Potential of *Lippia sidoides* essential oil in controlling *Agroathelia rolfsii*, the causal agent of sclerotium rot

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ABSTRACT. The fungus *Agroathelia rolfsii*, the causal agent of sclerotium rot (southern blight), is responsible for causing wilting and rot in the collar and roots of various agricultural crops. It is a highly destructive fungal pathogen that affects approximately 500 plant species. In addition to infecting virtually all dicotyledonous crops, this fungus can also infest some monocotyledons. Given this context, the objective of this study was to analyze the inhibition of mycelial growth of the fungus *A. rolfsii* in the presence of essential oil of *Lippia sidoides* (rosemary-pepper). The experimental design was completely randomized, with five replicates. After solidification of the culture medium (20 mL per plate), the treatments consisted of the surface application of different volumes of pure essential oil (0, 5, 25, 50, and 100 µL), corresponding to concentrations of 0; 0.25; 1.25; 2.5 and 5.0 µL mL^{-1} (0; 0.025; 0.125; 0.25 and 0.5 % v/v), uniformly distributed on the surface of the plates. Subsequently, 1 cm diameter mycelial discs of *A. rolfsii* were inoculated into the central part of the plates and incubated in a BOD-type chamber under controlled temperature conditions (28 ± 1 °C) and absence of light. Mycelial growth rate index (MGRI), percentage of mycelial growth inhibition (PGI), and average mycelial growth were evaluated. The data were subjected to analysis of variance, and the means were compared using Tukey's test (5%). The results demonstrated that, starting from a concentration of 1.25 µL mL^{-1} (25 µL), there was complete inhibition of *A. rolfsii* growth throughout the 12 days of evaluation. The concentration of 0.25 µL mL^{-1} (5 µL) showed an initial inhibition of 99 %, reduced to 78 % at the end of the period, possibly due to the volatilization of the active compounds. These results indicate that the essential oil of *L. sidoides* shows high potential in controlling *A. rolfsii*, representing a promising and sustainable alternative to the use of synthetic fungicides, especially in agricultural systems with low environmental impact.

Key-words: mycelial growth, alternative control, natural fungicide, essential oil.

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INTRODUCTION

The plant pathogen *Agroathelia rolfsii* (Sacc.) Redhead & Mullineux (2023) (= *Sclerotium rolfsii*) belongs to the Kingdom Fungi, Phylum Basidiomycota, Subphylum Agaricomycotina, Class Agaricomycetes, Subclass Agaricomycetidae, order Agaricales, Family Amylocorticiaceae, Genus *Agroathelia*, Specie *A. rolfsii*. Phylogenetic studies have demonstrated that the organism, historically known by the anamorph *S. rolfsii* and by the teleomorph *Athelia rolfsii*, belongs to the Order Amylocorticiales (Binder et al. 2010), and the name was changed to *A. rolfsii* (Sacc.) Redhead & Mullineux (Redhead and Mullineux, 2023). The current taxonomy can be found on MycoBank (www.mycobank.org).

The plant pathogen *A. rolfsii* exhibits a wide diversity of hosts, affecting approximately 500 plant species belonging to about 100 botanical families, including monocots and dicots (Aycock, 1966; Kator et al., 2015; You et al., 2025). This pathogen is responsible for causing necrosis in the collar and roots, as well as wilting and damping-off of seedlings in several economically important crops, such as tomato, pepper,

eggplant and peanut (Meena et al., 2023; Aljabali et al., 2025), common bean (Paparu et al., 2020) and wheat (Choppakatla et al., 2006), among others. Additional information on the host range of *A. rolfsii* can be found in specialized databases, such as the USDA-ARS Fungal Database (https://nt.ars-grin.gov/fungaldatabases/).

The plant pathogen A. *rolfsii* is a difficult pathogen to control due to its ability to form sclerotia approximately 1 mm in diameter, initially white and later dark brown (Pinheiro et al., 2010; Pan et al., 2024). These resistant structures give the fungus the ability to survive for long periods in the soil, even under adverse environmental conditions, making its management a major challenge in agricultural systems (Auler et al., 2013; Wang et al., 2023). Therefore, the management of diseases caused by *A. rolfsii* has been based primarily on preventive strategies, including the application of fungicides, with the aim of reducing direct and indirect damage to production. However, due to the pathogen's ability to form sclerotia and survive in the soil for long periods, isolated chemical control is often limited, making it necessary to adopt integrated management practices.

