Comunicación breve

Allelopathy of *Mesosphaerum suaveolens* (bamburral) front to seeds of *Pilosocereus gounellei* subsp. Gounellei (xique-xique)

Alelopatía de *Mesosphaerum suaveolens* (bamburral) frente a las semillas de *Pilosocereus gounellei* subsp. Gounellei (xique-xique)

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ABSTRACT

Introduction: Plants used for reforestation purposes, e.g. the cactus *Pilosocereus gounellei* (F.A.C.Weber) Byles and Rowley subsp. Gounellei, may undergo the allelopathic effect of other plants (mainly those commonly known as "unwanted"), e.g. *Mesosphaerum suaveolens* L. Kuntze. In other words, coexistence of different plant species may affect the ecological succession of one of them.

Objective: Evaluate the allelopathic action of essential oil from *M. suaveolens* on the germination of seeds of *P. gounellei* subsp. Gounellei.
Methods: Leaf essential oil was diluted at various concentrations (125-1000 µg/ml) and applied to seeds of *P. gounellei* subsp. Gounellei, accompanied by a control group. The seeds that germinated were counted and measurements were taken of some variables, such as percentage and germination, germination speed and T50.
Results: The oil was shown to delay or inhibit the germination of seeds of *P. gounellei* subsp. Gounellei at concentrations ≥1000 µg/ml.
Conclusions: The essential oil of *M. suaveolens* is capable of delaying or inhibiting the germination of seeds of *P. gounellei* subsp. Gounellei. Therefore, seeds of the cactus should not be sown in areas with a large population of *M. suaveolens*.
Keywords: *Hyptis suaveolens*; terpene; allelochemical; *P. gounellei.*

RESUMEN
Introducción: Las plantas utilizadas en la reforestación, como el cactus *Pilosocereus gounellei* (F.A.C.Weber) Byles y Rowley subsp. Gounellei, pueden sufrir efecto alelopático de otras plantas, ejemplo la *Mesosphaerum suaveolens* (L.) Kuntze. De modo que, la coexistencia de las plantas puede traer consecuencias a la sucesión ecológica de una de las especies.
Objetivo: Evaluar la acción alelopática del aceite esencial de *M. suaveolens* en la germinación de semillas de *P. gounellei* subsp. Gounellei.
Métodos: El aceite esencial obtenido de las hojas se diluyó a concentraciones variadas (125-1000 µg/ml) y se aplicó en las semillas de *P. gounellei* subsp. Gounellei, acompañados de un grupo de control. Se contabilizaron las semillas que germinaron y se midieron algunas variables como el porcentaje y la germinación, el índice de velocidad de germinación y el T_{50}.
Resultados: Se demostró que el aceite es capaz de retrasar e inhibir la germinación de las semillas de *P. gounellei* subsp. Gounellei en concentraciones ≥ 1000 µg/ml.
Conclusiones: El aceite esencial de *M. suaveolens* es capaz de retardar la germinación e inhibir la germinación de las semillas de *P. gounellei* subsp. Gounellei. Conocido esto, se debe evitar la siembra de semillas del cactus en lugares donde exista una gran población de *M. suaveolens*.
Palabras clave: *Hyptis suaveolens*; terpeno; aleloquímico; *P. gounellei.*

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Introduction

Allelopathy is a phenomenon in which certain plant species produce substances that act favorably or unfavorably in the development of other species.[1] From species that are produced have allelochemicals herbicides which are used in agriculture for

the control of weeds.[2] However, in reforestation programs, these compounds may be a problem, as they may delay the germination of other species.[3] Thus, it is important to know the plants that have allelopathic action and that may interfere in the development of plants used in reforestation, so that the cultivation of these plants in nearby areas is avoided.[4]

The allelopathic action is derived from the secondary metabolisms of plants, and may belong to the group of terpenes, phenolic compounds or nitrogen compounds.[1] In the case of terpenes, the main allelopathic agents, also called allelochemicals, are the essential oils, which are complex mixtures of mono- and sesquiterpenes. These can be released into the environment by volatilization and decomposition, in a way that affects the surrounding vegetation.[5,6]

Among the plants of the Caatinga, a seasonally dry tropical forest in Brazil, which are being used in reforestation, we can mention the representatives of the Cactaceae family due to their great importance to the local fauna during the dry season, as well as the use of their representatives in forage.[7] One species of this family *Pilosocereus gounellei* (F.A.C.Weber) Byles & Rowley subsp. Gounellei, is an endemic cactus of Brazil popularly known as xique-xique, is important for fauna and is highlighted by its fruits and flowers participating in the energy chain (Fig. 1).[8]

Fig. 1. Highlighted of mature fruit of *Pilosocereus gounellei* subsp. Gounellei.

