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Semisynthetic compounds for controlling *Colletotrichum lindemuthianum* on bean seeds

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Abstract

Anthracnose caused by *Colletotrichum lindemuthianum* is one of the main diseases that affect the bean crop. The use of semisynthetic compounds for controlling anthracnose aims at providing a higher balance to the ecosystem and a lower environmental impact. Based on this context, the objective of this work was: a) to carry out the prospection of compounds such as Phenyl S Citral, Phenyl Se Citronellal, and Citral at concentrations of 1, 0.5, 0.25, 0.125, 0.0625, 0.0312, 0.0156, 0.0078, and 0.0039%, which were modified from the essential oil of citronella and lemongrass, for controlling *C. lindemuthianum*; b) to evaluate the initial performance of seedlings and treat the incidence of *C. lindemuthianum* in bean seeds with Phenyl S Citral and Phenyl Se Citronellal at concentrations of 0.125 and 0.0625%. Phenyl Se Citronellal at 0.5% controlled 100% of mycelial growth and Phenyl S Citral at 0.5 and 1% controlled more than 50% of mycelial growth of *C. lindemuthianum*. The treatment with Phenyl S Citral and Phenyl Se Citronellal did not affect the physiological quality of bean seeds while increasing seedling development when using the 0.0625% concentration of Phenyl Se Citronellal. Treatment with Phenyl Se Citronellal at both concentrations decreased the incidence of *C. lindemuthianum* infection.

Keywords: anthracnose, Citral, Phenyl S Citral, Phenyl Se Citronellal, semisynthetic compounds

Introduction

Anthracnose, caused by *Colletotrichum lindemuthianum* (Sacc & Magnus) Lams.-Scrib, is the primary fungal disease affecting bean crops. It is predominantly transmitted through seeds and has a high destructive potential in regions with moderately low temperatures and high relative humidity (Nabi *et al.* 2024; Padder *et al.* 2017). The disease can cause yield losses of up to 100% in susceptible cultivars and can also compromise seed quality. Infected seeds have the potential to reduce the initial stand, and in more severe cases, they can lead to seedling death. In the early stages, this occurs because the seedling tissues are still forming, showing little lignification, making the seedlings more vulnerable to pathogen attack (Pereira *et al.* 2018).

Crop management practices aimed at disease control include a series of alternatives, such as crop rotation, the use of resistant cultivars, the application of synthetic fungicides for seed treatment, and aerial spraying. However, the sequential use of synthetic fungicides can lead to the selection of individuals resistant to specific chemical groups. Therefore, other strategies, such as the use of biofungicides (Confortin *et al.* 2019; Sivalingam *et al.* 2024), are important to reduce disease damage.

Biofungicides can be produced from the extracts and essential oils of citronella and lemongrass. They are naturally sourced substances studied by research groups in the pharmaceutical, food, and agricultural

sciences (da Silva *et al.* 2023). The complex composition of the bioactive compounds exhibits antifungal, antibacterial, insecticidal, and antimicrobial activity. Overall, their mode of action can induce the inhibition and death of microorganisms by disrupting the cell membrane, resulting in the inhibition of electron transport and affecting protein translocation, phosphorylation, and other enzymatic activities, or by inhibiting mycelial growth and spore germination (Lenardão *et al.* 2015).

A limitation in the use of essential oils is the wide variation in molecule composition. It is based on these challenges that innovative technologies involving the synthesis and chemical modification of natural compounds have been proposed for the control of many pathogens. The synthesis of semisynthetic compounds allows for the improvement of biological activities exhibited by natural and unmodified molecules, reducing the volatilization of essential oils and providing higher enhancement when applied to crops (Zhang *et al.* 2021; da Silva *et al.* 2023).

