

Article

Not peer-reviewed version

Essential Oils as an Antifungal Alternative to Control Several Species of Fungi Isolated of *Musa paradisiaca*: Part II

[MaritzaD. RuizMedina](#) * and [Jenny Ruales](#)

Posted Date: 27 January 2025

doi: 10.20944/preprints202501.1894.v1

Keywords: banana; essential oils; natural antifungals; postharvest diseases



Preprints.org is a free multidisciplinary platform providing preprint service that is dedicated to making early versions of research outputs permanently available and citable. Preprints posted at Preprints.org appear in Web of Science, Crossref, Google Scholar, Scilit, Europe PMC.

Copyright: This open access article is published under a Creative Commons CC BY 4.0 license, which permit the free download, distribution, and reuse, provided that the author and preprint are cited in any reuse.

Article

Essential Oils as an Antifungal Alternative to Control Several Species of Fungi Isolated of *Musa paradisiaca*: Part II

Maritza D. Ruiz Medina * and Jenny Ruales

Departamento de Ciencias de Alimentos y Biotecnología (DECAB), Escuela Politécnica Nacional (EPN)

* Correspondence: marilolita3@hotmail.com; Tel.: +593-62609240; +593-997666999

Abstract: Essential oils from oregano (*Origanum vulgare*), rosemary (*Salvia rosmarinus*), clove (*Syzygium aromaticum*), thyme (*Thymus vulgaris*), cinnamon (*Cinnamomum verum*), and basil (*Ocimum basilicum*) possess antifungal properties. This study aimed to evaluate their ability to inhibit the growth of fungi isolated from the rot of banana peel (*Musa paradisiaca*) to control or reduce fungal growth in bananas. The methodology involved preparing dilutions of essential oils and inoculating them onto Potato Dextrose Agar (PDA) medium amended with chloramphenicol to prevent bacterial contamination. Fungal species, including *Trichoderma* spp., *Aspergillus* spp., *Penicillium* spp., and *Fusarium* spp., were isolated, purified, and characterized both macroscopically and microscopically. Their growth was assessed *ex vivo* and the inhibition percentage was measured *in vitro*. The *ex vivo* analysis revealed that the severity of fungal infection, ranked from highest to lowest, was as follows: *Penicillium* spp., *Trichoderma* spp., *Fusarium* spp., and *Aspergillus* spp. The results showed that rosemary and basil oils did not inhibit fungal growth, while clove oil was effective at 800 ppm, cinnamon at 400 ppm, and oregano at 200 ppm against all four fungi. These findings suggest that certain essential oils, particularly clove, cinnamon, and oregano, have strong antifungal potential and could serve as eco-friendly alternatives to synthetic fungicides in banana postharvest disease management.

Keywords: banana; essential oils; natural antifungals; postharvest diseases

1. Introduction

Banana (*Musa paradisiaca*), a food of great importance in tropical regions and in many developing countries, plays an essential role in serving as a pillar for economic growth and social development of local communities, for its ability to maintain consistent production throughout the year [1]. Ecuador's banana production is essential to its economy and food security [2]. It contributes significantly to employment and represents approximately 2% of total PIB and 35% of agricultural PIB. The main producing provinces are Guayas, El Oro and Los Ríos, highlighting El Oro for its quality, while Guayas and Los Ríos for the major producers [2].

In production, various factors, both external and internal, affect the quality of the fruit during the post-harvest phase. External factors include environmental conditions such as relative humidity and temperature and among internal factors, metabolic changes and the presence of fungal pathogens are highlighted [2,3].

It is important to emphasize that fungal pathogens, in particular diseases associated with *Fusarium* spp., *Penicillium* spp., *Trichoderma* spp., y *Aspergillus* spp., represent the main causes of banana wilt [4,5]. These diseases manifest as a reduction in the firmness of the superficial tissues in the areas of the rachis and the banana crown, accompanied by changes in leaf coloration and cracks at the base of the pseudo stem [6]. This causes internal rot in the fingers of the banana, eventually resulting in the crown rot and, in more severe cases, the death of the fruit [7,8].

Traditionally, these diseases have been managed using synthetic fungicides. However, the growing interest in organic farming and the concerns about toxic residues in food have prompted the search for more sustainable alternatives [9]. In this context, essential oils derived from plants such as *Origanum vulgare*, *Salvia rosmarinus*, *Syzygium aromaticum*, *Thymus vulgaris*, and *Cinnamomum verum* have emerged as a promising alternative. These oils not only possess antimicrobial properties but have also been shown to extend the shelf life of fruits by providing fungitoxic effects and enhancing resistance to postharvest diseases [10,11].

Essential oils contain bioactive compounds such as terpenes and phenols, which are documented for their antimicrobial effects. These compounds can inhibit the growth of fungal pathogens, including those responsible for postharvest rot in bananas. Previous studies have demonstrated the antifungal efficacy of essential oils such as oregano, rosemary, and clove, although the results vary depending on the concentration, the type of fungus, and the storage conditions [12,13]. However, despite the promising results, there is limited research specifically evaluating the antifungal activity of these oils against *Fusarium* spp., *Penicillium* spp., *Trichoderma* spp., and *Aspergillus* spp. in bananas, especially under real postharvest conditions.

This investigation focuses on assessing the antifungal effectiveness of commercially available essential oils against fungal pathogens isolated from banana peels. The research helps in the development of sustainable alternatives to synthetic fungicides, promoting eco-friendly agricultural practices and enhancing post-harvest management [14]. Understanding the composition of the essential oils used is crucial to identifying the bioactive compounds responsible for the observed antifungal effects. Below is a detailed overview of the information and components found in each essential oil.

Essential oils of oregano and thyme share similar volatile compounds [15], such as the carvacrol, thymol and p-cymene, the rosemary contains alpha-pinene, 1.8 cineol, camphor and verbenone [16,17]. Clove oil, is characterized by its content of eugenol, acetyl eugenol and α and β caryophyllenes and the basil contains estragole, linalool, eugenol and methyl cinnamate [16], Cinnamon oil is rich in cinnamaldehyde and eugenol, which have demonstrated significant antifungal and bioactive properties [18].

The objective is to evaluate the potential of essential oils as an ecological alternative for controlling fungal diseases in bananas, promoting sustainable agriculture and organic production. Specifically, this study evaluates the antifungal efficacy of these oils against *Trichoderma* spp., *Penicillium* spp., *Aspergillus* spp., and *Fusarium* spp. isolated from *Musa paradisiaca* through of *in vitro* and *ex vivo*. The isolated fungi are detailed below:

Fusarium a filamentous genus known for its ability to adapt to diverse environmental conditions, is a major cause of vascular wilt in bananas, blocking the transport of water and nutrients, and reducing both the quality and quantity of fruit production [19]. In Ecuador is generally found the species *oxysporum*, *verticillioides* and *solani* that are studied to innovate strategies to avoid damage to the banana [20,21].

Penicillium distributed in various environments, some species have antagonistic activity against pathogens that can cause deterioration of the fruit in post-harvest [22]. *Penicillium citrinum*, *Penicillium expansum*, and *Penicillium digitatum* are frequently isolated from banana surfaces, and essential oils like mandarin have been shown to inhibit their growth [23,24].

Trichoderma is a genus of filamentous, cosmopolitan fungi is known for its biocontrol properties [25,26]. *Trichoderma* species produce antibiotics and secondary metabolites that inhibit phytopathogenic fungi [27]. India distributes certified species of *Trichoderma asperellum*, *T. atroviride*, *T. gamsii*, *T. hamatum*, *T. harzianum*, *T. polysporum*, *T. virens*, and *T. viride* and with genetic engineering made significant improvements to apply in industrial processes [25,28].

Aspergillus spp. including species such as *Aspergillus niger*, *Aspergillus flavus*, and *Aspergillus parasiticus*, are common contaminants in bananas during storage and transportation [29]. These fungi produce a variety of enzymes that decompose plant tissues and can also produce harmful aflatoxins,

which pose a risk to human health [29,30]. *Aspergillus parasiticus* is similar to *Aspergillus fravus*, this species can also produce aflatoxin and affect the quality and safety of bananas [31].

