Phytotoxic potential of aqueous leaf extract of *Tocoyena formosa* and *Rudgea viburnoides*[1]

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ABSTRACT

Plants have organic components produced as secondary metabolites able to positively or negatively interfere on normal development of other species. The objective of this research was to determine the phytotoxic effects of aqueous leaf extract from two species Rubiaceae family: *Tocoyena formosa* and *Rudgea viburnoides* on the germination and initial development of lettuce (*Lactuca sativa* L). The extract was obtained from mixing 50 g of fresh leaf and 500 mL of distilled water. The experiment was arranged in completely randomized design for each specie, subjected to five extract concentrations: 0, 25, 50, 75 and 100%; and 4 repetitions of 50 seed each. The bioassays were performed in laboratory at controlled temperature and luminosity during seven days. Results showed that the aqueous extract of both species do not affected the germination and the percent of abnormal lettuce seedlings. *R. viburnoides* showed no pronounced difference in relation to shoot length and dry biomass; however, the root length was reduced by 85%. The extract of *T. formosa* at higher concentrations, drastically reduce the dry biomass and length of shoots and roots. Therefore, both species have phytotoxic effect on seedling growth of lettuce being the length root the most affected variable.

Keywords: *Lactuca sativa* L.; bioassays; aqueous extract; germination.

INTRODUCTION

The Rubiacea family has 17 genera and 727 species endemic to the Brazilian flora (Barbosa *et al*., 2015). The genus *Tocoyena* with neotropical distribution has an important role as food for wild animals (Delprete, 2008). The species *Tocoyena formosa* (Cham. & Schltdl.) K. Schum popularly known as jenipapo-bravo, guamarú and genipapo-de-cavalo, is a woody species from the Brazilian cerrado distributed in rural physiognomies and cerradão (Silva Júnior & Santos, 2005). It is considered an ornamental tree with potential use for landscaping. Commonly used in the Northeast of Brazil as an analgesic and for the treatment of cough, cystitis, kidney and heart problems. Also, the extract of different parts of the *T. formosa* has acaricidal, antifungal and antinociceptive properties (Santos *et al*., 2013; Cesário *et al*., 2018a).

Another genus of Rubiaceae is *Rudgea*, widely distributed on the coast and on the Brazilian cerrado with species of recognized medicinal properties such as *Rudgea viburnoides* (Cham.) Benth., popularly known as porangaba, casca-branca, congonha-do-gentio and chá-do-bugre. It grows mainly on sloping, well-drained soils and sandy soils with low fertility, therefore has great ability to absorb nutrients from poor soils and it is an aluminum (Al) hyperaccumulating specie (Malta *et al*., 2016). It is popularly used in the Brazilian folk medicine for its properties as diuretic, hypotensive, blood depurative, anti-rheumatic, and antisyphilitic (Alves *et al.*, 2004; Galdino *et al*., 2017).

The harmful or beneficial effect that a plant (including microorganism) exerts on another, due to the production and release of chemical compounds to the environment,

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is known as allelopathy (Ferreira, 2004). These chemical compounds are secondary metabolites (allelochemicals) produced by plants that vary in quality and quantity from species to species influenced by different natural conditions to which the plants are exposed (Gatti *et al*., 2014). Resistance or tolerance to the allelochemicals is relatively specific, with more sensitive species than others, such as *Lactuca sativa* (lettuce) and *Lycopersicum esculentum* (tomato), which are commonly used in laboratory bioassays (Ferreira & Aquila, 2000). Most of these compounds are water soluble and active at low concentrations, representing a potential alternative for use as herbicides in natural biological control of weeds under sustainable agricultural practices (Chung *et al*., 2001; Batish *et al*., 2007). Although many processes are involved, since the identification and selection of the compound as a mode of action, dose, persistence in the soil, toxicity to human and profitability and commercial scale, the laboratory assays of phytotoxic effect may act as a starting point for production of new bioherbicides (Soltys *et al*., 2013).

In the chemical characterization of *R. viburnoides*, the compounds were identified in the leaf extracts with positive reaction to tannins, flavonoids (quercetin, rutin), triterpenes (viburgenin and arjungenin) and saponins (arjunglucoside I and trachelosperosides) (Young *et al*., 1988; Alves *et al*., 2004, Almeida, 2011). In the phytochemical profile of the leaves of *T. formosa*, the presence of condensed tannins, saponins, fenolic acids and flavonoids was revealed (gallic acid, catechin, chlorogenic acid, caffeic acid, ellagic acid, rutin, quercetin and luteolin) which support its medicinal benefits (Cesário *et al*., 2018a, Cesário *et al*., 2018b).