Exploring the biological activity of many compounds can be an effective way to control diseases in cultivated plants (Brun *et al.* 2022; Schmaltz *et al.* 2023). However, their efficiency in controlling agriculturally significant phytopathogens, and the optimal application method for crops, whether through spraying or seed treatment, is still undefined and requires further study. Nevertheless, seed treatment is considered one of the more environmentally efficient methods for pathogen control, given the small quantity of the product released into the environment. Additionally, some modified molecules may contain bioactives that enhance the initial performance of the crop, providing the plant with defensive capabilities (Aguiar *et al.* 2017).

Thus, treating seeds with semisynthetic compounds, as reported elsewhere (Zhang *et al.* 2021; Calderón-Santoyo *et al.* 2022), could potentially be beneficial in reducing the incidence of *Colletotrichum* spp. in seeds and promoting seedling development, either by reducing the inoculum transmitted by seeds or by strengthening the plant's defense against pathogen attacks in the early stages of cultivation. In addition to the beneficial effects, it is important to determine whether the concentration effective in controlling the fungus does not alter the physiological potential of the seeds after treatment. This study was conducted to screen for three semisynthetic compounds (Phenyl S Citral, Phenyl Se Citronellal, and Citral) for the control of the fungus *C. lindemuthianum*, as well as to evaluate the physiological and sanitary potentials of bean seeds treated with the best concentrations and compounds identified from the screening study.

Materials and Methods

Colletotrichum lindemuthianum and semisynthetic compounds

The fungal isolate of *C. lindemunthianum* belongs to the collection of LPSFF (FAEM-UFPel, Brazil). The isolate was collected in the municipality of Seberi, Brazil, and obtained from bean pods. The semisynthetic compounds belong to the Laboratory of Organic Chemistry at UFPel, Brazil, and they were synthesized from citronella (*Cymbopogon nardus*) and lemongrass (*Cymbopogon citratus*) oils. They were chemically modified in their structure: Phenyl S Citral, Phenyl Se Citronellal, and Citral.

Mycelial growth and sporulation

Mycelial growth and sporulation were evaluated to identify the compounds under fungal development. The experiments were conducted in a completely randomized design in a 3 × 9 factorial scheme, where factor A consisted of the compounds Phenyl S Citral, Phenyl Se Citronellal, and Citral. Factor C consisted of nine concentrations (w/v; g · 100 ml^{-1} or %): 1, 0.5, 0.25, 0.125, 0.0625, 0.0312, 0.0156, 0.0078, and 0.0039%, with four independent repetitions. Medium without compounds (negative control) and medium added with dimethyl sulfoxide (DMSO; positive control) were used as controls.

An aliquot of 100 µl of different bioactive compounds and concentrations was spread on plates containing solidified Mathur medium to assess mycelial growth. Subsequently, a disk (5 mm) of the fungus was transferred to the center of the plate from young colonies (10 days of growth). The plates were kept in an incubation room at 22 ± 1°C and a photoperiod of 12 h. The evaluations of mycelial growth were performed daily using a digital caliper. From these data, the percentage of mycelial growth inhibition (PIC) was calculated using Equation 1:

$$PIC\,(\%) = \\ = \left(1 - \frac{Mycelial\ growth\ in\ treatment}{Mycelial\ growth\ in\ negative\ control}\right) \times 100, \quad (1)$$

where: *Mycelial growth in treatment* is the mycelial growth in the presence of the tested compounds or DMSO; *Mycelial growth in negative control* is the mycelial growth in the control (medium without compounds).

For the evaluation of fungal sporulation under different compounds and concentrations, mycelial disks were uniformly taken from all colonies after 21 days of incubation. Spore counting was carried out using a Neubauer chamber (400X magnification). The

response variables (mycelial growth and sporulation) were subjected to mean comparison using Tukey's test for treatment comparisons. A 0.01% probability of error was considered to verify if there was a significant interaction.

Phytotoxicity in beans treated with semisynthetic compounds

From the results of the prospecting of semisynthetic compounds, concentrations that showed good performance without the risk of phytotoxicity were used in subsequent experiments to assess their effectiveness in controlling the fungus associated with bean seeds and improving seed physiological performance. For the initial performance of bean seedlings treated with Phenyl S Citral and Phenyl Se Citronellal, an experiment was conducted in a completely randomized design in a factorial scheme with four repetitions. The factors consisted of six treatments, a positive control with pyraclostrobin and thiophanate-methyl, and a negative control (without compounds). An analysis of variance was performed on the data, and when significant at a 5% probability level, a mean comparison was performed by Tukey's test at 95% confidence level.

