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RESEARCH ARTICLE

ALUMINUM AFFECTS SEED GERMINATION AND INITIAL GROWTH OF *SCHINUSMOLLE* L. SEEDLINGS

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ABSTRACT

Aluminum is the most abundant metal in the earth's crust and its toxicity potentially limits the growth of plants grown in acid soils. The phytotoxic effects of aluminum on the seed germination and initial growth of *Schinus molle* seedlings were investigated in the present study. The treatments consisted of six aluminum concentrations (0, 25, 50, 100, 150 and 200 mg L^{-1}) in the form of chloride ($AlCl_3$) with four replicates of 12 seeds each. The majority of the evaluated variables responded in a negative quadratic form to Al concentrations. Our results indicate that intermediate Al concentrations tested were more harmful than the highest in this experiment for most of the evaluated variables, which shows that *S. molle* seeds have the potential to overcome the harmful effects of Al and to germinate in environments with high concentrations of this metal.

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INTRODUCTION

Aluminum (Al^{3+}) is the third most abundant element in the lithosphere, surpassed only by oxygen and silicon, and makes up 8% of the constitution of the earth's crust (Rossielo; JacobNeto, 2006), due tothe fact that most primary and secondary minerals ofrocks, formed by weathering, correspond to aluminosilicates (Miguel *et al.,* 2010). This large amount of aluminum in soils can become toxic to living organisms. Its toxicity is widely recognized due to the inhibition of more than 200 important biological functions, which, in turn, causes serious adverse effects on plants, animals and humans (Exley and Mold, 2015). The degree of toxicity depends not only on the total concentration of Al, but also on the chemical form in which it is available, which is determined mainly by the pH of the medium (Kisnierienė andLapeikaitė, 2015). In plants Al toxicity is most significantly reflected in root development and activity (Yu *et al.,* 2011), which occurs due to the interference of this metal in the mechanisms of acquisition and transport of water and nutrients, as well as to cytological changes (Sivaguru *et al.,* 1999; Vasconcelos Filho, 2014).

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As water and nutrient absorption is affected, plant growth can also be adversely affected (Vitorello *et al.,* 2005).Thus, seed germination is a critical stage for plants and Al tolerance is a crucial factor for the establishment of plants that grow in acid soils (Machado *et al.,* 2015). Metals are the soil contaminants posing the greatest environmental risk. The ecological costs attributed to soil problems are very highand the number of contaminated areas continues to increase. Among the alternatives for the recovery of contaminated soils, we highlight phytoremediation, by which plants and their associated microbiota remove, contain, transfer, stabilize or render harmless the metals present in the soil. At the end of the 20th century, the potential of plants to clean contaminated environments was identified, where *Thlaspicaerulescens* and *Viola calaminaria* were the first documented plant species to accumulate high levels of metals in the leaves (Lasat, 2000). Plants that exhibit rapid growth, high biomass production, competitiveness, vigor and pollution tolerance are generally preferred in a phytoremediation process (Lamego and Vidal, 2007). Thus, forest species stand out for this purpose, due to their highbiomass production, the wide root system and the high capacity to store metals compared to herbaceous species (Capuana, 2011). *Schinusmolle* L., popularly known as Peruvian pepper, American pepper, or Peruvian peppertree, belongs to the Anacardiaceaefamily and is a native species of South America and Mexico, but may also occur in regions of India, Africa and Australia as an exotic plant (Lim, 2012; Silva-Luz and Pirani, 2013). In Brazil, it occurs in the southernstates (Paraná, Rio Grande do Sul and Santa Catarina) in several types of plant formations (Silva-Luz and Pirani, 2013). Its success as a cultivated plant is attributed to its high tolerance to drought, high temperatures, competition for nutrients and light, as well as its high growth rate and biomass production (Demelash *et al.,* 2003; Iponga *et al.,* 2008). These ecological characteristics make the species of the genus *Schinus*more widely used in restoration programs for degraded areas, since they adaptto different environmental conditions. In addition, Doganlar *et al*. (2012) reported that *S. molle* is able to accumulate elevated levels of Cu, Zn and Pb in anthropic areas and they attributed this capacity to the physiological and genetic mechanisms of this species. However, to our knowledge, there is no research on the tolerance of *S. molle* to Al. Therefore, the aim of this study was to verify the pattern of germination behavior and initial growth of *S. molle* exposed to different Al concentrations.

