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Etiology of trunk rot in the Enano Verde del Brasil coconut and *in vitro* efficacy of fungicides in Tabasco, Mexico

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ABSTRACT

Background/Objetive. Coconut palm (*Cocos nucifera*) is an important crop in Mexico. In Tabasco, the "Enano Verde del Brasil" (CEVB) variety has been introduced to produce green fruit for coconut water; however, pathological problems of unknown etiology have been observed. The objective of this study was to identify the fungi associated with trunk rot of CEVB coconut in Tabasco, Mexico, and to conduct a comparative *in vitro* evaluation of the efficacy of systemic and contact fungicides.

Materials and Methods. Fungal isolation in PDA medium was carried out from trunk, spear, and inflorescence samples showing necrosis. The isolates were initially characterized by morphological analysis. Two of them were selected for pathogenicity tests on CEVB seedlings and identified molecularly through the analysis of partial sequences of the ITS gene (ITS1-5.8S-ITS2 of nuclear rDNA). In addition, *in vitro* bioassays were conducted to compare the efficacy of 10 systemic and contact fungicides. Each fungicide was evaluated at three concentrations (high, recommended, and low), estimating their effect on spore germination (SG) and mycelial growth (MG).

Results. The isolates were identified as *Thielaviopsis paradoxa*. The pathogenicity tests confirmed *T. paradoxa* as the causal agent of necrosis in seedlings. The systemic fungicides propiconazole, carbendazim, and tebuconazole showed complete inhibition of MG and SG starting from the lowest dose evaluated (0.45×10^3, 1×10^3, and 0.5×10^3 ppm, respectively). The protectant fungicides copper hydroxide and captan completely inhibited SG at the lowest dose (1×10^3 and 1.5×10^3 ppm, respectively), although they only reached 80% inhibition of MG.

Conclusion. *T. paradoxa* is reported for the first time on CEVB in Mexico. This study expands the knowledge on fungi affecting CEVB in Tabasco and provides a basis for the selection of promising fungicides, whose efficacy should be validated in subsequent field studies.

Keywords: Carbendazim, *Cocos nucifera,* Propiconazole, Tebuconazole, *Thielaviopsis paradoxa*

INTRODUCTION

The coconut (*Cocos nucifera*) is a pantropical palm established in more than 80 countries. This fruit crop ranks among the ten most widely cultivated in the world, covering 11 million hectares. Mexico is considered one of the ten leading coconut-producing countries, with a planted area of 124.8 thousand hectares (FAOSTAT, 2024). In Tabasco, coconut cultivation has been one of the main agricultural activities (Ramos-Hernández *et al.*, 2018). In 2023, Tabasco produced 10 696 tons of coconut, equivalent to 4.4% of national production (SIAP, 2024). The versatility of *C. nucifera* makes it one of the most important plants, as nearly all its parts can be utilized—from the roots for medicinal purposes, the stem as a timber resource, and the leaves for handicrafts, to the flowers and fruits as food, and the fiber and shell of the fruit for manufacturing alternative materials for the automotive industry and for making kitchen utensils and crafts (Granados-Sánchez and López-Ríos, 2002; Montufar-Marcalla and Remache-Coyago, 2021). Nevertheless, the nut is the main raw material, used to obtain oil, biodiesel, nutraceutical products, coconut flour, copra, and shell (Marquínez-Marquínez *et al.*, 2020).

As with other tropical crops of global importance, coconut production has declined due to the incidence of pests and diseases. In the Caribbean and Gulf of Mexico, the most devastating disease affecting coconut-growing regions has been lethal yellowing (LYD), caused by *'Candidatus Phytoplasma palmae'* (Ortiz *et al.*, 2024). In Tabasco, besides LYD, other coconut diseases have been reported, including red ring (*Bursaphelenchus cocophilus*), wilt (*Thielaviopsis paradoxa* syn. *Ceratocystis paradoxa*), and bud rot (*Phytophthora* sp.) (Moscoso-Ramírez *et al.*, 2002). This situation has highlighted the need for further research on this crop to help restore the region's productive potential. Several coconut hybrids are currently being evaluated in Tabasco for their resistance to lethal yellowing. The variety "Enano Verde del Brasil" (CEVB) has been introduced for phytosanitary evaluation, with the aim of producing green fruit for coconut water. However, in Tabasco, as in other Mexican states, this variety has shown phytosanitary problems whose causes remain unknown.

One of the main health problems affecting adult CEVB plants is progressive wilting and trunk rot at the crown level, which leads to plant death. Symptoms also include yellowing, defoliation, necrosis of the bud, collapse of the spear leaf, and fruit abortion. Since no studies have yet identified the causal agent(s) of this disease in CEVB in Tabasco, the objective of this research was to identify the fungi associated with trunk rot of the "Enano Verde del Brasil" coconut in Tabasco, Mexico, and to perform a comparative *in vitro* evaluation of the efficacy of systemic and contact fungicides. The results of this study expand current knowledge of the fungal pathogens associated with this coconut variety and help guide the selection of fungicidal alternatives for future integrated management studies.

MATERIALS AND METHODS

Collection site. Necrotic tissue samples were collected from wilted palms in a CEVB plantation located in Ranchería Ojoshal, Cárdenas, Tabasco (18°14′057″ N, 94°00′18″ W). The plantation covers an area of three hectares and is five years old. From four diseased, fruit-bearing palms, samples were taken of trunk tissue with internal necrosis at the crown level, necrotic bud tissue, and necrotic inflorescence tissue. Each sample was placed separately in polyethylene bags and transported to the Laboratorio de Fitosanidad of the

División Académica de Ciencias Biológicas, Universidad Juárez Autónoma de Tabasco, for processing.

Isolation and purification. Fragments of plant material showing necrotic symptoms were cut into 5 × 5 mm sections, washed with sterile distilled water (SDW), and surface-disinfested with 2% sodium hypochlorite for 2 min. The samples were rinsed with SDW, allowed to dry for 30 min, and, under aseptic conditions, ten sections of diseased tissue were placed in Petri dishes containing potato dextrose agar (PDA) medium. The dishes were incubated at 25 ± 1 °C in darkness for 5 days to promote mycelial growth. Subsequently, a fragment of culture medium with fungal growth was transferred to another Petri dish with PDA to isolate different morphotypes. From each polysporic isolation, monospore isolates were obtained.

