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Effects of Aluminum and pH on Germination of *Echium plantagineum* L.

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Abstract

The objetive of this study was to identify the effects of aluminum concentrations and pH levels of solution on germination of *Echium plantagineum* L. seeds. Three different experiments were carried out in completely randomized design with four repetitions. In the first experiment, we used aluminum sulfate solutions in concentrations of 0.0; 0.3; 0.6; 1.0; 2.0; 3.0; 4.0; 5.0; 6.0 and 7.0 $cmol_c L^{-1}$ and in the second experiment, solutions with different pH, 3.0; 4.0; 5.0; 6.0; 7.0; 8.0; 9.0 and 10.0. In the third experiment, we carried out a factorial (4x4) with aluminum sulfate solutions (0.0; 2.0; 4.0 and 6.0 $cmol_c L^{-1}$) and pH (4.0; 5.0; 6.0 and 7.0). It was evaluated the germination of *Echium plantagineum* L. at four and 14 days after seeding (DAS), germination speed index, primary root length, aerial part and dry mass of seedlings. The presence of aluminum reduced the germination by 27 and 40% at four and 14 DAS, respectively, in concentrations superior to 3.0 $cmol_c L^{-1}$. The three growth parameters presented linear reduction with the increase of aluminum concentrations. In the second experiment, the solutions with pH of 3.0 and 10.0 provided increases in germination, length of root and aerial part, and little influence in the dry mass of seedlings. In the third experiment, there was significant interaction between the aluminum concentrations and pH levels of substrate. The presence of aluminum in the substrate presented toxic effect on germination of seeds, length of seedlings and dry mass. The pH of the solution has little effect in germination of seeds and in the growth of seedlings of *E. plantagineum*.

Keywords: boraginaceae, purple flower, weed, substrate, phytotoxicity

1. Introduction

Among the weed plants of economic importance we can cite *Echium plantagineum* L., an annual plant of cold season from Boraginaceae family, known vulgarly as purple flower or echium oil. It is a species of herbaceous size, native from Western Mediterranean, Portugal and Spain, and North Africa, considered an important weed plant in Australia, South Africa, Canada, New Zealand and South America (Weston et al. 2012; Florentine et al. 2018). It is present in southern Brazil, causing damages in agricultural crops and cultivated pastures. It is considered an aggressive weed plant, presenting fast growth, tolerant to drought, with high capacity of competition for water and nutrients, adaptation to variations of temperature and photoperiod, elevated production of leaf area and seeds, dormancy and several fluxes of emergence during the year (Piggin, 1976; Forcella; Wood & Dillon, 1986; Sharma & Esler, 2008; Konarzewski; Murray & Godfree, 2012). *E. plantagineum* also has elevated phenotypic plasticity, demonstrated by the variation of morphological characteristics: height of plant, size and mass of seeds, triggered in response to the variations of precipitation and temperature (Sharma & Esler, 2008; Konarzewski; Murray & Godfree, 2012).

This species has scorpioid inflorescence, presenting flowers with upper ovary, bicarpellary, gamocarpellary and tetralocular (formation of a secondary wall in the ovary), with an ovule by locule and gynobasic gynoecium, which in maturation of fruits, these ones separate themselves being united by the base of style (Souza & Lorenzi, 2012). This type of gynoecium form fruits denominated carcerulus (indehiscent dry fruit and one-seeded), whose seed remains united to the fruit, being a unit of dissemination and propagation (Moreira & Bragança, 2010).

The germination of seeds is an ordered sequence of metabolic activities divided in phases that result in the formation of a seedling, which are directly influenced by environmental conditions (Bewley & Black, 1994; Yamashita & Guimarães, 2011). A lot of plants have reduced percentage of germination and emergence due to difficulties of soaking, dormancy of seeds or by interference of biotic or abiotic factors (Vivian et al. 2008; Yamashita; Guimarães & Cavenaghi, 2008; Yamashita & Guimarães, 2011; Luz et al. 2014). Nevertheless, seeds of many species of plants, such as of weed plants, are tolerant to adverse environmental conditions, remaining with reduced metabolic activity (dormant), having capacity of germinating after favorable environmental stimulus (Rajjou & Debeaujon, 2008; Mahmood et al. 2016).

