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Potential of Phylloplane Fungi from Mangrove Plant (*Rhizophora apiculata* Blume) as Biological Control Agents against *Fusarium oxysporum* f. sp. *cubense* in Banana Plant (*Musa acuminata* L.)

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Abstract: Phylloplane fungi is a non-pathogenic fungi on the leaf surface that can be used to control plant diseases caused by pathogens. One of the most damaging banana plant diseases is fusarium wilt, caused by the pathogenic fungi *Fusarium oxysporum* f. sp. *Cubense* (*Foc*). Mangrove plant *Rhizophora apiculate* is widely distributed and is a high-diversity area where microorganisms that produce anti-microbial compounds flourish. This plant can be used as a biological agent. This study aims to determine the various phylloplane fungi available from mangrove plant *R. apiculata* leaves and their potential use against *Fusarium oxysporum* f. sp. *cubense* (*Foc*) in banana plants (*Musa acuminata* L.). All 20 phylloplane fungi were identified through DNA sequencing with identities of 83.88–100%; of those 20, 3 were found that have antagonistic potential against *Fusarium oxysporum* f. sp. *cubense* (*Foc*): *Lasiodiplodia theobromae* (67.43%), *Trichoderma harzianum* (66.65), and *Nigrospora sphaerica* (65.33%). In the in vivo tests, the best inhibition of disease incidence was shown by treatment with *Lasiodiplodia theobromae* (11.11%). The present study confirmed that phylloplane fungi isolated from *R. apiculata* can inhibit fusarium wilt disease in banana plant.

Keywords: phylloplane; *Rhizophora paiculata* leaf; *Fusarium oxysporum* f. sp. *cubense*; banana

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1. Introduction

Mangroves are an ecosystem with high biodiversity and within this ecosystem are microbes that have the biological potential to be applied to plants, especially as biological control agents, many types of fungi associated with plant are known to protect host plant from pathogen [1]. Mangrove ecosystems play many important roles, such as offering protection from storms, blocking strong winds, controlling erosion, and functioning as a breeding ground for several marine animals. Along with these physical functions, mangroves also function as a source of medicines because they are capable of producing various bioactive compounds, such as alkaloids, phenols, steroids, terpenoids, and tannins. They are also widely used as biopesticides because they have antimicrobial activity [2]. Extracts from the leaves and roots of mangrove have been reported to have anti-fungal activity [3]. Antimicrobial compounds produced in plant parts can also be produced by non-pathogenic microbes that live in plant areas and are useful in inhibiting pathogenic microbes and inducing SAR (*Systemic Acquired Resistance*) induced defenses in plant cells [4]. Phylloplane is the leaf surface area which is an ideal area for the development of antagonistic microorganisms

in regulating their life as well as being competitor against the development of pathogen by produce toxin [1].

Phylloplane fungi have been widely used in biological disease control [5]. Although biological control has advantages in terms of farmers' economy and the level of environmental damage [6] to plant tissues, the leaf surface is an ideal part of the plant because the microflora can survive easily given the available nutrients and the suitable temperature, humidity, and pH that leaves exude [4].

Rhizophora sp. is a mangrove species that is widely distributed and can be found in coastal areas in both tropical and subtropical regions, including in North Sumatra, Indonesia [7]. A review study by Nayak and Anandhu [8] found many phylloplane and endophyte fungi from *Rhizophora* sp. including *Aspergillus niger*, *Cochilobolus victoria*, *Colletotrichum* sp., *Curvularia lunata*, *Drechslera* sp., *Glomerella* sp. *Fusarium oxysporum*, *Penicillium chrysogenum*, *P. oxalicum*, *Phoma* sp., and *Sordaria* sp. In the results of their study, Hamzah et al. (2018) [7] stated that endophyte fungi from mangroves showed that *Phoma* sp. had an inhibition of 69.64% in the antagonist test for inhibiting the growth of the pathogenic fungi *Fusarium solani*.

Regarding the pathogenic fungi *Fusarium* sp., Fusarium wilt disease is one of the most recognized diseases in banana cultivation caused by the fungus *Fusarium oxysporum* f. sp. *cubense*. *Fusarium oxysforum* f. sp. *cubense* is considered to be the cause of fusarium wilt disease in banana plants, which is the most destructive disease for the plants. Banana plants wilt and die due to *Fusarium oxysporum* f. sp. *cubense* (*Foc*) on the plant's xylem [9]. The study by Goswami et al. (2021) [4] suggested that the banana plant is one of the most important crops in the world and has been planted in 120 tropical and subtropical countries.

Meanwhile, the potential of phylloplane to inhibit the growth of the antagonistic fungi *Fusarium* sp. is rarely reported. However, some phylloplane fungi from mangrove plants have the potential to be used against *Foc*. Therefore, the present study aimed to identify and test the potential phylloplane fungi *R. apiculata* from the mangrove plant as a biocontrol agent for fusarium wilt disease in banana plants caused by *Foc*.

2. Materials and Methods

2.1. Sampling

Samples were taken from three leaves (young, medium, and old leaves) and chosen randomly from three sites of the mangrove plant (*Rhizophora apiculata*) in August 2021 from two locations: Lubuk Kertang, Langkat Regency, North Sumatra, with coordinates (Site 1: 4°4′30.072 98°17′5.418″), (Site 2: 4°3′53.421″ 98°14′21.276″), and (Site 3: 4°3′35.196″ 98°16′0.882″); and Percut Sei Tuan, Deli Serdang Regency, North Sumatra with coordinates (Site 1: 3°43′38.106″ 98°46′16.068″), (Site 2: 3°44′14.634″ 98°46′12.858″), and (Site 3: 3°43′55″ 98°46′25″). The three sampling locations were used to conduct repeat sampling from the different sites in the surrounding area. Pathogenic fungi *Fusarium oxysporum* f. sp. *cubense* (*Foc*) were collected from one of the symptomatic plants in the village of STM Hulu, Deli Serdang Regency, with coordinates 3°12′15.706″ 98°43′13.020″ (see Figure 1).

2.2. Isolation of Phylloplane Fungal and Pathogenic Fungi

Isolation of the modified phylloplane fungi is conducted by cutting 1–2 cm of mangrove leaves and removing the remnants of the soil using sterile distilled water. The leaves are dried using filter paper and then the upper and lower surfaces of the leaves are glued to the PDA (potato dextrose agar). The leaves are then incubated at room temperature until fungal colonies grow, and each type of fungi that grows is purified on new PDA (*Potato Dextrose Agar*) medium [10]. The method used by Wibowo et al. [11] is conducted to isolate the *Foc*. The technique was modified by cutting parts of plant tissue that grew on the PDA medium and showed necrotic symptoms, then incubating them at room temperature for seven days. The resulting fungal colonies were observed, and those showing morphological characteristics of *Foc* were purified into new PDA medium.

