PLANTA DANINHA

SOCIEDADE BRASILEIRA DA CIÊNCIA DAS PLANTAS DANINHAS

SBCPD

<http://www.sbcpd.org>

ISSN 0100-8358 (print)
1806-9681 (online)

Article

Allelopathic Potential of Ethanolic Extract and Phytochemical Analysis of *Paspalum maritimum* Trind

*Potencial Alelopático do Extrato Etanólico e Análise Fitoquímica de **Paspalum maritimum** Trind*

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ABSTRACT - Allelopathy is defined as the ability of certain plants and microorganisms to interfere with the metabolism of other species through substances released into the environment, being an alternative to control weeds and diseases. In this context, this study aimed to evaluate the allelopathic potential and identify groups of secondary metabolites of *Paspalum maritimum* Trind. The species *Lactuca sativa*, *Digitaria insularis*, *Emilia coccinea*, and *Portulaca oleracea* were used as recipient plants. The ethanolic extract was obtained from shoot and root of donor species to evaluate the allelopathic potential. Potentially allelopathic effects were evaluated by means of germination tests, germination speed index, and initial seedling growth. A phytochemical analysis of the extract was also performed to identify the secondary metabolites. The ethanolic extract from both plant parts had an allelopathic effect on recipient species. The presence of condensed tannins, chalcones and aurones, flavonones, steroids, and saponins were detected in the most active fraction of the shoot of *P. maritimum*.

Keywords: allelopathy, ginger grass, secondary metabolites.

*RESUMO - Alelopatia é definida como a capacidade que determinadas plantas e microrganismos têm de interferir no metabolismo de outras espécies, por meio de substâncias liberadas no ambiente, podendo ser uma alternativa de combate às plantas daninhas e doenças. Nesse contexto, este estudo teve por objetivos avaliar o potencial alelopático e identificar os grupos de metabólitos secundários de **Paspalum maritimum** Trind. As espécies **Lactuca sativa**, **Digitaria insularis**, **Emilia coccinea** e **Portulaca oleracea** foram utilizadas como plantas receptoras. Para avaliar o potencial alelopático, foram obtidos o extrato etanólico da parte aérea e radicular da espécie doadora. Os efeitos potencialmente alelopáticos foram avaliados por meio de testes de germinação, índice de velocidade de germinação e crescimento inicial de plântulas. Foi realizada também a análise fitoquímica do extrato, para identificar os metabólitos secundários. O extrato etanólico, de ambas as partes da planta, causaram efeito alelopático nas espécies receptoras. Foi detectada, na fração mais ativa da parte aérea de **P. maritimum**, a presença de taninos condensados, chalconas e auronas, flavononas, esteroides e saponinas.*

Palavras-chave: alelopatia, capim-gengibre, metabólitos secundários.

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Received: August 27, 2017
Approved: March 8, 2018

Planta Daninha 2019; v37:e019184596

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Doi: 10.1590/S0100-83582019370100053

INTRODUCTION

The interference of weeds in the productivity of crops of economic interest has caused great losses, being necessary appropriate management of them to reduce damages. Therefore, despite the significant contribution of conventional weed control methods to improving crop productivity, certain challenges are also associated with them, making it increasingly necessary the use of diversified management options. Thus, the research with species that have allelopathic potential is increasingly in evidence from the perspective of its manipulation for practical applications in agriculture and thus be used in weed control (Jabran and Farooq, 2013; Zeng, 2014).

Chemical substances are usually extracted from crushed plant structures placed in contact with water or organic extractor (ethanol, acetone, ether, chloroform, etc.) and an extract containing allelopathic compounds is obtained after filtration (Pires and Oliveira, 2011). The demonstration of allelopathic effects has been carried out experimentally through the application of extracts of the donor species in order to verify the effects of allelochemicals in seeds or seedlings of recipient species. However, allelochemicals from various plant parts are the result of secondary metabolism and responsible for producing allelopathic effects on the metabolism of recipient species (Taiz and Zeiger, 2013).

A great variety of secondary metabolites with allelopathic potential, such as fatty acids, aromatic acids, phenolic acids, aldehydes, lactones, coumarins, quinones, terpenes, sesquiterpenes, phenols, tannins, alkaloids, flavonoids, terpenoids, and steroids, has been already identified (Tur et al., 2010; Rice, 2013). These metabolites are divided into three main groups, composed of phenolic compounds, terpenes, and alkaloids (Ferreira and Aquila, 2000; Rice, 2013). They affect various physiological processes, including seedling growth (Peer and Murphy, 2007; Buer and Djordjevic, 2009) and seed germination (Hemmerlin et al., 2003; Carmo et al., 2007).

In this context, *Paspalum maritimum* Trind, popularly known as ginger grass, stands out since it is one of the weed species that are notable for its high capacity to invade cultivated areas, forming pure colonies and quickly dominating the area, presenting possible allelopathic effects (Souza Filho, 2006; Correa et al., 2010; Souza Filho, 2010). Studies aiming at controlling weeds through the allelopathic phenomenon has allowed concluding that allelochemicals interfere with many of the primary metabolic processes and plant growth system. Therefore, this study aimed to evaluate the allelopathic potential and identify groups of secondary metabolites of *P. maritimum* Trind.

