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Leaf extract effects of *Vochysia divergens* on lettuce and tomato

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ABSTRACT

In laboratory bioassays, the phytochemical activity of leaf extracts (0, 2.5, 5, 10 and 20%) of *Vochysia divergens* Cambará (Pohl*)* was investigated on germination and growth of lettuce and tomato. In all concentrations, the pH of extract was 4.3 ± 0.3, electrical conductivity was 14.9 to 26.1 µScm-1 and soluble solids concentration (expressed in degrees Brix) was 0.8. The extract concentrations did not influence the lettuce seed germination but reduced the seedlings vigour. The development of primary roots and aerial part was inhibited. The tomato seeds germination was affected at 20% concentration. The vigour of germination was greatly reduced at all concentrations and the development of primary roots and aerial part was notably impeded. Chemical analysis of extract revealed presence of phenolic compounds (FC= 4.16 to 9.32 µg/g), tannins, flavonoids (F= 2.44 to 4.17 µg/g), coumarins, steroids and free triterpenes.

Key words: Allelochemical, allelopathy, cambara, germination, leaf extract, lettuce, phenolic compounds, tomato, *Vochysia divergens.*

INTRODUCTION

The Brazilian pantanal lies in natural ecosystems subjected to periodic flooding that leads to seasonal changes in the environment (32). Besides the biome has suffered anthropogenic changes in vegetation cover, allowing certain plant species (did not exist previously) to occupy areas (25). Many of these species are mono- dominant, such as *Vochysia divergens* Pohl (Vochysiaceae), a tree native to the Amazon rainforest and now colonizing flooded and managed grasslands in the Pantanal. It has spread rapidly in recent years, forming monospecific wooded areas locally called "cambarazais" (28). The presence of *V. divergens* indicates the environmental change and its expansion reduces the number of herbaceous and shrub species in the region i.e. reducing local biodiversity (24).

Formation and occupation probably occur from seeds of trees in nearby areas, and the causes of its extensive spread in Pantanal are not known (4). Perhaps the ecological and physiological characteristics favour its rapid spread and dominance in seasonally flooded areas (24), it grows fast under intense light, it is tolerant to prolonged flooding,

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seedlings keep their leaves intact even under submerged conditions and there is large production of seeds dispersed by wind and water (4).

V. divergens is native specie, but its allelopathic potential is not known. It stem bark contains *β*-sitosterol and fatty triterpenes (betulinic and sericic acid). The extract is antimicrobial and has negative effects on cell-division in these organisms (16,17). Hess and Monache (18) identified a new lupane triterpene, divergioic acid (2α,3β,6β-trihydroxy-lup-20(29)-en-28-oic acid), β-sitosterol, betulinic acid, sericic and 24-hydroxytormentic acids and the (28 → 1) β-D-Glucopyranosil ester from the bark of *V. divergens* (34). The ethanol extract from its trunk is antibacterial (34).

Allelochemicals and their effects vary in intensity, as their action is conditioned by numerous factors [soil, concentration, temperature and water conditions (21)]. Till now there are no *in-vitro* and *in-vivo* studies to report allopathic effects (14). Thus, *V. divergens* can be a model of succession for existing plants and the chemicals they have released into the environment (13). The study aimed to determine the allelopathic potential of fresh leaves of Cambará (*V. divergens*) and the compounds present in leaves.

MATERIALS AND METHODS

Leaves of *V. divergens* were sampled from different wooded areas in the grounds of Research Institute of the Pantanal, 'Santa Emilia' Farm (19°29'12.2 to 19°30'49.8 S and 55°35'28.5 to 55°42'37.9 W, altitude 128 m). The region is called 'Pantanal do Negro' in municipality of Aquidauana, Mato Grosso do Sul state, Brazil. The collected materials were kept in polythene bags and transported in humid chambers. One plant was identified, catalogued and registered (7677) and added to the Herbarium collection of Plant Morphology Laboratory, Anhanguera-Uniderp University.

Preparation of extracts

The plant material (fresh leaves) was processed in the turbulizer to prepare an alcohol-based solution (40 g plant material in 400 mL commercial ethanol (92^o) – extract 20%) (8). The extract was left in ultrasound bath for 60 min and allowed to settle in dark for 24 in cold room (16 °C). Thereafter, the extract was again placed in ultrasound bath for 60 min.; then it was filtered through a funnel lined with filter paper and cotton and the extract was collected in Beaker. The crude extract (20%) was diluted with ethanol to obtain the 10, 5 and 2.5% concentrations (v/v).