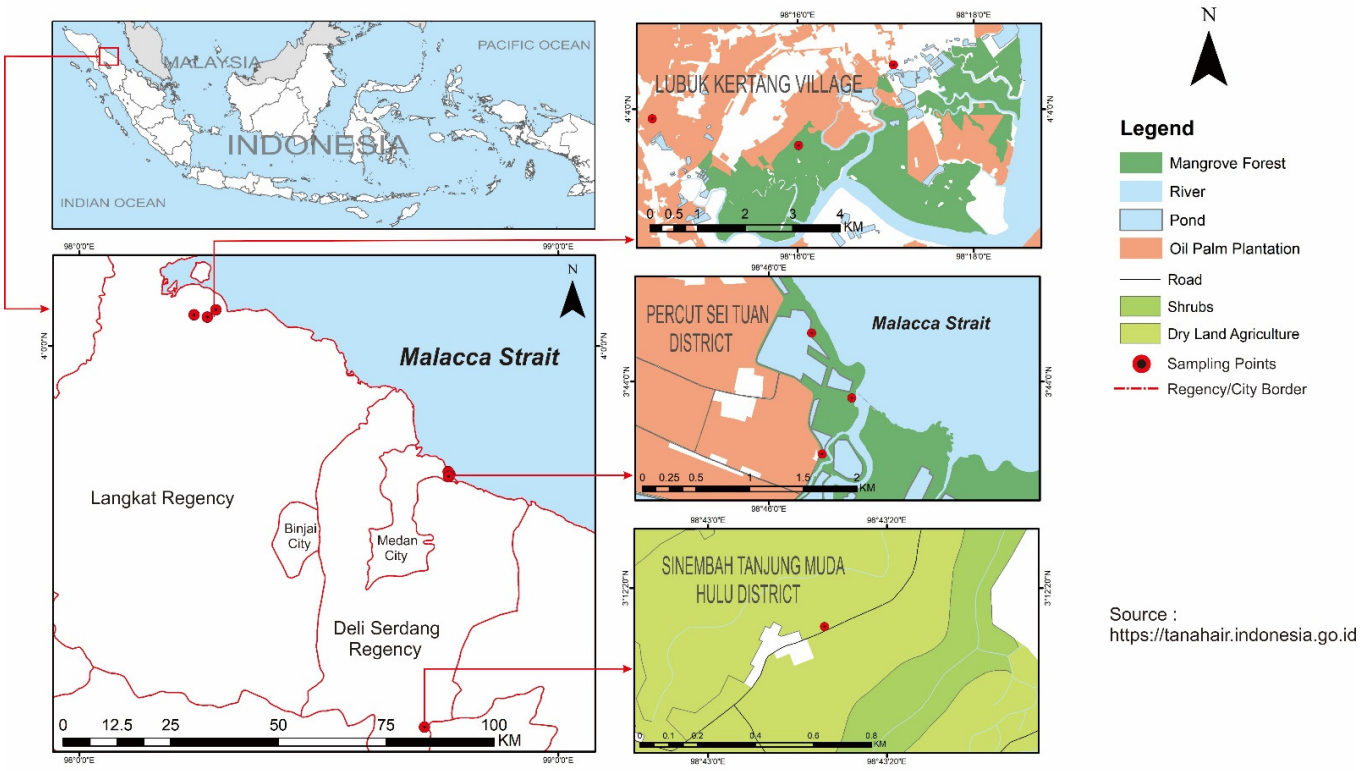

Figure 1. Sampling Location at Lubuk Kertang village, Percut Sei Tuan, and Sinembah Tanjung Muda, North Sumatra, Indonesia.

2.3. Postulate Koch Test

The postulate Koch test was carried out with the aim of seeing if the pathogenic fungi that were isolated from the field is a pathogen which, when tested on cultivated plants, causes the fusarium wilt symptoms in banana plants which would be used in this study. The test was conducted by inoculating the planting media or the two months old banana plant parts with the fungi *Foc* and observing the symptoms. The symptoms that appeared were required to be the same as those of plants infected by the pathogenic fungi *Foc*, where the pathogen had previously been isolated. After that, the symptomatic banana plants were isolated again.

2.4. DNA-Based Identification

Twenty isolates of phylloplane fungi that derived from two sampled locations, then identified by the molecular method, of which the first step is the isolation of the fungi's DNA. The extraction method used was the CTAB method from Sari (2019) [12]. PCR (*polymerase chain reaction*) amplification using universal primer ITS 1 (5′-TCC GTA GGT GAA CCT TGC GG-3′) and primer ITS4 (5′-TCC TCC GCT TAT TGA TAT GC-3′) [13] on PCR machine SensoQuest Labczler 48 was conducted, and the DNA template 3 µL plus 2.5 µL GoTaq Master, ddH_2O 3.5 µL, and primers ITS 1 and ITS 4 each 1 µL were used. The process includes several stages, with 1 repetition of pre-denaturation at 95 °C for 15 min, denaturation at 95 °C for 1 min, DNA attachment (annealing) at 56 °C for 30 s, and lengthening at 72 °C for 1 min. PCR products were run on 1% agarose gel to check for amplified products. The results of PCR products were analyzed in order to sequence the DNA samples. First, the results were aligned using the *software* UGENE ver 43.0. Then, the data were matched on the *National Center for Biotechnology Information* (NCBI) (http://www.ncbi.nlm.nih.gov accessed on 28 October 2022) to determine the similarity of nitrogenous bases with species already available in the gene bank found on the *Basic Local Alignment Search Tool Nucleotide* (BLASTN) website (http://blast.ncbi.nlm.nih.gov/blast.cgi

accessed on 30 October 2022). The results were then used to draw a phylogenetic tree analysis to determine the genetic distance between species using the *software* MEGA ver 5.2.

2.5. In Vitro Assays Antagonist Test

Tests of phylloplane fungi isolated from mangrove leaves against *Foc* were carried out macroscopically using the *dual culture* on PDA medium. Phylloplane fungi that were seven days old were cut with 4 mm diameter holes and then grown on PDA medium at a distance of 3 cm from the edge of a petri dish with a diameter of 9 cm. The *Foc* isolate was grown the same way as the phylloplane fungi isolates. *Foc* was also grown in petri dishes without biological agents to be used as a control. The process was repeated three times. Observations were made for seven days to calculate the inhibition based on the percentage of inhibition (P) according to the formula:

$$P = \frac{(r1 - r2)}{r1} \times 100\% \quad (1)$$

P = percentage of fungal inhibition against *foc*.

r1 = radius of the fungal pathogenic fungi *foc* that grows in the opposite direction to the antagonist fungus.

r2 = radius of the pathogenic fungus colony growing towards the antagonist fungus. The mechanism of inhibition was observed during the antagonist test [6].

The selection criteria were carried out on the proportion of inhibition where a value of >60% was determined as a selected isolate because an antagonistic fungus is categorized as potentially having inhibitory activity if the inhibition proportion exceeds 60% [14].

The interaction between the pathogenic fungi and the antagonist fungi was then observed using several types as follows: Type A: the two fungi grow together without any signs of interaction, Type B: there is an inhibition zone of <2 mm, Type C: one of the fungi inhibits, and the one that inhibits growth is not very influential, or the fungi is not affected but the growth of the inhibited species is disturbed significantly, Type D: there is an inhibition zone > 2 mm, Type E: inhibition occurs but the inhibition of growth is less significant/reduced, Type F: inhibition occurs, but the inhibiting species continues to grow toward or above the inhibited species [15].

2.6. Greenhouse Test of Phylloplane Fungal vs. Foc

Of the 20 isolates of phylloplane fungi, the three with the highest antagonistic activity against *Foc* in dual culture assays were used for greenhouse testing with Barangan Merah banana seedlings aged 2 months; first, the plant tissue was acclimatized in a greenhouse using a non-factorial completely randomized design (CRD) with 5 treatments: K^+ = without phylloplane fungi, K^- = untreated, A1 = *Trichoderma harzianum*, A2 = *Nigrospora sphaerica*, and A3 = *Lasiodiplodia theobromae*. Each treatment consisted of 12 plants and was repeated 4 times so that the total experimental unit was 5 treatments × 4 replicates × 12 plants = 240 plants. The number of samples that were randomly determined to be observed was 9 plants per treatment, so the number of sample plants was 5 treatments × 4 replicates × 9 sample plants = 180 sample plants. Observations were made for 8 weeks after inoculation with pathogenic fungi and biological agents.

Three phylloplane fungi were applied to banana plants with conidia density of 10^7/mL, which was calculated using a hemocytometer. Then, as much as 30 mL/plant of the phylloplane fungi and pathogenic fungi were applied. The plants were observed for disease incidence and severity from 1 week to 8 weeks after inoculation.

Disease incidence is calculated a week after *Foc* for two months. This method is based on [16], which has been modified using the formula:

$$P = \left(\frac{T1}{T2}\right) \times 100\% \quad (2)$$

where:

P = percentage of affected plants.

T1 = number of plants affected by each treatment.

T2 = number of plants observed.

The disease severity index was calculated on the leaves and tubers of banana plants. Leaf observations were observed every week up to eight weeks after inoculation using the following scale:

0 = no symptoms in plants (healthy plants); 1 = early yellowing of lower leaves; 2 = lower leaves turn yellow completely and younger leaves change color; 3 = all leaves turn yellow; 4 = plant dies [17].

The stems of plants were observed when the plants showed > 50% of symptoms on leaf observations on a scale of 5. If the plants did not show symptoms on the leaves, observations were made two months after inoculation by cutting the banana plant stems horizontally. Observations were made using the following scale: 0 = fully clean tubers; 1 = there is a color change in the form of spots on the plant tissue; 2 = black spots appear on 1/3 of plant tissue; 3 = black spots appear on 1/3–2/3 of plant tissue; 4 = black spots appear on >2/3 of plant tissue; 5 = black spots almost cover the plant tissue [17]. The value disease severity leaf and rhizome can be categorized into various types of resistance (see Table 1)

Table 1. Value of disease severity based on Emilda et al. (2020) [16].

Percentage of Disease %	Severity Index		
	Leaf	Rhizome	Resistance Category
0	1	1	Very resistant
20	1–2	1–2	Resistant
20–50	2–3	2–3	Moderate resistance
50–75	3–4	3–5	Susceptible
>75	>4	>5	Very susceptible

Disease on banana leaves and stems is then calculated using the following formula:

$$KP = \frac{\Sigma\,(n \times v)}{Z \times N} \times 100\% \quad (3)$$

Description:

KP: disease severity (%).

n: number of infected plants per attack category.

v: attack category score.

Z: highest score from the attack category.

N: number of plants observed.

2.7. Data Analysis

Data was analyzed statically using analysis of variance (ANOVA) and Tukey's HSD post-hoc test using IBM SPSS Statistics ver.26 software.

