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RESEARCH ARTICLE

The Effects of Cadmium Concentrations on Germination and Physiological Parameters in Tomato (*Solanum lycopersicum* Lam.)

Ömer Bingöl[1]✉ • Abdulhamit Battal[2] • Mehmet Emre Erez[3]

[1]Van Yüzüncü Yıl University, Faculty of Education, Department of Biology Education, Van/Türkiye
[2]Van Yüzüncü Yıl University, Faculty of Pharmacy, Department of Pharmaceutical Biotechnology, Van/Türkiye
[3]Van Yüzüncü Yıl University, Faculty of Science, Department of Molecular Biology and Genetics, Van/Türkiye

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ABSTRACT

Cadmium (Cd) is omnipresent trace element in environmental that is unessential in plants. Cd levels rise because of anthropogenic activity such as the combustion of fossil fuels, phosphate fertilizer manufacturing, mineral fertilizers, batteries technology. It is extremely toxic metal and reduces plant growth. In this context, the aim of this study was to investigate the effect of different concentrations (5/10/20/40 ppm) of Cd on germination of seeds and physiological effects in early developmental stage of tomato *Solanum lycopersicum* Lam. seedlings. 20 ppm (80%) and 40 ppm (83.3%) Cd concentrations caused significantly decrease in germination percentage. All Cd treatments were resulted with decrease in Vigor Index, especially in 20 ppm (42% decrease compared to control). Application of 5 ppm Cd caused decreases in chlorophyll and carotenoid contents in seedlings. Finally, significant decrease in protein content of 5 ppm, 10 ppm and 20 ppm treated seedlings were determined compared to control. As a conclusion, Cd negatively affected germination and physiological parameters of tomato in early developmental stage. Overall, these results indicate that Cd affects different physiologic processes and pathways according to concentration.

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1. Introduction

Some heavy metals (Cu, Zn, Fe, etc.) are micronutrients that essential in very small amounts for plant growth, but others (Cd, Co, Pb) are metals that have a toxic effect even at very low concentrations (Verbruggen et al., 2009). The average dietary cadmium (Cd) uptake by populations in low-income countries are below World Health Organization limits of concern. However, Cd intake increases in some developing countries; therefore, managing Cd transfer across the food chain is critical to limiting human exposure. Long-term exposure to Cd via air, water, soil, and food effect on nervous, and respiratory systems leads to cancer (Mahajan & Kaushal, 2018).

Cd toxicity is effective on plant growth because of its great mobility and assimilability. Cd enters plants through the roots and then transported by transporters through shoots and into vascular bundles (such as phloem and xylem) in the ionic state (Dong et al., 2019). The most obvious effect of Cd toxicity on plants is on photosynthesis. Iron (Fe^{3+}) reductase enzyme is inhibited by Cd induction and it has a major impact on the photosynthetic process and its components. Cd causes stomatal

✉Correspondence
E-mail address: omerbingol@yyu.edu.tr

closure and overall photosynthetic suppression by degradation of chlorophyll, which is essential for photosynthesis (Hasan et al., 2009).

One of the most important actions in the plant lifecycle is seed germination. Seed germination physiology is combination of different aspects as dormancy, imbibition, mineral uptake and hormone balance (Guilherme et al., 2015). Toxic amounts of Cd in plants restrict germination, affect growth and production, interfere with physiological processes in seedlings, and thereby reduce agricultural productivity (Raza et al., 2020).

Cd has been shown in recent research to limit seed germination via various mechanisms, despite the fact that some cultivars continue to germinate in the presence of high Cd concentrations. There are two proposed routes for effect of Cd on seed germination. First, Cd reduces hydrolyzing enzyme levels, starch mobilization, and seed imbibition, all of which have a negative impact on metabolic reactivation. Second, Cd can affect signaling pathway by Calcium (Ca), mitogen-activated protein kinases (MAPKs), and transcription factors (TFs). This interaction will naturally trigger the phytohormonal mechanism, especially gibberellic acid, auxin and stress hormone abscisic acid. All these interactions crucial in the seed germination process (Huybrechts et al., 2019).

Cd exposure influences not only germination but also seedling formation; nonetheless, investigations linking the relationship between Cd administration and physiological parameters during seedling formation are still scarce. In the study, germination and physiological parameters were analyzed to understand the responses of tomato seedling to different Cd concentrations.

2. Materials and Methods

2.1. Materials

All chemicals were purchased from Sigma-Aldrich, Thermo Fischer, Carlo Erba, Isolab and Duchefa.

