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Allelopathic interference of *Sapindus saponaria* root and mature leaf aqueous extracts on diaspore germination and seedling growth of *Lactuca sativa* and *Allium cepa*

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ABSTRACT – (Allelopathic interference of *Sapindus saponaria* root and mature leaf aqueous extracts on diaspore germination and seedling growth of *Lactuca sativa* and *Allium cepa*). *Sapindus saponaria* (soapberry) is a species that presents a great diversity of chemical compounds, such as saponins; however, few studies have examined the allelopathic effect of this species. Therefore, this study provides an evaluation of the allelopathic potential of aqueous extracts of the roots and mature leaves of *S. saponaria* on the germination of diaspores and seedlings growth of lettuce (*Lactuca sativa*) and onion (*Allium cepa*). The aqueous extract was prepared in the proportion of 100 g of dry plant material in 1,000 mL of distilled water (a concentration of 10% w v^{-1}), and diluted with distilled water to 7.5, 5.0 and 2.5% concentrations. The mature leaf extracts caused delay and decrease in the germination process of the lettuce and onion diaspores, with inhibitory effect concentration-dependent, while the root extracts showed no allelopathic effects on the germination process. Both extracts caused abnormalities and inhibited the growth of shoot and root seedlings.

Key words - allelopathy, lettuce, onion, soapberry

INTRODUCTION

During evolution and natural selection, plants have developed defense methods against herbivores, pathogens and environmental stress factors, as well as signaling processes to attract pollinators and dispersers (Wink 2003). Some of these events result from the action of organic compounds of low molecular weight originating in the primary carbon metabolism of plants, known as secondary metabolites (Hadacek 2002).

Plants release into the environment many secondary metabolites that may interfere with the conservation, dormancy and germination of seeds, as well as seedling growth and the vigor of mature plants (Wandscheer & Pastorini 2008). The release of these allelochemicals can occur in several ways such as volatilization, root exudation, leaching and decomposition of plant residues (Reigosa et al. 1999).

Allelochemicals may be present in all plant organs such as leaves, stems, roots, fruits, flowers and seeds, and their quantities vary from one tissue to another (Hedge & Miller 1990; Gatti et al. 2004; Grisi et al. 2012). Leaves appear to be the most consistent source of chemicals involved in phytotoxicity (Dorning & Cipollini 2006), producing the greatest allelopathic effects on target species (Cipollini & Dorning 2006; Tanveer et al. 2010).

Allelopathic compounds found in roots, however, have more direct access to belowground biomass than compounds found in leaves because roots are belowground organs while leaves are aerial organs (Wu et al. 2009). Root derived compounds have the ability to regulate the soil microbial community and the soil chemical and physical properties, affecting the growth of neighboring plant species (Ens et al. 2009). Additionally, the soil biotic and abiotic conditions also have the potential to determine the persistence and chemical transformation of these compounds (Inderjit 2001).

In recent decades, the role of allelopathy in natural and managed ecosystems has aroused the interest of many researchers. Allelopathy has been recognized as an important ecological process that influences the primary and secondary plant succession and the structure, composition and dynamics of native and cultivated plant communities (Reigosa et al. 1999). In forest science and ecology, there is a lack of knowledge concerning the allelopathic behavior of native tree species (Carmo et al. 2007) and this information can contribute to the management of agroforestry systems. Agroforestry systems are more balanced from the environmental and sustainability point of view, and tree species with allelopathic activity may play a crucial role in the stability

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of these systems, especially with respect to weed control (Grisi et al. 2012).

Sapindus saponaria L. (Sapindaceae), popularly known as soapberry, is a species that presents a great diversity of chemical compounds, such as saponins (Albiero 2001); however, few studies have addressed the allelopathic effect of this species. It is a native tree that is pioneer, evergreen or semi-deciduous, heliophytic and small (up to 8 m tall), which is used in landscaping and the recovery of degraded areas (Albiero 2001). In Brazil, it occurs from Pará to Rio Grande do Sul, with even distribution in the states of the north, northeast and midwest.

The objective of this study was to evaluate the allelopathic potential of different concentrations of aqueous extracts of root and mature leaf of *Sapindus saponaria* on the germination of diaspores and seedlings growth of lettuce (*Lactuca sativa* L.) and onion (*Allium cepa* L.).

MATERIAL AND METHODS

Leaves and roots of *Sapindus saponaria* were collected in São Carlos, SP (22°02' S and 47°52' W). The region is characterized by the Aw climate type (Köppen 1948), with dry winters (April to September) and wet summers (October to March). Mature leaves (with dark green color and papiraceous texture) were collected from 10 trees, while roots were collected from two.

