International Journal of Sciences

Research Article Volume 5 – November 2016 (11)

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

Mariana Saragioto Krause[1], Ana Flavia Schvabe Duarte[1], Francis José Zortéa Merino[1] Cristiane da Silva Paula[1], Marilis Dallarmi Miguel[1], Obdulio Gomes Miguel[1]

[1]Universidade Federal do Paraná, Departamento de farmácia, Av. Lothario Meissner, 632, Caixa Postal 19081, Jardim Botânico, CEP 80210-170, Curitiba- PR

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

Published at: http://www.ijsciences.com/pub/issue/2016-11/
DOI: 10.18483/ijSci.1142; Online ISSN: 2305-3925; Print ISSN: 2410-4477

Mariana Saragioto Krause (Correspondence)
msaragioto@yahoo.com.br
+55 41 9581-0660

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 & SERAFIMOV, 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), hydrocarbonetes, 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 25º 26'S and 49º 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

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

Statistical analysis was performed with SISVAR 5.3 (FERREIRA, 2000) and comparison of means was undertaken by Scott-Knott test at 5% probability.

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) E *Allium cepa* L. (onion) seeds.

	GVI					
	Lactuca sativa L.			*Allium cepa* L.		
Sample	250 µg/mL	500 µg/mL	1000 µg/mL	250 µg/mL	500 µg/mL	1000 µg/mL
CE	23.85 ± 1.77 ^{e}	22.25 ± 2.79 ^{e}	21.25 ± 0.46 ^{e}	19.60 ± 2.77 ^{e}	15.35 ± 1.63 ^{i}	14.82 ± 2.06 ^{i}
HF	17.20 ± 0.62 ^{i}	19.84 ± 1.97 ^{c}	19.19 ± 1.18 ^{c}	18.14 ± 2.82 ^{c}	14.96 ± 1.68 ^{i}	11.91 ± 1.76 ^{i}
CF	17.15 ± 0.96 ^{i}	15.82 ± 1.22 ^{i}	14.40 ± 0.10 ^{i}	18.89 ± 2.27 ^{e}	26.18 ± 1.80 ^{e}	16.00 ± 0.80 ^{i}
AF	17.90 ± 1.17 ^{i}	17.23 ± 1.64 ^{i}	14.71 ± 1.06 ^{i}	21.68 ± 0.82 ^{e}	19.07 ± 3.51 ^{e}	15.83 ± 2.65 ^{i}
RF	18.62 ± 0.43 ^{c}	20.13 ± 0.54 ^{c}	20.20 ± 1.58 ^{c}	20.14 ± 3.11 ^{e}	20.68 ± 1.40 ^{e}	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 ^{i}inhibiting or ^{e}stimulating germination velocity by Scott Knott test. Treatment means do not differ significantly from ^{c}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, glycoalkaloids of plants are found in AF and recent studies have revealed that several glycoalkaloids 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.

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.