The seeds of *Solanum lycopersicum* cultivar H2274 was used as plant material. Tomato seeds were surface sterilized with sodium hypochlorite (3%) for ten minutes. Seeds were washed with sterile distilled water for four or five times. Additionally, seeds were treated with ethanol (80%) for 30 or 40 seconds. Surface sterilized seeds were rinsed with sterile distilled water for several times. Sterilized seeds are used for germination and seedling for Cd toxicity tests.

2.2. Methods

2.2.1. Determination of cadmium effect on germination and Vigor Index test

Cadmium chloride ($CdCl_2$) was used for Cd exposure. Ten uniform seeds were placed in sterile petri dishes covered by double filter paper for each application as triplicate. Hoagland's solution was used for control, different Cd concentrations (5/10/20/40 ppm) were used to determine Cd effect on germination.

Germination percentage was calculated as following formula at the end of the fifth day when germination was completed in the control group;

Germination Percentage (%) = (Germinated seeds/Sowed seeds) * 100

The vigor level of each treated seed lot was calculated according to formula (Kumar et al., 2012);

Vigor Index = Seedling length (mm) x Germination percentage (%)

2.2.2. Determination of cadmium effect on seedling growth

Surface sterilized seeds were planted in plastic pots (including 180 ml of Hoagland's medium). Seven days old, seedlings were exposed to different Cd treatments (5/10/20/40 ppm) for five days. Seedlings were harvested at the 12^{th} day. Shoot and root length were measured by a ruler. Fresh weight was weighed for shoot and root tissues. Tissues were incubated at 72 °C for 48 hours to measure dry weight. Finally, Relative Water Content (RWC) was calculated following formula (Smart & Bingham, 1974):

RWC (%) = (Turgid weight-Fresh weight)/(Turgid weight-Dry weight) x 100

2.2.3. Determination of cadmium effect on pigments and protein content

Chlorophyll a, b and carotenoids were determined by using spectrophotometer (AE-S90-MD UV-VIS). Leaf samples (approximately 0.1 g) were homogenized using 80% acetone to measure pigment content. Chlorophyll and carotenoids contents were assessed by determining absorbance at 470, 645 and 662 nm (Arnon, 1949). Chlorophyll and carotenoids contents were calculated as mg/g fresh weight (Lichtenthaler & Wellburn, 1983).

Protein content of tomato seedlings was determined using the Bradford technique. 5 ml sodium phosphate buffer (pH 6.1), homogenized and centrifuged for 20 minutes at 2300 g. Bradford reagent was added to the supernatant, and the absorbance at 595 nm was measured. The absorbance was measured on a spectrophotometer using a standard curve produced with BSA (bovine serum albumin, concentration of 0.1 - 1%) (Bradford, 1976).

2.2.4. Statistical analysis

Data were presented as average and standard error of mean (SEM). Experiments were conducted as at least three independent replicates. GraphPad Prism 8 package program

one-way anova Fisher's LSD test was used to compare groups. P value lower than 0.05 was accepted as statistically significant.

3. Results and Discussion

3.1. Determination of Cadmium Effect on Germination

Cd exposure affected germination percentage of tomato plant. The highest germination percentage (96.7 %) was in the control group. 20 ppm (80%) and 40 ppm (83.3%) Cd concentrations caused significantly decrease in germination percentage compared to control (Figure 1A). The germination of soybean, lettuce, and sugar beet (*Beta vulgaris* L.) seedlings was reduced by 8.0%, 19.0%, and 18.0%, respectively, after exposure to 5 mg/L Cd (Li et al., 2013; Guilherme et al., 2015).

In addition, Cd treatments affected significantly Vigor Index parameters; the highest vigor index value was calculated in the control group (Figure 1B). Cd stress causes decrease in the release of starch from cotyledons due to the reduction in amylase activity (Kalai et al., 2016). The lower mobilization of storage in sorghum seeds (*Sorghum bicolor* L.) has been attributed to a restriction of hydrolyzing enzymes, specifically acid phosphatases, proteases, and –amylase so germination was affected (De Lespinay et al., 2010).