After collection, leaves and roots were dried at 40 °C for 72 hours and ground in an industrial mill. Aqueous extracts were prepared in the proportion of 100 g of dry plant material in 1,000 mL of distilled water (a concentration of 10% w v^{-1}). The extract was left to stand at 4 °C for 30 minutes and then, vacuum filtered (Gatti et al. 2004). The concentrated extract was collected in a beaker and diluted to 7.5, 5.0 and 2.5% with distilled water.

Osmotic potential and pH of the extract

An Analion pH meter (model PM608) was used to measure pH values, and an automatic osmometer (µOsmotte, model 5004) was used to measure molar concentrations. The osmotic potentials of the most concentrated extracts of leaves and roots were calculated.

Germination bioassays

Aqueous extracts of roots and mature leaves were applied to the cypselas (sensu Marzinek et al. 2008) of lettuce (*Lactuca sativa* 'Grand Rapids') and seeds of onion (*Allium cepa* 'Baia Periforme').

Bioassays were conducted in Petri dishes with two sheets of filter paper moistened with 5 mL of extract or distilled water (control). The experimental design was completely randomized, using five treatments and four replicates of 30 diaspores. The experiment was conducted in a germination chamber at 25 °C, under continuous fluorescent white lamps and an average irradiance of 12.26 ± 6.49 µmol m^{-2} s^{-1}, similar to the conditions adopted by Iganci et al. (2006) and Povh et al. (2007). The criterion selected to measure the germination process was embryo protrusion, and this criterion was evaluated every 12 hours during the first seven days of the experiment and at intervals of 24 hours thereafter until the stabilization of germination.

The relative frequency of germination, germinability, mean germination time, mean germination rate and synchrony of germination were calculated according to Ranal and Santana (2006).

To evaluate the influence of the osmotic potential of the extracts, germination bioassays with diaspores of lettuce and onions using polyethylene glycol 6000 (PEG 6000) solutions at -0.3, -0.2 and 0 MPa (control) were performed according to Villela et al. (1991). The experiments were conducted using the same methodology described for the germination bioassays.

Seedling growth

Lettuce and onion seedling growth was tested using diaspores previously germinated in distilled water. Only seedlings with 3 mm-long roots were selected and transferred to transparent plastic boxes (21.0 × 14.3 × 6.0 cm) containing, as substrate, filter paper moistened with 15 mL of water, leaf extract, or root extract.

The boxes were kept in a germination chamber at 25 °C, with a photoperiod of 12 hours and a mean irradiance of 13.38 ± 7.96 µmol m^{-2} s^{-1}. The experimental design was completely randomized, with four replicates of 20 seedlings. After seven days, the seedlings were classified as normal or abnormal, according to Brazil (1992). Dead seedlings were also counted. Seedling shoot and primary root lengths were measured in a random sample of 10 seedlings per replicate, using a caliper.

Statistical analysis

The data were subjected to normality (Shapiro-Wilk) and homogeneity (Levene) tests. When these two assumptions were met, an analysis of variance (ANOVA) was applied, followed by Tukey's test at a significance level of 0.05. In case of a lack of normality or homogeneity or both, the non-parametric Kruskal-Wallis and Dunn tests were applied for pairwise comparisons at a significance level of 0.05.

Linear or quadratic regression models were adjusted when the ANOVA *F* was significant. The goodness of the models was evaluated by the coefficient of determination (R^2). Linear regression equations were subjected to the parallelism (*F*-test) test with the null hypothesis being that the slopes of the equations were statistically equal, as described by Sokal and Rohlf (1997).

The data from germinability and mean germination rate were subjected to conjoint analysis, because the ratio between the larger and smaller residual mean square was not greater than 7 (Pimentel-Gomes 1990).

RESULTS

Sapindus saponaria mature leaf aqueous extracts significantly inhibited the process of germination of lettuce and onion diaspores. There was a linear decrease in the germinability (2.23 and 4.23% for each addition of 0.01 mg mL^{-1} of extract, for lettuce and onion, respectively) and in the mean germination rate (0.00079 $hour^{-1}$ for the lettuce and 0.00049 $hour^{-1}$ for the onion, per each additional 0.01 mg mL^{-1} of extract) (figure 1). As a result, there was a linear increase in the mean germination time of 2.79 and 3.51 hours for each addition of 0.01 mg mL^{-1} of extract, for lettuce and onion, respectively (figure 1). The synchrony decreased linearly by 0.0135 in lettuce cypselas and 0.017 in onion seeds, for each 0.01 mg mL^{-1} extract added, indicating a lack of overlapping germination at the same time at a concentration of 10% (figure 1). This asynchrony can be confirmed by the relative frequency of germination, given that in the control treatment, approximately 45% (lettuce) and 50% (onion) of the diaspores germinated in a single evaluation (24 and 48 hours, respectively), while at a concentration of 10% only 23% (lettuce) and 18% (onion) of the diaspores germinated (72 and 96 hours, respectively) (figure 2).