GROWTH						
Lactuca sativa L.						
	Radicle (mm)			Hypocotyl (mm)		
Sample	250 µg/mL	500 µg/mL	1000 µg/mL	250 µg/mL	500 µg/mL	1000 µg/mL
CE	1.38 ± 0.13 [c]	1.21 ± 0.17 [c]	0.62 ± 0.05 [i]	0.49 ± 0.04 [e]	0.46 ± 0.06 [c]	0.38 ± 0.07 [c]
HF	1.26 ± 0.09 [c]	1.00 ± 0.10 [i]	1.08 ± 0.07 [i]	0.42 ± 0.03 [c]	0.40 ± 0.04 [c]	0.41 ± 0.01 [c]
CF	1.24 ± 0.13 [c]	1.05 ± 0.12 [i]	0.75 ± 0.09 [i]	0.47 ± 0.01 [e]	0.42 ± 0.05 [c]	0.39 ± 0.03 [c]
AF	1.28 ± 0.05 [c]	1.01 ± 0.11 [i]	0.74 ± 0.04 [i]	0.53 ± 0.18 [e]	0.46 ± 0.06 [c]	0.52 ± 0.05 [e]
RF	1.11 ± 0.03 [i]	0.72 ± 0.04 [i]	0.46 ± 0.04 [i]	0.43 ± 0.02 [c]	0.35 ± 0.03 [i]	0.31 ± 0.03 [i]
Control		1,37 ± 0,11 [c]			0,45 ± 0,03 [c]	
Onion – *Allium cepa* L.						
	Radicle (mm)			Coleoptile (mm)		
Sample	250 µg/mL	500 µg/mL	1000 µg/mL	250 µg/mL	500 µg/mL	1000 µg/mL
CE	1.69 ± 0.29 [e]	1.45 ± 0.18 [c]	1.12 ± 0.16 [i]	2.45 ± 0.35 [c]	2.34 ± 0.31 [c]	1.88 ± 0.23 [i]
HF	1.64 ± 0.23 [e]	1.34 ± 0.13 [i]	1.69 ± 0.29 [e]	2.43 ± 0.20 [c]	2.19 ± 0.06 [i]	2.45 ± 0.35 [c]
CF	1.54 ± 0.41 [c]	1.77 ± 0.07 [e]	1.38 ± 0.12 [i]	2.68 ± 0.15 [c]	2.55 ± 0.11 [c]	2.00 ± 0.38 [i]
AF	1.77 ± 0.12 [e]	1.78 ± 0.14 [e]	1.58 ± 0.10 [c]	2.19 ± 0.72 [i]	2.67 ± 0.20 [c]	2.40 ± 0.14 [c]
RF	1.84 ± 0.06 [e]	1.65 ± 0.18 [e]	1.45 ± 0.10 [c]	2.60 ± 0.15 [c]	2.38 ± 0.36 [c]	2.50 ± 0.16 [c]
Control		1.51 ± 0.16 [c]			2.58 ± 0.20 [c]	

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 [e]stimulating germination velocity by Scott Knott test. Treatment means do not differ significantly from [c]control means.

Variation in growth stimulation of an organ and the inhibition of another may be due to allelochemical effects (AQÜILA *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 & Áquila 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/m L 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 (Jerônimo, 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.

RESPIRATION						
	Lettuce - *Lactuca sativa* L.			Onion – *Allium cepa* L.		
Sample	250 µg/mL	500 µg/mL	1000 µg/mL	250 µg/mL	500 µg/mL	1000 µg/mL
CE	0.20 ± 0.02 ^{e}	0.18 ± 0.04 ^{c}	0.19 ± 0.04 ^{c}	0.68 ± 0.03 ^{e}	0.12 ± 0.02 ^{c}	0,09 ± 0,01 ^{c}
HF	0.19 ± 0.03 ^{c}	0.16 ± 0.04 ^{c}	0.20 ± 0.05 ^{e}	0.08 ± 0.01 ^{c}	0.63 ± 0.06 ^{e}	0,09 ± 0,01 ^{c}
CF	0.19 ± 0.04 ^{c}	0.13 ± 0.02 ^{c}	0.11 ± 0.00 ^{i}	0.10 ± 0.01 ^{c}	0.17 ± 0.01 ^{e}	0,67 ± 0,06 ^{e}
AF	0.17 ± 0.02 ^{c}	0.19 ± 0.02 ^{c}	0.14 ± 0.02 ^{c}	0.07 ± 0.00 ^{c}	0.08 ± 0.00 ^{c}	0,08 ± 0,00 ^{c}
RF	0.15 ± 0.00 ^{c}	0.14 ± 0.00 ^{c}	0.14 ± 0.05 ^{c}	0.06 ± 0.01 ^{c}	0.10 ± 0.01 ^{c}	0,11 ± 0,01 ^{c}
Control	0.18 ± 0.03 ^{c}			0.08 ± 0.01 ^{c}		

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 ^{e}stimulating germination velocity by Scott Knott test. Treatment means do not differ significantly from ^{c}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

subsequent decrease in plant size.

