PLANTA DANINHA

SOCIEDADE BRASILEIRA DA CIÊNCIA DAS PLANTAS DANINHAS

<http://www.sbcpd.org>

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

Article

Leaf and Root Allelopathic Potential of the *Vernonanthura brasiliana*

Potencialidade Alelopática de Folha e Raiz de ***Vernonanthura brasiliana***

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ABSTRACT - The Brazilian native weed *assa-peixe* (*Vernonanthura brasiliana*) is considered one of the main invasive plant species of pasture areas in the Amazon. It is thus necessary to know their biology and their behavior in the environment, and to enhance the development of management strategies, minimizing the use of herbicides. The objective of this study was to test the allelopathic effect on the germination and growth of the bioindicator species *Lactuca sativa* from leaf and root extracts obtained from fresh and dry *V. brasiliana*. The experiment consisted of six treatments with concentrations of 0, 12.5, 25, 50, 100, and 200 mg mL^{-1}, using four replicates per treatment. The evaluated parameters were: germination percentage, first count, germination speed index, and shoot and root length. The aqueous extract of fresh *V. brasiliana* roots caused an allelopathic effect on all evaluated parameters, while the dry root extract only affected the germination speed index. Root growth inhibition and growth stimulation of lettuce seedlings were verified when submitted to dry and fresh root extracts. Extracts of *V. brasiliana* caused an allelopathic effect on the species *Lactuca sativa* L., where the aqueous extracts of dry leaves and fresh roots were the most influential.

Keywords: assa-peixe, *Lactuca sativa* L., allelopathy, germination.

*RESUMO - A espécie de planta daninha assa-peixe (**Vernonanthura brasiliana**) tem se destacado como uma das principais espécies vegetais invasoras de áreas de pastagens na Amazônia, tornando-se importante a necessidade da produção de informações acerca da sua biologia, visando o conhecimento de seu comportamento no ambiente e potencializando o desenvolvimento de estratégias de manejo, minimizando o uso de herbicidas. O presente estudo teve como objetivo testar o efeito alelopático na germinação e crescimento da espécie bioindicadora Lactuca sativa a partir de extratos de folhas e raiz obtidos de **V. brasiliana** em estado fresco e seco. O experimento consistiu de seis tratamentos, com concentrações de 0; 12,5; 25; 50; 100; e 200 mg mL^{-1}, utilizando quatro repetições por tratamento. Os parâmetros avaliados foram: porcentagem de germinação, primeira contagem, índice de velocidade de germinação e comprimento da parte aérea e radicular das plântulas. O extrato aquoso das raízes frescas de **V. brasiliana** provocou efeito alelopático em todos os parâmetros avaliados, enquanto o extrato de raízes secas só afetou o índice de velocidade de germinação. Verificou-se inibição no crescimento da parte radicular e estímulo no crescimento da parte aérea das plântulas de alface quando submetidas aos extratos das raízes secas e frescas. Extratos de **V. brasiliana** ocasionaram efeito alelopático sobre a espécie **Lactuca sativa** L., sendo os extratos aquosos das folhas secas e das raízes frescas os que apresentaram maior influência.*

Palavras-chave: assa-peixe, *Lactuca sativa* L., alelopatia, germinação.

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Received: July 8, 2018
Approved: January 21, 2019

Planta Daninha 2019; v37:e019208452

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Doi: 10.1590/S0100-83582019370100142

INTRODUCTION

Vernonanthura brasiliana L., species known as *assa-peixe*, has become one of the most important weeds of livestock environments in the entire Amazon, and especially in the southern Amazon, which includes southern Pará and northern Mato Grosso. It has turned production more costly given the use of large volumes of herbicides from the auxin mimics chemical group for their effective control.

Intensive application of herbicides, especially those with a sustained effect on the environment, is a major cause of devastation on the agricultural environment (Procópio et al., 2008). Picloram herbicides are persistent for long periods after their application, resulting in intensive discussions about their use (Franco et al., 2014).

However, there are no efficient alternative techniques for the management of weeds such as *V. brasiliana*, and the amount of information regarding these species in the livestock environment is little. Knowledge of their behavior and effective aggressiveness would be relevant to develop management strategies that reduce the intensive use of herbicides, especially those with a prolonged effect on the environment.