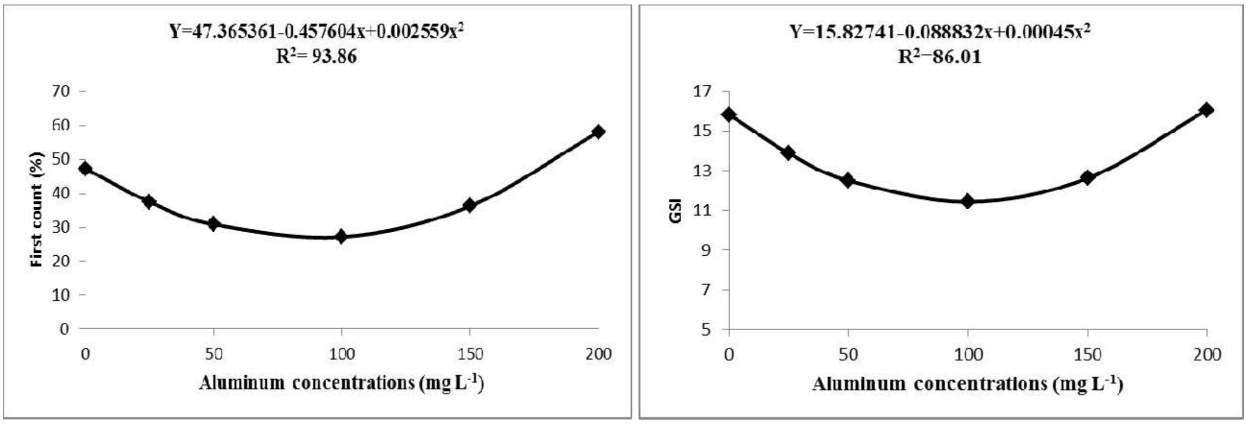
MATERIALS AND METHODS

The experiment was carried out at the Laboratory of the FepagroFlorestas Research Center in Santa Maria, RS, Brazil and is part of the project entitled "Fitorremediaçãomediadapor *Schinusmolle*napresençaouausência de fungosmicorrízicos e *Trichoderma* sp. emáreascontaminadasporalumínio". *Schinusmolle* seeds (lot 04/2014) belonging to the Renewable Forest Resources Bank, maintained at this research center, were used in this study. Amechanical scarification process was performedusing sandpaper 80 meshin a rotary drum for 150 seconds(as defined in previous tests); as well as a disinfestation with commercial hypochlorite (2.5%; v/v) and water at a 1:1ratio (v/v) plus two drops of liquid detergent per 100 mLof solution, for 5 minutes, followed by washing in distilled water. After that, the seeds were arranged on germitest paper in plastic boxes (gerbox - 11x11 x 3.5 cm).

The treatments consisted of six aluminum concentrations (0, 25, 50, 100, 150 and 200 mg L^{-1}) in the form of chloride ($AlCl_3$) with four replicates of 12 seeds each. Five milliliters of solution of the respective treatment were added to each replicate. The experiment was carried out in a growth room with a temperature of 25 °C ± 2, artificial photoperiod (16 hours light) and light intensity of 35 µmol m^{-2} s^{-1} obtained by cold white lamps. The treatment solutions were renewedevery four days, at a volume of 3 mL per gerbox. To evaluate thegermination potential, the germinated seeds were counted daily at the same time, during a period of 14 days, using root protrusion (2.0 mm) asthe germination criterion.The mean data from the first count (Percentage of germinated plants at 7th day) and accumulated germination (percentage of plants sprouted on the 14th day) were analyzed according to the Rules of Analysis of Seeds (Brazil, 2009); and the Germination Speed Index (GSI) was calculated using the formula: IVG = G1 / N1 + G2 / N2 + ... + Gn / Nn (Maguire, 1962), where G1, G2, Gn = number of seeds germinated at the first count, second count until the last count and N1, N2, Nn = number of days from sowing, first, second, until the last count. At 28 days of sowing, the number of seedlings was counted, the mean height of seedlings was measured using a millimeter ruler and the number of leaves per plantlet was counted. Aerial parts and roots were separated and the biomass was measured in an analytical scale; to obtain the dry weightdata the material was placed in a drying oven at 60 ± 3 ° C until reaching a constant mass. The experimental data were submitted to analysis of variance and when significant (P<0.05), regression analysis was performedwith the aid of the statistical program SISVAR 5.6 (Ferreira, 2011).

