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The Allelopathic Effect of Eucalyptus Leaf Extract on Grass Forage Seed[1]

Efeito Alelopático de Extrato Foliar de Eucalipto em Sementes de Gramíneas Forrageiras

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ABSTRACT - This study aimed to evaluate the allelopathic effect of various concentrations of an aqueous extract of eucalyptus leaves on *Urochloa decumbens* and *Panicum maximum* seeds. The extract was prepared from *Eucalyptus urograndis* leaves that were milled and mixed with distilled water in a 1:9 milled leaves: water ratio to obtain an extract with a defined concentration of 100%. In addition, dilutions of 50%, 25% and 12.5% were prepared, and a 0% dilution was used as a control. The experiment followed a completely randomized design, with four replicates, each of 50 seeds of *U. decumbens* and 50 seeds of *P. maximum*, arranged on filter paper moistened with each concentration of extract in a Gerbox plastic box. The results demonstrated the allelopathic potential of *E. urograndis* aqueous extracts applied to the seeds of *U. decumbens* and *P. maximum.* The 50% and 100% concentrations of leaf extract most strongly inhibited the germination, vigor and seedling growth of *U. decumbens* and *P. maximum*. The germination speed index and the root length were the characteristics that were most affected by the potentially allelopathic substances contained in the eucalyptus extracts at all concentrations.

Keywords: *Eucalyptus urograndis, Urochloa decumbens, Panicum maximum,* agroforestry system, germination, seedling performance.

RESUMO - Objetivou-se neste estudo avaliar o efeito alelopático de concentrações do extrato aquoso de folhas de eucalipto sobre sementes de ***Urochloa decumbens*** *e* ***Panicum maximum****. O extrato foi preparado utilizando folhas de* ***Eucalyptus urograndis*** *moídas, as quais foram colocadas em água destilada na proporção de 1:9, obtendo-se a concentração de 100% e as diluições de 50%, 25%, 12,5% e 0% (testemunha). O ensaio foi montado em delineamento inteiramente casualizado, com quatro repetições de 50 sementes de* ***U. decumbens*** *e* ***P. maximum****, dispostas sobre papel-filtro umedecido com as concentrações do extrato, em caixas do tipo gerbox. Os resultados indicam a existência de potencial alelopático de* ***E. urograndis*** *sobre as espécies* ***U. decumbens*** *e* ***P. maximum****. Efeitos mais pronunciados na inibição da germinação e redução do vigor e do crescimento de plântulas de* ***U. decumbens*** *e* ***P. maximum*** *foram observados nas concentrações de 50% e 100% do extrato foliar de eucalipto. O índice de velocidade de germinação e o comprimento da radícula foram as características mais afetadas pelas substâncias potencialmente alelopáticas do extrato de eucalipto, em todas as concentrações.*

Palavras-chave: *Eucalyptus urograndis, Urochloa decumbens, Panicum maximum,* sistemas agroflorestais, germinação, desempenho de plântulas.

INTRODUCTION

Agroforestry and silvopasture systems have been increasingly adopted in recent years as alternatives to traditional models of forestry and to facilitate the recovery and renewal of degraded pasture. These systems are attractive because they allow the amortization of the implementation costs through diversified production. These systems are considered to be multifunctional, as they allow the intensification of production through

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the integrated management of natural resources without environmental degradation (Porfírio da Silva, 2006). Furthermore, both systems can support forestry production combined with other products and according to the basic principles of sustainable management (Oliveira Neto et al., 2007).

The species of the genus *Eucalyptus* used in forestry are notable for their rapid growth and excellent adaptation to many types of environmental conditions and for the versatile uses of their wood (Oliveira Neto et al., 2010). The forage crops most widely used in agroforestry systems are species belonging to the genera *Urochloa* and *Panicum*. These species are preferred because of their moderate tolerance to shade, their traditional role in agriculture and their nutritional quality (Andrade et al., 2001).

Although eucalyptus is a rapidly growing tree, it is vulnerable to competition from weeds, primarily during the first two years after planting (Toledo et al., 2003). Thus, frequent weeding is required (Toledo et al., 2001). In monoculture, as well as in multifunctional systems, weed control is performed with chemical and mechanical methods, separately or in combination.

