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Aloysia citriodora essential oil: antimicrobial potential and induced resistance in controlling tomato black spot fungus

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Abstract The objective of this work was to evaluate the antimicrobial and biochemical activity of *Aloysia citriodora* essential oil in controlling *Alternaria linariae*. Antimicrobial activity was tested *in vitro* with 0.0, 0.8, 1.8 and 2.4 μL mL^{-1} dosages of essential oil, and mycelial growth and the growth inhibition were evaluated thereafter. Furthermore, 2.5 and 5.0 μL mL^{-1} essential oil doses were tested in vivo, and severity, control efficiency, diseased and healthy areas, total leaf area, number of leaves and plant height were also subsequently evaluated. Plants grown in pots in a protected environment were used for biochemical analyzes. A 2.4 μL mL^{-1} dose of *A. citriodora* essential oil inhibited *A. linariae* growth *in vitro* and a dose of 2.5 μL mL^{-1} reduced the severity of the disease. Essential oil application to tomato plants induced resistance against tomato black spot fungus, mainly due to β-1,3 glucanase enzyme activity. The increase in guaiacol peroxidase activity was more evident in the presence of the pathogen with or without any treatment. Thus, the use of the *A. citridiora* essential oil is a promising option for controlling black spot fungus and to induce resistance in tomato plants, thereby promoting efficient and sustainable agriculture.

Keywords *Alternaria linariae* · Alternative control · Biopesticide · *Solannum lycopersicum*

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Introduction

Tomato production reached a total area of 55,600 ha in Brazil in 2020, and approximately 65% of this area was destined for fresh market tomato cultivation. The production was 3.9 million tons, and although there has been a reduction in planted area and production, productivity per area has increased (IBGE, 2021). The tomato crop is affected by several diseases; among them is the black spot caused by *Alternaria solani*, being one of the most common and which has caused a reduction in the yield and quality of agricultural production. *A. solani*, as

it is widely known, was reclassified by Woudenberg et al. (2014) due to the size of its spores, while the *A. linearie* species mainly occurs in tomato crops in Brazil.

Plant disease control mainly occurs through chemical control; however, its indiscriminate use can result in environmental pollution, selecting pathogens which are resistant to the active principles used (Chaud, 2021), in addition to putting the farmer's and also final consumers' health at risk (Gaur & Sharma, 2010). Thus, we seek less toxic and efficient products in nature to control plant diseases.

An alternative is the use of *Aloysia citriodora* essential oil. This essential oil is predominantly composed of citral and presents in lower quantities limonene, citroneol, geraniol, α and β-pipene (Haber & Clemente, 2013). In addition, the essential oils has been gaining prominence in agriculture, as it has important characteristics such as antibacterial, antifungal and insecticidal activity. Essential oils can reduce/inhibit mycelial growth, spore production and germination in fungi (Vilela et al., 2009), and promote activation of the plant's defense mechanisms against pathogens (Dalio et al., 2020). The activation of these mechanisms may involve the production of reactive oxygen species (ROS), biochemical and structural changes.

A host plant also activates a wide variety of protection mechanisms in response to infection by pathogens through producing antimicrobial proteins, accumulating ROS and changes in the constitution of the cell wall (Murphy, 2013). The biochemical changes in the host plant due to the higher ROS production are responses which can be involved in resistance, while harmful in the case of susceptible hosts through signaling factors (Wrzaczek et al., 2010). Thus, both application of eliciting products and pathogen infection can alter biochemical attributes, which can be related to the host's resistance or susceptibility.

Tomato cultivation is economically important to the world, and the search for more sustainable tools to control common diseases, such as the black spot of tomatoes, drives this research line. Therefore, the objective of this work was to answer the following questions:

(i) What dose of *A. citriodora* essential oil controls *A. linariae in vitro*?

(ii) What dose of *A. citriodora* essential oil is most effective in controlling the black spot fungus in tomatoes?

(iii) Does *A. citriodora* essential oil induce resistance in tomato plants to control the black spot fungus?

Material and methods

The experiments were carried out in 2020 in the Federal University of Santa Maria (UFSM) on the Frederico Westphalen/RS campus, and the "Luiz de Queiroz" College of Agriculture (Esalq-USP)/SP. The *A. linariae* isolate was provided by the Federal University of Viçosa. *A. citriodora* essential oil used in the experiments was obtained by hydrodistillation of fresh leaves from plants grown in the experimental station of the UFSM.

Antimicrobial activity *in vitro* of *A. citriodora* essential oil in a. linariae isolate

A completely randomized experimental design was adopted using different *A. citriodora* essential oil doses (0.0, 0.8, 1.6 and 2.4 μL mL^{-1}) with five repetitions, with each repetition composed of a Petri dish.

