Pigeon pea initial growth under different concentrations of aluminum and commercial substrate

Gustavo Araújo Rodrigues[1], Breno de Jesus Pereira[2], Anacleto Ranulfo dos Santos[1], Francielle Medeiros Costa[1], Gilvanda Leão dos Anjos[1]

[1] Universidade Federal do Recôncavo da Bahia, Campus Cruz das Almas, Cruz das Almas, Bahia, Brasil. E-mail: r.gustavoaraujo@gmail.com, anacleto@ufrb.edu.br, fran-eng@hotmail.com, gilvandas218s2@hotmail.com

[2] Universidade Estadual Paulista, Campus Jaboticabal, Jaboticabal, São Paulo, Brasil. E-mail: brenojp93@gmail.com

ABSTRACT

The incorporation of organic substrate into the soil can reduce aluminum toxicity in pigeon pea (*Cajanus cajan* (L.) Millsp.) plants, making it a viable alternative for use by small farmers in acidic soil regions such as the northeast semi-arid regions of Brazil that contain toxic aluminum in the soil. The aim of this study was to evaluate the effect of increasing aluminum doses and different commercial substrate concentrations on the initial growth of the pigeon pea. The experiment was undertaken in a greenhouse using a completely randomized design with a 5×3 factorial scheme. The first factor consisted of five doses of aluminum (0, 13.5, 27, 54, and 108 mg L^{-1}) and the second factor consisted of three percentages of commercial substrate Vivato Slim Pro® (0%, 10%, and 20%), with five replicates per treatment, totaling 60 experimental units. The variables evaluated were plant height; total chlorophyll; shoot, root, and total dry matter; and root volume. The use of the commercial substrate attenuated the aluminum toxicity and favored the initial growth of pigeon pea plants, regardless of the concentration used. Thus, the aluminum was detrimental to the initial plant growth at all tested concentrations and showed more pronounced signs of toxicity on the root volume.

Keywords: *Cajanus cajan*, acid soils, toxicity.

Crescimento inicial do feijão guandu submetido a diferentes concentrações de alumínio e substrato comercial

RESUMO

A incorporação de substratos orgânicos no solo pode atenuar a toxidade causada pelo alumínio em plantas de feijão guandu (*Cajanus cajan* (L.) Millsp.), configurando-se numa alternativa viável a ser utilizada por pequenos agricultores em regiões como o Nordeste, que apresenta solos ácidos com presença de alumínio tóxico. Assim, o objetivo deste estudo foi avaliar o efeito de doses crescentes de alumínio e diferentes concentrações de substrato comercial sobre o crescimento inicial do feijão guandu. O experimento foi conduzido em casa de vegetação e organizado em delineamento inteiramente casualizado, em esquema fatorial 5×3. O primeiro fator consistiu em cinco doses de alumínio (0; 13,5; 27; 54 e 108 mg L^{-1}) e o segundo fator em três porcentagens de substrato comercial Vivato Slim Pro® (0%, 10% e 20%), com cinco repetições por tratamento, totalizando 60 unidades experimentais. Procedeu-se à análise das seguintes variáveis: altura da planta, clorofila total, massa seca da raiz, massa seca da parte aérea, massa seca total e volume da raiz. A utilização do substrato comercial atenuou o efeito tóxico do alumínio e favoreceu o crescimento inicial das plantas de feijão guandu, independentemente da concentração utilizada. A presença do alumínio foi prejudicial ao crescimento inicial das plantas em todas as concentrações testadas, apresentando sinais de toxidez mais pronunciados sobre o volume das raízes.

Palavras-chave: *Cajanus cajan,* solos ácidos, toxidade.

1. Introduction

Aluminum (Al^{3+}) toxicity is one of the most limiting factors for crop production. (Haridasan, 2008; Spehar and Souza, 2006), with approximately 40%–50% of arable soils in tropical and subtropical regions being acidic (Kochian et al., 2015; Jesus et al., 2016). Soil acidification is a natural process that occurs by H^+ liberation and consequent dissolution of toxic Al^{3+} species to soil solution (Rahman et al., 2018). The chemical alterations caused by this metal are one of the main factors that produce low crop productivity (Haridasan, 2008; Rahman et al., 2018) because the presence of Al^{3+} in soil solutions inhibits root elongation, reduces water and nutrient absorption, and negatively affects the biomass production of plants (Choudhary and Singh, 2011; Kopittke et al., 2015).

