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Essential Oils as an Antifungal Alternative to Control *Cladosporium* spp., *Lasiodiplodia* spp., *Colletotrichum* spp., *Fusarium* spp. and *Aspergillus* spp. Isolated of *Musa paradisiaca*

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Article

Essential Oils as an Antifungal Alternative to Control *Cladosporium* spp., *Lasiodiplodia* spp., *Colletotrichum* spp., *Fusarium* spp. and *Aspergillus* spp. Isolated of *Musa paradisiaca*

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[2] Facultad de Ingeniería en Ciencias Agropecuarias y Ambientales (FICAYA), Universidad Técnica del Norte (UTN)
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Abstract: Antifungal properties are found in essential oil extracts of oregano (*Origanum vulgare*), rosemary (*Salvia rosmarinus*), clove (*Syzygium aromaticum*), thyme (*Thymus*), cinnamon (*Cinnamomum verum*), basil (*Ocimum basilicum*), therefore, the ability to prevent or inhibit the growth of *Cladosporium spp.*, *Lasiodiplodia spp.*, *Colletotrichum spp.*, *Fusarium spp.*, and *Aspergillus spp.* was studied. *Cladosporium spp.*, *Lasiodiplodia spp. Colletotrichum spp. Fusarium spp.* y *Aspergillus spp.* fungi that were isolated and purified from the rot found on the banana peels (*Musa paradisiaca*). To perform a visual (macroscopic) analysis of the Petri dish containing the pure fungus and a more detailed (microscopic) analysis of the fungal spores to classify it by genus, then, dilutions are prepared to inoculate onto amended PDA medium with chloramphenicol. Weekly re-inoculations are performed until isolated and purified fungi are obtained, which are then evaluated for *in vitro* inhibition and *in vivo* growth, according to the results of the *in vivo* analysis, the fungi were classified from higher to lower severity in: *Colletotrichum spp.*, *Lasiodiplodia spp.*, *Aspergillus spp.*, *Fusarium spp.* and *Cladosporium spp* and based on fungal growth observations, it was found that basil and rosemary do not inhibit growth at the maximum concentration. Cinnamon inhibits at 400 ppm, rosemary halts fungal growth at 600 ppm, clove at 1000 ppm, and oregano at 800 ppm.

Keywords: *Origanum vulgare*; *Salvia rosmarinus*; *Syzygium aromaticum*; *Thymus*; *Ocimum basilicum*; *Cinnamomum*

1. Introduction

The origin of the banana is from India, Asia, it is an exotic and climatic fruit known as cambur, gualele, guineo, musacea, platano. In Ecuador, in El Oro province, Machala, has led exports since 1910 to the United States, Peru, and Chile, highlighting its quality, which is unmatched by fruits offered at lower prices from other countries [1]. Currently it is the largest exporter of banana with internationally recognized quality product with a productivity of 1500 boxes per hectare per year, it is consumed when the peel is yellow, being an important source of vitamins, potassium, carbohydrates, and minerals [2]. The rot in the banana increases in transport and the fungal growth decreases the quality of the fruit after harvest and produces the rot of the crown, known as "Crown rot" caused mainly by *Colletotrichum spp.* [3].

Oregano has bioactive compounds such as carvacrol, thymol, phenolic acids that have antimicrobial properties, antioxidants, cinnamon has cinnamaldehyde, eugenol, flavonoids that has anti-inflammatory, antioxidant, glucose regulation potential and the clove has Eugenol, acetyl eugenol, flavonoids acting as analgesic, antimicrobial, antioxidant [4,5]. Cinnamon essential oil was used as a bioactive compound to extend the lifetime of the strawberry as a post-harvest treatment [6].

Basil has bioactive compounds such as eugenol, Rosmarinus acid, flavonoids that generates antioxidant, anti-inflammatory properties, thyme has thymol, carvacrol, flavonoids that generates

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antibacterial properties, antioxidant, enhances digestion and rosemary acid Rosmarinus, carnosol, flavonoids with antioxidative, anti-inflammatory, potential neuroprotective effect [7–9].

The Ascomycetes family includes the genus of fungi *Cladosporium spp.* and are commonly found in indoor and outdoor environments, are microscopically distinguished by having septate hyphae and conidia in groups or chains, the conidia are black, olive green, or sometimes brown, and frequently form in chains, are globose or ellipsoidal in shape. the hyphae are dark, branched, septate, and have a rough, cottony, or velvety texture. They may be plant pathogens, sometimes affect humans causing respiratory problems and allergies, are used in biodegradation, although they do not have significant industrial applications [10,11].

Lasiodiplodia spp. causes rot in fruit crops and is another type of fungus that is part of the Ascomycetes, the conidia are spherical in shape and occasionally form in chains, the hyphae are septate and frequently contain conidia in their spores, the texture may be cottony or velvety, with colors ranging from brown to black, and microscopically, conidia in spores and septate hyphae can be observed. The main impact of this fungus is on fruits and crops, causing diseases such as fruit rot. Its impact on agriculture is significant, although it is not frequently used in biotechnology [12,13].

Anthracnose is a common disease in many plants and is caused by various fungal pathogens, including those in the genera *Colletotrichum spp.*, is classified within the Ascomycetes, microscopically the conidia appear in clusters or groups and have a cylindrical or ellipsoidal shape, the hyphae usually have conidia in their cluster and are septate, the texture may be velvety or cottony, and the color may be pink, red or orange. It is used to control pests in agriculture and is used in biopesticides [3,9]. Studies are being conducted on antifungal activity (*in vitro)* of plant extracts for the control of anthracnosis [14].

Plant diseases such as wilt and root rot are caused by *Fusarium spp.*, some species produce mycotoxins that are harmful to animals and humans. It is a fungus of the Ascomycetes or Basidiomycetes families, the conidia may be white, pink, red, or purple, are elongated, and frequently appear in chains. Macroconidia and microconidia can be observed in the septate hyphae, and the texture may be cottony, velvety, or powdery [15,16].

