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Leaching from leaves of *Sarcomphalus joazeiro* and *Cenostigma bracteosum* stimulate or inhibit the germination of *Mimosa caesalpiniifolia*?

O lixiviado das folhas de *Sarcomphalus joazeiro* e *Cenostigma bracteosum* estimula ou inibe a germinação de *Mimosa caesalpiniifolia*?

¿La lixiviación de hojas de *Sarcomphalus joazeiro* y *Cenostigma bracteosum* estimulan o inhiben la germinación de *Mimosa caesalpiniifolia*?

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Fernando dos Santos Araújo
ORCID: https://orcid.org/0000-0002-0605-1613
Universidade Federal da Paraíba, Brasil
E-mail: nandosantos005@hotmail.com
Josenilda Aprígio Dantas de Medeiros
ORCID: https://orcid.org/0000-0001-5759-6771
Universidade Federal do Rio Grande do Norte, Brasil
E-mail: josi-nilda@hotmail.com
Francival Cardoso Felix
ORCID: https://orcid.org/0000-0002-6518-5697
Universidade Federal do Paraná, Brasil
E-mail: felixfc@outlook.com.br
Luiz Augusto da Silva Correia
ORCID: https://orcid.org/0000-0002-1333-8364
Universidade Federal do Rio Grande do Norte, Brasil
E-mail: lasc.eng@gmail.com
Cibele dos Santos Ferrari
ORCID: https://orcid.org/0000-0003-2940-0190
Universidade Federal do Rio Grande do Norte, Brasil
E-mail: cibeferrari@hotmail.com
Mauro Vasconcelos Pacheco
ORCID: https://orcid.org/0000-0002-0447-9800
Universidade Federal do Rio Grande do Norte, Brasil
E-mail: pachecomv@hotmail.com

Abstract
Allelopathy is an ecological mechanism that influences the development of neighboring plants. The objective was to evaluate the allelopathic potential of *Cenostigma bracteosum* and *Sarcomphalus joazeiro* on seed germination and initial growth of *Mimosa caesalpiniifolia* seedlings. Seeds of this species were placed to germinate on paper towel substrate, and then moistened with extracts from dry leaf of *S. joazeiro* and *C. bracteosum* at 1.0; 2.5; 5.0 and 10.0% (w.v^{-1}), and control (0.0% - distilled water) at 25 °C. The variables evaluated: germination, germination speed index, primary root length and root system dry weight of the seedlings. Positive allelopathic effects of *S. joazeiro* leaf extracts were observed on the vigor of *M. caesalpiniifolia*; when used in low concentrations (up to 2.6%), *C. bracteosum* leaf extracts stimulated germination of *M. caesalpiniifolia* seeds and showed more severe toxic effects when exposed to high concentrations (5.0%). *S. joazeiro* leaf extracts favor the germination and vigor of *M. caesalpiniifolia* seedlings, while *C. bracteosum* leaf extracts cause phytotoxic effects on seed germination and initial growth of *M. caesalpiniifolia* seedlings from the concentration of 5%. Therefore, there are indications of benefits for regeneration or associated forest composition between *M. caesalpiniifolia* and *S. joazeiro*.
Keywords: Allelopathy; Phytotoxicity; Forest seeds; Dry forest.

Resumo
A alelopatia é um mecanismo ecológico que influencia o desenvolvimento das plantas vizinhas. O objetivo foi avaliar o potencial alelopático de *Cenostigma bracteosum* e *Sarcomphalus joazeiro* na germinação de sementes e no crescimento inicial de plântulas de *Mimosa caesalpiniifolia*. Sementes desta espécie foram colocadas para germinar em substrato papel toalha, umedecidas com extratos de folhas secas de *S. joazeiro* e *C. bracteosum* a 1,0; 2,5; 5,0 e 10,0% (p.v^{-1}), e controle (0,0% - água destilada) a 25 °C. As variáveis avaliadas: germinação, índice de velocidade de germinação, comprimento da raiz primária e massa seca do sistema radicular das plântulas. Efeitos alelopáticos positivos de extratos de folhas de *S. joazeiro* foram observados sobre o vigor de *M. caesalpiniifolia*; quando usados