MATERIAL AND METHODS

Botanical material

Recipient species

Lactuca sativa (lettuce), considered sensitive to allelopathy tests (Ferreira and Aquila, 2000), and the weed species *Digitaria insularis* (sourgrass), *Emilia coccinea* (scarlet tasselflower), and *Portulaca oleracea* (common purslane), which occur widely in various crops, were used as recipient species. These species were collected in areas of infestation, except for *L. sativa* (variety Veneranda), which was purchased from Feltrin Sementes®.

Donor species

Paspalum maritimum was selected as a donor species to evaluate the possible allelopathic potential. The procedures of collection and herborization of the plant sample were performed based on the methodologies of Cartaxo et al. (2010). The plant was collected in the experimental area of the Center of Agricultural Sciences (CECA/UFAL) in Rio Largo (AL), located at the geographical coordinates 9°27'56" S and 35°49'41" W. The botanical identification was carried out in the MAC Herbarium of the Institute of the Environment/AL and a voucher specimen was deposited under the No. 64,247.

Obtaining the extract

The extract was obtained from the shoot and root of *P. maritimum*, collected during the flowering stage. After collection, the material was washed in running water to remove impurities and shoot was separated from the root and taken to a forced air circulation oven at 60 °C until constant weight. After this process, the dehydrated material was ground in a Willey-type mill in a 30 mesh sieve until smaller fragments were obtained, resulting in a shoot and root material of 346 and 594 g, respectively. The obtained powder was stored in sealed containers and maintained at -20 °C until the moment of its use (Hagemann et al., 2010).

Root and shoot portions of the plant were submerged separately in 95% ethyl alcohol at a ratio of 1:5 (w/v), stirred and maintained at ambient temperature for 72 hours. Subsequently, the obtained extracts were filtered and the residue of the material was again extracted using the same solvent for 72 hours. This process was repeated several times until obtaining a clear extraction. The extracts from all extractions were collected and concentrated with the aid of a reduced pressure rotaevaporator at an average temperature of 50 °C, obtaining 16.72 and 13.91 g of extracts from the shoot and roots of *P. maritimum*, respectively (Al-Sherif et al., 2013).

Preparation of sample solutions

Concentrations of 1.25, 2.50, 5.00, 10.00, and 20.00 mg mL^{-1} were obtained through the solubilized crude extract by adding 0.1% dimethylsulfoxide (DMSO) and distilled water, being then submitted to ultrasonication until its complete solubilization and to avoid possible solubilizer interference. Water and DMSO were used as a control.

Liquid-liquid partition

The liquid-liquid partition was carried out with the crude ethanolic extract from the shoot of *P. maritimum* for all the solvents. Initially, 12.5 g of the crude extract was suspended with 250 mL of methanol in a separatory funnel and the solvent n-hexane was added for extraction (3 x 250 mL). After this process, hexane fraction was removed, being the methanol fraction suspended in 250 mL of water and partitioned with chloroform (3 x 250 mL). The chloroform fraction was removed and ethyl acetate (3 x 250 mL) was added to the methanol fraction with water. The four fractions resulting from this process were submitted to drying of the solvent in the reduced pressure rotaevaporator at an average temperature of 50 °C (Oliveira et al., 2012).

For determining the most active fraction of this partition, the closest concentration that inhibited 50% germination of *L. sativa* (2.5 mg mL^{-1}) was selected. Tests of germination, shoot length, and root length of seedlings of this species were conducted.

Phytochemical analysis

Aliquots of crude extracts and fractions from the partition with hexane, chloroform, ethyl acetate, and methanol of the shoot of *P. maritimum* were prepared to verify the existence of groups such as phenols, pyrogallic tannins, phlobaphenic tannins, anthocyanins, anthocyanidins, flavones, flavonols, xanthones, chalcones, aurones, flavononols, leucoanthocyanidins, catechins, steroids, flavonones, triterpenoids, and saponins by color change or precipitate formation, following the methodology of Mattos (1997).

Allelopathic activity

The experimental design was a completely randomized design with four replications. Each extract was considered an individual experiment at increasing concentrations, aiming at observing the allelopathic effect on different recipient species. The potentially allelopathic effects of the ethanolic extract of *P. maritimum* were evaluated by means of germination tests, germination rate index, and initial seedling growth.