The essential oils were incorporated into the BDA melting culture medium at 50 °C, together with the Tween 20© (for each 0.8 μL of essential oil, 0.05 mL of surfactant was added) and poured into Petri dishes (90 × 15 mm). Inside a laminar flow chamber, 5.0 mm mycelium discs of *A. linariae* were transferred to the center of the dishes containing their respective treatments. The Petri dishes were then incubated in a growth chamber with a 12 hours photoperiod at a temperature of 25 °C for 10 days.

On the tenth day of the experiment, the mycelial growth (MG) was evaluated with the aid of a digital caliper, obtaining two orthogonal measurements and calculating the mean of the MG. Then, it was possible to evaluate the growth inhibition percentage (GIP) from the mean values of the control treatment, calculated for each dosage in relation to the control values.

Potencial of *A. citriodora* essential oil in controlling the black spot in tomato plants

The experiment consisted of foliar applications of *A. citriodora* essential oil to control the tomato black spot fungus. Thus, Santa Clara cultivar tomato plants were grown in plastic pots with a capacity of 3.0 L filled with Plantmax® commercial substrate and kept in a greenhouse with daily irrigation.

The plants were sprayed 40 days after transplanting (fourth pair of true leaves visible) until leaf runoff. The applied solution consisted of the essential oil of *A. citriodora* at doses of 2.5 (D1) or 5.0 µL mL^{-1} (D2), the treatments are described in Table 1. Five plants were used per treatment, each plant characterized as a repetition.

Inoculation was carried out 72 hours after applying the essential oil with an *A. linariae* spore suspension containing 1×10^4 spores mL^{-1} until leaf runoff occurred. In the treatment [W + W], only distilled water was applied. After spore inoculation, the plants were kept in a humid chamber using humidifiers for 48 hours.

The plants became symptomatic on the 5th day after inoculation and then evaluated. Two leaves from the middle third of each plant were evaluated, scanned and submitted to the QUANT software program to obtain the following variables: disease severity, healthy and diseased leaf area. The control efficiency was calculated with the severity results in relation to the control [W + I]. Other growth variables such as number of leaves and plant height were also evaluated.

Table 1 Description of treatments used in tomato plants previously treated with water and without inoculation (W + W) and with doses of essential oil of *Aloysia citriodora* 2.5 µL mL^{-1} (D_1 + I), 5.0 µL mL^{-1} (D_2 + I) and water (W + I), both with inoculation (I) of the pathogen (*A. linariae*)

	-72 h	0 h
D_1 + I	2.5 µL mL^{-1}	Pathogen
D_2 + I	5.0 µL mL^{-1}	Pathogen
W + I	Water	Pathogen
W + W	Water	Water

Biochemical changes in tomato plants treated with *A. citriodora* essential oil to the control black spot

Tomato plants from the Santa Clara cultivar were used and grown in a greenhouse. The plants were then sprayed with a 2.5 µL mL^{-1} dose of *A. citriodora* essential oil (diluted in water + Tween 0.2 mL) at 15 days after transplanting, considered as a possible resistance inducer. The treatments are described in Table 2.

The plants treated as shown in Table 2 were used to evaluate the biochemical responses to the potential inducer (essential oil) with or without pathogen. The experiment was divided into two stages, as described below, due to experimental limitations.

The samples for biochemical analyzes were harvested in six moments: at the treatment application time (−72 h), and at the times 0, 12, 24, 48 and 96 hours after inoculation (hai) with or without pathogen. A completely randomized experimental design was adopted with three replications.

Preparation of the enzyme extract

The leaf samples were macerated with a mechanical homogenizer (Ultra-Turrax) and 15 mL of 0.1 M sodium phosphate buffer (pH 7.0) plus 1% (w/v) polyvinylpyrrolidone (PVPP) and 10 mM EDTA. The homogenate was centrifuged at 20,000 *g* for 20 min at 4 °C, and the obtained supernatant was considered the enzyme extract. The extract was transferred to Eppendorf tubes and used to quantify the total protein and to carry out the enzymatic reactions.

Table 2 Description of treatments used in two experiments for tomato plants previously treated with water and without inoculation (W + W) and with essential oil of *Aloysia citriodora* inoculated with the pathogen (*A. linariae*) (D_1 + I). For experiment II there was also spraying with water and inoculation of the pathogen (W + I)

	Experiment I		Experiment II	
	- 72 h	0 h	- 72 h	0 h
W + W	Water	Water	Water	Water
W + I	–	–	Water	Pathogen
D_1 + I	Essential oil	Water	Essential oil	Pathogen

Total protein quantification

Total proteins were determined by using the Bradford method (1976). Thus, each 20 µL of extract had 1000 µL of Bradford's reagent added (diluted in deionized water in the proportion of 1:5; v/v). The absorbance was subsequently read in a spectrophotometer at 595 nm after 5 minutes.

