Phytotoxic Analysis of Extract of Leaves of *Solanum megalochiton* Mart. Solanaceae on *Lactuca sativa* L. and *Allium cepa* L.

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**ABSTRACT:** The phytotoxic effect of crude ethanol extracts and fractions of leaves of *Solanum megalochiton* Mart., Solanaceae on the germination, initial growth and respiration of *Lactuca sativa* L. (lettuce) and *Allium cepa* L. (onion) is analyzed. They were placed on 9.0-cm-diameter petri plates with filter paper 6 (Whatmann®). Assay was performed in a totally randomized design. Crude extract and fractions have a phytotoxic activity, with greatest effect on remaining fraction. Results show stimulation and inhibition on germination, growth and respiration. *S. megalochiton* contains metabolites with phytotoxic activity capable of affecting germination, growth and respiration of the species under analysis, with a potential for discovering new natural herbicide compounds.

**Keywords:** germination, growth, respiration, bioassay, Atlantic Rainforest.

**INTRODUCTION:**

Plants produce bioactive metabolites from their secondary metabolism and these metabolites contribute towards their survival or the development of defense mechanisms. They may be released into the environment through leaching, volatilization and root exudation, and they may cause modifications in the development of other plants (AGARWAL et al., 2002; ALVES et al., 2004; BORGHETTI, 2004; SERAFIMOV, 2005).

A species’s germination and growth changes may be caused by several effects at primary development level. Ferreira & Aquila (2000) and Maraschin-Silva & Aquila (2006) underscore, among these effects, changes in the permeability of membranes, DNA transcription and translation, functioning of other secondary messengers, respiration due to oxygen sequestration, conformation of enzymes and receptors and all the above factors combined. Einhellig (2002) lists other factors that may cause alteration in the plant’s germination and growth, among which may be mentioned changes in the characteristics of cell morphology, interference in cell cycle (replication, protein synthesis, mitosis, cell mechanisms), changes in phyto-hormonal activity, disorder of the energy metabolism (respiration and photosynthesis), problems in water balance and in the function of the stomata, inhibition in pigment synthesis and blockage of the function of several enzymes.

Since resistance or tolerance to bioactive metabolites of a plant is characteristically species specific, certain plants are more prone for tests on phytotoxic activity. The species *Lactuca sativa* L. (lettuce) and *Allium cepa* L. (onion) are species which indicate phytotoxic activities. They are not merely sensitive to low concentrations of phytotoxic compounds but also demonstrate fast and uniform germination and a linear growth which is scarcely sensitive to pH variations (GABOR & VEATH, 1981; SOUZA et al., 2007).

Analysis of plants with phytotoxic capacity contributes towards new alternatives for the managements of weeds and decreases the need for insecticides, nematicides and traditional herbicides in...
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agricultural production. Plants featuring phytotoxic activities are more selective and biodegradable and less polluting than traditional herbicides. Several research works on the phytochemistry of vegetal species have investigated vegetal extracts, fractions and their respective secondary metabolites which affect the development of other species. This is due to the use of allelopathy not merely as an in vitro assay for the tracing of metabolites but particularly as a biological screening for applied phytochemistry (MACIAS et al., 2000).

Solanum megalochiton Mart., a species of the family Solanaceae, commonly called joá-velame in Brazil, mainly occurs in regions covered by dense and mixed ombrophile forests, woods, forest edges or clearings and thickets, at altitudes up to 900m (MENTZ et al., 2004; SAMPAIO, 2013). It is widely distributed in Brazil, ranging from the northeastern region (Alagoas, Bahia), to the mid-western (Federal District, Goiás, Mato Grosso) and southeastern regions (Espírito Santo, Minas Gerais, Rio de Janeiro, São Paulo) to the southern region (Paraná, Rio Grande do Sul, Santa Catarina), mainly in savannah areas and in the Atlantic Rainforest (STEHMANN, 2014). Since the biomes are representative of Brazilian flora and due to the great devastation in the Atlantic Rainforest, studies on the species of these areas are important and highly relevant.

