Chemical composition and phytotoxic activity of *Lippia origanoides* essential oil on weeds

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Abstract

Studies in the area of allelopathy in agriculture have increased due to search on alternative methods of weed control compared to conventional herbicides. Thus, the objective of this work was to evaluate the chemical composition and phytotoxicity of the *Lippia origanoides* essential oil on weeds of the species *Bidens subalternans*, *Euphorbia heterophylla* and *Macroptilium lathyroides*. The essential oil was extracted from the leaves of *L. origanoides* by means of hydrodistillation and diluted in the concentrations of 0.01 to 1%. The chemistry composition was determined by a gas chromatograph coupled with a mass spectrometry. Phytotoxic activity was evaluated in pre and post-emergence by tests of germination, seedling growth, chlorophyll content and cellular respiration. The results demonstrated an essential oil rich in monoterpenes, mainly oxygenated, with camphor as the major compound. In general, both germination and seedling development were entirely inhibited by essential oil, decreasing with increasing concentrations. The concentrations 0.5 and 1.0% totally inhibited the germination of *B. subalternans*, but did not affect the germination of *M. lathyroides*. The essential oil sprayed on the weed leaves had no effect on chlorophyll content but was able to induce visible injuries such as necrosis and chlorosis. Only the cellular respiration of *E. heterophylla* was affected by the essential oil. All the phytotoxic effects observed are due to the high content of monoterpenes in the essential oil, mainly the oxygenates, and also the concentration used. Therefore, the *L. origanoides* essential oil have bioherbicidal potential for the tested species.

Keywords: Allelopathy; Phytotoxicity; Weeds; Bioherbicide.

Introduction

Lippia origanoides Kunth. is a species of the Verbenaceae family native to South America and some Central American countries (Vicuña et al., 2010), commonly used as a culinary spice or remedy for gastrointestinal disorders (Pascual et al., 2001; Oliveira et al., 2014). Its essential oil has a wide variation in chemical composition, and is currently classified in 5 chemotypes (A, B, C, D and E) according to its main constituents (Dos Santos et al., 2004; Silva et al., 2009; Stashenko et al., 2010; Vega-Vela et al., 2013; Ribeiro et al., 2014).

The essential oils are natural, volatile and complex compounds that are normally extracted from plants found in countries of the tropics and the Mediterranean, where they represent a fundamental part of herbal medicines. Great part of medicinal plants produce essential oils as a result of secondary plant metabolism. The main characteristic of essential oils is the strong odor (Bakkali et al., 2008).

The utilization of essential oils in agriculture is an alternative to conventional methods of pest, phytopathogen and weed control (Ootani et al., 2013). Weeds cause serious economic damage to agriculture, by means of competition with crops for available resources, including solar radiation, nutrients,

water and soil space. The weeds reduce production and impair the quality of the final product (Oliveira Jr. et al., 2011; Ootani et al., 2013).

The chemical control of weeds, made through the application of herbicides, is the most used method due to its advantages of efficiency, selectivity and cost benefit (Oliveira Jr. et al., 2011). However, with the intensification of herbicide application, the number of weed species resistant to these products is systematically increasing (Oliveira Jr. et al., 2011; Ootani et al., 2013).

In the current scenario of agriculture in Brazil, with the increasing number of weed species resistant to herbicides, studies related to allelopathy become increasingly necessary. According to Ferreira and Aquila (2000), the activity of allelochemicals has been used as an alternative to the use of herbicides, insecticides and nematicides.

Allelopathy is the process, by which products of secondary metabolism of a particular vegetable are released, preventing the germination or development of other plants (Alves et al., 2004). Research in the field of allelopathy are increasingly necessary to understand better action of allelochemicals. Investigations of plants with allelopathic

activity may be useful in the search for phytotoxins with the potential to compose new agrochemicals (Rosado et al., 2009; Amri et al., 2013). Thus, the objective of this work was to evaluate the chemical composition of the essential oil of *Lippia origanoides* and its phytotoxic activity in *Bidens subalternans*, *Euphorbia heterophylla* and *Macroptilium lathyroides*, in laboratory and greenhouse.

Results and discussion

Physical and chemical characterization of essential oil

The essential oil of *L. origanoides* presented light yellow color, with clear appearance and mild camphor odor, with a density of 1.28g.mL^{-1} at 25 ºC, and an average yield of 2.44%.

The chemical analysis by GC-MS identified 37 components in the essential oil that represent 91.58% of the total identified (Table 01). The oil is composed mainly of monoterpenes (58.7%), followed by sesquiterpenes (32.63%) and other compounds (0.25%). The oxygenated monoterpenes are present in a greater amount (40%), with camphor (34.04%) as the main constituent, whereas the monoterpenes hydrocarbon represent only 18.7%, which is constituted mainly by camphene (10.99%). The hydrocarbon sesquiterpenes represent 24.71% and have mainly β-bisabolene (10.8%), and the oxygenated sesquiterpenes constitute 7.92%, with caryophyllene oxide (2.81%), as the main component (Table 01).

