

**Chromatographic profile and allelopathic potential of the essential oil of *Acritopappus confertus* (Gardner) R. M. King & H. Rob. (Asteraceae)**

**Perfil cromatográfico e potencial alelopático do óleo essencial de *Acritopappus confertus* (Gardner) R. M. King & H. Rob. (Asteraceae)**

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**Jeane Dantas Sousa**

ORCID: <https://orcid.org/0000-0001-6377-7712>

Regional University of Cariri, Brazil

E-mail: [jeane.dantas@urca.br](mailto:jeane.dantas@urca.br)

**Tiago Rodrigues Leite**

ORCID: <http://orcid.org/0000-0002-2575-1420>

Regional University of Cariri, Brazil

E-mail: [tiago.urca85@gmail.com](mailto:tiago.urca85@gmail.com)

**Karina Vieiralves Linhares**

ORCID: <https://orcid.org/0000-0001-6567-3271>

Regional University of Cariri, Brazil

E-mail: [karina\\_linhares@yahoo.com](mailto:karina_linhares@yahoo.com)

**Josué Dantas de Sousa**

ORCID: <https://orcid.org/0000-0002-0312-4362>

Regional University of Cariri, Brazil

E-mail: [josue.dantasjds@hotmail.com](mailto:josue.dantasjds@hotmail.com)

**José Weverton Almeida Bezerra**

ORCID: <https://orcid.org/0000-0002-0966-9750>

Federal University of Pernambuco, Brazil

E-mail: [weverton.almeida@urca.br](mailto:weverton.almeida@urca.br)

**Marcos Aurélio Figueiredo dos Santos**

ORCID: <https://orcid.org/0000-0002-3409-5242>

Regional University of Cariri, Brazil

E-mail: [marcos.figueiredo@urca.br](mailto:marcos.figueiredo@urca.br)

**Isabella Hevily Silva Torquato**

ORCID: <https://orcid.org/0000-0001-6476-2045>

Regional University of Cariri, Brazil

E-mail: [isabella.torquato@urca.br](mailto:isabella.torquato@urca.br)

**Aline Augusti Boligon**

ORCID: <https://orcid.org/0000-0002-6001-1313>

Federal University of Santa Maria, Brazil

E-mail: [alineboligon@hotmail.com](mailto:alineboligon@hotmail.com)

**Janete Souza Bezerra**

ORCID: <https://orcid.org/0000-0002-2674-0790>

Regional University of Cariri, Brazil

E-mail: [Janete.bezerra@urca.br](mailto:Janete.bezerra@urca.br)

**Nathália Barbosa Campos**

ORCID: <https://orcid.org/0000-0003-1805-6216>

Regional University of Cariri, Brazil

E-mail: [campos.b.natalia@gmail.com](mailto:campos.b.natalia@gmail.com)

**Maria Arlene Pessoa da Silva**

ORCID: <https://orcid.org/0000-0001-8148-5350>

Regional University of Cariri, Brazil

E-mail: [arlene.pessoa@urca.br](mailto:arlene.pessoa@urca.br)

**Abstract**

The present study aims to test the *Acritopappus confertus* (Gardner) R. M. King & H. Rob. essential oil allelopathic potential on the germination and initial development of *Cenchrus echinatus* L. and *Lactuca sativa* L. seedlings, in addition to identifying and quantifying chemical constituents. The chemical composition analysis was performed by gas chromatography (GC-FID) and gas chromatography coupled to mass-spectrometry (GC-MS). For the allelopathic assays, the essential oil was emulsified with dimethylsulfoxide (DMSO) in a 1:1 ratio, and diluted in distilled water to obtain 0.001, 0.01, 0.10, 0.25, 0.50, 0.75 and 1% c. The control consisted of 1% aqueous DMSO solution. Treatments were performed in five repetition with 20 seeds each. The pH of the oils were measured and adjusted to a scale of 6 to 7. Assays were conducted in a germination chamber at 25 °C with a 12h photoperiod. Seed germination, Germination Speed Index (GSI), caulicle and radicle length were analyzed.

The data were subjected to analysis of variance and the means were compared by Tukey test ( $p < 0.05$ ), through the ASSISTAT. The constituents mycene,  $\beta$ -pinene and limonene stood out the most in chemical analysis. The essential oil did not influence *C. echinatus* and *L. sativa* seed germination, however it influenced GSI and seedling development. The effects observed herein may be due to chemical constituents found in the studied species, which may act in an isolated or combined manner.