Research has already shown that plants of the genus Solanum have phytotoxic effects and inhibit the germination and growth of other plants (AGARWAL et al., 2002; SERAFIMOV et al., 2005; ALEKSIEVA & SERAFIMOVA, 2008). Due to their great diversity in chemical compounds such as glycoalkaloids, glucoside steroids (SUN et al., 2010), flavones and flavonoids (SILVA et al., 2003), hydrocarbonates, terpenes, fatty acids (ALIERO et al., 2006) and steroidal saponins (ZHOU et al., 2006), Solanum plants have phytotoxic capacities.

Current analysis evaluates the velocity index of germination, growth and respiration of L. sativa and A. cepa resulting from direct contact with crude ethanol extract and hexane, chloroform, ethyl acetate, remnants fractions of leaf of S. megalochiton.

MATERIALS AND METHODS

S. megalochiton leaves were collected in Curitiba PR Brazil in November 2012, at 25° 26’S and 49° 14’W, altitude 930 m. A sample was identified by the herbarium of the Municipal Botanic Garden Museum of Curitiba and vouchered as MBM 384849. Samples were dried and the leaves were ground. Crude ethanol extract (CE) was obtained from 500g of vegetal material in ethanol 70% by modified Soxhlet apparatus (CARVALHO et al., 2007). The CE was employed to obtain fractions by liquid/liquid partitioning technique with increasing polarity solvents resulting in hexane (HF), chloroform (CF), ethyl acetate (AF) and hydro-alcohol or remnant (RF) fractions. Extract and fractions were concentrated by rotation evaporator and dried in a warm bath (60°C). Assays were performed with CE, HF, CF, AF and RF (RECH et al. 2015).

The phytotoxic activity of crude extracts and fractions of leaves of S. megalochiton was assessed on seeds of target sensitive species L. sativa and A. cepa to identify the treatment with the highest phytotoxicity rates (MACIAS et al. 2000). Petri dishes (diameter 9.0 cm) with filter paper n. 6 (Whatmann®) were previously autoclaved and received a solution of 5.0 mL of samples (CE, HF, CF, AF and RF) at concentrations 250, 500, 1000 μg/mL and control solution (purified water). Analyses were performed in quadruplicate. Thirty seeds of the target species (L. sativa and A. cepa) were planted, following Brasil (2009). The plates were then placed in a germination chamber (BOD) with relative humidity at (±80%) and at constant temperature (25°C) for germination and growth.

Daily analysis for primary root protrusion (every 12 hours for lettuce; every 24 hours for onion) was undertaken to report seed germination velocity index (GVI). To avoid false germination, seeds with root protrusion of at least 50% of seed size were considered germinated (LABORIAU, 1983). Assay was concluded when germination failed to occur in three consecutive days. GVI was calculated for each replication according to number of germinated seeds, divided by the number of germination days, till the last day of germination (Macias et al., 2000; Hoffmann et al., 2007; Lima et al., 2011; RECH et al., 2015).

Growth test comprised maintenance of the material in a germinator (five days for lettuce and twelve days for onion) and the verification of results after the removal of 10 samples from each plate. Radicle and hypocotyl size of each sample was measured by millimeter paper (BARNES et al., 1987).

Respiration capacity was assessed at the end of the assay: ten plants were removed and their radicles separated. The latter were placed in contact with triphenyl-tetrazolium chloride solution 0.6% (p/v) in a phosphate buffer 0.05 M (pH 7.0) at 40°C for 12 h. The material was then washed twice in distilled water and placed in contact with ethanol 95% (v/v) and maintained in a warm bath at 95°C for 15 minutes or until dried. After cooling, it was placed again in contact with ethanol 95% (v/v) and read by spectrophotometer at 530 nm. The test was made in

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triplicate for each sample. The method was based on the reduction of triphenyl-tetrazolium chloride solution by dehydrogenase enzymes and the emergence of formazan (STEPONKUS; LANPHEAR, 1967).

RESULTS AND DISCUSSION

Table 1 shows the effect of different concentrations of extract and fractions of S. megalochiton and control on germination velocity index (GVI) of L. sativa and A. cepa seeds.

