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TOXIC EFFECTS OF CADMIUM ON *IN VITRO* SEED GERMINATION AND SEEDLING GROWTH OF *SORGHUM BICOLOR* (L.) MOENCH

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Abstract

Heavy metal toxicity is one of the limiting factors for plant growth and yield. With the increasing formation of lethal heavy metals through natural and anthropogenic reasons, agricultural crops are frequently affected with this stress. Moreover at present considerable knowledge is necessary particularly in molecular aspects of crop heavy metal biology for sustainable agriculture development. In the present investigation, we aimed to study the effects of cadmium (Cd) on sorghum seed germination along with seedling growth under *in vitro* conditions. Initially we estimated the percentage of seed germination using various concentrations of cadmium. In addition seedlings growth was also analyzed via shoot and root development in both treated and control samples. Cadmium severely damages the seed germination and seedlings growth when compared to controls at higher doses. Estimation of chlorophyll content was also carried out using cadmium treated and untreated sorghum seedling samples to know the biomass values. We found the drastic reduction of total chlorophyll content at high concentrations of cadmium indicates the noxious nature of this particular heavy metal. Present work may be beneficial to sorghum cultivation and also useful for future research on plants growing in cadmium rich regions.

Keywords: Heavy metal, Cadmium, Sorghum, Chlorophyll, Seedlings, *In vitro*

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INTRODUCTION

Crop yield mainly depends on both biotic and abiotic stress factors. Heavy metal stress is known for several decades apart from other abiotic stresses such as drought, salinity, extreme temperature and light [1]. Heavy metals are generally categorized broadly into two groups based on the usage i.e. essential and non-essential [2]. Some of the heavy metals including copper (Cu), iron (Fe), manganese (Mn), zinc (Zn) etc., are nutritionally essential for living organisms. Moreover certain essential heavy metals when cross their critical level may also cause metabolic disorders and growth inhibition in most of the plant species [3]. In addition non-essential heavy metals such as cadmium (Cd), mercury (Hg), chromium (Cr), lead (Pb) etc., are not used due to their noxious nature and they may become hazard to growth and development of plants, animals including human beings. Apart from natural source, bypassing the anthropogenic wastes such as domestic wastes, sewage sludge and industrial effluents on agricultural land is frequent practice and as a result these toxic metals can be concentrated in the soil [4]. In general heavy metal and plant species interact in a specific manner which depends on various environmental factors [5]. The excessive uptake of these heavy metals from the soil can create problems such as low biomass accumulation which in turn affects yield levels in any plant species. So it is very essential to gain knowledge on impacts of various heavy metals on growth and development particularly for certain crop plants. Cadmium is one of the noxious elements which bagged seventh rank among top twenty toxic elements and enters in to the environment from heating systems, power stations, urban traffic including lead and zinc mining activity [6, 7]. It is highly portable element from soil to plants and its uptake and transportation in plant was also depends on pH of the soil [8]. Few reports stated the adverse impact of cadmium on seed germination and seedling growth of wheat crop [9].Alterations in the mineral nutrition of pea seedlings was noticed when it exposed to cadmium [10]. Involvement of cadmium in ROS formation and differential antioxidative response was observed in *Pisumsativum* [11]. Adverse effect of cadmium on wheat growth and development was also noticed as mentioned above [12]. Over-accumulation of cadmium affects the other activities such as suppression of nitrate absorption by inhibiting nitrate reductase activity [13]. Levels of proline and antioxidative system in cadmium treated bean was studied and proved the lethal nature of this heavy metal [14]. Similarly variations in photosynthetic capacity and antioxidative nature in two mustard cultivars with cadmium treatment was also demonstrated [15]. Recently cadmium-mediated growth damage was observed particularly in roots

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of safflower [16]. Cadmium also induce membrane damage and over-accumulation of lipid peroxidation products in seeds and ultimately delays the germination [17]. In addition cadmium also reduces the water and mineral uptake in various plants including pulses [18]. Our group also demonstrated lethal nature of cadmium on *in vitro* horse gram seeds and seedling growth [19]. Uptake and sequestration of cadmium by Cd hyper-accumulator plant *Sedum alfredii* was explained very recently [20]. Hence a detailed study about the effects of heavy metals in various plant species, especially food crops is very much necessary. *Sorghum bicolor* generally called as sorghum is the fifth most important cereal crop and used as food, syrup, animal fodder and biofuel (ethanol) production [21]. Certain cultivars of sorghum has ability to grow even under drought, salinity, low soil fertility and high temperature conditions.Previously few of the *in vivo* or pot based heavy metal studies have been demonstrated using different sorghum cultivars [22-25]. Here in the present work, we studied the impact of cadmium on sorghum seed germination and seedling growth under *in vitro* conditions which is more reliable than *in vivo* conditions.