**Keywords:** Bioherbicides; Natural environment; Secondary metabolism; Vegetables.

### Resumo

O presente estudo tem por objetivo testar o potencial alelopático do óleo essencial de *Acritopappus confertus* (Gardner) R. M. King & H. Rob. sobre a germinação e desenvolvimento inicial de plântulas de *Cenchrus echinatus* L. e *Lactuca sativa* L, além de identificar e quantificar os constituintes químicos. A análise da composição química foi realizada através da cromatografia gasosa (GC-FID) e Cromatografia gasosa acoplada ao espectro de massa (GC-MS). Para o teste alelopático, o óleo essencial foi emulsionado com Dimetilsulfoxido (DMSO) na proporção 1:1, e dissolvido em água destilada a 0,001, 0,01, 0,10, 0,25, 0,50, 0,75 e 1%. O controle foi constituído por uma solução aquosa de DMSO a 1%. Os tratamentos constaram de cinco repetições com 20 sementes. Foi aferido o pH das concentrações e ajustado para uma escala de 6 a 7. Os testes foram conduzidos em câmara de germinação a 25°C com fotoperíodo de 12 h. Foi analisada a germinação das sementes, Índice de Velocidade de Germinação (IVG), comprimento do caulículo e radícula. Os dados foram submetidos à análise de variância e as médias comparadas pelo teste de Tukey ( $p < 0.05$ ), através do ASSISTAT. Na análise química os constituintes que mais se destacaram foram mirceno,  $\beta$ -pineno e limoneno. O óleo essencial não influenciou a germinação das sementes de *C. echinatus* e *L. sativa*, mas afetou o IVG e o desenvolvimento das plântulas. Os efeitos observados possivelmente devem-se a ação dos constituintes químicos encontrados na espécie em estudo, os quais podem atuar isoladamente ou em conjunto.

**Palavras-chave:** Bioherbicidas; Ambiente Natural; Metabolismo Secundário; Vegetais.

### Resumen

El presente estudio tiene como objetivo probar el potencial alelopático del aceite esencial de *Acritopappus confertus* (Gardner) R. M. King & H. Rob acerca de la germinación y el desarrollo inicial de plántulas de *Cenchrus echinatus* L. y *Lactuca sativa* L., además de

identificar y cuantificar los constituyentes químicos. El análisis de la composición química fue realizada a través de cromatografía gaseosa (GC-FID) y cromatografía gaseosa acoplada al espectro de masa (GC-MS). Para la prueba alelopática el aceite esencial emulsionado con Dimetilsulfóxido (DMSO) en la proporción 1:1 y disuelto en agua destilada a 0,001, 0,01, 0,10, 0,25, 0,50, 0,75 y 1%. El control se constituyó por una solución acuosa de DMSO a 1%. Los tratamientos constaron de cinco repeticiones con 20 semillas. Foi medido el pH de las concentraciones y ajustado para una escala de 6 a 7. Las pruebas se realizaron en una cámara de germinación a 25°C con fotoperíodo de 12h. Fue analizada la germinación de las semillas, el Índice de Velocidad de Germinación (IVG), la longitud del tallo y radícula. Los datos fueron sometidos a análisis de varianza y a las medias comparadas mediante la prueba de Turkey ( $p < 0.05$ ) a través del ASSISTAT. En el análisis químico los constituyentes que más se destacaron fueron mirceno,  $\beta$ -pineno y limoneno. El aceite esencial no influyó en la germinación de las semillas de *C. echinatus* y *L. sativa*, mas afectó el IVG y el desarrollo de las plántulas. Los efectos observados posiblemente se deben a la acción de los constituyentes químicos encontrados en la especie en estudio, los cuales pueden actuar separados o en conjunto.

**Palabras clave:** Bioherbicidas; Ambiente Natural; Metabolismo Secundario; Vegetales.

## 1. Introduction

Allelopathy can be defined as an important mechanism in which living or dead plants release chemical compounds which can interfere in a negative or positive way with the growth and development of other plants. In a natural environment, allelopathy plays an important role in dominance, plant succession and plant community formation, being an important colonization strategy of many exotic species in natural communities (Li et al., 2011; Oliveira, Gualtieri, Domínguez, Molinillo & Montoya, 2012).

Many secondary metabolites, such as phenolic acids, flavonoids, tannins and others, possess allelopathic activity. Such compounds, denominated as allelochemicals, promote biochemical interactions between plants when released into the environment (Fiorenza et al., 2016; Borella, Tur, & Pastorini, 2010).

A wide variety of allelochemicals synthesized and stored in different plant cells, either free or combined with other molecules, are released into the environment under natural conditions as a response to biotic and abiotic stress factors. Such release may also occur

through volatilization, leaching, root exudation, plant residue decomposition and burlap leaching (Maraschin-Silva & Aquila, 2006b; Pires & Oliveira, 2011; Oliveira et al., 2012).

Allelochemical action can affect plant growth, interfering with cell division and elongation processes, modifying the synthesis of a plant's main constituents and carbon distribution in cells, they may also affect hormones which play an important role in growth regulation, as well as interfering with enzymes, increasing or inhibiting their activities (Pires & Oliveira, 2011).