Among the factors that influence the process of establishment and colonization of weed plants in determined environments, the characteristics of soil, such as the presence of aluminum and pH, play predominant role (Monquero et al. 2012). The aluminum is one of the main mineral constituents of soil, being dissolved in solution of soil mainly in the form of Al^{+3} ions, under conditions of acid pH and low content of organic matter, being toxic to the plants through the inhibition of growing of roots and accumulation in cell wall (Ezaki et al. 2008; Rodrigues et al. 2017). When the aluminum is absorbed by the roots of plants in its toxic form (Al^{+3} ions), occurs interference in the cell division of the root apices and, consequently, inhibition of the growth of primary and secondary roots (Ryan et al. 2011).

The germination of seeds of weed plants and the success in infestation of new environments can also be affected by soil pH. The response of plants to pH varies among the species, and many of them tolerate an interval of pH between 5.0 and 10.0, however, studies show that some weed plants can germinate outside of this range (Javaid & Tanveer, 2014). The tolerance to a broad range of pH is common in weed plant species, and it allows invading diverse environments (Mobli; Ghanbari & Rastgoo, 2018). Despite the fact that in soils with low pH and high concentration of aluminum it occurs germination of seeds, the aluminum can induce the formation of pectin compounds, resulting in the loss of elasticity of cells and interruption of cell division, evolving to the seedling death (Yamashita & Guimarães, 2011). This way, the ability of adaptation of weed plants to environments with variations of pH and aluminum changes according to the species, modifying also the population dynamics and, consequently, the capacity of competition (Monquero et al. 2012).

It is important to consider ecologic and agriculturally the effect of these ones on the germination and growth of seeds, once that the majority of the Brazilian soils have medium to high acidity (Monquero et al. 2012). Before that, the understanding of germination of seeds of weed plant species and the relation with environmental factors are important to know the ecological behavior of species, as well as the capacity of adaptation to new environments (Vivian et al. 2008; Florentine et al. 2018). Thus, allowing the establishment of efficient systematic strategies of management, seeking the reduction of the bank of seeds in soil in cultivated areas. In this context, the present study had as objective to investigate the effect of concentrations of aluminum and the levels of pH of the solution on the germination of *E. plantagineum* seeds.

2. Materials and Methods

The experimental work was conducted in Didactic and Seed Research Laboratory from Phytotechny Department, Rural Sciences Center (Laboratório Didático e de Pesquisa em Sementes do Departamento de Fitotecnia, Centro de Ciências Rurais) of Universidade Federal de Santa Maria, in Santa Maria, RS.

The fruits of *E. plantagineum* were collected manually in crop with history of infestation by this weed plant (±20 plants m^{-2}), located in the municipality of Restinga Seca, RS (29° 51' 29" South latitude and 53° 31' 41" West longitude and altitude 72 m). The collecting area is characterized by the soybean cultivation (*Glycine max*) in summer and ryegrass (*Lolium multiflorum*) in winter.

We collected only dark-colored fruits that could easily detach from the mother plant, having, apparently, physiological maturity. The same ones passed through a manual cleaning process, dried at the shade for five days, and stored in Kraft paper bags at room temperature and dry place (laboratory) until carrying out the experiments.

The experiments were conducted in germination chamber of Biochemical Oxygen Demand (B.O.D.) type at temperature of 20 °C and photoperiod of 24 hours of artificial light inside the chamber (30 μmol m^{-2} s^{-1} of illuminance) We used the completely randomized experimental design (RED) with four repetitions of 50 diaspores (carcerulus: concresced fruit with the seed) totaling 200 by treatment.