Aspergillus spp. is found in food production, are used in the production of organic acids, enzymes and antibiotics, produce aflatoxins, which are toxic and carcinogenic, may also be opportunistic pathogens that infect people with weakened immune systems, are of the Ascomycetes family, microscopically, the conidia may be in chains or clusters, septate hyphae and the mycelium may be dense and abundant, the colonies may be green, black, white and yellow and the texture may be smooth, velvety, or cottony [17,18].

2. Materials and Methods

2.1. Isolation and Purification of Microorganisms

It is based on the scheme of Figure 1., to prepare the amended medium, PDA medium is made according to the supplier's specifications, and then amended with 0.5 g/L of chloramphenicol, which prevents bacterial growth, allowing for the study of fungi only [19].

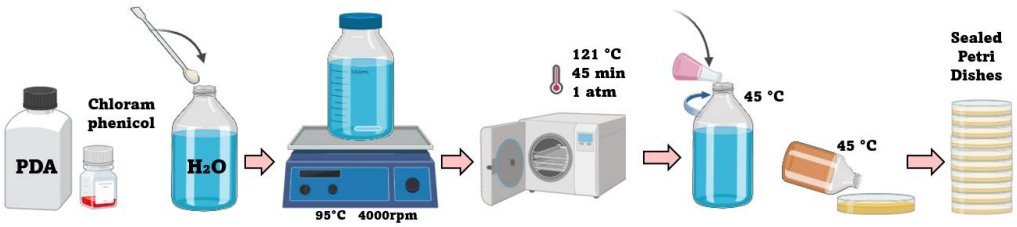

Figure 1. Preparation of the amended medium with chloramphenicol (0.5 g/L), to obtain pure fungi responsible for the rot present in banana peels.

Figure 2 shows the process for obtaining the initial dilution, which begins by decomposing the fruit to take a piece of the peel, rinsing it with sterile water twice, and discarding the used water [3].

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The pieces of fruit peel affected by the presence of pathogens are placed in an Erlenmeyer flask with 200 mL of a 0.05% (v/v) Tween 80 solution and stirred in the vortex for approximately 2 minutes.

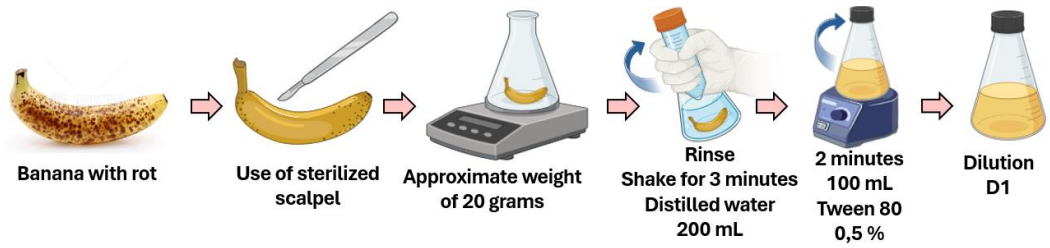


Figure 2. Preparation of the initial dilution containing the microorganisms causing banana rot.

From dilution 1, four dilution series are prepared, using a 0.1% preparation of the initial solution to stir each dilution. Then, 0.1 mL of each dilution is plated onto Petri dishes with PDA to observe the macroscopic and microscopic morphology [3]. Figure 3 provides the diagram for preparing the dilutions used to analyze the growth.

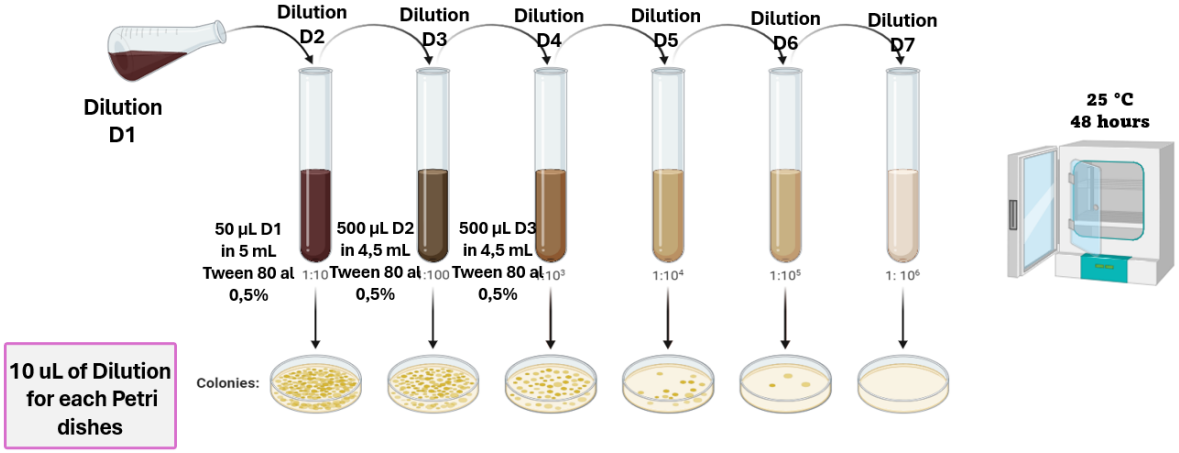


Figure 3. Preparation of serial dilutions for plating 10µL on amended PDA medium.

The pathogen was visibly isolated on a Petri dish with PDA medium, which was incubated at 25°C for 7 days. A colony was picked with a sterile loop and subculture weekly until the strains were purified.

2.2. Macroscopic Identification

The purified fungi, three replicates were evaluated each week after plating the isolated pathogens. Their macroscopic characteristics were recorded and compared with bibliographic information from books and guides on fungal morphology to identify the pathogen's genus. During the analysis, aspects such as colony shape, elevation, edges, and appearance were considered.

2.3. Microscopic Identification

The fungus obtained from the culture was examined in triplicate under the microscope using adhesive tape to collect the aerial mycelium and adhere it to a slide. The slide was observed under a microscope with 40X and 60X magnification lenses [5]. The evaluation focused on the hyphae, mycelium, spores, and the microscopically observed structures.

 doi:10.20944/preprints202408.2122.v1

2.4. In Vivo Fungal Activity

After the morphological characterization, the pathogens *Cladosporium spp., Lasiodiplodia spp. Colletotrichum spp. Fusarium spp.* and *Aspergillus spp.* which were isolated from the banana (*Musa paradisiaca*), were evaluated.