Morphological identification. Fungi were identified to the species level based on their reproductive structures and the descriptions provided by Soytong *et al.* (2015) and Tzeng *et al.* (2010). For this purpose, isolates were grown in Petri dishes containing PDA medium for 14 days. In addition, a spore suspension of 5×10^6 was inoculated onto 8-mm PDA disks to obtain microscopic structures of each isolate. All inoculated disks were incubated at 25 ± 1 °C under a 12-h light/dark photoperiod in a humid chamber for 5 days. Morphological characteristics were observed under a light microscope. Photodocumentation and micrometry were performed using ZEN lite® software.

Molecular identification and phylogeny. Genomic DNA was extracted from the mycelium of two isolates (M2-18 and M3-16) following the CTAB method (Doyle and Doyle, 1990). DNA concentration was estimated by spectrophotometry (NanoDrop 2000, Thermo Scientific®). Partial sequences of the ITS gene (ITS1-5.8S-ITS2 region of nuclear rDNA) were obtained and amplified by PCR. Amplification was performed using the universal primers ITS5 (5'-GCA AGT AAA AGT CGT AAC AAG G-3') and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3'). PCR conditions were as follows: 94 °C for 2 min; 40 cycles of 94 °C for 30 s, 55 °C for 45 s, and 72 °C for 90 s; with a final extension at 72 °C for 4 min. Amplification reactions were carried out in 25 µL volumes containing 1× buffer (5 µL), 1.5 mM $MgCl_2$ (2 µL), 0.2 mM dNTPs (2 µL), 0.4 µM reverse primer (IDT) (2 µL), 0.4 µM forward primer (IDT) (2 µL), 1 unit of Taq DNA polymerase (Promega) (0.2 µL), Milli-Q^2-grade water (9.8 µL), and 20 ng of DNA (2 µL). DNA quantification was performed by spectrophotometry (NanoDrop 2000, Thermo Scientific®). Amplicons of 512 and 532 bp were sequenced at Psomagen (USA). The resulting sequences were compared with those in the National Center for Biotechnology Information (NCBI) GenBank database using the BLAST tool.

Sequences were aligned using MUSCLE (Edgar, 2004) implemented in MEGA-X (Kumar *et al.*, 2018) and concatenated with SeaView 4.7 (Gouy *et al.*, 2010) for phylogenetic analysis. To identify the most appropriate nucleotide substitution model, jModelTest v2 (Darriba *et al.*, 2012) was used. Phylogenetic analysis was performed with IQ-TREE v2 (Minh *et al.*, 2020) using the Maximum Likelihood algorithm. This analysis was conducted through the CIPRES Science Gateway v3.3 (Miller *et al.*, 2011). Phylogenetic trees were visualized and edited with iTOL (Letunic *et al.*, 2021). Final species identification was based on morphological, morphometric, and molecular characteristics. Accepted synonymy followed Index Fungorum (www.indexfungorum.org) and MycoBank (www.mycobank.org).

Pathogenicity test on *Cocos nucifera* seedlings. For the pathogenicity tests, isolates M2-18 and M3-16 and three-month-old CEVB seedlings obtained under controlled nursery conditions were used. Three plants were inoculated per isolate, and the experiment was conducted in duplicate. A suspension of 1×10^6 spores mL^{-1} of each isolate was prepared and applied by infiltration 5 cm above the base of each seedling. The plants were then placed in humid chambers at 25 ± 1 °C under a 12-h light/dark photoperiod for three days. Observations were made every three days for 20 days, and symptoms at the inoculation sites were recorded. In addition, three plants were used as controls, which were infiltrated only with SDW.

Fungicide efficacy assays. Systemic and protectant fungicides were tested to preliminarily evaluate their comparative efficacy under *in vitro* conditions. The effectiveness of the fungicides was assessed based on spore germination (SG) and mycelial growth (MG) inhibition of two *T. paradoxa* isolates (M2-18 and M3-16). The assays were performed using poisoned plates (90 × 15 mm) following the method of Ayika *et al.* (2024). Since no fungicides are specifically registered for the control of *T. paradoxa* in coconut, the products evaluated were selected based on their recommended use against fungal diseases in tropical fruit crops such as cacao, citrus, and avocado, and against related pathogens. Commercial availability was also considered. Fungicide concentrations were calculated from the manufacturer's recommended field dose and expressed in parts per million (ppm). Each commercial fungicide was added to the medium at 50 °C before pouring into Petri dishes. Three doses per fungicide were tested: the manufacturer's recommended dose, one higher dose, and one lower dose (Table 1). All doses were expressed in units of $\times 10^3$ ppm.

Table 1. Fungicides and doses evaluated *in vitro* against *Thielaviopsis paradoxa.*

Active Ingredient	Commercial Name	Manufacturer	Mode of Action	Dose (ppm)[y]		
				0.5x	1 [z]	1.5x
Oil of *Melaleuca alternifolia*	Timorex gold	Syngenta	Protective	$0.83x10^3$	$1.66x10^3$	$2.49x10^3$
Copper oxychloride	Oximet	Cuprosa	Protective	$1.5x10^3$	$3x10^3$	$4.5x10^3$
Captan	Captan 500	Mexfer	Protective	$1.5x10^3$	$3x10^3$	$4.5x10^3$
Copper hydroxide	Hidromet	Cuprosa	Protective	$1x10^3$	$2\ x10^3$	$3x10^3$
Propiconazole	Tilt 250	Syngenta	Systemic	$0.46x10^3$	$0.93x10^3$	$1.4x10^3$
Difenoconazole + cyprodinil	Inspire gold	Syngenta	Systemic	$0.21+0.62x10^3$	$0.43+1.25\ x10^3$	$0.65+1.87x10^3$
Tebuconazole	Folicur 250	Bayer	Systemic	$0.5x10^3$	$1x10^3$	$1.5x10^3$
Carbendazim	Prozycar	AgroLucava	Systemic	$1x10^3$	$2x10^3$	$3\ x10^3$
Thiabendazole	Tecto 60	Syngenta	Systemic	$0.12x10^3$	$0.24x10^3$	$0.36x10^3$
Mandipropamid + mefenoxam	Pergado cocoa	Syngenta	Systemic	$0.31+0.25x10^3$	$0.62+0.5\ x10^3$	$0.93+0.75x10^3$

[y]ppm = parts per million; [z]Commercial dose.