The discovery of new allelochemicals has been used as an alternative to conventional herbicide usage for the control of invasive plants. Conventional herbicides have increased the risk of environmental contamination, causing changes in invasive species population and increasing resistance to these compounds. In this sense, studies carried out with allelochemicals may contribute to new bioherbicide discoveries, either for direct use or as possible precursors for the synthesis of new agrochemicals (Oliveira et al., 2012). Miranda et al. (2015a) reinforce that bioherbicide use contributes to the development of more sustainable agriculture.

According to Ootania et al. (2017), the essential oils present in plants can be used for the production of herbicides, since they are more specific and less harmful to the environment. The authors draw attention to the phytotoxic effects of essential oils such as herbicidins, insecticides, fungitoxins, in addition to the allelopathic effects, responsible for interfering in the germination, growth and development processes of other plants.

The bioassays carried out in the laboratory have proved to be an essential tool in studies aimed at determining the allelopathic potential of different plant species, whether native, naturalized, exotic or cultivated (Costa et al., 2019).

Weeds have been used as receptor species in allelopathic assays and, several authors, like Pires and Oliveira (2011), confirm the allelopathic influences of such species, which in many cases affect native and cultivated plants, causing delay or impediment germination, reduced growth and interference in the symbiosis process of cultures.

Through allelopathic potential and constituent analysis, new alternatives for spontaneous and cultivated plant management may be observed, in addition to allowing diversification in agricultural crops (Silva et al., 2011; Gusman, Vieira & Vestena, 2012).

Within this context, several pieces of research have proven the allelopathic action in species of botanical substances, such as Poaceae, Euphorbiaceae, Lamiaceae, Asteraceae, among others. (Oliveira, Belinelo, Almeida, Aguilar, & Vieira Filho, 2011; Miranda et al., 2015b; Leite, Silva, M. Santos, A. Santos & Costa, 2015; Alencar et al., 2015). Asteraceae,

one of the most numerous groups within the Angiosperms, has approximately 24,000 species grouped into more than 1,600 genera comprising mostly small herbs and shrubs, and rarely trees. Roughly 98% of their genera are composed of small plants, found in all habitat types, being more frequently found in mountainous tropical regions in South America (Verdi, Brighente & Pizzolatti, 2005; Funk, Susanna, Stuessy & Robinson, 2009; Judd, Campbell, Kellogg, Stevens & Donoghue, 2009).

The *Acritopappus* R.M. King & H. Rob. genus is composed of 17 shrub and small tree species, endemic in the Northeast and Southeast regions of Brazil. The genus diversity is restricted to the states of Bahia and Minas Gerais, with only the *Acritopappus confertus* (Gardner) R.M. King and H. Rob. and *Acritopappus longifolius* (Gardner) R. M. King & H. Rob. species having a wider distribution, however without exceeding the country's Northeast and Southeast region limits (Bautista, Ortiz & Rodríguez-Oubiña, 2011).

Species of the genus *Acritopappus* have been studied from the phytochemical point of view, having been observed the presence of several chemical compounds such as diterpene monoterpenes and diverse sesquiterpenes (Ferreira, Marturano, Carollo & Oliveira, 2011; Giacomini et al., 2005; Lima et al., 2005), however, few studies relate these compounds to their allelopathic potential.

Based on the aforementioned, the present study aims to test the *A. confertus* essential oil allelopathic potential on the germination and development of *Cenchrus echinatus* L. and *Lactuca sativa* L. seedlings, in addition to identifying and quantifying chemical constituents.

## 2. Methodology

The present study is characterized a experimental research with a quantitative approach. In the experimental research, the variables to be analyzed are selected, establishing the form of control over them, in addition to investigating the implications on the object of study in pre-established situations. As the researcher exercises control over the variables, flaws and biases can be eliminated, thus ensuring greater reliability in his results (Fontelles, Simões, Farias & Fontelles, 2009).

In quantitative methods, collections of numerical data are performed through measurements of quantities, generating data that are examined by mathematical techniques such as percentages, statistics, probabilities and equations (Pereira, Shitsuka, Parreira, & Shitsuka, 2018).

## 2.1 Botanical material collect and identification

In order to extract the essential oil, fresh *A. confertus* leaves were collected from a Cerrado area in the Araripe National Forest, located at 7° 28'92" S and 39° 54'11" W, 933m altitude, in the morning.

The collections were authorized by the Biodiversity Authorization and Information System (SISBIO) of the Ministry of the Environment (MMA), Chico Mendes Institute of Biodiversity Conservation (ICMBio) under number 53674-1, issued on the 22 April 2016. The research was registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SISGEN) under number AE88FAA.