Urochloa decumbens and *Panicum maximum* are desirable components in agroforestry systems. However, these species can become problematic if they are not well managed. They can have significant impacts as weeds because they are highly aggressive and show strong competitive ability during the initial stages of growth of eucalyptus (Toledo et al., 2000; Cruz et al., 2010). Thus, appropriate management measures should be adopted to reduce the effects of competition among species.

A forestry practice widely used in integrated systems is artificial pruning. This practice is followed to improve stem form and wood quality. Moreover, it opens a route for solar radiation to reach the understory, where the forage plants occur. After pruning, the branches and leaves are allowed to remain among the rows of eucalyptus. It is believed that these materials can produce allelopathic effects on weeds or forage plants. Such effects may involve, e.g., the abnormal growth of the seedlings or the inhibition of seed germination. These allelopathic effects ensure that the development of the eucalyptus is free from interference.

Due to the complexity of these mixed systems, knowledge about the interactions of their components is essential, and allelopathy is an important factor that should be investigated. Allelopathy is defined as an inhibitory or beneficial effect, direct or indirect, of a plant on another plant through the production and exudation of chemical compounds (Rice, 1984).

Many previous studies have demonstrated the allelopathic effects of *Eucalyptus* spp. on various crops (Alves et al., 1999; Willis, 1999; Ferreira & Áquila, 2000; Ferreira et al., 2007; Yamagushi et al., 2011). However, there is no information about the allelopathic effects of *Eucalyptus* spp. on forage species, and studies of allelopathic interactions in agroforestry systems are lacking. Accordingly, this study aims to address these topics.

The objective of this study was to evaluate the allelopathic effects of concentrations of an aqueous extract of eucalyptus leaves on *Urochloa decumbens* and *Panicum maximum*.

MATERIALS AND METHODS

Leaves of one-year-old *Eucalyptus urograndis* were collected from the base of the canopy of 20 trees. The leaves were dried in a forced-air circulation drying oven (65 ± 3 °C) for 48 hours and ground in an analytical mill. A 75-gram sample of the ground leaves was then collected. A total of 675 mL of distilled water was added to the sample to obtain a solution with a 1:9 ratio of ground leaves to water. The solution was allowed to rest for 24 hours and then passed through filter paper to obtain an extract containing water-soluble compounds. This extract was a 100% stock solution containing the extracted compounds and represented the potential amount able to influence one grass seed on field. Aliquots from this solution were diluted to obtain concentrations of 50%, 25% and 12.5%. The effects of these concentrations were compared to the effects of a distilled-water control (designated 0%).

Urochloa decumbens and *Panicum maximum* seed were used (germination below 80%). A total of 50 seeds were placed on germitest paper in a plastic Gerbox. At each concentration, the paper was moistened with the selected test solution to achieve a weight of 2.5 times the original paperweight. The experiments were performed in a germinator at 20 ºC for 16 hours (without light) and at 35 ºC for eight hours (with light) per day according to the Regras para Análise de Sementes (RAS) specifications (Brasil, 2009).

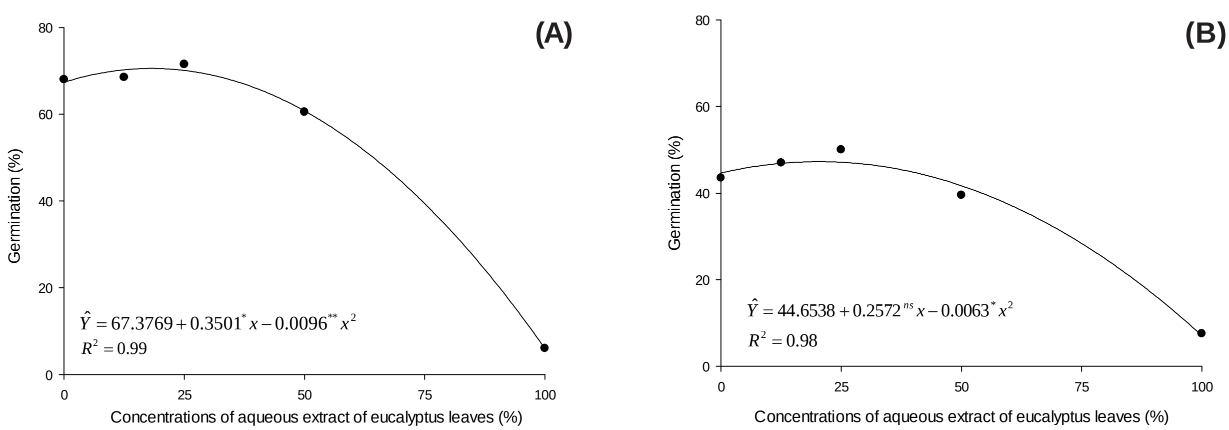
Normal seedlings were counted daily up to the seventh and tenth day for *U. decumbens* and *P. maximum*, respectively. The germination speed index (GSI) was calculated according to Maguire (1962), and normal seedlings resulting from the first germination were counted. The radicle length and shoot length of ten plants were evaluated for each replication on the first germination count with a digital caliper.

