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Phytotoxic Potential of Benzyl Salicylate and Benzyl Benzoate on Bioindicator and Invasive Species

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Abstract

The growing number of invasive species in agricultural areas reduces productivity and results in production losses. The need to discover new compounds with herbicidal activity increases as cases of resistance of invasive plants to herbicides rise. The aim of this study was to evaluate the phytotoxic potential of benzyl salicylate and benzyl benzoate upon the growth of *Triticum aestivum* coleoptiles and on the initial growth of *Lactuca sativa*, *Lycopersicon esculentum*, *Allium cepa*, *T. aestivum, Euphorbia heterophylla*, and *Megathyrsus maximus*. For the *T. aestivum* coleoptile bioassays, the treatments used the concentrations of 10^{-3} M, 3×10^{-4} M, 10^{-4} M, 3×10^{-5} M, and 10^{-5} M; while for the initial growth bioassays the concentrations of 10^{-3} M, 10^{-4} M, and 10^{-5} M were used. Both compounds presented a minimum of 89% growth inhibition on *T. aestivum* coleoptiles in all concentrations. Both compounds inhibited the growth of the root system and shoot of *A. cepa* and *E. heterophylla* at all concentrations. The species most affected by both compounds in all evaluated parameters was *E. heterophylla*. For the benzyl benzoate, the inhibition of the roots of *E. heterophylla* were statistically equivalent to those obtained with the herbicide. Regarding benzyl salicylate, the root inhibition in this species in the 10^{-4} M and 10^{-5} M treatments did not differ statistically from the herbicide in the same concentrations. Benzyl salicylate and benzyl benzoate are compounds that presented phytotoxic activity on *E. heterophylla* and for the first time the phytotoxic effect of these compounds on invasive species is reported.

Keywords: allelochemicals, phytotoxicity, secondary compounds

1. Introduction

The increase in the number of invasive species in agricultural areas causes a reduction in the productivity of agricultural crops resulting in economic losses (Lamego et al., 2013). These plants, besides competing for space with the crop, also compete for nutrients, water, light (Galon et al., 2018), and may also host pests and diseases common to the crop (Alvino et al., 2011; Vasconcelos, Silva, & Lima, 2012). The need to discover the herbicidal potential of new compounds increases as cases of resistance to commercial products rise. Brazil is the fifth country with the largest number of herbicide-resistant species, with 50 plant species, behind the United States (165), Australia (95), Canada (68), and France (55) (Heap, 2020). These numbers increase more and more due to bad agricultural practices such as the recurrent use of the same herbicide or herbicides with the same mechanism of action in the same agricultural area. This leads to the selection of herbicide-resistant invasive plant biotypes for a particular mechanism of action.

Guinea grass (*M. maximus*) (Jacq.) B. K. Simon and S. W. L. Jacobs is a Poaceae with intense seed production and high dispersion capacity (Costa, Matallo, Carvalho, & Rozanski, 2002). This species is highly invasive and is considered one of the most aggressive species in Brazil (Mantoani & Torezan, 2016), being difficult to control

(Aimar & Durigan, 2001) and frequently found in sugarcane crops, causing reduced productivity (Correia, Gomes, & Perussi, 2012).

Another species that impairs agricultural production is wild poinsettia (*E. heterophylla* L.), a Euphorbiaceae characterized by high competitive capacity (Oliveira Jr. et al., 2011). This species causing losses in quality and productivity in agricultural systems (Kern et al., 2009). *E. heterophylla* can occur in several crops of agricultural importance, including soybean (Carvalho, Bianco, & Guzzo, 2010; Lopes Ovejero et al., 2013), corn (Cury et al., 2012), sugar cane (Marques, Martins, Costa, & Vitorino, 2012), and beans (Cury et al., 2011; Machado et al., 2015). It is a hard to control invasive plant, presenting a fast-initial development and great genetic variability (Trezzi, Machado, & Xavier, 2014). In addition, due to mutations in the gene that codes the acetolactate synthase (ALS) enzyme, this is a multi-resistant herbicide species. It inhibits the enzyme protoporphyrinogen oxidase-Protox, acetolactate synthase-ALS (Trezzi et al., 2011; Xavier, Trezzi, Oliveira, Vidal, & Brusamarello, 2018), being recently reported as resistant to the herbicide glyphosate (inhibitor of the enzyme 5-enolpyruvate-chiquimate-3-phosphate synthase-EPSPS) (Adegas, Gazziero, Oliveira Jr., Mendes, & Rodrigues, 2020).

Thus, the search for compounds that have new mechanisms of action, as well as improvements in their application, are essential for the control of invasive plants (Grigolli, Pereira, Peñaherrera, Santos, & Ferreira, 2011). An alternative is the use of compounds from the secondary metabolism of plants, which are called allelochemicals, and are associated with plant defense strategies. When released into the environment these compounds may positively or negatively affect the development of other plant species, in the process known as allelopathy (International Allelopathy Society-IAS, 2020; Alves, Medeiros Filho, Innecco, & Torres, 2004).

Essential oils are secondary metabolites from different parts of plants that have a complex chemical composition (Oussalah, Caillet, Saucier, & Lacroix, 2007). Due to their volatility, they can be used to emit chemical communication signals and as defense mechanisms (Saito & Scramin, 2000). Its effects have been studied mainly due to phytotoxic properties, and uses such as bactericides (Hussain, Anwar, Sherazi, & Przybylski, 2008), insecticides (Coitinho, Oliveira, Gondim Júnior, & Câmara, 2010), herbicides (Souza Filho, Guilhon, & Santos, 2010), and fungicides (Guimarães, Cardoso, Sousa, Andrade, & Vieira, 2011).

Among the variety of compounds with these characteristics that can be extracted from plants, the aromatic esters benzyl salicylate ($C_{14}H_{12}O_3$) and benzyl benzoate ($C_{14}H_{12}O_2$) deserve to be highlighted because they have part of their unexplored potential. Benzyl salicylate is a benzyl ester of salicylic acid widely used in industry, being used as a fragrance fixer in the cosmetic, personal hygiene, and household cleaning areas (Lapczynski et al., 2007). This compound is found naturally in plants such as *Polianthes tuberosa* L. (International Fragrance Association-IFRA, 2009) and *Ocotea pulchella* Nees and Mart. (Candido et al., 2016).

Benzyl benzoate is a compound frequently used in the pharmaceutical industry as an active ingredient for medicines used in the treatment of scabies (Salavastru, Chosidow, Boffa, Janierv, & Tiplica, 2017) and pediculosis (Salavastru, Chosidow, Janierv, & Tiplica, 2017). In addition, it is used as an insect repellent (Knowles, 1991), solvent, in the perfumery and cosmetics industry (Pybus & Sell, 2006), as well as in the composition of pesticides (Johnson et al., 2017). This compound can be found naturally in the plants *Citharexylum spinosum* L. (El Ayeb-Zakhama, Sakka-Rouis, Flamini, Jannet, & Harzallah-Skhiri, 2017), *Magnolia champaca* L. (Sá, Meneses, Araújo, & Oliveira, 2017), and *O. pulchella* (Candido et al., 2016).

Although there are studies reporting the importance of these compounds in several areas, studies on the phytotoxic potential of benzyl salicylate and benzyl benzoate in plant species are still insufficient. Highlighting the need for studies on their properties and mode of action, in invasive and cultivated plants. Candido et al. (2016), isolated these two compounds from *O. pulchella* plants and evaluated their phytotoxic activity on *T. aestivum* coleoptiles and on the germination and initial growth of *L. esculentum* seedlings, verifying its phytotoxic potential. Thus, the potential of these compounds as growth inhibiting agents for some plant species is emphasized. Therefore, this work aimed to evaluate the phytotoxic potential of these compounds on the initial growth of the bioindicator species *L. sativa*, *L. esculentum*, *A. cepa*, and *T. aestivum* and of the invasive species *E. heterophylla* and *M. maximus* in order to evaluate if any of these compounds have greater phytotoxicity than the herbicide (a.i. oxyfluorfen).

2. Materials and Methods

The work was developed at the Laboratory of Ecophysiology of Reproduction and Phytochemical Studies, Department of Botany, Center for Biological and Health Sciences-CCBS, at the Federal University of São Carlos, in the city of São Carlos-SP, Brazil. Both compounds are commercial products that were purchased from a local supplier (benzyl salicylate 98% from Sigma-Aldrich and benzyl benzoate 99% from Neon Commercial Ltda). All experiments were carried out in a climate-controlled chamber type B.O.D under controlled conditions.

2.1 Preparation of Solutions

The pure benzyl salicylate and benzyl benzoate compounds were pre-solubilized in dimethyl sulfoxide (DMSO) with a concentration of 5 $\mu L.mL^{-1}$ and diluted in a buffer solution (pH = 5.6) containing citric acid monohydrate (1.05 g L^{-1}), potassium hydrogen phosphate trihydrate (2.9 g L^{-1}), and 2% sucrose according to the recommendations of Macías, Lacret, Varela, Nogueiras, and Molinillo (2010).

In all bioassays three compounds were evaluated: benzyl salicylate, benzyl benzoate, and positive control, commercial Goal® herbicide (a.i. oxyfluorfen 240 g L^{-1}). In the *T. aestivum* coleoptile bioassay, five concentrations were used for each compound: 10^{-3} M (treatment 1), 3×10^{-4} M (treatment 2), 10^{-4} M (treatment 3), 3×10^{-5} M (treatment 4), and 10^{-5} M (treatment 5), totaling 15 treatments. In the initial seedling growth bioassays, three concentrations were used for each compound: 10^{-3} M (treatment 1), 10^{-4} M (treatment 2), and 10^{-5} M (treatment 3), totaling nine treatments. In addition, in each bioassay, one negative control was performed with buffer solution and DMSO (5 $\mu L\ mL^{-1}$).