Seeds of the bean cultivar "IPR Tuiuiú" were used for the experiments. The seeds were surface-disinfested with 70% (v/v) ethyl alcohol, followed by 1% (v/v) sodium hypochlorite and distilled, sterilized water. After the initial germination and health test, a germination rate of 88% and zero incidence of *C. lindemuthianum* were obtained. They were then subjected to the artificial inoculation process.

The semisynthetic compounds used were Phenyl S Citral and Phenyl Se Citronellal at concentrations of 0.125 and 0.0625%. The application of the compounds was done through seed treatment. The process was carried out manually in polyethylene plastic bags. For this, 0.08 ml of each semisynthetic compound and 1.92 ml of water were homogenized, and 0.04 kg of seeds were added, followed by manual shaking of the bags for 2 minutes until the product was completely adhered to the seed. Then, the bags containing the treated seeds were opened, and the seeds were allowed to dry at 20°C for 48 h. In the control treatment, the seeds received only 2 ml of sterilized water.

In the evaluation of the initial development of the crop, the following variables were measured: germination, first count of the germination test (FCG), germination speed index (GSI), total seedling length, shoot length, root length, total dry mass, shoot dry mass, root dry mass, root fresh mass, shoot fresh mass, and total fresh mass. Physiological quality was assessed to check for phytotoxicity in the initial development of the seedlings.

The germination evaluation was performed with four replications of 50 seeds per treatment, sown on germitest paper rolls, moistened with distilled water, and placed in a germinator at 25 ± 2°C. The count of normal seedlings was carried out on the fifth and ninth days after starting the experiment, and the results were expressed in percentages. The FCG test was performed together with the germination test.

The seedling emergence evaluation was performed with 200 seeds per treatment, distributed in four replications of 50 seeds in each tray. Vermiculite was moistened with water, following the field capacity of 60% retention, and kept moist as needed. The treatments were kept in a controlled environment at 25 ± 1°C and a photoperiod of 12 hours. The evaluation was conducted daily for 15 days after sowing (DAS). In the final emergence, the seedlings with expanded primary leaves were considered to have a normal emergence, with results expressed in percentages. The daily emergence evaluation data were used to determine the GSI (Equation 2):

$$GSI = \sum_{i=1}^{n} \frac{E_i}{N_i} \quad (2)$$

where: E_i – the number of normal seedlings at DAS i; N_i – the number of days from sowing at ith evaluation.

The determination of shoot length, root length, and total length was performed similarly to the germination test. It was evaluated on the fifth day after starting the experiment, in four subsamples of 10 seedlings for each treatment. The rolls were placed in a germinator at 25 ± 2°C. Measurements were taken with the aid of a graduated ruler in millimeters, and the root length was obtained by subtracting the total length from the shoot length. The results were expressed in centimeters. Shoot dry mass, root dry mass, and total mass were determined on the normal seedlings evaluated in the length test. The roots and shoots of the seedlings from each repetition were separated and dried in an oven at 70°C until a constant weight was obtained, which took 72 hours.

The evaluation of the incidence of *C. lindemuthianum* in bean seeds treated with Phenyl S Citral and Phenyl Se Citronellal was similar to the previous experiment but with two levels of pathogen infection. The experiment was subjected to an analysis of variance in a factorial design, with mean comparison by Tukey's test at a 95% confidence level. Considering the normality of the residues by the Shapiro-Wilk test with significant interaction, there was a breakdown of the interaction for the comparison of treatments within each level and of the levels within each treatment.

For the artificial inoculation of seeds, the CL16 isolate of *C. lindemuthianum* was used. The fungus was transferred to Petri dishes containing Mathur culture medium, osmotically modified with sucrose solute.