Recent allelopathic studies have shown that aqueous extracts of species from Rubiaceae family have phytotoxic effects on the germination and initial growth of different target species (Frescura *et al*., 2013; Oliveira *et al*., 2014b). Therefore, the main objective of the present study was to evaluate if aqueous leaf extracts of *T. formosa* and *R. viburnoides* present a phytotoxic effect on the germination and initial growth of *Lactuca sativa* L., at different concentration.

MATERIALS AND METHODS

The experiment was carried out at the Seed Laboratory of the Faculty of Agronomy and Animal Science of the Federal University of Mato Grosso (UFMT), Cuiabá campus.

Leaves of *Tocoyena formosa* and *Rudgea viburnoides* were collected in a preserved area from Chapada dos Guimarães, Mato Grosso, located in the Central west of Brazil, between the geographic coordinates 15º10' - 15º30' S 55º40' - 56º00 W. The climate of the region is type Aw (Climate of Cerrado), according to Köppen classification.

The plant was identified and the material was collected from reproductive plants (7-8 age) in the month of august 2018, between 8:00 and 10:00 hours.

For the preparation of the aqueous extract, the adult and fresh leaves of each species were previously washed and disinfected for 5 minutes with 10 mL of 2.5% Sodium Hypochlorite, diluted in 500 mL of distilled water. Then, the leaves were rinsed with distilled water and gently dried with paper towel. The leaves were grounded in a blender in the proportion of 50 g of leaves per 500 ml of cold distilled water (Pires & Oliveira 2011). The extract was filtered using filter paper and stored in amber glass bottles at 18 °C until used in the bioassays.

A completely randomized experimental design with five treatments, consisting of concentrations of leaf extract, obtained by dilution in distilled water, was used (0% - control, 25%, 50%, 75% and 100%). Each contained four replications of 50 of lettuce seeds (*Lactuca sativa* L. cv Veneranda) obtained from commercial store.

pH was determined with a pH-meter and the electrical conductivity (EC) was measured by the conductivity meter. From the EC values, the osmotic potential (PO) was determined using the formula proposed by Ayers & Westcot (1985): Osmotic potential in atmosphere (ATM) = - 0.36 * electrical conductivity (EC). The ATM data was transformed to Osmotic Potential (MPa).

The seeds were placed in clear plastic boxes (11 x 11 x 3 cm) over two sheets of blotting paper moistened with each treatment, using the amount of 2.5 times the mass of the substrate. The boxes were capped and sealed with film paper, and kept in a BOD type germination chamber, at constant temperature of 20 °C and photoperiod of 12 hours during seven days.

Every 24 hours the number of seeds germinated were recorded for a period of 5 days (period in which stabilization occurred). Seeds with 2 mm radicular protrusion were considered germinated according to the concept of physiological germination. The percentage of germination was calculated by the expression: $G = (N/A) * 100$, where: N = number of germinated seeds, A = total number of seeds placed to germinate.

The Germination Speed Index (GSI) was determined according to Maguire (1962) by the expression: $GSI = (G1/N1) + (G2/N2) + ... + (Gn/Nn)$, where: G1, G2, Gn = number of seeds germinated at the first count, at the second count and at the last count; and N1, N2, Nn = number of days of sowing the first, second and last count.

Seedlings were classified as normal or abnormal according to the specifications of Brasil (2009). Abnormal plants were those that showed no potential to continue their development, with rotten, absent, totally atrophied root or aerial systems. Therefore, seedlings with small defects, such as limited or small damage, and delayed growth in the root system were considered normal.

At the end of germination test (5 days after the experiment), normal lettuce seedlings were evaluated for shoot length: root transition region up to cotyledon insertion, and root length: shoot transition region up to the apex of the root; with the aid of a graduated ruler in centimetres. In order to determine the dry mass, the normal seedlings of each replicate were dried in the oven with forced air circulation at 65 °C, where it remained until reaching constant weight. After this period, the samples were placed to cool in desiccators for approximately 60 minutes and weighed in an analytical balance (0,001 g).