The yield of *L. origanoides* essential oil was considered good, since, values above 1.0% are considered good for application in industrial scale (Ribeiro et al., 2014). However, the major compounds present in the essential oil differ from the results already reported for the species. There are currently 5 chemotypes reported in the literature for the *L. origanoides* essential oil based on their main constituents: chemotype A characterized by p-cymene, α- and β-felandren, limonene and 1,8-cineol (Stashenko et al., 2010); chemotype B by carvacrol (Dos Santos et al., 2004; Oliveira et al., 2007; Teles et al., 2014; Sarrazin et al., 2015); chemotype C by thymol (Rojas et al., 2006; Stashenko et al., 2010; Arango-Bedoya et al., 2012; Vega-vela et al., 2013); chemotype D by 1,8-cineol (Silva et al., 2009) and chemotype E by (E)-methyl cinnamate and (E)-nerolidol (Ribeiro et al., 2014).

The essential oil chemical composition may vary due to place, time of year, plant age and collection time. For example, Teles et al. (2014) for the carvacrol chemotype verified that the rainy season and plant growth of *L. origanoides* significantly reduced the thymol and carvacrol contents. In this same work, camphor and camphene contents increased with plant age (tabulated data only, not discussed). These are the same chemical constituents found in the present study as majority compounds. Ribeiro et al. (2014) also verified great variation in the chemical constituents of the chemotype E according to the time/month of collection. They discovered the possibility of scheduling the collection according to the desired chemical interest.

Another *L. origanoides* essential oil with camphor aroma, collected in the state of Maranhão, Brazil, has been reported by Silva et al. (2009), but having as main component the 1,8 cineol, followed by α-terpinol. In this work, *L. origanoides* also presented similar odor to the mentioned study, but with camphor, camphene and β-bisabolene as majority compounds.

The variation found in the essential oil of the present study is probably due to the large levels of genetic diversity found for *L. origanoides*, and can be even compared to other species of the same genus (Vega-Vela et al., 2013), and also to the alterations of its chemical composition due to the environmental factors, circadian, collection point and plant age (Stashenko et al., 2010; Vega-Vela et al., 2013; Ribeiro et al., 2014; Teles et al., 2014).

Phytotoxic effect on weed seed germination

In general, both the germination and germination speed index (GSI) of the weeds were reduced with increasing concentration of essential oil of *L. origanoides* (Figure 01 A and B), except *M. lathyroides*, whose germination was not affected by the tested concentrations (Figure 01A).

In germination, the species *B. subalternans* showed the greatest reduction in the percentage of emergence (r^2 = 0.924), mainly in the 0.5 and 1.0% concentrations, which reduced the number of seeds germinated in 96.10 and 100%, respectively. In *E. heterophylla*, the maximum inhibition of germination (r^2 = 0.863) was observed in the concentration 1.0%, which reduced the number of germinated seeds in 52.94% (Figure 01A).

Several works have already proved the efficacy of essential oils as inhibitors of weed germination, especially, with increasing concentration. For example, *Citrus aurantifolia* oil (0.10 – 1.50 mg.ml^{-1}) reduced the germination of *Avena fatua*, *Echinochloa crus-galli* and *Phalaris minor* (Fagodia et al., 2017). The essential oils of *Eucalyptus citriodora* (1%) and *Cymbopogon nardus* (1%) significantly reduced the germination of *Cenchrus echinatus* and *Digitaria horizontalis* compared to the control (Ootani et al., 2017).

Higher reductions in GSI of *B. subalternans* (r^2 = 0.985) were observed at the 0.5 and 1.0% concentrations, which reduced the germinative index by 93.75 and 100%, respectively. On the other hand, the maximum reductions in GSI of *E. heterophylla* and *M. lathyroides* was observed at the 1.0% concentration that reduced the germination speed index of the seeds in 61.15 and 59.7%, respectively (Figure 1B). Ferreira and Aquila (2000) stated that, sometimes the allelopathic action does not act directly on the germination, but on another process, such as the speed of germination. This explains why the germination of *M. lathyroides* was not affected by the concentrations of the essential oil of *L. origanoides*, but the germination speed index showed reducing effect at all concentrations.

In addition to the concentration, the phytotoxic potential of each essential oil can be explained by its chemical composition. Oils with high content of oxygenated monoterpenes are more efficient to inhibit the germination compared to those that contain higher amount of hydrocarbon monoterpenes (Vokou et al., 2003; Kordali et al., 2007; De Martino et al., 2010). In this study, we found higher content of oxygenated monoterpenes, with a ketone (camphor) as the major compound. Studies show that ketones have a high rate of germination inhibition (Vokou et al., 2003; De Martino et al., 2010).