To evaluate the effectiveness of the fungicides on SG, 10-day-old fungal cultures were used to prepare a 5×10^6 spores mL^{-1} suspension. Petri dishes containing PDA medium with the selected fungicide concentrations were divided into four equal sections. In the

center of each quadrant, 30 µL of the spore suspension was deposited, and each aliquot was covered with a sterile coverslip to facilitate observation. The dishes were incubated at 25 ± 0.5 °C. Five replicates per dose were established, and a control treatment with five replicates under the same experimental conditions was included. The germination percentage was determined by observing 100 spores per treatment and replicate. Observations were made every 2 h during the first day and subsequently every 24 h, concluding when the control treatment reached 90% germination. This experiment was conducted in duplicate.

To evaluate the effectiveness of the fungicides on MG, 5-mm-diameter fragments were taken from the margins of 10-day-old fungal cultures. These disks were placed at the center of Petri dishes containing PDA medium with the corresponding fungicide concentrations. Five replicates per dose were established, and a control treatment with five replicates was included. The dishes were incubated at 25 ± 0.5 °C. Mycelial growth was recorded every 24 hours by measuring colony diameter in two perpendicular directions per dish, using the inoculation point as a reference. Measurements were concluded when the control treatment completely covered the surface of the medium. The experiment was conducted in duplicate.

Statistical analysis. Based on the SG and MG data, fungicide effectiveness was calculated using Abbott's formula (1925), obtaining the percentage of mycelial growth inhibition (MGI) and spore germination inhibition (SGI). Percentage data for MGI and SGI were transformed using the arcsine square root of the proportion to meet normality assumptions. The transformed data were then subjected to ANOVA. When significant differences were detected ($P \leq 0.05$), a multiple mean comparison test was performed using Tukey's method ($P \leq 0.05$). All analyses were conducted using SAS software, version 9.0 (SAS Institute, Cary, NC, USA).

RESULTS

Description of field symptoms. The first symptoms observed were necrotic lesions on the sheaths of the basal leaves, specifically at their point of attachment to the trunk (Figure 1A). This necrosis progressively extended along the rachis and was accompanied by yellowing, wilting, and gradual leaf death (Figure 1F). Affected leaves bent just above the necrotic area (Figure 1A) and remained attached to the trunk, forming a "skirt" of leaves (Figure 1F), which could be easily detached. As necrosis advanced upward, younger leaves became affected, resulting in progressive, ascending wilting (Figure 1F). Most leaves eventually collapsed and bent downward, leaving only the crown leaves in an upright position. On the trunk (stipe), at crown height, necrotic tissue was observed at the points of leaf insertion (Figure 1B), extending into the inner part of the stem (Figure 1C). In plants with advanced wilting, fermentation of the bud (heart) and necrosis of the spear leaf were detected (Figure 1D). As the bud disintegrated, the crown collapsed, even though the terminal leaves remained green (Figure 1G). After crown collapse, the dry leaves detached, and the trunk began to rot downward (Figure 1H). During disease progression, necrosis also appeared on spathes and inflorescences (Figure 1E), along with fruit abortion at different developmental stages. No exudation or bleeding symptoms were observed on the stem.

Figure 1. Symptoms associated with trunk rot of *Cocos nucifera* "Enano Verde del Brasil" on the coast of Tabasco, Mexico. **A)** Necrosis on the sheath of basal leaves at the point of attachment to the trunk; **B)** Necrosis at the leaf insertion point on the stipe; **C)** Progression of necrosis toward the interior of the trunk; **D)** Necrosis of the spear leaf; **E)** Necrosis on spathes and inflorescences; **F)** Wilted leaves and "skirt" of leaves attached to the trunk; **G)** Crown collapse with terminal leaves still green; **H)** Downward trunk rot.

Morphological identification. Five fungal isolates were obtained from necrotic tissues of four adult CEVB plants. Three isolates were obtained from the trunk (M2-18, M2-14, M2-07), one from the bud (M3-16), and one from the inflorescence (M3-02). The isolates developed colonies with dark gray to black mycelium on PDA medium under dark conditions at 25 °C (Figure 2A). When grown on PDA disks in a humid chamber at 25 ± 1 °C under a 12-h light/dark photoperiod, the isolates produced conidiophores, generally single and straight, ranging in color from hyaline to light brown (Figure 2B). The conidiophores produced primary and secondary phialoconidia in chains, cylindrical to oval in shape, hyaline to light brown, with thick walls and smooth surfaces. Their dimensions were 5.0–14.1 µm in length and 3–6 µm in width, with an average of 8.4 ± 1.5 × 4.1 ± 0.7 µm and a length-to-width ratio of 2 (n = 100) (Figure 2C, D, E). The aleuroconidia were oval, dark brown, and thick-walled. Their size ranged from 11.7–22.2 µm in length and 7.7–12.2 µm in width, with an average of 16.3 ± 3.2 × 9.6 ± 1.1 µm and a length-to-width ratio of 1.6 (n = 100) (Figure 2F, G). These morphological characteristics are consistent with those described by Soytong *et al.* (2015) and Tzeng *et al.* (2010) for the species *Thielaviopsis paradoxa*.

