

Phytotoxicity of *Piper marginatum* Jacq. essential oil on detached leaves and post-emergence of plants¹

Fitotoxicidade do óleo essencial de *Piper marginatum* Jacq. em folhas destacadas e em pós-emergência de plantas

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HIGHLIGHTS:

Piper marginatum essential oil has phytotoxic activity on lettuce, pepper, tomato, and *Spermacoce verticillata*.

Piper marginatum essential oil at 1% concentration completely destroyed the tested plants' leaves.

In post-emergence, the *Piper marginatum* essential oil concentrations assessed had no effect on plant development.

ABSTRACT: Essential oils used as bioherbicides are a promising alternative in agriculture. This study aimed to assess the phytotoxic potential of *Piper marginatum* essential oil through an *in vitro* study with detached and post-emergence leaves of three commercial species (lettuce, pepper, and tomato) and a weed (*Spermacoce verticillata*). The experiments were conducted in a randomized design with six treatments that consisted of four concentrations of essential oil (0.001, 0.01, 0.1, and 1% [v/v]) and two control treatments (distilled water, and 1% polysorbate 80 in distilled water). The *P. marginatum* essential oil had a phytotoxic effect on the leaves of the experimental species. The highest essential oil concentration resulted in greater toxicity effects on tomato, lettuce, and pepper leaves. The 0.1% essential oil concentration completely destroyed the *S. verticillata* leaves. Seven days after application, the *P. marginatum* essential oil did not show adverse effects on plant growth in the post-emergence phase. It is recommended to investigate its possible phytotoxic activity in other phases of plant development to understand its mode of action and potential as an alternative to developing bioproducts for weed control.

Key words: *Spermacoce verticillata*, bioherbicide activity, Piperaceae, sustainability

RESUMO: Óleos essenciais usados como bioherbicidas são uma alternativa promissora na agricultura. O objetivo deste trabalho foi avaliar o potencial fitotóxico do óleo essencial de *Piper marginatum*, através de estudo *in vitro* com folhas destacadas e em pós-emergência de três espécies comerciais (alface, pimentão e tomate) e uma planta daninha (*Spermacoce verticillata*). Os experimentos foram conduzidos em delineamento casualizado com seis tratamentos que consistiram em quatro concentrações de óleo essencial (0,001; 0,01; 0,1 e 1% [v/v]), e dois tratamentos controle (água destilada, e 1% de polissorbatato 80 em água destilada). O óleo essencial de *P. marginatum* apresentou efeito fitotóxico sobre as folhas das espécies experimentais. A maior concentração de óleo essencial resultou em maiores efeitos de toxicidade nas folhas de tomate, alface e pimentão. A concentração de 0,1% de óleo essencial foi suficiente para destruir completamente as folhas de *S. verticillata*. Sete dias após a aplicação, o óleo essencial de *P. marginatum* não evidenciou efeitos adversos no crescimento das plantas na fase pós-emergência. Recomenda-se investigar sua possível atividade fitotóxica em outras fases do desenvolvimento vegetal, para entender o seu modo de ação e potencial como alternativa no desenvolvimento de bioprodutos para controle de plantas daninhas.

Palavras-chave: *Spermacoce verticillata*, atividade bioherbicida, Piperaceae, sustentabilidade

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INTRODUCTION

In agriculture, the intensive use of herbicides to control weeds can harm the environment and human health and favor the development of weed resistance (Gomes et al., 2019). In this way, new strategies are needed to control these species. Plant essential oils (EOs) are one of the natural and promising alternatives for developing bioherbicides since they are natural products and easily degrade when introduced into the environment (Han et al., 2021).

Several studies have demonstrated the potential of EOs in weed control, presenting satisfactory results. For example, the *Artemisia fragrans* Willd. EO effectively reduced germination and growth of *Convolvulus arvensis* L. (Pouresmaeil et al., 2020). Another study showed that the *Carlina acaulis* L. EO presented phytotoxic activity against adult plants of *Bidens pilosa* L., causing leaf necrosis, dehydration (reduction in relative water content), decrease in total leaf area, and increase in the dry weight/fresh weight ratio, as well as damaging the photosynthetic machinery and reducing the plant photosynthetic efficiency (Álvarez-Rodríguez et al., 2023).