A second trial was conducted simultaneously with the first trial to minimize possible contamination. The germination percentages resulting from the tested concentrations of aqueous extract of eucalyptus leaves were evaluated. The normal seedlings of both species were counted at 7, 14 and 21 days after the application of the treatments. For *P. maximum* only, a further count was performed at 28 days. The final count was determined according to Brasil (2009). The normal seedlings were separated, placed in kraft paper bags and dried in an oven with forced air circulation at 70 ºC. The weight of dry matter per seedling was then determined. The percentage of abnormal seedlings was determined at the end of the trial.

A completely randomized experimental design was used. The design comprised five extract concentrations (0, 12.5, 25, 50 and 100%) and four replications. An analysis of variance ($p<0.05$) and a regression analysis were used to analyze the data. Equations and models were selected based on the coefficient of determination, the regression coefficient and the biological phenomenon to be analyzed.

RESULTS AND DISCUSSION

All analyzed variables showed quadratic or linear responses to the concentrations of the test extracts. The initial count of normal seedlings of both species in the germination test showed a quadratic response to concentration, with an initial growth increase at lower concentrations and a drastic reduction of the percentage of normal seedlings at higher concentrations (Figure 1). Based on the fitted curves, the promotion of growth of *U. decumbens* and *P. maximum* by the extracts extended to concentrations of 24.58% and 13.66%, respectively. The growth then decreased at higher concentrations.



ns, ** and * non significant, significant to 1 and to 5% of probability, respectively.

Figure 1 **-** The percentage of normal seedlings of *Urochloa decumbens* (A) and *Panicum maximum* (B) counted daily up to the seventh and tenth day, respectively.

Eucalyptus species are known to produce inhibitory substances that affect the growth of other plant species (Rizvi et al., 1999). These allelopathic substances are exuded by the eucalyptus plants and can benefit or damage the growth of other plants (Sobrero et al., 2004). Souza Filho & Alves (2000) evaluated the allelopathic potential of acapu (*Vouacapoua americana*) plants against two types of pasture weeds, malícia (*Mimosa pudica*) and malva (*Urena lobata*). The results of their study showed that germination and primary root length were decreased by the treatment. Bedin et al. (2006) found that aqueous extracts of fresh and dry leaves of *Eucalyptus citriodora* had no effect on tomato seed germination. Tomato plants are sensitive to allelopathic substances. However, the concentrations of the extracts used in the Bedin et al. (2006) study were lower than the concentrations used in the current study and did not show allelopathic inhibition of seeds.

The final counts of germination showed that the tested extracts had negative effects on both forage species. Based on the fitted curves, germination increased at concentrations up to 18.23% and 20.41%, respectively, for *U. decumbens* and *P. maximum*. Germination then decreased to 6.39% in *U. decumbens* and 7.37% in *P. maximum* at an extract concentration of 100% (Figure 2). *U. decumbens* was found to be sensitive to an aqueous extract of *Vernonia polyanthes*, which reduced germination to 69.59% at a concentration of 100% (Marcos Filho et al., 1996). An understanding of allelopathic effects is important to determine suitable husbandry practices and management for crops to prevent the interference of allelopathic substances with crop growth and production (Gatti et al., 2004).

In the current study, the tested extracts produced an increase in abnormal seedlings. The percentage of abnormal seedlings increased with increasing concentrations of the extract. *U. decumbens* showed an increase in the percentage of abnormal seedlings at extract concentrations greater than 8.99%. The percentage of abnormal seedlings in *U. decumbens* was greater than 60% at a 100% concentration of eucalyptus extract, whereas the percentage of abnormal seedlings in *P. maximum* was greater than 35% at this concentration (Figure 3).

