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Chemical constitution and allelopathic effects of *Curcuma zedoaria* essential oil on lettuce achenes and tomato seeds

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Abstract

The contamination of food, water, and soils by herbicides, as well as the growing resistance of weeds to these products, has increased demands for alternative methods of weed control that have less impact on the environment and human health. *Curcuma zedoaria* (zedoary), a species of the Zingiberaceae family, is a promising plant for alternative weed control as it produces an essential oil with herbicidal action. We evaluated the chemical composition of *C. zedoaria* essential oil and its allelopathic effects on the vigor and germination of lettuce achenes and tomato seeds. The essential oil was extracted from the plant rhizome by hydrodistillation and subjected to gas chromatography-mass spectrometry to identify its component chemical compounds. Bioassays to evaluate allelopathic potential were performed in gerbox-type containers maintained in a refrigerated biochemical oxygen demand incubator at 25°C. The treatments consisted of five essential oil concentrations (0.00, 0.25, 0.50, 0.75, and 1.00%) and two bioindicators (lettuce and tomato). *C. zedoaria* essential oil comprises monoterpene hydrocarbons (4.63%), oxygenated monoterpenes (25.88%), sesquiterpene hydrocarbons (21.95%), and oxygenated sesquiterpenes (47.54%). The major compounds encountered were 1.8-cineole (15.75%) and *epi*-curzerenone (18.20%). We believe that the isolated effect of these compounds or the synergism between them may have influenced the observed results. Germination was inhibited in both bioindicator species, but the percentage inhibition was greater in lettuce achenes. Germination rates decreased in lettuce and tomato with increasing concentrations of essential oil, and bioindicator growth was slowed. The root system was more heavily damaged than the hypocotyl, especially in tomato compared to lettuce. These preliminary results indicate that *C. zedoaria* essential oil has allelopathic effects on seed germination and seedling growth and thus shows potential for weed control.

Keywords: bioassay, bio-herbicide, germination inhibition, 1.8-cineole, *epi*-curzerenone.
Abbreviation: GC-MS_chromatography-mass spectrometry; GSI_germination speed index; MS_identification based on comparison of mass; Na_2SO_4_Sodium sulfate; RI_retention indices; SISVAR_computer statistical analysis system.

Introduction

Curcuma zedoaria Roscoe is an Asian species belonging to the Zingiberaceae family (Pinto and Graziano, 2003) that produces numerous secondary metabolic compounds of potential utility, including curcumenol (Singh et al. 2013), isocurcumenol (Lakshmi et al. 2011), and eucalyptol (Rahman et al., 2012). This species is known to have various uses and applications; according to Lobo et al. (2009), *C. zedoaria* plants have medicinal properties as expectorants, diuretics, and dyspeptics as well as uses as an insecticide (Tagliari et al. 2010), antioxidant (Mau et al. 2003; Singh et al. 2013), analgesic (Navarro et al. 2002), antitumor agent (Lakshmi et al. 2011; Pal et al. 2015), and have antimicrobial effects on the fungi *Aspergillus* sp., *Candida* sp., and *Tricophyton* sp. (Nicoletti et al., 2003). Additionally, these plants can be used in landscaping projects and ornamentation. Their exuberant inflorescences can be marketed in the form of bouquets (Pinto and Graziano, 2003).

The high yields of essential oils produced by the rhizomes of *C. zedoaria* and their chemical compositions have been well described in the literature, but the allelopathic actions of Zingiberaceae extracts are still poorly understood. Allelopathy is a phenomenon by which secondary metabolites synthetized by fungi, viruses, microorganisms, and plants influence biological and agricultural systems as stimulants or inhibitors (Farooq et al. 2011) and pesticides (Rizvi et al. 1992). In general, allelopathic compounds result from the secondary metabolism of phenols, terpenoids, and alkaloids (Grisi et al. 2014), but can also be synthesized, to a

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lesser extent, by the primary metabolism of fatty acids (Wu et al., 2006). According to Weir et al. (2004) and Soltys et al. (2013), allelochemicals can influence electron movements in plants during photosynthesis, thus affecting photosystem II efficiency, chlorophyll accumulation, cell division, and cell elongation, and can influence growth hormones, inhibit enzymes of target species, and function, in some cases, as commercial herbicides.

Among the defensive chemicals used in global agriculture, herbicides are the most sought after (Pradnya et al., 2015) because weeds compete with cash crops for water and nutrients (Pradnya et al., 2015), and annual losses of 12% are calculated due to their presence in crop areas (Grosso et al., 2010). Herbicides are systematically applied to resolve this issue and represent the major weed control technique used worldwide (Farooq et al. 2011). The use of these chemicals can, however, negatively impact the environment by contaminating soils, water resources, and forests (Farooq et al. 2011), and be detrimental to human health (Mostafalou and Abdollah, 2013). Another aggravating factoris the resistance that weeds develop to the active ingredients of agricultural defensive products, largely due to their indiscriminate use, making weed control progressively more difficult and costly (Heap, 2014). Thus, new active ingredients and biomolecules for weed control that demonstrate high selectivity and are less aggressive on the environment and human health are being investigated continually (Qasem, 2010a).