In the field, as well as the cactus *Cereus jamacaru* DC subsp. Jamacaru,[4] it is observed that the species *P. gounellei* subsp. Gounellei does not occur frequently in the same place as some weeds of allelopathic potential. Among these species is *Mesosphaerum suaveolens* (L.) Kuntze, an herbaceous plant known as bamburral and lavender-brava belonging to the Lamiaceae Family.[9,10] The representatives of this species are used in folk medicine for the treatment of several diseases, among them the treatment of respiratory diseases, this is due to the presence of the essential oil present in the leaves of the species.[6,11]

It has been demonstrated that the presence of *M. suaveolens* in an environment causes a negative impact on the vegetation, in a way that reduces the number of species occurring in that place, diversity, richness and uniformity.[12,13] In this way, the allelopathy of the species can contribute with this, since it presents allelopathic action in the *C. jamacaru* cactos.[4]

Considering these aspects it is important to investigate whether the above-mentioned annual plant may interfere with *P. gounellei* subsp. Gounellei reforestation programs, with the purpose of this research was to evaluate the *M. suaveolens* essential oil allelopathy on the germination of *P. gounellei* subsp. Gounellei.

Methods

Plant Material

The mature fruits of *P. gounellei* subsp. Gounellei were collected in a preserved Caatinga area, in the municipality of Quixelô, Ceará, with coordinates of Lat: -6º21'5.396", Long: -39º27'1.424" and altitude of 245 m, in the period of June 2016. The fruits were pulped to obtain the seeds. After obtaining the seeds, they were conditioned in glass bottles until the moment of the allelopathic tests. The material was identified by José Bezerra Weverton Almeida pressed and deposited in the Herbarium Caririense Dárdano de Andrade Lima – HCDAL under voucher #13,623.

As for the leaves of *M. suaveolens*, these were collected in the morning (09:00 h) in March 2015 under coordinates of Lat: -6°15'43.0056", Long: -39°16'2.5926" and altitude of 193 m of altitude. Fertile branches were treated according to the collection procedures, and later deposited in the same herbarium, under voucher #12.104.

Extraction of essential oil

The essential oil of *M. suaveolens* was extracted from dried leaves, submitted to hydrodistillation in Clevenger apparatus. After collection, the leaves were crushed into small pieces (150 g) and filled into a 1 L volumetric flask, where 2 L of distilled water was added. The flask was coupled to the Clevenger apparatus under the heating mantle and the temperature adjustment was carried out until the water boiled. After boiling, the 2 h time of the extraction cycle was started. At the end of each extractive cycle, the oil contained in the apparatus was collected with the aid of a pipette and stored in amber and refrigerated bottles. After extraction, sodium sulphate was used to remove the aqueous phase present in the essential oil.[6,14]

Allelopathic activity of essential oil

The seeds of *P. gounellei* subsp. *gounellei* were first selected and immersed in 5% hypocorite for five minutes, followed by washing under running water for the same time. Subsequently, the essential oil was diluted in 1% Dimethylsulfoxide (DMSO) to avoid interference with germination, and subsequently diluted in different concentrations (125-1000 µg/ml) to perform the allelopathic bioassays. All solutions were adjusted for pH, while osmolality levels were determined.

The concentrations used in this study are based on the fact that *M. suaveolens* has an annual leaf fall of 3 tons of leaves per hectare, and as its oil has a yield of 0.153% of dry weight, it will have a ratio of about 459 µg of oil per ml of soil.[4]

The tests were performed in quatruplicatas with 25 seeds per unit, totaling 100 seeds per treatment. The seeds were distributed in Petri dishes lined with two sheets of germitest paper moistened with 3 ml of distilled water. Afterwards, the plates were packed in Biochemical Oxygen Demand (B.O.D.) chambers with 12-hour photoperiod at a constant temperature of 30 °C.[15] The readings were performed every 24 hours for 7 days, where the seeds were considered as germinated when their rootlets had a size of 2 mm in length.

Variables analyzed

The percentage of germination (PG), germination speed index (GSI) according to Maguire's,[16] and T_{50} were analyzed.

Statistical analysis

For the statistical analysis of the data, the mean (± standard deviation) was used and the GraphPadPrism 6 software was analyzed with one-way ANOVA analysis, followed by the Tukey test ($p<0.05$).

Results

Chemical physical analysis

According to table 1, the essential oil of *M. suaveolens* showed a higher pressure proportional to the concentration. At 1000 µg/ml the oil had a pressure of -0.404 MPa. As for the pH natural product presented as being basic.

Table 1. Physicochemical parameters of *Mesosphaerum suaveolens* essential oil

Concentration (µg/mL)	pH measured	Adjusted pH	Osmolarity (MPa)
125	9.61	6.89	-0.027
250	9.52	6.73	-0.074
500	9.25	7.18	-0.154
1000	4.68	6.87	-0.404

Percentage of germination

Concerning the percentage of germination of the cactus seeds under study, it is possible to observe that at concentrations ≤500 µg/ml, the essential oil does not significantly affect the germination. However, at the highest concentration used in this study, 1000 µg/ml, it was demonstrated that there was a negative allelopathic action of the oil, since only 51.25% of the seeds germinated, whereas in the control group 90% of the seeds had their germination effected (Fig. 2).