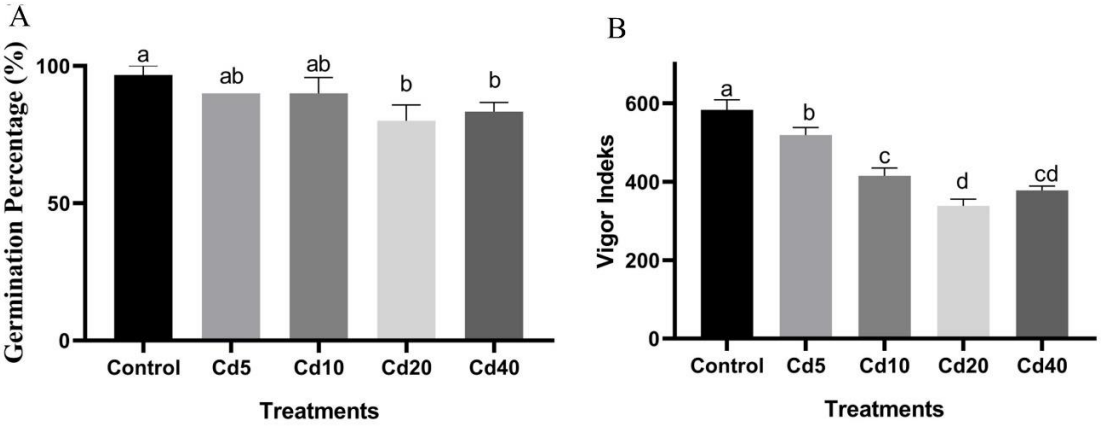

Figure 1. Germination percentage and vigor index of tomato seeds exposed to different Cd concentrations. Different lowercase letters on the columns indicate significantly difference between treatments, P<0.05.

3.2. Determination of Cadmium Effect on Seedling Growth

The highest root length was measured in 5 ppm Cd treated tomato seedlings. Probably, tomato seedlings adapted to low concentration of Cd. On the other hand, 10 ppm, 20 ppm and 40 ppm Cd treatments caused significantly decreases in root length compared to control (Figure 2A). The highest shoot length was measured in control seedlings. The lowest shoot length was measured in 40 ppm treated seedlings. Cd treatments caused significantly decreases in shoot length according to control (Figure 2B).

Our results indicated that Cd affected on seedling growth. Especially shoot lengths were more effected by increase in Cd concentrations. Root and shoot length are most sensitive end-points and considered good indicators for metal toxicity (Ahmad et al., 2011).

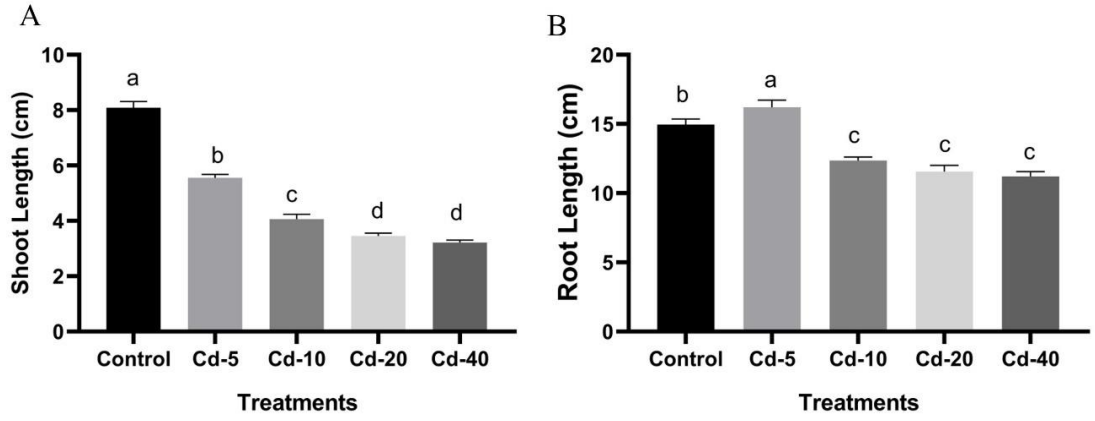

Figure 2. Root and shoot length measurements (cm) exposed to different Cd concentrations. Different lowercase letters on the columns indicate significantly difference between treatments, P<0.05.

3.3. Determination of Cadmium Effect on Pigments and Protein Content

Cd is a toxic heavy metal that can have detrimental effects on plant photosynthesis (Gallego et al., 2012). Considering the effects of chlorophyll in seedling development; 5 ppm Cd treatment caused significantly decrease in pigment content compared to control. (Figure 3A). Cd toxicity has an effect on plants by limiting carbon fixation and lowering chlorophyll content and photosynthetic activity (Gallego et al., 2012). The interaction between Cd and chlorophyll production reduces chloroplast density and causes chlorosis in oilseed crops such as rapeseed (Baryla et al., 2001).