Guaiacol peroxidase enzyme activity

Guaiacol peroxidase activity was determined by measuring the guaiacol conversion to tetraguaiacol. A mixture made up of 30 µL of the protein extract and 870 µL of the reaction solution was carried out in test tubes on ice. The reaction solution was composed of dibasic potassium phosphate, citric acid, commercial guaiacol and 30% H_2O_2. The tubes were kept in a water bath for 10 minutes at a temperature of 30 °C. After this period, the tubes were returned to the ice and the reaction was stopped by adding 100 µL of 0.5% H_2SO_4. Next, 870 µL of the reaction solution +30 µL of the buffer were used in the reference cell (Kar & Mishra, 1976). The guaiacol peroxidase enzyme activity was read at 470 nm in a spectrophotometer.

β-1,3 glucanase activity

Two tubes were used for each reaction treatment (A and B), with 150 µL of laminarin (1.0 mM sodium phosphate buffer pH 5.0 + laminarin) and 100 µL of the protein extract added to both tubes. Next, 125 µL of the ADNS reagent was added to tube A (Solution A: 2.5 g of ADNS in 75 ml of water + Solution B: 4 g of sodium hydroxide in 50 ml of water +75 g of sodium tartrate). All reactions were performed on ice. The blank tube received only 100 µL of laminarin +100 µL of 1.0 mM potassium phosphate pH 5.0. The tubes were transferred to a water bath at 40 °C for 2 hours. After this period, 125 µL of ADNS was added to tubes B and to the blank, and then they were heated in a water bath at 95 °C for reaction for 5 minutes. The tubes were cooled on ice and the samples were read on a spectrophotometer at 540 nm. It was then possible to calculate the enzyme specific activity by using the amount of protein and the equation generated on the standard glucose curve, according by (Miller, 1959) methodology.

Quantification of free phenols

The leaves were lyophilized for 48 hours to determine the phenols. The lyophilized samples were weighed and 50 mg was placed inside Falcon tubes with a capacity of 15 mL. The tubes received 10 mL of 70% ethanol and remained in a water bath for 1.5 h at a temperature of 70 °C. The free phenols were quantified based on the methodology of Hillis & Swain (1959), and chlorogenic acid (5.0 µg μL^{-1}) was used for the standard curve. The reading was performed in a spectrophotometer at 725 nm after 1 hour.

Statistical analysis

The obtained data were submitted to analysis of variance (ANOVA) and the means were compared by using the Tukey test at 5% probability of error when significant ($p \leq 0.05$). Residual normality tests were performed and the data were normalized by box-cox when the assumptions were not followed ($p \geq 0.05$).

Principal component analysis (PCA) was used. The data were standardized in the PCA by dividing the difference between each sample and the arithmetic mean of the variable of interest by the standard deviation of the variable. The components were selected according to the eigenvalues from the correlation matrix and according to the presence of components with eigenvalues greater than 1 (Kaiser, 1974).

Pearson's correlation was investigated. All analyzes were performed by using the R program (version 4.0). The regression analysis and all graphs were made by using the GraphPad Prism software (version 9.0).

Results

Antimicrobial activity *in vitro* of *A. citriodora* essential oil against Alternaria linariae

The analysis of variance showed a significant interaction between the essential oil x dose factors for the mycelial growth and growth inhibition variables. There was a reduction in mycelial growth as the *A. citriodora* essential oil dose increased (Fig. 1A),

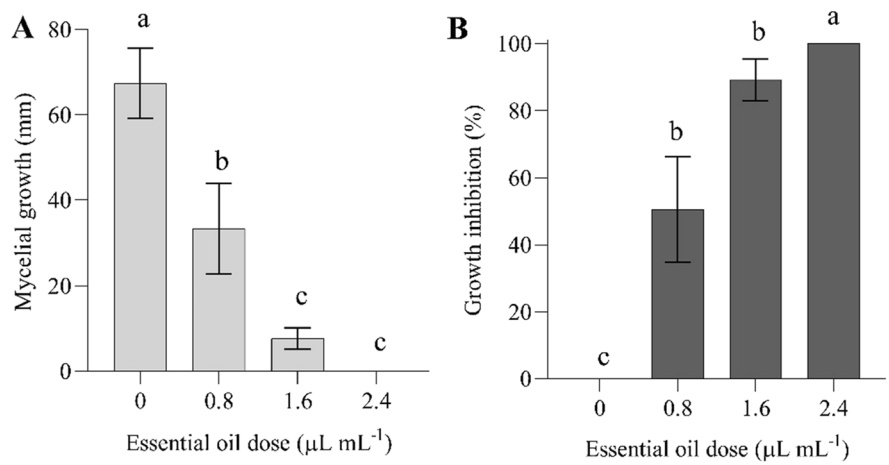

Fig. 1 **A** Mycelial growth and **B** growth inhibition of *A. linariae* in the presence of different *Aloysia citriodora* essential oil concentrations. Equal lower case letters in the same columns indicates that the values do not differ statistically by the Tukey test at 5% probability

while a total mycelial growth inhibition occurred at the dose of 2.4 µL mL^{-1} (Fig. 1B).

