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Phytotoxic activity of crude aqueous extracts and fractions of young leaves of *Sapindus saponaria* L. (Sapindaceae)

Patrícia Umeda Grisi[1,4], Sônia Cristina Juliano Gualtieri[1], Marli Aparecida Ranal[2] and Denise Garcia de Santana[3]

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ABSTRACT

The aim of this study was to evaluate the phytotoxic potential of aqueous extract of young leaves of *Sapindus saponaria* L. (soapberry) on the diaspore germination and seedling growth *Lactuca sativa* L. (lettuce) and *Allium cepa* L. (onion), as well as to determine, by bioassay-guided fractioning, whether the fractionated extracts of those leaves are phytotoxic to *Triticum aestivum* L. (wheat) coleoptiles. The aqueous extract was prepared using 100 g of dried plant material dissolved in 1000 ml of distilled water, resulting in a concentration of 10.0%. Distilled water was added in order to obtain dilutions of 7.5%, 5.0%, and 2.5%. The extraction was carried out with young leaves (in powder form) and organic solvents of various polarities. We fractioned the ethyl acetate extract using column chromatography. The phytotoxic potential of the aqueous extract of young leaves *S. saponaria* varied according to the receiving species and the concentration-dependent inhibitory effect. The ethyl acetate extract, specifically fraction 6 (57-70), had the greatest inhibitory effect on the elongation of wheat coleoptiles, indicating that the compounds responsible for the phytotoxic effect reside within this fraction.

Key words: *Allium cepa* L., *Lactuca sativa* L., phytotoxicity, soapberry, *Triticum aestivum* L

Introduction

Sapindus saponaria L. (Sapindaceae), popularly known as the soapberry, is a native, pioneer, evergreen or semi-deciduous, heliophytic tree species, small in size, used in landscaping and in recovery plans for areas of degradation. It is typically found in humid areas, including the northern, northeastern and central-west regions of Brazil (Albiero *et al.* 2001). The wood is moderately heavy, hard and compact. Soapberry wood also has low natural durability and is used in civil construction. The soapberry fruit contains high levels of saponins and is used in fabric laundering, as well as for treating ulcers, skin sores and inflammation (Pelegrini *et al.* 2008).

Studies using high-performance liquid chromatography with ultraviolet detection and mass spectrometry have shown that the major glycosides present in soapberry fruit are saponins derived from the triterpenes hederagenin and oleanolic acid, as well acyclic sesquiterpene oligoglycosides. Using these methods of analysis, 30 saponins and 63 acyclic sesquiterpene oligoglycosides have been detected (Murgu & Rodrigues-Filho 2006; Pelegrini *et al.* 2008). Saponins display diverse behaviors, such as molluscicidal, and piscicidal, anti-inflammatory, analgesic, expectorant, antioxidant, antiviral, antibacterial and antifungal activity (Sparg *et al.* 2004).

Some secondary metabolites of plants play a fundamental role in the defense interactions with predators, pathogens and competitors (Croteau *et al.* 2000), which characterizes allelopathic interactions mediated by phenols, terpenes, alkaloids, polyacetylenes, fatty acids and peptides, among others. These substances can be produced in any plant organ, although in very low concentrations and with characteristics intrinsic to the plant, such as, for example, species and age (Rizvi & Rizvi 1992; Gatti *et al.* 2004). Most of these secondary metabolites exhibit biological activity and have been used in the pharmaceutical and agrochemical industries (Hamburger & Hostettmann 1991; Vyvyan 2002; Pelegrini *et al.* 2008).

Allelochemicals affect various metabolic processes in organisms, including changes in membrane permeability, ion absorption (Gniazdowska & Bogatek 2005) and inhibition of electron transport in respiration and photosynthesis (Abrahim *et al.* 2000), as well as changes in enzyme activities (Singh *et al.* 2009) and inhibition of cell division

[1] Universidade Federal de São Carlos, Departamento de Botânica, São Carlos, SP, Brazil
[2] Universidade Federal de Uberlândia, Instituto de Biologia, Uberlândia, MG, Brazil
[3] Universidade Federal de Uberlândia, Instituto de Ciências Agrárias, Uberlândia, MG, Brazil
[4] Author for correspondence: patriciaumeda@hotmail.com

(Teerarak *et al.* 2010). Because of these changes, allelopathy is recognized as an important ecophysiological process in ecosystems, influencing primary and secondary plant succession, structure, composition and dynamics of native or cultivated plant communities (Rizvi & Rizvi 1992).