After transferring the fungus, the plates were incubated in a growth chamber at 22 ± 1°C and a 12 h photoperiod for 7 days. Subsequently, the bean seeds were randomly distributed on plates that were completely colonized by the fungus. The treatment containing non-inoculated seeds was placed only on the modified culture medium for 96 h at 22°C and a 12 h photoperiod. Then, the seeds were removed and distributed on filter paper at a controlled temperature of 20°C for 48 h, where they could return to their initial water content .

After the inoculation period, the seeds were disinfested and evaluated for initial sanitary quality, following the Rules for Seed Analysis. Based on this result, batches with different levels of infection by *C. lindemuthianum* were prepared, corresponding to 4% (N1) and 14% (N2). Subsequently, the seeds were subjected to the treatment process with the selected semisynthetic compounds and concentrations identified in the previous study. The incidence of *C. lindemuthianum* in bean seeds was determined by the paper roll method, using four replicates of 50 seeds. The evaluation was performed on the seventh day after incubation by observing dark necrotic lesions on the cotyledons and hypocotyl. The results were expressed as a percentage of incidence.

Results

Mycelial growth inhibition

All concentrations evaluated in this study showed significant differences between compounds, achieving 100% efficiency of mycelial growth inhibition with Phenyl Se Citronellal at 0.5% (Table 1). Starting at a concentration of 0.0078%, the treatment with the semisynthetic compound Phenyl Se Citronellal exhibited the highest inhibitions in the mycelial growth of *C. lindemunthianum*. When exposed to concentrations of 0.25% and 1% of the Phenyl Se Citronellal treatment, the fungus showed growth below 30%. A similar pattern occurred for the concentration of 0.125%, with a reduction in mycelial growth of 67.45%. Additionally, the semisynthetic compound Phenyl S Citral at concentrations of 1 and 0.5% showed control in the fungal mycelial growth superior to 50%. The growth at the highest tested concentration (1%) suggests the adaptation of the fungus to the semisynthetic compound. However, its potential in controlling *C. lindemuthianum* became evident with total inhibition at a concentration of 0.5% by the end of the evaluation period.

For the treatments with the three semisynthetic compounds, concentrations of 0.0312% and 0.0625% did not differ statistically (Table 1). In the subsequent concentrations (0.125, 0.25, 0.5, and 1%), Phenyl Se Citronellal stood out, differing from the other two semisynthetic compounds. For the control treatment with DMSO, concentrations ranging from 0.0039 to 0.0312% showed a negative percentage, indicating that the fungus had slightly higher growth than the control.

Sporulation

A significant variation in spore production capacity was seen (Table 2). The treatment with DMSO exhibited the fewest sporulations at the concentrations of 0.0039, 0.0312, 0.0625, and 0.125%. The semisynthetic compound Phenyl Se Citronellal inhibited the sporulation phase and presented an average number of spores lower than 3×10^4. This result was also expected, as the evaluation of mycelial growth showed 100% inhibition.

In the treatment with Citral, it was observed that as the concentration increased, there was an increase in the average sporulation. This effect is opposite to the inhibition of mycelial growth. This also occurred for the treatment with Phenyl S Citral at concentrations of 0.25 and 1%.

Table 1. Inhibition (%) of the growth of *Colletotrichum lindemuthianum* subjected to different concentrations of DMSO, Phenyl S Citral, Phenyl Se Citronellal, and Citral

	Concentration [%]								
	0.0039	0.0078	0.0156	0.0312	0.0625	0.125	0.25	0.5	1
	Inhibition [%]								
DMSO	–0.12 ab	–1.54 c	–7.83 c	–2.20 b	3.40 b	1.88 c	3.11 c	3.46 c	9.28 c
Phenyl S Citral	–2.63 b	3.93 bc	4.90 b	11.01 a	44.77 a	41.36 b	40.06 b	50.60 b	54.91 b
Phenyl Se Citronellal	3.07 ab	21.23 a	23.29 a	10.84 a	46.23 a	67.45 a	74.73 a	100.00 a	77.98 a
Citral	6.10 a	6.08 b	4.86 b	15.77 a	41.32 a	42.91 b	42.28 b	45.23 b	48.38 b
P-value	0.0063*	<0.001**	<0.001**	<0.001**	<0.001**	<0.001**	<0.001**	<0.001**	<0.001**

