UNIVERSIDADE FEDERAL RURAL DO SEMI-ÁRIDO
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
PROGRAMA DE PÓS-GRADUAÇÃO EM FITOTECNIA
MESTRADO EM FITOTECNIA

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CHARACTERIZATION AND AGGRESSIVITY OF *Fusarium* spp. **ASSOCIATED WITH ROOT AND STEM ROT IN** *Carica papaya* **IN NORTHEAST BRAZIL**

MOSSORÓ
2025

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Dissertação apresentada ao Mestrado em Fitotecnia do Programa de Pós-Graduação em Fitotecnia da Universidade Federal Rural do Semi-Árido como requisito para obtenção do título de Mestre em Fitotecnia.

Linha de Pesquisa: Fitopatologia

Orientador: Profa. Dsc. Márcia Michelle de Queiroz Ambrósio

Co-orientador: Prof. Dsc. Washington Luis da Silva

MOSSORÓ

2025

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586c Silva, Jarlan Lucas dos Santos.
CHARACTERIZATION AND AGGRESSIVITY OF Fusarium spp. ASSOCIATED WITH ROOT AND STEM ROT IN Carica papaya IN NORTHEAST BRAZIL / Jarlan Lucas dos Santos Silva. - 2025.
62 f. : il.

Orientadora: Márcia Michelle de Queiroz Ambrósio.
Coorientadora: Washington Luis da Silva.
Dissertação (Mestrado) - Universidade Federal Rural do Semi-árido, Programa de Pós-graduação em Fitotecnia, 2025.

1. Papaya. 2. Fusariosis. 3. Diagnose. 4. Soil-borne Pathogens. I. Ambrósio, Márcia Michelle de Queiroz, orient. II. da Silva, Washington Luis, co-orient. III. Título.

Ficha catalográfica elaborada por sistema gerador automáto em conformidade com AACR2 e os dados fornecidos pelo) autor(a).
Biblioteca Campus Mossoró / Setor de Informação e Referência
Bibliotecária: Keina Cristina Santos Sousa e Silva
CRB: 15/120

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Linha de Pesquisa: Fitopatologia

Defendida em: 25 / 02 / 2025.

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AGRADECIMENTOS

Agradeço primeiramente a Deus, por ter me dado saúde, determinação, forças e proteção sempre.

Agradeço à minha família em especial minha mãe Francisca Francineide da Silva, que sempre me proporcionou o melhor que conseguiu, desejando me oferecer uma melhor qualidade de vida e uma boa educação. Muito obrigado pelo amor, carinho e incentivo ao longo dessa jornada.

Agradeço à UFERSA e o Programa de Fitotecnia pela oportunidade de experienciar o curso de pós-graduação e me proporcionar tanta vivência e conhecimento.

Agradeço a Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) pela concessão da bolsa e incentivo a pesquisa.

Agradeço imensamente à minha orientadora, professora Márcia Michelle de Queiroz Ambrósio, por sempre me incentivar a dar o meu melhor, por desejar o crescimento profissional e pessoal de todos os alunos e por proporcionar continuamente todo o suporte necessário.

Agradeço também ao meu coorientador, professor Washington Luis da Silva, pela coorientação e pelos ensinamentos durante esse período, e por me oferecer a oportunidade de trabalhar em um grande centro de pesquisa, o que contribuiu imensamente para o meu desenvolvimento pessoal e profissional.

Agradeço aos professores do curso de Fitotecnia que me transmitiram seus conhecimentos e experiências ao longo do mestrado.

Agradeço a Banca Examinadora, Jailma Suerda Silva de Lima, Dauri José Tessmann e César Júnior Bueno pela participação e contribuições para aperfeiçoamento deste trabalho.

Agradeço ao grupo de pesquisa do Laboratório de Microbiologia e Fitopatologia, onde conheci pessoas que me ajudaram grandemente não só de forma profissional e institucional, mas também de forma pessoal, as quais foram: Louise, Ana Paula, Tatianne, Janderson, Vinícius, Elisandra, Juliano, Rebeca, Vitória, Silvan, Vanuza, Jemerson, Fernando, Breno, Atarissis, e Maria Helena.

Agradeço aos meus amigos que conheci durante o período no exterior que me ajudaram a vivenciar da melhor forma essa experiencia, as quais foram: Monique, Vitctória, Paula, Neuzi, Juliana, William, Joe, Talisson, Jewell, Rania, Ravi, Regan e Raquel.

Agradeço aos meus amigos que diretamente ou indiretamente fizeram parte da minha história. Muito obrigado!

ABSTRACT

Papaya (*Carica papaya* L.) is a crop of great socioeconomic importance in Northeastern region of Brazil. However, soil-borne pathogens (SBP) cause significant losses to papaya growers in the medium and long term. The control of diseases caused by SBP is difficult due to the lack of resistant cultivars and commercial products recommended by the Ministério da Agricultura, Pecuária e Abastecimento (MAPA), to be used in soil and, due to the lack of information on the etiological agents related to these diseases. Stem and root rot of papaya caused by the *Fusarium solani* complex (FSSC) has been previously reported in Brazil. Still, the *Fusarium* species belonging to this complex have not been properly characterized. This study aims to molecularly and morphologically characterize the *Fusarium* isolates associated with papaya stem and root rot in Ceará (CE) and Rio Grande do Norte (RN) states. Papaya growers in semiarid Northeastern Region of Brazil have reported losses of almost 50%, due to problems with SBP. To diagnose the causal agents of root rot and stem rot in papaya plants, we collected samples of roots and stems of papaya plants in commercial fields exhibiting yellowing, wilting, and collapse. Twenty-five *Fusarium* isolates were obtained from six sampled production areas of Aracati (CE), Apodi (RN), Baraúna (RN) and Caraúbas (RN). Koch's postulates were performed to confirm the pathogenicity of these isolates on one month old papaya seedlings, and all inoculated plants showed symptoms two months after inoculation. At the end of the experiments, stem and root rots were evaluated using a disease scoring scale: 1 = no symptoms, 2 = less than 10% symptoms, 3 = between 10 and 30%, 4 = between 31 and 50%, 5 = more than 50%, and 6 = dead plant. Treatments were compared by the non-parametric Van der Warden statistical test. The EF-1α (elongation factor 1-alpha) and RPB2 (second largest subunit of RNA polymerase) genes from isolates were partially amplified by PCR and sequenced. A maximum-parsimony phylogenetic tree was constructed with the concatenated partial sequences. Five species were identified causing this disease in the region: *F. falciforme* (FSSC 3+4), *F. petroliphilum* (FSSC 1), *F. pernambucanum* (FIESC 17), *F. sulawesiense* (FIESC 16) and *F. delphinoides* (FDSC). Among these species, the most aggressive was *F. delphinoides* followed by *F. pernambucanum*, *F. falciforme* and *F. petroliphilium*. The least aggressive species was *F. sulawesiense*. The findings of this work will help papaya growers to understand the causal agents of papaya stem and root rot and assist in the development of new management strategies for this crop.