Although essential oils from oregano, basil, thyme, clove, cinnamon, and rosemary have a potent antifungal effect, their phytotoxicity must be carefully monitored. Excessive or misdosed use of these oils can cause damage to plants, such as chlorosis, necrosis, and reduced root growth, especially at concentrations above 1%. It is recommended to use safe concentrations and conduct preliminary tolerance tests on specific plant species before large-scale application [32,33].

In conclusion, this study investigates the potential of essential oils as antifungal agents against common pathogens in bananas. By promoting sustainable agriculture and reducing reliance on chemical fungicides, this research supports the development of more eco-friendly, effective strategies for postharvest disease management.

2. Materials and Methods

In this research it was used *Musa paradisiaca*, harvested Ecuadorian banana, treaties and considerations for export. A sample of bananas was taken and exposed to ambient conditions of approximately 25°C temperature until signs of rot were observed. Bananas showed at least 50% of the symptoms of the presence of fungi in the banana peel [34].

2.1. Isolation and Purification of Microorganisms

To the isolation of pathogenic fungi from infected banana peels was performed using Potato Dextrose Agar (PDA) from Difco™ as the culture medium. To prepare the medium, 39 g of PDA was dissolved in 1 L of distilled water and sterilized by autoclaving. This medium is used for fungal propagation due to its ability to support fungal growth [35]. To inhibit bacterial growth, chloramphenicol (Merck, Ecuador) was added at a final concentration of 0.5 g/L. The antibiotic was incorporated to prevent bacterial contamination during the fungal isolation process [36]. The prepared medium was then poured into sterile Petri dishes and allowed to solidify at room temperature.

Samples were collected from 30 infected bananas, with approximately 20 g of visibly affected peel removed from each. The peels were submerged in sterile distilled water and manually agitated for 2 minutes [37]. Subsequently, the samples were rinsed twice with sterile water to remove any residual contaminants. For fungal spore extraction, the banana peel fragments were placed into 200 mL Erlenmeyer flasks containing a 0.05% (v/v) aqueous solution of Tween 80. This surfactant aids in releasing the spores from the infected plant tissues, facilitating fungal isolation [38]. The solution was vortexed for 1-2 minutes to ensure homogeneity. Four serial dilutions were prepared by diluting each sample with 0.1% of the previous dilution in each successive series [39].

The resulting dilutions were inoculated onto Petri plates containing PDA medium supplemented with chloramphenicol at 0.05%. 0.1 mL of each dilution was applied to the plates, which were incubated at 25°C. The plates were examined every 48 hours to monitor fungal colony development. Emerging colonies were selected using a sterile loop to avoid cross-contamination, and the colonies were then subcultured weekly onto fresh PDA plates to have pure cultures [40].

2.2. Morphological Identification

Once pure fungi were obtained, macroscopic analysis was conducted to assess the characteristics of the fungal colonies, including shape, color, and texture. For the identification of the causative agents affecting the banana, the upper and lower surfaces of the Petri dishes were observed macroscopically, considering the morphological similarity obtained through direct comparisons. The pure cultures were examined in triplicate over a period of two weeks after inoculation with the isolated pathogens [41]. Their macroscopic characteristics were recorded and compared with bibliographic information from books and descriptive guides of fungal morphology to identify the

genus of the pathogen [37,42]. Aspects such as colony shape, elevation, edges and appearance of pure fungi were considered.

Microscopic examination was performed using conventional methods, to observe the fungal reproductive structures, including hyphae, conidia, and spores. To visualize the microscopic and specialized structures, a piece of adhesive tape was used to collect the aerial mycelium of the fungus and it was mounted on a microscope slide [37]. The plate was examined using a compound microscope with a 40X and 60X objective lens, this analysis was performed in triplicate. The evaluation was based on the observation of hyphae, mycelium, spores and other microscopic structures present.

2.3. Molecular Identification Through DNA Sequencing

Molecular identification of fungi isolated from *Musa paradisiaca* peel was performed through DNA sequencing. Genomic DNA was extracted from pure fungal colonies using the commercial Invitrogen kit, following the manufacturer's instructions. The quality and quantity of the extracted DNA were evaluated using spectrophotometry with a Nanodrop spectrophotometer and by performing 1% agarose gel electrophoresis to assess DNA integrity [43].

The ribosomal DNA fragment corresponding to the Internal Transcribed Spacer (ITS) region was amplified using the universal primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'). The PCR amplification was carried out under optimal conditions in a thermocycler, and the amplified products were visualized on a 1.5% agarose gel stained with ethidium bromide under UV light to confirm successful amplification of the ITS region [36,43].

Purified amplicons were sent to Macrogen Inc. for sequencing. The obtained sequences were analyzed using BioEdit software for alignment and subsequent comparison with public databases, such as NCBI GenBank, using the BLASTn algorithm. The fungal species were identified based on a $\geq 98\%$ similarity to deposited sequences in the database.

2.4. Ex Vivo Fungal Activity

After completing the macroscopic and microscopic characterization of the pathogens, the *ex vivo* antifungal activity was assessed. The pathogens were isolated and purified from banana peel samples and included *Trichoderma* spp., *Aspergillus* spp., *Penicillium* spp., and *Fusarium* spp., which had been stored for 7 to 10 days [44]. These fungi were selected from a total of 15 due to their relevance in postharvest diseases of bananas during storage

The growth index was calculated based on the development of these fungus on fruit, with 20 samples used for each fungus. The inoculum concentration was adjusted to 10^6 conidia/mL to maintain a constant infection [14]. Fungal growth diameter was measured weekly for up to 5 weeks post-inoculation, providing a comprehensive timeline of the pathogen's progression and activity. The severity of infection was classified based on the growth diameter of the fungal colonies, with all the purified pathogens considered in the analysis [43]. This classification provides valuable data on their interaction with the banana peel under controlled conditions [44].

2.5. In Vitro Antifungal Activity with Essential Oils

The antifungal efficacy of essential oils derived from six species: oregano (*Origanum vulgare*), rosemary (*Salvia rosmarinus*), clove (*Syzygium aromaticum*), thyme (*Thymus vulgaris*), cinnamon (*Cinnamomum verum*), and basil (*Ocimum basilicum*) was assessed *in vitro* using a standard laboratory procedure [44]. Essential oils were extracted through steam distillation, a commonly employed method for obtaining bioactive compounds from plants. Oregano oil was sourced from dried leaves, cinnamon oil from the dried bark, and clove oil from dried flower buds. For rosemary, basil, and thyme, essential oils were extracted from fresh leaves and flowers and all oils were purchased from commercial suppliers.

To prepare the solutions, each essential oil was diluted in a 0.05% Tween 80 solution to form a homogeneous emulsion, concentrations of 200, 400, 600, 800, and 1000 ppm [45]. These emulsions were added to cooled Potato Dextrose Agar (PDA) medium before solidification. The medium was subsequently inoculated with fungal pathogens and were incubated at 25°C to assess the inhibitory effects of the essential oils on fungal growth. The experiments were conducted in quadruplicate, and the inhibition percentage was evaluated every 48 hours to determine the most effective concentration of each oil.

A negative control was included, consisting of PDA medium containing 0.05% Tween 80 but no essential oil. This control allowed for the comparison of fungal growth in the absence of treatment, serving as a baseline to assess the antifungal activity of the essential oils [46]. The experimental design employed a 6 × 5 mixed-factor model, where the independent variables included the six types of essential oils and five concentration levels, and the dependent variable was the percentage of growth inhibition. Statistical analyses were performed to identify the concentration of each essential oil that effectively inhibited fungal growth.

The results of this study provide valuable insights into the potential use of plant-derived essential oils as natural anti-fungal agents. By evaluating a diverse range of essential oils at varying concentrations, the study aims to identify the most potent oils for future applications in biocontrol and agriculture.

3. Results

3.1. Morphological Identification

Table 1 shows the pure fungi isolated from banana peel rot on selective medium (PDA + Chloramphenicol) and stored in PDA at approximately 25°C, with macroscopic images of the front and reverse sides of *Trichoderma* spp., *Penicillium* spp., *Aspergillus* spp., and *Fusarium* spp.

Table 1. Macroscopy images of (a) *Trichoderma* spp, (b) *Penicillium* spp., (c) *Aspergillus* spp., (d) *Fusarium* spp., considering the appearance of the front side and (e) *Trichoderma* spp., (f) *Penicillium* spp., (g) *Aspergillus* spp. (h) *Fusarium* spp. of reverse side.