*significant at 1% probability by the F-test; **significant at 0.1% probability by the F-test. Means followed by the same letters in each column do not differ from each other by Tukey's test at 95% confidence level

Table 2. Spore production rate of *Colletotrichum lindemuthianum* subjected to different concentrations of DMSO, Phenyl S Citral, Phenyl Se Citronellal, and Citral

	Concentration [%]								
	0.0039	0.0078	0.0156	0.0312	0.0625	0.125	0.25	0.5	1
	Spore production rate [spores × 10^4]								
DMSO	0.19	0.16	0.13	0.31	0.22 b	0.65 b	1.56 c	2.50 b	7.81 bc
Phenyl S Citral	0.94	0.37	0.72	1.19	0.50 b	3.44 b	34.79 a	8.87 ab	38.61 a
Phenyl Se Citronellal	0.34	0.09	0.00	1.22	2.63 b	2.72 b	1.19 c	0.00 b	2.93 c
Citral	0.31	0.25	0.25	0.59	12.28 a	13.66 a	16.62 b	14.75 a	16.75 b
P-value	0.99 ns	0.99 ns	0.99 ns	0.99 ns	**0.003***	**0.002***	**<0.001****	**<0.001****	**<0.001****

ns – not significant at 5% probability by the F-test; *significant at 1% probability by the F-test; **significant at 0.1% probability by the F-test. Means followed by the same letters in each column do not differ from each other by Tukey's test at 95% confidence level

Physiological bean quality

Figure 1 presents the results obtained for the initial performance of bean seedlings concerning physiological bean quality variables. The seeds used were from the "Tuiuiú" cultivar and were treated with Phenyl S Citral and Phenyl Se Citronellal at two concentrations based on the previous experiments: 0.125% (C1) and 0.0625% (C2). Normal seedlings from the germination test, as verified by the variables of FCG and germination, showed no significant difference between treatments, indicating that they were not influenced by the treatments or the different concentrations.

The GSI, root length, shoot length, and fresh root mass showed significant differences (Table 3). The highest GSI of 58.26 was observed for the treatment with DMSO at a concentration of 0.125%. The lowest GSI of 51.51 was observed for the treatment with Phenyl S Citral at a concentration of 0.0625%, while for

