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Allelopathy in five species of *Erythroxylum*

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ABSTRACT. The objective of this study was to determine the allelopathic potential of *Erythroxylum rosuliferum* O.E. Schulz; *Erythroxylum stipulosum*; *Erythroxylum cuneifolium* (Mart) O.E. Schulz; *Erythroxylum vacciniifolium* Mart. and *Erythroxylum barbatum* O.E. Schulz. Bioassays were performed to examine the seed germination and initial growth of seedlings of *Lycopersicon esculentum* Mill (tomato) and *Allium cepa* L. (onion) in Petri dishes following exposure to an ethanolic extract of fresh leaves from the test species at various concentrations. The growth of the tomato and onion seedlings was inhibited with the extracts of the species tested, and significant alterations in the development of the shoots and primary roots were observed. These results suggest that the five species that were evaluated have allelopathic properties.

Keywords: allelopathic properties, germination, development of seedlings, cerrado, chapada do Araripe.

Alelopatia em cinco espécies de *Erythroxylum*

RESUMO. O objetivo deste estudo foi determinar o potencial alelopático de *Erythroxylum rosuliferum* O.E. Schulz; *Erythroxylum stipulosum*; *Erythroxylum cuneifolium* (Mart) O.E. Schulz; *Erythroxylum vacciniifolium* Mart. e *Erythroxylum barbatum* O.E. Schulz. Foram realizados bioensaios sobre a germinação de sementes e o crescimento inicial de plântulas de *Lycopersicon esculentum* Mill (tomate) e *Allium cepa* L. (cebola) em placas de Petri, submetidos ao extrato etanólico das folhas frescas das espécies doadoras em várias concentrações. O crescimento das plântulas de tomate e cebola foi inibido pelos extratos das espécies testadas, observando-se alterações significativas no desenvolvimento dos caulículos e radículas. Os resultados sugerem que as cinco espécies avaliadas possuem propriedades alelopáticas.

Palavras-chave: propriedades alelopáticas, germinação, desenvolvimento de plântulas, cerrado, chapada do Araripe.

Introduction

For thousands of years, researchers have been interested in the effects that particular organisms exert on other organisms. Plants are the principal focus of current research, as particular relationships are more evident among these organisms.

According to Lovett and Ryuntyu, (1992), allelopathy is a biochemical interaction considered to be a defensive chemical adaptation in plants and an environmental stress factor for many species. Allelochemicals are released into the environment, which stimulate or inhibit the germination of seeds and/or development of other surrounding plants (RODRIGUES; LOPES, 2001).

In a majority of cases, allelopathy refers to the negative and/or harmful effects on isolated species, populations or even surrounding communities. Today, it is known that the action of these compounds can also be positive and favorable to the recipient, acting in the ecological process for the regeneration or succession of plant species (BRASS, 2009).

Allelopathic studies represent the alternative and biological search for natural phytotoxins and synthetic derivatives for use as natural herbicides because these compounds show specific action and have a less harmful impact on the environment (CHOU, 1999).

The genus *Erythroxylum* P. Browne includes approximately 230 species (PLOWMAN; HENSOLD, 2004). A total of 130 Brazilian species have been listed, which generally occur in forests and cerrado environments (BIERAS; SAJO, 2004). In Northeast Brazil, 66 species and one variety have been identified, among which 25 (37.31%) were recorded only in this region. The occurrence of representatives of Erythroxylaceae in semi-arid regions of Brazil has been described (PLOWMAN; HENSOLD, 2004).

Considering the occurrence of the species of *Erythroxylum* in the chapada do Araripe and the lack of knowledge concerning their allelopathic potential, the aim of this study was to determine the allelopathic potential of five species of *Erythroxylum*

growing in cerrado areas in chapada do Araripe, Ceará State, Brazil with regard to the germination and initial growth of *Lycopersicon esculentum* Mill (tomato) and *Allium cepa* L (onion). These data might be useful in future studies concerning the control of weeds.