The inhibition index was calculated in quadruplicate based on the measurement corresponding to the diameter of fungal growth to assess the severity of the identified pathogens. In the Figu[re 4 provides the diagram for obtaining a desired concentration of 10^6 conidia/mL of each inoculum.

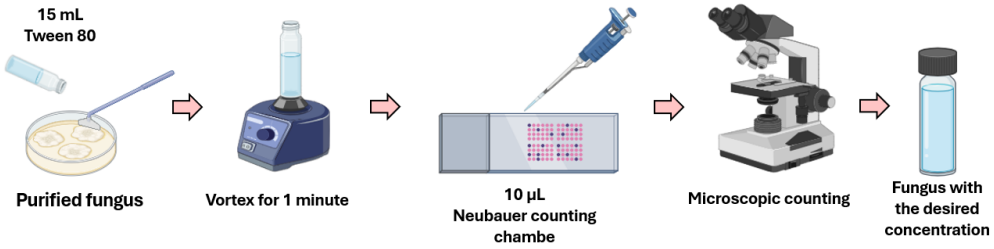


Figure 4. Diagram for obtaining a concentration of 10^6 conidia/mL of each inoculum.

The fungal growth diameter on the days following inoculation was the response variable studied, based on each fungal genus, the most severe pathogen was determined. Figure 5 provides the diagram for the inoculation of the fungi on the banana.

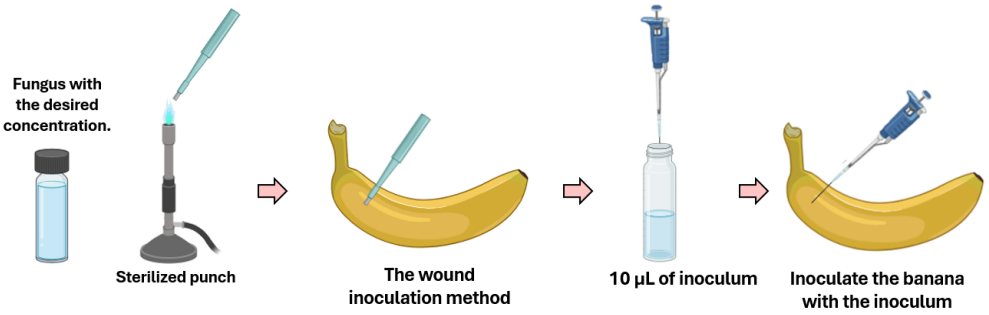


Figure 5. Inoculation of the fungi with a concentration of 10^6 conidia/mL.

2.5. In Vitro Antifungal Activity with Essential Oils

Antifungal activity was analyzed under controlled laboratory conditions using essential oils of oregano (*Origanum vulgare*), rosemary (*Salvia rosmarinus*), clove (*Syzygium aromaticum*), thyme (*Thymus*), cinnamon (*Cinnamomum verum*), and basil (*Ocimum basilicum*). PDA media were prepared with concentrations of 200, 400, 600, 800, and 1000 ppm of each essential oil, and a control without essential oil was included (Figure 6).

The solutions with the specific concentrations of essential oil were solidified for the inoculation of the pathogens maintained at 25°C in the incubator to identify the most effective concentration. The prepared Petri dishes were visually inspected in triplicate every 48 hours to determine the percentage of inhibition, and the effectiveness of the essential oil used.

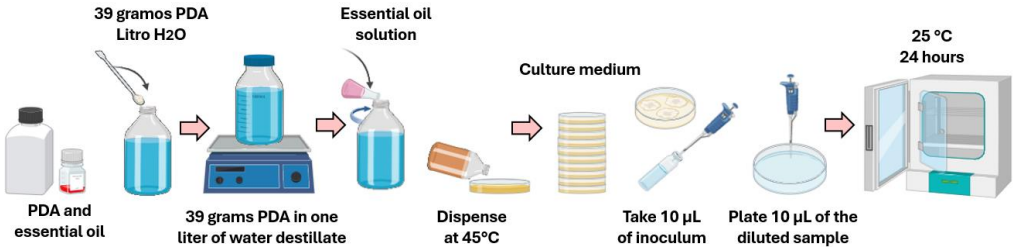


Figure 6. Preparation scheme of the medium with established essential oil concentrations.

 doi:10.20944/preprints202408.2122.v1

3. Results

3.1. Macroscopic Identification

Table 1 shows the macroscopic comparison of purified fungi by characteristics such as colony shape, appearance, elevation, shore, surface and color of both the front and reverse.

3.2. Microscopic Identification

Table 2 presents the aerial mycelium of *Cladosporium spp., Lasiodiplodia spp., Colletotrichum spp., Fusarium spp.,* and *Aspergillus spp.* The observations were made using 40X and 60X microscope lenses. The table evaluated the hyphae, mycelium, spores, and appearance in an analysis of their morphological characteristics.

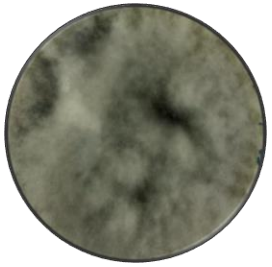
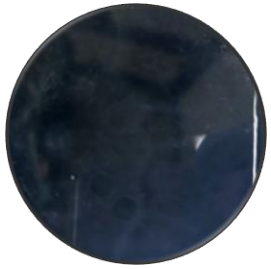
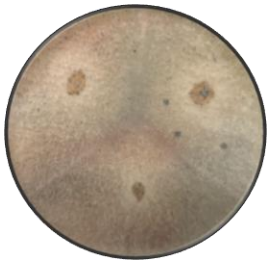
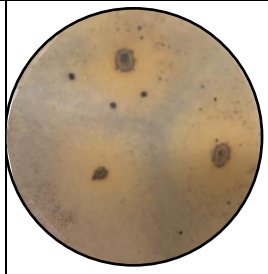
3.3. In vivo fungal activity

The severity of fungal infection was assessed through an *in vivo* analysis. Figure 7 illustrates the evaluation of fungal growth across 20 banana samples, which were monitored over a period of 6 weeks. The analysis focused on identifying and assessing the growth of various fungal strains, including *Cladosporium spp., Lasiodiplodia spp., Colletotrichum spp., Fusarium spp.,* and *Aspergillus spp.*

The results provide insights into the impact of these fungal strains on the bananas over the study period, highlighting differences in growth and infection severity of the different fungal species.

 doi:10.20944/preprints202408.2122.v1

Table 1. Macroscopic characterization of **(a)** *Cladosporium spp.*, **(b)** *Lasiodiplodia spp.*, **(c)** *Colletotrichum spp.*, **(d)** *Fusarium spp.* and **(e)** *Aspergillus spp.* considering appearance of upper side and lower side.