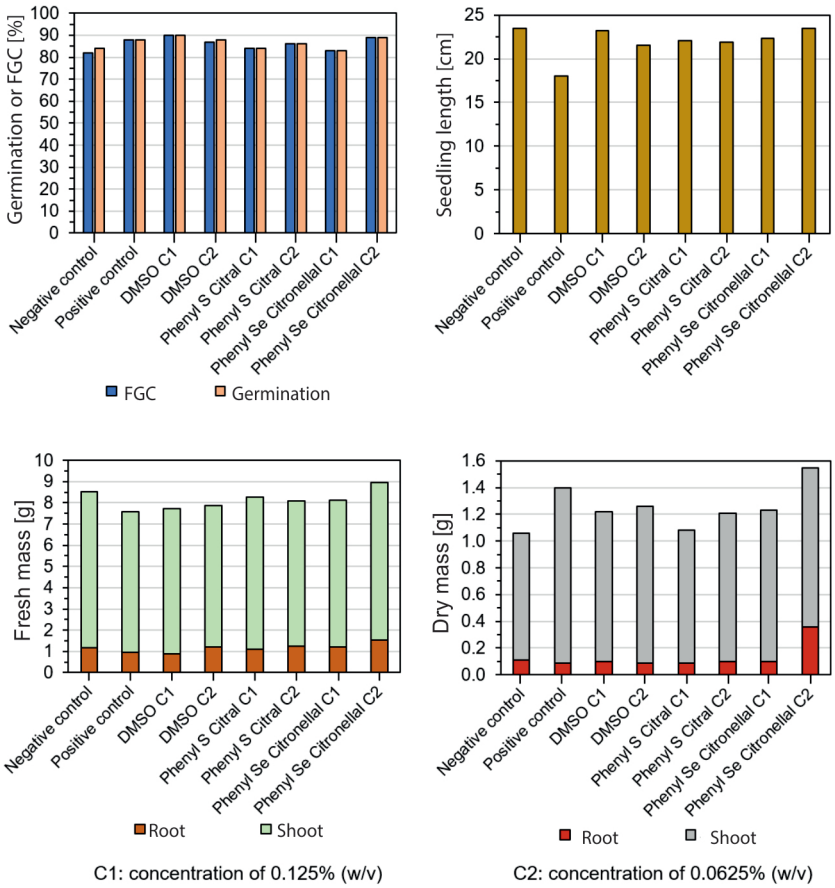

Fig. 1. Germination of bean seeds and dry mass, fresh mass and lengths of seedlings from seeds treated with Phenyl S Citral and Phenyl Se Citronellal at concentrations of 0.125% (C1) and 0.0625% (C2)

Table 3. Average of the physiological quality variables of bean seeds and seedlings from seeds treated with Phenyl S Citral and Phenyl Se Citronellal at concentrations of 0.125% (C1) and 0.0625% (C2)

	Root length [cm · seedling^{-1}]	Shoot length [cm · seedling^{-1}]	Root fresh mass [g · seedling^{-1}]	GSI (–)
Negative control	14.95 ab	9.16 a	1.16 ab	57.45 ab
Fungicide (positive control)	11.29 b	6.73 b	0.94 b	56.13 ab
DMSO – C1	14.80 ab	8.45 ab	0.90 b	58.26 a
DMSO – C2	13.07 ab	8.43 ab	1.20 ab	55.09 ab
Phenyl S Citral – C1	13.77 ab	8.25 ab	1.11 ab	53.16 ab
Phenyl S Citral – C2	14.30 ab	7.61 ab	1.26 ab	51.51 b
Phenyl Se Citronellal – C1	14.67 ab	7.68 ab	1.23 ab	54.17 ab
Phenyl Se Citronellal – C2	16.26 a	7.23 ab	1.55 a	54.83 ab
P-value	0.050*	0.012*	0.012*	0.030*

GSI – germination speed index; *significant at 5% probability by the F-test. Means followed by the same letters in each column do not differ from each other by Tukey's test at 95% confidence level

the remaining treatments, including the control, there was no difference between them, of which the values ranged from 53.16 to 57.45. For the variables root length, shoot length, and root fresh mass, the treatment with synthetic fungicide significantly reduced seedling growth and development, indicating a negative effect on vigor expression. With the application of commercial fungicide, the root length, shoot length, and root fresh mass were 11.29 cm, 6.73 cm, and 0.94 g, respectively. Although no statistical difference was reached between Phenyl Se citronellal and the negative control, the root length was 16.26 cm for the Phenyl Se citronellal at a concentration of 0.0625%.

When comparing treatments for each incidence level (Table 4), fewer symptoms of *C. lindemuthianum* on the cotyledons and hypocotyls of bean seedlings were observed for treatments with Phenyl Se Citronellal at both concentrations and Standak with 4% fungus infection. The *C. lindemuthianum* incidences were 2.0 and 2.5% at concentrations of 0.125% and 0.0625%, respectively. For the 14% infection level, the lowest incidences were observed for the treatments with Phenyl Se Citronellal (4.0%) and Phenyl S Citral (4.0%) at a concentration of 0.0625%.

