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Research Article

Melaleuca Essential Oil (*Melaleuca alternifolia cheel*) in the Control of Beans Diseases

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Abstract

Bean cultivation is vital to the global food and economy, especially in Brazil. Facing challenges from diseases that affect production, it is crucial to seek new strategies to maintain productivity and sustainability. *Melaleuca alternifolia,* known as the tea tree due to its medicinal properties, has little explored potential in controlling diseases in bean plants. The objective of this work was to evaluate the effectiveness of tea tree essential oil in controlling diseases in bean cultivation. *In vitro* tests were carried out to evaluate bacterial growth, at concentrations of (0.0%, 0.05%, 0.1%, 0.5%, 1% and 2%). And antibiogram with the bacteria *Xanthomonas axonopodis* pv. *phaseoli*, in different concentrations (0.0%, 0.05%, 0.1%, 0.5%, 1%, 2% and 3%). For the fungus *Pseudocercospora griseola,* sporulation tests were carried out, using direct and indirect methods, at concentrations of (0.0%, 0.05%, 0.1%, 0.5%, 1%, 2%, and 3%). Furthermore, for the fungus *Colletotrichum lindemuthianum*, mycelial growth tests were carried out with the same concentrations. The experiments took place *in vivo,* with a completely randomized statistical design, involving five replications per treatment and concentrations varying from (0.0%, 0.05%, 0.1%, 0.5%, 1% and 2%). Disease incidence was assessed using a diagrammatic scale, disease severity, Area under the Disease Progress Curve (AACPD) and Area under the Incidence Progress Curve (AACPI). Melaleuca Essential Oil (EO) inhibited the development of fungi and bacteria in *in vitro* tests starting at 0.5%. *In vivo*, Melaleuca Essential Oil (EO) showed a significant reduction in the incidence and severity of the disease from 0.5% in both fungi and bacteria. Melaleuca EO can be an effective alternative for disease control in bean cultivation.

More Information

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Keywords: Plant health; Phytopathogens; Antibiogram; Sporulation; Mycelial growth

Introduction

Both nationally and globally, beans play a fundamental role in cuisine, culture and the agricultural economy. In Brazil, it is a central element in the diet, contributing significantly to food security and involving millions of farmers, generating jobs throughout the production chain [1,2]. Belonging to the genus *Phaseolus,* with around 55 species distributed worldwide, beans stand out for their nutritional relevance, providing protein, complex carbohydrates, dietary fiber, B vitamins, iron, calcium and other minerals. In addition, the crop plays a vital role in fixing nitrogen in the soil [3,4].

In addition to its nutritional importance, beans rank third among the most consumed foods worldwide. The world's largest producers are Myanmar, India, Brazil, the United States, Mexico and Tanzania, with production in these countries accounting for more than 60% of the total [5,6]. In Brazil, production is estimated at around 2.982 million tons in the 2022/23 season, down 0.2% on the previous year [7].

The bean crop is susceptible to various pathogens, including fungi, bacteria, viruses and nematodes, which can cause significant losses in production. Among these pathogens, the fungus *Colletotrichum lindemuthianum*, which causes anthracnose, is one of the most important. Damage can reach 100% of the harvest, damaging both grain quality and productivity. If the seeds are contaminated, the losses can be even greater Siqueira, et al. [8]. This disease affects the entire aerial part of the plant, causing lesions on the leaves and damaging pod production. It spreads via wind and rain and is most common at temperatures of 13 to 27°C with humidity above 91%, especially in temperate and subtropical climates Alves, et al. [9].

Another impactful disease is common bacterial blight, caused by the agent *Xanthomonas axonopodis* pv. *phaseoli*, which can cause losses of up to 70% of the crop and is transmitted by seeds, rainwater, irrigation, temperature and high humidity Telaxka, et al. [10]. Bacterial blight on beans, caused by infection through stomata and wounds, affects

the entire plant, from seeds to leaves. The watery lesions on the stem develop into red cracks, accompanied by visible bacterial exudate, while on the pods, watery spots with yellowish exudate compromise the germination and vigor of young seeds. The pathogen thrives at temperatures between 9 and 49°C, preferring 28 to 32°C, common in bean-growing regions. Production losses range from 10 to 75%, impacting photosynthetic capacity and resulting in plant breakage, as well as seed contamination [11,12].

Angular spot of the bean, caused by the fungus *Pseudocercospora griseola,* which can cause losses of up to 80% in production, can also cause losses to the same extent as bacterial growth and is difficult to control, since its spread occurs mainly through air currents Schmildt, et al. [13]. Angular spot on bean plants causes circular lesions with dark brown halos on the leaves, leading to premature defoliation and damaging production Viecelli, et al. [14]. The reddish-brown circular lesions on the pods affect the seeds and are favored by temperatures between 16 and 28°C, with an optimum of around 24°C, and alternating periods of high and low relative humidity, as well as the action of the wind, which is crucial for dissemination GUAZINA, et al. [15]. The fungus begins infection with the germination of spores, occurring 3 to 7 days after inoculation, between 8 and 32°C, forming germ tubes that penetrate the leaves, with visible symptoms 8 to 12 days later, when the intracellular region develops Viecelli, et al. [16].