Fungus		Macroscopic characteristics						
Upper side	Lower side	Shape	Appearance	Elevation	Shore	Surface	Color	
							Top	Bottom
 a		Irregular round shape	Velvety, cottony, powdery	Elevated, crater-like	Wavy and irregular	Rough and cottony	Green	Green
 b		Round, spreading	Woolly and cottony	Planar and slightly elevated	Irregular and diffuse	Slightly rough, rugged, and hairy	Black	Black
 c		Radial from the inoculation point	White to beige mycelium with black spots	Planar or slightly elevated	Slightly lobed, irregular	Often smooth or rough	Pigmented beige	Pigmented beige

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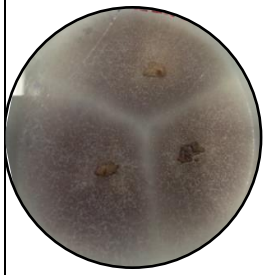
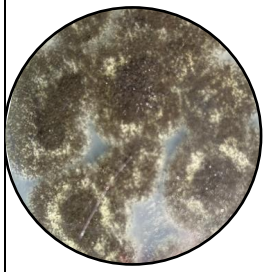
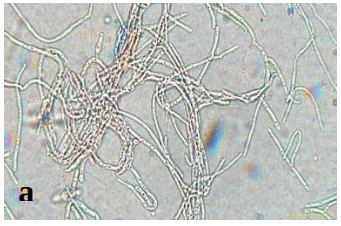
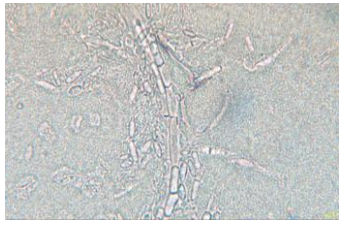
Fungus		Macroscopic characteristics						
Upper side	Lower side	Shape	Appearance	Elevation	Shore	Surface	Color	
							Top	Bottom
 d		Radial, expanding irregularly	Cottony, purple	Planar or slightly elevated	Irregular, fringed, and serrated	Rough, coarse, and cottony	White and purple	White and purple
 e		Round and irregular	Velvety, powdery, sporulating	Slightly elevated	Defined, fringed, and wavy	Rough, dense spore layer	Initially white, then turns black	White

Table 2. Microscopic characterization of **(a)** *Cladosporium spp.*, **(b)** *Lasiodiplodia spp.*, **(c)** *Colletotrichum spp.*, **(d)** *Fusarium spp. spp.* and **(e)** *Aspergillus spp.* observed under the microscope.

Fungus		Microscopic characteristics			
40 X	60 X	Hyphae	Mycelium	Spores	Appearance
 a		Septate and branched	Density and pigmentated	Elliptical conidia linked in long chains	Septate hyphae with conidia linked in chains

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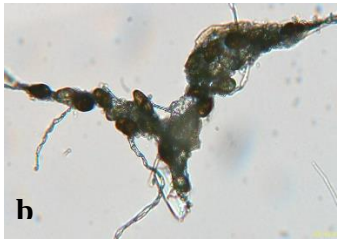
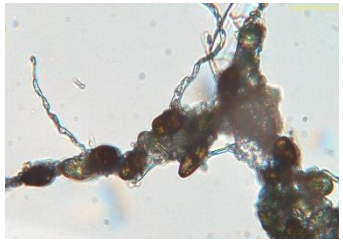
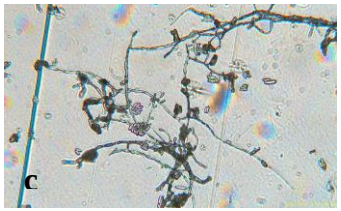
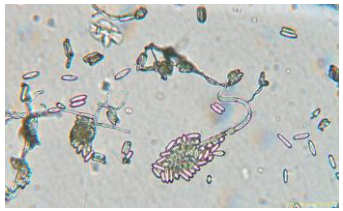
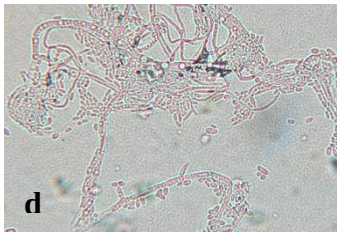
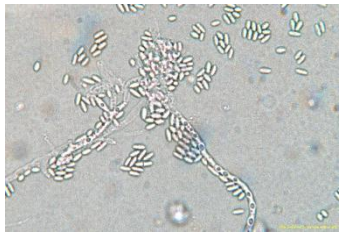
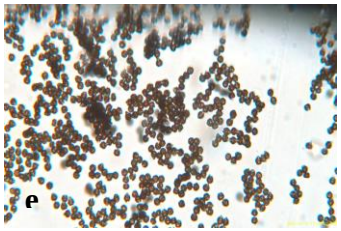
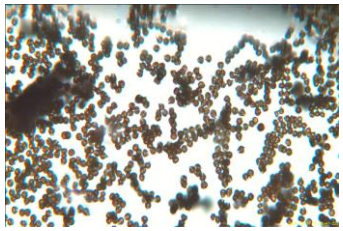
Fungus		Microscopic characteristics			
40 X	60 X	Hyphae	Mycelium	Spores	Appearance
 b		Septate	Dense mycelium	Oval conidia produced on conidiophores	Network of septate hyphae with conidiophores
 c		Septate and branched	Dense mycelium with a network of conidia	Elongated oval spores	Conidia in short chains on the conidiophores
 d		Hyaline, septate hyphae	Dense mycelium	Fusiform asexual spores from conidiophores	Conidia observed on the conidiophores
 e		Septate, thin, and hyaline	White, dense mycelium	Spherical or slightly ellipsoidal	Thin conidiophores with conidia arranged around them

