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ARTICLE

Evaluation of seed germination development and initial growth of cotton plants exposed to cadmium

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Resumen

Evaluación de la germinación de semillas y desarrollo inicial de las semillas de algodón expuestas al cadmio

Esta investigación evaluó los efectos fitotóxicos del cadmio en el desarrollo germinativo y el crecimiento inicial de *Gossypium hirsutum* L. Se evaluaron los efectos ecotoxicológicos de 13 concentraciones experimentales de cadmio (0.0-50.0 mM) sobre variables de respuesta como porcentaje de germinación (IVG), tiempo promedio de germinación (TMG) y longitud de la raíz de las plántulas. Las semillas de *G. hirsutum* expuestas al cadmio mostraron una disminución en el porcentaje de germinación y un aumento en el número de plántulas anormales con las concentraciones 18.0 y 3.0 mM, respectivamente, además de inhibir el crecimiento de la raíz y elevar el TMG de la exposición a 0,5 mM, reduciendo el IVG. *G. hirsutum* es una especie tolerante al cadmio durante su desarrollo germinativo.

Palabras clave: Contaminación; *Gossypium hirsutum*; Metal pesado.

Abstract

This research evaluated the phytotoxic effects of cadmium on the germination development and initial growth of *Gossypium hirsutum* L. The ecotoxicological effects of 13 experimental concentrations of cadmium (0.0-50.0 mM) on variables like germination percentage, GSI, average germination time (AGT) and root length were evaluated. *G. hirsutum* seeds exposed to cadmium showed a decrease in germination percentage and an increase in the number of normal plantules from the concentrations 18.0 and 3.0 mM, respectively. It also inhibited root growth and increased the AGT from the exposure to the concentration 0.5 mM, reducing the GSI. *G. hirsutum* is tolerant to cadmium during its germinal stage.

Key words: Contamination; *Gossypium hirsutum*; Heavy metal.

Introduction

Cotton (*Gossypium hirsutum* L.), belonging to the family Malvaceae, is a species cash crop cultivated in various regions of the world. It has a great importance for Brazilian economy as an agricultural product (Sousa, 2010), and its fibers generate sources of income for local and national farmers (Pereira *et al.* 2012).

For farmers, the analysis of seed quality and how seeds behave when sown is of great importance (Ferreira & Novembre, 2016). Therefore, studies on seed germination are important to improve the morphological and physiological knowledge of embryos and plantules by analyzing especially the environmental conditions to which they are exposed (Gordin *et al.* 2012).

Environmental factors influence the germination and development of the seedlings (Guilherme *et al.* 2015). The presence of chemical contaminants, such as toxic heavy metals, in the substrate may interfere with or inhibit important physiological events in organisms.

Pollution of soils by heavy metals originates mainly from human activities such as industry, mining and agriculture. Cadmium is one of the heavy metals that cause harm to plants and animals (Melo *et al.* 2014). The effects of cadmium on plants vary with exposure time to metal, and may cause morphological, physiological and structural changes in plants, inducing enzymatic and metabolic changes (Augusto *et al.* 2014) or inhibiting the development of the embryonic axis and roots (Guimarães *et al.* 2008).

The presence of cadmium in the soil in a concentration that causes significant inhibitory effects on plants may be a source of chemical stress limiting a good agricultural productivity.

Several studies have reported the influence of cadmium on cotton (Ozyigit *et al.*, 2013; Chen *et al.*, 2015; Farooq *et al.*, 2016; Liu *et al.* 2016; An *et al.*, 2020). In general, these studies report biochemical, anatomical and genetic effects in organs of already developed and exposed plantules. Most of them do not report cadmium effects on germination and initial establishment of this species, so that the limiting effects of cadmium on the establishment of the species can be known. Thus, knowing the amounts of cadmium potentially toxic to agricultural crops, such as cotton, is important because they can guide decision-making on the appropriate choice of the species to be cultivated in a soil with an indication of contamination by this metal.