For advances in technological terms and to increase the potential applicability of the results of allelopathy, it is necessary to identify the fractions or allelochemicals responsible for phytotoxic activity, as well as to identify which compounds have potential for use as growth regulators and herbicides (Dayan & Duke 2006). Therefore, certain methods, such as bioguided fractionation, are being used to study new phytotoxic compounds. Bioguided fractionation consists of performing bioassays at each step of fractionation, in order to determine which fractions display the highest level of biological activity (Macías *et al.* 2006).

The phytochemical composition of the fruit of *S. saponaria* is known to have important pharmaceutical properties (Guarim Neto *et al.* 2000; Pelegrini *et al.* 2008). However, few studies have made reference to the phytochemical analysis of the leaves of the species (Grisi *et al.* 2012). Consequently, the objective of this study was to assess the phytotoxic potential of the aqueous extract of the young leaves of *S. saponaria* on diaspore germination and seedling growth of lettuce (*Lactuca sativa* L.) and onion (*Allium cepa* L.), as well as to analyze, through bioguided fractionation, the phytotoxic effects of its fractions on the elongation of *Triticum aestivum* L. (wheat) coleoptiles.

Materials and methods

In November of 2008, young leaves of *S. saponaria* were collected from 10 trees in the city of São Carlos, Brazil (22°02'S; 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). The morphological criterion used to identify the young leaves was the light green coloration and and texture of the membranous type.

After collection, the leaves were dried at 40°C for 72 h and ground in an industrial mill to obtain dried plant material. The aqueous extract was prepared at a ratio of 100 g of dried plant material to 1000 ml of distilled water, in a final concentration of 10.0%. The extract remained at rest for 30 min at 4°C and was then filtered using an electric vacuum pump coupled to a Buchner funnel lined with filter paper (Gatti *et al.* 2004). The resulting extract was collected in a beaker and distilled water was added, making dilutions of 7.5%, 5.0% and 2.5%.

Osmotic potential and pH of the extract

The pH of the aqueous extract was measured with a pH meter (PM608; Analion, Ribeirão Preto, Brazil) and molar concentration was measured with an automatic osmometer (5004 MICRO-OSMETTE; Precision Systems, Natick, MA, USA). The osmotic potentials of the most concentrated extracts of young leaves were calculated.

Germination bioassay

The four concentrations obtained from the aqueous extract of young leaves were applied to the cypselas (*sensu* Marzinek *et al.* 2008) of lettuce (*L. sativa* cv. Grand Rapids) and seeds of onions (*A. cepa* cv. Baia Periforme). Four replicates of 30 diaspores were seeded in Petri dishes over two sheets of filter paper moistened with 5 ml of the aqueous extract or distilled water (control treatment). Each dish was covered with a transparent plastic film to prevent evaporation. The experiment was conducted in a germination chamber at 25°C under constant white fluorescent light and average irradiance of 12.26 ± 6.49 μmol m^{-2} s^{-1}, as recommended by Iganci *et al.* (2006) and Povh *et al.* (2007). The experimental design was completely randomized. Readings were taken every 12 h during the first seven days, and every 24 h until germination had stabilized, using the embryo protusion as criterion for germination. We evaluated the initial, final and mean germination time, germinability, mean germination rate, rate (Maguire's index), coefficient of variation of the germination time, uncertainty and synchronization (Ranal & Santana 2006).

To evaluate the osmotic effect of the extract obtained, we performed a germination bioassay with lettuce and onion diaspores in solutions of polyethylene glycol 6000 (PEG 6000) at −0.2 and −0.3 MPa, as well as the control (0 MPa), following recommendations by Villela *et al.* (1991). The experiment was performed using the same methodology described for the germination bioassay.

The term "diaspore" has been used throughout the text to refer to both types of dispersion units studied. When the lettuce and onion were referred to individually, the specific name of their diaspores were used; in this case, cypsela and seed, respectively.

Growth bioassay

The dispersion units of lettuce and onion were germinated in distilled water, and only seedlings with up to 3 mm of primary roots were transferred into clear plastic boxes (21.0 × 14.3 × 6.0 cm) containing, as a substrate, filter paper moistened with 15 ml of distilled water (control) or aqueous extracts of young leaves at the same concentrations adopted for the germination test. The boxes were kept in a growth chamber at 25°C, with a photoperiod of 12 hours and mean irradiance of 13.38 ± 7.96 μmol m^{-2} s^{-1}. Four replicates of 20 seedlings were used in a completely randomized experimental design. After seven days, the seedling shoots and primary root lengths were measured in a random sample of 10 seedlings per replicate, using a caliper. The seedlings were classified as normal or abnormal, according to the specifications established by Brasil (2009), and dead seedlings were quantified.