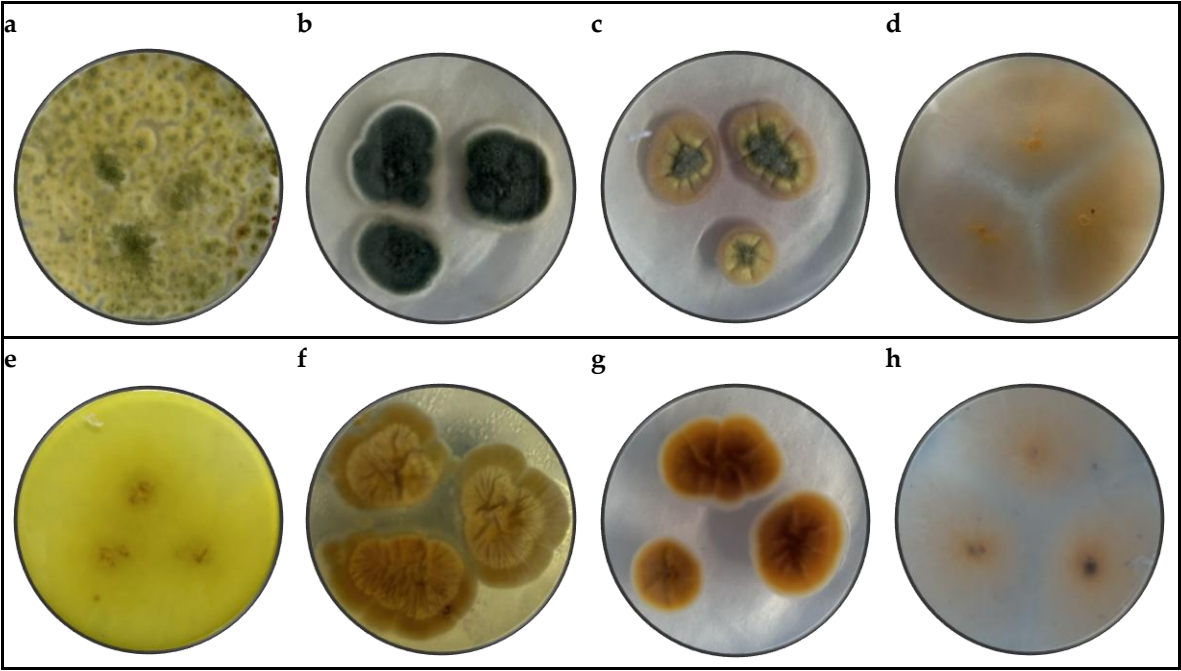
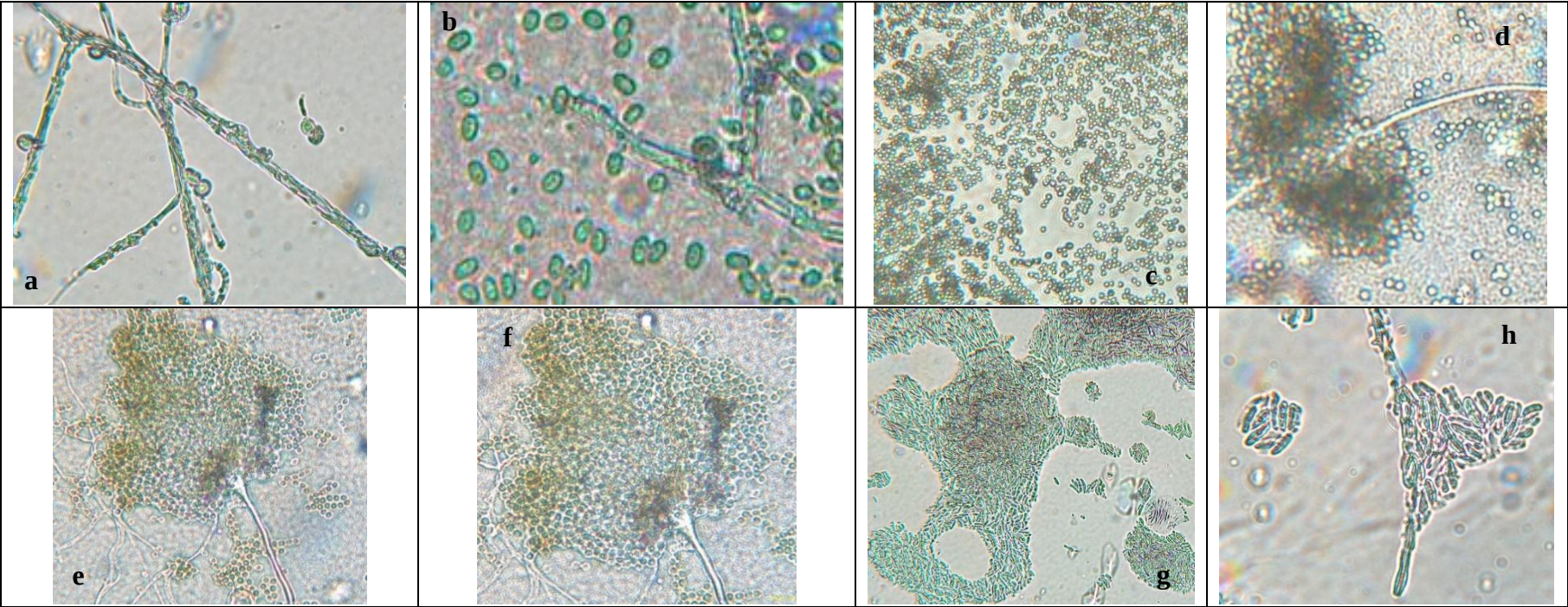


Table 2 presents the aerial mycelium of the fungi. These observations provide visual information that complements the morphological analysis and microscopic images observed under 40X and 60X magnifications.

Table 2. Microscopy images of (a) *Trichoderma* spp, (c) *Penicillium* spp., (e) *Aspergillus* spp., (g) *Fusarium* spp. to 40 X and (b) *Trichoderma* spp., (d) *Penicillium* spp., (f) *Aspergillus* spp. (h) *Fusarium* spp. to 60 X.



Trichoderma spp., including *T. asperellum*, *T. harzianum*, and *T. koningii*, are characterized by ellipsoidal conidia grouped together and thin, septate hyphae [47]. The colonies exhibit rapid growth and are typically green, with a circular shape, rough surface, regular edges, and slightly elevated contours [48]. The texture varies from fuzzy to cottony, reflecting their adaptability to different growth conditions.

Penicillium spp. displays septate hyphae of variable length, forming structures that resemble plumes. The colonies range in color from white to green or blue, with a velvety surface and well-defined edges. The characteristic brush-like arrangement of conidia is a defining feature of this genus, enabling clear morphological differentiation from other fungi [22].

Aspergillus spp. is distinguished by septate hyphae and conidiophores bearing long, ellipsoidal conidia arranged in characteristic plumose structures. The colonies exhibit a range of colors, typically yellow to green, with a rugged surface and a texture resembling tufts. Edges are regular or slightly wavy, and the colony elevation often shows a cratiform pattern [31].

Fusarium spp. is characterized by fusiform, cylindrical conidia arranged in chains or clusters, supported by septate hyphae. The colonies appear pink, red, or orange, with a cottony or velvety texture, diffuse edges, and a flat or slightly elevated surface. These macroscopic and microscopic features distinguish *Fusarium* spp. from other genera [21].

3.2. Molecular Identification Through DNA Sequencing

Table 3 presents a summary of the results from sequencing the ITS region of fungi isolated from banana peels. It lists the identified fungal organisms, the genetic fragments utilized for sequencing, and the percentage similarity derived from comparison with the database. The sequences exhibited a high degree of similarity ($\geq 98\%$), which supports the reliability of the fungal genera and species identification in the analyzed samples.

Table 3. Sequencing results for fungi isolated from the samples of *Musa paradisiaca*.

