JOSYELEM TIBURTINO LEITE CHAVES

POTENCIAL BIO-HERBICIDA DE *Vanillosmopsis arborea* Baker

LAVRAS, MG

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Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Agronomia/Fisiologia Vegetal, para obtenção do título de Doutor.

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LAVRAS, MG
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APROVADA em 17 de dezembro de 2020.
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DEDICATÓRIA

Dedico aos meus pais, Estanislau e Bernadete, meus maiores incentivadores.

Aos meus sobrinhos, Yuri, Renan, Sofia e Estevan, por iluminarem o meu caminho e serem a força nos piores momentos.

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RESUMO

O uso indiscriminado de herbicidas sintéticos no controle de plantas daninhas pode resultar em severos danos ao ambiente e à saúde humana. Uma alternativa é o uso de bio-herbicidas produzidos a partir de metabólitos secundários de plantas, cujos compostos apresentam potencial aleloquímico. Os óleos essenciais contêm aleloquímicos que afetam o crescimento das plantas-alvo por meio da modificação de processos fisiológicos essenciais para a germinação e crescimento destas. O potencial alelopático do óleo essencial de *Vanillosmopsis arborea* Baker (candeeiro), planta endêmica da Chapada do Araripe, Crato, Ceará, Brasil, foi relatado na supressão da germinação de espécies modelo sendo, portanto, um candidato à prospecção de bio-herbicida. Porém não se conhece a ação deste óleo na pré e pós-emergência de plantas daninhas e cultivadas. Este potencial pode estar relacionado ao seu componente majoritário, o sesquiterpeno α-bisabolol, ou à sinergia de todos os seus componentes. Desta forma, faz-se necessário conhecer a ação do óleo essencial e a ação isolada do α-bisabolol em plantas daninhas e espécies cultivadas. Assim, objetivou-se com este trabalho investigar se, e como o óleo essencial de *V. arborea* e o α-bisabolol afetam a pré e pós-emergência em distintas espécies. O óleo essencial de *V.* arborea foi extraído de sua madeira pelo processo de hidrodestilação e a molécula α-bisabolol foi adquirida comercialmente na Sigma-Aldrich-Merck®. Ambos foram diluídos em água deionizada para se obter as concentrações (0,125; 0,25; 0,5; 0,75; e 1%). No capítulo 1, o óleo essencial foi aplicado nas sementes de espécies alvo (*Bidens pilosa* L., *Cenchrus echinatus* L., *Cyperus disfformis* L., *Desmodium tortuosum* (Sw.) DC. e *Senna occidentalis* (L.) Link) e não-alvo (*Lactuca sativa* L. e *Oryza sativa* L.). A Percentagem de germinação (PG), índice de velocidade de germinação (IVG), e comprimento do caule e raiz de diferentes espécies foram afetados significativamente pelos tratamentos. As espécies apresentaram diferentes níveis de sensibilidade ao óleo, sendo as espécies daninhas *B. pilosa*, *C. echinatus*, e *C. disfformis*, as mais sensíveis e a espécie cultivada, *O. sativa*, a menos sensível. O índice mitótico das células do meristema das raízes de *L. sativa* foi reduzido pela ação do óleo essencial de *V. arborea*. No segundo capítulo foram aplicadas diferentes concentrações do óleo ou do α-bisabolol nas sementes de duas espécies selecionadas no experimento anterior. o óleo essencial reduziu o PG e IVG, atividade da α-amilase e induziu um desbalanço redox em sementes da espécie daninha *Senna occidentalis*. O α-bisabolol induziu um estresse oxidativo em plântulas de *S. occidentalis* reduzindo o crescimento e induzindo necrose nestas. As sementes e plântulas da espécie cultivada *O. sativa* apresentaram um desbalanço redox, mas em menor proporção que a espécie daninha. No terceiro capítulo, a concentração 0.5% do óleo ou do α-bisabolol foram aplicados em plantas das espécies selecionadas no experimento 1. Os tratamentos induziram redução da fotossíntese nas plantas de *S. occidentalis*. Porém as plantas *O. sativa* foram minimamente influenciadas pelos tratamentos. Diante desses resultados conclui-se que óleo essencial de *V. arborea* e o α-bisabolol apresentaram ação seletiva e promissora para uso como bio-herbicida.

Palavras-chave: Plantas daninhas; óleo essencial; α-bisabolol, fitotóxico; citotóxico; sistema antioxidante

ABSTRACT

The indiscriminate use of synthetic herbicides to control weeds can result in severe damage to the environment. An alternative is the use of bioherbicides produced from secondary plant metabolites, which compounds have allelochemical potential. Essential oils contain allelochemicals that affect the growth of target plants, through the modification of physiological processes that are essential for their germination and growth. The allelopathic potential of the essential oil from *Vanillosmopsis arborea* Baker ("candeeiro"), an endemic plant from Chapada of Araripe, Crato, Ceará, Brazil, has been reported in suppressing the germination of model species; and it is therefore a candidate for prospecting for bioherbicide. However, the action of this essential oil in the pre and post-emergence of weeds and crops is unknown. This potential may be related to its major component, the sesquiterpene α-bisabolol, or to the synergy of all its components. Thus, it is necessary to know the action of the essential oil and the isolated action of α-bisabolol on weeds and crops. Hence, this work aimed to investigate if and how the essential oil of *V. arborea* and α-bisabolol affect pre and post-emergence in different species. The essential oil of *V. arborea* was extracted from its wood by the hydrodistillation process and the α-bisabolol molecule was obtained commercially from Sigma-Aldrich-Merck®. Both were diluted in deionized water to obtain the concentrations (0.125; 0.25; 0.5; 0.75; and 1%). In chapter 1, the essential oil was applied to the seeds of target (*Bidens pilosa* L., *Cenchrus echinatus* L., *Cyperus disfformis* L., *Desmodium tortuosum* (Sw.) DC. and *Senna occidentalis* (L.) Link) and non-target species (*Lactuca sativa* L. and *Oryza sativa* L.). The germination percentage (GP), germination speed index (GSI), and shoot and root length of different species were significantly affected by the treatments. The species showed different levels of sensitivity to the essential oil, *B. pilosa*, *C. echinatus*, and *C. disfformis* were the most sensitive and the cultivated species *O. sativa* the least sensitive. The mitotic index of cells from roots meristem of *L. sativa* was reduced by the action of the essential oil of *V. arborea*. In the chapter II, different concentrations of essential oil or α-bisabolol were applied to the seeds of two species selected in the previous experiment. The essential oil reduced the GP, GSI and α-amylase activity and induced a redox imbalance in seeds of the weed species *Senna occidentalis*. α-bisabolol induced oxidative stress in *S. occidentalis* seedlings, reducing growth and inducing necrosis. The seeds and seedlings of the cultivated species *O. sativa* showed a redox imbalance, but with a lesser extent than the weed species. In the chapter III, the 0.5% concentration of oil or α-bisabolol was applied to the species selected in experiment 1. The treatments induced a reduction in photosynthesis in *S. occidentalis* plants. However, *O. sativa* plants were minimally influenced by the treatments. It is concluded, that the essential oil of *V. arborea* and α-bisabolol showed a selective and promising action for its use as a bioherbicide.

Keywords: Weeds; Essential oil; α-bisabolol; Phytotoxic; Cytotoxic, Antioxidant system

SUMÁRIO

PRIMEIRA PARTE

1. INTRODUÇÃO

A utilização de herbicidas sintéticos é uma alternativa no controle de plantas daninhas, por outro lado, está relacionado a riscos potenciais ao ambiente. Além disso, o número de espécies daninhas resistentes aos herbicidas tem crescido, reduzindo assim a eficiência desses produtos. Uma alternativa seria o uso de herbicidas produzidos a partir dos metabólitos de plantas, tais como os óleos essenciais. Estes têm sido foco de várias pesquisas no âmbito agrícola pelo seu potencial no controle de plantas daninhas e pela busca por uma maior segurança alimentar e menores riscos ao meio ambiente. O primeiro passo na prospecção de um bio-herbicida consiste na avaliação da atividade fitotóxica, traduzida em variáveis morfofisiológicas nas espécies-alvo (PUIG et al., 2018).

Do ponto de vista fisiológico, os bio-herbicidas produzidos a partir de óleos essenciais podem causar danos em diferentes alvos no metabolismo da planta daninha. Dentre os prejuízos estão danos ao DNA, modificação de processos bioquímicos, acréscimo na produção de espécies reativas de oxigênio, supressão do metabolismo antioxidante, redução na atividade da α-amilase, mudanças na estrutura e mitose celular, redução da fotossíntese, dentre outros (RADHAKRISHNAN; ALQARAWI; ABD-ALLAH, 2018).

Estudos têm sido desenvolvidos com o óleo essencial de *Vanillosmopsis arborea* Baker (candeeiro), família Asteraceae, planta de porte arbóreo endêmica da Chapada do Araripe, Crato, Ceará. O óleo essencial de *V. arborea* possui valor econômico e medicinal e seu uso tem sido investigado como anti-inflamatório, antibacteriano, antifúngico, analgésico e anti-leishmania (LORENZI; MATOS, 2008; COLARES et al., 2013; MARCO et al., 2015). O α-bisabolol, componente majoritário do óleo essencial de *V. arborea*, é um sesquiterpeno utilizado em cosméticos e apresenta propriedades farmacológicas (MARCO et al., 2015).

O óleo essencial de *V. arborea* possui também atividade alelopática, podendo influenciar negativamente a germinação e o crescimento de plântulas de diferentes espécies, onde o α-bisabolol é citado como responsável por essa característica (MARCO et al., 2015). Porém os estudos com esta molécula e como esse óleo essencial pode afetar os processos fisiológicos nas plantas-alvo na pré ou pós emergência são escassos, sendo estes necessários para estudos preliminares de substâncias com ação bio-herbicida.

Dessa forma, hipotetiza-se que o óleo essencial de *V. arborea* pode ser utilizado como alternativa no controle de plantas daninhas, por possuir ação na redução da taxa de germinação e/ou crescimento de plântulas. Além disso, acredita-se que o óleo essencial de *V. arborea* e o metabólito α-bisabolol possuem ação similar no comprometimento da atividade de enzimas

amilolíticas de sementes, indução de estresse oxidativo em sementes e plântulas, modificação da morfologia e divisão celular, além de afetarem os processos fotossintéticos que podem levar a danos oxidativos em plantas de diferentes espécies de daninhas.

As plantas utilizadas em estudos preliminares para a validação de bio-herbicidas são plantas-alvo (espécies daninhas) e as plantas não alvo (cultivadas). Dentre as plantas daninhas de interesse econômico, que se propagam por sementes e disseminadas por todo o território brasileiro estão a *Bidens pilosa* L. (picão preto), *Cenchrus echinatus* L. (carrapicho), *Cyperus disfformis* L. (tiririca), *Desmodium tortuosum* (Sw.) DC. (Pegapega) e *Senna occidentalis* (L.) Link (matapasto). As espécies cultivadas *Oryza sativa* L. e *Lactuca sativa* L., mono e dicotiledônea, respectivamente, são utilizadas em ensaios fitotóxicos por serem modelos para esse tipo de experimento e assim avaliar os efeitos em organismos não-alvo.

Assim, objetivou-se neste trabalho determinar se o óleo essencial de *Vanillosmopsis arborea* e a molécula de composição majoritária, α-bisabolol, possuem ação bio-herbicida na pré e pós-emergência em distintas espécies. No capítulo 1, o objetivo foi avaliar o potencial bio-herbicida do óleo essencial de *V. arborea* na germinação e crescimento de plântulas de espécies-alvo (daninhas) e não-alvo (cultivadas) e o potencial citotóxico deste óleo essencial nas células meristemáticas de raízes de *Lactuca sativa*. No capítulo 2 o objetivo foi avaliar o efeito do óleo essencial de *V. arborea* e da molécula de α-bisabolol em ação simulada na pré-emergência de sementes e plântulas de *Senna occidentalis* e *Oryza sativa.* A ação do óleo e da molécula foi avaliada por meio da germinação, atividade da α-amilase e metabolismo antioxidante. No capítulo 3, o objetivo foi avaliar o potencial bio-herbicida do óleo essencial de *V. arborea* e da molécula α-bisabolol na pós-emergência de *O. sativa* e *S. occidentalis,* no que se refere à fotossíntese e estresse oxidativo.

2. REFERENCIAL TEÓRICO

A Chapada do Araripe possui 972.605,18 de hectares de extensão territorial que se dividem entre os Estados do Ceará, Pernambuco e Piauí no Nordeste brasileiro e compreende Biomas como Caatinga e Cerrado. Uma parte do território da Chapada é área de proteção ambiental e de preservação, assim como é o caso da Floresta Nacional do Araripe, localizada no município de Crato-CE, com o intuito de preservar a alta biodiversidade, com endemismos e descrição de novas espécies, diversos tipos de hábitats, sítios de fósseis, um extenso lençol freático e várias nascentes (DNPM, 1996, CASTRO, 1996, NOVAIS; LAURINDO, 2014, ICMbio, 2020).

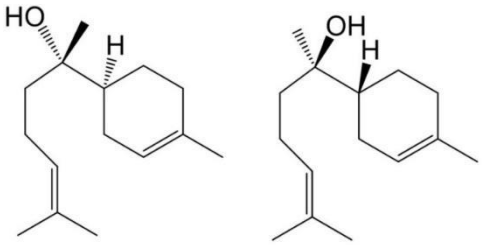
A biodiversidade da Chapada do Araripe compreende espécies vegetais de interesse econômico e medicinal, por produzirem uma vasta quantidade de metabólitos secundários. Estes são divididos em classes, como os terpenoides, compostos nitrogenados e fenólicos (KESSLER; KALSKE, 2018). Esses compostos possuem ação na atração de polinizadores, contra patógenos ou predadores, na proteção contra estressores ambientais e na colonização de ambientes (BÖTTGER et al., 2018). Dentre os metabólitos secundários têm-se os óleos essenciais, constituídos de uma mistura de compostos, majoritariamente mono e sesquiterpenos voláteis, além de fenólicos dos tipos benzenóides e fenilpropanóides (ASBAHANI et al., 2015). Os óleos essenciais possuem diversas propriedades biológicas, tais como farmacológica, citotóxica, bactericida, fungicida e inseticida, constituindo assim fonte para o desenvolvimento de novos produtos (BASER; BUCHBAUER, 2016).

Dentre as plantas endêmicas da Chapada do Araripe, está a *Vanillosmopsis arborea*, popularmente conhecida como candeeiro (Fig. 1) e que apresenta propriedades medicinais. É uma arvoreta que pode chegar a cerca de quatro metros, possui tronco com uma casca espessa, de forte odor, e sua madeira é considerada de boa qualidade. O seu caule é rico em óleo essencial de alto valor econômico, devido seus constituintes químicos (LORENZI; MATOS, 2008).

Figura 1 – Espécie *Vanillosmopsis arborea*, Crato-CE.

O óleo essencial de *V. arborea* possui entre 70-95% do α-bisabolol ((−)-6-Methyl-2-(4-methyl-3-cyclohexen-1-yl)-5-hepten-2-ol), a molécula de composição majoritária. Este é um álcool sesquiterpênico monocíclico (Fig. 2), também conhecido como levomenol (PERBELLINI et al., 2004; MARCO et al., 2015). Suas atividades foram citadas por Albertti e colaboradores (2018) como anti-inflamatório, antifúngico, antibacteriano, gastro-protetor, possui efeitos citotóxicos em células tumorais e vem sendo testado no tratamento de leishmaniose (COLARES et al., 2013). No mercado estético, esta molécula, pode ser utilizada em cosméticos e bálsamos (MARCO et al., 2015).

Figura 2 – Estrutura química do α-bisabolol.



(-)-α-bisabolol (+)-α-bisabolol

Óleos essenciais têm sido estudados no controle de plantas daninhas, pois uma grande variedade de compostos altamente fitotóxicos é derivado da via dos terpenos (DHIFI et al., 2016). Esses possuem ação alelopática, termo, originário do grego allelon = mútuo e pathos = prejuízos que foi proposto por Hans Molish em 1937 (LATIF; CHIAPUSIOX; WESTON, 2017). Utilizado para caracterizar a influência no crescimento e desenvolvimento de espécies-

alvo como as plantas daninhas (SOUZA FILHO; ALVES, 2002; SCHANDRY; BECKER, 2020). As plantas daninhas apresentam rápido crescimento, competem por água, luz e nutrientes com essas espécies cultivadas, reduzindo a produtividade e aumentando os custos de produção das culturas de interesse. O controle das plantas daninhas é realizado a partir de tratos culturais mecanizados e do uso de herbicidas sintéticos, porém o uso destes químicos pode causar riscos à saúde humana e ao meio ambiente. Além disso o uso excessivo destes também pode induzir uma resistência das plantas daninhas à herbicidas sintéticos específicos, proporcionando prejuízos econômicos e dificultando o controle dessas plantas (BHADORIA, 2011).

Segundo Puig et al. (2018) o uso de aleloquímicos fitotóxicos pode ser uma ferramenta eficaz no controle de plantas daninhas. Pois estes permitem um manejo efetivo da produção agrícola com poucos riscos de contaminação do ambiente devido à alta degradabilidade dos aleloquímicos (BHADORIA, 2011; CHENG; CHENG, 2015). Os efeitos fitotóxicos dos aleloquímicos ocorrem em diversos processos vegetais, entre estes, estão aqueles relacionados diretamente com a germinação, crescimento e desenvolvimento de plântulas (RADHAKRISHNAN; ALQARAWI; ABD-ALLAH et al., 2018).

Na germinação, os aleloquímicos podem inibir a respiração mitocondrial através do bloqueio do transporte de elétrons que resulta em uma menor produção de ATP, aumento da produção de espécie reativas de oxigênio (EROS) (EINHELLIG, 2004; CHUNG et al., 2018) Que resulta na perturbação das membranas da mitocôndria, aumento da peroxidação lipídica e supressão do sistema antioxidante, levando à deterioração de sementes (EINHELLIG, 2004; GNIAZDOWSKA; BOGATEK, 2005; PERGO; ISHII-IWAMOTO, 2011; CHUNG et al., 2018). O crescimento das plantas pode ser afetado através de modificações citológicas, como as perturbações na forma, estrutura e divisão de cromossomos das células vegetais, incremento de anormalidades nucleares, aumento do número de vacúolos e rompimento da parede celular (PAWLOWSKI et al., 2012; CHENG; CHENG, 2015). Esses efeitos citotóxicos de óleos essenciais incluem a indução da morte celular por ativação de processos de apoptose e/ou necrose, parada do ciclo celular e perda da função de organelas essenciais (SHARIFI-RAD et al 2017). Todas essas modificações no crescimento podem induzir um incremento de EROS e consequente estresse oxidativo nas plantas. O processo de hidrólise de amido é sensível aos aleloquímicos, pois a atividade da α-amilase pode ser reduzida durante o estabelecimento de plântulas de espécies daninhas, prejudicando assim o crescimento inicial dessas (HEGAB et al., 2008; RADHAKRISHNAN; ALQARAWI; ABD-ALLAH, 2018).

Além da germinação e crescimento inicial, pode-se verificar efeitos na fotossíntese. Diferentes aleloquímicos como os terpenos podem inibir o fotossistema II proporcionando um

bloqueio da cadeia de transferência de elétrons (CTE) entre a plastoquinona A e B (SCAVO; RESTUCCIA; MAUROMICALE, 2018). O bloqueio da CTE do cloroplasto pode aumentar a quantidade de EROS e proporcionar um estresse oxidativo em plantas na pós-emergência. Alguns aleloquímicos também podem ser precursores da biossíntese de clorofila, e como tais, podem proporcionar um aumento no conteúdo de clorofila quando aplicados em baixas concentrações, ou, quando em altas concentrações, causar inibição de intermediários da síntese (protoporfirina, Mg-protoporfirina) e da enzima Mg-quelatase, induzindo a degradação da clorofila (KANCHAN; JAYACHANDRA, 1980; YANG et al., 2002; ZHOU; YU, 2006). Portanto, os estudos com os óleos essenciais na fisiologia vegetal são importantes para a prospecção de bio-herbicidas.