For identification of the species under study, 5 branches in the reproductive phase, with flowers and/or fruits were collected. These were herborized, treated and identified using identification keys and incorporated into the collection at the Caririense Dárdano de Andrade-Lima Herbarium (HCDAL) of the Regional University of Cariri (URCA), under registration number 12.462.

## 2.2 Essential oil extraction

For essential oil extraction, the hydrodistillation technique using a Clevenger type apparatus was employed.

For the extraction of essential oil, the hydrodistillation technique was used, using the Clevenger type apparatus. For that, 649g of leaves were crushed and placed in 3 portions in a 3 liter glass bottle, in which 1.5 liters of distilled water were added for each extraction, totaling 4.5 liters (Matos, 1997). Two hours after the start of the extraction process, during which it was observed to be exhausted, the essential oil was collected, packed in an amber bottle, labeled and kept refrigerated until the moment of use.

The yield of essential oil was calculated from the mass of the leaves (649g) and the mass of the extracted oil (2,23219g) through the equation:

$$Yield = \frac{2,23219g}{649g} \times 100 = 0,34\%$$

## **2.3 Essential oil chemical composition analysis**

### **2.3.1 Gas chromatography (GC-FID)**

The analyzes were performed following the protocol described by Cunha et al. (2015) using a gas chromatography (GC) system with an Agilent Technologies System 6890N GC-FID equipped with a DB-5 capillary column (30m x 0.25mm, film thickness 0.25mm) coupled to a FID detector. The injector and detector temperatures were set to 280°C. Helium was used as the carrier gas at a flow rate of 1.0 mL/min. The thermal programmer consisted of 50-300 °C at a rate of 5 °C/min. Two sample replicates were processed the same way. The constituents relative concentrations were calculated based on the CG peak areas without correction factors. The *A. confertus* essential oil injection volume was 1 µL.

### **2.3.2 Gas chromatography coupled to mass spectrometry (GC-MS)**

Gas chromatography coupled to mass spectrometry (GC-MS) analysis were performed on an Agilent Technologies AutoSystem XL GC-MS system operating in EI mode at 70 eV, equipped with a split/splitless injector (250°C). The transfer line temperature was 280 °C. Helium was used as the carrier gas (1.0 mL/min) and the capillary columns used were HP 5MS (30m x 0.25mm, film thickness 0.25mm) and HP Innowax (30m x 0.32mm id, film thickness 0.50mm). The temperature program was the same as that used for GC analyzes. The Essential oil injection volume was 1 µL (Cunha et al., 2015).

### **2.3.3 Chemical constituents identification**

The *A. confertus* essential oil chemical constituents identification was performed based on retention indices (RI), determined with n-alkanes homologous series references, C<sub>7</sub>-C<sub>30</sub>, under identical experimental conditions, comparing with mass spectra library (NIST and Wiley) and with mass spectra literature according to Adams (1995).

The relative quantities of individual components were calculated based on the GC peak areas (FID response).

## 2.4 Allelopathic activity

The *A. confertus* leaf essential oil influence on seed germination, Germination Speed Index (GSI) and initial *C. echinatus* L. and *L. sativa* L. seedling development using diverse concentrations was tested.

The essential oil was emulsified with dimethyl sulfoxide (DMSO) at a 1:1 proportion and then dissolved in distilled water to obtain solutions at the concentrations of 0.001, 0.01, 0.10, 0.25, 0.50, 0.75 and 1%. The control group consisted of 1% aqueous DMSO solution. The pH of the solutions in their various concentrations were adjusted to a range between 6.0 and 7.0 due to high acidity. The recipient species seeds were distributed in sterilized petri dishes, having three sheets of filter paper moistened with distilled water as a substratum (at a ratio of 1 gram of paper to 3 mL of distilled water). Each treatment consisted of five replicates with 20 seeds totaling 140 seeds per treatment. After sowing, 3 mL of the oil solution were distributed in two filter paper sheets at each established concentration and placed in the plate cover, adopting the indirect contact methodology (Leite et al., 2015).

The experiments were conducted in a BOD-type germination chamber at 25 °C with a 12-hour photoperiod for five days for *C. echinatus* seeds and seven days for *L. sativa* seeds.

## 2.5 Statistical analysis

For germination data, caulicular and radicular development, an analysis of variance (ANOVA) was performed where Tukey's test was applied at a 5% probability for comparison between the samples. All statistical analyzes were performed using the ASSISTAT version 7.7 beta program.

## 3.3. Results and Discussion

### 3.1 Essential oil chemical analysis

The fresh *A. confertus* leaf essential oil presented a yield of 0.34%. Chemical analysis allowed the identification and quantification of 12 compounds, representing 89.51% of the total chemical composition. Myrcene (49.16%),  $\beta$ -pinene (17.09%) and limonene (8.73%) were identified as the major constituents, corresponding to 74.98% of the total oil composition (Table 1).