2.2 Phytotoxicity Bioassays

2.2.1 *Triticum aestivum* Coleoptile Bioassays

The bioassays were performed with wheat diaspores (*T. aestivum* L: Poaceae), BRS 264 cultivar, pre-twinned in plastic boxes covered with 2 sheets of filter paper, moistened with distilled water (±12 mL) and stored in a B.O.D. for 72 hours at 24±1 °C in the absence of light.

After the time spent in the B.O.D. chamber, the coleoptiles were cut in a Van der Weij guillotine and the apical part (2 mm) was discarded as recommended by Hancock, Barlow, and Lacey (1964). After this stage, the coleoptiles were cut into 4 mm segments and used in bioassays (Macías et al., 2010). The assembly of this experiment was carried out in a closed environment with green safety light (Nitsch & Nitsch, 1956).

Three repetitions were used in each treatment, each one being composed of five coleoptiles of *T. aestivum*, kept in test tubes containing 2 mL of solution. The samples, properly identified, were kept at 25±1 °C, in the dark, and with a constant rotation of 1.4 rpm for 24 h, in order to guarantee the homogeneity of the contact between the plant material and the solution. After that period the coleoptiles were removed, photographed, and measured using the Image J® 1.8.0 software (Macías et al., 2010).

To evaluate the effect of the compounds on the initial growth of invasive plants, two invasive target species were used: the eudicotyledon wild poinsettia (*E. heterophylla* L. Euphorbiaceae) and the monocotyledon guinea grass (*M. maximus* J. Poaceae). In addition, four target bioindicator species were also employed, two eudicotyledons, lettuce (*L. sativa* L. Asteraceae) and tomato (*L. esculentum* L. Solanaceae); and two monocotyledons, wheat (*T. aestivum* L. Poaceae) and onion (*A. cepa* L. Amaryllidaceae). These species are considered indicators of phytotoxic activity because they present important characteristics such as rapid and uniform germination and sensitivity to phytotoxic substances even in low concentrations (Ferreira & Áquila, 2000; Souza Filho et al., 2010).

The diaspores were placed in plastic boxes for germination and moistened with distilled water. After the emergence of the primary root (2 mm) and the identification of positive gravitropism, the diaspores were considered germinated (Brazil, 2009). Then, the diaspores that germinated were transferred to transparent plastic boxes (10.5 × 6 × 4.5 cm) containing two sheets of filter paper moistened with 6 mL of each solution.

Each treatment contained four replicates with ten seedlings of the target species. The boxes with the respective treatments were identified, closed, and kept in a germination chamber at 27±1 °C, under a 12-hour photoperiod, according to Inoue et al. (2010). After eight days, the material was removed from the germination chamber, and measurements were taken of the length of the shoot and the main root (for eudicotyledons *L. sativa*, *L. esculentum*, and *E. heterophylla*) and of the shoot and the longest root (for monocotyledons *T. aestivum, A. cepa*, and *M. maximus*).

This step was performed with the aid of the Image J® 1.8.0 software. In addition, in the presence of abnormalities, they were photographed, counted, and characterized as described by Brazil (2009).

2.3 Statistical Analysis

The data obtained were submitted to the Shapiro-Wilk normality test using the Past v.2.17c software (Hammer, Harper, & Ryan, 2001). Normal data were subjected to Analysis of Variance (ANOVA), followed by the Tukey test at 5% significance with the aid of the Sisvar software (Ferreira, 2011). Non-normal data were submitted to the Kruskal-Wallis non-parametric test followed by the Dunn test at 5% significance with the aid of the Past v.2.17c software.

3. Results

Strong inhibitory activity was observed for both compounds on the growth of *T. aestivum* coleoptiles in all tested concentrations. These results were statistically equivalent to the data obtained with the herbicide in treatments at concentrations of 10^{-3} M, 3 × 10^{-4} M, and 10^{-4} M. In the five concentrations tested for both evaluated compounds (10^{-3} M, 3 × 10^{-4} M, 10^{-4} M, 3 × 10^{-5} M, and 10^{-5} M), coleoptile growth was inhibited compared to the negative control. The herbicide presented a greater decrease in the inhibition of coleoptile growth especially at concentrations of 3 × 10^{-5} M and 10^{-5} M (54% and 51%, respectively), differing significantly from the treatments with benzyl salicylate and benzyl benzoate, while the same compounds showed no reduction in phytotoxic activity at the five concentrations tested (Figure 1).

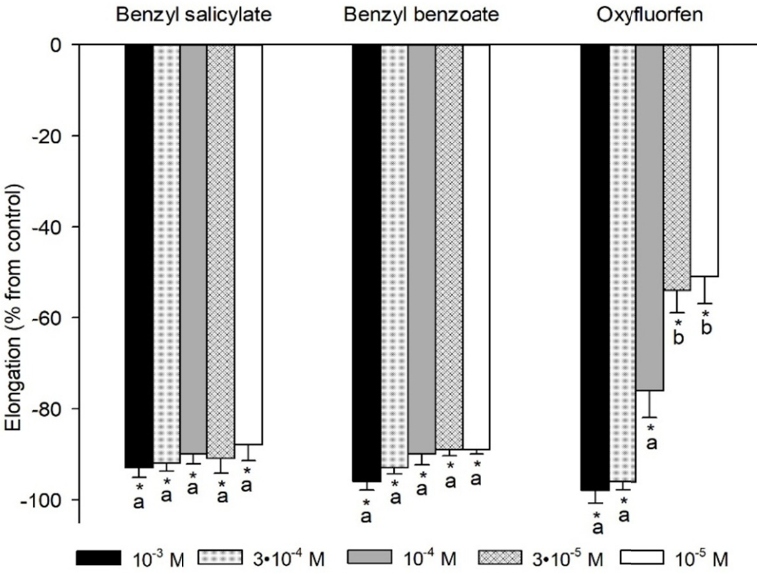

Figure 1. Percentage inhibition of benzyl salicylate, benzyl benzoate, and herbicide on the growth of *T. aestivum* coleoptiles. Values expressed as a percentage of inhibition compared to the negative control. (*) Means differ significantly from the negative control by the Tukey test at 0.05 significance. Means followed by the same letter do not differ significantly from the positive control at the same concentration, by the Tukey test at 0.05

The results obtained in the initial growth bioassay for the bioindicator species *L. sativa* showed a significant inhibitory activity of benzyl salicylate in the plant shoot and root, at the three concentrations tested. However, the treatments with benzyl benzoate resulted in shoot inhibition at 10^{-3} M and 10^{-4} M concentrations. For the root system there was no significant difference in any of the tested concentrations compared to the negative control in this species. Regarding the root system, all the inhibition values obtained with both compounds in this species were lower than those obtained with the herbicide. However, in the shoot, the 10^{-5} M treatment with benzyl salicylate was not statistically different from the treatment with the herbicide at the same concentration (Figure 2).

For the bioindicator species *L. esculentum*, treatments with benzyl salicylate showed no significant difference from the negative control in the shoot at the three concentrations tested. On the other hand, treatments with this compound resulted in a decrease in root growth, at the three concentrations. However, treatments with benzyl benzoate significantly inhibited the growth of the shoot of this species at concentrations 10^{-3} M, and 10^{-4} M. In the roots showed reduced growth in all treatments tested compared to the negative control (Figure 2).

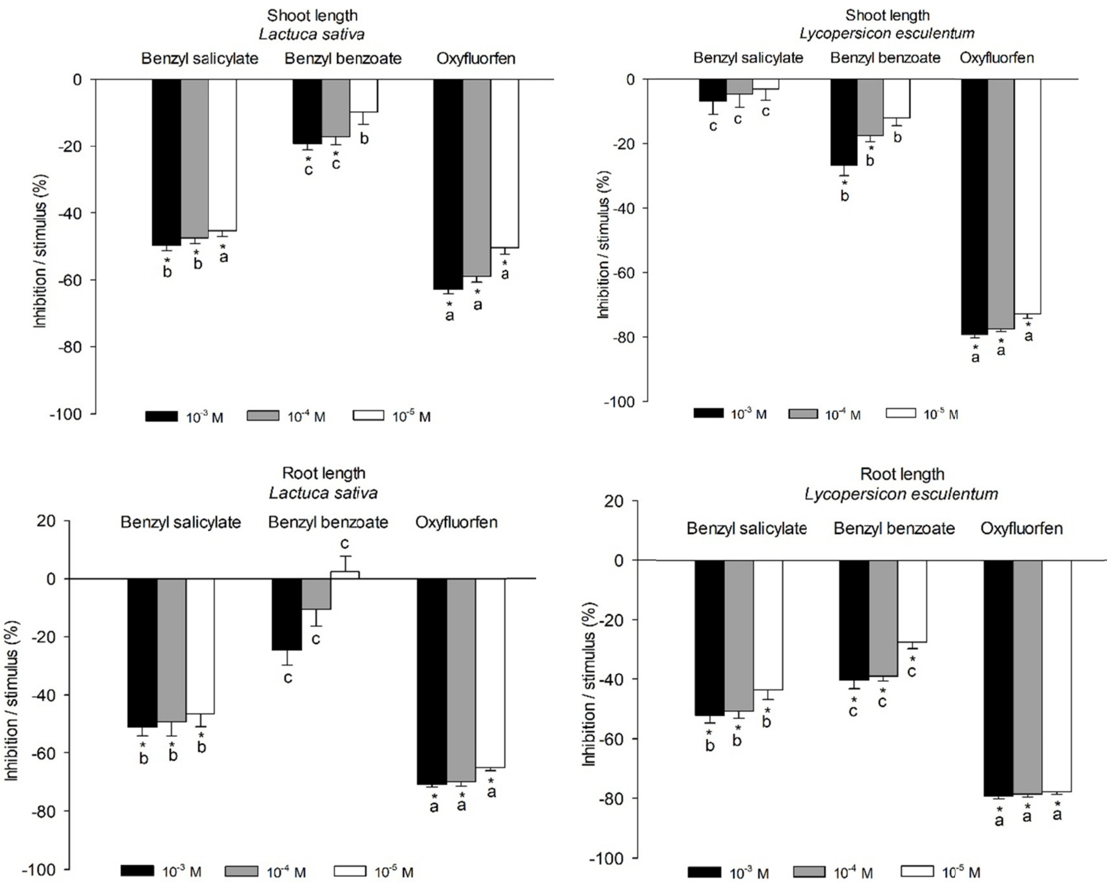

Figure 2. Percentage of inhibition promoted by the action of benzyl salicylate, benzyl benzoate, and herbicide on the growth of roots and shoot of the bioindicator species *L. sativa* and *L. esculentum*. Values expressed as a percentage of inhibition/stimulus with respect to the negative control. (*) Means differ significantly from the negative control by the Tukey test at 0.05 significance. Means followed by the same letter do not differ significantly from the positive control at the same concentration, by the Tukey test at 0.05