Moreover, the *Eucalyptus falcata*, *Eucalyptus sideroxylon*, and *Eucalyptus citriodora* EOs showed a phytotoxic potential effect on germination and growth (shoot and root of germinated seeds) of the weeds *Sinapis arvensis* and *Phalaris canariensis*. They also showed herbicidal efficacy over the post-emergence stage (Amri et al., 2023). Species of the genus *Piper* have assumed a prominent position in research in chemistry and biotechnology (Oliveira et al., 2020). Among them, *Piper marginatum* Jacq., popularly known in Brazil as *malvaíscó*, *pimenta do mato*, and *caapeba cheirosa* (Autran et al., 2009), have shown several biological activities of their EO, including the antioxidant activity (Feitosa et al., 2023), insecticide (Ayres et al., 2021), fungicide (Santos et al., 2011), acaricide (Ayres et al., 2023), ovicidal (Guedes et al., 2020), larvicide (Pereira Filho et al., 2021), antimicrobial (Santos et al., 2021), antibacterial (Carvalho et al., 2022) and antiparasitic (Macêdo et al., 2020). However, there is still a lack of information on its phytotoxic activity.

The use of *P. marginatum* EO can be expanded through new research, such as developing formulations for weed control in crops. Thus, this study aimed to evaluate the phytotoxic potential of *P. marginatum* EO through an *in vitro* study with detached and post-emergence leaves of three commercial species (lettuce, pepper, and tomato) and a weed (*Spermacoce verticillata*).

MATERIAL AND METHODS

Expanded leaves from adult *P. marginatum* plants were randomly collected in an urban area in the municipality of Itacoatiara, state of Amazonas, Brazil (3° 08' 41.56" S, 58° 26' 56.35" W, and an average altitude of 28 m). Data collection was conducted in the morning. Fertile branches were herborized and deposited in the Herbarium of the Center for Higher Studies of Itacoatiara - CESIT, belonging to the State University of Amazonas (UEA), under number 0034. The species identification was confirmed through determination

keys and comparative analysis in a collection analyzed by plant family experts.

The collected leaves were immediately scraped and subjected to hydrodistillation using a modified Clevenger (Silveira et al., 2012) apparatus coupled to a round-bottom flask with a capacity of 5 L for three hours. Extractions were conducted in triplicates, with approximately 500 g of material in each flask. The OE obtained was stored in an amber glass bottle, sealed, and stored at -4 °C until the experiments were conducted.

The studies on phytotoxicity of the *P. marginatum* essential oil (EO) in lettuce (*Lactuca sativa* L.), pepper (*Capsicum annuum* L.), tomato (*Solanum lycopersicum* L.), and the weed *Spermacoce verticillata* L. were conducted between September 2021 and June 2022. The choice of these species was based on their specific features. Lettuce and tomato are recognized as indicators of allelopathic activity due to their rapid and uniform germination and sensitivity to low allelochemicals concentrations, making it easier to detect phytotoxic effects (Ferreira & Aquila, 2000; Alves et al., 2004). Pepper was included to diversify the evaluation, considering its closer taxonomic relation with tomato. *S. verticillata* was chosen as a weed representative, highlighting the importance of these plants' control in the study of EO phytotoxicity.

The first stage of the study consisted of a bioassay of the phytotoxic effect of *P. marginatum* EO on detached leaves, being conducted in the laboratory (3° 08' 34" S, 58° 25' 53" W, and an average altitude of 25.2 m). The second stage was a post-emergence bioherbicide test in a greenhouse (3° 08' 31" S, 58° 25' 54" W, and an average altitude of 21.73 m), both conducted in the experimental field of the Federal University of Amazonas (UFAM).

The bioassay of the phytotoxic effect of *P. marginatum* OE on detached leaves was conducted in a completely randomized design with six treatments and four replicates. EO concentrations were prepared using 1% polysorbate 80 (Tween 80) as the surfactant, and the final volume was obtained with distilled water, with the following concentrations: 0.001, 0.01, 0.1, and 1% (v/v). These concentrations were based on the methodology described by Rosado et al. (2009). Furthermore, two controls were included in the test: 1) 1% polysorbate 80 in distilled water and 2) distilled water only.