Table 1. Effect of different concentrations of crude ethanol extract and hexane, chlorophorm, ethyl acetate and residual fractions of leaves of Solanum megalochiton, Mart. Solanaceae and control on germination velocity index (GVI) of Lactuca sativa L. (lettuce) and Allium cepa L. (onion) seeds.

| Sample | Lactuca sativa L. | Allium cepa L. |
|--------|------------------|----------------|
|        | 250 µg/mL | 500 µg/mL | 1000 µg/mL | 250 µg/mL | 500 µg/mL | 1000 µg/mL |
| CE     | 23.85 ± 1.77 ^c | 22.25 ± 2.79 ^c | 21.25 ± 0.46 ^c | 19.60 ± 2.77 ^c | 15.35 ± 1.63 ^c | 14.82 ± 2.06 ^c |
| HF     | 17.20 ± 0.62 ^c | 19.84 ± 1.97 ^c | 19.19 ± 1.18 ^c | 18.14 ± 2.82 ^c | 14.96 ± 1.68 ^c | 11.91 ± 1.76 ^c |
| CF     | 17.15 ± 0.96 ^c | 15.82 ± 1.22 ^c | 14.40 ± 0.10 ^c | 18.89 ± 2.27 ^c | 21.18 ± 0.88 ^c | 16.00 ± 0.80 ^c |
| AF     | 17.90 ± 1.17 ^c | 17.23 ± 1.64 ^c | 14.71 ± 1.06 ^c | 20.36 ± 1.02 ^c | 19.07 ± 3.51 ^c | 15.83 ± 2.65 ^c |
| RF     | 18.62 ± 0.43 ^c | 20.13 ± 0.54 ^c | 20.20 ± 1.58 ^c | 20.14 ± 3.11 ^c | 20.68 ± 1.40 ^c | 16.73 ± 2.57 ^c |
| Control | 20.16 ± 1.35 ^c | 17.80 ± 1.50 ^c |            |         |         |         |

Germination Velocity Index (GVI), crude ethanol extract (CE), hexane fraction (HF), chlorophorm fraction (CF), ethyl acetate fraction (AF), residual fraction (RF). Means ± standard deviation. Treatment means differ significantly (p < 0.05) when compared to control means, inhibiting or stimulating germination velocity by Scott Knott test. Treatment means do not differ significantly from control means.

Analysis of the phytotoxic effects of CE, HF, CF, AF and RF of leaves of S. megalochiton on the seeds of L. sativa show interference on GVI inhibiting and stimulating germination.

CF and AF samples decreased the number of lettuce achenes germinated per experimental day when compared to control following concentration increase 250, 500 and 1000 µg/mL. Greatest germination inhibition occurred with dose 1000 µg/mL in CF and AF. Table 1 shows that inhibition reached 28 and 27% respectively when compared with the germination of the control group. In fact, glycoalcaloids of plants are found in AF and recent studies have revealed that several glycoalcaloids normally found in plants of the genus Solanum may alter the development of other plants by stimulating or inhibiting their germination (GUNTNER et al., 1997; GUNTNER et al., 2000, SUN et al., 2010). Further, HF at dose 250 µg/mL was also inhibiting (14%). According to Ferreira and Borguetti (2004), GVI rate is directly proportional to the vigor of lettuce achenes, or rather, the lower the GVI, the less is the seeds vigor.

CE stimulated germination at dose 250 µg/mL provided the best results, with 18% more germinated achenes when compared to control. The literature has scanty reference to the above-mentioned stimulating effect, although the information below is highly relevant to current analysis. Aquila et al. (1999) reported the phenomenon when they evaluated the phytotoxic activity of Achyrocline satureioides (Lam) DC. In another study, Gorla et al. (1997) also reported a 25% stimulating effect on the growth of radicles of tomato plants when they evaluated the phytotoxic activity of Drimis winteri extract, however, in this study, increase in concentration caused inhibitory activity.

