

Potential for allelopathy in fresh leaves of the Cambará during the germination in lettuce seeds.

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RESUMO - Este trabalho teve como objetivo verificar a existência de potenciais alelopáticos no extrato da folha fresca de cambará através da germinação de sementes de alface e formação inicial de radículas, com coletas no Pantanal do Negro, Mato Grosso do Sul. Para os bioensaios de germinação de alface como planta alvo, foram utilizados os extratos foliares nas concentrações de 4, 8, 12, 16 e 20%, obtidos através da diluição do extrato a 20%. Na análise fitoquímica foi detectado a presença de compostos fenólicos, taninos, saponinas e cumarinas. A taxa de germinação não foi afetada, porém o vigor de germinação foi afetado negativamente nas concentrações de 12, 16 e 20%. Os extratos, nas diferentes concentrações, também prejudicaram o desenvolvimento da radícula, indicando que existem substâncias químicas inibidoras e revelando potencial alelopático para as folhas frescas desta espécie.

Palavras-chave: *Vochysia divergens*, Vochysiaceae, Pantanal, aleloquímico, compostos fenólicos.

ABSTRACT (Potencial alelopático de folhas frescas de cambará na germinação de sementes de alface) - The current work seeks to verify the existence of allelopathy in fresh Cambará leaves, via the germination and initial formation of radicles in lettuce seeds. Leaf samples of the Cambará (*Vochysia divergens*) were collected from the *Rio Negro* ('Black River') region of the Pantanal, in *Mato Grosso do Sul*. For the bio-testing (relating to the germination of our target plant, the lettuce), we used leaf samples in the following concentrations: 4, 8, 12, 16 and 20% - all diluted to 20%. Through phytochemical analysis, were able to detect the presence of phenols, tannins, saponins and coumarins. The rate of germination was unaffected, however in concentrations of 12%, 16% and 20%, respectively, the strength of germination was adversely effected. In addition, the development of radicles was notably impeded in samples of various concentrations, thus indicating the presence of chemical inhibitors and thereby revealing the potential for allelopathy in fresh leaves of this species.

Key words: *Vochysia divergens*, Vochysiaceae, Pantanal, allelochemical, phenolic compounds.

INTRODUCTION

Allelopathy is the capacity in plants to produce chemical substances which, when transferred, are able to influence the development of adjacent vegetation (Rice, 1984).

Allelochemicals discovered in various plant species can be used to create synthetic agrochemicals, which maintain agricultural production in a more sustainable and ecologically friendly manner. Many allelopathic substances offer great potential in terms of the bio-control of damaging herbs (Chung et al., 2001). Souza-Filho and Alves (2002) suggest that allelopathic substances could be used directly in the formulation of bio-herbicides.

Amongst native species which have, as yet, received little attention in terms of their potential for allelopathy, is the *Vochysia divergens* [Pohl], commonly known as Cambará; pertains to the family Vochysiaceae and occurs throughout the Brazilian states of Goiás, Mato Grosso and Mato Grosso do Sul (Lorenzi, 2000). The chemistry of the species constituting the family *Vochysiaceae*, is not well understood (Mayworm et al., 2000). Due to the fact that studies showing the potential of *V. divergens* leaves were not forthcoming, as far as the presence of secondary class metabolites are concerned, our present research seeks to verify the allelopathic potential of fresh Cambará leaves and subject them to a phytochemical analysis.

MATERIAL AND METHODS

Leaves of the *V. divergens* were taken from different specimens from wooded areas around the rio correntoso (River Correntoso). The locale is in a region called 'Pantanal do Negro' in the municipality of Aquidauana, Mato Grosso do Sul, Brazil (19°29'12,2 to 19°30'49,8 S and 55°35'28,5 to 55°42'37,9 W), November, 2009. The collected materials were placed in polythene sacks and transported in damp chambers. One of the branches was herborized (classified) and the excicate sent to the herbarium (herbar), catalogued and registered (7677) and incorporated into the existing collection.

The plant material (leaves) were used to prepare a water-based (aqueous) solution (to 20%), via a turbulizer (Brandt et al., 2009). The sample was then submitted to phytochemical analysis and used to test for allelopathy. Phytochemical 'prospecting' was carried out via humidification, according to colorimetric testing and/or the chemical precipitation methods as adapted by Matos (1997) and by Wagner and Bladt (1995). The results were compared and contrasted, observing any alteration in colour or precipitation (Costa, 2001).

The water-based (aqueous) sample, after being filtered, was further diluted by concentrations of 16%, 12%, 8% and 4%, and these samples were then used to test for allelopathy.

Bio-testing was facilitated by using lettuce seeds (*Lactuca sativa* L.). These were placed onto a Petri dish, each one containing 25 seeds resting on humidified paper filters with 10 ml of extract accordingly. In total, 100 seeds were tested (four Petri dishes per test). The 'control-model', which also had 100 seeds separated into four Petri dishes (25 per dish), was humidified with distilled water. The seeds were placed in BOD germination chambers at 20 °C (± 2 °C), with 12 hours of light. They were evaluated daily and the experiment was finalized on the third day.

The parameters so analyzed were as follows: percentage of germination, average time of germination (ATG) and the indice of velocity of germination (IVG) – (this is in addition to the average size of the radicle by way of 40 showings of each treatment on the third day of testing, according to the paquimeter precision). The experiment was installed in entirely random design/scheme – six treatments. The data was analyzed and anything of significance was passed through Tukey's test, to a level of 5% ($p < 0,05$).

RESULTS AND DISCUSSION

The chemical analysis, in reference to secondary metabolites, indicated the presence of phenol compounds, tannins, coumarins and saponins in the aqueous extract of *V. divergens*.

The results obtained indicate that the aqueous extract did not represent an influence in the germination of lettuce seeds, as they were statistically equal to the 'control-model' (Table 1). In relation to ATG, significant differences occurred between different treatments, with germination occurring over the largest time period when in concentrations of 12, 16 and 20%. In relation to IVG, the results also indicated that concentrations of 12, 16 and 20% had a negative effect on germination strength.

The development of radicles (ASR) was negatively affected by all treatments, being statistically different to the control model (Table 1); with average sizes lower or equal to 4.0 mm, thus indicating the presence of chemical substances which affect cell-division in radicles.

The reduction of growth in lettuce seeds, treated with aqueous extracts in concentrations of 4, 8, 12, 16 and 20%, suggests that the effects of allelopathy in these extracts might be linked to the harmful effects of constituent chemicals present, as well as by phenol compounds and tannins. Phenol compounds correspond to a secondary class of metabolites in which one encounters the main compounds thought to harness allelopathic activity. The mechanisms of allelopathy may also be mediated by the presence of coumarins and saponins.

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Table 1 - Average value in percentage terms of germination (%G), average time of germination (ATG), indice of velocity of germination (IVG) and average size of radicle (ASR) of lettuce seeds treated with aqueous extracts in concentrations of 4%, 8%, 12%, 16% and 20% at a temperature of 20° C.

Treatments	% G	AVG	IVG	ASR (mm)
4%	99 a	2.1 a	12.2 a	4.0 b
8%	95 a	2.2 ab	11.7 ab	3.0 b
12%	97 a	2.4 b	10.8 bc	2.9 b
16%	99 a	2.4 b	11 bc	3.1 b
20%	94 a	2.5 b	10.3 c	2.9 b
Control	100 a	2.0 a	12.1 a	12 a

Means followed by same letters in a column do not differ (Tukey test, $p = 0.05$)