We carried out three experiments to evaluate the effect of aluminum and pH in germination of seeds. The tests were conducted in transparent acrylic boxes of gearbox type (11.0 x 11.0 x 3.5 cm), under three sheets of germitest paper, moistened in quantities 2.5 times the mass of dry paper (Brasil, 2009). In the first one, the germitest paper was moistened with aqueous solutions of aluminum sulfate ($Al_2(SO_4)_3$ 18 H_2O) (P.M.= 666), testing the concentrations 0.0; 0.3; 0.6; 1.0; 2.0; 3.0; 4.0; 5.0; 6.0 and 7.0 $cmol_c$ L^{-1}, adapted from Gallon (2015). In the second experiment, the germitest paper was moistened with distilled water adjusted with NaOH or HCl, both 0.1N, at different levels of pH: 3.0; 4.0; 5.0; 6.0; 7.0; 8.0; 9.0 and 10.0.

From these experiments, in which we evaluate the effect separated of aluminum and pH, we noted interference in germination of *E. plantagineum.* Before that, we carried out the third experiment studying the interaction of both, using the same seed lot of the previous experiments. This one, was organized in a 4 x 4 factorial form, being the first factor, concentrations of aluminum sulfate (0.0; 2.0; 4.0 and 6.0 $cmol_c$ L^{-1}) and the second, levels of pH of solution (4.0; 5.0; 6.0 and 7.0), according to procedure carried out in the previous experiments.

The analyzed variables were germination of seeds at four and 14 days after seeding (DAS) and germination speed index (GSI) during 14 days, considering germinated seed when occurred the protrusion of primary seed (≥ 2 mm) according to Bewley & Black (1994). The percentage of germination was determined according to the methodology described by Labouriau & Valadares (1976) and the GSI according to Maguire (1962). It was also evaluated the length of primary root, aerial part and dry mass of seedlings at 14 DAS according to Nakagawa (1999).

The data were submitted to analysis of variance by the F test ($p<0.05$) through the statistical program SISVAR (Ferreira, 2011). Before the analysis of variance, seeking to meet the assumptions of normality of errors and homogeneity of variances, for the variables in percentage, the data were transformed for arc-sine $\sqrt{\%/100}$ and the lengths of root of first and second experiment for $\sqrt{x}$. For the variables evaluated in the first and second experiment we carried out analysis of polynomial regression and presentation of results obtained in graphics. In the third experiment, the deployment of interaction between the concentrations of aluminum and levels of pH, the averages were compared by the Scott-Knott test, in 0.05 of error probability.

3. Results and Discussion

In the first experiment, the results indicated that the germination of *E. plantagineum* seeds was influenced by the concentrations of aluminum in solutions. Thus, insofar as it increased the concentrations of aluminum, the germination at 4 and 14 DAS decreased, until the concentrations of 4.3 and 4.6 $cmol_c\ L^{-1}$, respectively, occurring adjustment of a second degree polynomial for the variables of germination at 4 and 14 DAS and GSI (Figure 1A and 1B). We observed that the presence of aluminum in the substrate interfered both in germination at four and at 14 DAS, having reduction of 27 and 40%, respectively, in concentration of 3.0 $cmol_c\ L^{-1}$ of aluminum, in comparison with the control without the presence of aluminum, whose values of germination found were 19 and 27% at four and 14 DAS, respectively (Figure 1A). As well as the germination, the GSI was also influenced by the concentrations of aluminum, occurring lower GSI (3.4) in concentration of 4.5 $cmol_c\ L^{-1}$, with reduction of 29% in comparison to the control (Figure 1B), increasing in the higher concentrations. This way, besides the aluminum reduces the germination, it also reduced the germination speed, demonstrating the toxic effect for the *E. plantagineum* seeds.

