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Allelopathy of Aromatic Species on the Germination of *Cereus jamacaru* DC. subsp. *jamacaru* (Cactaceae)

José Weverton A. Bezerra[1,2], Marcos Aurélio F. dos Santos[1], Marcos V. Meiado[3], Karina V. Linhares[1], Aline A. Boligon[4], Cicero dos S. Leandro[1], Maria Daniele P. Rodrigues[1], Ana Karolina F. Silva[1], Danúbio L. da Silva[1], Janete de S. Bezerra[1], Viviane B. da Silva[1] & Maria Arlene P. da Silva[1]

[1] Regional University of Cariri, Crato, CE, Brazil

[2] Federal University of Pernambuco, Recife, PE, Brazil

[3] Federal University of Sergipe, Professor Alberto Carvalho Campus, Itabaiana, SE, Brazil

[4] Federal University of Santa Maria, Santa Maria, RS, Brazil

Correspondence: José Weverton A. Bezerra, Federal University of Pernambuco, Recife, PE, CEP: 50670-901, Brazil. Tel: 55-889-8144-7371. E-mail: weverton.almeida@urca.br

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Abstract

Cereus jamacaru DC subsp. *jamacaru*, has been suffering from severe anthropic pressure, in addition, when their seeds are dispersed, some end up not germinating due to the action of allelochemicals. Therefore, the present study was to evaluate the allelopathic effect of the essential oil (EO) from four species over *C. jamacaru* germination, as well as to identify their constituents. Four plants were selected for EO extraction (*Mesospherum suaveolens* (L.) Kuntze, *Lantana montevidensis* (Spreng.) Briq., *Lantana camara* L., and *Tarenaya spinosa* (Jacq.) Raf.) and the chemical analysis was performed by GC-MS. In order to evaluate the allelopathic activity of the EO's, the *C. jamacaru* seeds were treated with the EO's. The results showed that the EO's presented heterogeneity in their composition, with *M. suaveolens* presenting the highest number of constituents (44), followed by *L. camara* (26), *T. spinosa* (23) and *L. montevidensis* (22). All the oils negatively affected the *C. jamacaru* germination percentage in a concentration-dependent manner. Regarding the GVI, the *M. suaveolens*, *L. montevidensis* and *L. camara* OEs significantly decreased this index at all analyzed concentrations. Based on the results obtained, it is suggested that *C. jamacaru* should not be sown close to the aforementioned aromatic species in reforestation programs.

Keywords: allelochemicals, cactus, essential oil, Mandacaru

1. Introduction

The Cactaceae family is native to the Americas where a high species richness occurs (Judd, Campbell, Kellogg, Stevens, & Donoghue, 2009). This taxon is very important in the maintenance of ecosystems, as in the case of the Caatinga, a Dry Tropical Forest located in the Northeastern region of Brazil, which given the availability of various resources (fruits, nectar, pollen and water) from the local fauna, directly participates in the food chain (Cavalcante, Teles, & Machado, 2013; Gomes, Meiado, Quirino, & Machado, 2016). *Cereus jamacaru* DC. subsp. *jamacaru* (Cactaceae) is an endemic species from Brazil known as "mandacaru" (Cavalcante et al., 2013; Menezes, Taylor, & Loiola, 2014) and is a pioneer in the colonization of arid and inhospitable environments, especially outcrops. Birds indulged in their fruits, thus being the main animals responsible for the dispersion of its seeds through endozoochory (Gomes, Quirino, & Araujo, 2014).

Despite its importance, this is a species threatened by intense anthropization of its natural environment and by the fact that, when its seeds are released into the environment, some are unable to germinate due to several factors (Meiado, Albuquerque, Rocha, Rojas-Aréchiga, & Leal, 2010). One of these factors is the allelopathic action caused by secondary compounds, allelochemicals, produced by surrounding plants. These metabolites belong to the phenolic compounds, nitrogen compounds and terpenes groups (Gachon, Langlois-Meurinne, & Saindrenan, 2005). Within the terpene group, volatile terpenes, also referred to as essential oils, may act through different mechanisms of action against seeds in order to impair water assimilation, nutrient uptake, protein synthesis and germinative biochemical processes (Oliveira Junior, Constantin, & Inoue, 2011).