Material and methods

Fresh adult leaves were utilized to evaluate the allelopathic effects of *Erythroxylum rosuliferum* O.E. Schulz (bandeirinha), *Erythroxylum stipulosum* Plowman (carrasco), *Erythroxylum cuneifolium* (Mart) O.E. Schulz (carrasquinho), *Erythroxylum vacciniifolium* Mart. (catuaba), and *Erythroxylum barbatum* O.E. Schulz (cururu). The plants were collected in chapada do Araripe along the Crato/Exu highway at the geographic coordinates of 07°17'810"S and 39°32'683"W at an altitude of 928 m. The receptor species *Lycopersicon esculentum* Mill (tomato) and *Allium cepa* L (onion) were cultivated using seeds obtained from local stores. The experiments were conducted from June to August of 2010 in the Applied Botany Laboratory (LBA) at the Universidade Regional of Cariri (URCA).

The crude ethanolic extract (CEE) was prepared using 99.3% ethanol (500 g fresh leaves in 3 L of ethanol) at an ambient temperature for seven days with periodic mixing. After filtration, the solvent was removed using a rotary evaporator. The CEE of the species tested was dissolved in 66% ethanol (MAZZAFERA, 2003) at a proportion of 1:1 to obtain a stock solution of 100%. From this stock solution, dilutions were prepared to obtain final concentrations of 6.25, 12.5, 25, and 50%. The solutions were added to the plates at their initial pH and adjusted to 6.0 (MACIAS et al., 2000) with 0.1 N KOH and 5% HCl using a pH meter.

The germination assays were conducted in Petri dishes (9.0 cm in diameter) lined with two layers of germitest paper. Subsequently, 3 mL of diluted extract or 66% ethanol (control) were distributed evenly. The plates were left open for 48 h for the complete evaporation of the alcohol (MAZZAFERA, 2003). A total of 20 seeds were placed in the receptor species, randomly distributed with five repetitions, and 3 mL of distilled water were added. The distilled water was utilized according to the method of Macias et al. (2000), where the volume of solution should not exceed 0.2 mL per seed. The Petri dishes containing the seeds were sealed with plastic film to assure a closed system and placed in a germination chamber (BOD) at a constant temperature (25°C) and a 12-h photoperiod, which was adequate for the receptor

species. Germination was evaluated every 24h for seven days and was measured as the 2-mm emergence of the primary root. The percentage germination (G) was calculated according to Labouriau and Valadares (1976), the germination speed index (GSI) was calculated according to Maguire (1962), and the number of germinated seeds was recorded daily. The following formulas were used:

$$G = (N/A).100,$$

where:

N - total number of seeds germinated;

A - number of seeds placed to germinate.

$$GSI = (\sum ni)/(\sum ni.ti),$$

where:

Ni = number of seeds germinated within a defined time interval (ti - 1) - (ti)

The data for the length of the stem and primary root were obtained during the germination period. Among the 20 seeds that formed each plot (Petri dish), five seedlings were evaluated to obtain the mean values for the lengths of the shoots and primary roots. These parameters were determined using a digital pachymeter.

The experiments were conducted with a complete randomized block design utilizing a 6 x 2 x 2 factorial scheme, which consisted of the control treatment plus five concentrations in each experiment, two situations (initial pH and adjusted pH) and two plant species (tomato and onion) with five repetitions. The data were recorded and archived on an Excel 2007 spreadsheet for the subsequent comparison of the means using the statistics package ASSISTAT 7.5 beta, where the means were submitted to analysis of variance (ANOVA) and Tukey's test to determine significance at the 1 and 5% level. To determine the presence or absence of certain classes of secondary metabolites, phytochemical tests were conducted following the method of Matos (1997) based on a color change or formation of precipitate after the addition of specific reagents. The phytochemical prospecting of the extracts was performed in the Chemistry and Natural Products Laboratory at the Universidade Regional of Cariri (URCA).