Evaluation of germination (G)

These tests were carried out in transparent plastic gerbox-type boxes with a lid (11 x 11 x 4 cm) with two sheets of germitest paper. The paper was moistened with 6 mL of each extract and 30, 50, and 200 seeds of *L. sativa* (cultivar Baba de Verão), *D. insularis*, *E. coccinea*, and *P. oleracea* was placed on it, respectively. These boxes were maintained in a BOD (Biochemical Oxygen Demand) chamber at 25 °C and 12 hour photoperiod for *L. sativa* and at an alternating temperature of 20 to 30 °C and 12 hour photoperiod for the weed species *D. insularis*, *E. coccinea*, and *P. oleracea*. The seeds were evaluated every 24 hours to verify root protrusion, according to Borghetti and Ferreira (2004). At the end of the experimental time, 7 days of sowing for *L. sativa* and *D. insularis* and 14 days for *E. coccinea* and *P. oleracea*, seedlings were evaluated for the shoot and root development and classified as normal or abnormal (Brasil, 2009).

Evaluation of the germination rate index (GRI)

The data obtained in the daily counts performed in the germination test were used to calculate GRI. The data were tabulated and calculated according to the Maguire (1962) equation:

$$GRI = (N1/E1) + (N2/E2) + \ldots + (Nn/En)$$

where GRI is the germination rate index, E1, E2, and En are the number of seedlings in the first, second, and last count, and N1, N2, and Nn is the number of days of sowing in the first, second, and last count.

Evaluation of seedling length

The seedling length was evaluated together with the germination test using normal seedlings. Shoot (SL) and primary root length (RL) were evaluated by means of a millimeter ruler. Osmotic potential of extracts was measured using a Wescor osmometer. A germination bioassay was performed with recipient species submitted to treatments with solutions of polyethylene glycol 6000 (PEG-6000) at concentrations of osmotic potentials corresponding to those of the extract.

Statistical analysis

The original data were submitted to analysis of variance at 5 and 1% probability by the F test and the means of treatments were compared by the Tukey's test using the SISVAR statistical program. The data were transformed into $\sqrt{(x + 0.5)}$ (Barbin, 2003). The data referring to the concentrations were verified through the application of the F test on the analysis of variance. When the effect of the extract was significant in determining the LC_{50} (inhibitory concentration equivalent to 50% of effect in relation to the control), the data were adjusted to the three-parameter logistic nonlinear regression model (Streibig, 1988) by the program Sigmaplot:

$$y = \frac{a}{\left[1 + \left(\frac{x}{b}\right)\right]^2}$$

where y is the percentage of control, x is the extract concentration, and a, b, and c are the estimated parameters of the equation so that a is the amplitude between the maximum and minimum point of the variable, b is the concentration that provides 50% response of the variable, and c is the slope of the curve around b.

The logistic model has advantages since one of the terms of the equation (b) is an estimate of the LC_{50} value (Christoffoleti, 2002). Because it is a good parameter used to determine the 50% controlling dose in herbicides, it was adapted to determine this concentration in allelopathy. LC_{50} is the concentration of extract that provides 50% of control or reduction of growth of the recipient species (Christoffoleti, 2002; Christoffoleti and López-Ovejero, 2008).

The values of control were considered 100% and the results of each variable of the other treatments were calculated in relation to the control:

$$VR\ (\%) = 100 \times V / Cm$$

where VR is the variable in relation to the control (%), V = analyzed variable (G, GRI, SL or RL), and Cm is the mean of the control treatment (%).

RESULTS AND DISCUSSION

Extracts of *P. maritimum* presented values of osmotic potential between -0.015 and -0.126 MPa (Table 1). The tested PEG-6000 solutions (-0.0 and -0.2 MPa) had no influence on the percentages of germination of recipient species, showing that the effects are possibly due to the allelochemicals contained in the extract (data not shown).

Table 1 - Osmotic potential of the ethanolic extract of the shoot and root of *C. ensiformis*

Concentration (mg mL^{-1})	Shoot extract	Root extract
	Ψs (Mpa)	
0.00	-0.015	-0.015
1.25	-0.089	-0.064
2.50	-0.105	-0.059
5.00	-0.104	-0.077
10.00	-0.106	-0.076
20.00	-0.104	-0.126

Ethanolic extract

Dose-response curves clearly represent the patterns observed in the species *L. sativa*, *D. insularis*, *E. coccinea*, and *P. oleracea* when submitted to the ethanolic extract of *P. maritimum*. The equations used to describe the response of different species as a function of concentrations were satisfactory since most of them presented a coefficient of determination above 95% (Table 2).

Shoot and root ethanolic extracts of *P. maritimum* showed a possible allelopathic potential for the analyzed species. As extract concentration increased, there was a decrease in germination for all recipient species. The species *L. sativa* and *D. insularis* exposed to shoot (Figure 1A) and root extracts (Figure 1B) of *P. maritimum* had the lowest concentration for germination, with an LC_{50} of 3.28 and 5.55 mg mL^{-1} and 1.74 and 2.80 mg mL^{-1} (Table 2), respectively. The root extract of *P. maritimum*, when compared to that of the shoot of the same species, presented a lower LC_{50} for the percentage of germination of recipient species (Table 2).