Bioassays have been used to determine if a given species has allelopathic potential for use in weed control (Qasem, 2010b; Albuquerque et al. 2011), and there are a number of specific methodologies used in that testing. The principal methodologies employ substances isolated from essential oils or plant extracts (Farooq et al. 2011) that are applied to reference bioindicator species responsive to allelochemicals, such as lettuce, tomato, and sesame (Qasem, 2010b; Albuquerque et al. 2011). Determining the chemical constitution of the tested material is also an important step in those bioassays, to identify the specific allelopathic substances involved (Verdeguer et al. 2011; Magalhães et al., 2012). The most widely used analytical methods are high-performance liquid chromatography and GC-MS gas chromatography-mass spectrometry (Zuzarte and Salgueiro, 2015). Allelopathy studies of Zingiberaceae family are rare, and little is known about *C. zedoaria*, except that it is well-adapted to tropical conditions, is easily propagated, and produces essential oils with yields varying from 0.38% to 1.4% (Angel et al. 2014). If the potential of this species as a bio-herbicide were confirmed, its use could minimize environmental impacts and threats to human health.

If *C. zedoaria* essential oil functions as a bio-herbicide, it should inhibit seed germination and seedling vigor in bioindicator plants. Bioassays with essential oils have shown that they have considerable influence on germination and seedling vigor and affect the root system mainly. According to Magalhães et al. (2013), the essential oil of *Cymbopogon citratus* was capable of depressing lettuce achene germination by more than 70%. Zahed et al. (2010) also observed that the essential oil from *Schinus moll* fruits completely inhibited germination and root elongation in *Triticum aestivum* L. when applied at concentrations of 10 and 30µ/ml. According to Mutlu et al. (2011), the essential oil of *Nepeta meyeri* Benth. reduced the germination of eight different weed species by more than 50% when applied at a concentration of 0.01%; Iwamoto et al. (2012) likewise reported phytotoxic effects on seedling growth by depressing their antioxidant systems. Verifying the allelopathic potentials of plants is only the first step. Further research is needed to isolate the bioactive molecules (Siddique and Ismail 2013), confirm real control of target species, perfect large-scale production, determine component stability in agricultural systems (Qasem, 2010a), and verify any impact on the environment and human health (Farooq et al. 2011). Bioassays represent a rapid and inexpensive manner for identifying species producing allelopathic substances (Ferreira and Aquila, 2000) and for examining the actions of those compounds on the physiology, morphology, and biochemical processes of target plants (Qasem, 2010a).

In this context, the present study was aimed at assessing the allelopathic effects of the essential oil of *Curcuma zedoaria* on the vigor and germination of lettuce achenes and tomato seeds, and determining its chemical composition.

Results and Discussion

Chemical composition of the essential oil of C. zedoaria

The chemical composition of *C. zedoaria* essential oil is shown in Table 1 and Fig.1. Seventy-nine constituents were detected, of which 72 were identified by gas chromatography-mass spectrometry analysis and listed by their elution times in Table 1. Oxygenated sesquiterpenes were the principal compounds, comprising (47.54%) of the oil, mainly *epi*-curzerenone (18.20%); the second most significant class of terpenes was oxygenated monoterpenes (25.88%), represented principally by 1.8-cineole (15.76%) and camphor (5.61%). Studies conducted by Singh et al. (2013) showed that *C. zedoaria* essential oil was composed mainly of curzerenone (31.6%), germacrone (10.8%), and camphor (10.3%). The essential oil extracted from the rhizomes of *C. zedoaria* from northwestern India (Purkayastha et al. 2006) contained curzerenone (22.3%) as the principal constituent, followed by 1.8-cineole (15.9%) and germacrone (9.0%).

The chemical compositions of essential oils can vary widely according to the region in which the plants are grown, local climatic conditions, and plant physiologies (Ootani et al., 2013). The rhizomes in the present study were collected in the Austral winter, during normal plant dormancy; it is known that this plant produces leaves and inflorescences during the summer, thus, the essential oil composition would be expected to be different at that time. The substances found in *C. zedoaria* essential oil are reported here according to their allelopathic effects on the diaspores of bioindicator species and on weeds, being mainly oxygenated monoterpenes, 1.8-cineole, and camphor.

1.8-Cineole is found in species of the genus *Eucalyptus* and is known to inhibit the development of *C. zedoaria* (Qiu et al. 2010), principally in its adult phase. It reduced the germination of lettuce achenes by more than 80% in the present work; this same compound also produced promising results in inhibiting the development of species of the genus *Brassica*. According to Koitabashi et al. (1997), 1.8-cineole almost completely inhibits the germination of *Brassica campestris* at concentrations of 130 µM (solution) and 400 µM (gas). Nishida et al. (2005) likewise observed that 1.8-cineole inhibited germination and seedling development in *B. campestris*.

Camphor and terpinen-4-ol were reported by Grosso et al. (2010) as inhibitors of the germination and development of sweet corn, lettuce, peas, and wheat. Vokou et al. (2003) observed that 2.5 µL of dihydrocarvone significantly reduced the germination of *Lactuca sativa* seeds. Analyses of 47 monoterpenes of different classes by same authors showed that non-oxygenated compounds were less effective