Keywords: Papaya; Fusariosis; Diagnose; Soil-borne Pathogens.

RESUMO

O mamoeiro (*Carica papaya* L.) é uma cultura de grande importância socioeconômica no Nordeste do Brasil, no entanto as perdas ocasionadas por patógenos habitantes do solo (PHS) têm acarretado prejuízos a médio e longo prazo para os produtores. O controle de doenças ocasionadas por PHS, é difícil devido a falta de cultivares resistentes e produtos comerciais recomendados pelo Ministério da Agricultura, Pecuária e Abastecimento (MAPA), para ser utilizado no solo, e pela falta de informações acerca dos agentes etiológicos relacionados a estas doenças. A podridão causada pelo complexo *Fusarium solani* (FSSC) já foi relata no Brasil, no entanto, não foi caracterizada. O objetivo do trabalho foi caracterizar molecularmente, e morfologicamente, isolados de *Fusarium* associados a podridão do mamoeiro nos estados do Ceará (CE), e Rio grande do Norte (RN). Produtores de mamão da região semiárida do Brasil relataram perdas de aproximadamente 50% em áreas de produção, devido aos problemas com PHS. Para diagnosticar os agentes causais da podridão radicular, foram coletadas amostras de raízes e caules de mamoeiros, em campos comerciais cujas plantas exibiam amarelecimento, murcha e colapso. Vinte e cinco isolados de *Fusarium* foram obtidos de seis áreas de produção amostradas nos municípios, Aracati (CE), Apodi (RN), Baraúna (RN) e Caraúbas (RN). Os postulados de Koch foram realizados para confirmar a patogenicidade desses isolados em mudas de mamoeiro (um mês após a semeadura), e todas as plantas inoculadas apresentaram sintomas 2 meses após a inoculação. No final dos experimentos, as podridões de caules e raízes foram avaliadas usando uma escala de notas: 1 = sem sintomas, 2 = menos de 10% dos sintomas, 3 = entre 10 e 30%, 4 = entre 31 e 50%, 5 = mais de 50%, e 6 = planta morta. Os tratamentos foram comparados pelo teste de Van der Warden. Os genes EF-1α (fator de elongação 1-alfa) e RPB2 (segunda maior subunidade da RNA polimerase) dos isolados foram parcialmente amplificados por PCR, e sequenciados. Uma árvore filogenética de máxima parcimônia foi construída com as sequências parciais concatenadas. Cinco espécies foram identificadas causando podridão no sistema radicular do mamoeiro, *F. falciforme* (FSSC 3+4), *F. petroliphilum* (FSSC 1), *F. pernambucanum* (FIESC 17), *F. sulawesiense* (FIESC 16), e *F. delphinoides* (FDSC). Entre estas espécies, a mais agressiva foi *F. delphinoides* seguida por *F. pernambucanum, F. falciforme,* e *F. petroliphilum*. A espécie menos agressiva foi *F. sulawesiense.* Os achados deste trabalho ajudarão os produtores a entender os agentes causais da podridão radicular do mamoeiro, auxiliando no desenvolvimento de novas estratégias de manejo para essa cultura.

Palavras Chaves: Mamão; Fusariose; Diagnóstico; Patógenos habitantes do solo

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1. General introduction

Carica papaya Linn, belonging to the Caricaceae family, is native to tropical America. The plant has a weak and unbranched stem, exuding white latex and with dense foliage grouped at the top. It grows quickly, reaching up to 20 meters in height (Varisha et al. 2013). The leaves have been traditionally used to treat many diseases, such as malaria, dengue fever, and jaundice, in addition to having immunomodulatory and antiviral activities (Vijay, 2014). Papaya fruit is a rich source of nutrients such as provitamin A, carotenoids, vitamin C, B vitamins, lycopene, minerals, and dietary fiber (Oliveira, 2021). Both the leaves and fruits of *C. papaya* contain carotenoids, such as β-carotene and lycopene, and have medicinal properties, such as anti-inflammatory, hypoglycemic, anti-infertility, and wound healing (Vijay, 2014).

The importance of papaya cultivation in national fruit production is remarkable, with Brazil ranking as the fourth-largest global producer, cultivating 26,839 hectares in 2023 (FAO, 2024). This crop not only generates jobs and income but also plays a crucial role in the social and economic sectors. The demand for labor is constant throughout the year, with activities ranging from cultivation practices to harvesting and marketing, besides the renewal of plantations every 2 to 3 years (Oliveira, 2021). Papaya is a key crop in Brazilian agribusiness and a major product in the country's fresh fruit exports. The state of Bahia is the leading papaya producer in Brazil, with an estimated production of 354.525 tons in 2023 (IBGE, 2024). This crop also has great social importance, directly employing over 33,000 people in Brazil each year (Serafini et al., 2021; Gonçalves, Leonardo, Pereira Filho, 2022).

Stem and root rot is a disease complex becoming prevalent in papaya production areas worldwide. Normally this disease is associated with infection by the oomycetes *Pythium* and *Phytophtora*. These diseases manifest in many ways, including rot symptoms in the root system, collar rot, or gummosis (Zakaria, 2023). In periods of excessive humidity and high

temperatures, especially in clay and poorly drained soils, plant decline and collapse occur. Furthermore, the fruits can also be affected by the disease (Santos Filho et al., 2009).

Papaya stem and root rot has also been associated with *Fusarium* species. These soil-borne fungi are adapted to different environmental conditions and can develop at temperatures ranging from 5 ºC to 40 ºC (Panwar et al., 2016). Their conidia can spread through wind, rain, or even by humans. Many *Fusarium* species are known to produce survival structures called chlamydospores, which allow the fungi to survive in the soil for more than ten years without a host (Leslie & Summerell, 2008; Gabrekiristos & Demiyo, 2020). Furthermore, it has several hosts, including plants, animals, insects, and humans. The pathogen is commonly known to cause severe losses to a large array of crops such as tomato (*Lycopersicon esculentum* L.), banana (*Musa acuminata* Colla.), cabbage (*Brassica oleracea* var. *capitata* L.), pea (*Pisum sativum* L.), chickpea (*Cicer arietinum* L.), melon (*Cucumis melo* L.), cotton (*Gossypium hirsutum L.*), spinach (*Spinacia oleracea*), basil (*Ocimum basilicum* L.), beans (*Phaseolus vulgaris* L.), carnation (*Dianthus caryophyllus*), chrysanthemum (*Chrysanthemum*), and watermelon [*Citrullus lanatus* (Thunb.) (Matsum & Nakai)] (Gabrekiristos & Demiyo, 2020). Furthermore, this fungus has other importances, such as the capacity of these fungi to produce mycotoxins in grains and cereals, such as trichothecenes, zearalenone, and fumonisins, which can cause problems to human health (Summerell & Leslie, 2011).