Results and discussion

Germination

The crude ethanolic extract (CEE) of *E. stipulosum* leaves at concentrations of 6.25, 12.5 and 100% did not affect the germination of *L. esculentum* seeds (tomato) in tests with the initial

and adjusted pH. However, at concentrations of 25 and 50%, a significant inhibition of germination was observed compared with the control in tests with adjusted pH values. The extracts of *E. rosuliferum*, *E. cuneifolium* and *E. barbatum* did not influence the germination of *L. esculentum* and *A. cepa* seeds, as the mean germination did not show significant differences with any of the treatments compared with the control at an initial and adjusted pH. The ethanolic extract of *E. vacciniifolium* did not interfere with the germination of *L. esculentum* seeds. However, the germination of *A. cepa* seeds was inhibited in the test with adjusted pH at concentrations of 25, 50 and 100%. According to Ferreira and Áquila (2000), allelochemicals can interfere with the process of germination during both pre- and post-emergence.

Silva et al. (2006) showed that the ethanolic extract of *Stryphnodendron adstringens* leaves had no effect on the germination of corn (*Zea mays*) and bean (*Phaseolus vulgaris*) but did inhibit the germination of “picão” (*Bidens pilosus*) by 1/3 compared with the controls.

According to Ferreira and Áquila (2000) allelopathic interference is more common in the growth of the seedlings and development of the adult plants than in germination. Silva et al. (2006) studied 15 native tree species of the Cerrado, where only four showed an allelopathic effect, thereby inhibiting the germination of lettuce seeds.

Germination is a parameter that is least sensitive to allelochemicals, but its experimental quantification is much simpler because for each seed, the phenomenon is clear whether germinated or not (FERREIRA; ÁQUILA, 2000).

Germination speed index (GSI)

The extracts of *Erythroxylum rosuliferum*, *E. vacciniifolium* and *E. barbatum* affected the GSI of the seeds of *Lycopersicon esculentum*. The seeds exposed to *E. rosuliferum* extract at an initial pH showed a decrease in GSI at all concentrations compared with the control. However, at an adjusted pH, there was a decrease in this index with 100% extract. The extract of *E. barbatum* decreased the germination rate of the seeds at all concentrations at an adjusted pH. For *E. vacciniifolium* Mart, the extract at 25% with an initial pH and at 6.25% with an adjusted pH caused an increase in GSI, while a significant delay in germination was observed at 100% with an adjusted pH.

Fernandes et al. (2007) obtained similar results, where treatment with the lowest concentration of *Merostachys multiramea* Hackel (2.5%) extract caused

the most rapid germination of *Araucaria angustifolia* (Bert.) Kuntze seeds even compared with the water-only treatment. The more concentrated extract (10%) caused slower germination.

Piña-Rodrigues and Lopes (2001) observed that the extracts of *Mimosa caesalpiniaefolia* Benth did not affect the germination percentage but did reduce the germination rate of “ipe amarelo” (*Tabebuia alba* (Cham.) Sandw), seeds.

The analysis of the results of Silva et al. (2006) showed that the application of crude ethanolic extract and the HF (hexane fraction) and EAF (ethyl acetate fraction) of leaves of *Dicranopteris flexuosa* (Schrad.) Underw. Negatively affected the GSI of lettuce but had no effect on the germination ability.

The extracts of *E. rosuliferum*, *E. cuneifolium* and *E. barbatum*, when tested on *Allium cepa* seeds, showed no statistically significant effects either on germination or GSI with initial and adjusted pH values. However, treatment with *E. stipulosum* extract at concentrations of 12.5 and 25% (under initial and adjusted pH) lowered the GSI, and the administration of *E. vacciniifolium* accelerated germination at concentrations of 50% with an initial pH and at 12.5, 25 and 50% with an adjusted pH.

Biometry of the shoot and primary root

The initial growth of the seedlings was more sensitive compared with germination. Treatment with *E. rosuliferum* extracts significantly affected the length of the shoot of tomato seedlings, increasing growth by 50% (initial pH) and at initial concentrations of 6.25, 12.5 and 25% (adjusted pH); at 100%, the effect was phytotoxic. *E. stipulosum* showed a negative allelopathic effect at concentrations of 25 and 50% (initial pH) and 100% (adjusted pH); however, the lowest extract concentration (6.25%) accelerated stem growth. With respect to the *E. vacciniifolium* extract, all concentrations had a negative influence with the exception of 12.5 and 25% concentrations in tests with adjusted pH, which did not differ statistically from the control. *E. barbatum* showed a positive allelopathic effect, showing accelerated stem development at all concentrations, except the lower extract concentrations with initial pH, which did not differ statistically from the control.

