasing metal concentrations. Various Chromosomal aberrations were recorded viz., scattering, stickiness, precocious movement, laggards, bridges, c-metaphase, etc. Among the various types of chromosomal aberrations encountered, stickiness was dominant among all. On comparison Pb was found to be more genotoxic followed by Cd and Cr, respectively.

KEYWORDS: Active Mitotic Index, Total Abnormality Percentage, Genotoxicity, *Cuminum cyminum* L., Heavy metals

Expanding population leads to shrinking of natural resources and many problems which were less dominant earlier have become more prominent now a days. Pollution is one such problem which is increasing steadily because of myriad sources, of which, heavy metals pollution in particular is more hazardous because the effects of these metals are very detrimental on human health. Heavy metals are generally referred to as those metals which possess a specific density of more than 5 g/cm³ and adversely affect the environment and living organisms (Jarup 2003). Heavy metals have relative high density and show their deteriorating effects even at miniscule concentrations. The term 'Heavy metals' is misnomer has been questioned over many years and all attempts were failed to replace it (Armah *et al.* 2014). Activities like mining, soil erosion, natural weathering of earth's crust, industrial effluent, sewage discharge, insects or disease control agents applied to crops are some sources of heavy metals (Morais *et al.* 2012).

Among all heavy metals cadmium (Cd), Chromium (Cr), and lead (Pb) are highly toxic and hazardous heavy metals, which are released into environment by anthropogenic activities. Cadmium at extreme levels causes Itai-itai disease and at low levels over prolonged period causes high blood pressure, sterility among males, kidney damage and flu disorders (Baird 1999). Chromium, particularly Cr (IV) reported to cause lung cancer. Lead causes serious health disorders such as anaemia, kidney disease and affects the nervous system (Crosby 2002). The evidence from studies in humans shows adverse neurotoxic effects besides cancer occurring at very low concentrations of lead. Plants are the ideal biological material to test the toxicity of heavy metals as they are in direct contact with soil and water from which they can accumulate heavy metals. In case of plants, the

permeability of cell membrane, the biochemical activities at the macromolecular level and the regular growth and reproduction of cells are negatively affected (Tuna *et al.* 2001).

Cumin (*C. cyminum*) is small annual herbaceous plant. In India, cumin is grown as major spice and well known for its economical and medicinal properties. The use of aromatic medicinal herbs to relieve and treat many human diseases has been increased in world because of their mild feature and low side effects (Abu-Darwish 2009). Cumin seeds are prominently considered as carminative, eupeptic, antispasmodic, and astringent. In Unani system of medicine, the fruits of *C. cyminum*, were used for the treatment of corneal opacities, ulcers, boils, styes and to relieve cough and inflammation (Shivakumar *et al.* 2010)

Metal contamination in herbal drugs continues during their transportation and storage at herbal shops where these are exposed to environmental pollution, dust, and heavy metals because of unhygienic storage conditions, and become toxic in nature. Such contaminated herbs are one of the major potential sources of heavy metal accumulation in the human organs and systems, because these are not only utilized as herbal medicines and food supplements, but many of them are consumed as condiments in daily routine (Hina *et al.* 2011).

Mitotic study is of great significance because the root tips are often the first to be exposed to chemicals spread in nature, in the soil and water (Samuel *et al.* 2010). Henceforth assessment constituting mitotic study shall assist in obtaining an insight to the cytogenetic level. The present study has been conducted to reveal the comparative toxic impact of Cadmium (CdCl₂), lead nitrate [Pb (NO₃)₂] and Chromium oxide (Cr₂O₃) on the root tips of cumin (*Cuminum cyminum* L.) for examining the effect that these metals have on the mitotic stages as

Fig. 1. Mitotic chromosomes of *Cuminum cyminum* L.

A: Normal Prophase, B: Normal Metaphase, C: Normal Anaphase, D: C- metaphase, arrow shows c-metaphase, E: Unorientation at metaphase, F: Scattering at metaphase, G: Precocious movement, arrow shows precocious chromosome, H: Clumping at metaphase, I: Stickiness at metaphase, J: Scattering at anaphase, K: Unorientation with broken bridge at anaphase, arrows show the broken bridge, L: Stickiness at anaphase. Scale bar shows 10 μ m.

well as the on the chromosomal architecture.