Potential of *A. citriodora* essential oil in controlling black spot in tomato plants

The analysis of variance ($p \leq 0.05$) showed significant interaction for the essential oil x dose factors for the diseased and healthy leaf area, severity, control efficiency and number of leaves. The D1 *A. citriodora* essential oil promoted a reduction in the diseased leaf area, although without statistically differing from the control treatment [W + I]. On the other hand, in the tomato plants that were treated with [D1 + I] the healthy leaf area was approximately 70% higher than that of the [D2 + I] treatment (Fig. 2A). The treatment

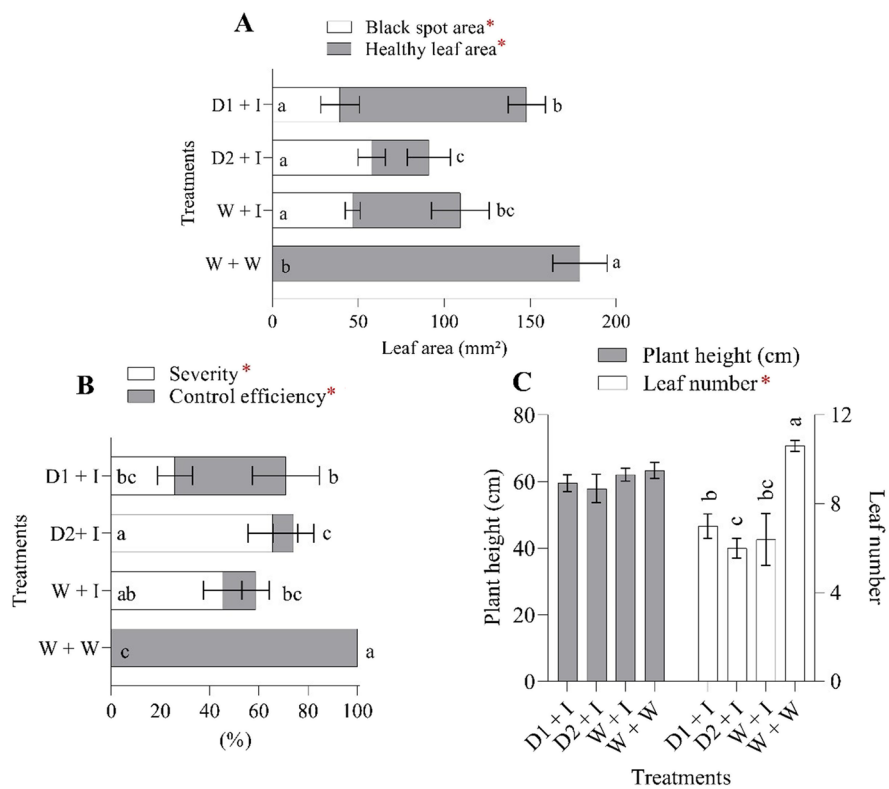

Fig. 2 Effect of *Aloysia citriodora* essential oil in the control of *A. linariae* in tomato and growth of the plants. **A** Diseased and healthy leaf area; **B** severity and disease control; **C** plant height and number of leaves. Plants treated with the essential oil in different concentrations [D_1 + I], [D_2 + I] or not [W + I], [W + W]. D_1 and D_2 corresponding to doses 2,5 µL mL^{-1} and 5,0 µL mL^{-1} of *A. citriodora* essential oil. Same lower case letters in the same column do not differ by the Tukey test at 5% probability

[D1 + I] generated 45% of control efficiency in relation to the control treatment [W + I], while the treatment [D2 + I] compared to the control treatment [W + I] was only 8%.

The highest severity values and lowest efficiency of control were observed with [D2 + I] (Fig. 2B). The [D1 + I] was superior to [D2 + I] treatment in relation to healthy area (108.5mm^2), control efficiency (45%) and number of leaves (7.4). There were no significant differences in plant height (Fig. 2C).

Biochemical changes in tomato plants treated with *A. citriodora* essential oil to control the black spot fungus

The analysis of variance showed significance for the enzymes analyzed in some sampling times. The guaiacol peroxidase enzyme activity did not change between the treatments carried out in experiment I (Fig. 3A). However, there was a difference between treatments for experiment II at 96 hours after inoculation (hai), when [D1 + I] and [W + I] showed higher peroxidase activity, differing statistically from [W + W] (Fig. 3B).

