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Effect of cadmium stress on seed germination and seedling morpho-physiological growth parameters of barnyard millet (*Echinochloa frumentacea* Link)

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Abstract

Cadmium (Cd) is a heavy metal, which is seen in the contaminated soils and severely affects the growth and development of plants in recent years. The study on the seed germination and morpho-physiological growth characteristics of barnyard millet (*Echinochloa frumentacea* Link) cultivar CO (KV) 2 treated with different concentrations (50, 100, 150, 200 and 250 mg/kg of soil) of Cd were evaluated at 15th, 30th and 45th day of interval. The findings of this research demonstrate that the maximum dosage of Cd (250 mg/kg of soil) affects the germination percentage (65%) of barnyard millet. Seedling vigor index has been negatively influences a drop in germination percentage. Increasing concentrations of Cd reveals the growth of root and shoot length and the quantity of fresh and dry weight affected. The phytotoxicity percentage of roots and shoots also increases with increasing concentrations of Cd, whereas the tolerance index level decreases with increasing concentrations of Cd. In root and shoot, the relative growth index was reduced in higher concentration of Cd. The relative water content remains high in the initial stages of leaf development and declines when the leaf matures. From this study, it was found that the increase in the concentration of Cd leads to decrease the germination percentage and morpho-physiological growth parameters as compared to control.

Keywords

Barnyard millet, cadmium, heavy metals, phytotoxicity, seed germination

Introduction

Over many decades, heavy metal contamination has led to severe environmental pollution in modern human society because of industrialization, use of chemical pesticides and fertilizers in agriculture, widespread mining, metal smelting, excessive reliance on fossil fuels and the production of batteries and other metal goods all contribute to global warming (1). Therefore, natural biogeochemical cycles have been disrupted (2, 3), resulting in pollution of air, water and soil. Accumulations of heavy metals pose a threat to the ecological, dietary and environmental balances (4, 5). Heavy metals in soil may pose serious health and environmental risks to people and the ecosystem via the food chain, polluted groundwater, phytotoxicity and decreased crop production contributing to food insecurity (6). Biological creatures, such as human beings, animals and plants, are all susceptible to the cytotoxic, genotoxic and mutagenic impacts of heavy metal pollution (7, 8).

Cadmium (Cd) is one of the most poisonous heavy metals, causing both plant and human health problems (9). Many stress symptoms in plants are caused by high levels of Cd in soil and these include stunted root development,mineral feeding difficulties and glucose metabolism impairments (10), all of which may dramatically reduce biomass output. Removing excess heavy metals from water and soil is easier with phytoremediation (the use of plants in metal extraction) (11). Cadmium toxicity harms plants by disturbing their physiological systems as a whole, as is well-known (12). Heavy metals have been shown to increase the production of reactive oxygen species (ROS) in plants. Damage to the membrane, peroxidation of lipids and enzyme inactivation are all consequences of the production of ROS, which interact with proteins, lipids, nucleic acids and other molecules (13). That is cadmium toxicity disturbs the equilibrium between the synthesis of antioxidants and generation of ROS and enhances ROS accumulation in plants, which stimulates oxidative stress. Over-accumulation of ROS in plants alters the synthesis of proteins and lipids, affects enzymatic activity that leads to lipid peroxidation and reduces cell division to negatively impact crop productivity (13).

Millets may tolerate several heavy metals and this tolerance is linked to a reduction in metal translocation from the shoots to the leaves (14). Especially, barnyard millet can produce large number of seeds and grow better than other crops in adverse environments, for example, contaminated soils (14, 15). In temperate and warm locations, barnyard millet is a prevalent weed, especially in China, Korea and Japan (16). “Sawa” is also a prevalent name for it in India's northern regions, including from Kashmir to Sikkim, where it is grown abundantly (17). In southern states, it is mostly grown in Tamil Nadu andhra Pradesh and Karnataka (18). Coimbatore, Namakkal, Ramanathapuram, Vilupuram, Madurai, Salem, Dindugal and Erode districts in Tamil Nadu are the primary regions where it is grown (19). Barnyard millet (*Echinochloa frumentacea* Link) belongs to the Poaceae family (Fig. 1A, B) and it is a self-pollinated crop. Various Asian tribal people raise it for food and fodder under the common name “Indian barnyard millet” (16). When compared to other cereals, such as rice and wheat, barnyard millet is a critical source of protein, crude fibre, low fat and carbohydrate content, vitamins and minerals (20-22). Antinutritional compounds such phenolic acids, flavonoids and tannins (23), all of which are effective sources of antioxidants, are found in it. Interestingly, there is increased evidence showing that barnyard millet may play an important role in improving heavy metal-contaminated soil (15).

