

## EFFECTS OF ALUMINUM ON SEED GERMINATION AND INITIAL GROWTH OF PHYSIC NUT SEEDLINGS

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**ABSTRACT:** Aluminum ( $Al^{3+}$ ) toxicity is one of the major factors that limit plant growth and crop yield in acid soils. The phytotoxic effects of aluminum on the seed germination and initial growth of physic nut (*Jatropha curcas* L.) seedlings were investigated in the present study. Physic nut seeds were subjected to five levels of aluminum [0 (control), 20, 40, 60 and 80 mg L<sup>-1</sup>], using the solution-paper method, with eight replications. The experimental unit consisted in a germitest paper roll with 25 physic nut seeds. Treatments were evaluated based on the following tests: first count of germination test (7 days), total germination (12 days) and initial growth of physic nut seedlings (12 days). The addition of 80 mg L<sup>-1</sup> of Al reduced the first count of germination test (79%), total germination (90%), shoot length (20%), shoot dry matter (30%), root length (76%) and root dry matter (83%) of physic nut seedlings compared to the Al-free control. The addition of high Al concentrations severely restricted the seed germination and initial growth of physic nut. Phytotoxic effects of Al is more accentuated on the root growth than on shoot growth of seedlings. The data suggest that the physic nut is a susceptible species to high concentrations of Al during the phase of seed germination and early growth.

**KEY WORDS:** *Jatropha curcas*, aluminum toxicity, stress, root growth.

## EFEITOS DO ALUMÍNIO NA GERMINAÇÃO DAS SEMENTES E NO CRESCIMENTO INICIAL DAS PLÂNTULAS DE PINHÃO-MANSO

**RESUMO:** A toxicidade de alumínio ( $Al^{3+}$ ) é um dos principais fatores que limitam o crescimento e a produtividades das culturas em solos ácidos. Este estudo teve como objetivo avaliar os efeitos fitotóxicos do alumínio na germinação das sementes e no crescimento inicial das plântulas de pinhão-mansó (*Jatropha curcas* L.). As sementes de pinhão-mansó foram expostas a cinco níveis de Al [0 (controle), 20, 40, 60 and 80 mg L<sup>-1</sup>], com oito repetições. As unidades experimentais foram constituídas de rolos de papel germitest com 25 sementes de pinhão-mansó. Os tratamentos foram avaliados com base nos testes de primeira contagem da germinação (7 dias), germinação (12 dias) e crescimento inicial das plântulas de pinhão-mansó (12 dias). A adição de 80 mg L<sup>-1</sup> de Al reduziu a primeira contagem da germinação (79%), a germinação final (90%), comprimento da parte aérea (20%), matéria seca da parte aérea (30%), comprimento das raízes (76%) e a matéria seca das raízes (83%) de pinhão-mansó em comparação as plântulas sem exposição ao Al. A adição de altas concentrações de Al reduziu severamente a germinação das sementes e o crescimento inicial das plântulas. Os efeitos fitotóxicos do Al foram mais acentuados no crescimento das raízes do que no crescimento da parte aérea das plântulas. Os dados sugerem que o pinhão-mansó é uma

espécie sensível à alta concentração de Al durante a fase de germinação das sementes e crescimento inicial.

**PALAVRAS-CHAVE:** *Jatropha curcas*, toxicidade do Al, estresse, crescimento das raízes.

## INTRODUCTION

Physic nut (*Jatropha curcas* L.) is a perennial species, native from tropical America and belongs to the family Euphorbiaceae. The other important members of this family include castor oil plant (*Ricinus communis*), cassava (*Mahihot esculenta*) and rubber tree (*Hevea brasiliensis*). This species is widely distributed in the arid and semiarid areas of South America and in all tropical regions (KING et al., 2009). In the last years, it has received special attention due to its high seed oil content and quality, which can be converted into biodiesel by the industry (ARRUDA et al., 2004; KUMAR; SHARMA, 2008).