Besides providing information about weed biology, allelopathy research incorporates these natural defense strategies, whose allelochemicals have been used as an alternative to herbicides, insecticides, and nematicides. Most of these substances come from secondary metabolism, since they represented some advantage in plant evolution against microorganisms, insects and other pathogens or predators, either inhibiting their action or stimulating plant growth or development (Khanh et al., 2006).

There is a possibility of controlling invasive species through allelochemicals (Santos and Rezende, 2008). Natural compounds have several advantages over synthetic compounds, such as greater water solubility, absence of halogenated molecules, and shorter half-life (Duke et al., 2000).

Based on the interaction of allelopathic chemicals, the objective of this study was to analyze the allelopathic potential of aqueous extracts of *assa-peixe* leaves and roots on lettuce seed germination.

MATERIALS AND METHODS

The collection of botanical material from *Vernonanthura brasiliana* was carried out in Alta Floresta-MT, at coordinates 09º50'43.7"S and 056º04'33.2"W. The samples were deposited in the Southern Amazon Herbarium (HERBAM), Campus I - Alta Floresta/MT, under Voucher: Nishimuta, H. A. 01 - 27/08/2013.

The experimental design adopted for the experiment was completely randomized, consisting of a 2 x 2 x 6 factorial scheme. The factors evaluated were two plant parts (leaf and root), in two states (fresh and dry), and six concentrations (0; 12.5, 25, 50, 100, and 200 mg mL^{-1}), having four replications of 30 seeds per treatment.

The aqueous extracts of *V. brasiliana* were obtained from the collection of fresh and dried leaves and roots.

To extract the fresh material, it was grounded in a blender and sifted on filter paper immediately after collection, using 12.5, 25, 50, 100 and 200 mg mL^{-1} concentrations.

The test species was lettuce (*Lactuca sativa* L.), and the bioassays were performed in a germination chamber with a controlled temperature of 25 ºC (± 2 ºC), in a photoperiod of 12 hours. The seeds were placed in transparent gerbox acrylic boxes, lined with two sheets of germitest paper, previously autoclaved and moistened with 5 mL of each treatment extract. A control treatment was also used, where the germitest paper was moistened only with distilled water. Four replications of 30 seeds were used for each of the five concentrations of the extract plus control group, totaling 96 gerbox boxes (fresh and dried leaf, and root extract).

According to the "Rules for Seed Analysis" (Brasil, 2009), to assess seed vigor the first count test (FC) was performed at four days, and for germination percentage (GP) the count occurred at

seven days. Daily counts were performed for the germination speed index (GSI), as proposed by Maguire (1962).

On the seventh day of germination, lettuce seedlings were measured using a digital caliper, evaluating shoot length (SL), and root length (RL). Forty seedlings per treatment were evaluated. Seedling selection was random.

Results were submitted to variance analysis (ANOVA), and means were grouped by Scott-Knott test (5% probability). A regression analysis was also performed, which was done with the aid of the SISVAR 5.3 statistical program (Ferreira, 2011).

RESULTS AND DISCUSSION

Aqueous extract of fresh *assa-peixe* did not affect FC or GP, yet GSI was influenced, showing a negative effect on 100 mg mL^{-1}, and 200 mg mL^{-1} (Table 1). According to Ferreira and Borghetti (2004), often the allelopathic effect does not occur on germinability (final percentage of germination in time), but rather on germination speed or another process parameter.

Table 1 - First count (FC), germination percentage (GP), and germination speed index (GSI) of lettuce seeds submitted to different concentrations of aqueous extracts of fresh and dry leaves of *Vernonanthura brasiliana* L.