3. Results

3.1. Fungal Phylloplane from Mangrove

A total of 20 fungi were isolated, consisting of nine families collected from leaves in two location mangrove areas, as sources of fungal phylloplane. All isolates identified by the molecular method and DNA sequences compared with NCBI (National Center for Biotechnology Information) through BLAST showed 83.88–100% similarity to a known sequence. A total of 18 isolates were matched and 2 isolates were not match with the data in the gene bank. The fungi included *Trichoderma harzianum* strain

QTYC64, *Trichoderma harzianum* strain WJF52, *Nigrospora sphaerica, Lasiodiplodia theobromae*, *Scopulariopsis brevicaulis*, *Curvularia* clavata, *Fusarium solani*, *Fusarium incarnatum*, *Fusarium oxysporum*, *Neopestaliopsis clavispora, Nigrospora* sp., *Nigrospora* sp. JS24, *Nigrospora oryzae*, *Pestaliopsis microspora, Pestaliopsis* sp., *Aspergillus versicolor, Mycoterium* sp., *Penicillium oxalicum*, and two unidentified species (Figure 2). The experiment resulted in a reconstructed phylogenetic tree with bootstrap lengths ranging from 51 to 100, as shown in (see Figure 3).

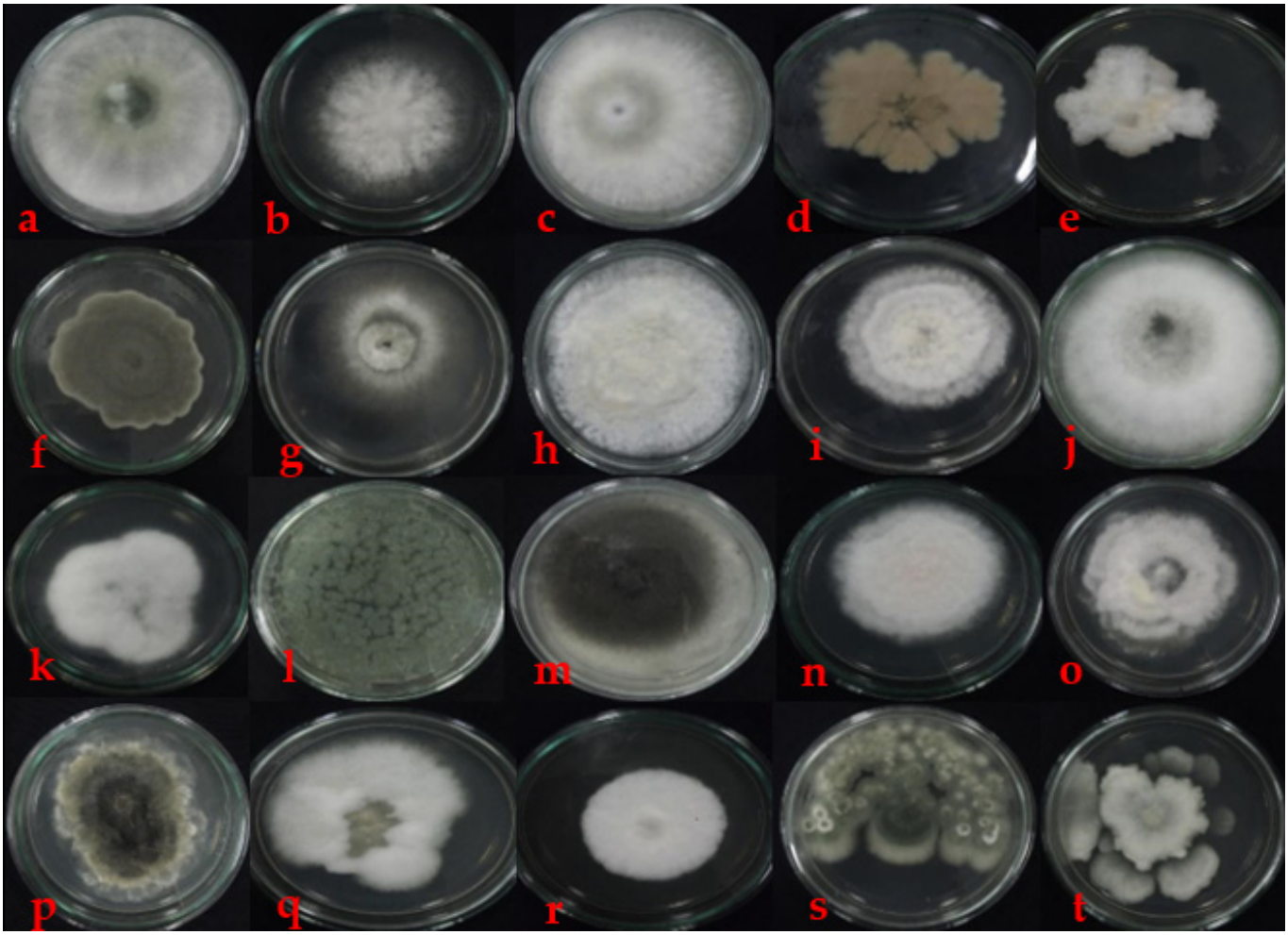

Figure 2. Colonies of Phylloplane Fungi (**a**) *Trichoderma harzianum* strain QTYC64; (**b**) *Nigrospora sphaerica*; (**c**) *Lasiodiplodia theobromae*; (**d**) *Scopulariopsis brevicaulis*; (**e**) Unidentified; (**f**) *Curvularia clavata*; (**g**) *Fusarium solani*; (**h**) Unidentified; (**i**) *Neopestalotiopsis clavispora*; (**j**) *Nigrospora* sp.; (**k**) *Fusarium oxysporum*; (**l**) *Aspergillus versicolor*; (**m**) *Nigrospora singularis*; (**n**) *Nigrospora* sp. JS24; (**o**) *Pestalotiopsis microspora*; (**p**) Pestalotiopsis sp.; (**q**) Fusarium incarnatum; (**r**) Myrothecium sp.; (**s**) *Penicillium oxalicum*; (**t**) *Trichoderma harzianum* strain WJF52.

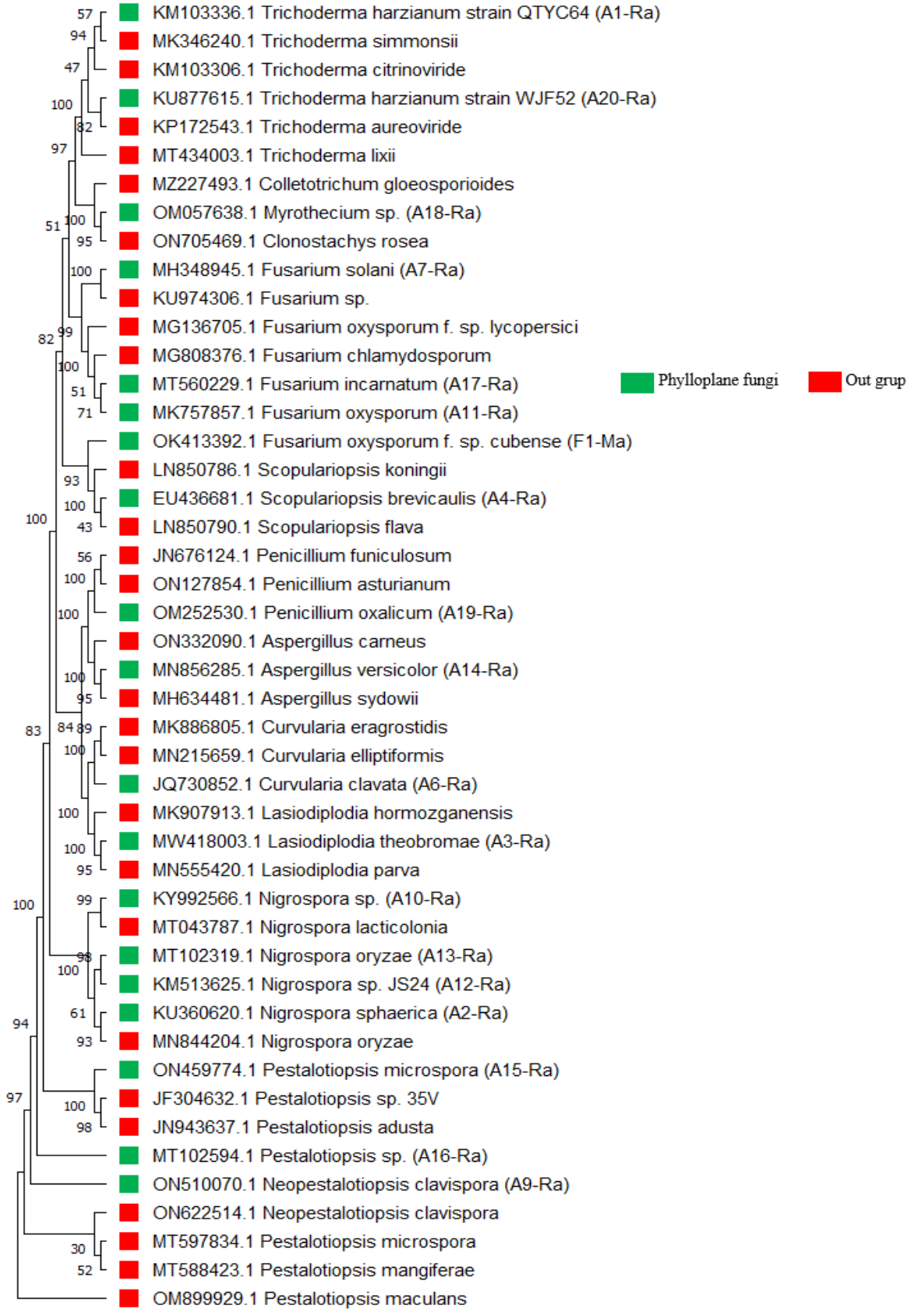

Figure 3. Phylogenetic tree of phylloplane fungi.