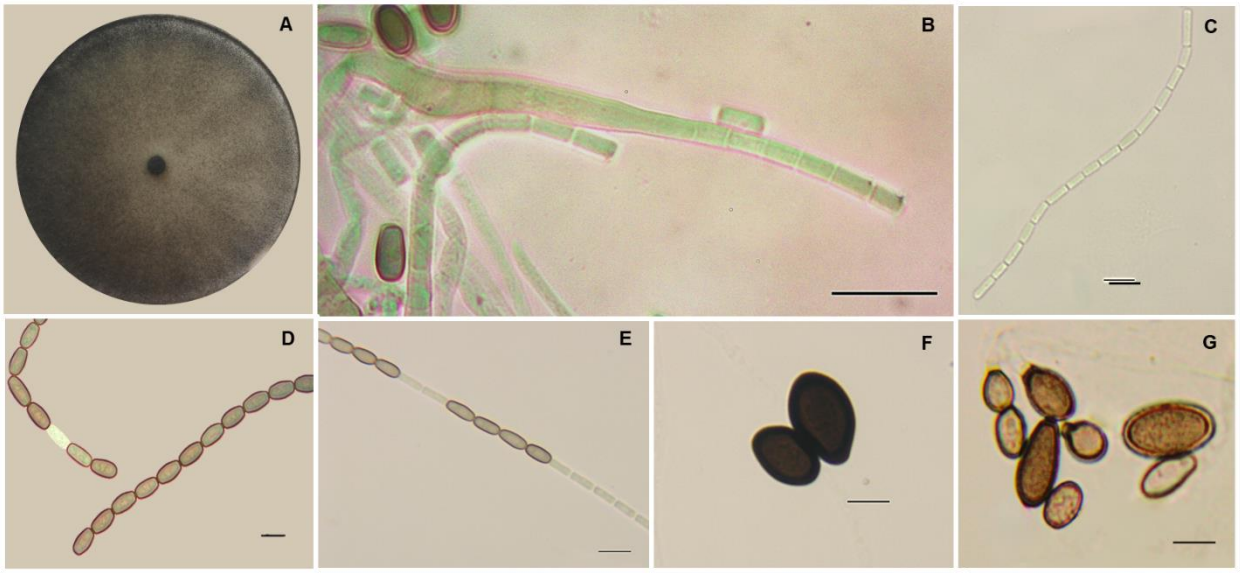

Figure 2. Morphological characteristics of *Thielaviopsis paradoxa.* **A)** Fifteen-day-old colony grown on PDA medium; **B)** Conidiophores; **C)** Primary phialoconidia in chains; **D, E)** Primary and secondary phialoconidia in chains; **F, G)** Aleuroconidia. Scale bars: **B** = 20 µm; **C**, **D**, **E**, **F**, **G** = 10 µm.

Molecular identification and phylogeny. The ITS region sequences of isolates M2-18 (532 bp, GenBank accession number: PQ643222) and M3-16 (512 bp, GenBank accession number: PQ643223) showed 99.8% and 100% identity, respectively, with sequences reported for *T. paradoxa* (GU358207, GU358206) from *C. nucifera* in Taiwan (Tzeng *et al.*, 2010). The phylogenetic tree constructed for *T. paradoxa* isolates M2-18 and M3-16 showed significant maximum-likelihood support values separating our isolates from other *Thielaviopsis* species and grouping them with *T. paradoxa* (*Ceratocystis paradoxa*) (Ceratocystidaceae) (Figure 3).

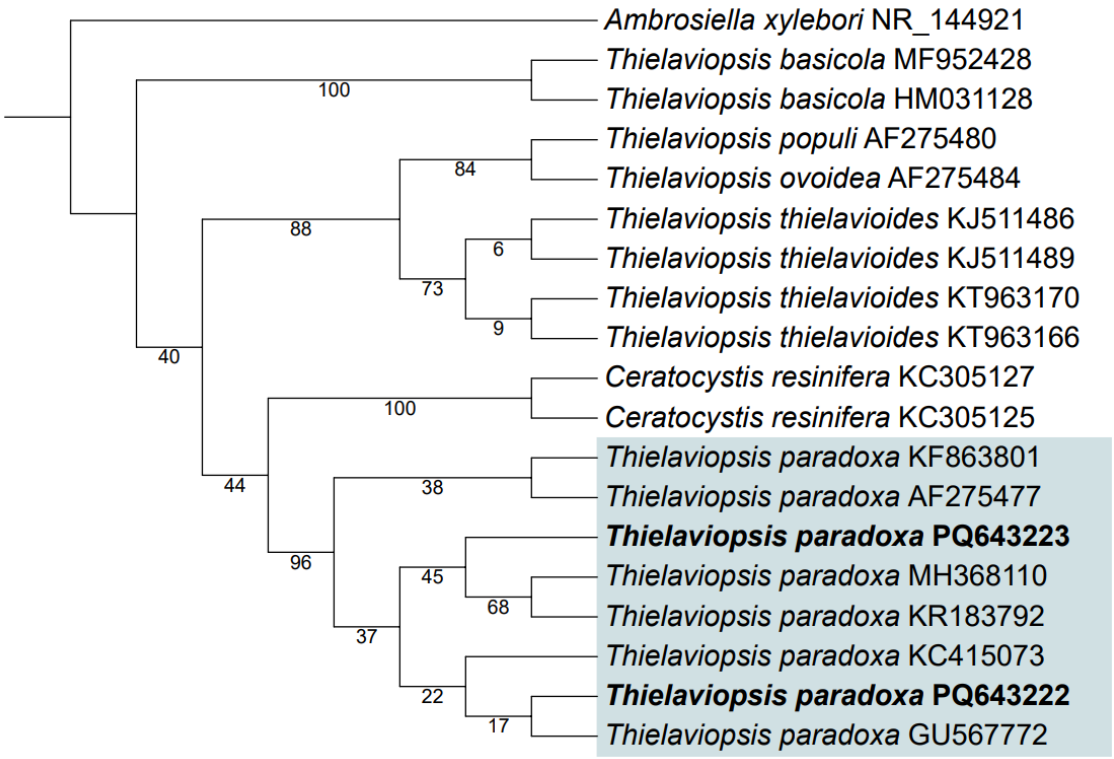

Figure 3. Maximum-likelihood phylogenetic tree constructed using a dataset of partial sequences of the internal transcribed spacer regions 1 and 2 (ITS1 and ITS2) and the intervening 5.8S rDNA subunit for isolates M2-18 (GenBank accession number: PQ643222) and M3-16 (GenBank accession number: PQ643223) of *Thielaviopsis paradoxa*, obtained from trunk and bud samples of *Cocos nucifera* "Enano Verde del Brasil" exhibiting trunk rot on the coast of Tabasco, Mexico. Sequence data for other *Thielaviopsis* (*Ceratocystis*) strains and species were obtained from GenBank.