Germination bioassays

In bioassays, lettuce seeds (*Lactuca sativa* L.) or 'maravilha quatro estações' and tomato seeds (*Lycopersicum esculentum* Mill.) or 'Santa Clara' were used. Five mL extract at four concentrations (20, 10, 5 and 2.5%) were applied in Petri dishes (7 cm dia) lined with two 'germitest' papers and left to stand for total evaporation of ethanol. Afterwards, 25 seeds were placed in dishes and the substrate was irrigated again with 5 mL distilled water and compared with control (0%). In control, the dishes with 5 mL alcohol were left to stand for total evaporation of ethanol; afterwards the substrate was irrigated with 5 mL distilled water. In total, 100 seeds were tested (4 replicate Petri-dishes or 4 replications per test). The seeds were placed in BOD germination chambers at 20 °C (lettuce) and 25 °C (tomato), with a variable of ± 2 °C, for a photo-period of 12 hours of light. Germinated seeds were counted daily for 7 days. The seeds were considered germinated when they showed about 2 mm of root protrusion. The parameters recorded were: germination (%), average time of germination in days (ATG) and the germination speed index (GSI) (23).

Growth bioassays

Transparent boxes (11x 11x 3 cm) were lined with two sheets of germitest paper and received 10 mL of extract at test concentrations (20, 10, 5 and 2.5%). The boxes were left open for total evaporation of ethanol and then the substrate was moistened again with 10 mL distilled water and 10 pre-germinated seeds were placed on the paper in the boxes, with four replicates each. In control group, the boxes with 10 mL of ethanol were left open for total evaporation of ethanol; the substrate was re-moistened with 10 mL distilled water and seedlings were placed on two 'germitest' papers). The boxes were maintained in BOD germination chambers (20 and 25^{o}C), respectively, for lettuce and tomato seeds. Ten day after sowing; stems length was measured (mm), considering the stem of each plant to be apex of shoot and root length (mm) at the neck of the plant to the meristematic apex of root system.

Phytochemicals

Phytochemicals were determined by humidification (crude extract - 20%), as per colorimetric testing and/or the chemical precipitation methods of Matos (22) and Wagner and Bladt (35). The ethanolic extracts were used to quantify the flavonoids and total phenolis. The flavonoids were determined based on method of Peixoto Sobrinho *et al.* (27). The total phenolic (FT) compounds were determined based on the Folin-Ciocalteu method, as per method of Sousa *et al.* (33). The pH (pH DM-20, Digimed) and electrical conductivity (EC DM3, Digimed) of ethanolic extracts were determined. The concentration of soluble solids was determined using a refractometer and results were expressed in degrees Brix corrected to 20 °C (Model RTD-45, Refractometer).

Statistical analyses

The experiment was done in random omised design. The data were analyzed using statistics programme called BioEstat 5.0, and anything of significance was passed through Tukey's test, to a level of 5% ($p<0.05$).

RESULTS AND DISCUSSION

pH, Electrical Conductivity and Soluble Solids

The pH of all concentrations was 4.3±0.3. The pH of leaf extracts of *V. divergens* did not influence the germination and early seedling growth of test crops. Lettuce is insensitive to differences in pH in a wide range of variations (29). Only extremes of acidity or alkalinity [pH 3.0 or less (very acid) or higher than or equal to 9.0 or 11 (extremely alkaline)] exert depressive effects on seed germination and root growth (12).

The electrical conductivity was 14.9, 15.0, 16.8 and 26.1 μScm^{-1}, respectively, for 2.5, 5, 10 and 20% concentrations. The electrical conductivity of extracts also did not affect the germination. As per Carmello (9) the process of germination and growth were maintained between 25.3 to 173.5 μScm^{-1}. The electrical conductivity (EC) can also estimate the ionic strength of extract and consequently the osmotic potential (36), as per our EC data, the osmotic potential was low.

There was no difference between the tested concentrations in soluble solids contents, values equal to 0.8. The soluble solids concentration indicated that the amount of sugar was low and the osmotic potential of extract did not interfere with germination and growth.