Organism	Fragment	% identity
<i>Fusarium longicornicola</i>	ITS	99.08 %
<i>Penicillium expansum</i>	ITS	99.76 %
<i>Trichoderma pseudokoningii</i>	ITS	99.18 %
<i>Aspergillus flavus</i>	ITS	99.33 %

3.3. Fungal Activity Ex Vivo

The severity of fungal infection in banana samples was assessed through *ex vivo* analysis, where 20 banana samples were monitored over a 6-week period. Fungal growth was evaluated for various species, including *Trichoderma* spp., *Penicillium* spp., *Aspergillus* spp., and *Fusarium* spp., with Figure 1 providing a visual representation of this growth over time.

The analysis utilized an ANOVA approach to examine the impact of different treatments and concentrations on fungal development. Statistically significant differences were observed between treatments ($p < 0.05$), suggesting that both the type of treatment and its concentration have a notable influence on fungal growth. These findings offer valuable insights into the dynamics of fungal infections in bananas, underlining the variation in growth rates and infection severity across different fungal species. The results highlight the importance of treatment strategies in controlling fungal growth and emphasize the differential susceptibility of banana samples to these pathogens during the study period.

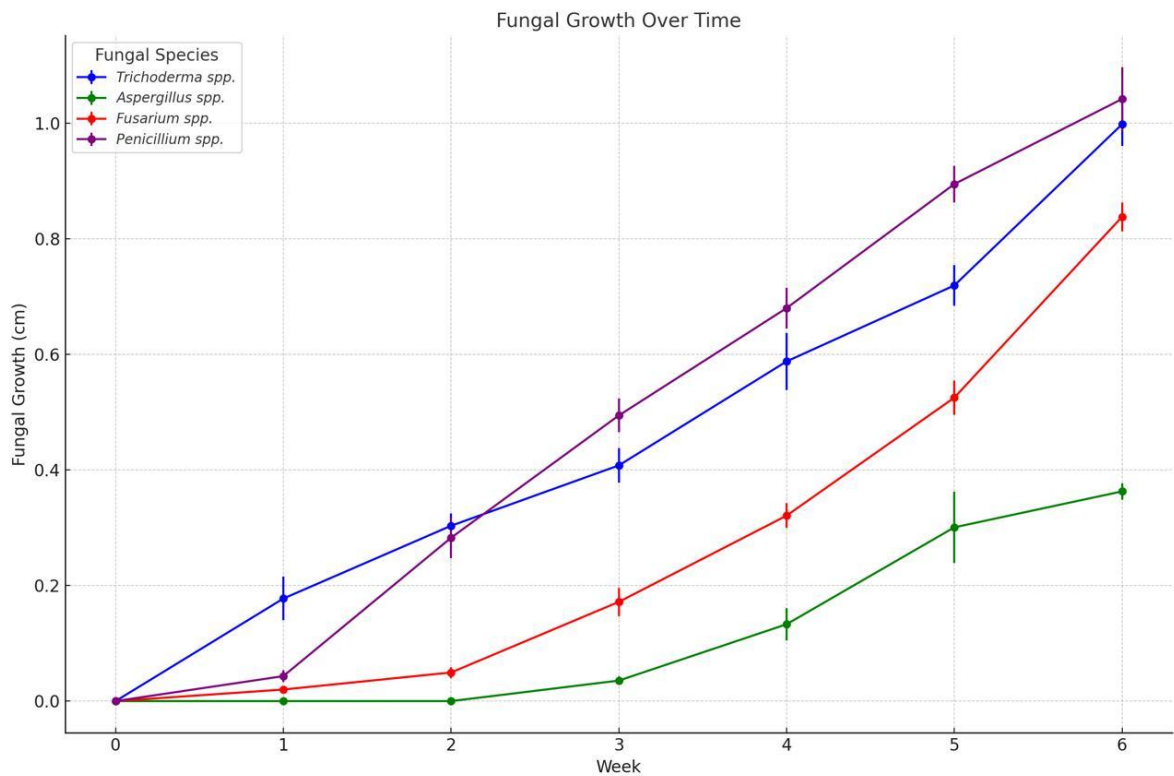


Figure 1. Fungal growth (cm) during 6 weeks in 20 banana samples inoculated with *Trichoderma* spp., *Penicillium* spp., *Aspergillus* spp., and *Fusarium* spp., stored at 13°C and 95% HR approximately.

3.4. In Vitro Antifungal Activity with Essential Oils

Figures 2–5 show the growths *in vitro* de *Trichoderma* spp., *Penicillium* spp., *Aspergillus* spp., in medium PDA with 200 ppm, 400 ppm, 600 ppm, 800 ppm and 1000 ppm of oregano, basil, cinnamon rosemary, thyme and clove.

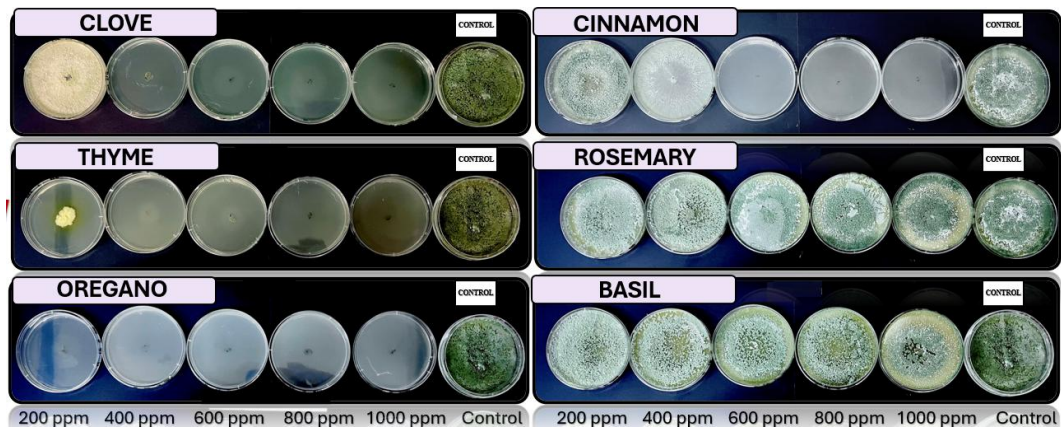


Figure 2. Analysis *in vitro* of *Trichoderma* spp. in PDA medium with essential oils of basil, cinnamon, clove, oregano, rosemary, and thyme 200, 400, 600, 800 y 1000 ppm, stored to 25°C (n=4).

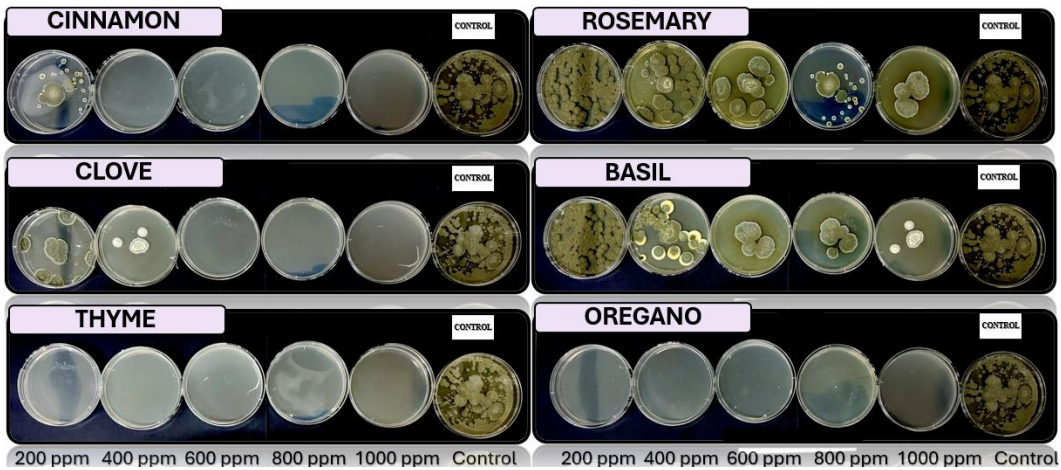


Figure 3. Analysis *in vitro* of *Penicillium* spp. in PDA medium with essential oils of basil, cinnamon, clove, oregano, rosemary, and thyme 200, 400, 600, 800 y 1000 ppm, stored to 25°C (n=4).