MATERIALS AND METHODS

Seed procurement Seeds of *Cuminum cyminum* L. var. *Cumin-4* were procured from Centre for Research on Seed Spices, Jagudan, Gujarat, India.

Treatment The seeds were kept soaked for sometime in distilled water followed by Sodium Hypochloride solution for two minutes. Thereafter the seeds were thoroughly washed with running water. Presoaked seeds were allowed to germinate, and then the root meristems were treated with freshly prepared aqueous solution of Cadmium chloride, Lead nitrate and Chromium oxide for 3 hours, at

Table 1. Effect of Pb (NO₃)₂, CdCl₂ and Cr₂O₃ on active mitotic index and total abnormality percentage of *Cuminum cyminum* L.

Concentration	AMI % (Mean ± SE)			TAB % (Mean ± SE)		
	Cr	Cd	Pd	Cr	Cd	Pd
Control	12.29±0.10 ^a	12.29 ±0.10 ^a	12.29±0.10 ^a	-	-	-
50 ppm	11.72±0.25 ^{ab}	10.88±0.42 ^b	9.21±0.55 ^b	1.78±0.34 ^c	2.27±0.21 ^d	3.65±0.64 ^c
100 ppm	10.55±0.18 ^{bc}	8.71±0.46 ^c	8.27±0.63 ^b	2.50±0.20 ^c	4.49±0.36 ^c	5.51±0.64 ^{bc}
150 ppm	9.75±0.92 ^c	7.04±0.61 ^d	6.68±0.71 ^c	4.85±0.36 ^b	6.07±0.29 ^b	7.31±0.81 ^b
200 ppm	7.51±1.30 ^d	5.97±0.25 ^d	5.00±0.08 ^c	7.20±0.22 ^a	9.22±0.27 ^a	11.20±0.33 ^a

AMI: Active Mitotic Index, TAB: Total Abnormality Percentage

Means followed by lower case letter are statistically significant at $p < 0.05$ in Duncan's Multiple Range Test (DMRT).

different concentrations viz. 50ppm, 100ppm, 150ppm and 200ppm. Some root meristems were kept in distilled water for control in each set. Experiment was performed in 7 replicates. After 3 hours, metal treated roots were washed thoroughly with distilled water. Then, these root meristems were fixed in Carnoy's fixative (Glacial Acetic Acid : Absolute Alcohol = 1 : 3) along with respective control. After 24 hours, the fixed treated root meristems were preserved in Absolute Alcohol and then cytological preparations were carried out.

Mitotic preparation The roots were taken in 1N HCl and hydrolyzed in water bath maintained at 60°C. Then the roots were washed under running water to remove excess of HCl. Now, the roots were stained using 2 % Acetocarmine for 30 minutes. Squash preparation was done for mitotic observation. Observed cells were snapped under Nikon Research Electron Microscope using PCTV vision software at 40X resolution. Mitotic indices were calculated by

$$\text{Active Mitotic index (AMI) \%} = \frac{\text{Total no. of dividing cell}}{\text{Total no. of cell observed}} \times 100$$

Statistical analysis Statistical analysis was performed using the SPSS 16.0 software. A one way analysis of variance (ANOVA) and Duncan's multiple range test ($p \leq 0.05$) were performed and the graph was plotted by using Sigma plot 10.0 software.

RESULTS AND DISCUSSION

In cumin, diploid chromosome number has been found to be $2n = 14$. The cytological observations unveiled the mitotic index in control set was 12.29 ± 0.10^a % showing normal chromosomal morphology at metaphase (Fig.1 B) and normal separation at anaphase 14 : 14 (Fig.1 C). In experiment, root meristems treated with (Cr₂O₃), (CdCl₂) and Pb(NO₃)₂ solutions create check in path of

chromosomal movement. On increasing the concentrations, the chromosomal abnormalities increased whereas mitotic index decreased (Table2).

