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Antagonistic Activities of Fungal Endophytes from *Rhizophora mucronata* Lamk. against *Fusarium oxysporum* Under Altered Nutrient Levels

Jacob JKS[1,2,4] and dela Cruz TEE[2,3,4]*

[1]*Department of Biological Sciences, College of Arts and Sciences, Isabela State University, Echague 3309 Isabela, Philippines*
[2]*The Graduate School, University of Santo Tomas, Manila 1008 Philippines*
[3]*Department of Biological Sciences, College of Science, University of Santo Tomas, Manila 1008 Philippines*
[4]*Fungal Biodiversity, Ecogenomics and Systematics-Metabolomics (FBeS) Group, Research Center for the Natural and Applied Sciences, University of Santo Tomas, 1008 Manila, Philippines*

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Abstract

Mangrove ecosystems harbor diverse fungal endophytes that are increasingly recognized as sources of bioactive metabolites with potential applications in sustainable agriculture. However, their antagonistic roles against phytopathogens under variable environmental conditions remain poorly understood. This study investigated the antagonistic activities of 16 fungal endophytes isolated from the mangrove *Rhizophora mucronata* against the fungal phytopathogen *Fusarium oxysporum*. These mangrove fungal endophytes, representing the genera *Pestalotiopsis*, *Neopestalotiopsis*, and *Pseudopestalotiopsis*, were isolated from both disturbed and undisturbed mangrove ecosystems and evaluated for antifungal activities using a dual culture assay. Our results revealed diverse antagonistic interactions, with Types C (inhibition upon contact) and E (mutual inhibition with subsequent overgrowth) being the most frequently observed, occurring in 9 of the 16 mangrove fungal endophytic isolates. Interaction Types B (mutual inhibition upon contact or at a distance) and D (mutual inhibition between fungi at a distance with clear zones larger than 2 mm) were also observed among the tested mangrove fungal endophytic isolates. The percent inhibition of *F. oxysporum* by the mangrove fungal endophytes ranged from 68% to 86%, with *Pseudopestalotiopsis* sp. isolated from undisturbed sites displaying the highest antagonism. We further examined these interactions under altered phosphate and zinc concentrations in the growth medium. Low phosphate levels enhanced fungal inhibition, promoting Type C interactions, whereas elevated phosphate concentrations reduced antagonism, shifting interactions toward Type E. Modifications to zinc concentration produced more consistent inhibitory effects, with interactions primarily classified as Types C and E. These findings suggest that environmental factors, particularly mineral availability, play a critical role in shaping the antagonistic potential of mangrove fungal endophytes against phytopathogens, underscoring their promise as sustainable biocontrol agents.

Keywords – bioassay – co-cultivation – *Fusarium oxysporum* – phytopathogen – mangrove fungi

Introduction

Mangroves in South and Southeast Asia account for 41% of the global mangrove population

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Corresponding Author: Thomas Edison E. dela Cruz – e-mail – tedelacruz@ust.edu.ph

(Singh 2020). Approximately 70 distinct mangrove species belonging to 17 families are recognized, with the Rhizophoraceae family being the largest, comprising of the genera *Bruguiera*, *Ceriops*, *Kandelia*, and *Rhizophora* (Tomlinson 1986, Polidoro et al. 2010). Among these, *Rhizophora* is the most widely distributed, thriving in tropical and subtropical coastal regions, particularly in the Indo-West Pacific (Yan et al. 2016). This genus includes six true species and one hybrid, with *R. apiculata*, *R. mucronata*, and *R. stylosa* particularly common in the Indo-Malaya region (Duke et al. 2007).

Rhizophora mucronata, locally known as *bakawan lalaki* in the Philippines, is characterized by broadly elliptic to oblong leaves and yellowish stalked flowers (Qin & Boufford 2007, Setyawan & Ulumuddin 2012). Traditionally, its bark and leaves have been used for their astringent and antiseptic properties, with reported efficacy against bacterial infections, ulcers, and inflammation and other pharmacological applications (Roy et al. 2023). Beyond the plant itself, attention has increasingly turned to its associated microorganisms. Endophytic fungi isolated from *R. mucronata*—including species of *Alternaria, Fusarium, Nigrospora, Pestalotiopsis, Phoma, and Xylaria*—have shown antagonistic activity (Hamzah et al. 2018) and have yielded novel secondary metabolites such as acropyrone, bicytosporone D, waol acid, and pestalotiopene (Klaiklay et al. 2012). Because marine fungi are exposed to unique ecological pressures, they often activate specialized metabolic pathways, producing structurally distinct compounds that support adaptation and survival (Debbab et al. 2011, de Souza Sebastianes et al. 2013, El-Bondkly et al. 2021). Such traits make mangrove-associated fungi attractive candidates for natural product discovery and bioprospecting.