Physic nut grows in environments with constraining conditions, such as reduced rainfall, high temperatures, poor soil conditions, where most of the agriculturally important plant species are not able to grow satisfactorily (FRANCIS et al., 2005). However, to achieve high yield levels, plant requires fertile soils and good physical conditions (KUMAR; SHARMA, 2008). According to Arruda et al. (2004), in acid soils with pH below 4.5, roots of physic nut do not grow. Thus, the acidity correction and soil fertility are critical for success and profitability in this crop (LAVIOLA; DIAS, 2008; SOUZA et al., 2011; BALOTA et al., 2012). This finding becomes even more relevant because the main producing regions of physic nut in Brazil are located in acid soils, characterized by low base saturation and high aluminum ( $Al^{3+}$ ) levels, sufficient to alter the seed germination and normal growth of plants.

Aluminum toxicity is considered one of the main factors limiting plant growth in acidic soils of tropical regions, mainly by inhibiting root growth (GIANNAKOULA et al., 2008). In Brazil, toxic Al levels are present in 60% of areas with agricultural potential (SÁNCHEZ; SALINAS, 1981). Thus, knowledge and selection of species less susceptible to the deleterious effects of Al is an alternative to the deployment of these crops in agricultural areas with these fertility conditions.

Several researches have been conducted using nutrient solution to determine the perennial species' tolerance to Al (BRACCINI et al., 1998; TECCHIO et al., 2006; STOLF et al., 2008; MATTIELLO et al., 2008; NAING et al., 2009; MACEDO et al., 2011; STEINER et al., 2012; LANA et al., 2013). However, there are no studies that evaluated the effects of Al on the physic nut seed germination. Aluminum toxicity manifests initially by reduction of the root elongation rate after contact with the solution containing Al (HARTWIG et al., 2007), and drastic reduction in the plant shoot growth (BEUTLER et al., 2001). Phytotoxic effects of Al on roots include reduction in the dry matter yield, number and length of lateral roots, and root area, which are often associated with increases in the mean root diameter and root volume (BARCELÓ; POSCHENRIEDER, 2002; HARTWIG et al., 2007).

Seed germination is a critical stage in the history of plants and Al tolerance during germination is crucial for the establishment of plants that grow in acid soils. This research was carried out to investigate the effects of different aluminum levels on seed germination and initial growth of physic nut (*Jatropha curcas* L.) seedlings, using the solution-paper method.

## MATERIAL AND METHODS

The experiment was conducted at the Seed Analysis Laboratory of the Department of Agronomy, Integrated College of Ourinhos (FIO), located in the city of Ourinhos, São Paulo State (SP), Brazil (24°55'20" S, 49°54'24" W, and altitude of 480 m). Physic nut seeds, based on homogeneous size and weight, were surface sterilized for 5 minutes with sodium hypochlorite solution (2%, v/v). Afterwards, seeds were subjected to five levels of aluminum [0 (control), 20, 40, 60 and 80 mg L<sup>-1</sup> of Al], using the solution-paper method. Aluminum solution was prepared using aluminum sulfate [Al<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub>.16H<sub>2</sub>O].

The experimental design was completely randomized with eight replications. The experimental unit consisted in a germitest paper roll with 25 seeds of physic nut.

*Germination test:* 25 seeds were distributed in two sheets of germitest paper, properly moistened with the aluminum solution of each treatment, in a volume equivalent to 2.7 times the weight of the dry paper. Then, the seeds were covered with a third sheet of paper and rolled up. The eight rolls, corresponding to the replicates, were grouped by treatment and then placed in the germinator at 25 °C in an upright position to germinate. Evaluations were performed at 7 (first count of germination test) and 12 days (total germination percentage) after the test installation as previously described by Oliveira et al. (2014). The results expressed in percentage of normal seedlings, according to the recommendations of Seed Analysis Rules - RAS (BRASIL, 2009).

*Shoot and root length:* the shoot and root length was measured in 10 normal seedlings randomly obtained after count of the total germination (14 days) using meter scale. The results were expressed in centimeter (cm).

*Shoot and root dry matter:* 10 normal seedlings randomly obtained at 14 days were separated into shoot and roots. The plant parts were removed carefully and washed with deionized water, dried in a forced air circulation oven for four days at 65 °C, and then weighed. The results were expressed in mg seedling<sup>-1</sup>.