Chemical extraction

We used 600 g of powder *S. saponaria* young leaves and 6000 ml of organic solvents. In a glass container with a cover, we performed extraction with the following solvents, in ascending order by polarity: hexane, dichloromethane, ethyl acetate, acetone and methanol. For each solvent, the container was kept in an ultrasonic bath for 30 min. The mixture was then filtered through a Buchner funnel lined with filter paper and coupled to a vacuum pump. The extract was evaporated in a rotary evaporator under reduced pressure at 40°C. This extraction was repeated three times for each solvent (Macías *et al.* 2010). Thus, the extracts obtained after filtering and drying were hexane, dichloromethane, ethyl acetate, acetone and methanol.

Wheat coleoptiles bioassay

Wheat caryopses (*T. aestivum* cv. BRS Camboatá) were sown in plastic boxes lined with two sheets of filter paper (moistened with 10 ml of distilled water) and covered with aluminum foil. The boxes were maintained in the dark at 25°C for 72 h. Shoots were placed in a Van der Weij guillotine and the apical meristem (2 mm) was cut and discarded. The next 4 mm of the coleoptile sections were removed and used for bioassay. All manipulations were performed under green safelight (Macías *et al.* 2010).

The solutions were prepared with 10 mg of each extract, pre-dissolved in 5 μL ml^{-1} of dimethyl sulfoxide and diluted in a phosphate-citrate buffer (250 ml distilled water, 5 g sucrose, 0.2625 g citric acid and 0.725 g dibasic potassium phosphate, pH 5.6), at concentrations of 800, 400 and 200 ppm. In test tubes were added 2 mL of the solutions containing five wheat coleoptiles (Macias *et al.* 2010). We used a negative control, containing buffer solution and dimethyl sulfoxide, and a positive control, containing nicosulfuron herbicide (40 g L^{-1}).

The tubes were randomly distributed among the different treatments and were maintained at 25°C in the dark under a constant rotation of 0.25 rpm, with three repetitions per treatment (Macias *et al.* 2010). After 24 h, the coleoptiles were removed from the tubes and measured using the image digitization program (Image-Pro plus; Media Cybernetics, Silver Spring, MD, USA).

Chromatographic fractionation

The ethyl acetate extract obtained from direct solvent extraction, selected based on the wheat coleoptile bioassay, was submitted to fractionation on a chromatography column containing common silica as the stationary phase, under atmospheric pressure. For the mobile phase (Fig. 1), we used hexane 100 (250 ml); hexane/acetone at 95:5 (1400 ml), 90:10 (1100 ml), 80:20 (1500 ml), 70:30 (1000 ml), 60:40 (1000 ml), 40:60 (1000 ml), and 20:80 (900 ml); acetone 100 (500 ml); and methanol 100 (500 ml).

This procedure yielded 129 fractions, which were grouped in accordance with data obtained through thin layer chromatography, resulting in a total of 10 fractions (Fig. 1). Each chromatoplate was observed under ultraviolet light at wavelengths of 254 and 365 nm, and revealed with *Oleum* (a solution containing 10 ml of sulfuric acid, 200 ml of acetic acid and 40 ml of distilled water), and then heated to 150°C. The resulting fractions were subjected to bioassay with wheat coleoptiles, as previously described.

Statistical analysis

The data were subjected to normality (Shapiro-Wilk) and homogeneity (Levene) tests. When those 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.

Polynomial or exponential regression models were adjusted when the ANOVA *F* was significant. The goodness of the models was evaluated by its coefficient of determination (R^2). For the variables that showed no significant differences between treatments, the means were represented in the figures with their standard deviations.