The pigeon pea (*Cajanus cajan* (L.) Millsp.) is a leguminous plant with a high potential of grain production that is found in tropical and subtropical countries (Marin et al., 2004) and is grown in approximately 7 million hectares worldwide (FAOSTAT, 2018). The pigeon pea is a multipurpose plant that can be used in human and animal alimentation, as a forage crop, and in degraded soil recuperation. It can also be used as a green fertilizer via nitrogen symbiotic fixing (Azevedo et al., 2007; Sri et al., 2016).

In Brazil, pigeon pea cultivation occurs mainly through agriculture, in the northeast semi-arid regions, where farmers only have access to limited technology and inputs (Santos et al., 2000; Azevedo et al., 2008). The pigeon plant is an important source of income for the farmers in this region, with it being adapted and tolerant of the climatic conditions in these regions (Farias et al., 2013) as well as to the acidic soils that contain toxic aluminum (Singh et al., 2011).

The incorporation of organic matter into soils can attenuate the toxicity caused by aluminum in plants via Al^{3+} complexation with organic acids, converting them into stable and nontoxic molecules (Haynes and Mokolobate, 2001; Pavinato and Rosolem, 2008). Organic substrate use during pigeon pea growth could represent a viable alternative for use by small farmers, with the aim to reduce aluminum toxicity in soil acid regions, which are characteristic in some northeast regions of Brazil. Therefore, the hypothesis for the present study was that the use of commercial substrate (CS) would reduce the aluminum toxicity during the initial growth of the pigeon pea plant.

Thus, the aim of the present study was to evaluate the effect of increasing aluminum doses and different commercial organic substrate concentrations on the initial growth of the pigeon pea.

2. Material and Methods

The experiment was performed from December 2017 to January 2018 in a greenhouse at the Soil and Plant Mineral Nutrition sector, at the Federal University of Recôncavo of Bahia (12° 39′ 32″ S; 39° 5′ 9″ W), located in Cruz das Almas City, Bahia State, Brazil.

The treatments were performed using a completely randomized design, in a 5 × 3 factorial scheme. The first factor was five doses of aluminum (0, 13.5, 27, 54, and 108 mg L^{-1} $AlCl_3 \cdot 6H_2O$) and the second factor was three concentrations of the organic substrate Vivato Slim Pro® (0%, 10%, and 20%). There were five replicates per treatment, totaling 60 experimental units.

For seedling formation, *C. cajan* cultivar IAPAR 43 seeds were sown in plastic trays containing washed sand. The trays were arranged on benches in an arched-roof greenhouse covered with a transparent, low-density polyethylene film and anti-aphid screens along the sides.

At 15 days after sowing, when the first permanent leaf pair had formed, the seedlings were transplanted to 3.0 dm^3 pots and filled with washed sand and the CS Vivato Slim Pro® in the proportions previously described. The substrate was composed of pine bark, fine charcoal, water, and phenolic foam. The additive fertilizer and limestone content that were present in the substrate were 1.20% and 0.20%, respectively, and the pH and C.E (mS/cm) values were 5.6 and 1.2, respectively. Each pot represented one experimental parcel, with one plant transplanted per pot.

The nutritional requirements of the plants were supplied by the application of Hoagland and Arnon (1950) nutritive solution, modified according to the Al^{3+} treatments. For the first seven days after transplanting, only a dilute 50% solution was applied to establish the seedlings. The Al^{3+} source that was used was a hexahydrate aluminum trichloride solution ($AlCl_3$ $6H_2O$). It was applied once, one week after the seedling establishment period, at the treatment concentrations previously described. The plants were manually irrigated daily using a watering can.

At 35 days after transplanting, the following variables were analyzed: plant height (cm), total chlorophyll content (ICF), root dry weight (g), shoot dry weight (g), total dry weight (g), and root volume (cm^3).

A millimeter ruler was used to measure the plant height (by measuring the distance from the neck to the apex of the plants). The total chlorophyll content was determined using a Clorofilog CFL 1030 instrument. The root and shoot dry weights were determined after drying the material in an oven at 65°C and then weighing the roots and shoots on an electronic analytical balance to an accuracy of 0.001 g.

Total dry weight was obtained by summing the shoot and root dry weights. Root volume was determined by the volume difference observed after root immersion in a graduated cylinder.