In the bioindicator species *A. cepa,* a significant percentage of inhibition was observed compared to the negative control both in the shoot and in the root with all treatments of both compounds. In this species, all the values of root and shoot inhibition using both compounds were statistically different from those obtained with the herbicide (Figure 3).

For the bioindicator species *T. aestivum*, significant root inhibition was observed with the treatments with both compounds at the concentrations of 10^{-3} M and 10^{-4} M compared to the negative control. When compared to the herbicide, the treatments of both compounds were statistically different. In the shoot, the application of both compounds significantly inhibited the growth at all concentrations compared to the negative control. Regarding the positive control, all the treatments with both compounds showed significant statistical difference from the herbicide (Figure 3).

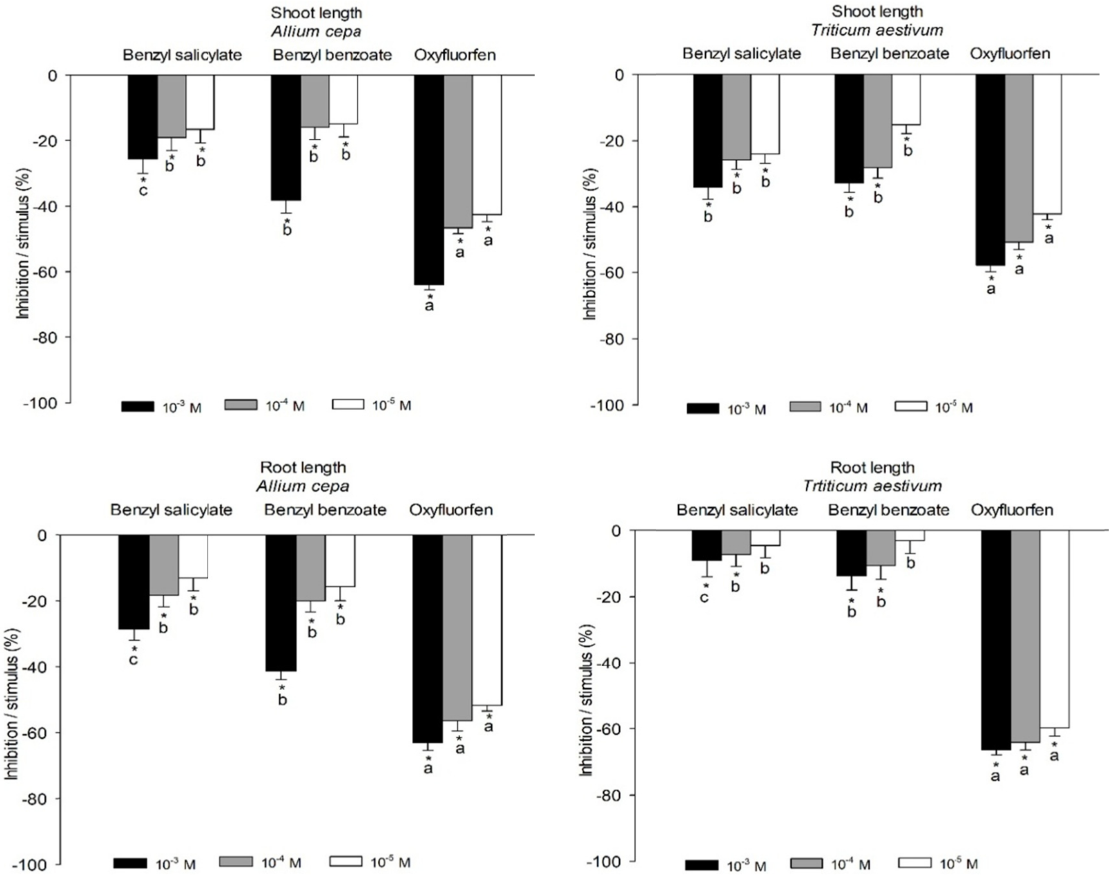

Figure.3 Effect of benzyl salicylate, benzyl benzoate, and herbicide on the growth of roots and shoot of the bioindicator species *A. cepa* and *T. aestivum*. Values expressed as a percentage of inhibition/stimulus compared to the negative control. (*) Means differ significantly from the negative control by the Tukey test at 0.05 significance. Means followed by the same letter do not differ significantly from the positive control at the same concentration, by the Tukey test at 0.05

For the invasive species *E. heterophylla*, the application of both compounds had a significant inhibitory effect on the growth of the shoot and roots at all concentrations compared to the negative control. In the shoot, the three compounds significantly inhibited growth at all concentrations tested. For the root system, all treatments with benzyl benzoate were not statistically different from the treatments with the herbicide at the same concentrations. For benzyl salicylate, the treatments 10^{-4} M and 10^{-5} M did not differ statistically from the herbicide treatments at the same concentrations (Figure 4).

Benzyl salicylate significantly reduced the shoot growth of *M. maximus* at the highest concentration (10^{-3} M). This compound also reduced the root growth at the three concentrations compared to the negative control. On the other hand, treatments with benzyl benzoate significantly reduced the shoot growth of this species at concentrations of 10^{-3} M and 10^{-4} M. For the root part, there was a significant reduction in growth only when the treatment with the highest concentration was applied, compared to the negative control. All the inhibition values of the shoot and the root obtained with the application of both compounds in this species proved to be statistically inferior to those obtained with the herbicide (Figure 4).

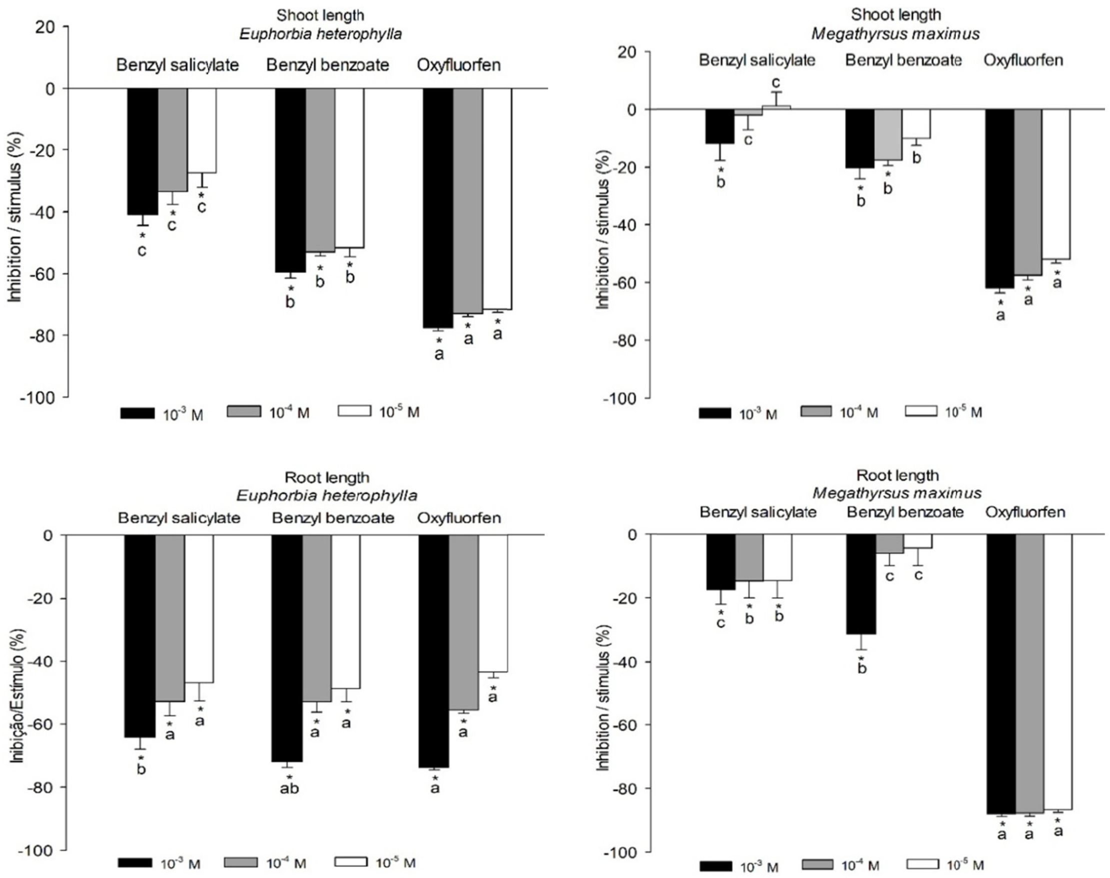

Figure 4. Effect of benzyl salicylate, benzyl benzoate, and herbicide on root and shoot growth of invasive species *E. heterophylla* and *M. maximus*. Values expressed as a percentage of inhibition/stimulus compared to the negative control. (*) Means differ significantly from the negative control by the Tukey test at 0.05 significance. Means followed by the same letter do not differ significantly from the positive control at the same concentration, by the Tukey test at 0.05

E. heterophylla was the species that presented the highest percentage of abnormalities with the application of both compounds at all tested concentrations (Figure 5). In this species, the application of both compounds showed a statistical difference in all concentrations in relation to the negative control. However, only treatments with the highest concentration of benzyl benzoate showed a significant difference in the species *L. esculentum*, *L. sativa* and *M. maximus* (Figure 5). It should be noted that in treatments with the herbicide, there were no abnormalities in the target species due to the high percentage of recorded dead seedlings.