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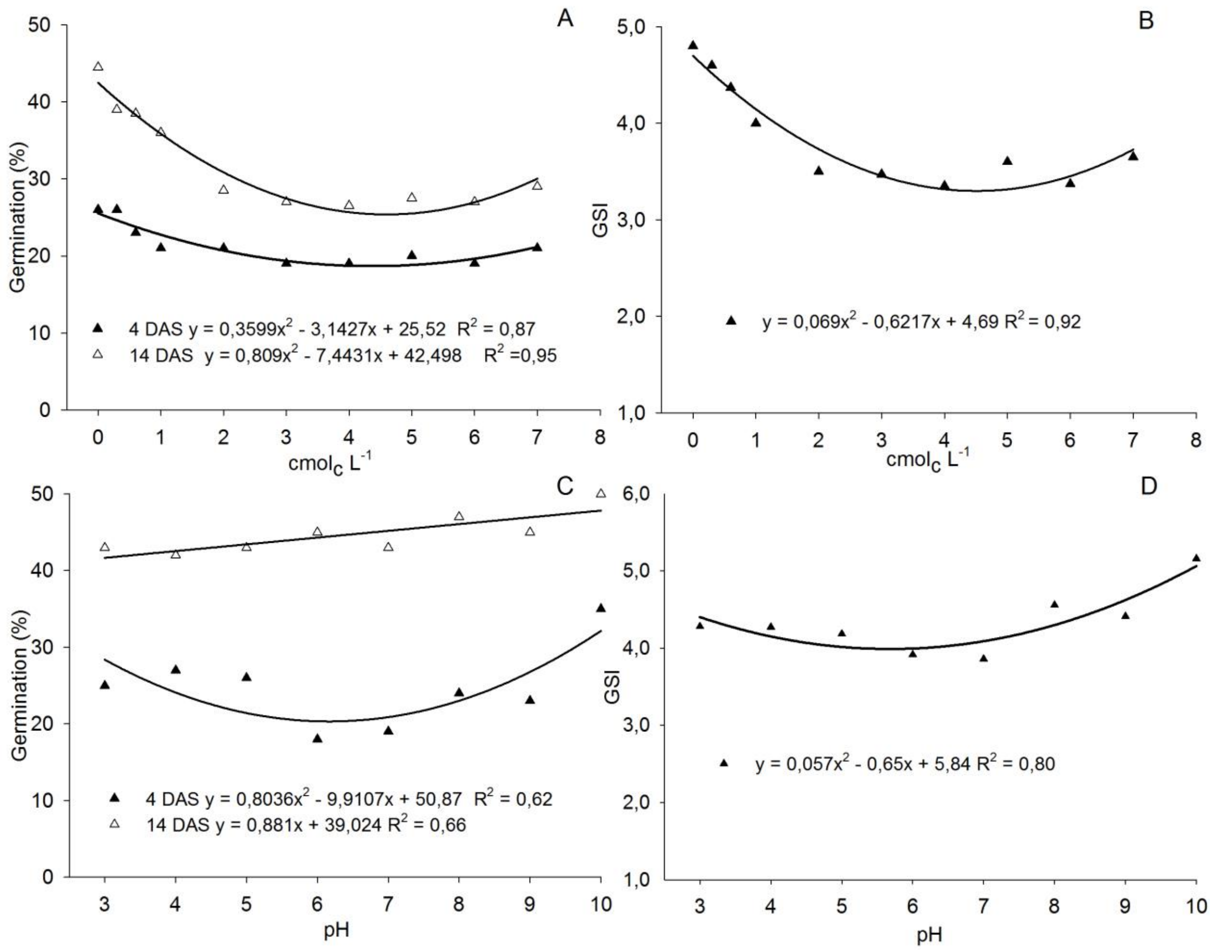

Figure 1. Germination at four and 14 DAS (days after seeding) and germination speed index (GSI) of seeds of *Echium plantagineum* exposed to concentrations (cmol$_c$ L^{-1}) of aluminum sulfate (A and B) and levels of pH (C and D)

Nevertheless, even with the reduction in germination by the toxic effect of aluminum, this was superior to 25%, and it can be considered elevated for a weed plant, demonstrating the capacity of adaptation of this to that adverse conditions (Zhao et al. 2017). Similar results were observed in *Conyza bonariensis* and *C. canadenses*, when the seeds were put to germinate in moistened substrate with solutions of aluminum from zero to two cmol$_c$ L^{-1}, occurring reduction of germination in concentrations superior to 1.5 cmol$_c$ L^{-1} (Yamashita & Guimarães, 2011). The aluminum also had toxic effect in other species, such as: *Conyza sumatrensis*, *Echinochloa crus-galli*, *Amaranthus plameri* and *Sonchus oleraceus*, (Ezaki et al. 2008), *Triticum aestivum* (Zhang et al. 2010), *Ricinus communis* (Silva et al. 2014), *Galianthe chodatiana*, *Borreria latifolia* and *Richardia brasiliensis* (Gallon, 2015), *Jatropha curcas* (Machado et al. 2015) and *Hancornia speciosa* (Rodrigues et al. 2017).