Para verificar o potencial bio-herbicida é necessário a utilização de espécies modelo sensíveis aos aleloquímicos, plantas daninhas e espécies importantes para a agricultura. Os aleloquímicos podem ser específicos para cada espécie e ainda podem ser atribuídos à existência de receptores exclusivos nas diferentes espécies (HOSNI et al., 2013). Inicialmente, os estudos preliminares de bio-herbicidas, são realizados em laboratórios e casas de vegetação para excluir os efeitos ambientais da interação de indivíduos (TUR et al., 2012). As diretrizes da OECD (Organization for Economic Co-operation and Development) para ensaios com químicos, cita a *Oryza sativa* L. e a *Lactuca sativa* L., mono e dicotiledôneas, respectivamente, como espécies modelo (OECD, 2003). Dentre as espécies daninhas, que podem se desenvolver em praticamente todo o território brasileiro, podem causar prejuízos econômicos em diversos cultivos e são propagadas por sementes, temos:

- *Bidens pilosa* L.: Planta herbácea, família Asteraceae, popularmente conhecida como picão preto e carrapicho de duas pontas. Possui crescimento ereto e rápido, resistente às mais diversas adversidades com alta produção de sementes e mecanismos eficientes de dispersão e longevidade (BARTOLOME; VILLASEÑOR; YANG, 2013; BARROS et al., 2017).
- *Cyperus disfformis* L.: Pequena erva ereta, família Cyperaceae, conhecida como tiririca e propagação exclusivamente por via sexuada (LORENZI, 2014).
- *Cenchrus echinatus* L.: Planta herbácea da família Poaceae, conhecida popularmente como capim carrapicho, planta anual que possui reprodução via sementes (LORENZI, 2014).
- *Desmodium tortuosum* (Sw.) DC.: Planta da família Fabaceae, conhecida popularmente por carrapicho e erva de mendigo, trevo da Flórida, considerada planta daninha de porte ereto, pode atingir até 80 cm de altura e produz alta quantidade de biomassa (LORENZI; MATOS, 2008).

- *Senna occidentalis* (L.) Link: planta perene subarbustiva, lenhosa da família Fabaceae, conhecida popularmente como mata-pasto ou fedegoso, crescimento rápido podendo chegar até 2 m de altura (LORENZI, 2014).

Neste contexto e diante de todas as atividades descritas para o óleo essencial de *V. arborea* e seu componente majoritário o α-bisabolol, justifica-se a elaboração de estudos como potenciais bio-herbicidas com esta espécie.

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SEGUNDA PARTE – MANUSCRITOS

Capítulo 1: Chemical composition of *Vanillosmopsis arborea* essential oil and its use as potential bioherbicide in the pre-emergence of target and non-target species

Manuscrito nas normas do periódico Ecotoxicology and Environmental Safety (Fator de impacto: 4,8

Capítulo 2: Comparative physiological effects of *Vanillosmopsis arborea* essential oil and α-bisabolol on a weed and a crop species in pre-emergence

Manuscrito nas normas do periódico Industrial Crops and Products (Fator de impacto: 4,2)

Capítulo 3: Could the essential oil of *Vanillosmopsis arborea* and the sesquiterpene α-bisabolol induce changes in photosynthesis and oxidative system in a weed and a crop species in the same way?

Manuscrito nas normas do periódico Journal of Agricultural and Food Chemistry (Fator de impacto: 4,1)

Chemical composition of *Vanillosmopsis arborea* essential oil and its use as potential bioherbicide in the pre-emergence of target and non-target species

Abstract

The aim of this work was to describe the bioherbicidal potential of the essential oil of *Vanillosmopsis arborea* in the germination and growth of seedlings of target and non-target species. In addition, the cytotoxic potential of the essential oil in *Lactuca sativa* meristematic cells was evaluated. The essential oil of *V. arborea* was extracted, its metabolites were identified and quantified and it was diluted in deionized water in the concentrations 0.125, 0.25, 0.5, 0.75, and 1% (deionized water was used as control). The solutions or water were used to moisten the germination paper where the seeds of the target and non-target species were sown. Eleven metabolites were found, with α-bisabolol being the major component (around 93%). The germination percentage, germination speed index, the shoot and the root length of target species were significantly reduced by the treatments. The percentage of germination and growth of the weeds *Bidens pilosa*, *Cenchrus echinatus*, and *Cyperus disfformis* were inhibited by about 90% in all concentrations of the essential oil. The non-target species, *Oryza sativa*, was less sensitive to the application of the treatments compared to target species. The germination percentage of this species was not significantly reduced by essential oil. The mitotic index of the meristem cells from the roots of *L. sativa* was reduced by the action of the essential oil of *V. arborea*. Therefore, the essential oil of *V. arborea* reduced the germination and growth of several target species in a species-dependent way without inducing negative effects on non-target species. It suggests a promising and selective action on weeds of this essential oil as a bioherbicide.

Keywords: Allelopathy; Weeds; Cytotoxic; Sesquiterpene.

1. Introduction

Weeds interfere in the cultivation of crop species, increasing production costs and, impairing crop yields (Baghel et al., 2020). These losses may reach about 90%, according to the type of cultivation and the weed (EMBRAPA, 2020). Thereby, it is necessary to perform weed control to minimize losses. Weed management is carried out by chemical and non-chemical methods, being the chemicals the most used in the conventional agricultural system (Moss et al., 2019). The indiscriminate use of chemicals, such as synthetic herbicides, is one of the sources of environmental contamination, affecting food security and causing risks to human health. (Moss et al., 2019; Powles & Yu, 2010).

The use of bioherbicides, produced from plants or microorganisms, has been emerging as an 'environmentally friendly' alternative. Plants are potential candidates for the prospection of bioherbicides due to the production of biologically active substances with diverse chemical nature, as the secondary metabolites (Dayan & Duke, 2014). These 'natural products' have several functions in the survival of plants and their communication with the environment (Böttger et al., 2018).

Essential oils are produced by plants and rich in metabolites with different biological activities, such as allelochemical action. This way, essential oils can affect several physiological processes in target plants, such as modification of biochemical processes, suppression of enzymatic activity, changes in the cell cycle, which may result in the inhibition of seed germination, growth and development of plants (Aragão et al., 2015; Laosinwattana et al., 2018; Mutlu et al., 2011; Radhakrishnan et al., 2018; Tohidi et al., 2019). However, the effects of essential oils in non-target plants are also needed to be investigated due to avoiding negative interferences in crop species.

Vanillosmopsis arborea Baker ("candeeiro"), from Asteraceae, is an endemic tree plant from Chapada do Araripe, Crato-CE, Brazil. The shoot of the plants produces an essential oil of high economic and medicinal value. Candeeiro's essential oil has been investigated in studies, and it has been reported as anti-inflammatory, antibacterial, antifungal, analgesic and, antileishmanial action (Colares et al., 2013; Marco et al., 2015).

The essential oil of *V. arborea* has also been mentioned for its allelopathic action on model species (Marco et al., 2015), but its action on weeds and mode of action is still unknown. The shape, structure and division of chromosomes could be affected by the metabolites present in essential oils, such as volatile monoterpenes, eucalyptol, and camphor. These compounds act on root tip cells inducing or suppressing the cell elongation process. Moreover, they induce nuclear abnormalities and increasing the number of vacuoles, which influence the growth of

target species (Cheng & Cheng, 2015; Pawlowski et al., 2012). Essential oils are been commonly used to control weed species. The first phase to prove the bioherbicide efficiency is the evaluation of cytotoxic activity, translated into morpho-physiological parameters in the target species (Puig et al., 2018). Therefore, the use of cytogenetic tests that involve changes in the mitotic activity of meristematic cells, i.e., number of dividing cells (mitotic index), and the number of chromosomal aberrations, is an essential approach for evaluating a bioherbicidal potential and its mode of action in target (weeds) and non-target species (Aragão et al., 2015; Campos et al., 2008). In this way, *Lactuca sativa* root tips are being suggested as model for toxicological studies (Grant, 1994; Andrade-Vieira et al., 2012).

Thus, it is hypothesized that the essential oil of *V. arborea* inhibits seed germination and reduces the seedling growth of different target species through changes in the cell cycle. Moreover, the non-target plants could not be sensible to the negative effects of candeeiro´s essential oil. Therefore, this work aimed to evaluate the bioherbicide potential of the essential oil of *V. arborea* in the germination and seedling growth of target and non-target species. And also evaluate the cytotoxic potential of this essential oil in the meristematic cells of *L. sativa* roots.

2. Materials and Methods

2.1 Plant material, extraction, analysis, and dilution of the essential oil

*Vanillosmopsis arborea´*s wood was collected at Chapada do Araripe in the municipalities of Crato – CE, Brazil (7°07'39"S 39°25'32"W) in December, 2019. The wood from the lateral branches was preferentially removed from three donor plants aiming not severely compromising its growth and development.

The plant material was prepared in the Interdisciplinary Laboratory in Natural Products of the Federal University of Cariri, where it was cut, divided into 10 portions of 500 g, emerged

in 2.5 L of distilled water, and forwarded to a Clevenger type hydrodistillation, where the essential oil was extracted by the methodology of Alencar et al. (1987). In brief, the plant material was heated releasing essential oil that is vaporized, cooled in a condenser, and collected.

The essential oil obtained from each portion of 500 g was mixed, transferred to a sealed glass container, protected from light, and stored at 4° C. The oil components were identified by gas chromatography coupled to the mass spectrophotometer (GC-MS), model GCMS-QP2010 Ultra, Shimadzu brand, and RTX-5MS column and capillary with 5% diphenyl/ 95% dimethyl polysiloxane, with 30 m in length and 0.25 mm diameter, according to the methodology described by Adams (2007).

For dilution to obtain the concentrations applied in the experiments, the pure essential oil (100%) was diluted in heated deionized water at 40ºC to obtain the concentrations 0.125, 0.25, 0.50, 0.75, and 1%. Each concentration was considered a treatment and the distilled water the negative control.

2.2 Target and non-target species

The non-target species, *Lactuca sativa* L. seeds cv. Monica were obtained in an agricultural house in the municipalities of Lavras-MG and the *Oryza sativa* L. seeds cv. Caçula, harvest 2019 were assigned by the Genetics and Plant Breeding sector at the Federal University of Lavras (UFLA).

The seeds of *Bidens pilosa* L. and *Cenchrus echinatus* L. were collected in the rural area of Crato-CE (7°14'11"S 39°22'08"W). The seeds of *Cyperus disfformis* L. were collected at UFLA (21°13'36"S 44°58'53"W). The seeds of *Desmodium tortuosum* (Sw.) DC. and *Senna occidentalis* (L.) Link were collected in Ijaci-MG (21°10'08"S 44°54'52"W).

2.3 Bioassay

Seeds of *C. difformis* and *S. occidentalis* were submerged in sulfuric acid (96%) for 5 and 20 minutes to break physical dormancy (Delachiave & De Pinho, 2003), respectively, following of washes with deionized water to remove any traces of the acid. For *D. tortuosum* seeds the physical dormancy were break scarifying them with sandpaper number 180 (Montanha et al., 2017). The seeds of all species used in the bioassay were disinfected in a solution of 2.5% sodium hypochlorite (NaClO) and detergent for 15 minutes and then washed three times in distilled water.

The seeds were disposed into Petri dishes with germination paper. In each Petri dish, the paper was moistened with 4 mL of each concentration of essential oil applied in the study. The Petri dishes were kept in germination chambers with optimal temperature and photoperiod for each species during the experiment. *B. pilosa* and *L. sativa*, temperature was of 25 ºC and 24 hours of darkness, *O. sativa*, 25 ºC and 12 hours of light 40µM photons m^{-2} s^{-1}, *C. echinatus*, *D. tortuosum* and *S. occidentalis*, 30 ºC and 12 hours 40µM photons m^{-2} s^{-1}, *C. disfformis* an alternating temperature of 30/20 ºC light/dark with 16 hours of light 40µM photons m^{-2} s^{-1}.

The duration of the experiment was determined by germination stabilization (two consecutive days without increasing germination percentage in control) of each target and non-target species. The number of germinated seeds were taken every 24 hours. Germination was considered in seeds with radicles of at least 2 mm in length. The percentage of germination (GP) and germination speed index (GSI) were calculated by the methodology of Ranal et al. (2009). The shoot and root elongation in seedlings were measured with the aid of a ruler, but it was performed only in experimental plots that had at least five seedlings.

The cytogenetic analysis including the mitotic index (MI), chromosome and nuclear alterations were carried out in *L. sativa* root tips. After treatment (96h), roots were collected and fixed in ethanol and acetic acid solution (3:1 v/v). The slides were prepared and analyzed

according to dos Santos et al. (2018) with adjustment. In brief, the roots were hydrolyzed in hydrochloric acid (1N) and stained with Schiff reactive. Then, the root meristem was cut, kept in acetic orcein dye (2%) for 20 minutes, and squashed. For counting and detecting abnormalities/aberrations, around 600-800 cells for repetition (one slide), were analyzed in a light microscope (Zeiss® with Image A2 and AxioCam) in a total augmentation of 400×.

The mitotic index (MI) was calculated as the number of cells in division for the total number of cells in the slides, and the aberrations were calculated as the fraction in percentage of the number of cells with chromosomal changes for the total number of cells.

2.4 Seed viability after bioassay

The non-germinated seeds from each treatment (essential oil concentration and water) were subjected to a viability test. The remnants seeds of the species *B. pilosa*, *C. echinatus,* and *C. disfformis* were transferred from treatments with essential oil to Petri dishes containing two discs of filter paper moistened with deionized water and kept in germination chambers in the same condition of the experiments. The germinated seeds were considered viable. After germination stabilization, the non-germinated seeds were submitted to the tetrazolium test. The viability test with tetrazolium salt was not possible to be performed with seeds of *C. disfformis* due to the size of the seeds of this species (1 mm, mean).

For tetrazolium salt test, the seeds of *L. sativa* and *O. sativa* were cut with the aid of tweezers and scalpel and stained with 2-3-5 triphenyltetrazolium chloride solution 0.5 and 1%, respectively, in dark flasks and kept in BOD at 30 °C for 3 hours, following the methodology by Brasil (2009). The coat of *B. pilosa* and *S. occidentalis* seeds were removed, and the staining was carried out with a solution of 1 and 0.5% tetrazolium salt, respectively, kept in BOD at a temperature of 30º C for one hour in the dark. The seeds of *C. echinatus* were cut longitudinally, emerged in 0.5% tetrazolium solution, and kept in BOD at a temperature of 30º C for 3h in the

dark. After the staining time, the seeds were washed in tap water and evaluated for the location and the intensity of the red and pink color of the seeds structures to classify them as viable. Non-colored seeds were considered dead (França-Neto & Krzyzanowski, 2019).

2.5 Experimental design and statistical analysis

The experiments were conducted in a completely randomized design, with six treatments, five concentrations of the essential oil of *V. arborea*, and the negative control, deionized water. Each treatment had 5 repetitions, thus totaling 30 experimental plots of 25 seeds each, per species. An independent experiment was carried out for each target and non-target species.

The data were analyzed in the statistical software RBio© (BHERING, 2017) and were submitted to the Shapiro-Wilk normality test, analysis of variance (ANOVA), and when significant by the F test at 5% probability, they were submitted to regression analysis. All equations, R^2 values, and p values were shown in a table at Supplementary Material (Suppl. Mat. T1.) Then, the germination percentage, the germination speed index, and the length of the shoot and root of all species and concentrations were used to group the species by similarities and differences through a multivariate cluster analysis (UPGMA) based on Euclidean distance in percentage.

The data of index mitotic and, chromosomal aberrations were analyzed for the Shapiro-Wilk normality test, analysis of variance (ANOVA) and when significant by the F test at 5% probability, they were submitted to Tukey test at 5%.

3. Results

3.1 Chemical composition of *V. arborea* essential oil

The CG-MS analysis of the essential oil of *V. arborea* identified eleven compounds (Table 1), distributed between the classes of terpenes (96.96%) and phenolics (2.14%), totalizing 99.10% of the components of this essential oil. The major components of the oil were α-bisabolol (93.57%), eugenol (2.14%), bisabolol oxide (1.48%), elemicin (0.67%), and eucalyptol (0.65%).

Table 1. Percentage of *Vanillosmopsis arborea* essential oil components identified for CG-MS.

Percentage (%)	Components
0.1	3-butenyl propyl ether
0.09	3,3-dimethyl-2-hexanone
0.65	Eucalyptol
0.09	Terpineol
2.14	Eugenol
0.21	cis-Caryophyllene
0.67	Elemicin
0.3	(-)-Spathulenol
1.48	Oxide bisabolol
93.57	α-Bisabolol
0.15	β-Chamigrene
0.51	Eremanthine
99.96	Total

3.2 Germination test

The germination percentage (GP) was significantly ($p \leq 0.05$) influenced by the essential oil of *V. arborea* in all target species (Fig. 1). The effects the essential oil reduces the GP in a dose-dependent manner in *L. sativa* seeds without effect in *O. sativa*. The data were adjusted

in two-degree polynomial for all species. The highest concentration of the essential oil (1%) was the most effective in reducing the PG of *L. sativa*, *S. occidentalis,* and *D. tortuosum* seeds, decreasing about 85, 61, and 27% the GP, respectively. While for *C. disfformis,* and *C. echinatus* the lowest concentration (0.125%) reduced seed germination in 95 and 87%, respectively. The GP of the seeds of *B. pilosa* was completely inhibited by all concentrations of essential oil of *V. arborea*, which did not allow the analysis of the subsequent variables.

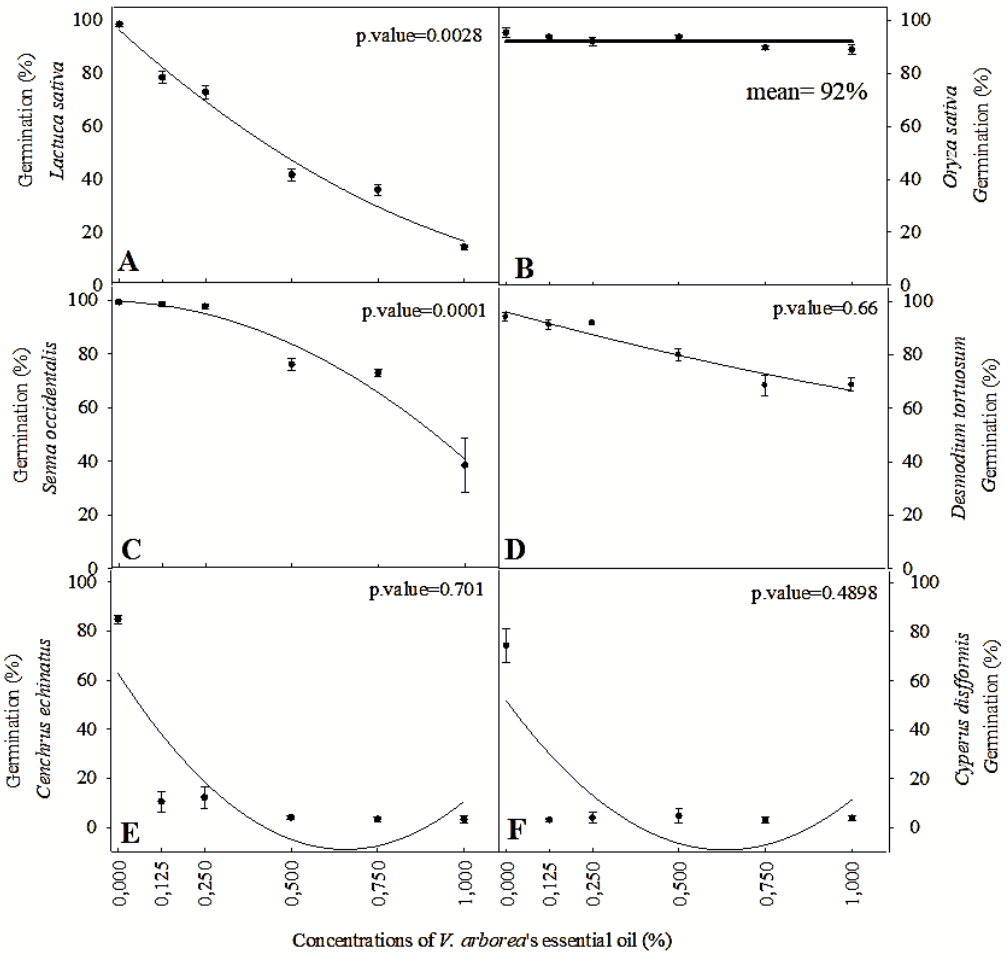

Fig. 1. Percentage of seed germination submitted to different concentrations of essential oil of *Vanillosmopsis arborea.* (A) *Lactuca sativa*. (B). *Oryza sativa*. (C) *Senna occidentalis.* (D) *Desmodium tortuosum.* (E) *Cenchrus echinatus*. (F) *Cyperus disfformis*.