In many works, the allelopathic effect of pure essential oils is attributed to the organic compounds (monoterpenes or

sesquiterpenes) present in larger amounts (Ismail et al., 2012; Laosinwattana et al., 2018) or to the major compound (Alves et al., 2004; Abd El-Gawad, 2016). However, it is important to observe that the interactions between the chemical components found in the essential oil can act in a synergistic or antagonistic mode (Vokou et al., 2003; Souza Filho et al., 2010; Fagodia et al., 2017). Thus, the inhibitory activity of the essential oil on the species tested can be attributed to the major compound (camphor) or the interaction between the present compounds.

Phytotoxic effect on weed seedling growth and development

The *L. origanoides* essential oil showed inhibitory effect on the growth and development of all seedlings as a function of the concentration increase for the variables analyzed (Figure 02 and 03). In this study, all seedlings were affected by the essential oil, differently from the effect on germination. This can be explained by the fact that seedlings are more sensitive to the action of allelochemicals in relation to germination (Ferreira and Aquila, 2000; Tigre et al., 2012; Laosinwattana et al., 2018). This is because, germination utilizes the seed's own reserves, and some allelochemicals may stay retained only in the integument (Tigre et al., 2012). The root length was affected in all tested weeds, but only *B. subalternans* adjusted to the quadratic model (r^2 = 0.890), with a more efficient reduction in 0.5 and 1.0% concentrations that decreased root growth by 52.02 and 50%, respectively (Figure 02A).

Hypocotyl length was significantly affected in all seedlings, but *E. heterophylla* did not fit any model. *B. subalternans* (r^2 = 0.773) and *M. lathyroides* (r^2 = 0.779) showed higher reductions in concentrations 0.5 and 1.0%. *B. subalternans* reduced by 65.15 and 70.91%, and *M. lathyroides*, 91.46 and 91.56%, according to the respective concentrations (Figure 02B).

The action of the allelochemicals on seedling growth usually presents a higher rate of inhibition in the development of the root in relation to the aerial part of the plant (Kaur et al., 2010; Abd El-Gawad, 2016; Fagodia et al., 2017). In this study we observed opposite results, with the aerial part of the plant more inhibited than the root. Grichi et al. (2016) found similar results on essential oil of *E. lemanium* (25-100 μL.mL^{-1}) and verified that the growth of the aerial part of the weeds was more inhibited than the one of the roots. Synowiec et al. (2016) found that the essential oil of *C. sativa* preferentially inhibited the growth of the hypocotyl of *Echinochloa crus-galli*.

The effect that the essential oils on reduction of growth is probably due to the decrease of the mitotic activity in the apical meristems (Fagodia et al., 2017). These authors verified that the essential oil of *C. aurantifolia* reduced the mitotic index and caused the appearance of cells with chromosomal alterations in roots of *A. cepa* with the concentration increase. These effects may be responsible for the reduction of weed growth.

The leaf area of all seedlings tested adjusted to the quadratic model. The concentrations 0.5 and 1.0% showed the highest inhibitory effects for all the species tested, with reductions of 52.32 and 57.59% for B. subalternans (r^2 = 0.719), 70.87% for *E. heterophylla* (it remained practically constant from of 0.5%) (r^2 = 0.812), and 93 and 92.87% for

M. lathyroides (r^2 = 0.938). *M. lathyroides* showed the highest rate of leaf area reduction as a function of the increase in essential oil concentration (Figure 3A).

The weed dry weights presented quadratic behavior (r^2 = 0.893 and 0.911), except for *E. heterophylla*, which did not fit any model. As in the other analyzed variables, concentrations of 0.5 and 1.0% presented higher reductions, with a decrease of 52.14 and 57.69% for *B. subalternans* and 86.91 and 85.69% for *M. lathyroides*, respectively (Figure 3B).

For example, variables such as leaf area and dry mass of seedlings are not often reported in allelopathy studies, since the main interferences occur mainly in root and shoot growth. However, some studies cite and demonstrate that allelochemicals also affect these variables. The essential oil of *A. indica* reduces the dry weight of *B. pilosa*, *C. occidentalis*, *A. viridis* and *E. crus-galli* (Batish et al., 2012). Tigre et al. (2012) verified that the lichen extracts of *C. verticillaris* reduced the leaf area up to 65.42%. In the present study, the reductions that occurred in both the root system and aerial part and leaf area as a function of the increase of the essential oil directly influenced the seedling dry weights, as reported by Miranda et al. (2015).

Although *E. heterophylla* and *M. lathyroides* did not fit any regression model for some variables, these species presented important reduction values of the analyzed variables as a function of the increase of the *L. origanoides* essential oil concentration, particularly in the higher concentrations (Table 02).

Although some growth of the seedlings under study at the highest concentrations tested was still observed, the dosages of 0.5 and 1.0% of the essential oil killed all *B. subalternans* and *M. lathyroides* seedlings. A similar effect was observed for *E. heterophylla* from the 0.1% concentration. The seedlings showed fragile characteristics in their morphology and darkened roots (Figure 4).

