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Effect of Cadmium Chloride on Seed germination, Seedling growth parameters, and proline content in Maize (*Zea mays*)

Bhushan P. Bhusare[1], Mukund S. Ambawade[2], Akshay S Kshatriya[3], Mukul M. Barwant[4], Anuradha K. Mhaske[5]

[1]Department of Life Science (School Of Science), Sandip University Nashik-422213, Maharashtra, India.
[2]Department of Microbiology, P.G.K.M's Haribhai V. Desai College, Pune -411002, Maharashtra, India.
[3]Department of Botany, Savitribai Phule Pune University, Pune -411007, Maharashtra, India.
[4]Department of Botany Sanjivani Arts ,Commerce Science College Kopargoan- 423601, Maharashtra, India.
[5]Department of Biotechnology, Modern College of Agricultural Biotechnology, Paud, Pune- 412108, Maharashtra, India.
***Corresponding author:**
E.mail:bhushanbhusare@sandipuniversity.edu.in

Abstract: Present investigation was undertaken in order to determine the effect of different concentrations of Cadmium Chloride ($CdCl_2$) on morphological and biochemical responses of *Zea Mays.* For this, different concentrations (20, 40, 60, 80 and 100 mg L^{-1}) of Cadmium Chloride were supplemented to the seeds of maize. Morphological observations were collected in terms of percentage of seed germination, shoot length, and root length. Highest percentage of seed germination (92.8%) and maximum shoot length (18.4±0.9 cm) was observed on 40 mg L^{-1} of $CdCl_2$ when compared with control (seed germination- 85.7 %, shoot length- 17.1±0.7 cm). However 20 mg L^{-1} of $CdCl_2$, found to be best for highest length of roots (9.2±0.3 cm) as compared to control (8.7±0.4 cm). Higher amount of total chlorophyll (61.6 µg/ml) was recorded at 20 mg/l of $CdCl_2$. The proline content increases significantly with increasing the concentration of $CdCl_2$. The present study confirmed the potential use of maize in phytoextraction of heavy metal.
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Keywords: Cadmium, Chlorophyll, Maize, Proline, *Zea mays*

Abbreviations: Cadmium (Cd); Cadmium Chloride ($CdCl_2$)

1. Introduction

Maize (*Zea mays*) is the third most important crop globally, and in India third largest grown crop after wheat, and rice (Ramirez-Cabral et al. 2017). It is counted as one of the major food source for the world as it is source of nutrient supplement for humans and animals. Globally, maize considered as Queen of cereals and miracle crop due to its high productivity (Younas et al. 2020). Annual worldwide production of maize is about 600 million metric tons with an area of more than 118 million hectares (Arain 2013). It is a high-yield commodity crop, from 2000 to 2014; average harvested area was 157 million hectares with 781 mega tonnes of production (Jones and Thornton 2003; Bassu et al. 2014). In most of the developing countries, maize is vital crop to ensure global food security (Ahmed et al. 2020). Besides this, it is used as a source of basic raw material for protein, starch oil and alcoholic beverages productions and more, recently as potential fuel (Nuss and Tanumihardjo 2010). Furthermore, forage of maize is used for biogas production (Ammani et al. 2013). In tropical areas, it mainly cultivates in rainy areas whereas in kharif, it contributes more than 80% of annual production and remaining production get in summer and Rabi season, from irrigated lands (Zhang et al. 2012).

From last few decades, heavy metals have attracted much attention worldwide because of their nonbiodegradability and high accumulating ability in living things, thus posing threat to humans and ecosystem (Nagajyoti et al. 2010; Ali et al. 2015; Rizwan et al. 2017a). Cadmium (Cd) is one of the toxic trace elements present in the environment (Rizwan et al. 2012; Ali et al. 2014a). Different human activities such as agricultural practices and mining activities mainly responsible for entry of Cd in an ecosystem (Ali et al. 2014b; Murtaza et al. 2015). Cd may negatively affects the different organs of humans hence considered one of the human carcinogens (Gallego et al. 2012; Ansari et al. 2015). Cadmium enters into the food chain mainly through food crops grown in Cd-contaminated soil (Harris and Taylor 2001; Basnet et al. 2014; Fontes et al.