The aim of this research was to evaluate the phytotoxic effects of cadmium on the germination and initial growth of cotton seeds.

Materials and methods

The study was conducted at the Laboratory for Environmental Research (LAPAM) at the Federal University of Campina Grande, in the campus of Patos, Paraíba state, between September and December 2015.

Cotton seeds (variety BRS-286) were obtained from the Brazilian Agricultural Research Company (EMBRAPA), Campina Grande, Paraíba state. Its pre-treatment consisted in delinting with sulfuric acid (1 L of H_2SO_4 P.A./6.5 kg of seeds) for 5 minutes, followed by a wash in distilled water and neutralization of the residual acid with sodium bicarbonate ($NaHCO_3$ at 10%) for 40 seconds, and drying in the sun for 30 minutes (Lopes *et al.* 2006). Delinted seeds were disinfected with sodium hypochlorite (NaClO at 2.5%) for 15 minutes and subjected to a germination viability test (Brasil, 2009).

Upon evaluating the effect of cadmium, 13 experimental concentrations (0.0, 0.5, 1.0, 3.0, 6.0, 9.0, 12.0, 15.0, 18.0, 21.0, 30.0, 40.0 and 50.0 mM) were analyzed in quadruplicate with 25 seeds in each plate, totaling 1,400 seeds. This concentration range was defined from the experimental concentration ranges reported in studies with this metal and confirmed to be relevant in a pilot experiment with germination at concentrations of 0.5, 5.0, 25.0; 50.0, and 75.0 mM.

Seeds were immersed for 30 minutes in 20 mL of each experimental concentration and sown in Petri dishes (Ø 150 mm) over a sterilized double layered germitest paper (105 ºC for 120 min), dampened with 8 mL of distilled water. The plates were sealed with plastic film to prevent loss of moisture.

Experimental cadmium solutions were prepared using cadmium sulfate ($CdSO_4.8H_2O$), keeping the pH of the solution at approximately 6.0. The control experiment contained only distilled water.

The experiment was conducted under laboratory conditions (on the bench), where the treatments were exposed to the photoperiod 8h light and 12h dark, under white fluorescent light. The room temperature was monitored twice daily and varied 28±2 ºC with an average of 60% relative humidity.

The following variables were analyzed: germination speed index (GSI), average germination time (AGT), percentage of germination and abnormal plantules, root growth and biomass production with fresh and dry matter (Maguire, 1962).

There was a daily monitoring of the count of germination and abnormalities of plantules (BRASIL, 2009). The root length was measured with a digital caliper at the beginning of germination (48 hours) and when the germination percentage became constant (120 h).

Root lengths were obtained by measuring 8 plantules in each plate, totaling 32 seedlings per experimental concentration, and were expressed in millimeters. Fresh and dry matters were determined according to BRASIL (2009). The dry matter was obtained after drying at 105 ºC and was expressed in grams.

The results were statistically evaluated using the statistical software SPSS 20.0. When necessary, data were transformed in arc-sine $[(x+0.5)/100]^{0.5}$ to ensure the homoscedasticity of data, which were evaluated by two-way analysis of variance (ANOVA) followed by a Tukey test to determine the statistical significance of the difference between the variances. The analysis of the growth of seedling parts over time was performed using the polynomial regression model. For all statistical analyses, a significance level of $p<0.05$ was adopted.

Results and discussion

The viability of the analyzed seeds reported average values of 94±5.0%. According to Brazil (2009), this value confirms that the seeds are viable for germination.

G. hirsutum seeds showed a significant decrease in germination percentage when exposed to the cadmium concentration 21.0 mM, with a 10% decrease compared to control, achieving a decrease of 82% in the exposure to the concentration 50 mM (Fig. 1).