A study conducted in Bihar, India (Singh & Kumar, 2015), identified papaya root rot caused by *Fusarium solani* as an emerging disease. *F. solani*, a soil-borne pathogen, infects seedlings, resulting in rotting and wilting of the young plants. The progression of root rot is accelerated after rainfall and affects all papaya varieties and growth stages, potentially causing significant yield losses of up to 95%. Later studies identified *Fusarium falciforme* as a causal agent of papaya root rot in India and Mexico (Gupta et al., 2019; Vega-Gutiérrez et al., 2019) besides *F. verticillioides* in Mexico (Vega-Gutiérrez et al., 2023). Similar to root

rot, stem rot affects young papaya plants, where the infected stem becomes soft and develops black or brown lesions, which become the focal point of the rot (Zakaria, 2023).

In Brazil, although *Phytophthora palmivora* and *Pythium aphanidermatum* are the main pathogenic causes of this disease (Zakaria, 2023), stem rot can also be caused by the *Fusarium solani* complex (Correia et al., 2013). Besides this report, there are no other studies that demonstrate the *Fusarium* species that cause root rot in papaya trees in Brazil (Silva et al. 2025).

Many *Fusarium* species are soil-borne pathogens, and they can be transmitted through contaminated seeds, seedlings, and agricultural equipment, which is particularly relevant for diseases like vascular wilt, root rot, and stem rot (Lin et al., 2013). To control the disease spread, cultural practices are recommended, such as avoiding poorly drained soils, successive planting of papaya plants in the area, and injuries to the stems and fruit. Biological control using antagonistic fungi has proven effective in many crops and is also an option for papaya if applied correctly to the management (Tavares, 2009; Bedine et al., 2022). Although there are recommendations for chemical products to control *Fusarium* in fruits, there is a lack of products with good efficacy and no such recommendation in Brazil, for managing the pathogen in the field regulated by the Ministério da Agricultura Pecuária e Abastecimento (Oliveira, 2021; AGROFIT, 2025).

Deepening our understanding of *Fusarium* species that infect major tropical fruit crops is crucial to improving the effectiveness of management strategies related to these pathogens. Furthermore, identifying specific pathogenic *Fusarium* species is essential for initiating control trials with commercial products targeting the correct causal agent, as well as for breeding programs aimed at identifying resistance genes against these pathogens.

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3. Chapter I: Characterization and aggressivity of *Fusarium* spp. associated with root and stem rot in *Carica papaya* in Northeast Brazil

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4. Abstract

In the past five years, papaya farmers in the Northeastern Region of Brazil have been reporting major losses in fruit production, up to 50% in some years, due to diseases caused by soil-borne pathogens. To diagnose the causal agents of this disease's complex in the region (root and stem rots), we collected root and stem samples from papaya plants in commercial fields exhibiting yellowing, wilting, and collapse. Fifteen *Fusarium* isolates were obtained from six production areas sampled and Koch's postulates were carried out to confirm the pathogenicity of these isolates. Five species were identified causing this disease in the region: *F. falciforme* (FSSC 3+4), *F. petroliphilum* (FSSC 1), *F. pernambucanum* (FIESC 17), *F. sulawesiense* (FIESC 16), and *F. delphinoides* (FDSC). Among these species, the most aggressive was *F. delphinoides* followed by *F. pernambucanum*, *F. falciforme*, *F. petroliphilum*, and the less aggressive was *F. sulawesiense*. Our findings will aid the development of strategies to manage these disease complexes to help farmers reduce the damage caused by these pathogens in papaya in Brazil and other papaya production areas in the world.

Keywords: Papaya; Fusariosis; Diagnose; Soil-borne Pathogens.

5. Introduction

Papaya (*Carica papaya* L.) is one of the major fruit crops in Brazil, which has been extensively explored by various sectors such as the food, pharmaceutical, drinks, and cosmetic industries (Oliveira & Meissner Filho, 2021; Serafini et al., 2021; Ávila-Hernández et al., 2023). Brazil is one of the largest producers and the biggest exporter of papaya in the world, accounting for over US$ 53 million in sales (HF BRASIL, 2024). This crop also has great social importance in producing regions as it is cultivated year-round, directly employing over 33,000 people in Brazil (Serafini et al., 2021; Gonçalves, Leonardo, Pereira Filho, 2022). Furthermore, papaya is a semi-perennial crop, and the orchard needs to be renewed every 2 years for intensive production, generating and maintaining jobs for longer than some other agricultural sectors (Oliveira & Meissner Filho, 2021).

The production of papaya is directly affected by diseases, such as root and stem rot, which was reported to cause more than 90% of losses in yield in Bihar, India (Singh & Kumar, 2015). Although the main pathogens associated with stem and root rot belong to the genera *Phytophthora* and *Pythium*, this disease complex can also be caused by species of the *Fusarium* genus (Correia et al. 2013; Koul et al., 2022, Silva et al. 2025). *Fusarium* is an emerging pathogen in papaya fields, and infected plants develop rots quickly after rain. This disease affects all development stages of the plant, and all varieties of papaya are susceptible to this pathogen group (Zakaria, 2023). Most species within the genus *Fusarium* can be classified as hemibiotrophic, that is, organisms whose infection initially resembles pathogens that depend on a living host (biotroph) but eventually cause necrosis of the host cells (necrotrophic) (MA et al., 2013). The symptoms in papaya are yellowing, wilting, and leaf drops, along with browning and

rotting of the roots, which often extend to the stem and can cause the plants to collapse (Gupta et al., 2019).

In Mexico *F. falciforme* and *F. verticillioides* were reported as cause agents of root and stem rot in papaya (Vega-Gutiérrez et al., 2019, Vega-Gutiérrez et al., 2023). In India, *F. falciforme* was also reported to cause the same diseases (Gupta et al., 2019). In Brazil, the first report of papaya stem rot was made by Correia et al. (2013). The researchers reported that the causal pathogen was a species of the *Fusarium solani* species complex (FSSC). However, they couldn't reach the species level of those isolates related to the disease. Recently, two species have been reported in the Northeast Region of Brazil, *F. falciforme* and *F. pernambucanum*, causing stem and root rot in papaya plants (Silva et al., 2025).

In this study, we aimed to identify and characterize the morphology and aggressiveness of isolates of *Fusarium* that cause papaya root and stem rot in the Northeast Region of Brazil. We hypothesized that more species are associated with this disease's complex than what has been reported in the literature. We also believed that the initial inoculum for this disease complex originated from melon plants that are cultivated in the papaya production areas of the the Northeast Region of Brazil.

