EFFECTS OF CADMIUM AND SELENIUM ON CELL DIVISION AND CHROMOSOMAL ABERRATIONS IN *Allium sativum* L.

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Abstract. The effects of $CdCl_2$ and Na_2SeO_3, administered singly and in combination, were studied on cell division and chromosomal aberrations in root tip cells of *Allium sativum* L. The frequency of dividing cells was reduced significantly and the number of chromosomal aberrations was enhanced significantly following combined treatment with Cd and Se as compared with treatment with the individual salts. The type of interaction and consequent effects on cytotoxicity depended mainly on the relative concentrations of the two salts used.

1. Introduction

Cadmium is a heavy metal pollutant known for its phytotoxic effect (Fox, 1974; Nriagu, 1980; Mukherjee *et al.*, 1984; Jana *et al.*, 1987). The essential trace element Se is a metalloid which interacts with a number of toxic heavy metals such as Pb, Hg, Ag, methylmercury, Tl, As and Cd and renders these substances less toxic (Nordberg, 1976; Shamberger, 1985). Particularly Se is known to protect a wide range of organisms from Cd toxicity (Early and Schnell, 1981). Data on such studies on plants are relatively meagre. In the present investigation we attempt to analyze the effects of different concentrations of the heavy metal Cd and the metalloid Se singly, and the effects of combinations of Cd and Se on cell division and chromosomal aberrations in garlic, *Allium sativum* L. roots.

2. Materials and Methods

2.1. Plant material

Bulbs of *Allium sativum* L. single clove variety, each weighing 2 to 4 g, procured in bulk from an established nursery, were chosen as test system due to their sensitivity to changes in environmental conditions (Kihlman, 1974).

2.2. Treatment with $CdCl_2$ and Na_2SeO_3

Bulbs (10 in number) were kept with the roots immersed in Hoagland's solution pH 7 (control) or graded buffered Hoagland's solutions (pH 7), containing $CdCl_2$ and Na_2SeO_3, separately or in combinations, for 24 hr at $30 \pm 2°$ C. The solutions were also aerated artificially. The excised root tips were then washed with distilled water, and fixed

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in acetic-ethanol (1 : 3). Slides were prepared following the standard acetic-orcein squash schedule (see Sharma and Sharma, 1980). Approximately 1000 cells were scanned for the frequencies of dividing and aberrant cells.

Accumulation of Cd in root tissue was measured by an Atomic Absorption Spectrophotometer. Fresh root tissue was dried in oven at 60 to 80 °C. To 1 g of the sample, 5 mL of triacid mixture (conc. HNO_3, H_2SO_4, and 60% $HClO_4$; 9 : 2 : 1) were added. The mixture was heated until a clear solution was obtained. The digested material was then diluted to 100 mL with glass distilled water and prepared for observation in Perkin–Elmer 303 AAS. The mean values with standard deviation are given in tables. Duncan's multiple range test was used to determine the significance at 95% confidence limits.

3. Results and Discussion

The principal effects of Cd and Se were of two types and involved either spindle disturbances (Group I) with consequent disturbed division or alterations in the chromosome structure (Group II) including breaks and gaps. The frequency of dividing cells at 0.5 mg L^{-1} of Cd was lower than control data following exposure for 24 hr (Table I). At 5 mg L^{-1} of Cd the mitotic frequency decreased significantly from that of control. The percentage of aberrant cells was, however, significantly higher than that of control. It was related to the doses used, with the Group I type of aberrants predominant over Group II type.

TABLE I

Effects of concentrations of $CdCl_2$ and Na_2SeO_3 on root tip cell division of *Allium sativum* at 24 hr exposure

Treatment with concentration (mg L^{-1})	Dividing cell (%)[a]		
		Observed inhibition	Sum of individual inhibition
Control (0)	7.561 ± 1.34 a	–	–
Cd (0.50)	6.802 ± 1.45 a	0.759	–
Cd (5.00)	5.608 ± 1.20 b	1.953	–
Se (0.05)	7.458 ± 1.08 a	0.103	–
Se (0.50)	6.205 ± 2.04 a	1.356	–
Cd (0.50) + Se (0.05)	5.514 ± 0.93 b	2.047	0.862
Cd (5.00) + Se (0.05)	5.133 ± 1.70 b	2.428	2.056
Cd (0.50) + Se (0.50)	5.134 ± 1.27 b	2.427	2.115
Cd (5.00) + Se (0.50)	5.077 ± 0.81 b	2.554	3.309

[a] Values in a vertical column followed by the same letter are not significantly different at 5% level as determined by Duncan's multiple range test. Each value is the mean of 10 samples ± S.D.