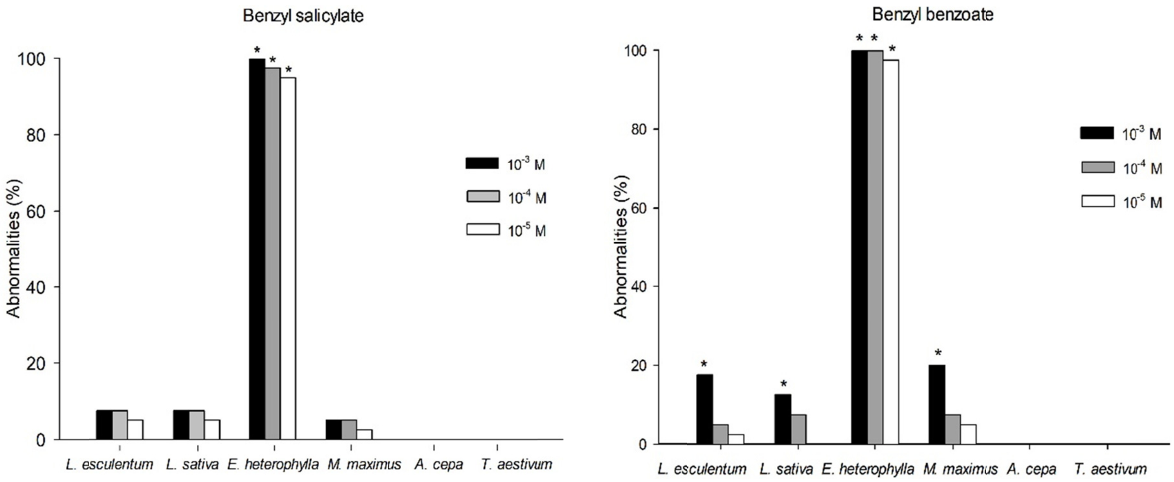

Figure 5. Effect of benzyl salicylate and benzyl benzoate compounds on the percentage of abnormalities in *L. esculentum*, *L. sativa*, *E. heterophylla*, *M. maximus*, *A. cepa*, and *T. aestivum* species. (*) Means differ significantly from the negative control by the Dunn's test at 0.05 significance

As for the characterization of the abnormalities, *E. heterophylla* was the most affected species. This phenomenon was observed with the application of both compounds. When treatments with benzyl salicylate were used nine abnormalities were verified. Of these, root necrosis (RN), hypocotyl winding (HW), and the poorly developed main root (PDMR) may be highlighted (Figures 6 and 7). Treatments with benzyl benzoate resulted in seven different abnormal parameters, among these the poorly developed main root (PDMR) and hypocotyl winding (HW) stand out (Figures 6 and 7).

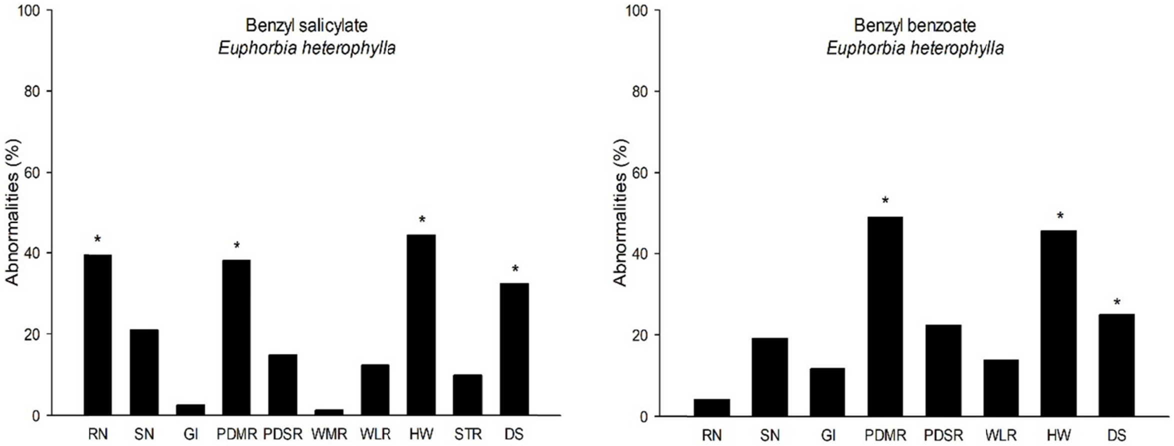

Figure 6. Percentage of abnormalities found, types of anomalies, and dead seedlings identified in treatments with benzyl salicylate and benzyl benzoate in the species *E. heterophylla*. RN (root necrosis), SN (shoot necrosis), GI (gravitropic inversion), PDMR (poorly developed main root), PDSR (poorly developed secondary root), WMR (without main root), WLR (without lateral root), HW (hypocotyl winding), STR (short and thick root) and DS (dead seedling). (*) Means differ significantly from the negative control by the Dunn's test at 0.05 significance

Figure 7. Types of abnormalities found in treatments with benzyl salicylate and benzyl benzoate in the species *E. heterophylla*. Root necrosis (A), gravitropic inversion (B), shoot necrosis (C poorly developed main root (D), poorly developed secondary root (E), without main root (F), without lateral root (G), hypocotyl winding (E), short and thick root (C), and dead seedling (H)

Among the evaluated species, the bioindicator species and the invasive species *M. maximus* were more tolerant to the compounds used, presenting the lowest percentages of abnormalities and dead seedlings (Table 1).

Table 1. Percentage of anomalies and dead seedlings found in the species *M. maximus*, *L. sativa*, *L. esculentum, A. cepa*, and *T. aestivum* in treatments with benzyl salicylate and benzyl benzoate

		Anomalies and dead seedlings (%)						
Compound	Species	RN	AN	GI	PDMR	WMR	HW	DS
Benzyl salicylate	*M. maximus*	-	0.92	1.83	-	0.92	0.92	9.17
	L. sativa	6.67	-	2.5	-	-	-	-
	L. esculentum	3.48	1.74	-	1.74	-	-	4.17
	A. cepa	-	-	-	-	-	-	1.6
	T. aestivum	-	-	-	-	-	-	-
Benzyl benzoate	*M. maximus*	-	1.67	-	3.33	5.83	-	-
	L. sativa	6.67	-	-	-	-	-	-
	L. esculentum	-	4.35	-	2.61	-	1.74	-
	A. cepa	-	-	-	-	-	-	4.16
	T. aestivum	-	-	-	-	-	-	-

Note. RN (root necrosis), AN (aerial necrosis), GI (gravitropic inversion), PDMR (poorly developed main root), WMR (without main root) HW (hypocotyl winding), and DS (dead seedling).

For the percentage of dead seedlings, in the species *E. heterophylla* the treatments with benzyl salicylate in concentrations 10^{-3} M and 10^{-4} M showed a statistical difference from the negative control. In the *M. maximus* species, only the application of the highest concentration of this compound showed a statistically significant difference (Figure 7). However, when the tests were performed with benzyl benzoate, the application of the 10^{-3} M and 10^{-5} M treatments showed a significant difference from the negative control only in the species *E. heterophylla* (Figure 7). Finally, in the treatments with the herbicide, only the application of the lowest concentration showed no statistical difference in the species *T. aestivum* (Figure 7).