The use of fungicides as a strategy in the management of *A. rolfsii* in various crops presents several disadvantages, such as increased production costs, negative environmental impact due to the use of synthetic formulations, the emergence of resistant fungi, limited contact action, phytotoxicity, and risks to human health and non-target organisms, such as insects and other fungi (Santos et al., 2023; Huang et al., 2025). Due to environmental concerns and the increasing resistance of plant pathogen, the demand for more sustainable alternatives is growing. In this context, control through plant extracts or compounds has gained prominence, being increasingly explored as a way to minimize these challenges (Santos et al., 2023).

Among the great diversity of species with medicinal potential is the genus *Lippia sp.*, which has about 200 species, including herbs, shrubs, and small trees belonging to the Verbenaceae family (Terblanché et al., 1996). Among the prominent species is *L. sidoides* Cham. (rosemary pepper), a perennial shrubby plant popularly known as pepper rosemary. It is a native deciduous plant of the Caatinga biome, with aromatic leaves and intense biological potential (Guimarães et al., 2015; Rocha et al., 2022; Santos et al., 2024).

The plant *L. sidoides* stands out for the diverse biological activities attributed to its essential oil, making it a promising source of bioactive compounds. Studies report that *L. sidoides* exhibits antifungal, antibacterial, and insecticidal properties (Guimarães et al., 2015). Its antimicrobial effects are mainly associated with the presence of thymol, one of its major constituents, which demonstrates inhibitory and bactericidal action against Gram-positive and Gram-negative bacteria (Pinheiro, 2021). Furthermore, research involving thymol confirms its healing, anti-inflammatory, and antifungal properties (Rocha et al., 2022). More recent evidence also highlights its effectiveness in modulating the intestinal microbiota of *Danio rerio* (Cardoso et al., 2024), as well as its antimicrobial activity against clinically relevant pathogens such as *Candida albicans* (C. P. Robin) Berkhout (1923) and *Staphylococcus aureus* Rosenbach (1884) (Santos et al. 2024). This evidence expands the possibilities for applying *L. sidoides* essential oil in biological control. Given the already recognized potential of this species, this work aims to analyze the percentage of mycelial growth inhibition (PGI) of the fungus *A. rolfsii* in the presence of *L. sidoides* essential oil.

MATERIALS AND METHODS

The experiment was conducted in the Multipurpose Natural Sciences Laboratories (LMCN) of the Federal Institute of Goiás – Posse Campus (latitude 14°04'56'' S, longitude 46°22'40'' W and altitude of 811 meters). The essential oil of *L. sidoides* was obtained by steam distillation, supplied by the company Flor da Pedra Essencial, which certifies the plant material of origin, the degree of purity (100 %) and the distillation date (March 2025), adopted as a traceability parameter for the product.

The steam distillation extraction method, the plant material, and the purity grade of the tested essential oil (100 % pure) were certified by the supplier Flor da Pedra Essencial. The supplier guaranteed the chemical composition of the essential oil (Table 1), analyzed by a gas chromatograph (Agilent, Model MSD5977B) coupled to mass spectrometry.

The fungi *A. rolfsii* was obtained from the Mycology collection of the Microbiology and Phytopathology Laboratory of IF Goiano – Campus Urutaí. The micelial Sclerotia activation was performed by plating on PDA (Potato-Dextrose-Agar) culture medium in a laminar flow hood, followed by incubation in a BOD " *Biochemical Oxygen Demand* " chamber for 96 hours.

The assay was conducted in sterile glass Petri dishes (90 × 15 mm) containing 20 mL of PDA, to evaluate the effects of essential oils on plant pathogen fungi. After the medium solidified, different volumes of pure essential oil (0, 5, 25, 50, and 100 µL), corresponding to concentrations of 0, 0.25, 1.25, 2.5, and 5.0 µL mL^{-1} (0, 0.025, 0.125, 0.25, and 0.5 % v/v), were applied to the surface of the medium and uniformly distributed using a Drigalski loop. This procedure was adopted to favor the homogeneous diffusion of the

extract on the surface of the culture medium, allowing direct contact with the fungal mycelium, according to the methodology of Kirby and Bauer (Bauer et al. 1966), adapted by Ferreira et al. (2020). Subsequently, *A. rolfsii* mycelial discs (1 cm in diameter) were inoculated into the center of the plates, which were then incubated in a BOD-type chamber under controlled temperature (28 ± 1 °C) and dark conditions.