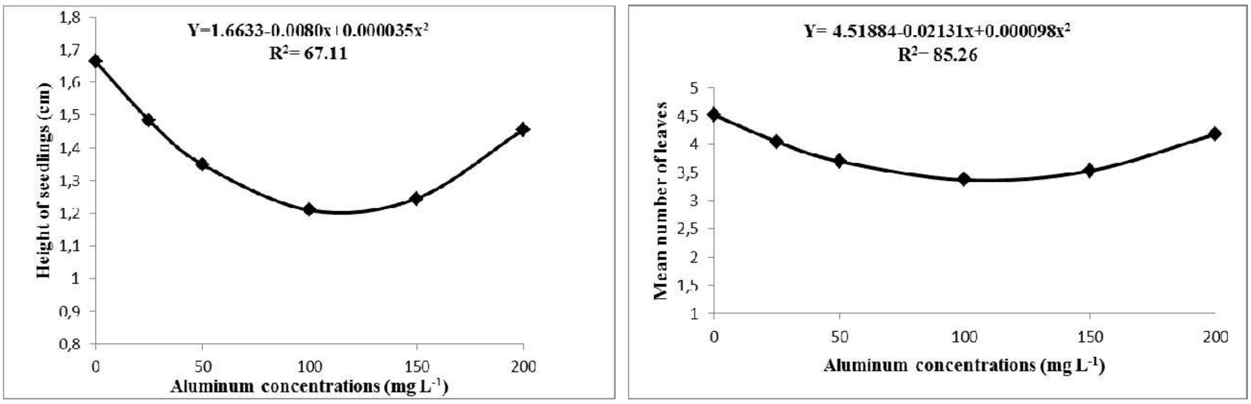
RESULTS AND DISCUSSION

Most of the evaluated variableswere affected by aluminum concentrations (Fig. 1, 2 and 3).Both the first count and germination speed index (GSI) presenteda negative quadratic response to Al treatments (Fig. 1A and 1B). Only accumulated germination was not significantly influenced by Al supply(data not shown). Seed germination is a complex process which begins with water absorption to activate enzyme proteins (Roshani *et al.,* 2014). The whole germination process is regulated by the hormonal equilibrium of the species and environmental conditions (Atici *et al*., 2005). Heavy metal stress is a major abiotic stress which can affect seed germination (Casierra-Posada *et al.,* 2009). Besides the effects on the reduction of water absorption, some studies have shown that heavy metalsinterfere with hormonal equilibrium balance, for example, decreasing endogenous cytokinins and GA_3 content in germinating seeds (Atici *et al*., 2005; Roshani *et al.,* 2014). There are few studies that investigate the effects of Al on seedgermination and results have beenvariable. Seed germination and initial growth of physic nut seedlings was drastically reduced by the addition of high concentrations of Al (Machado *et al.,* 2015). Differently, some studies showed that concentrations up to60 or 75 mg L^{-1} do not affect germination, vigor, or initial growth on onion and corn seeds, respectively (Stefanello *et al.,* 2016; Milane *et al.,* 2014). In our study, intermediate concentrations tested (50 to 150 mg L^{-1}) were more harmful than the highest ones for the first count and GSI. Although the influence of Al on the germination of some species is recognized, Lima and Copeland (1990), while working withdifferent germplasms of wheat, reported that seed germination was less sensitive to Al than seedling growth. The effects of Alon plant growth are observed primarily in the root system, particularly with interference in the elongation and division of meristematic cells (Rengel and Zhang, 2003; Kochian *et al.,* 2004; Sivaguru *et al.,* 2013). Consequently the absorption of water and nutrients is reduced, thereby reducing growth and plant biomass production (Inostroza-Blancheteau *et al.,* 2012). Similarly to that observed for the germination parameters, the variables of initial growth of *S. molle*seedlings alsopresented anegative quadraticresponse to Al treatments (Fig. 2A and 2B). For example, height of seedlings and meannumber of leaves were highly impaired by Al concentrations in a quadratic way.In relation to the height of the seedlings, the concentration of 100 mg L^{-1} of Al caused a decrease of about 30%, which was greater than that caused by higher Al concentrations (200 mg L^{-1}). Among the effects of Al on plant shoot, the reduction in plant height, leaf area and dry matter yield are the most commonly reported (Lana *et al.,* 2013). Evaluating the effects of aluminum concentrations on the initial growth of physic nut, Machado *et al*. (2015) observed lower shoot growth, especially athigher Al levels. Similarly Santos *et al*. (2010) reported significant reductions in root, shoot and total growth, number of leaves, and meandry weightof arugula plants at increasing concentrations of aluminum up to 60 mg L^{-1}. Moreover, dry weightof both roots and shoot significantly decreasedin response to Al concentrations in a quadratic way (Fig. 3A and 3B).