Table 1. Chemical composition of the essential oil of *Curcuma zedoaria.*

Peak	[A]Compounds	Area (%)	[a]RI	Methods of identification
	Monoterpene hydrocarbons			
1	tricyclene	0.06	900	a,b
2	*α*-thujene	0.05	904	a,b
3	*α*-pinene	0.85	930	a,b
4	camphene	1.39	1003	a,b
5	sabinene	0.27	1009	a,b
6	*β*-pinene	1.55	1015	a,b
7	myrcene	0.41	1021	a,b
8	*α*-terpinene	0.05	1025	a,b
	Oxygenated monoterpenes			
9	1.8-cineole	15.76	1039	a,b
13	nonanone	0.14	1087	a,b
14	*trans*-sabinene	0.33	1089	a,b
15	*cis*-limonene oxide	0.05	1120	a,b
16	camphor	5.61	1127	a,b
17	camphenehydrate	0.06	1142	a,b
18	isoborneol	1.51	1146	a,b
19	borneol	0.60	1150	a,b
20	terpineol	0.45	1179	a,b
21	*α*-terpineol	1.03	1181	a,b
22	*cis*-carveol	0.11	1221	a,b
23	carvone	0.15	1256	a,b
24	isogeraniol	0.08	1276	a,b
	Sesquiterpene hydrocarbons			
25	*δ*-elemene	1.05	1336	a,b
26	*cis*-*α*-copaene	0.29	1350	a,b
27	*β*-elemene	4.43	1384	a,b
28	*α*- gurjunene	0.20	1400	a,b
29	caryophyllene (E)	0.05	1421	a,b
30	*γ*- elemene	1.12	1427	a,b
31	*α*-guaiene	0.33	1432	a,b
32	aromadendrene	0.28	1436	a,b
33	*trans*-*β*-farnesene	0.18	1447	a,b
34	*α*-humulene	3.14	1448	a,b
35	*allo*-aromadendrene	0.30	1458	a,b
36	germacrene D	2.42	1462	a,b
37	*β*- selinene	0.67	1474	a,b
38	*α*- selinene	4.68	1476	a,b
39	curzerene	1.07	1481	a,b
40	bicyclogermacrene	0.08	1502	a,b
41	*β*- guaiene	0.34	1526	a,b
42	*γ*-cadinene	0.24	1531	a,b
43	*δ*- cadinene	0.07	1532	a,b
44	selina 3,7 diene	0.08	1535	a,b
45	germacrene B	0.94	1546	a,b
	Oxygenated sesquiterpenes			
46	spathulenol	0.15	1551	a,b
47	caryophyllene oxide	0.12	1560	a,b
48	*γ*-elemeneepoxy	0.27	1561	a,b
49	globulol	0.61	1567	a,b
50	viridiflorol	0.69	1573	a,b
51	*epi*-curzerenone	18.20	1607	a,b
52	curzerenone	0.54	1626	a,b
53	Caryophylla-3,8-dien-beta-ol	0.31	1627	a,b
54	*α*- muurolol	2.02	1636	a,b
55	*β*- eudesmol	0.82	1638	a,b
57	n.i	0.45	1639	a,b
58	valerenol	0.79	1641	a,b
59	n.i	0.41	1650	a,b
61	α- santalol	0.60	1650	a,b
62	n.i	0.61	1670	a,b
63	cedren-13-ol	1.39	1673	a,b
64	germacrone	3.29	1678	a,b
65	n.i	0.29	1705	a,b
67	farnesol (2E,6Z)	0.33	1708	a,b
68	valerenal	0.35	1720	a,b
69	n.i	0.32	1727	a,b
70	curcumenol	1.43	1735	a,b
71	(-)- elema1,3,11(13)- trien- 12ol	2.77	1735	a,b
72	lanceol (Z)	1.01	1735	a,b
74	n.i	0.51	1786	a,b
76	azuleno [6,5-b] furan -2(3h)-one	3.56	1809	a,b
77	isocurcumenol	4.78	1868	a,b
78	n.i	0.18	1871	a,b
79	isovelleral	0.74	1891	a,b

Total Identified (%) 97.23; Compound groups (%): Monoterpene hydrocarbons 4.63; Oxygenated monoterpenes 25.88; Sesquiterpene hydrocarbons 21.95; Oxygenated sesquiterpenes 47.54.

[a]**RI=** Identification based on retention index (RI) using n-alkane C7 - C26 on an Agilent HP-5MS column; [b]**MS=** identification based on comparison of mass spectra using Wiley 275 libraries. [A]Compounds listed in order of elution from DB-5 column HP-5MS. n.i. = not identified.

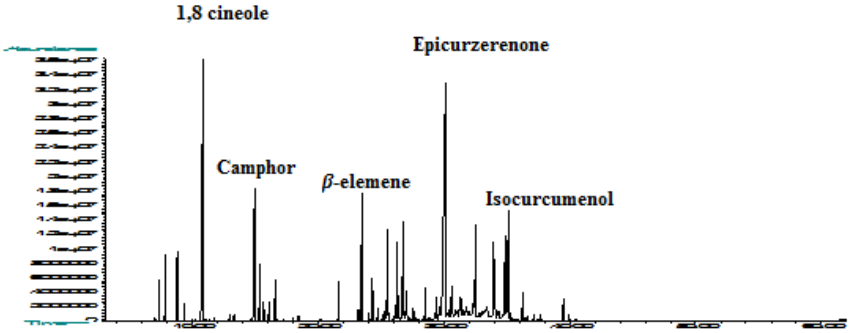

Fig 1. Chromatogram obtained by GC-MS of the essential oil of *C. zedoaria*, indicating the principal compounds.