Souza Filho (2006) compared the allelopathic effects of the shoot and root of *P. maritimum* on *Mimosa pudica* (Fabaceae), *Senna obtusifolia* (Fabaceae), *Pueraria phaseoloides* (Fabaceae), and *Brachiaria brizantha* cv. Marandu (Poaceae) and observed a higher inhibition of their germination with the shoot extract. The root extract, on the other hand, caused a higher inhibition of the root length of seedlings, differing from the results obtained in our study, which presented a higher inhibition of germination with the root extract of *P. maritimum*.

In addition, the shoot and root of *P. maritimum* showed allelopathic effect, corroborating the study of Borges et al. (1994), who found that all plant organs have the potential to store allelochemicals, but their chemical nature and quantity may vary with plant age and organ, as well as other factors. The quantity and paths by which they are released differ from species to species (Taiz and Zeiger, 2013). Therefore, species present different allelopathic responses of compounds from different organs of the same plant (Souza Filho et al., 2010).

For the germination rate index (GRI), there was an interaction between the concentrations of ethanolic extract and recipient species for all the analyzed extracts (Figure 2). For all recipient species, seed germination rate decreased as concentrations increased. The species *E. coccinea* and *D. insularis* had the lowest LC_{50} (1.78 and 2.90 mg mL^{-1}) (Table 2) for the shoot and root extracts of *P. maritimum*, respectively (Figure 2A and B, respectively). Similarly, Wandscheer and

Table 2 - Estimates of the parameters a, b, and c and coefficient of determination (R^2) of the log-logistic model for germination (G), germination rate index (GRI), shoot length (SL), root length (RL), and dry matter (DM) of seeds of recipient species using shoot (ST) and root (RT) ethanolic extract of *P. maritimum*

Variable	Plant part	Recipient species	Parameter[1]			R[2]	F[3]
			a	b	c		
G	ST	*L. sativa*	104.12**	3.287**	5.95**	0.99	574.06
		D. insularis	84.59**	5.55**	4.73	0.97	32.18
		E. coccinea	93.37**	6.54**	3.16*	0.99	92.41
		P. oleracea	99.54**	5.56**	12.14	0.99	9875.42
	RT	*L. sativa*	100.04**	1.74**	4.52**	0.99	10890.47
		D. insularis	92.40**	2.80*	2.21	0.96	19.31
		E. coccinea	99.69**	3.95*	1.15*	0.96	21.83
		P. oleracea	90.35*	4.70	1.54	0.93	11.15
GRI	ST	*L. sativa*	101.69**	3.89**	2.75**	0.99	1328.74
		D. insularis	84.94**	7.49**	4.82	0.97	32.04
		E. coccinea	100.06**	1.78*	1.14*	0.99	81.30
		P. oleracea	97.13**	5.77	12.34	0.99	681.86
	RT	*L. sativa*	93.09**	3.86**	7.72	0.99	181.58
		D. insularis	93.72**	2.90*	2.03	0.97	24.90
		E. coccinea	96.94**	5.62*	1.17*	0.97	23.94
		P. oleracea	92.13**	9.62**	3.95	0.98	57.21
SL	ST	*L. sativa*	81.19**	5.5672*	5.89	0.96	21.46
		D. insularis	84.24**	6.56*	2.78	0.96	19.05
		E. coccinea	95.76**	5.025	0.96	0.92	8.44
		P. oleracea	82.62**	5.91	10.21	0.97	27.27
	RT	*L. sativa*	79.00**	11.42	5.93	0.96	17.88
		D. insularis	81.12**	6.00	15.71	0.97	23.88
		E. coccinea	84.43**	11.61	8.09	0.95	13.96
		P. oleracea	98.13*	1.99	0.80	0.92	8.72
RL	ST	*L. sativa*	100.12**	2.86**	2.14**	0.99	164.66
		D. insularis	97.70**	2.67*	1.42*	0.98	53.20
		E. coccinea	99.82**	1.13	0.76	0.98	41.65
		P. oleracea	96.58**	5.742	13.46	0.99	971.11
	RT	MOS[4]	99.26**	1.81*	1.26*	0.98	41.68

[1] Model: y = y0 + a/(1 + (x/b) c); [2] Coefficient of determination of the regression curve; [3] F-value for the non-linear regression; [4] Means of the species; ** Significant at 1% probability ($p<0.01$); * Significant at 5% probability ($0.01 \leq p<0.05$).

Pastorini (2008) observed that root and leaf extracts of *Raphanus raphanistrum* (Brassicaceae) caused changes in GRI of seeds of *L. sativa* at all extract concentrations.

The delay in germination rate is an indicator of the allelopathic effect on cell elongation and division. The lower the GRI is, the longer the time required to germinate. Hoagland and Williams (2004) have observed that it can occur through the activation of cellular detoxification mechanisms. They also observed that the time required for the activation of this mechanism retards the germination.

Ferreira and Borghetti (2004) reported that the allelopathic effect might not occur on the percentage of germination but on other processes of plant development. Ferreira and Aquila (2000) also demonstrated the fact that germination is less sensitive to secondary metabolites than seedling growth.