5 ppm, 10 ppm and 20 ppm Cd treatments caused significantly decrease in carotenoid content according to control (Figure 3B). Cd mainly affects photosynthesis metabolism and its pigments, carotenoids and chlorophyll synthesis (Rafiq et al., 2014).

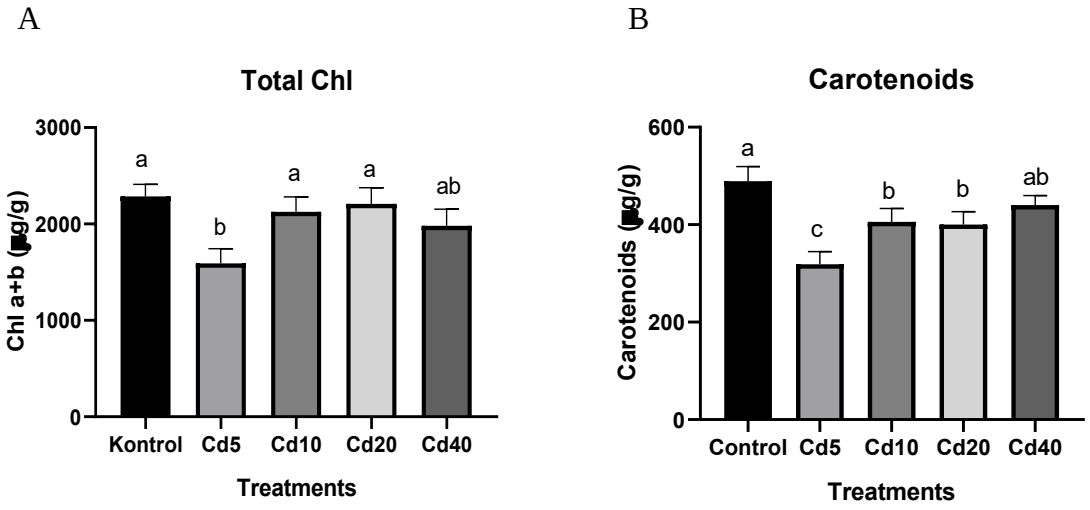

Figure 3. Chlorophyll and carotenoid contents of tomato seedlings exposed to different Cd concentrations. Different lowercase letters on the columns indicate significantly difference between treatments, P<0.05.

The highest protein content was determined in control seedlings. 5 ppm (1301 µg/g FW), 10 ppm (1070 µg/g FW) and 20 ppm (1368 µg/g FW) Cd treatments caused significantly decrease in protein content according to control (1697 µg/g FW) (Figure 4). Metal pollution disrupts plant metabolism through interactions with enzymes and biochemical events that occur within the plant (Ashraf et al., 2011).

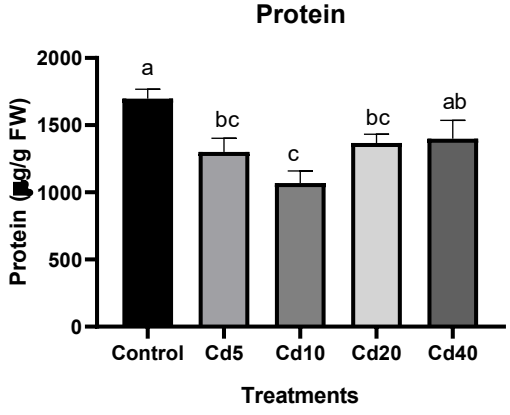

Figure 4. Protein content of tomato seedlings exposed to different Cd concentrations. Different lowercase letters on the columns indicate significantly difference between treatments, P<0.05.

Cd related DNA damage destroys cell membranes and nucleic acids, damages photosynthetic proteins, and reduces protein synthesis, all of which have an impact on organism growth (Abbas et al., 2017). Protein expression of numerous metabolic pathways was negatively affected by Cd entrance in plants. Several proteins abundance decreased associated by oxidative stress response to Cd toxicity (Haider et al., 2021).

4. Conclusion

In conclusion, Cd has reached extremely serious and alarming levels in terms of food safety all over the world, especially with the development of technology. High Cd concentration in plants, which are primary producers, delays their development and photosynthetic activity, which reduces crop productivity. In addition, Cd appears to have a serious effect on germination by acting on enzymes and on early development stage Cd effect primary carbon metabolism and oxidative stress response mechanism, caused to the appearance of chlorosis and changes in protein profile especially in youngest leaves and reduction in growth and yield. However, bioremediation technologies used to decontaminate Cd, understanding Cd accumulator plant mechanisms, and selecting resistant species may provide economically viable and environmentally options for remediating Cd-contaminated soil.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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