Fungal endophytes reside asymptomatically within host plant tissues, providing benefits such as enhanced tolerance to biotic and abiotic stresses (Potshangbam et al. 2017, Akram et al. 2023) and eliciting chemical responses (Hartley et al. 2015). Their diversity varies among leaf types (Douanla-Meli et al. 2013). Their bioactivities include antimicrobial, antioxidant, and biotechnological applications (Torres & dela Cruz 2013, Moron et al. 2018, Apurillo et al. 2019, Ramirez et al. 2020, Jacob et al. 2023). One important ecological role of fungal endophytes is antagonism, which is defined as the ability of one organism to adversely affect another and which may involve competition, mycoparasitism, or production of extracellular metabolites (Rajani et al. 2021, Akram et al. 2023, Jamilano-Llames & dela Cruz 2025). Dual culture or co-cultivation assays provide a rapid and effective means to assess such antagonism under laboratory conditions, simulating microbial interactions that may occur in nature (Pecundo et al. 2021).

Despite increasing reports of bioactive metabolites from mangrove fungal endophytes, little is known about their direct antagonistic activities against fungal phytopathogens, particularly under variable nutrient conditions. Nutrient availability such as phosphate and zinc levels can strongly influence fungal physiology, including secondary metabolism and antagonistic responses, yet these ecological aspects remain underexplored in mangrove-associated fungi. Here, we investigated 16 fungal endophytes isolated from *Rhizophora mucronata* for their antagonistic activities against the phytopathogen *Fusarium oxysporum*. Specifically, we examined how alterations in phosphate and zinc concentrations influence their inhibitory interactions in dual culture assays. By addressing this gap, our study provides new insights into how mineral availability modulates the antagonistic potential of mangrove fungal endophytes, with implications for their ecological roles and future applications in the sustainable biological control of plant diseases.

Materials & Methods

The mangrove fungal endophytes

Sixteen representative mangrove fungal endophytes, comprising five isolates of *Pestalotiopsis*, five isolates of *Neopestalotiopsis*, and six isolates of *Pseudopestalotiopsis*, previously isolated and characterized by Jacob et al. (2023) were used in this study (Table 1). Ten of these mangrove fungal endophytes were isolated from an undisturbed (ND) mangrove forest while six mangrove fungal endophytic isolates were from a mangrove forest disturbed by aquaculture practices (D). These fungi are among the most prevalent taxa as described in our earlier study (Jacob et al. 2023).

Evaluation of antagonistic activities using dual culture assay

Following the protocol outlined by Pecundo et al. (2021), the 16 mangrove fungal endophytes were evaluated for ability to inhibit the mycelial growth of the fungal phytopathogen *F. oxysporum*. In this assay, fungal agar discs (6 mm in diameter) were prepared from colony margin of 7-day old cultures of the fungal pathogen and the mangrove fungal endophytes. Each mangrove fungal endophyte mycelial agar disc was placed on the left side of a 90-mm Petri plate containing half-strength Potato Dextrose Agar (PDA, Carl Roth GmbH, Karlsruhe, Germany), with the fungal pathogen disc positioned on the right side of the plate opposite of mangrove fungal endophyte. Control plates consisted of PDA inoculated with *F. oxysporum* alone, representing uninhibited pathogen growth, as employed in the original assay design of Pecundo et al. (2021). The dual cultures were replicated three times, and observations were recorded over a seven-day period. To assess the extent of mycelial growth inhibition, the radial growth of the test pathogen in both the control plate (r1) and in the dual culture plates (r2) was measured. Subsequently, the percentage mycelial growth inhibition of the antagonist was calculated using the formula $I\% = [(r1 - r2)/r1] \times 100$.