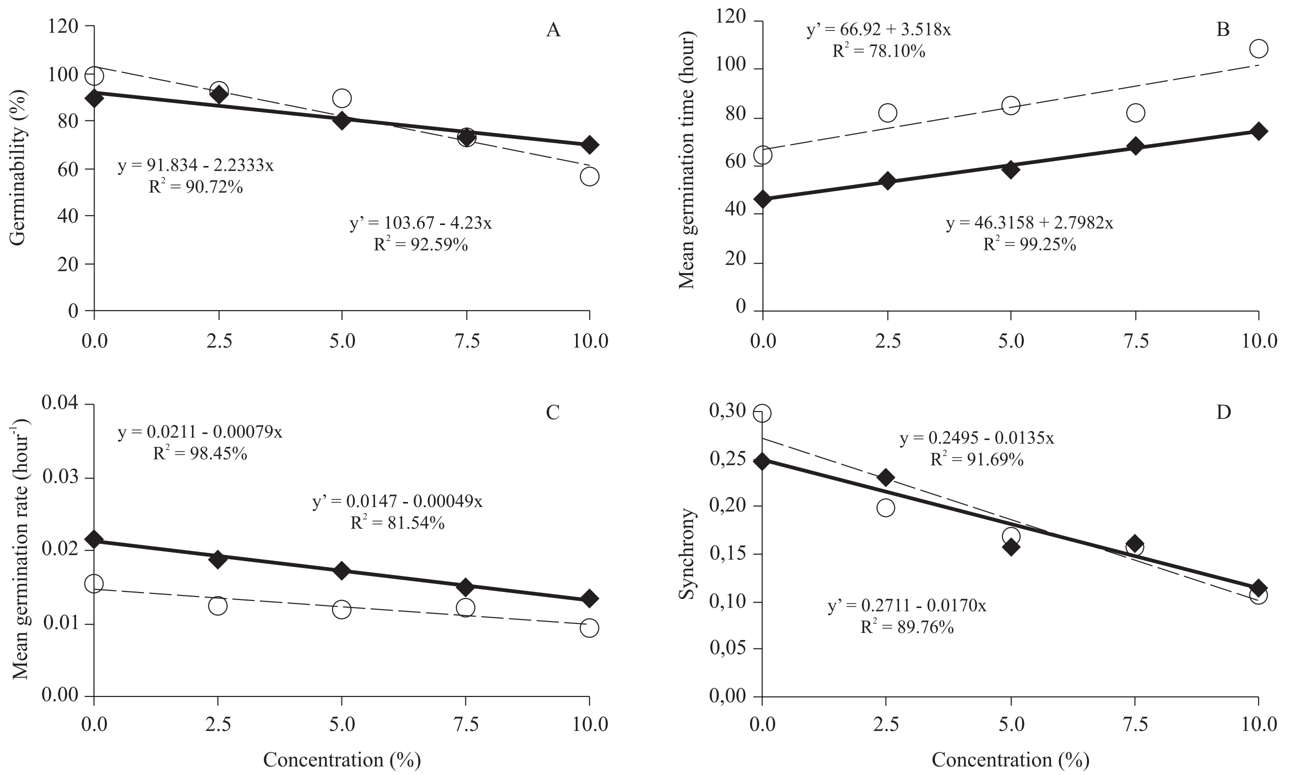

Figure 1. Germinability (A), mean germination time (B), mean germination rate (C) and synchrony (D) of *Lactuca sativa* (lettuce) and *Allium cepa* (onion) diaspores under the action of different mature leaf extract concentrations of *Sapindus saponaria* (◆ = lettuce (y); ○ = onion (y')).

Comparison of the linear regressions showed that for the germinability and mean germination rate of the diaspores treated with mature leaf extracts, the largest decrements occurred for onion seeds. The mean germination time and synchrony of both species had the same increment trends (figure 1, table 1).

Leaf extracts induced abnormalities in the seedlings. The percentage of normal seedlings of lettuce and onion ranged from 96.25% (control) to 0% (10% concentration) and from 95% (control) to 1.25% (10% concentration), respectively (figures 3 and 4). For both species, the largest number of dead seedlings was recorded at the 10% concentration (13.75% for lettuce and 21.25% for onion). Seedlings of lettuce and onion had the lowest shoot length (9.13 mm for lettuce and 12.11 mm for onion) at concentrations of 7.66 and 10%, respectively.