Given the absence of *C. jamacaru* subsp. *jamacaru* together with aromatic plants, we hypothesized that some plant species may have an allelopathic effect on the germination and/or development of this species. Within the aromatic plants that occur in the Caatinga, some have a wide distribution as well as a high population density, such as *Mesospherum suaveolens* (L.) Kuntze (Lamiaceae), *Lantana montevidensis* (Spreng.) Briq. (Verbenaceae), *Lantana camara* L. (Verbenaceae), and *Tarenaya spinosa* (Jacq.) Raf (Cleomaceae). These species, during the drought periods lose their leaves, the site of essential oil synthesis, and therefore, the soil where they fall will contain several allelochemicals (Li, Wang, Ruan, Pan, & Jiang, 2010). In the study by Sharma, Batish, Singh, Jaryan and Kohli (1995), it has been shown that Mesosphaerum suaveolens adversely impacts the number of species, diversity, richness and plant uniformity, so that infestation of this plant may compromise the succession of other plants in the community.

Considering the aspects related to this research, the objective of this study was to evaluate the allelopathic effect of the essential oil (EO) from four Caatinga species on *C. jamacaru* subsp. *jamacaru* germination, as well as to identify the chemical compounds present in these oils, in order to suggest potential actions to increase the efficiency of restoration strategies for environments in which the *C. jamacaru* subsp. *jamacaru* species is present.

2. Materials and Methods

2.1 Botanic Material Collection

To obtain the seeds, mature fruits were collected from 15 *C. jamacaru* subsp. *jamacaru* specimens in May 2016, from the Quixelô City - CE (06º24′85.9″ S, 39º27′87.3″ W). On this occasion, plant materials with reproductive parts which were pressed, identified and deposited in the Caririense Dárdano de Andrade-Lima Herbarium (HCDAL) of the Regional University of Cariri (URCA) were also collected and stored under the record number 12.513.

M. suaveolens (HCDAL 12.104), *L. montevidensis* (HCDAL 12.377), *L. camara* (HCDAL 12,609) and *T. spinosa* (HCDAL 12,625) samples were also collected from the same city in areas where *C jamacaru* subsp. *jamacaru* specimens were also present. Leaves for essential oil extraction were collected in January 2017, at 09:00±30 hrs, and left to dry in the shade.

2.2 Essential Oil Extraction

Essential oil (EO) extraction was carried out in a hydrodistillation system, as proposed by Matos (2009), with modifications. For this, the donor species dry leaves were ground to increase the contact surface and obtain a higher essential oil (EO) yield. In the hydrodistillation system, 200 g of the dried leaves from each donor species together with 4 liters of distilled water were conditioned and the mixture was boiled for 2 hours. The oil was collected with a glass pipette and stored in a refrigerator at -10 °C until the chemical analyzes and the allelopathy tests were performed.

2.3 Chemical Composition Analysis

Essential oil chemical composition analysis was carried out by Gas Chromatography coupled to Mass Spectrometry (CG/MS), using a Shimidzu QP2010 Series equipment to identify the constituents, possibly responsible for the allelopathic action. The Rtx-5MS type capillary column, measuring 30 m in length by 0.25 mm in diameter and 0.25 μm in film thickness was used. Helium gas at a rate of 1.5 mL/min was used as a carrier. The injector temperature was 250 °C and the detector temperature was 290 °C. The essential oil was diluted in a 1:200 proportion in chloroform, with 1 μL being injected. The mass spectrophotometer had its ionization energy adjusted to 70 eV and the indentification of the individual components was based on its mass spectrum fragmentation, according to its NIST Mass 08 spectral library, retention indices and comparison with published data (Adams, 1995).

2.4 Allelopathic Activity of the Essential Oils

Initially, *C. jamacaru* subsp. *jamacaru* seeds were immersed in 5% hypochlorite for 5 minutes, and washed in running water for the same time for complete sterilization. The essential oils obtained were first diluted in Dimethyl sulfoxide (DMSO) at a 1% DMSO percentage to avoid interference with germination, and were subsequently diluted to the 1000, 500, 250 and 125 μg/mL concentrations for the realization of the allelopathic bioassays. The concentrations used in this study are based on the fact that the species present variations in the leaf drop rates, and that *M. suaveolens* is the one with the greatest contribution in leaf dry biomass in relation to the other aromatic species, with a rate of 3 sheets tonelas per hectare, and as its oil has a yield of 0.153% dry weight yield, will have a ratio of around 459 μg of oil per ml of soil, whereas the leaves are in an engagement with depth of 1 cm from soil (Bezerra et al., 2017).

Each treatment consisted of four replicates with 25 seeds each, totaling 100 seeds per treatment. The assays were assembled on Petri dishes, lined with two germitest paper sheets, moistened with 3 mL distilled water (Meiado, Rojas-Aréchiga, Siqueira-Filho, & Leal, 2016). The plates were maintained in a *Biochemical Oxygen Demand* (B.O.D.) germination chamber, with a 12 hour light/dark photoperiod at a constant temperature of 30 °C, as according to Meiado et al. (2010). Observations were performed every 24 hours for 7 days, where seeds were considered to be germinated when their radicles achieved at least 1 mm in length.