3.2. Postulate Koch Test

After eight weeks of observation, banana plants showed symptoms of fusarium wilt, which was identified by the older leaves turning yellow, and when the rhizome of the banana was split vertically, black spots were visible (see Figure 4).

Figure 4. Koch's postulate test (**a**) External Symptoms (**b**) Internal Symptoms.

3.3. Antagonism of Fungal Phylloplanes against Foc

3.3.1. In Vitro Assays

From the results of the in vitro antagonist test on the 20 fungal isolates, it was found that three isolates had the potential to inhibit the growth of the pathogenic fungi *Foc.* The three isolates were *Lasiodiplodia theobromae*, *Trichoderma harzianum,* and *Nigrospora sphaerica* (see Figure 5), with 67.43%, 66.65%, and 65.33% inhibition in seven days. In contrast, the fungi species with the lowest percentage of inhibition was *Scopulariopsis brevicaulis*, with an average inhibition of 20%. The in vitro antagonist test results were analyzed using the ANOVA analysis test and Tukey's further test (see Table 2).

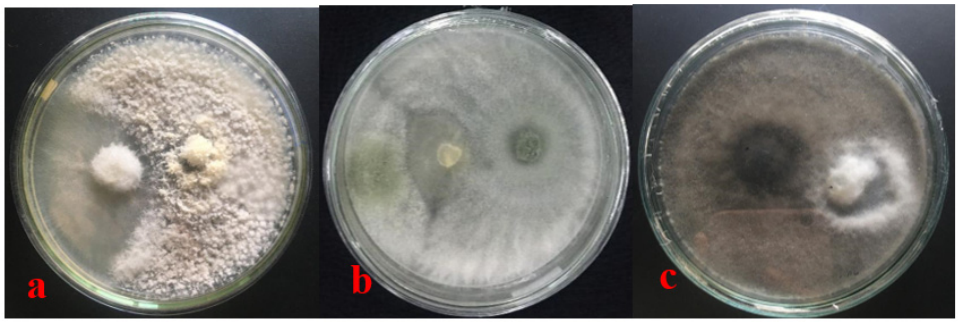

Figure 5. Inhibition (%) 7th day (**a**) *Nigrospora sphaerica* (**b**) Trichoderma harzianum (**c**) Lasiodiplodia theobromae.

From Tukey's further test, the three types of potential fungi, namely *Lasiodiplodia theobromae*, *Trichoderma harzianum* strain QTYC64, and *Nigrospora sphaerica* were not significantly different from *theobromaioediplodia*, *Fusariumsolani*, *Fusarium oxysporum*, A5 (Unidentified), A8 (Unidentified), *Myrothecium* sp., *Neopestalotiopsis clavispora,* and *Scopulariopsis brevicaulis*. On the other hand, *Trichoderma harzianum* strains QTYC64 and *Nigrospora sphaerica* were significantly different from *Fusarium oxysporum*, A5 (Unidentified), A8 (Unidentified), *Myrothecium* sp., *Neopestalotiopsis clavispora* ($p = 0.000$).

Table 2. Average Percentage of Inhibitory of Phylloplane Fungi against *Fusarium oxysporum* f. sp. *cubense* in seven days.

Fungus	Percentage of Inhibition (%)
Lasiodiplodia theobromae	67.43 ^{c} ± 2.66
Trichoderma harzianum strain QTYC64	66.65 ^{bc} ± 14.352
Nigrospora sphaerica	65.33 ^{bc} ± 3.850
Fusarium incarnatum	48.95 ^{abc} ± 1.391
Curvularia clavata	48.83 ^{abc} ± 17.806
Nigrospora sp.	48.54 ^{abc} ± 8.174
Pestalotiopsis microspora	47.33 ^{abc} ± 3.055
Nigrospora sp.	44.09 ^{abc} ± 2.297
Pestalotiopsis sp.	43.78 ^{abc} ± 10.633
Nigrospora singularis	43.36 ^{abc} ± 8.474
Penicillium oxalicum	43.30 ^{abc} ± 13.702
Trichoderma harzianum strain WJF52	42.00 ^{abc} ± 4.163
Aspergillus versicolor	38.94 ^{abc} ± 6.075
Fusarium solani	37.44 ^{ab} ± 13.722
Fusarium oxysporum	35.34 ^{a} ± 12.055
A5 (Not significant similarity found)	29.78 ^{a} ± 0.773
A8 (Not significant similarity found)	26.28 ^{a} ± 14.144
Myrothecium sp.	25.19 ^{a} ± 2.468
Neopestalotiopsis clavispora	24.17 ^{a} ± 13.687
Scopulariopsis brevicaulis	20.00 ^{a} ± 5.814

Remarks: The same letter in the same column shows no significant effect; different letters in the same column indicate a significantly different effect (0.05%). The nucleotide sequence data of our DNA barcode collection have been deposited in the GenBank/EMBL/DDBJ nucleotide sequence database with the accession numbers: A1_Ra (OP854605), A2_Ra (OP854606), A3_Ra (OP854607), A4_Ra (OP854608), A6_Ra (OP854609), A7_Ra (OP854610), A9_Ra (OP854611), A10_Ra (OP854612), A11_Ra (OP854613), A12_Ra (OP854614), A13_Ra (OP854615), A14_Ra (OP854616), A15_Ra (OP854617), A16_Ra (OP854618), A17_Ra (OP854619), A18_Ra (OP854620), A19_Ra (OP854621), A20_Ra (OP854622), and F1_Ma (OP854623).

3.3.2. Greenhouse Test of Fungal Phylloplanes vs. Foc

After eight weeks of inoculation, there was no significant difference (p = 0.060) in the yellowing of leaves. In the eighth week, the highest disease incidence was in the treatment K^+ (without phylloplane fungi) with an average of 100%, and the lowest disease incidence was in the treatment of *Trichoderma harzianum* with an average of 63.88% (see Table 3) However, there was a significant difference between the K^+ treatment and other treatments at weeks five, six, and seventh week. In treatment, K^- leaves of plants that experienced senescence in the last three weeks of observation did not experience subsequent chlorosis symptoms.

There was no significant difference in banana plants in the first to the fourth week, but in the fifth to eighth week there was a significant difference (p = 0.000) between the K^+ (without phylloplane fungi) and other treatments. In eight weeks, the highest score on disease severity was in the K^+ with an average of 48.60%, while the lowest average was in the *Trichoderma harzianum*, with an average of 16.66% (see Table 4).

Table 3. Incidence of Fusarium Wilt on Banana Plant Leaves 1–8 Weeks after Inoculation (%).

Treatment	Weeks							
	1	2	3	4	5	6	7	8
K^+	2.77 ^{a}	5.55 ^{a}	16.66 ^{a}	16.66 ^{a}	22.22 ^{b}	52.77 ^{b}	100.00 ^{a}	100.00 ^{b}
K^-	0.00 ^{a}	0.00 ^{a}	5.55 ^{a}	0.00 ^{a}	0.00 ^{a}	8.33 ^{a}	22.22 ^{a}	69.43 ^{a}
Trichoderma harzianum	0.00 ^{a}	0.00 ^{a}	5.55 ^{a}	8.33 ^{a}	0.00 ^{a}	11.11 ^{a}	25.55 ^{a}	63.88 ^{a}
Nigrospora sphaerica	0.00 ^{a}	0.00 ^{a}	5.55 ^{a}	16.66 ^{a}	2.77 ^{a}	11.11 ^{a}	33.33 ^{a}	69.44 ^{a}
Lasiodiplodia theobromae	0.00 ^{a}	0.00 ^{a}	2.77 ^{a}	8.33 ^{a}	2.77 ^{a}	5.55 ^{a}	20.81 ^{a}	80.55 ^{a}

Description: numbers followed by the same letter notation in the same column show no significant difference in the Tukey test level at a 5% level.