The Allelopathic Index (IR) suggested by Williamson & Richardson (1988) was calculated, according to: IR = 1 - C/T (T e” C) or RI= T/C - 1 (T < C) where C = control germination speed (0%) and T = Treatment speed germination.

The results were subjected to ANOVA, and means were compared by Tukey test at 5% of probability. Polynomial regressions were adjusted, and the choice was based on significance ($p < 0.05$) and on the coefficient of determination (R2). The analysis was carried out using the statistical program SISVAR (Ferreira, 2010).

RESULTS AND DISCUSSION

It was verified that the pH values of the aqueous leaf extracts of the germination bioassays ranged from 4.95 to 5.12 for the species *Rudgea viburnoides* and from 4.99 to 5.01 for *Tocoyena formosa*, considered acidic when compared to the control (pH 7). The osmotic potential obtained for the aqueous leaf extract of *R. viburnoides* and *T. formosa* at different concentrations varied between -0.007 and -0.024 MPa respectively, being similar values in the two species (Table1).

According to Gatti *et al*., (2004), solutions with osmotic potential smaller or close to -0.2 MPa do not interfere in the germination of lettuce seed. The importance of pH control and the osmotic concentration of the crude extracts is essential in germination tests in laboratory, because there may be osmotically active substances in them that influence pH such as aminoacids, sugars and organic acids inhibiting germination and masking the allelopathic effect (Ferreira & Aquila, 2000).

The physical-chemical parameters evaluated were within the limits considered adequate for seed germination indicating that at pH values below 3 or above 10, germination could be affected or completely inhibited (Chachalis & Reddy, 2000). Therefore, it was considered that both the acidity and the osmotic potential of the aqueous extracts did not interfere in the observed results.

The germination ranged from 88 to 100%, with no significant difference between doses for the two species evaluated. In addition, there was no difference on the speed germination (GSI) calculated in the concentrations evaluated for both *R. viburnoides* and *T. formosa*, the results varied between 21.1 and 23.9; and the percentage of abnormal seedlings ranged from 0 to 12%, being not different from the control (distilled water).

Germination is the parameter less sensitive to the effect of the allelochemicals, which could be influenced by the season of the year that the collection of the material was made and the distribution of the germination curve. Thus, the allelopathic effect does not always affect germination, the generational behaviour of the germination curve, which is developed using the germination speed index (GSI), is important in the identification of anomalies in a normal germination development.

In this sense, germination should be monitored at intervals shorter than 24 hours, so it is recommended every 8 or 12 hours (Ferreira & Aquila, 2000).

Table 1: Hydrogen potential (pH) and Osmotic Potential (MPa) from aqueous leaf extract of *Rudgea viburnoides* and *Tocoyena formosa*, Cuiabá, MT. 2018

Species	**Extract concentration**	**pH**	**(MPa)**
	0%	7.00	-0.0010
	25%	5.12	-0.0076
Rudgea viburnoides	50%	5.02	-0.0127
	75%	4.95	-0.0180
	100%	5.00	-0.0237
	0%	7.00	-0.0010
	25%	4.99	-0.0077
Tocoyena formosa	50%	5.01	-0.0131
	75%	5.00	-0.0182
	100%	5.00	-0.0240

In this sense, germination should be monitored at intervals shorter than 24 hours, so it is recommended every 8 or 12 hours (Ferreira & Aquila, 2000).

According to Silveira *et al*. (2012), there was no significant difference in germination, Mean Germination Time and Germination Speed Index (GSI) in the concentrations of the stem extract of *M. tenuiflora* on

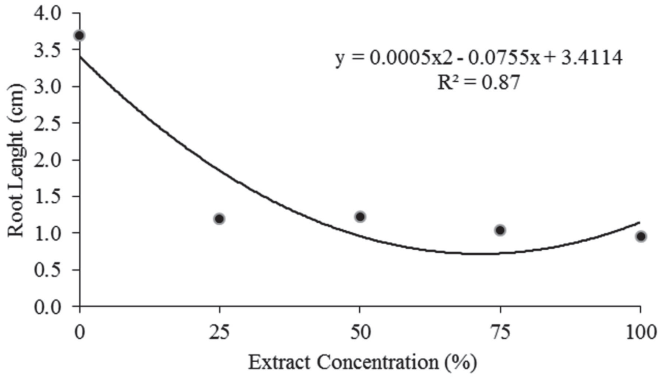

Figure 1: Root length of the initial growth of lettuce seedlings germinated at different concentrations of leaf extracts of *Rudgea viburnoides*. Cuiabá, MT 2018.