Similarly, to GP, the germination speed index (GSI) of the seeds of target species (Fig. 2) was significantly affected by the increasing concentration of the essential oil of *V. arborea* in treatments solutions. The increasing concentrations of the essential oil reduced the GSI of

the seeds of *L. sativa* and *S. occidentalis* in a similar pattern by more than 50% from 0.5% concentration. Interestingly, the concentrations 0.125 and 0.25% increased the GSI in the species *D. tortuosum*. The GSI to *C. echinatus* and *C. disfformis* was almost totally reduced since the smaller concentration.

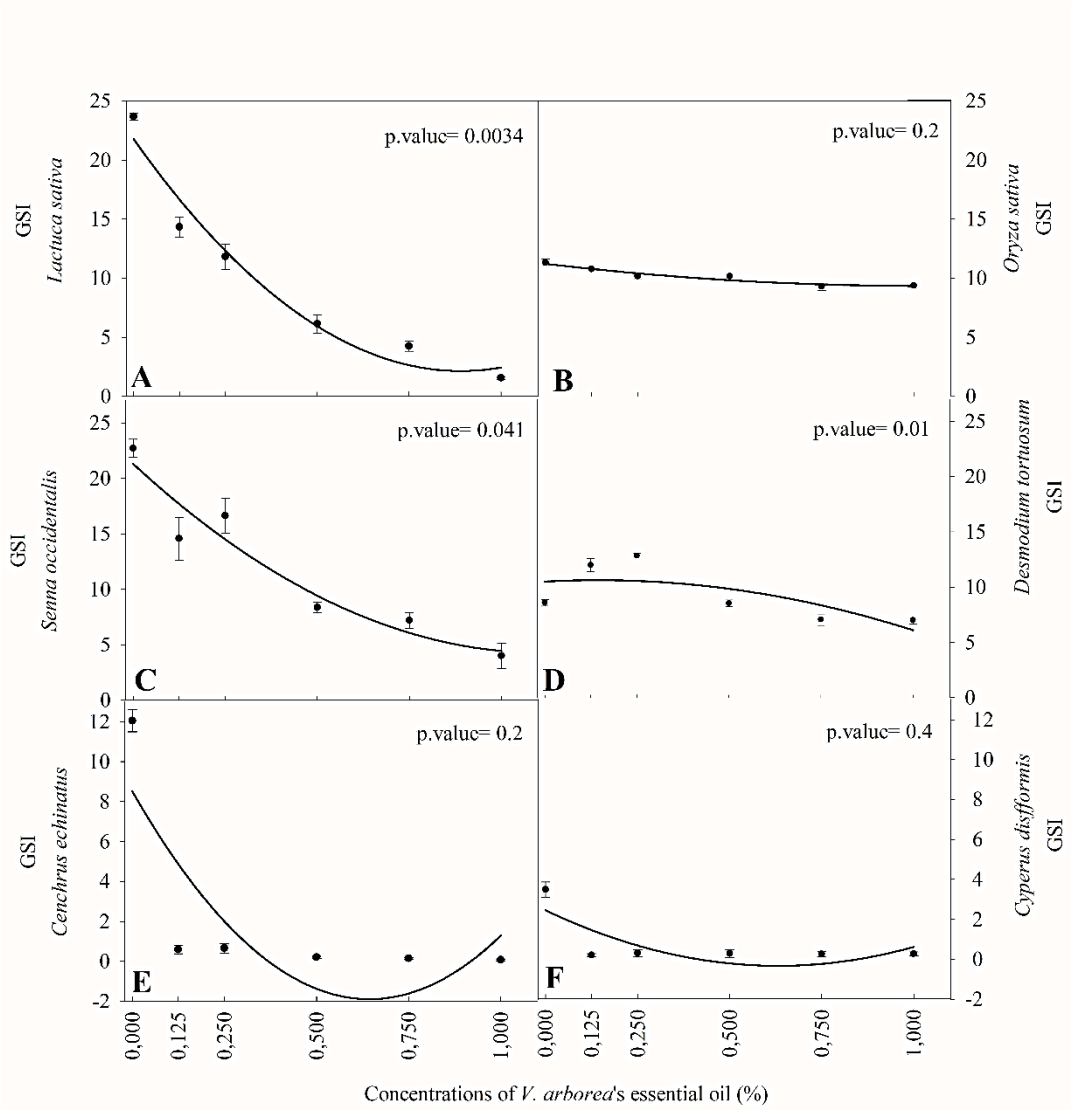

Fig. 2. Germination speed index (GSI) of seeds submitted to different concentrations of essential oil of *Vanillosmopsis arborea*. (A) *Lactuca sativa*. (B). *Oryza sativa*. (C) *Senna occidentalis*. (D) *Desmodium tortuosum*. (E) *Cenchrus echinatus*. (F) *Cyperus disfformis*.

3.3 Seedling growth

The seedlings size of the target and non-target species was significantly influenced by all treatments (Fig. 3 and 4). The length of the shoot and root of *L. sativa* seedlings was reduced

with increasing concentrations. At a concentration of 1% in this species most seedlings were underdeveloped, which did not allow the measurements of size. The root size of *S. occidentalis* (Fig. 3) was larger when treated with 0.125% of the essential oil of *V. arborea*, but this did not provide a greater seedling length (Fig. 3), and the shoot size was reduced by about 50% from the concentration of 0.125%. The size of *O. sativa*, *D. tortuosum,* and *C. echinatus* seedlings (Fig. 3 and 4) was reduced in all concentrations of the essential oil of *V. arborea*, whereas for C. *echinatus,* from the concentration of 0.5% onwards, the reduction was 100 %. The application of the essential oil of *V. arborea* completely inhibited the growth of *C. disfformis* seedlings (Fig. 4).

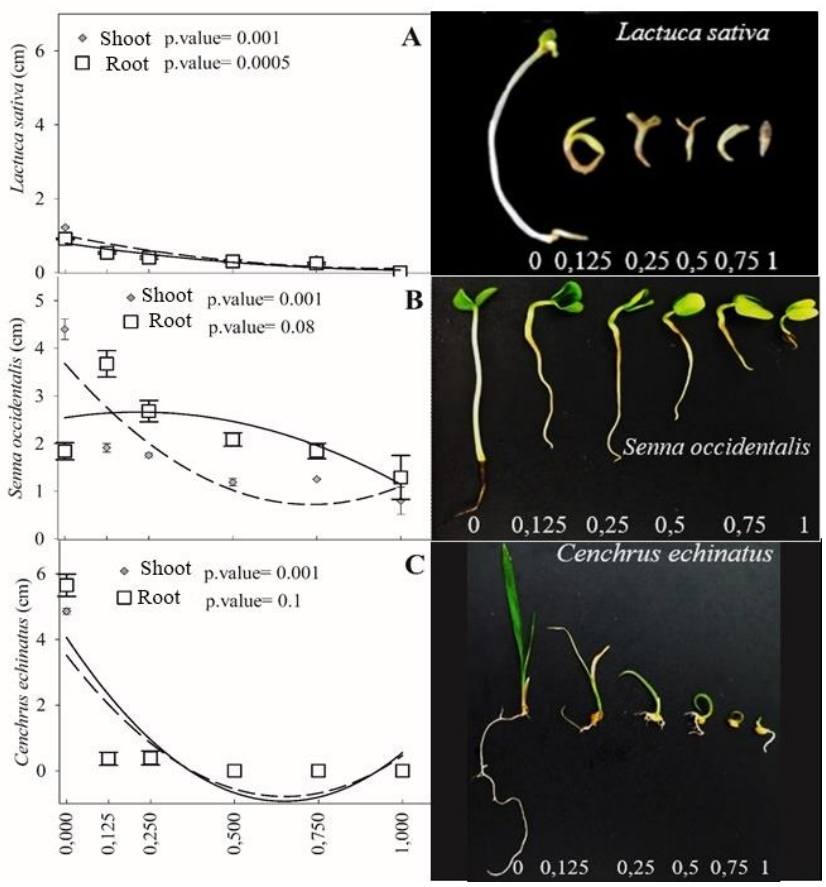

Fig. 3. Shoot and root length of seedlings submitted to different concentrations of essential oil of *Vanillosmopsis arborea.* Morphology of the seedlings submitted to different concentrations of essential oil of *Vanillosmopsis arborea* (A) *Lactuca sativa*. (B) *Senna occidentalis*. (C) *Cenchrus echinatus*.

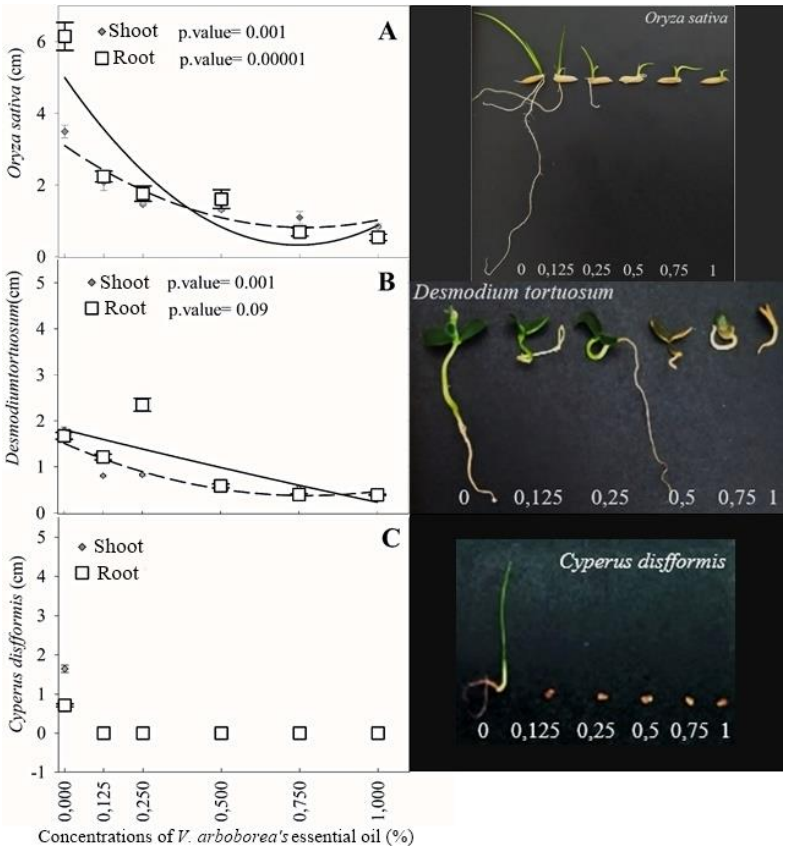

Fig. 4. Shoot and root length of seedlings submitted to different concentrations of essential oil of *Vanillosmopsis arborea.* Morphology of the seedlings submitted to different concentrations of essential oil of *Vanillosmopsis arborea* (A) *Oryza sativa*. (B) *Desmodium tortuosum*. (C) *Cyperus disfformis*.

The species were grouped according to its degree of sensitivity to the application of the essential oil of *V arborea* (Fig. 5). Two distinct group were observed, being *O. sativa* and *B. pilosa* had the more dissimilarity. The model species *L. sativa* showed medium sensitivity to treatments, being close to both groups. The species *O. sativa* and *S. occidentalis* showed differences in the reduction of GP and GSI, however for the other variables considered in the analysis of the cluster there was a similar pattern of reduction, thus these species were separated into the same group. The species of the group *C. disfformis*, *C. echinatus*, and *B. pilosa* were the most sensitive to the treatments.

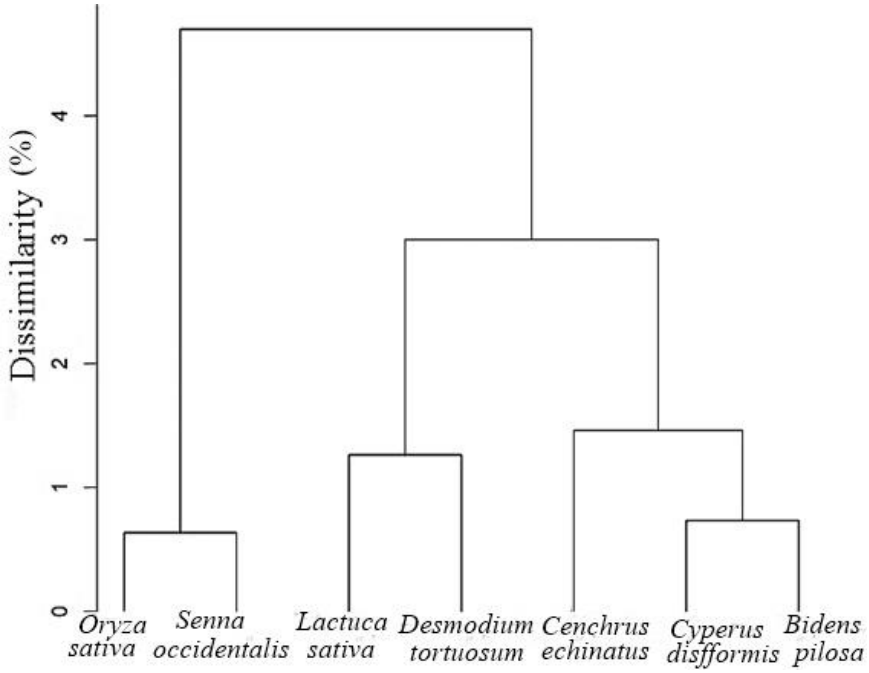

Fig. 5. Grouping analysis of target and non-target species treated with essential oil of *Vanillosmopsis arborea* and based on the variables germination percentage, germination speed index, and length of shoot and root.

3.4 Mortality and abnormality of the seedlings

Seed mortality of target species and of the non-target species *L. sativa* were significantly influenced by the treatments, but the non-target specie *O. sativa* not was affected by treatments (Fig. 6). The results showed enhancement of the percentage of dead seeds with increasing concentrations of essential oil in *L. sativa, S. occidentalis,* and *D. tortuosum* seeds. About 83 and 84% of dead seeds were counted in the target species *B. pilosa* and *C. echinathus*, respectively, in all concentrations of essential oil. The seeds of *C. disfformis* transferred to Petri dishes containing deionized water did not germinate, therefore, it was not possible to assess its mortality.

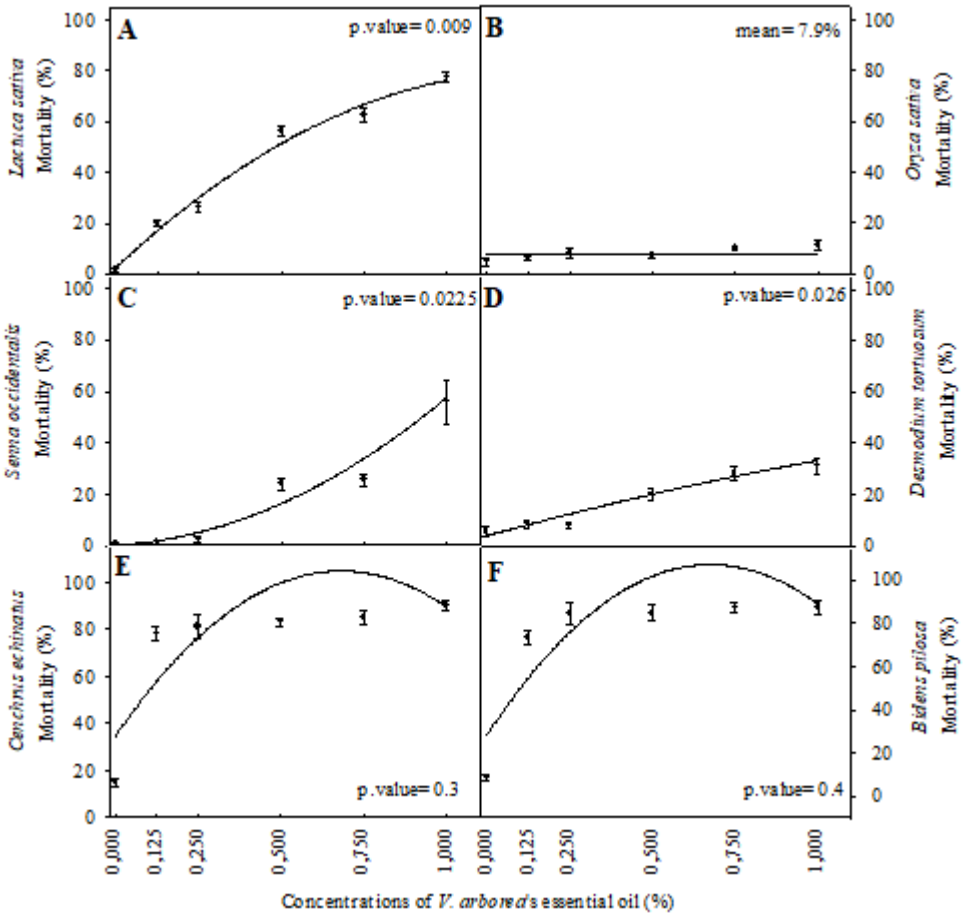

Fig. 6. Seeds mortality after submission to different concentrations of essential oil of *Vanillosmopsis arborea.* (A) *Lactuca sativa*. (B). *Oryza sativa*. (C) *Senna occidentalis*. (D) *Desmodium tortuosum*. (E) *Cenchrus echinatus*. (F) *Cyperus disfformis*.

After treatments, the target species *L. sativa*, *O. sativa*, *S. occidentalis*, and *C. echinatus* showed necrotic and abnormal seeds and seedlings (Fig. 7). Moreover, it was observed seeds with an aqueous phase that prevented a rupture of the tegument besides chlorotic seedlings or without roots and shoot. For the target species *S. occidentalis*, seedlings treated with 1% of the essential oil of *V. arborea* started showing necrosis immediately after germination.

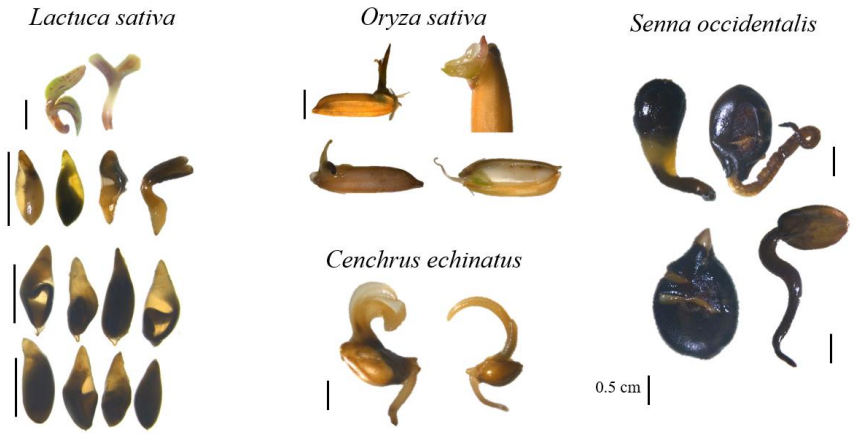

Fig. 7. Seeds and seedlings of different target species submitted to 1% of the essential oil of *Vanillosmopsis arborea.* Bars = 0.5cm.