MATERIALS AND METHODS

Surface Sterilization of Seed Material

In the present study locally available seeds of *Sorghum bicolor* cultivar (M35-1) were used and the all experiments were carried out under *in vitro* conditions. Properly washed glassware including decontaminated vessels were used for *in vitro* experiments to avoid contamination. Moreover without surface sterilization of seed materials we cannot avoid the contamination of the cultures. Before seed sterilization, glass and plastic ware which are essential for inoculation were autoclaved and later transferred to laminar air flow chamber. In addition 70% ethanol and 0.1% mercuric chloride were also prepared for seed sterilization. Surface sterilization of seed material was carried out initially with 70% ethanol for 1 min and later 0.1% mercuric chloride ($HgCl_2$) for 10 min as a routine process and finally rinsed with autoclaved distilled water for 3 to 4 times. Wet seeds were blotted dry on sterile filter paper before inoculating them on cadmium media.

Media Preparation and Inoculation

The media which were used in the present study does not contain any nutrients and all the media consists distilled water along with cadmium [($CdCl_2.H_2O$) (Cadmium chloride monohydrate)] with different concentrations. Heavy metal stocks were prepared by using standard formula and from these stocks the media was prepared in ppm. In the present research we selected 1, 10, 20, 50 and 100 ppm concentrations, keeping tap water (T.W) and distilled water (D.W) media without any heavy metals as controls (Table-1).

Table 1: List of the cadmium media composition

Sl. No.	Media Combinations	Cd in ppm
1	Tap water + 0.8% Agar + pH 5.7-5.8	T.W
2	Distilled water + 0.8% Agar + pH 5.7-5.8	D.W
3	Distilled water + 0.8% Agar + pH 5.7-5.8	1
4	Distilled water+ 0.8% Agar + pH 5.7-5.8	10
5	Distilled water+0.8% Agar + pH 5.7-5.8	20
6	Distilled water+0.8% Agar + pH 5.7-5.8	50
7	Distilled water+0.8% Agar + pH 5.7-5.8	100

For all the experiments, pH of the media was 5.7 to 5.8 and0.8% agar was added to all the media for solidification. Later all the media were boiled to dissolve agar and then about 15 ml of media was dispensed into each test tube and media were autoclaved at 121°C for 15 min. Sterilized conditions were maintained in laminar air flow chamber by switching on the ultra violet lamp for half an hour before inoculation and also chamber was smeared with 70% ethanol. All the inoculated cultures were incubated in a culture room at 25 ± 2°C with 16 h photoperiod. The percentage of seed germination along with seedling growth was monitored at regular intervals.

Estimation of Percentage of Seed Germination, Seedling Growth, and Chlorophyll Contents

The inoculated seeds on cadmium media along with controls were incubated for 14 days and on 15th day the seedlings were observed for percentage of germination. The percentage of seed germination was calculated using visual observation. Later root and shoot lengths were also calculated by using centimeter scale. All the calculations part was performed using excel program. In the present work, estimation of chlorophyll contents were also carried out using Arnon's method [26]. One gram of fresh leaf materials of both controls and cadmium treated seedlings were ground with using 80% acetone and the homogenate was centrifuged at 5000 rpm for 5min and the supernatant was saved and the residue was re-extracted with 80% acetone up to the formation of pale yellow residue. Then the supernatant final volume will make upto 100ml with 80% acetone and absorbance values were read at 645 and 663nm in a UV-Spectrophotometer. Total chlorophyll content was estimated using standard Arnon's formula.