Results and discussion

The aqueous extract of *S. saponaria* young leaves showed phytotoxic effect on the germination of lettuce and onion diaspores. The extract had a strong inhibitory effect on the lettuce: at concentrations of 7.5% and 10.0%, no cypsela germinated (Fig. 2 and Tab. 1). The estimated maximum

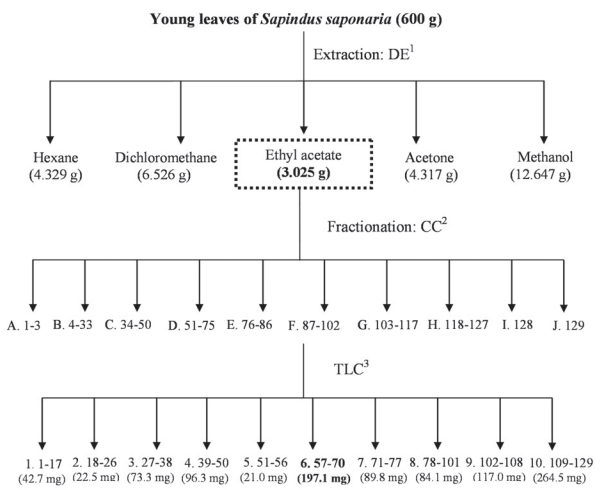

Figure 1. Fractioning and related yields of the powder of young leaves of *Sapindus saponaria* L. [1]Direct extraction. [2]Column chromatography. (A) hexane (100%); (B) hexane/acetone (95:5); (C) hexane/acetone (90:10); (D) hexane/acetone (80:20); (E) hexane/acetone (70:30) (F) hexane/acetone (60:40); (G) hexane/acetone (40:60); (H) hexane/acetone (20:80); (I) acetone (100%); (J) methanol (100%). [3]Thin-layer chromatography. The extracts and fractions in bold are those with the highest levels of phytotoxic activity in bioassays.

final germination time of onion seeds was 169 h in extract at a concentration of 4.11%, and there was a linear reduction in germinability of 8% for each addition of 0.01 mg ml^{-1} of extract. The coefficient of variation of the germination time (12.13%), as was uncertainty (0.73 bits) and the rate of onion seeds (0.02666 seeds h^{-1}) were lower at a concentration of 10.0%, indicating late germination and greater homogeneity of germinated seeds (Fig. 2). Other variables showed no significant differences between the concentrations (Fig. 2 and Tab. 1). Lettuce cypselas are widely used in allelopathy tests and are known for their sensitivity to plant extracts, which have the potential not only to reduce germinability but also to slow mean germination rate and other germination process variables (Wandscheer & Pastorini 2008; Scrivanti 2010, Souza *et al.* 2010).

In growth bioassays, the absence of normal seedlings of lettuce treated with extract of *S. saponaria* young leaf, can be seen at concentrations of ≥ 2.5% (Fig. 3), while for the onion from the 5% concentration (Fig. 4). In addition, we observed a linear increase of 6.05% of dead lettuce seedlings for each 0.01 mg ml^{-1} of extract added, and the proportion of dead onion seedlings reached its maximum estimated (91.32%) at a concentration of 12.55% (Fig. 3 and 4). The lowest shoot length for lettuce and onion seedlings were recorded at estimated concentrations of 7.30% and 7.59%, respectively (Fig. 3), whereas the lowest root length was recorded at concentrations of 6.95% and 7.09%, respectively (Fig. 4).

The main features found among abnormal lettuce and onions seedlings treated with *S. saponaria* young leaf extracts were the rotting, necrosis, complete atrophy or total absence of roots and shoots. These anomalies have been recorded in studies of lettuce seedlings treated with extracts of *Aristolochia esperanzae* Kuntze (Gatti *et al.* 2004) and *Esenbeckia leiocarpa* Engl. (Souza *et al.* 2010). The root system was more sensitive to allelochemicals than shoot seedling Root sensitivity to allelochemicals is well documented in the literature, as it is one of the characteristics that best indicates the phytotoxicity of plant extracts (Inoue *et al.* 2010; Souza *et al.* 2010; Grisi *et al.* 2012). This can be attributed to the fact that root growth is characterized by high metabolic rates and the roots are therefore highly susceptible to environmental stress, such as allelochemicals in the substrate (Cruz-Ortega *et al.* 1 998).

We found that *S. saponaria* young leaf extracts had a pH of 6.21, with an osmotic potential of −0.32 MPa. Although extreme acidity or alkalinity have been shown to have a negative effect on germination and seedling development (Souza Filho *et al.* 1996), we observed no such effect related to the pH of the extract.

In PEG-6000 solutions with osmotic potentials of 0, −0.2 and −0.3 MPa, respectively, 89%, 86% and 82% of lettuce cypselas germinated, compared with 90%, 89% and 86% of onion seeds. Considering that solutions with osmotic potential of −0.3 MPa did not significantly influence the germinability of these diaspores, we can infer that the reduction in the germination occurred mainly due to the presence of substances with phytotoxic activity in these extracts. Our decision to perform chemical extraction and fractionation of the powder of *S. saponaria* young leaves was based on these results, which indicated that the aqueous extract of the leaves is phytotoxic to lettuce and onions, inhibiting diaspore germination and seedling growth.