Disease management in modern agriculture is generally carried out through chemical control, posing threats to the environment and human health Toillier, et al. [17]. In response, it is crucial to look for sustainable alternatives to reduce crop diseases while minimizing the use of chemical inputs. One viable option includes the use of substances extracted from plants, such as essential oils and hydrosols, which are effective in controlling diseases and inducing defense mechanisms in plants. These alternatives are degradable and less aggressive to the environment, benefiting small producers in particular [18,19].

Melaleuca, also known as Tea Tree and belonging to the Myrtaceae family, has been the subject of studies in recent years, with emphasis on the essential oil extracted from its leaves, which has remarkable antimicrobial activity Castro, et al. [20]. The main composition of this oil is terpinen-4-ol, 1.8-cineol, α-terpinene, γterpinene, α-pinene, β-pinene, α-terpineol, pcimene and sesquiterpenic alcohols, which make up around 90% of the oil, but can vary according to the fresh weight of the plant used Chagas, et al. [21]. However, the quality of Melaleuca essential oil is determined by the proportions of the compounds terpinen-4-ol and 1,8-cineol, which must be at least 30% and at most 15% of each other, and most of these components have inhibitory properties against fungi and bacteria [22,23]. The main antifungal constituent is α-terpineol (*M. alternifolia*).

However, there are few studies showing its use in controlling bean diseases such as bacterial blight, angular spot and anthracnose [24,25]. The aim of this work was to evaluate the *in vitro* and *in vivo* effect of *Melaleuca alternifolia* essential oil in protecting against bacterial blight, anthracnose and angular spot, as well as analyzing the effectiveness of its emulsion in protecting bean plants.

Materials and methods

The experiment was carried out at the Phytopathology Laboratory and in a greenhouse at the Federal University of the Southern Frontier, Laranjeiras do Sul *Campus*, Paraná, Brazil. The location of the region is 25° 24' 28'' S 52° 24' 58'' W, at an altitude of 840 meters. The region's climate is temperate, with an average annual rainfall of 1800 to 2000 mm/year. We used commercial melaleuca essential oil (EO) of Australian origin extracted by steam distillation of the leaves [26]. The composition of the oil is described in a technical report and is made up of the following constituents: α-terpinen-4-ol (40%), cineol (1%), α-terpinene (3%), α-pinene (4%), γterpinene (22%), α-terpinene (11%), α-terpinolene (3%) and pcimene (3%).

Samples of diseased plants were collected from bean plantations in the municipality of Laranjeiras do Sul/PR. The pathogens were identified using an optical microscope and culture media with Nutrient Broth Agar - M001-500G for bacteria and Nutrient Potato Dextrose Agar for fungi Pathmanathan, et al. [27]. Subsequently, the pathogens were incubated to grow in the BOD at a temperature of 25°C in the dark. To use the EO, 5 mL of the essential oil was emulsified with 5 mL of Tween 80, i.e. in a 1:1 ratio, resulting in an initial emulsion with a concentration of 10,000 µl/mL of EO for the *in vitro* tests. For the greenhouse tests, 100 mL of distilled water, 50 µL of essential oil and 100 mL of Tween 80 were emulsified for each treatment, resulting in an initial emulsion with a concentration of 1000 µl/mL of EO, considered to be 100%. The mixture was homogenized in a magnetic stirrer at 500 rpm for 10 minutes.

Under *in vitro* conditions, a test was carried out to evaluate the *Melaleuca alternifolia* EO emulsion on the pathogens *Pseudocercospora griseola, Xanthomonas axonopodis* pv. *phaseoli* and *Colletotrichum lindemuthianum.* The treatments consisted of diluting the EO emulsion in different concentrations (0.0%, 0.05%, 0.1%, 0.5%, 1%, 2% and 3%). To analyze the germination of *Pseudocercospora griseola* spores, 40 µL of a spore suspension calibrated at 1 x 105 conidia mL-1 were used, plus 40 µL of the solution of each treatment. This mixture was placed in an ELISA test plate, which was incubated in a Biochemical Oxygen Demand (BOD) incubator at 25 ºC in the dark for 20 hours. After this period, germination was stopped by applying 5µL of lactophenol cotton blue.

The germination percentage was determined by counting 100 spores per repetition under an optical microscope, with

each repetition consisting of one well of the ELISA plate, with each treatment consisting of 4 repetitions. Spores with visible hyphae were considered germinated. In addition to the direct evaluation, indirect evaluations were carried out. In this process, a filter paper was placed at the bottom of the Petri dish. A microscope slide was then added and the evaluation was carried out in the same way as the direct analysis.

For the mycelial growth of *Colletotrichum lindemuthianum,* the emulsified EO was diluted in the same concentrations as mentioned above. This solution was incorporated into the Potato Dextrose Agar (BDA) culture medium before being poured into Petri dishes. The treatments were inoculated with a 5 mm disk of the pathogen's mycelium and kept in a Biochemical Oxygen Demand (BOD) incubator at 26 °C. Horizontal and vertical mycelial growth measurements were taken on the 5th, 7th and 9th days to obtain representative averages. Measuring the mycelial growth of *Colletotrichum lindemuthianum* using the indirect method was similar, except that the essential oil was applied to the lids of the Petri dishes. Four replicates were carried out and a 4 cm^2 piece of gauze was fixed to the Petri dishes, which was evaluated in a similar way to the direct method.