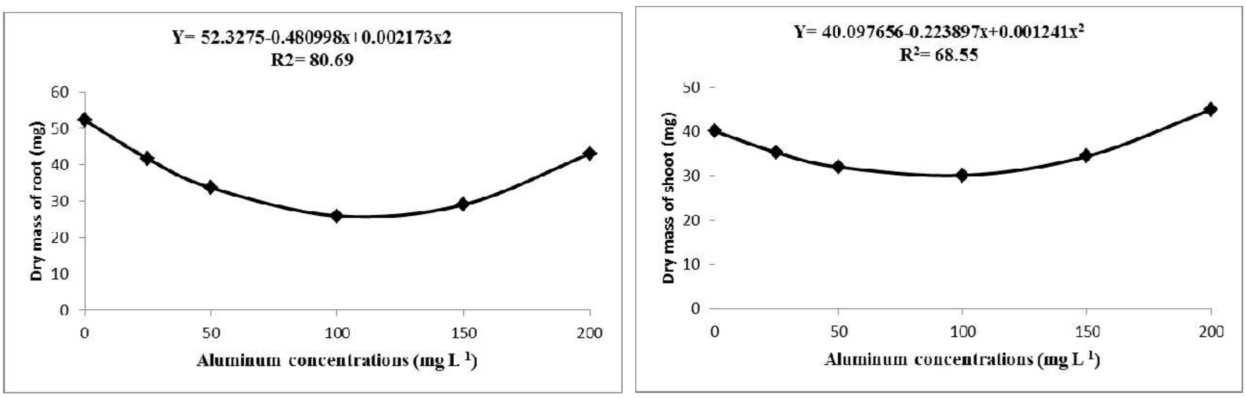
(A) (B)

Figure 1. First count (A) and Germination speed index (B) of *Schinusmolle* seed at different aluminum concentrations



(A) (B)

Figure 2. Height (A) and number ofleaves (B) of *Schinusmolle*seedlingsat different aluminum concentrations



(A) (B)

Figure 3. Dry weight of roots (A) and shoot (B) of *Schinusmolle*seedlings at different aluminum concentrations

The biomass responses are a reflection of the effects of Al on germination and initial seedling growth. Most of wheat varietiesanalyzed by Alamgir and Akhter (2009) presentedreduction of root and shoot dry mass under Al treatments, withthe most severe damage in the roots. These authors agree that in addition to other growth variables, biomass can also be a criterion for classifying varieties with respect to Al tolerance. Toxicity of Al and other metals occurs at varying levels and depends on the concentration, plant species, stage of development, period of exposure and the organ and tissue of the plant (Benavides *et al.,* 2005; Stefanello *et al.,* 2016). Thus, the negative quadratic response observed for almost all variables evaluated in this experiment may indicate the possible activation of some mechanism of tolerance to this metal in *S.molle* plants. It is possible that atcertain Al concentrations, some signaling route is triggered at this stage of growth, to relieve the negative effects of this metal. To prove this hypothesis further studies are needed. It is important to remember that the Al concentrations used in this study are higher than those normally found in the solution of acid soils.High concentrations are important since this test brings preliminary results of a larger project that seeks to reach conclusions in regarding phytoremediation.

Conclusion

Under the conditions and for the variables evaluated in this experiment, the intermediate concentrations of Al were more damaging than the highest ones, showing that *S. molle*seeds have the potential to overcome the harmful effects of Al and to germinate in environments with high concentrations of this metal.

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