The various extracts, obtained through the direct extraction of the powder of *S. saponaria* young leaves with organic solvents, had varying yields (Fig. 1). Extracts with the highest polarity, such as that obtained with methanol (12.647 g), showed the highest yields. This might be due to the presence of free sugars, glycosylated products and hydrosoluble proteins (Stacciarini-Seraphin 2001) in the young leaves of the species.

As can be seen in Fig. 5, the extracts that showed significant inhibitory effect, compared to the negative control, were those obtained with ethyl acetate or acetone, and a similar effect was observed for the positive control (herbicide). The maximum inhibition recorded was 72% (at 800 ppm) for the ethyl acetate extract and 54% (at 400 ppm) for the acetone extract. Therefore, we selected the ethyl acetate extract for use in the column chromatography fractionation. In addition, the inhibitory effect of the ethyl acetate extract on the elongation of wheat coleoptiles was similar to that of the herbicide, which presented 72% inhibition at a dilution of 800 ppm. For most of the extracts tested, we observed no dose-dependent relationship, which can be explained by their low solubility in aqueous media.

As can be seen in Weston *et al.* (1989) and An *et al.* (2000), ethyl acetate has often been used to extract phytotoxins from plant residues. The ethyl acetate fraction obtained from the ethanol extract of *Senna occidentalis* (L.) Link had effects on the germination and growth of lettuce, tomato and onion that are similar to those obtained with commercial herbicides (Cândido *et al.* 2010). In addition, Souza Filho *et al.* (2006) showed that the ethyl acetate fraction from the leaves of *Myrcia guianensis* (Aubl.) DC. has an inhibitory effect on the germination of *Mimosa pudica* L..

From the ethyl acetate extract, we obtained 11 fractions (Fig. 1), which were subjected to bioassay with wheat coleoptiles. There was significant inhibition in relation to the negative control in the fractions 4 (at 800 ppm), 6 (at 200, 400 and 800 ppm), 7 (at 200, 400 and 800 ppm), 8 (at 200 ppm), 9 (at 200, 400 and 800 ppm) and 10 (at 200, 400 and 800 ppm). In terms of the mean length of wheat coleoptiles, fraction 6 showed the highest inhibitory activity (5.83 mm), similar to that of the herbicide (5.50 mm), indicating that the fraction contains organic compounds that are phytotoxic (Fig. 6).

In fractions 8, 9 and 10, the shortest wheat coleoptile lengths (5.20 mm, 5.99 mm and 6.01 mm, respectively) were recorded at the 200 ppm dilution (Tab. 3), indicating that phytotoxic activity is greater at lower dilutions. Phytotoxic potential can