**Table 1.** *Acritopappus confertus* (Gardner) R. M. King & H. Rob. essential oil constituents

Compounds	RI <sup>a</sup>	RI <sup>b</sup>	Essential Oil
$\alpha$ -Pinene	937	935	2.38
Sabinene	978	978	0.24
$\beta$ -Pinene	983	981	17.09
Myrcene	987	989	49.16
<i>p</i> -Cymene	1026	1023	0.15
Limonene	1029	1031	8.73
$\beta$ -Cimene	1051	1050	0.08
$\beta$ -Cubebene	1390	1391	5.62
$\beta$ -caryophyllene	1418	1418	1.04
$\alpha$ -Humulene	1454	1459	0.95
$\delta$ -Eudesmol	1631	1630	0.47
$\beta$ -Eudesmol	1652	1652	3.60
<b>Total Identified (%)</b>			<b>89.51</b>

<sup>a</sup>Experimental retention index (based on n-alkane C7-C30 homologous series).

<sup>b</sup>Literature retention index (Adams, 1995). Source: Authors.

Lima et al. (2005) performed studies on the chemical composition of *A. confertus* leaf volatile oils and identified the occurrence of monoterpenes composing 81.0% of the total, with myrcene (52.0%) as its main component, followed by  $\beta$ -pinene (16.8%) and limonene (8.2%). These results corroborate with those of the present study.

In other species of Asteraceae, monoterpenes were also found. Negreiros, Pawlowski, Soares, Motta and Frazzon (2016) analyzed the chemical composition of the essential oil of two species of the Asteraceae family, and found  $\beta$ -pinene in leaves of *Heterothalamus alienus* (Spreng.) O. Kuntze (40, 8%) and *Heterothalamus psiadioides* Less. (44.65%), Limonene (6, 86% and 6.50%) and Mirceno (1.09% and 1.91%), respectively.

Miranda, Cardoso, Batista, Rodrigues and Figueiredo (2016) analyzed the chemical constitution of the essential oil of the leaves of *Tithonia diversifolia* (Hemsl.) A.Gray (Asteraceae) and found  $\beta$ -pinene (38.3%) as its main components,  $\alpha$ -pinene (28.6%) and limonene (8.8%). In the species *Baccharis dracunculifolia* DC. (Asteraceae), Limonene (30.9%) and  $\beta$ -pinene (14.5%) were characterized as predominant compounds. Paroul et al. (2016) verified the *Baccharis trimera* (Less.) DC (Asteraceae) essential oil constituents and found the monoterpene  $\beta$ -pinene (23.28%) as the majoritarian.

According to Miranda et al. (2014) monoterpenes are the main essential oil constituents with allelopathic activity, which directly affect plant germination and growth,

causing morphological and physiological changes, thus interfering with respiratory chain, mitosis, cell membranes, cuticular cells, transpiration, lipid peroxidation and microtubules.

Moreover, Miranda et al. (2015b) state it is of paramount importance to perform comparative analyzes of essential oil allelopathic effects and their major constituents.

### 3.2 Essential oil Ph

Essential oil pH values varied between 5.0 and 5.5 for the 0.5% concentration, and in very acidic ranges between 4.8 and 4.7 for the 0.75 and 1% concentrations, respectively; these values were adjusted to the 6 range to prevent interferences with seedling growth and development (table 2).

**Table 2.** PH values for Essential Oil concentrations of fresh leaves of *Acritopappus confertus* (Gardner) R. M. King & H. Rob.

Concentrations (%)	Normal	Adjusted
0,001	5.5	6.9
0,01	5.1	7.0
0,10	5.3	6.6
0,25	5.0	6.8
0,50	5.2	6.7
0,75	4.8	7.0
1	4.7	7.0

Source: Authors.

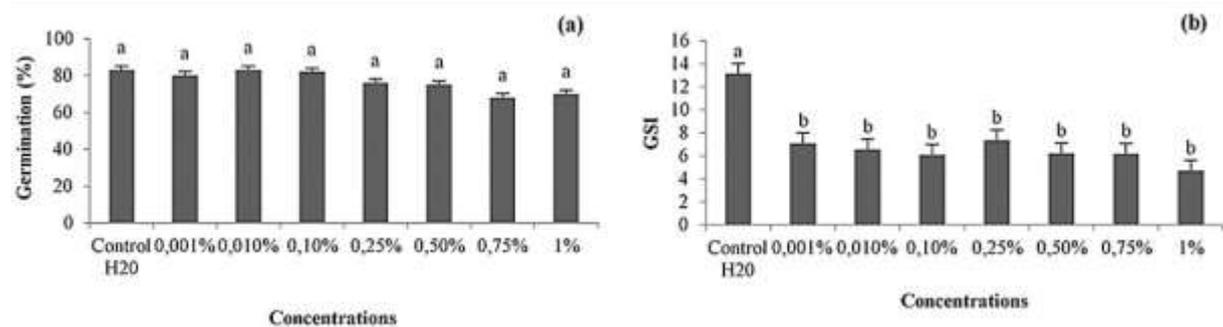
According to Oliveira, Diógenes, Coelho and Maia (2009) pH values between 5.37 and 6.81 do not interfere with seedling germination and are therefore suitable for the development of most species. For Maraschin-Silva and Aquila (2006a), pH values between 5.0 and 7.0 for aqueous extracts from native species are suitable for germination and lettuce growth.