RESULTS AND DISCUSSION

The *in vitro* seed germination and seedling growth in cadmium media along with controls were studied under identical culture conditions. Sufficient sorghum seed germination was observed in both the controls which indicate that there was no seed dormancy problem. In addition, there was a

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significant reduction in seed germination in high doses of cadmium and specifically at 100 ppm we did not found any germination indicates the severity of this toxic element (Fig. 1). In both control media percent of seed germination was 94.1 and 86.3 and at 50 ppm cadmium concentration only 9.5 percent of germination was observed (Fig. 1 and Table-2). The negative correlation between the higher doses of cadmium and percentage of germination is mainly due to toxicity of cadmium. Li et al. [27] also observed the drastic decreased seed germination in *Arabidopsis* in high concentration of cadmium treatment.

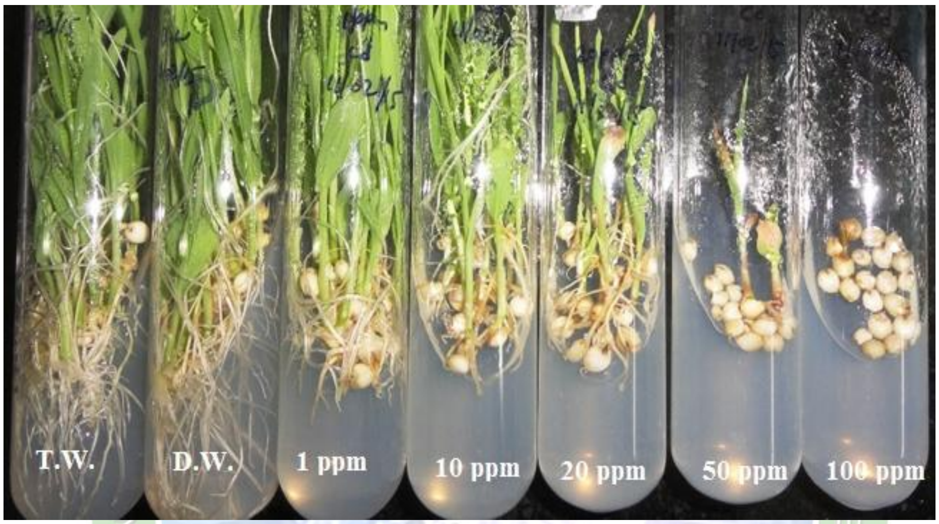

Fig.1: Impact of cadmium on sorghum seed germination and seedling growth
T.W.-Tap water and D.W.-Distilled water (control media)

Cadmium affects the growth and development based on the dose and plant species. Similar decreased seed germination in high doses of cadmium treatment was also noticed in alfalfa [28].

Table 2: Impact of cadmium on sorghum seed germination

Sl. No	Concentration of cadmium (in PPM)	Percentage of seed germination along with S.E.
1	T.W. Control	94.1 ± 0.2
2	D.W. Control	86.3 ± 0.3
3	1	84.2 ± 0.3
4	10	57.0 ± 0.7
5	20	53.8 ± 0.7
6	50	09.5 ± 0.5
7	100	-

The values above represented are mean of 3 replicates and experiment was conducted thrice

T.W.-Tap water medium, D.W.-Distilled water medium, S.E.-Standard Error

In addition we also estimated the shoot and root lengths of seedlings and found phytotoxic nature of cadmium. After 14 days of incubation, 15^{th} day we collected the data of lengths of both shoot

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and roots of sorghum seedlings. Increasing cadmium concentrations exhibited decreasing length of shoots and roots when compared to control samples (Fig. 2). Shoot lengths were gradually declined in seedlings grown in high concentration of cadmium. Specifically there was no shoot growth and development was observed after 10 ppm concentration. Probably it was due to inhibition of root growth from 10 ppm onwards, which in turn decreases shoot length also (Fig. 2). These results are correlated with the results of different crops species done by various researcher in different plants [12, 14, 29, 30]. Moreover inhibition of seed germination of mustard crop was observed with high cadmium doses [31]. Similarly wheat seed germination and seedling growth was reduced with high concentration of cadmium [9, 32]. Here in our observation increased concentration of cadmium also decreases drastically the lengths of roots (Fig. 2). Drastic reduction of root lengths were observed at higher doses of cadmium and similar toxic nature of cadmium was also proved in milk thistle [33]. These results again proved the toxic nature of cadmium with sorghum seedlings. Heavy metal treated sorghum seedlings were further examined using biochemical methods including estimation of chlorophyll contents to know the biomass values. Studies of these biochemical parameters help us to know the biological damage done by various doses of cadmium. Interestingly total chlorophyll content was decreased in higher doses of cadmium except at 10 ppm where there was a sudden increase in total chlorophyll content was noticed (Fig. 3).Probably 10 ppm is critical concentration for sorghum specifically for photosynthesis process. Overall total chlorophyll contents were decreased with increasing concentration of cadmium in agreement with the previous works [20, 34, 35].