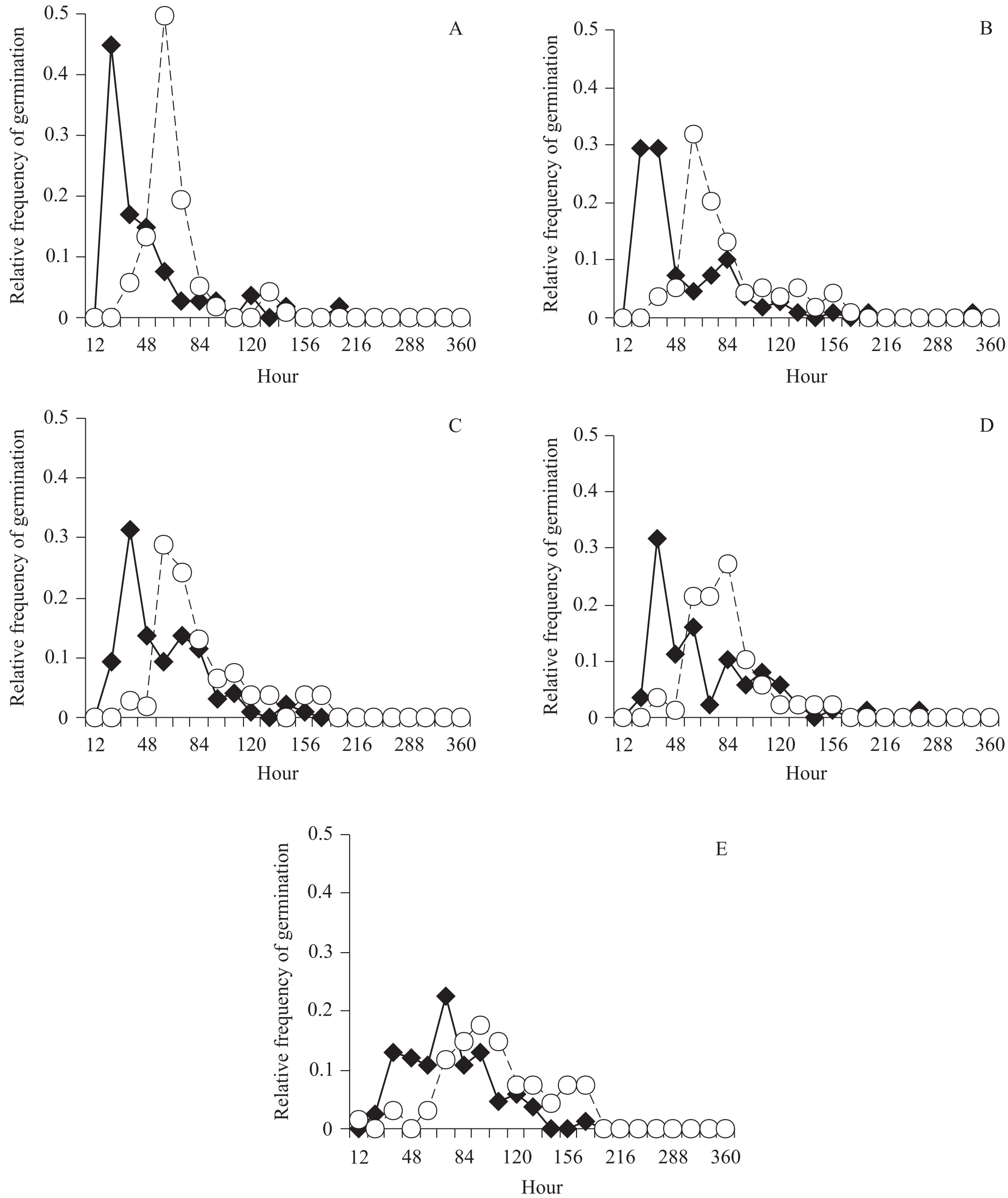

Figure 2. Relative frequency of germination of *Lactuca sativa* (lettuce) and *Allium cepa* (onion) diaspores treated with different mature leaf extract concentrations of *Sapindus saponaria* (◆ = lettuce; ○ = onion; A = control; B = 2.5%; C = 5%; D = 7.5%; E = 10%).

The lowest root length (3.57 mm for lettuce and 2.39 mm for onion) was recorded at concentrations of 7.59 and 10% for seedlings of lettuce and onion, respectively (figures 3 and 4).

The variables analyzed for the germination process of lettuce cypselas treated with root extracts showed no significant differences among treatments (table 2).

The effect of the root extract was significant for some variables of the germination process of onion seeds, but their values did not fit in the regression models (table 2). The highest mean germination time (86.49 hours), and consequently the lowest mean germination rate (0.0117 hours^{-1}) were observed at the 5% concentration (table 2).