[1] Red arrows indicate the inoculation time in Figs. B, D and F;
[2] Lower case letters compare treatments at the time indicated by the Tukey test at 5% probability of error.

The β-1,3 glucanase enzyme activity changed by the treatments in both experiments. The [D_1 + I] treatment increased the enzyme activity at 12 hai, statistically differing from [W + W] in experiment I (Fig. 3C). Although there was no statistical difference, it was verified that there was a new increase of enzyme activity at 96hai in the treatment where

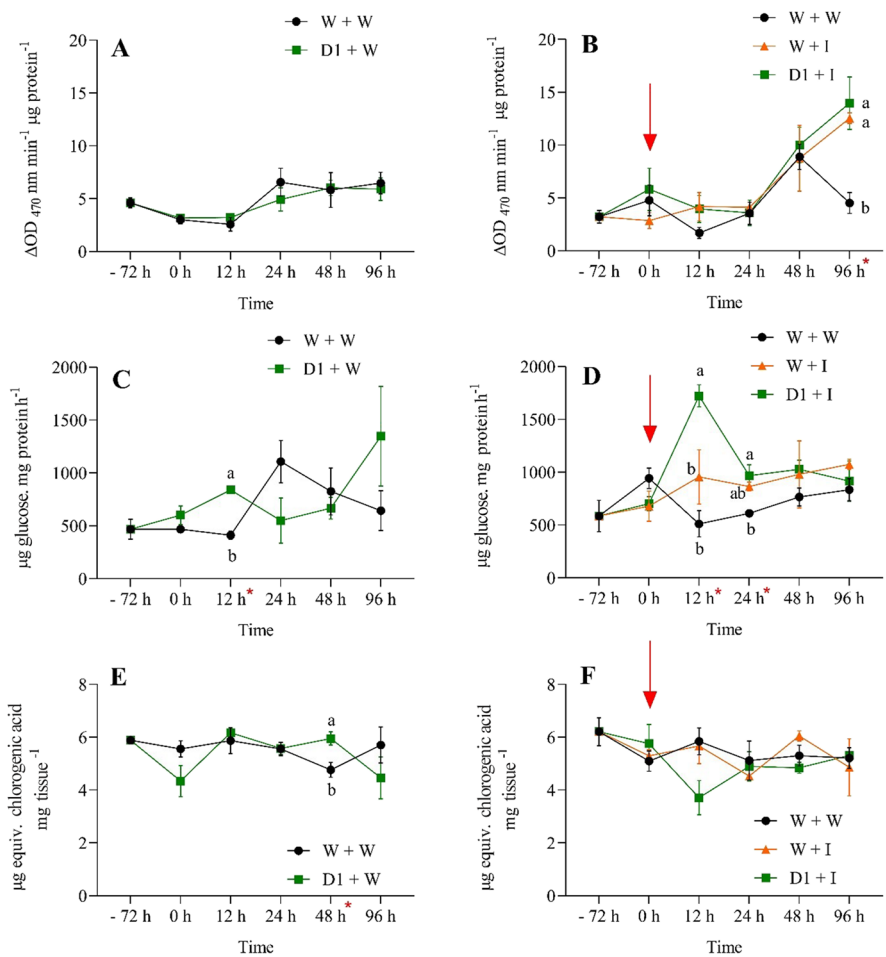

Fig. 3 Biochemical changes in tomato plants treated with the essential oil of *Aloysia citriodora* for the control of black spot disease. Guaiacol peroxidase (**A**-**B**) and β-1,3 glucanase (**C**-**D**) enzyme activities, and accumulation of free phenolic compounds (**E**-**F**), in experiments I and II, respectively

the essential oil was applied. For experiment II, the [D_1 + I] treatment provided higher β-1,3 glucanase enzymatic activity at 12 hai, differing from the other treatments. The [D_1 + I] essential oil treatment continued to promote higher enzyme activity at 24hai, differing statistically from the [W + W] control (Fig. 3D).

Regarding the free phenols, the [D_1 + W] treatment promoted a higher accumulation of these compounds at 48 h when compared to [W + W] (Fig. 3E). There was a trend in the reduction of free phenols at some times in the [D1 + W] essential oil treatment, although with variable values. There were no significant differences between treatments when plants were inoculated with the pathogen (Fig. 3F).

We investigated phenolic compounds and was analyzed the results together through principal components analysis (PCA) in order to understand the biochemical changes that the treatments promoted and how they behave after application of the oil or inoculation with the pathogen (Fig. 4).