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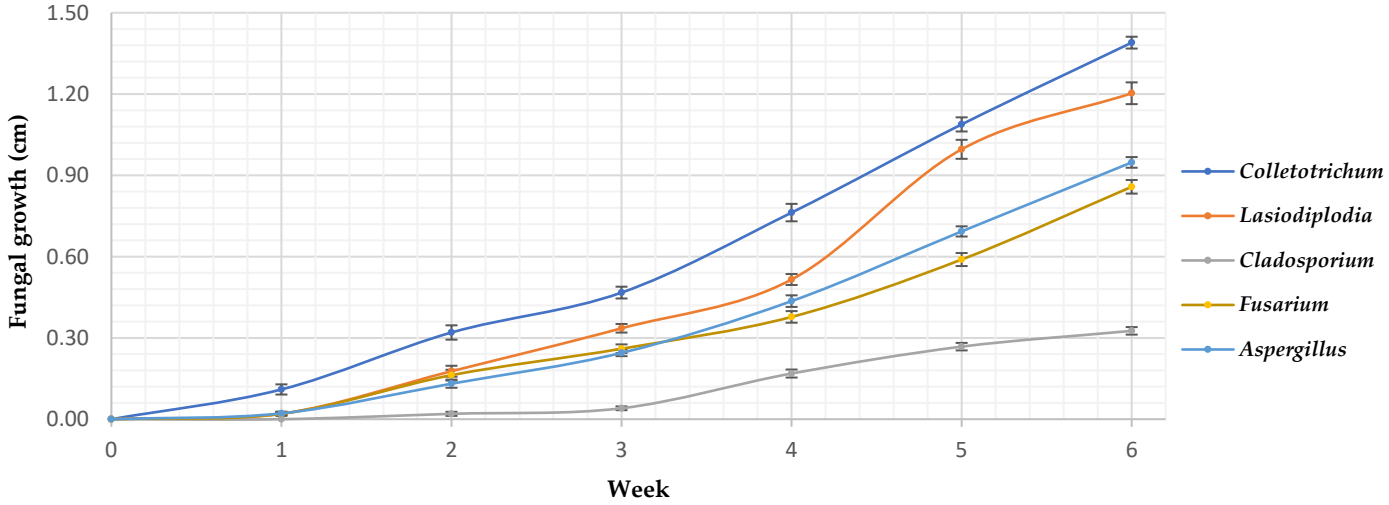

Figure 7. Fungal growth (cm) for 6 weeks in 20 banana samples inoculated with *Cladosporium spp., Lasiodiplodia spp. Colletotrichum spp., Fusarium spp.* and *Aspergillus spp.,* stored at 13°C and 95% HR.

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3.4. In Vitro Antifungal Activity with Essential Oils

Figures 8–12 illustrate the growth of *Cladosporium spp., Lasiodiplodia spp., Colletotrichum spp., Fusarium spp.*, and *Aspergillus spp*. on PDA medium with essential oil concentrations of 200, 400, 600, 800, and 1000 ppm. The essential oils evaluated include oregano, basil, cinnamon, rosemary, thyme, and clove.

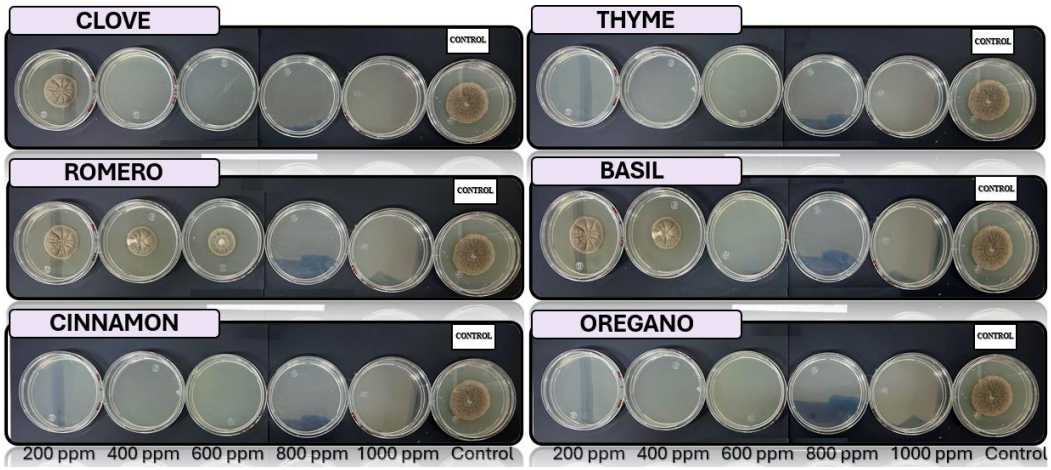

Figure 8. *In vitro* growth analysis of *Cladosporium spp.* on PDA medium with basil, cinnamon, clove, oregano, rosemary, and thyme essential oils at 200, 400, 600, 800, and 1000 ppm, stored at 25°C (n=4).

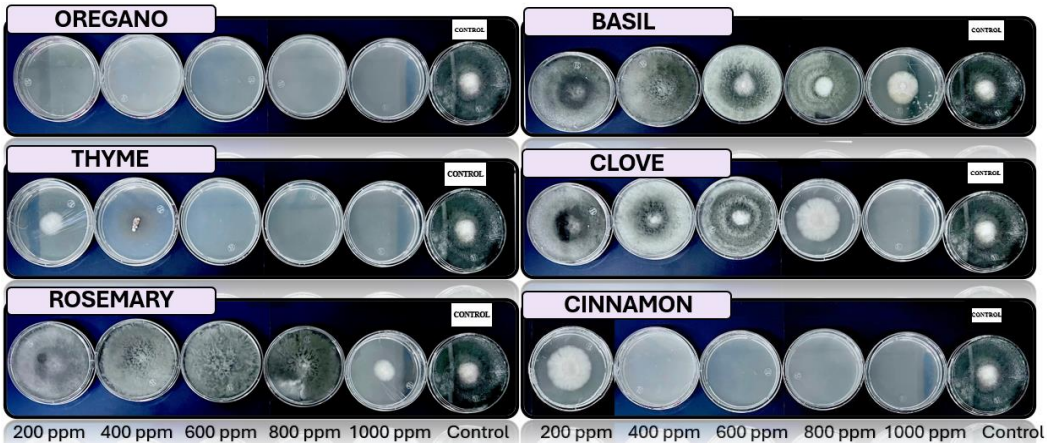

Figure 9. *In vitro* growth analysis of *Lasiodiplodia spp.* on PDA medium with basil, cinnamon, clove, oregano, rosemary, and thyme essential oils at 200, 400, 600, 800, and 1000 ppm, stored at 25°C (n=4).