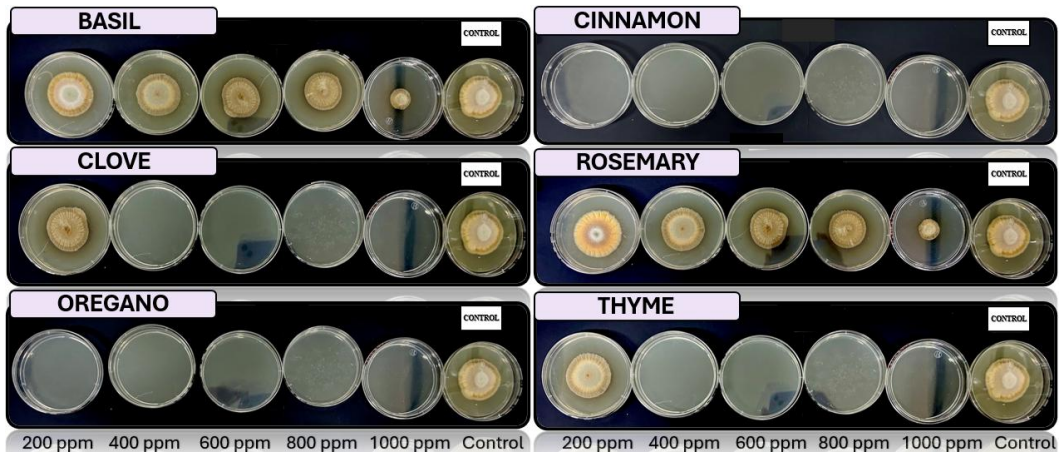


Figure 4. Analysis *in vitro* of *Aspergillus* spp. in PDA medium with essential oils of basil, cinnamon, clove, oregano, rosemary, and thyme 200, 400, 600, 800 y 1000 ppm, stored to 25°C (n=4).

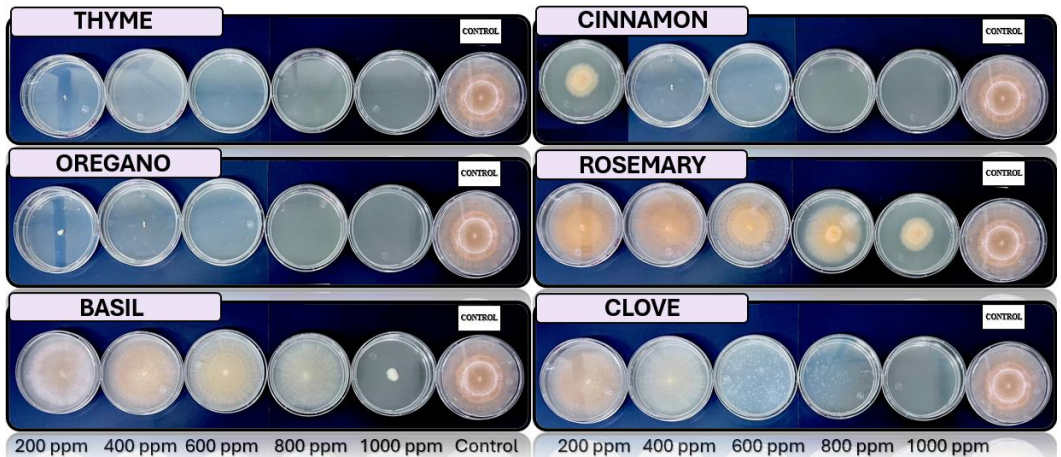


Figure 5. Analysis *in vitro* of *Fusarium* spp. in PDA medium with essential oils of basil, cinnamon, clove, oregano, rosemary, and thyme 200, 400, 600, 800 y 1000 ppm, stored to 25°C (n=4).

4. Discussion

4.1. Morphological Identification

In Tables 1 and 2, the results of the macroscopic and microscopic analysis are presented and detailed below.

Trichoderma spp. grows rapidly, with colonies that vary in color (white, green, yellow, or orange) and texture (cottony, velvety, or granular) depending on growth conditions. Microscopically, it produces branched conidiophores with small, oval or cylindrical conidia that form in chains or clusters. A characteristic odor may be present but is not a reliable feature [49]. *Fusarium* spp. has networks of filaments (hyphae), conidia (asexual and sexual spores or ascospores). In the microscope, the phialide is generally thin, with a bottle – shaped that can be simple or branched; short or long, they can have different characteristics depending on their species [50].

Aspergillus spp. is characterized by a prominent columella and highly organized plumose structures, where conidia emerge symmetrically. This distinct arrangement differentiates from *Penicillium* and *Fusarium*, which have simpler conidiophore structures. *Penicillium* spp. can cause stains and discoloration on the peel of the banana, affecting its appearance and quality, *expansum* can affect the bananas during storage, causing rotting and loss of firmness, while *digitatum* is more common in citrus fruits but can affect bananas if are stored in wet and warm storage conditions, causing rotting and stains on the peel [51]. To prevent the presence of this fungus should be handled properly during the storage and transport of the fruit.

The macroscopic and microscopic analysis of the fungal colonies, in combination with DNA sequencing, revealed distinct characteristics that facilitated accurate identification of the species. The following discussion outlines the macroscopic features observed for *Fusarium longicornicola*, *Penicillium expansum*, *Trichoderma pseudokoringii*, and *Aspergillus flavus*.

Fusarium longicornicola exhibited typical characteristics of the *Fusarium* genus, including pink to orange colonies with a cottony texture and regular edges. The colony surface was flat or slightly elevated, which is consistent with the morphological described [52]. The conidia, arranged in chains, were observable under the microscope. The macroscopic features, including the color and texture, provided reliable indicators for the identification of *Fusarium longicornicola* when compared to other species.

Penicillium expansum showed green colonies with white edges and a powdery, flat surface, which are characteristic of many *Penicillium* species, particularly those associated with decaying fruits and vegetables [53]. The white edge and powdery surface were key distinguishing features that aligned with the macroscopic description of *Penicillium expansum*. The appearance supports its identification, which may have similar macroscopic features but differ in detail such as colony color or edge definition.

Trichoderma pseudokoringii include green colonies with a rough, granular surface and slightly elevated, regular edges. These features, common in many *Trichoderma* species [49], were consistent with the macroscopic description of *T. pseudokoringii*. The growing of the colonies and the distinctive texture helped confirm its identification. The rough surface and elevated edges serve as distinguishing features when compared to other [27].

Aspergillus flavus exhibited yellow-green colonies with a rough surface and cratiform elevation, consistent with the known morphological traits of *A. flavus* [31,54]. The coloration and rough texture of the colonies made it easy to differentiate *A. flavus* from other *Aspergillus* species. The cratiform elevation, a distinct feature, further facilitated the identification. The yellow-green color and texture are particularly indicative of the species.

4.2. Molecular Identification Through DNA Sequencing

The DNA sequencing of fungi isolated from *Musa paradisiaca* samples provided highly reliable identification of the species through the ITS region, with all isolates showing a percentage identity

above 98% [43]. These results validate the initial morphological analysis and emphasize the precision of molecular techniques in fungal taxonomy.

Fusarium longicornicola was identified with a 99.08% identity in the ITS region, confirming its classification within the *Fusarium* genus. This high similarity aligns with the macroscopic features previously observed, such as the pink to orange colonies with cottony texture and regular edges. The combined findings provide strong evidence of its presence in the samples analyzed.

Penicillium expansum showed the highest percentage identity (99.76%) among the isolates, reflecting its genetic distinction. The molecular data corroborates its characteristic macroscopic traits, such as green colonies with white edges and a powdery, flat surface. The DNA sequencing and morphological analysis solidifies its identification and highlights its significance of *Musa paradisiaca* samples, association with fruit decay.

Trichoderma pseudokoningii exhibited a 99.18% identity, supporting its classification aligns with its observed rapid growth and green colonies with a granular texture. The genetic match enhances confidence in identification, particularly given the importance of *Trichoderma* species in biological control and agricultural systems.

Aspergillus flavus was identified with a 99.33% identity, confirming its classification. The sequencing results are consistent with their distinctive morphologies, such as yellow-green colonies with a rough surface and cratiform elevation. This high genetic identity underscores the reliability of ITS sequencing for distinguishing *A. flavus*, a species of economic and health significance due to its ability to produce aflatoxins.

The DNA sequencing results validate morphology observations and demonstrate the utility of the ITS region in accurately identifying fungal species. The high percentage identities across all isolates confirm the effectiveness of combining morphological and molecular approaches for the comprehensive study of fungal biodiversity in *Musa paradisiaca* samples.