Reigosa et al. (1999) stated that the effects of allelochemicals on the different physiological processes of a plant are dependent on the concentration, causing activation at low concentrations and inhibition at high concentrations.

In their work with some native species, including *Erythroxylum argentinum*, Maraschin-Silva and Áquila (2006) noted significant increases in the hypocotyl development of lettuce seedlings when exposed to the leaf extract of *E. argentinum* notably, the extract utilized in these experiments exhibited a pH that was considered ideal for allelopathic tests.

No statistically significant differences were observed in relation to the length of the shoots of *Allium cepa* seedlings compared with the control.

According to Chon et al. (2000), the roots are generally more sensitive to the substances present in the extracts when compared with the other seedling structures, which is potentially related to the direct and prolonged contact of the roots with the extract (allelochemicals) (CHUNG et al., 2001) and/or to a distinct physiological response between structures (FERREIRA; ÁQUILA, 2000).

The present study showed an increase in the length of the primary roots of tomato seedlings exposed to 6.25% *E. rosuliferum* extract, while at a concentration of 100% no growth inhibition was observed when compared with the control in a test with initial pH. In the test with adjusted pH, the primary roots exposed to a concentration of 25% exhibited increased development. Maraschin-Silva and Áquila (2006) showed that the leaf extract of *Erythroxylum argentinum* also reduced the size of the primary roots of lettuce seedlings with increasing extract concentration compared with the control treatment.

The extract of *E. stipulosum* inhibited the development of the primary roots of tomato seedlings at a concentration of 6.25% in the test with adjusted pH, whereas treatment with 100% extract accelerated root development. These results are contrary to the effects observed in relation to the shoot when the seeds are treated with the same extract. However, Ferreira and Áquila (2000) observed that the effects of allelochemicals vary depending on the organ of the plant, where they might inhibit development in one organ and induce small increases in another.

The *E. cuneifolium* extract in the test with adjusted pH at concentrations of 6.25 and 12.5% had phytotoxic effects on the roots compared with the control. However, treatments with other concentrations did not result in any alteration compared with the control.

D'Abrosca et al. (2001) obtained 24 compounds from *Sambucus nigra* and tested them for allelopathic

properties in three different species, namely, *Lactuca sativa*, *Raphanus sativa* and *Allium cepa*, using different concentrations. These authors observed diverse results showing varying degrees of stimulation and inhibition of primary root growth in the three species.

The *E. stipulosum* and *E. vacciniifolium* extracts significantly affected the development of the primary root of onion. In the test with initial pH, positive allelopathic activity was demonstrated with low concentrations of 6.25 and 12.5%. In the test with adjusted pH, the same result was obtained only with a concentration of 6.25% for the *E. stipulosum* extract. The *E. vacciniifolium* extracts showed inhibition at concentrations of 6.25 (initial pH) and 12.5, 25, 50 and 100% (adjusted pH) compared with the control. Consistent with the results presented here, Peres et al. (2004) observed that extracts of *Adiantopsis radiata* (L.) Fee, *Adiantum serratodentatum* Humb. and Bonpl. ex Willd and *Pteris denticulata* Sw. (Pteridaceae) inhibited the primary root growth of onion seedlings.

Ranal (2006) and Ferreira (2004) proposed that the interference in the growth of a seedling is a response of metabolism. The alterations that occur in the seedlings exposed to extracts at different concentrations could result from effects regarding cell membrane permeability, DNA transcription and translation, second messenger function, respiration, oxygen sequestration, enzyme conformation, or even combinations of these factors.

From an ecological point of view, the inhibition of the growth of the seedling after germination is a more efficient mechanism of selection than preventing the germination of the competitor because the descendants would be eliminated through the death of the individuals, the disappearance of competitor DNA, or in less severe cases, the delay of growth or germination. In the last case, the ontogenic results are similar because if the development of one species is hampered, the favored species can establish its progeny, avoiding the pressure of competition (JACOBI; FERREIRA, 1991).