Additionally, the interactions between the cultured fungi during this confrontation assay were categorized into six types based on the degree of inhibition and growth behavior (Pecundo et al. 2021), providing insights into the dynamics of their fungal interactions. The type of interactions described in this study are as follows: Type A - where the test fungi exhibit mutual intermingling of mycelial growth with no visible signs of antagonism or inhibition; Type B - characterized by mutual inhibition upon contact or at a distance, often resulting in a very minimal clear zone, typically less than 2 mm; Type C - marked by the inhibition of one fungus upon contact, while the inhibited species continues to grow at a significantly reduced rate, and the inhibitor species may experience a slight reduction or remain unchanged in growth rate; Type D - involving mutual inhibition between fungi at a distance, leading to the production of significantly clear zones larger than 2 mm; Type E - in which one fungus inhibits another upon contact, with the inhibitor species continuing to grow at a reduced rate within the inhibited colony; and Type F - where one species inhibits another upon contact or at a distance, with the inhibitor species continuing to grow at an unchanged rate, either through or over the inhibited fungus.

Evaluation of antagonistic activities under reduced or elevated mineral content

To further elucidate the interaction between fungi as defined by their natural habitat, six mangrove fungal endophytes were selected, comprising *P. protearum* (2 isolates), *Ps. curvatispora* (2 isolates), *N. clavispora*, and *Neopestalotiopsis* sp. These isolates were deliberately chosen to represent both non-disturbed (three isolates) and disturbed (three isolates) mangrove sites, ensuring comparable species from the two habitat conditions (Table 2). A modified antagonistic challenge test between these six mangrove fungal endophytes and *F. oxysporum* was conducted as described above but with modifications. First, we used Basal Medium (BSL, composition: glucose, 10 g/L; zinc oxide, 1 g/L; ammonium sulfate, 1 g/L; potassium chloride, 0.20 g/L; dipotassium hydrogen phosphate, 0.10 g/L; magnesium sulfate, 0.20 g/L; agar, 15 g/L) instead of half-strength Potato Dextrose Agar. Second, the Basal Medium was supplemented with zinc oxide (for zinc solubilization) and tricalcium phosphate (for phosphate solubilization). Finally, we varied the concentration of zinc and phosphates to simulate reduced or elevated mineral content as would be observed in the natural environment. These modifications were informed by the findings of Jacob et al. (2023), which demonstrated that changes in zinc and phosphate availability significantly affected the growth of mangrove fungal endophytes. Elevated mineral levels were set to threefold (3×) the standard concentration, while reduced levels were set to one-third (0.33×) of the standard. For the phosphate solubilization assay, standard tricalcium phosphate (TCP) was 5 $g \cdot L^{-1}$, elevated was 15 $g \cdot L^{-1}$ (3×), and reduced was 1.67 $g \cdot L^{-1}$ (0.33×). For the zinc solubilization assay, standard zinc oxide (ZnO) was 1 $g \cdot L^{-1}$, elevated was 3 $g \cdot L^{-1}$ (3×), and reduced was 0.33 $g \cdot L^{-1}$ (0.33×). All concentrations are reported in $g \cdot L^{-1}$ and rounded to two decimal places where appropriate. Measurements of colonial growth and the type of interactions were evaluated as described previously.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics version 31 (IBM Corp. USA, https://www.ibm.com/products/spss-statistics). Percent inhibition values were expressed as mean ± standard deviation (SD). Differences between fungal endophytes isolated from disturbed (D) and undisturbed (ND) mangrove sites were evaluated using an independent-sample two-tailed t-test. A significance level of $\alpha = 0.05$ was applied. Results were interpreted as significant at $p < 0.05$ (*), highly significant at $p < 0.01$ (**), and not significant when $p > 0.05$ (ns).