Periotto, Perez and Lima (2004) state pH values at extreme conditions such as acidity or alkalinity can negatively affect seedling growth and development. In these cases a pH adjustment to 6.0, the appropriate range for germination and allelopathic effect verification, is indicated (Macias, Gallindo & Molinillo, 2000). As stated by Ferreira and Áquila (2000), pH verification is extremely important in studies addressing allelopathic action since extracts may contain sugars, amino acids and organic acids capable of masking such effect, due to pH interference.

### 3.3 Essential oil allelopathic effects

In the bioassays carried out with the *A. confertus* leaf essential oil, no significant statistical differences were observed regarding to *C. echinatus* seed germination when compared to the control (Figure 1a), but it caused a delay in the Germination Speed Index of *C. echinatus* seeds at all concentrations tested, when compared to the control (Figure 1b).

**Figure 1.** (a) Percentage of germination and (b) Germination Speed Index (GSI) of *Cenchrus echinatus* seeds exposed to different *Acritopappus confertus* fresh leaf essential oil concentrations. Equal letters do not differ statistically by Tukey's test at 5% probability.



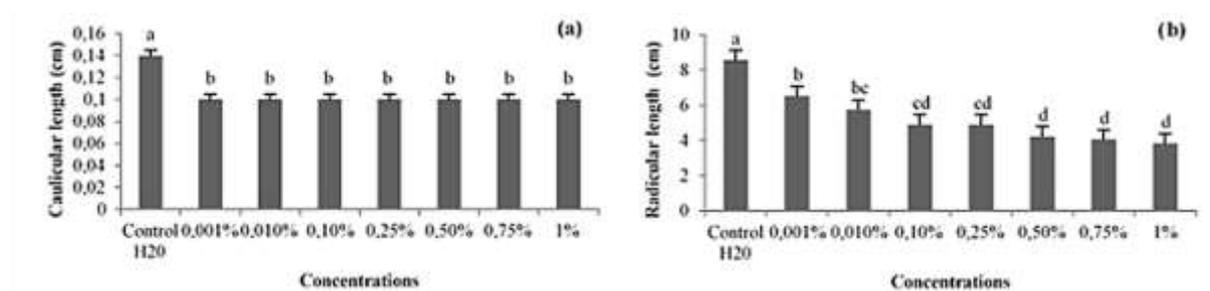
Source: Authors.

Rosado et al. (2009) state that even without allelochemical interferences in the final germination percentage, it is possible that these compounds cause changes in the germination pattern through differences in seed germination speed and synchrony.

Such effect in natural environment may be important so competition from a donor species with other functionally recipient species is avoided. In this context, Gatti, Perez and Ferreira (2007) reported this condition could represent an important ecological factor since plants which germinate in a slower manner may have a reduced size, which contributes to them being more vulnerable to stress, and present few conditions for resource competition.

*C. echinatus* seedling stem length at all concentrations tested was significantly reduced compared to the control (Figure 2a). Significant inhibitory effects on root growth and elongation were also observed, this effect being more evident at higher concentrations (0.50, 0.75 and 1%) (Figure 2b).

**Figure 2.** Average (a) Caulicular length and (b) radicle length of *Cenchrus echinatus* seedlings exposed to different *Acritopappus confertus* fresh leaf essential oil concentrations. Equal letters do not differ statistically by Tukey's test at 5% probability.



Source: Authors.

The significant reduction in stem length may be due to certain compounds present in the essential oil of the species under study. Other authors using distinct species whose chemical constitution possess compounds in common to those found in the *A. confertus* essential oil, such as Souza Filho, Bayma, Guilhon and Zoghbi (2009a) when evaluating the *Ocimum americanum* L. essential oil inhibitory effects over *Mimosa pudica* Benth. and *Senna obtusifolia* (L.) H.S. Irwin & Barneby hypocotyl and root development, where the authors attributed these results to the presence of several chemical compounds with allelopathic potential, especially limonene, camphor and linalool.