CONCLUSION

S. megalochitom 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.

REFERENCES

1. AGARWAL, A.; GAHLOT, A.; VERMA, R.; RAO, P. Effects of weed extracts on seedling growth of same varieties of wheat. **Journal of Environmental Biology**, v. 23, p.19-23, 2002.
2. ALEKSIEVA, A.; SERAFIMOV, P.L. A study of allelopathic effect of *Amaranthus retroflexus* (L.) and *Solanum nigrum* (L.) in different soybean genotypes. **Herbologia**, v. 9, n. 2, p. 47-58, 2008.
3. ALIERO, A.; ASEKUN, O.; GRIERSON, D.; AFOLAYAN, A. Chemical composition of the hexane extract from the leaves of *Solanum pseudocapisicum*. **Asian Journal of Plant Sciences**, v. 5, n. 6, p. 1054-1056, 2006.
4. ALVES, M.C.S.; MEDEIROS FILHO, S.; INNECO, R.; TORRES, S.B. Alelopatia de extratos voláteis na germinação de sementes e no comprimento da raiz de alface. **Pesquisa Agropecuária Brasileira,** Brasília, v. 39, p. 1083-1086, 2004.
5. AQUILA, M.E.A. Preliminary observation on allelopathic activity in *Achyrocline satureiodes* (Lam.) DC. **Acta Horticulturae,** p.383-388, 1999.
6. BARNES, J.P.; PUTNAM, A.R. Role of benzoxazinones in allelopathy by rye *(Secale cereale).* **Journal of Chemical Ecology**, v. 13, p. 889-906, 1987.
7. BATISH, D.R.; SINGH, H.P.; KOHLI, R.K.; SAXENA, D.B.; KAUR, S. Allelopathic effects of parthenin against two weedy species, *Avena fatua* and *Bidens pilosa*. **Environmental and Experimental Botany**, v. 47, p. 149 – 155, 2002.
8. BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. Regras para análise de sementes. Brasília: Mapa/ACS; 2009. 398 p.
9. BORGHETTI, F.; PESSOA, D.M.A. Autotoxicidade e alelopatia em sementes de *Solanum lycocarpum* St. Hil. (Solanaceae). In: L.L. Leite & C.H. Saito (orgs.). **Contribuição ao conhecimento ecológico do cerrado**. Editora da UnB, Brasília, DF, p. 54-58, 1997.
10. CHON, S. U.; COUTTS, J. H.; NELSON, C. J. Effects of light, growth media and seedling orientation on bioassays of alfalfa autotoxicity. **Agronomy Journal,** v. 92, p. 715-720, 2000.
11. CHUNG, I.M.; AHN, L.K.; YUN, S.J. Assessment of allelopathic potential of barnyard grass (*Echinochloa crus-gall*) on rice (*Oriza sativa* L.) cultivars. **Crop Protection**, v. 20, p. 921-928, 2001.
12. EINHELLIG, F.A. The physiology of allelochemical action: Clues and Viewa. In: REIGOSA, M.; PEDROL, N. (Ed.). Allelopathy from Molecules to Ecosystems. **Vigo**: Universidade de Vigo, p.1-23, 2002.
13. FERREIRA, A.G.; AQUILA, M.E. Alelopatia: uma área emergente da ecofisiologia. **Revista Brasileira de Fisiologia Vegetal**. v. 12, p. 175-204, 2000.
14. FERREIRA, A.G.; BORGHETTI, F. **Germinação: do básico ao aplicado**. Porto Alegre: Artmed, 2004.
15. FERREIRA, D.F. Sistema de análises de variância para dados balanceados (SISVAR). Pacote computacional. Lavras: UFLA; 2000.
16. GABOR, W. E.; VEATCH, C. Isolation of phytotoxin from quackgrass (*Agropyon repens*) rhizomes. **Weed Science**, Champaign, v. 29, p. 155-159, 1981.