The present study was performed to enhance our understanding of the ability of crops to grow in the presence of metallic stress and to improve our knowledge of plant reactions when they are exposed to various concentration levels of heavy metal contamination. Here, we studied the seed germination and morpho-physiological growth parameters of barnyard millet with the effect of different concentrations of heavy metal cadmium.

Fig. 1A

Fig. 1B

Fig. 1. (A) Habit of barnyard millet and (B) inflorescence of barnyard millet.

Materials and Methods

Seed procurement and experimental site

The seeds of a popular cultivar of barnyard millet, namely CO (KV) 2 (seed lot number: EF 79; date: April 15, 2021; germination percentage: 96%), were procured from the Centre for Excellence in Millets, Tamil Nadu Agricultural University, Athiyandal, Thiruvannamalai, Tamil Nadu, India. A pot experiment was carried out in Nursery, Department of Botany, Madras Christian College (Autonomous), Chennai, Tamil Nadu, India.

Soil preparation

The sandy loam soil was used for this experiment. Each pot contains 5 kg of air-dried soil. The soil was analyzed for specific general characteristics such as soil type, color, pH, odor, minerals and the trace of heavy metals, moisture content, hardness, permeability, salinity, temperature and soil water content.

Screening test (paper cup assay)

The seeds were screened for their relative tolerance to the heavy metal Cd as $CdCl_2$ under varying concentration levels, such as 0, 50, 100, 150, 200, 250, 300, 350, 400, 450 and 500 mg/kg of soil. Fifteen seeds were grown in each cup and monitored daily to note their morphological characteristics and the plant growth was observed for 10 days. Based on the results (i.e., growth of the plants when compared with control), the concentrations of “50, 100, 150, 200 and 250 mg/kg” of soil were finalized for further studies.

Pot experiment

After analysis of soil and screening test, seeds are surface-sterilized with 0.1% mercuric chloride for 2 min and washed with water. In each pot, 15 sterilized seeds were sown. Before sowing the seeds, the different concentrations of Cd (50, 100, 150, 200 and 250 mg/kg of soil) in the form of $CdCl_2$ were mixed thoroughly with soil, however, the soil without Cd was treated as a control. The plants were grown under natural photoperiod for throughout the study period (i.e. 45 days). During the growth period (July to August), plants were regularly monitored for any morphological changes and phytotoxicity symptoms. All pots were watered based on the field capacity of the soil. Four replicas were maintained for each treatment.

Sample collection

For the study of morpho-physiological parameters, such as germination percentage, length of root and shoot, fresh weight (FW) and dry weight (DW) of root and shoot as well as percent phytotoxicity, tolerance index (TI), relative growth index (RGI) and relative water content (RWC), the plant samples were collected every 15th, 30th and 45th day of interval. In order to remove the soil particles that had been accumulated on the plants, they were first removed from the soil. Then, using a fused blotting paper, the water droplets were dried. Various morpho-physiological characteristics were measured in the early hrs of the morning.

Seed germination

In order to prevent fungal infection, the seeds of barnyard millet were treated for 3 min with 5% sodium hypochlorite and then washed with distilled water. The soil without Cd was kept as a control while 15 seedlings grew on a paper cup with different concentrations of Cd as mentioned above. At 27 °C, the germination test was carried out. Ten days of daily seed germination tallied, after which there was no more seed germination. The normal germination percentage of barnyard millet is 96% (24). Bewley and Black's method for calculating germination percentage was used to determine the number of seeds that germinated successfully on the 10th day (25).

$$\text{Germination percentage (\%)} = \frac{\text{number of seeds germinated}}{\text{number of seeds sown}} \times 100$$

Seedling vigor index

According to Dhindwal et al. (26), seedling vigor index was computed using the following formula: root and shoot length on the 10th day of germination.

$$\text{Seedling vigor index} = \text{mean root length} + \text{mean shoot length} \times \text{\% germination}$$

Root and shoot length

To measure the length of each portion, a scale was used to separate the seedlings into roots and shoots. At the root-stem intersection, the shortest leaf tip was used to calculate the stem's height. The root and shoot length were measured in centimeters.