However, despite a good germination, the presence of normal plantules was observed at a

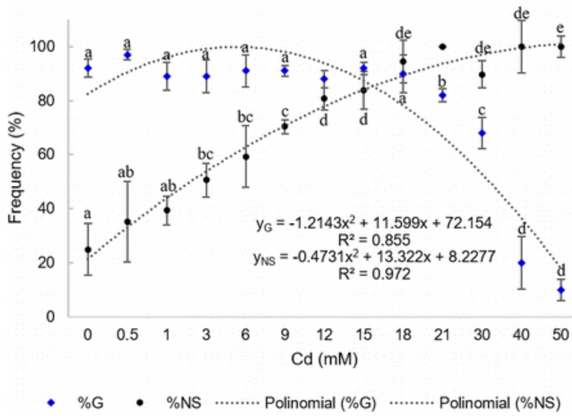

Figura 1. Porcentaje de germinación (%G) y plántulas normales (%NS) de semillas de *Gossypium hirsutum* expuestas a diferentes concentraciones de cadmio.

Figure 1. Germination percentage (%G) and percentage normal plantules (%NS) of *Gossypium hirsutum* seeds exposed to different cadmium concentrations.

high frequency, significantly different from the control ($F=10.972$, $p<0.001$), from the concentration 3.0 mM (50.6%), reaching 100% of abnormal plantules at the concentration 21 mM, respectively. The types of abnormalities observed and their percentage were often (Fig. 2) short and coarse primary roots (56.5%) and thin and weak primary roots (27.6%).

There was a significant correlation among cadmium concentrations to the decrease in germination percentage, and the increase in the number of abnormal plantules (Fig. 1).

The variables GSI and AGT were negatively influenced by the presence of cadmium, recording a significant decrease from the concentrations 0.5 mM and 30 mM, respectively (Table 1). The polynomial regression analyses confirmed the correlations between the change in GSI and AGT according to the increase in the concentration of cadmium.

The root growth, after 24 hours of sowing, decreased ($F=10.972$, $p<0.0001$) from the concentration 0.5 mM. This change was most evident after 72 hours of exposure (Fig. 3), with a 50% inhibition of root growth from the concentration 3.0 mM in relation to the control, reaching 100% of inhibition in plantules exposed to the concentration 40.0 mM.

These results suggest that plantules susceptible to cultivation in the field with a soil contaminated by cadmium may have a decreased root growth and consequently a reduced ability to absorb nutrients and soil moisture. This may hinder the

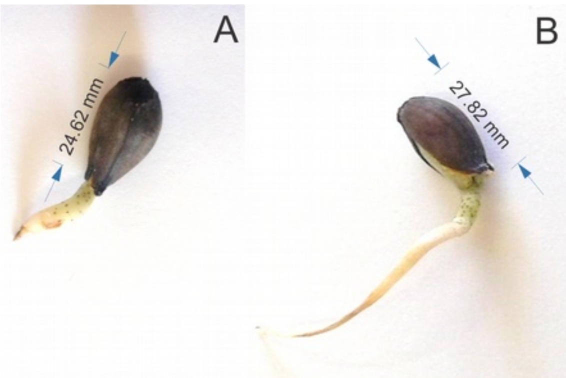

Figura 2. Imágenes de los tipos de anormalidades observadas en plántulas de *Gossypium hirsutu*m. **A:** raíces primarias cortas y gruesas; **B:** raíces primarias finas y débiles.

Figure 2. Images of the types of abnormalities observed in *Gossypium hirsutum* plantules. **A:** short and coarse primary roots; **B:** thin and weak primary roots.

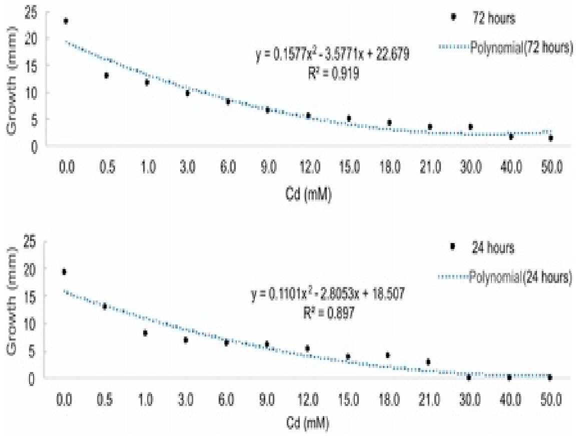

Figura 3. Crecimiento de las raíces después de 24 hy 72 h de la siembra de semillas de *Gossypium hirsutum*.