Ribeiro and Lima (2012) in studies using *Citrus sinensis* L. (Rutaceae) showed that the essential oil from this species, majorly constituted of limonene (84.2% of the total chemical composition), inhibited the length of *Euphorbia heterophylla* L. and *Ipomoea grandifolia* (Dammer) O'Donnell weed caulicles. According to the authors, the high limonene concentration in the species's essential oil has been speculated as the main reason for the observed bioactivity.

Figure 2b shows that the essential oil of *A. confertus* negatively affected the roots of the seedlings of *C. echinatus*, since it significantly inhibited their growth and development in all tested concentrations. Studies have shown that roots are more sensitive to allelochemicals than other plant structures (Souza Filho, Guilhon & Santos, 2010).

Ribeiro and Lima (2012) verified inhibitory effects on *E. heterophylla* L. roots exposed to the *C. sinensis* L. bark essential oil, whose main component is limonene. In addition, lesions and malformations were also observed in the roots, some of which were serious enough to kill seedlings.

According to Duschatzky et al. (2007) studies on the *Heterothalamus alienus* (Spreng.) Kuntze and *H. psiadioides* Less. (Asteraceae) essential oil chemical analysis,

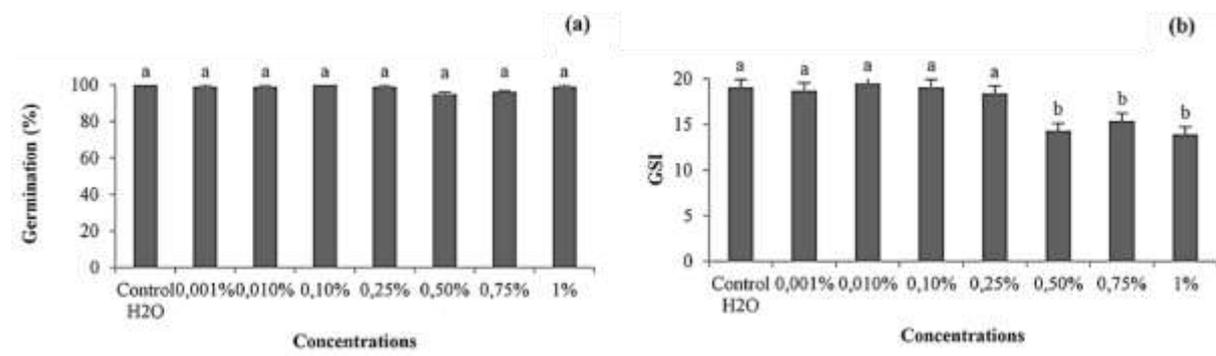
indicated  $\beta$ -pinene as the major constituent. When evaluating the oil cytotoxicity of these two species, Schmidt-Silva, Pawlowski, Santos, Zini and Soares (2011) observed a reduction in the mitotic index of *L. sativa* (lettuce) and *A. cepa* (onion) meristematic cells, resulting in chromosomal abnormalities such as chromosomal adhesion, c-mitosis and micronucleus formation. Such effects may be associated with a reduction in root length.

Allelochemicals have the ability to interfere in primary metabolic processes and plant growth, where the actions of these compounds can directly affect cell division and elongation, which are extremely important processes to ensure plant growth and development of the plants (Pires & Oliveira, 2011).

According to Maia et al. (2011) studies have pointed out that monoterpenes are capable of causing significant damage to the membranes and respiratory processes of some plants, since these substances can be absorbed through their secretory structures. Rosado et al. (2009) reported that monoterpenes are capable of altering the structure and function of membranes, impeding cell growth and performance.

Regarding *L. sativa* seeds, the *A. confertus* essential oil did not interfere with germination at any of tested concentrations (Figure 3a). As for GSI, no statistical differences in relation to the control were found at the lowest concentrations (0.001, 0.01, 0.10 and 0.25%). However, at the highest concentrations (0.50, 0.75% and 1%), a delay in *L. sativa* seed GSI with statistical differences when compared to control was observed (Figure 3b).

**Figure 3.** (a) Percentage of germination and (b) Germination Speed Index (GSI) of *Lactuca sativa* seeds exposed to different *Acritopappus confertus* fresh leaf essential oil concentrations. Equal letters do not differ statistically by Tukey's test at 5% probability.



Source: Authors.