The percentage of normal seedlings of lettuce and onion treated with root extracts decreased from 97.5% (control) to 1.25% (10% concentration) and from 97.5% (control) to 30% (concentration 10%), respectively. The percentage of dead seedlings of onion increased

Table 1. *F*-test comparing the linear regression angular coefficients derived from the germination process of *Lactuca sativa* (lettuce) and *Allium cepa* (onion) diaspores treated with mature leaf extracts of *Sapindus saponaria*.

Measurement (unit)	Equation[1]	$F_{calculated}$
G: germinability (%)	y = 91.83 – 2.23x y' = 103.67 – 4.23x	18.50*
$\bar{t}$: mean germination time (hour)	y = 46.31 + 2.79x y' = 66.92 + 3.51x	1.32 ns
$\bar{v}$: mean germination rate ($hour^{-1}$)	y = 0.0211 – 0.00079x y' = 0.0147 – 0.00049x	11.85*
Z: synchrony	y = 0.2495 – 0.0135x y' = 0.2711 – 0.0170x	2.22 ns

[1] H_0: $\beta_1 - \beta_2 = 0$, where β_1 and β_2 are the parameters of the angular coefficients of the equations (y = *Lactuca sativa*; y' = *Allium cepa*; ns = angular coefficients for the two species do not differ significantly; * = angular coefficients for the two species differ significantly ($F_{calculated} > F_{tabulated}$)).

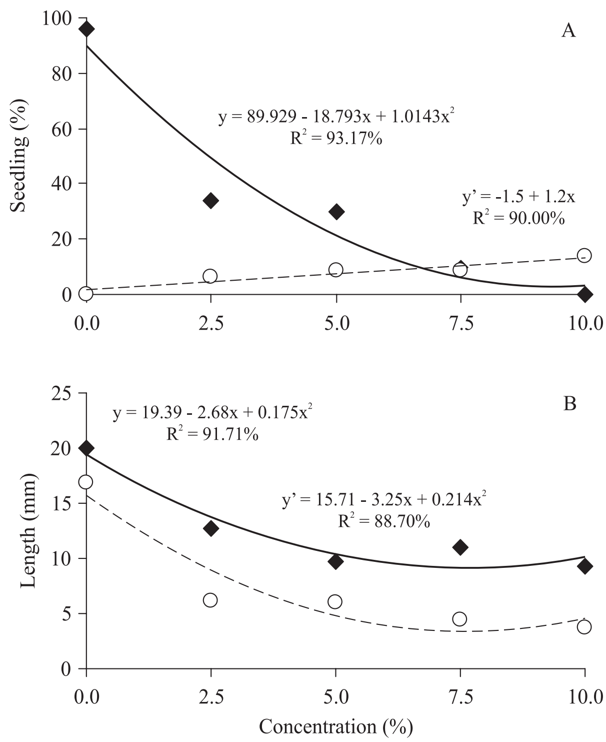

Figure 3. Percentage of normal and dead seedlings (A) and shoot and root length (B) of *Lactuca sativa* (lettuce) treated with mature leaf extracts of *Sapindus saponaria* in different concentrations (◆ = normal (y) and ○ = dead (y') in A; ◆ = shoot lenght (y) and ○ = root lenght (y') in B).

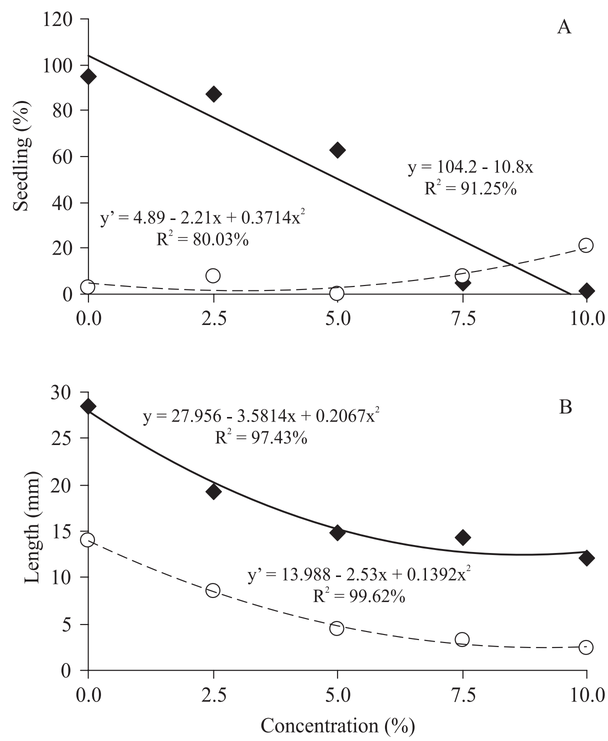

Figure 4. Percentage of normal and dead seedlings (A) and shoot and root length (B) of *Allium cepa* (onion) treated with mature leaf extracts of *Sapindus saponaria* in different concentrations (◆ = normal (y) and ○ = dead (y') in A; ◆ = shoot lenght (y) and ○ = root lenght (y') in B).