17. GATTI, A.B.; PEREZ, S.C.J.G.A.; LIMA, M.I.S. Atividade alelopática de extratos aquosos de *Aristolochia esperanzae* O. Kuntze na germinação e no crescimento de *Lactuca sativa* L. e *Raphanus sativus* L. **Acta Botânica Brasílica**, v. 18, n. 3, p. 495-472, 2004.
18. GORLA, C.M.; PEREZ, S.C.J.G.A. Influência de extratos aquosos de folhas de *Miconiaalbicans* Triana, *Lantana camara* L., *Leucaena leucocephala* (Lam) de Wit e *Drimyswinteri* Forst, na germinação e crescimento inicial de sementes de tomate e pepino. **Revista Brasileira de Sementes**, v.19, n.2, p.260-265, 1997.
19. GUNTNER, C.; GONZALEZ, A.; REIS, R.; GONZALEZ, G.; VAZQUEZ, A.; FERREIRA, F.; MOYNA, P. effect of *Solanum* glycoalkaloids on potato aphid, *Macrosiphum euphorbiae.* **Journal of Chemical Ecology**, v. 23, n. 6, 1997.
20. GUNTNER, C.; VÁZQUEZ, A.; LEZ, G.G.; USUBILLAGA, A.; FERREIRA, F.; MOYNA, P. Effect of *Solanum* glycoalkaloids on potato aphid, *Macrosiphum euphorbiae*: PART II. **Journal of Chemical Ecology**, v. 26, v. 5, 2000.
21. HOFFMANN, C.E.F.; NEVES, L.A.D.; BASTOS, C.F.; WALLAU, G.L. Atividade alelopática de *Nerium oleander* L. e *Dieffenbachia picta Schott* em sementes de *Lactuca sativa L.* e Bidens pilosa L. **Revista de Ciências Agroveterinária**, v. 6, n. 1, p.:11-21, 2007.
22. JERONIMO, C.A. Alelopatia de extratos de folhas de *Solanum lycocarpum* St. Hil. no crescimento inicial e na síntese proteica de plântulas de *Sesamun indicum* L. Monograph. faculdade da Terra de Brasília, Recanto das Emas, p.37, 2003.
23. LABORIAU, L.G. **A germinação das sementes**. Secretaria Geral da Organização dos Estados Americanos, Washington, p. 170, 1983.
24. LIMA, C.P.; CUNICO, M.M.; TREVISAN, R.R.; PHILIPPSEN, A.F.; MIGUEL, O.G.; MIGUEL, M.D. Efeito alelopático e toxicidade frente à *Artemia salina* Leach dos extratos do fruto de *Euterpe edulis* Martius. **Acta Botânica Brasilica**, v. 25, n. 2, p. 331-336, 2011.
25. MACIAS, A.F. *et al.* Search for a standard phytotoxic bioassay for allelochemicals. Election of standard target species. **Journal of Agricultural and Food Chemistry**, v. 48, n. 6, p. 2512-21, 2000.
26. MALHEIROS, A.; PERES, M.T.L.P. Alelopatia**:** interações químicas entre espécies. In: YUNES, R. A.; CALIXTO, J. B. Plantas medicinais sob a ótica da química medicinal moderna. **Chapecó**: Argos, p. 503-523, 2001.
27. MARASCHIN-SILVA, F.; AQUILA, M.E.A. Potencial alelopático de espécies nativas na germinação e crescimento inicial de Lactica sativa L. (Asteraceae). **Acta botanica Brásílica**, v. 20, n. 1, p. 61-69, 2006.
28. MENTZ, L.A.; OLIVEIRA, P.L. Solanum (Solanaceae) na Região Sul do Brasil. Pesquisas, Botânica, n. 54. Instituto Anchietano de Pesquisas, p.327, 2004.
29. OLIVEIRA, S.C.C. Alelopatia em *Solanum lycocarpum* St. Hil. (Solanaceae). Master´s Dissertation, Universidade de Brasília, Brasilia, p.78, 2003.
30. PIRES, N.M.; SOUZA, I.R.P.; PRATES, H.T.; FARIA, T.C.L.; PEREIRA FILHO, I.A.; MAGALHAES, P.C., Efeito do extrato aquoso de leucena sobre o desenvolvimentos, índice mitótico e atividade da peroxidase em plântulas de