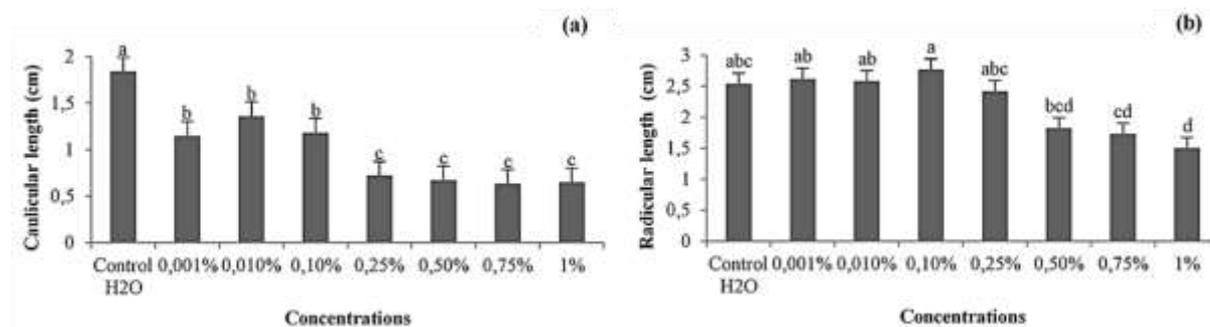
Miranda et al. (2015a) observed a significant GSI reduction in *L. sativa* seeds exposed to the *Cymbopogon citratus* (DC.) Stapf (lemon grass) fresh leaf essential oil as

concentrations increased, observations which are attributed to the presence of myrcene as one of the major compounds in this species.

Allelochemical concentration and the response threshold of the recipient species are factors which determine the action of a compound. Its inhibitory effects are closely associated with the sensitivity of the affected species, plant physiological processes and environmental conditions (Souza Filho, Vasconcelos, Zoghbi & Cunha, 2009c).

*A. confertus* significantly inhibited the caulicular length of lettuce seedlings at the various tested concentrations, with greater inhibition being observed as concentrations increased (Figure 4a). Root length was negatively affected by the 0.50% concentration (Figure 4b).

**Figure 4.** Mean (a) Caulicular length and (b) radicle length of *Lactuca sativa* seedlings exposed to different *Acritopappus confertus* fresh leaf essential oil concentrations. Equal letters do not differ statistically by Tukey's test at 5% probability.



Source: Authors.

In view of the analyzed data, it is observed that the compounds present in the essential oil of *A. confertus* cause negative influences on the stem growth of lettuce seedlings. The studies developed by Pawlowski Kaltchuk-Santos, Zini, Caramão and Soares (2012) on the cytogenotoxic activities of *Schinus terebinthifolius* Raddi and *Schinus molle* L. (Anacardiaceae) demonstrate possible interference of chemical compounds on the meristematic cells of *L. sativa* and *A. cepa*, where they observed a significant reduction in the mitotic index of the cells of the said recipient species, which showed high rates of chromosomal abnormalities and it is likely that this effect is due to the presence of limonene and  $\beta$ -pinene in the donor species.

Regarding radicular development, the oil stimulated *L. sativa* radicular growth at the 0.001, 0.01, 0.10% concentrations, while the 0.25% concentration did not display an effect when compared to the control group, however, from the 0.50% concentration upwards

radicular length inhibition was observed, presenting statistical differences only in the concentration of 1% (Figure 4b). Such results may be due to the fact that an allelochemical can be both inhibitory and stimulatory depending on the concentration in which they are found and their combination (Rice, 1984; An, Johnson & Lovette, 1993).

Silva, Overbeck and Soares (2014) observed the *H. psiadioides* Less. (Asteraceae) essential oil inhibitory effect over *L. sativa* and *A. cepa* seedling aerial and root length. According to these authors, the phytotoxic effects of this species can be attributed to the interaction of their chemical components, which reveal the presence of monoterpenes, mainly  $\beta$ -pinene,  $\Delta^3$ -carene e limonene.

The allelopathic effects observed in the present study can be attributed to the constituents identified in the *A. confertus* leaf essential oil.

Souza Filho, Guilhon, Zoghbi and Cunha (2009b) state the presence of major constituents in essential oils may explain the allelopathic effects over the germination and growth of the receptive plant.

#### 4. Conclusion

Myrcene,  $\beta$ -Pinene and limonene were the most outstanding compounds in the *A. confertus* leaf essential oil chemical analysis. The negative interference observed in the Germination Speed Index and seedling development of *L. sativa* and *C. echinatus* may be due to the combined or isolated action of these compounds. However, further studies are needed to purify and isolate these compounds, to investigate the specific constituents that cause the allelopathic effects verified in the bioassays performed.

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**Percentage of contribution of each author in the manuscript**

Jeane Dantas Sousa - 35%  
Tiago Rodrigues Leite - 5%  
Karina Vieiralves Linhares - 5%  
Josué Dantas de Sousa - 5%  
José Weverton Almeida Bezerra - 5%  
Marcos Aurélio Figueiredo dos Santos - 5%  
Isabella Hevily Silva Torquato - 5%  
Aline Augusti Boligon - 5%  
Janete Souza Bezerra - 5%  
Nathália Barbosa Campos - 5%  
Maria Arlene Pessoa da Silva - 20%