Mycelial growth was evaluated 48 hours after the experiment was set up, and subsequently every 24 hours until the fungus completely covered the surface of the medium in the control treatment. Colony diameter was determined by averaging two perpendicular measurements obtained with a digital caliper, with values expressed in centimeters. These data were used to estimate the percentage of mycelial growth inhibition (PGI %), according to Edgington, Knew, and Barron (1971), and the mycelial growth rate index (IVCM), according to Oliveira (1991).

The experimental design adopted was completely randomized (CRD), with five treatments and five repetitions, totaling 25 experimental units. The treatments consisted of the following concentrations of *L. sidoides* essential oil, obtained from the application of different volumes on 20 mL of culture medium: T0 (0 µL mL^{-1}; 0 % v/v), T1 (0.25 µL mL^{-1}; 0.025 % v/v), T2 (1.25 µL mL^{-1}; 0.125 % v/v), T3 (2.5 µL mL^{-1}; 0.25 % v/v) and T4 (5.0 µL mL^{-1}; 0.5 % v/v).

The data were subjected to analysis of variance (ANOVA) and, when significant by the F-test ($p < 0.05$), regression models were fitted as a function of time. The means of IVCM were compared using Tukey's test at a 5 % probability level. All statistical analyses were performed using R software, version 4.4.3 (R Core Team, 2025).

RESULTS AND DISCUSSION

Mycelial growth rate index was reduced in all treatments containing essential oil throughout the 12-day evaluation period. The control treatment showed the highest average growth rate (0.476 cm day^{-1}), differing statistically from the treatments containing essential oil.

Although the lowest concentration tested (0.25 µL mL^{-1}) showed an IVCM of 0.14 cm day^{-1}, it did not differ statistically from the concentrations of 1.25, 2.5, and 5.0 µL mL^{-1}, in which no mycelial growth was observed (Table 1). This result indicates that even the lowest concentration exerts a strong inhibitory effect, although insufficient to completely prevent fungal development over time.

The potential antimicrobial activity of *L. sidoides* essential oil has already been tested in several biological contexts, such as: clinically relevant oral pathogens (Botelho et al., 2007), respiratory tract microorganisms (Veras et al., 2012), intestinal microbiota (Cardoso et al., 2024), and it also exhibits larvicidal activity (Carvalho et al., 2003). These results demonstrate that *L. sidoides* essential oil has a broad spectrum of action and high efficacy against various pathogenic microorganisms. This corroborates the results found in the present study for *A. rolfsii*, a plant pathogen microorganism.

Other species of the genus *Lippia* Schauer are studied for their plant pathogen potential, such as *Lippia gracilis*, which showed total percentage of mycelial growth inhibition (PGI %) of fungi *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. 1884, *C. musae* (Berk. & M.A. Curtis) Arx (1957), *C. fructicola Prihast* L. Cai & K.D. Hyde 2009, *C. asianum* L. Cai & K.D. Hyde 2009, *Alternaria alternata* (Fr.) Keissl. (1912), *A. brassicicola* (Schwein.) Wiltshire 1947, *Fusarium solani* (Mart.) Sacc. (1881), *F. oxysporum* f. sp. *Cubense* W.C. Snyder & H.N. Hansen (1940) and *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl. (1909) (França, 2019).

Table 1. Mycelial growth rate index (IVCM, cm day^{-1}) of *Agroathelia rolfsii* under different concentrations of *Lippia sidoides* essential oil, over 12 days of incubation.

Treatment	Volume applied (µL)	Concentration (µL/ mL^{-1}; % v/v)	IVCM cm/day
T0	0	0 µL mL^{-1}; 0% v/v	0.476 ± 0.03a
T1	5	0.25 µL mL^{-1}; 0.025% v/v	0.14 ± 0.35 b
T2	25	1.25 µL mL^{-1}; 0.125% v/v	0 ± 0.0 b
T3	50	2.5 µL mL^{-1}; 0.25% v/v	0 ± 0.0 b
T4	100	5.0 µL mL^{-1}; 0.5% v/v	0 ± 0.0 b

Values are expressed as mean ± standard deviation (n = 5). Means followed by different letters differ significantly according to Tukey's test ($p \leq 0.05$).