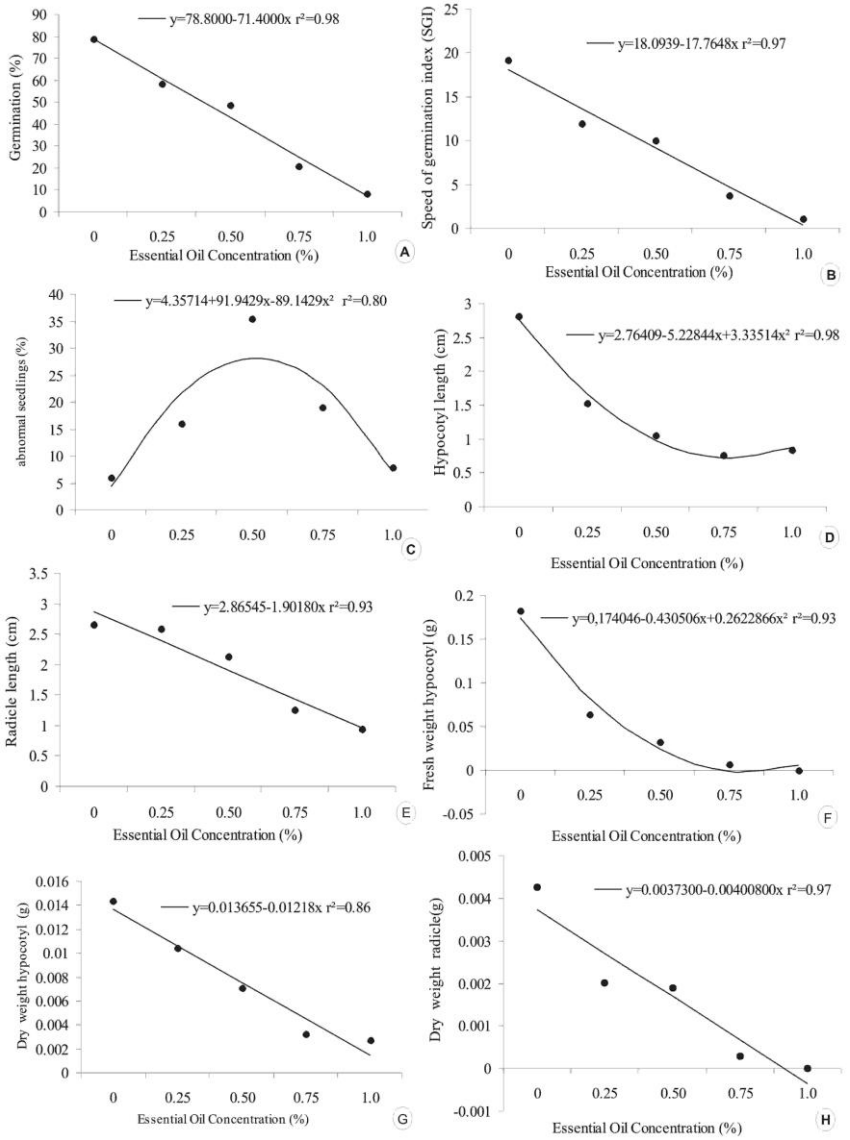

Fig 2. Germination (%) (A), Speed of germination index (SGI) (B), Abnormal seedlings (%) (C), Hypocotyl length (D), Radicle length (E), Fresh weight hypocotyls (F), Dry weight hypocotyls (G), and Dry weight radicle (H) of lettuce seedlings exposed to increasing concentrations of the essential oil of *C. zedoaria*.