The data were subjected to analysis of variance via the F test using the R statistical program (R Core Team, 2015). For significant variables, a compare means test was used (Tukey at 5% probability level) to verify the effect of the substrate. The effects of the different aluminum doses were analyzed using polynomial regression. A model that used the highest R^2 values was adopted and the results were graphed using SigmaPlot software.

3. Results and Discussion

There was a significant interaction between the Al^{3+} doses and CS concentrations on the total chlorophyll content of *C. cajan* plants. However, there were only isolated effects of each factor on plant height, root volume (RV), shoot dry weight (SDW), root dry weight (RDW), and total dry weight (TDW).

Verifying the interactive effect of the CS concentrations at each Al^{3+} dose on the variable interaction, the total chlorophyll content in the plants grown in the 10% CS treatment was significantly higher than those grown in the treatment without CS (0%) for all tested Al^{3+} doses. However, when the 20% CS treatment was applied, the total chlorophyll content was higher only for the 0, 13.5, and 54 mg L^{-1} Al^{3+} compared to the 0% CS treatment (Table 1). Therefore, the use of CS, in the studied concentrations, attenuated the harmful effects of aluminum on the plants, allowing greater chlorophyll biosynthesis (Rahman et al., 2018).

The interaction of Al^{3+} doses at each CS concentration showed that the increasing Al^{3+} doses resulted in an inverse quadratic regression curve adjustment for the total chlorophyll content of plants grown without CS (0%) and a negative linear line adjustment for the 20% CS treatment (Figure 1). Therefore, the increased aluminum doses were harmful to the total chlorophyll content of the plants, even though a CS had been used. Between 0 mg L^{-1} and the maximum dose (108 mg L^{-1}) of Al^{3+}, there was approximately 30% reduction in the total chlorophyll content of plants grown in the 20% CS treatment. There was no regression adjustment for total chlorophyll content in the 10% CS treatment (Figure 1).

Visual symptoms caused by Al^{3+} toxicity were observed in the leaves of *C. cajan* plants, with chlorosis occurring following necrosis on the tips of the oldest leaves. According to Pereira et al. (2006), aluminum may cause oxidative damage by inhibiting aminolevulinic dehydratase acid, which is important for chlorophyll biosynthesis. This explains the decrease in the total chlorophyll content with the increase in Al^{3+} dose and the damage caused by the aluminum to the leaves of the plants.

Analysis of the isolated effect of the aluminum doses showed that there was an inverse quadratic regression curve for Al^{3+} doses on *C. cajan* plant height (Figure 2A). Plants grown with 54 mg L^{-1} of Al^{3+} had a decrease in growth of approximately 14% compared to the treatment without Al^{3+}. Therefore, the decrease in plant height was associated with chlorophyll content reduction (Figure 1), which consequently reduced the photosynthetic activity of the plants. Al^{3+} toxicity can cause injuries during chloroplast formation and function, damaging the thylakoid membrane structure, and causing a decrease in the CO_2 assimilation rate (Konrad et al., 2005).

Regarding the effect of the different Al^{3+} doses on plant root volume, increasing the Al^{3+} dose resulted in a negative linear regression (Figure 2B). The Al^{3+} harmful effect on plant roots was more evident at the maximum dose (108 mg L^{-1}), decreasing approximately 42% of the root volume compared to the 0 mg L^{-1} Al^{3+} dose. The inhibition of the development of the root system is one of the most characterized symptoms of aluminum toxicity that has been described in the literature. This symptom occurs because of the direct inhibition of cell stretching, which is caused by Al^{3+} bonding at the rhizodermal cell wall and external cortex (Kopittke et al., 2015).

Table 1 – Mean values for the interaction between aluminum doses and commercial substrate concentrations (CS) on total chlorophyll content of *Cajanus cajan* plants [1].

Aluminum doses (mg L^{-1})	Total chlorophyll (ICF) [2]		
	20% CS	10% CS	0% CS
0	44.66 a	43.08 a	33.83 b
13.5	48.50 a	46.69 a	39.28 b
27	38.11 b	45.64 a	35.54 b
54	39.45 a	40.85 a	27.26 b
108	31.17 b	39.43 a	30.91 b