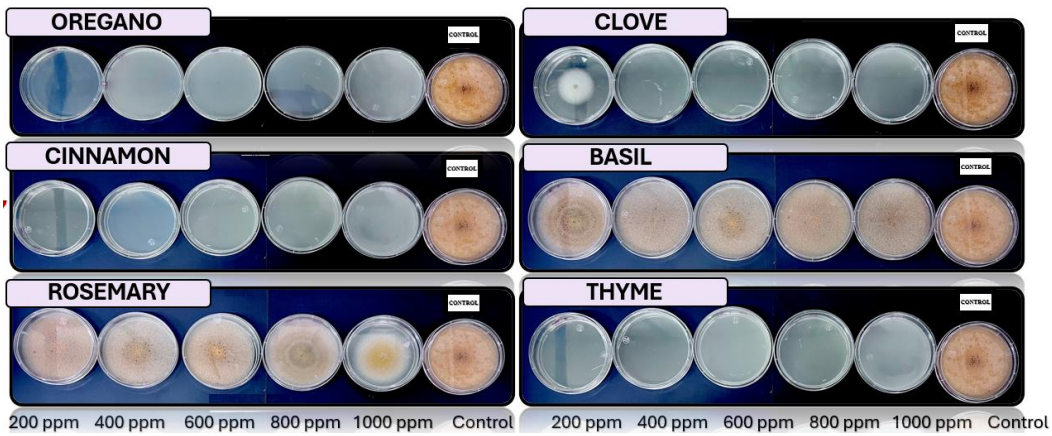

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Figure 10. *In vitro* growth analysis of *Colletotrichum spp.* on PDA medium with basil, cinnamon, clove, oregano, rosemary, and thyme essential oils at 200, 400, 600, 800, and 1000 ppm, stored at 25°C (n=4).

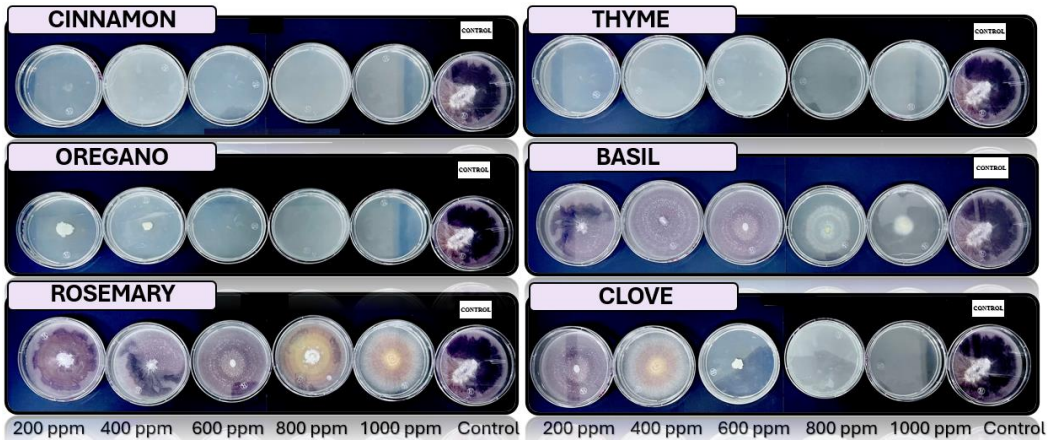


Figure 11. *In vitro* growth analysis of *Fusarium spp.* on PDA medium with basil, cinnamon, clove, oregano, rosemary, and thyme essential oils at 200, 400, 600, 800, and 1000 ppm, stored at 25°C (n=4).

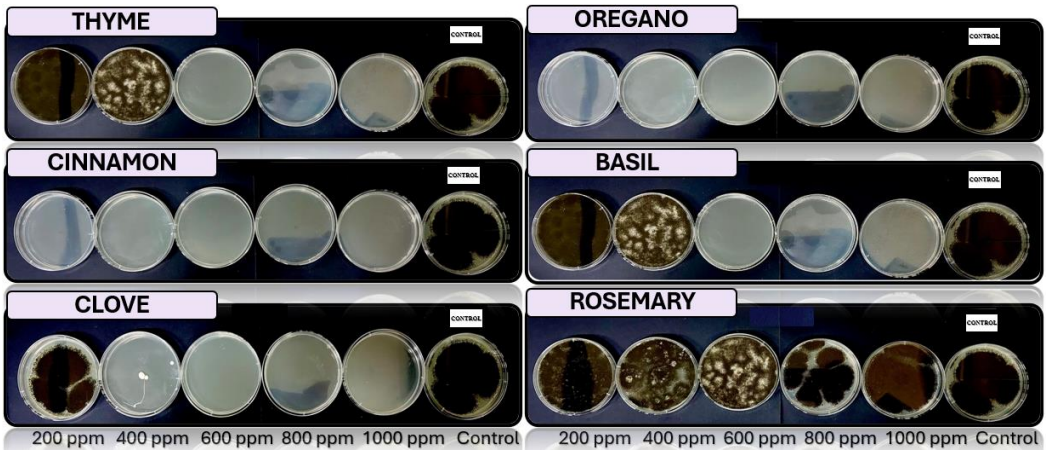
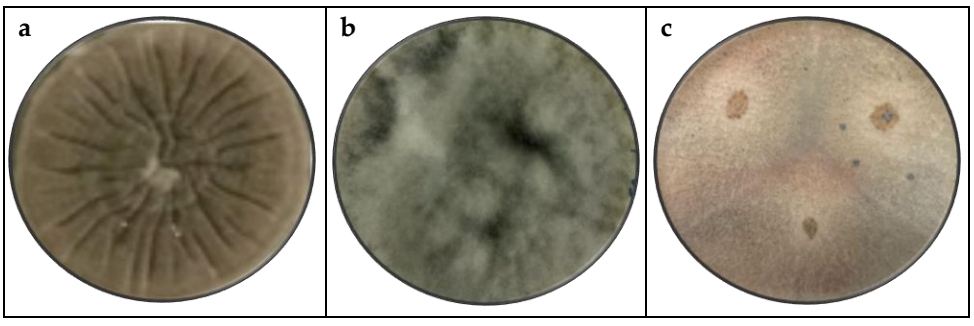