Extract fresh	Concentration (mg mL^{-1})	FC	GP	GSI
Fresh leaves	0	96.67 aA	97.50 aA	28.57 aA
	12.5	98.33 aA	98.33 aA	28.69 aA
	25	99.17 aA	99.17 aA	28.15 aA
	50	99.17 aA	99.17 aA	27.44 aA
	100	99.17 aA	99.17 aA	24.96 bA
	200	97.50 aA	97.50 aA	9.00 cA
Dry leaves	0	97.50 aA	97.50 aA	28.75 aA
	12.5	95.83 aA	97.50 aA	21.78 bB
	25	99.17 aA	99.17 aA	20.96 bB
	50	92.50 aA	95.00 aA	14.62 cB
	100	95.83 aA	97.50 aA	14.39 cB
	200	68.33 bB	70.00 bB	7.72 dA

Lower case letters compare the concentrations (control; 12.5; 25; 50; 100; and 200 mg mL^{-1}) for each extract type separately. Capital letters compare the control and extracts of fresh and dry leaves at the same concentrations. Equal letters do not differ from each other by the Scott-Knott test ($p<0.05$).

Ferreira and Aquila (2000) attribute these changes in germination patterns to possible results on membrane permeability, DNA transcription and RNA translation, cellular respiration (oxygen sequestration by phenols), enzymes and receptors conformation, or even the combination of these factors.

There was a greater influence of the aqueous extract of dried leaves than of green leaves on lettuce seeds (bioindicator species) (Table 1). According to Singh et al. (2005), it is probable that the integrity of cell membranes was undone while the material was dried, facilitating inhibitor(s) release to the medium. Moreover, fresh leaves have more water in the tissues (Ferreira and Borghetti, 2004).

According to Borella et al. (2010), negative allelopathic effects resulting from substances in araticum extracts found in both germination and early radish growth were more significant in dry leaf extracts, as pH effects and osmotic potential on such results.

There was a greater influence of the aqueous extract of dried leaves than of green leaves on lettuce seeds (bioindicator species) (Table 1). Similarly, Goetze and Thomé (2004) also found that tobacco and eucalyptus extracts slowed seed germination of three types of vegetables, especially those with dry leaves, without germination.

Dried plant material may have a greater influence due to non-destruction of allelochemicals while drying (Singh et al., 2005). It is believed that the greatest effect of extracts from dry plant material is possibly associated with the use of larger leaves to obtain the extract at the desired concentration (Goetze and Thomé, 2004).

In the interaction between fresh and dried leaves, they differed in both FC and GP in the highest concentration (200 mg mL^{-1}), but not in GSI. There were differences only in concentrations of 12.5; 25; 50; and 100 mg mL^{-1} for this variable (Table 1).

In the development of lettuce seedlings treated with aqueous extract of fresh *V. brasiliana* leaves, RL showed significant growth decrease as concentrations were increased (Table 2). However, SL was stimulated at all concentrations, showing higher and statistically different means than control. The 25 and 50 mg mL^{-1} concentrations had the highest averages (Table 2). In the initial development of *Lactuca sativa*, *Lycopersicum esculentum* and *Zea mays* treated with *Capaifera sabulicola* ethanolic extracts, there were possible divergences in studies involving allelopathic effects. This may be related to different factors, such as concentrations and extract polarity (alcoholic or aqueous), photochemical analyses characteristics of each species evaluated, exposure time, and tissue type versus absorption efficiency (Linhares Neto et al., 2014). Thus, the type of extraction may be more effective in selecting specific sets of organic molecules of greater or less affinity with the solvents used for extract preparation (ethanol, methanol, distilled water, etc.).

Lactuca sativa seedling RL reduction was also reported by Alves et al. (2004), when they were subjected to the action of the essential oils of cinnamon, rosemary pepper and citronella grass, especially with increasing concentrations. These results resemble those found in this study.

In allelopathic studies involving weed physiology, there was a reduction in apical growth of both shoot and root cultivated plants due to AIA (indolylacetic acid) and GA (gibberellic acid) hormone inhibition, due to allelochemicals in these species (Quayyum et al., 2000; Cavalcante et al., 2018). This statement underscores the importance of developing studies aiming at a better understanding of how invasive plants act against cultivable plants and, above all, to develop effective strategies for controlling and/or inhibiting such agronomically undesirable actions.