Fresh and dry weight

FW and DW values were promptly recorded for all plants (treated and untreated) at every sample stage (i.e., 15th, 30th and 45th day of interval) after roots and shoots were carefully separated and their FW was recorded immediately. For DW, a hot air oven at 80 °C was used to dry the roots and shoots for 48 hrs, after which they were stored in small labelled paper covers (27). FW and DW were recorded in grams.

Percent phytotoxicity

Cadmium's phytotoxic impact on root and shoot growth was measured using the standard method (28).

$$\text{\%Phytotoxicity} = \frac{\text{root or shoot length of control} - \text{root or shoot length of treated}}{\text{root or shoot length of control}} \times 100$$

Tolerance index

TI of the mean root and shoot length of each concentration was determined based on the standard formula (29).

$$\text{Tolerance index (\%)} = \frac{\text{mean root or shoot length of treated}}{\text{mean root or shoot length of control}} \times 100$$

Relative growth index

In root and shoot, concentrations of RGI were determined using the technique of Paliouris and Hutchinson (30).

$$\text{Relative growth index (\%)} = \frac{\text{average dry weight of root or shoot of treated}}{\text{average dry weight of root or shoot of control}} \times 100$$

Relative water content

According to the standard formula (31), 1 g of leaf disc was prepared from the control and Cd-treated plants. Discs were immersed in 10 ml of water and kept for 3 hr at room temperature. After the samples had been blot dried, the turgor weight was calculated. At 65 °C, the samples were then over-dried until they had reached a stable weight The following formula was used to determine RWC from the DW of the samples.

$$\text{Relative water content (\%)} = (\text{fresh weight} - \text{dry weight})/\text{turgid weight} - \text{dry weight} \times 100$$

Statistical analysis

The data were given in the form of the mean ± standard error (SE). Measurements were taken on each of the four replicas. ANOVA was performed on the data using SPSS version 22.0.

Results and Discussion

The present study provides the detailed information about the effect of different concentrations of Cd stress (50, 100, 150, 200 and 250 mg/kg of soil) on the growth parameters of barnyard millet which includes seed germination, seedling vigor index, the length of root and shoot, FW and DW of root and shoot, phytotoxicity, TI, RGI and RWC.

Effect of cadmium on seed germination

Seed germination was decreased in the screening test when Cd contents were elevated and plant development was substantially hampered at these elevated levels (Figs. 2, 3). That is why Cd values from 50 mg/kg of soil to 250 mg/kg of soil were selected for the current investigation. For the barnyard millet cultivar CO (KV) 2, Cd had a significant impact on seed germination and subsequent growth. As the Cd content rose, the seed germination percentage reduced as well. Control had the highest rate of seed germination (100%), while the lowest rate of seed germination was reported at 250 mg/kg soil (65%), followed by 200 (75%) and 150 (80%) and 100 (85%) and 50 (90%) mg/kg soil (Fig. 4). When Cd is present in seed embryos, the breakdown of the seed's reserve food supply might reduce germination. The germination of seed, the germination index and the seedling vigor index of several crops were all reduced by Cd stress, as has been reported before (32-35). The capacity of the seed to germinate in a medium containing Cr is an indication of the tolerance level of the seed to Cr (36). Inhibition or restriction of amylase a and b activity, which hydrolyzes starch into sugar required by growing embryos, is thought to be the cause of Cr's reduced seed germination. As a result of the increase in protease activity seen as a result of the increased Cr stress, Cr-treated seeds were less able to germinate (37).

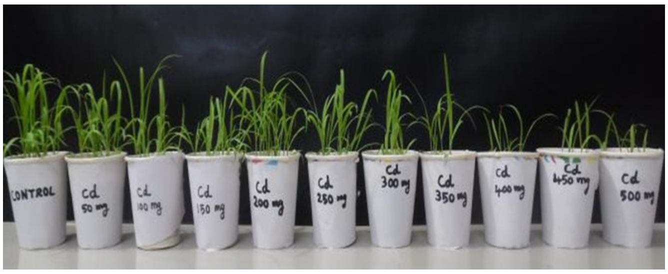

Fig. 2. Screening of different concentrations of cadmium on seed germination.