linearly 4.62% for each addition of 0.01 mg mL^{-1} extract, while for lettuce the highest percentage was 8.75% at a concentration of 10% (figures 5 and 6). The shoot length of lettuce seedlings was lowest (2.90 mm) at a concentration of 7.7%, while for onion seedlings there was a linear decrease of 6.61 mm for each addition of 0.01 mg mL^{-1} extract. The lowest root length for seedlings of lettuce (0.62 mm) and onion (0.61 mm) was observed at estimated concentrations of 7.27 and 7.31%, respectively (figures 5 and 6).

The results obtained from different organs of *Sapindus saponaria* were compared by conjoint analysis. The mature leaf extracts caused the highest inhibitory effect on germinability and the greatest reduction in the

Table 2. Germination of *Lactuca sativa* (lettuce) and *Allium cepa* (onion) diaspores treated with root extracts of *Sapindus saponaria* in different concentrations. (G = germinability; t = mean germination time; $\bar{v}$ = mean germination rate; Z = synchrony; W = Shapiro-Wilk test; boldfaced values indicate residuals normality ($P > 0.01$); ^{1}F = Levene's test; boldfaced values indicate homogeneity between variances ($P > 0.01$); ^{2}F = Snedecor's test; boldfaced values indicate significant difference among concentrations (ANOVA; $P < 0.05$); P = probability).

Measurement (unit)	Control	2.5%	5.0%	7.5%	10.0%	Statistics W (P)	^{1}F (P)	^{2}F (P)
Lettuce								
G (%)	91.67 ± 4.30 a	89.17 ± 5.00 a	89.17 ± 5.00 a	85.00 ± 4.30 a	84.17 ± 10.32 a	0.9397 (0.2479)	1.402 (0.2808)	1.026 (0.4255)
t (h)	45.96 ± 5.23 a	43.45 ± 3.00 a	49.26 ± 6.35 a	49.49 ± 6.35 a	47.51 ± 09.28 a	0.9374 (0.2248)	1.077 (0.4025)	0.547 (0.7041)
v (h^{-1})	0.0220 ± 0.0028 a	0.0231 ± 0.0016 a	0.0207 ± 0.0033 a	0.0204 ± 0.0024 a	0.0216 ± 0.0036 a	0.9797 (0.9144)	0.665 (0.6259)	0.565 (0.692)0
Z	0.2375 ± 0.0701 a	0.2155 ± 0.0172 a	0.2082 ± 0.0729 a	0.1832 ± 0.0219 a	0.2889 ± 0.1526 a	0.8989 (0.0403)	2.670 (0.0731)	0.924 (0.4756)
Onion								
G (%)	93.33 ± 6.08 a	98.33 ± 1.92 a	85.83 ± 11.98 a	90.83 ± 8.33 ab	91.67 ± 3.33 ab	0.9577 (0.5063)	2.843 (0.0615)	1.537 (0.242)0
t (h)	66.37 ± 5.81 a	64.98 ± 0.72 a	86.49 ± 11.50 b	77.47 ± 6.22 ab	77.04 ± 5.81 ab	0.9831 (0.9538)	2.025 (0.1424)	6.609 (0.0028)
v (h^{-1})	0.0152 ± 0.0013 a	0.0154 ± 0.0002 a	0.0117 ± 0.0016 b	0.0130 ± 0.0011 ab	0.0130 ± 0.0009 ab	0.9768 (0.8701)	2.430 (0.0932)	7.647 (0.0014)
Z	0.2496 ± 0.0789 a	0.2672 ± 0.0327 a	0.2101 ± 0.0891 a	0.2451 ± 0.0400 ab	0.2433 ± 0.0566 ab	0.9437 (0.2935)	0.782 (0.5545)	0.428 (0.7863)

Means followed by the same in each line are not significantly different based on the Dunn or Tukey tests at 0.05% probability.

