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The Effects of Cadmium on Seed Germination, Root Development and Mitotic of Root Tip Cells of Lentil (*Lens culinaris* Medik)

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Abstract: In this study, the effects of cadmium on the seed germination, root development and mitosis division of root tip cells of lentil (*Lens culinaris* Medik) were studied. For this purpose, lentil seed were germinated into various concentrations of $CdCl_2$ (0.0625, 0.125, 0.250, 0.500 and 1.00 mM) at 23-24°C for 72 h. In conclusion, it was determined that the germination of seeds and the growth of roots inhibited and mitotic abnormalities increased with the increasing of Cd concentration.

Key words: Cadmium · *Lens culinaris* · germination · mitosis

INTRODUCTION

The heavy metals pollution is a big problem for the large parts of the world. They usually occur as a result of natural and anthropogenic activities such as volcanic, industrial and mining, exhaust gases of vehicles and using of agricultural pesticides and fertilizers. In recent years, various aspects of the effects of heavy metals on plants have been studied in many researches.

The concentration and type of heavy metal and the species of plant are very important for these sorts of studies. Because it is well known that different plants behave very selective to the elements in their surrounding and the threshold dose of any heavy metal may vary according to the plant species and the kind of heavy metal. Especially, it is necessary to do these sorts of studies on the many different plant species. Since, different plant species show different adaptive mechanisms to the environmental stress factors.

Cadmium mainly is an unnecessary element for both plants and animals and it also has toxic effect for them when its concentration is exceeded a limit. Generally, it makes negative effect on their metabolisms by doing some reactions with enzymes [1, 2]. In recently, many researches have been carried out on the effects of cadmium on the plants. They showed that cadmium inhibited the germination of seeds, the development of root and body of plants [3, 4] and caused to the chlorophyll mutation in very high concentrations [5]. In addition, they were reported that cadmium caused to the reduction of mitotic index in root cells [6], chromosomal abnormalities and micronucleus formation [7-9], disorders in nucleus structure [10] and abnormalities in DNA and RNA synthesis [11, 12]. All these data shows that cadmium delay the normal growth periods of plants. It also cause to the considerable amounts of crop lost by inhibiting their genetic potentials.

In recent years, the consumption of lentil as an alternative food has been increased due to contain the higher proportion of protein and vitamin. In addition to this, animal protein is very expensive for the people living in the undeveloped and developing countries. Animal protein has also being preferred less by the people living in the developed countries due to its higher cholesterol content and increasing tendency of people to the vegetable foods. In 2001 Indian, Canada and Turkey have had 38, 19 and 13% of the lentil planting fields. These there countries have had 70% of the lentil planting fields on the world. Especially in the undeveloped and developing countries, more food requirements of the people due to rapid increase of the population have been increased to the demand for relatively cheapest leguminosae. In order to obtain these increased food demands of people, the planting fields for leguminosae have continuously increased on the world for last 20 years. In 2001 lentil which has a very important place into the edible leguminosae in Turkey has covered 30% of total leguminosae planting fields [13]. This study is therefore aimed to determine the effects of different cadmium concentrations on the germination of seeds, development of root and mitosis division of root tip cells in lentil (*Lens culinaris* Medik) which is a very important

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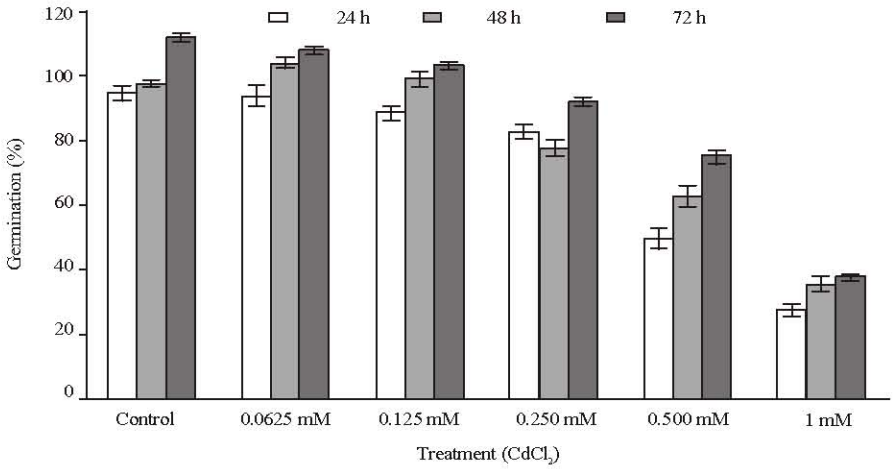

Fig. 1: The effects of different concentrations of $CdCl_2$ on the germination of lentil seeds

agricultural plant. We have not observed any studies on this subject as a result of detail literature investigation. So this study will be a light for the future studies.