Germination and Growth

Germination (%) indicated that the extract (10 and 20%) showed significant inhibition with negative influence on the germination of lettuce and tomato seeds; but 2.5 and 5% were statistically equal to the 'control-model' (Tables 1 and 2, Figs 1 and 2). The obtained results indicate that the stratum tested has allelochemicals that interfere in the process of germination.

Table 1. Effects of *V. divergence* extracts on germination (%), germination speed index (GSI), average time of germination (ATG) and elongation primary root and lettuce (*Lactuca sativa* L.)

Concentrations (%)	Germination (%)	GSI	ATG (days)	Growth (mm)	
				Root	Stems
0	86 a	9 a	2.6 a	18.3 a	7.5 a
2.5	82 ab	3.5 b	2.6 a	11.8 b	2.9 b
5	77 ab	2.1 b	3.9 b	11 b	3.6 b
10	75 b	2.3 b	3.3 b	8.1 c	4 b
20	75 b	2.1 b	3.6 b	7 c	4.1 b

* Means followed by same letter in columns do not differ statistically among themselves by Tukey test ($p > 0.05$).

The allelopathic potential of certain species of the Vochysiaceae family, in rocky scrubland and parts of the Pantanal region in Mato Grosso do Sul state, has been studied; with regard to work carried out on *Qualea parviflora* [Mart.], where aqueous extracts of leaves (1, 3 and 5%) were used to evaluate the germination and initial development of invasive species such as *Bidens pilosa* [L.], *Digitaria horizonatalis* [Willd.], *Melinis minutiflora* [P. Beauv.] and the cultivated species *Zea mayas* [L.]. Only at the highest concentrations did there occur a delay in the growth-peak and germination of the species under evaluation (2).

Germination speed index (GSI) indicated that all concentrations had a negative effect on germination vigor (tomato and lettuce), showing a large reduction for the average values from the concentration of 2.5% (Table 1 and 2), for tomato and lettuce.

Average time of germination (ATG), for lettuce, significant differences occurred between different treatments, with germination occurring over the longest time period, with the average germination affected by the concentration of 5% compared with the control group (Table 1), delaying the germination time for almost four days. Extracts increased the average germination of tomato at concentration of 2.5%, indicating greater allelopathic effect of extract (Table 2).

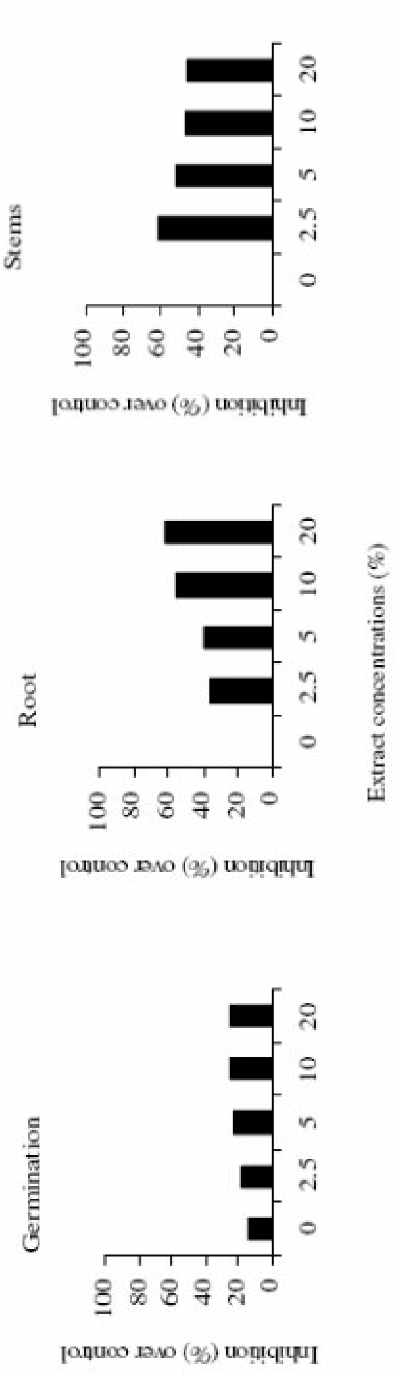

Figure 1. Inhibitory effects of *V. divergence* extracts on germination and seedling growth of lettuce.