In this sense, ethanolic extracts from the shoot and root of *P. maritimum* contributed to decreasing shoot length in a more intensely way as the concentration increased (Figure 3), indicating that the allelopathic effect of plant extract is dependent on the concentrations. For the shoot of *P. maritimum* (Figure 3A), *E. coccinea* showed a lower LC_{50} when compared to the

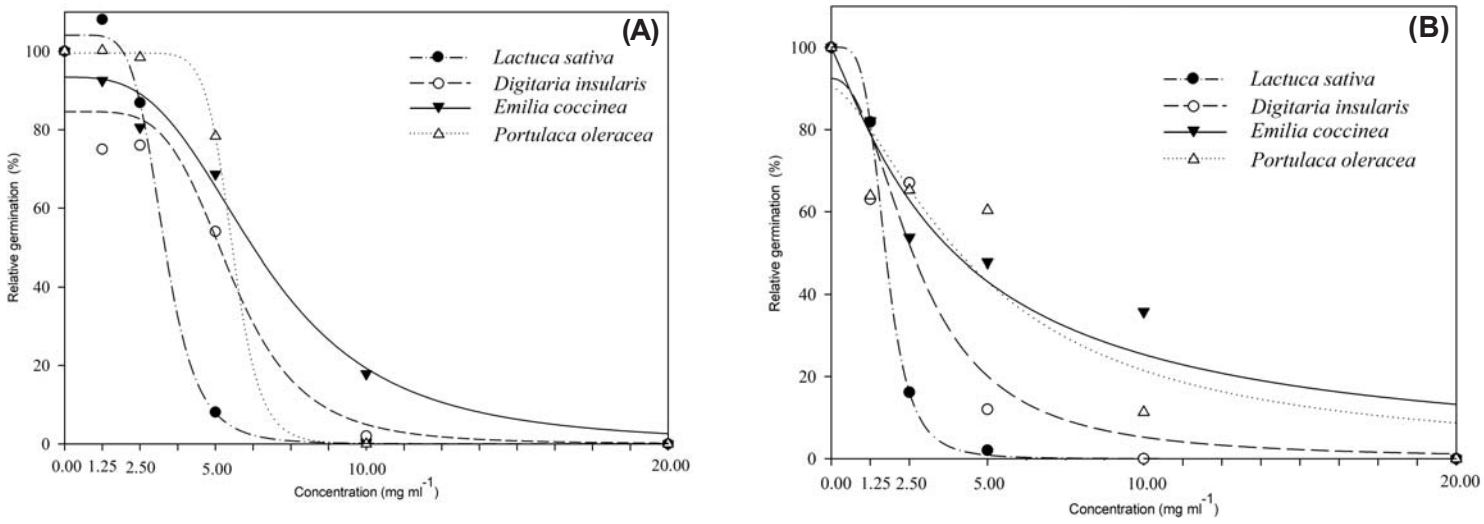

Figure 1 - Percentage of germination of recipient species as a function of increasing concentrations of ethanolic extract of the shoot (A) and root (B) of *P. maritimum*.

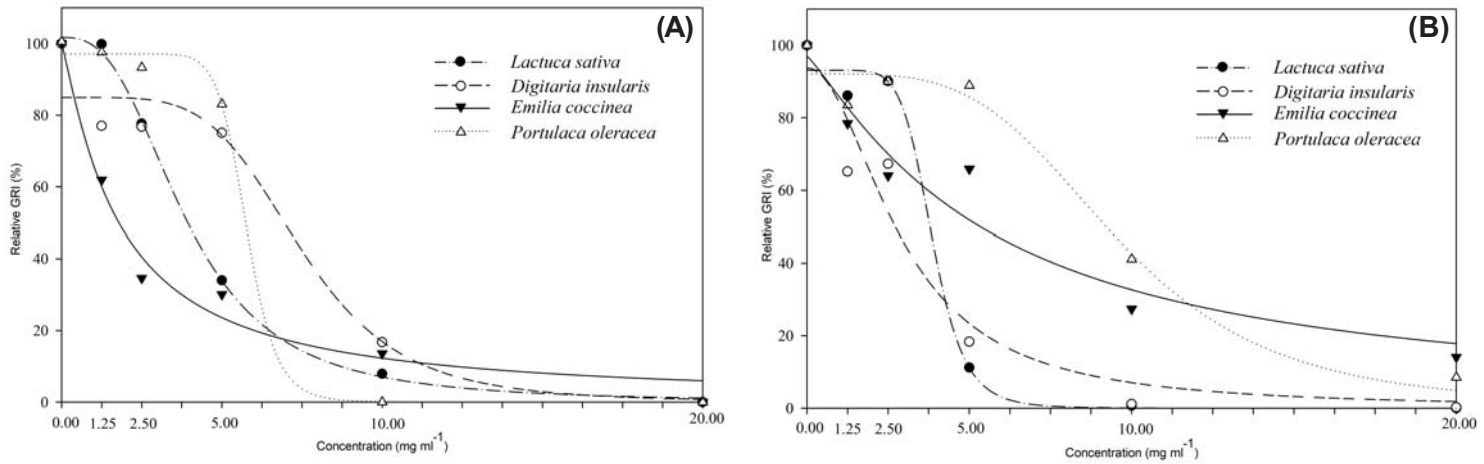

Figure 2 - Germination rate index (GRI) of seedlings of recipient species as a function of increasing concentrations of ethanolic extract of the shoot (A) and root (B) of *P. maritimum*.