Table 4. Severity of Fusarium Wilt on Banana Leaf Plant 1–8 Weeks after Inoculation (%).

Treatment	Weeks							
	1	2	3	4	5	6	7	8
K^+	0.69 ^{a}	1.38 ^{a}	4.16 ^{a}	6.24 ^{a}	7.63 ^{b}	21.52 ^{b}	40.27 ^{b}	48.60 ^{b}
K^-	0.00 ^{a}	0.00 ^{a}	2.08 ^{a}	0.00 ^{a}	0.00 ^{a}	2.08 ^{a}	5.55 ^{a}	18.67 ^{a}
Trichoderma harzianum	0.00 ^{a}	0.00 ^{a}	1.38 ^{a}	2.08 ^{a}	0.00 ^{a}	2.77 ^{a}	7.63 ^{a}	16.66 ^{a}
Nigrospora sphaerica	0.00 ^{a}	0.00 ^{a}	1.38 ^{a}	4.16 ^{a}	0.69 ^{a}	2.77 ^{a}	9.72 ^{a}	24.30 ^{a}
Lasiodiplodia theobromae	0.00 ^{a}	0.00 ^{a}	0.69 ^{a}	2.08 ^{a}	1.38 ^{a}	2.08 ^{a}	7.63 ^{a}	25.69 ^{a}

Description: numbers followed by the same letter notation in the same column show no significant difference in the Tukey test at a 5% level.

Discoloration of rhizome caused by *Foc* (see Figure 6) got results of observations on the degree of rhizome discoloration on banana weeds after eight weeks of inoculation showed that, in the disease incidence observation, the K^+ (without the phylloplane fungi) was significantly different from the other treatments, and the *Trichoderma harzianum* was different from the K^+ (without the phylloplane fungi) and K^- (without the treatment) (Figure 6). The highest average value of disease incidence was in the K^+ (without phylloplane fungi), with an average of 77.77%, and the lowest average disease incidence was in *Lasiodiplodia theobromae*, with an average of 11.11% (p = 0.000) (see Table 5).

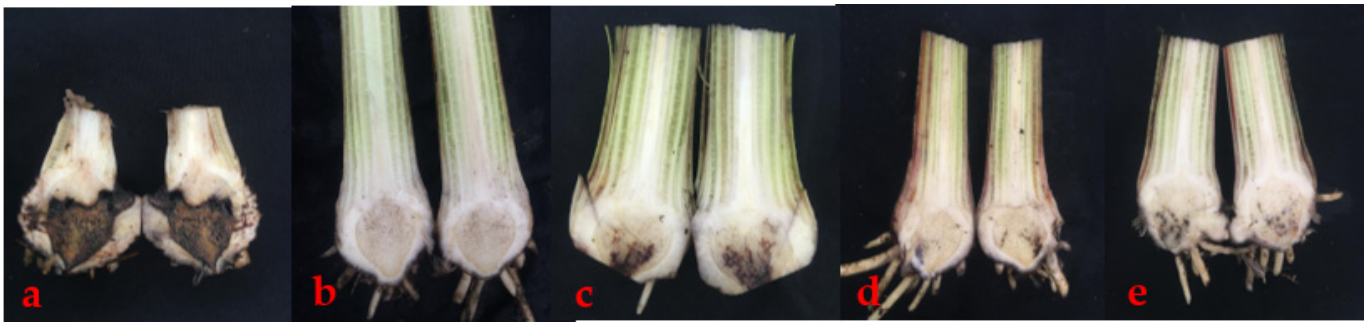

Figure 6. Effect of fungal phylloplane from mangrove on the degree of rhizome discoloration caused by Fusarium wilt (**a**) K^+ (Without fungal phylloplane), (**b**) K^- (untreated), (**c**) *Trichoderma harzianum*, (**d**) *Nigrospora sphaerica* (**e**) *Lasiodiplodia theobromae*.

However, for the disease severity observation, the three phylloplane fungi showed no significant difference with the K^- (without treatment) but were significantly different from the K^+ (without the phylloplane fungus). The disease severity value with the highest average was in the K^+ (without the phylloplane fungus), with an average of 26.10%. The lowest was in the *Nigrospora sphaerica*, with an average of 2.77% (p = 0.000) (see Table 5).

Table 5. Incidence and Severity of Fusarium Wilt on Banana Rhizome Eight Weeks after Inoculation (%).

Treatment	Incidence	Severity
K^+	77.77 ^{c}	26.10 ^{b}
K^-	0.00 ^{a}	0.00 ^{a}
Trichoderma harzianum	30.55 ^{b}	6.80 ^{a}
Nigrospora sphaerica	16.66 ^{ab}	2.77 ^{a}
Lasiodiplodia theobromae	11.11 ^{ab}	3.88 ^{a}

Description: numbers followed by the same letter notation in the same column show no significant difference in the Tukey test at a 5% level.

4. Discussion

4.1. Fungal Phylloplanes from Mangroves

Lee and Hyde (2002) [18] identified two phylloplane groups, namely residential and casual. Residential fungi are usually able to grow and reproduce on the surface area without affecting the host, while casual fungi only occupy the leaf surface area without developing. In this work, the fungal phylloplane that were found in almost all sampling locations were suspected to be permanent or residential fungi, while those found in only one location were suspected of casual.

Molecular identification of phylloplane fungi uses their DNA base sequence to determine the type of each fungus from genus to species. DNA was amplified by PCR (Polymerase Chain Reaction) technique using primers ITS 1 (5′-TCCGTTAGGTGAACCTGCGC-3′) and ITS 4 (5′-TCCTCCGCTTATTGATATGC-3′) in the ribosomal DNA. Primer ITS 1 and ITS 4 are widely used by researchers in fungi identification because they have the ability to amplify conserved areas in fungi [19].

A total of 20 isolates of the fungal phylloplane that were obtained from *R. apiculata* leaves were phylum Ascomycota. The phylum Ascomycota is known to be the largest fungal phylum, consisting of two-thirds of fungal species worldwide [20–22]. The most commonly isolated fungi in this study were from the class of *Sordariomycetes*, including *Nigrospora sphaerica, Nigrospora* sp., *Nigrospora singularis, Scopulariopsis brevicauli, Fusarium solani, Fusarium oxysporum, Fusarium incarnatum, Neopestalotiopsis, Pestalotiopsis microspora, Pestalotiopsis* sp., *Aspergillus versicolor*, and *Myrothecium* sp. Therefore, *Sordariomycetes* are one of the most diverse classes of fungal species in the phylum Ascomycota. Meanwhile, the most dominant genera were *Nigrospora* and *Fusarium*, with 3 of each species present in the 20 isolates. The population and diversity of fungal species vary depending on their habitat, where they have an ecological role in the nutrient cycle and act as decomposers of organic matter [23].

4.2. Antagonism of Fungal Phylloplanes against Foc

4.2.1. In Vitro Assay

There are three mechanisms used by antagonist fungi in inhibiting the growth of antagonistic fungi: competition, antibiosis, and parasitism. Antagonistic fungi can use one or more of these mechanisms to inhibit the growth of pathogens. As suggested by Thambugala et al., (2020) [24], antagonist fungi can utilize mechanisms directly or indirectly, including antibiotic mechanisms (metabolite compounds produced by antagonist agents), mycoparasitism (antagonistic agents obtain nutrition from pathogenic fungi), induced resistance (plant defense responses against pathogen attack), and competition (for space and nutrients).

In vitro antagonist tests conducted with the dual culture method show that 3 of the 20 phylloplane fungi have the potential to inhibit the pathogenic fungi *Fusarium oxysporum* f.sp. *cubense* (*Foc*); the highest average was *Trichoderma harzianum, Nigrospora sphaerica*, and *Lasiodiplodia theobromae*. An in vitro study conducted by Nayak et al., (2020) [25] stated that *Trichoderma harzianum* could inhibit the growth of *Fusarium oxysporum* f. sp. *cubense* (*Foc*) by 82.02%. *Trichoderma* sp. is a species of fungus that can grow very fast. *Trichoderma* sp. inhibits

the growth of Fusarium through mycoparasitism [25,26]. In addition, *Trichoderma* sp. also produces metabolites that can inhibit the growth of *Alternaria* sp. and *Fusarium* sp. so this fungi has the potential to be used as a pathogenic biocontrol agents with good prospects [26]. Many studies have stated that *Nigrospora sphaerica* has anti-fungal properties [27] and is a biocontrol agent for pathogenic fungi *P. infestans* and *P. capsici* because it can inhibit the mycelium and the zoospore germination process [28].