Data were submitted to analysis of polynomial regression, and significant equations ( $p \leq 0.05$ ) with the higher coefficient of determination were adjusted. All analyses were performed using SigmaPlot 11.0 software for Windows (Systat Software, Inc., San Jose, CA, USA).

## RESULTS AND DISCUSSION

The first count of germination test (7 days) and total germination percentage (12 days) of physic nut seeds decreased progressively with increasing Al concentrations (Figure 1). First count of germination test reduced from 45.8% in the control treatment (aluminum-free) to minimum of 9.4% when the seeds were exposed to 80 mg L<sup>-1</sup> of Al, indicating mean reduction of 79% (Figure 1A). Total germination percentage reduced from 73.0% in the Al-free control to minimum of 7.2% when the seeds were exposed to 80 mg L<sup>-1</sup> of Al, indicating mean reduction of 90% (Figure 1B). When the seeds were exposed to high concentrations of Al, practically there was no condition for seed germination. These data suggest that physic nut is a susceptible species to the deleterious effects of high Al concentrations during the seed germination process. Seed germination is a critical stage in the life cycle of plants. This is a determining factor for the success of plant growth in acid soils. Therefore, the establishment of physic nut in agricultural areas with high concentrations of water-soluble and exchangeable Al should be avoided. However, it is emphasized that the Al concentrations used in this study are higher than those normally found in the solution of acid soils. In the majority of acid soils,

the concentration of water-soluble Al is less than  $10 \text{ mg L}^{-1}$  or  $0.11 \text{ cmol}_c \text{ L}^{-1}$ . According to MacLeod and Jackson (1967), the soluble Al concentration ranging from  $3.5$  to  $5.0 \text{ mg L}^{-1}$  is sufficient to severely restrict growth of several agricultural crops.

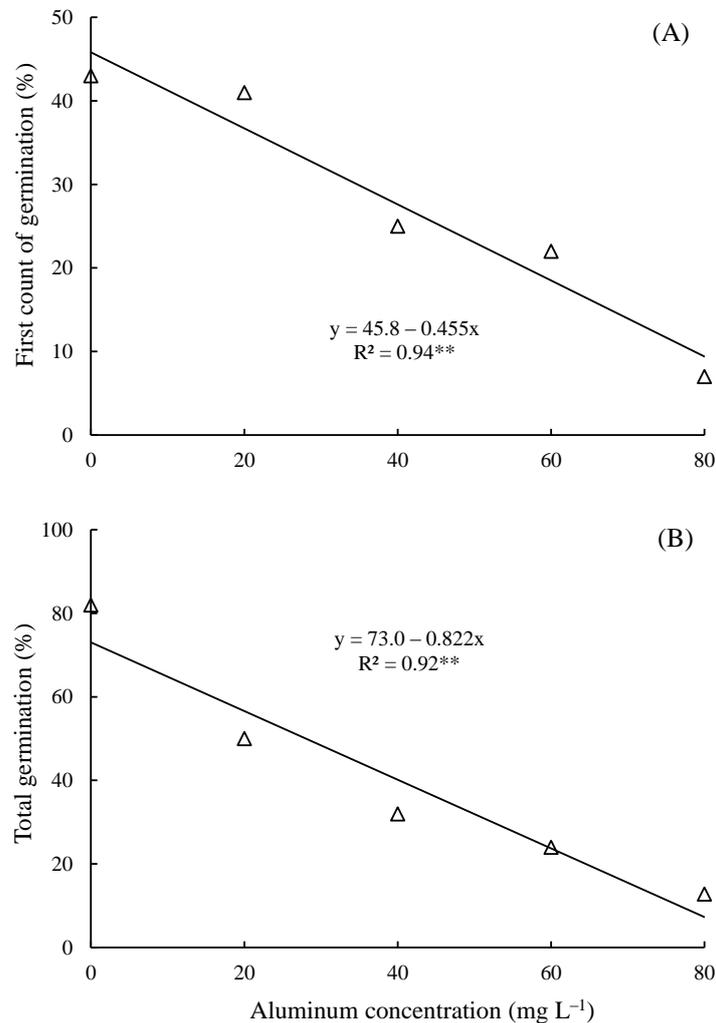


Figure 1. Effects of the aluminum solution concentration in the first count of germination test – 7 days (A) and total germination percentage – 12 days (B) of physic nut (*Jatropha curcas* L.) seeds. Data refer to mean values (n = 8). \*\*: statistical significance at 1%.