2014; Martos et al. 2016). If concentrations of Cd are higher in the plants, it caused toxicity at physiological, morphological, and molecular levels (Erdem et al. 2012; Baycu et al. 2017; Qayyum et al. 2017). By using the several mechanisms such as binding with cell wall, compartmentalization of Cd in inactive parts, chelation and enhancement in certain enzymes activities and activation of genes responsible for metal transport, plant tolerate the certain amount of Cd (Rizwan et al. 2016b, c; Martos et al. 2016). However, excess Cd overcome the defense system of plants and caused toxicities at various levels (Gallego et al. 2012). Therefore, it is necessary to deal with the problems associated with of Cd contamination (Younas et al. 2020). Also, Cadmium (Cd) contamination has posed an increasing challenge to environmental quality and food security. In recent years, phytoremediation has been particularly scrutinized because of its cost-effectiveness and environmental friendly potential, especially the use of metal-hyperaccumulating plants to extract or mine heavy metals from polluted soils (Shanyinget al., 2017). Hence the present study aimed to determine the effect of the cadmium chloride on the morphological photosynthetic pigments, and proline content in *Zea mays*.

2. Materials and Methods

2.1 Plant material and Soil sample

The seeds of Maize were collected from local seed market of Pune, MH-India. The soil was collected from agricultural land of Pune, MH-IndiaSeeds were washed under running tap water for 5 min. Then the seeds were surface sterilized with 0.1% (w/v) HgCl2 solution for 5 min and washed five times with sterile distilled water. The surface sterilized seeds were kept for germination experiment.

2.2 Treatments

The different concentration of (20, 40, 60, 80 and 100 mg L^{-1}) of $CdCl_2$ was prepared by taking the known amount of $CdCl_2$ dissolved in water. Treatments were given as control, 20, 40, 60, 80 and 100 mg L^{-1} concentration of $CdCl_2$ to the seeds which kept for germination in soil.

2.3 Chlorophyll and proline determination

Chlorophyll was extracted by the Hiscox and Israelstam (1979) method and estimated using the equation of Arnon (1949). The proline was determined from the leaves by the method described by Bayat and Moghadam in 2019 (Bayat and Moghadam 2019).

2.4 Statistical analysis

For morphological responses, total seven replicates taken in each experiment and experiment repeated three times and results were expressed as Mean+SD.

3. Results and Discussion

3.1 Effect of Cadmium Chloride on percentage seed germination, length of shoots, and length of roots of *Zea mays*

Cadmium is not an essential nutrient for plants and it is normally toxic (Wang et al. 2009). The germination percentage shoots length, and root length of *Zea mays* was significantly affected at the highest concentration $CdCl_2$. In control and 20 mg L^{-1} of $CdCl_2$ concentration, germination percent values were 85.7% and 78.5%. The highest germination percentage was observed at 40 mg L^{-1} of $CdCl_2$ i.e. 92.8%. The percentage was reduced again to 57.1% at 60 mg L^{-1} of $CdCl_2$ concentration. However at the highest concentrations of $CdCl_2$ (80 and 100 mg L^{-1}), the percentage of seed germination drastically reduced i.e. 28.5% and 14.2% (Table 1). Similar types of results was reported by Bavi et al. (2011) in pea plants. Bhusare et al (2018) observed that at higher concentration of exogenously applied plant growth regulators negatively affected on percentage of seed germination in *D. lanata* (Bhusare et al. 2018). Lower seed germination percentage was reported in chickpea at higher concentration of *Alternanthera* weed extract (Bhusare et al. 2017). The maize plant has been even shown to accumulate certain heavy metals such as Cd (Kimenyu et al, 2009). If plant accumulates higher cadmium, it may affect the growth and development of plants such as reduction of enzymatic activity (Ouarili et al., 1997; Van Assche & Clijsters, 1990), poor growth, low biomass, cell death and inhibition of growth (Sanitá-diToppi and Gabbrielli 1999; Popova et al. 2009; Xu et al. 2009). Our results confirmed that cytotoxic effects of Cd in cells were concentration dependent and followed a distinct time course (Fojtova and Kovarik 2000). We have observed that 40 mg L^{-1} of $CdCl_2$ showed best response for shoot length (18.4±0.9 cm) as compare to control (17.1±0.7 cm) and 20 mg L^{-1} of $CdCl_2$ (16.7±0.4 cm), while 16.3±0.7 cm shoot length was seen at 60 mg L^{-1} of $CdCl_2$ concentration and it was again to decrease 13.6±0.7 cm and 11.9±1.2 cm when 80 mg L^{-1} and 100 mg $L^{-1}$of $CdCl_2$ was used (Table 1; fig 1). Shelke et al. (2019) reported the negative effect of sodium chloride on *Lathyrus sativus* shoot length (Shelke et al. 2019). In case of root length (9.2±0.3), 20 mg L^{-1} of $CdCl_2$ found to be effective than control (8.7±0.4) and other used concentrations (Table 1). The decrease of plant growth is indicator of metal toxicity (Ernst *et al.*, 1992). Plant root can be absorbed the Cd within few hours of its application in root medium and transported very easily to other parts of the plants (Ghoshroy & Nadakavukaren, 1990; Rauser & Meuwly, 1995). Low nutrient and water uptake may showed inhibition of root growth when Cd supplemented in a medium. In case of shoot growth, low rate of transpiration may be a reason when cadmium contain in media (Chen et al., 2003).