The essential oils have the ability to cause loss of membrane integrity, causing leakage of solutes (Kaur et al., 2010; Amri et al., 2013) and altering biochemical and physiological processes (Kaur et al., 2010). Membrane rupture is one of the main factors that determine cell injury and may result in death of the cells (Singh et al., 2006). Therefore, the seedling death observed in the present study may have occurred due to increase in solute leakage and changes in the permeability of the membrane caused by the *L. Origanoides* essential oil. In addition, this phenomenon is also involved in the inhibition of root growth, since both are significantly correlated (Ismail et al., 2012).

Similar to germination, the phytotoxic action on seedling growth depends on the chemical composition of the volatile oils and the concentration used. The phytotoxic effect observed in the growth of all the seedlings is probably due to the higher concentration of oxygenated monoterpenes present in the essential oil and its high herbicidal power (Vokou et al., 2003; Kordali et al., 2007; De Martino et al., 2010). Almarie et al. (2016) verified that the essential oils of the plants with higher content of monoterpenes presented greater phytotoxicity in relation to the others.

Some studies have also reported the great phytotoxic potential of camphor in the growth of seedlings with the ability to inhibit germination and seedling growth (Vokou et al., 2003; De Martino et al., 2010).

Table 1. Chemical composition of the essential oil of leaves of *L. origanoides.*

Organic compounds	Constituents	R.I.c	R.I.l	%
Hydrocarbon monotherpene	Tricyclene	932	926	0.06
	α-Pinene	944	939	1.68
	Camphene [2]	961	954	10.99
	Sabinene	982	975	0.1
	β-Pinene	986	979	1.25
	Myrcene	996	990	0.96
	p-Cymene	1033	1024	0.11
	Limonene	1039	1029	2.6
	γ-Terpinene	1067	1059	0.05
	Terpinolene	1095	1088	0.9
Total				18.7
Oxygenated monotherpene	1,8-Cineole	1041	1031	0.41
	Linalool	1107	1096	0.03
	E-Tagetone	1141	1144	0.06
	Camphor [1]	1162	1146	34.04
	Borneol	1179	1169	3.54
	Verbenone	1219	1205	0.29
	E-Carveol	1230	1216	0.41
	Carvone	1255	1243	1.22
Total				40
Hydrocarbon sesquiterpene	β-Elemeno	1388	1376	1.31
	α-Copaene	1402	1390	0.7
	β-Caryophyllene	1436	1419	4.01
	β-Copaene	1439	1432	0.18
	α-E-Bergamotene	1448	1434	2.4
	α-Humulene	1465	1454	0.81
	E-β-Farnecene	1476	1456	0.39
	Germacrene-D	1495	1485	1.39
	Viridiflorene	1502	1496	0.69
	(E,E)-α-Farnesene	1513	1505	1.02
	β-Bisabolene [3]	1523	1505	10.75
	δ-Cadinene	1538	1523	1.06
Total				24.71
Oxygenated sesquiterpene	Hedycaryol	1566	1548	1.46
	Caryophyllene oxide	1601	1583	2.81
	Pogostol	1674	1653	2.13
	Epi-β-Bisabolol	1690	1671	0.4
	Shyobunol	1713	1689	1.12
Total				7.92
Others	Acetophenone	1075	1065	0.12
	(2E,6E)-Farnesyl acetate	1848	1846	0.13
Total				0.25
Total identified				91.58

R.I.c: Retention Index calculated; R.I.l: Retention Index of the literature; %: Percentage of each chemical constituent. Numbers 1, 2 and 3: order of the main compounds.

Table 2. Mean values of the effect of *L. origanoides* essential oil on *E. heterophylla* and *M. lathyroides.*

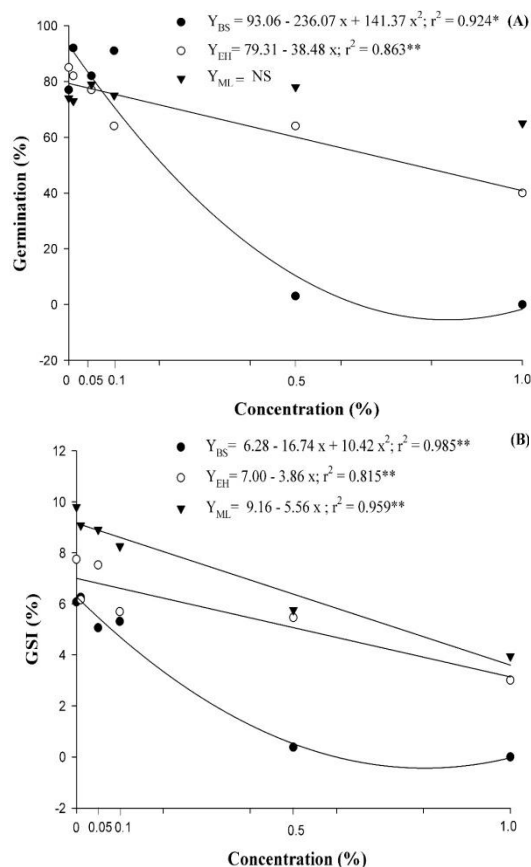| Concentration (%)	E. heterophylla			M. lathyroides
	Root (mm)	Leaf area (mm)	Dry weight (mg)	Root (mm)
0	52.2167	48.4583	12.05	13.6375
0.01	31.375	25.5833	7.1	11.525
0.05	30.375	24.6708	6.6	9.4375
0.1	20.275	20.7083	3.325	8.6125
0.5	20.7775	15.1417	2.15	7.75
1	22.5	14.8417	2.1	8.0375