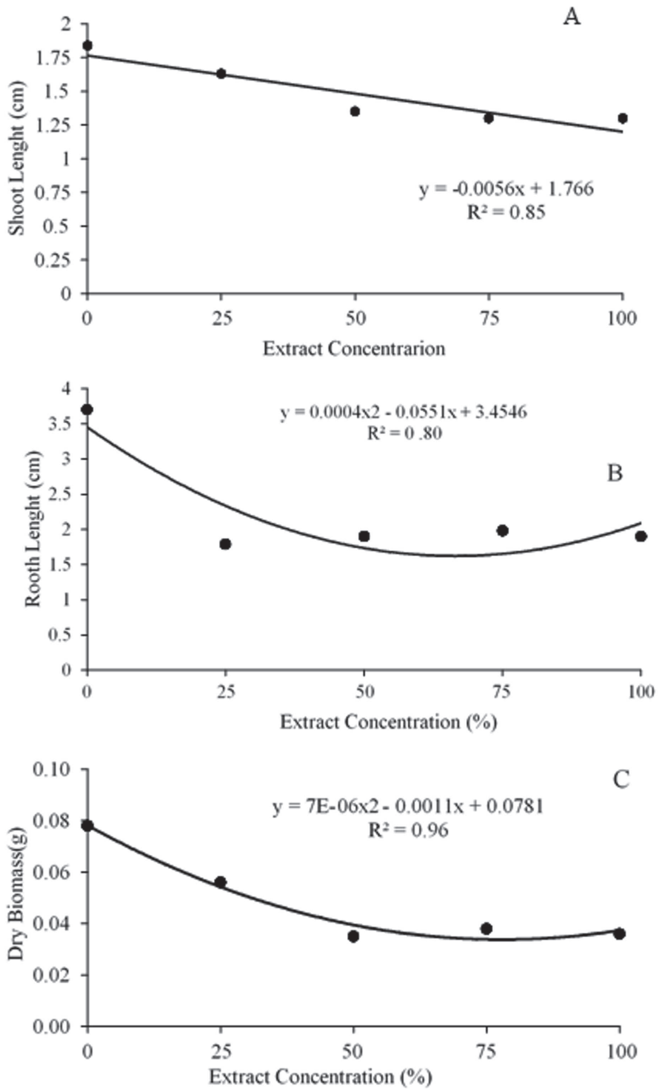

Figure 2: Shoot length (A), root length (B) and dry biomass (C) of initial growth of lettuce seedlings germinated at different concentrations of leaf extracts of *Tocoyena formosa.* Cuiabá, MT 2018.

lettuce germination; but showed a phytotoxic effect on seedling initial development. Also, in another study by Oliveira *et al.* (2014a) with aqueous stem extract of the *Pouteria ramiflora*, lettuce germination was not influenced even though the root length was reduced more than 80%.

The initial development of lettuce seedlings was affected by aqueous leaf extracts in both species. For *R. viburnoides* the root length was reduced by 74% from the extract concentration of 25% in relation to the control treatment (0%), with minimal influence between the lengths with the increase of extract concentrations. This variation can be explained by the polynomial regression model of second degree with coefficient of determination (R2) greater than 86% (Figure 1). However, there was no difference in shoot length in the different treatments for the *R. viburnoides* species. Similar results were demonstrated by Oliveira *et al.* (2015) when evaluating extracts of *Helianthus annuus*, *Brachiaria brizanthae* and *Sorghum bicolor* was reduced the growing of root of different target species.

For *T. formosa,* both root and shoot length were reduced on contact with extracts. Smaller shoot length (Figure 2A) was observed, at higher concentration reaching the linear model with the coefficient of determination (R^2) of 85%. However, the root length (Figure 2B) was affected more by the extract, than the shoot, in relation to the control, and the maximum values were reduced from 52% for root and 30% for shoot. This reduction in length was reflected in the dry seedling biomass, which was decreased as the aqueous extract concentration increased (Figure 2C).