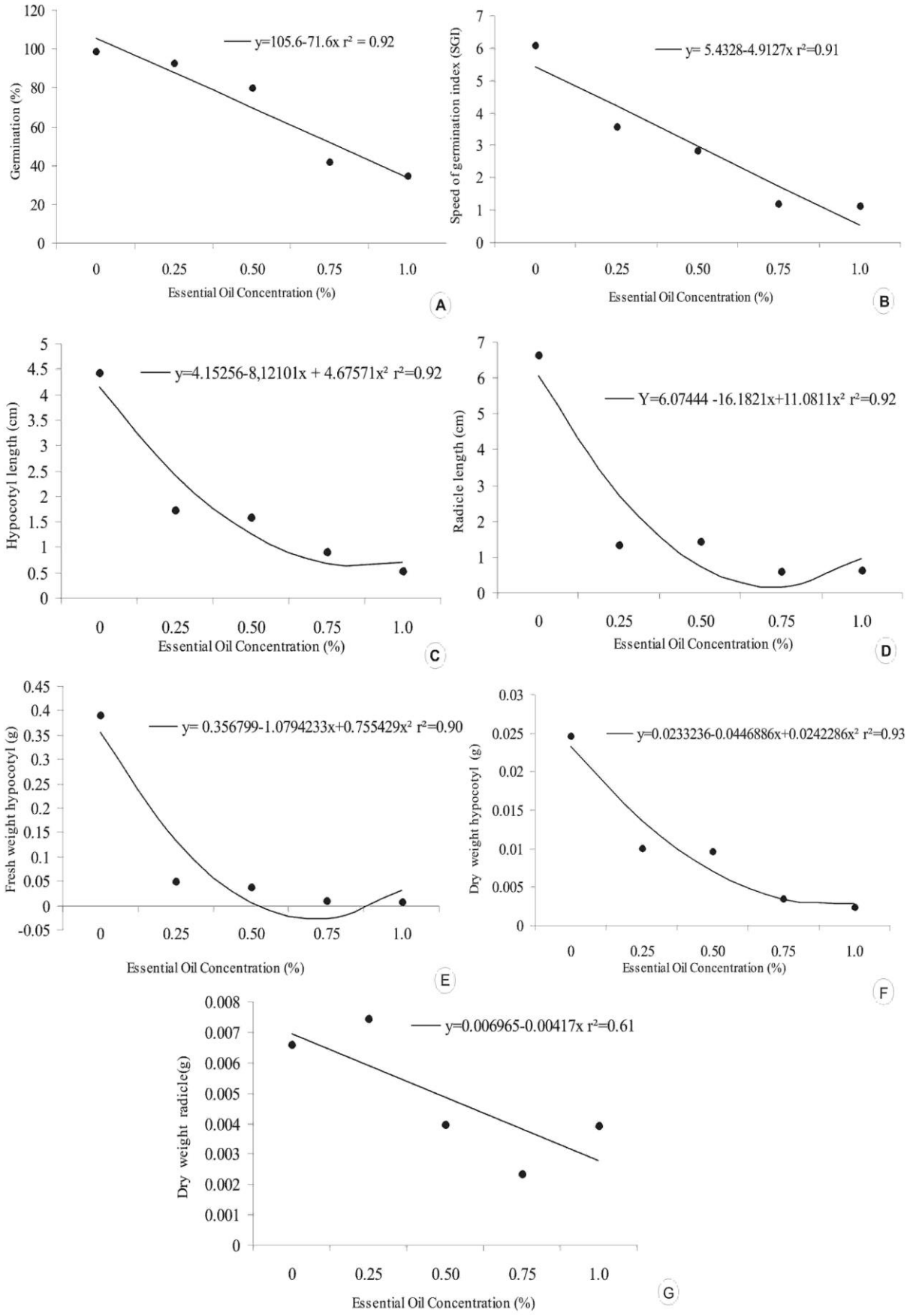

Fig 3. Germination (%) (A), Speed of germination index (SGI) (B), hypocotyl length (C), radicle length (D), fresh weight hypocotyls (E), dry weight hypocotyls (F), and dry weight radicle (G) of tomato seedlings exposed to increasing concentrations of the essential oil of *C. zedoaria*.

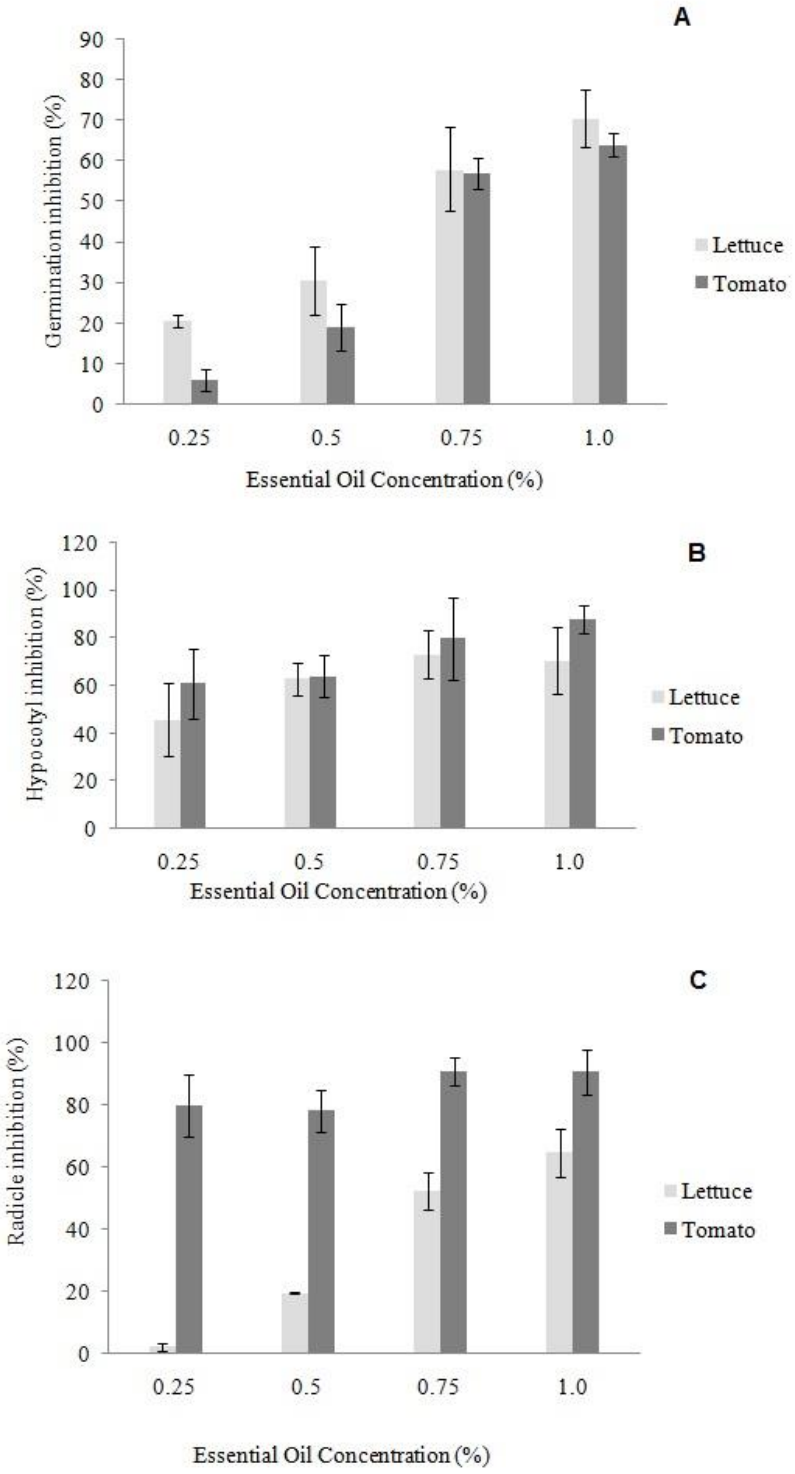

Fig 4. Effects of Germination inhibition (%) (A), Hypocotyl inhibition (%) (B), and Radicle inhibition (%) (C), of the essential oil of *C. zedoaria* on lettuce and tomato.

than oxygenated monoterpenes at inhibiting germination and seedling development.