Tables 1 and 2 present the mean germination rates, GSI and biometry of the shoots and primary roots of the receptor species when exposed to extracts of *E. rosuliferum* (bandeirinha); *E. stipulosum* (carrasco), *E. cuneifolium* (carrasquinho); *E. vacciniifolium* (catuaba) and *E. barbatum* (cururu).

Table 1. Effect of the ethanolic leaf extracts of *Erythroxyllum rosuliferum* O.E. Schulz, *Erythroxyllum stipulosum* Plowman, *Erythroxyllum cuneifolium* (Mart) O.E. Schulz, *Erythroxyllum vacciniifolium* Mart. and *Erythroxyllum barbatum* O.E. Schulz on *Allium cepa* L. seeds and seedlings.

H	Bioassay (%)	<i>Erythroxyllum rosuliferum</i>	<i>Erythroxyllum stipulosum</i>	<i>Erythroxyllum cuneifolium</i>	<i>Erythroxyllum vacciniifolium</i>	<i>Erythroxyllum barbatum</i>
pH _i	0	15 a	18 a	18 a	16.2 a	17 a
	6.25	17 a	16.8 a	16.8 a	16.2 a	17,6 a
	12.5	15.4 a	17.2 a	17.2 a	17.4 a	18,4 a
	25	15.8 a	16.8 a	16.8 a	16,8 a	16,8 a
	50	14.6 a	16.4 a	16.4 a	17,2 a	17 a
pH _f	100	17.4 a	18.6 a	18.6 a	15,8 a	16,4 a
	6.25	15.2 a	16.8 a	16.8 a	18 a	16,4 a
	12.5	16.4 a	17.2 a	17.2 a	15,6 a	17,2 a
	25	17.4 a	15.2 b	15.2 b	15,8 a	16,2 a
	50	17.2 a	15.2 b	15.2 b	15.4 a	17,2 a
pH _i	100	16.2 a	17.4 a	17.4 a	15,8 a	15,6 a
	0	0.320 a	0.316 a	0.316 a	0.314 ab	0,396 a
	6.25	0.312 abc	0.309 a	0.309 a	0.309 ab	0,396 a
	12.5	0.294 d	0.309 a	0.309 a	0.314 ab	0,395 a
	25	0.299 cd	0.312 a	0.312 a	0.316 a	0,393 a
pH _f	50	0.304 bcd	0.318 a	0.318 a	0.303 ab	0,395 a
	100	0.311 abc	0.305 a	0.305 a	0.305 ab	0,392 a
	6.25	0.317 ab	0.315 a	0.315 a	0.318 a	0,305 b
	12.5	0.319 ab	0.310 a	0.310 a	0.312 ab	0,293 b
	25	0.320 a	0.312 a	0.312 a	0.311 ab	0,305 b
pH _i	50	0.319 ab	0.317 a	0.317 a	0.313 ab	0,301 b
	100	0.312 abc	0.305 a	0.305 a	0.299 b	0,305 b
	0	2.68 ab	2.50 abc	2.50 abc	2.63	2,08 b
	6.25	2.64 ab	2.58 abc	2.58 abc	2.01 a	2,08 b
	12.5	2.60 ab	2.60 abc	2.60 abc	2.42 ab	2,12 b
pH _f	25	2.70 ab	2.38 bc	2.38 bc	2.51 ab	2,27 ab
	50	3.00 a	2.38 bc	2.38 bc	2.37 ab	2,37 ab
	100	2.46 ab	2.55c	2.55c	2.04 ab	2,25 ab
	6.25	3.21 a	3.06 a	3.06 a	2.36 ab	2,35 ab
	12.5	3.06 a	2.62 abc	2.62 abc	2.66 a	2,70 a
pH _i	25	3.14 a	2.88 ab	2.88 ab	2.89 a	2,68 a
	50	2.57 ab	2.46 abc	2.46 abc	2.38 ab	2,70 a
	100	2.05 b	2.04 c	2.04 c	1.70 b	2,53 ab
	0	7.14 ab	6.86 ab	6.86 ab	7.56 a	6,47 a
	6.25	7.93 a	6.57 ab	6.57 ab	6.51 a	6,10 a
pH _f	12.5	7.29 ab	6.76 ab	6.76 ab	7.59 a	6,20 a
	25	6.68 ab	6.44 ab	6.44 ab	7.18 a	5,80 a
	50	6.99 ab	6.93 ab	6.93 ab	7.28 a	7,02 a
	100	6.24 b	6.01 ab	6.01 ab	5.92 a	5,78 a
	6.25	7.25 ab	5.54 b	5.54 b	7.96 a	6,66 a
pH _i	12.5	7.18 ab	6.40 ab	6.40 ab	7.00 a	7,02 a
	25	8.22 a	6.94 ab	6.94 ab	6.90 a	7,22 a
	50	7.59 ab	7.33 ab	7.33 ab	7.32 a	6,78 a
	100	7.26 ab	8.07 a	8.07 a	6.18 a	6,04 a