em baixas concentrações (até 2,6%), os extratos de folhas de *C. bracteosum* estimularam a germinação de sementes de *M. caesalpiniifolia* e apresentaram efeitos tóxicos mais severos quando expostos a altas concentrações (5,0%). Extratos de folhas de *S. joazeiro* favorecem a germinação e o vigor de plântulas de *M. caesalpiniifolia*, enquanto os extratos de folhas de *C. bracteosum* causam efeitos fitotóxicos na germinação das sementes e crescimento inicial de plântulas de *M. caesalpiniifolia* a partir da concentração de 5%. Portanto, há indícios de benefícios para a regeneração ou composição florestal associada entre *M. caesalpiniifolia* e *S. joazeiro*.
Palavras-chave: Alelopatia; Fitotoxicidade; Sementes florestais; Floresta seca.

Resumen
La alelopatía es un mecanismo ecológico que influye en el desarrollo de las plantas vecinas. El objetivo fue evaluar el potencial alelopático de *Cenostigma bracteosum* y *Sarcomphalus joazeiro* sobre la germinación de semillas y el crecimiento inicial de plántulas de *Mimosa caesalpiniifolia*. Semillas de esta especie se colocaron para germinar sobre sustrato de papel toalla, y luego se humedecieron con extractos de hoja seca de *S. joazeiro* y *C. bracteosum* a 1,0; 2,5; 5,0 y 10,0% (p.v^{-1}) y control (0,0% - agua destilada) a 25 °C. Las variables evaluadas: germinación, índice de velocidad de germinación, longitud de raíz primaria y peso seco del sistema radical de las plántulas. Se observaron efectos alelopáticos positivos de extractos de hojas de *S. joazeiro* sobre el vigor de *M. caesalpiniifolia*; cuando se usaron en concentraciones bajas (hasta 2.6%), los extractos de hojas de *C. bracteosum* estimularon la germinación de semillas de *M. caesalpiniifolia* y mostraron efectos tóxicos más severos cuando se expusieron a concentraciones altas (5,0%). Los extractos de hojas de *S. joazeiro* favorecen la germinación y vigor de las plántulas de *M. caesalpiniifolia*, mientras que los extractos de hojas de *C. bracteosum* provocan efectos fitotóxicos sobre la germinación de semillas y el crecimiento inicial de plántulas de *M. caesalpiniifolia* a partir de la concentración del 5%. Existen indicios de beneficios para la regeneración o composición forestal asociada entre *M. caesalpiniifolia* y *S. joazeiro*.
Palabras clave: Alelopatía; Fitotoxicidad; Semillas forestales; Bosque seco.

1. Introduction

Allelopathy is considered an ecological mechanism that influences primary and secondary plant succession, community formation, plant dominance, and crop management and productivity (Chou, 1999; Almeida-Bezerra et al., 2020). Species that produce allelopathic compounds generally have a greater competitive capacity than those that do not have this mechanism (Silva et al., 2019).

Allelopathic compounds are by-products of primary and secondary metabolism released into the environment by plants which interfere in each other's development in a beneficial or detrimental way, among which can be mentioned terpenes, tannins, phenolic and nitrogen compounds (Taiz & Zeiger, 2017; Almeida-Bezerra et al., 2020). Their release into the environment occurs through leaching from living and dead plant tissues, root exudation, tissue decomposition, and volatilization (Reigosa, Pedrol & González, 2005). Affecting ecosystem dynamics, structure, composition, and interaction between plants (Rodrigues et al., 2020).