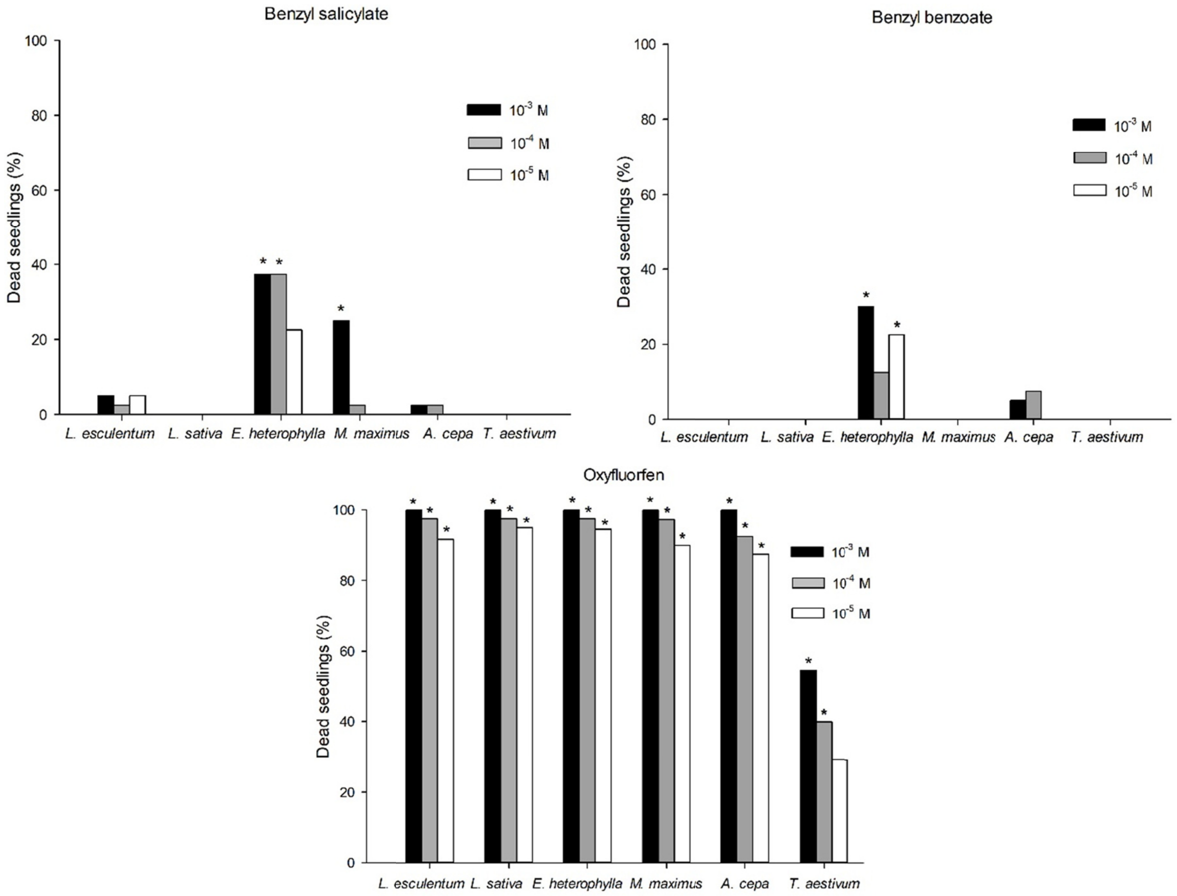

Figure 7. Effect of the benzyl salicylate and benzyl benzoate compounds on the percentage of dead seedlings found in the species *L. esculentum*, *L. sativa*, *E. heterophylla*, and *M. maximus*. (*) Means differ significantly from the negative control by the Dunn's test at 0.05 significance

4. Discussion

Some secondary metabolites are important for plant-environment interaction, for instance, allelochemicals may interfere with other plants (Kroymann, 2011). Essential oils have their phytotoxic potential often associated with the content of monoterpenes. This because they affect the growth of plants, causing morphological and physiological changes (Miranda et al., 2014). Inserted in this group of secondary metabolites, esters are characterized by acting as active ingredients with a phytotoxic nature (Tello, 2014).

In the present work, the phytotoxic effect of the esters benzyl salicylate and benzyl benzoate on the development of *T. aestivum* coleoptiles was observed. For the first time its phytotoxic effect on the initial growth of invasive species is reported. Positive results were obtained mainly in the species *E. heterophylla*. These compounds can be potential selective herbicides, since they more effectively inhibited the growth of the species *E. heterophylla*, an invasive species that presents multiple resistance to Protox, ALS, and EPSPS inhibiting herbicides (Trezzi et al., 2011; Xavier et al., 2018; Adegas et al., 2020). This makes this plant an invasive species that is difficult to control, resulting in significant in productivity losses in agricultural systems (Kern et al., 2009).

In the bioassays with *T. aestivum* coleoptiles, the results of the present study showed better uniformity in the inhibition values. This mainly in the lower concentrations (3×10^{-5} M and 10^{-5} M), when compared to the results of Candido et al. (2016), who evaluated the phytotoxic effect of these compounds on the growth of *T. aestivum* coleoptiles (Table 2). This difference may be attributed to the fact that these authors worked with compounds extracted from *O. pulchella*, ignoring their level of purity. Or due to the decrease in the activity of the compounds along the chromatographic fractionations. This may also take place due to the chemical modification or degradation of the active compounds (Dayan & Duke, 2006). In contrast, the compounds used in this work are synthetic compounds with a purity level above 98%.

Table 2. Percentage of growth inhibition of *T. aestivum* coleoptiles under the action of benzyl salicylate and benzyl benzoate compounds.

Concentration	1*	2*
10^{-3} M and 3×10^{-4} M	> 90%	≥ 93%
10^{-4} M	> 80%	≥ 90%
3×10^{-5} M and 10^{-5} M	< 40%	≥ 89%

Note. 1 * values reported in the literature (Candido et al., 2016) and 2 * values obtained in the present study.

Other authors used pure compounds extracted from different plant species and found their phytotoxic effects on the growth of *T. aestivum* coleoptiles (Anese, Jatobá, Grisi, Gualtieri, Santos, & Berlinck, 2015; Miranda et al., 2015; Jatobá et al., 2016; Silva et al., 2017). Thus, they confirmed that the initial growth bioassays of *T. aestivum* coleoptiles are a classic and efficient method to evaluate the phytotoxic activity of compounds belonging to the secondary plant metabolism (Hancock et al., 1964). Bioassays using *T. aestivum* coleoptiles have the advantage of being fast and sensitive to a wide variety of bioactive substances (Nepomuceno, 2011). In addition, they are widely used because they have proved to be able to evaluate the inhibition or growth stimulus when exposed to phytotoxic substances (Accarini, 2016).

In the initial growth bioassays of *L. esculentum* both compounds inhibited root growth at all concentrations. While shoot growth was significantly inhibited only by benzyl benzoate at concentrations of 10^{-3} M and 10^{-4} M. These results corroborated those of Candido et al. (2016) who tested the same compounds and observed root and shoot inhibition of this species at the highest concentration (10^{-3} M). However, the data of these authors presented a notable decrease in root growth inhibition as the concentrations decreased, as well as stimulation of shoot growth. While the results of the present work remained uniform over the different concentrations.

In general, the four bioindicator species showed uniform inhibition values between treatments with the application of the same compound, even at the lowest concentrations. Corroborating data from the literature that tested different pure compounds from plant extracts. The authors used these bioindicator species and reported root and shoot inhibition mainly at the highest concentration tested. They also reported that there was a rapid decrease in inhibition when the compounds used were diluted, and in some cases, growth stimulus was observed (Miranda et al., 2015; Silva et al., 2017).

This may emphasize what was previously mentioned. The high purity of the compounds used in this work has interfered in this observed difference, since the data in the literature are related to compounds isolated from plant species. The methods employed to isolate these compounds may leave them with impurities (Leite, 2009). Compared to a synthetic compound with a high degree of purity, such as the ones employed in this study. Other factors may have contributed to the differences observed, such as the variety of the species used, the temperature at which the experiments were conducted, the length of the experiments, as well as the type of compound used. This shows that the response of the plant to secondary metabolites is a species-specific characteristic, in which some species being more sensitive to certain compounds or molecules than others (Ferreira & Áquila, 2000).

Among the invasive species, *M. maximus* was the least affected by both compounds, with the roots being the most affected parameter, in accordance with the results of Candido (2016). Nevertheless, this author, who used leaf extracts of *O. pulchella* containing benzyl salicylate and benzyl benzoate in their composition, observed a greater susceptibility of the *M. maximus* roots to these extracts. On the other hand, *E. heterophylla* obtained a high percentage of inhibition both in the root and in the shoot, being as efficient as the herbicide mainly in the root system. Confirming the results of Candido (2016), who observed the same inhibitory effect on this plant species, mainly in shoot.

In addition, the same author mentioned that the length reduction of *E. heterophylla* roots caused by leaf extracts of *O. pulchella* is due to the decrease in the elongation of metaxylem cells. The presence of benzyl salicylate and benzyl benzoate may have resulted in a lower absorption of water and minerals by the roots. Since the action of the allelochemicals is more evident on these structures, because they have a direct contact with the solution of allelochemicals (Lupini, Sorgonà, Miller, & Abenavoli, 2010). However, Carvalho, Carvalho, Abbade Neto, & Teixeira (2014) highlighted that this condition makes plants more sensitive, since their elongation depends on cell divisions. Once inhibited they affect the normal development of the plant, resulting in a reduction of the roots and stems of the seedlings (Bogatek, Gniazdowska, Zakrzewska, Oracz, & Gawronski, 2005; Kenany & Darier, 2013).

Compounds with phytotoxic activity can inhibit cell elongation. This might be associated with changes in the concentration of plant hormones such as cytokinins and auxins, since these are of vital importance in the development of roots, vascular differentiation, and in the gravitropism of plants (Gatti, Ferreira, Arduin, & Perez, 2010; Grisi, Gualtieri, Anese, Pereira, & Forim, 2013).

Cell growth in plants is dependent on the mitotic process. When cell divisions are compromised during germination, most seedlings die or present abnormalities (Imatomi, Novaes, & Gualtieri, 2013). Some allelochemicals, besides affecting seedling growth, can completely inhibit the formation of normal seedlings (Juchem, Lauxen, Silva, Denardin, & Sobral, 2013). Causing morphological changes such as root atrophy (Formigheiri et al., 2018), little or no root development, necrotic apexes, and hypocotyl winding, which can impair the emergence of the seedlings from the soil (Denardin et al., 2018).

The compounds benzyl salicylate and benzyl benzoate induced the appearance of several abnormalities and dead seedlings in the species tested with a higher incidence in *E. heterophylla*. Corroborating the results obtained by Candido (2016), who observed several abnormalities in this species, with the highest percentage of anomalies found in the root system, using *O. pulchella* leaf extracts.