[1] Means followed by different letters in a line differ statistically according to Tukey's test at the 5% probability level.
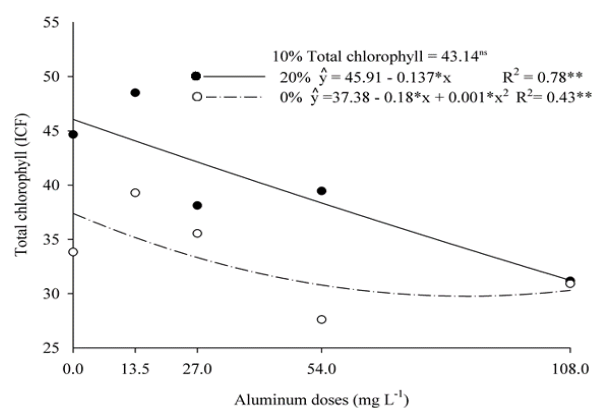[2] ICF = Falker Chlorophyll Index

Figure 1 – Regression of aluminum doses effect on total chlorophyll content (ICF) of *Cajanus cajan* plants at each commercial substrate concentration. ** = significant with 1% probability using the F test. ns = not significant via the F test.
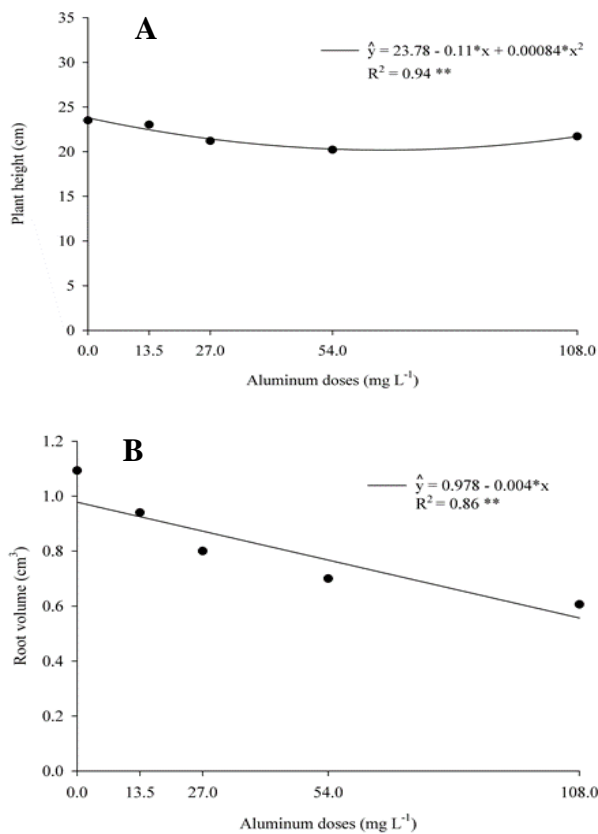
A

$$\hat{y} = 23.78 - 0.11*x + 0.00084*x^2$$
$$R^2 = 0.94 **$$

B

$$\hat{y} = 0.978 - 0.004*x$$
$$R^2 = 0.86 **$$

Figure 2 – Regression of the isolated effect of different aluminum doses on plant height (A) and root volume (B) of *Cajanus cajan* plants. ** = significant with 1% probability using the F test.

The results of the isolated effect of Al^{3+} doses on SDW, RDW, and TDW showed that there was an inverse quadratic regression curve for these three variables. The minimum values calculated showed that the lowest accumulation of SDW, RDW, and TDW occurred at 73, 92, and 82 mg L^{-1} of Al^{3+}, respectively (Figures 3A, 3B, and 3C). Therefore, at these concentrations, aluminum was more harmful to the accumulation of plant weight. The weight reduction of plants can result from the nutritional imbalance caused by the presence of Al^{3+}, which decreases the absorption and transport of plant essential elements, such as calcium and magnesium (Bhalerao and Prabhu, 2013).

The analysis of the isolated effect of CS concentrations showed that the plants grown in the 20% CS treatment were significantly taller and had a higher shoot dry weight and total dry weight than those grown in the 10% or without CS (0%) treatments (Table 2). The use of 20% CS led to increases of approximately 67%, 168%, and 138% for plant height, SDW, and TDW, respectively, compared to the treatment without CS (0%). For RDW and RV, the plants grown in the 10% and 20% CS treatments had values that were significantly higher than those without CS (0%) (Table 2).