The first two principal components in the PCA there selected for experiments II, explaining 66.83% and 59.63% of the data variation, respectively (Fig. 4) and I. The first principal component explained 46.32% of the data variance in experiment I. In this experiment, it they founded that the accumulation of [W + W] phenols is main grouped as a function of 0 and 12 h. Peroxidase and β-1,3 glucanase enzyme activities are mainly grouped from 24 to 96 hours. There is a negative relationship between the accumulation of [W + W] phenols and the [W + W] β-1,3 glucanase activity. The greatest accumulation of [D1 + W] phenols occurs at 48 hours, as verified by principal component 2 (20.51% of the explained variance) (Fig. 4A).

For experiment II, the [D1 + I] peroxidase, [W + I] peroxidase and [W + I] β-1,3 glucanase enzyme activities were mainly grouped as a function of 48 to 96hai in principal component 1 (34.92% of the explained variance). The greatest [D_1 + I] β-1,3 glucanase activity occurs at 12 hai, which corroborates with the data shown in Fig. 3D. With inoculation, the plant altered its metabolism and activated defense mechanisms such as enzyme activity in order to defend itself (Fig. 4B).

The greatest accumulation of [W + I] phenols and [D_1 + I] phenols was observed at -72 h and at 0 hai, respectively, demonstrating a reduction in phenolic compounds during experiment II. Greater [W + W] phenol activity occurs at 24hai. The

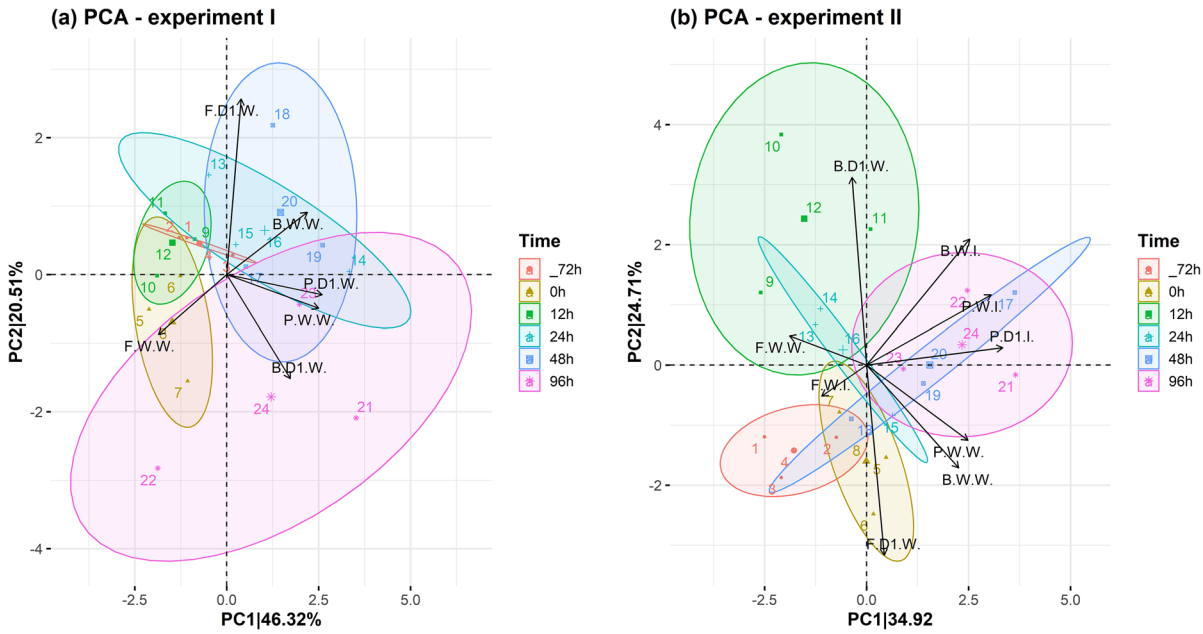

Fig. 4 Principal component analysis (PCA) for biochemical analyzes for the enzymes peroxidase (P), β-1,3 glucanase (B) and phenolic compounds (F), and in the treatments: [W + W], [D_1 + W] first experiment I; [W + W], [W + I] and [D_1 + I] according to experiment II, in tomato plants treated with the essential oil of *Aloysia citriodora* for the control of the black spot. The contours were classified according to the evaluation times

[W + I] phenol activity is negatively related to the [W + I] peroxidase enzyme, in the same way that [W + W] phenols are inversely related to [W + W] peroxidase and [W + W] β-1,3 glucanase. [D_1 + I] phenol activity is negatively related to the [D_1 + I] β-1,3 glucanase enzyme, and its distribution was best explained by principal component 2 (24.71% of the explained variance) (Fig. 4B).

The correlation analysis showed a lack of significance between the variables analyzed in the second experiment, with a low and negative correlation between the beta and phenol variables.