milho. **Revista Brasileira de Visiologia Vegetal**, v. 14, n. 1, p. 55-65, 2001.

31. RECH, K.S., SILVA, C.B.;. KULIK, J.D.; DIAS, J.F.G.; ZANIN, S.M.W.; KERBER, V.A; OCAMPOS, F.M.M.; DALARMI, L.; SANTOS, G.O.; SIMIONATTO, E.; LIMA, C.P.; MIGUEL, O.G.; MIGUEL, M.D. ***Croton argenteus* preparation inhibits initial growth, mitochondrial respiration and increase the oxidative stress from *Senna occidentalis* seedlings.** Anais da Academia Brasileira de Ciências, v. 87, n. 2, 2015.
32. REIGOSA, M.J.; SÁNCHEZ-MOREIRAS, A.; GONZÁLEZ, L. Ecophysiological approach in allelopathy. **Critical Reviews in Plant Sciences**, v. 18, n. 5, p. 577-608, 1999.
33. RICE, E.L. **Allelopathy**. 2ª ed. New York, Academic Press, 1984.
34. SAMPAIO, V. S. **O Gênero Solanum L. (Solanaceae) na Floresta Atlântica ao norte do Rio São Francisco.** Master´s Dissertation. Universidade Federal de Pernambuco. Recife, PE, 2013.
35. SERAFIMOV, P.; SABEV, V.; GOLUBINOVA, I. Inhibiting effect of water extract from black nightshade (*Solanum nigrum* L.) on the initial germinability of soybean seeds. Collection of scientific reports from the jubilee scientific conference on selection and technological aspects of production and processing of soybeans and other legumes, 08/09/2005 - **Pavlikeni**, p. 100-106, 2005.
36. SILVA, T.; CARVALHO, M. & BRAZ-FILHO, R. Ocorrencia de flavonas, flavonóis e seus glicosideos em especies do genero *Solanum* (Solanaceae). **Química Nova, n. 26, v.** 4, p. 517-522, 2003.
37. SONG, F. M.; ZHENG, Z.; CHUN, G. X. Role of active oxygen and membrane lipid peroxidation in plantpathogegen interactions. **Plant Physiology Journal**. v. 32, p. 377-385, 1992.
38. SOUZA, C. S. M.; SILVA, W. L. P.; GUERRA, A. M. N. M.; CARDOSO, M. C. R.; TORRES, S. B. Alelopatia do extrato aquoso de folhas de aroeira na germinação de sementes de alface. **Revista Verde de Agroecologia e Desenvolvimento Sustentável**, v. 2, p. 96-100, 2007.
39. STEHMANN, J. R.; MENTZ, L. A.; AGRA, M. F.; VIGNOLI-SILVA, M.; GIACOMIN, L.; RODRIGUES, I. M. C. Solanaceae in Lista de Espécies da Flora do Brasil. Jardim Botânico do Rio de Janeiro. Available at <http://reflora.jbrj.gov.br/jabot/floradobrasil/FB14810>. on 17/10/2016.
40. CARVALHO, J. L. S.; MIGUEL, M. D.; MIGUEL, O. G.; DADALT, R. C. Processo de obtenção de extratos hidroalcoólico, extratos secos e derivados do agrião (*Nasturtium officinale*) e espécies medicinais afins, com modificações introduzidas em equipamento Soxhlet para aplicações na industria , área farmacêutica, cosmética, alimentícia e afins. Brasil, PI 0601703-7A, 2007.
41. STEPONKUS, P. L.; LANPHEAR, F. O. Refinement of the triphenyl tetrazolium chloride method of determining cold injury**. Plant Physiology**, v. 10, p. 1423-6, 1967.
42. SUN, F.; LI, S.; HE, D.; CAO, G.; NI, X.; TAI, G.; ZHOU, Y.; WANG, D. Effects of glycoalkaloids from *Solanum* plants on cucumber root growth. **Phytochemistry**, v. 71, p. 1534-1538, 2010.
43. ZHOU, X.; HE, X.; WANG, G.; GAO, H.; ZHOU, G.; YE, W. & YAO X. Steroidal saponins from *Solanum nigrum*. **Journal of Natural Products**, v. 69, p. 1158-1163, 2006.