In contrast, *Lasiodiplodia theobromae* is a species of *Botryosphaeriaceae* that is most commonly found in nature and has more than 500 host plant species [29] and research on secondary metabolites produced by *Lasiodiplodia theobromae* has been conducted more frequently. *Lasiodiplodia theobromae* produces secondary metabolites from both biotic and abiotic stimuli that can live in plants as endophytic fungi and have a defense function. For example, one of the secondary metabolites produced by *Lasiodiplodia theobromae* is *Depsidones,* which has anti-fungal activity and can inhibit the growth of the pathogenic fungi *Aspergillus terreus* and *Fusarium oxysporium* [29].

Research conducted by [30] demonstrates that *Lasiodiplodia theobromae* is one of the most common fungi found in the mangrove species *R. stylosa* and *R. mucronata*. Furthermore, a study conducted by [29] showed results where *Lasiodiplodia theobromae* became the fungus with the highest inhibitory value against the pathogenic fungi *Foc* TR4. Furthermore, *lasiodiplodia theobromae* which have been fermented produce secondary metabolites in the form of jasmonic acid [31]. This jasmonic acid has a role in plant defense gene induction against pathogens and pests [32]. As stated by [9] banana plants carry out a defense response to *Foc* triggered by the presence of salicylic acid and jasmonic acid/ethylene.

Nigrospora sp. species are fungi that produce various chemical compounds and bioactive substances. Nigrospora fungi isolated from land and sea areas each produce metabolites, especially polyketides, divided into monophenyl turunan, quinone, pyran, furanone derivative and others. These metabolites have various biological activities such as enzyme inhibitors, phytotoxic, anti-fungal, anti-bacterial, anti-viral, anti-oxidant, and others [9] Nigrospores isolated from the roots of *Moringa oleifera* have been shown to produce bioactive compounds with anti-fungal activity, while these compounds include *griseofulvin*, *dechlorogriseofulvin*, and *mullein* [33].

Several species of *Trichoderma* spp. can produce anti-fungal compounds that can indirectly affect the growth of other fungi [34,35]. For example, *Trichoderma parareesei* had the highest inhibitory ability against FocTR4 with an average of 96%, while *T. harzianum* had a medium inhibitory ability with an average of 70.67%, which means that the species of *Trichoderma* spp. have different abilities against FocTR4 pathogens [35]. The same thing was also found in [36] which showed that the highest inhibition of *Trichoderma* spp. against *Fusarium oxysporum* f. sp. *cubense* at the in vitro test with direct opposition to *T. koningii* with an average inhibition of 45.70%, while *T. harzianum* with an average inhibition of 43.60% had an intermediate inhibitory ability, followed by *T. piluliferum* with an average inhibition of 40.10% with the lowest inhibitory ability. This indicates that several species of *Trichoderma* sp. have different abilities against different species of fusarium pathogens.

4.2.2. Greenhouse Test of Fungal Phylloplanes vs. Foc

Symptoms of fusarium wilt can be observed by yellowing and wilting of the leaves beginning from the oldest leaves but leaf yellowing can also occur without any disease infection because of leaf senescence. Common symptoms of fusarium wilt disease infection in banana plants include yellowing and wilting of plants. Pathogenic fungi enter plants through roots [37]. From there, they enters the epidermis, the space inside plant cells, and quickly form microconidia, macroconidia, and chlamydospores [38]. Hyphae that develop in the spaces between plant cells invade the tissue parts of the cortex and pass through the endodermis to the xylem vessels [39]. Xylem vessels are blocked through very fast sporulation. This disrupts water absorption, causing the host plant to wilt. Infected host plants show yellowing of the leaves, beginning with the older leaves. Over time, the leaves fall, hanging on the plant's stem. Only a few leaves that are still new stand upright. Then,

there is a change in color on the banana weevil when it is split. Finally, there is a brown color, and plants that wither over time eventually die [37].

K^- (without treatment) can show yellowing leaves, but they are assumed to be due to plant senescence. Leaf senescence is the final process of leaf development and is a process of programmed cell death that is induced depending on the age of the plant and is caused by various environmental factors [40,41]. Although leaf senescence is an ongoing process, and the older leaves are replaced by emerging young leaves, cell structure, metabolism, and gene expression change during the leaf senescence process [42]. However, in infected plants, some enzyme activities such as phenylalanine ammonia-lyase (PAL), peroxidase (POD), and polyphenol oxidase (PPO) activity increased in addition to soluble phenolic compounds found in higher amounts in the leaves of infected plants [41]. This is associated with plants trying to recover from or create disease resistance because of a pathogen attack [43]. Structural and biochemical changes in plants go hand in hand with senescence. Pathogenic fungi residing in the vascular tissue of host plants secrete phytotoxin compounds that cause the plant to respond as a whole, which can trigger changes in host plant cells [44]. The phytotoxin compound found and produced in every Fusarium species is fusaric acid [45,46]. The presence of fusaric acid in plants causes accelerated leaf senescence [47]. Fusaric acid interacts with other secondary metabolites, such as moniliformin, fumonisin, and mycotoxins, to increase toxicity [48]. Fusaric acid secreted by the Fusarium in the xylem tissue of the host plant will be carried by water to the leaves. This fusaric acid will disrupt membrane balance, decrease mitochondrial activity, and interfere with oxygen uptake and ATP formation [49]. This fusaric acid activity triggers an increase in the speed of leaf senescence in banana plants [47].

Mangroves are coastal biotopes that develop in the intertidal zone and are tolerant to various types of extreme environmental stresses [50,51] and as host diverse fungal communities, making them a potential bioactive natural product source [30] The diversity of fungi species can also be influenced by various environmental factors, such as temperature, humidity, rainfall, wind direction, wind speed, and dew. Phylloplane microorganisms are also microorganisms that usually live in soil and air [52]. On the mangroves *Rhizophora mucronata* and *Avicennia marina*, phylloplane fungi were also found, such as *Aspergillus* sp., *Acromium* sp., *Alternatia alternata*, *Fusarium* sp., *Rhizopus stolonifer*, *Cladosporium* sp., *Curvularia* sp., *Penicillium* sp., *Glycladium* sp., *Memnoniella* sp., *Mucor hiemalis*, *Mycelia sterilia*, *Trichoderma viride*, *Paecilomyces* sp., and *Phoma* sp. [52].

Symptoms may change as a result of the host response because the affected plant may induce some resistance from the host plant. The mechanism of host plant resistance can be caused by the stimulation of non-pathogenic biotic agents that produce compounds that are useful in defense against pathogen. Biological control of plant disease phenomena such as fusarium wilt in banana plants has become a significant concern because pesticide use has resulted in economic losses, environmental damage, and health and safety issues for farmers [53]. In general, the different processes of biological agents/biocontrols are referred to as mycoparasitism, which produces substances such as enzymes or antibiotics and induces host plants to defend and compete for nutrients and the scope of growth [54–58]. For example, *Trichoderma* sp., *Aureobasidium* sp., *Fusarium* sp., and *Penicillium* sp. against plant pathogens induce SAR (systemic acquired resistance) through mycoparasitism [35,56,59].

Thakur and Harsh (2014) [5] reported that, on the surface of leaves, phylloplane fungi live in habitats where nutrients are available and where there is an appropriate environment for antagonistic fungi to develop so that they can inhibit microbial growth by producing antibiotics or toxins. As well as phylloplane, fungi produce chemicals that can protect host plants from infection with pathogens that cause various diseases that affect plants [60,61].

This study confirmed that phylloplane fungi isolated from mangrove *R. apiculata* leaves have potential inhibit fusarium wilt disease of banana and for further studies, the optimization of application to bananas and testing use in other cultivar is required to see the potencial and identify metabolites from phylloplane fungi. Phylloplane microbes have an important role because they have anti-bacterial and anti-fungal activities [62].

Antimicrobial compounds produced by phylloplane fungi can directly induce systemic resistance (SAR) in host plants, and this event can inhibit pathogens [63].

5. Conclusions

Out of the 20 fungal isolates obtained from mangrove plant *R. apiculata*, three had antagonistic potential against *Fusarium oxysporum* f. sp. *cubense* (*Foc*) in the in vitro test. They had an average inhibition of *Lasiodiplodia theobromae* (67.43%), *Trichoderma harzianum* (66.65), and *Nigrospora sphaerica* (65.33%), and in the in vivo test, the best inhibition value on disease incidence in shown in the treatment of *Lasiodiplodia theobromae* (11.11%).