The *in vitro* antibacterial activity was evaluated against *Xanthomonas axonopodis* pv. *phaseoli*, which causes bacterial blight. The treatments consisted of different concentrations (0.0%, 0.05%, 0.1%, 0.5%, 1% and 2%) and 5 replicates. The concentration of the bacterial suspension (Csb) was assessed in Agar broth liquid culture medium, in sterile 5 mL tubes. These tubes received the treatments and an aliquot of 50 µL of bacterial suspension with 108 CFU.mL-1, kept under constant agitation in an Orbital Shaker Incubator at 27 °C for 48 hours. Subsequently, the absorbance at 580 nm was determined using a spectrophotometer. Each treatment included an additional repetition without the addition of the bacterial aliquot, serving as the basis for calibrating the spectrophotometer when reading the treatment repetitions.

To evaluate the *in vitro* antibiogram test with *Xanthomonas axonopodis* pv. *phaseoli,* which causes bacterial blight, the treatments consisted of different concentrations (0.0%, 0.05%, 0.1%, 0.5%, 1%, 2% and 3%), carried out in four replicates. The concentration of the bacterial suspension (Csb) was assessed in solid and liquid Agar broth culture media, using sterile 5 mL tubes. Distilled water and Tween 80% were used as the control group. Germ paper disks were autoclaved and distributed, five disks per treatment. Next, a bacterial suspension of 100 µL with 108 CFU.mL-1 was prepared in test tubes containing saline solution (0.85%) with an absorbance of approximately 0.100 at 625 nm, under constant agitation in an Orbital Shaker Incubator at 27 °C for 10 minutes. Afterwards, 8 µL of the suspensions were applied to each disk. The plates were incubated at 27 °C for 48 hours until the bacterial colonies grew. The inhibition halos were then assessed using a ruler.

Different concentrations (0.0%, 0.05%, 0.1%, 0.5%, 1%, 2% and 3%) were used to analyze the germination of Pseudocercospora griseola spores *in vitro*. 100 µL of a calibrated spore suspension with a concentration of 1 x 105 conidia mL-1 was applied to the medium on the microscope slide. The samples were then incubated in a Biochemical Oxygen Demand (BOD) chamber at 25 ºC for a period of 48 hours. The percentage of germinated spores was determined by counting 100 spores per repetition under an optical microscope. Each repetition consisted of a "well" on the ELISA plate, with each treatment consisting of 4 repetitions. Spores with visible hyphae were considered germinated. For the indirect group, the procedure was the same, except that only the essential oil was placed on the lids, along with a piece of gauze fixed to the Petri dish lids.

Under *in vivo* conditions, a bioassay was conducted for each bean pathogen in a greenhouse. A phytotoxicity test was carried out on a group of plants to determine the ideal dose of the EO emulsion that would not cause damage to the plants, this dose being considered the strongest for the subsequent treatments in the experiment. The EO emulsion was diluted in different concentrations (0.0%, 0.05%, 0.1%, 0.5%, 1%, 2% and 3%). The treatments were applied by spraying the entire aerial part of the bean plants.

The soil was first sieved and then placed in the pots and moistened before sowing. IPR Tuiuiu black bean seeds were used for the anthracnose and bean bacterial growth treatments, due to the fact that this species is susceptible to these diseases. On the other hand, BRS Esteio was used for the angular spot test for the same reason. Plastic 3.6 liter pots were used, with 5 replicates for each treatment. The concentrations used were 0.0%, 0.05%, 0.1%, 0.5%, 1% and 2%. To prepare the substrates, 60% soil and 40% fertile soil were used to fill each pot. Four seeds were sown in each pot, depending on the cultivar. They were irrigated every two days at a temperature of 25 ± 2 ºC and relative humidity of approximately 73%. Between the V3 and V4 stages of the crop, an emulsion was made with a ratio of 100 mL of distilled water, 50 µL of essential oil and 100 µL of Tween 80. From this emulsion, different concentrations were applied to the leaves: 0.0%, 0.05%, 0.1%, 0.5%, 1.0% and 2.0%.

In the *Pseudocercospora griseola* and *Colletotrichum lindemuthianum* bioassay, a spore suspension (1 x 10^4 conidia mL^{-1}) containing the respective disease was sprayed on the plants until it reached the point of running, and this procedure was carried out 72 hours after the treatments were applied. The plants were then kept in a humid chamber for 20 hours. In the *Xanthomonas axonopodis* pv. *phaseoli* bioassay, the bacterial suspension (1 x 10^8 CFU.mL^{-1}) was inoculated by spraying the plants until they ran down, 72 hours after the treatments were applied. The plants were kept in a humid chamber for 20 hours.