Fig. 3. Seedling growth of barnyard millet with different concentrations of cadmium on the 10th day.

Effect of cadmium on seedling vigor index

Barnyard millet's seedling vigor index has suffered because of a decline in germination percentage and early seedling development. At 250 mg/kg of soil, the seedling vigor index decreased significantly (1370) and the greatest seedling vigor index was reported in the control group (2390). Seedling vigor index dropped from 1998 at 50 mg/kg of soil to 891 at 250 mg/kg of soil in the experimental groups (Fig. 5). It is crucial to assess seed physiological potential throughout the different phases of seed formation via the evaluation of seedling vigor index. It is also necessary to measure seedling vigor parameters in order to assess the metabolic status of seeds and to determine the susceptibility of seeds to various stresses. In order for seeds to perform their fundamental activities in both favourable and unfavourable environmental situations, a variety of variables influence their physiological potential, including seed germination and seedling vigor index (38).

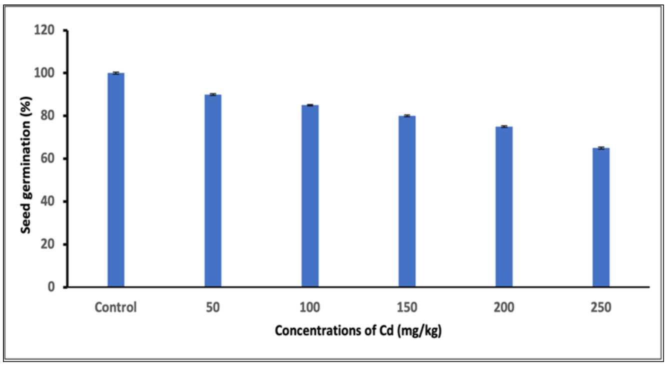

Fig. 4. Effect of cadmium on the germination percentage of barnyard millet. Vertical bars represent mean ± SE.

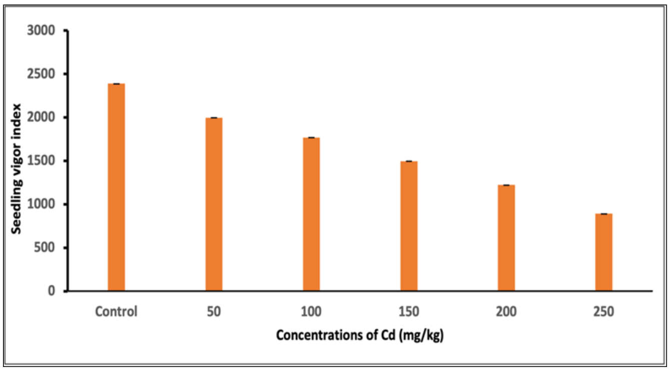

Fig. 5. Effect of cadmium on seedling vigor index of barnyard millet. Vertical bars represent mean ± SE.

Effect of cadmium on root and shoot length

Barnyard millet roots and shoots were measured every 15th, 30th and 45th day of interval. When compared to the control, root and shoot lengths were significantly reduced in all stages of seedling development. On the 15th day, 250 mg/kg of soil (5.2 cm) had the shortest root length, whereas control had the longest root length (9.4 cm). On the 15th, 30th and 45th day of the experiment, the shoot length was measured as 14.5, 48.5 and 77.5 cm in the control group, whereas the shoot length was measured as 8.5, 27.9 and 44 cm in the 250 mg/kg group (Fig. 6A, B). According to the findings, root length decreased more rapidly than shoot length did. Cd may have been retained in the root owing to cross-linking of Cd to carboxyl groups of the cell wall (39) and/or contact with thiol residues of soluble proteins (40).