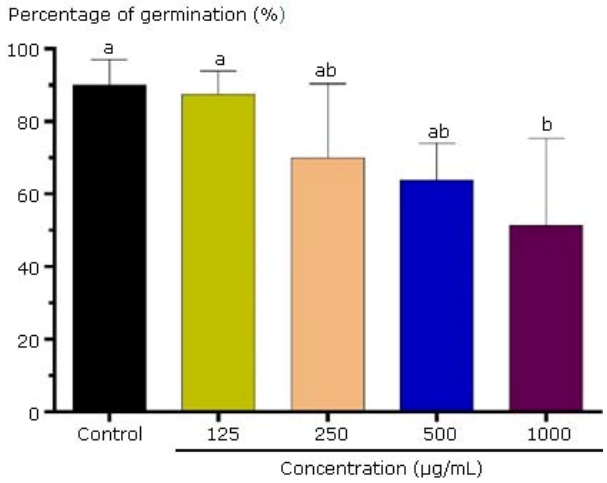

Fig. 2. Percentage of seeds of *P. gounellei* subsp. Gounellei germinated in front of the essential oil of *M. suaveolens*. Bars with different letters differ statistically by the Tukey test at 5%.

Germination speed index (GSI) and T_{50}

As regards the GSI, it was observed that, as well as the percentage of germination, only the concentration of 1000 µg/ml had an influence on the germination of the seeds. While the other concentrations did not differ statistically from the control group (Table 2). As for T_{50}, this index uses the number of seeds germinated in the treatment. From this, it calculates the time that 50% of these seeds took to germinate. In this way, it was demonstrated that the oil of *M. suaveolens* is not able to modify the time that the seeds took to germinate.

Table 2. Germination Speed Index (GSI) and T_{50} of the seeds of *P. gounellei* subsp. *gounellei* front to the essential oil of *M. suaveolens*

Concentration (µg/ml)	GSI	T_{50} (days)
0 (Control)	3.76±0.31a	4.40±0.14a
125	3.86±0.30a	4.03±0.27a
250	2.90±0.88ab	4.43±0.12a
500	2.75±0.43ab	4.31±0.40a
1000	2.04±0.99b	4.64±0.59a

Means with different letters in the same column differ statistically by the Tukey test at 5%.

Discussion

The terpenes present in the leaves of *M. suaveolens* have a negative allelopathic effect on the seeds of *P. gounellei* subsp. Gounellei, this may be related to the action of the main constituents of this oil, which was already characterized in the study by Bezerra, *et al*.[6] As the main constituents are ß-Caryophyllene (18.57%), the sabinene (15.99%) and spathulenol (11.09%), in the case of the former, it is an important volatile sesquiterpene and is also a allelochemical that influences the growth of neighboring plants.[16]

The oils' allelopathic effects may be due to the action of the major constituents or by a synergism from all or some of the EO's chemical constituents.[17] The EO's allelochemical mechanisms of action are varied and may have effects similar to those from flavonoids, which may act as ion channel regulators involved in oxidative phosphorylation or may close these channels, thus preventing ions from flowing through the cytoplasmic membrane. In addition, when these constituents are in high concentrations in the intracellular medium, they may hyperpolarize such membranes, altering ATP pump functioning and, consequently, preventing germination.[18]

In addition to the terpenes, it was shown that *M. suaveolens* leachates exhibit allelopathic action, as in the study by Maiti, *et al*.[19] which demonstrated that the leachate inhibited the germination of *Vigna radiata* (L.) R. Wilczek. Among the constituents which are present in weed species studied, there is a diterpene suaveolic acid.[20] This diterpene can liberate itself in the environment along with other terpenes by means of the decomposition of its aerial parts and to cause allelopathy in other species, in a way that will affect the structure of the environment.[10]

Bezerra, *et al*.[4] demonstrated that weed oil is able to inhibit and retard the germination at concentrations ≥125 µg/ml of cactus seeds of *Cereus jamacaru* subsp. Jamacaru, an endemic cactus of Brazil, so that the action of this weed species can bring several damages to this taxon of great importance in the Brazilian Northeast. Thus, it is evidenced that the essential oil of *M. suaveolens* is able to retard the germination and inhibit the germination of the seeds of *P. gounellei* subsp. Gounellei. With this in mind, one should avoid sowing cactus seeds in places where there is a large population of *M. suaveolens*.

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Conflict of interests

The authors do not declare conflict of interests.

Authors´ contribution

All the authors participated in the research, in the writting and the final revision or the article.