Well-expanded leaves of lettuce, pepper, and tomato were sectioned at the base of the petiole after 15 days of growth, and *S. verticillata* when it had four definitive leaves. Treatments were applied immediately after sectioning at the base of the petiole. The sectioned petiole was wrapped with cotton and soaked in 2 mL of treatment solutions with an automatic pipette. The leaves were placed in seed germination boxes in a germination chamber at 25 °C and a 12/12 hour photoperiod (Toderio et al., 2019). Phytotoxicity was evaluated three, six, and nine days after the application (DAA) of treatments, based on the visual scale adapted from Frans et al. (1986) (Table 1).

In the bioassay to evaluate post-emergence phytotoxicity, the same species as in the bioassay on detached leaves were evaluated.

The experimental design was completely randomized with six treatments (two controls: 1% polysorbate 80 (Tween

Table 1. Scale to evaluate phytotoxicity over plants

%	Main categories	Description of the plant phytotoxicity
0	No effect	No harm or reduction
10		Mild discoloration or atrophy
20	Light effect	Some discoloration or atrophy
30		More pronounced injury but not lasting
40		Moderate injury, usually with recovery
50	Moderate effect	Longer lasting injury, doubtful recovery
60		Lasting injury, no recovery
70		Heavy injury, stand reduction
80	Severe effect	Plant close to destruction
90		There are rarely any plants left
100	Total effect	Complete destruction of the plant

Source: Adapted from Frans et al. (1986)

80) in distilled water and 2) distilled water only, and four concentrations of essential oil) and ten replicates.

Each experimental unit consisted of a 200 mL polyethylene recipient filled with commercial substrate Vivatto Slim Plus™ composed of pine bark (biostabilized), vermiculite, charcoal mill, water, phenolic foam, and additives. The additives included 1.50% fertilizers and 0.20% soil conditioners. The fertilizers consisted of 1.31% NPK formulation (4-14-8) and 0.19% simple superphosphate, while the soil conditioner contained calcium silicate (18.6% Ca) and magnesium (8.7% Mg). Three seeds were sown per pot, and after presenting a pair of definitive leaves, thinning was done, leaving the most vigorous seedling for the treatment application. Irrigation was daily.

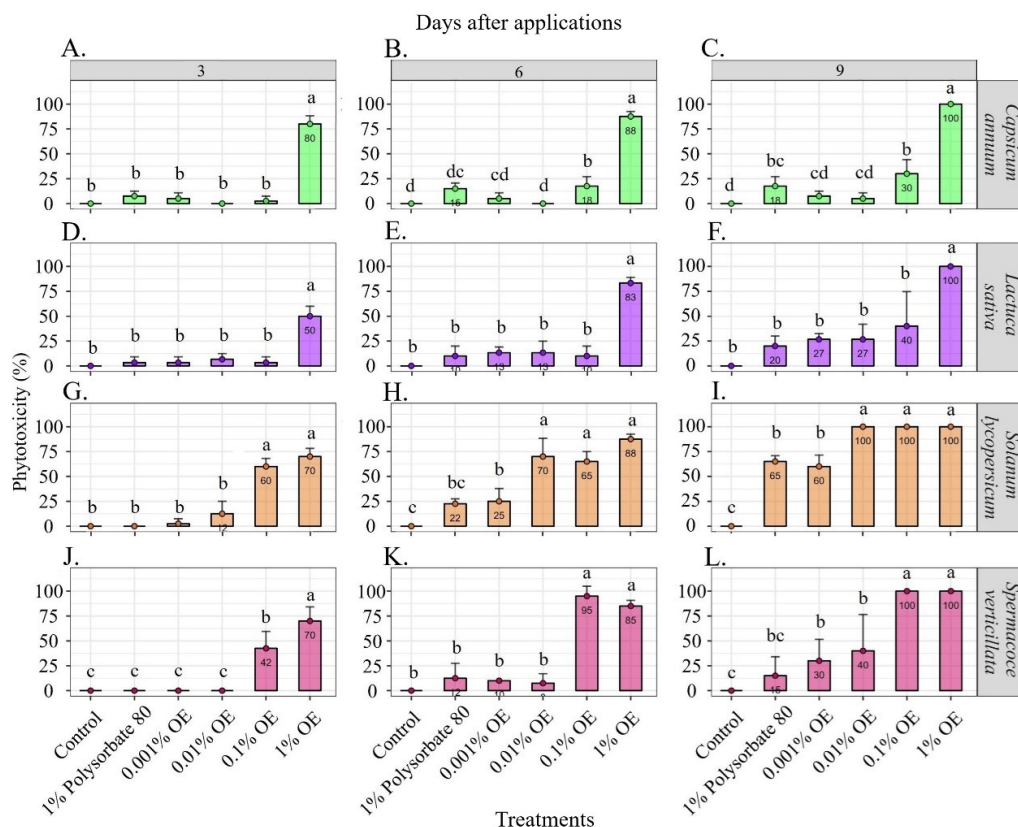
The treatments were applied 15 days after emergence (DAE), except for *S. verticillata*, where the treatments were