CE and fractions also showed inhibitory and stimulating activities in the germination of A. cepa seeds. HF fraction at concentration 1000 µg/mL (33% inhibition) demonstrated the greatest inhibitory activity, followed by CE at concentration 1000 µg/mL with a 16% inhibition. Other samples were also inhibiting, such as CE at 500 µg/mL and CF and AF at 1000 µg/mL.

AF at concentration 250 µg/mL had the highest stimulating effect on the germination of A. cepa seeds, followed by RF at concentration 500 µg/mL, respectively with 21 and 16% germination percentage. Several other extract and fractions doses also revealed stimulating activities, such as CE with dose 250 µg/mL, CF at 250 and 500 µg/mL, AF at 500 µg/mL and RF at 250 µg/mL.

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Table 2 shows that the growth of *L. sativa* plants at concentration 250 µg/mL of CE stimulated hypocotyl growth, whereas concentration 1000 µg/mL inhibited radicle growth.

Table 2. Effect of different concentrations of crude ethanol extract and hexane, chlorophorm, ethyl acetate and residual fractions of leaves of *Solanum megalochiton*, Mart. Solanaceae on the growth of radicle, hypocotyl and coleoptile of *Lactuca sativa* L. and *Allium cepa* L. seeds.

| Sample | Radicle (mm) | Hypocotyl (mm) | Coleoptile (mm) |
|--------|-------------|----------------|-----------------|
|        | 250 µg/mL   | 500 µg/mL      | 1000 µg/mL      | 250 µg/mL      | 500 µg/mL      | 1000 µg/mL      |
| CE     | 1.38 ± 0.13c | 1.21 ± 0.17c   | 0.62 ± 0.05c    | 0.49 ± 0.04c   | 0.46 ± 0.06c   | 0.38 ± 0.07c   |
| HF     | 1.26 ± 0.09c | 1.00 ± 0.10c   | 1.08 ± 0.07i    | 0.42 ± 0.03c   | 0.40 ± 0.04c   | 0.41 ± 0.01c   |
| CF     | 1.24 ± 0.13c | 1.05 ± 0.12c   | 0.75 ± 0.09c    | 0.47 ± 0.01c   | 0.42 ± 0.05c   | 0.39 ± 0.03c   |
| AF     | 1.28 ± 0.05c | 1.01 ± 0.11c   | 0.74 ± 0.04i    | 0.53 ± 0.18c   | 0.46 ± 0.06c   | 0.52 ± 0.05c   |
| RF     | 1.11 ± 0.03i | 0.72 ± 0.04i   | 0.46 ± 0.04i    | 0.43 ± 0.02c   | 0.35 ± 0.03i   | 0.31 ± 0.03i   |
| Control| 1.37 ± 0.11c | 0.45 ± 0.03c   |                  |                |                |                |

**GROWTH**

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Crude ethanol extract (CE), hexane fraction (HF), chlorophorm fraction (CF), ethyl acetate fraction (AF), residual fraction (RF). Means ± standard deviation. Treatment means differ significantly (p < 0.05) when compared to control means ‘i’inhibiting or ‘c’stimulating germination velocity by Scott Knott test. Treatment means do not differ significantly from control means.

Variation in growth stimulation of an organ and the inhibition of another may be due to allelochemical effects (AQUILA et al., 1999). Albeit not so common, other research works have already described this effect, perhaps due to the activity of phytotoxic compounds on phytohormones. Reigosa et al. (1999) reported that the effects of phytotoxic compounds in different physiological processes of a plant depend on concentration or they may be expected to be, enhancing activations at low concentrations and inhibitions at high ones. A study by Pires et al. (2001) suggests that slight interference on the aerial section may be due to the use of nutritional reserves of plant seeds within this development stage.

RF at 1000 µg/mL, with 0.46 mm growth, caused the greatest inhibition in the radicle growth of *L. sativa*, followed by CE within the same concentration with 0.62 mm growth. The above represents 67 and 55% growth inhibition, respectively, when compared to the growth of control plants. All fractions at 250 µg/mL remained statistically similar to control. Only CE at 250 µg/mL inhibited the growth of lettuce radicle. Several studies have shown that allelochemicals-caused changes in the radicle are the best indicators of the phytotoxic capacity of the extracts (CHON et al., 2000; FERREIRA & ÁQUILA, 2000; OLIVEIRA, 2003; GATTI et al., 2004).