The osmolarity and pH were analyzed. The latter was calibrated and adjusted to neutral ranges, between 6.5 and 7.5, when necessary with 0.1 mol/L KOH and 5% HCl solutions, since in allelopathic assays it is important that pH ranges are not very acidic or alkaline as these may be harmful to seed germination (Periotto, Perez & Lima, 2004). The osmolarity values were measured with a PZL-1000 Osmometer.

2.5 Analyzed Variables

2.5.1 Germination Percentage (GP)

The germination percentage (GP) - number of seeds that generate normal seedlings, with preserved essential structures, such as a radicular system (primary root), aerial part (hypocotyl), terminal buds and cotyledons were verified. The following formula was used:

$$GP = N/Nt \times 100 \quad (1)$$

Where, N refers to the number of germinated seeds and Nt refers to the total number of seeds sown.

2.5.2 Germination Velocity Index (GVI)

Maguire's (1962) formula was adopted to determine the GVI:

$$GVI = E^1/N^1 + E^2/N^2 + ... + E^n/N^n \quad (2)$$

Where, E^1, E^2 and E^n are the number of normal seedlings emerged computed at the first, second and last counts, respectively; and N^1, N^2 and N^n are the number of days from sowing to the first, second and last count.

2.5.3 Mean Germination Time (Tm)

To evaluate the mean emergence time, the formula proposed by Edmond and Drapala (1958) was used:

$$Tm = E^1T^1 + E^2T^2 + ... + E^nT^n/E^1 + E^2 + ... + E^n \quad (3)$$

Where, Tm is the average time required for the species to reach maximum germination; E^1, E^2 and E^n corresponds to the number of seedlings emerged at times T^1, T^2 and T^n.

2.6 Statistical Analysis

For the statistical analysis of the data, the mean (±standard deviation) were used and analyzed using the GraphPadPrism 6 software with a two-way analysis of variance (two-way ANOVA), followed by Tukey's test ($p < 0.05$).

3. Results

3.1 Phytochemical Analysis

The pH value of the oils varied according to the plants. For the osmolarity, osmotic pressures were proportional to the concentrations, that is, the higher the concentration, the higher the osmotic pressure the oil presented. The *M. suaveolens* EO presented the highest osmotic pressure at the concentration of 1000 µg/mL. Thus, it was evident that this oil presents a large number of dissolved solutes (Table 1).

Table 1. Physicochemical parameters for the different concentrations of the *Mesosphaerum suaveolens* (L.) Kuntze (Lamiaceae), *Lantana montevidensis* (Spreng.) Briq. (Verbenaceae), *Lantana camara* L. (Verbenaceae) and *Tarenaya spinosa* (Jacq.) Raf. (Cleomaceae) essential oils

Tested Species	Concentration (µg/mL)	pH measured	Adjusted pH	Osmolarity (MPa)
Mesosphaerum suaveolens	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
Lantana montevidensis	125	6.01	6.65	-0.02
	250	5.23	7.41	-0.065
	500	5.52	6.61	-0.152
	1000	4.87	7.25	-0.304
Lantana camara	125	4.68	6.76	-0.015
	250	6.2	6.87	-0.052
	500	5.93	6.74	-0.124
	1000	5.43	6.74	-0.268
Tarenaya spinosa	125	4.56	6.54	-0.024
	250	5.89	6.93	-0.054
	500	9.54	6.71	-0.131
	1000	4.91	6.74	-0.281

3.2 Essential Oil Chemical Composition

The results indicate that the oils from all the species are rich in mono- and sesquiterpenes. Moreover, the compositions were shown to be very heterogenous, with the *M. suaveolens* EO being the one with the highest number of constituents (44 constituents), followed by the *L. camara* (26 constituents), *T. spinosa* (23 constituents) and *L. montevidensis* (22 constituents) EOs (Table 2).

No major components, those with greater than 20% composition, were found. However, within the *M. suaveolens* EO secondary constituents, three occurred in greater quantity: β-Cariophilene (18.57%), sabinene (15.99%) and spatulenol (11.09%), totalling 45.58% of the EO constitution. This oil, for being largely heterogenous, presented a total of 26 trace components (below 1%), with (Z)-β-ocimene (0.07%), α-Cupene (0.09%) and δ-cadidene (0.09%) being the constituents which presented the lowest constitution percentage (Table 2).