Means followed by the same letter in each bioassay do not differ statistically ($p < 0.05$). pH_i: initial pH, pH_f: final pH. GSI: germination speed index.

Table 2. Effect of the ethanolic leaf extracts of *Erythroxyllum rosuliferum* O.E. Schulz, *Erythroxyllum stipulosum* Plowman, *Erythroxyllum cuneifolium* (Mart) O.E. Schulz, *Erythroxyllum vacciniifolium* Mart. And *Erythroxyllum barbatum* O.E. Schulz on *Lycopersicon esculentum* Mill. seeds and seedlings.

pH	Bioassay (%)	<i>Erythroxyllum rosuliferum</i>	<i>Erythroxyllum stipulosum</i>	<i>Erythroxyllum cuneifolium</i>	<i>Erythroxyllum vacciniifolium</i>	<i>Erythroxyllum barbatum</i>
pH _i	0	8,6 a	7,6 a	6,4 a	8 a	8,2 a
	6.25	9 a	9,6 a	5,6 a	7,8 a	8,8 a
	12.5	7,8 a	9,2 a	9 a	9,2 a	8,8 a
	25	8,8 a	8,4 a	9,8 a	9,2 a	8,8 a
	50	8,4 a	8 a	8,4 a	8 a	9,8 a
pH _f	100	7,8 a	6,8 a	9,2 a	8,8 a	9,2 a
	6.25	6,8 a	7,8 a	7,2 a	8,4 a	9,6 a
	12.5	8,4 a	8 a	7,2 a	7,4 a	9,4 a
	25	7,2 a	7,8 a	6,4 a	6,4 b	9,2 a
	50	5,8 a	5,8 a	7,2 a	4,6 b	8,2 a
pH _i	100	6 a	7,6 a	8 a	5,8 b	7,8 a
	0	0.371 a	0.339 a	0.352 a	0.311 a	0,339 a
	6.25	0.356 a	0.316 b	0.358 a	0.322 a	0,348 a
	12.5	0.366 a	0.337 a	0.352 a	0.316 a	0,362 a
	25	0.306 a	0.319 b	0.361 a	0.316 a	0,340 a
pH _f	50	0.345 a	0.342 a	0.354 a	0.363 b	0,346 a
	100	0.342 a	0.344 a	0.350 a	0.323 a	0,371 a
	6.25	0.325 a	0.316 b	0.332 a	0.347 a	0,338 a
	12.5	0.335 a	0.337 a	0.361 a	0.363 b	0,316 a
	25	0.306 a	0.319 b	0.355 a	0.368 b	0,317 a
pH _i	50	0.345 a	0.342 a	0.346 a	0.383 b	0,318 a