Allelochemicals can cause changes in the plant growth and development such as cellular alterations, changes in the enzymatic activities of the phenylpropanoid pathway, reduction of leaf expansion and root elongation, reduction of the photosynthetic rate, disintegration of the cap, increase in the diameter of the vascular cylinder, and premature lignification of the metaxylem and cell wall. This may impair the nutrient absorption ability, the expansion of cells, and the ability to sustain plant growth (Soltys et al., 2011; Granã et al., 2013; Ferro et al., 2015; Bido et al., 2018).

The high percentage of abnormalities found in *E. heterophylla* in the present study, mainly in the root system, may reveal the action of both compounds on this species. Also, some of the abnormalities observed may seriously compromise the development of the plant. The growth of the root system is characterized by a high metabolic activity and for this reason, the roots are particularly more susceptible to stress by allelochemicals. Some studies have indicated that the impairment of root system growth by the action of allelochemicals is associated with premature lignification of cell walls (Politycka & Mielcarz, 2007; Bubhna et al., 2011).

Abnormal seedlings are those with a damaged or absent primordial structure, that present fragile development or are so deteriorated that their normal development is compromised (Brazil, 2009). The evaluation of abnormalities in seedlings is a fundamental parameter in detecting the phytotoxic effects of allelochemicals. Since they can induce the appearance of abnormalities, thus inhibiting their normal development (Ferreira & Aquila, 2000).

In general, the four bioindicator species and the invasive *M. maximus* presented a low percentage of abnormalities, as well as a low or zero percentage of dead seedlings, as a result of the compounds used. No pattern was observed regarding the presence of abnormalities between monocots and eudicots, with *E. heterophylla* being the species most sensitive to treatments with both compounds.

Candido (2016) found abnormalities in the seedlings of *M. maximus* and *E. heterophylla*, when subjected to the action of *O. Pulchella* extracts. Favaretto (2018) described some abnormalities such as root necrosis, absence of secondary roots, and short and thick roots in *L. sativ*a seedlings, when treated with different concentrations of catechin, caffeic acid, and vanillic acid. Likewise, Melo et al. (2017) found abnormalities in this species when treated with different concentrations of *Curcuma zedoaria* essential oil. However, the same authors reported *that L. esculentum* seedlings did not show changes in the percentage of abnormal seedlings when exposed to the same treatments with this essential oil. This information shows that tolerance or resistance to secondary metabolites is a species-specific characteristic, in which some species are more sensitive to certain compounds or molecules than others (Ferreira & Áquila, 2000), as observed in the results of the present study.

Information on the mode of action of some allelochemicals on plants is scarce. Since these substances affect several functions and cause many side effects, making them difficult to distinguish from other effects (Goldfarb, Pimentel & Pimentel, 2009). Phytotoxic substances may or may not inhibit germination, besides inducing the appearance of abnormal seedlings. Therefore, the evaluation of seedling abnormalities is an important tool for the identification of phytotoxic substances (Ferreira & Áquila, 2000; Alves, Oliveira, França, Alves, & Pereira, 2011).

Oliveira Jr., Pereira, Muller, and Matias (2014), mentioned that some compounds may be toxic for one species and not have the same effect on another one, even acting as a stimulant (Almeida, 1988). It is important to mention that results obtained in the laboratory involving phytotoxic activity may be different under natural conditions, since the simultaneous occurrence of biotic and abiotic factors may interfere with the results (Formagio et al., 2010).

5. Conclusions

This study provided knowledge on the effect of the tested compounds upon plant species, reporting for the first time their effect on invasive species. The results obtained showed that the benzyl salicylate and benzyl benzoate compounds have phytotoxic potential. This effect was more evident in *E. heterophylla* since it was the most affected species according to all the evaluated parameters, and in some parameters, the inhibitory effects of both compounds have proved to be as efficient as the herbicide, therefore confirming the phytotoxic potential of both compounds.

References

Accarini, R. B. (2016). *Atividade fitotóxica de extratos foliares de Piptocarpha rotundifolia (candeia) (Less.) Baker-Asteraceae* (Master's thesis, Universidade Federal de São Carlos, Brasil). Retrieved from https://repositorio.ufscar.br/bitstream/handle/ufscar/8474/DissRBA.pdf

Adegas, F. S., Gazziero, D. L. P., Oliveira Jr., R. S., Mendes, R. R., & Rodrigues L. J. (2020). *Euphorbia heterophylla: Um novo caso de resistência ao glifosato no Brasil* (p. 5). Embrapa soja. Comunicado técnico 98, Londrina, PR.

Aimar, F. F. P. J., & Durigan, J. C. (2001). Controle de capim-colonião na cultura da cana-de-açúcar com herbicidas aplicados em pré-emergência. *Revista Brasileira de Herbicidas, 2*(3), 125-132. https://doi.org/10.7824/rbh.v2i3.361

Almeida, F. S. (1988). *Alelopatia e as plantas* (Circular, 53, p. 68). Londrina: IAPAR.

Alves, L. L., Oliveira, P. V. A., França, S. C., Alves, P. L. C., & Pereira, P. S. (2011). Atividade alelopática de extratos aquosos de plantas medicinais na germinação de *Lactuca sativa* L. e *Bidens pilosa* L. *Revista Brasileira de Plantas Medicinais, 13*(3), 328-336. https://doi.org/10.1590/S1516-057220 11000300012

Alves, M. C. S., Medeiros Filho, S., Innecco, R., & Torres, S. B. (2004). Alelopatia de extratos voláteis na germinação de sementes e no comprimento da raiz de alface. *Pesquisa Agropecuária Brasileira, 39*(11), 1083-1086. https://doi.org/10.1590/S0100-204X2004001100005

Alvino, C. A., Gricio, L. H., Sampaio, F. A., Girotto, M., Felipe, A. L. S., Junior, C. E. I., … Lima, F. C. C. (2011). Interferência e controle de plantas daninhas nas culturas agrícolas. *Revista Científica Eletrônica de Agronomia, 20*.

Anese, S., Jatobá, L. J., Grisi, P. U., Gualtieri, S. C. J., Santos, M. F. C., & Berlinck, R. G. S. (2015). Bioherbicidal activity of drimane sesquiterpenes from *Drimys brasiliensis* Miers roots. *Industrial and Products, 74*, 28-35. https://doi.org/10.1016/j.indcrop.2015.04.042

Bido, G. S., Silva, H. A., Bortolo, T. S. C., Maldonado, M. R., Marchiosi, R., dos Santos, W. D., & Ferrarese-Filho, O. (2018). Comparative effects of L-DOPA and velvet bean seed extract on soybean lignification. *Plant Signaling & Behavior, 13*(4), e1451705. https://doi.org/10.1080/15592324.2018.1451705

Bogatek, R., Gniazdowska, A., Zakrzewska, W., Oracz, K., & Gawronski, S. W. (2005). Allelopathic effects of sunflower extracts on mustard seed germination and seedling growth. *Biologia Plantarum, 50*(1), 156-158. https://doi.org/10.1007/s10535-005-0094-6

Brazil, Ministério da Agricultura, Pecuária e Abastecimento. (2009). *Secretaria da Defesa Agropecuária* (p. 395). Regras para a análise de sementes. Brasília, Brazil.

Bubna, G. A., Lima, R. B., Zanardo, D. Y. L., dos Santos, W. D., Ferrarese, M. L. L., & Ferrarese-Filho, O. (2011). Exogenous caffeic acid inhibits the growth and enhances the lignification of the roots of soybean (*Glycine max*). *Journal of Plant Physiology, 168*(14), 1627-1633. https://doi.org/10.1016/j.jplph.2011.03.005

Candido, L. P. (2016). *Busca de extratos e compostos ativos com potencial herbicida e inseticida nas espécies Davilla elliptica St. Hill e Ocotea pulchella Nees & Mart* (Doctoral dissertation, Universidade Federal de São Carlos, São Carlos, SP, Brazil). Retrieved from https://repositorio.ufscar.br/bitstream/handle/ufscar/8003/TeseLPC.pdf

Candido, L. P., Varela, R. M., Torres, A., Molinillo, J. M. G., Gualtieri, S. C. J., & Macías, F. A. (2016). Evaluation of the Allelopathic Potential of Leaf, Stem, and Root Extracts of *Ocotea pulchella* Nees et Mart. *Chemistry & Biodiversity, 13*, 1058-1067. https://doi.org/10.1002/cbdv.201500501

Carvalho, L. B., Bianco, S., & Guzzo, C. D. (2010). Interferência de *Euphorbia heterophylla* no crescimento e acúmulo de macronutrientes da soja. *Planta Daninha, 28*, 33-39. https://doi.org/10.1590/S0100-83582010000100004

Carvalho, W. P., Carvalho, G. J., Abbade Neto, D. O., & Teixeira, L. G. V. (2014). Alelopatia de extratos de adubos verdes sobre a germinação e crescimento inicial de alface. *Bioscience Journal, 30*, 1-11.