Table 2 - Growth characteristics of *Lactuca sativa* L., submitted to different concentrations of aqueous extracts of fresh and dry leaves of *Vernonanthura brasiliana* L. RL (root length) and AL (aerial length)

Extract fresh	Concentration (mg mL^{-1})	RL	AL
Fresh leaves	0	46.63 a	23.85 d
	12.5	23.20 b	31.17 b
	25	19.64 c	38.78 a
	50	13.26 d	39.47 a
	100	6.73 e	30.16 b
	200	3.71 f	27.14 c
Dry leaves	0	45.55 a	24.03 b
	12.5	17.80 b	35.73 a
	25	10.81 c	34.18 a
	50	6.65 d	33.91 a
	100	7.24 d	24.14 b
	200	4.18 e	11.00 c

Means followed by the same letters in the column do not differ from each other by the Scott-Knott test ($p<0.05$).

There was a reduction in RL averages in all treatments with the use of aqueous extract of *V. brasiliana* dry leaves. There was a decrease in root growth with the increase of aqueous extract concentration, yet 50 and 100 mg mL^{-1} concentrations did not differ from each other (Table 2).

Lettuce seedling SL was stimulated at concentrations between 12.5 and 50 mg mL^{-1} of the aqueous extract of dried leaves when compared to the control. Only the higher concentration of this extract caused SL inhibition in the lettuce seedling (Table 2).

By subjecting lettuce seeds to germination with *Ziziphus joazeiro* fruit extract, root growth and shoot growth were inhibited (Oliveira et al., 2009). Lettuce seedling roots were inhibited by all concentrations of *C. americanum* leaf and root extracts (Ribeiro et al., 2009).

In the evaluation of lettuce seedlings submitted to concentrations of *Dipteryx odorata* extracts, root development was more affected than that of shoots, showing a reduction in concentration of 0.19 mg mL^{-1}, compared to control (Mano et al., 2006). This was also reported by Tur et al. (2010)

in lettuce and tomato seedlings under aqueous extracts of *Duranta repens*, which found the occurrence of abnormal seedlings with atrophied primary roots, absence of secondary roots and root necrosis when submitted to higher concentrations.

The initial growth of any seedling is more sensitive than germination because, regardless of species type, seed germination is considered a discrete process, whether or not it germinates (Ferreira and Aquila, 2000).

The sensitivity of the root system to the action of allelochemicals, compared to that of the shoot, due to longer contact time with aqueous extracts, may more effectively compromise root development (Hoffmann et al., 2007).

Fresh and dried leaf extracts caused significant allelopathic effects from 12.5 mg mL^{-1}, proving to be effective inhibitors of the initial development process of sensitive plants. These results reinforce the need to develop more specific research on the action of allelochemicals in *assa-peixe*, especially as they might act to inhibit other invasive plants. Effects of aqueous extracts obtained from fresh and dried leaves in relation to lettuce seed GSI can be seen in Figure 1. The GSI decreased with increasing quadratic concentration of aqueous extracts.