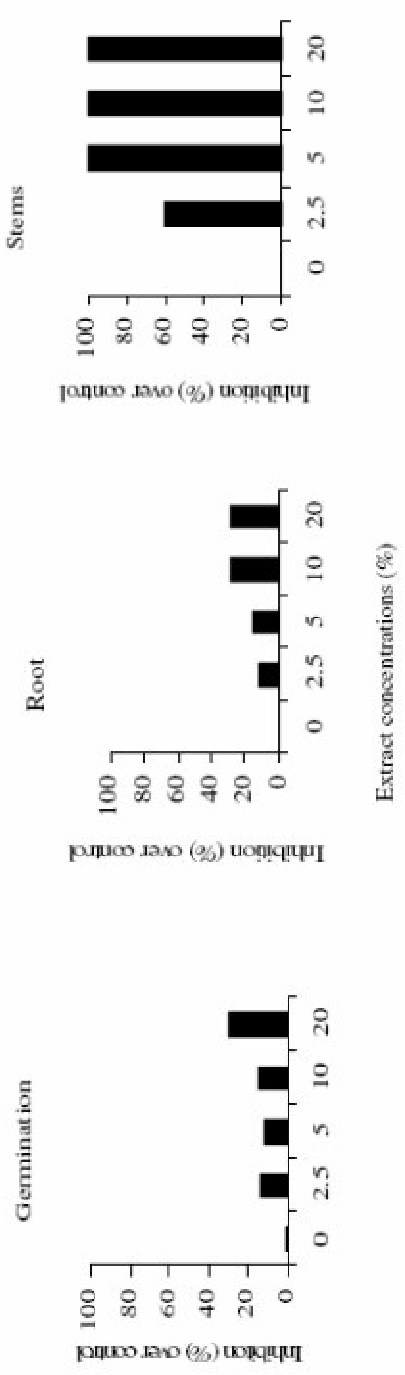

Figure 2. Inhibitory effects of *V. divergence* extracts on germination and seedling growth of tomato.

Table 2. Average value in percentage terms of germination (%), germination speed index (GSI), average time of germination (ATG) and average size of primary root and aerial part of tomato (*Lycopersicum esculentum* Mill.) seeds treated with extracts at concentrations of 0% (control - water), 2.5, 5, 10 and 20%, temperature of 25 °C

Concentrations (%)	Germination (%)	GSI	ATG (days)	Growth (mm)	
				Root	Stems
0	99 a	9.1 a	3 a	5 a	4.8 a
2.5	86 ab	5.1 b	4.2 b	4.4 ab	1.9 b
5	88 ab	5 b	4.5 bc	4.2 ab	0 c
10	85 b	4.5 bc	4.9 c	3.6 b	0 c
20	70 b	3.3 c	5.5 d	3.6 b	0 c

* Means followed by same letter in columns do not differ statistically among themselves by Tukey test (p> 0.05).

Where seeds had germinated in high numbers, the above concentrations affected GSI and ATG – distributing the germination over a larger time frame. It can be inferred that the larger concentrations of metabolites, at these concentrations (2.5, 5, 10, 20%), was a negative factor in the germination speed and vigor of the seeds.

Significant inhibitory effects were also observed for the seedling growth medium for stem and root system for the two target species (Table 1 and 2, Figs. 1 and 2). In lettuce (Table 1, Fig. 1) seedling growth (stem and root) was inhibited by the extract of lowest concentration (2.5%).

The aerial part of tomato seedlings was also inhibited by the lowest concentration (2.5%), and extracts of 5, 10 and 20% prevented the development of the stems. Root growth was more inhibited at concentrations of 10 and 20% (Table 2, Fig. 2).

According to Ferreira and Borghetti (13), germination is less sensitive to allelochemicals than is the growth of seedlings, because allelopathic substances can induce the appearance of abnormal seedlings – radicle decay being one of the most common symptoms of this. Thus, very often, allelopathy does not affect germination potential *per se*, but rather it affects the vigor and/or velocity of germination as well as other parameters. The results obtained for the germination bioassays in this study are consistent with the considerations made, where germination is less affected by allelochemicals, since the percentage of germination was little affected by the extracts in the two target species.

Dry root mass or parts exposed to air such as the length of the lower stem or radicle, is the parameter most used when evaluating allelopathy effects on growth. The seedling growth may be more sensitive to allelopathic substances, as these can induce the appearance of abnormal seedlings, and necrosis of the radicle is among the most common symptoms (15,21). The growth of seedlings in the present study was negatively affected at all concentrations of extracts compared with the control (0%).