In the second experiment, different from aluminum, the germination of *E. plantagineum* seeds at 14 DAS presented direct linear effect; however, there was germination in pH of 3.0 to 10.0, indicating that the pH may not be a limiting factor for germination of this weed plant.

The germination at 14 DAS was superior to 40% in the studied pH range, occurring linear increase inside this range (Figure 1C). Yet, the germination at four DAS, resulted in greater sensitivity to pH near the neutrality (pH 6.0 and 7.0), presenting squared tendency, being the lower germination (18%) observed in pH 6.2. In contrast, the extreme values of pH presented greater germination at four DAS, with values near to 27 and 35%, for the lowest and highest studied pH, respectively (Figure 1C).

The results of GSI corroborated with the germination at four DAS, in which we observed lower GSI in pH 6.0, already in levels of basic pH, we verified higher GSI, demonstrating the preference of germination in basic pH, in which besides occurring greater germination, this one occurs in a faster way (Figure 1D). The capacity of germinating throughout a broad range of pH supports the premise that *E. plantagineum* is adapted to several conditions and types of soil, having capacity of invading various environments and present wide geographic distribution (Florentine et al. 2018). Similar results were found by Zhao et al. (2017) when working with different pH of solution in *Alopecurus aequalis* seeds, obtaining germination of 27% (lower) in pH 4.0 (acid) and above 95% in pH range from 7.0 to 10.0 (alkaline), suggesting the preference of this species to alkaline pH, but having capacity of adaptation to acid soils.

These results corroborated with the ones found by Florentine et al. (2018), in work performed with *E. plantagineum* seeds in Australia, in which the authors did not find significant effect of pH, in the range from 4.0 to 10.0, of substrate in germination, obtaining germination superior to 80%. These characteristics are common in many weed plant species, such as: *Emex spinosa* and *E. australis* (Javaid & Tanveer, 2014), *Galenia pubescens* (Mahmood et al. 2016), *Sophora alopecuroides* (Nosratti et al. 2017), *Parthenium hysterophorus* (Bajwa; Chauhan & Adkins, 2017) and *Alyssum linifolium* (Mobli; Ghanbari & Rastgoo, 2018).

The figure 2 shows the effect of aluminum in the length of primary root, aerial part (Figure 2A) and dry mass of seedlings (Figure 2B), observing linear decreases in these parameters. The root growth was the most impaired, with reduction of 74% in the length of roots in concentration of 7.0 $cmol_c\ L^{-1}$ in comparison to control. Similar results occurred for the length of aerial part and dry mass of seedlings, with reduction of 63 and 26%, respectively, in greater concentration (7.0 $cmol_c\ L^{-1}$) in relation to control.

The adjustment of linear model for length of root and aerial part (Figure 2C), and squared for dry mass of seedlings (Figure 2D) was significant in function of the levels of pH. The acid pH (3.0) of solution was harmful to the length of primary root and aerial part, yet in basic pH (10.0) we observed increase of 44 and 41% in these parameters, respectively, in comparison to pH 3.0. Similar results occurred for dry mass of seedling, however with increment of only 28% of pH 5.4 to 10.0. Nevertheless, even occurring influence of pH in the length of root and aerial part and dry mass of seedlings, the same ones presented values superior to 0.8 cm and 1.5 mg pl^{-1}, respectively, not being observed mortality of seedlings. This way, in all levels of studied pH occurred formation of normal seedlings, confirming the capacity of tolerance of this species to variation of pH. This can indicate that the pH of soil is not a limiting factor to germination and establishment of *E. plantagineum*, as well as the adjustment of pH of soil, in an isolated form, using liming, may not be an efficient strategy of suppression or stimulus of this weed plant (Florentine et al. 2018).