Fig 1. Effect of *L. origanoides* essential oil on germination (A) and germination speed index (B) on *B. subalternans* (BS), *E. heterophylla* (EH) and *M. lathyroides* (ML). For p <0.05 (*), p <0.01 (**) and not significant (NS).
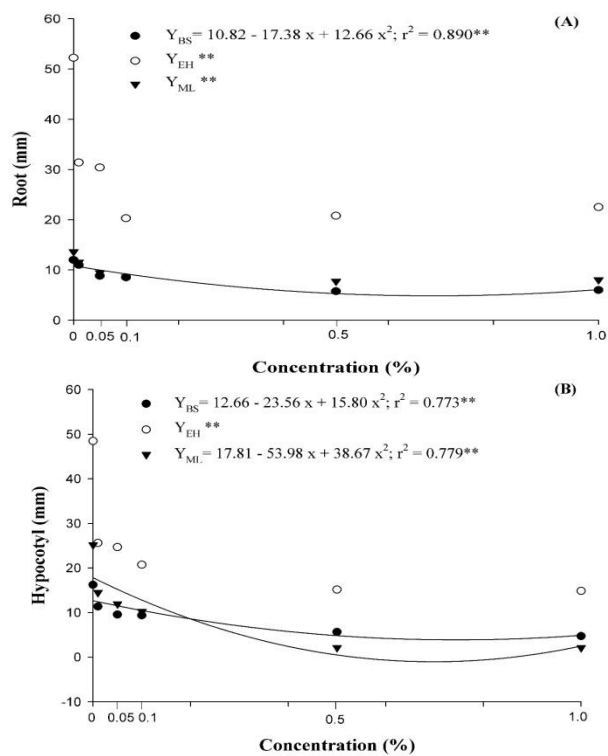

Fig 2. Effect of *L. origanoides* essential oil on root length (A) and hypocotyl (B) on *B. subalternans* (BS), *E. heterophylla* (EH) and *M. lathyroides* (ML). For p <0.05 (*), p <0.01 (**) and not significant (NS).

$Y_{BS} = 12.14 - 16.23\,x + 10.62\,x^2;\ r^2 = 0.719^*$ (A)
$Y_{EH} = 17.19 - 35.16\,x + 24.15\,x^2;\ r^2 = 0.812^{**}$
$Y_{ML} = 33.02 - 101.33\,x + 71.55\,x^2;\ r^2 = 0.938^{**}$

$Y_{BS} = 5.26 - 8.23\,x + 5.51\,x^2;\ r^2 = 0.893^{**}$ (B)
$Y_{EH}\ ^{**}$
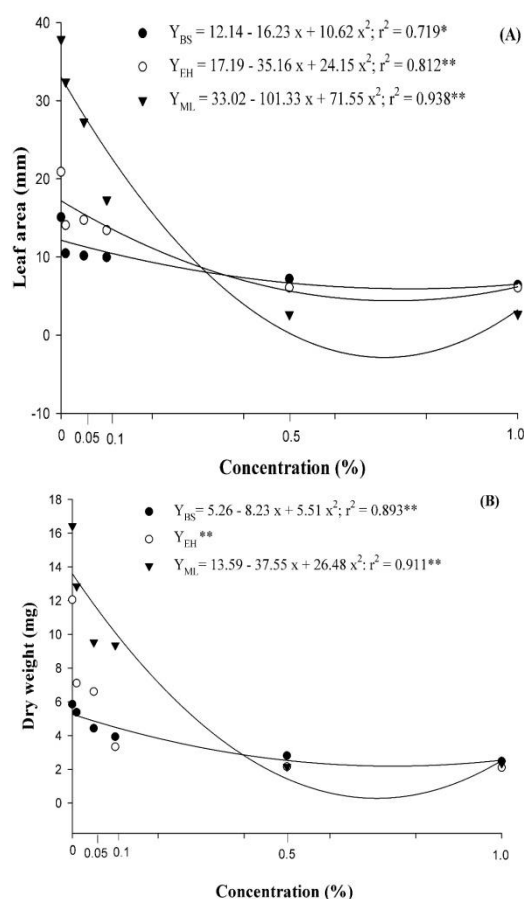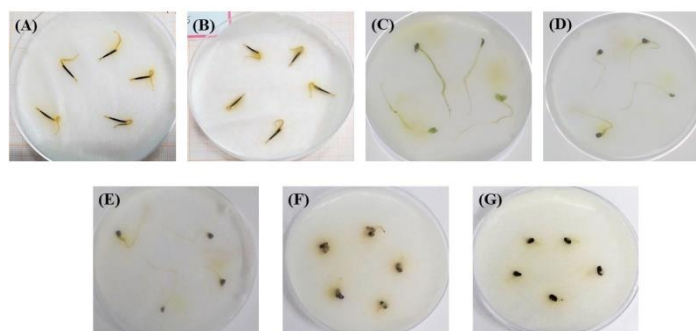$Y_{ML} = 13.59 - 37.55\,x + 26.48\,x^2;\ r^2 = 0.911^{**}$