6. Materials and Methods

6.1 Sampling

Samples of roots and stems of papaya plants showing rot symptoms were collected in five farms in the Rio Grande do Norte state (municipalities of Apodi and Baraúna) and one in the Ceará state (municipalities of Aracati). The collection was performed between August and September of 2023. Papaya plants showed symptoms like yellowing, wilting, collapse of the plants, and necrotic tissues on the stem and roots

(Figure 1). The samples were analyzed at the Laboratório de Microbiologia e Fitopatologia of the Universidade Federal do Semi-Árido (UFERSA).

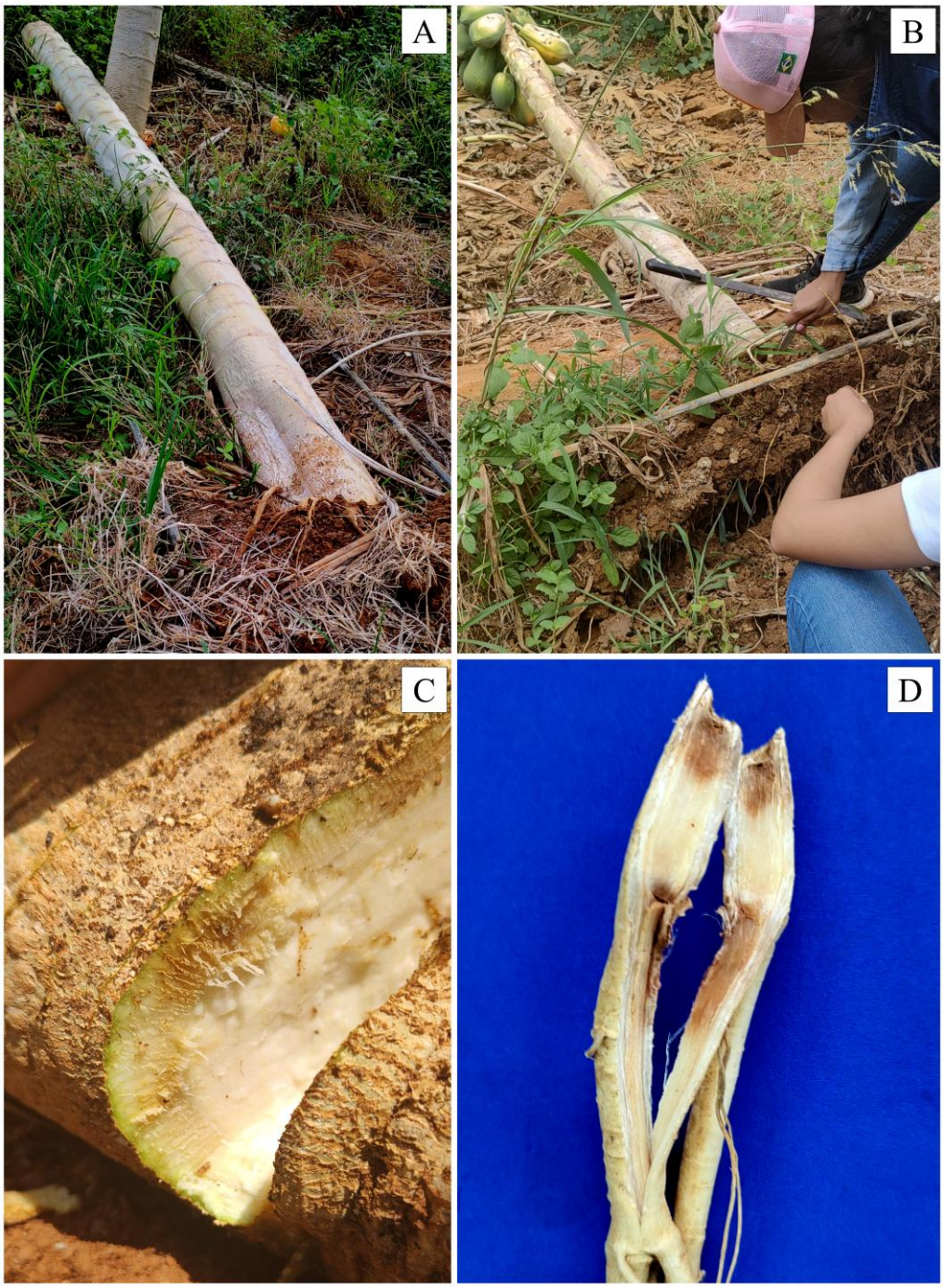


Figure 1: Sampling of papaya root and stem tissues showing symptoms of rot from trees with yellowing, wilting, and collapse (A-D).

6.2 Isolation

Small fragments of symptomatic tissues were surface disinfected in Ethanol 70 % (1 s) and NaClO 2.5 % (60 s) and rinsed in sterilized water. The fragments were placed in Petri dishes with potato dextrose agar (PDA) + tetracycline 0.05% and incubated in a Biochemistry Oxygen Demand incubator (BOD) at 28 ± 2 °C with a 12-hour photoperiod for five days. A spore solution (0.85% NaCl, 0.1% Tween 20) was made for each isolate. Each isolate was transferred to water agar Petri dishes, and after spore germination, a single spore was transferred to PDA plates. Monosporic cultures were obtained from 15 *Fusarium* isolates and preserved in sterilized water (Castellani, 1939).

6.3 DNA extraction, PCR amplification, and Sequencing

Mycelia from individual monosporic colonies growing on PDA were collected and ground with a mortar and pestle in liquid nitrogen. Genomic DNA was extracted using the Plant/Fungi DNA isolation kit (NORGEN BIOTEK CORP.®, Thorold, ON, Canada). DNA concentration and purity were measured using NanoDrop 2000 (Thermo Fisher, Waltham, MA) and adjusted to a final concentration of 50 ng/µL per sample.

The translation elongation factor 1 alpha (EF-1α) and the second largest subunit of RNA polymerase (RPB2) genes were partially amplified by PCR using primers pairs Ef1/Ef2 (O'Donnell et al. 1998) and 5F2/7cR (Liu et al. 1999; Sung et al. 2007), respectively. Amplicons were checked in 1.5% agarose gel, Sanger sequenced at Keck's Lab at Yale University, New Haven - CT, and the sequences deposited on GenBank (Table S1).