Results

Our study revealed that three selected mangrove fungal endophytes, namely *Pseudopestalotiopsis curvatispora, Neopestalotiopsis clavispora*, and *Pestalotiopsis microspora*, isolated from *R. mucronata* in an undisturbed area, exhibited a Type B interaction (Table 1). This interaction involved mutual inhibition of the pathogenic fungus *Fusarium oxysporum* (Figure 1). Conversely, Type C interaction was observed for *N. egyptiaca* (isolated from a disturbed site), and *Ps. curvatispora* and *P. microspora* (both species from an undisturbed area). This type of interaction characterized by inhibition of *F. oxysporum* upon contact with the mangrove fungal endophyte while the mangrove fungal endophyte continued to grow at a significantly reduced rate. Type D interaction, characterized by mutual inhibition between fungi at a distance, resulting in clear zones larger than 2 mm, was observed in three mangrove fungal endophytes from the disturbed area, namely *P. protearum*, *Ps. curvatispora*, and *Neopestalotiopsis* sp., as well as in one strain of *P. protearum* from the undisturbed area. Notably, six mangrove fungal endophytic isolates exhibited Type E interaction (mutual inhibition upon contact followed by continued overgrowth of the endophyte). For instance, *Pseudopestalotiopsis* sp. mutually inhibited the colony of *F. oxysporum* upon contact but continued to grow at a reduced rate and subsequently advanced over the colony of *F. oxysporum*.

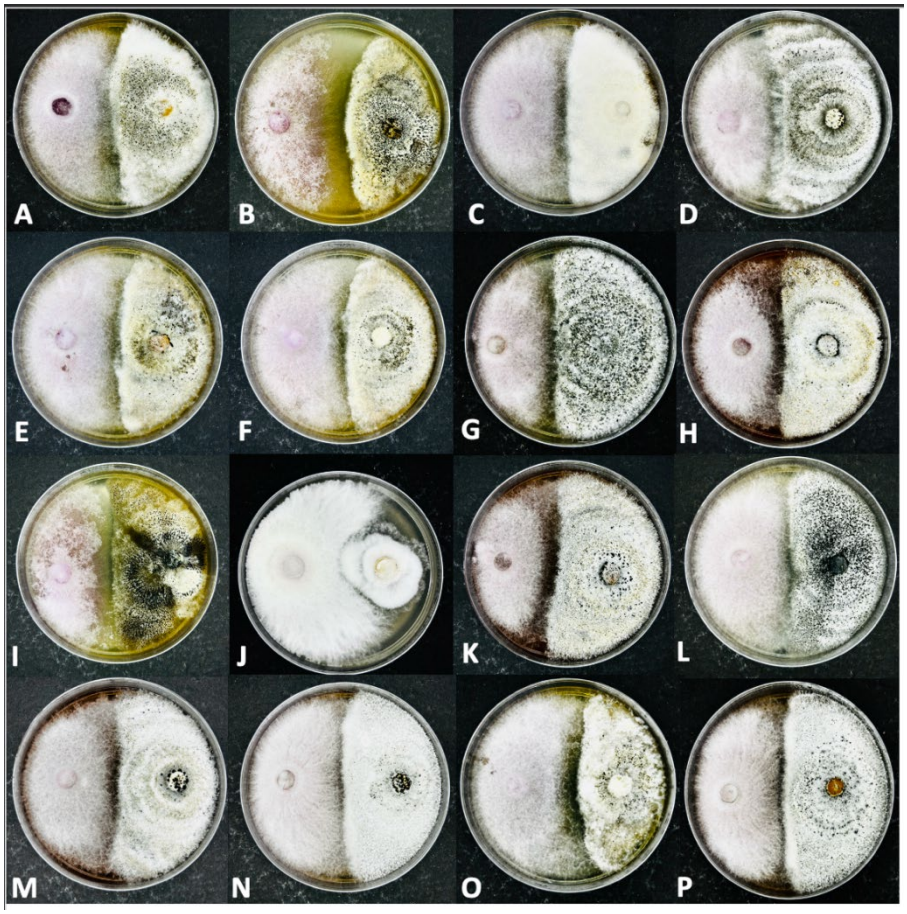

Fig. 1 – Dual culture assays showing interaction between *Fusarium oxysporum* and selected fungal endophytes. Images are labeled A–P, with A – F corresponding to D1 to D6 isolates and G – P

corresponding to ND1 to ND10 isolates as listed in Table 1: A, *N. rhizophorae* (D1); B, *N. egyptiaca* (D2); C, *P. protearum* (D3); D, *Pestalotiopsis* sp. (D4); E, *Ps. curvatispora* (D5); F, *Neopestalotiopsis* sp. (D6); G, *Pseudopestalotiopsis* sp. (ND1); H, *Pseudopestalotiopsis* sp. (ND2); I, *Ps. curvatispora* (ND3); J, *Ps. curvatispora* (ND4); K, *Pseudopestalotiopsis* sp. (ND5); L, *N. clavispora* (ND6); M, *N. clavispora* (ND7); N, *P. microspora* (ND8); O, *P. microspora* (ND9); P, *P. protearum* (ND10). Petri plate has 90-mm diameter.