The germination speed index showed a greater decrease in both forage species. The index decreased to values near zero in *U. decumbens* and *P. maximum* as the concentration of the extract increased (Figure 4). Germination is less sensitive to allelopathic substances than seedling growth, and allelopathic substances can produce abnormal characteristics in seedlings. Root necrosis is a common symptom associated with allelopathic effects. An increased number of abnormalities were observed at higher concentrations of the eucalyptus extract.

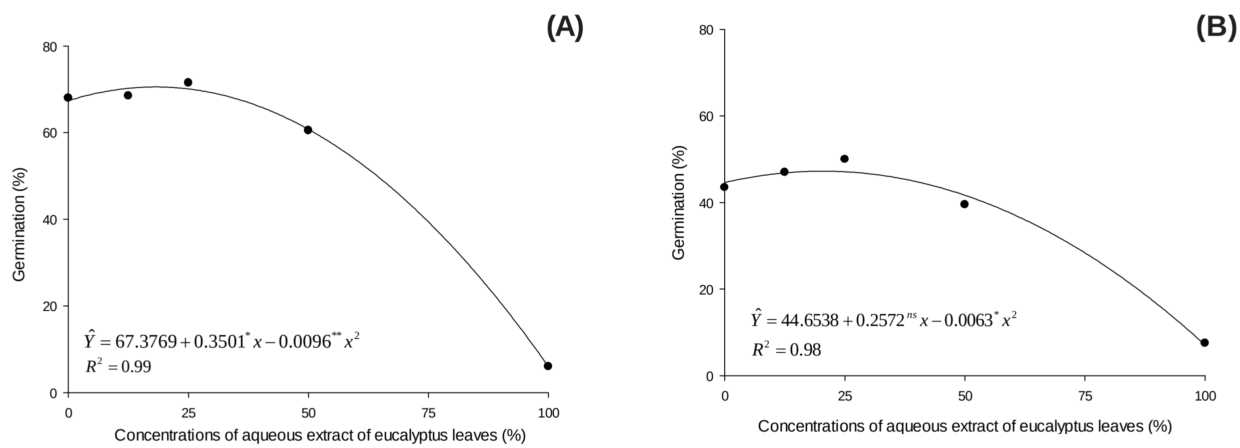


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Figure 2 - The germination percentage of *Urochloa decumbens* (A) and *Panicum maximum* (B) seed resulting from concentrations of aqueous extract of eucalyptus leaves.

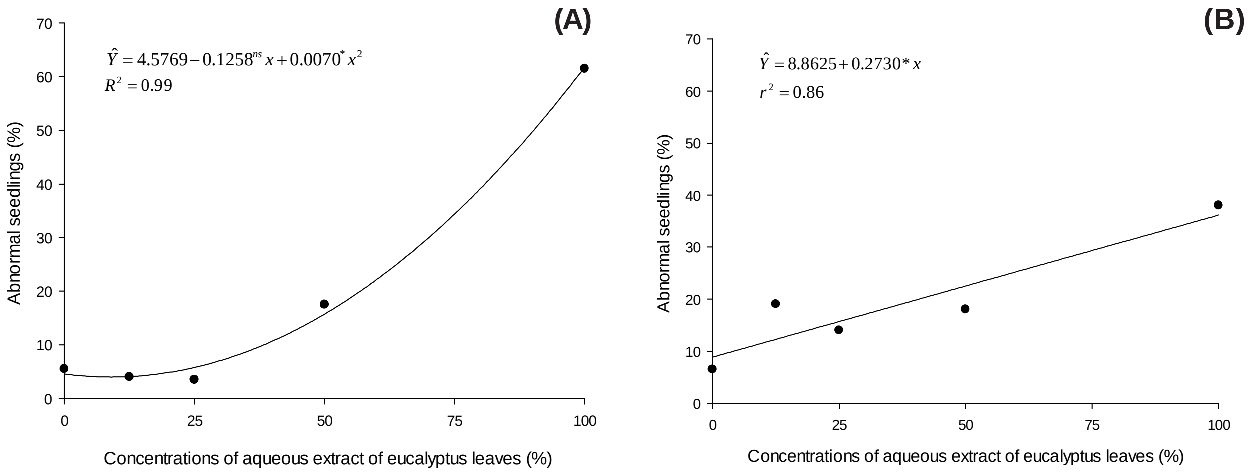

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Figure 3 **-** The abnormal seedlings percentage of *Urochloa decumbens* (A) and *Panicum maximum* (B) resulting from concentrations of aqueous extract of eucalyptus leaves.