6.4 Phylogenetic analysis

Multiple alignments of nucleotide sequences were generated using the sequences in the ClustalW tool (Thompson et al., 1994), implemented in MEGA X software (Kumar et al., 2018). Reference sequences of *Fusarium* species from both EF-1α and RPB2

were extracted from the GenBank database and added to the alignments (Table S2). The sequences aligned consisted of a total of 634 bp for EF-1α, 785 bp for RPB2, and 1419 bp for EF-1α + RPB2 (concatenated sequences). Using the Maximum Parsimony (MP) method, a phylogenetic tree was built using concatenated sequences. The MP tree was obtained using the Subtree-Pruning-Regrafting (SPR) algorithm (Nei & Kumar, 2000) with search level 1 in which the initial trees were obtained by the random addition of sequences (10 replicates). Analyses were conducted in MEGA X (Kumar et al. 2018) with 1000 bootstraps of support.

6.5 Morphological characterization

Isolates were individually cultivated in different culture media for the study of each fungal isolate morphology. PDA was used to evaluate the colony growth (10 days), spezieller nährstoffarmer Agar (SNA) was used for measuring and evaluating the micromorphological characters (10 days), and carnation leaf agar (CLA) was used to assess the ability of the isolates to produce sphorodochia (30 days). The reproductive structures were analyzed based on color, size, shape, number of septa, and arrangement of spores on hyphae, plates were incubated in BOD with 28 ± 2 °C and a 12-hour photoperiod (Leslie and Summerell, 2006).

6.6 Pathogenicity test and fungal isolates aggressiveness

Ten *Fusarium* isolates previously identified, (Table S3) (Silva et al., 2025) and preserved in the laboratory of phytopathology and microbiology at UFERSA, were used together with the 15 isolates obtained in this study in the evaluations conducted herein.

To perform the pathogenicity test, each isolate was cultivated in the sterilized substrate (74% manure tanned, 24% washed sand, and 2% commercial oat) for seven days to produce the inoculum (Lefèvre & Souza, 1993). The inoculum was mixed on

autoclaved soil in a dosage of 36g. L^{-1} and incubated for one day in a greenhouse. Then, 45-day-old papaya seedlings (hybrid Tainung 01) were transferred to the pots and cultivated for 60 days (greenhouse conditions 37 ± 2 °C and 44 ± 2% RU). Sterilized substrate was incorporated into the autoclaved soil as a control (mock) treatment in this assay. There were five replicates per isolate, and the mock assay was conducted twice and carried out in a completely randomized design.

At the end of the experiments, the severity of root rot and stem rot was evaluated using a modified scoring scale (Hernández-Montiel, et al., 2013): 0 = no symptoms, 1 = less than 10% of symptoms, 2 = between 10 and 30%, 3 = between 31 and 50%, 4 = more than 50%, and 5 = dead plant. The aggressiveness of each species was determined by the average of the severity of the isolates within each species. The fungi were reisolated from symptomatic tissues and reidentified based on morphology and sequencing to fulfill Koch's postulates.

The data of severity were tested by the Shapiro–Wilk normality test ($p \leq 0.05$) and were compared using the Van der Waerden nonparametric test ($p \leq 0.05$) (Hay et al. 2023). Analyses were performed in R version 3.6.1 using the R package agricolae v1.3-7 in R studio (R Core Team, 2021).

7. Results

7.1 Molecular identification of *Fusarium* spp.

Morphology and phylogenetic analyses based on the concatenated partial sequences of the EF-1α and RPB2 genes (with a minimum bootstrap support of 98%) revealed that five species (*F. falciforme, F. petroliphilum, F. pernambucanum, F. sulawesiense,* and *F. delphinoides*), cause root and stem rot in papaya plants in the Northeast Region of Brazil (Figure 2).