Table 1 also shows AMI % and TAB % are inversely related to each other. AMI % reduced from 11.72 ± 0.25^{ab} (50ppm) to 7.51 ± 1.30^d (200ppm) in Chromium treated sets, whereas in Cadmium treated sets it declined from 10.88 ± 0.42^b (50ppm) to 5.97 ± 0.25^d (200ppm) and 9.21 ± 0.55^b (50ppm) to 5.00 ± 0.08^c (200ppm) in Lead treated sets. Fig. 2 shows the comparative trend of AMI % against the increasing concentrations of heavy metals.

Heavy metals induced various chromosomal abnormalities as shown in Fig. 1 such as c- metaphase (Fig. 1D), unorientation at metaphase (Fig. 1E), scattering (Fig. 1F), precocious movement (Fig. 1G), clumping (Fig. 1H), stickiness at metaphase (Fig. 1H), scattering (Fig. 1J), un-orientation with broken bridge (Fig. 1K), stickiness at anaphase (Fig. 1L) and so on.

In case of chromium treated sets TAB % shows elevation from 1.78 ± 0.34 (50ppm) to 7.20 ± 0.22 (200 ppm) and in Cadmium, it increases from 2.27 ± 0.21 (50 ppm) to 9.22 ± 0.27 (200ppm) while in Lead, it shows major increase from 3.65 ± 0.64 (50ppm) to 11.20 ± 0.33 (200ppm) (Table 1). In all the treated sets; stickiness percentage was recorded as the predominant chromosomal abnormality as shown in Table 2.

Heavy metals adversely affect the growth and development of roots. The Active Mitotic Indices reflects the frequency of cell division and it is regarded as important parameter while determining the rate of root growth (Liu *et al.* 1992). Genotoxicity of heavy metals is reflected by the gradual decrease in Active Mitotic Indices as concentration of doses increased (Fig. 2). These metals are the potent clastogenic as they are responsible for the reduction in the Mitotic Index and a wide range of chromosomal aberrations. They also induce the chromosomal breakage and structural deformity; there action on chromosome is generally regarded to involve an action on DNA (Grant 1978; Chauhan and Sundararaman

Table 2. Comparative account of Metaphasic and Anaphasic abnormalities induced by Cr_2O_3 , CdCl_2 and $\text{Pb}(\text{NO}_3)_2$ in root meristems of *Cuminum cyminum* L.