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References

1. Anandhu, R. Studies on Phylloplane and Endophytic Fungi Isolated from Mangrove Plants and Their Bioactive Compounds. Ph.D. Thesis, Department of Botany, KMCPGS, Pondicherry University, Puducherry, India, 2016.
2. Patra, J.K.; Mohanta, Y.K. Antimicrobial compounds from mangrove plants: A pharmaceutical prospective. *Chin. J. Integr. Med.* **2014**, *20*, 311–320. [CrossRef]
3. Bose, S.; Bose, A. Antimicrobial activity of *Acanthus ilicifolius* (L.). *Indian J. Pharm. Sci.* **2008**, *70*, 821–823. [CrossRef]
4. Goswami, S.; Goel, N.; Majumdar, R.S. Phylloplane microbes impact host physiology: A review. *J. Plant Prot. Res.* **2021**, *61*, 213–221. [CrossRef]
5. Thakur, S.; Harsh, N.S.K. Phylloplane fungi as biocontrol agent against Alternaria leaf spot disease of (Akarkara) *Spilanthes oleracea*. *Biosci. Discov.* **2014**, *5*, 139–144.
6. Chauhan, R.; Navneet; Gautam, S.S. Potential antagonistic phylloplane fungi from Stevia rebaudiana Bert. as bio-control of aerial blight disease caused by Rhizoctonia solani. *Indian Phytopathol.* **2019**, *72*, 177–180. [CrossRef]
7. Hamzah, T.N.T.; Lee, S.Y.; Hidayat, A.; Terhem, R.; Faridah-Hanum, I.; Mohamed, R. Diversity and Characterization of Endophytic Fungi Isolated From the Tropical Mangrove Species, Rhizophora mucronata, and Identification of Potential Antagonists Against the Soil-Borne Fungus, Fusarium solani. *Front. Microbiol.* **2018**, *9*, 1707. [CrossRef] [PubMed]
8. Nayak, B.K.; Anandhu, R. Biodiversity of phylloplane and endophytic fungi from different aged leaves of medicinal mangrove plant species. *Avicennia Mar. J. Pharm. Sci. Res.* **2017**, *9*, 6–9.
9. Dita, M.; Barquero, M.; Heck, D.W.; Mizubuti, E.S.G.; Staver, C.P. Fusarium Wilt of Banana: Current Knowledge on Epidemiology and Research Needs Toward Sustainable Disease Management. *Front. Plant Sci.* **2018**, *871*, 1468. [CrossRef]
10. Abo-Elyousr, K.A.M.; Abdel-Hafez, S.I.I.; Abdel-Rahim, I.R. Isolation of *Trichoderma* and Evaluation of their Antagonistic Potential against *Alternaria porri*. *J. Phytopathol.* **2014**, *162*, 567–574. [CrossRef]
11. Wibowo, A.; Subandiyah, S.; Sumardiyono, C.; Sulistyowati, L.; Taylor, P.; Fegan, M. Occurrence of Tropical Race 4 of *Fusarium oxysporum* f. sp. cubense in Indonesia. *Plant Pathol. J.* **2011**, *27*, 280–284. [CrossRef]
12. Sari, W.; Rosmeita, C.N. Identifikasi molekuler cendawan entomopatogen Beauveria Bassiana dan Metarhizium anisopliae asal isolat Cianjur. *Pro-STek* **2020**, *1*, 1–9. [CrossRef]
13. White, T.J.; Bruns, T.; Lee, S.; Taylor, J.W. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Proto-Cols: A Guide to Methods and Applications*; Innis, M., Gelfand, D., Sninsky, J., White, T., Eds.; Academic Press Inc.: New York, NY, USA, 1990; Volume 64, pp. 315–322.
14. Hartanto, E.S.; Heni, K. Uji antagonis 5 isolat trichoderma dari rizosfer pinus sp terhadap pertumbuhan cendawan colletotricum sp penyebab penyakit antraknos pada cabai secara in vitro. In *Prosiding Symbion (Symposium Biology Education. Program Study Biology Education. FKIP*; Ahmad Dahlan University: Yogyakarta, Indonesia, 2016; pp. 205–212.
15. Wheeler, K.A.; Hocking, A.D. Interactions among xerophilic fungi associated with dried salted fish. *J. Appl. Bacteriol.* **1993**, *74*, 164–169. [CrossRef] [PubMed]

16. Emilda, D.; Sutanto, A.; Sukartini; Jumjunidang. Application of salicylic acid to induce disease resistance against fusarium wilt on banana. *IOP Conf. Ser. Earth Environ. Sci.* **2020**, *468*, 012026. [CrossRef]
17. Catambacan, D.; Cumagun, C. Weed-Associated Fungal Endophytes as Biocontrol Agents of *Fusarium oxysporum* f. sp. *cubense* TR4 in Cavendish Banana. *J. Fungi* **2021**, *7*, 224. [CrossRef]
18. Lee, O.H.K.; Hyde, K.D. Phylloplane fungi in Hong Kong mangroves: Evaluation of study methods. *Mycologia* **2002**, *94*, 596–606. [CrossRef]
19. Legiastuti, T.S.; Aminingsih, T. Identifikasi Cendawan Endofit Menggunakan Teknik Polymerase Chain Reaction. *J. Fitopatol. Indones.* **2012**, *8*, 31–36. [CrossRef]
20. Lutzoni, F.; Kauff, F.; Cox, C.J.; McLaughlin, D.; Celio, G.; Dentinger, B.; Padamsee, M.; Hibbett, D.; James, T.Y.; Baloch, E.; et al. Assembling the fungal tree of life: Progress, classification, and evolution of subcellular traits. *Am. J. Bot.* **2004**, *91*, 1446–1480. [CrossRef]
21. Schoch, C.L.; Sung, G.-H.; López-Giráldez, F.; Townsend, J.P.; Miadlikowska, J.; Hofstetter, V.; Robbertse, B.; Matheny, P.B.; Kauff, F.; Wang, Z.; et al. The Ascomycota Tree of Life: A Phylum-wide Phylogeny Clarifies the Origin and Evolution of Fundamental Reproductive and Ecological Traits. *Syst. Biol.* **2009**, *58*, 224–239. [CrossRef]
22. Naranjo-Ortiz, M.Á.; Gabaldón, T. Fungal evolution: Diversity, taxonomy and phylogeny of the Fungi. *Biol. Rev.* **2019**, *58*, 224–239. [CrossRef]
23. Naikwade, P.; Mogle, U.; Sankpal, S. Phyloplane mycoflora associated with Mangrove plant Ceriops tagal (Perr.). 2012. Available online: https://jsrr.net/Vol%202%20No.%201/Naikwade85-87.pdf (accessed on 28 October 2022).
24. Thambugala, K.M.; Daranagama, D.A.; Phillips, A.J.L.; Kannangara, S.D.; Promputtha, I. Fungi vs. Fungi in Biocontrol: An Overview of Fungal Antagonists Applied Against Fungal Plant Pathogens. *Front. Cell. Infect. Microbiol.* **2020**, *10*, 604923. [CrossRef]
25. Nayak, D.; Mishra, M.; Pradhan, B.; Sharma, K.K. Evaluation of some bio-control agents in in vitro control of *Fusarium oxysporum* f. sp. cubense, an incitant of banana panama wilt. *J. Pharmacogn. Phytochem.* **2020**, *9*, 751–753.
26. Napitupulu, T.P. In vitro evaluation of Trichoderma harzianum strains for the control of *Fusarium oxysporum* f. sp. cubense. *Plant Pathol. Quar.* **2019**, *9*, 152–159. [CrossRef]
27. Mmbaga, T.; Gurung, S.; Maheshwari, A. Screening of Plant Endophytes as Biological Control Agents against Root Rot Pathogens of Pepper (*Capsicum annum* L.). *J. Plant Pathol. Microbiol.* **2018**, *9*, 3. [CrossRef]
28. Kim, J.C.; Choi, G.J.; Park, J.H.; Kim, H.T.; Cho, K.Y. Activity against plant pathogenic fungi of phomalactone isolated from Nigrospora sphaerica. *Pest Manag. Sci.* **2001**, *57*, 554–559. [CrossRef] [PubMed]
29. Salvatore, M.M.; Alves, A.; Andolfi, A. Secondary Metabolites of *Lasiodiplodia theobromae*: Distribution, Chemical Diversity, Bioactivity, and Implications of Their Occurrence. *Toxins* **2020**, *12*, 457. [CrossRef]
30. Zhou, J.; Diao, X.; Wang, T.; Chen, G.; Lin, Q.; Yang, X.; Xu, J. Phylogenetic diversity and antioxidant activities of culturable fungal endophytes associated with the mangrove species Rhizophora stylosa and R. mucronata in the South China Sea. *PLoS ONE* **2018**, *13*, e0197359. [CrossRef]
31. Eng, F.; Haroth, S.; Feussner, K.; Meldau, D.; Rekhter, D.; Ischebeck, T.; Brodhun, F.; Feussner, I. Optimized Jasmonic Acid Production by Lasiodiplodia theobromae Reveals Formation of Valuable Plant Secondary Metabolites. *PLoS ONE* **2016**, *11*, e0167627. [CrossRef]
32. Wasternack, C.; Hause, B. Jasmonates: Biosynthesis, perception, signal transduction and action in plant stress response, growth and development. An update to the 2007 review in Annals of Botany. *Ann. Bot.* **2013**, *111*, 1021–1058. [CrossRef]
33. Zhao, J.H.; Zhang, Y.L.; Wang, L.W.; Wang, J.Y. Bioactive secondary metabolites from Nigrospora sp. LLGLM003, an endophytic fungus of the medicinal plant Moringa oleifera Lam. *World J. Microbiol. Biotechnol.* **2012**, *28*, 2107–2112. [CrossRef]
34. Al-Ani, L.K.T. Trichoderma: Beneficial Role in Sustainable Agriculture by Plant Disease Management. In *Plant Microbiome: Stress Response*; Springer: Berlin/Heidelberg, Germany, 2018; pp. 105–126. [CrossRef]
35. Al-ani, L.K.T.; Albaayit, S.F.A. Antagonistic of some Trichoderma against *Fusarium oxysporum* sp. f. cubense tropical race 4 (FocTR4). *Eurasia Proc. Sci. Technol. Eng. Math.* **2018**, *2*, 35–38.
36. Sudantha, I.M. Characterization and virulence of *Fusarium oxysporum* f. sp. cubense cause wilt disease in banana plants and its biological control using endophytic fungi Trichoderma spp. At West Nusa Tenggara, Indonesia. *IOP Conf. Ser. Earth Environ. Sci.* **2021**, *886*, 1. [CrossRef]
37. Ghag, S.B.; Shekhawat, U.K.; Ganapathi, T.R. Fusarium wilt of banana: Biology, epidemiology and management. *Int. J. Pest Manag.* **2015**, *61*, 250–263. [CrossRef]
38. Li, C.; Chen, S.; Zuo, C.; Sun, Q.; Ye, Q.; Yi, G.; Huang, B. The use of GFP-transformed isolates to study infection of banana with *Fusarium oxysporum* f. sp. cubense race 4. *Eur. J. Plant Pathol.* **2011**, *131*, 327–340. [CrossRef]
39. Yadeta, K.A.; Thomma, B.P.H.J. The xylem as battleground for plant hosts and vascular wilt pathogens. *Front. Plant Sci.* **2013**, *4*, 1–12. [CrossRef] [PubMed]
40. Yoshida, S. Molecular regulation of leaf senescence. *Curr. Opin. Plant Biol.* **2003**, *6*, 79–84. [CrossRef] [PubMed]
41. Dong, X.; Xiong, Y.; Ling, N.; Shen, Q.; Guo, S. Fusaric acid accelerates the senescence of leaf in banana when infected by Fusarium. *World J. Microbiol. Biotechnol.* **2014**, *30*, 1399–1408. [CrossRef]
42. Pyung, O.L.; Hyo, J.K.; Hong, G.N. Leaf senescence. *Annu. Rev. Plant Biol.* **2007**, *58*, 115–136. [CrossRef]