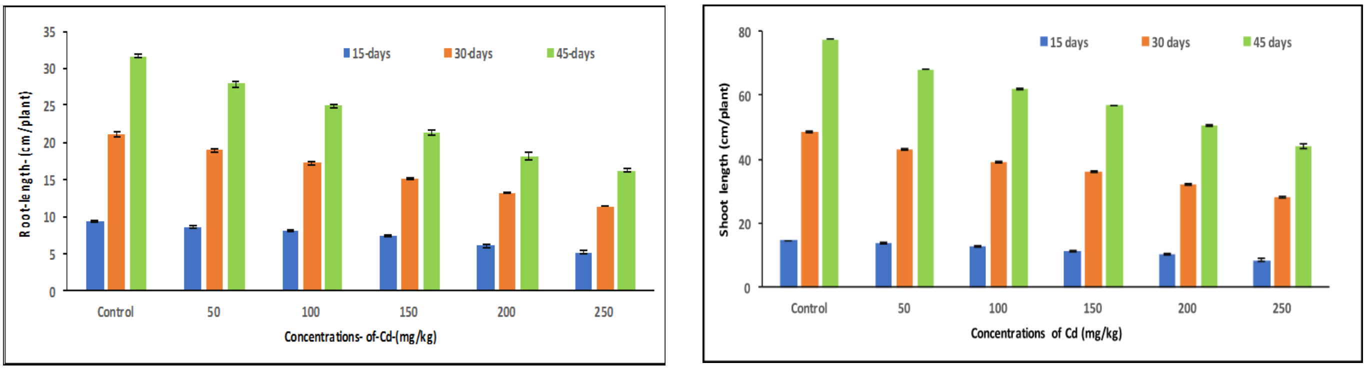

Fig. 6A **Fig. 6B**

Fig. 6. Effect of cadmium on (A) root length and (B) shoot length of barnyard millet at different stages of seedling growth. Vertical bars represent mean ± SE.

Effect of cadmium on the fresh and dry weight of root and shoot

In all stages of seedling growth, the FW and DW of root and shoot were reduced with increasing concentrations of Cd. In FW of root and shoot, the recorded decrease was found in the concentration of 250 mg/kg (0.022 and 0.091 g/plant respectively) (Fig. 7A, B). The DWs of both the root and the shoot were very low. At 250 mg/kg of soil, the root and shoot DWs of 15-day-old plants were 0.0079 and 0.059 g/plant respectively (Fig. 8A, B). Exposure to Cd decreased root and shoot lengths as well as FW and DW significantly (41). A reduction in root and shoot lengths and weights was also observed and noted a similarity to the effects of Cd in other plant species like cucumber (42). According to one report, plants' responses to metal toxicity may be measured using these growth alterations (43). In addition, the degree of Cd toxicity in plants varies with respect to growing circumstances and experiment setting, as well as the amount of Cd available, the duration of exposure and the age of plants (44).

Effect of cadmium on phytotoxicity

Many organisms are poisoned by cadmium, which is not required for plant development. The phytotoxicity percentage of roots and shoots treated with Cd rose in the present research as Cd concentrations in the barnyard millet cultivar CO (KV) 2 rose. 250 mg/kg of soil was discovered to be the highest phytotoxic percent value for roots and shoots. Root phytotoxicity was 8.51% at 50 mg/kg soil and 44.68% at 250 mg/kg soil on the 15th day, but in shoots, the lowest was found at 50 mg/kg soil (6.2%) and the highest was discovered at 250 mg/kg soil (41.37%) on the 15th day (Fig. 9A, B). Roots had a higher phytotoxicity % than barnyard millet shoots. It was found that Cr concentration enhanced the proportion of phytotoxicity in the roots and shoots of sour orange (45). The 200-ppm Cr concentration was determined to have the greatest percentage of phytotoxicity in roots and shoots. In roots, 50 ppm had a phytotoxicity of 39.3% and 200 ppm of 90.4%, whereas in shoots, 50 ppm had a