Treatment	Concentration	Metaphasic abnormalities (Mean $\pm$ SE)					Anaphasic abnormalities (Mean $\pm$ SE)					Other (Mean $\pm$ SE)
		Sc	St	Un	Pr	Cm	Sc	St	Un	Lg	Br	
Cr	50 ppm	0.12 $\pm$ 0.12	0.76 $\pm$ 0.11	0.28 $\pm$ 0.14	-	-	0.12 $\pm$ 0.12	0.28 $\pm$ 0.14	0.18 $\pm$ 0.18	-	-	-
	100 ppm	0.33 $\pm$ 0.16	0.56 $\pm$ 0.07	0.18 $\pm$ 0.18	0.38 $\pm$ 0.20	-	0.38 $\pm$ 0.20	0.15 $\pm$ 0.15	0.18 $\pm$ 0.18	-	0.15 $\pm$ 0.15	0.18 $\pm$ 0.18
	150 ppm	0.28 $\pm$ 0.14	1.08 $\pm$ 0.08	0.12 $\pm$ 0.12	0.28 $\pm$ 0.14	0.52 $\pm$ 0.34	0.43 $\pm$ 0.26	0.75 $\pm$ 0.09	0.67 $\pm$ 0.25	0.12 $\pm$ 0.12	0.28 $\pm$ 0.14	0.28 $\pm$ 0.14
	200 ppm	1.15 $\pm$ 0.65	1.43 $\pm$ 0.04	0.28 $\pm$ 0.14	0.12 $\pm$ 0.12	0.86 $\pm$ 0.26	0.92 $\pm$ 0.13	1.31 $\pm$ 0.09	0.32 $\pm$ 0.32	0.35 $\pm$ 0.19	0.28 $\pm$ 0.14	0.12 $\pm$ 0.12
Cd	50 ppm	-	1.05 $\pm$ 0.10	0.16 $\pm$ 0.16	-	-	0.16 $\pm$ 0.16	0.67 $\pm$ 0.12	0.21 $\pm$ 0.21	-	-	-
	100 ppm	0.14 $\pm$ 0.14	1.23 $\pm$ 0.24	0.26 $\pm$ 0.16	0.40 $\pm$ 0.22	0.14 $\pm$ 0.14	0.18 $\pm$ 0.18	1.05 $\pm$ 0.14	0.18 $\pm$ 0.18	0.40 $\pm$ 0.22	0.40 $\pm$ 0.22	0.14 $\pm$ 0.14
	150 ppm	0.75 $\pm$ 0.08	1.35 $\pm$ 0.08	0.27 $\pm$ 0.13	0.47 $\pm$ 0.06	0.44 $\pm$ 0.17	0.62 $\pm$ 0.15	0.87 $\pm$ 0.14	0.32 $\pm$ 0.17	0.27 $\pm$ 0.13	0.44 $\pm$ 0.17	0.27 $\pm$ 0.13
	200 ppm	0.93 $\pm$ 0.17	2.19 $\pm$ 0.17	0.63 $\pm$ 0.09	0.63 $\pm$ 0.09	0.29 $\pm$ 0.14	0.70 $\pm$ 0.16	1.80 $\pm$ 0.10	0.79 $\pm$ 0.10	0.63 $\pm$ 0.09	0.50 $\pm$ 0.06	0.13 $\pm$ 0.13
Pb	50 ppm	0.44 $\pm$ 0.23	1.33 $\pm$ 0.25	0.13 $\pm$ 0.13	-	-	0.31 $\pm$ 0.16	1.15 $\pm$ 0.21	0.27 $\pm$ 0.27	-	-	-
	100 ppm	0.59 $\pm$ 0.09	1.49 $\pm$ 0.20	0.28 $\pm$ 0.14	0.28 $\pm$ 0.14	0.31 $\pm$ 0.16	0.41 $\pm$ 0.22	1.21 $\pm$ 0.07	0.31 $\pm$ 0.16	0.31 $\pm$ 0.16	0.15 $\pm$ 0.15	0.13 $\pm$ 0.13
	150 ppm	0.48 $\pm$ 0.03	2.24 $\pm$ 0.20	0.48 $\pm$ 0.03	0.30 $\pm$ 0.15	0.48 $\pm$ 0.03	0.14 $\pm$ 0.14	2.04 $\pm$ 0.48	0.30 $\pm$ 0.15	0.32 $\pm$ 0.16	0.16 $\pm$ 0.16	0.32 $\pm$ 0.16
	200 ppm	0.84 $\pm$ 0.10	4.19 $\pm$ 0.24	0.21 $\pm$ 0.21	0.68 $\pm$ 0.12	0.47 $\pm$ 0.26	0.51 $\pm$ 0.27	3.03 $\pm$ 0.60	0.37 $\pm$ 0.19	0.47 $\pm$ 0.26	0.21 $\pm$ 0.21	0.16 $\pm$ 0.16

Sc- Scattering, St- Stickiness, Un- Unorientation, Pr- Precocious movement, Cm- C-metaphase, Lg-Laggard formation, Br- Bridge

Fig. 2. Comparative account of active mitotic index (AMI %) for Cr_2O_3 , CdCl_2 and $\text{Pb}(\text{NO}_3)_2$ in root meristems of *Cuminum cyminum* L.

1990).

In our experimental investigation, the highest (TAB %) was recorded in Pb treated sets followed by Cd and Cr, respectively (Fig. 3). Stickiness was found to be more prominent abnormality in Pb and Cd treated sets. Stickiness may be defined as the physical adhesion involving mainly proteinaceous matrix of chromatin material (Patil and Bhatt 1992). Stickiness has been reported to be a result of partial dissociation of nucleoproteins and alteration in the pattern of organization of chromosomes (Evans 1962) or due to disturbances in cytochemically balanced reactions (Jayabalan and Rao 1987). The chromosomal stickiness could also be observed at high frequency owing to the disturbance in nucleic acid metabolism of the cell (Chidambaram *et al.*, 2006)