43. Préstamo, G.; Manzano, P. Peroxidases of Selected Fruits and Vegetables and the Possible Use of Ascorbic Acid as an Antioxidant. *Hortscience* **2019**, *28*, 48–50. [CrossRef]
44. Czymmek, K.J.; Fogg, M.; Powell, D.H.; Sweigard, J.; Park, S.-Y.; Kang, S. In vivo time-lapse documentation using confocal and multi-photon microscopy reveals the mechanisms of invasion into the Arabidopsis root vascular system by *Fusarium oxysporum*. *Fungal Genet. Biol.* **2007**, *44*, 1011–1023. [CrossRef]
45. Bacon, C.W.; Porter, J.K.; Norred, W.P.; Leslie, J.F. Production of fusaric acid by Fusarium species. *Appl. Environ. Microbiol.* **1996**, *62*, 4039–4043. [CrossRef]
46. Gapillout, I.; Milat, M.-L.; Blein, J.-P. Effects of fusaric acid on cells from tomato cultivars resistant or susceptible toFusarium oxysporum f. sp.Lycopersici. *Eur. J. Plant Pathol.* **1996**, *102*, 127–132. [CrossRef]
47. Rahayu, G.; Maulana, I. Widodo Endobacterial symbiont of *Fusarium oxysporum* f. sp. cubense and the pathogenicity of their symbiosis towards banana plantling. *IOP Conf. Ser. Earth Environ. Sci.* **2020**, *457*, 012051. [CrossRef]
48. Chen, Z.; Luo, Q.; Wang, M.; Chen, B. A Rapid Method with UPLC for the Determination of Fusaric Acid in Fusarium Strains and Commercial Food and Feed Products. *Indian J. Microbiol.* **2017**, *57*, 68–74. [CrossRef] [PubMed]
49. Dong, X.; Ling, N.; Wang, M.; Shen, Q.; Guo, S. Fusaric acid is a crucial factor in the disturbance of leaf water imbalance in Fusarium-infected banana plants. *Plant Physiol. Biochem.* **2012**, *60*, 171–179. [CrossRef] [PubMed]
50. Basyuni, M.; Baba, S.; Kinjo, Y.; Oku, H. Salinity increases the triterpenoid content of a salt secretor and a non-salt secretor mangrove. *Aquat. Bot.* **2012**, *97*, 17–23. [CrossRef]
51. Inafuku, M.; Basyuni, M.; Oku, H. Triterpenoid modulates the salt tolerance of lanosterol synthase deficient Saccharomyces cerevisiae, GIL77. *Saudi J. Biol. Sci.* **2018**, *25*, 1–9. [CrossRef] [PubMed]
52. Shaukat, S.S.; Zafar, H.; Ahmed, W.; Khan, M.; Zaidi, S. Diversity of Phylloplane Mycobiota of Avicennia Marina (Forssk.) Vierh. and Rhizophora Mucronata Poir. At Indus Delta, Sindh Coast, Pakistan. *Int. J. Biol. Biotech* **2013**, *10*, 45–51.
53. Bubici, G.; Kaushal, M.; Prigigallo, M.I.; Cabanás, C.G.-L.; Mercado-Blanco, J. Biological Control Agents Against Fusarium Wilt of Banana. *Front. Microbiol.* **2019**, *10*, 616. [CrossRef]
54. Suárez-Estrella, F.; Arcos-Nievas, M.; López, M.; Vargas-García, M.; Moreno, J. Biological control of plant pathogens by microorganisms isolated from agro-industrial composts. *Biol. Control* **2013**, *67*, 509–515. [CrossRef]
55. Yan, J.; Broughton, S.; Yang, S.; Gange, A. Do endophytic fungi grow through their hosts systemically? *Fungal Ecol.* **2015**, *13*, 53–59. [CrossRef]
56. Busby, P.E.; Ridout, M.; Newcombe, G. Fungal endophytes: Modifiers of plant disease. *Plant Mol. Biol.* **2016**, *90*, 645–655. [CrossRef] [PubMed]
57. Lecomte, C.; Alabouvette, C.; Edel-Hermann, V.; Robert, F.; Steinberg, C. Biological control of ornamental plant diseases caused by *Fusarium oxysporum*: A review. *Biol. Control* **2016**, *101*, 17–30. [CrossRef]
58. Cheong, S.L.; Cheow, Y.L.; Ting, A.S.Y. Characterizing antagonistic activities and host compatibility (via simple endophyte-calli test) of endophytes as biocontrol agents of Ganoderma boninense. *Biol. Control* **2017**, *105*, 86–92. [CrossRef]
59. De Silva, N.I.; Brooks, S.; Lumyong, S.; Hyde, K.D. Use of endophytes as biocontrol agents. *Fungal Biol. Rev.* **2019**, *33*, 133–148. [CrossRef]
60. Innerebner, G.; Knief, C.; Vorholt, J.A. Protection of Arabidopsis thaliana against Leaf-Pathogenic Pseudomonas syringae by Sphingomonas Strains in a Controlled Model System. *Appl. Environ. Microbiol.* **2011**, *77*, 3202–3210. [CrossRef]
61. Ritpitakphong, U.; Falquet, L.; Vimoltust, A.; Berger, A.; Métraux, J.; L'Haridon, F. The microbiome of the leaf surface of Arabidopsis protects against a fungal pathogen. *New Phytol.* **2016**, *210*, 1033–1043. [CrossRef] [PubMed]
62. Mazinani, Z.; Zamani, M.; Sardari, S. Isolation and identification of phyllospheric bacteria possessing antimicrobial activity from Astragalus obtusifolius, Prosopis juliflora, Xanthium strumarium and Hippocrepis unisiliqousa. *Avicenna J. Med. Biotechnol.* **2017**, *9*, 31–37. [PubMed]
63. Lindow, S.E.; Brandl, M.T. Microbiology of the Phyllosphere. *Appl. Environ. Microbiol.* **2003**, *69*, 1875–1883. [CrossRef] [PubMed]