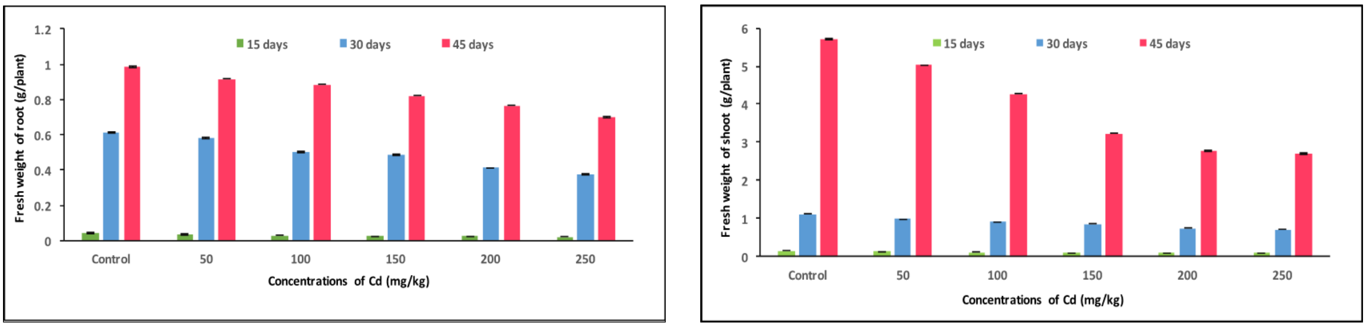

Fig. 7A **Fig. 7B.**

Fig. 7. Effect of cadmium on the fresh weight of (A) roots and (B) shoots of barnyard millet at different stages of seedling growth. Vertical bars represent mean ± SE

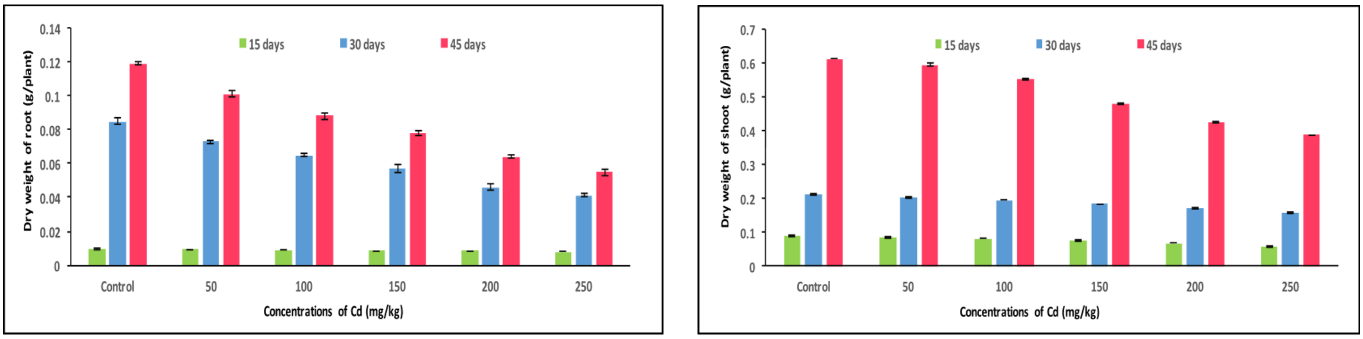

Fig. 8A **Fig. 8B**

Fig. 8. Effect of cadmium on the dry weight of (A) roots and (B) shoots of barnyard millet at different stages of seedling growth. Vertical bars represent mean ± SE.

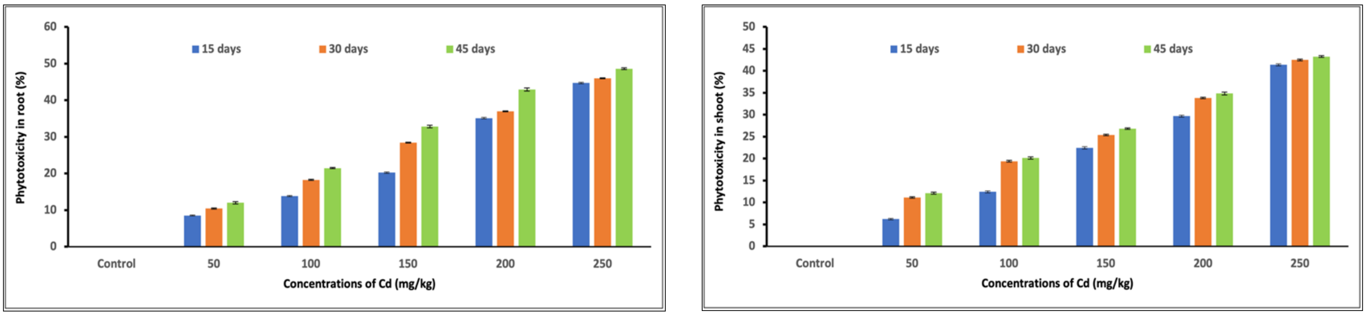

Fig. 9A **Fig. 9B**

Fig. 9. Effect of cadmium on the percent phytotoxicity in (A) roots and (B) shoot of barnyard millet at different stages of seedling growth. Vertical bars represent mean ± SE

phytotoxicity of 66.02% and 200 ppm of 97.67%.