In the case of the growth development of *L. sativa* hypocotyl, RF at concentration 1000 µg/mL, with mean growth at 0.31 mm, caused the greatest inhibition, followed by concentration 500 µg/mL with mean growth 0.35 mm, or rather, 31 and 22% inhibition respectively, when compared to the development of control plants. Although the above result may be due to the stimulus caused by allelochemicals, it may also be related to low growth of the plants radicles which affected the development of hypocotyls.

AF at concentration 250 µg/mL caused the greatest hypocotyl stimulus with mean growth 0.53 mm, followed by concentration of 1000 µg/mL, with mean growth 0.52 mm, or rather, 17 and 15% stimulus when compared to control. HF remained statistically similar to control at all concentrations 250, 500 and 1000 µg/mL.

Decrease of the hypocotyl-radicle axis when in contract with the sample is generally the most reported alteration in growth (AQUILA, 1999; RODRIGUES, 2002). Current assay showed decrease
and increase of plant growth. Growth decrease is normally more observed in the development of the radicle since it has the greatest contact with the sample in the filter paper (CHUNG et al., 2001). As may be seen in the assay, the radicles developed as those in control or developed growth inhibition. There was no growth stimulus for lettuce radicles. Extract and fractions inhibited the growth of lettuce radicle at concentration 1000 µg/mL whilst all RF concentrations inhibited the growth of lettuce radicle.

Radicles revealed the highest sensitiveness to allelochemicals when compared to hypocotyls, already registered by several authors (Chon et al. 2000; Ferreira & Aquí 2000; Batish et al. 2002). Whereas the radicle segment showed a 66% inhibition, the greatest inhibition in hypocotyls merely reached 31%. Growth stimulus and inhibition may be observed in radicles in A. cepa plants. CE at concentration 1000 µg/mL caused the greatest inhibition in radicle growth with a mean growth of 1.12 mm, or 25% inhibition. Concentration at 250 µg/mL of extract and fractions generally stimulated the group of onion radicle; only CF at the same concentration failed to stimulate growth and remained statistically similar to control. RF at concentration 250 µg/mL caused the greatest radicle growth with an increase of 1.84 mm, or a 21% stimulus. RF concentrates the greatest amount of glycoalkaloids, corroborating studies by Sun et al. (2003) who reported stimulus for cucumber growth caused by glycoalkaloids isolated from Solanum plants.

Growth of the hypocotyl was inhibited by CE at concentration 1000 µg/mL and also caused the greatest growth inhibition of onion coleoptile with mean growth 1.88 mm between plants, followed by CF at 1000 µg/mL with 2 mm, or rather, 27 and 22% inhibition respectively. Studies on Solanum lycocarpum extract did not reveal any difference in the development of sesame coleoptile (Jeronimo, 2003). Growth stimulus was not reported for this assay. Data are given in Table 2.

There are currently more than 10000 allelochemicals derived from plants secondary metabolism (MALHEIROS; PERES, 2001). Interferences on other plants are normally observed due to the synergism of two or more compounds and not merely to an isolated compound (Sun et al., 2010). Growth inhibition of A. cepa radicle and coleoptile caused by CE may be due to the fact that the sample has the greatest number of chemical compounds which together caused the most important result in current assay.

Stimulus caused by CE at concentration 250 µg/mL and by HF at concentration 1000 µg/mL occurred in the respiration of lettuce radicles. CF at concentration 1000 µg/mL inhibited respiration of radicles. Strong stimulus of respiration of A. cepa radicle was reported for the samples tested. Stimulus was mainly observed in contact with EC at concentration 250 µg/mL, HF and CF at concentration 1000 µg/mL and CF at concentration 500 µg/mL. Table 3 provides the necessary data.