After 10 days, disease symptoms began to appear on the bean plants. Evaluations were carried out every 7 days over the course of a month, following the same interval for the severity analysis. Data was collected from the first to the fourth assessment, and cumulative calculations were made to represent the progress of the disease over time. This data was then used to calculate the Area Under the Disease Progress Curve (AACPD). For the calculations of the Area Under the Incidence Progress Curve (AACPI) and the Area Under the Severity Progress Curve (AACPS), the formula used was the following Santos, et al. [28]:

(AACPD): $AACPD = \sum[(y^1 + y^2/2).(t^2 - t^1)]$

Where (y^1) and (y^2) represent the consecutive evaluations at times (y^1) and (y^2), respectively.

Disease severity was assessed using a diagrammatic scale, disease incidence and the Area under the Disease Progress Curve (AACPD). To analyze the severity of anthracnose in beans, a diagrammatic scale of scores from 1 to 7 was used, as described by FEIJÓ, et al. [29], as shown in (Table 1). Grades were assigned to each of the plants in the plot according to the degree of the disease, and then the average of the plots was calculated using the values in the statistics program. In the case of bean spot, a grading scale was used covering the range from 1 to 9, drawn up by LIMA, et al. (2013), according to (Table 2). For bean bacterial blight, we used a scale ranging from 1 to 6, developed by BARBOSA [31], as described in (Table 3).

In order to assess the incidence of *Pseudocercospora griseola, Colletotrichum lindemuthianum and Xanthomonas axonopodis* pv. *phaseoli,* the number of affected and unaffected leaves in each repetition of each treatment was counted according to Santos, et al. [28].

All bioassays were conducted in a completely randomized design (DIC), with 4 and 5 replicates for the *in vitro* and *in vivo* treatments, respectively. The results were evaluated using analysis of variance and then regression analysis or Tukey's test of means at 5% according to the characteristics of the data, with the support of the Sisvar computer program [32].

Table 1: Scale of scores for assessing the symptoms of anthracnose caused by *C. lindemuthianum* in bean plants [29].

Notes	Severity of the disease
1	Absence of symptoms;
2	Up to 1% of the veins showing necrotic spots noticeable only on the underside of the leaves;
3	Greater frequency of the leaf symptoms described in the previous grade, up to 3% of the veins affected;
4	Up to 1% of the veins showing noticeable necrotic spots on both sides of the leaves;
5	Higher frequency of the leaf symptoms described in the previous grade, up to 3% of the veins and pods affected;
6	Necrotic spots on the veins, noticeable on both sides of the leaves, presence of some lesions on the stems, branches and petioles and on the pods;
7	Necrotic spots on most of the veins and on a large part of the adjacent mesophyll tissue that breaks off. Presence of abundant lesions on the stems, branches and petioles and on the pods.

Table 2. Scale of scores to evaluate the symptoms of angular spot caused by *P. griseola* in bean plants [30].

Notes	Severity of the disease
1	Absence of symptoms;
2	0.1% to 0.5% of area with lesions;
3	0.6% to 4% area with lesions;
4	4.1% to 7.0% area with lesions;
5	7.1% to 16% area with lesions;
6	16.1% to 26% area with lesions;
7	26.1% 32% area with lesions;
8	32.1% to 38% area with lesions;
9	38.1% to 60% area with lesions.

Table 3: Scale of scores to evaluate the symptoms of bacterial blight caused by *X. axonopodis* pv. *phaseoli* in bean plants [31].

Notes	Severity of the disease
1	Green leaves and no symptoms;
2	Slight yellowing on the cut;
3	Yellowing extending slightly to the area between cuts;
4	Necrosis in the region of the cuts and yellowing concentrated throughout the region between cuts;
5	Same aspect observed in note 4, but with yellowing in the region above the upper cut and below the lower cut;
6	Leaf completely damaged.

Results and discussions

The results of the *in vitro* experiment on bacterial growth in different concentrations of tea tree essential oil (Figure 1A) showed a decrease in absorbance values, indicating a reduction in bacterial multiplication and development. The reduction in bacterial growth was directly proportional to the increase in the concentration of essential oil, with total inhibition of bacterial growth occurring at concentrations of 1% and 2%. There was a significant reduction in the 0.5% concentration of EO, around 72.8%. This pattern may be associated with the higher concentration of active compounds in melaleuca oil which play a crucial role in inhibiting bacterial growth Hillen, et al. [33]. The presence of compounds such as terpenoids, terpinen-4-ol and cineol in *M. alternifolia* has been associated with antimicrobial and anti-inflammatory activities. These compounds can disrupt the cell membranes of bacteria, interfering with vital processes and leading to inhibition of bacterial growth RAMOS, et al. [34].

The antibiogram activity of *X. axonopodis pv. phaseoli,* as shown in (Figure 1B), resulted in the inhibition of bacterial growth halos at concentrations of 0.5%, 1%, 2% and 3%, these being the most significant, and the higher the concentration of the EO, the larger the inhibition halo. This implies that although bacterial inhibition may increase with increasing concentrations of the oil, there is a point at which higher concentrations may not result in a proportional increase in

inhibition. This can be influenced by various factors, such as wind, relative air temperature, humidity, etc. Correa, et al. [35]. This information can be crucial in agricultural practice, where the application of substances such as essential oils needs to ensure maximum efficiency in preventing diseases. In addition, lower concentrations are more economically and ecologically viable, since excessive use of essential oils can also be harmful to the environment MARQUES, et al. [36].