In forest species, recent studies were conducted in *Araucaria angustifolia* (Bertol.) Kuntze (Araucariaceae) (Silveira, Hosokawa, Nogueira & Weber, 2014), *Blepharocalyx salicifolius* (Kunth) O. Berg (Myrtaceae) (Habermann, Pontes, Pereira, Imatomi & Gualtieri, 2016), and *Caryocar brasiliense* Cambess. (Caryocaraceae) (Araújo, Madalão, Jakelaitis, Costa & Almeida, 2018), aiming to demonstrate allelopathic potential on seeds germination and vigor.

In Seasonally Dry Tropical Forest located in the Semi-arid region of Brazil, known as 'Caatinga" (Queiroz et al., 2017), there is still a lack of information on the phytosociological structure, ecological succession processes and natural regeneration, making it relevant to understand the processes that influence regeneration of these environments for plant restoration purpose and biodiversity conservation. In semiarid regions, studies with *Senna cearensis* Afr. Fern. (Fabaceae) (Torquato et al., 2020) and interactions between *Pityrocarpa moniliformis* (Benth.) Luckow & R.W. Jobson (Fabaceae) and *Cynophalla hastata* (Jacq.) J. Presl (Capparaceae) on the *Mimosa tenuiflora* (Willd.) Poir. (Fabaceae) were evaluated for the allelopathic effect (Silva et al., 2020).

As the vegetation of a given area may have a succession model conditioned to the preexisting plants and the chemicals they have released in the environment (Ferreira & Aquila, 2000), it is important to estimate the allelopathic potential of the species that make up the ecosystem. Among the autochthonous species found in “Caatinga”, *Cenostigma bracteosum* (Tul.) Gagnon & G.P. Lewis, *Sarcomphalus joazeiro* (Mart.) Hauenshild, and *Mimosa caesalpiniifolia* Benth. can be cited.

C. bracteosum (Fabaceae) is a tree-sized species and with high economic potential (Santos et al., 2012; Mendonça, Passos, Victor-Junior, Freitas & Souza, 2014). This species has a high capacity for regrowth and rapid growth, revealing potential for recovering degraded areas (Maia, 2012). The species has been observed in associations of plants that develop in stony soils and in humid plains (Chaves et al., 2015).

S. joazeiro (Rhamnaceae) does not lose its leaves during the dry season, always remaining green (Costa, 2018). This species has several uses such as in popular medicine, in cosmetics and toothpaste, as well as being a source animal food during drought periods (Oliveira, Coelho, Maia, Diógenes & Medeiros-Filho, 2012; Lorenzi, 2016) and in beekeeping (Coelho, Maia, Oliveira & Diógenes, 2011). *M. caesalpiniifolia* (Fabaceae) (Costa-Filho, Valeri & Cruz, 2013) is a species that has exploited (Freitas, Pinto, Praxedes, Nogueira & Ribeiro, 2011; Nogueira, Ribeiro, Freitas, Matuoka & Souza, 2012) for its wood and energy use (Costa-Filho et al., 2013). In addition, the species plays an important role in the recovery degraded areas since it is a nitrogen fixing plant (Nogueira et al., 2012).

Thus, the objective was to evaluate the allelopathic potential of *C. bracteosum* and *S. joazeiro* on seed germination and initial seedling growth of *M. caesalpiniifolia*.

2. Methodology

2.1 Leaf extracts

The *S. joazeiro* and *C. bracteosum* leaves as well as *M. caesalpiniifolia* seeds were collected in a dry forest/rainforest transition area (5°53'07.6 "S and 35°22'13.7" W), Macaíba, Rio Grande do Norte State, Brazil.

The extracts were prepared with 100 g of fresh *S. joazeiro* or *C. bracteosum* leaves were ground in a blender with 900 mL distilled water to obtain an aqueous extract with a concentration of 10.0% (w.v^{-1}). The extracts were obtained from successive dilutions of this concentration at 5.0, 2.5 and 1.25% (w.v^{-1}), which were then used to moisten the substrate used in germination.