Effect of cadmium on tolerance index

A high TI was found in the shoots of 15-day-old plants, but a low one in the roots. However, the TI was concentrated in the roots of 30- and 45-day-old plants. In roots, TI was highest in the control, followed by 50 (91.4%) mg/kg of soil on the 15th day and lowest at 250 (51.41%) mg/kg of soil on the 45th day. In shoots, a TI of 93.7% was detected on the 15th day in 50 mg/kg of soil, whereas a TI of 56.77% was discovered on the 45th day in 250 mg/kg of soil (Fig. 10A, B). When the plant develops, the TI of the roots will be higher than the TI of the shoots. Tolerant plants are frequently exclusionary, preventing heavy metals from entering and moving from the roots to the shoots (46). The root of *Virola surinamensis* has a high concentration of Cd, which suggests the plant's ability to absorb and retain Cd from the solution, especially in the roots, which in turn indicates the exclusion and chelation of the metal in the root system's cellular and subcellular compartments. As a tactic to protect the plant's photosynthetic equipment and also raise its tolerance to Cd, this may result in a restricted movement of Cd from the root to the shoot (47). When it comes to other tree species, it was their roots where the highest concentration of Cd was found (48, 49). Plant cell wall components, such as thiols (50) and other chemicals, such as glutathione (51),metallothioneins and phytochelatins (52), induce the retention of Cd in roots by binding to the metal. Root cell walls from plants exposed to metal (53), such as *V. surinamensis*, were found to contain some of these chemicals, suggesting the root cell wall may have functioned as an obstacle for Cd translocation, supporting larger concentrations of metal there. Due to the lignification, the cell membrane is less permeable, forming a barrier against Cd input and the lignified metal bonds with the metal (54).

Effect of cadmium on relative growth index

In roots and shoots, RGI was reduced in the higher concentrations of Cd at 15th, 30th and 45th day of interval. Apart from control, the maximum RGI was observed in 50 (96.9%) mg/kg of soil in the root of the 15-day-old plant, whereas the minimum RGI was noticed in 250 (46.2%) mg/kg of soil in the root of the 45-day-old plant (Fig. 11A). In shoots, the highest RGI was found in 50 (96.2%) mg/kg of soil of the 30-day-old plant and the lowest RGI was recorded in 250 (61.4%) mg/kg of soil of the 45-day-old plant (Fig. 11B). The high amount of Cd in the soil leads to many toxic symptoms in plants, such as diminished growth, particularly growth of the root (55), hindrances in mineral nutrition and carbohydrate metabolism (10) and may hence significantly bring down biomass production.

Effect of cadmium on relative water content

When compared to a control, the RWC was lower at the highest concentration. All of the Cd-treated plants showed a lower decrease percentage than the control. As a drought-resistant plant, it's reasonable to assume that barnyard millet also avoids cell water loss by closing its stomata. In the early stages of leaf formation, RWC stays high, but as dry matter accumulates and the leaf develops, it decreases. On the 15th day, the RWC was 91.8% in control, while 89.4% in 50 mg/kg of soil, followed by 100 (87.2%), 150 (85.1%), 200 (83.2) and 250 (81.1) mg/kg of soil. The least RWC was observed in 250 (78.2%) mg/kg of soil on the 45th day (Fig. 12). It has been shown that the plant's RWC may be maintained at a high level at low water potential by osmotic adjustment (56) Furthermore, it was found that RWC is a significant measure for assessing the water position in plants because it indicates the balance between the supply of water to leaf tissue and the transpiration rate (57). Reduction in RWC is linked to a decrease in plant vigor, which has been studied in a variety of plants (58).