The *L. montevidensis* species presented only two major components: β-caryophyllene (34.96%) and germacrene D (25.49%), which together totalled 60.45% of the EO's chemical content. As for the secondary constituents, 11 chemical components were identified, however, bicyclogermacrene (9.78%) and α-copaene (5.05%) were the ones with the highest prevalence. While the trace constituent (E)-caryophyllene (0.08%) presented with the lowest percentage (Table 2).

In the *L. camara* essential oil, (E)-caryophyllene (24.28%) was the chemical constituent identified as the major compound. While bicyclogermacrene (15.92%), germacrene D (11.83%) and Valencene (8.37%) were the three secondary constituents with the highest percentage, making up 36.12% of the EO constitution. Regarding the trace components identified by chromatography, 10 compounds were totalled, with camphene (0.09%) being the lowest percentage constituent (Table 2).

The *T. spinosa* EO was the only one which presented a diterpene (C_{20}), phytol (27.19%), as its major component. As secondary constituents, chromatography identified α-farnesen (11.56%) and eugenol (7.34%) with greatest predominance. In addition, a total of 8 trace components present in this oil, with methyl benzoate (0.07%) and γ-cadinene (0.09) being identified at an oil concentration below 1% (Table 2). Some constituents, such as β-caryophyllene, allo-aromadendrene, germacrene D, espatulenol and caryophyllene oxide, were present in all the oils.

Table 2. Chemical composition of the *Mesosphaerum suaveolens* (L.) Kuntze, *Lantana montevidensis* (Spreng.) Briq., *Lantana camara* L. and *Tarenaya spinosa* (Jacq.) Raf. Essential oils

Constituents	RI[a]	RI[b]	Chemical Composition			
			M. suaveolens	*L. montevidensis*	*L. camara*	*T. spinosa*
α-Thujene	989	931	1.09	-	-	-
α-Pinene	940	939	0.85	-	0.20	0.25
Sabinene	976	976	15.94	1.09	6.05	-
β-Pinene	980	980	2.11	-	0.51	-
Myrcene	994	991	0.26	-	0.28	0.74
δ-2-Carene	999	1001	0.49	-	-	-
α-Felandrene	1006	1005	1.38	-	-	2.63
α-Terpinene	1019	1018	1.05	-	0.07	-
p-Cimene	1030	1029	0.76	-	2.86	3.51
Limonene	1031	1031	5.19	-	-	-
1-8-Cineol	1037	1033	3.04	-	-	-
(Z)-β-Ocimene	1041	1040	0.07	-	0.73	-
(E)-β-Ocimene	1055	1050	0.12	-	0.97	-
γ-Terpinene	1060	1061	2.97	-	1.85	-
t-Sabinene hydrate	1068	1068	0.61	0.56	0.15	-
Linalool	1095	1098	0.43	3.40	-	1.59
Cis-p-Menth-2-en-1-ol	1123	1121	0.28	-	-	-
t-Sabinol	1139	1140	0.15	-	-	-
4-Terpineol	1178	1177	6.82	-	-	-
p-Cymen-8-ol	1183	1183	0.23	-	-	-
α-Terpineol	1191	1189	0.94	-	1.10	-
δ-Elemene	1335	1338	1.17	-	-	-
α-Cupene	1377	1376	0.09	5.05	1.01	-
β-Elemene	1390	1391	0.78	2.61	1.52	-
β-Cedrene	1416	1417	0.14	-	-	-
β-Caryophyllene	1421	1418	18.57	34.96	3.69	1.62
β-Gurjunene	1433	1432	0.23	-	-	-
γ-Elemene	1435	1433	1.44	-	-	0.61
Aromadendrene	1439	1439	0.32	-	-	-
α-humulene	1453	1454	1.17	2.56	4.03	-
Allo-aromadendrene	1461	1462	0.40	1.35	2.35	-
γ-Muurolene	1477	1477	0.28	-	-	-
Germacrene D	1481	1480	5.21	25.49	11.83	0.34
β-selinene	1486	1485	0.89	-	-	-
Bicyclogermacrene	1501	1488	7.52	9.78	15.92	-
γ-Cadinene	1512	1513	0.36	-	-	0.09
δ-Cadidene	1525	1520	0.09	0.13	-	-
Germacrene B	1559	1556	0.27	-	-	-
Spathulenol	1576	1576	11.09	2.23	2.13	6.12
Caryophyllene Oxide	1580	1581	3.18	3.94	2.78	9.07
Globulol	1582	1583	0.62	-	-	-
Cubenol	1641	1642	1.07	-	-	-
β-Eudesmol	1649	1649	0.13	-	-	-
α-Cadinol	1656	1653	0.45	-	-	-
Camphene	953	951	-	0.56	-	-
p-Cimene	1026	1026	-	0.67	-	-
Terpinolene	1088	1079	-	1.15	5.48	-
c*is*-Linalool Oxide	1074	1074	-	0.53	-	-