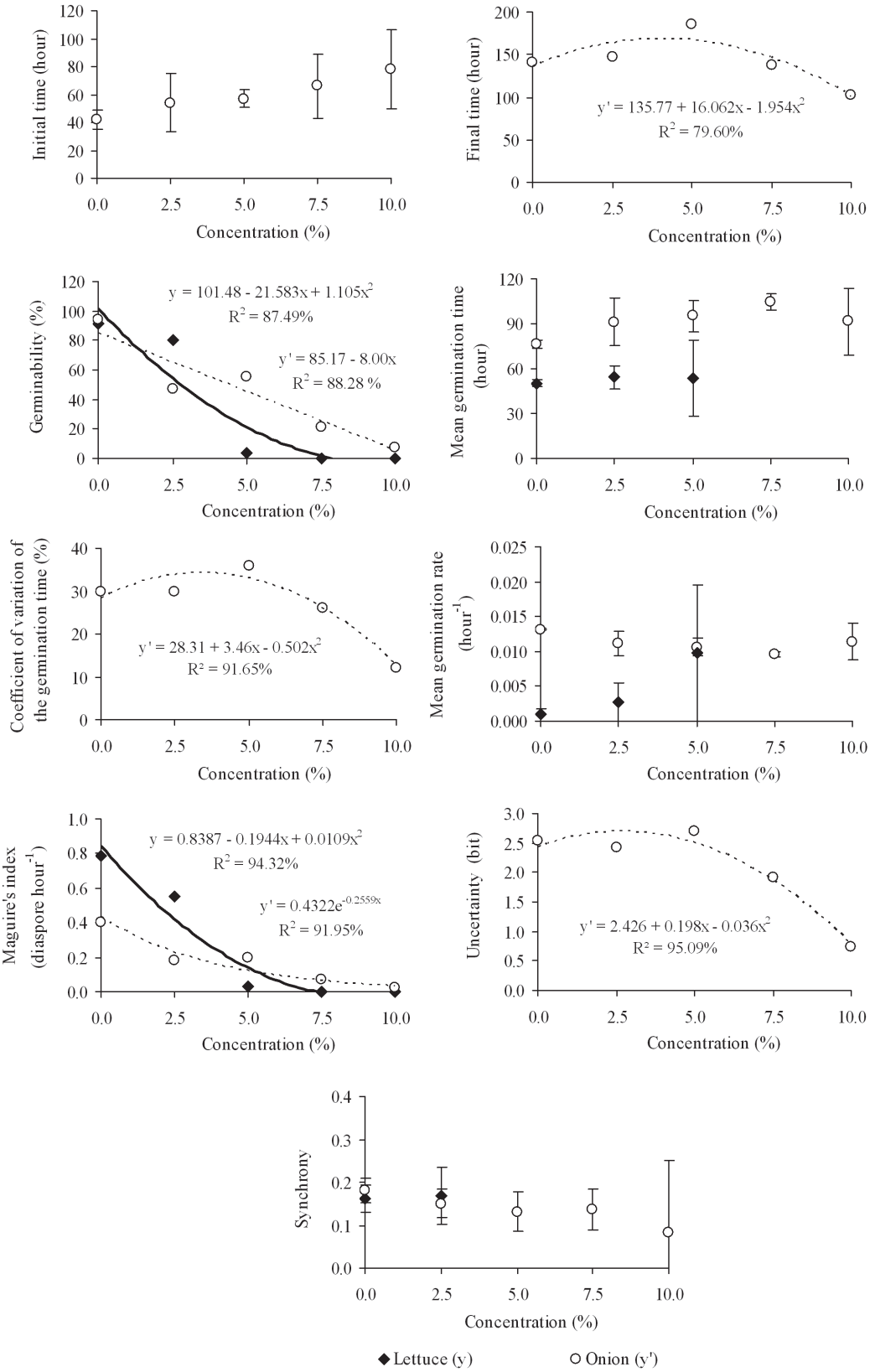

Figure 2. Initial and final germination time, germinability, mean germination time, coefficient of variation of the germination time, mean germination rate, rate, uncertainty and synchrony of germination process of *Lactuca sativa* L. (lettuce) and *Allium cepa* L. (onion) diasporess treated with aqueous extract of young leaves of *Sapindus saponaria* L. in different concentrations.

Table 1. Germination of *Lactuca sativa* L. (lettuce) and *Allium cepa* L. (onion) diaspores treated with aqueous extract of young leaves of *Sapindus saponaria* in different concentrations.

Measurement (unit)	Control	2.5%	5.0%	7.5%	10.0%	Statistics			
						*W** (*P*)	*F*** (*P*)	*F**** (*P*)	*H***** (*P*)
L. sativa									
T_i (h)	15.00 ± 6.00 a	24.00 ± 0.00 a	48.00 ± 16.97 b	---	---	**0.8861** (0.1461)	24.967 (0.0007)		**7.286** (0.01)
^1T_f (h)	186.00 ± 60.00 b	111.00 ± 24.74 ab	60.00 ± 33.94 a	---	---	**0.8248** (0.0285)	**1.746** (0.2426)	**6.313** (0.0271)	
t̃ (h)	50.18 ± 2.22 a	54.15 ± 8.07 a	54.00 ± 25.45 a	---	---	**0.9682** (0.8641)	25.894 (0.0006)		0.151 (0.8628)
CV_t (%)	76.22 ± 18.64 b	47.68 ± 8.15 b	16.67 ± 0.00 a	---	---	**0.9112** (0.2764)	**1.013** (0.4108)	**15.79** (0.0025)	
v̄ (h^{-1})	0.0200 ± 0.0009 a	0.0188 ± 0.0027 a	0.0208 ± 0.0098 a	---	---	**0.9608** (0.7828)	36.527 (0.0002)		0.182 (0.8375)
I (bit)	2.6492 ± 0.1650b	2.5817 ± 0.4766 b	1.5850 ± 0.0000 a	---	---	**0.9902** (0.9963)	36.348 (0.0002)		**4.418** (0.0109)
Z	0.1620 ± 0.0311 a	0.1694 ± 0.0671 a	---	---	---	**0.9793** (0.9596)	**1.943** (0.2128)	0.039 (0.8492)	
A. cepa									
T_i (h)	42.00 ± 6.93 a	54.00 ± 20.78 a	57.00 ± 6.00 a	66.00 ± 22.98 a	78.00 ± 28.57 a	**0.9502** (0.3819)	**3.514** (0.0325)	1.955 (0.1535)	
t̃ (h)	76.26 ± 2.80 a	91.19 ± 15.93 a	95.10 ± 10.46 a	104.61 ± 5.22 a	91.75 ± 22.28 a	**0.9484** (0.3551)	**3.808** (0.0249)	2.331 (0.1032)	
v̄ (h^{-1})	0.0131 ± 0.0001 a	0.0112 ± 0.0017 a	0.0106 ± 0.0013 a	0.0096 ± 0.0004 a	0.0114 ± 0.0026 a	**0.9662** (0.6699)	5.025 (0.009)		8.067 (0.0892)
Z	0.1816 ± 0.0278 a	0.1503 ± 0.0332 a	0.1302 ± 0.0460 a	0.1364 ± 0.0475 a	0.0833 ± 0.1667 a	0.8028 (0.0006)	**4.433** (0.0145)		5.246 (0.263)