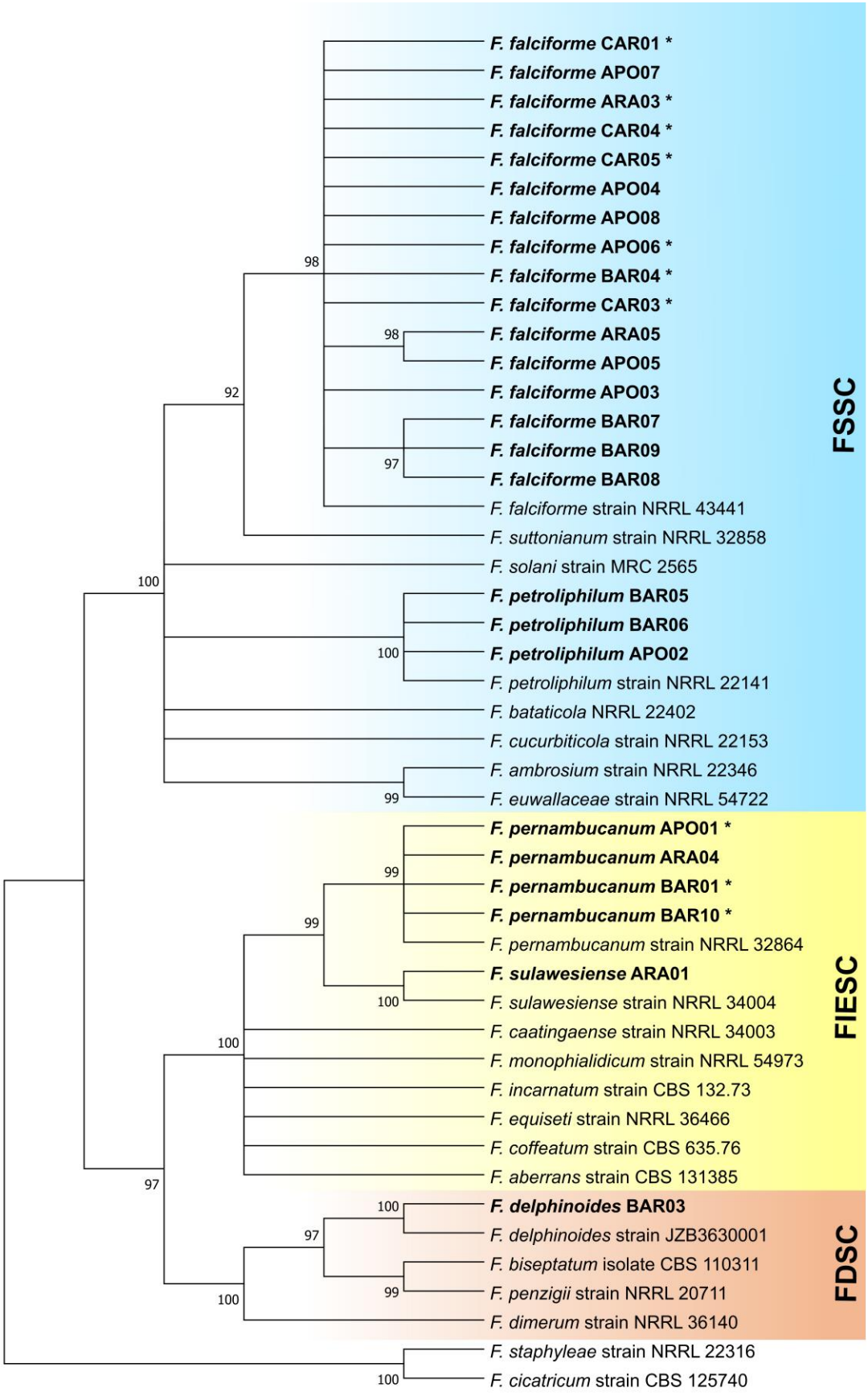

F. falciforme CAR01 *
F. falciforme APO07
F. falciforme ARA03 *
F. falciforme CAR04 *
F. falciforme CAR05 *
F. falciforme APO04
F. falciforme APO08
F. falciforme APO06 *
F. falciforme BAR04 *
F. falciforme CAR03 *
F. falciforme ARA05
F. falciforme APO05
F. falciforme APO03
F. falciforme BAR07
F. falciforme BAR09
F. falciforme BAR08
F. falciforme strain NRRL 43441
F. suttonianum strain NRRL 32858
F. solani strain MRC 2565
F. petroliphilum BAR05
F. petroliphilum BAR06
F. petroliphilum APO02
F. petroliphilum strain NRRL 22141
F. bataticola NRRL 22402
F. cucurbiticola strain NRRL 22153
F. ambrosium strain NRRL 22346
F. euwallaceae strain NRRL 54722
F. pernambucanum APO01 *
F. pernambucanum ARA04
F. pernambucanum BAR01 *
F. pernambucanum BAR10 *
F. pernambucanum strain NRRL 32864
F. sulawesiense ARA01
F. sulawesiense strain NRRL 34004
F. caatingaense strain NRRL 34003
F. monophialidicum strain NRRL 54973
F. incarnatum strain CBS 132.73
F. equiseti strain NRRL 36466
F. coffeatum strain CBS 635.76
F. aberrans strain CBS 131385
F. delphinoides BAR03
F. delphinoides strain JZB3630001
F. biseptatum isolate CBS 110311
F. penzigii strain NRRL 20711
F. dimerum strain NRRL 36140
F. staphyleae strain NRRL 22316
F. cicatricum strain CBS 125740
FSSC
FIESC
FDSC

Figure 2: Maximun-parsimony tree built with concatenate partial sequences of the genes EF-1α + RPB2 (1000 bootstrap), from 15 isolates obtained in this study (bold letter), 10 isolates from a previous work (followed by an asterisk) (Silva et al., 2025) and reference sequences from GenBank of *Fusarium solani* species complex (FSSC), *Fusarium incarnatum-equiseti* species complex (FIESC), *Fusarium dimerum* species complex (FDSC) and *Fusarium staphylae* species complex (FSTSC). Isolates APO03, APO04, APO05, APO07, APO08, ARA05, BAR07, BAR08, and BAR09 were grouped in *F. falciforme* clade (FSSC 3+4) with 98% of Bootstrap support. Isolates APO02, BAR05, and BAR06 were grouped in the *F. petroliphilum* clade (FSSC 1) with 100% Bootstrap support. Isolate BAR03 was grouped with *F. delphinoides* clade with 100% Bootstrap support. Isolate ARA01 was grouped with *F. sulawesiense* clade (FIESC 16) with 100% Bootstrap support. Isolate ARA04 was grouped with *F. pernambucanum* clade (FIESC 17) with 99% Bootstrap support.

Most of the isolates grouped with *F. falciforme* (ARA05, APO03, APO04, APO05, APO07, APO08, BAR07, BAR08, and BAR09) (FSSC 3 +4). Other species less frequently isolated were grouped with *F. petroliphilum* (APO02, BAR05, and BAR06) (FSSC 1), *F. pernambucanum* (ARA04) from *Fusarium incarnatum-equiseti* species complex (FIESC 17), *F. sulawesiense* (ARA01) (FIESC 16), and *F. delphinoides* (BAR03) from *Fusarium dimerum* species complex (FDSC).

The area sampled in this study can be observed in the map of the region (municipalities of Apodi, Aracati and Baraúna) (Figure 3). All species found in this study were present in Baraúna. The most frequent isolate in this study was *F. falciforme*, which was the only one present in all areas sampled in this and previous studies in melon and papaya crops. The other species less frequent were *F.*

pernambucanum, F. sulawesiense, and *F. delphinoides,* with just one isolate per species, and all of them collected from farms in Baraúna.

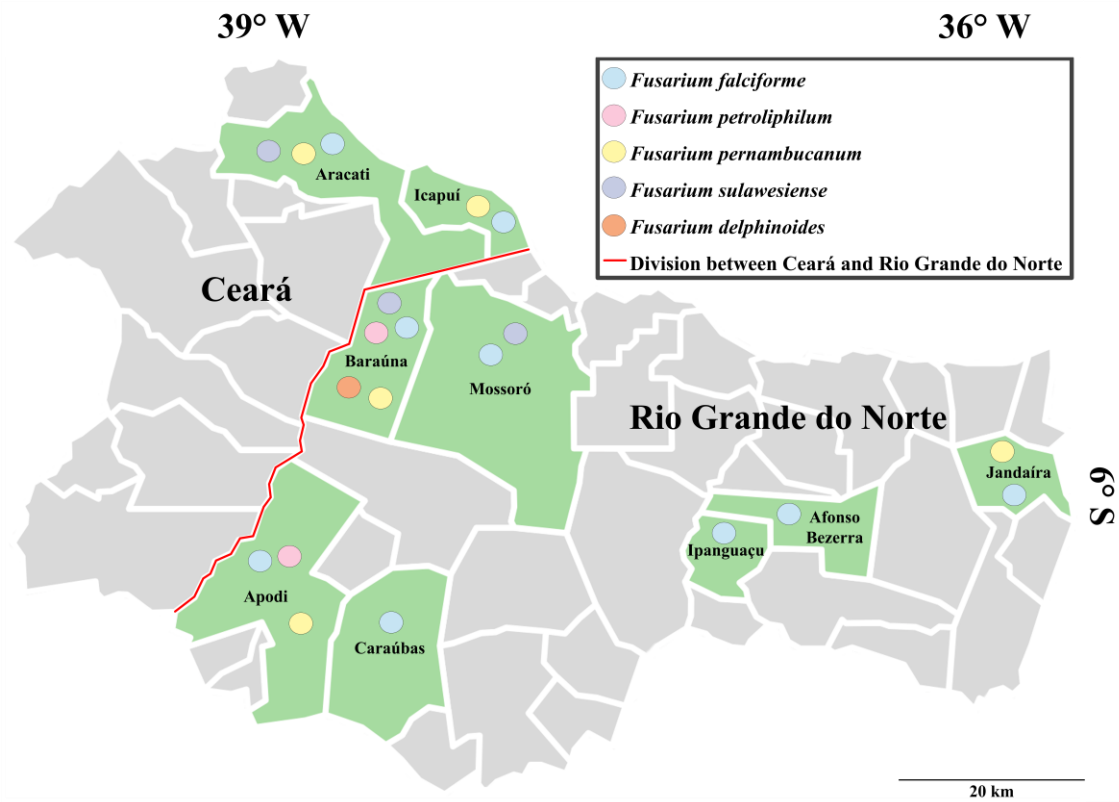


Figure 3: Map of the region with the presence of the species found in this and previous studies in melon and papaya crops. The areas sampled in this study were from Apodi, Baraúna, and Aracati municipalities in Brazil. *Created with MapChart.