Bioassays with lettuce

Both bioindicator species were responsive to the essential oil of *C. zedoaria* at various concentrations. The only parameter of lettuce achenes that did not demonstrate any alteration when exposed to the essential oil was the fresh weight of the radicles; similarly, the only parameter of tomato seeds that did not demonstrate alterations when exposed to the essential oil was the percentage of abnormal seedlings. No examples of accelerated gravitropism, root necrosis, or the presence of lateral roots were observed as a result of exposure to the essential in either bioindicator species used in the present study. Significant differences ($p \leq 0.05$) were observed in the means of the other variables. Regression equations indicated linear effects on the characters analyzed, with drastic

reductions of the measured parameters and restricted seedling development being observed at certain concentrations.

Linear models showed the best adjustment for germination and the germination speed index (GSI) of lettuce achenes. The initial germination percentage was approximately 80% and decreased to almost 0% as the essential oil concentrations of *C. zedoaria* increased (Fig. 2A). The intervals between germination events were longer than those seen with the control (Fig. 2B).

The bioassays performed with tomato seeds showed that *C. zedoaria* essential oil had depressive effects on germination and seedling development. Seed germination and the GSI of tomato seeds decreased in a linear manner with incremental concentrations of essential oil. The initial germination percentage was near 100% but decreased to approximately 40% at the highest essential oil concentrations (Fig. 3A). The GSI likewise slowed and became uneven with increasing concentrations of the essential oil (Fig. 3B).

Numerous studies have reported the oxygenated monoterpenes 1.8-cineole, camphor, borneol, and terpinen-4-ol as being responsible for inhibiting the germination of the seeds of *B. campestris* (Yoshimura et al. 2011), *L. sativa*, and *Allium cepa* (Vokou et al. 2003; Pawlowski et al. 2013), *Portulaca oleracea* (Verdeguer et al. 2011), and many other plant species. The responses of the evaluated characteristics were dependent on the essential oil concentrations applied and the contact mode with the seeds (Macías et al. 2004).

The effects of herbicides on weeds can occur by interrupting the formation of microtubules, cell division, or the production of amino acids, thus inhibiting germination and seedling development (Hatzios, 2012). The modes of action of oxygenated monoterpenes on cell division have been confirmed, and 1.8-cineole is known to inhibit mitosis and interrupt cell proliferation in the root apical meristem of *B. campestris* by inhibiting DNA replication (Koitabashi et al. 1997). Yoshimura et al. (2011) likewise observed decreases in the mitotic indices and difficulties in starch absorption by BY-2 cells and protoplasts of *Nicotiana tabacum* when exposed to different concentrations of cineole.

It is likely that other germination mechanisms are being affected, such as the interruption of ATP synthesis for cell respiration and synthesis processes, and hormone transport within cell membranes and mitochondria (Weir et al., 2004; Iwamoto et al., 2012). Camphoris considered responsible for mitochondrial uncoupling in soybean hypocotyls, resulting in negative impacts to early seedling development (Abrahim et al., 2003). High concentrations of 1.8-cineole have also been observed to inhibit mitochondrial respiration in isolated onion root cells, which therefore interrupts all stages of their mitotic division (Macías et al., 2004).

With respect to the formation of abnormal seedlings, *C. zedoaria* essential oil produced deformed seedlings that could barely survive under natural conditions, even after apparently normal germination. The seedlings developed normally when exposed to ≤0.51% of the essential oil; above that concentration, however, severe abnormalities were observed, such as deformities of the roots and cotyledons or their complete absence (Fig. 2C). Some tomato seedlings showed abnormalities after exposure to *C. zedoaria* essential oil, but these effects were not very severe. Magalhães et al. (2013) likewise observed a high percentage of abnormal seedlings (56.7% and 75%) generated from lettuce achenes exposed to *Lippia sidoides* essential oil concentrations of 0.75% and 1.0%, respectively.

There are many reports in the literature concerning the effects of essential oils on seedling length. Root systems are more commonly affected than shoots (Pukclai and Kato-Noguchi, 2012; Alves et al. 2014), possibly because they are the first plant organs to come into contact with and absorb allelochemicals (Salam and Kato-Noguchi, 2010). The present study demonstrated that the actions of the essential oil were different for hypocotyl and radicle structures. Lettuce hypocotyl lengths decreased exposure to ≤0.78% essential oil, but a slight increase in growth was again observed at higher concentrations (Fig. 2D). The linear model was found to be the best fit for root length, as the roots became shorter with increasing concentrations of essential oil. Initially, mean root length was observed to be ~3cm; at the end of the assay, the mean root length was only 1cm after exposure to the highest concentrations of essential oil (Fig. 2E).

Bioassays with tomato

Tomato seedling hypocotyl lengths were reduced after exposure to ≤0.86% essential oil, with slight growth being observed at higher concentrations (Fig. 3C). Similar effects were observed with radicle length, which was reduced by exposure to≤0.73% essential oil; above that concentration, root development continued normally (Fig. 3D). In this specific case, the action of *C. zedoaria* essential oil seems to be related not only to cell proliferation (mitosis) but also to cell elongation. This issue has been addressed by some researchers (Yoshimura et al. 2011; Iwamoto et al. 2012), although additional investigation will be necessary due to the complexity of the question. Furthermore, allelochemicals can mutually interact, causing complex plant responses (Macías et al. 2004).

Cell wall loosening processes mediated by expansins occur during the initial phases of cell elongation (Wang and Ruan, 2013) induced by auxins (Weir et al., 2004; Rechenmann, 2010). Essential oils may act in two distinct manners: through the synthesis of auxin-inhibiting enzymes or by interrupting the action of that hormone (Depuydt and Hardtke, 2011) directly on expansins, restricting their cell wall-loosening functions (Rechenmann, 2010). Caffeic acid and other polyphenols have been found to increase peroxidase, catalase, and indoleacetic acid oxidase activities, thus altering the amounts of free auxins in plants (Macías et al. 2004). Phenolic compounds have been associated with decreased hormone and enzyme levels, but these were not quantified in the present study – although the possible effects of monoterpenes on those factors cannot be dismissed.