Averages followed by the same letter in the line do not differ in Tukey's or Dunn's tests (significance level, 0.05); t_i:initial time; t_f: final time; t̃: mean germination time; CV_i: coefficient of variation of the germination time; v̄: mean germination rate; *U*: uncertainty; *Z*: synchrony

W* – Shapiro-Wilk test, boldfaced values indicate residuals normality (*P*>0,01); F** – Levene's test statistic, boldfaced values indicate homogeneity between variances (*P*>0,01); F*** – Snedecor's test; boldfaced values indicate significant difference among concentrations (ANOVA, *P*<0.05); ****H – Kruskal-Wallis test, boldfaced values indicate significant difference between concentrations (p<0.05); *P:* probability

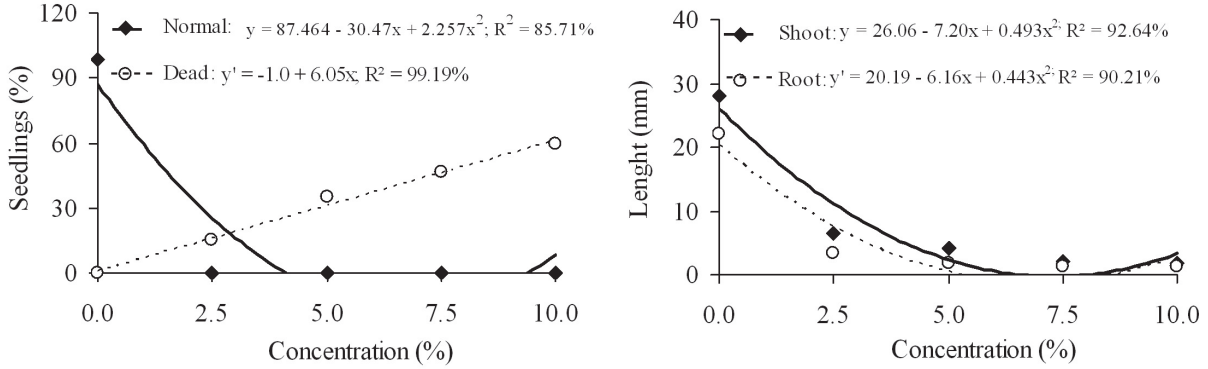

Figure 3. Percentage of normal and dead seedlings and shoot and root length of *Lactuca sativa* L. (lettuce) treated with aqueous extract of young leaves of *Sapindus saponaria* in different concentrations.

