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Cadmium's effects on germination and growth in *Dianthus deltoides*: an *in vitro* culture approach

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Abstract
Heavy metals, such as cadmium, pose a real threat to the health and balance of ecosystems and have a significant impact on organisms and plants. The use of biotechnology to assess toxicity levels in plants, as well as to set species-specific toxicity thresholds, may be a feasible method for determining effects and also for creating new genotypes more adapted to environmental conditions and changes. In this research, we focused on the impact of cadmium on germination and growth processes in *Dianthus deltoides* using cadmium concentrations of 1,124, 11,24 and 112,4 mg/l. The experiment was conducted by growing the plants under in *vitro* conditions in MS medium supplemented with different cadmium concentrations over three time periods of 7, 14 and 21 days. The results showed a decrease in root growth and leaf area and a complete inhibition of the germination process at the highest cadmium concentrations, highlighting the toxic impact that cadmium can have on plants and organisms in general. A noteworthy finding in concentration of 11,24 mg/l revealed a negative impact of cadmium. Although, that effect wasn't immediately observable in the initial analysis period, the prolonged exposure to cadmium concentrations resulted in significant differences in root growth and leaf area compared to the control ($p < 0.05$). These results underscore the plants response to varying cadmium levels and underscore the significance of exposure duration. Specifically, the findings suggest that the toxic effects of cadmium may not manifest immediately but intensify with prolonged exposure. Thus, by using biothenology and *in vitro* cultures, toxicity phenomena as well as heavy metal effects can be determined.

Keywords: Dianthus deltoides, cadmium toxicity, In vitro culture, heavy metal stress

Introduction

Heavy metals are a real danger to physical and mental health and have a significant impact on organisms and plants [4]. We usually do not realize we are exposed to these toxic metals until the toxicity reaches a critical level [17]. Exposure to heavy metals can cause cardiovascular problems, myocardial infarction, stroke and neurological conditions [5]. Cadmium toxicity is another important issue. It enters the environment through the air, binds stably to the soil and reaches the soil mainly through industrial activities and fertilizer use. Humans take up cadmium through food such as liver, mushrooms, shellfish and others [9]. Exposure can have serious consequences for the lungs, liver, kidneys and bone system [23]. To avoid the negative impact of heavy metals, it is essential to be aware of the sources of exposure and to take preventive measures [21]. Cleansing the body of such toxins requires chelation therapy, and monitoring metal levels in the body can help maintain a healthy balance [16, 24, 29].

Cadmium induces oxidative stress [8], leading to the generation of reactive oxygen species (ROS) [2, 8, 19, 30].

Studies indicate that the phenomenon of inducing oxidative reactions leads [3,7] to the vacuolization process [12,20] reflected by a decrease in mitotic indices [6,15] and a reduction in plant growth [28]. Cadmium is one of the most toxic heavy metals, with different alert and intervention limits depending on the country, ranging from 1-10 mg/kg in residential areas. Unacceptable limits begin even at concentrations of 2 mg/kg in

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Europe [10]. The variability of these limits is likely due to environmental conditions, which play an essential role in accentuating toxic effects [27]. However, one thing is clear: the long-term accumulation of cadmium and its presence as a result of pollution [11, 27] can have extremely serious consequences for both health and the ecosystem [19, 31, 32].

In the field of health, biotechnology aims to develop technology-based products or processes related to energy, nutrition [25], and health [26], with the goal of optimizing the development of new products. This objective includes reducing dependence on animal toxicity testing by adopting validated in vitro tests, processes that are sensitive, reproducible, and financially accessible.

In our research, we aim to use the in vitro biotechnological method for testing cadmium toxicity [13], using the species Dianthus deltoides, also known as the deltoid carnation. As a perennial plant belonging to the Caryophyllaceae family and being popular in gardens, borders, and rocky areas [14], its adaptability to diverse growth conditions renders it valuable in toxicity tests [24], providing relevant information on substance impact on the environment. Remarkably, this species exhibits high drought tolerance [22], adding durability and robustness to laboratory experiments. This characteristic makes Dianthus deltoides suitable for assessing the effects of chemical substances and potential hazards on local flora and fauna.

Material and Method

Preparation of biological material

For the experiment, we used 20 seeds of *Dianthus deltoides*, which were sterilized by surface disinfection in 70% ethyl alcohol for 10 seconds. This was followed by immersion in a solution of 0.1% mercuric chloride added with Tween for 5 minutes, followed by repeated washing with sterile distilled water.

Preparing the culture environment

To prepare the culture medium, we used Murashige and Skoog (MS) medium supplemented with 3% sucrose and 0.82% agar, with pH adjusted to 5.8 by adding NaOH for 20 minutes. Cultures were maintained in a culture chamber at an air temperature of 24 ± 1˚C under controlled lighting. Pouring of the culture medium was carried out in glass flasks of 115 ml capacity.