4.3. Ex Vivo Fungal Activity

The inhibition rate was evaluated by measuring fungal growth from 20 inoculations of each fungus to assess the severity of the pathogens. The *ex vivo* growth of fungal species isolated from *Musa paradisiaca* was monitored over six weeks, as shown in Figure 1. The data demonstrates distinct growth patterns among the fungal species (*Trichoderma pseudokoningii*, *Aspergillus flavus*, *Fusarium longicornicola*, and *Penicillium expansum*), highlighting their varying rates of colonization and adaptation.

Trichoderma pseudokoningii exhibited the slowest growth among the species, with minimal increases in fungal colony size during the first three weeks. By week 6, the colony size reached approximately 0.4 cm. The initial slow growth may reflect the competitive nature of *Trichoderma* spp. as a biological control agent, requiring time to adapt and establish in the substrate [49]. However, its accelerated growth in later weeks suggests its ability to utilize the available nutrients efficiently once adapted.

Aspergillus flavus displayed moderate growth, showing a consistent increase in colony size throughout the six weeks and reaching approximately 0.8 cm by the end of the observation period. This growth pattern aligns with its documented ability to colonize plant materials, particularly in nutrient-rich environments such as stored grains and fruits [55,56]. The steady growth indicates *A. flavus*'s adaptability and efficiency in utilizing *Musa paradisiaca* as a substrate.

Fusarium longicornicola demonstrated a growth rate between that of *Trichoderma pseudokoningii* and *Aspergillus flavus*. By week 6, the colony size was approximately 0.6 cm. The intermediate growth rate is consistent with the ecological behavior of *Fusarium* spp., which thrives under specific conditions but does not dominate rapidly in environments with competing organisms [46,52].

Penicillium expansum showed the highest growth rate among the species, reaching over 1 cm by week 6. This rapid growth reflects its opportunistic nature and strong ability to colonize decaying organic matter, including fruits like *Musa paradisiaca* [23,53,57]. The powdery texture and efficiency in nutrient utilization are characteristic of *P. expansum*, enabling its dominance in the substrate.

4.4. In Vitro Antifungal Activity with Essential Oils

The *ex vivo* experiments with essential oils at concentrations (200, 400, 600, 800, and 1000 ppm) demonstrated distinct antifungal efficacy across fungal species (*Trichoderma pseudokoningii*, *Penicillium expansum*, *Aspergillus flavus*, and *Fusarium longicornicola*). The results emphasize the importance of essential oils as natural antifungal agents in agricultural and food preservation applications.

Trichoderma pseudokoningii displayed a response to cinnamon and clove oils, were most effective at higher concentrations (800–1000 ppm), showing significant inhibition of fungal growth, probably due to the phenolic compounds such as cinnamaldehyde and eugenol that disrupt fungal cell membranes [49]. Basil and rosemary oils exhibited moderate inhibition at all concentrations, while oregano and thyme oils showed minimal effects. These findings align with previous studies highlighting the potential of *Trichoderma pseudokoningii* as a biological control agent when combined with natural compounds [58].

The growth of *Penicillium expansum* was highly sensitive to cinnamon and clove oils, with complete inhibition observed at 800–1000 ppm. Basil and rosemary oils provided moderate inhibition across all concentrations, while thyme and oregano oils had limited effects. The pronounced sensitivity of *Penicillium expansum* to phenolic compounds suggests their potential use in controlling post-harvest diseases in fruits and vegetables [59].

Aspergillus flavus showed significant growth inhibition when treated with cinnamon and clove oils, particularly at higher concentrations (800–1000 ppm). Thyme and oregano oils provided moderate inhibition, while basil and rosemary oils were less effective. The susceptibility of *Aspergillus flavus* to these oils underscores their utility in controlling fungal contamination and reducing aflatoxin production [60].

Fusarium longicornicola demonstrated varied responses to essential oils. Cinnamon and clove oils showed moderate inhibition at concentrations above 600 ppm, while basil and rosemary oils exhibited consistent but limited effects across all concentrations. Oregano and thyme oils had minimal inhibitory activity. These results suggest that *Fusarium longicornicola* may require combined treatments, such as the integration of essential oils with chemical fungicides or other biological agents, to achieve effective control [61].

Cinnamon and clove oils exhibited the highest antifungal activity across all species, particularly at higher concentrations. Their effectiveness is attributed to the high phenolic content, which disrupts fungal cell structures and inhibits growth [62,63]. Basil and rosemary oils demonstrated moderate antifungal activity, suggesting their potential for combined use with other antifungal agents [64]. Thyme and oregano oils exhibited limited antifungal effects, particularly against *Fusarium longicornicola* and *Trichoderma pseudokoningii*, highlighting the need for higher concentrations or combinations with other treatments.

The Table 3 demonstrates that essential oils, particularly cinnamon and clove, have significant potential as eco-friendly antifungal agents. Further research should focus on optimizing their use in agricultural systems and evaluating their synergistic effects with other control methods to enhance efficacy.

Table 3. Evaluation of antifungal activity *in vitro* of *Trichoderma* spp., *Penicillium* spp., *Aspergillus* spp. and *Fusarium* spp. use essential oil of oregano, rosemary, clove, thyme, cinnamon and basil.

Essential oil	Fungus	Concentration [ppm]				
		200	400	600	800	1000
Cinnamon	<i>Trichoderma</i> spp.	+	+	-	-	-
	<i>Penicillium</i> spp.	+	-	-	-	-
	<i>Aspergillus</i> spp.	+	-	-	-	-
	<i>Fusarium</i> spp.	-	-	-	-	-
Clove	<i>Trichoderma</i> spp.	+	-	-	-	-
	<i>Penicillium</i> spp.	+	+	+	+	-
	<i>Aspergillus</i> spp.	+	-	-	-	-
	<i>Fusarium</i> spp.	+	+	-	-	-
Basil	<i>Trichoderma</i> spp.	+	+	+	+	+
	<i>Penicillium</i> spp.	+	+	+	+	+
	<i>Aspergillus</i> spp.	+	+	+	+	+
	<i>Fusarium</i> spp.	+	+	+	+	+
Oregano	<i>Trichoderma</i> spp.	-	-	-	-	-
	<i>Penicillium</i> spp.	+	-	-	-	-
	<i>Aspergillus</i> spp.	-	-	-	-	-
	<i>Fusarium</i> spp.	+	-	-	-	-
Rosemary	<i>Trichoderma</i> spp.	+	+	+	+	+
	<i>Penicillium</i> spp.	+	+	+	+	+
	<i>Aspergillus</i> spp.	+	+	+	+	+
	<i>Fusarium</i> spp.	+	+	+	+	+
Thyme	<i>Trichoderma</i> spp.	+	-	-	-	-
	<i>Penicillium</i> spp.	+	-	-	-	-
	<i>Aspergillus</i> spp.	+	-	-	-	-
	<i>Fusarium</i> spp.	-	-	-	-	-

5. Conclusions

The characterization of the fungus by order of severity identified *Penicillium expansum*, *Trichoderma pseudokoningii*, *Fusarium longicornicola*, and *Aspergillus flavus* as the fungus affecting *Musa paradisiaca* during postharvest.

In the *in vitro* analysis of essential oil efficacy, specifically, *Penicillium expansum* was controlled by 400 ppm of cinnamon, oregano, and thyme. *Trichoderma pseudokoningii* was inhibited by 200 ppm

of oregano, 400 ppm of clove and thyme, and 600 ppm of cinnamon. *Fusarium longicornicola* was most effectively managed with 200 ppm of cinnamon and thyme, and 400 ppm of oregano. *Aspergillus flavus* was controlled by 200 ppm of oregano, and 400 ppm of cinnamon, clove, and thyme.

These findings suggest that essential oils, especially cinnamon, clove, oregano, and thyme, are potent natural alternatives to synthetic fungicides. They offer an eco-friendly solution for managing fungal infections in bananas, promoting sustainability in agricultural practices.

Author Contributions: All the mentioned authors have significantly contributed to the development and writing of this article. All authors have read and accepted the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: Data will be made available upon reasonable request.