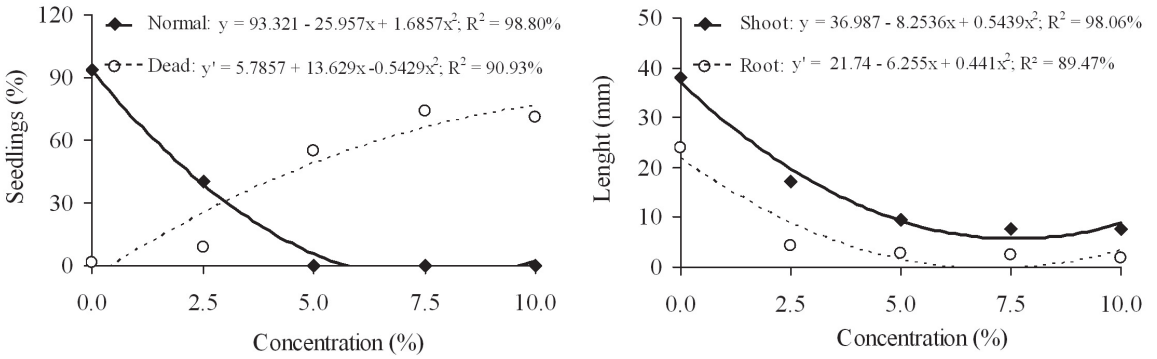

Figure 4. Percentage of normal and dead seedlings and shoot and root length of *Allium cepa* L. (onion) treated with aqueous extract of young leaves of *Sapindus saponaria* in different concentrations.

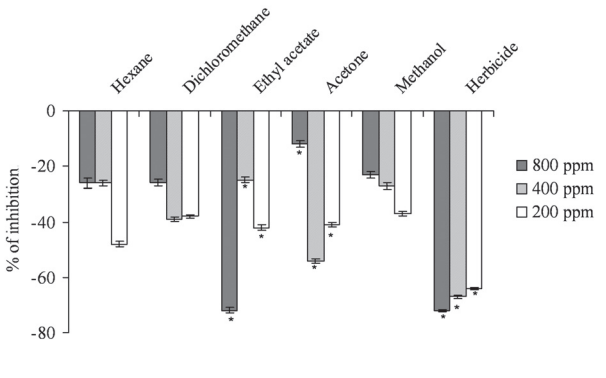

Figure 5. Percentage of inhibition, over negative control, of the length (mm) of wheat coleoptiles treated with different extracts obtained by extracting powder from the young leaves of *Sapindus saponaria* L. Vertical bars represent standard deviations. *Significant difference vs. negative control.

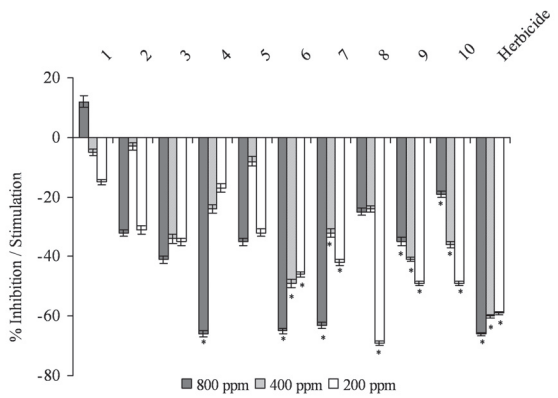

Figure 6. Percentage inhibition/stimulation, over negative control, for the fractions resulting from column chromatography fractionation, on the length *Triticum aestivum* L. (wheat) coleoptiles. Vertical bars represent standard deviations. *Significant difference vs. negative control.

vary as a function of the concentration of the solutions tested. Some authors claim that the action of allelochemical substances is not very specific and that the same substance can perform several functions, depending on its concentration and form of translocation (Maraschin-Silva & Aquila 2005).

Young leaves, for lack of physical protection, such as thorns and trichomes, often depend on the production of secondary metabolites to defend themselves against environmental aggression, such as that of herbivores. Because many species defend themselves metabolically in their juvenile stages of development (Bryant & Julkunen-Tiitto 1995), we can assumed that the concentration of allelochemicals is higher in young leaves than in mature leaves. This also applies to *S. saponaria*, Grisi *et al.* (2012) having reported that the phytotoxicity of young leaves of this species is greater than that of mature leaves. Other studies have also shown

that the inhibitory effect decreases as age increases, being highest at the early stages of plant development (Kobayashi *et al.* 2,008; Marchi *et al.* 2008). These results suggest that the maturation stage influences the production of allelochemicals and that phytochemical studies using young leaves may be more promise in the search for new substances.

There is a need for further fractionation using fraction 6 (57-70) of the ethyl acetate extract obtained from the powder of *S. saponaria* young leaves. Such studies should employ column chromatography, as well as purification with high-performance liquid chromatography and proton nuclear magnetic resonance, in order to identify the class of compounds responsible for the phytotoxic effect observed. However, it is important to note that bioguided fractionation is a useful and essential tool for the isolation of active compounds.

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