7.2 Morphological characterization

Herein, we isolated five species of *Fusarium* belonging to three different species complexes. Both species showed aerial mycelia with conidia grouped in false heads at the ends of the monophialides. The microconidia were ovoid to reniform with one septum per conidia. Macroconidia were visible in sporodochia and falcate with 2 to 4 septa. The chlamydospores were globose, single, or in chains, with a spiculated cell wall, arranged in the end and the middle of hyphae. The color of sporodochia was cream to white. The colony of *F. falciforme* was cottony and beige, with a yellowish center,

and white edges. Some isolates presented concentric rings that alternated from the middle to the edges of the colony (Figure 4A and F). The colony of *F. petroliphilum* was white, with a grayish center, cottony, and uniform. On the reverse side of the plate, the middle of the colony had a brownish-orange pigment (Figure 4B and G).

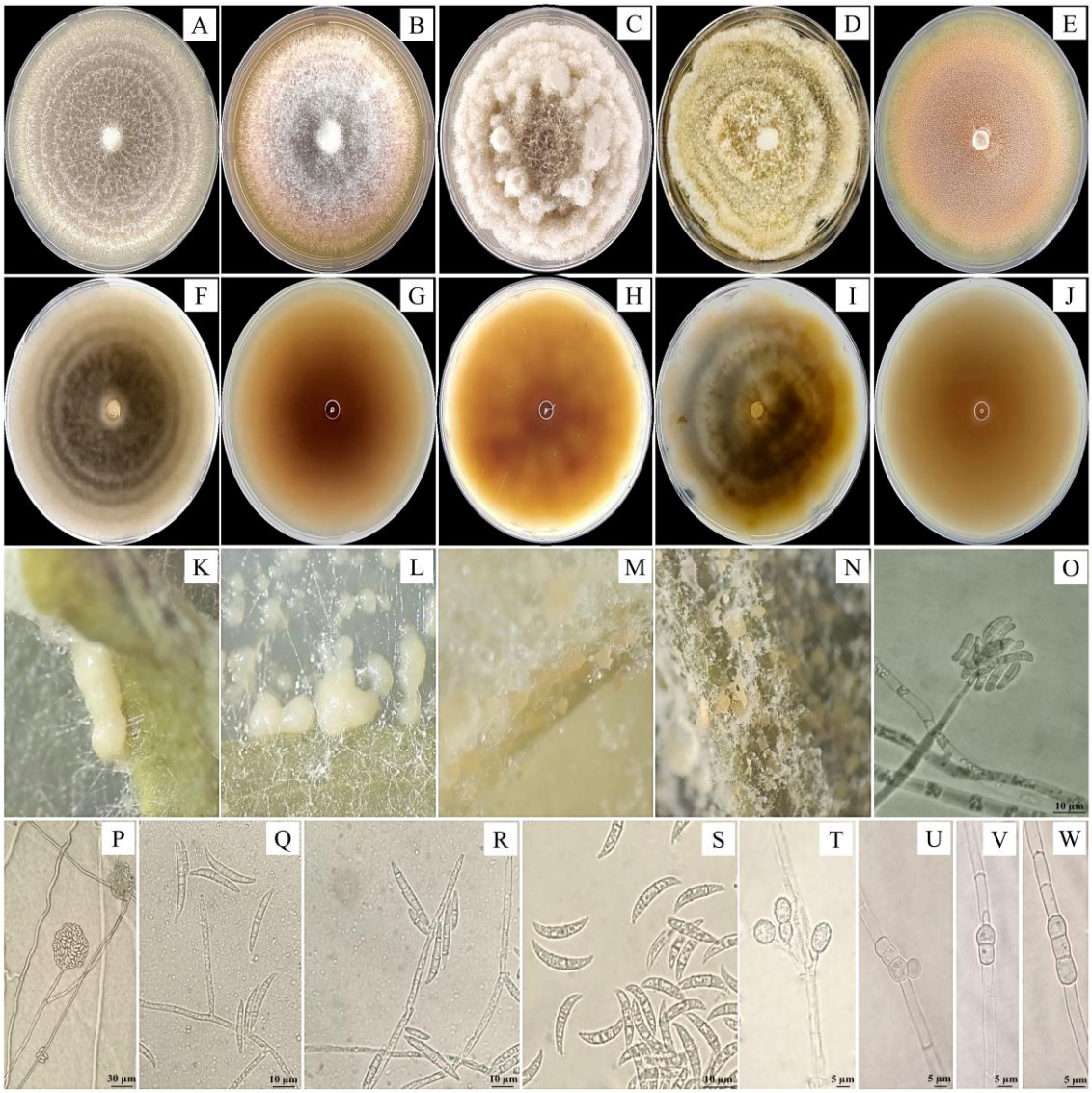


Figure 4: Morphology of the species isolated in this study. Upside and reverse-side colony of *F. falciforme* (A and F), *F. petroliphilum* (B and G), *F. pernambucanum* (C and H), *F. sulawesiense* (D and I), and *F. delphinoides* (E and J). Sphorodochia of *F. falciforme* (K), *F. petroliphilum* (L), *F. pernambucanum* (M), and *F. sulawesiense* (N). Conidia in monophialides of *F. falciforme* (O), *F. petroliphilum* (P), *F. pernambucanum*

(Q), and *F. sulawesiense* (R). Free conidia of *F. delphinoides* (S). Chlamydospores of *F. falciforme* (T), *F. petroliphilum* (U), *F. pernambucanum* (V), and *F. sulawesiense* (W).

The species *F. pernambucanum* (Figure 4C and H) and *F. sulawesiense* (Figure 4D and I) belonging to FIESC were morphologically identical and could only be differentiated by sequence similarities and phylogenetic analysis. Both species had an abundant cottony colony in the beginning with an orange-light vibrant color. After 20 days, the color becomes darker, getting an olive-green tone. Conidia in aerial mycelia and sporodochia were falcate-shaped with 2 to 4 septa. Conidia in aerial mycelia were produced in monophialides and polyphialides with and without branches. Chlamydospores were rare, globose, and single, with a smooth cell wall, arranged in the middle of hyphae. Sporodochia were pale orange on carnation leaves.