Acknowledgments: The authors thank the support of DECAB – Escuela Politécnica Nacional.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Galan V, Rangel A, Lopez J, Hernandez JBP, Sandoval J, Rocha HS. Propagación del banano: técnicas tradicionales, nuevas tecnologías e innovaciones. Rev Bras Frutic 2018;40. <https://doi.org/10.1590/0100-29452018574>.
2. Ruiz Medina MD, Ruales J. Postharvest Alternatives in Banana Cultivation. 2024. <https://doi.org/10.20944/preprints202402.0783.v1>.
3. Capa Benítez LB, Alaña Castillo TP, Benítez Narváez RM. Importancia de la producción de banano orgánico. Caso: Provincia de El Oro, Ecuador. Revista Universidad y Sociedad 2016;8:64–71.
4. Mata Anchundia D, Suatunce Cunuhay P, Poveda Morán R. Análisis económico del banano orgánico y convencional en la provincia Los Ríos, Ecuador. Avances 2021;23:419–30.
5. Aguilar-Ancota R, Arévalo-Quinde CG, Morales-Pizarro A, Galecio-Julca M, Aguilar-Ancota R, Arévalo-Quinde CG, et al. Hongos asociados a la necrosis de haces vasculares en el cultivo de banano orgánico: síntomas, aislamiento e identificación, y alternativas de manejo integrado. Scientia Agropecuaria 2021;12:249–56. <https://doi.org/10.17268/sci.agropecu.2021.028>.
6. Guzmán O. El nemátodo barrenador (Radopholus similis [COBB] Thorne) del banano y plátano 2011;1.
7. Smith JA, Johnson RH, Thompson L. Fungal pathogens of banana: Current understanding and management strategies. Journal of Tropical Agriculture 2018;52:1–10. <https://doi.org/10.1007/jta.2018.04023>.
8. Solis EM, Montoya CG. Fusarium wilt in bananas: A critical review of fungal pathogens and control methods. Tropical Plant Pathology 2017;42:124–35. <https://doi.org/10.1007/s40858-017-0146-9>.
9. Castellanos D, Algecira N, Villota C. Aspectos relevantes en el almacenamiento de banano en empaques con atmósferas modificadas. 2011 2011;12:114–34.
10. Garcia M, Pérez J, Valero M. Antifungal properties of essential oils in postharvest disease control of tropical fruits. International Journal of Food Science 2021;23:250–8. <https://doi.org/10.1016/j.ijfs.2020.11.021>.
11. Boulous M, Lacey M. Role of essential oils in food preservation: A review. Food Control 2020;111:203–12. <https://doi.org/10.1016/j.foodcont.2020.106946>.
12. Khan SH, Rehman S. Therapeutic and antifungal potentials of essential oils from plants. Natural Products Research 2020;34:1045–51. <https://doi.org/10.1080/14786419.2019.1685932>.
13. Zambonelli A, Guerrini L. Essential oils and their antifungal properties. Fungal Biology Reviews 2021;35:21–32. <https://doi.org/10.1016/j.fbr.2021.01.003>.
14. Mesa V a. M, Marín P, Ocampo O, Calle J, Monsalve Z, Mesa V a. M, et al. Fungicidas a partir de extractos vegetales: una alternativa en el manejo integrado de hongos fitopatógenos. RIA Revista de Investigaciones Agropecuarias 2019;45:23–30.
15. Barrera Necha LL, García Barrera LJ. Actividad antifúngica de aceites esenciales y sus compuestos sobre el crecimiento de Fusarium sp. aislado de papaya (Carica papaya). Revista Científica UDO Agrícola 2008;8:33–41.

16. López Luengo MT. El romero. Planta aromática con efectos antioxidantes. *Offarm* 2008;27:60–3.
17. Flores-Villa E, Sáenz-Galindo A, Castañeda-Facio AO, Narro-Céspedes RI, Flores-Villa E, Sáenz-Galindo A, et al. Romero (*Rosmarinus officinalis* L.): su origen, importancia y generalidades de sus metabolitos secundarios. *TIP Revista especializada en ciencias químico-biológicas* 2020;23. <https://doi.org/10.22201/fesz.23958723e.2020.0.266>.
18. Ruiz M, Ávila J, Ruales J. Diseño de un recubrimiento comestible bioactivo para aplicarlo en la frutilla (*Fragaria vesca*) como proceso de postcosecha 2016;17:276–87.
19. Jeri R, H C. Consideraciones epidemiológicas para el manejo de la marchitez por *Fusarium* (*Fusarium oxysporum* f. sp. cubense) del banano en la región central del Perú 2012.
20. Magdama F, Monserrate-Maggi L, Serrano L, García Onofre J, Jiménez-Gasco M del M. Genetic Diversity of *Fusarium oxysporum* f. sp. cubense, the *Fusarium* Wilt Pathogen of Banana, in Ecuador. *Plants* 2020;9:1133. <https://doi.org/10.3390/plants9091133>.
21. Tapia C, Amaro J. Género *Fusarium*. *Revista Chilena de Infectología* 2014;31:85–6. <https://doi.org/10.4067/S0716-10182014000100012>.
22. Srinivasan R, Prabhu G, Prasad M, Mishra M, Chaudhary M, Srivastava R. *Penicillium*. In: Amaresan N, Senthil Kumar M, Annapurna K, Kumar K, Sankaranarayanan A, editors. *Beneficial Microbes in Agro-Ecology*, Academic Press; 2020, p. 651–67. <https://doi.org/10.1016/B978-0-12-823414-3.00032-0>.
23. Velásquez MA, Álvarez RM, Tamayo PJ, Carvalho CP. Evaluación in vitro de la actividad fungistática del aceite esencial de mandarina sobre el crecimiento de *Penicillium* sp. *Ciencia y Tecnología Agropecuaria* 2014;15:7–14.
24. Timmermann LA, Lopez C, Becker B. Inhibitory effects of thyme and oregano oils on fungal spore formation. *International Journal of Fungal Biology* 2019;29:32–40. <https://doi.org/10.1016/j.fbr.2019.01.003>.
25. Schuster A, Schmoll M. Biology and biotechnology of *Trichoderma* | *Applied Microbiology and Biotechnology* 2010. <https://link.springer.com/article/10.1007/s00253-010-2632-1> (accessed August 4, 2024).
26. Brotman Y, Kapuganti JG, Viterbo A. *Trichoderma*. *Current Biology* 2010;20:R390–1. <https://doi.org/10.1016/j.cub.2010.02.042>.
27. Hernández-Melchor DJ, Ferrera-Cerrato R, Alarcón A, Hernández-Melchor DJ, Ferrera-Cerrato R, Alarcón A. *Trichoderma*: Importancia agrícola, biotecnológica y sistemas de fermentación para producir biomasa y enzimas de interés industrial. *Chilean Journal of Agricultural & Animal Sciences* 2019;35:98–112. <https://doi.org/10.4067/S0719-38902019005000205>.
28. Palou L. Control integrado no contaminante de enfermedades de poscosecha (CINCEP). Nuevo paradigma para el sector español de los cítricos. *Levante Agrícola* 2011;173–83.
29. Chang PK, Horn BW, Abe K, Gomi K. *Aspergillus*: Introduction. *Encyclopedia of Food Microbiology*: Second Edition, Elsevier Inc.; 2014, p. 77–82. <https://doi.org/10.1016/B978-0-12-384730-0.00010-0>.
30. Pinto E, Silva C, Costa L. Eugenol as an antifungal agent: Mechanisms and applications. *Journal of Applied Microbiology* 2018;124:1089–99. <https://doi.org/10.1111/jam.13748>.
31. Rokas A. *Aspergillus*. *Current Biology* 2013;23:R187–8. <https://doi.org/10.1016/j.cub.2013.01.021>.
32. Burt S. Essential oils: Their antibacterial properties and potential applications in foods—A review. *International Journal of Food Microbiology* 2004;94:223–53. <https://doi.org/10.1016/j.ijfoodmicro.2004.03.022>.
33. Suppakul P, Miltz J, Sonneveld K, Bigger SW. Antimicrobial properties of basil and its possible application in food packaging. *Journal of Agricultural and Food Chemistry* 2003;51:3197–207. <https://doi.org/10.1021/jf021038t>.
34. Vargas-Fernández JP, Wang-Wong A, Muñoz-Fonseca M, Vargas-Fernández JP, Wang-Wong A, Muñoz-Fonseca M. Microorganismos asociados a la enfermedad conocida como pudrición suave del fruto de banano (*Musa* sp.) y alternativas de control microbiológicas y químicas a nivel in vitro *. *Agronomía Costarricense* 2022;46:61–76. <https://doi.org/10.15517/rac.v46i2.52046>.
35. Suárez L, Rangel A. Aislamiento de microorganismos para control biológico de *Moniliophthora roreri* 2013;62.