MATERIALS AND METHODS

In this study, lentil (*Lens culinaris* Medik) seeds were obtained from Agriculture Directorate of Elazig City. Cadmium solutions were prepared from $CdCl_2$ (Sigma) by using of double distilled water (pH 6.3). The experiment was conducted in a temperature adjustable plant growth cabinet.

Lentil seeds were soaked in 150 mL solution of 0.0625, 0.125, 0.250, 0.500 and 1.00 mM Cd concentrations and kept in plant growth cabinet for 4 h at 23-24°C in dark. Only double distilled water was used for control group. Then 30 of swollen lentil seed for each Cd solution were planted in 11 mm diameter petri dishes with the double layer filter paper. Two replicates were made for each concentration. Planted petri dishes were filled with 9 mL of different Cd concentrations. Control groups were filled with only double distilled water. They were covered and kept into plant growth cabinet for 72 h in dark. Petri dishes were checked and added with 1 mL of Cd solutions for a period of 24 h. At the same time, the germination ratios of seeds were also determined by radicula formation bases. At the end of 72 h, the root lengths of the germinated seeds were measured with a millimetric ruler.

The root tips of germinated seeds were cut and kept into paradichlorobenzene for 4 h. Then they were fixed in acetic acid-alcohol (1:3) for 24 h. Fixed root tips were transferred in 70% alcohol and stored in the fridge.

For mitotic preparation, root tips were removed from alcohol and washed with tap water. After hydrolyzing with 1 N HCl in an etüve for 17 min at 60°C, they were dyed with Feulgen reactive for 1 h [14]. Then root tips were kept in tap water for 15 min. Finally the last parts of root tips which dyed very densely were cut and their crushing preparates in 45% acetic acid were made.

Statistical analysis was performed based on SPSS (version 10.0) program. To detect the significance of differences ($p<0.01$ or $p<0.05$) of variables, a multiple comparison (LSD) test was performed. All data are expressed as the mean±SD (n).

RESULTS

The germination percentages of lentil seeds exposed to different concentrations of $CdCl_2$ for 72 h are given in Fig. 1. This figure shows that seed germination was inhibited with increase of Cd concentration. In comparison with control group, two lowest concentrations (0.0625 and 0.125 mM) caused only 10% decrease of seed germination ($p>0.05$), but 0.250, 0.500 and 1.00 mM concentrations of Cd caused to the 23, 37.5 and 68.3% decreases of seed germination ($p<0.05$) respectively.

Figure 2 shows the root lengths of lentil seeds exposed to different concentrations of $CdCl_2$ for 72 h in dark. As it is shown in Fig. 2, the root development of lentil seedlings was gradually inhibited with increase of the Cd concentrations ($p<0.05$). Especially root development of lentil seedling exposed to 1.00 mM concentration of Cd inhibited around 95% compared with control ($p<0.01$).

In order to determination of the effects of the different Cd concentrations on mitosis division of root tip cells of the lentil plant, the type of chromosomal

Table 1: Mitotic effects of Cd in root tip cells of lentil plant

Treatment $CdCl_2$ mM	Number of cells	Mitotic İndex	Normal dividing cells in	Anormalous dividing cells				
				Micronucleus	c-mitosis	Multipolar anaphase	Anaphase bridge	Telophase bridge
Control	1500	42.73	630	1	5	4	1	-
0.0625	1500	38.60	540	7	17	6	5	4
0.125	1500	35.00	457	12	23	15	8	10
0.250	1500	26.00	310	13	43	12	6	6
0.500	1500	23.20	264	16	35	18	8	7