Fig 3. Effect of *L. origanoides* essential oil on leaf area (A) and dry weight (B) on *B. subalternans* (BS), *E. heterophylla* (EH) and *M. lathyroides* (ML). For p <0.05 (*), p <0.01 (**) and not significant (NS).

Fig 4. Undeveloped (dead) seedlings in the largest concentrations of essential oil of *L. origanoides.* Seedlings of *B. subalternans* at concentrations 0.5% (A) and 1.0% (B). Seedlings of *E. heterophylla* at concentrations 0.1 (C), 0.5% (D) and 1.0% (E). Seedlings of *M. lathyroides* at concentrations 0.5% (F) and 1.0% (G).
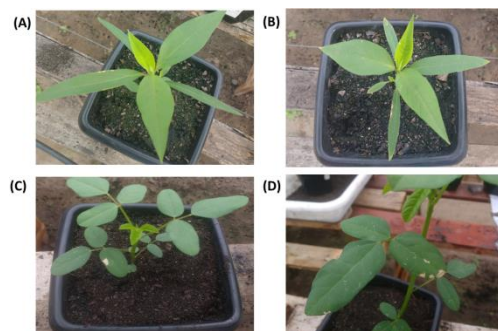

Fig 5. Visible injuries 24 hours after spraying with the essential oil of *L. origanoides*. Necrosis on leaf margin of *E. heterophylla* at concentrations 0.5% (A) and 1.0% (B). Necrosis on leaf margin and chlorotic points on leaves of *M. lathyroides* at concentrations 0.5% (C) and 1.0% (D).
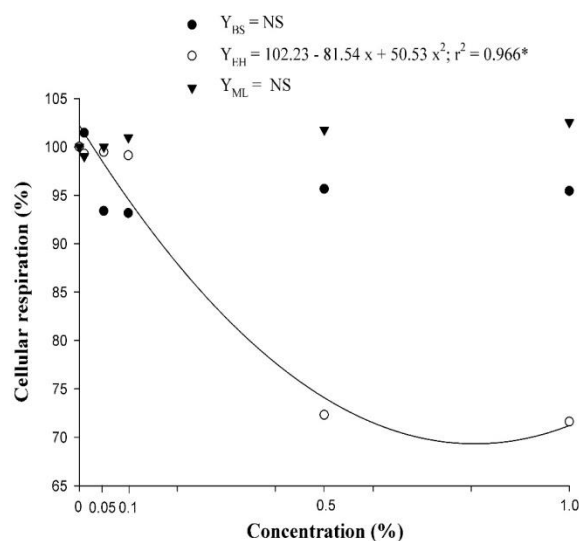
Fig 6 Effect of *L. origanoides* essential oil on cellular respiration in *B. subalternans* (BS), *E. heterophylla* (EH) and *M. lathyroides* (ML). For p <0.05 (*), p <0.01 (**) and not significant (NS).

Some allelopathic substances stimulate seedling growth at small dosages or only cause partial reductions, and inhibit only in the highest concentrations (Vokou et al., 2003; Tigre et al., 2012; Cakir et al., 2016; Laosinwattana et al., 2018). This probably explains why the lower concentrations of the *L. origanoides* essential oil used in the present study, generally, presented few reductions in seedling growth, causing maximum inhibition only at the highest concentrations.

Chlorophyll content on weed leaves

The essential oil presented no effect on chlorophyll content in any of the weeds analyzed but was able to induce different levels of visible injuries in the leaves of *E. heterophylla* and *M. lathyroides*, including necrosis of leaf edges and chlorosis points in the leaf blades in the first 24 hours after application, mainly for the highest concentrations, 0.5 and 1.0% (Figure 5). Kaur et al. (2010) observed that in concentrations greater than 4% of the *Artemisia scoparia* oil, the main visible symptoms in weeds were necrosis, chlorosis and leaf wilting, mainly in the margins. *B. subalternans* showed no lesions at any of the concentrations tested.