Table 4. Comparison of averages of *Colletotrichum lindemuthianum* incidence for the interaction between inoculation levels and treatments at concentrations of 0.125% (C1) and 0.0625% (C2)

	Treatment	
	4%	14%
	C. lindemuthianum incidence [%]	
Negative control	3.5 bB	13.5 abcA
Fungicide (positive control)	2.0 aA	7.5 bcA
Phenyl S Citral – C1	3.5 abA	7.0 cA
Phenyl S Citral – C2	3.0 bB	4.0 abA
Phenyl Se Citronellal – C1	2.0 bA	12.5 cA
Phenyl Se Citronellal – C2	2.5 aB	4.0 aA
P-value	<0.0010*	0.0104**

*significant at 0.1% probability by the F-test; **significant at 5% probability by the F-test.
Means followed by the same letters, lowercase in each column and uppercase in each row, do not differ from each other by Tukey's test at 95% confidence level

Discussion

Regarding the semisynthetic compound Citral, there was a higher percentage of inhibition of mycelial growth as the concentration of the compound increased. Although it was effective in inhibiting the growth of *C. lindemunthianum* at all studied concentrations, its efficiency in control did not reach 50%. The main component of lemongrass essential oil is citral, and the antifungal activity is a prominent property of this oil (Dangol *et al.* 2023). Therefore, other minor components in the oil can be responsible for this bioactivity. Overall, for all treatments, there was a trend between lower concentrations and percentages of fungal inhibition, indicating lower control efficiency, which makes sense. This trend is because essential oils are volatile, and the more diluted they are, the lower the action of the major components due to the vola tilization and dilution of the oil.

The direct activity of essential oils and vegetal extracts on phytopathogens is broad and encompasses various genera of fungi (Confortin *et al.* 2019, 2021; Stegmayer *et al.* 2022). In the control of post-harvest diseases, essential oils have been demonstrated to be

effective against anthracnose in crops such as guava, papaya, banana, and mango, whether diluted, combined, or coated (De Araújo *et al.* 2018). Lemongrass, citronella, and thyme, at concentrations of 12.5% and 6.25%, completely inhibited the mycelial growth of *C. gloeosporioides* (Ramos *et al.* 2016). The advantage of our work in comparison to the previously mentioned ones is the use of a lower concentration (0.5%) to reach complete mycelial growth inhibition. Citral and citronellal can act by disrupting the integrity of the cell wall and membrane permeability, leading to physiological changes and causing cytotoxicity, as well as altering hyphal morphology. The inhibitory effect may be associated with their effects on mycelia, reducing sugar, soluble protein, chitinase activity, and pyruvate content (Rong-Yu *et al.* 2014).

Although the fungal colony's growth was higher than the control at some concentrations, there was no spore production. This behavior can be attributed to the adaptability of the fungus to the environment to which it was subjected. It is important to highlight that mycelial growth is the vegetative part of the fungal colony responsible for carrying nutrients and continuing the subsequent processes of the fungus's biological cycle, such as providing support for fruiting bodies or propagules for reproduction. In the next stage, spore production occurs to ensure the survival and perpetuation of the species through dissemination in the environment. However, when subjected to adverse conditions, the fungus tends to produce more spores as a survival strategy, but these spores are not always viable, thus not guaranteeing their germination.

Considering the pathogen's sporulation, its rate influences its dispersal capacity, affecting the spatial distribution of the species. Additionally, it is reported in the literature that *C. lindemuthianum* has limited dispersal capacity (Mota *et al.* 2016), occurring preferentially between different parts of the same plant and occasionally between adjacent plants. The isolate of *C. lindemuthianum* exhibited low sporulation, most likely due to the effect of semisynthetic compounds. It is noteworthy that the preferred mode of sporulation for this species is through pods. Not all plant extracts exhibit significant fungicidal effects. Therefore,studies like this one are important to present the effects of specific essential oils on fungal development.

The root length and fresh root mass data demonstrated an increase in seedling development in response to treatment with semisynthetic compounds, indicating a positive influence on seedling vigor expression through enhanced bean seedling growth. The optimal concentration was achieved with 0.0625% of the compound Phenyl Se Citronellal. This generated an expected increase in seedling vigor of 8 and 33%, respectively, compared to the negative control. However, for the variable shoot length, there was no increase among the treatments.