3.2 Effect of Cadmium Chloride on chlorophyll, and proline content

The total amount of chlorophyll was increased when the soil treated with different concentration of $CdCl_2$ which showed positive photosynthetic activity. Chlorophyll content significantly increased (61.6 and 52.7 µg/ml) at 20 and 80 mg L^{-1} of $CdCl_2$ while 40 and 60 mg L^{-1} $CdCl_2$ concentration, total chlorophyll content (49.2 µg/ml and 16.4 µg/ml) get decreased. In control and 100 mg L^{-1} of $CdCl_2$ concentration the total chlorophyll was recorded 50.3 µg/ml and 16.6 µg/ml. Applications of $CdCl_2$ in plants inhibited the growth of cells. Because when cadmium enters in cell, it inhibits and disturbs various biochemical and physiological processes (Bavi et al. 2011). It includes photosynthetic disorders (Vassilev & Yordanov, 1997), closing of stomata (Barcelo & Poschenrieder, 1990), and nutrient uptake inhibition (Sanita di Toppi & Gabbrielli, 1999). However, it has been reported in tolerant plants that chlorophyll content increases or does not change significantly in responses to metals treatments (Burzyn´ski and Buczek 1994; Stiborova et al. 1986). In case of proline content, the proline content increases significantly with increasing the concentration of $CdCl_2$. The proline content was 16.2, 16.8, 17.1, 17.9 and 18 (mg g-1 fw) were recorded in 20, 40, 60, 80 and 100 mg/l of $CdCl_2$ respectively which is higher than control (15.9 mg g-1 fw). Dramatic accumulation of proline is a common physiological response in plants exposed to various abiotic stresses. Accumulation of proline could be due to *de novo* synthesis, decreased degradation, lower utilization, or hydrolysis of proteins (Kaur and Asthir 2015). A very high content of cellular proline has been documented due to its increased synthesis and decreased degradation under a variety of stress conditions in many plant species (Szabados and Savoure 2009). Dinakar et al. (2008) reported that with increasing Cd concentrations, proline levels increased in *Arachis hypogaea* seedling tissues (leaves and roots) in response to 25 days of exposure. Schat et al. (1997) suggested that proline accumulation in plant tissues under Cd stress is due to the decrease in the plant water potential, and therefore, this accumulation could be related to the water equilibrium。

Table 1: **Effect of Cadmium Chloride on seed germination and seedling growth of *Zea mays***

Sr.no	Cadmium Chloride (mg/l)	Seed germination (%)	Shoot length (cm)	Root Length (cm)
1	Control	85.7	17.1±0.7	8.7±0.4
2	20	78.5	16.7±0.4	9.2±0.3
3	40	92.8	18.4±0.9	8.5±0.3
4	60	57.1	16.3±0.7	7.4±0.4
5	80	28.5	13.6±0.7	6.9±0.6
6	100	14.2	11.9±1.2	5.1±0.3

Fig 1: Effect of Cadmium Chloride on growth of *Zea mays*

4. Conclusions

We have recorded the increasing percentage of seed germination, shoot length, and root length at lower concentrations of $CdCl_2$ treatment. Our results confirmed that cytotoxic effects of cadmium in cells were concentration dependent. Decrease in percentage seed germination, length of shoot, and length of root could be indicators of Cd toxicity in maize. Maximum content of proline at higher concentration confirmed cadmium stress in maize. It can be concluded that the maize could successfully grow on Cd contaminated soils at some level.

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