Both the fresh and dry weights of lettuce seedling radicles and hypocotyls demonstrated linear behaviors, with lower weights being observed with increasing essential oil concentrations (Fig. 2, F, G, and H). The hypocotyl became extremely reduced in the presence of 1% essential oil, and its dry weight was almost zero at end of the assay (Fig. 2G). The fresh weight of the tomato hypocotyl was also extremely reduced by exposure to ≤0.71% essential oil; above that concentration, limiting effects diminished, and the seedlings reinitiated fresh weight gains (Fig. 3E). Similar effects were observed with the dry weight of the hypocotyl, which decreased at concentrations ≤0.92% of essential oil, although above that concentration, the hypocotyls again reinitiated dry matter accumulation (Fig. 3F). The dry matter of roots decreased by half in comparison with the control when exposed to 1% essential oil (Fig. 3G).

The weight gains were mainly related to cell proliferation (mitosis) followed by cell expansion (Rechenmann, 2010). The root systems of the lettuce and tomato controls showed several lateral and secondary roots that were not observed at the highest *C. zedoaria* essential oil concentrations, probably affecting the final dry weights of those roots. Weir et al.

(2004) noted that damage to cell membranes decreases growth and the final weights of roots and shoots. Camphor, *a*-pinene, and limonene strongly affect the respiratory activities of mitochondria by damaging the cell membranes of those organelles; monoterpenes are capable of penetrating and modifying bacterial membranes (Cristiani et al., 2007). Studies have demonstrated that allelochemicals can alter the final weights of seedlings. Pukclai and Kato-Noguchi (2012) examined the concentrations of extracts of *Amomum krervanh* needed to decrease the dry weights of sprouts and roots of various plants, and found that *L. sativa* was the most responsive species.

Inhibitory effect of C. zedoaria essential oil on lettuce and tomato

When the percentages of germination inhibition of the two bioindicator species at different *C. zedoaria* essential oil concentrations were compared, the germination of lettuce achenes was found to be more strongly impacted than seen with tomato seeds, demonstrating that lettuce was the most sensitive bioindicator (Fig. 4A). An initial germination inhibition of 30% was observed for lettuce achenes and 25% for tomato seeds, but both more than doubled at higher concentrations; the inhibition of lettuce achenes upon exposure to 1% essential oil was almost 75%, whereas the inhibition of tomato seed germination was almost 65%. Ferreira and Borguetti (2004) reported that allelochemical effects on germination are not always observed, but *C. zedoaria* essential oil appears to be a promising inhibitor of germination as it was effective on both bioindicators in the present study.

Hypocotyl inhibition was observed to be similar at all concentrations tested for both bioindicator species. Their general aspects were similar, although tomato hypocotyls appeared more damaged by *C. zedoaria* essential oil than did those of lettuce achenes. *C. zedoaria* essential oil at 0.25% limited tomato hypocotyl growth by almost 50%, and lettuce hypocotyl growth by almost 60% (Fig. 4B). At 0.75% concentration, lettuce hypocotyl inhibition rose to approximately 70% (Fig. 4B), while tomato hypocotyl inhibition was close to 90%.

The inhibition of tomato radicle lengths was high for all tested concentrations of the essential oil (Fig. 4C), with initial values close to 80%, increasing to 90% at higher concentrations, indicating that tomato radicles were more sensitive to *C. zedoaria* essential oil than lettuce radicles (Fig. 4C). At an essential oil concentration of 0.25%, lettuce radicle inhibition was approximately 2%, at 0.5% concentration, it rose to 20%, and at the highest concentration, radicle inhibition was 65%. Plant species become responsive to certain compounds because of failures in their defense mechanisms or through damage caused by allelochemicals (Macías et al. 2004). These variations result from complex interactions between the plant and those types of compounds, and it is difficult to define standardized behaviors (Vokou et al. 2003; Souza Filho et al. 2009). Each plant species has its own genetic constitution, and the nature of the compounds (and their concentrations) can interact with plants through different pathways, resulting in diverse responses being observed in assays.

Similar findings were reported by Rosado et al. (2009), who demonstrated that the root systems of tomatoes were approximately four times shorter (in comparison with the root systems of lettuce) when exposed to a 0.01% aqueous extract of basil. Tur et al. (2010) also noted drastic reductions in the growth of tomatoes radicles (compared to lettuce radicles) when exposed to extracts of *Duranta repens.* Neto et al. (2014), however, reported that ethanol extracts of *Copaifera sabulicola* were more harmful to root and hypocotyl growth in lettuce than in tomatoes.

Materials and Methods

Plant materials and botanical identifications

C. zedoaria plants were obtained from the medicinal plant garden at the Paranaense University Educational Herbarium; a voucher specimen is deposited there under number 2400 (collected at 23°46'60"S × 53°16'60"W, at 391 m a.s.l.). The rhizomes were harvested in August 2015 (Austral winter), during the dormancy period of those plants (Rinne et al., 2010; Ding and Nilsson, 2016).

Essential oil collection and chemical composition

The rhizomes were cleaned, sliced, and dried at room temperature, and subsequently ground to a particle size of 850 μm (Frighetto et al., 2005). The dried and ground plant material was then subjected to hydrodistillation for 2 hours. The oil obtained was filtered with Na_2SO_4 and stored at −10°C (Lai et al., 2004). After total evaporation of the solvent, five extraction cycles were performed and the oil yield (%) was calculated.