Martins, et al. [37] observed total inhibition of the growth of *Macrophomina phaseolina* and *Sclerotinia sclerotiorum* from a concentration of 0.2% of melaleuca EO. In the case of controlling Alternaria *radicina* and *A. dauci*, [38] managed to completely inhibit mycelial growth from a concentration of 0.5%.

There was a significant reduction in the germination of spores of the fungus *P. griseola* at concentrations of 0.5%, 1%, 2% and 3% of *M. alternifolia* essential oil (Figure 2A), accompanied by a decrease in the length of germ tube sizes (Figure 2B). The study by SILVA, et al. (2019) consistently demonstrated that the highest concentrations of *M. alternifolia essential* oil have a direct impact on inhibiting the germination of *Pseudocercospora griseola* spores. According to NASCIMENTO, et al. [39], essential oils have the ability to prevent spore germination by intervening in various metabolic processes, such as protein synthesis, RNA production and respiratory activity. In this way, these oils can play an effective role as fungicidal agents.

The antifungal activity of *M. alternifolia* essential oil has been reported in several studies Costa, et al. [40]. The presence of compounds such as terpinen-4-ol, γterpinene, α-terpinene, α-terpineol, β-pinene, α-terpinolene, pcimene and 1,8-cineol in the essential oil have known antimicrobial properties, including antifungal activity Portella, et al. [41]. These compounds can interact with the lipid components of the cell membranes of fungal spores and inhibit their development. However, it is important to consider that very high concentrations of the essential oil can have phytotoxic effects on the target plants, which can limit its applicability in practice [42].

The sporulation test of the fungus *P. griseola* showed that no spores formed at the different concentrations of *M. alternifolia* essential oil. The control obtained a concentration of 46 x 10^4 spores/mL. Although the phytopathogenic fungus showed vigorous growth in the natural environment, the presence of the essential oil resulted in complete inhibition. On the other hand, growth occurred normally in the control plant without the essential oil. The sporulation of the *P. griseola* fungus plays a crucial role in the spread of the disease, as the spores are released into the environment and can infect new host plants. The absence of spore formation in the different concentrations of the essential oil is important to evaluate its potential [43,44].

In the mycelial growth test at different concentrations of *M.*

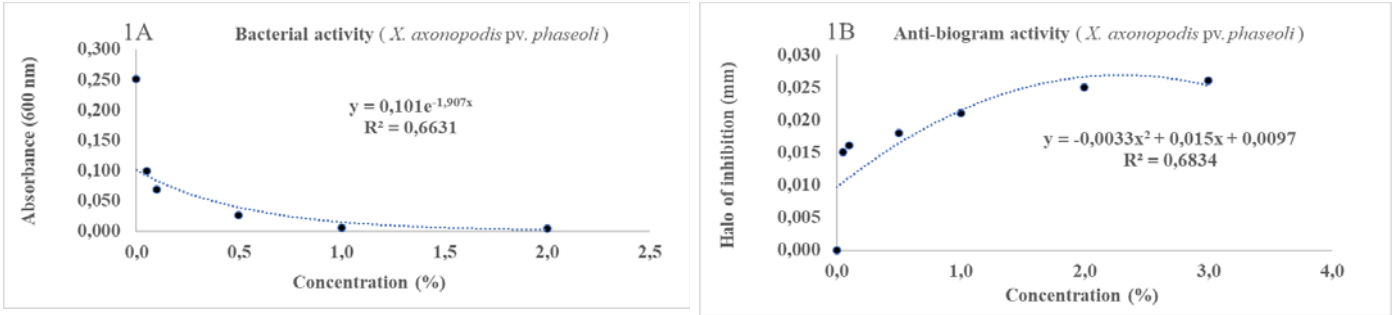

Figure 1: Bacterial activity (1A) and anti-biogram activity of *X. axonopodis* pv. *phaseoli* (1B) under different concentrations of *M. alternifolia* essential oil.