36. Salazar E, Hernández R, Tapia A, Gómez-Alpizar L. Identificación molecular del hongo *Colletotrichum* spp., aislado de banano (*Musa* spp) de la altura en la zona de Turrialba y determinación de su sensibilidad a fungicidas poscosecha. *Agronomía Costarricense* 2012;36:53–68.
37. Morales R, Henríquez G. Aislamiento e identificación del moho causante de antracnosis en *musa paradisiaca* l. (plátano) en cooperativa san carlos, el salvador y aislamiento de mohos y levaduras con capacidad antagonista. *Crea Ciencia Revista Científica* 2021;13:84–94. <https://doi.org/10.5377/creaciencia.v13i2.11825>.
38. Toure N, Yaye T. Antifungal efficacy of plant-derived essential oils in controlling post-harvest banana disease. *Tropical Fruit Research* 2023;18:56–64. <https://doi.org/10.1007/s51813-023-1067-2>.
39. Tortora GJ, Funke BR, Case CL. Introducción a la microbiología. Ed. Médica Panamericana; 2007.
40. Vanegas J Santamaría, González N Comba, Mancilla XC Pérez. Manual de Microbiología General: Principios Básicos de Laboratorio. Editorial Tadeo Lozano; 2014.
41. Suárez Contreras LY. Identificación molecular de aislamientos de *Moniliophthora roreri* en huertos de cacao de Norte de Santander, Colombia. *Acta Agronómica* 2016;65:51–7. <https://doi.org/10.15446/acag.v65n1.47994>.
42. Aguilar Armijos JS. Identificación del hongo fitopatógeno *Phoma* spp. aislado a partir de plantas de uvilla (*Physalis peruviana* L.) en localidades de zona norte y centro-norte de la serranía ecuatoriana 2020.
43. Castro J, Guzmán M. Identificación molecular de hongos asociados a frutos de banano en Costa Rica mediante la región ITS y análisis de secuencias. *Revista de Biología Tropical* 2012;60:45–58.
44. Aguilar-Ancota R, Apaza-Apaza S, Maldonado E, Calle-Cheje Y, Rafael-Rutte R, Montalvo K, et al. Control in vitro e in vivo de *Thielaviopsis paradoxa* y *Collettrichum musae* cn biofungicidas en frutos de banano orgánico. *Manglar* 2024;21:57–63. <https://doi.org/10.57188/manglar.2024.006>.
45. Ruiz-Medina M, Ruales J. Essential Oils as an Antifungal Alternative to Control *Fusarium* spp., *Penicillium* spp., *Trichoderma* spp. and *Aspergillus* spp. 2024. <https://doi.org/10.20944/preprints202408.1987.v1>.
46. Aquino-Martínez JG, Vázquez-García LM, Reyes-Reyes BG. Biocontrol in vitro e in vivo de *Fusarium oxysporum* Schlecht. f. sp. *dianthi* (Prill. y Delacr.) Snyder y Hans. con Hongos Antagonistas Nativos de la Zona Florícola de Villa Guerrero, Estado de México. *Revista mexicana de fitopatología* 2008;26:127–37.
47. Savín-Molina J, Hernández-Montiel LG, Ceiro-Catasú W, Ávila-Quezada GD, Palacios-Espinosa A, Ruiz-Espinoza FH, et al. Caracterización morfológica y potencial de biocontrol de especies de *Trichoderma* aisladas de suelos del semiárido. *Revista mexicana de fitopatología* 2021;39:435–51. <https://doi.org/10.18781/r.mex.fit.2106-7>.
48. Cayotopa-Torres J, Arevalo L, Pichis-García R, Olivera-Cayotopa D, Rimachi-Valle M, Kadir KJMD. New cadmium bioremediation agents: *Trichoderma* species native to the rhizosphere of cacao trees. *Scientia Agropecuaria* 2021;24:155–60. <https://doi.org/10.17268/sci.agropecu.2021.017>.
49. Harman GE, Kubicek CP. *Trichoderma* And *Gliocladium*: Basic Biology, Taxonomy and Genetics. Vienna, Austria: CRC Press; 2002.
50. Ortiz E, Riascos D, Angarita M, Castro O, Rivera C, Romero D, et al. Tópicos taxonómicos para el estudio del género *Fusarium* 2020;33:61–6.
51. Smith J, Henderson R. *Mycotoxins and Animal Foods*. 1er ed. Glasgow, Scotland: CRC Press; 1991.
52. Leslie JF, Summerell BA. *The Fusarium Laboratory Manual*. Ames, Iowa: Blackwell Publishing; 2006.
53. Samson RA, others. *Common Aspergillus species*. Springer; 2011. https://doi.org/10.1007/978-94-007-0215-4_7.
54. Pitt JI, Hocking AD. *Fungi and Food Spoilage*. Springer; 2009. <https://doi.org/10.1007/978-1-4419-0407-0>.
55. Pitt JI. Mycotoxins: Fumonisin. In: Motarjemi Y, editor. *Encyclopedia of Food Safety*, Waltham: Academic Press; 2014, p. 299–303. <https://doi.org/10.1016/B978-0-12-378612-8.00192-X>.
56. Mendez RM, others. *Aspergillus flavus*: Aflatoxigenic species of importance. *Food Control* 2011;22:1472–84. <https://doi.org/10.1016/j.foodcont.2011.03.004>.
57. Abadías M, Teixidó N, Usall J, Viñas I. Evaluation of alternative strategies to control postharvest blue mould of apple caused by *Penicillium expansum*. *International Journal of Food Microbiology* 2008;122:25–31. <https://doi.org/10.1016/j.ijfoodmicro.2007.11.048>.

58. Acurio Váscone RD, España Imbaquingo CK, Acurio Váscone RD, España Imbaquingo CK. Aislamiento, caracterización y evaluación de *Trichoderma* spp. como promotor de crecimiento vegetal en pasturas de Raygrass (*Lolium perenne*) y trébol blanco (*Trifolium repens*). LA GRANJA Revista de Ciencias de la Vida 2017;25:53–61. <https://doi.org/10.17163/lgr.n25.2017.05>.
59. Boulanger R, Liu Y, Jiang Z. Synergistic effects of essential oils against fungal pathogens: Mechanisms and applications. Food Control 2021;123:107–18. <https://doi.org/10.1016/j.foodcont.2021.107118>.
60. Choi YH, Kim H, Lee S. The antifungal activity of essential oils: Mechanisms and applications in plant disease management. Phytopathology Research 2018;44:89–102. <https://doi.org/10.1186/s42055-018-0032-2>.
61. Zhao S, Zhang Z, Wei Y. Mechanisms of antifungal action of cinnamaldehyde. Phytochemistry 2020;173:112112. <https://doi.org/10.1016/j.phytochem.2020.112112>.
62. Farias APP, Monteiro O dos S, da Silva JKR, Figueiredo PLB, Rodrigues AAC, Monteiro IN, et al. Chemical composition and biological activities of two chemotype-oils from *Cinnamomum verum* J. Presl growing in North Brazil. J Food Sci Technol 2020;57:3176–83. <https://doi.org/10.1007/s13197-020-04288-7>.
63. Sadiq MB, Hameed S, Tufail S. Role of essential oils in the inhibition of fungal pathogens. Microbial Pathogenesis 2021;160:105113. <https://doi.org/10.1016/j.micpath.2021.105113>.
64. Cavalcante HA, Filho AC, Rocha F. Essential oils as alternative antifungal agents. Mycology 2019;60:358–66. <https://doi.org/10.1080/21501203.2019.1607866>.

Disclaimer/Publisher's Note: The statements, opinions, and data contained in all publications are solely those of the individual authors and contributors and not of MDPI or the editor. MDPI and the editor disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions, or products referred to in the content.