3.5 Mitotic index

The cell cycle of root tips from the seedlings of *L. sativa* was significantly influenced by the treatments (Table 2). The concentrations of essential oil, 0.125, 0.25, and 0.5%, reduced the mitotic index by about 46, 31, and 21%, but the other concentrations did not interfere in this parameter. The number of chromosomal aberrations increased in all concentrations of essential oil of *V. arborea*, with a concentration of 0.75% leading to a higher amount of aberrations (Table 2). Among the chromosomal changes, there were found aneugenic and clastogenic ones, with chromosomal bridges in anaphase, not oriented chromosomes in metaphase and C-metaphase (Fig. 8).

Table 2. Cell cycle alterations were observed in *L. sativa* meristematic root cells after exposure to the different concentrations of *V. arborea*'s essential oil.

Concentrations	MI (%)	Chromosomal aberrations (%)
0	10.6426a	0.363992e
0.125	5.7373c	0.984724d
0.25	7.2633b	1.483848c
0.5	8.3986b	1.958139bc
0.75	10.0929a	2.122215a
1	10.3048a	1.830132ab

CV (%)	8.96	13.15
p.value	$8.172e^{-10}$	$5.5579e^{-14}$

*means followed by the same letter, in the column, do not differ by Tukey's test at 5% probability

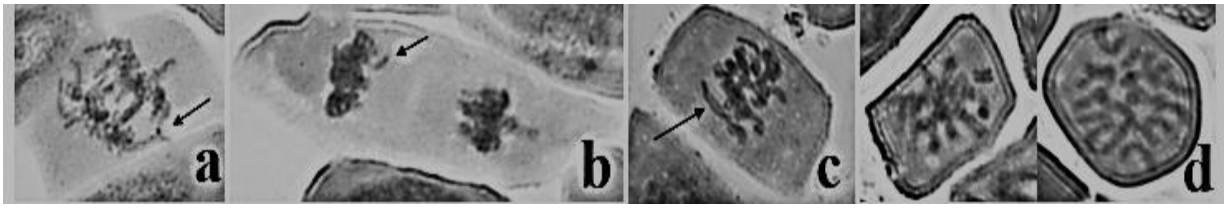

Fig. 8. Cell cycle alterations observed in *L. sativa* meristematic root cells after exposure to the *V. arborea*'s essential oil. (a) chromosomal bridge; (b) non-oriented chromosome; (c) non-oriented chromosome; (d) C-metaphase.

4. Discussion

The phytotoxic potential of *V. arborea* essential oil is due to its components and its synergistic action which resulted in the inhibition of the germination percentage, germination speed index and seedling length is complex and can involve several compounds (Bhowmik & Inderjit, 2003). The main components of the essential oil of *V. arborea* (terpenes and phenolics) have varied chemical composition. Has been characterized in other studies and the pattern of metabolites content was like found here, except for metabolite eucalyptol (Marco et al., 2015).

The majority component found in *V. arborea* essential oil, α-bisabolol, a sesquiterpene (Latif et al., 2017). It is present in other species of economic interest, such as *Pulicaria somalensis* and *Chamomilla recutita*, which have 5.3 and 15.2% of this metabolite and only in *V. arborea* was mentioned as possible responsible for the allelopathic potential (Marco et al., 2015; Synowiec et al., 2017).

The reduction of seed germination in the target species induced by allelochemicals is considered a secondary effect of several interferences in the metabolism of the species, i.e., inhibition of cell division, respiration, the modification of the cell membrane permeability, increase of reactive oxygen species (ROS), suppression of the oxidative metabolism, and oxidative stress (Radhakrishnan et al., 2018; Scavo et al., 2018). Considering the chemical characterization of α-bisabolol as sesquiterpene, it is suggested an interaction with

313 phytohormones, ROS, and oxidative stress. Nevertheless, due to the percentage of α-bisabolol
314 in *V. arborea* essential oil, the reduction of the GP could be linked to this metabolite. Increase
315 in ROS and membrane disturbance has been reported in seeds treated with eugenol and its by-
316 products causing the reduction in percentage of germination (de Oliveira et al., 2016; Sueko et
317 al., 2020).

318 The initial growth of the target and non-target species was also significantly influenced
319 by the negative effects of the treatments in roots. Indeed, the roots are in contact with
320 allelochemicals, that could explain their sensibility. The reduction in the number of dividing
321 cells (MI) in the root tip of *L. sativa*, at 0,125% of the essential oil of *V. arborea* demonstrates
322 its cytotoxic activity (Andrade-Vieira et al., 2012). The reduction of cell division impairs the
323 roots growth of the of the target plants since the cell division, growth, and differentiation are
324 interconnected and overlap during the development of the plant (Santos et al., 2018; Harashima
325 & Schnittger, 2010). The different types of changes in chromosomes can decrease cell division
326 inducing death, if they become not repaired damage by cellular mechanisms. C-metaphase is
327 characterized by spindle disorders that paralyze the cell cycle. The chromosomal bridges, the
328 most frequent alteration induced by the action of "candeeiro" oil, and non-oriented
329 chromosomes are more commonly found in anaphase and telophase and can induce the
330 appearance of micronuclei and represent DNA damage. Thus, due to the universal structure of
331 DNA, the results described in the model plant, *L. sativa*, may be extrapolated to other organisms
332 (Andrade-Vieira & Silveira, 2018).

333 The α-bisabolol metabolite has been studied for its cytotoxic effect on animal and fungal
334 cells, reducing cell division and increasing chromosomal aberrations (Rigo et al., 2019; Rottini
335 et al., 2015). Therefore, it can be affirmed that the cytotoxic action of the essential oil of
336 *V. arborea* is mostly due to α-bisabolol, and also probably the synergy action with this molecule
337 with other compounds.

However, the target species, *S. occidentalis* and *D. tortuosum* showed an increase in the size of the roots in the lowest concentrations of essential oil, followed by a reduction in the shoot. These results may suggest a hormonal imbalance of auxin and cytokinin induced by sesquiterpene allelochemical, competing with the biosynthetic pathway of the hormones, as well as strigolactones (Araniti et al., 2017; Meléndez-Martíneza, et al., 2019). However, more studies are needed to investigate the role of the main bioactive molecules in this essential oil with the hormonal balance of the target species, mainly of the metabolite α-bisabolol that belongs to the sesquiterpenes class.

The target and non-target species showed different levels of sensitivity to the treatments and, therefore, with the results was not possible to determine a pattern of response among monocots and dicots species or of those from the same botanical family (Asteraceae, Fabaceae, and Poaceae). For this reason, the results suggest the essential oil of *V. arborea* could provide different degrees of weed control when used as a bioherbicide. However, the effectiveness of the weed control is not entirely related to the death of these plants but also the reduction of their growth, allowing the development of crops reducing competition with weed species (Almarie, 2016). Indeed, as reported here, *O. sativa* seeds was not sensible to *V. arborea* essential oil on reducing seed germination. Weeds and the model species *L. sativa* showed more severe effects in reducing GP, GSI, shoot and root length, and increasing the number of abnormal plants. These results suggest a selective action of this essential oil among crop and weed species. This way, the ability of the essential oil of *V. arborea* to reduce the growth of seedlings of the target species in all concentrations may promote the control of weeds.

5. Conclusion

The essential oil of *V. arborea* reduces the growth and development of several target species in a specific pattern for each species studied. This way, it indicates a promising and selective action of this essential oil as a bioherbicide. This study demonstrated that the weed

364 species, *B. pilosa, C. echinatus* and *C. disfformis,* are highly sensitive to the essential oil of
365 *V. arborea* no matter the concentration used, totally inhibiting the germinability and initial
366 growth of them. Regarding weed species, *S. occidentalis* and *D. tortuosum*, the 0.5%
367 concentration of essential oil is effective in reducing the growth and initial development. The
368 essential oil of *V. arborea* has a cytotoxic action by altering the cell cycle and inducing
369 aberrations that can cause cell death, and consequently reduced the growth of the target species.
370 Finally, due to the composed almost entirely of α-bisabolol of essential oil of *V. arborea*, the
371 effects on germination and growth reported can be caused by this molecule. Therefore, the
372 potential bioherbicidal action suggested by *V. arborea* essential oil is related to this major
373 compound, but further investigations with the isolated molecule need to be carried out to prove
374 this action.
375
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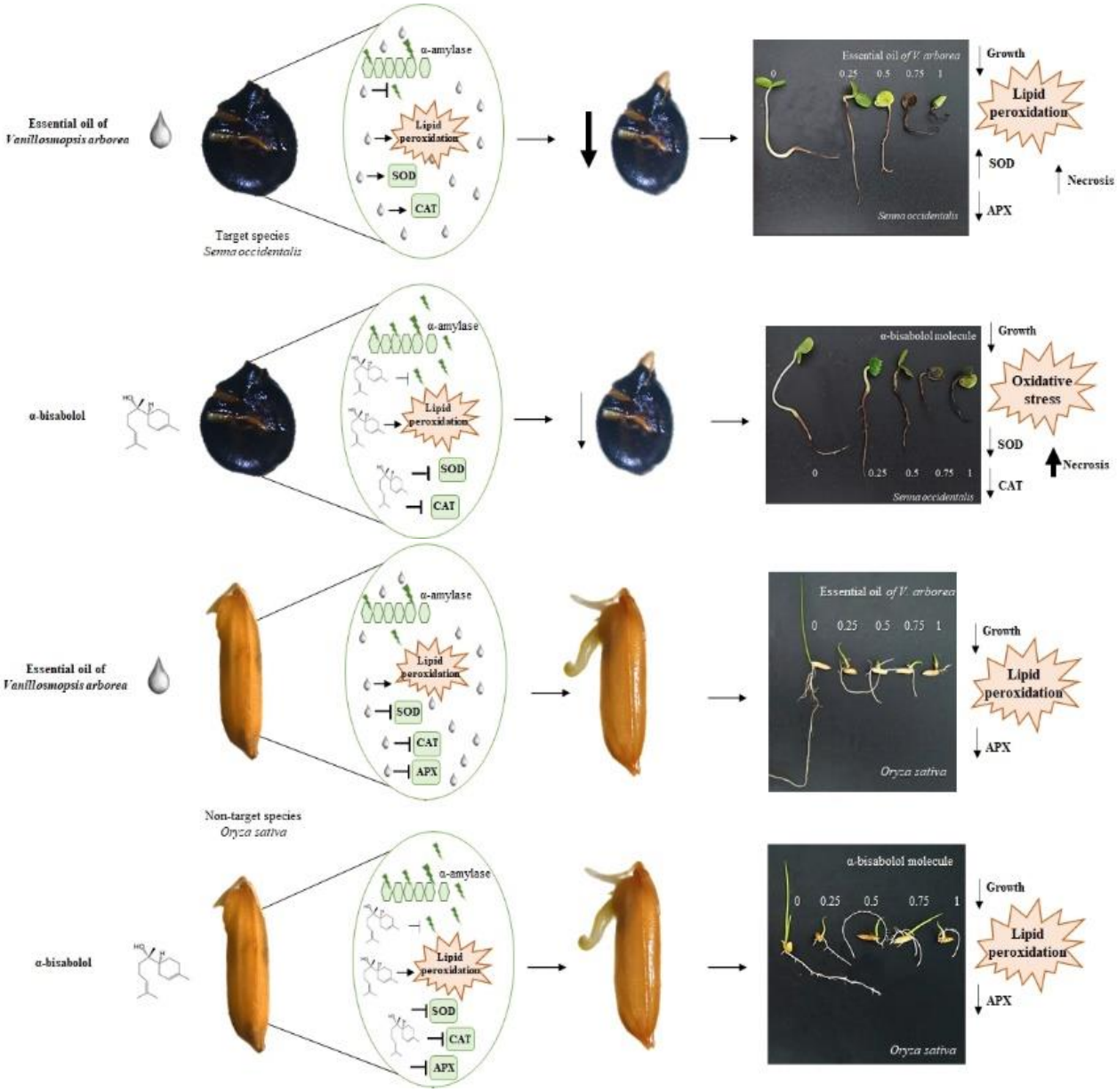
Comparative physiological effects of *Vanillosmopsis arborea* essential oil and α-bisabolol on a weed and a crop species in pre-emergence

Graphic abstract



Abstract

The aim of the work was to evaluate the effect of both *Vanillosmopsis arborea* essential oil and the α-bisabolol molecule in the germination rate, α-amylase activity, and the antioxidant metabolism of *S. occidentalis* and *O. sativa* seeds and seedlings. The essential oil of *V. arborea* and α-bisabolol were diluted and applied to seeds of *S. occidentalis* and *O. sativa*. The essential oil reduced the percentage and speed of germination of *S. occidentalis* seeds, reduced α-amylase activity and induced a redox imbalance in seeds of this species. α-bisabolol induced oxidative stress in *S. occidentalis* seedlings, reducing growth and increasing necrosis. The treatments induced a redox imbalance in the seeds and seedlings of the cultivated species *O. sativa*, but to a lesser extent than the weeds. The essential oil of *V. arborea* and the α-bisabolol molecule showed a promising phytotoxic effect on the germination and initial growth of *S. occidentalis* seedlings. The mode of action of the essential oil consists of inhibiting the activity of the enzyme α-amylase, increasing the ROS and lipid peroxidation. The molecule reduces growth through of inducing oxidative stress in seedlings, suggests a greater role of this in post-emergence.

Keywords: Bioherbicide; *Senna occidentalis*; *Oryza sativa,* Lipid peroxidation; Allelochemical

1. Introduction

Essential oils are constituted of volatile secondary metabolites and its composition is related to genetics, evolution and the environment in which the plant is inserted (Bakkali et al., 2008; Figueiredo et al., 2008). Therefore, essential oils have different biological functions and can be sources for the formulation of new products (Bakkali et al., 2008). The metabolites of essential oils are commonly related to weed control (Raveau et al., 2020). Due to the presence of substances capable of inducing disturbances in the metabolism of other species (Dhifi et al 2016; Macias et al., 2006). In addition, essential oils can influence different targets in the metabolism of weeds, thus increasing the efficiency of the management of these species (Araniti et al., 2018; Grul'ová et al., 2020). Alterations in the metabolism of weeds induced by allelochemicals can occur in the pre and post-emergence, that is, in the germination and initial growth.

Among the species that produce essential oil with high potential in controlling weeds, there is *Vanillosmopsis arborea* Baker ("candeeiro"), an endemic tree of Chapada do Araripe,

Crato-CE, Brazil (Colares et al., 2013; Marco et al., 2015). The essential oil of *V. arborea* has α-bisabolol as its main component (around 90%) (Cap. 1). The α-bisabolol is a sesquiterpene with pharmacological properties widely used in cosmetics (Marco et al., 2015). In previous studies, it was reported that *V. arborea* essential oil reduced the growth and development of several weed species, besides inducing changes in the cell cycle and increasing chromosomal aberrations in the plant model *Lactuca sativa* (see chapter 1).

It was suggested that the effects caused by *V. arborea* essential oil are related to its major component (see chapter 1). However, studies with the use of the isolated α-bisabolol molecule contrasting with the essential oil are necessary for the advancement of the formulation of a bioherbicide. Also, the understanding of the physiological effect of both α-bisabolol and the essential oil in weed and non-target species must be reached. In this premise, it is hypothesized that the essential oil of *V. arborea* and the α-bisabolol molecule act similarly reducing the germination and initial growth of target plants. It is supposing that this reduction occurs through of the increasing of reactive oxygen species (ROS), leading to damage by lipid peroxidation in seeds and seedlings and suppressing the activity of amylolytic enzymes.

Whereas in the germination, essential metabolic processes start with the imbibition of dry seeds, increasing cellular respiration, and providing the metabolic energy (Johnson, 2003). In contrast, this process produces ROS; it is well documented that during germination the ability to regulate the levels of ROS is essential for the success of radicle protrusion. (El-Maarouf-Bouteau e Bailly, 2008; El-Maarouf-Bouteau et al., 2013; Pergo e Ishii-Iwamoto, 2011;). Several allelochemicals can modify the redox system of cells by overproducing ROS due to the inhibition of mitochondrial O_2 consumption (respiration), resulting in seed deterioration due to enhanced lipid peroxidation (Chung et al., 2018; Einhellig, 2004; Gniazdowska e Bogatek, 2005; Pergo; Ishii-Iwamoto, 2011).

The low molecular weight of the allelochemicals allows them to cross cell membranes, changing their composition and fluidity leading to the leakage of ions and cytoplasmic molecules, reducing the capacity of ATP production and inducing loss of mitochondrial potential (Sharifi-Rad et al., 2017). The production of ROS in seedlings plays a fundamental role in growth by cell signaling and interaction with phytohormones (Mishra et al., 2018). However, in excess, they can cause damage to lipids, proteins, and DNA. It has been reported that the decrease in growth induced by allelochemicals occurs due to the overproduction of ROS, suppression of the activity of antioxidant enzymes, consequently, causing oxidative stress (Werrie et al., 2020). And the hydrolysis of starch is a sensitive process of allelochemical action, which can reduce the activity of α-amylase during the establishment of seedlings of weeds species, thus impairing their initial growth (Hegab et al., 2008; Radhakrishnan et al., 2018).

The species commonly used in the validation of metabolites with bioherbicidal potential are weeds and non-target species, such as crops. In this work, there were used seeds and seedlings of the weed *Senna occidentalis* (L.) Link and the crop *Oryza sativa* L. These species were selected for their several responses to the percentage of germination and growth at different concentrations of the essential oil of *V. arborea* (see chapter 1). Therefore, this work aimed was to determine if both *V. arborea* essential oil and the α-bisabolol molecule reduce the germination rate, inhibit α-amylase activity, and induce changes in the antioxidant metabolism of *S. occidentalis* and *O. sativa* seeds and seedlings.

2. Materials and Methods

2.1 Plant material, extraction, and analysis of essential oil of *V. arborea*

The essential oil of *Vanillosmopsis arborea* was extracted from its wood, collected in Chapada do Araripe, Crato - CE, Brazil (7 ° 07'39 "S 39 ° 25'32" W). The essential oil was extracted from the *V. arborea* wood in Clevenger hydrodistillator type (See chapter 1). The

main components identified on *V. arborea* essential oil were α-bisabolol (93.57%), eugenol (2.14%), bisabolol oxide (1.48%), elemicin (0, 67%) and eucalyptol (0.65%) (see chapter 1).

2.2 Preparing of essential oil of *V. arborea* and α-bisabolol solutions

The sesquiterpene α-bisabolol ((−)-6-Methyl-2-(4-methyl-3-cyclohexen-1-yl)-5-hepten-2-ol) molecule was purchased from Sigma-Aldrich-Merck®. The essential oil (100%) or α-bisabolol molecule was diluted in deionized water at 40ºC to obtain the concentrations 0.25, 0.50, 0.75, and 1%. Deionized water was used as control. The pH of the solutions was between 5.5-6.2.

2.3 Non-target and Target species

The seeds of the non-target species *O. sativa*, cv. Caçula, harvest 2019 were assigned by the Genetics and Plant Breeding from the Federal University of Lavras (UFLA). The seeds of the target species *S. occidentalis* were collected from 20 plants in the municipalities of Ijaci-MG in geographic coordinates 21°10'08"S 44°54'52" W from a natural population.

2.4 Bioassay

The seeds of *S. occidentalis* were submitted to physical dormancy breaking treatment by with sulfuric acid (96%) for 20 minutes, and then washing with deionized water three times to remove any traces of the acid (Delachiave e Pinho, 2003). The seeds of the non-target and target species were surface disinfected in a solution of 2.5% sodium hypochlorite (NaClO) and detergent for 15 minutes and then washed three times in distilled water before germination assays.

After diluting, 4 mL of each concentration of the essential oil or α-bisabolol were utilized to moisten the germination paper in Petri dishes where the seeds were sown. The entire experiment was carried out in germination chambers adjusted with optimal temperature and photoperiod of each species, 25 ºC for *O. sativa* and 30 ºC for *S. occidentalis,* 12 hours of light 40µM photons m^{-2} s^{-1}.