The regression analysis showed significance for the variables β-1,3 glucanase and free phenols in the second experiment. Dismembering the treatments, it was found that the treatment with essential oil [D_1 + I] was the one that most contributed to the negative relationship between the variables analyzed, proving the assumptions of the work (Fig. 5).

Discussion

Similar studies corroborate the results obtained in this work in which there is a total inhibition of phytopathogens *in vitro* with the use of low essential oil concentrations of the *Aloysia* genus. Fontana et al. (2020) observed the fungicidal potential of four essential oils, including *A. citriodora* oil, showing high fungistatic activity against *Sclerotinia sclerotiorum* and *Fusarium* spp. The authors verified mycelial growth inhibition from the dose of 0.8 and 0.6 μL mL^{-1} of the essential oil, respectively.

The results obtained also agree with other studies with the *Alternaria* spp. fungus being controlled with essential oils from *Pinus elliottii* and *Pinus taeda* (Tomazoni et al., 2014), *Lippia alba* (Tomazoni et al., 2016), *Eucalyptus staigeriana, Eucalyptus globulus* and *Cinnamomum camphora* (Tomazoni et al., 2017), and *Lippia origanoides, Lippia citriodora* and *L. alba* (Lozada et al., 2012).

The treatment [D1 + I] reduced black spot disease symptoms in tomato plants around 45%. Figures similar to those found by Silva et al. (2014) with essential oil of *Aloysia gratissima*, in the pathosystem *Glycine max* x *Phakopsora pachyrhizi*, which demonstrated a reduction in disease severity of up to 41%.

The fact that [D2 + I] did not perform well probably is due to the dose x phytotoxicity factor.

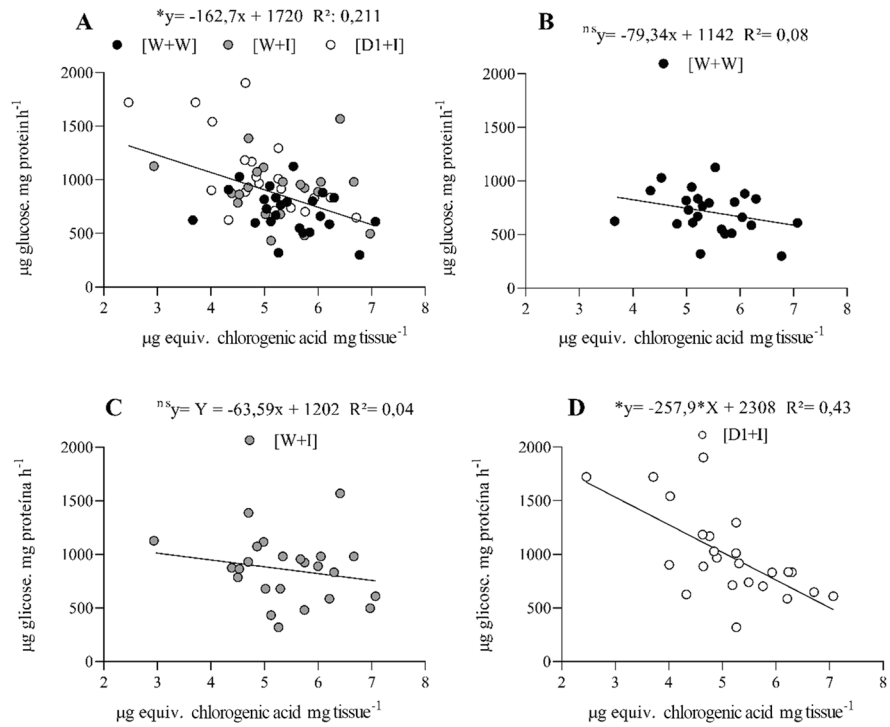

Fig. 5 Linear regression between the variables β-1,3 glucanase and free phenols, describing the relationship between all treatments (**A**), with [W + W] (**B**), [W + I] (**C**) and [D1 + I] (**D**), in tomato plants treated with the essential oil of *Aloysia citriodora* for the control of black spot fungus. $^{*, ns}$ significant and non-significant at 5% probability of error, respectively

Lorenzetti et al. (2018) mention that the efficiency results by using essential oils depend on the dose and application method, and the result can be negative when a high dosage is used.

A. citriodora essential oil has monoterpenoids in its composition, considering citral as major constituent of this species (Fontana et al., 2021). Thus, it is possible that the fungicidal action presented *in vitro* is related to these chemical constituents. Monoterpenes comprise the group of compounds identified as providing the highest inhibitory potential, as they cause changes in the structure and function of fungal membranes, thereby preventing hyphal growth and activity (Custodio et al., 2009).