The growth of physic nut seedlings was negatively affected by the Al levels (Figure 2). The shoot length (Figure 2A) and shoot dry matter (Figure 2C) of physic nut seedlings decreased progressively with increasing Al solution concentrations. Shoot length reduced from  $14.9 \text{ cm}$  in the Al-free control to minimum of  $11.9 \text{ cm}$  with the addition of  $80 \text{ mg L}^{-1}$  of Al, indicating mean reduction of 20% (Figure 2A). Shoot dry matter reduced from  $72.8 \text{ mg seedling}^{-1}$  in the Al-free control to minimum of  $51.2 \text{ mg seedling}^{-1}$  when the seedlings were exposed to  $80 \text{ mg L}^{-1}$  of Al, indicating mean reduction of 30% (Figure 2C). Steiner et al. (2012) found that the addition of  $40 \text{ mg L}^{-1}$  of Al reduced plant height of physic nut at 54% compared to the Al-free control. In grapevine, Tecchio et al. (2006) found decreases of 81 and 85% for the plant height and shoot dry matter, respectively, when the plants were exposed to  $30 \text{ mg L}^{-1}$  of Al. Phytotoxic effects of this metal on the initial growth of physic nut seedlings reflected in lower shoot growth, especially in higher Al levels. Among the effects of Al on

plant shoot the reduction in the plant height, leaf area and dry matter yield are the most commonly reported (LANA et al., 2013).