Table 1. Co-cultivation assay of 16 selected mangrove fungal endophytes against *F. oxysporum* in PDA.

Isolate Code	Source	Species Name	Percent inhibition (%I) Values are mean ± SD.	[a]Type of interaction
D1	D	*N. rhizophorae*	74.28 ± 1.55	E
D2	D	*N. egyptiaca*	72.18 ± 1.85	C
D3	D	*P. protearum*	75.65 ± 0.26	D
D4	D	*Pestalotiopsis* sp.	73.79 ± 1.13	E
D5	D	*Ps. curvatispora*	74.79 ± 0.86	D
D6	D	*Neopestalotiopsis* sp.	71.69 ± 0.52	D
ND1	ND	*Pseudopestalotiopsis* sp.	82.09 ± 0.60	E
ND2	ND	*Pseudopestalotiopsis* sp.	86.40 ± 1.96	E
ND3	ND	*Ps. curvatispora*	75.36 ± 1.32	B
ND4	ND	*Ps. curvatispora*	73.44 ± 0.76	C
ND5	ND	*Pseudopestalotiopsis* sp.	80.33 ± 0.86	E
ND6	ND	*N. clavispora*	73.36 ± 1.57	B
ND7	ND	*N. clavispora*	71.98 ± 2.31	E
ND8	ND	*P. microspora*	75.07 ± 2.69	B
ND9	ND	*P. microspora*	68.92 ± 1.09	C
ND10	ND	*P. protearum*	73.72 ± 1.69	D

[a]Type of fungal interaction for each isolate against the fungal pathogen and the percentage of growth inhibition (%I) ± standard deviation (SD). Origin: D = disturbed by aquaculture practices; ND = undisturbed. Statistical differences between disturbed (D) and undisturbed (ND) isolates were determined using independent-sample *t*-test: *t = –1.50, p= 0.162* (ns, not significant).

Percent inhibition ranged from 68.92 ± 1.09% to 86.40 ± 1.96%, with the strongest inhibition observed with *Pseudopestalotiopsis* sp. from an undisturbed mangrove site (Table 1). On average, mangrove fungal endophytic isolates from disturbed (D) site recorded a mean inhibition of 73.73 ± 1.37%, while those from undisturbed (ND) site showed 76.37 ± 5.42%. An independent-sample t-test revealed no significant difference between the two groups (t = –1.50, df = 14, p = 0.162, ns), suggesting comparable antagonistic performance of isolates from both habitats.

Given the presence of minerals such as phosphate and zinc in our study sites, we challenged our selected mangrove fungal endophytes with the fungal pathogen under elevated or reduced mineral content. With a Basal Agar medium, modified by adding a trace or high amounts of tricalcium phosphate and zinc oxide corresponding to the results we obtained from our earlier water analysis, we conducted co-culture assay. We selected six mangrove fungal endophytes for this assay, as shown in Table 2.

The six mangrove fungal endophytes exhibited clear differences in their antagonistic activities against *F. oxysporum* under varying phosphate (PO_4) levels (Table 2). Under phosphate-modified conditions (Table 2), mangrove fungal endophytic isolates from disturbed site (*P. protearum*, *Ps. curvatispora*, *Neopestalotiopsis* sp.) exhibited consistently stronger inhibition of *F. oxysporum* than