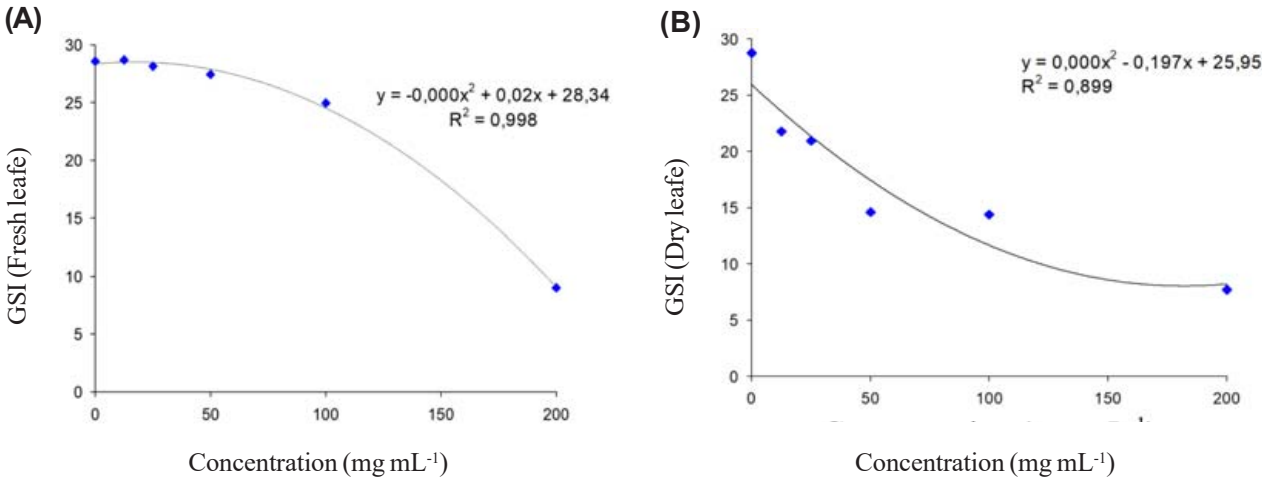

Figure 1 - *Lactuca sativa* germination speed index (GSI) exposed to different concentrations of aqueous extracts of *Vernonanthura brasiliana*. (A) fresh leaf; (B) dry leaf.

Changes that occur in both the germination curve and the GSI indicate interference with metabolic reactions, which culminates in germination (Ferreira and Borguetti, 2004). Hoffmann et al. (2007) also observed GSI reduction when they submitted *L. sativa* and *B. pilosa* seeds to aqueous extracts of leaves of *Nerium oleander* and *Dieffenbachia picta*.

Maraschin-Silva and Aquila (2006) observed a longer period of time for *L. sativa* achenes germination when they analyzed the allelopathic potential of *Cecropia pachystachya*, *Peltophorum dubium*, *Psychotria leiocarpa*, *Sapium glandulatum* and *Sorocea bonplandii* specie.

The aqueous extract of fresh *V. brasiliana* roots, at concentrations between 25 and 200 mg mL^{-1}, negatively affected the variables FC, GP and GSI, and the means differed from the control. In turn, the aqueous extract of dried roots only affected the GSI at the concentration of 200 mg mL^{-1} (Table 3).

Borella and Pastorini (2009) obtained similar results with *Phytolacca dioica* on *Lycopersicum esculentum* and *Bidens pilosa*, as they observed reduced germination and GSI, with the highest concentrations (4 and 8%) of aqueous extracts of *P. dioica*. Similarly, lettuce germination decreased with increasing concentration of *Machaerium acutifolium* root extracts on the same bioindicator species, where there was total inhibition under 100% concentration of the extract (Povh et al., 2007).

Analyzing the development of lettuce seedlings treated with aqueous extract of fresh and dried roots of *V. brasiliana*, there was inhibition of root growth and stimulation of shoot growth

Table 3 - First count (FC), germination percentage (GP) and germination speed index (GSI) of lettuce seeds submitted to different concentrations of aqueous extracts of fresh and dry roots of *Vernonanthura brasiliana*

Aqueous extract	Concentration (mg mL^{-1})	FC	GP	GSI
Fresh root	0	97.50 aA	98.33 aA	28.92 aA
	12.5	98.33 aA	98.33 aA	29.25 aA
	25	94.16 bA	95.00 bA	27.42 bA
	50	98.33 aA	99.17 aA	29.36 aA
	100	94.16 bA	95.00 bA	27.69 bA
	200	94.16 bA	95.83 bA	23.50 cA
Dry root	0	95.00 aA	97.50 aA	28.07 aA
	12.5	94.17 aA	95.83 aA	27.60 aA
	25	95.83 aA	97.50 aA	28.01 aA
	50	96.67 aA	97.50 aA	28.05 aA
	100	96.67 aA	97.50 aA	26.47 aA
	200	89.16 aA	90.00 aA	22.50 bA

Lower case letters comparing the concentrations (control; 12.5; 25; 50; 100; and 200 mg mL^{-1}) for each extract type separately. Capital letters comparing control and fresh and dried root extracts at the same concentrations. Equal letters do not differ from each other by the Scott-Knott test ($p<0.05$).

(Table 4). Only at the concentration of 200 mg mL^{-1}, dry root extract negatively affected SL (Table 4). Borella et al. (2010) also found a decrease in RL with increasing concentration of fresh leaf extract of *Rollinia sylvatica*.

It was consolidated in graphical representation of the GSI of *L. sativa* seeds, which were submitted to different concentrations of the aqueous extract of fresh and dried *V. brasiliana* roots (Figure 2). It was also verified that the effects were similar in both extract types, where there was greater reduction effect in the concentrations of 200 mg mL^{-1}.

