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Toxic effect of cadmium on germination, seedling growth and proline content of Milk thistle (*Silybum marianum*)

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ABSTRACT

Cadmium (Cd) is one of the most common toxic metals present in the environment that induces various toxic effects in plants even at low doses. Medicinal plants may be easily contaminated with heavy metals during growing period. A laboratory experiment was conducted out to determine the effects of cadmium on seed germination percentage, seedling growth parameters and proline content of Milk thistle (Silybum marianum) medicinal plant at Biotech Research Center of University of Zabol in Iran during 2010. The seedlings were treated with concentrations of 0, 100, 200, 400 and 600 mg L^{-1} of cadmium chloride. The experiment was done in a completely randomized design with four replications. The parameters were measured included of: germination percentage, germination rate, root and shoot length, soot/root length ratio, seedling fresh weight and proline content of leaves. Our results indicated that a significant inhibitory effect was observed at all levels of cadmium compared to control. Increasing the concentration of cadmium to 600 mg L^{-1} showed a significant decrease in seed germination, shoot and root length and fresh weight of seedlings, while shoot/root length ratio and proline content increased by increasing Cd concentration.

Key words: Milk thistle (*Silybum marianum*), Cadmium, Germination, Seedling growth, Proline.

INTRODUCTION

Currently, contamination of soil in cultivated fields with toxic heavy metals such as cadmium, copper, nickel and zinc has emerged as a new challenge to agriculture [1]. Medicinal plants may be easily contaminated during growth, development and processing. Lead, cadmium, chromium, nickel, arsenic and mercury are the most common toxic metals that have become a matter of concern due to the reports of their contamination in various herbal preparations and herbal ingredients [2]. The process of metal uptake and accumulation by different plants depend on the concentration of available metals, solubility sequences and the plant species growing on these environments [3].

Cadmium is a non-essential trace element for plant growth and induces various toxic effects on plants including leaf chlorosis, alteration in the activity of many key enzymes, inhibits the growth of root and shoot and productivity, affects nutrient uptake and homeostasis and disturb plant water status [4-6]. Cadmium is also known to induce a burst of reactive oxygen species (ROS) in plant tissues, leading to oxidative stress [7-8]. Furthermore, Cd toxicity leads to protein degradation through amino acid metabolism resulting in decreased plant growth [9]. Liu *et al*. [10] evaluated the effects of Cd on *Lonicera japonica* Thunb and concluded that this medicine plant accumulated high concentrations of Cd in roots and shoots, which increased edible security risk of the plant. In addition, the chlorophyll content showed slightly increase in plants exposed to low concentration of Cd, but decreased due to higher Cd concentrations. Pasquale *et al*. [10] considered the effects of cadmium on the growth and pharmacologically active constituents of the *Coriandrum sativum* L. medicinal plant and reported that plants grown under different levels of cadmium showed a significant reduction in the length of shoots and roots, a yellowing and ultrastructural alterations of the leaves and a significant decrease in the essential oil composition. Zheng *et al*. [12] evaluated the effects of cadmium on growth and antioxidant responses of *Glycyrrhiza uralensis* seedlings and found that the uptake of cadmium in different tissues of seedlings increased with increasing Cd concentrations, however, most Cd accumulation found in the radicles. They also observed that increased cadmium concentrations lead to decreased shoot elongation and seedling biomass.

Milk thistle (*Silybum marianum*) is a member of the Asteraceae (Compositae) family cultivated in agriculture. The achenes, i.e. fruits of this medicinal plant are commonly used to make medicines; they are the raw material for isolation of different substances with liver-protection activity [13].

The objective of this study was to examine the responses of a medicinal plant (*Silybum marianu*m) to toxic effect of different concentrations of cadmium.