Inhibition of root growth should be considered satisfactory to verify the existence of phytotoxic potential. These results were congruent with those obtained by Gusman *et al.* (2012) in bioassays with aqueous leaf extracts of different medicinal plants on the initial growth of lettuce that demonstrated a more pronounced allelopathic effect on root length than on aerial part. Chung *et al.* (2001) suggest that the difference between these effects is due to the direct and more prolonged contact of the root with the medium containing the allelochemical compounds, than the other parts of the plant. On the other hand, Magiero *et al.* (2009) found that the root is the organ most sensitive to the effect of the allelochemicals because its elongation depends on the cell division that, if inhibited, the normal development of the seedling is considerably compromised.

In general, the effects caused by allelochemicals tend to be concentration-dependent, that is, they tend to be more pronounced at higher concentrations, and this tendency is observed in the initial growth bioassays in recent studies with leaf extract of *Kielmeyera coriacea, Canavalia ensiformis* and *Anacardium humile*, on *L. sativa* (Santos *et al*., 2015; Pereira *et al*., 2018; Matias *et al*., 2018).

Based on the phytotoxic characterization of *T. formosa* and *R. viburnoides* detailed above, it is possible to associate the phytotoxic effects observed in the study with the type

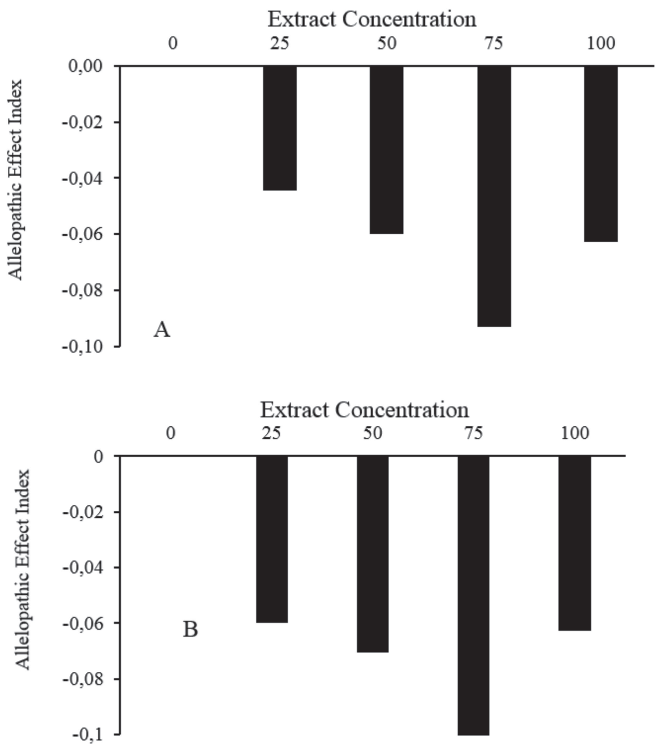

Figure 3: Influence of the extract of *Rudgea viburnoides* (A) and *Tocoyena formosa* (B) on the Allelopathic Index (IR) in achenes of *Lactuca sativa* L. Cuiabá, MT 2018.

of allelochemicals present in the aqueous extract. Gallic acid is an allelochemical with a strongly inhibitory effect on root length and shoot; however, catechin inhibits plant growth to a lesser extent as reported by Iqbal *et al.* (2003) that evaluated the allelopathic potential of buckwheat (*Fagopyrum esculentum*) on weeds.

On the other hand, Golisz *et al*. (2007) demonstrated that rutin at high concentrations has a strong inhibitory effect on lettuce seedlings growth; therefore, it is important to know the amount of the compound present in the species studied to determine the allelochemical responsible for the observed phytotoxic effect.

The allelopathic index (IR) of the extract of the two species was inhibitory (Figure 3A and B), being higher in the extract concentration of 75%. For both extracts as the concentration of 25% to 75% was increased, greater inhibitions were evidenced; however, in the 100% concentration the allelopathic index was reduced.

In order to evaluate the allelopathic index (IR) of *Tagetes erecta* L. on the germination of several crops, Quispe *et al*. (2010) observed that, as the concentration of the aqueous extract increased, had a greater inhibitory effect on lettuce, cucumber and radish germination; however, it had a stimulating effect on beans. Therefore, the concentration of the extract is of paramount importance in this type of experiments.

CONCLUSION

The aqueous leaf extracts of both species, *Tocoyena formosa* and *Rudgea viburnoides* did not affected any parameter of the germination, however it cause phytotoxicity on the initial growth of lettuce seedlings, being the root the part most sensible to the allelochemicals.

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