The experiment lasted until germination stabilization (two consecutive days without increasing germination percentage in control) of each target species. The germinated seeds were counted every 24 hours considering 2 mm of the radicle. The germination percentage (GP) and germination speed index (GSI) were calculated by the methodology of Ranal et al. (2009). The shoot and root of the seedlings were measured with the aid of a ruler, but only experimental plots that had at least five seedlings were considered for these measurements.

2.5 Sampling

The imbibition curve was carried out with species for determining the timing of seeds sampling. During imbibition, five replicates of 25 seeds were weighed until the radicle protrusion. The seeds were collected in the phase II of germination triphasic pattern (seeds completely imbibed). The *S. occidentalis* and *O. sativa* seeds were collected at 10 and 12 hours, respectively, of the imbibition curve. Regarding the seedlings, they were collected at the end of the experiment. The seeds or seedlings were frozen in liquid nitrogen (N_2) and stored at -80° C until biochemical analysis.

2.6 Biochemical analysis

The α - amylase activity was performed using 200 mg of seeds macerated with cold deionized water. The supernatant was heated for 15 min at 70 °C, mixed with 1% soluble starch dissolved in sodium acetate buffer at pH 5.6. The mixture was incubated for 15 min at 40 ° C

and boiled for 5 min with 3,5-dinitrosalicylic acid. The samples were read in spectrophotometer at 540 nm with maltose as a reducing sugar standard (Miller, 1959 modified by Liu et al., 2018).

The H_2O_2 quantification was performing with 100 mg of seeds or 200 mg seedlings were macerated in liquid N_2 and homogenized with 0.1% trichloroacetic acid. The supernatant was used for reaction with potassium phosphate buffer and potassium iodide. The readings were performed on a spectrophotometer at 390 ηm and the H2O2 content was subsequently calculated from the standard curve (Velikova et al., 2000).

The extension of lipid peroxidation was measured by the amount of malondialdehyde (MDA). Samples of 100 mg of seeds were macerated in liquid N_2 and homogenized with ethyl alcohol (80%), centrifuged and the supernatant collected three times (Du e Bramlage, 1992). 200 mg of seedlings were homogenized with 0.1% trichloroacetic acid, centrifuged and the supernatant collected (Buege e Aust, 1978). The supernatant was used for reaction with thiobarbituric acid 0.5 or 0.65% with trichloroacetic acid 10 or 20%, for seedlings or seeds, respectively, in a water bath at 95º C for 30 minutes and cooled for 10min in ice. The readings were performed on a spectrophotometer at 440, 532, and 600 ηm (Buege e Aust, 1978; Du e Bramlage, 1992).

Regarding antioxidant enzymes, the extraction for catalase (CAT), superoxide dismutase (SOD) and ascorbate peroxidase (APX) activities were performed with 200 mg of seeds or seedlings macerated in N_2, homogenized with 1.5 ml of phosphate buffer, EDTA and ascorbic acid (Biemelt et al., 1998). Protein quantification was performed using the Bradford method (Bradford, 1976). CAT activity was performed according to Havir and McHale et al. (1987), SOD activity as described by Giannopolitis and Ries (1977), and APX according to the methodology by Nakano and Asada (1981).

158 **2.7 Statistical analysis**

159 The experiments were conducted in a completely randomized design, in a two-way
160 analysis of variance with ten treatments, two sources of potential bioherbicides (α-bisabolol and
161 essential oil of *V. arborea*), and five concentrations (0, 0.25, 0.50, 0.75, 1%). Each treatment
162 had 5 repetitions, totaling 50 experimental plots of 25 seeds each, per species. An independent
163 experiment was carried out for both target and non-target species.

164 The data were analyzed using the statistical software RBio© (BHERING, 2017) and
165 were submitted to the Shapiro-Wilk normality test, analysis of variance (ANOVA). When
166 significant by the F test at 5% probability, the data were submitted to regression analysis or to
167 Tukey test set at 5% of probability.

168

169 **3. Results**

170 **3.1 Physiological responses of essential oil of *V. arborea* and α-bisabolol in seeds**

171 The germination percentage (GP) of *S. occidentalis* seeds was significantly affected (p
172 <0.05) by the application of the essential oil of *V. arborea* (Fig. 1). The highest concentration
173 (1%) used in this experiment caused a reduction of about 50% in this variable. However, the
174 use of the α-bisabolol molecule did not significantly reduce the germination percentage. The
175 germination speed index (GSI) of *S. occidentalis* was decreased by about 50% since the lowest
176 concentration (0.25%) of the essential oil of *V. arborea*, however, the greatest reduction
177 induced by the α-bisabolol was 30% in the concentration 1%. The GP of *O. sativa* seeds was
178 not significantly affected ($p \geq 0.05$) by the treatments (Fig. 1), but the use of essential oil and α-
179 bisabolol reduced about 30% the GSI in the concentration 1%.

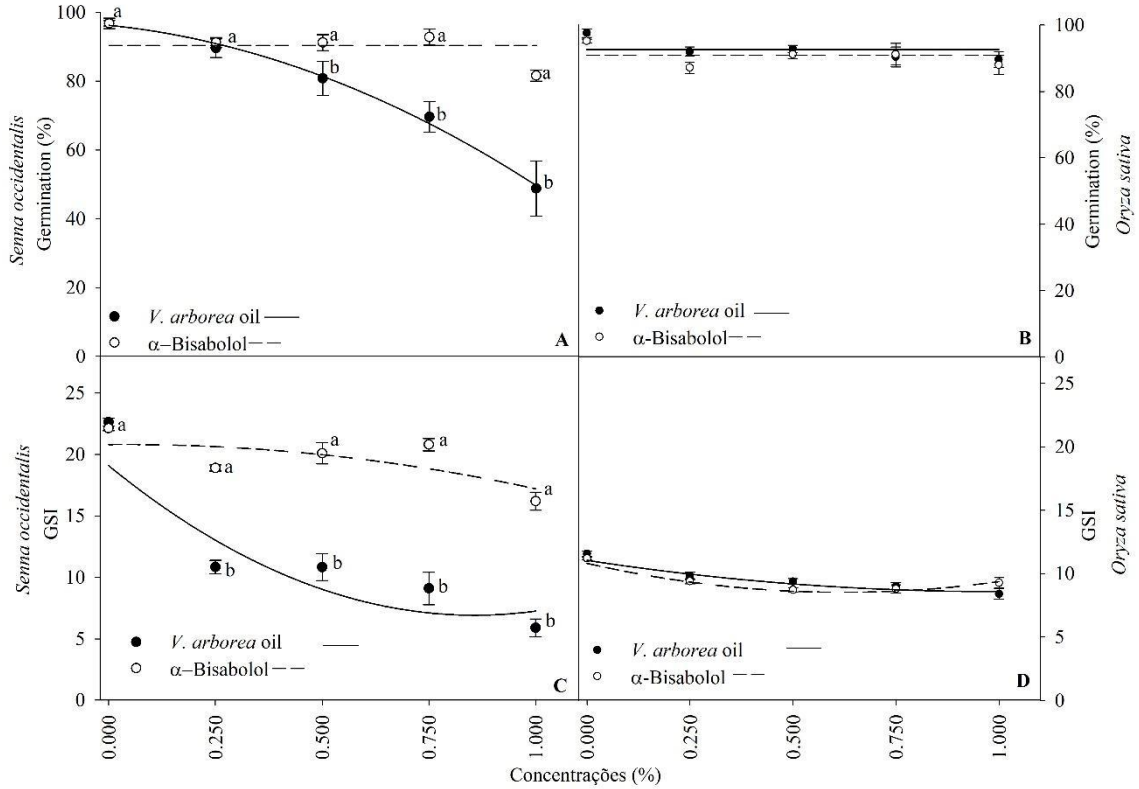


Figure 1.: Germination percentage and germination speed index of *Senna occidentalis* (A; C) and *Oryza sativa* (B; D) seeds submitted to different concentrations of the essential oil of *Vanillosmopsis arborea* and α-bisabolol molecule. The equations are compiled in Supplementary Material.

The essential oil of *V. arborea* reduced the activity of α-amylase (Fig. 2) of *S. occidentalis* seeds in all concentrations, mainly regarding 0.25%, that showed the greatest reduction (60%). The α-bisabolol molecule reduced the activity of α-amylase in all concentrations, except for 0.25%. The use of essential oil did not significantly influence the α-amylase of *O. sativa* seeds, but the molecule reduced (40%) the activity at the concentration of 0.25% and increased (80%) at 1%.

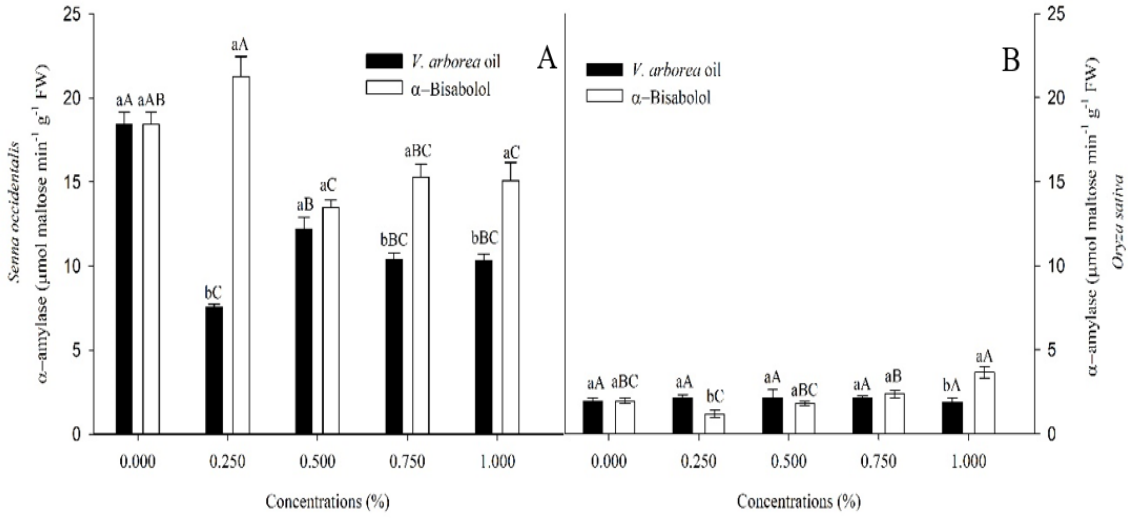


Figure 2.: α-amylase activity of *Senna occidentalis* (A) and *Oryza sativa* (B) seeds in different concentrations of the essential oil of *Vanillosmopsis arborea* and α-bisabolol. * Lowercase compare *V. arborea* essential oil and α-bisabolol. Uppercase compares concentrations of oil or α-bisabolol.

The essential oil of *V. arborea* increased the amount of H_2O_2 in *S. occidentalis* seeds (Fig. 3) at 0.25 and 1%, but the contrary was observed by α-bisabolol application. The essential oil also increased malondialdehyde (MDA) in the seeds of *S. occidentalis* by about 150% at 0.25 and 0.75% (Fig. 3), but the MDA concentration was reduced around 40% at 1%. The α-bisabolol induced enhanced MDA concentrations in both species (Fig. 3). Regarding antioxidant enzymes, the treatment of *V. arborea* oil induced increasing activities of SOD, CAT and APX in *S. occidentalis* seeds at the highest concentrations (Fig. 3). It was not observed in the treatments with α-bisabolol, in which only the concentration of 0.5% increased the activity of the three enzymes (Fig. 3). The lowest concentrations of the oil increased the levels of H_2O_2 in *O. sativa* seeds with no effect of MDA concentration, contrary to the observed for the treatments with α-bisabolol. There was a depletion in CAT, APX and SOD activities at 0.25% in both *V. arborea* oil and α-bisabolol (Fig. 3).

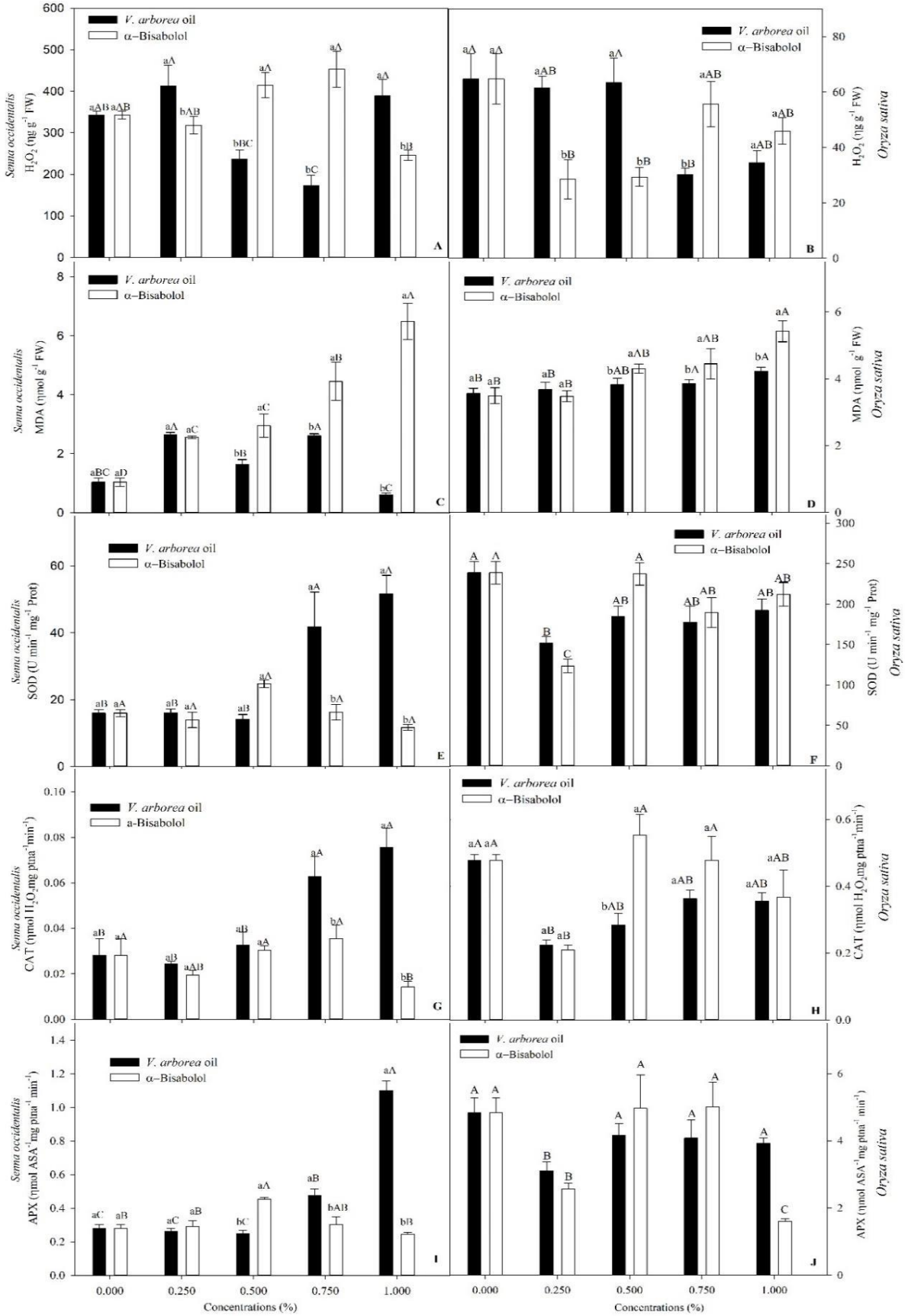


Figure 3: Content of of H_2O_2, malondialdehyde (MDA), and the activity of the antioxidant enzymes superoxide dismutase (SOD), ascorbate peroxidase (APX), and catalases (CAT) of *Senna occidentalis* (A; C; E; G; I) and *Oryza sativa* (B; D; F; H; J) seeds submitted to different concentrations of the essential oil of *Vanillosmopsis*

arborea and α-bisabolol. * Lowercase compare V. arborea essential oil and α-bisabolol. Uppercase compares concentrations of oil or α-bisabolol.

3.2 Physiological responses of essential oil of *V. arborea* and α-bisabolol in seedlings

The shoot and root size of *S. occidentalis* seedlings were significantly affected ($p < 0.05$) by the treatments (Fig. 4). The shoot size was strongly reduced from the concentration of 0.25% in both treatments, the essential oil of *V. arborea* and the α-bisabolol. The concentration of 0.25% increased the root size of *S. occidentalis* seedlings as treated with oil as with α-bisabolol. However, in both treatments, a decrease of seedling root of about 50% was observed at 1%. Seedlings of *S. occidentalis* showed necrosis when treated with 0.75 and 1% of the essential oil, and with 0.5, 0.75 and 1% of α-bisabolol (Fig. 4).

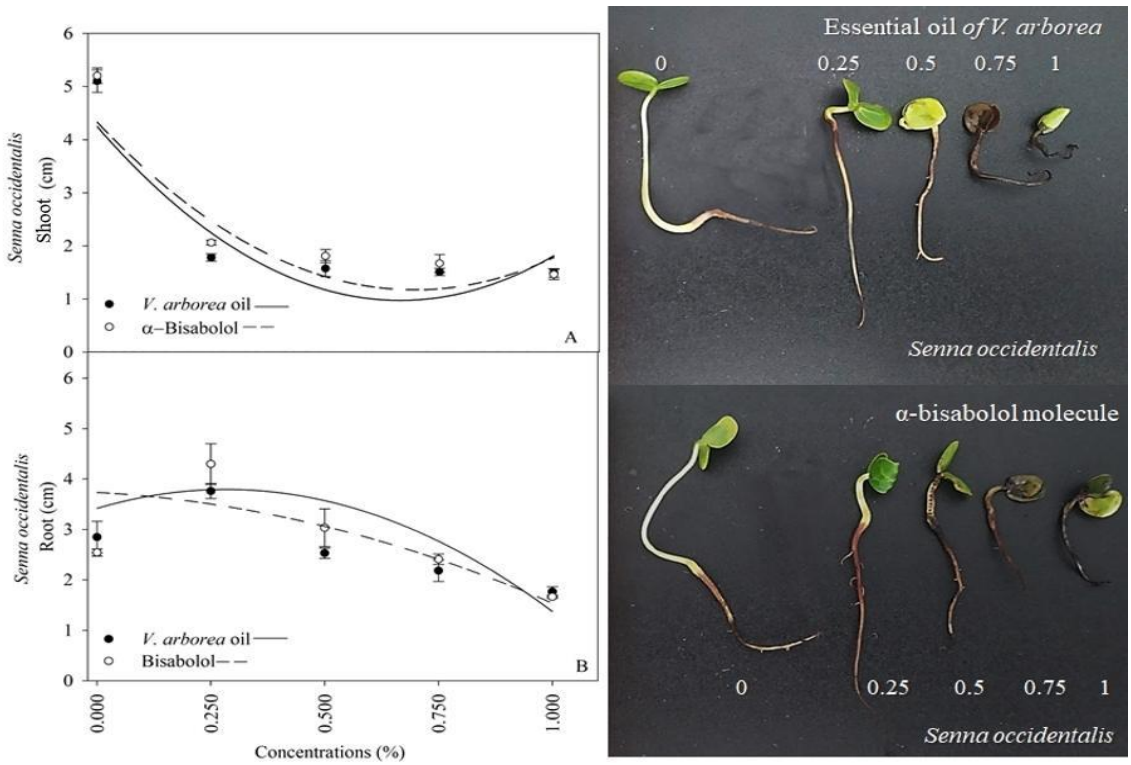


Figure 4: Length and morphology of shoot (A) and root (B shoot of *Senna occidentalis* seedlings submitted to different concentrations of the essential oil of *Vanillosmopsis arborea* and α-bisabolol molecule. The equations can be seen at Supplementary Material.

The root and shoot size of *O. sativa* seedlings was significantly affected ($p < 0.05$) by the treatments (Fig. 5). The essential oil caused more drastic reduction than α-bisabolol of the shoot size in all concentrations tested in *O. sativa* seedlings. The concentration of 1% of the

essential oil and α-bisabolol showed the greatest reduction of *O. sativa* shoot. The root size of *O. sativa* seedlings was also affected by the treatments. However, the essential oil caused a huge reduction compared to α-bisabolol with the increasing of the concentrations (Fig. 5).