Pathogenicity test on *Cocos nucifera* seedlings. The pathogenicity test conducted on CEVB seedlings confirmed the pathogenicity of the two evaluated *T. paradoxa* strains (M3-16 and M2-18), isolated from necrotic stem and bud tissues. The seedlings exhibited progressive internal necrosis starting from the inoculation site. Necrotic lesions extended upward toward the bud and downward along the seedling, causing wilting 15 days after inoculation (Figure 4A, B). Seedlings from the control treatment showed no visible disease symptoms during the evaluation period (Figure 4C). The inoculated isolates were successfully re-isolated from necrotic tissues, fulfilling Koch's postulates.

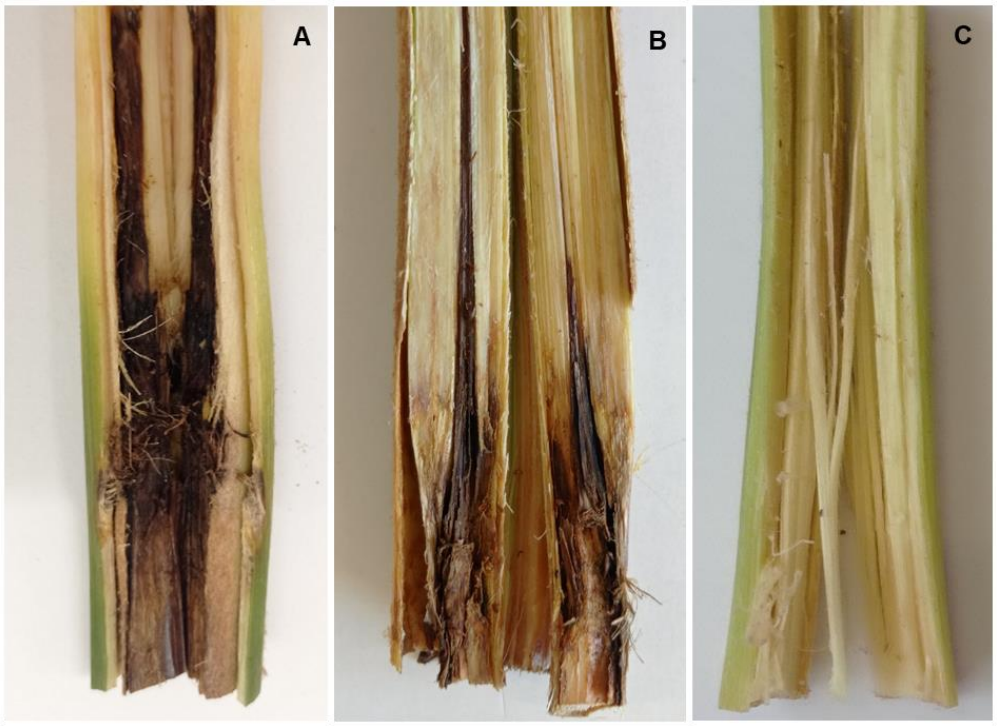

Figure 4. Pathogenicity tests on coconut seedlings "Enano Verde del Brasil". **A, B**) Necrosis caused by *Thielaviopsis paradoxa*, strains M2-18 and M3-16, respectively. **C**) Control.

***In vitro* evaluation of fungicides on growth and germination.** All ten fungicides, both systemic and contact, were effective against the two *T. paradoxa* isolates at the different doses tested (Table 2). Significant differences ($P < 0.0001$) were found among fungicides in both mycelial growth inhibition (MGI) and spore germination inhibition (SGI) of *T. paradoxa* strain M2-18. The systemic fungicides propiconazole, carbendazim, and tebuconazole inhibited 100% of both mycelial growth and spore germination at all three evaluated doses. Similarly, thiabendazole and difenoconazole + cyprodinil achieved total inhibition of mycelial growth at all doses, although they reached 100% inhibition of germination only at the highest dose. No significant differences were observed at the medium dose (Table 2). In contrast, the systemic fungicide mandipropamid + mefenoxam showed no effectiveness against the mycelial growth of *T. paradoxa* (M2-18) at any of the tested doses (Figure 5F); however, it achieved spore germination inhibition ranging from 40 to 97%.

Regarding the effectiveness of the protectant fungicides against *T. paradoxa* (M2-18), *Melaleuca alternifolia* oil and copper hydroxide inhibited 100% of mycelial growth at the highest evaluated dose (Table 2). Captan showed 81–83% inhibition of mycelial growth at all three doses, while copper oxychloride reached 81% inhibition only at the highest dose. In addition, captan and copper hydroxide achieved 100% inhibition of spore germination at all doses. The protectant fungicides with the lowest effectiveness for both mycelial growth (MG) and spore germination (SG) were *M. alternifolia* oil and copper oxychloride (Figure 5G). For *T. paradoxa* strain M3-16, significant differences ($P < 0.0001$) were observed in fungicide effectiveness for both MG and SG. Nevertheless, the response to systemic and contact fungicides was similar to that observed for strain M2-18 (Table 2).

Table 2. Effectiveness of systemic and contact fungicides on mycelial growth and conidial germination of two *Thielaviopsis paradoxa* strains (M2-18 and M3-16) isolated from *Cocos nucifera*, var. Enano Verde del Brasil, on the coast of Tabasco, Mexico.