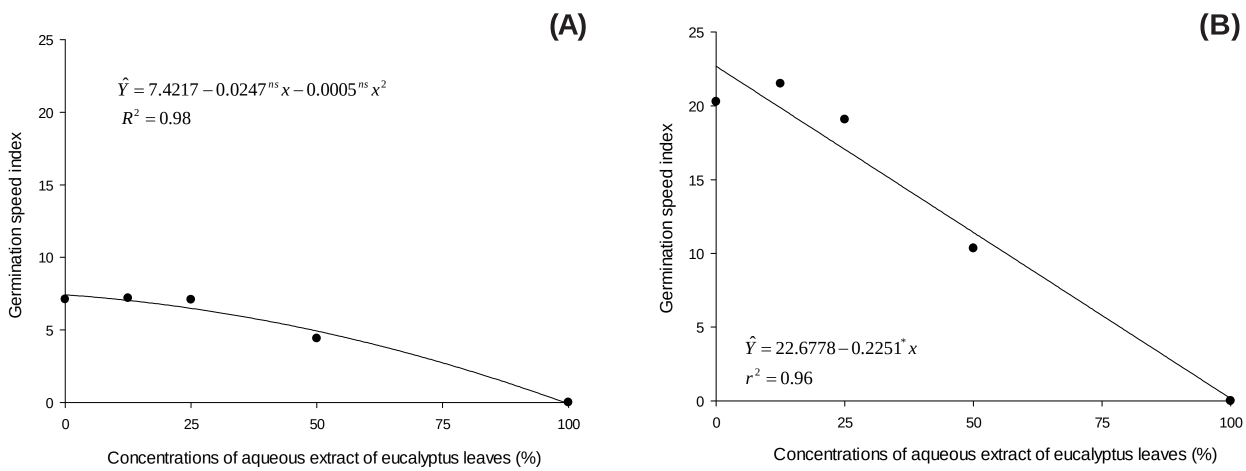

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Figure 4 **-** The germination speed index of *Urochloa decumbens* (A) and *Panicum maximum* (B) seeds resulting from concentrations of aqueous extract of eucalyptus leaves.

Generally, necroses were observed in the distal region of the roots, and the roots were thicker or exhibited signs of atrophy. According to Jacobi & Ferreira (1991), the inhibition of seedling growth after germination is, in an ecological context, a more efficient mechanism than the prevention of germination because it can actually eliminate the affected species.

The shoot length of *U. decumbens* seedlings was positively affected by the eucalyptus extract. The maximum length obtained was 2.97 cm seedling^{-1}, at a concentration of 58% (Figure 5A). However, shoot length in *P. maximum* and *U. decumbens* was stimulated at extract concentrations up to 50%. A decrease of 71.4% in shoot growth relative to the control was obtained with the 100% concentration of the extract (Figure 5B). Yamagushi et al. (2011) showed that an aqueous extract of *Eucalyptus globulus* influenced the initial growth of crops, inhibiting the growth of shoots and roots.