Coitinho, R. L. B. C., Oliveira, J. V., Gondim Júnior, M. G., & Câmara, C. A. G. (2010). Persistência de óleos essenciais em milho armazenado, submetido à infestação de gorgulho do milho. *Ciência Rural, 40*(7), 1492-1496. https://doi.org/10.1590/S0103-84782010005000109

Correia, N. M., Gomes, L. P., & Perussi, F. J. (2012). Control of *Brachiaria decumbens* and *Panicum maximum* by S-metolachlor as influenced by the occurrence of rain and amount of sugarcane straw on the soil. *Acta Sci. Agron., 34*(4). https://doi.org/10.4025/actasciagron.v34i4.14676

Costa, E. A. D., Matallo, M. B., Carvalho, J. C., & Rozanski, A. (2002). Eficiência de nova formulação do herbicida oxyfluorfen no controle de plantas daninhas em área de *Pinus caribea* morelet var. hondurensis barr. et Golf. *Revista Árvore, 26*, 683-689. https://doi.org/10.1590/S0100-67622002000600005

Cury, J. P., Santos, J. B., Silva, D. V., Carvalho, F. P., Braga, R. R., Byrro, E. C. M., & Ferreira, E. A. (2011). Produção e partição de matéria seca de cultivares de feijão em competição com plantas daninhas. *Planta Daninha, 29*(1), 149-158. https://doi.org/10.1590/S0100-83582011000100017

Cury, J. P., Santos, J. B., Silva, E. B., Byrro, E. C. M., Braga, R. R., Carvalho, F. P., & Silva, D. V. (2012). Acúmulo e partição de nutrientes de cultivares de milho em competição com plantas daninhas. *Planta Daninha, 30*(2), p. 287-296. https://doi.org/10.1590/S0100-83582012000200007

Dayan, F. E., & Duke, S. O. (2006). Clues in the search for new herbicides. In M. J. Reigosa, N. Pedrol, L. González (Eds.), *Allelopathy—A Physiological Process with Ecological Implications* (pp. 63-83). Netherlands, Springer. https://doi.org/10.1007/1-4020-4280-9_3

Denardin, R. B. N., Kraemer, A., Corona, J. M., Bernardi, D., Sobral, L. S., & Moura, N. F. (2018). *Potencial alelopático de extratos de folhas e frutos verdes de feijão de porco (Canavalia ensiformis (L.) DC.)* (Vol. 13, No. 1). X Congresso Brasileiro de Agroecologia, Anais, Brasília.

El Ayeb-Zakhama, A., Sakka-Rouis, L., Flamini, G., Jannet, H. B., & Harzallah-Skhiri, F. (2017). Chemical composition and allelopathic potential of essential oils from *Citharexylum spinosum* L. grown in Tunisia. *Chem Biodivers, 14*(4), e1600225. https://doi.org/10.1002/cbdv.201600225

Favaretto, A. (2018). *Identificação e avaliação de compostos bioativos de capim-annoni em plantas daninhas* (Doctoral dissertation, Universidade de Passo Fundo, Brazil). Retrieved from http://tede.upf.br/jspui/bitstream/tede/1552/2/2018AdrianaFavaretto.pdf

Ferreira, D. F. (2011). SISVAR: A computer statistical analysis system. *Ciência e Agrotecnologia, 35*(6), 1039-1042. https://doi.org/10.1590/S1413-70542011000600001

Ferreira, G. A., & Áquila, M. E. A. (2000). Alelopatia: Uma área emergente da ecofisiologia. *Revista Brasileira de Fisiologia Vegetal, 12*, 175-204, Edição Especial.

Ferro, A. P., Marchiosi, R., Siqueira-Soares, R. C., Bonini, E. A., Ferrarese, M. L. L., & Ferrarese-Filho, O. (2015). Effects of cinnamic and ferulic acids on growth and lignification of maize roots. *J Allel Inter, 1*, 29-38.

Formagio, A. S. N., Masetto, T. E., Baldivia, D. S., Vieira, M. C., Zárate, N. A. H., & Pereira, Z. V. (2010). Potencial alelopático de cinco espécies da família Annonaceae. *Revista Brasileira de Biociências, 8*(4), 349-354.

Formigheiri, F. B., Bonome, L. T. S., Bittencourt, H. V. H., Leite, K., Reginatto, M., & Giovanetti, L. K. (2018). Alelopatia de *Ambrosia artemisiifolia* na germinação e no crescimento de plântulas de milho e soja. *Revista de Ciências Agrárias, 41*(3), 729-739. https://doi.org/10.19084/RCA18074

Galon, L., Santin, C. O., Andres, A., Basso, F. J. M., Nonemacher, F., Agazzi, L. R., … Fernandes, F. F. (2018). Competitive Interaction Between Sweet Sorghum with Weeds. *Planta Daninha, 36*, 1-13. https://doi.org/10.1590/s0100-83582018360100053

Gatti, A. B., Ferreira, A. G., Arduin, M., & Perez, S. C. G. A. (2010). Allelopathic effects of aqueous extracts of *Aristolochia esperanzae* O. Kuntze on development of *Sesamum indicum* L. seedlings. *Acta Botânica Brasílica, 24*, 454-461. https://doi.org/10.1590/S0102-33062010000200016

Goldfarb, M., Pimentel, L. W., & Pimentel, N. W. (2009). Alelopatia: relações nos agroecossistemas. *Tecnol. & Ciên. Agropec., 3*(1), 23-28.

Graña, E., Sotelo, T., Díaz-Tielas, C., Araniti, F., Krasuska, U., Bogatek, R., … Sánchez-Moreiras, A. M. (2013). Citral induces auxin and ethylene-mediated malformations and arrests cell division in *Arabidopsis thaliana* roots. *Journal Chemical Ecology, 39*, 271-282. https://doi.org/10.1007/s10886-013-0250-y

Grigolli, J. F. J., Pereira, F. C. M., Peñaherrera, L. C., Santos, E. A., & Ferreira, M. C. (2011). Controle de *Euphorbia heterophylla* com mesotrione e óleos para diferentes pontas de pulverização. *Revista Brasileira de Herbicidas, 10*(3), 266-276. https://doi.org/10.7824/rbh.v10i3.127

Grisi, P. U., Gualtieri, S. C. J., Anese, S., Pereira, V. C., & Forim, M. R. (2013). Efeito do extrato etanólico de *Serjania lethalis* no controle de plantas daninhas. *Planta Daninha, 31*(2), 239-248. https://doi.org/10.1590/S0100-83582013000200001

Guimarães, L. G. L., Cardoso, M. G., Sousa, P. E., Andrade, J., & Vieira, S. S. (2011). Atividade antioxidante e antifúngica do óleo essencial da capim-limão e citral. *Revista Ciência Agronômica, 42*(2), 464-472. https://doi.org/10.1590/S1806-66902011000200028

Hammer, O., Harper, D. A. T., & Ryan, P. D. (2001). Past: Paleontological Statistic software package for education and data analysis. *Paleontologia Eletronica, 4*(1), 1-9.

Hancock, C. R., Barlow, H. W. B., & Lacey, H. J. (1964). The east malling coleoptile straight growth test method. *Journal of Experimental Botany, 15*(1), 166-17. https://doi.org/10.1093/jxb/15.1.166

Heap, I. (2020). *The International Herbicide-Resistant Weed Database*. Retrieved March 4, 2020, from http://www.weedscience.org/Pages/GeoChart.aspx

Hussain, A. I., Anwar, F., Sherazi, S. H. T., & Przybylski, R. (2008). Chemical composition, antioxidant and antimicrobial activities of basil (*Ocimum basilicum*) essential oils depends on seasonal variations. *Food Chemistry, 108*(3), 986-995. https://doi.org/10.1016/j.foodchem.2007.12.010

IAS (International Allelopathy Society). (2020). Retrieved January 12, 2020, from https://allelopathy-society.osupytheas.fr/about/

IFRA (International Fragance Association). (2009). *Standards, including amendments as of October 14th, 2009*. International Fragrance Association, Brussels.

Imatomi, M., Novaes, P., & Gualtieri, S. C. J. (2013). Interspecific variation in the allelopathic potential of the family Myrtaceae. *Acta Botanica Brasilica, 27*, 54-61. https://doi.org/10.1590/S0102-33062013000100008

Jatobá, L. J., Varela, R. M., González, J. M., Din, Z. U., Gualtieri, S. C. J., Rodrigues-Filho, E., & Macías, F. A. (2016). Allelopathy of Bracken Fern (*Pteridium arachnoideum*): New Evidence from Green Fronds, Litter, and Soil. *Plos One, 11*, e0161670. https://doi.org/10.1371/journal.pone.0161670

Johnson, W., Bergfeld, W. F., Belsito, D. V., Hill, R. A., Klaassen, C. D., Liebler, D. C., … Andersen, F. A. (2017). Safety Assessment of Benzyl Alcohol, Benzoic Acid and its Salts, and Benzyl Benzoate. *International Journal of Toxicology, 36*(3), 5S-30S. https://doi.org/10.1177/1091581817728996

Juchem, F., Lauxen, G. A., Silva, F. B., Denardin, R. B. N., & Sobral, L. S. (2013). Potencial alelopático de diferentes extratos de frutos de adubos verdes sobre a germinação de sementes de couve. *Cadernos de Agroecologia, 8*(2).

Kenany, E. T., & Darier, S. M. (2013). Suppression effects of *Lantana camara* L. aqueous extracts on germination efficiency of *Phalaris minor* Retz. and *Sorghum bicolor* L. (Moench). *Journal of Taibah University for Science, 7*(2), 64-71. https://doi.org/10.1016/j.jtusci.2013.04.004

Kern, K. A., Pergo, E. M., Kagami, F. L., Arraes, L. S., Sert, M. A., & Ishii-Iwamoto, E. L. (2009). The phytotoxic effect of exogenous ethanol on *Euphorbia heterophylla* L. *Plant Physiology and Biochemistry, 47*(2), 1095-1101. https://doi.org/10.1016/j.plaphy.2009.07.002

Knowles, C. O. (1991). Benzyl Benzoate. In Jr. W. J. Hayes & Jr. E. R. Laws (Eds.), *Handbook of Pesticide Toxicology* (Volume 3: Classes of Pesticides). Academic Press, San Diego.