Heavy metals make highly reactive complexes which directly interact with DNA, Histone or Non Histone proteins, causing chromosomal deformities and making them to be sticky (Kumar and Rai 2007). Malfunctioning of one or two types of specific non histone proteins, which were involved in chromosomal organization and in chromatid separation are also responsible for chromosomal stickiness (Gaulden 1987). Sticky chromosome could not move towards equator lines or poles of anaphase and cause improper division in cell cycle ultimately cause lethality of the cell and affecting the growth of roots. Due to disturbance in spindle apparatus by these metals, there is irregular spreading of chromosomes over the cells (Oladele *et al.* 2013). Pb and Cr form complexes with the tubulin proteins by binding with its carbonyl group of peptide chains and by this mechanism Pb and Cr bind to tubulin molecules and it may change their structure

leading to spindle dysfunction (Mishra *et al.* 2012).

Spindle activity is very important for normal cell division. Abnormal behavior of spindle activity leads to many abnormalities in chromosomes during cell division such as unequal distribution, bridge, C-metaphase, laggards, precocious movement etc. The high doses of Chromium supply have a toxic effect on cell division which attributes different types of abnormalities due to loss of microtubule of spindle fibres (Pickett-Heaps and Trimothy 1982; Katz and Salem 1993). Chemical breakage of the protein moiety of nucleoprotein backbone may be one of the possible reasons of precocious movement (Patnaik 1984). According to Levan (1938), C-metaphase can be described as inactivated spindle followed by random scattering of condensed Chromosome.

In the present study, the chromosomal bridge was reported in all treated sets. Bridges might be formed due to the sticky behavior of chromosomes which could not move towards the poles at anaphase (Kumar and Rai 2007). The breaks at the same locus and their lateral fusion might have also led to the formation of dicentric chromosomes (Rai and Kumar 2010). It has been reported that the dicentric chromosomes were involved in the formation of bridges which are formed by the inversion of chromosome. The dicentric chromosomes were pulled equally towards both poles at anaphase and bridges were formed (Anis *et al.* 1998).

Laggard chromosomes depend upon the moving speed and process of an individual chromosome differing from normal ones (Qian 2004). Rivetta *et al.* (1997), found that Cd binds to CAM calmodulin and competes with Cd in

Fig. 3. Comparative account of total abnormality percentage (TAB %) for Cr_2O_3 , CdCl_2 and $\text{Pb}(\text{NO}_3)_2$ in root meristems of *Cuminum cyminum* L.

these bindings. This might be explanation of mitotic abnormalities caused by Cd (Liu *et al.* 2003).

According to Rodriguez (2011), Cr. can induce blockage of cell cycle at G2/M checkpoint due to severe DNA degradation, and giving the cells extra time to either repair the damage (Ó Conell and Cimprich 2005) or activate an apoptosis (Santos and Rodriguez 2012), which might have led to the chromosomal aberrations. Garcia-Leston *et al.* (2010) suggested that Pb has the ability to replace the calcium/zinc in enzymes involved in DNA processing and repairing and enhancing the genotoxicity when combined with other DNA damaging agents.

Cr acts as prophase poison by exerting deleterious effects on the enzymatic activity of ribonuclease and inhibits the progressive cell division cycle (Jayaprakash *et al.* 1994).

CONCLUSION

The present study reveals the genome damaging effects of heavy metals on *Cuminum cyminum* L. Heavy metals furthermore induce various genotoxic effects which may cause deleterious changes in genetic material of plants. The impact of Pb was found to be more genotoxic followed by Cd and Cr, respectively. Heavy metals are extremely toxic elements and they could reduce the mitotic activity and induce many types of chromosomal anomalies. Soil and water polluted with these heavy metals not only effect crop but also reduce the productivity of vegetable crops which are directly consumed by humans and causing great impact on human health. Hence, it is a matter of concern that pollutant from

various industries should be properly treated before draining into water sources. So, the concentration of heavy metals should be optimized to a level which is less harmful for the crop plants which ultimately led to the increment in the yield of crop.

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