Symptoms observed as necrosis and chlorosis in the sprayed leaves are due to the herbicidal property of the essential oils, which cause damage or injury by contact (Tworkoski, 2002; Kaur et al., 2010; Ootani et al., 2017). This explains why in the present study only the sprayed leaves had the symptoms of necrosis and chlorosis. The necrosis in the leaves is probably due to the leaf loss of cell membrane integrity caused by the essential oil. Kaur et al. (2010) obtained similar results for the visible injuries and concluded that wilting weeds after foliar application the *A. scoparia* essential oil may have occurred due to electrolyte leakage.

Weed cellular respiration

The cellular respiration of weeds was tested as a function of *L. origanoides* essential oil concentration and only affected *E. heterophylla* (r^2 = 0.996) (Figure 6). The relationship between cellular respiration and essential oil concentration showed greater inhibition in the highest concentrations, 0.5 and 1.0%, where the reduction reached 27.69 and 28.4% in relation to the control, respectively.

Respiratory activity may be affected by both the chemical composition of the essential oil and the time of exposure of the plants to the allelochemicals (Kaur et al., 2010). In addition, it has been reported that monoterpenes affect cellular respiration acting as decoupled agents in oxidative phosphorylation (Abrahim et al., 2000). Consequently, essential oils and their compounds reduce the cellular respiration of weeds, interfering with the energy metabolism of the plant and impairing its growth (Singh et al., 2009).

Materials and methods

Plant materials

Fresh leaves of *L. origanoides* Kunth were collected from adult plants of natural occurrence in the municipality of Montes Altos, Maranhão, Brazil (5°50'06.7"S 47°16'06.0"W), between November 2017 and January 2018, from 8:00 AM to 10:00 AM. A specimen was identified by Professor Dr. Fátima Salimena and deposited in the Herbarium Leopoldo Krieger (CESJ) of the Federal University of Juiz de Fora (UFJF), with registration number CESJ 71577.

Seeds were collected in wastelands in the State of Maranhão, Brazil, and they passed by process of dormancy breaking, according to the Rules for Seed Analysis (Brasil, 2009). The disinfection was made in 1% sodium hypochlorite solution for 5 minutes and, soon after, they were washed in distilled water and stored at 25 ºC until the moment of the analyzes.

Extraction of essential oil

The essential oil was extracted from the fresh leaves of *L. origanoides* by hydrodistillation, using a Clevenger apparatus (Teles et al., 2014). In each extraction, a mass of 100 g of fresh leaves was used for 1,000 ml of water (ratio 1:10) at

100 °C for 3 hours. At the end of the extraction, the oil volume was collected, dehydrated in anhydrous sodium sulfate and stored in amber glass bottles, under refrigeration of approximately 4 °C until analysis.

Assessment of physical and chemical characterization of essential oil

For the determination of the physical and chemical characteristics, the average of 3 extractions was used. The yield was calculated on the basis of the dry weight of the sample, by the formula:

$$Yield\ (\%) = \frac{Volume(ml) * density\left(\frac{g}{ml}\right)}{dry\ mass\ of\ the\ sample\ (g)} * 100.$$

The density was determined by means of a 2 ml pycnometer, at 25 °C. The color and appearance were determined visually, and the odor through the aroma of volatile oil.

The chemical analysis was performed on gas chromatograph coupled to a mass spectrometer (CG-MS) using a QP2010 Plus (Shimadzu), helium (He) chromatograph as carrier gas. A Factor Four/VF-5 ms capillary column, with 30 m long, 0.25 mm internal diameter and 0.25 mm film thickness was used. The flow of the carrier gas was 1 mL.min^{-1}.

The initial oven temperature was 60 °C. After constant heating for 2 minutes, it was increased at a constant ratio of 2 °C per minute, up to 110 °C, then at a ratio of 3 °C per minute up to 150 °C, then a 15 °C per minute to 290 °C, with a final isotherm of 290 °C for 17 minutes. The temperatures of the injector and detector were respectively 250 °C and 310 °C. The injection mode was split and the injection volume was 1 µL. Mass spectra were produced by electron impact (70 eV).

The quantitative analysis of the chemical components was determined in a gas chromatograph coupled to an HP5890 Series II flame ionization detector (CG-DIC), under the same operating conditions as GC-MS, except for injector temperatures (220 °C) and for the detector (250 °C). The constituents of the essential oil were identified by comparison of the mass spectra and retention index of the compounds with the information in the literature (Adams, 2007), and with the standards in the Nist08 library of the computer in the apparatus.

The retention indices were determined with a standard solution of n-alkanes (C_8-C_{20}) injected under the same chromatographic conditions of the sample (Van Den Dool and Kratz, 1963). The percentage of each chemical constituent was obtained by means of the integral of the area of the peaks in relation to the total area of the constituents of the sample.