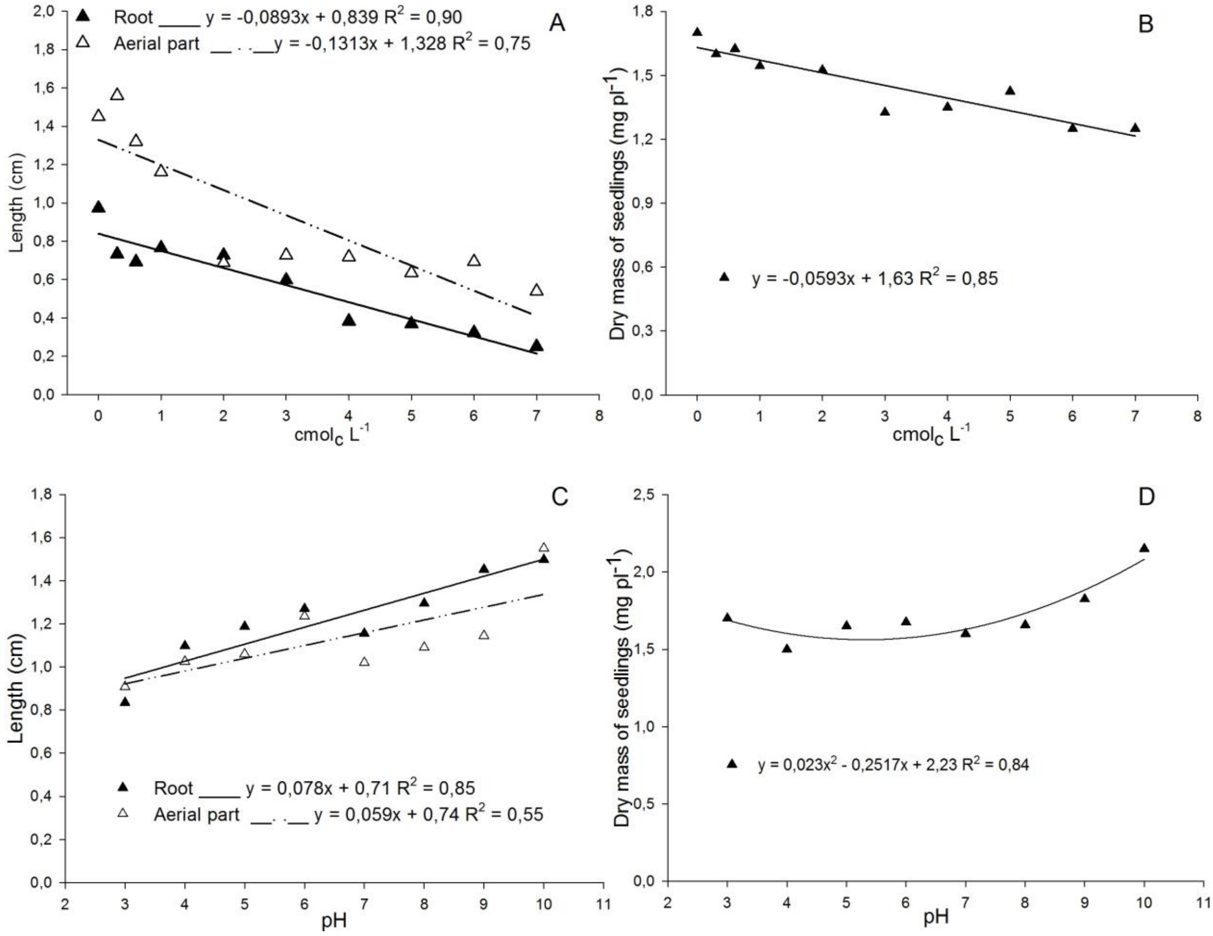

Figure 2. Length of root, aerial part (cm) and dry mass of seedlings (mg plant^{-1}), at 14 days after seeding, coming from seeds of *Echium plantagineum* exposed to concentrations (cmol$_c$ L^{-1}) of aluminum sulfate (A e B) and levels of pH (C and D)