Fungicide	Dose (ppm)[x]	Strain M2-18		Strain M3-16	
		ICM (%)[y]	IGE (%)[y]	ICM (%)[y]	IGE (%)[y]
Control	0	----[z]	----[z]	----[z]	----[z]
Propiconazole	0.46x10^3	100 ±0a	100 ±0a	100 ±0a	100 ±0 a
	0.93x10^3	100 ±0 a	100 ±0a	100 ±0a	100 ±0a
	1.40x10^3	100 ±0 a	100 ±0a	100 ±0a	100 ±0a
Thiabendazole	0.12x10^3	100 ±0 a	77.7 ±6c	100 ±0a	84.7 ±6.4cd
	0.24x10^3	100 ±0a	98.2 ±2ab	100 ±0a	100 ±0a
	0.36x10^3	100 ±0a	100 ±0a	100 ±0a	100 ±0a
Carbendazim	1x10^3	100 ±0a	100 ±0a	100 ±0a	100 ±0a
	2x10^3	100 ±0a	100 ±0a	100 ±0a	100 ±0a
	3x10^3	100 ±0a	100 ±0a	100 ±0a	100 ±0a
Tebuconazole	0.5x10^3	100 ±0a	100 ±0a	100 ±0a	100 ±0a
	1x10^3	100 ±0a	100 ±0a	100 ±0a	100 ±0a
	1.5x10^3	100 ±0a	100 ±0a	100 ±0a	100 ±0a
Difenoconazole + cyprodinil	0.21+ 0.62 x10^3	100 ±0a	92.9 ±2.9b	100 ±0a	67.8 ±10.1def
	0.43+1.25 x10^3	100 ±0a	96.4 ±4.1ab	100 ±0a	84.7 ±6.4cd
	0.65+1.87 x10^3	100 ±0a	100 ±0a	100 ±0a	93.2 ±3.9bc
Mandipropamid + mefenoxam	0.31+0.25 x10^3	0 ±0f	40.2 ±11.4d	0 ±0g	44.1 ±8.5gh
	0.62+0.5 x10^3	0 ±0f	50.9 ±6d	0 ±0g	63.6 ±5.7efg
	0.93+0.75 x10^3	0 ±0f	97.3 ±3.4ab	0 ±0g	98.3 ±3.3ab
Copper oxychloride	1.5 x10^3	59.6 ±5.6e	22.3 ±12.8e	39.3 ±2.4f	44.1 ±26.1gh
	3 x10^3	58.7 ±2.4e	38.4 ±6de	42.6 ±1.4f	46.6 ±9.7fg
	4.5 x10^3	81.0 ±1.4c	79.5 ±3.4c	84 ±0.7c	58.5 ±8.4fg
Captan	1.5 x10^3	81.6 ±1.4c	100 ±0a	77.4 ±2.2d	100 ±0a
	3 x10^3	83.6 ±2.0c	100 ±0a	84 ±1.2c	100 ±0a
	4.5 x10^3	82.6 ±0.9c	100 ±0a	84.7 ±1.7c	100 ±0a
Oil of *Malaleuca alternifolia*	0.83 x10^3	1.2 ±2.5f	1.78 ±2f	0 ±0g	25.4 ±14.6h
	1.66 x10^3	71.3 ±6.9d	4.46 ±5.3f	68.3 ±9.5e	59.3 ±7.3fg
	2.49 x10^3	100 ±0 a	32.1 ±9.2de	100 ±0a	83.1 ±4.7cde
Copper hydroxide	1x10^3	84.2 ±0.1 c	100 ±0a	86.4 ±1.9c	100 ±0a
	2x10^3	89.7 ±0.1b	100 ±0a	90.8 ±0.7b	100 ±0a
	3x10^3	100 ±0a	100 ±0a	100 ±0a	100 ±0a

ICM = Mycelial growth inhibition; IGE = Spore germination inhibition; x ppm = parts per million; y Means followed by the same letter within the same column are not significantly different (Tukey, $P \leq 0.05$); z Due to the nature of Abbott's formula, these values were not calculated.