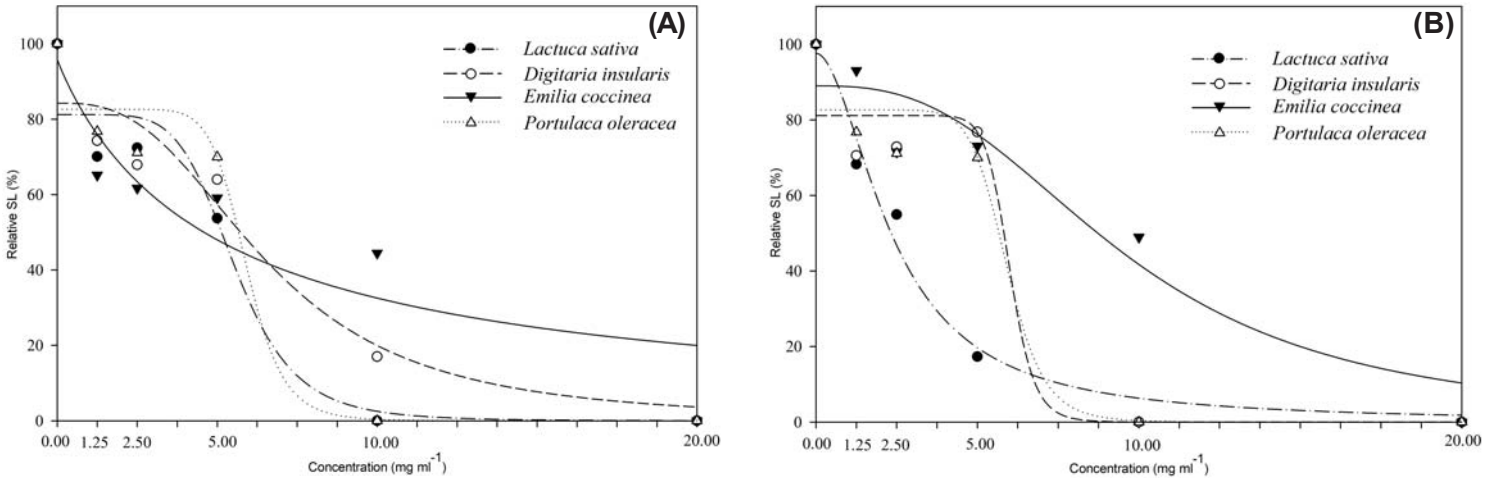

Figure 3 - Shoot length (SL) of seedlings of recipient species as a function of increasing concentrations of ethanolic extract of the shoot (A) and root (B) of *P. maritimum*.

other recipient species (LC_{50} = 5.02 mg mL^{-1}) (Table 2). Among all the analyzed recipient species, *L. sativa* had the lowest LC_{50} (2.39 mg mL^{-1}) for the root extract of *L. maritimum* (Figure 3B), which was the most sensitive species for seedling length. Differences in sensitivity among recipient species are common in studies addressing allelopathy (Marinov-Serafimov, 2010).

Seedlings also showed a reduction in root length under the influence of concentrations of the ethanolic extract (Figure 4). With the shoot extract of *P. maritimum* (Figure 4A), *E. coccinea* was more sensitive regarding the root length of seedlings, presenting an LC_{50} of 1.12 mg mL^{-1}, while *P. oleracea* required a higher concentration for the reduction of root length, with an LC_{50} of 5.74 mg mL^{-1} (Table 2). For the root of *P. maritimum*, the decrease in root length of seedlings was proportional to the increase in concentrations (Figure 4B).