Growth reduction in lettuce and tomato (root and aerial part), treated with extracts, suggests that the effects of allelopathy might be linked to the harmful effects of constituent chemicals present, as well as by phenolic compounds and tannins – chemicals which, in accordance with Peres et al. (26), have this effect.

The aqueous extract of leaves and fruits of *Q. parviflora*, at concentrations of 1%, reduced radicle growth by more than 50%, as well as inducing a lateral development of roots in sesame seedlings (*Sesamum indicum* L.) (7). Silva *et al.* (31), working with

Qualea grandiflora Mart., also indicated that the extract obtained from leaves of this species has an inhibiting effect on germination, showing that some species of the family Vochysiaceae have allelopathic potential.

Phytochemistry

The phytochemical screening of *V. divergens* fresh leaves indicated the presence of phenolic compounds (moderately positive= ++), tannins (Strongly positive= +++), flavonoids, coumarins, steroids and free triterpenes (weakly positive= +). In the determination of phenolic compounds (FC), values of the ethanoholic extract were 4.16 (±0.02), 4.21 (±0.001), 4.26 (±0.008) and 9.32 (±0.01) µg/g, respectively for the concentrations of 2.5, 5, 10 and 20%, while the content of total flavonoids were 2.44 (±0.01), 2.47 (±0.001), 2.49 (±0.003) and 4.17 (±0.07) µg/g, respectively for the concentrations of 2.5, 5, 10 and 20%.

The chemistry of the species constituting the family *Vochysiaceae* is still not well understood (10). As for the *Vochysia* genus (which comprises 105 different species), the presence of polyphenols (ellagic acid and its derivatives) has been noted, together with anthraquinones (*anthracenedione*, *dioxoanthracene*) in the bark of *V. acuminata* [Bongard] and *V. tyrsoidea* [Pohl] (11) trunk. Flavonoids were identified in the leaves of *V. cinnamomea* [Pohl] and *V. tucanorum* [Mart.] (20), and also in the fruit of *V. guianensis* [Poir], aside from the presence of alkaloids (6).

For the *Vochysia divergens* species, phenolic compounds, tannins and coumarins were not cited in work developed with tree bark by Hess et al. (16,17) and Hess and Monache (18). In other species of the genus, only the presence of coumarins was not recorded. Species of the genus *Vochysia* are rich source of terpenoids (19, 40), particularly the triterpenes found in bark of *V. vismiaefolia* [Spruce] (3) and of *V. pacifica* [Cuatrec] (38). Triterpenes were also identified in the leaves and fruit of *V. ferruginea* [Mart], although β-sitosterol was derived only from its leaves (40). These steroids were also noted in *V. vismiaefolia* (3).

Phenol compounds correspond to a secondary class of metabolites in which one encounters the main compounds thought to harness allelopathic activity; ranging from simple phenols to the more complex structure innate in tannins (29). Phenols act by diminishing the extension and the elasticity of the cellular wall, as well as by blocking mitochondrial respiration (37). Among the secondary metabolites, phenolic compounds are common in species of the Vochysiaceae family (30), but no data were found on the content of total phenolics and total flavonoids for species of this family.

The effects of allelopathy may also be mediated by the presence of coumarins (5). Coumarins are regarded as potent inhibitors both in the growth of plants and in the germination of seeds (29,39). According to Abenalovi *et al.* (1), this occurs due to the ability of coumarins to block mitosis, thereby reducing the entrance for water, the consumption of oxygen and the capacity for the seed interior to retain electrolytes.

The alterations in germination, or primary root growth, might be the result of allelochemical effects on the permeability of membranes, transcription and translation of DNA; the functioning of secondary messengers; respiration, for loss of oxygen (phenols); the conformation of enzymes and receptors, or even by a combination of these factors (21,29).

Allelochemical cytological structures can affect concentrations of hormones, membrane permeability, mineral absorption, respiration, protein synthesis, enzyme activity, water relations, causing changes in genetic material among others (21,37).

More specifically, studies using plants of the *Vochysia*, in order to verify allelopathic activity, are still in their initial stage, a fact which justifies the continuation of the present study in employing extracts with different grades of polarity, especially with studies on the species *V. divergens*, monodominant in large areas of the Pantanal, an indication of its competitiveness and potential allelopathic action in natural environments.

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