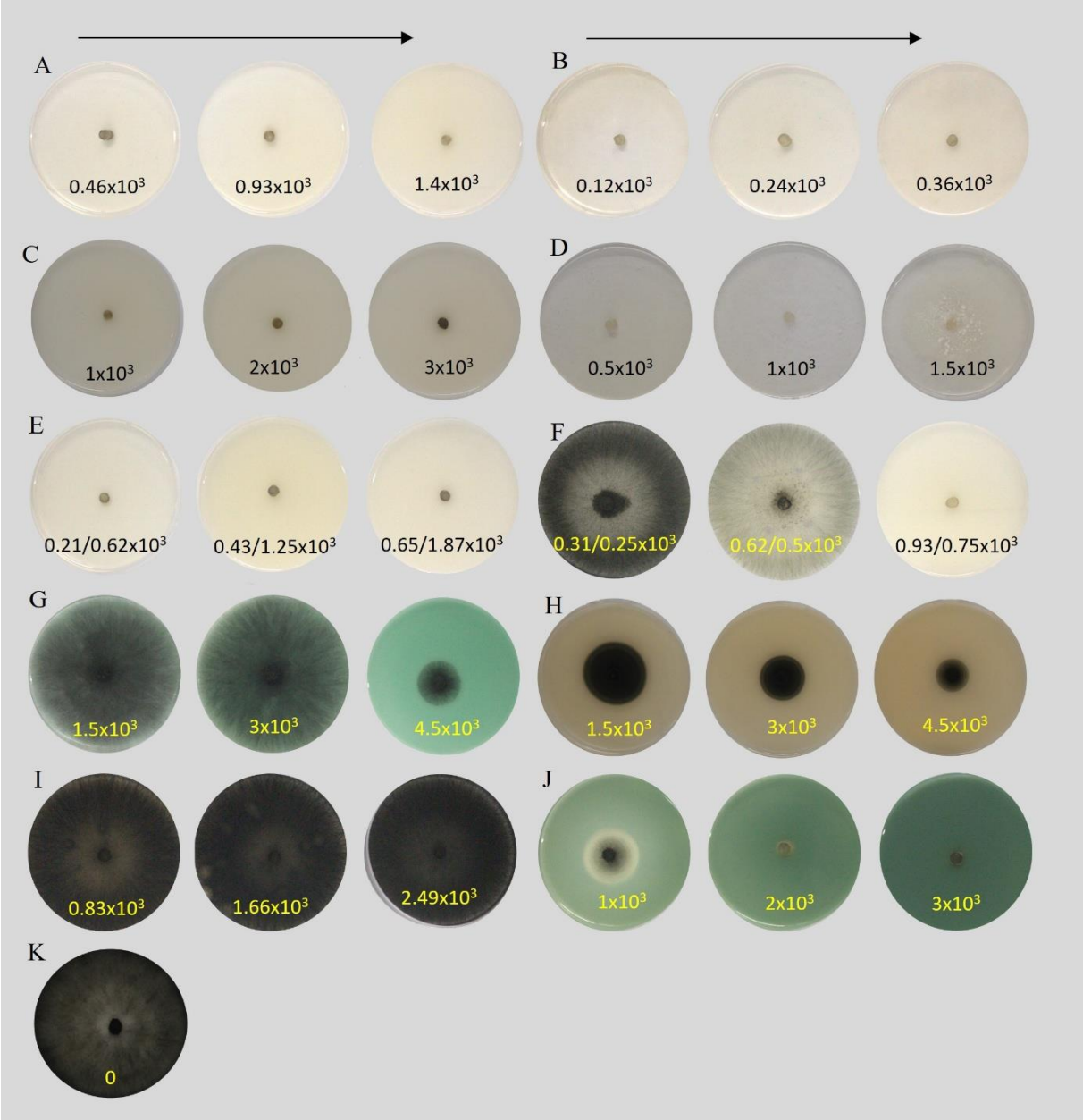

Figure 5. *In vitro* effect of systemic and contact fungicides on *Thielaviopsis paradoxa.* **A)** Medium with propiconazole; **B)** Medium with thiabendazole; **C)** Medium with carbendazim; **D)** Medium with tebuconazole; **E)** Medium with difenoconazole + cyprodinil; **F)** Medium with mandipropamid + mefenoxam; **G)** Medium with copper oxychloride; **H)** Medium with captan; **I)** Medium with *Melaleuca alternifolia* oil; **J)** Copper hydroxide; **K)** Control. Numbers within each image indicate the dose in parts per million (ppm). All doses are expressed in units of $\times 10^3$.

DISCUSSION

Characterization of the fungal isolates collected from CEVB palms showing trunk rot symptoms revealed the presence of *Thielaviopsis paradoxa*. This species has previously been reported as a pathogen of *C. nucifera* in Mexico (Moscoso-Ramírez *et al.*, 2002). However, this is the first report of *T. paradoxa* causing wilt in the "Enano Verde del Brasil" coconut variety in Mexico. *Thielaviopsis paradoxa* has been described as the causal agent of the "stem bleeding" disease of *C. nucifera* in various parts of the world, and this disease is considered economically important in coconut production (Tzeng *et al.*, 2010; Yu *et al.*,

2012). According to Ayika *et al.* (2024), *T. paradoxa* is a fungal pathogen responsible for causing trunk and bud rot in palms and can colonize all palm tissues, including roots, trunk, leaves, inflorescences, and fruit. In the present study, *T. paradoxa* was isolated from trunk, bud, and inflorescence tissues, with the highest frequency of occurrence in the trunk.

In Brazil, Warwick and Passos (2009) documented an outbreak of "stem bleeding" disease in dwarf coconut, identifying *T. paradoxa* as the causal agent. In Tabasco, Mexico, Moscoso-Ramírez *et al.* (2002) reported the incidence of coconut wilt caused by *Ceratocystis* sp. in the "Alto del Pacífico" and "Enano Malayo" varieties, as well as in a hybrid derived from these two progenitors. Most of the symptoms observed in the present study coincide with those described by Moscoso-Ramírez *et al.* (2002) and Warwick and Passos (2009); however, in this study, the "stem bleeding" symptom was not observed. Recently, Gangadhara-Naik *et al.* (2022) identified *T. paradoxa* as the causal agent of inflorescence necrosis in *C. nucifera* in India; this symptomatology had already been reported in Brazil in 1986. Tzeng *et al.* (2010) also reported *Ceratocystis paradoxa* causing basal rot of immature *C. nucifera* fruits under field conditions in Taiwan; however, necrosis and abortion of immature fruits in the field had already been described in Brazil in 1985. Pinho *et al.* (2013) confirmed the presence of *C. paradoxa* (*T. paradoxa*) as the causal agent of internal rot in immature *C. nucifera* fruits during postharvest in Brazil. According to these authors, *T. paradoxa* had already been detected in the sap of *C. nucifera* and may represent a route for the pathogen's dissemination to inflorescences and immature fruits.

According to Yu *et al.* (2012) and Ayika *et al.* (2024), *T. paradoxa* requires wounds to enter the tissue and cause infection, which is an important consideration for proper plantation management. Practices that minimize plant injury during handling, avoid excessive pruning, and eliminate inoculum sources are therefore essential. However, it is also necessary to determine the effectiveness of fungicides against *T. paradoxa* to support the development of effective disease management strategies (Ayika *et al.*, 2024).

The efficacy test of systemic and contact fungicides against *T. paradoxa* strains isolated from *C. nucifera* revealed that the systemic fungicides propiconazole, carbendazim, and tebuconazole were the most effective, achieving 100% inhibition of both mycelial growth (MG) and spore germination (SG) at all three evaluated doses. Similarly, the systemic fungicides thiabendazole and difenoconazole + cyprodinil achieved 100% inhibition of MG and SG starting from the commercial dose.

The effectiveness of tebuconazole observed in this study is consistent with the findings of Coelho *et al.* (2010), who reported 100% *in vitro* efficacy of this fungicide in inhibiting the mycelial growth (MG) of *T. paradoxa* isolated from *C. nucifera* in Brazil. In the present work, tebuconazole was effective even at doses lower than the commercial rate and also proved effective in inhibiting spore germination (SG). Likewise, the high efficacy of propiconazole agrees with the results of Irabi *et al.* (2018), who reported 100% inhibition of MG by this fungicide against *T. paradoxa* isolated from date palm (*Phoenix dactylifera*) in Sudan. In this study, propiconazole also demonstrated effectiveness at doses below the commercial rate and effectively inhibited SG. These fungicides are effective at low concentrations, as confirmed in the present research. Both propiconazole and tebuconazole belong to the triazole chemical group, which disrupts cell membrane formation by inhibiting methylation in the biosynthesis of ergosterol (Marzi *et al.*, 2022).