Similar results were verified in the third experiment (Tables 1 and 2), in which there was significant interaction between pH and concentrations of aluminum in substrate for all the variables ($p<0.05$). We observed in general way (Table 1), greater germination at four DAS in absence of aluminum and in levels of pH 5.0 and 6.0, with 46 and 49%, respectively and in lower concentration of aluminum (2.0 cmol$_c$ L^{-1}) in levels of pH 4.0 and 5.0 with 46 and 50% of germination, respectively. At 14 DAS there was greater germination near 70%, in absence of aluminum, independent of pH and in lower concentration of aluminum (2.0 cmol$_c$ L^{-1}) in levels of pH 4.0; 5.0 and 6.0. Yet, the lowest percentage of germination (near to 25%) at four DAS, were obtained in the greatest studied pH (7.0) and in the concentrations of aluminum 4.0 and 6.0 cmol$_c$ L^{-1}. At 14 DAS there was lower germination, approximately 60%, in concentrations of aluminum 4.0 and 6.0 cmol$_c$ L^{-1} and levels of pH above 4.0.

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This way, with the increase of the concentrations of aluminum and levels of pH, there was decrease of germination. In the same way, it occurred for GSI, in which in the lowest concentration and absence of aluminum and in the lowest levels of pH presented greater GSI, near 8.0, indicating faster germination. Yet, in greater concentrations of aluminum and greater levels of pH, the GSI was lower, near 6.5, and we can associate the slow germination to the toxic effect of aluminum (Table 1).

Table 1. Germination at four and 14 DAS (days after seeding), germination speed index (GSI) and dry mass of seedlings (mg plant^{-1}) of seeds of *Echium plantagineum* exposed to concentrations of aluminum sulfate (cmol$_c$ L^{-1}) and levels of pH

	Concentrations of aluminum sulfate (cmol$_c$ L^{-1})							
	Germination 4 DAS (%)				Germination 14 DAS (%)			
pH	0.0	2.0	4.0	6.0	0.0	2.0	4.0	6.0
4.0	37 bC[1]	50 aA	43 aB	47 aA	70 ^{nsns}	71 a	66 a	68 a
5.0	46 aA	46 bA	34 bB	36 bB	69 A	68 aA	59 bB	60 bB
6.0	49 aA	43 bB	35 bC	33 bC	70 A	70 aA	60 bB	61 bB
7.0	37 bA	31 cB	26 cC	25 cC	71 A	59 bB	62 bB	59 bB
CV (%)	4.83				3.78			
pH	GSI				Dry mass (mg pl^{-1})			
4.0	7.4 bB	8.4 aA	7.7 aB	8.4 aA	1.6 ^{ns}A	1.6 bA	1.7 ^{ns}A	1.4 ^{ns}B
5.0	8.0 aA	8.2 aA	6.9 bB	6.7 bB	1.7 A	1.8 aA	1.4 B	1.2 C
6.0	8.5 aA	7.8 aB	6.8 bC	6.6 bC	1.8 A	1.5 bB	1.6 B	1.3 C
7.0	8.0 aA	6.4 bB	6.5 bB	6.4 bB	1.8 A	1.5 bB	1.5 B	1.3 C
CV (%)	6.41				9.95			

[1]Averages followed by different letters, lowercase in column and uppercase in line, differ among them by the Scott-knott test (p-valor<0,05); CV: coefficient of variation; ns: non-significant.

For the dry mass of seedlings (Table 1), we observed greater values, around 1.7 mg pl^{-1}, in absence of aluminum independent of pH and in the concentration of 2.0 $cmol_c$ L^{-1} of aluminum and pH 4.0 and 5.0 with 1.6 and 1.8 mg pl^{-1}, respectively.