The observed antifungal effect can be directly associated with the chemical composition of the essential oil of *L. sidoides* (Table 2), characterized by the predominance of monoterpenic phenolic compounds, especially thymol (61.83 %) and methylthymol (6.20 %), in addition to monoterpenes such as p-cymene (7.58 %) and sesquiterpenes such as humulene (5.57 %) and β-caryophyllene (3.67 %). Phenolic compounds, such as thymol, are widely recognized for their high antifungal activity, acting on the destabilization of the cell membrane, increasing permeability and leakage of intracellular constituents, leading to cell death (Burt, 2004; Hyldgaard et al., 2012).

Furthermore, *p*-cymene, although exhibiting limited antimicrobial activity when isolated, can act synergistically with phenolic compounds, potentiating the action of thymol by facilitating its penetration into the cell membrane (Ultee et al., 2002). The sesquiterpenes present also contribute to the biological activity, potentially acting in the structural disorganization of fungal cells. These mechanisms explain the high inhibition observed in this study, especially at higher concentrations.

Table 2. Rosemary pepper (*Lippia sidoides*) essential oil, with identification of compounds, relative percentages (%) and respective chemical classes.

Compound	(%)	Chemical class
α-Tujeno	0.79	Monoterpene
α-Pinene	0.81	Monoterpene
Camphene	0.75	Monoterpene
β-Myrcene	2.00	Monoterpene
α-Terpinene	0.48	Monoterpene
p-Cymene	7.58	Aromatic monoterpene
Limonene	0.47	Monoterpene
Eucalyptol	0.45	Oxygenated monoterpene
γ-Terpinene	1.59	Monoterpene
α-Fenchene	0.65	Monoterpene
Camphor	1.42	Oxygenated monoterpene
Terpinen-4-ol	0.39	Oxygenated monoterpene
Methylthymol	6.20	Monoterpene phenol
Thymol	61.83	Monoterpene phenol
Thymol acetate	0.21	Monoterpene ester
β-Caryophyllene	3.67	Sesquiterpene
cis-α-Bergamotene	0.26	Sesquiterpene
Humulene	5.57	Sesquiterpene
γ-Muurolene	0.17	Sesquiterpene
Germacrene D	0.61	Sesquiterpene
γ-Amorphene	0.91	Sesquiterpene
β-Bisabolene	0.91	Sesquiterpene
7-epi-α-Selinene	0.96	Sesquiterpene
δ-Cadinene	0.27	Sesquiterpene
α-Bisabolene	0.25	Sesquiterpene

Source: Chromatography analysis, provided by Flor da Pedra Essencial.

The percentage of mycelial growth inhibition (PGI %) confirmed the dose-dependent effect of the essential oil (Figure 1). The concentration of 0.25 µL mL^{-1} promoted an initial inhibition of approximately 99 %, reducing to about 78 % at the end of the experimental period. This behavior suggests a loss of efficacy over time, possibly associated with the volatilization of the active compounds, a common characteristic of essential oils. On the other hand, the concentrations of 1.25, 2.5, and 5.0 µL mL^{-1} promoted total percentage of mycelial growth inhibition (PGI) throughout the experimental period (Figure 1), demonstrating the effectiveness of these concentrations in controlling the pathogen.

The mycelial growth curves reinforce this behavior (Figure 2), showing continuous growth in the control treatment, while the highest concentrations maintained zero growth. The concentration of 0.25 µL mL^{-1} showed delayed growth, indicating mycelial escape, suggesting that this concentration is not sufficient to guarantee long-lasting control. These results are corroborated by the visual analysis of mycelial growth (Figure 3), in which abundant development is observed in the control treatment and progressive reduction with increasing concentration of the essential oil, culminating in a total absence of growth at concentrations from 1.25 µL mL^{-1} onwards.

Figure 1. Percentage of inhibition of *Agroathelia rolfsii* mycelial growth over 12 days (48 to 288 h) under different concentrations of *Lippia sidoides* essential oil.