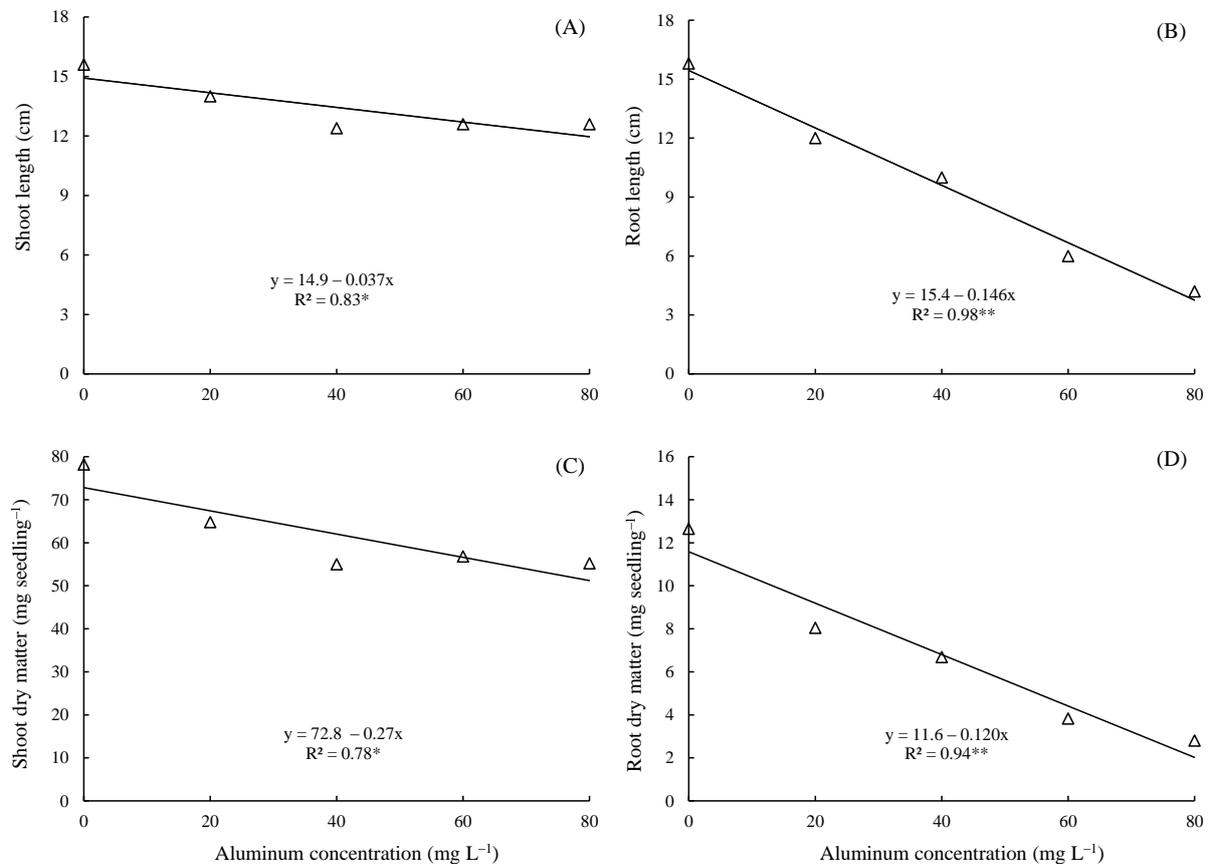


Figure 2. Effects of the aluminum solution concentration on the shoot length (A), root length (B), shoot dry matter (C) and root dry matter (D) of physic nut (*Jatropha curcas* L.) seedlings. Data refer to mean values (n = 8). \* and \*\*: statistical significance at 5% and 1%, respectively.

The root length (Figure 2B) and root dry matter (Figure 2D) of physic nut seedlings decreased progressively with increasing of Al concentrations. Root length reduced from 15.4 cm in the Al-free control to minimum of 3.7 cm with the addition of 80 mg L<sup>-1</sup> of Al, indicating mean reduction of 76% (Figure 2B). Root dry matter reduced from 11.6 mg seedling<sup>-1</sup> in the Al-free control to minimum of 2.0 mg seedling<sup>-1</sup> in the concentration of 80 mg L<sup>-1</sup> Al, indicating mean reduction of 83% (Figure 2D). When the plants were exposed to high concentrations of Al, practically there was no root growth. The main phytotoxic effect of Al is the inhibition of root growth, where roots become short and thick (BARCELÓ; POSCHENRIEDER, 2002). Other deleterious effects of Al on roots include reduction in the dry matter yield, number and length of lateral roots, and root area, which are often associated with increases in the mean root diameter and root volume (BARCELÓ; POSCHENRIEDER, 2002; HARTWIG et al., 2007).

In coffee plants, Braccini et al. (1998) reported that the percentage reduction in the dry matter of shoots and roots were more appropriate characteristics to classify the coffee genotypes according to Al tolerance. These authors found that when the reduction in the dry matter of shoots and roots was greater than 40%, the genotype in question was classified as

sensitive to Al. Based on these data and the results presented here, we infer that the genotype of physic nut used in this study is sensitive to Al toxicity.

In general, the deleterious effects of Al were most evident in the roots and can be attributed to the low mobility of this metal in the plant (GIAVENO et al., 2001). The damage to the structure of roots – increase in diameter and decrease in the permeability of root cells – accentuates the phytotoxic effects of Al on the root system (BARCELÓ; POSCHENRIEDER, 2002). The effects of Al on roots are well documented in the literature, and the reduction in root growth (elongation and cell division) of susceptible species has been considered the main effect of toxic levels of Al (EPSTEIN; BLOOM, 2006). Consistent with this interpretation, Samac and Tesfaye (2003) found that the primary site of the toxic action of Al is the distal part of the transition zone at the roots' apex, where the cells are entering the elongation phase. Inhibition of root growth is the most visible symptom of Al toxicity in plants (SAMAC; TESFAYE, 2003; HARTWIG et al., 2007).

## CONCLUSIONS

Seed germination and initial growth of physic nut seedlings was drastically reduced by the addition of high concentrations of aluminum.

Phytotoxic effects of aluminum is more accentuated on the root growth than on shoot growth of physic nut seedlings.

The physic nut is a susceptible species to high concentrations of aluminum during the phase of seed germination and early growth.

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