Camphor	1143	1141	-	0.78	-	-
Terpinen-4-ol	1177	1174	-	0.17	0.23	-
(E)-Caryophyllene	1418	1423	-	0.08	24.28	-
Valencene	1491	1489	-	1.58	8.37	-
α-Cadidene	1538	1538	-	0.45	0.18	-
Camphene	953	951	-	-	0.09	1.09
Methyl benzoate	1091	1087	-	-	-	0.07
Thymol	1290	1287	-	-	-	0.95
Eugenol	1356	1329	-	-	-	7.34
α-Humulene	1452	1439	-	-	-	2.08
α-Farnesene	1503	1503	-	-	-	11.56
Hexadecane	1600	1601	-	-	-	3.85
Eudesmol	1615	1618	-	-	-	4.19
Caryophyllene Acetate	1700	1704	-	-	-	0.13
N-Hexadecanoic acid	1984	1985	-	-	-	3.56
Phytol	2065	2049	-	-	-	27.19
Incensole	2158	2172	-	-	-	5.73
Total (%)			**99.97**	**99.12**	**98.66**	**94.31**

Note. Relative proportion of essential oil constituents expressed as a percentage.

[a]Relative Retention Index (Adams, 1995).

[b]Experimental Retention Index (based on the homologous *n*-alkane C_7-C_{30} series).

3.3 Germination Percentage

All the analyzed oils displayed a significant negative allelopathic effect on the *C. jamacaru* subsp. *jamacaru* germination percentage. In addition, germination percentage is inversely proportional to the concentration of the oils (Figure 1).

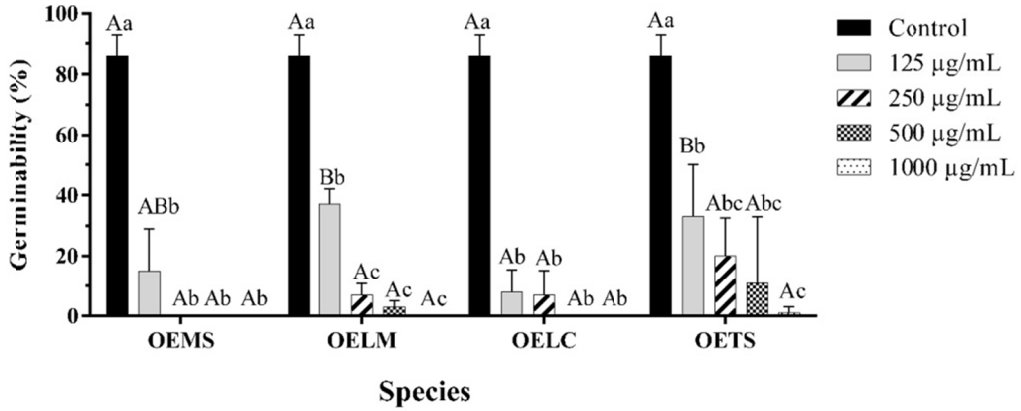

Figure 1. Germinability of *Cereus jamacaru* DC. subsp. *jamacaru* (Cactaceae) seeds against different concentrations (µg/mL) of the essential oils from (OEMS) *Mesosphaerum suaveolens* (L.) Kuntze (Lamiaceae), (OELM) *Lantana montevidensis* (Spreng.) Briq. (Verbenaceae), (OELC) *Lantana camara* L. (Verbenaceae) and (OETS) *Tarenaya spinosa* (Jacq.) Raf. (Cleomaceae). Capital letters compare the same essential oil concentration between different species. Lowercase letters compare the different concentrations from the same species

The *M. suaveolens* EO had a high allelopathic effect as it completely inhibited seed germination at 250, 500 and 1000 µg/mL concentrations over the 7 days, whereas a low germination percentage (with only 20±9.79%) was observed at the 125 µg/mL concentration. For the control group, a total of 86±6.92% of seeds germinated (Figure 1).

The *L. montevidensis* EO also presented negative allelopathic activity against the *C. jamacaru* subsp. *jamacaru* seeds. The 1000 μg/mL concentration completely inhibited seed germination during 7 days. In addition, the higher the EO's concentration, the lower the percentage of seeds were germinated. In the remaining concentrations, germination was observed, however, at low percentages such as in the group with the lowest concentration (125 μg/mL), where only 37±5.03% of seeds were germinated, less than half the germination percentage of the control group (Figure 1).

As for the *L. camara* EO, at concentrations ≥ 500 μg/mL, this inhibited *C. jamacaru* subsp. *jamacaru* seed germination, since no germination was observed for 7 days. At concentrations ≤ 250 μg/mL, some seeds germinated, however the percentage was low, such as at 125 μg/mL of the EO where only 8.0±7.3% of the seeds germinated (Figure 1).