According to Heynes and Mokolobate (2001), the positive results obtained from using CS are related to the reduction of toxic aluminum species (Al^{3+} and $AlOH^{2+}$) due to the complexation of those species by organic acids. These binders are generally organic acids that form carboxylic and phenolic functional groups that are capable of complexing aluminum at stable and nontoxic molecules (Franchini et al., 2001; Ebeling et al., 2008). Furthermore, the presence of limestone in the CS, even at low percentages, may have influenced the reduction of the aluminum toxic species.
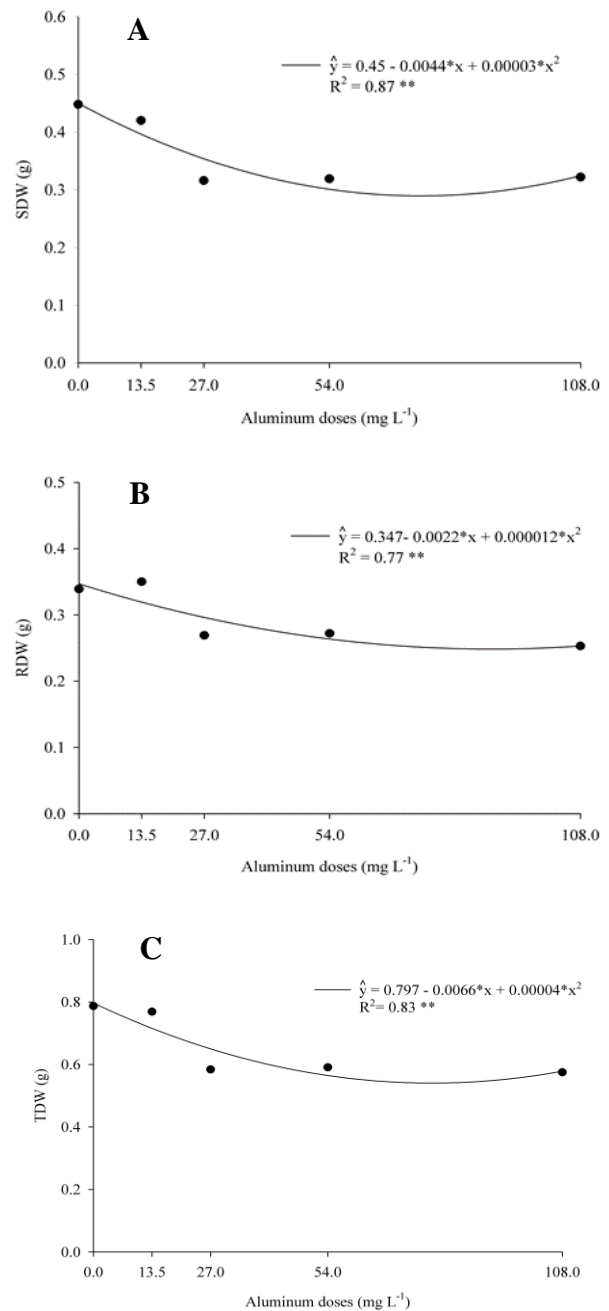

A

$$\hat{y} = 0.45 - 0.0044*x + 0.00003*x^2$$
$$R^2 = 0.87 **$$

B

$$\hat{y} = 0.347 - 0.0022*x + 0.000012*x^2$$
$$R^2 = 0.77 **$$

C

$$\hat{y} = 0.797 - 0.0066*x + 0.00004*x^2$$
$$R^2 = 0.83 **$$

Figure 3 – Regression of the isolated effect of different aluminum doses on shoot dry weight (SDW) (A), root dry weight (RDW) (B), and total dry weight (TDW) (C) of *Cajanus cajan* plants. ** = significant with 1% probability using the F test.

Table 2 – Mean values for the isolated effect of commercial substrate (CS) on plant height, shoot dry weight (SDW), root dry weight (RDW), total dry weight (TDW), and root volume of *Cajanus cajan* plants [1].

% CS	Plant height (cm)	SDW (g)	RDW (g)	TDW (g)	RV (g)
20%	26.12 a	0.51 a	0.35 a	0.86 a	0.94 a
10%	23.98 b	0.39 b	0.36 a	0.76 b	1.00 a
0%	15.66 c	0.19 c	0.17 b	0.36 c	0.52 b

[1] Mean followed by different letters in a column differ statistically according to Tukey's test at the 5% probability level.

4. Conclusions

The use of a CS reduced the toxic effect of aluminum and favored the initial growth of pigeon pea plants, independent of the concentration used.

The presence of aluminum was harmful to the initial growth of pigeon pea plants at all tested concentrations and showed more pronounced signs of toxicity on the root volume.

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