Although many essential oils and their compounds exhibit biological activities on the most diverse microorganisms, little is known about their action mechanisms. The capacity of essential oils to diffuse through the cell wall and the plasma membrane of pathogens is due to their hydrophobic constitution and antioxidant properties which can alter polysaccharide, fatty acid and phospholipid structures, causing the structure to rupture (Mendes et al., 2017). Kumar et al. (2008) report the importance of the hydrophobicity of essential oils and their constituents, thus being able to interact with the cell membrane lipid layer of pathogens, causing changes in their structures and making them less selective, which can cause leakage of the ions and other cellular constituents.

Apparently, the activity of the peroxidase enzyme is increased when inoculation occurs, both in the application of *A. citriodora* essential oil and with water. Natural products also can promote these biochemical changes, such as rosemary extract sprayed on soybean seedlings for controlling *Macrophomina phaseolina* which activated the peroxidase enzyme in two different times (Lorenzetti et al., 2018). Freddo et al. (2016) observed that the use of *A. citriodora* essential oil for treating cucumber seeds increased peroxidase enzyme activity linearly as the essential oil dose increased. Hendges (unpublished 2019) verified an increase in peroxidase enzyme activity in tomato plants with the use of bergamot, citronella and melaleuca essential oils in order to induce resistance in tomato against the black spot fungus.

Resistance induction against bacterial spot in tomato, caused by *Xanthomonas campestris*, has already been reported for other essential oils, such as tea tree (Dalio et al., 2020). This authors found satisfactory results in the plant response, with an increase in the β-1,3 glucanase and peroxidase enzymes (Dalio et al., 2020), thus showing the use of essential oil can induce resistance in tomato plants.

Peroxidase is associated with the plant's defense mechanism, since it can be involved in plant tissue lignification, H_2O_2 oxidation and hydroxycinnamic alcohol polymerization as one of its main functions (Stangarlin et al., 2011). The highest peroxidase enzyme activity occurred in the second experiment when the plants were treated with water or essential oil, and inoculated, demonstrating that the plants quickly activate their secondary metabolism in the presence of a stressor (inoculum) to fight against them.

The β-1,3 glucanase enzyme has direct action on structural components of some fungi in which the cell walls are composed of β-glucans (Stangarlin et al., 2011). As the main function of this structure is to help maintain the rigidity and integrity of the fungus cell walls, plants which express this enzyme are able to defend themselves and reduce the infectious process. Thus, the increase in the activity of the β-1,3 glucanase enzyme at 12 h (experiment I) and at 12 and 24 hai (experiment II), proves the action of *A. citriodora* essential oil in inducing tomato plant resistance against *A. linariae.*

Tomato plants that received essential oil application showed an accumulation of phenolic compounds at 48 h in the experiment I. Phenolic compounds have also been reported to eliminate low molecular weight ROS (Tariq et al., 2019). In addition, the induction of phenolic compounds can be of crucial importance for restricting expansion of the pathogenic fungus, constituting another defense mechanism (Tariq et al., 2019). It is important to understand the metabolic route for resistance/susceptibility of each culture and hybrid, disease and each resistance inducer, and to know that this can vary over time and in the environment.

In the principal components analysis, we were able to raise some informations about the altered metabolic routes for our experiment:

a. The accumulation of phenolic compounds occurs inversely to the enzymatic activity;
b. The β-1,3 glucanase enzyme strongly assists in controlling tomato black spot fungus, being acti-

vated quickly (12 hai) when the plant is under attack.

c. Enzymatic activity is higher in inoculated plants, remaining active longer.

A study by Awan et al. (2018) showed that tomato plants which are resistant to black spot fungus increase their total phenolic compounds at 15 days after inoculation, which is positively correlated with antioxidant enzyme activity. However, plants susceptible to the disease were not showed a positive correlation between antioxidant enzymes with phenolic compounds (Awan et al., 2018).

New chemical compounds from different plants have been discovered in recent times which are able of controlling phytopathogens (Silva et al., 2009). Thus, the essential oil of *A. citriodora* can be considered as a potential plant metabolite, acting directly against the pathogen and possible in the induction of resistance.

Conclusions

A 2.4 µL mL^{-1} dose of *A. citriodora* essential oil totally inhibits *A. linariae* growth *in vitro*. At a dose of 2.5 µL mL^{-1} the essential oil was able to partially control the tomato black spot disease, reducing the severity of the disease. Increases in the β-1,3 glucanase enzyme activity may indicate the occurrence of the resistance induction phenomenon. Thus, the use of *A. citriodora* essential oil is a promising option for controlling the black spot disease in tomato plants, and thus contributing to a sustainable agriculture.

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Declarations

Conflict of interest No funding was received to assist with the preparation of this manuscript and the authors have no competing interests to declare that are relevant to the content of this article.

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