Figure 12. *In vitro* growth analysis of *Aspergillus spp.* on PDA medium with basil, cinnamon, clove, oregano, rosemary, and thyme essential oils at 200, 400, 600, 800, and 1000 ppm, stored at 25°C (n=4).

4. Discussion

4.1. Macroscopic Identification

Figure 13 shows the initial isolation of pathogens obtained from perceptibly affected banana peels, which were plated on PDA and incubated at 25°C. The figure displays the purified fungi that are implicated in banana rot.



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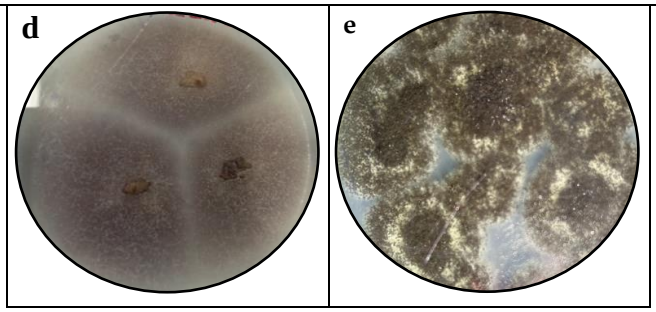

Figure 13. Fungi isolated and purified from banana peel rot, inoculated on selective medium (PDA with chloramphenicol), and stored on PDA at a 25°C in the incubator, **(a)** strain of *Cladosporium spp.*, **(b)** *Lasiodiplodia spp.*, **(c)** *Colletotrichum spp.*, **(d)** *Fusarium spp. spp.* and **(e)** *Aspergillus spp.*.

This macroscopic analysis suggests that the fungus is of the genus *Cladosporium spp.* due to its powdery appearance, green color on both the front and reverse sides, and its velvety, cottony, and cratiform (crater-like) appearance [20], *Lasiodiplodia spp.* based on the macroscopic characteristics, which report a woolly appearance with a black to gray color, rapid growth, and a rough surface. These features are similar to those reported when evaluating banana crown rot [21], *Colletotrichum spp.* have mycelium that is pigmented beige with a slightly raised, planar elevation, and has been identified as the most significant fungal agent in banana [22].

Postharvest deterioration of bananas is due to the presence of *Fusarium spp.* of various species that have been studied previously [15,23], this species exhibits a white and purple coloration with irregular edges and a cottony appearance, *Aspergillus spp.* have various applications in biotechnology, including the secretion of organic acids, proteins, enzymes, and secondary metabolites due to their prolific nature the genus is suspected based on its appearance on PDA, where it presents as round black spores with a rough texture, black coloration on the front, and white on the reverse [24].

4.2. Microscopic Identification

Based on the results of the macroscopic analysis and the comparison of microscopic characteristics with bibliographic references, the identification is *Cladosporium spp.* for septate and branched hyphae, and conidia that are generally ellipsoidal and are found in long chains [20], *Lasiodiplodia spp.* the identification is based on the black coloration, the ability to form multicellular oval conidia, and the septate hyphae, with conidia located at the apex of the conidiophore [21].

Colletotrichum spp. With high pathogenic potential, they have oval and elongated conidia arranged in short chains along the conidiophore, with septate and branched hyphae [25], *Fusarium spp.* is characterized by conidia that can be unicellular or multicellular, with a varied shape, including fusiform and ellipsoidal. Due to the diversity of species, sequencing is necessary to ensure accurate analysis and identification, and *Aspergillus spp.* is distinguished by having septate, thin, and hyaline hyphae, with dense white and black mycelium, and spores that are spherical or slightly ellipsoidal.

4.3. In Vivo Fungal Activity

After the macroscopic and microscopic characterization, the fungal strain was evaluated of *Cladosporium spp.*, *Lasiodiplodia spp. Colletotrichum spp. Fusarium spp.* and *Aspergillus spp.* To establish the calculated inhibition index for 20 samples based on the corresponding measurement of the circular growth diameter of the fungi, in order to assess the severity of the fungus [26].

The response variable studied was fungal growth over the 6 weeks following inoculation. To further analyze the fungal genera, the most severe agent was determined by statistically analyzing the obtained results using Stat graphics Centurion XV.

Figure 14 presents the analysis of means. The p-value of the F-test is less than 0.05, indicating a statistically significant difference between the means of the 5 variables with a 95.0% confidence level. It is concluded that the pathogens were assessed in the following order of severity, from highest to lowest: *Colletotrichum spp.*, *Lasiodiplodia spp.*, *Aspergillus spp.*, *Fusarium spp.* and *Cladosporium spp.*.