those from undisturbed site. At low phosphate, mangrove fungal endophytic isolates from disturbed site showed inhibition of 78.59–82.56% (all Type C interaction), whereas mangrove fungal endophytic isolates from undisturbed site ranged from 70.69% to 77.59%, with some (*Ps. curvatispora* and *N. clavispora*) displaying Type E interaction. This difference was significant (t = 4.26, p = 0.008). At standard phosphate, inhibition values varied more widely among mangrove fungal endophytic isolates derived from disturbed mangrove site (64.09–80.28%, mainly Type E), while mangrove fungal endophytic isolates from undisturbed mangrove site clustered more narrowly (69.19–70.57%, all Type C); the difference between groups was not significant (t = 1.21, p = 0.28, ns). At high phosphate, mangrove fungal endophytic isolates from disturbed area again outperformed those from undisturbed area (76.78–82.24% vs. 70.34–72.03%), with interaction types classified as C or E; here the difference was highly significant (t = 5.12, p = 0.004). These results indicate that mangrove fungal endophytic isolates from disturbed site maintained significantly higher inhibition under both phosphate-depleted and phosphate-enriched conditions, while inhibition was comparable at standard phosphate levels.

Table 2. Co-cultivation assay of six mangrove fungal endophytes against *F. oxysporum* grown in P-modified BSL medium.

Source	Species Name	Percent inhibition (I%) Values are mean ± SD.	Type of interaction
	LOW PO_4		
D	*P. protearum*	82.56 ± 0.56	C
D	*Ps. curvatispora*	81.84 ± 1.70	C
D	*Neopestalotiopsis* sp.	78.59 ± 0.35	C
ND	*P. protearum*	70.97 ± 2.52	C
ND	*Ps. curvatispora*	70.69 ± 0.46	E
ND	*N. clavispora*	77.59 ± 0.58	E
	Low PO_4 (t = 4.26, p = 0.008, CI (D – ND) = 0.79, 15.03 **)		
	STANDARD PO_4		
D	*P. protearum*	76.83 ± 0.88	E
D	*Ps. curvatispora*	64.09 ± 2.49	E
D	*Neopestalotiopsis* sp.	80.28 ± 0.97	C
ND	*P. protearum*	70.57 ± 0.44	C
ND	*Ps. curvatispora*	69.96 ± 0.68	C
ND	*N. clavispora*	69.19 ± 1.84	C
	Standard PO_4 (t = 1.21, p = 0.28, CI (D – ND) = –9.89, 17.54 ns)		
	HIGH PO_4		
D	*P. protearum*	76.78 ± 0.95	E
D	*Ps. curvatispora*	77.35 ± 2.84	C
D	*Neopestalotiopsis* sp.	82.24 ± 0.45	C
ND	*P. protearum*	70.34 ± 1.27	C
ND	*Ps. curvatispora*	70.89 ± 1.65	E
ND	*N. clavispora*	72.03 ± 1.52	E
	High PO_4 (t = 5.12, p = 0.004, CI (D – ND) = 2.70, 12.71 **)		

The results revealed a consistent pattern: mangrove fungal endophytic isolates from disturbed site exhibited significantly stronger inhibition of *F. oxysporum* under low and high phosphate

conditions, while inhibition was comparable between mangrove fungal endophytic isolates from disturbed and undisturbed sites under standard phosphate levels. These findings suggest that phosphate availability strongly influences antagonistic interactions, with mangrove fungal endophytic isolates from disturbed area maintaining higher inhibitory capacity under nutrient-stressed or enriched conditions.

In the zinc-modified BSL medium, inhibition of *F. oxysporum* by the six mangrove fungal endophytes remained relatively stable across low, standard, and high zinc concentrations, ranging narrowly from 70.44% to 80.87%, with no consistent dose-dependent trends (Table 3). For instance, *Ps. curvatispora* (ND) showed its highest inhibition under low Zn (80.87%) but lowest at standard Zn (70.44%). Across all zinc levels, interaction types were limited to C and E, with no emergence of other categories. Statistical comparisons between MFE isolates from disturbed and undisturbed sites confirmed the absence of significant differences under low Zn ($t = –0.84$, $p = 0.418$, ns), standard Zn ($t = –1.56$, $p = 0.148$, ns), and high Zn ($t = –0.91$, $p = 0.377$, ns). These results indicate that zinc availability exerted only minor effects on antagonistic activity, as the MFE isolates from both sites maintained comparable inhibition regardless of zinc concentration.

Table 3. Six mangrove fungal endophytes against *Fusarium oxysporum* grown in Zn-modified BSL medium.