In the analysis of the allelopathic potential of forage turnip plant extracts on lettuce seed germination, it was found that, for GSI parameter, there was a delay in the germination process, with consequent adjustment to the quadratic model in all root extract concentrations (Nery et al., 2013). However, the most pronounced effect was observed at 100% concentration, which recorded the lowest GSI values.

Results of the action of aqueous extract on lettuce seeds showed that GSI had a quadratic relationship with the different *Mikania glomerata* concentrations. The increase in concentration led to a decrease in this variable (Souza, 2005).

Table 4 - Lettuce growth characteristics, submitted to different concentrations of aqueous extracts of fresh and dried roots of *Vernonanthura brasiliana*. RL (root length) and AL (aerial length)

Aqueous extract	Concentration (mg mL^{-1})	RL	AL
Fresh root	0	38.48 a	28.28 d
	12.5	34.68 b	33.35 c
	25	35.44 b	36.08 b
	50	23.95 c	43.32 a
	100	24.51 c	42.14 a
	200	13.38 d	42.35 a
Dried root	0	48.20 a	31.46 c
	12.5	34.37 b	35.57 b
	25	26.80 c	41.06 a
	50	14.64 d	43.14 a
	100	7.73 e	31.90 c
	200	3.54 f	18.11 d

Means followed by the same letters in the column do not differ from each other by the Scott-Knott test ($p<0.05$).

The aqueous extracts of fresh and dried *V. brasiliana* leaves and roots showed inhibitory effect on lettuce seedlings RL and stimulatory effect on SL. In general, the roots have more pronounced responses to phytochemicals in the extracts, compared to the other seedling structures, since they are in direct and prolonged contact with the allelochemicals and also by the physiological distinction of each structure (Chon et al., 2000; Chung et al., 2001).

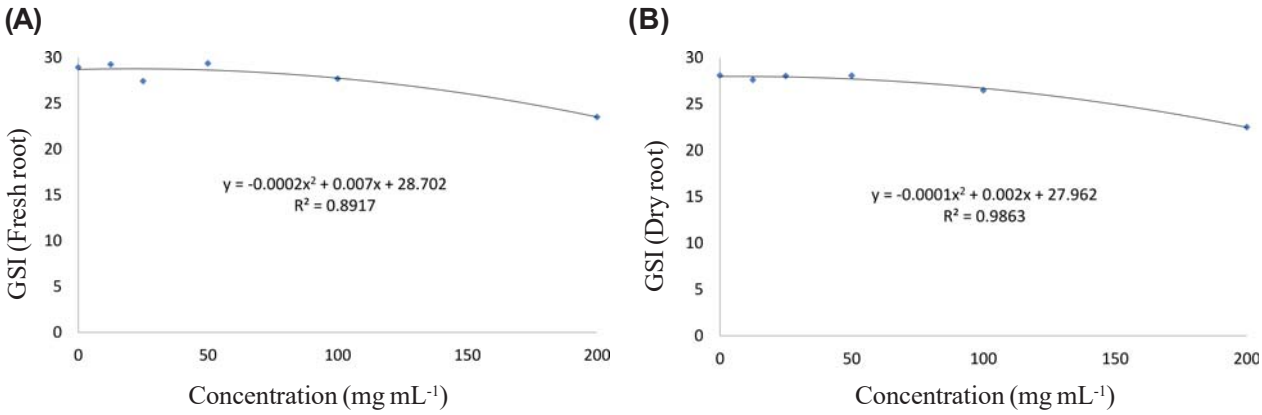

Figure 2 *- Lactuca sativa* germination speed index (GSI) exposed to different concentrations of aqueous extracts of *Vernonanthura brasiliana*. A) Root - fresh; B) Root - dry.

The fresh root extract of *V. brasiliana* proved to have a negative allelopathic effect on FC, GP and GSI characteristics at concentrations of 25, 100 and 200 mg mL^{-1}, while the dry root extract showed a significant GSI reduction only with the highest concentration.

Finally, it can be verified that the aqueous extracts of the dried leaves and fresh roots of the *Vernonanthura brasiliana* species have greater allelopathic effects on lettuce seedling germination and growth, *ergo*, a species with allelopathic potentiality.

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