The *M. caesalpiniifolia* seeds were scarified to overcoming dormancy (Bruno, Alves, Oliveira & Paula, 2001) and disinfested with 2.5% sodium hypochlorite solution for 5 min. The germination substrate (paper substrate - Germitest®) were moistened in the amount equivalent to 2.5 times the weight paper substrate (Brasil, 2013), with the following solutions that corresponded to the five treatments: distilled water (0.0% - control); and leaf extracts at concentrations of 1.25; 2.5; 5.0 and 10.0% (w.v^{-1}). The paper sheets were organized as rolls, packed into transparent plastic bags, and incubated in a Biochemical Oxygen Demand (BOD) germinator under a constant temperature of 25 °C and photoperiod of 8 h for seven days.

2.2 Evaluated variables

The variables evaluated: germination (%) - the germinated seeds that originated normal seedlings were counted on the 7th day after sowing (Brasil, 2013); germination speed index (GSI) - daily counting of normal seedlings was performed until the 7th day after sowing and calculated according to the equation proposed by Maguire (1962); primary root length (cm seedling^{-1}) - the primary root length of normal seedlings was measured using a ruler graduated in millimeters at the end of the experiment; root system dry weight (mg.seedling^{-1}) - the root system of normal seedlings was packed into Kraft paper bags and placed in a dry oven with forced air circulation regulated at a constant temperature of 60 ºC until reaching constant weight. The seedlings were removed from the dry oven and weighed on an analytical scale (0.001 g).

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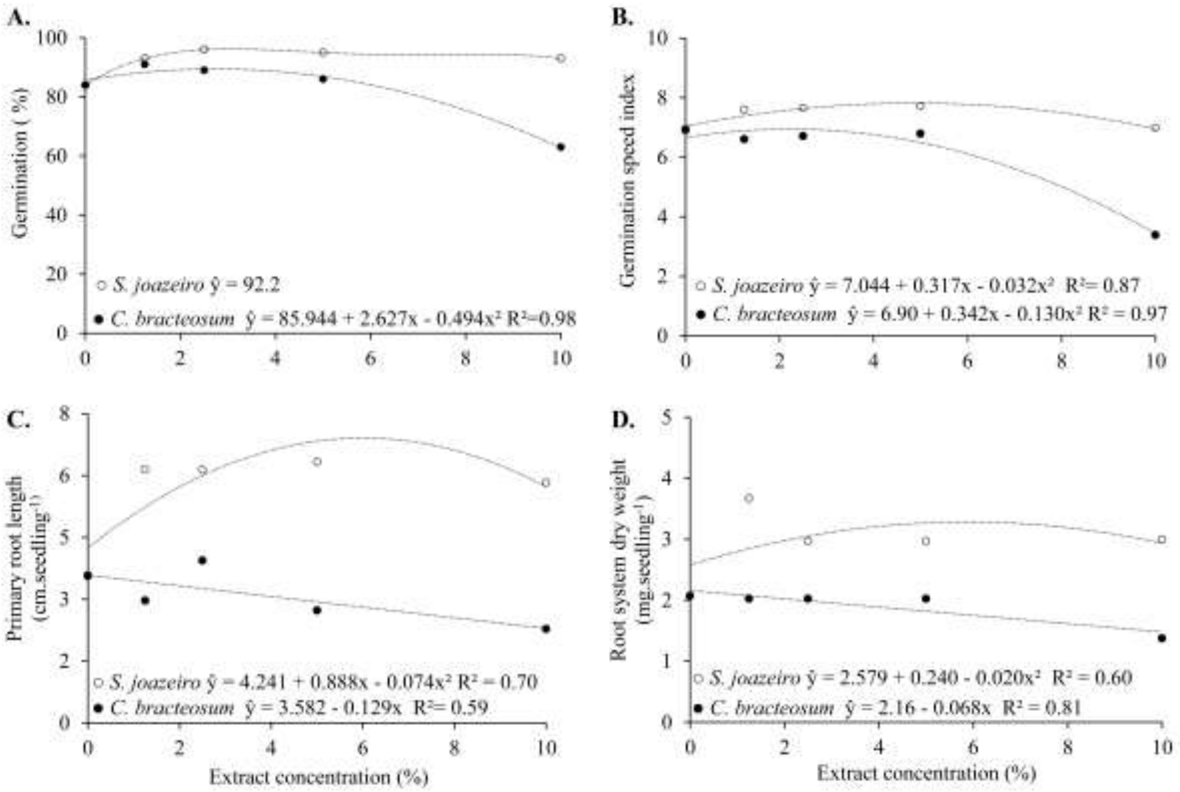
2.3 Experimental design