To create experimental variants, we added different concentrations of cadmium to the culture media using an initial stock solution of 0,01 mmol/l concentration (1124.1 mg/l of Cd). In order to create the experimental variants of cadmium to stock solution concentrations was diluted to 0,00001 (1,124 mg/l, value below the signal intervention in soil concentration [10]), 0,0001 (11,24 mg/l, value above the industrial concentration of Cd of 10 mg/l [10]) and 0,001 mmol/l (112,4 mg/l) of Cd (Figure 1).

V1- control V2- 1,124 mg/l V3- 11,24 mg/l V4-112,4 mg/l

Figure 1. **Experimental variants used in an in vitro culture approach**

Experimental design

The experiment included 5 replicates in three experimental periods (7, 14 and 21 days) for each variant used in which we analyzed the in vitro growth process of Dianthus deltoides seeds on culture media enriched with heavy metal solutions, focusing in particular on cadmium (V2-V4). We compared this growth with that of seeds grown on culture media without any enrichment (V1 - control). The main objective of this study was to determine the growth dynamics of roots of Dianthus sp. species in cadmium containing solutions, compared to a control group grown in control medium with water.

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Statistical calculations

The measurement results underwent scrutiny via ANOVA analysis at a significance level of $p < 0.05$, aiming to discern the impact of cadmium concentrations on the growth of both root systems and leaf areas across three evaluation periods. Additionally, the Tukey test was used to determine and to compare the variations associated with the utilized concentrations on growth. All statistical calculations were executed using the RStudio software platform.

Results and Discussion

Results obtained under in vitro conditions show the toxicity of cadmium over all three time periods ($p < 0.05$). Thus, at the highest cadmium concentration (V4), the seeds did not show germination under those in vitro conditions, unlike the control and the V2 and V3 variants.

As for the influence of cadmium on root growth in the experimental variants, it was inhibited for all three time periods analyzed, the effect being directly proportional to cadmium concentration ($p < 0.05$) (table 1).

Table 1. Analysis of Variance (ANOVA) on the Effect of Cadmium Concentrations on Root Length at Various Time Periods

Source		SS	df	S2	F	Mr
Concentration	Period 1	4.790	3	1.597	92.943	***
	Period 2	18.064	3	6.021	139.846	***
	Period 3	50.355	3	16.785	79.896	***
Error	Period 1	0.275	16	0.017		
	Period 2	0.689	16	0.043		
	Period 3	3.361	16	0.210		
Total	Period 1	19.331	20			
	Period 2	65.551	20			
	Period 3	189.153	20			

In the first analysis period, at 7 days, root length was similar for both the control and V2 and V3, in contrast to V4, where seeds showed no germination. In the second period, at 14 days, significant differences from the control group were evident, with the exception that V3 showed lower root length values.

In the evaluation of the results of the third period, at 21 days, the effect of cadmium influence became evident in the case of variants V3 and V4 ($p < 0.05$), while the values obtained for the control variant and V2 were similar ($p > 0.05$) (Figure 2).

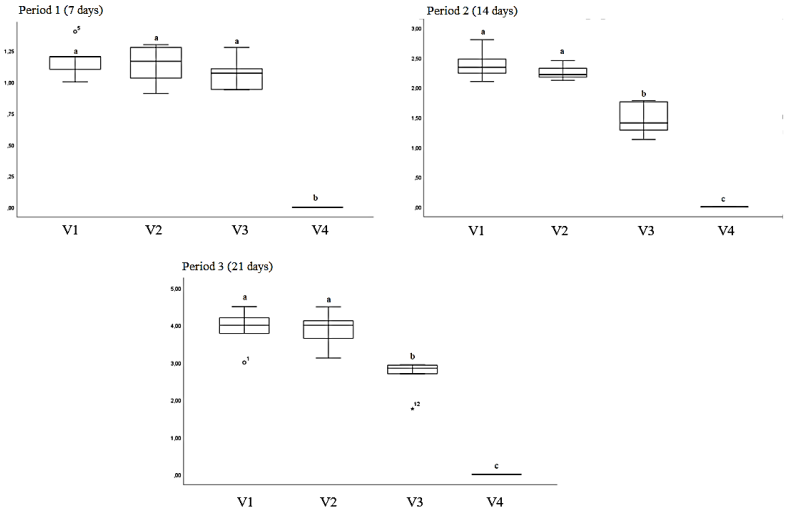

Figure 2. **Results of cadmium influence on *Dianthus deltoides* root growth: V1 - control, V2 - 1.124 mg/l Cd, V3 - 11.24 mg/l Cd and V4 - 112.4 mg/l Cd**

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As regards leaf growth capacity, similarly to the situation found for roots, there is a significant difference in growth and its influence due to the toxic effect of cadmium ($p < 0.05$). It hinders growth during all periods evaluated, and the differences are significant compared to the values obtained for the variant (Table 2).