The results obtained with carbendazim are consistent with those of Nambiar and Iyer (1991), who reported 100% inhibition of *T. paradoxa* mycelial growth (MG) isolated from *C. nucifera* in India. The dose reported by these authors (0.01 g a.i. L^{-1}) was lower than

those evaluated in the present study. Martínez *et al.* (1997) also reported the effectiveness of carbendazim in controlling the bud rot complex (*T. paradoxa*) of oil palm (*Elaeis guineensis*) under field conditions in Colombia. According to Zhou *et al.* (2023), carbendazim is a systemic fungicide belonging to the benzimidazole chemical group that acts by inhibiting nuclear division and β-tubulin synthesis, thereby halting cell multiplication and fungal growth.

The efficacy of thiabendazole observed in this study is also consistent with the findings of Coelho *et al.* (2010), who reported 100% inhibition of *T. paradoxa* mycelial growth (MG) isolated from *C. nucifera*. Furthermore, this study demonstrated the effectiveness of this fungicide against spore germination (SG) and confirmed its efficacy at doses below both the commercial rate and those evaluated by Coelho *et al.* (2010). According to Zhou *et al.* (2016), thiabendazole belongs to the benzimidazole chemical group and acts by interfering with mitosis and cell division through the inhibition of β-tubulin microtubule formation.

The effective action of difenoconazole observed in this study is consistent with Coelho *et al.* (2010), who reported 100% efficacy of this fungicide in inhibiting the mycelial growth (MG) of *T. paradoxa*. In the present research, doses lower than the commercial rate and those tested by Coelho *et al.* (2010) were used; however, in this study, the fungicide was applied in combination with cyprodinil. Ayika *et al.* (2024) reported high efficacy of difenoconazole mixed with pydiflumetofen against *T. paradoxa* isolated from *C. nucifera* in Florida. Cyprodinil has shown effectiveness against *Botrytis cinerea* isolated from apple (*Malus domestica*) (Sholberg *et al.*, 2003). Currently, there are no reports on the effectiveness of cyprodinil against *T. paradoxa* isolated from *C. nucifera*. Like propiconazole and tebuconazole, difenoconazole is a typical triazole with high efficiency and broad-spectrum activity; it acts by inhibiting demethylase and blocking fungal ergosterol biosynthesis. Cyprodinil, on the other hand, is a fungicide belonging to the anilinopyrimidine class that inhibits methionine biosynthesis and the secretion of hydrolytic enzymes, as well as germ tube elongation and mycelial growth (Qin *et al.*, 2023).

These results suggest that some fungicides exhibit a strong inhibitory effect on *Thielaviopsis paradoxa* under controlled conditions. However, the findings of this *in vitro* assay should be interpreted as a preliminary comparative assessment, since the concentrations used were estimated based on commercially recommended field application rates. Therefore, additional validation under field or greenhouse conditions is required.

The contact fungicides showed lower effectiveness against *T. paradoxa* compared with the systemic fungicides, with copper oxychloride being the least effective. The low efficacy of this fungicide is consistent with the findings of Irabi *et al.* (2018), who reported limited activity of copper oxychloride against *T. paradoxa* isolated from date palm (*P. dactylifera*) in Sudan. In contrast, copper hydroxide achieved 90% inhibition of *T. paradoxa* mycelial growth (MG) starting at the commercial dose and reached 100% inhibition at the highest evaluated dose (3 g a.i. L^{-1}). Moreover, this fungicide inhibited 100% of spore germination (SG) at the lowest dose (1 g a.i. L^{-1}), demonstrating its potential as a preventive agent. According to Gaviria-Hernández *et al.* (2013), copper hydroxide is a contact fungicide with multisite activity, low toxicity, and a low risk of resistance development, acting mainly during spore germination. At the cellular level, it reacts with sulfhydryl, hydroxyl, amino, and carboxyl groups, inactivating them and thereby disrupting the respiratory chain. Its efficacy against various fungal species makes it a useful chemical tool for preventing fungal

diseases in crops. To date, no studies have specifically evaluated the use of copper hydroxide against *T. paradoxa*.

The effectiveness of captan (82.3%) against the mycelial growth (MG) of *T. paradoxa* isolated from CEVB in Tabasco agrees with the findings of Chakrabarty *et al.* (2013), who reported 91% efficacy against the MG of *T. paradoxa* isolated from *C. nucifera* in India. In the present study, captan also demonstrated 100% effectiveness in inhibiting spore germination (SG), highlighting its potential as a preventive fungicide against *T. paradoxa*. Captan is a contact fungicide effective in controlling various plant diseases (Tutunaru *et al.*, 2024). Its mode of action involves interference with cellular respiration and reaction with sulfhydryl enzymes, leading to the formation of thiophosgene—a compound toxic to fungal cells (Ackermann *et al.*, 2000). Other systemic and contact fungicides that have shown 100% inhibition of *T. paradoxa* MG include benomyl, carboxin, and thiophanate-methyl (Coelho *et al.*, 2010; Irabi *et al.*, 2018).

CONCLUSIONS

This study focused on the morphological and molecular characterization of fungi associated with wilt and trunk rot of the "Enano Verde del Brasil" (CEVB) coconut in Tabasco, Mexico, as well as on the *in vitro* evaluation of fungicides with potential for its control. *Thielaviopsis paradoxa* was identified as the causal agent of the observed symptoms, representing the first record of this species associated with CEVB in Tabasco, Mexico. This finding is a significant contribution to the phytosanitary diagnosis of the crop, as it enables more precise implementation of monitoring, prevention, and management measures. Pathogenicity tests confirmed the fungus's ability to induce necrosis in seedlings under controlled conditions. Furthermore, the *in vitro* assays identified propiconazole, carbendazim, and tebuconazole as highly effective, achieving complete inhibition of *T. paradoxa* mycelial growth and spore germination at the lowest tested doses (0.45×10^3, 1×10^3, and 0.5×10^3 ppm, respectively). This information provides a useful foundation for selecting promising chemical treatments, which should be further validated under field conditions as part of integrated disease management strategies.

Conflict of interest

The authors explicitly declare that there is no conflict of interest regarding this research.

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