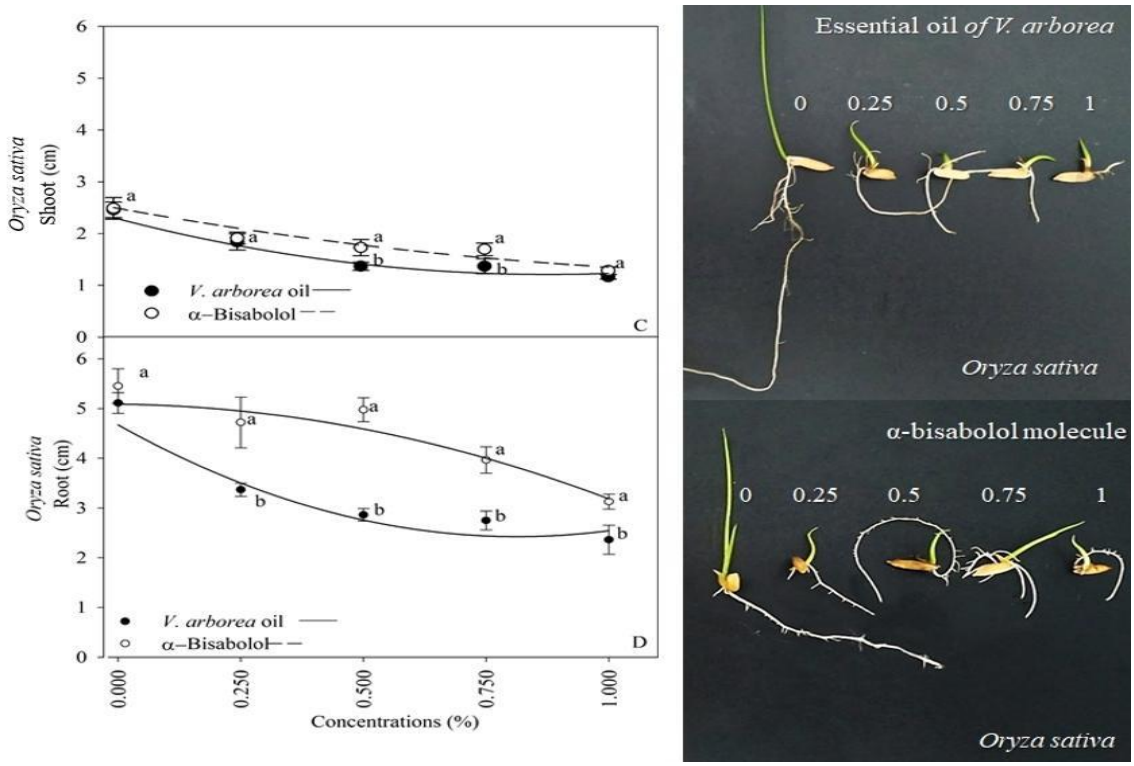
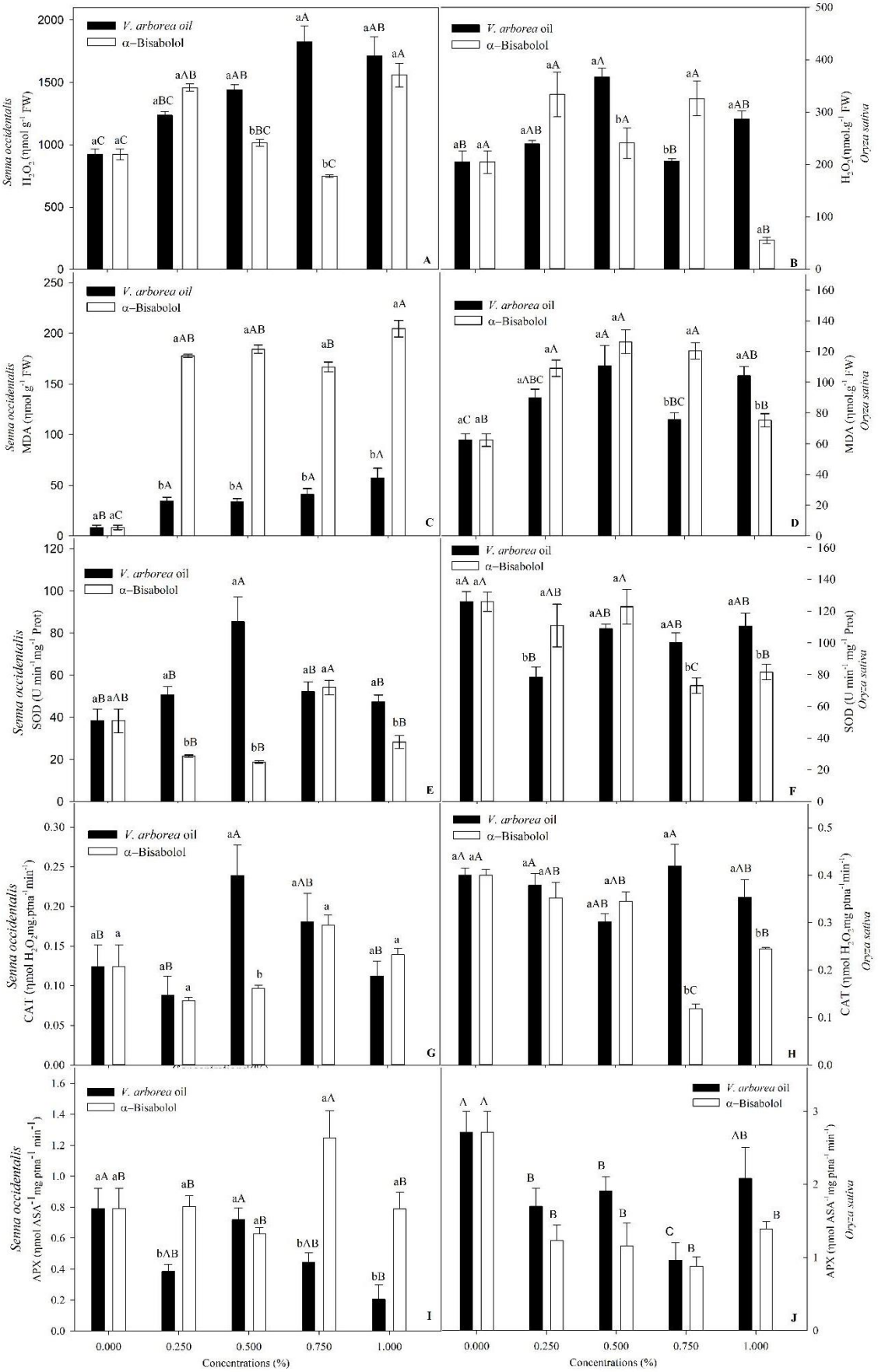


Figure 5: Morphology and length of shoot (A) and root (B) of *Oryza sativa* seedlings submitted to different concentrations of the essential oil of *Vanillosmopsis arborea* and α-bisabolol molecule.

The use of the essential oil of *V. arborea* induced a progressive increase of up to 90% and 500% in the amount of H_2O_2 and MDA, respectively in seedlings of *S. occidentalis* (Fig. 6). The content of MDA in all concentrations of α-bisabolol was increased. The concentrations of the essential oil of *V. arborea* induced different responses of the antioxidant enzymes in *S. occidentalis* seedlings (Fig. 6). An increment of the activity of SOD and CAT at 0.5% of essential oil was observed. The enzyme activity SOD and CAT were reduced by the action of the α-bisabolol at 0.25 and 0.5%. At the concentration of 0.75%, the activity of the enzymes SOD, CAT, and APX was enhanced by about 40, 40, and 50%, respectively, in *S. occidentalis* seedlings (Fig. 6).

The amount of H_2O_2 and MDA in *O. sativa* seedlings was significantly influenced ($p < 0.05$) by the treatments (Fig. 6). Increased in H_2O_2 concentration was verified at the doses of 0.5 and 1% of the essential oil of *V. arborea,* corresponding an increment of 80 and 40%, respectively. The treatments with α-bisabolol showed an increase of H_2O_2 concentration around 50% at 0.25 and 0.75%. The MDA content of *O. sativa* seedlings increased following the concentrations until 0.5% in both α-bisabolol and essential oil. The use of the essential oil of *V. arborea* and α-bisabolol molecule had few influences in dose-response in antioxidant enzymes of *O. sativa* seedlings. The reductions were observed mainly in APX activity (Fig. 6).


V. arborea oil
α–Bisabolol
Senna occidentalis
Oryza sativa
H2O2 (ηmol g-1 FW)
MDA (ηmol g-1 FW)
SOD (U min-1 mg-1 Prot)
CAT (ηmol H2O2 mg ptna-1 min-1)
APX (ηmol ASA-1 mg ptna-1 min-1)
A
B
C
D
E
F
G
H
I
J
Concentrations (%)
0.000
0.250
0.500
0.750
1.000

257

Figure 6: Levels of H_2O_2, malondialdehyde (MDA), and enzyme activity of superoxide dismutase (SOD), ascorbate peroxidase (APX), and catalase (CAT) of *Senna occidentalis* (A; C; E; G; I) and *Oryza sativa* (B; D; F; H; J) seedlings submitted to different concentrations of the essential oil of *Vanillosmopsis arborea* and α-bisabolol molecule. * Lowercase compare *V. arborea* essential oil and α-bisabolol. Uppercase compares concentrations of oil or α-bisabolol.

4. Discussion

This work brings a comparative physiologic action of *V. arborea* essential oil and its majority component, α-bisabolol, in two species in pre-emergence, a weed (*S. occidentalis*) and a crop (*O. sativa*). The action of the essential oil of *V. arborea* was more efficient then α-bisabolol in the inhibiting and delay the germination of the target species of bioherbicide. This reduction and delay in germination may be related to the presence of other metabolites with allelochemical potential in the essential oil of *V. arborea*, such as eucalyptol and eugenol, and their synergistic action with α-bisabolol (Einhellig, 2002; Govêa et al., 2020; Silva et al., 2020; Taban et al., 2018). The reduction of α-amylase enzyme activity slows down the starch hydrolysis process and, consequently, reduces the production of energy necessary for germination and initial growth of the seedlings (Hegab et al., 2008; Mahakham et al., 2017), which is in line with germination velocity index.

The α-bisabolol molecule was more effective in reducing the capacity of seedlings growth than seed germination in both species. This suggests the changes induced by the essential oil of *V. arborea* in the *S. occidentalis* seedlings are related to the α-bisabolol present in this oil. Interestingly, at 0.25% concentration of α-bisabolol, the increased root: shoot ratio of *S. occidentalis* seedlings suggests a hormonal imbalance. The growth of the root is controlled by the action of hormones, mainly auxin, which is transported from cell to cell (Overvoorde et al., 2010). The effects of a sesquiterpene was already reported by Aratini et al (2017) in which the morphology of the *Arabidopsis* root was changed due to modifications in the pattern of auxin distribution.

In *S. occidentalis* seeds, the treatments with α-bisabolol induced more lipid peroxidation comparatively to the essential oil without, however, decreasing germinability. Membrane losses

by oxidative damage are related to increased fluidity and solute leakage (Sharma et al., 2012; Sharifi-Rad et al., 2017). However, it has been discussed that in some species loss of viability is not always associated only with lipid peroxidation (Bailly et al., 2008). On the other hand, the essential oil at the highest concentrations, induced enhanced SOD, CAT, and APX activities, probably decreasing the losses by lipid peroxidation. Impairing of antioxidant system could induce changes in the essential role of ROS as a signal in the plant metabolism over an "oxidative window" (El-Maarouf-Bouteau e Bailly, 2008). In the concentration 1% of the essential oil in *S. occidentalis* seeds, the increase of ROS was probably controlled by high activity of the SOD, CAT, and APX enzymes in the seeds, but this high investment in defense can harm other functions necessary for germination. Also, changes in antioxidant metabolism induced by allelochemicals can still cause disturbances in the hormonal balance of the seeds (Bogatek e Gniazdowska, 2007). All of these changes provided oxidative damage that could cause cell death by activating processes of apoptosis and/or necrosis, and loss of function of essential organelles it is a mechanism associated with essential oils (Sharifi-Rad et al., 2017). However, it is reasonable to suggest that the impairment of germination of *S. occidentalis* seeds treated with the essential oil could be related to the oxidation of other kind of targets, such as proteins (i.e., storage proteins) and DNA. In the seeds of *O. sativa,* the reduction in hydrogen peroxide in practically all concentrations of the treatments, and the significant increase in lipid peroxidation induced by α-bisabolol at 1% concentration, which can be explained by the reduction in APX activity.

The *S. occidentalis* seedlings treated with different concentrations of the molecule showed oxidative stress, proven by a much higher amount of MDA than the control and the seedlings treated with essential oil. The seedlings of *S. occidentalis* do not dissipate a high content of peroxide that occupies a consumption of this ROS in lipid peroxidation. It is also possible that were produced other reactive species, such as superoxide anion (O^{2}-) and hydroxyl

radical (*OH) that can induce damage oxidative (Sharma et al., 2012). Besides, there was inhibition of the activity of the enzymes SOD, CAT, and APX. The oxidative stress, cause cell death, increase necrosis in various parts of the plant, reaching the entire plant (Fig. 3), reducing growth and eventually causing the plant death (Araniti et al., 2018; Sharma et al., 2012). This increasing and high lipid peroxidation and, consequently, oxidative stress has been occurring since the seeds treated by α-bisabolol, thus suggesting a non-recovery of these plants. Regarding for *O. sativa* seedlings, the antioxidant system was suppressed and consequently increased the lipid peroxidation, which reduced the length of the *O. sativa* seedlings. But, contrary to what happened with *S. occidentalis*, this lipid peroxidation was not so high, thus providing a greater chance of recovery of *O. sativa* seedlings.

5. Conclusion

The essential oil of *V. arborea* and α-bisabolol molecule showed a phytotoxic effect promising in the reducing of germination percentage and growth of *S. occidentalis* seedlings. The mode action of essential oil consists of inhibiting the activity of the enzyme α-amylase, increasing ROS and lipid peroxidation. This was more aggressive in the inhibition of the parameters evaluable depending of stage of development of the target plant. Its due to the synergistic action of the essential oil metabolites with the α-bisabolol molecule. Regarding the molecule reduces growth occurs by inducing oxidative stress in seedlings, it suggests a greater role of this in the post-emergence. The 0.5% concentration of the essential oil and the molecule are effective in reducing the growth and initial development of *S. occidentalis*. The specie non-target, *O. sativa* showed small sensibly to the treatments. There is an action selective of essential oil of *V. arborea* and the α-bisabolol molecule. Further studies are needed to investigate the application of the essential oil and molecule in the pos-emergence of weeds.

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Could the essential oil of *Vanillosmopsis arborea* and the sesquiterpene α-bisabolol induce changes in photosynthesis and oxidative system in a weed and a crop species in the same way?

Abstract

The aim of this work was to evaluate the bioherbicidal potential of the essential oil of *Vanillosmopsis arborea* and α-bisabolol molecule in the post-emergence of *Oryza sativa* and *Senna occidentalis*. The essential oil of *V. arborea* and α-bisabolol were diluted in concentration of 0.5%. and sprayed in plants, according to the treatments. The variables analyzed were gas exchange, chlorophyll fluorescence, pigment content and biochemical analyzes. The essential oil reduced the photosynthesis of *O. sativa* and increased lipid peroxidation. Both treatments reduced the photosynthesis of *S. occidentalis* and caused damage to the shoot. The oil reduced the chlorophyll and increase of lipid peroxidation in *S. occidentalis*. Some physiological processes of *S. occidentalis* plants were affected, but the crop species, *O. sativa,* was minimally influenced. It is suggested that in post-emergence there is a selective action and promising of the essential oil of *V. arborea* and the molecule α-bisabolol as a bioherbicide.

Key words: bioherbicide, 'candeeiro', chlorophyll fluorescence, post-emergence, oxidative stress.

1. Introduction

The quantity and quality of agricultural products applied in crop species is constantly affected by weeds plants[1]. Usually, synthetic herbicides are used to control these biotic agents, but the risks to non-target organisms, such as crops, soil microbiome, air, and for aquifers, are diverse and sometimes harmful[2–4]. Thus, it is necessary to search for "environmentally-friendly" products that increase food security and can reduce damage to the environment. An alternative considered environmentally safe in the management of weeds are the bioherbicides, produced from metabolites of plants or microorganisms[5].

These bioherbicides may be formulated from secondary metabolites of plants, known as allelochemicals also found in essential oils, due to the potential they have to influence growth and development of the surrounding species[6,7]. They can be used to negatively interfering with the life cycle of plants, thereby increasing its efficiency in weed control[6]. Moreover, the use of

allelochemicals can bring ecological benefits reducing soil contamination due to the high degradability of these compounds into the environment[8–10].

The main components of essential oils which are often responsible for their biological properties, are able to interacting with the plasma membrane of the target species[11, 12]. In general, essential oils are composed by a mixture of terpenes; indeed, a huge number of terpenes has already been reported as allelochemical action[13, 14]. The effect of essential oil on the metabolism of the target species can occur in a pre or post-emergence, inducing damage to the seeds, affecting germination, and reducing plants growth, causing injury [6, 11].

Vanillosmopsis arborea Baker is an endemic species of Chapada do Araripe, Crato-CE, Brazil, that produces essential oil in its stem with high economic and medicinal value. The essential oil of *V. arborea* is usually composed by terpenes and phenolics, and the sesquiterpene α-bisabolol composes about of 70-90%[15]. The essential oil of *V. arborea* has already been characterized by its phytotoxic and cytotoxic potential (See chapter 1). Moreover, it was recently reported with ability to inhibit the germination and growth of model species and weeds, besides reducing the cellular division and increasing the chromosomal aberrations in meristematic cells of the root of *Lactuca sativa* L (see chapter 1). These results suggested a selective and promising action of this oil due to its the majority component, the α-bisabolol.

Therefore, an investigation of the influence and mode of action of the essential oil of *V. arborea* and the α-bisabolol molecule on the initial growth (early post-emergence) of species was carried out. It was reported that essential oil causes a greater influence in the germination than the α-bisabolol. The initial growth of seedlings was reduced in a similar way by essential oil and α-bisabolol, but with greater induction of oxidative stress was caused by the α-bisabolol application. Therefore, the previous results suggest a larger action in physiology of target plants (weeds) in post-emergence. In this premise, it is hypothesized that the essential oil of *V. arborea*

and the α-bisabolol molecule act similarly in inhibiting photosynthesis and, consequently, inducing oxidative stress in the target species.

Whereas in the post-emergence, allelochemicals can interfere in several process, such as photosynthesis, water and nutrient uptake, and antioxidant metabolism[6]. The changes in the chlorophyll content can occur by inhibit their synthesis, induce its degradation or both an integrated way[16]. Changes in chlorophyll can modify electron transport, consequently affecting photosynthesis. The allelochemicals reduce the photosynthesis through damage to the photosystem II and to protein D1, changing the stomatal conductance[10, 17]. Considering the chloroplast as a site of reactive oxygen species (ROS) production and by the numerous modifications induced by allelochemicals, it is expected a significant increase in the production of ROS as a result of allelochemicals application. ROS overproduction modify cellular homeostasis if no efficient scavenging system is being performed, causing oxidative damage in plants[18]. Thus, changes in plant growth and redox status are modes of action that can guarantee an effective control of weeds.

In the present study, in consonance of the previous studies, the target species of a bioherbicide chosen was *Senna occidentalis* (L.) Link ("matapasto"). *Senna occidentalis* is often considered a weed, growing in several territories and influencing the yield of pastures and cereal crops, such as *Oryza sativa* L. [19-21]. Moreover, ingesting large amounts of its seeds can be toxic to animals[20]. Similarly, the non-target species of a bioherbicide (crop species) chosen was *Oryza sativa* L., one of the most largely consumed food by humans. Considering that non-target species could absorb bioherbicides during application, it is necessary to use crops to understand the physiological effect of bioherbicides on non-target species. Therefore, the aim of this work was to determine whether the essential oil of *V. arborea* and of the α-bisabolol molecule cause changes in photosynthesis and induce oxidative stress in plants *O. sativa* and *S. occidentalis*.