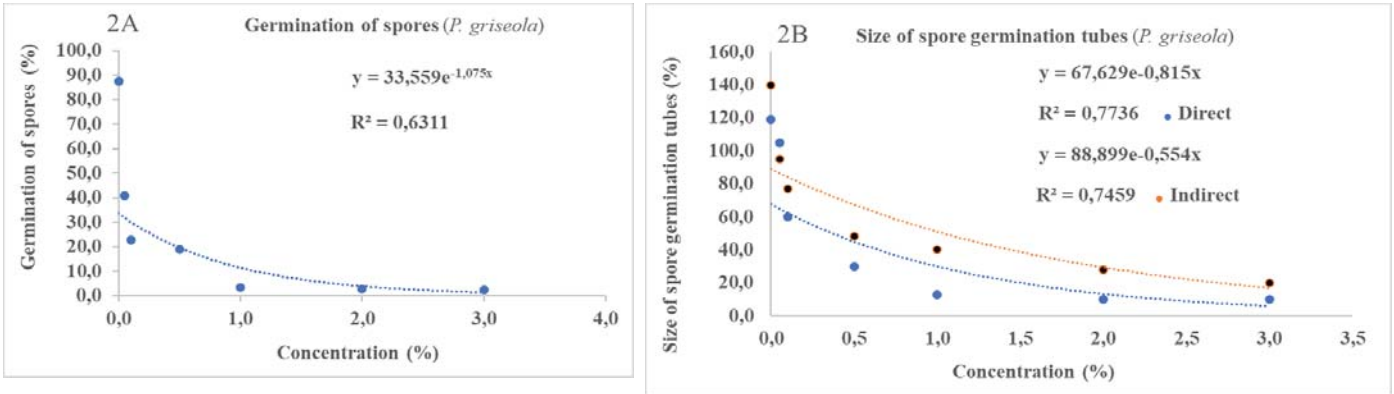

Figure 2: Germination of spores (2A) and size of spore germination tubes (2B) of *P. griseola* by the direct and indirect method in different concentrations of *M. alternifolia* essential oil.

alternifolia essential oil, there was a decrease in the mycelial growth of the fungus *C. lindemuthianum* directly proportional to the increase in oil concentration (Figure 3A). Other studies have shown the antifungal potential of *M. alternifolia* essential oil on various fungi [45,46] and this work has shown its effectiveness in controlling *C. lindemuthianum.* Further strengthening the biological properties of this essential oil, it is suggested that it could be a viable and ecologically friendly alternative for controlling fungi in agriculture. In the sporulation test of the fungus *C. lindemuthianum* (Figure 3B), carried out with various concentrations of *M. alternifolia* essential oil, it was observed that from the concentration of 0.5% there was total inhibition of spore growth.

On the other hand, in the indirect treatment, the complete reduction occurred from the application of 1% of the essential oil (Figure 3B). Other research has also highlighted the potential of *M. alternifolia* essential oil to combat phytopathogenic diseases, showing its ability to inhibit the fungus *C. lindemuthianum* at different concentrations. This additional information is relevant and points to the promising possibility of developing disease control strategies based on natural products [6,47].

For the *in vivo* experiment in the greenhouse, it is initially important to highlight common bacterial blight in the bean plant in relation to the area under the disease incidence and severity progress curve (AACPI and AACPS) of the *C. lindemuthianum* fungus. Significant differences were observed from the 0.5% concentration when compared to the control group, both in terms of incidence and severity of the disease, as shown in (Figure 4A and Figure 4B). The results of this PUVAČA, et al. study [48] show that *M. alternifolia* EO is effective against bacteria of the *Xanthomonas* genus. In addition, it prevents these bacteria from growing and reproducing.

The scanning electron microscopy technique identified cell collapse and denaturation after exposure to extracts of natural products. Based on this evidence, it is plausible to infer that the mechanism of action of essential oils in relation to microorganisms is associated with modifying the permeability of the cytoplasmic membrane, resulting in cell death [49,50]. Essential oils, due to their chemical complexity, offer antifungal control through various constituents that act simultaneously on different targets. These properties confer advantages compared to synthetic fungicides, reducing the likelihood of resistance by phytopathogens RUSSO, et al. [51].

The anthracnose results show a significant difference between all the treatments, in contrast to the control group. This highlights the positive impact of *M. alternifolia*

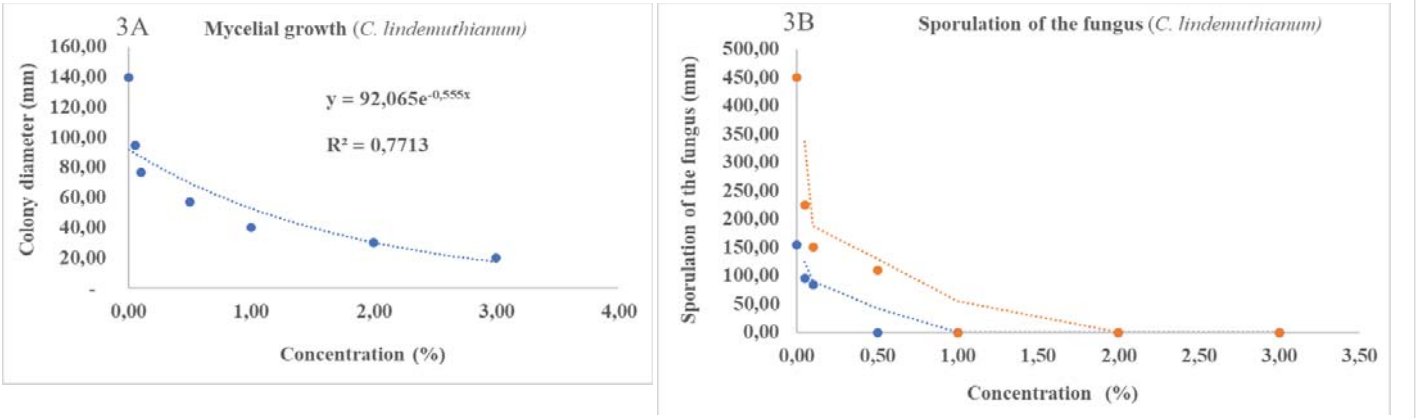

Figure 3: Mycelial growth of the fungus (3A) and sporulation of the fungus (3B) *C. lindemuthianum,* in different concentrations of *M. alternifolia* essential oil.