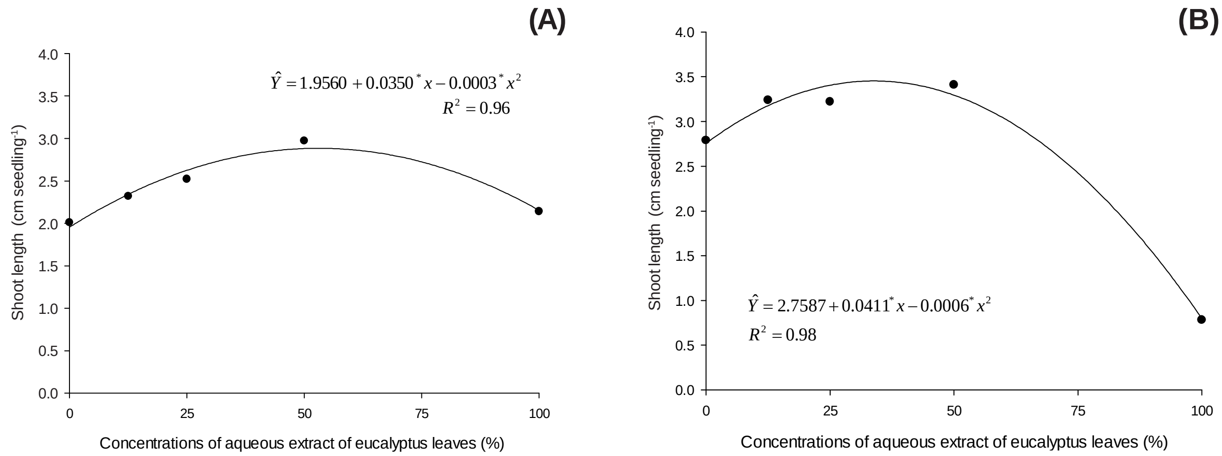

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Figure 5 **-** The shoot length of seedlings of *Urochloa decumbens* (A) and *Panicum maximum* (B) seeds resulting from concentrations of aqueous extract of eucalyptus leaves.

Increased extract concentrations produced more marked results.

The eucalyptus extract showed potential allelopathic effects on the radicle length of both forage species (Figure 6). *U. decumbens* seedlings showed a negative linear effect, with reductions in radical length of approximately 10, 19, 37 and 74% at concentrations of 25, 50, 75 and 100%, respectively (Figure 6A). The radicle length of *P. maximum* was affected negatively. The decrease was up to 3.56 cm seedling^{-1} (85%) at an extract concentration of 100% (Figure 6B).

The current study and previous studies found allelopathic effects on germination, on vigor and on seedling growth. Several studies have found that the damage to seedling growth was more severe than the damage to seed germination (Bedin et al., 2006; Iganci et al., 2006). However, other studies have found that extracts of leaves of species used in forestry inhibit the germination, germination speed index and growth of crops (Jacobi & Ferreira, 1991; Goetze & Thomé, 2004). No previous studies have addressed the allelopathic potential of eucalyptus extract in forage species, and research is lacking on the use of the associated allelopathic compounds in agroforestry systems.

A regression analysis found a quadratic relationship between seedling dry matter in both forage species and increases in the concentration of the aqueous extract of eucalyptus leaves. Negative effects were only found up to 75 and 50% concentration in *U. decumbens* and *P. maximum*, respectively (Figure 7).

A stimulatory effect at low concentrations of extract and a toxic effect at high concentrations, as observed in several variables in this study, may be related to a phenomenon termed hormesis. This phenomenon was first discussed by Southam & Erlick (1943), who showed that certain substances that are toxic at high concentrations can stimulate or promote growth at low concentrations. The hormetic effects of pesticide applications on crops are well known (Wagner et al., 2003; Cedergreen et al., 2004; Cedergreen et al., 2005; Silva et al., 2012). According to Duke et al. (2006), allelochemicals are known to induce hormesis. Exposure to allelochemicals can involve various feedback mechanisms whose behavior depends on the specific substance and on the species subjected to the exposure. The mechanism can be a physiological effect, e.g., an increase in growth to facilitate escape from a chemical stressor, as verified in this study. Allelochemicals can alter the allocation of resources and reduce the performance of plants in their environment, directly influencing competition among species in an ecosystem