MATERIALS AND METHODS

In order to investigation the effects of cadmium concentrations on seed germination and seedling characteristics of Milk thistle (*Silybum marianum*) an experiment was carried out in Biotech Research Center of University of Zabol, Zabol, Iran. Seed samples were collected from Dehdasht city, Kohgiluyeh and Boyer-Ahmad province, Iran and were used in this study. Prior to germination, the seeds of Milk thistle were surface-sterilized with 3% Formaldehyde for 10 minutes and washed several times with distilled water. 25 healthy and uniform sized seeds were selected and sown at equal distance in a petri dish lined with filter paper. Seeds were treated under 0, 100, 200, 400 and 600 mg L^{-1} of cadmium concentration solutions. 10 ml of test solution was added to each petri dish and kept inside the germination cage. The petri dishes were kept moist by regularly adding 5ml of test solution. The petri dishes were covered with a net and kept under room temperature and light condition. The experiment was laid out as a completely randomized design (CRD) with four replications.

During the experiment, germinated seeds were counted daily and seed germination was assessed by the final cumulative percentage of germination at the end of the tests. Here, a seed was considered as germinated when its root length was longer more than 2 mm. The number of germinated seeds per time was presented as germination rate. After 7 days, ten seedlings of each petri dish were sampled randomly and shoot and root length measured using a ruler (against a black background). Fresh weight was also evaluated by weighting of these ten seedlings. For the analysis of free proline, the fine powder of freeze-dried plant tissues (0.2 g) was treated with 5ml of 3% sulphosalicylic acid and and incubated for 10 minutes at 100 °C. The supernatant (2 ml) was added to a solution of 2 ml of glacial acetic plus 2 ml of 2.5% (w/v) acidic ninhydrin, and kept at 100 °C for 25 minutes. After the liquid was cooled down, it was added to 4 ml of toluene. The photometric absorbance of the toluene extract was read photometrically at 520 nm.

Finally, the data collected were statically analyzed for variance using SAS software. The mean values were compared by applying Duncan's multiple range test at 5% probability level.

RESULTS AND DISCUSSION

Results obtained from the variance analysis showed that cadmium levels negatively affected seed germination and other measured parameters of Milk thistle seedlings significantly at 0.01 level of probability (Table 1).

Under cadmium stress, seed germination percentage reduced significantly as compared to control. Control showed the highest, whereas 600 mg L^{-1} of cadmium concentration resulted to the lowest value of seed germination (Table 2). Shafiq *et al*. [14] indicated that Cd can cause significant reduction in seed germination percentage of *Leucaena leucocephala*. Decrease in seed germination of plant can be attributed to the accelerated breakdown of stored nutrients in seed and alterations of selection permeability properties of cell membrane, due to negative effects of heavy metals [14]. The present results suggested that the germination rate gradually decreased with increasing cadmium concentrations compared to the control. Germination rate was significantly lowered in 600 mg L^{-1} cadmium treatment (1.07) as compared to control (9.54) and it decreased continuously with increase in concentration of Cd (Table 2). The negative effect of cadmium on germination rate of *Triticum aestivum* L. seedlings has been reported by Munzuroglu and Geckil [15].

Shoot length was consistently reduced with increasing concentrations of cadmium but high reduction was observed at concentration of 600 mg L^{-1} cadmium (0.65 cm) as compared to control (4.227 cm) and other treatments (Table 2). The reduction in shoot length could be attributed to the adverse affect of heavy metal on the reduction in meristematic cells present in this region and some enzyme contained in cotyledon and endosperm and thereby, reduction the cell elongation and cell expansion [16]. Also, Cd in cells gets associated with cell walls and middle lamella and increases the cross-linking between the cell wall components, resulting in the inhibition of the cell expansion growth [18]. Street *et al*. [19] reported that low concentration of cadmium significantly reduced shoot length of *Merwilla natalensis* medicinal plant compared to control treatment.

Application of different concentrations of cadmium caused a reduction in root length of Milk thistle (*Silybum marianum*). Data on Table 2 illustrate that the maximum and statistically significant depression in root length (0.14 cm) was recorded from treatment of Cd at 600 mg L^{-1} concentration, while control had the highest value (2.44 cm). The rate of decline in root length was more pronounced under Cd treatment compared to shoot length. Roots are the first organs in contact with the toxic metal ions, and the inhibition of root elongation caused by heavy metals may be due to metal interference with cell division (including inducement of chromosomal aberrations and abnormal mitosis) and cell elongation [20-22].