Even occurring germination in all levels of pH and concentrations of aluminum, in a general way, there was lower growth of aerial part and root in the greatest concentrations of aluminum independent of pH, besides observing greater mortality of seedlings during the test (Table 2). We observed greater length of aerial part in absence of aluminum in levels of pH 5.0 and 7.0 with 1.46 and 1.39 cm, respectively, and in concentration of 2.0 $cmol_c$ L^{-1} of aluminum and pH 4.0; 6.0 and 7.0, with approximately 1.0 cm. Similar result occurred for the length of root, in which we observed the greatest values in absence of aluminum in the substrate, near 1.0 cm, independent of pH, reducing the length of root in the presence of aluminum.

Table 2. Length of aerial part (cm) and length of root (cm) at 14 days after seeding (DAS), coming from seeds of *Echium plantagineum* exposed to concentrations of aluminum sulfate ($cmol_c$ L^{-1}) and levels of pH

	Concentrations of aluminum sulfate ($cmol_c$ L^{-1})							
	Length of aerial part (cm)				Length of root (cm)			
pH	0.0	2.0	4.0	6.0	0.0	2.0	4.0	6.0
4.0	1.27 bA[1]	1.06 aB	0.68 cC	0.50 cD	1.49 aA	0.75 ^{ns}B	0.64 ^{ns}B	0.30 ^{ns}C
5.0	1.46 aA	0.88 bC	1.05 aB	0.76 bC	0.95 bA	0.63 B	0.56 B	0.49 B
6.0	0.92 cA	1.00 aA	0.93 bA	0.75 bB	1.35 aA	0.74 B	0.59 B	0.45 B
7.0	1.39 aA	1.02 aB	0.89 bB	0.93 aB	1.09 bA	0.51 B	0.53 B	0.44 B
CV (%)	9.99				21.12			

[1]Averages followed by different letters, lowercase in column and uppercase in line, differ among them by the Scott-knott test (p-value<0.05); CV: coefficent of variation; ns:non-significant.

This way, the aluminum presented itself toxic to germination and initial growth of *E. plantagineum*, because in concentrations superior to 2.0 $cmol_c$ L^{-1}, the growth of primary structures was compromised, presenting necrosis in the extremities of roots that evolved, in some cases, for the death of seedling. This can be due to alterations in division and/or impediment of elongation of cells from the embryonic axis, compromising the protrusion of primary root, occurring morphological abnormalities and of development (Ezaki et al. 2008; Rodrigues et al. 2017). Besides that, the toxic effect of aluminum results in loss of cell elasticity and oxidative stress, through the accumulation of reactive species to oxygen (ERO), as the superoxide anion (O_2) and hydrogen peroxide (H_2O_2) (Zhang et al. 2010). Nevertheless, even presenting toxic effect to germination and initial growth, approximately 50% of seeds presented germination and formation of seedling in concentrations superior to 2.0 $cmol_c$ L^{-1} of aluminum independent of the level of pH.

In literature, we find reports of many species of weed plants that presented tolerance to variations in concentration aluminum and pH (Javaid & Tanveer, 2014; Mahmood et al. 2016; Rodrigues et al. 2017; Florentine et al. 2018; Mobli; Ghanbari & Rastgoo, 2018). Similar results were observed in this study, in which *E. plantagineum* can present adaptive plasticity to variations of aluminum and pH. Nevertheless, the use of association of management strategies such as liming, direct planting, using of vegetal covering and chemical control can help in the control or minimize the infestation of *E. plantagineum*, contributing to reduce the bank of seeds of the soil.

4. Conclusion

The presence of aluminum in substrate had toxic effect on germination of seeds, length of primary root and aerial part, and dry mass of seedling of *E. plantagineum.*

The germination of seeds of *E. plantagineum* was little affected by the substrate pH, and this is not a limiting factor to germination.

Even with the toxic effect of aluminum in germination of seeds of *E. plantagineum* and initial growth of seedlings, more than 50% of seeds germinate and present formation of normal seedling, independent of the substrate pH.

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