Source	Species Name	Percent inhibition (I%) Values are mean ± SD.	Type of interaction
	LOW Zn		
D	*P. protearum*	78.26 ± 1.04	C
D	*Ps. curvatispora*	74.71 ± 0.49	C
D	*Neopestalotiopsis* sp.	73.49 ± 0.97	C
ND	*P. protearum*	74.87 ± 1.30	E
ND	*Ps. curvatispora*	80.87 ± 0.43	E
ND	*N. clavispora*	78.68 ± 0.79	C
	Low Zn ($t = –0.84$, $p = 0.418$, CI (D – ND) = –8.94, 3.63 ns)		
	STANDARD Zn		
D	*P. protearum*	79.42 ± 1.47	E
D	*Ps. curvatispora*	79.90 ± 0.75	E
D	*Neopestalotiopsis* sp.	74.63 ± 1.67	C
ND	*P. protearum*	73.73 ± 0.75	E
ND	*Ps. curvatispora*	70.44 ± 0.54	E
ND	*N. clavispora*	73.17 ± 1.19	E
	Standard Zn ($t = –1.56$, $p = 0.148$, CI (D – ND) = –3.1, 9.6 ns)		
	HIGH Zn		
D	*P. protearum*	77.46 ± 1.17	C
D	*Ps. curvatispora*	74.54 ± 1.93	C
D	*Neopestalotiopsis* sp.	71.87 ± 2.46	C
ND	*P. protearum*	71.54 ± 0.63	E
ND	*Ps. curvatispora*	77.70 ± 0.65	E
ND	*N. clavispora*	71.64 ± 1.63	E
	High Zn ($t = –0.91$, $p = 0.377$, CI (D – ND) = –6.22, 8.21 ns)		

Discussion

Antibiotics and lytic enzymes are bioactive metabolites with a broad range of applications, particularly in crop–pathogen control (Mishra et al. 2020). It has been reported that endophytic microorganisms found in plants can produce novel and diverse bioactive metabolites, making them a potential source for new secondary metabolites (Burragoni & Jeon 2021, Wen et al. 2022). Consequently, there is substantial potential in exploring the bioactive compounds produced by plants, their associated microbes, and even marine organisms for the discovery of novel agrochemicals.

In our study, we conducted a co-cultivation assay to evaluate whether fungal endophytes associated with mangrove leaves could serve as potential biocontrol agents against *F. oxysporum*, the causal agent of root rot in asparagus and other economically important crops. Research conducted by Pecundo et al. (2021) underscored that fungal endophytes derived from native and endemic plants possess the capacity to produce bioactive compounds. For instance, Catambacan & Cumagun (2021) isolated fungal endophytes from weeds and assessed their antagonistic activities against *F. oxysporum* f. sp. *cubense*, the pathogen responsible for wilt in Cavendish bananas. Furthermore, Abro et al. (2019) investigated various fungal endophytes from different plant species in China for their efficacy against *F. oxysporum* f. sp. *cucumerinum*, the causal agent of cucumber wilt. Similarly, Gonzalez and Estefania (2020) successfully identified species of *Neopestalotiopsis* and *Pestalotiopsis* that effectively inhibited *Cryphonectria parasitica*.

Interestingly, in our study, several species of *Pseudopestalotiopsis* and *Pestalotiopsis* displayed strong antagonistic activity (≥70%) against *F. oxysporum*, exhibiting interaction types C and E. Interaction Type C, where colonies intermingled without overgrowth, and Type E, where one isolate consistently overgrew the other, indicate distinct competitive strategies among the isolates. This phenomenon may be attributed to the production of lytic enzymes by *Pseudopestalotiopsis*, which can degrade the cell wall of the pathogen, allowing the inhibitor species to colonize the pathogen's environment. Such interactions align with the findings reported by Hamzah et al. (2018) and Pecundo et al. (2021). Additionally, various fungal endophytes may release antibiotics during co-cultivation, potentially with fungicidal effects on specific fungi.