Kroymann, J. (2011). Natural diversity and adaptation in plant secondary metabolism. *Current Opinion in Plant Biology, 14*, 246-251. https://doi.org/10.1016/j.pbi.2011.03.021

Lamego, F. P., Ruchel, Q., Kaspary, T. E., Gallon, M., Basso, C. J., & Santi, A. L. (2013). Habilidade competitiva de cultivares de trigo com plantas daninhas. *Planta Daninha, 31*(3), 521-531. https://doi.org/10.1590/S0100-83582013000300004

Lapczynski, A., Mcginty, D., Jones, L., Bhatia, S., Letizia, C. S., & Letizia, A. M. (2007). Fragrance material review on benzyl salicylate. *Food and Chemical Toxicology, 45*, S362-S380. https://doi.org/10.1016/j.fct.2007.09.036

Leite, F. (2009). Impurezas de degradação. *Scientia Chromatographica, 1*(2), p. 63-72.

Lopes Ovejero, R. F., Soares, D. J., Oliveira, W. S., Fonseca, L. B., Berger, G. U., Soteres, J., & Christoffoleti, P. J. (2013). Residual herbicides in weed management for glyphosate-resistant soybean in Brazil. *Planta Daninha, 31*(4). https://doi.org/ 10.1590/S0100-83582013000400021

Lupini, A., Sorgonà, A., Miller, A. J., & Abenavoli, M. R. (2010). Short-term effects of coumarin along the maize primary root axis. *Plant Signaling & Behavior, 5*, 1395-1400. https://doi.org/10.4161/ psb.5.11.13021

Machado, A. B., Trezzi, M. M. I., Vidal, R. A., Patel, F., Cieslik, L. F., & Debastiani, F. (2015). Rendimento de grãos de feijão e nível de dano econômico sob dois períodos de competição com *Euphorbia heterophylla*. *Planta Daninha, 33*(1). https://doi.org/10.1590/S0100-83582015000100005

Macías, F. A., Lacret, R., Varela, R. M., Nogueiras, C., & Molinillo, J. M. G. (2010). Isolation and phytotoxicity of terpenes from *Tectona grandis*. *Journal of Chemical Ecology, 36*(4), 396-404. https://doi.org/10.1007/s10886-010-9769-3

Mantoani, M. C., & Torezan, J. M. D. (2016). Regeneration response of Brazilian Atlantic Forest woody species to four years of *Megathyrsus maximus* removal. *Forest Ecology and Management, 359*, 141-146. https://doi.org/10.1016/j.foreco.2015.10.004

Marques, R. P., Martins, D., Costa, S. I. A., & Vitorino, H. S. (2012). Densidades de palha e condições de luminosidade na germinação de sementes de *Euphorbia heterophylla*. *Semina: Ciências Agrárias, 33*(3), 867-872. https://doi.org/10.5433/1679-0359.2012v33n3p867

Melo, S. C., Sa, L. E. C., Oliveira, H. L. M., Trettel, J. R., Silva, P. S., Goncalves, J., … Magalhaes, H. M. (2017). Chemical constitution and allelopathic effects of *Curcuma zedoaria* essential oil on lettuce achenes and tomato seeds. *Australian Journal of Crop Science, 11*(7), 906-916. https://doi.org/10.21475/ajcs.17.11.07.pne562

Miranda, C. A. S. F., Cardoso, M. G., Carvalho, M. L. M., Figueiredo, A. C. S., Nelson, D. L. N., Oliveira, C. M., … Albuquerque, L. R. M. (2014). Chemical composition and allelopathic activity of *Parthenium hysterophorus* and *Ambrosia polystachya* weeds essential oils. *American Journal of Plant Sciences, 5*(9), 1248-1257. https://doi.org/10.4236/ajps.2014.59137

Miranda, M. A., Varela, R. M., Torres, A., Molinillo, J. M., Gualtieri, S. C. J., & Macías, F. A. (2015). Phytotoxins from *Tithonia diversifolia*. *J. Nat. Prod., 78*, 1083-1092. https://doi.org/10.1021/acs.jnatprod.5b00040

Nepomuceno, M. (2011). *Intervalo de dessecação de Urochloa ruziziensis (R. German & Evrard) Crins e seu efeito alelopático na cultura da soja RR* (Doctoral dissertation, Universidade Estadual Paulista, Jaboticabal, SP, Brazil). Retrieved from https://repositorio.unesp.br/bitstream/handle/11449/105267/nepomuceno_mp_dr_jabo.pdf

Nitsch, J. P., & Nitsch, C. (1956). Studies on the growth of coleoptiles and first internod sections. A new, sensitive, straight-growth test for auxins. *Plant Physiology, 31*(2), 94-111. https://doi.org/10.1104/pp.31.2.94

Oliveira Jr., R. S., Carneiro, J. C., Constantin, J., Santos, G., Martini, P. E., Francischini, A. C., & Osipe, J. B. (2011). Aplicações isoladas ou associadas de diuron, oxyfluorfen e prometryne para o controle de *Euphorbia heterophylla*. *Planta Daninha, 29*(3), 635-645. https://doi.org/10.1590/S0100-83582011000300018

Oliveira, A. K. M., Pereira, K. C. L., Muller, J. A. I., & Matias, R. (2014). Análise fitoquímica e potencial alelopático das cascas de *Pouteria ramiflora* na germinação de alface. *Horticultura Brasileira, 32*, 41-47. https://doi.org/10.1590/S0102-05362014000100007

Oussalah, M., Caillet, S., Saucier, L., & Lacroix, M. (2007). Inhibitory effects of selected plant essential oils on the growth of four pathogenic bacteria: *E. coli* O_{157}:H_7, *Salmonella typhimurium*, *Staphylococcus aureus* and *Listeria monocytogenes*. *Food Control, 18*(5), 414-420. https://doi.org/10.1016/j.foodcont.2005.11.009

Politycka, B., & Mielcarz, B. (2007). Involvement of ethylene in growth inhibition of cucumber roots by ferulic and p-coumaric acids. *Allelopathy Journal, 19*, 451-460.

Pybus, D. H., & Sell, C. S. (2006). The Chemistry of Fragrances. *From Perfumer to Consumer* (2nd ed.). RSC Publishing, London. https://doi.org/10.1039/9781847555342

Sá, A. G. A., Meneses, A. C., Araújo, P. H. H., & Oliveira, D. (2017). A review on enzymatic synthesis of aromatic esters used as flavor ingredients for food, cosmetics and pharmaceuticals industries. *Trends in Food Science & Technology, 69*, 95-105. https://doi.org/10.1016/j.tifs.2017.09.004

Saito, M. L., & Scramin, S. (2000). *Plantas aromáticas e seu uso na agricultura* (Documentos 20, p. 48). Embrapa Meio Ambiente. Jaguariúna: EMPRABA.

Salavastru, C. M., Chosidow, O., Boffa, M. J., Janier, M., & Tiplica, G. S. (2017). European guideline for the management of scabies. *J Eur Acad Dermatol Venereol, 31*(8), 1248-53. https://doi.org/10.1111/jdv.14351

Salavastru, C. M., Chosidow, O., Janier, M., & Tiplica, G. S. (2017). European guideline for the management of pediculosis pubis. *J Eur Acad Dermatol Venereol, 3*(9), 1425-1428. https://doi.org/10.1111/jdv.14420

Silva, B. P., Nepomuceno, M. P., Varela, R. M., Torres, A., Molinillo, J. M. G., Alves, P. L. C. A., & Macías F. A. (2017). Phytotoxicity Study on *Bidens sulphurea* Sch. Bip. as a Preliminary Approach for Weed Control. *J. Agric. Food Chem., 65*, 5161-5172. https://doi.org/10.1021/acs.jafc.7b01922

Soltys, D., Rudzińska-Langwald, A., Kurek, W., Gniazdowska, A. Sliwinska, E., & Bogatek, R. (2011). Cyanamide mode of action during inhibition of onion (*Allium cepa* L.) root growth involves disturbances in cell division and cytoskeleton formation. *Planta, 234*, 609-621. https://doi.org/10.1007/s00425-011-1429-5

Souza Filho, A. P. S., Guilhon, G. M. S. P., & Santos, L. S. (2010). Metodologias empregadas em estudos de avaliação da atividade alelopática em condições de laboratório: Revisão crítica. *Planta Daninha, 28*(3), 689-697. https://doi.org/10.1590/S0100-83582010000300026

Taiz, L., Zeiger, E., Moller, I., & Murphy, A. (2017). *Fisiologia e desenvolvimento vegetal* (6th ed., p. 888). Porto Alegre: Artmed.

Tello, C. C. (2014). Las Plantas alelopáticas. *Ciencias de la Vida*. Retrieved from http://repositorio.espe.edu.ec/handle/21000/9218

Trezzi, M. M., Machado, A. B., & Xavier, E. (2014). Soja: Impacto do leiteiro e estratégias de manejo. *Cultivar, 183*, 8-10.

Trezzi, M. M., Xavier, E., Vidal, R. A., Portes, E. D. S., Vottri, M., & Lamego, F. P. (2011). Teste rápido de imersão foliar de *Euphorbia heterophylla* para confirmação de resistência a herbicidas inibidores da Protox e da ALS. *Planta Daninha, 29*, 901-12. https://doi.org/10.1590/S0100-83582011000400021

Vasconcelos, M. C. C., Silva, A. F. A., & Lima, R. S. (2012). Interferência de plantas daninhas sobre plantas cultivadas. *Agropecuária Científica no Semiárido, 8*(1), 01-06. https://doi.org/10.30969/acsa.v8i1.159

Xavier, E., Trezzi, M. M., Oliveira, M. C., Vidal, R. A., & Brusamarelo, A. P. (2018). Activity of Antioxidant Enzymes in *Euphorbia heterophylla* Biotypes and their Relation to Cross Resistance to ALS and Protox Inhibitors. *Planta Daninha, 36*, 1-14. https://doi.org/10.1590/s0100-83582018360100138

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