applied when they had four definitive leaves. A manual pressurized sprayer was used for the application, with 50 mL of treatment solutions applied (Brun et al., 2016). Seven days after application, plant damage was estimated as a percentage reduction in growth compared to untreated controls, where 100% represents complete death and 0% no effects (Frans et al., 1986). The following variables were also measured: plant height (cm) and shoot and root dry mass (mg). Dry mass was obtained after drying in an oven with air circulation of 40 L at 70 °C until reaching constant weight. A Semi-Analytical Balance (Bel 0.001 g, 310 g L303iH) was used to measure weight.

For statistical analyses, assumptions of analysis of variance were checked: normality with the Shapiro-Wilk test, visualization with the Q-Q plot, and homoscedasticity using the Bartlett test. The means were compared by the Tukey test. The variables' height' of lettuce and pepper, 'shoot dry weight' of lettuce, "root dry weight" of *S. verticillata*, as well as phytotoxicity of lettuce, pepper, tomato, and *S. verticillata* did not meet the assumptions, so they were subjected to Kruskal-Wallis non-parametric analysis of variance. For the analyses, the R software version 4.2.3 (R Core Team, 2023), at $p \leq 0.05$.

RESULTS AND DISCUSSION

Applying different concentrations of *P. marginatum* EO showed a phytotoxic effect on all species studied. On the first day of evaluation, there were injuries from 50 to 80% caused by EO at 1%, among the species studied (Figures 1A, D, G, and J). These values increased six days after application, ranging from



Bars with the same letter do not differ according to the Tukey test ($p \leq 0.05$)

Figure 1. Effect of *Piper marginatum* essential oil (EO) concentrations on the percentage of phytotoxicity in detached leaves of pepper (*C. annuum*) (A, B, and C), lettuce (*L. sativa*) (D, E, and F), tomato (*S. lycopersicum*) (G, H, and I), and *S. verticillata* (J, K, and L) at three, six, and nine days after the application of the treatments

83 to 88% (Figures 1B, E, H, and K). At nine DAA, all species showed 100% phytotoxicity at 1% (Figures 1C, F, I, and L). However, at a concentration of 0.1% EO, only *S. verticillata* and tomato (*S. lycopersicum*) showed 100% phytotoxicity (Figures 1L and I). Pepper (*C. annuum*) and lettuce (*L. sativa*) showed, respectively, 30 and 40% phytotoxicity (Figures 1C and F).

In tomatoes, concentrations of 0.01, 0.1, and 1% of *P. marginatum* EO caused 100% phytotoxicity at nine DAA, resulting in complete plant destruction on the ninth day after application (Figures 1I and 2A).

The concentration of 1% EO also caused strong phytotoxicity effects on the leaves of pepper (Figures 1A, B, C, and 2C) and lettuce (1D, E, F, and 2B) on all days evaluated. EO at a concentration of 0.1% was capable of causing more foliar damage in *S. verticillata* on the 6 and 9th day of evaluation. This concentration was sufficient to cause leaves death (Figures 1K, L, and 2D).