During the dual culture assay, we also observed that some mangrove fungal endophytic isolates, particularly *Pseudopestalotiopsis* and *Pestalotiopsis*, produced visible pigmentation at the interaction zones. While our study did not directly characterize these pigments, their occurrence is noteworthy since pigment production in fungi has been associated with ecological functions such as stress tolerance and microbial antagonism (Dufossé et al. 2014, da Costa Souza et al. 2016, Hamzah et al. 2018). Previous studies reported that fungal pigments, including melanins, phenazines, and quinones, can contribute to antifungal activity by protecting hyphae from enzymatic degradation or by exerting direct antimicrobial effects (Premalatha et al. 2012, Teixeira et al. 2012). Thus, although speculative, the pigment production observed in our assays may indicate secondary metabolite activity that complements the antagonistic responses of mangrove fungal endophytes (Afroz Toma et al. 2023, Espín-Sánchez et al. 2023). Future studies should chemically characterize these pigments to confirm their roles in antagonism.

Under unfavorable environmental conditions—such as low moisture, extreme pH, and elevated UV exposure—many fungi secrete extracellular pigments that act as protective compounds against abiotic stress and microbial competitors (Score et al. 1997, Tudor et al. 2013, Douanla et al. 2015). Recent studies confirm this adaptive role: environmental factors like nutrient limitation, temperature extremes, pH shifts, and light wavelengths significantly modulate the biosynthesis of fungal pigments such as melanins, polyketides, and carotenoids (Lin & Xu 2022, Afroz Toma et al. 2023). This response is often associated with a metabolic shift: when essential nutrients become scarce, fungal mycelia redirect their biochemical processes toward secondary metabolism rather than structural growth, leading to the production of metabolites that manifest as pigmentation (Isaac 1994). Such pigments may act as a defense mechanism, protecting the mycelia from enzymatic degradation by other microorganisms.

In our experiment, the six selected mangrove fungal endophytes exhibited distinct responses to varying phosphate and zinc levels. Phosphate limitation is known to trigger a metabolic shift from

primary to secondary metabolism in filamentous fungi, enhancing the synthesis of antifungal metabolites and hydrolytic enzymes involved in competitive interactions (Deshmukh et al. 2020. In phosphate-modified media, mangrove fungal endophytic isolates from disturbed site showed significantly stronger inhibition under both low and high phosphate conditions, while inhibition levels were comparable between site types at standard phosphate. Interaction types also varied with phosphate: Type C predominated under low phosphate, while mixed Type C/E patterns emerged under high phosphate. These results suggest that phosphate availability is a key ecological factor influencing antagonistic performance of mangrove fungal endophytes, possibly linked to their ability to produce lytic enzymes and secondary metabolites (Deshmukh et al. 2020, Bibi et al. 2020). This interpretation is consistent with previous reports that phosphate accumulation can increase host susceptibility to pathogens such as *Magnaporthe oryzae* (Campos-Soriano et al. 2020), whereas phosphate deficiency in *Verticillium dahliae* has been linked to enhanced resistance (Lou et al. 2021).

By contrast, zinc-modified media produced narrower inhibition ranges (70–81%) and similar interaction types (C and E), with no significant differences between mangrove fungal endophytic isolates from disturbed and undisturbed mangrove sites. Although some isolates showed slight fluctuations in inhibition across zinc levels, these changes were not statistically meaningful. This indicates that zinc supplementation did not markedly alter antagonistic potential, underscoring that zinc is a less important factor compared to phosphate in shaping these interactions.

Conclusively, the observed differences between mangrove fungal endophytic isolates from disturbed and undisturbed mangrove sites underscore the influence of habitat conditions on the functional capacity of mangrove fungal endophytes. Notably, mangrove fungal endophytic isolates from disturbed sites exhibited higher antagonistic activity under phosphate stress and enrichment, suggesting adaptive traits that may allow them to thrive in nutrient-variable environments. This contrasts with mangrove fungal endophytic isolates from undisturbed sites, which showed narrower inhibition ranges and more consistent interaction patterns. Such results reflect broader ecological dynamics where habitat quality and disturbance history shape endophyte function and secondary metabolism. Mangrove-derived fungi are known to be prolific producers of bioactive compounds with antimicrobial properties (Deshmukh et al. 2020), and their enzymatic and metabolic flexibility may provide resilience under fluctuating nutrient conditions (Bibi et al. 2020). Taken together, our results suggest that the ecological integrity and nutrient dynamics of mangrove habitats directly influence the antagonistic potential of associated fungal endophytes, reinforcing their value as promising candidates for sustainable biocontrol applications.

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Accessibility of data

The original data presented in this study are available. Inquiries can be directed at the corresponding author.

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