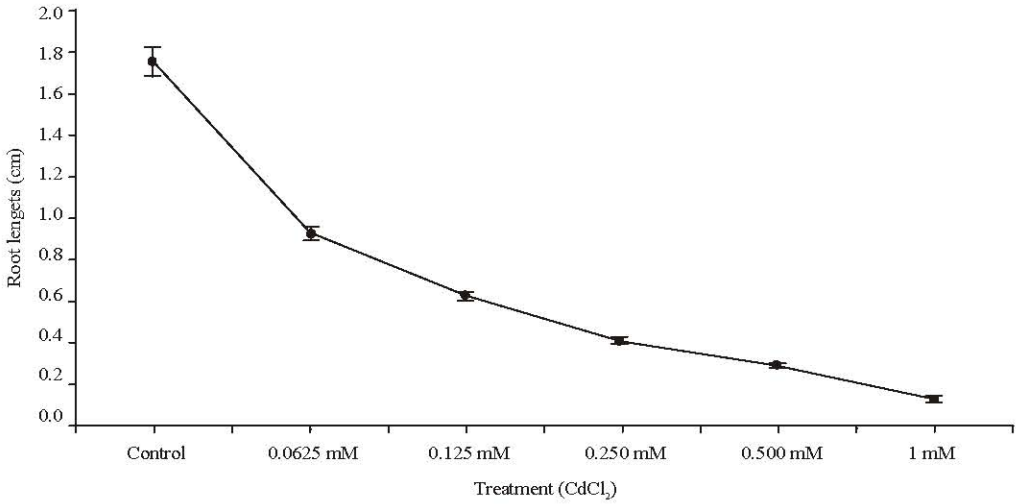

Fig. 2: The root lengths of lentil seedlings exposed to different concentrations of $CdCl_2$ for 72 h in dark during the germination

abnormalities and their distribution according to phases were determined in 1500 cells from each concentration (Table 1). The cell division was observed in only 579 of 1500 cells from root tips of lentil seedlings exposed to 0.0625 mM Cd. The abnormalities observed in this concentration were the micronucleus in 7 cells at prophase, c-mitosis in 17 cells at metaphase, the multipolar anaphase in 6 cells at anaphase, bridge formation in 5 cells at anaphase and bridge formation in 4 cells at telophase stages. The cell division was determined in only 525 of 1500 cells from root tips of lentil seedlings exposed to 0.125 mM Cd. The abnormalities occurred in this concentration were the micronucleus in 12 cells at prophase, c-mitosis in 23 cells at metaphase, multipolar anaphase in 15 cells at anaphase, bridge formation in 8 cells at anaphase and bridge formation in 10 cells at telophase stages. The cell division was determined in only 390 of 1500 cells from root tip cells of lentil seedlings exposed to 0.250 mM Cd. The abnormalities found in this concentration were the micronucleus in 13 cells at prophase, c-mitosis in 43 cells at metaphase, multipolar anaphase in 12 cells and the bridge formation in 6 cells at anaphase and bridge formation in 6 cells at telophase stages. The cell division in root tip cells of lentil seedlings exposed to 0.500 mM Cd was observed in only 348 of 1500 cells. The abnormalities of this concentration were the micronucleus in 16 cells at prophase, c-mitosis in 35 cells at metaphase, multipolar anaphase in 18 cells and bridge formation in 8 cells at anaphase and bridge formation in 7 cells at telophase stages. In the control group, cell division was observed in 641 of 1500 cells and the abnormalities occurred in control group were the micronucleus in 1 cells at prophase, c-mitosis in 5 cells at metaphase, multipolar anaphase in 4 cells and bridge formation in 1 cell at anaphase stages (Table 1).

Mitotic index which indicate to the cell division frequency was determined for each Cd concentration (Table 2). As it is shown in Table 2, mitotic index decreased with the increase of Cd concentration ($p<0.05$).

At the end of mitotic observations, abnormalities occurred in root tip cells of lentil (*Lens culinaris*) during cell division period are given in Fig. 3.