Potential phytotoxic effects varied according to the EO concentration and the species studied, with 1% EO causing 100% phytotoxicity at nine DAA in all crops assessed. In *S. verticillata* and tomato, 100% phytotoxicity was also observed at lower concentrations (0.1% in *S. verticillata* and 0.01 and 0.1% in tomato) at nine DAA.

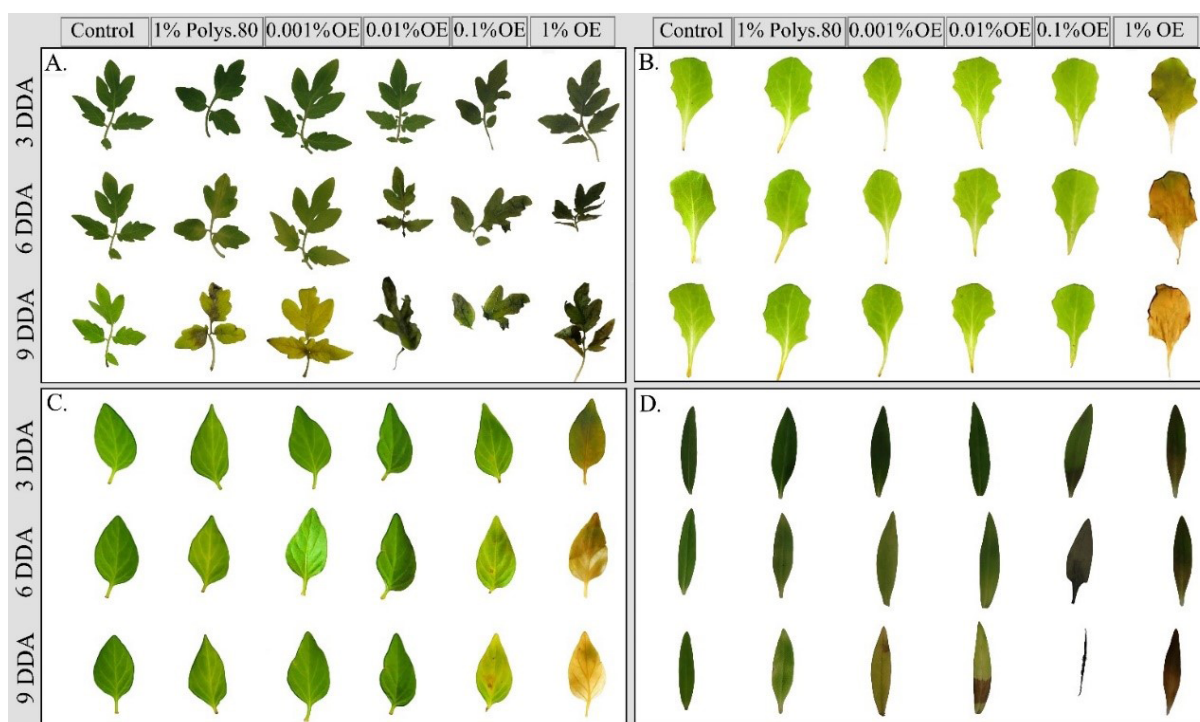
The phytotoxic effect of *P. marginatum* EO can be related to the presence of one or more compounds and their concentrations. When evaluating the chemical composition of the EO obtained from *P. marginatum* leaves in Itacoatiara, Amazonas, Ayres et al. (2021) identified 25 constituents, with hydrocarbon sesquiterpenes being the most prevalent, followed by phenylpropanoids, hydrocarbon monoterpenes, and oxygenated sesquiterpenes. The majority of constituents in the sample were: 3,4-methylenedioxypropylphenone (10.4%), bicyclogermacrene (10.1%), and germacrene

D (9.9%) (Ayres et al., 2021). Considering the Pará state (Brazil), the EO extracted from this same species presented 3,4-methylenedioxypropylphenone (22.90%), δ -3-carene (10.19%), trans-caryophyllene (9.67%), and spathulenol (6.89%) as main constituents (Macêdo et al., 2020). Exalatacin (9.12%), α -pinene (8.45%), α -phellandrene (6.97%), β -pinene (6.51%), (E)-caryophyllene (5.71%), limonene (4.98%), (E)-asarone (3.53%), and two other undetermined compounds (accounting for 22.19% and 5.50%), were the main constituents of EO obtained from *P. marginatum* in the state of Pernambuco, Brazil (Guedes et al., 2020).