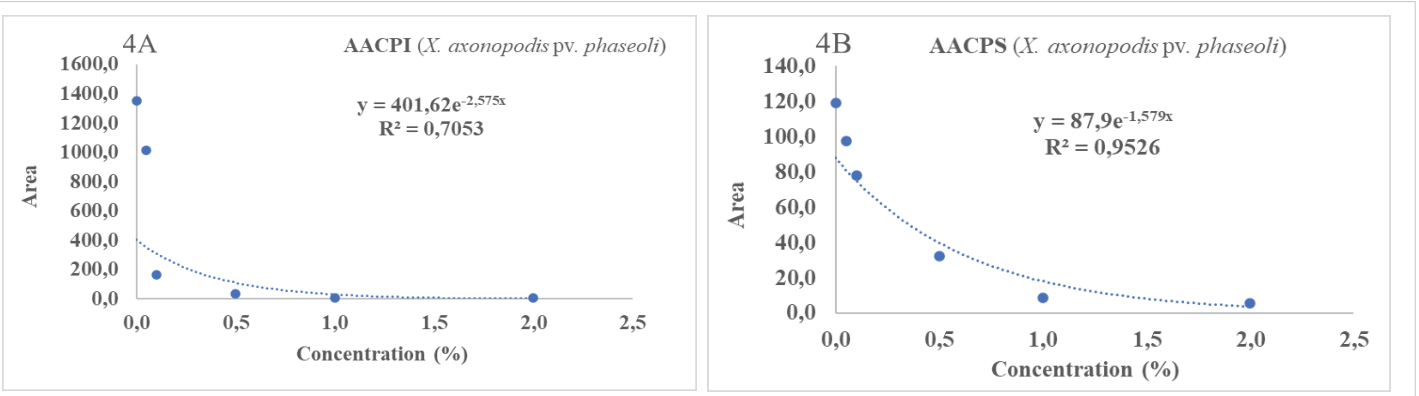

Figure 4: Area under the Disease Incidence Progress Curve (AACPI (4A)) and Area under the Disease Severity Progress Curve (AACPS (4B)) of bacterial blight on bean plants, under different concentrations of *M. alternifolia* essential oil.

essential oil in reducing the spread of anthracnose in bean plants, as shown in (Figure 5A and Figure 5B). However, at a concentration of 0.5% of EO, there was a reduction in both the severity and incidence of the disease. A study by Quintão, et al. [52] also found a positive effect of tea tree oil in inhibiting different species of phytopathogenic fungi. In the case of γterpinene, it reduces the viability of fungi, making them less able to reproduce and cause infections in plants.

The fat-soluble nature of essential oils makes it possible for them to interact with cell structures that have a lipid composition. This phenomenon results in increased membrane permeability, causing an electrolyte imbalance and, consequently, leading to cell death [53,54]. Melaleuca oil has shown effective antifungal action against *Sclerotinia sclerotiorum* and *Fusarium sp.* The 0.25% concentration of the oil provided 100% control of *S. sclerotiorum* and around 70% control of the *Fusarium* isolates under *in vitro* conditions. Growth inhibition of *S. sclerotiorum* and *Fusarium sp*. was evident with 0.5% of the essential oil, indicating the potential of this species for formulating agricultural biofungicides [55,56] point out that the formation and stability of oils, composed of volatile elements, are influenced by the ambient temperature. The volatilization of oil constituents, combined with their susceptibility to light, heat and humidity, emerges as a crucial factor that can compromise the effectiveness of these oils in controlling plant diseases.

The effects of angular spot on bean plants in relation to AACPI and AACPS were significantly different between the treatments compared to the control group, as illustrated in (Figure 6A and Figure 6B). The presence of compounds in *M. alternifolia* EO, such as α-terpinene, plays a vital role in preventing spore germination and inhibiting the reproduction of various microorganisms, such as bacteria, fungi and viruses. In addition, this compound exerts an important protective action for plants [57].

The various biological activities linked to essential oils are intrinsically linked to their composition, the concentration of the main components and any synergistic interactions with minor elements POWERS, et al. [58]. In the study conducted by BARBOSA, et al. [59], a concentration of 50 $\mu L.L^{-1}$ of melaleuca essential oil was found to be effective in controlling *Colletotrichum musae.* In addition, Souza, et al. [24] reported a significant 99% reduction in the mycelial growth of *Cercospora beticola Sacc.* through the application of 1% tea tree essential oil. And also at a concentration of 0.8%, it proved effective in combating *cercosporiosis i*n beet. An additional study carried out by Mariano, et al. [60] at melaleuca EO concentrations of 0.50% to 1.17% showed efficacy in controlling *Aspergillus sp.* on sunflower seeds.