Table 3. Effect of different concentrations of crude ethanol extract and hexane, chlorophorm, ethyl acetate and residual fractions of leaves of Solanum megalochiton, Mart. Solanaceae on the respiration radicle, hypocotyl and coleoptile of Lactuca sativa L. (lettuce) and Allium cepa L. (onion) seeds.

| Sample | Lettuce - Lactuca sativa L. | Onion – Allium cepa L. |
|--------|-----------------------------|-------------------------|
|        | 250 µg/mL | 500 µg/mL | 1000 µg/mL | 250 µg/mL | 500 µg/mL | 1000 µg/mL |
| CE     | 0.20 ± 0.02<sup>±</sup>    | 0.18 ± 0.04<sup>±</sup> | 0.19 ± 0.04<sup>±</sup> | 0.68 ± 0.03<sup>±</sup> | 0.12 ± 0.02<sup>±</sup> | 0.09 ± 0.01<sup>±</sup> |
| HF     | 0.19 ± 0.03<sup>±</sup>    | 0.16 ± 0.04<sup>±</sup> | 0.20 ± 0.05<sup>±</sup> | 0.08 ± 0.01<sup>±</sup> | 0.03 ± 0.06<sup>±</sup> | 0.09 ± 0.01<sup>±</sup> |
| CF     | 0.19 ± 0.04<sup>±</sup>    | 0.13 ± 0.02<sup>±</sup> | 0.11 ± 0.00<sup>±</sup> | 0.10 ± 0.01<sup>±</sup> | 0.17 ± 0.01<sup>±</sup> | 0.67 ± 0.06<sup>±</sup> |
| AF     | 0.17 ± 0.02<sup>±</sup>    | 0.19 ± 0.02<sup>±</sup> | 0.14 ± 0.02<sup>±</sup> | 0.07 ± 0.00<sup>±</sup> | 0.08 ± 0.00<sup>±</sup> | 0.08 ± 0.00<sup>±</sup> |
| RF     | 0.15 ± 0.00<sup>±</sup>    | 0.14 ± 0.00<sup>±</sup> | 0.14 ± 0.05<sup>±</sup> | 0.06 ± 0.01<sup>±</sup> | 0.10 ± 0.01<sup>±</sup> | 0.11 ± 0.01<sup>±</sup> |
| Control| 0.18 ± 0.03<sup>±</sup>    | 0.08 ± 0.01<sup>±</sup> | 0.08 ± 0.01<sup>±</sup> | 0.08 ± 0.01<sup>±</sup> | 0.08 ± 0.01<sup>±</sup> | 0.08 ± 0.01<sup>±</sup> |

Crude ethanol extract (CE), hexane fraction (HF), chlorophorm fraction (CF), ethyl acetate fraction (AF), residual fraction (RF). Means ± standard deviation. Treatment means differ significantly (p < 0.05) when compared to control means ’inhibiting or ’stimulating germination velocity by Scott Knott test. Treatment means do not differ significantly from ‘control means.

Allelochemicals can affect the germination and growth of plants, and they have great influence on cell respiration to (RICE, 1984; REIGOSA et al., 1999). Allelochemicals may interfere at several stages of cell respiration, at one or more levels, depending on the response given (CHON et al., 2000).

Song, Zheng and Chun (1992) underscore that alterations in normal physiological processes reduce photosynthesis and contribute towards plants’ growth reduction. The above has been reported in onion plants with a great increase in respiration and the
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subsequent decrease in plant size.

CONCLUSION

S. megalochiton has a phytotoxic activity on Lactuca sativa (lettuce) and Allium cepa (onion) since extract and fraction caused changes in germination, growth and respiration velocity. Identification of phytotoxic compounds may contribute towards the discovery of new natural compounds with phytoherbicide capacity. Therefore, the isolation and identification of secondary metabolites of S. megalochiton are greatly promising on future studies. Since RF provided the most relevant results, it is actually the fraction in which secondary compounds become more interesting for the isolation and identification process and in future tests as phytoherbicides. Current assay reveals that a highly accessible native plant may be employed as a non-toxic method for the ecosystem and may reduce the use of herbicides in the environment.

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