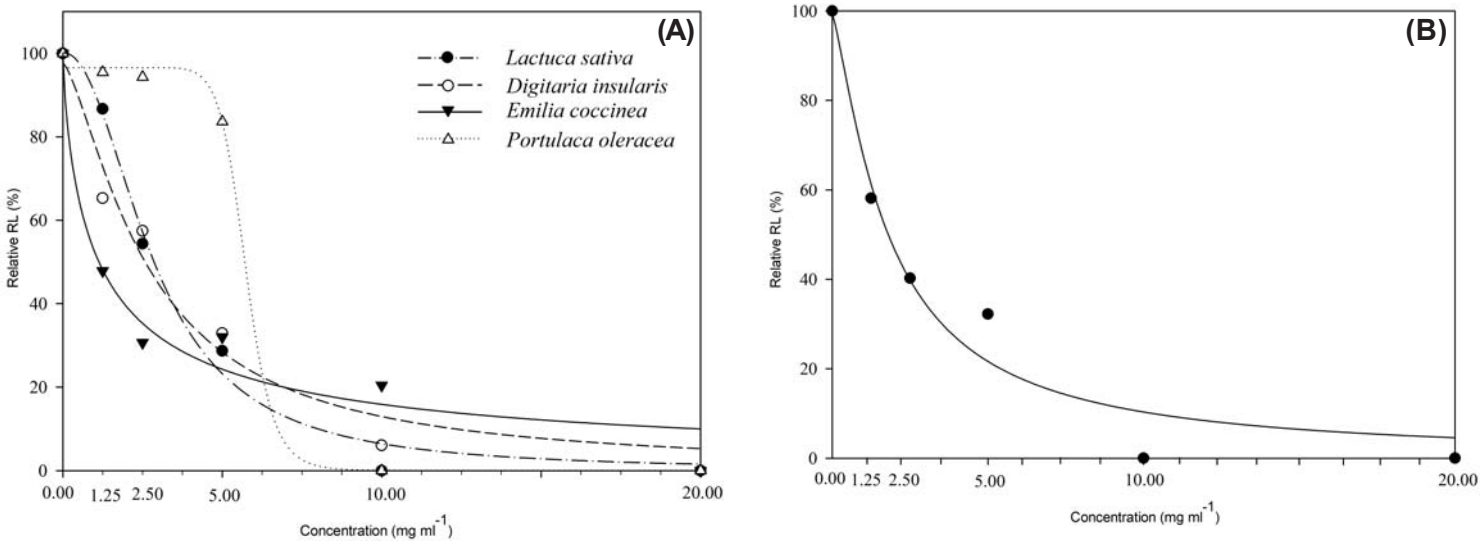

Figure 4 - Root length (RL) of seedlings of recipient species as a function of increasing concentrations of the shoot (A) and root (B) of *P. maritimum*.

Moreover, a higher reduction was observed in the root length when compared to the shoot length of seedlings. The species *L. sativa*, *D. insularis*, *E. coccinea*, and *P. oleracea* showed a reduction in root length of seedlings under the shoot extract of *P. maritimum,* with values of LC_{50} of 2.86, 2.67, 1.13, and 5.742 mg mL^{-1}, respectively, while the reduction of the shoot length of seedlings presented values of LC_{50} of 5.56, 6.56, 5.025, and 5.91 mg mL^{1}, respectively (Table 2). Roots are usually more sensitive to substances present in extracts when compared to other seedling structures due to their direct and prolonged contact with the extract (allelochemical) and/or a different physiological response between structures (Ferreira and Aquila, 2000).

When analyzing the data together, the highest concentrations among all variables were 7.49 and 11.61 mg mL^{-1} for the ethanolic extracts of the shoot and root of *P. maritimum*, respectively (Table 2). Therefore, these concentrations are recommended for the control of recipient species since they affected any of the variables (germination, GRI, and seedling length).

Liquid-liquid partition

The means of the three variables (G, SL, and RL) showed that the partitions with the highest percentage of germination inhibition and length reduction of seedlings of *L. sativa* were those extracted with the solvents chloroform, ethyl acetate, and methanol (Table 3).

Several studies have reported the ability of the fraction ethyl acetate to extract possible allelopathic compounds. In this sense, Santos et al. (2011) evaluated the germination of *Cassia tora* (Fabaceae), *M. pudica* (Fabaceae), and *Cassia occidentalis* (Fabaceae) submitted to hexane, dichloromethane, ethyl acetate, and methanol extract of *Calopogonium mucunoides* (Fabaceae) and observed that the extract obtained with ethyl acetate was more active in inhibiting seed germination.

Table 3 - Percentage of germination (G), shoot length (SL), and root length (RL) of the species *L. sativa* submitted to the crude, hexane, chloroform, ethyl acetate, and methanol extract and control of the shoot of *P. maritimum*

Type of extract	G	SL	RL	Mean
Control	100.00 a	100.00 a	100.00 a	100.00 a
Crude	86.86 a	72.39 ab	54.39 ab	71.21 b
Hexane	39.70 b	51.95 bc	43.49 b	45.05 c
Chloroform	16.76 c	20.46 d	52.94 ab	30.05 cd
Ethyl acetate	18.52 c	35.36 cd	26.14 b	26.68 d
Methanol	46.76 b	36.92 cd	20.43 b	34.70 cd
CV	9.99	9.44	19.68	8.93

Means followed by the same letter do not differ statistically from each other by the Tukey's test at 5% probability.

Silva et al. (2010) also found that the fraction ethyl acetate of *Hydrocotyle bonarienses* (Araliaceae) inhibited the germination and growth of the shoot and root of *L. sativa*, *Lycopersicon esculentum* (Solanaceae), *Allium cepa* (Alliaceae), and *Triticum aestivum* (Poaceae) and presented higher contents of phenolic compounds. Thus, our results are in agreement with the information found in the literature, in which the fraction ethyl acetate of the shoot of *P. maritimum* has secondary metabolites that affect some physiological process during the germination of *L. sativa*, compromising its development.