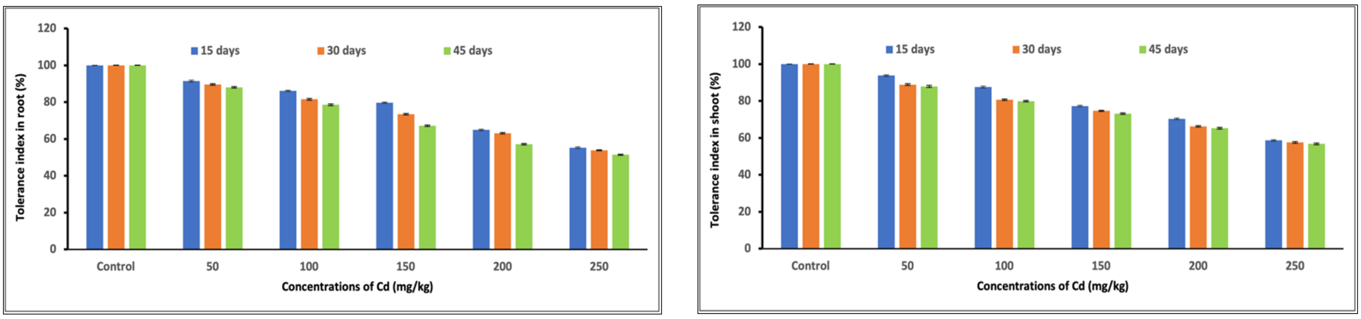

Fig. 10A **Fig. 10B**

Fig. 10. Effect of cadmium on tolerance index in (A) roots and (B) shoots of barnyard millet at different stages of seedling growth. Vertical bars represent mean ± SE.

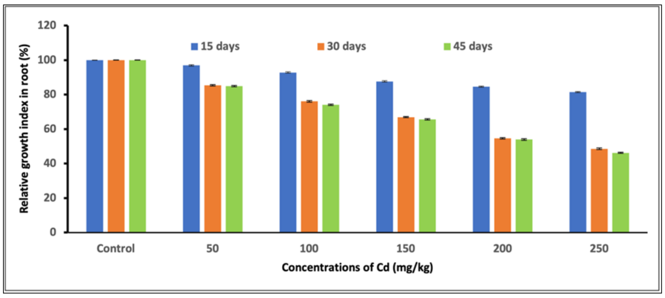

Fig. 11A

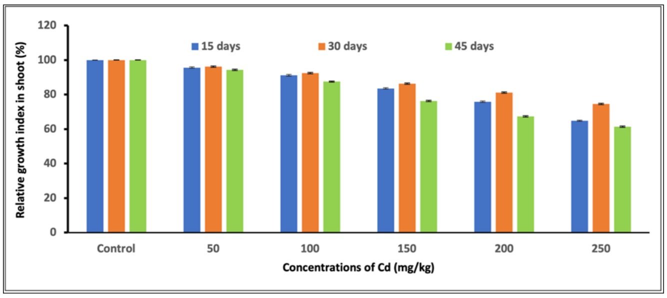

Fig. 11B

Fig. 11. Effect of cadmium on relative growth index in (A) roots and (B) shoots of barnyard millet at different stages of seedling growth. Vertical bars represent mean ± SE.

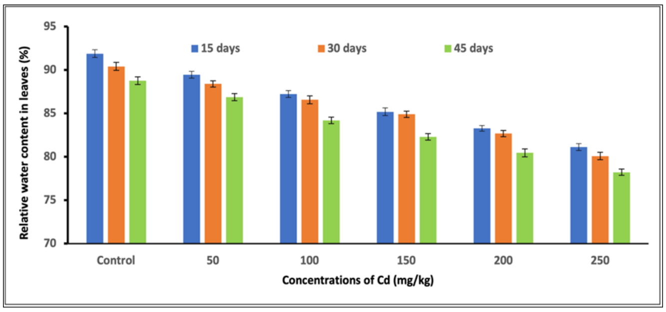

Fig. 12

Fig. 12. Effect of cadmium on relative water content in leaves of barnyard millet at different stages of seedling growth. Vertical bars represent mean ± SE.

Conclusion

The heavy metal Cd is major concern which affects germination, growth and yield in crop plants. It has become inevitable to study the effect of such heavy metals like Cd to understand whether they are tolerant or susceptible they are against this metal. In this investigation, an attempt was made to understand the effect of different concentrations of Cd on the seed germination and morpho-physiological changes in barnyard millet. Analysis of the data revealed that the increase in the concentration of Cd leads to decrease in their activity by inhibiting the growth and development of barnyard millet because of the high toxicity level of Cd. Cadmium stress causes morpho-physiological alterations and affects the shoot and root metabolic systems. Further investigation will help us to understand the accumulation of Cd in leaves or seed so that it can be utilized as feed or food for consumption.

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Authors contributions

KR and SR conceived and designed the study and prepared the manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues : None.

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