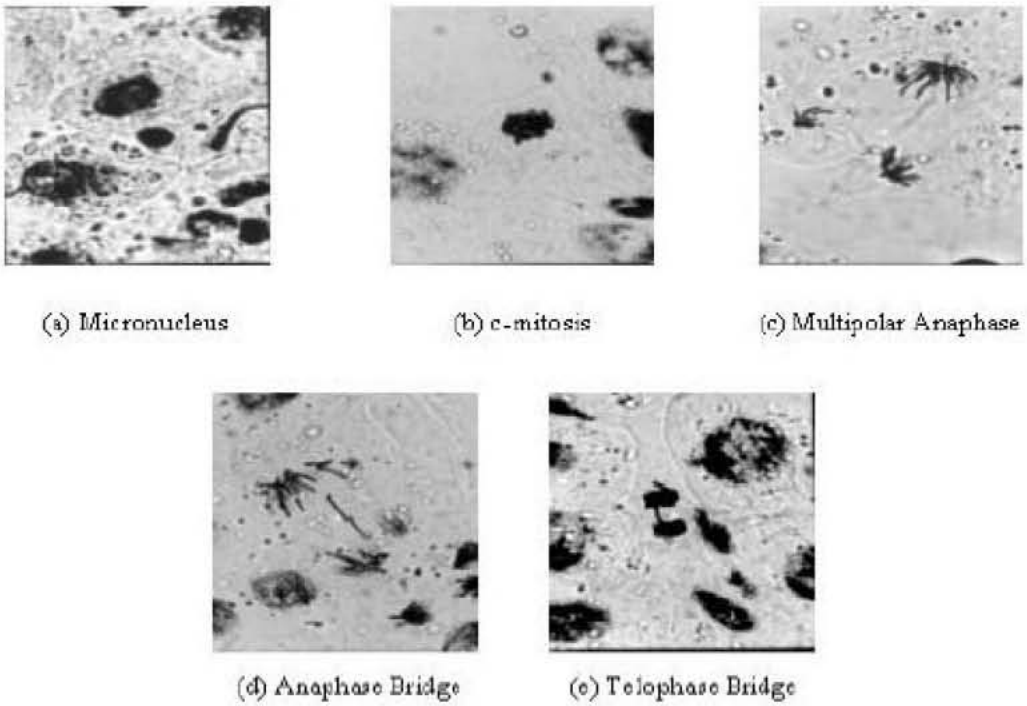

Fig. 3: Mitotic effects of $CdCl_2$ on root-tip cells of lentil (*Lens culinaris*)

DISCUSSION

The reduced germination and inhibition of root and body development on *Sorghun bicolour* L. exposed to different Cd concentrations were reported [15]. The toxic level of Cd caused to very short lateral root formation resulted with a compact root system formation [16]. Cadmium reduced the germination of *Lolium perene* seeds [17, 18]. The germination of *Lens esculate* L. seeds exposed to various concentrations of Cd (10-100 mg/L) have gradually reduced with the increase of Cd concentration [19]. 10^{-6} and 10^{-5} M concentrations of Cd have increased the root development in garlic (*Allium sativum* L.), but 10^{-3} and 10^{-2} M concentrations have reduced [20]. The germination of *Helianthus annuus* seeds have been gradually inhibited by Cd, Al, Cu, Ni, Pb and Zn metals with respect to increase of metal concentrations and exposure time [3]. $CdCl_2$ and $HgCl_2$ (0.05-50 mM) have reduced the seed germination and the shoot development with increase of metal concentrations and exposure time [21]. The different concentrations of Cd (0.5-20 ppm) have reduced to the root development and mitotic index and caused to the some abnormalities such as c-mitosis and bridge formation at anaphase, micronucleus formation and refraction, fusion and less development of chromosomes [6]. The various concentrations of $CdCl_2$ (10^{-7}- 10^{-1}) have caused to the following abnormalities in *Staria italica* and *Hordeum vulgera* species; c-mitose, chromosomal refractions, chromatid bridges, residual chromosome and micronucleus formation [22]. It was also reported that the different concentrations of various metals have caused to the c-mitosis, chromatid bridges and fusion and abnormalities in nucleus and nucleolus of *Allium cepa* [23].

The results of the present study showed that the seed germination and root development in lentil plant was gradually reduced with the increase of Cd concentration. We also found that the root growth was more sensitive to the cadmium stress than seed germination. In addition, it was observed that cadmium reduced to the mitotic index and caused to some abnormalities in lentil plant. This study also showed that lentil like other studied plants was not a tolerant plant to the cadmium pollution. The findings of the present study were found quite similar to the results of other studies mentioned above.

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