Phytochemical analysis

The preliminary identification of the chemical groups present in the extract is important because it allows a low cost and fast initial screening (Mattos, 1997), thus directing the best method for extracting these compounds. The results of the phytochemical analysis of the shoot extract of *P. maritimum* showed that the crude ethanolic extract and hexane and chloroform fractions had a higher extraction capacity of secondary metabolites, presenting in common the condensed tannins, catechins, flavonones, steroids, and saponins (Table 4). However, these fractions did not present the highest capacity to inhibit germination and seedling growth of recipient species (Table 3).

Positive results could be observed in all fractions of *P. maritimum* for condensed tannins, flavonones, and saponins (Table 4). Catunda et al. (2002) also observed the presence of phenols, condensed tannins, flavonones, and saponins in the shoot of *Cyperus rotundus* (Cyperaceae).

Table 4 - Phytochemical analysis of the crude ethanolic extract (CE) and fractions of hexane (HEX), chloroform (CHL), ethyl acetate (ETL), and methanol (MET) from the shoot of *P. maritimum*

Secondary metabolites	CE	HEX	CHL	ETL	MET
Phenols	-	-	-	-	-
Hydrolyzable tannins	-	-	-	-	-
Condensed tannins	+	+	+	+	+
Anthocyanin and anthocyanidin	-	-	-	-	-
Flavones, flavonols, and xanthones	-	-	+	-	-
Chalcones and auronas	-	-	-	+	-
Flavononols	+	+	-	-	+
Leucoanthocyanidins	-	-	-	-	-
Catechins	+	+	+	-	-
Flavonones	+	+	+	+	+
Flavonols, flavonones, and xanthones	-	-	-	-	-
Steroids	+	+	+	+	-
Triterpenoids	-	-	-	-	-
Saponins	+	+	+	+	+

(-) negative result and (+) positive result.

The crude ethanolic extract and hexane fraction of *P. maritimum* had the same secondary metabolites (condensed tannins, flavononols, catechins, flavonones, steroids, and saponins). In the chloroform fraction of *P. maritimum*, the detected flavonoids were flavones, flavonols, xanthones, catechins, and flavonones. Phenolic compounds and terpenes (steroids and saponins) were also found in this fraction. Similar to this result, Gomes et al. (2011) performed phytochemical tests with extracts of *Cymbopogon citratus* (Poaceae) and identified the presence of tannins, alkaloids, and flavonoids (flavones and flavonols).

The fraction ethyl acetate of *P. maritimum* showed the presence of phenolic compounds (condensed tannins and flavonones) and terpenes (steroids and saponins) (Table 4). Chalcones and auronas, belonging to the flavonoids, were detected only in this fraction. The presence of chalcones may be a possible explanation for the solvent ethyl acetate be more active in inhibiting germination and seedling length (Table 3). Some flavonoids, such as chalcones, are capable of increasing the levels of reactive oxygen species (Kumar et al., 2010). Bitencourt et al. (2007) verified inhibition of germination of *M. pudica* and *S. obtusifolia* in the presence of chalcones, indicating that these substances have an allelopathic activity.

The presence of flavonoids was detected in all analyzed fractions of *P. maritimum* (Table 4). Flavonoids are the most natural compounds found in plants and have functions of pigmentation, attraction or repellency to herbivores, antimicrobial actions, interference in the germination of the pollen tube, protection against UV radiation, influence in the transport of auxins, capture reactive oxygen species, and have allelopathic effects, being able to inhibit plant growth (Peer and Murphy, 2007; Edwards et al., 2008; Agati and Tattini, 2010).

Steroids were detected in all fractions (Table 4), except for the methanol fraction. This occurred because they are apolar compounds, appearing only in apolar solvents such as hexane, chloroform or, to a lesser extent, solvents of intermediate polarity (ethyl acetate). Studies have shown that steroids have an allelopathic activity in the species *Oryza sativa* (Poaceae) (Macías et al., 2006) and *Moutabea guianensis* (Polygalaceae) (Ripardo Filho et al., 2012).

Saponins were also found in all fractions (Table 4). Similarly, Bertoldi et al. (2009) identified the presence of saponins and flavonoids in *Avena sativa* (Poaceae) but found that only flavonoids exerted an allelopathic activity, inhibiting the germination of seeds of *L. sativa*.

In this sense, the ethanolic extract of the shoot and root of *P. maritimum* had an allelopathic effect. The ethanolic extract of the shoot with a concentration of 7.49 mg mL^{-1} affected the germination and seedling growth. For the ethanolic extract of the root, this concentration is 11.41 mg mL^{-1}. With the alterations of the recipient species tested in this experiment, the allelopathic activity was possibly due to the presence of secondary metabolites belonging to the group of flavonones, condensed tannins, chalcones, steroids, and saponins identified in the ethyl acetate fraction. These changes may be related to an isolated action or to synergistic or antagonistic changes with other substances.

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