The analysis of the essential oil was carried out in a gas chromatograph (Agilent 7890 B, Agilent Technologies, Santa Clara, CA) coupled to a mass spectrometer (Agilent 5977 A, Agilent Technologies).The gas chromatograph was equipped with an Agilent HP-5MS UI capillary column (30 m × 0.250 mm × 0.25 μm; Agilent Technologies) run under the following conditions: injector temperature of 250°C, injection volume of 2 μL in splitless mode (valve time, 0.5 min), initial column temperature of 35°C heated gradually to 60°C with a 1°C/min climb rate, then heated to 200°C with a 8°C/min climb rate, then heated to 280°C at 15°C/min, and subsequently held for 5 min at 280°C. The carrier gas (helium) flow was set at 1 mL min^{-1}. The temperatures of the transfer line, ion source, and quadrupole were 280°C, 230°C, and 150°C, respectively.

The mass spectra were obtained in the range of 40 to 550 (*m/z*) provided through scan mode, with a solvent delay time of 3 min. The compounds were identified based on comparisons of their retention indices. Their electron ionization-mass spectra were also compared with the Wiley 275 library spectra (Adams, 2012).

Sample preparation

The essential oil was emulsified with polysorbate 80 at five different concentrations (0.0%, 0.25%, 0.50%, 0.75%, and 1.0%). Two independent assays were performed with lettuce achenes and with tomato seeds. A completely randomized design was used for the five concentrations of *C. zedoaria* essential oil (0.0%, 0.25%, 0.50%, 0.75%, and 1.0%), using four repetitions of 50 achenes of lettuce (summer curly cultivar) per gerbox, and 25 tomato seeds (cultivar Hy-color 312). A 2.0% aqueous solution of polysorbate 80 was used as the control treatment.

Bioassay performance

The bioassays were performed in the Plant Production Laboratory of the Paranaense University according to the methodology described by Seed Analysis Rules (Brasil, 2009)

and Magalhães et al. (2013), with adaptations. The 4-ml treatment solutions (0.0%, 0.25%, 0.50%, 0.75%, and 1.0%) were applied (using a micropipette) to the gerbox-type containers (11 × 11 × 3 cm) lined with filter paper that had been moistened with a volume of water equivalent to 2.5 times the mass of the dry paper. The lettuce achenes and tomato seeds were distributed into the gerbox-type containers and subsequently transferred to the biochemical oxygen demand germination room at 25°C under dark conditions (as the two indicator seed types are neutral photoblastic). When necessary, the filter papers were again moistened with 4 ml of deionized and autoclaved water.

Analysis of achenes and seeds

Germinated lettuce achenes and tomato seeds were counted daily until the 7th and 14th day after sowing, respectively (Brasil, 2009), considering germination as the protrusion of 0.1 mm of the primary root. After those periods of 7 days for lettuce and 14 days for tomato, the following characteristics were evaluated: germination; the GSI according to Maguire (1962); radicle and hypocotyl lengths, measured using a millimeter ruler; and dry masses of the root and hypocotyl, obtained after drying at 65°C to a constant weight in a forced circulation oven. Additionally, we visually inspected the seedlings for root necrosis; the presence of lateral roots; abnormal seedlings; and altered gravitropic movements. Seedlings were considered normal or abnormal according to Seed Analysis Rules (Brasil, 2009).

Data were subjected to analysis of variance ($p<0.05$) and the means were compared by polynomial regression ($p<0.05$) according to Banzatto and Kronka (2006), using SISVAR software (a computer statistical analysis system) (Ferreira et al., 2011). Bar graphs were used to illustrate and compare germination inhibition (in percent) and the lengths of the hypocotyls and radicles (in centimeters) of the two bioindicator species.

Conclusion

Based on the results of this research, we conclude that *C. zedoaria* essential oil presents potential allelopathic and bio-herbicide use for invasive plant species because the bionic plants (lettuce and tomato) tested were shown to be sensitive to the compounds present in the essential oil. While *C. zedoaria* has been extensively researched for medicinal purposes, there are only a few reports regarding other purposes; hence this study highlights the unprecedented nature of this research. Our studies have revealed that oxygenated sesquiterpenes are the main components of *C. zedoaria* essential oil. The main constituent of the oil was epi-curzerenone, followed by oxygenated monoterpenes (1.8-cineole). Both bioindicator species used showed reductions in germination and seedling vigor when exposed to the essential oil. A 1% concentration of *C. zedoaria* essential oil was more damaging to lettuce seedlings, whereas a 0.75% concentration limited the growth of tomato seedlings. The roots of the tomato seedlings suffered greater inhibition than those of lettuce seedlings at all essential oil concentrations.

However, as the compounds act on the physiology and biochemistry of plants, triggering the observed responses is still unknown. We propose some hypotheses such as the reduction of the process of cell division, interruptions in the synthesis of ATP, in the breathing process, and in the synthesis of hormones, especially the auxins that are linked to the rooting in the plants. These hypotheses should be investigated in future research. Sequence studies that consider stability and action on invasive plants should also be conducted. It is necessary to determine in which type of class (monocotyledons and dicotyledons) *C. zedoaria* essential oil has more action. Finally, in the face of the demand for products that control invasive plants that are less toxic to the environment and human health, essential oils have been pointed out as excellent alternatives because they generally present low toxicity and are not cumulative in the environment. In addition, the resistance of invasive plants to many commercial molecules has been a challenge in food production by increasing production costs and harm to producers. Therefore, essential oils can contribute in solving this problem.

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