Figure 3. Root growth after 24 h and 72 h from the sowing of *Gossypium hirsutum* seeds.

Concentration	GSI	AGT
0.0	14.27 ± 2.03^{b} (14.2)	2.00 ± 0.34 (17.0)
0.5	12.58 ± 0.83^{ab} (6.6)	2.49 ± 0.24^{ab} (9.4)
1.0	12.02 ± 2.15^{ab} (17.9)	2.39 ± 0.38^{ab} (16.1)
3.0	11.71 ± 0.71^{ab} (6.1)	2.38 ± 0.16^{ab} (6.6)
6.0	11.63 ± 1.00^{ab} (8.6)	2.46 ± 0.14^{ab} (5.7)
9.0	11.02 ± 1.20^{ab} (10.9)	2.55 ± 0.17^{ab} (6.8)
12.0	11.67 ± 1.59^{ab} (13.7)	2.28 ± 0.21^{ab} (9.4)
15.0	10.46 ± 1.27^{ab} (12.2)	2.65 ± 0.14^{ab} (5.3)
18.0	10.85 ± 0.71^{ab} (6.6)	2.46 ± 0.07^{ab} (2.9)
21.0	10.83 ± 1.91^{ab} (17.7)	2.27 ± 0.27^{ab} (12.0)
30.0	5.29 ± 0.67^{ab} (12.8)	3.61 ± 0.12^{ab} (3.3)
40.0	1.63 ± 0.31^{a} (18.7)	3.88 ± 0.33^{a} (8.6)
50.0	0.66 ± 0.30^{a} (34.6)	4.21 ± 1.18^{a} (28.1)
Polynomial equation	$y = -0.1254x^2 + 0.8184x + 11.793$	$y = 0.0233x^2 - 0.19x + 2.602$
R^2	$R^2 = 0.8648$*	$R^2 = 0.7867$*
F ANOVA	34.369*	12.232*

Mean ± SD (percent coefficient of variation)

Different letters indicate significant difference among values in the same column (p<0.05). *Significant at p<0.001

Tabla 1. Índice de velocidad de germinación (GSI) y tiempo promedio de germinación (AGT) de semillas de algodón expuestas al cadmio. Experimentar.

Table 1. Germination speed index (GSI) and average germination time (AGT) of cotton seeds exposed to different concentrations of cadmium.

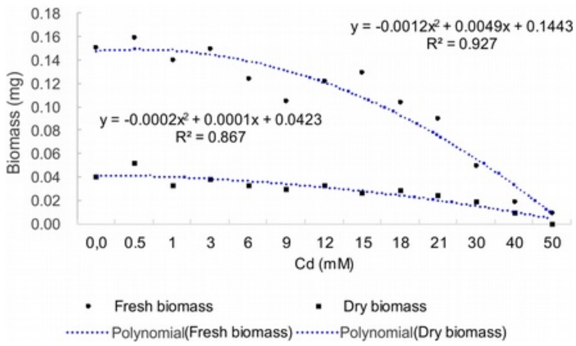

Figura 4. Influencia del cadmio en la producción de biomasa fresca y seca de plántulas de *Gossypium hirsutum*.

Figure 4. Cadmium influence on the production of fresh and dry biomass of *Gossypium. hirsutum* plantules.

growth and establishment of the plants.

Cadmium also influenced the biomass production. Compared to the control, there was a significant (p<0.001) reduction in fresh (F=26.499) and dry mass (F=1,526.28), where this effect was observed from 6.0 mM, respectively (Fig. 4). Linear regression analysis confirms high correlation between reduction of biomass and increasing concentrations of cadmium (Fig. 4).