The *T. spinosa* EO showed the lowest negative allelopathic activity when compared to the other oils, since some seeds germinated at the highest concentration (1000 μg/mL), despite obtaining low germination percentages. Moreover, in the 125 μg/mL treatment, germination was observed in 33±17% of seeds, which, although the seeds were shown to be more tolerant to this oil, the EO still promoted a high negative allelopathic action (Figure 1). It is noteworthy that the different oil concentrations inhibited germination in different ways, as the *L. camara* EO had a more negative allelopathic effect than the *L. montevidensis* and *T. spinosa* EOs at the 125 μg/mL concentration (Figure 1; Table 3).

Table 3. Two-way ANOVA results for the germination percentage (%), germination velocity index and mean germination time of *Cereus jamacaru* DC. subsp. *jamacaru* (Cactaceae) seeds against different concentrations (μg/mL) of the *Mesosphaerum suaveolens* (L.) Kuntze (Lamiaceae), *Lantana montevidensis* (Spreng.) Briq. (Verbenaceae), *Lantana camara* L. (Verbenaceae) and *Tarenaya spinosa* (Jacq.) Raf. (Cleomaceae) essential oils

	F	gl	p
Germination Percentage (%)			
Species	6.5821	3.60	0.0009
Concentration	271.5362	4.60	< 0.0001
Species × Concentration	2.2629	12.60	0.0191
Germination Velocity Index			
Species	3.6347	3.60	0.0175
Concentration	51.014	4.60	< 0.0001
Species × Concentration	0.978	12.60	0.5198
Mean Germination Time (days)			
Species	113.512	3.60	< 0.0001
Concentration	351.4481	4.60	< 0.0001
Species x Concentration	59.1192	12.60	< 0.0001

3.4 Germination Velocity Index (GVI)

The essential oils from the four plants analyzed affected the germination speed of *C. jamacaru* subsp. *jamacaru* seeds in a dose-dependent manner, such that the higher the EO concentration, the slower the germination speed.

The *L. camara* EO was the one which most affected the GVI of *C. jamacaru* subsp. *jamacaru* seeds at the lowest tested concentration of 125 μg/mL which was 0.38±0.36. Similarly, the *M. suaveolens* and *L. montevidensis* oils negatively affected the GVI in all the concentrations. For *T. spinosa*, the EO from this species significantly affected the GVI only at concentrations ≥ 500 μg/mL (Table 4).

Table 4. Germination Velocity Index (GVI) of *Cereus jamacaru* DC. subsp. *jamacaru* seeds against essential oils from *Mesosphaerum suaveolens* (L.) Kuntze, *Lantana montevidensis* (Spreng.) Briq., *Lantana camara* L. and *Tarenaya spinosa* (Jacq.) Raf

Concentration µg/mL	OEMS	OELM	OELC	OETS
125	0.71±0.60b	1.34±0.76b	0.38±0.36b	1.69±0.77ab
250	0±0b	0.31±0.24bc	0.42±0.49b	1.14±0.72ab
500	0±0b	0.11±0.07c	0±0b	0.62±1.25b
1000	0±0b	0±0c	0±0b	0.03±0.07b
Control (H_2O)	2.64±0.87a	2.64±0.87a	2.64±0.87a	2.64±0.87a

Note. Means followed by the same letter column do not differ from each other at 5% probability by Tukey's test. OEMS: *Mesosphaerum suaveolens* (L.) Kuntze essential oil; OELM: *Lantana montevidensis* (Spreng.) Briq. essential oil; OELC: *Lantana camara* L. essential oil; OETS: *Tarenaya spinosa* (Jacq.) Raf. essential oil.

3.5 Mean Germination Time (Tm)

Although the oils affected the germination percentage and the germination velocity index of *C. jamacaru* subsp. *jamacaru* seeds, those which germinated were not significantly affected with respect to the mean germination time. Even though germination was inhibited at concentrations ≥ 250µg/mL, the *M. suaveolens* EO at the 125 µg/mL concentration had a Tm of 5.16±0.81, not differing from the control group (Tm: 4.97±0.54). The same occurred with the other essential oils (Figure 2).