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pH	Bioassay (%)	<i>Erythroxylum rosuliferum</i>	<i>Erythroxylum stipulosum</i>	<i>Erythroxylum cuneifolium</i>	<i>Erythroxylum vacciniifolium</i>	<i>Erythroxylum barbatum</i>
pH _i	100	0.341 a	0.343 a	0.344 a	0.320 a	0.358 a
	0	0.42 a	0.22 a	0.20 a	0.50 a	0.33 a
	6.25	0.37 a	0.52 a	0.41 a	0.26 a	0.36 a
	12.5	0.36 a	0.40 a	0.39 a	0.55 a	0.19 a
	25	0.36 a	0.52 a	0.34 a	0.58 a	0.30 a
pH _f	50	0.51 a	0.35 a	0.39 a	0.38 a	0.48 a
	100	0.40 a	0.35 a	0.36 a	0.71 a	0.40 a
	6.25	0.54 a	0.55 a	0.18 a	0.55 a	0.55 a
	12.5	0.68 a	0.53 a	0.48 a	0.54 a	0.45 a
	25	0.42 a	0.62 a	0.30 a	0.38 a	0.41 a
pH _i	50	0.34 a	0.39 a	0.40 a	0.49 a	0.46 a
	100	0.50 a	0.44 a	0.84 a	0.34 a	0.53 a
	0	0.60 a	0.42 a	0.42 a	0.72 a	0.57 a
	6.25	0.64 a	0.73 b	0.60 a	0.49 b	0.56 a
	12.5	0.57 a	0.70 b	0.55 a	0.68 a	0.55 a
pH _f	25	0.61 a	0.60 a	0.50 a	0.72 a	0.64 a
	50	0.69 a	0.51 a	0.56 a	0.63 a	0.70 a
	100	0.61 a	0.44 a	0.50 a	0.63 a	0.60 a
	6.25	0.65 a	0.69 b	0.45 a	0.60 a	0.61 a
	12.5	0.75 a	0.49 a	0.50 a	0.44 b	0.69 a
pH _i	25	0.56 a	0.66 a	0.42 a	0.44 b	0.55 a
	50	0.52 a	0.48 a	0.60 a	0.53 b	0.58 a
	100	0.48 a	0.48 a	0.68 a	0.42 b	0.52 a

Means followed by the same letter in each bioassay do not differ statistically ($p < 0.05$). pH_i: initial pH, pH_f: final pH. GSI: germination speed index.

Phytochemical analysis

The genus *Erythroxylum* is characterized by the presence of alkaloids of the tropane group, including cocaine, a natural alkaloid produced by *E. coca*, which has been used as a local anesthetic in minor surgeries (GRIFFIN; LIN 2000) and is currently sold illegally in large urban centers to a considerable drug-dependent population.

The detection of compounds of secondary metabolism through phytochemical analysis in the extracts of fresh leaves of the species investigated in this study revealed the presence of tannins, phenols, flavonoids and alkaloids (Table 3).

Table 3. Classes of secondary metabolites in the ethanolic extracts of the fresh leaves of *Erythroxylum rosuliferum* O.E. Schulz; *Erythroxylum stipulosum* Plowman; *Erythroxylum cuneifolium* (Mart) O.E. Schulz; *Erythroxylum vacciniifolium* Mart. and *Erythroxylum barbatum* O.E. Schulz.

Species	Classes of secondary metabolites			
	Tannins	Phenols	Flavonoids	Alkaloids
<i>Erythroxylum rosuliferum</i>	+	-	+	+
<i>Erythroxylum stipulosum</i>	-	+	+	+
<i>Erythroxylum cuneifolium</i>	-	+	+	+
<i>Erythroxylum vacciniifolium</i>	+	-	+	+
<i>Erythroxylum barbatum</i>	-	+	+	-

(+): Present; (-): Absent

Chemical substances, such as terpenes, phenolic compounds, cumarins, flavonoids, alkaloids, glycosides, tannins and quinones, originating from the secondary metabolisms of plants could initiate beneficial or harmful effects on other plants or other organisms (PINÃ- RODRIGUES; LOPES, 2001).

Phenolic compounds correspond to a class of secondary metabolites, which include the majority of compounds with allelopathic activity, ranging from simple phenols to tannins of complex structure (RICE, 1984).

However whether the presence of these compounds in plant extracts accounts for these allelopathic effects has not been determined, as the test results only determine their presence or absence. Future studies are necessary to isolate the chemical constituents to identify the compounds responsible for the allelopathic action because not all of these compounds act as allelochemicals.

Conclusion

The allelopathic potential of the ethanolic extract of *Erythroxylum* leaves varies with the target species and was more evident in the seed germination and seedling development of *Lycopersicon esculentum* than in *Allium cepa*.

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