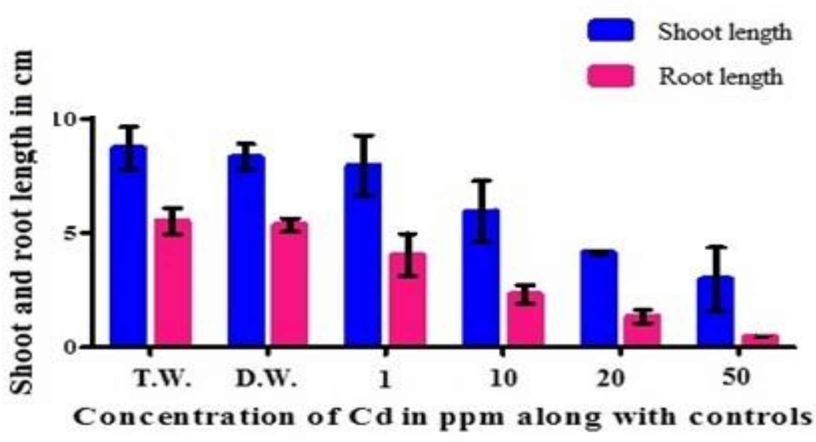

Fig. 2: Impact of cadmium on sorghum seedling growth

The values above represented are mean of 3 replicates and experiment was conducted thrice

T.W.-Tap water medium D.W.-Distilled water medium

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The bars indicate standard error

Precisely this work may be useful for further molecular research to know the cadmium receptors in plants. It is more important that disposal of cadmium wastes in agricultural land should be restricted for better crop yield.

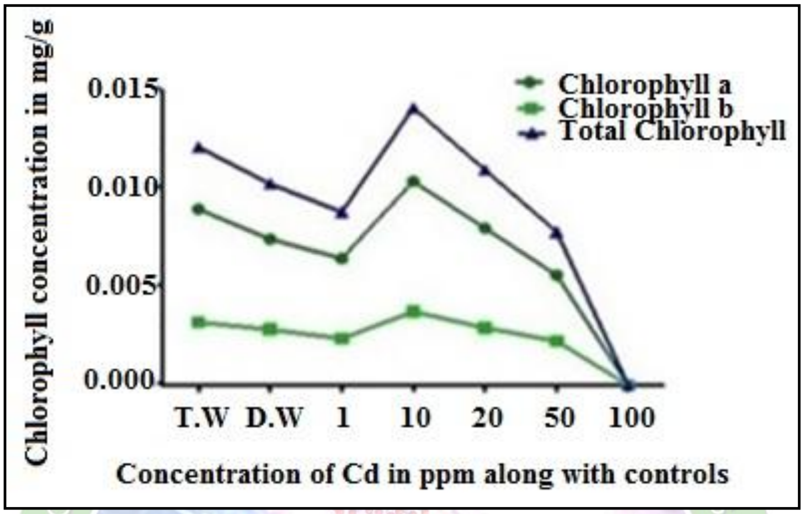

Fig.3: Chlorophyll estimation using cadmium treated and control sorghum seedlings

The values above represented are mean of 3 replicates and experiment was conducted thrice

T.W.-Tap water medium D.W.-Distilled water medium

Chlorophyll contents were mentioned in mg per gram fresh weight of material

CONCLUSIONS

Present work deals with the *in vitro* morphological, physiological and biochemical aspects of cadmium stress on sorghum seed germination and seedling growth, which is framework for the scope for future research on phytoremediation. In *in vitro* experiments, cadmium negatively affects the sorghum seed germination, seedling growth and biomass values when compared to control plants. This work may be useful to know the insights into the mechanism of cadmium stress and their impacts on plant growth and development.

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