Studies with *G. hirsutum* also recorded efects on the germination depending on cadmium doses (Aycicek *et al.* 2008a; Aycicek *et al.* 2008b). Several studies reported the toxic effects of cadmium to others crops. In the evaluation of the toxic effects of cadmium in *Sinapis arvensis* L., a decrease in seed germination percentage (5.6%) was observed for the concentration 1.0 mM (Heidari & Sarani, 2011).

In a study by Tao *et al.* (2015), involving several crop species exposed to cadmium (0.0-3.2 mM), it showed significant variations in the percentage of germination. In relation to root length, these species, when exposed to the concentration 3.2 mM, underwent the following growth decrease percentages: soybean (*Glycine max* (L.) Merr.): 25%, mung bean (V*igna radiata* L.): 20%, beans (*Vigna angularis* (Willd.) Ohwi & Ohashi): 27%, lentils (*Lablab purpureus* L.): 38.5%, sweet pea (*Lathyrus odoratus* L.): 23%, and black beans (*Dumasia villosa* DC.): 33%. The results reported here for *G. hirsutum* seeds reveal that it is more tolerant than these crop species.

The presence of abnormalities in the roots of *G. hirsutum* seedlings exposed to cadmium are similar to the results reported by Wilhelm *et al.*

(2015), who observed a higher occurrence of abnormal seedlings in *Triticum aestivum* L. when exposed to cadmium, and a decrease in root length of seedlings from the concentration 0.12 mM.

The inhibition of root growth is one of plants' responses to exposure to cadmium, and occurs more rapidly than other physiological responses (Gonçalves *et al.* 2009). This toxic effect is observed in roots, being reported for several species of plants, such as *Spinacia oleracea* L. (Hosseini *et al.* 2012), *Cicer arietinum* L. (Faizan *et al.* 2011), *T. aestivum* (Guilherme *et al.* 2015) and *Solanum tuberosum* L. (Gonçalves *et al.* 2009).

The reduction of biomass by seedlings of *G. hirsutum* caused by cadmium was not significant in studies reported by Ozyigit *et al.* (2013) for fresh and dried aerial part (leaves) exposed to 0.1 and 0.2 mM cadmium. However, under this concentration, cultivars (cv. NDM9 and cv. GXM3) showed reduced leaf growth by 45.2% and 40.8%, respectively (Liu *et al.*, 2016). However significant intolerance was observed in roots of *G. hirsutum* ((ZMS-49) at 0.5 mM (Khan *et al.* 2013).

Studies with other species show similar effect trends such as *T. aestivum* (Guilherme *et al.* 2015), *Jatropha curcas* (Chaves; Souza, 2014), *Phaseolus vulgaris* L. (Santos *et al.* 2013) and *Brassica juncea* (L.) Hook.f. & Thomson (Augusto *et al.* 2014).

Most plants have a low tolerance to cadmium and higher levels of metals in roots than in shoots (Guimarães *et al.* 2008). The tolerance to cadmium of many plant species is related to the compartmentalization of the metal in the cellular vacuole (Hartke, 2012).

According to Guimarães *et al.* (2008), the ability plants to survive and prevail in environments rich in heavy metals, where most plants fail to survive, provides a study model system able to detect the mechanisms contributing to tolerance related to transport of metals.

According to Sá *et al.* (2011), cadmium affects photosynthesis, respiration and the activity of enzymes associated with the antioxidant system of the plant. These effects cause a reduction in the capacity to absorb micronutrients (Guimarães *et al.* 2008) and water, hindering growth and decreasing the thickness of root tissues proportionally to increasing doses of cadmium.

Conclusions

Cadmium is a heavy metal toxic even at low concentrations. Cadmium cause toxic effects on the initial development of *G. hirsutum* reducing the percentage of germination and root length at concentration 1.0 mM.

The identification of plants tolerant to cadmium allows developing a cultivar that does not concentrate contaminating metals in plant parts and seeds consumed by humans and animals.

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