Table 1. Analyses of variance (ANOVA) for germination, seedlings growth and proline content of Milk thistle as affected by different concentrations of cadmium

S.O.V	d.f.	Seed germination	Germination rate	Shoot length	Root length	Seedling weight	Shoot/root length ratio	Proline
Treatment	4	1785.2**	44.64**	8.20**	3.39**	0.004**	4.76**	1.026**
Error	12	91.2	2.99	0.57	0.11	0.0002	5.67	0.117
C.V (%)		7.33	11.5	10.1	10.9	1.84	20.9	6.02

*** = Significant at 1% level of probability.*

Table 2. Mean comparison for germination, seedlings growth and proline content of Milk thistle as affected by different concentrations of cadmium

Cadmium (mg L^{-1})	Seed germination (%)	Germination rate	Shoot length (cm)	Root length (cm)	Fresh weight (g)	Shoot/root length ratio	Proline (mg g^{-1} DW)
0	68a	9.54a	4.27a	2.43a	0.12a	1.70c	1.33d
100	47b	5.38b	2.75b	0.95b	0.09b	2.95b	1.54c
200	36c	3.43c	1.97c	0.60c	0.07c	3.38b	1.65c
400	23d	2.23d	1.12d	0.30d	0.05d	3.79ab	2.07b
600	14e	1.07e	0.65e	0.14e	0.04e	4.64a	2.61a

Means followed by same letter in each column are not significantly different at the 5% level.

The fresh weight of Milk thistle seedlings showed inhibitory effect with increasing concentration of cadmium in comparison with the control. This parameter was significantly higher in control (0.119 g) and gradually decreased with enhanced Cd concentration. The inhibitory effect of cadmium was greater at 600 mg L^{-1} concentration than other treatments, so that the lowest value (0.038 g) was observed in this treatment (Table 2). Similar result of fresh weight decrease due to application of cadmium was also reported by Zheng *et al*. [12] about *Glycyrrhiza uralensis* seedlings. Reduction in seedling growth of Milk thistle, provided evidence that, the metal elements like cadmium if present in excess amount are responsible for producing toxic effects which reduced plant development [16]. Cd also alters the water relation in plants, causing a physiological drought stress [17].

Results presented in Table 2 reveal that shoot/root length ratio was higher in Cd treatments as compared to control and increased significantly along with the increase in Cd concentration in the solution. The data recorded on this parameter revealed that treatment of cadmium at 100, 200, 400 and 600 mg L^{-1} concentrations increased shoot/root length ratio to 2.9, 3.4, 3.8 and 4.6, respectively as compared to control (1.7). Our results are in confirmation with that of Houshmandfar and Moraghebi [16] who observed higher shoot/root length ratio of sunflower seedlings in Cd treatments. In this study, heavy metal treatment drastically increased the proline content of leaves. The content of proline in Milk thistle leaves increased by 15.8, 24.1, 58 and 96.2%, as compared to the control plants, respectively with increasing Cd concentrations (Table 2). Proline accumulation is as an indicator of environmental stress such as heavy metal stress. The free proline has been found to chelate Cd ion in plants and form a non toxic Cd-proline complex [23]. Similar result of increasing proline content by cadmium was also reported by Zengin and Munzuroglu [24] in the case of sunflower.

CONCLUSION

Obtained results of this study revealed that cadmium had a toxic effect on germination percentage, germination rate and seedling growth of Milk thistle (*Silybum marianum*). All Concentrations of this heavy metal tested negatively affected germination percentage and germination rate of seeds as compared with the control. The root and shoot growth of Milk thistle treated with Cd seriously inhibited and increase in Cd concentration caused a gradual decrease of them. Root growth was affected more by Cd than was shoot growth. The proline content showed slightly increase in seedlings exposed to cadmium toxicity.

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