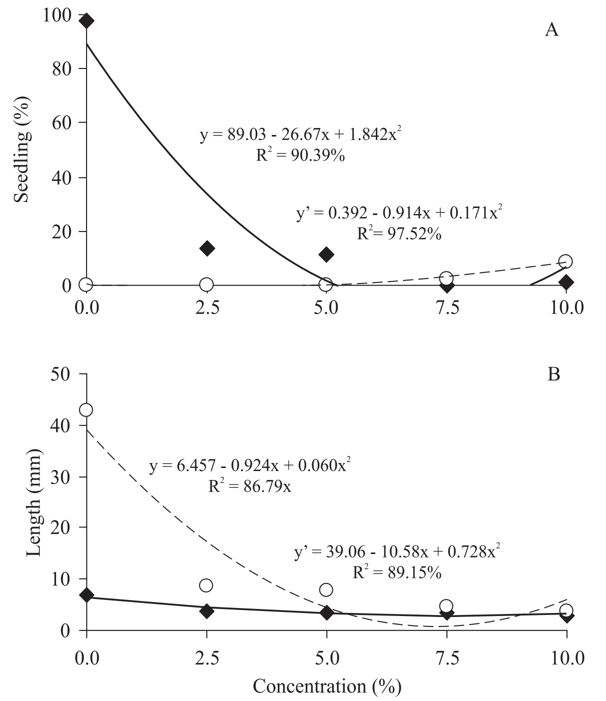

Figure 5. Percentage of normal and dead seedlings (A) and shoot and root length (B) of *Lactuca sativa* (lettuce) treated with root extracts of *Sapindus saponaria* in different concentrations (◆ = normal (y) and ○ = dead (y') in A; ◆ = shoot lenght (y) and ○ = root lenght (y') in B).

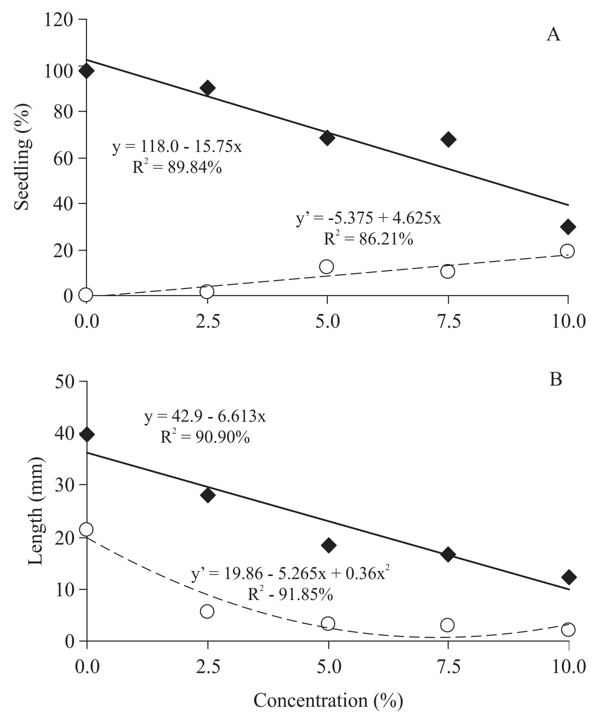

Figure 6. Percentage of normal and dead seedlings (A) and shoot and root length (B) of *Allium cepa* (onion) treated with root extracts of *Sapindus saponaria* in different concentrations (◆ = normal (y) and ○ = dead (y') in A; ◆ = shoot lenght (y) and ○ = root lenght (y') in B).

mean germination rate of the lettuce and onion diaspores, while the lowest effect on germination process was observed for the root extracts (figure 7).

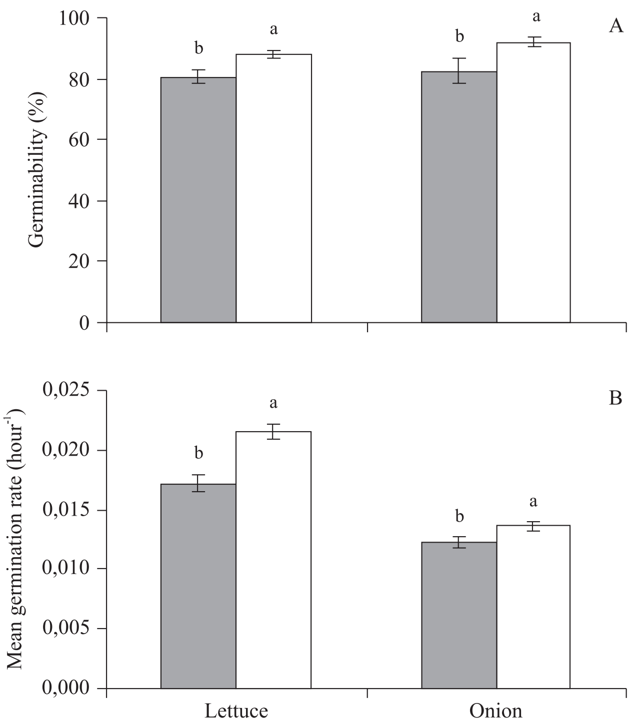

Figure 7. Germinability (A) and mean germination rate (B) of *Lactuca sativa* (lettuce) and *Allium cepa* (onion) diaspores treated with root and mature leaf extracts of *Sapindus saponaria*. Means followed by the same small letters for organs do not differ significantly by Tukey's test at 0.05% probability. Vertical bars represent the standard error of the mean (■ = leaf; □ = root)