Essential oils constitute a complex mixture of substances that can vary in modes of action under plants. Maes et al. (2021) report that EO can rupture the cuticle and cause desiccation or burning of young tissues. It can also affect photosynthesis and inhibit mitochondrial respiration, alter enzymatic and phytohormonal regulation, alter water status, induce microtubule rupture and genotoxicity, induce reactive oxygen and nitrogen species, and alter cell membrane properties and interactions.

Han et al. (2021) evaluated the phytotoxic effect of *Ambrosia artemisiifolia* EO and observed lesions such as discoloration, chlorosis, necrosis, and complete wilting in *Poa annua*, *Setaria viridis*, *Amaranthus retroflexus*, and *Medicago sativa*, when subjected to 5.00 mg mL⁻¹ of OE. Chlorosis occurs due to reduced chlorophyll content, which may be associated with inhibiting chlorophyll synthesis and/or its degradation (Han et al., 2021).

Concentrations of 2, 4, and 6% of *Artemisia scoparia* EO caused lesions on *Achyranthes aspera*, *Cassia occidentalis*, *Parthenium hysterophorus*, *Echinochloa crus-galli*, and *Ageratum conyzoides*, such as chlorosis, necrosis, and complete wilting in plants depending on the species and concentration



Polys- Polysorbate 80; DAA- Days after application; OE- Essential oil

Figure 2. Visual effects of the application of *Piper marginatum* essential oil concentrations in detached leaves of tomato (*S. lycopersicum*) (A), lettuce (*L. sativa*) (B), pepper (*C. annuum*) (C), and *S. verticillata* (D) at three, six, and nine days after application

of EO applied (Kaur et al., 2010). Symptoms ranged from chlorosis to reduced chlorophyll content and wilting due to excessive electrolyte leakage due to the disruption in membrane integrity (Kaur et al., 2010). The symptoms severity promoted by EOs can be related to their ability to rupture the membrane, triggering increased permeability, solutes leakage, and constant stomata opening (Ootani et al., 2017).

None of the EO concentrations in tomatoes significantly affected the growth variables studied, and neither promoted significant leaf damage (Table 2). In lettuce and *S. verticillata*, only phytotoxicity responded significantly to the application of EO in the concentration of 1%, causing an average of 62 and 10% of foliar damage, respectively (Table 2). The main symptoms of phytotoxicity in plants were chlorosis and necrosis.

Awojide et al. (2021), analyzing four concentrations (1, 2, 3, and 4 mL L⁻¹) of *Piper nigrum* EO on corn, tomato, and *Vigna unguiculata*, identified a 100% phytotoxic effect in *V. unguiculata* under the concentration of 2 mL L⁻¹, complete toxicity in corn with 3 mL L⁻¹, and a foliar effect of 40% in tomato seedlings when exposed to the highest concentration (4 mL L⁻¹) of EO tested. According to Kaur et al. (2010) and Ootani et al. (2017), the use of EO in post-emergence on the leaf surface can cause necrosis and chlorosis in sprayed leaves due to the herbicidal properties that some EOs have. The concentration of OE also influences the type and severity of the damage; concentrations greater than 4% of *Artemisia scoparia* EO, for example, caused necrosis, chlorosis, and wilting of leaves (Kaur et al., 2010).

In pepper, the shoot dry mass and phytotoxicity were not significantly affected by EO at any of the concentrations assessed. The largest EO concentration reduced plant height and root dry mass compared to the 0.001 and 0.01% concentrations. Nevertheless, they showed no statistical difference compared to the 0.1% concentration, the control, and the treatment with 1% polysorbate 80 in both variables (Table 2).

Studies indicate a higher phytotoxic potential of EOs in the weeds post-emergence. Pouresmaeil et al. (2020) found that *Artemisia fragrans* EO, especially at the 4% concentration, reduced several growth parameters, including the shoot, root length, as well as the fresh and dry weight of the shoot and root of *Convolvulus arvensis*. Ootani et al. (2017) observed that a concentration of 20% of EO derived from *Cymbopogon nardus* and *Eucalyptus citriodora* reduced by more than 50% the shoot and root dry mass of *Digitaria horizontalis* and *Cenchrus echinatus*. The authors concluded that the phytotoxic effects of these EOs and the citronellal compound are probably associated with impaired photosynthesis and respiration, significantly impacting metabolic processes in germination, seedling development, and growth in later plant stages.