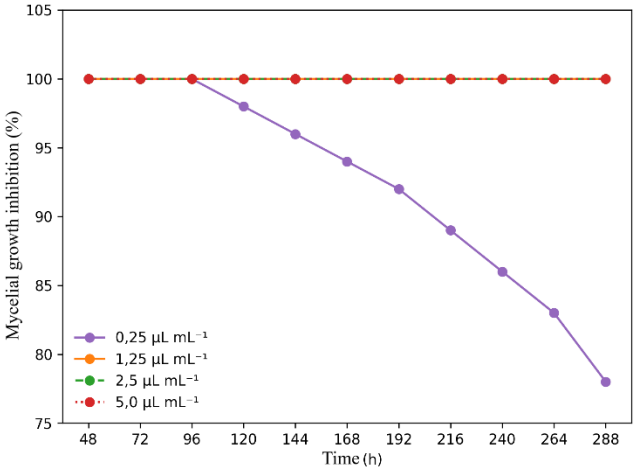
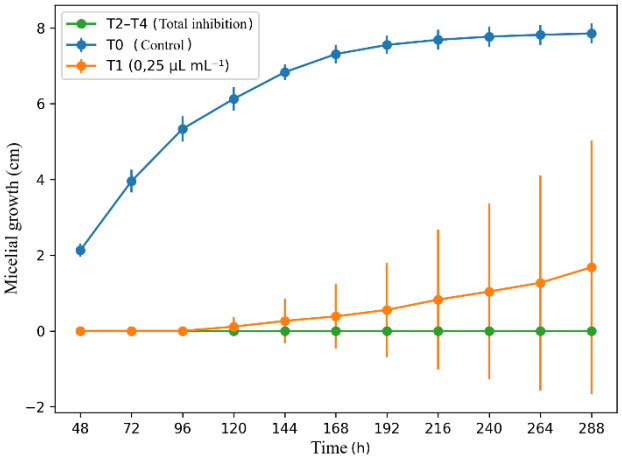


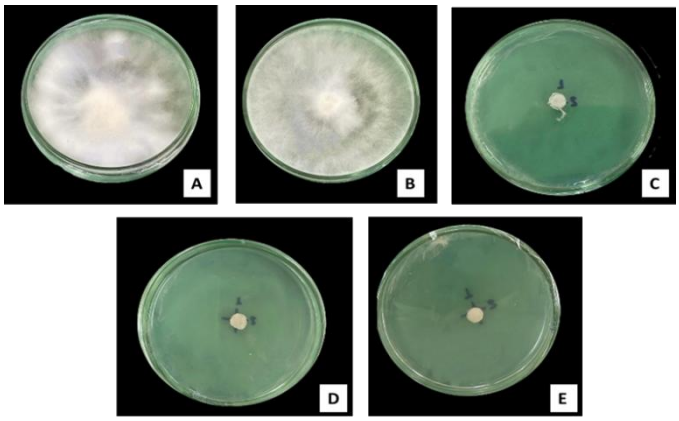
Figure 2. Mycelial growth of *Agroathelia rolfsii* over 12 days (48 to 288 h) under different concentrations of *Lippia sidoides* essential oil. The points represent the mean (n = 5), and the bars indicate the standard deviation.



Similar results have been reported for species of the genus *Lippia*, such as *Lippia gracilis*, which showed total inhibition of several plant pathogen fungi, including species of *Colletotrichum* sp., *Fusarium* sp., and *Alternaria* sp. (França, 2019). Furthermore, previous studies demonstrate that the essential oil of *L. sidoides* has a broad antimicrobial spectrum, including action on human and environmental pathogens (Botelho et al.,

2007; Veras et al., 2012; Cardoso et al., 2024), corroborating the results observed in this study for a plant pathogen fungus. Thus, the essential oil of *L. sidoides* exhibits a dose-dependent antifungal effect, with concentrations equal to or greater than 1.25 µL mL^{-1} being sufficient to promote complete and sustained percentage of mycelial growth inhibition (PGI %) of *A. rolfsii*.

Figure 3. Mycelial growth of *Agroathelia rolfsii* in PDA culture medium supplemented with different concentrations of *Lippia sidoides* essential oil, after 12 days of incubation. A: control (0 µL mL^{-1}); B: 0.25 µL mL^{-1}; C: 1.25 µL mL^{-1}; D: 2.5 µL mL^{-1}; E: 5.0 µL mL^{-1}.



CONCLUSION

Rosemery pepper (*L. sidoides*) essential oil demonstrated antifungal potential in controlling the mycelial growth of *Agroathelia rolfsii*, with a concentration-dependent effect. Concentrations equal to or greater than 1.25 µL mL^{-1} promoted complete percentage of mycelial growth inhibition (PGI %) under the experimental conditions of this study, while the lowest concentration showed a significant initial effect, but with a reduction over time.

This behavior may be associated with the chemical composition of essential oil, especially the high proportion of phenolic compounds, such as thymol, known for their antifungal activity. Therefore, the essential oil of *L. sidoides* represents a promising alternative in the management of *A. rolfsii*, especially in sustainable agricultural systems. However, further studies, including comparisons with commercial fungicides and evaluations under field conditions, are needed to validate its efficacy and practical applicability.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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