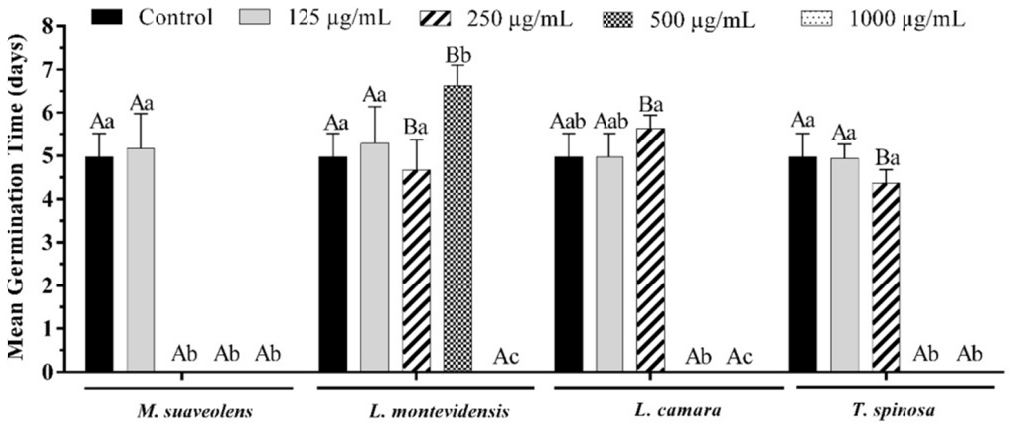

Figure 2. Mean germination time of *C. jamacaru* DC. subsp. *jamacaru* seeds subjected to the OEMS, OELM, OELC and OETS. Mean (±standard deviation). Capital letters compare the same essential oil concentration between different species. Lowercase letters compare different concentrations from the same species

4. Discussion

In this study, *C. jamacaru* DC. subsp. *jamacaru* seeds obtained an average germination time of 4.9 days, which may be justified by the distribution of this species, native to the semi-arid region of Brazil, a region characterized by sporadic rains in which the available water time in superficial soils is reduced and the seeds need to germinate in a few days in order to make the most of the available water for their metabolic processes (Rito, Rocha, Leal, & Meiado, 2009). This average germination time may represent a pattern for some Cactaceae species such as *Pilosocereus gounellei* (F.A.C.Weber) Byles & G.D. Rowley. (xique-xique) (Abud et al., 2012), *Pilosocereus arrabidae* (Lem.) Byles & Rowley (Facheiro-da-praia) (Martins et al. 2012) and *Discocactus zehntneri* Britton & Rose subsp. *Zehntneri* (Meiado et al., 2016). Whereas, other cacti species have a higher Tm, as cited in the study by Meiado et al. (2016), for *Facheiroa squamosa* (Gürke) P.J.Braun & E. Esteves Pereira (Facheiro-preto), *Harrisia adscendens* (Gürke) Britton and Rose (Rabo-de-raposa) and *Melocactus ernestii* Vaupel subsp. *ernestii*. There are few cacti which require more than ten days to germinate under suitable conditions such as *Pilosocereus pentaedrophorus* (J.F.Cels) Byles & G.D. Rowley subsp. *pentaedrophorus* (mandacaru-de-veado),

from the Atlantic Forest, an ecosystem which is subjected to less water stress, compared with the Cerrado and the Caatinga (Meiado et al., 2016).

For allelopathic tests, osmolarity levels should be very close to 0 MPa since solute levels negatively and significantly interfere with seed germinability (Góis, Torres, & Pereira, 2008). High osmolarity levels exert a harmful osmotic pressure between the solution and the seeds, consequently, water does not penetrate the seeds and in this way it inhibits or slows down germination (Ortiz, Gomes, Urbano, & Strapasson, 2014). However, in the evolutionary scale, cacti developed strategies to survive in arid environments where a high rate of soil water evaporation and low drainage predominate, resulting in the accumulation of solutes such as salts, thus, seeds from these plants are generally tolerant to salt stress (Góis et al., 2008).

C. jamacaru subsp. *jamacaru* tolerate high saline stress, that is, they are halotolerant which can be observed in a study by Meiado et al. (2010) who found a percentage of germinated seeds, not differing from the control group, which were subjected to an osmotic potential of up to -0.6 MPa. Thus, with respect to allelopathic activities, all the essential oils presented a negative allelopathic activity against *C. jamacaru* subsp. *jamacaru* seeds. since the results are not attributable to the osmotic pressures exerted by the oils' solutes, given that the highest osmotic pressure exerted was 0.404 MPa from the *M. suaveolens* EO at the 1000 µg/mL concentration.

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 (Simões, Schenkel, Mello, Mentz, & Petrovick, 2017). 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 (Martino et al. 2012).