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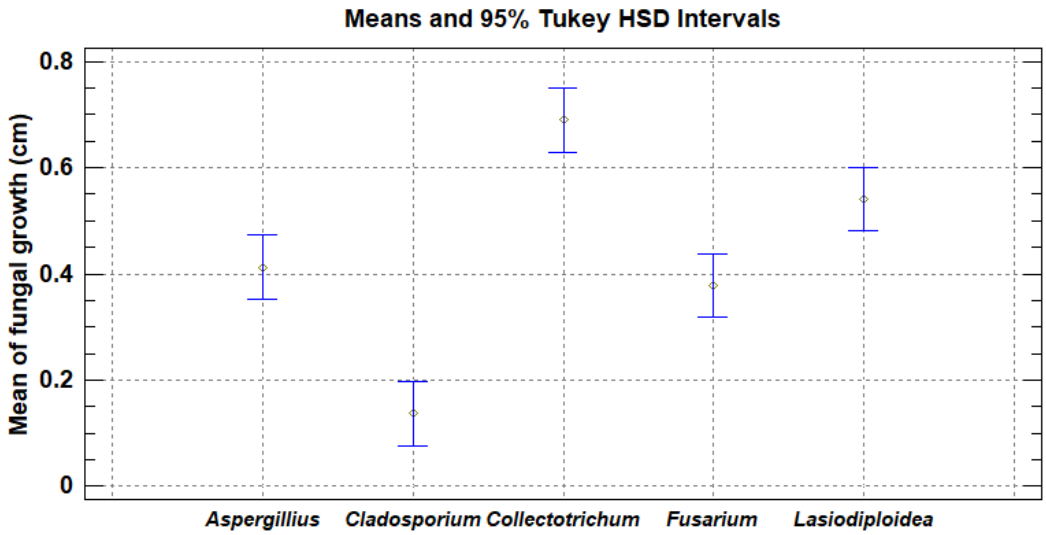

Figure 14. Mean diameter (cm) of fungal decay circles in banana samples inoculated with *Cladosporium spp., Lasiodiplodia spp. Colletotrichum spp., Fusarium spp.* and *Aspergillus spp.* for 6 weeks, stored at 13°C and 95% HR (n=20).

4.4. In Vitro Antifungal Activity with Essential Oils

The potential of essential oils as antifungal agents was evaluated *in vitro* to oregano (*Origanum vulgare*), rosemary (*Salvia rosmarinus*), clove (*Syzygium aromaticum*), thyme (*Thymus*), cinnamon (*Cinnamomum verum*) and basil (*Ocimum basilicum*), from commercial stores and extracted through steam distillation, were evaluated for their antifungal potential. Solutions were prepared using PDA (Potato Dextrose Agar) medium with varying concentrations of essential oils 200, 400, 600, 800 y 1000 ppm.

The different concentrations were meticulously formulated to assess their antifungal efficacy against the targeted fungal strains. Each concentration was mixed into the PDA medium to ensure uniform distribution before solidifying in petri dishes for subsequent testing. A control without essential oil was included for comparison.

The most effective concentration was identified through the analysis conducted in four replicates, and the Petri dishes were examined every 48 hours to determine the percentage of inhibition and effectiveness of the essential oil at the established concentration [27].

Table 3 presents the *in vivo* analysis of antifungal activity, demonstrating that a concentration of 400 ppm of cinnamon essential oil effectively inhibits the growth of all five fungal species tested.

Table 3. Evaluation of antifungal activity *in vitro* of essential oil against *Cladosporium spp., Lasiodiplodia spp., Colletotrichum spp., Fusarium spp.* and *Aspergillus spp*. strain, using oregano, rosemary, clove, thyme, cinnamon and basil essential oils.

Essential oil	Fungus	Concentration [ppm]				
		200	400	600	800	1000
Cinnamon	*Cladosporium spp.*	+	-	-	-	-
	Lasiodiplodia spp.	-	-	-	-	-
	Colletotrichum spp.	-	-	-	-	-
	Fusarium spp.	-	-	-	-	-
	Aspergillus spp.	-	-	-	-	-
Clove	*Cladosporium spp.*	+	-	-	-	-

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	Lasiodiplodia spp.	+	+	+	+	-
	Colletotrichum spp.	+	-	-	-	-
	Fusarium spp.	+	+	+	-	-
	Aspergillus spp.	+	+	-	-	-
Basil	*Cladosporium spp.*	+	+	-	-	-
	Lasiodiplodia spp.	+	+	+	+	+
	Colletotrichum spp.	+	+	+	+	+
	Fusarium spp.	+	+	+	+	+
	Aspergillus spp.	+	+	-	-	-
Oregano	*Cladosporium spp.*	-	-	-	-	-
	Lasiodiplodia spp.	-	-	-	-	-
	Colletotrichum spp.	-	-	-	-	-
	Fusarium spp.	+	+	+	-	-
	Aspergillus spp.	-	-	-	-	-
Rosemary	*Cladosporium spp.*	+	+	+	-	-
	Lasiodiplodia spp.	+	+	+	+	+
	Colletotrichum spp.	+	+	+	+	+
	Fusarium spp.	+	+	+	+	+
	Aspergillus spp.	+	+	+	+	+
Thyme	*Cladosporium spp.*	-	-	-	-	-
	Lasiodiplodia spp.	+	+	-	-	-
	Colletotrichum spp.	-	-	-	-	-
	Fusarium spp.	-	-	-	-	-
	Aspergillus spp.	+	+	-	-	-

5. Conclusions

During the characterization process, the fungal strains were assessed and ranked based on their severity, from the most severe to the least severe. This ranking was determined through of an evaluation of the analysis *in vivo* report: *Colletotrichum spp.*, *Lasiodiplodia spp.*, *Aspergillus spp.*, *Fusarium spp.* y *Cladosporium spp.* The *in vitro* antifungal activity analysis reveals that a concentration of 400 ppm of cinnamon essential oil is effective in inhibiting the growth of all five fungal species examined.

In the *in vitro* study to evaluate the efficacy of essential oils, cinnamon was effective at 400 ppm, thyme at 600 ppm, and clove at 1000 ppm, while basil and rosemary did not inhibit the growth of the analyzed pathogens. Specifically: *Colletotrichum spp.* was controlled with 200 ppm of oregano, thyme, 400 ppm of cinnamon, and 1000 ppm of clove. *Lasiodiplodia spp.* was controlled with 200 ppm of cinnamon, oregano, 600 ppm of thyme, and 1000 ppm of clove. *Aspergillus spp.* was controlled with 200 ppm of cinnamon, oregano, 600 ppm of clove, basil, and thyme. *Fusarium spp.* was controlled with 200 ppm of cinnamon, thyme, 800 ppm of clove, and oregano. *Cladosporium spp.* was controlled with 200 ppm of oregano, thyme, 400 ppm of cinnamon, clove, 600 ppm of basil, and 800 ppm of rosemary.

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