Other species of the genus *Piper* have shown inhibitory effects on seed germination and initial seedling development. Souza Filho et al. (2009) investigated the inhibition effects of the *Piper hispidinervium* EO on germination and development of the radicle and hypocotyl of two weeds (*Mimosa pudica* and *Senna obtusifolia*). The authors found that an EO concentration of 1% promoted greater inhibitions. Pinheiro et al. (2016) described the harmful effects of *Piper callosum*

Table 2. Plant height (cm), shoot dry mass (SDM) (mg), root dry mass (RDM) (mg), and phytotoxicity (phytotox.) (%) in tomato (*S. lycopersicum* L.), lettuce (*L. sativa* L.), pepper (*C. annuum* L.), and *S. verticillata* L.

	Treatment	Plant height (cm)	SDM (mg)	RDM (mg)	Phytotox. (%)
<i>Solanum lycopersicum</i>	Control	15.0 a	152.2 a	39.2 a	0 a
	1% Polysorbate 80	15.9 a	134.5 a	27.4 a	4 a
	0.001% OE	16.1 a	138.8 a	34.4 a	3 a
	0.01% OE	16.4 a	163.2 a	40.0 a	3 a
	0.1% OE	17.2 a	161.6 a	33.6 a	0 a
	1% OE	17.0 a	155.4 a	31.4 a	1 a
	CV (%)	32.8	35.8	51.0	-
<i>Lactuca sativa</i>	Control	7.5 a	75.6 a	26.7 a	0 d
	1% Polysorbate 80	9.5 a	99.8 a	24.2 a	5 cd
	0.001% OE	9.5 a	107.0 a	31.0 a	5 cd
	0.01% OE	8.1 a	77.3 a	29.0 a	9 bc
	0.1% OE	8.2 a	80.2 a	23.9 a	20 b
	1% OE	8.7 a	84.2 a	19.1 a	62 a
	CV (%)	-	-	43.2	-
<i>Capsicum annuum</i>	Control	5.0 ab	33.7 a	6.6 ab	0 a
	1% Polysorbate 80	4.8 ab	32.9 a	6.8 ab	6 a
	0.001% OE	5.4 a	43.7 a	12.5 a	0 a
	0.01% OE	5.2 a	41.9 a	12.5 a	6 a
	0.1% OE	4.6 ab	34.6 a	9.1 ab	6 a
	1% OE	4.1 b	27.0 a	2.0 b	5 a
	CV (%)	-	11.5	66.6	-
<i>Spermacoce verticillata</i>	Control	6.6 a	18.6 a	4.8 a	0 b
	1% Polysorbate 80	6.3 a	18.1 a	5.5 a	0 b
	0.001% OE	6.2 a	15.8 a	5.8 a	0 b
	0.01% OE	6.7 a	18.1 a	2.1 a	0 b
	0.1% OE	6.8 a	20.0 a	5.1 a	0 b
	1% OE	5.7 a	18.0 a	2.9 a	10 a
	CV (%)	17.0	9.2	-	-

OE - Essential oil; Means followed by the same letter in the columns do not differ by the Tukey test ($p \leq 0.05$)

and *Piper aduncum* extracts in different concentrations on the germination and initial development of lettuce seedlings. Lustosa et al. (2007) found that *Piper aduncum* and *Piper tectoniifolium* extracts inhibited germination and radicle growth of lettuce seedlings, especially at the highest concentration (5%).

The possible phytotoxic activity of *P. marginatum* EO can be better investigated in other phases of plant development, providing a more in-depth understanding of its action and potential as an alternative in developing bioproducts for weed control.

CONCLUSIONS

1. *Piper marginatum* essential oil did not have harmful effects on plant growth in the post-emergence phase.
2. The effects promoted by *Piper marginatum* essential oil were more pronounced in leaves under *in vitro* conditions, being tomato the species most susceptible.

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