For *M. suavoelens*, the allelopathic action may be attributed to a synergistic action from the β-Caryophyllene (18.57%), sabinene (15.99%) and spatulenol (11.09%) secondary constituents. In the study by Miranda, Cardoso, Carvalho, Figueiredo and Andrade (2015), β-caryophyllene constituted 13.2% of the *Hedychium coronarium* J. Koenig (lírio-do-brejo) EO and showed relatively low negative allelopathic effects on *Lactuca sativa* L. (lettuce) seeds., which are sensitive to allelochemicals. However, this chemical component needs other components to act on seed germination. Whereas, sabinene is a cyclic hydrocarbon monoterpene which, according to Sikkema, Bont, and Poolman (1995), causes alterations in cell membrane structure and function, preventing growth and cellular activities, therefore, the action observed in this study may be related to a synergistic action of the aforementioned constituents.

As for the *M. suaveolens* EO chemical composition, its constitution is variable according to the plant origin, which is evidenced in the study by Benelli, Flamini, Canale, Cioni, and Conti (2012), where the EO of individuals from the same species, collected in Italy, showed variations in constitution percentage and in main constituents, with emphasis on sabinene (34.0%), β-caryophyllene (11.2%) and terpinolene (10.7%), where in the present study the latter constituent is not present in the EO obtained. In the study by Rodrigues et al. (2012) the main constituents obtained from leaves in the Minas Gerais (BR) state were trans-caryophyllene (18.15%), beta-elemene (7.26%) and caryophyllene oxide (6.97%). Despite EO being obtained from specimens collected in Brazil, when compared to the EO in this study, the observed composition varied considerably. These differences may be justified by the different cultivation forms, harvesting periods, climatic stress and, especially, the geographic origin of the plant (Noudjou et al., 2007).

The *L. montevidensis* essential oil also negatively affected the germination of *C. jamacaru* subsp. *jamacaru* seeds. This fact can be attributed to the two major sesquiterpenes present, β-caryophyllene and germacrene D. In the study by Ayeb et al. (2016), the allelochemical action of the *Tipuana tipu* (Benth.) Kuntze essential oil against *L. sativa* seeds was verified to be due to these two sesquiterpenes.

The *L. camara* allelopathic effect was also recorded by Kong, Wang, Zhang, Zhang and Hu (2006) which, according to the authors, this effect was due to pentacyclic triterpenoid (C_{30}) compounds, Lanthanum A and Lanthanum B. However, in our study, the allelopathic action observed is attributed to volatile terpenes, since the mentioned triterpenoids are not present in our essential oils.

The seed germination inhibitory effect promoted by the *T. spinosa* essential oil over *C. jamacaru* subsp. *jamacaru* seeds is justified by its major constituent phytol, which is a cytotoxic and genotoxic diterpene responsible for inducing apoptosis in plant cells, as well as nuclear anomalies and pro-oxidative effects (Islam et al., 2017). This constituent is also present in other species from the Cleomaceae family, such as in *Cleome serrata* (Mcneil, Porter, & Williams, 2012). With this, the sowing of *C. jamacaru* subsp. *jamacaru* seeds should

not be performed near these two species, as well as other species that present the diterpene phytol as an oil constituent.

In the northeastern semi-arid region, plants are subjected to several abiotic stresses, such as high temperatures, high radiation rate and water stress during their growth and development. Therefore, there is an increase in the production and release of secondary metabolites, especially volatile terpenes (Oliveira Junior et al., 2011). Thus, allelochemical release in the medium will be greater at times of prolonged drought and high temperatures for *L. montevidensis*, *L. camara* and *T. spinosa*, since these species present leaves during the dry season, while *M. suaveolens* is not established in this period as it is an annual species which grows and reproduces during the rainy season (Bezerra et al., 2017).

However, although the volatile terpenes analyzed here may be detrimental to the *C. jamacaru* subsp. *jamacaru* ecological succession, some EO's may be beneficial to seeds from this plant aiding the perpetuation of the species (Hillen et al., 2012), such as the *Pimpinella anisum* L. and *Cymbopogon winterianus* Jowitt EOs. These oils can, in addition to controlling the pathogens present in seeds, they can increase their germinability, that is, they present positive allelopathy (Mata, Araujo, Nascimento, Souza, & Viana, 2009). Thus, a better understanding of the species occurring in the Caatinga and those which produce allelochemicals will aid the development of *C. jamacaru* subsp. *jamacaru* conservation strategies, in addition to limiting the spread of weeds, thus preserving the native plants.

5. Conclusion

In reforestation programs, *C. jamacaru* subsp. *jamacaru* seeds should not be sown near *M. suaveolens*, *L. montevidensis*, *L. camara* and *T. spinosa* since these species release essential oils in the environment with potential negative allelopathic activity, affecting *C. jamacaru* subsp. *jamacaru* seeds. Such allelochemicals may impair the ecological succession of the cactus under study.

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