Table 2. Analysis of variance (ANOVA) on the effect of Cadmium concentrations on leaf area at various time periods

Source		**SS**	df	S2	F	Mr
Concentration	Period 1	8.119	3	2.706	239.214	***
	Period 2	44.581	3	14.860	41.907	***
	Period 3	95.713	3	31.904	184.599	***
Error	Period 1	0.181	16	0.011		
	Period 2	5.674	16	0.355		
	Period 3	2.765	16	0.173		
Total	Period 1	32.236	20			
	Period 2	167.212	20			
	Period 3	363.350	20			

In the first phase of the 7-day investigations no significant differences were observed between V1-V3. However, the influence of cadmium, especially at the specific concentration in V3, became more evident in the subsequent 14 and 21 day phases. In this context, the higher cadmium concentrations in the V4 variant led to a complete inhibition of the germination process (Figure 3).

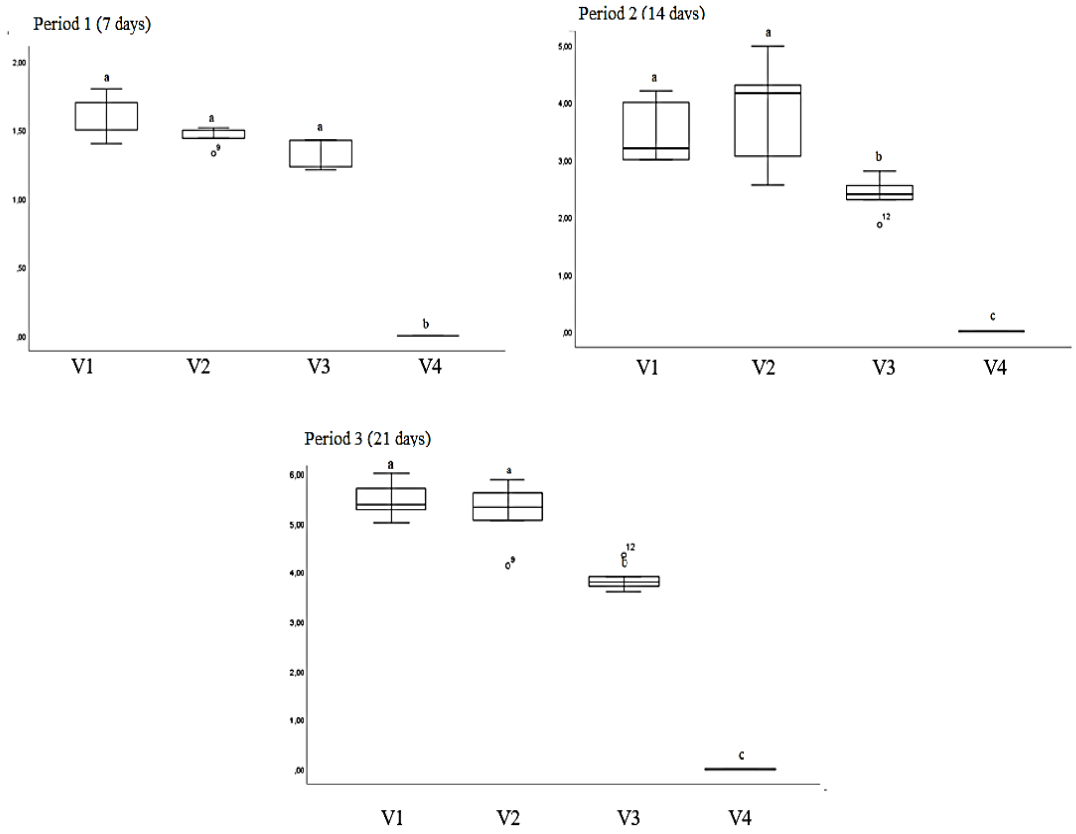

Figure 3. Results of cadmium influence on Dianthus deltoides leaf area growth: V1- control, V2-1.124 mg/l Cd, V3-11.24 mg/l Cd and V4—112.4 mg/l Cd.

From the analysis of the results obtained, the toxic effect of cadmium is evident especially at the V2

concentration, where seed germination was completely inhibited. A negative influence of cadmium was also found at lower concentrations in V3. However, it is important to note that this effect was not immediately observable in the first period of analysis. However, as the period of exposure to cadmium concentrations increased, the negative impact became increasingly evident, with significant differences in root growth and leaf area between V3 and the control ($p < 0.05$), as shown in figures 2 and 3. The results reflect the response of the plants to different cadmium concentrations and highlight the importance of the duration of exposure. In particular, the results indicate that the toxic effects of cadmium may not manifest immediately but become more pronounced as the plant is exposed over a longer period.

Also, the finding that there were no significant differences in the lowest concentration highlights the variability of plant response to specific cadmium concentration. This observation highlights that the effect of cadmium manifests itself differently depending on concentration and duration of exposure, underlining the need for a complex approach in assessing the impact of toxic substances on plants.

Conclusions

In conclusion, the results obtained in this study indicate a significant effect of cadmium on plant germination and growth processes, and the importance of the duration of exposure to this toxic substance, especially at lower concentrations, which causes inhibition of root growth and leaf area.

The observation of no significant differences at the lowest cadmium concentration underlines the variability of plant response depending on the specific cadmium concentration, while highlighting the need for long-term monitoring of cadmium concentrations to control toxic effects and protect the environment.

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