The pH values obtained for the root and mature leaf extracts varied between 6.25 and 6.55 and the osmotic potential between -0.20 and -0.25 MPa, respectively. In PEG-6000 solutions, 89, 86 and 82% of cypselas of lettuce germinated at osmotic potentials of 0, -0.2 and -0.3 MPa, respectively while 90, 89 and 86% of onion seeds germinated at these potentials.

DISCUSSION

The evaluation of pH and the molar concentration of crude extracts is important given that osmotically active substances such as sugars and amino acids that influence the ion concentration (Ferreira & Áquila 2000) may affect the results of germination and seedling growth (Gatti et al. 2004). Considering that the solutions with osmotic potential of -0.3 MPa did not interfere with in the germination of lettuce and onion diaspores, it may be inferred that the reduction in the germinability of diaspores was due specifically to the presence of substances with allelopathic activity in the extracts of *Sapindus saponaria*.

The *Sapindus saponaria* leaf extracts were phytotoxic, with an inhibitory effect on diaspore germination, causing morphological abnormalities, loss of vigor and mortality of lettuce and onion seedlings. The germination process of both species was inhibited, showing concentration-dependent inhibitory effects. The same effect has been recorded for other species of donor plants (Povh et al. 2007, Wandscheer & Pastorini 2008, Scrivanti 2010, Souza et al. 2010).

The greatest inhibitory effect observed for root allelochemicals of *Sapindus saponaria* was on root and shoot elongation and not on germination of the lettuce and onion diaspores. Some studies confirm the low allelopathic effects of root extracts on the germination of target species (Gatti et al. 2004, Marchi et al. 2008, Sodaeizadeh et al. 2009, Ashrafi et al. 2009). However, during seedling development, the root extract causes significant morphological changes (Hernández-Terrones et al. 2007), especially in root growth (Carmo et al. 2007, Wandscheer & Pastorini 2008).

The toxic effects of aqueous extracts of some species of Gleicheniaceae, visualized as size reduction and necrosis in lettuce roots is similar to the damage caused by natural detergents such as saponins (Smith & Vieira 2000). Saponins are present in *Sapindus saponaria* extracts, as reported by Pellegrini et al. (2008), and acts to reduce the respiratory rate through lowering the diffusion of oxygen through the seed coat, inhibiting the germination process and the plant growth (Maraschin-Silva & Áquila 2005).

The ability of *Sapindus saponaria* extracts to inhibit the root development of other species is an important ecological aspect that favours the dominance of this species in the environment (Ferreira & Áquila 2000). According to Iganci et al. (2006), root inhibition under the influence of allelopathic compounds is a consequence of cell division inhibition, membrane permeability and the activation of enzymes.

Allelopathic activity varies depending on the concentration of the extract, target species and plant tissues from which chemicals are extracted (Sodaeizadeh et al. 2009). According to previous reports, inhibitory effects on diaspore germination and seedling growth increases with the concentration of extracts (Batish et al. 2007, Ashrafi et al. 2009, Zhang et al. 2009). Wandscheer & Pastorini (2008), by analyzing the allelopathic effect of leaves and roots of *Raphanus raphanistrum* L. on diaspores and seedlings of lettuce and tomato, found that the extracts were more active at higher concentrations, as was also observed in the present study.

Each organ of the plant may contribute to allelopathic effects. However, the leaf is the most metabolically active plant organ, with higher concentration (Sodaeizadeh et al. 2009) and diversity of allelochemicals (Ribeiro et al. 2009, Tanveer et al. 2010). Similar results were reported by Gatti et al. (2004) and Wu et al. (2009), showing that the leaf extracts of *Aristolochia esperanzae* Kuntze and *Mikania micrantha* Kunth have greater allelopathic effect than their respective root extracts on the germination process of target species.

The difference in the allelopathic effects of the *Sapindus saponaria* root and mature leaf extracts may be related to the different concentrations of allelochemicals or chemical composition among the extracts, as discussed by Dorning & Cipollini (2006) and Batish et al. (2007). Accordingly, the stronger allelopathic effect of the leaves on the target species of vegetables can significantly promote success in weed control. Therefore, the leaf of *Sapindus saponaria* can be considered as an important organ with active principles for the development of natural herbicides.

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