2. Materials and Methods

2.1 Plant material, extraction, analysis and dilution of essential oil of *Vanillosmopsis arborea*

The wood from *V. arborea* was removed of lateral branches from a nature population localized in the Chapada do Araripe in the municipality of Crato – CE, Brazil (7°07'39"S 39°25'32"W). The collection of plant material was carried out in December 2019 and sent to the Interdisciplinary Laboratory in Natural Products of the Federal University of Cariri, where it was cut and portioned in parts of 500 g. The essential oil was extracted using a Clevenger type hydrodistillator and was mixed and placed in a glass container, protected from light and refrigerated (4 ºC) until use in the experiment (See chapter 1).

The pure essential oil (100%) was heated and diluted in deionized water at 40 ºC to obtain the concentration of 0.5%. The analysis of the essential oil was carried out by gas chromatography coupled to the mass spectrophotometer (GC-MS) and was revealed that the main components of this essential oil were α-bisabolol (93.57%), eugenol (2.14%), bisabolol oxide (1.48%), elemicin (0.67%) and eucalyptol (0.65%) (see chapter 1).

2.2 α-bisabolol solution

The sesquiterpene α-bisabolol ((−)-6-Methyl-2-(4-methyl-3-cyclohexen-1-yl)-5-hepten-2-ol) was purchased commercially from Sigma-Aldrich-Merck KGaA. The dilution of 0.5% of α-bisabolol followed the same dilution method as the essential oil of *V. arborea*.

2.2 Non target and Target species

The seeds of the non-target species *Oryza sativa* cv. Caçula, harvested in 2019, were assigned by the Genetics and Plant Breeding sector at the Federal University of Lavras (UFLA).

The seeds of the target weed specie *Senna occidentalis* were collected from a population of 20 plants in the municipally of Ijaci-MG (21°10'08"S 44°54'52"W).

2.3 Bioassay

The seeds of *S. occidentalis* were submitted to physical dormancy breaking treatment by submerging in sulfuric acid (96%) for 20 minutes and then washed with deionized water to remove any traces of the acid[22]. The seeds of the non-target and target species were disinfected in a solution of 2.5% sodium hypochlorite (NaClO) and detergent for 15 minutes and then washed three times in distilled water.

The experiment was conducted in a greenhouse, and the seeds were sown in 0.8L pots containing a mixture of soil (dystrophic Red Latosol) and sand (1:1). Twenty days after emergence, the thinning was carried out and two plants per pot were maintained. The imposition of the treatments in plants of *O. sativa* and *S. occidentalis* was carried out 45 and 120 days after emergence, respectively. Critical period of competition among rice plants and weeds[23]. The essential oil of *V. arborea* or the α-bisabolol molecule, both at 0.5% (as defined in previous experiment), or deionized water (control), were pulverized in the plants at a volume of 4 mL. After performing the treatments, the plants were accompanied daily. The duration of the experiment was five days for *O. sativa* and 40 hours for *S. occidentalis*, which was determined by the deterioration of the treated plants (leaf necrosis).

2.4 Growth parameters

The leaf number was performed at the end of the experiment considering all the leaves formed (totally or not expanded). The dry matter was considered at the end of the experiment in which the plants were collected, washed in deionized water, separated in root and shoot, and maintained at 60 °C in oven until constant weight.

130

131 **2.5 Photosynthesis and chlorophyll fluorescence parameters**

132 Gas exchange was measurement before and after the imposition of treatments. Regarding
133 *O. sativa,* the assimilation of CO_2 (*A*) was measured in May of 2020 and in the period of 9-12h
134 in a close system considering the entire plant. It was used the CO_2 Gas Analyzer (SBA-5, PP
135 Systems, Amesbury, USA), according to method of Sestak et al[23], described in Mitchell[24]. A
136 chamber with a metallic structure covered with transparent plastic and a volume of 0.028486
137 m^3 was used. The plant was placed in the chamber and the chamber was completely sealed to
138 avoid the effects of changes in temperature and/or CO_2 concentration. After stabilization of the
139 initial CO_2 concentration (400±10 ppm), the drop in CO_2 concentration was measured for 5
140 minutes. From this, the following equation was used to calculate CO_2 assimilation in a closed
141 system[24] (Equation 1). The correction of the photosynthesis was performed by ambient
142 temperature (30±2 ºC) and standard pressure (STP is 0 °C or 273K and 1 atm or 0.1013 MPa)[24].
143 The leaf area was measured with Easy Leaf Area app.

144 Equation 1:

145 $$A = \frac{C_1 - C_2}{T_1 - T_2} \, x \, \mathrm{V} \, x \, \frac{1}{\mathrm{L}}$$

146 Where:
147 A = CO_2 assimilation (μmol m^{-2} s^{-1});
148 C_1 and C_2 = CO_2 concentration at times T_1 and T_2;
149 V = total volume of the system;
150 L = leaf area.
151 The *S. occidentalis* plants was not large enough for the method of quantifying gas exchange
152 in entire plant, thus this were determined using the LI-6400xt infrared gas analyzer (Li-Cor
153 Inc., Lincoln, NE, USA). Inside the leaf cuvette (6400-40, Li-Cor Inc, USA), the leaf tissue was
154 exposed to photosynthetic active radiation of 1200 μmol m^{-2} s^{-1}, CO_2 concentration of 400 μmol

mol^{-1}, and flux of 500 µmol s^{-1}. The leaf area was adjusted with the ImageJ. Evaluations were carried out in July 2020 and in the period of 9-12h and ambient temperature of 27 ± 2 ºC.

The chlorophyll a fluorescence was measured, right after gas exchange averages, by the MultispeQ spectrophotometer (PhotosynQ), by methodology of Kuhlgert et al[25]. Using the Photosynthesis RIDES new SPAD DMK protocol which is available on the PhotosynQ platform under the Bilabilol project title (https://photosynq.org/projects/bilabilol).

2.6 Pigments concentration

The photosynthetic pigments were determined with 50 mg of leaf tissue discolored with acetone (80% v/v) and the concentration of chlorophylls a, b and carotenoids were analyzed using leaf extract and quantified in spectrophotometer, according to Lichtenthaler and Buschmann[26].

2.7 Biochemical analyses

Biochemicals analyses were performed on the shoot and roots, thus, at the end of the experiment, the plants were collected, washed in deionized water, separated in shoot and roots, frozen in liquid nitrogen and stored at -80 ºC. The quantity of H_2O_2 was measurement using 50 mg of shoot or root, macerated in liquid N_2, homogenized with 0.1% trichloroacetic acid. The samples were centrifuged, the supernatant collected and used to quantify H_2O_2 with potassium phosphate buffer (10mM and pH 7) and potassium iodine (KI). At the end, readings were performed on a spectrophotometer at 390 nm[27].

The quantification of MDA (malondialdehyde, an extension of lipid peroxidation), was performed with 50 mg of shoot or root, macerated in liquid N_2, homogenized with 0.1% trichloroacetic acid, centrifuged at 4 °C and 12 000g and the supernatant collected. The supernatant and a solution of thiobarbituric acid with trichloroacetic acid were taken in a water

bath at 95 ºC for 30 minutes. At the end, readings were performed on a spectrophotometer at 532 and 600 ηm[28].

The extraction for enzymatic analysis of catalase, superoxide dismutase, and ascorbate peroxidase was carried out with 100 mg of shoot or root macerated in N_2, in which the samples were homogenized with 1.5 ml of phosphate buffer, (100mM and pH 7.8), Ethylenediaminetetraacetic acid (EDTA) and ascorbic acid[29]. Protein quantification was performed using the Bradford method[30]. Quantification of catalase was performed using the methodology described by Havir and McHale[31], the superoxide dismutase by the methodology described by Giannopolitis and Ries[32], and for ascorbate peroxidase it was used the methodology described by Nakano and Asada[33].

2.5 Statistical analysis

The experiments were conducted in a completely randomized design with three treatments: two potential bioherbicides (the essential oil of *V. arborea* and the α-bisabolol molecule) and the control (deionized water), with seven replications with four plants each. Of these two plants were used for gas exchanges analysis and two for biochemical analyses. An independent experiment was carried out for target (*S. occidentalis*) and non-target (*O. sativa*) species. The data were analyzed using the statistical software RBio©[34] and were submitted to the Shapiro-Wilk normality test, analysis of variance (ANOVA) and when significant by the F test at 5% probability the means were compared by 5% Tukey test.

3. Results

The growth of the target and non-target species here analyzed due to the pulverization of potential bioherbicides, the *V. arborea* essential oil and the α-bisabolol, showed significant

differences. The accumulation of dry mass of the shoot of the two species was influenced significantly by the application of treatments. The dry mass of the shoot of *S. occidentalis* plants was reduced in 40% using the α-bisabolol. The α-bisabolol induce more damage the shoot in the *S. occidentalis* plants (Fig. 1). For *O. sativa* plants there was an increase of 10% and a decrease of 15% in the dry mass of the shoot by pulverization of the α-bisabolol and essential oil, respectively. The roots, dry mass (Fig. 1) of the plants *O. sativa* and *S. occidentalis* were not significantly affected by the treatments. The number of leaves (Fig. 1) of the *O. sativa* plants was not influenced by the treatments, but the treatment with α-bisabolol reduced the number of leaves of *S. occidentalis* plants. The ratio shoot-root of *O. sativa* plants not was affected by treatments, but for *S. occidentalis* plants there was decrease of this ratio by essential oil and α-bisabolol (Fig. 1).

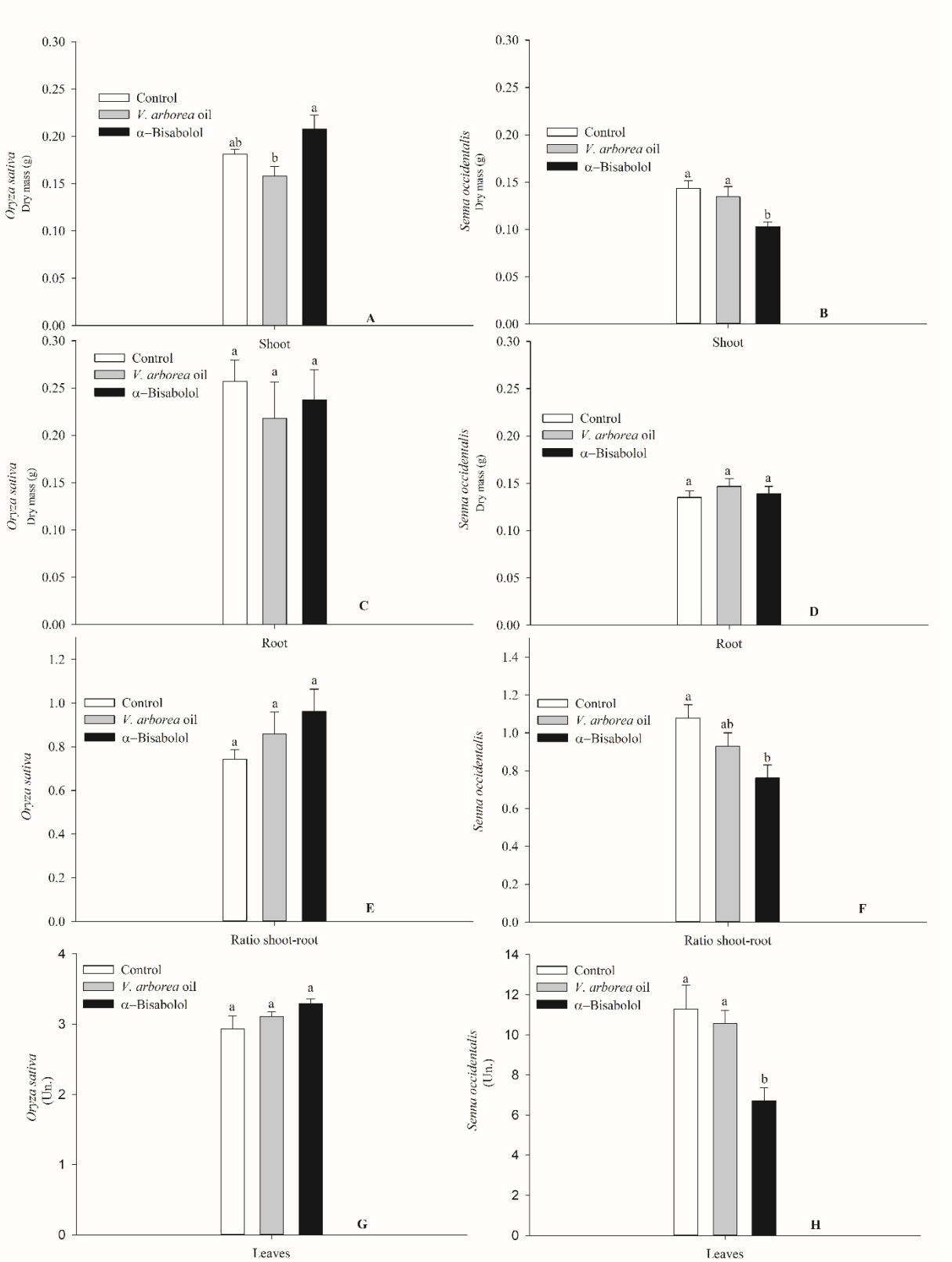

Figure 1: Dry mass, shoot-root ratio and number of leaves of *Oryza sativa* (A; C; E; G) and *Senna occidentalis* (B; D; F; H) plants subjected to the pulverizing of *Vanillosmopsis arborea* essential oil and α-bisabolol molecule at 0.5% concentration. Bars are means ± standard error. Distinct letters show statistically differences among means in shoot, root, shoot-root ratio or number of leaves by Tukey test set at 5% of probability.

The essential oil of *V. arborea* induced injury and small chlorotic spots in the shoot of *O. sativa* plants (Fig. 2). The shoot of *S. occidentalis* plants showed injury after the application of essential oil and α-bisabolol molecule (Fig. 2), but the α-bisabolol induced a greater damage.

Figure 2: Morphology of *Oryza sativa* (A) and *Senna occidentalis* (B) plants subjected to the pulverization of *Vanillosmopsis arborea* essential oil and α-bisabolol molecule. Bar= 1cm

The photosynthesis of the *O. sativa* and *S. occidentalis* plants was significantly influenced ($p<0.05$) by the treatments (Fig. 3). For *O. sativa*, essential oil reduced photosynthesis by about 30%, whereas for *S. occidentalis* this variable was decreased by 90 and 98% by essential oil and α-bisabolol, respectively.

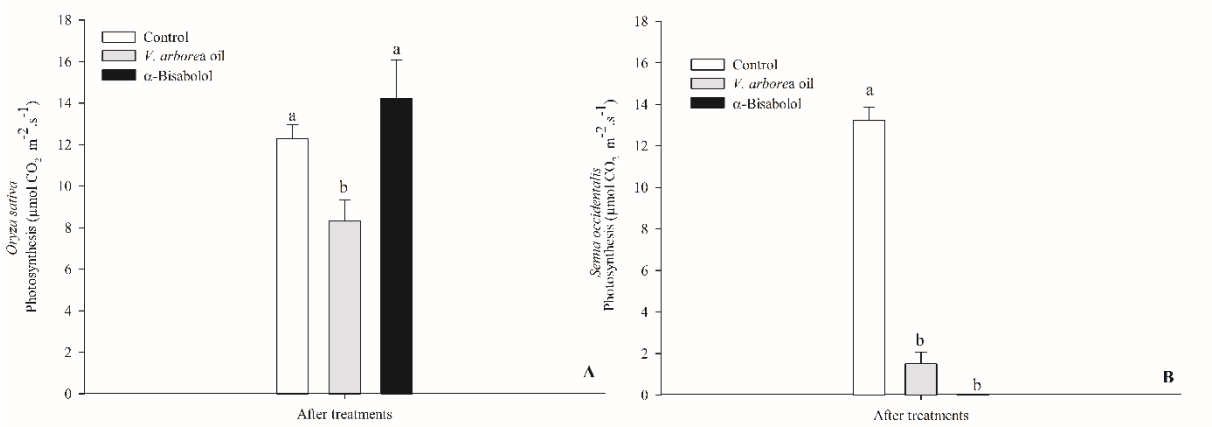

Figure 3: Photosynthesis (µmol CO_2 $m^{-2}s^{-1}$) of *Oryza sativa* (A) and *Senna occidentalis* (B) plants subjected to the pulverizing of *Vanillosmopsis arborea* essential oil and α-bisabolol molecule at 0.5% concentration. Five days and 40 hours after treatments for *Oryza sativa* and *Senna occidentalis*, respectively. Bars are means ± standard error. Distinct letters show statistically differences among means in before treatments or after treatments by Tukey test set at 5% of probability.

The essential oil of *V. arborea* reduced the efficiency of photosystem II and electron transport rate (ETR) in plants of *O. sativa* (Fig. 4). The essential oil of *V. arborea* and α-bisabolol molecule reduced the efficiency of photosystem II in plants of *S. occidentalis* (Fig. 4). And the α-bisabolol reduced the ETR.

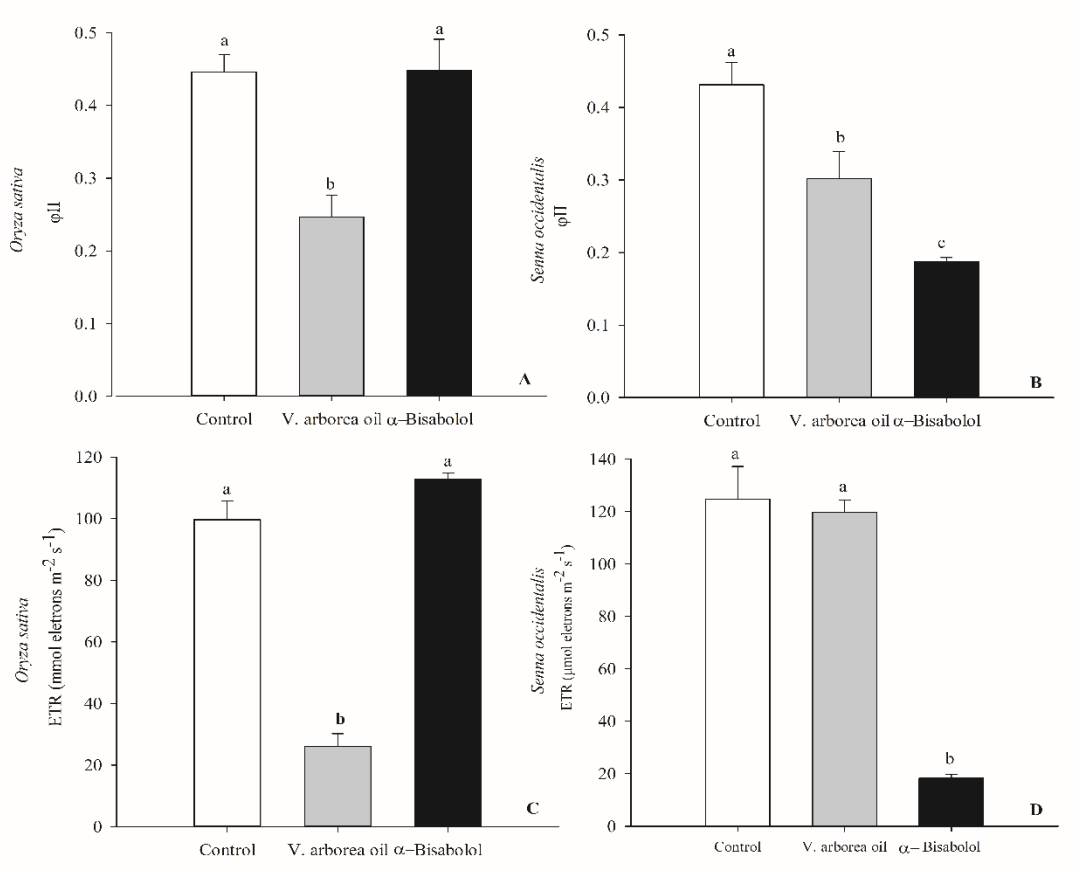

Figure 4: Photosystem II efficiency and electron transport rate of *Oryza sativa* (A; C) and *Senna occidentalis* (B; D) plants subjected to the pulverizing of *Vanillosmopsis arborea* essential oil and α-bisabolol molecule at 0.5% concentration. Bars are means ± standard error. Distinct letters show statistically differences among means in photosystem II efficiency, basal non-radiative decays, non-photochemical quenching and electron transport rate by Tukey test set at 5% of probability.