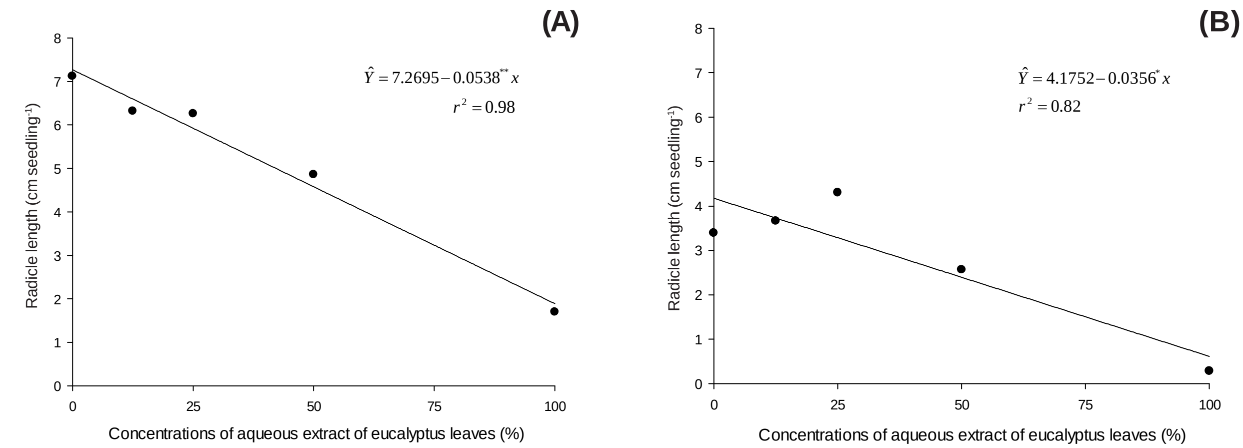

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Figure 6 **-** The radicle length of seedlings of *Urochloa decumbens* (A) and *Panicum maximum* (B) seeds resulting from concentrations of aqueous extract of eucalyptus leaves.

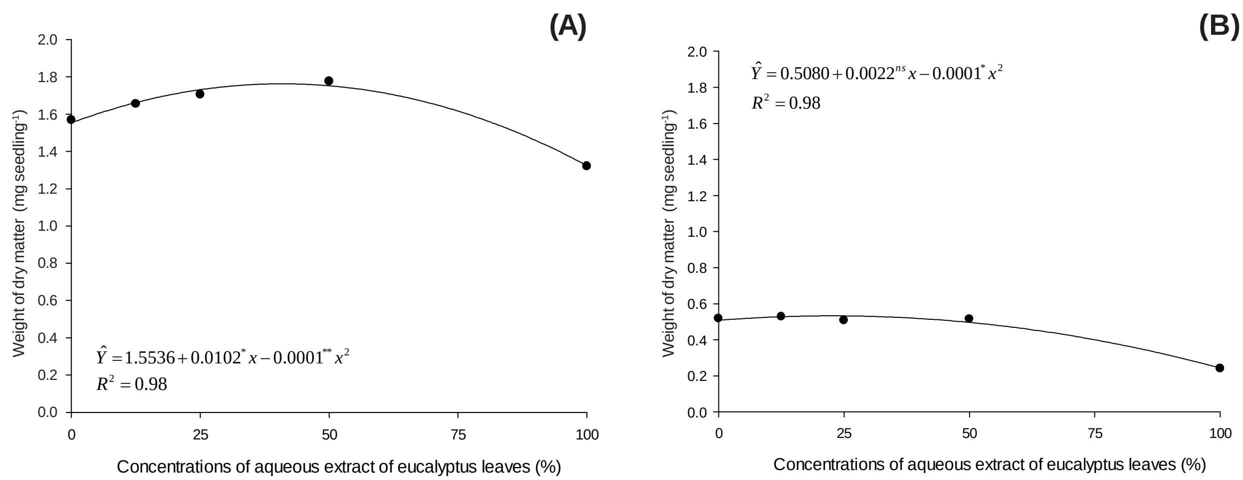

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Figure 7 **-** The weight of dry matter per seedlings of *Urochloa decumbens* (A) and *Panicum maximum* (B) resulting from concentrations of aqueous extract of eucalyptus leaves.

(Duke et al., 2006). Accordingly, an improved understanding of allelopathy can improve the potential use of allelopathic substances to control crops in weed management.

The results of this study show that the potential allelopathic effects of an aqueous extract of the leaves of *E. urograndis* can influence all the evaluated characteristics of germination and initial seedling growth. Except the shoot growth of *U. decumbens* did not showed different, despite a growth with 50% of extract concentration. The most marked effects on the inhibition of germination, the reduction of vigor and the reduction of the initial growth of seedlings of *U. decumbens* and *P. maximum* were observed for 50% and 100% concentrations of the eucalyptus extract. Among the evaluated characteristics, the germination speed index and radicle length were most negatively affected by the potentially allelopathic substances contained in all concentrations of the eucalyptus extract.

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