TABLE II

Effects of concentrations of $CdCl_2$ and Na_2SeO_3 on mitotic cells of *Allium sativum* at 24 hr exposure

Treatment with concentration ($mg\ L^{-1}$)	Aberrant cell (%)[a]								
	Total			Group I			Group II		
		Observed increase	Sum of individual increase		Observed increase	Sum of individual increase		Observed increase	Sum of individual increase
Control (0)	0.082 ± 0.020 a	–	–	0.076 ± 0.030 a	–	–	0.006 ± 0.001 a	–	–
Cd (0.50)	0.152 ± 0.030 b	0.070	–	0.145 ± 0.009 b	0.069	–	0.007 ± 0.001 b	0.001	–
Cd (5.00)	0.305 ± 0.090 c	0.223	–	0.155 ± 0.020 bc	0.079	–	0.013 ± 0.008 c	0.007	–
Se (0.05)	0.121 ± 0.080 b	0.039	–	0.119 ± 0.070 b	0.043	–	0.002 ± 0.001 d	0.004	–
Se (0.50)	0.140 ± 0.008 b	0.126	–	0.131 ± 0.060 b	0.055	–	0.009 ± 0.003 bc	0.003	–
Cd (0.50) + Se (0.05)	0.221 ± 0.060 d	0.129	0.109	0.193 ± 0.070 c	0.117	0.112	0.028 ± 0.010 d	0.021	0.005
Cd (5.00) + Se (0.05)	0.370 ± 0.080 c	0.288	0.262	0.220 ± 0.060 c	0.144	0.122	0.150 ± 0.007 f	0.144	0.011
Cd (0.50) + Se (0.50)	0.393 ± 0.080 c	0.0311	0.169	0.300 ± 0.080 d	0.224	0.124	0.093 ± 0.002 e	0.087	0.004
Cd (5.00) + Se (0.50)	0.426 ± 0.150 c	0.344	0.349	0.327 ± 0.090 d	0.251	0.271	0.099 ± 0.010 e	0.093	0.010

[a] Values in a vertical column followed by the same letter are not significantly different at 5% level as determined by Duncan's multiple range test. Each value is the mean of 10 samples ± S.D.

TABLE III

Accumulation of Cd in root tissue of *Allium sativum* at 24 hr exposure

Treatment with concentration (mg L^{-1})	Cd accumulated (mg kg^{-1} WW)[a]
Control (0)	–
Cd (1)	0.080 ± 0.001
Cd (10)	0.456 ± 0.002
Cd (0.5) + Se (0.05)	0.065 ± 0.040
Cd (5) + Se (0.05)	0.317 ± 0.070
Cd (0.5) + Se (0.5)	0.050 ± 0.010
Cd (5) + Se (0.5)	0.232 ± 0.090

[a] Data represent mean ± S.E. for n = 3

A few breaks could be recorded (Table II). At 0.05 and 0.5 mg L^{-1} of Se the mitotic frequency decreased with the duration of treatment but was not significant when compared to the control. The percentage of aberrant cells was related to the dose for both the concentrations used and was significantly high. Breaks and gaps, that is, aberrations of the Group II type were high in numbers but not higher than the frequency of Group I type aberrations.

Following combined treatment with

(i) Cd (0.5 mg L^{-1}) and Se (0.05 mg L^{-1});
(ii) Cd (5 mg L^{-1}) and Se (0.05 mg L^{-1});
(iii) Cd (0.5 mg L^{-1}) and Se (0.5 mg L^{-1}); and
(iv) Cd (b mg L^{-1}) and Se (0.5 mg L^{-1});

the frequencies of dividing cells were significantly lower than those of control and Se or Cd (0.5 mg L^{-1}) treated sets. The aberrations induced were significantly higher following the combined treatment than that recorded in control or individual salts. The types of aberrations noted mainly involved chromosomal alterations (Group I). The frequencies of gaps and breaks were higher than that of control and Cd (0.5 mg L^{-1}) or Se treated sets.

In the combination sets (i), (ii), and (iii) the decrease in divisional frequency as compared to the control, was greater than the sum of the decrease induced by individual salts over control. The enhancement in the frequency of aberrant cells in the combination sets was also greater than that induced by the individual salts over control. Thus, synergistic effect between Cd and Se at these combinations on the divisional frequency and increase in aberrant cells, was observed. The combination set 5 mg L^{-1} Cd with 0.5 mg L^{-1} Se gave an antagonistic interaction with respect to these parameters. In this case, the inhibition of divisional frequency or the increase in aberrant cells were less than the sum of individual inhibitions or increase over control.

Simultaneous treatment with other metal pollutants in plants gave either additive

(Dhir, 1985; Mukherjee and Sharma, 1987), syngergistic (Allison and Dzialo, 1981; Whitton and Snehalata, 1982; Jana and Choudhuri, 1984), or antagonistic effects (Pietilainen, 1975; Keul *et al.*, 1979). Certain metals like Hg, Cu, Zn, Cd, and Pb were more toxic in combination with each other than when present alone (Gachter, 1976). Interactions between Pb and Cd on primary production of phytoplankton indicated antagonism when concentration of Pb exceeded that of Cd while synergism was observed in solution where the concentration of Cd was greater than that of Pb (Pietilainen, 1975).

The concentration of Cd retained by the roots recorded after 24 hr of exposure revealed that the amount of Cd was more when administered individually than when given in combination with Se (Table III). That the effects were more or less physical was confirmed when the addition of ions of another metal further increased the toxicity. Hydroponic experiments with corn showed increased toxicity of Cd by the addition of Zn to the nutrient solution (Malone *et al.*, 1978). Atomic absorption spectroscopic studies show that though the amount of Cd retained in the roots in the combination sets, was lower than Cd given alone, the level of cytotoxicity was further enhanced. Probably the metals when given in combination disrupt cell division through acting on other metabolites or through ionic disbalance.

An overall assessment confirms that in all combination experiments the total effect was higher than the effect of either chemical given alone though in proportionate concentrations. The effects of Cd and Se on cell division and chromosomal aberrations may be synergistic or antagonistic depending on the relative concentrations of the two metallic salts used.

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