For *O. sativa* plants, only the amount of chlorophyll b was significantly affected by the treatments ($p < 0.05$) and the application of essential oil reduced it by 20%. However, for plants of *S. occidentalis* the concentration of chlorophyll a, b and carotenoids were reduced by the use of essential oil, but the chlorophyll a/b ratio was not statistically different from the control. Regarding *S. occidentalis*, the use of the α-bisabolol induced an inverse relationship for the amount of pigments, since chlorophyll b and carotenoids increased by 40 and 15%, respectively, and the chlorophyll a/b ratio decreased by 30%.

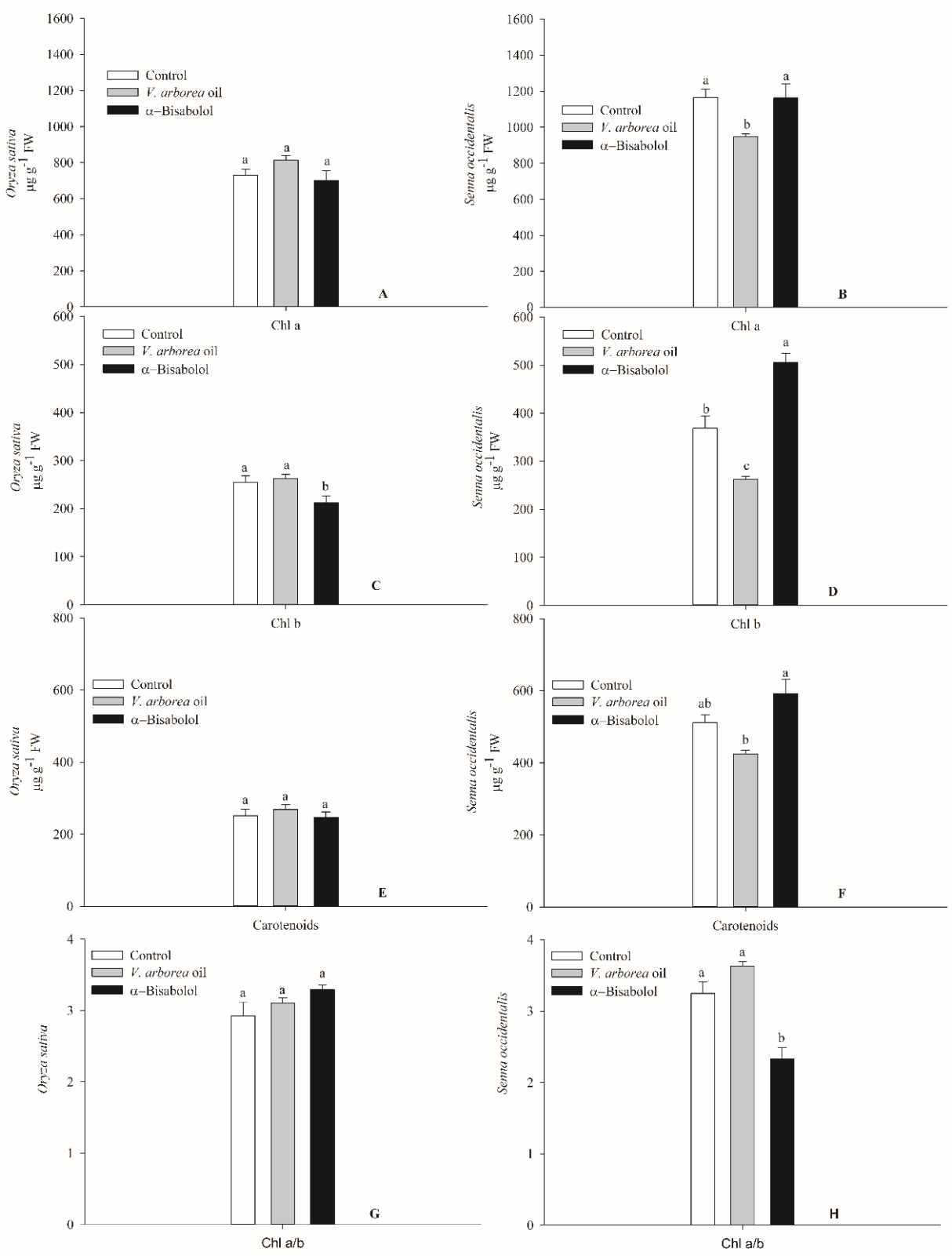

Figure 5: Quantification of chlorophyll a and b, carotenoids and chlorophyll a/b of *Oryza sativa* (A; C; E; G) and *Senna occidentalis* (B; D; F; H) plants subjected to the pulverizing of *Vanillosmopsis arborea* essential oil and α-bisabolol molecule at 0.5% concentration. Bars are means ± standard error. Distinct letters show statistically differences among means in chl a, chl b, carotenoids and chl a/b by Tukey test set at 5% of probability.

The amount of H_2O_2 (Fig. 6) was reduced in the *O. sativa* shoot treated with the essential oil and the α-bisabolol, but the amount of MDA (Fig. 6) was increased by about 75 and 45% with these treatments, respectively. The amount of H_2O_2 in the *S. occidentalis* plants was not significantly influenced by the treatments. The essential oil increased in 10% the quantity of the MDA in shoot. In the roots of the *O. sativa* and *S. occidentalis* plants there was a decrease significative of the MDA with use of the α-bisabolol.

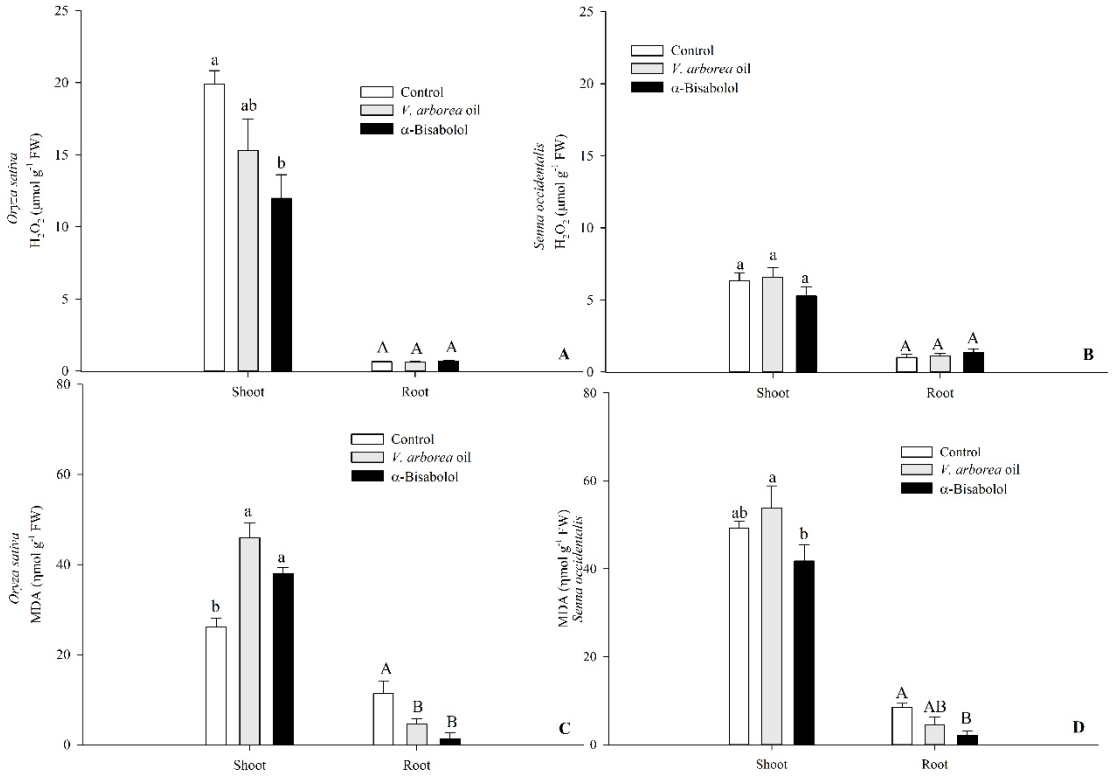

Figure 6: Quantification of H_2O_2 and malondialdehyde in plants of *Oryza sativa* (A; C) and *Senna occidentalis* (B; D) plants subjected to the pulverizing of *Vanillosmopsis arborea* essential oil and α-bisabolol molecule at 0.5% concentration. Bars are means ± standard error. Distinct letters show statistically differences among means in shoot and root by Tukey test set at 5% of probability.

The activity of the SOD, CAT and APX enzymes (Fig. 7) in the *O. sativa* shoot were reduced in about 40, 40 and 35%, respectively, by pulverization of the essential oil and an increase in the activity of SOD and APX by the α-bisabolol. In the roots of *O. sativa* only the activity of SOD was significantly influenced (Fig. 7), with a decrease of 35% induced by essential oil. The pulverization of α-bisabolol molecule increased activity of the SOD, CAT and

APX enzymes (Fig. 7) in the *S. occidentalis* plants, by the use of the essential oil of *V. arborea*, it was verified a reduction in the activity of these enzymes in *S. occidentalis* shoot.

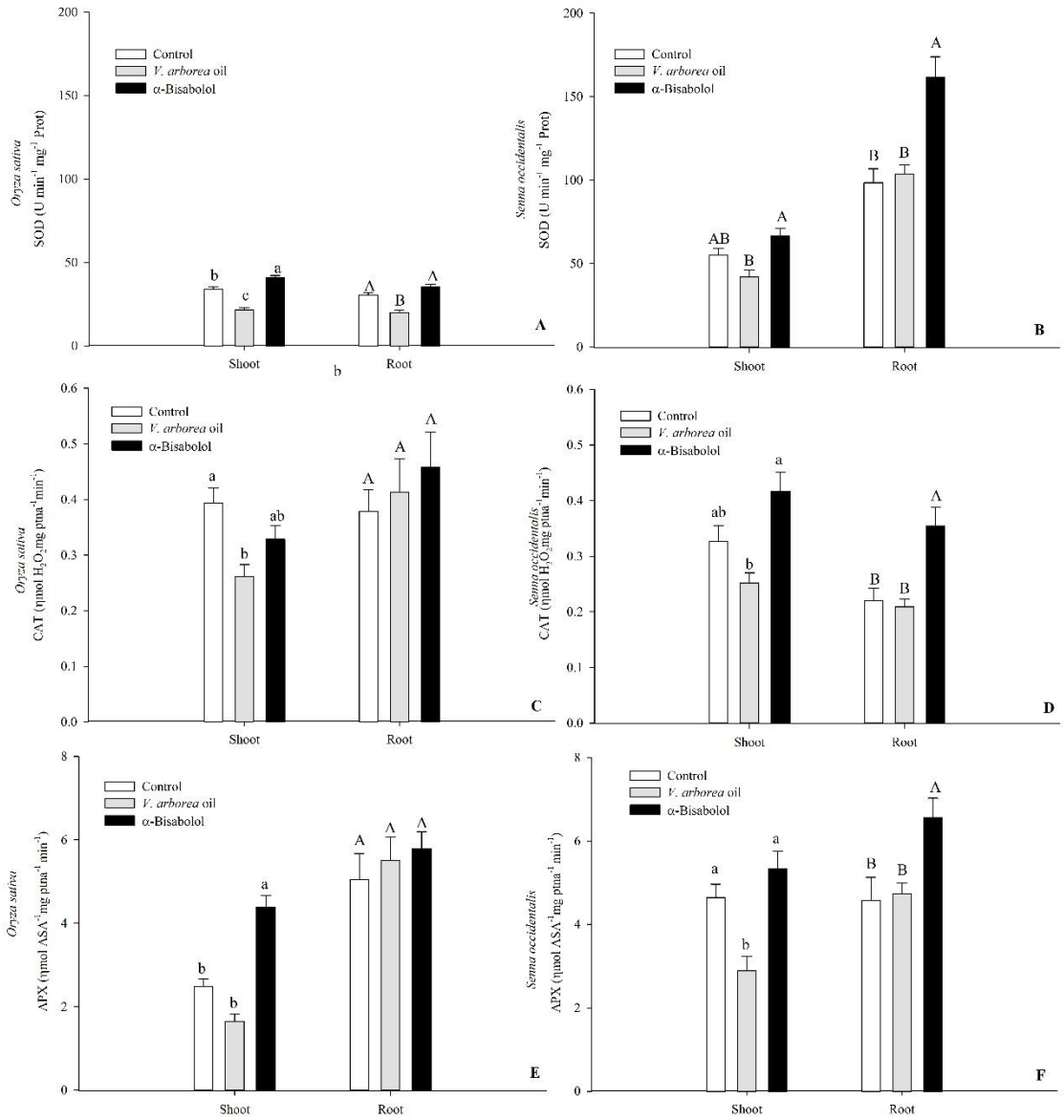

Figure 7: Enzyme activity of superoxide dismutase, catalases and ascorbate peroxidase of *Oryza sativa* (A; C; E) and *Senna occidentalis* (B; D; F) plants subjected to the pulverizing of *Vanillosmopsis arborea* essential oil and α-bisabolol molecule at 0.5% concentration. Bars are means ± standard error. Distinct letters show statistically differences among means in shoot and root by Tukey test set at 5% of probability.

4. Discussion

The application of the essential oil of *V. arborea* and the α-bisabolol molecule in plants induced greater changes in the physiology of the weed *S. occidentalis* than in the crop species *O. sativa,* consequently, the weed deteriorated faster. It thus demonstrates selectivity, the ability to eliminate weeds without affecting the crop[35, 36]. The changes in the photosynthesis of the *O. sativa* plants were induced by the synergistic action of all essential oil metabolites, once the use of the α-bisabolol molecule did not influence this process[37]. The reduction of electron transport in *O. sativa* plants may be related to inhibition of oxidation of the quinones, primary acceptor of electrons and blockage of the electron transport chain (ETC), thus inducing changes in photosystem II[10, 16]. The inhibition of this process decreases photosynthesis[38]. However, these modifications were not sufficient to drastically reduce the photosynthesis of these plants, suggesting that the dry mass of *O. sativa* plants was not affected during the evaluated time.

For the plants of *S. occidentalis*, the essential oil and α-bisabolol induced the reducing carbon fixation, but with some differences in the process. The reduction in photosynthesis and light energy utilization in plants treated with essential oil of *V. arborea*, might be related to the with decreasing in the content of chlorophyll *a* and *b*, this is a common response at allelochemicals and that can occur by inhibition in chlorophyll synthesis or induction of its degradation[39]. This reduction can influence the harvesting of light energy and electron transfer in the center of reaction, inhibiting the activity of enzymes, and thus decreasing ATP synthesis[10]. All these changes could favor an accumulation of unfixed CO_2 and, thus, induced disturbances in the stomata, providing a decrease in photosynthesis[40]. However, until the time of collection its modifications were not sufficient for reduce the number of leaves and the dry mass of *S. occidentalis* plants.

The reduction in photosynthesis in *S. occidentalis* plants treated with α-bisabolol molecule is due to the inhibits the electron transport rate, which reduce the efficiency of

316 photosystem II and decreasing ATP. Unlike of the treatment with essential oil there was more
317 amount of chlorophyll b and consequent reduction in the Chl a/b ratio. There are two antenna
318 systems in the photosystem, the internal antenna complex, which doesn't contain Chl b and the
319 light-harvesting complex, that contain Chl b. Thus, the smaller Chl a/b ratio may be related to
320 the degradation of internal antenna complexes as they are more sensitive than the light-
321 harvesting complexes[41]. The use of α-bisabolol induced the drying of leaves of *S. occidentalis*
322 plants, causing them to fall and, consequently, to reduce the dry mass of the plants.

323 The increase in lipid peroxidation in the shoot of the *O. sativa* plants treated with
324 essential oil it suggests that the H_2O_2 produced lead to oxidative damage and, consequently,
325 inhibited the activity of the enzymes SOD, CAT and APX[42]. The use of the α-bisabolol
326 molecule in the *O. sativa* plants may have induced a depolarization of the cell membrane,
327 increasing its permeability and lipid peroxidation, causing thus, a leakage of cell content and
328 consequently death[18; 43-46]. The increase in lipid peroxidation of shoot of the *S. occidentalis*
329 plants treated with essential oil of *V. arborea* is due to inhibition of the activity of the
330 antioxidant system enzymes associated with the changes in the photosynthesis process[6, 47]. The
331 lower lipid peroxidation in plants treated with α-bisabolol is due to increase of carotenoids,
332 antioxidant pigment and the activity of the enzymes SOD and CAT[42]. In this way, it was
333 possible to determine that the essential oil *V. arborea* and α-bisabolol molecule have the ability
334 of influence physiological process essentials in the growth of the plants.

335

336 **5. Conclusion**

337 The evaluate physiological processes of *S. occidentalis* plants were affected. The mode
338 of action of essential oil of *V. arborea* and the α-bisabolol molecule includes changes in the
339 photosynthetic system, in pigment concentration and in the antioxidant system. The cultivated
340 species, *O. sativa*, was minimally influenced by the treatments. Thus, the results showed here

indicate that in post-emergence there is a selective action and promising of the essential oil of *V. arborea* and the molecule α-bisabolol as a bioherbicide. The rapid deterioration of *S. occidentalis* plants by action of α-bisabolol molecule indicate that it could be classified as a contact bioherbicide. However, more deep studies are needed to explain the main mode of action of both essential oil and α-bisabolol in other target and non-target species regarding the prospection of a promisor bioherbicide.

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CONSIDERAÇÕES FINAIS

O óleo essencial de *Vanillosmopsis arborea* apresentou ação fitotóxica, inibindo a germinação e o crescimento de diversas espécies daninhas e em vários níveis de sensibilidade, sugerindo um controle eficiente das espécies-alvo. A análise do óleo essencial e da molécula α-bisabolol trouxeram resultados inovadores na elucidação do modo de ação destes em espécies alvo e não-alvo. A ação inibitória do óleo essencial de *V. arborea* foi verificada na germinação e crescimento inicial, bem como na pós-emergência nas espécies deste estudo. A ação do óleo essencial na pós-emergência pode estar relacionada à molécula α-bisabolol, seu componente majoritário, pois ao ser utilizada isoladamente apresentou um padrão similar ao óleo. O modo de ação do óleo e da molécula nas plantas da espécie-alvo incluem a modificações no sistema fotossintético, no conteúdo de pigmentos e no balanço redox dessas plantas. Todas essas alterações proporcionaram uma deterioração mais rápida da espécie alvo e, especialmente, mais acentuada com a aplicação da molécula α-bisabolol isolada. Em relação a espécie cultivada, *Oryza sativa* apresentou uma menor sensibilidade a ação fitotóxica deste óleo e do α-bisabolol.

Os resultados aqui apresentados são promissores para o uso do óleo de *V. arborea* e da molécula α-bisabolol como bio-herbicidas, sendo que o α-bisabolol pode ser utilizada para a produção de um bio-herbicida de contato. Dessa forma a espécie de interesse pode ser mais competitiva durante as etapas iniciais do desenvolvimento frente às daninhas por meio do uso do óleo de *V. arborea*. Esses potenciais bio-herbicidas podem ainda ser mais seguro para o ambiente e saúde humana, proporcionando assim uma maior segurança alimentar. No entanto, para confirmação deste potencial ainda são necessários estudos que visem o entendimento da degradação destes compostos no solo, sua relação na cadeia alimentar e que utilizem outras espécies alvo e cultivadas para aprofundamento do modo de ação. Além disso, estudos de métodos eficientes da propagação da espécie *V. arborea* podem proporcionar uma maior utilização desta comercialmente evitando o extrativismo predatório, e impulsionar, assim, um maior desenvolvimento regional.