Despite many reports on the use of essential oils and their derivatives, most studies focus on the initial in vitro stages, while in vivo research tends to be applied to fruits and leaves (Sefu *et al.* 2015; Rabuske *et al.* 2023). In the case of essential oils in seed treatments, among the factors that interfere with seed germination, those originating from the secondary metabolism of plants stand out. These compounds can be detrimental to nutrient uptake, protein synthesis, water assimilation, and biochemical processes related to germination, among other factors. This limitation is a significant consideration when applying essential oils and plant extracts to seed treatments.

In this work, the marked reduction in the incidence of *C. lindemuthianum* in treated seeds confirms the antifungal action of the semisynthetic compound Phenyl Se Citronellal. When comparing treatments within the inoculation levels, Phenyl Se Citronellal showed higher control over the incidence of the fungus at a concentration of 0.125% for the 4% infection level. Furthermore, the efficiency of fungal control decreased as the percentage of inoculum increased.

Anthracnose is one of the most important diseases affecting bean crops. Seeds serve as a primary source of initial inoculum, allowing the fungus to survive for extended periods in infected seeds (Marcenaro and Valkonen 2016; Pereira *et al.* 2018). The incidence of anthracnose symptoms in seedlings evaluated on germination paper rolls ranged from 2.0 to 13.5%. This behavior is likely due to the fungus infecting the seeds internally, given that surface disinfection was initially performed.

The symptomatic seedlings exhibited small and depressed lesions with brown discoloration on the cotyledons. On the hypocotyl, elongated and depressed lesions with a dark coloration were observed. Among the treatments, the best initial performance of the seedlings was observed for the semisynthetic compound Phenyl Se Citronellal at both concentrations, resulting in vigorous seedlings with good germination. This outcome may be related to the presence of the micronutrient selenium, which contributes to improved seed performance. A lower incidence of symptoms was also observed with this treatment. Compared to other compounds, its efficiency in pathogen control may be associated with the presence of the monoterpene citronellal. "IPR Tuiuiú" is considered a cultivar susceptible to the fungus, leading to variations in seed health.

The use of Phenyl Se Citronellal and Phenyl S Citral is an interesting alternative to enhance plant resistance

against pathogen attacks, as one of their components, citronellal, has shown effectiveness in disease control across various crops (Quintana-Rodriguez *et al.* 2018). However, the effects of Phenyl Se Citronellal are not well-known during the initial development of bean crops or in disease control in seedlings. Therefore, the findings obtained in this study are important information in order to move forward in ongoing projects and research.

Conclusions

In this study, seed treatment with semisynthetic compounds helped in the growth of bean ("IPR Tuiuiú" cultivar) seedlings and reduced the incidence of anthracnose. Disease control through seed treatment provides benefits for early plant growth and reduces disease incidence in the initial stages of bean cultivation. Phenyl S Citral and Phenyl Se Citronellal contributed to mitigating damage caused by *C. lindemuthianum*, prompting seedlings to respond favorably to the supply of these compounds. Therefore, the use of these compounds appears to be a promising measure in an integrated management system for disease control. Phenyl Se Citronellal, at a concentration of 0.5%, was able to control 100% of the mycelial growth of *C. lindemuthianum*. Phenyl S Citral controlled over 50% of the fungal mycelial growth at concentrations of 0.5 and 1%. Thus, seed treatment with the semisynthetic compounds Phenyl Se Citronellal and Phenyl S Citral not only reduced the incidence of *C. lindemuthianum* (isolate CL16) but also promoted higher growth of bean seedlings. This research emphasizes the need for in vivo trials to recommend the use of semisynthetic compounds in seed treatment. There is a considerable variation in effects depending on the chosen essential oil, the concentration of oil used for treatment, the plant species treated with the compound, and the target pathogen. The potential use of semisynthetic compounds in seed treatment as an alternative to plant disease management is evident.

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