The experiment was conducted under a completely randomized design consisting five treatments (0.0, 1.25, 2.5, 5.0 and 10%) with four replications of 25 seeds each. Therefore, the study is characterized as quantitative according to Pereira, Shitsuka, Pereira and Shitsuka (2018). Data were submitted to analysis of variance and polynomial regression, with the significant model ($p < 0.05$ by the F-test) and the highest coefficient of determination (R^2) being selected. The analyzes were performed using Assistat statistical software, version 7.7 (Silva & Azevedo, 2016). For determining the extract concentration, the maximum points of the regression equations were estimated through the derivative of “Y” in relation to “X”.

3. Results and Discussion

There are increase in *M. caesalpiniifolia* seed germination percentage up to the concentration of 2.6% (Figure 1A). Then from this concentration there was a reduction in germination, with a marked decrease from 5.0% of the *C. bracteosum* leaf extract concentration. Regarding *S. joazeiro*, there was no statistical difference between the studied concentrations for germination (Figure 1A).

Figure 1 - Germination (A), germination speed index (B), primary root length (C), and root system dry weight (D) of *M. caesalpiniifolia* seeds submitted to different concentrations of *S. joazeiro* and *C. bracteosum* aqueous dry leaf extracts.



Fonte: Authors.

These results corroborate those presented by Oliveira et al. (2012) in which none of the extract concentrations prepared from *S. joazeiro* leaves were phytotoxic to lettuce seeds (*Lactuca sativa* L.), interfering with their viability. However, Coelho et al. (2011) observed that there was reduced germination when testing extracts prepared from *S. joazeiro* seeds. These results indicate that active principles responsible for the allelopathic effects of this species are possibly distributed in distinct plant organs. The saponin presence can be observed on inner plants bark (floem) (Higuchi et al., 1984), while n-alkaline and triterpenoids are observed in leaves (Oliveira & Salatino, 2000).

The saponins are water-soluble glycosides or insoluble polymers from secondary metabolism that act in defense against pathogens and herbivores, protection against ultraviolet radiation and reduced growth of neighboring plants (Taiz & Zeiger, 2017). While terpenoids are volatile compounds that act in biochemical signaling and plant establishment (Rice, 2012; Almeida-Bezerra et al., 2020).

Even though the *S. joazeiro* extracts did not negatively interfere in the seed germination percentage, they caused reduced vigor evaluated by the germination speed index from the concentration of 4.9%, for which the maximum germination speed was obtained (Figure 1B). This is because the allelochemicals have a greater effect on germination speed and synchrony than on the final percentage (Ferreira, 2004). *S. joazeiro* leaf extract presents saponins, flavonoids, phenols, and tannins, with these being the allelochemicals most likely responsible for this result (Brito et al., 2015).

The extracts dry leaf of *C. bracteosum* caused phytotoxic effects on seed germination (Figure 1A) and initial growth (Figures 1B, 1C and 1D) of *M. caesalpiniifolia* seedlings at 5.0% concentration. In addition, aqueous extract with fresh leaf of *C. bracteosum* did not affect the germination of *M. caesalpiniifolia* seeds, but it exerted a negative effect on the physiological quality of the seedlings of this species (Medeiros, Correia, Santos, Ferrari & Pacheco, 2018). Aqueous extracts of *P. moniliformis* leaves also do not affect the germination of *M. caesalpiniifolia* seeds, although they have a negative allelopathic effect on the speed and growth of seedlings (Pacheco et al., 2017).