Evaluation of the phytotoxic effect on weed seed germination

The assessment of the phytotoxic effect of *L. origanoides* essential oil on weed germination and growth *(B. subalternans, E. heterophylla* and *M. lathyroides*) was performed in the laboratory, using sterilized Petri dishes 9 cm in diameter, coated with 2 filter papers (Whatman n. 1) moistened with the essential oil concentrations (0%, 0.01%, 0.05%, 0.1%, 0.5% and 1%), with 4 replicates each. The essential oil was emulsified with the Tween 80® surfactant in the ratio 1:1 (v/v), diluted in distilled water and a 1% Tween

80® solution was used as control (Alves et al., 2004; Abd El-Gawad, 2016).

In the germination bioassay, each plate received 3 ml of the solutions and 25 seeds of the tested species. The Petri dishes were kept in a Mangelsdorf type germination chamber (MA4000) with a temperature of 25 ± 2 ºC and photoperiod of 12 hours. Seedling counts were performed daily for 10 days, by removing the seeds germinated (radicle 2 mm) to determine the germination percentage (GP) and the germination speed index (GSI), according Maguire (1962):

$$GP(\%) = \frac{n^o germinated}{Total} * 100$$

$$GSI(\%) = \left(\frac{G1}{N1} + \frac{G2}{N2} + \cdots \frac{Gn}{Nn}\right) * 100$$

G1, G2, ... Gn = number of seeds germinated on the day; N1, N2, ... Nn = number of days after sowing.

Assessment of weed seedling growth and development

In the seedling growth experiment, the seeds were placed to germinate in Petri dishes lined with filter paper moistened with 3 ml of distilled water for 3 days, and then, transferred to the treatments. Each Petri dish received 4 ml of the concentrations tested, where 5 pre-germinated seeds were placed, except for *E. heterophylla*, where 4 seeds were placed. The plates were wrapped in PVC film rolls and placed in a Mangelsdorf germination chamber (MA4000) under the same conditions used in the germination test. After 10 days, the root, hypocotyl, leaf area and dry weight of the seedlings were measured (Abd El-Gawad, 2016).

Greenhouse (Post-emergency application)

The weed seeds were seeded in plastic trays filled with commercial Basaplant® substrate (peat, correctives, vermiculite, charcoal and *Pinus* bark). When the plants were in the stage of 2 leaves they were transferred to plastic pots filled with the same substrate and with a capacity of 1 L each.

The application of the treatments was performed when the plants were in the four-leaf stage, by means of a manual sprayer, covering the entire aerial part of the plants (Kaur et al., 2010; Ootani et al., 2017). After 10 days, the chlorophyll content and cellular respiration of leaves were determined.

Estimation of the weed chlorophyll content

Chlorophyll content present in the weeds was analyzed with a portable SPAD 502 PLUS chlorophyll meter, Minolta, Japan (Almarie et al., 2016; Grichi et al., 2016), in the period from 7:30 AM to 9:00 AM. Three leaves were sampled in each plant, with 3 measurements per leaf. Measurements were performed before spraying with essential oil on day 0, and after spraying, on days 1, 3, 5, 7 and 10.

Evaluation of weed cellular respiration

The leaf cellular respiration analysis was done according to the method described by Steponkus and Lanphear (1967), by means of reducing triphenyl tetrazolium hydrochloride - TTC to formazan.

Six leaf disks of 5 cm diameter were removed from each plant and transferred to test tubes containing 3 mL of 0.6% (w / v) TTC solution in 0.05 mol.L^{-1} phosphate buffer (pH 7.0). The tubes were placed in vacuum desiccators for 2 hours, and were placed in a water bath at 30 °C for 15 hours. The TTC solutions were drained from the test tubes and the foliar samples washed once in distilled water. The leaf samples were extracted by adding 7 mL of 95% (v / v) ethanol in each tube and placed in a water bath (± 100 °C) for 5 minutes. The ethanol solutions were transferred to other tubes and cooled to room temperature and 10 mL of 95% (v / v) ethanol was added to each solution for spectrophotometer reading (Spectro 590) at wavelength 530 nm (Steponkus and Lanphear, 1967).

Statistical analysis

The experimental design adopted in all experiments was completely randomized. The results obtained in each bioassay were submitted to analysis of variance (ANOVA) followed by regression (linear or quadratic), with the determination coefficient calculated based on the square sum of the treatment. The data were analyzed by means of the software SisVar 5.6 (Ferreira, 2011).

Conclusion

The essential oil of *L. origanoides* found in this study showed a good yield with mild camphor odor and having as main compounds, camphor, camphene and β-bisabolene. The phytotoxic activity of the essential oil presented better results under laboratory conditions in relation to the greenhouse studies for the tested concentrations. Both the germination and the seedlings were affected by the essential oil, with seedlings more sensitive to the allelochemicals. In the post-emergence application, the essential oil affected only the cellular respiration of *E. Heterophylla.* However, it did not affect the chlorophyll content of the tested species, but was able to induce visible injuries such as chlorosis and necrosis for the highest concentrations, except for *B. subalternans*. The essential oil presented herbicidal potential for the species tested. All the phytotoxic effects observed are due to the high content of monoterpenes in the essential oil, particularly the oxygenates, as well as the concentration used.

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