Among others, silver nanoparticles (AgNPs) offer a promising alternative for controlling plant diseases, including those affecting crops such as beans. Their antimicrobial

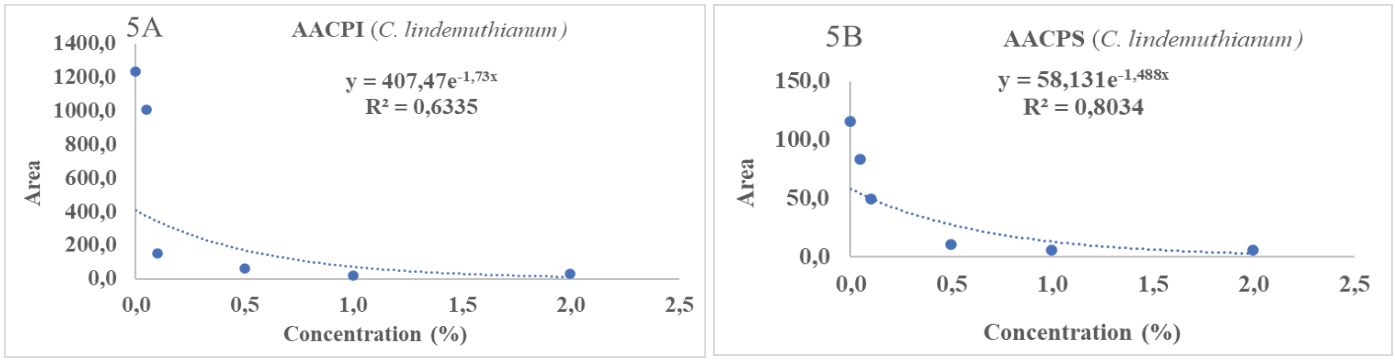

Figure 5: Area under the Disease Incidence Progress Curve (AACPI (5A)) and Area under the Disease Severity Progress Curve (AACPS (5B)) of anthracnose in bean plants, under different concentrations of *M. alternifolia* essential oil.

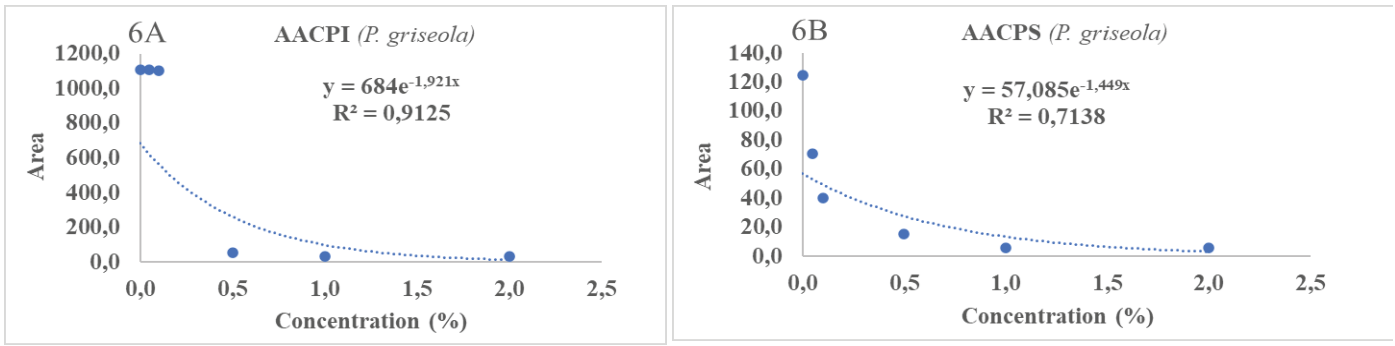

Figure 6: Area under the Disease Incidence Progress Curve (AACPI (6A)) and Area under the Disease Severity Progress Curve (AACPS (6B)) of angular spot on bean plants, under different concentrations of *M. alternifolia* essential oil.

efficacy can reduce dependence on conventional pesticides while mitigating environmental impacts. The eco-friendly biosynthesis of AgNPs using biomolecules minimizes environmental risks. However, biosafety challenges and studies on interactions with soil and plants are needed to ensure their safe and effective use in sustainable agricultural practices [61].

Despite the positive results in controlling bean diseases, the study of melaleuca essential oil faces challenges of generalization of results, limited experimental scales and the need for more research in field conditions and over the long term Ferreira, et al. [62].

Conclusion

This study investigated the effects of melaleuca essential oil on the control of diseases affecting the bean plant. Tests carried out *in vitro* proved the efficacy of the essential oil, indicating inhibitory effects on the fungi that cause angular blotch, anthracnose and the bacteria responsible for bacterial blight. In all tests, the 0.5% concentration of EO had a significant effect on inhibiting the growth of fungi and bacteria in beans. The experiments conducted *in vivo* in the greenhouse showed equally promising results. The essential oil treatments showed significant effects in combating phytopathogenic fungi and bacteria, resulting in a notable reduction, at a concentration of 0.5% EO, in the incidence and severity of bean diseases.

It is important to note that the Area under the Disease Incidence Progress Curve (AACPI) and the Area Under the Disease Severity Progress Curve (AACPS) were also significantly affected by the treatments at a concentration of 0.5% EO, showing the positive impact of melaleuca essential oil in containing these diseases. Therefore, this study highlights the potential of this essential oil as a promising tool for effective disease management in bean cultivation, with important implications for sustainable agriculture and agro-ecological production. It is recommended that future studies explore the efficacy of the oil in different bean varieties and environmental conditions, carry out extended studies to assess sustainability and ecological impacts, and investigate practical applications considering economic viability in agriculture.

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