Regarding the allelopathic potential of *C. bracteosum*, it is presumed that the extract's toxicity on the germination of. *M. caesalpiniifolia* seeds is due to the presence of tannin, since this is a phenolic compound abundant in the leaves of this species (Gonzaga-Neto et al., 2001). Phenolic compounds are present in plant decomposition products in the soil that cause widespread cytotoxicity and physiological damage to neighboring plants, such as reduced plant growth and photosynthetic capacity, and impaired absorption of ions, water and mineral nutrients (Rice, 2012; Almeida-Bezerra et al., 2020).

The *C. bracteosum* extracts also interfered in the germination speed (Figure 1B), where its reduction occurred from the concentration of 1.3%. In a bioassay performed by Ribeiro et al. (2012), the reduced germination speed indicated a synchrony loss in the metabolic reactions of germination, demonstrating the inhibition of lettuce seed vigor when treated with *Stryphnodendron adstringens* (Mart.) Coville (Fabaceae) leaf extracts. These changes also indicate interference of the allelochemicals in metabolic reactions during germination (França, 2008).

Also, *C. bracteosum* extracts reduced the primary root length of *M. caesalpiniifolia* (Figure 1C) from the 1.0% concentration. These results are due to the deleterious effects of allelochemicals that are more drastic on root metabolism, especially during initial plant growth, which is characterized by high metabolism and sensitivity to environmental stresses (Cruz-Ortega, Anaya, Hernández & Laguna, 1998).

It is also verified in Figure 1C that the primary root length of *M. caesalpiniifolia* seedlings increases as the *S. joazeiro* extract concentration increases to 6.0%. It is a positive allelopathic effect, since the extracts also optimized the germination percentage (Figure 1A), as well as the germination speed index (Figure 1B) of the *M. caesalpiniifolia* seeds.

These results indicate that the natural regeneration of *M. caesalpiniifolia* can be benefited by *S. joazeiro* if they are used in the composition of mixed forest plantations for forest restoration.

However, it can be observed that when under high concentrations (from 6.0%). The *S. joazeiro* extract negatively affects the initial growth of the *M. caesalpiniifolia* seedlings, being more expressive over the primary root length (Figure 1C) than the dry weight (Figure 1D), since root length has been one of the most sensitive variables in detecting allelopathic effects in seedlings of forest species.

Few studies report the growth stimulus of one plant relative to the other by allelopathy, with detrimental allelopathic effects being more common than beneficial effects (Rice, 2012). However, Reigosa, Sánchez-Moreiras & González (1999) emphasized that each physiological process has a different response to certain doses of each allelopathic substance, corroborating the results found in the present study.

The influence of the extracts on seed germination and root growth of *M. caesalpiniifolia* seedlings suggests the existence of relevant allelopathic substances in *S. joazeiro* and *C. bracteosum* leaves. We verify that there was both positive and negative stimulated on the germination process.

Positive allelopathic effects on germinative performance were verified up to the concentration of 2.6% of leaf extracts, while negative effects were intensified with increasing leaf extract concentrations of both species in the present study.

This information can help to understand ecological processes among these species in the vegetation of dry forests. In addition, the obtained results can be used for the correct population size of the studied species if they are chosen to compose agroforestry systems or mixed plantations for forest restorations.

4. Conclusion

S. joazeiro leaf extracts favor the germination and vigor of *M. caesalpiniifolia* seedlings, while *C. bracteosum* leaf extracts cause phytotoxic effects on seed germination and initial growth of *M. caesalpiniifolia* seedlings from the concentration of 5%. Therefore, there are indications of benefits for regeneration or associated forest composition between *M. caesalpiniifolia* and *S. joazeiro*. In addition, new studies in field must be carried out to verify the effect of interaction of *S. joazeiro* on the growth and development of *M. caesalpiniifolia*.

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