The species *F. delphinoides* (Figure 4E and J), belonging to FDSC, had a sparse growth of the colony, with a pale orange color on both sides of the plate. Conidia were ellipsoids or curved, with 1 or no septum, produced in short monophialides in the middle or the end of the hypha. Chlamydospores and sporodochia were absent in this isolate.

7.3 Pathogenicity test and aggressiveness evaluation

In both experiments, the pathogenicity of all isolates in papaya plants was confirmed. After 60 days, inoculated plants showed symptoms like wilting, yellowing, leaf fall, necrosis on roots and stem, and a visible reduction of root volume. Re-isolation was performed from necrotic tissues for reidentification by morphology and sequencing to fulfill Koch's postulates. The mock treatment plants were asymptomatic and showed a greater volume of roots than the plants infected with the fungal isolates.

The isolates that caused the most severe symptoms in both experiments belong to *F. falciforme, F pernambucanum,* and *F. delphinoides* species, with an average severity of

4.2, 5.1, and 5.2 in experiments 1 and 4.25, 4.9 and 5.2 in experiment 2 for each species, respectively (Figure 5). The isolates ARA05, CAR04, APO04, APO06, APO08, and BAR07 (*F. falciforme*), caused a severity of around 5, highlighting the isolate APO08, which caused the death of three replicates in experiment 1.

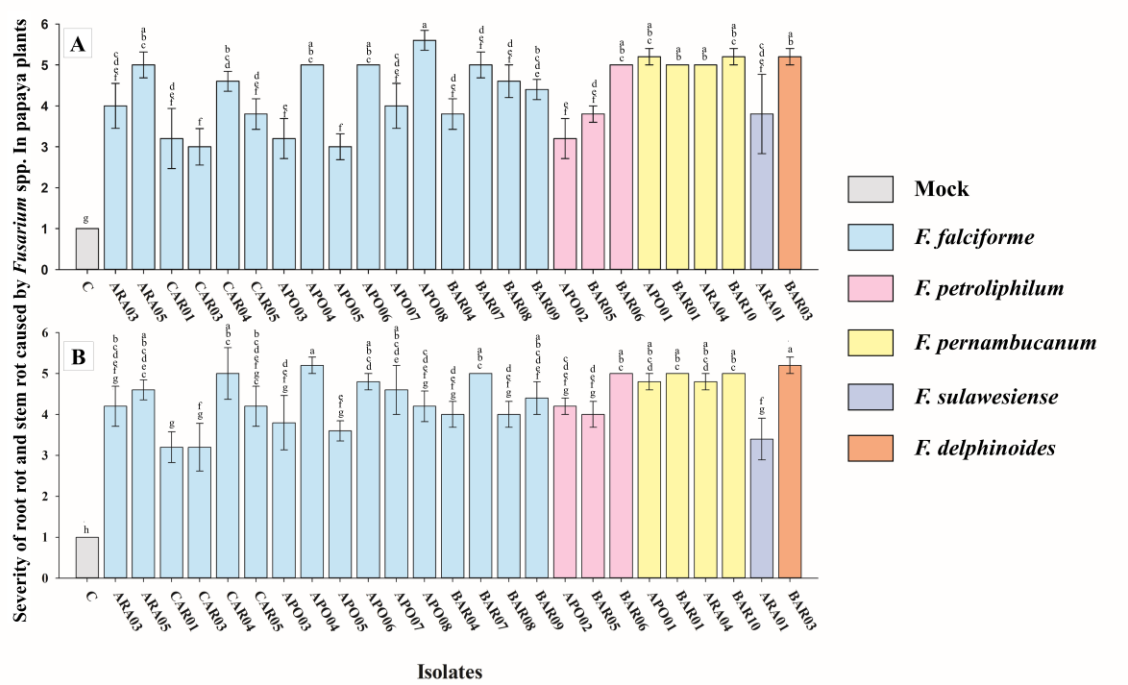


Figure 5: Severity of root rot and stem rot caused by isolates of *Fusarium* spp. in papaya plants two months after inoculation. Experiment 1 (A) and experiment 2 (B). Error bars in the columns are the standard error. Score scale: 0 = no symptoms, 1 = less than 10% of symptoms, 2 = between 10 and 30%, 3 = between 31 and 50%, 4 = more than 50%, and 5 = dead plant. Different letters above columns mean significant differences between isolates by Van der Waerden test ($p \leq 0.05$) (Table S4).

All isolates (APO01, BAR01, ARA04, and BAR10) of *F. pernambucanum* species caused severe symptoms in the papaya plants, with scores around 5 in both experiments. Also, the isolates BAR06 (*F. petroliphilum*) and BAR03 (*F. delphinoides*), showed a severity score of 5 and 5.2 respectively, in both experiments. However, the isolates CAR01, CAR03, APO03, APO05 (*F. falciforme*), and ARA01 (*F. sulawesiense*) caused

less aggressive symptoms in the plants in both experiments. The plants inoculated with those isolates showed symptoms with a score of around 3.

Despite the isolates of each species showing different aggressiveness levels between them, it's noticeable that the species *F. pernambucanum* and *F. delphinoides* were the most aggressive in this study. Although *F. falciforme*, *F. sulawesiense,* and *F. petroliphilum* showed less aggressiveness based on the symptoms they caused, they still caused some level of necrosis in the roots and stems of papaya plants.

8. Discussion

In this study, we characterized the main *Fusarium* species causing root and stem rot in papaya plants in the northeast of Brazil. Some farmers reported losses of about 30% in total papaya production and about 50% of the incidence of disease with high severity, often leading to plant death. Root and stem rots of papaya are frequently related to oomycetes, *Phytophthora*, and *Pythium* genera, and the disease incidence is common in the rainy season (Zakaria, 2023). In this study, we sampled many fields during the dry season, when the farmers reported plants with severe yellowing, wilting, and necrosis in the root system. After the analysis of infected tissues, we observed only *Fusarium* spp. and obtained 15 isolates belonging to three complex species (FSSC, FIESC, and FDSC). Although Correia et al. (2013) have reported that FSSC causes root and stem rot in papaya trees in Brazil, and *F. falciforme* was detected (Silva et al., 2025), that belongs to the same species complex and *F. pernambucanum* (FIESC 17), in our study we identified three other *Fusarium* species causing this disease complex in the region.

The FSSC is a widespread species complex known to group soil-borne pathogens that cause root diseases in many plant species. *F. falciforme* has already been reported to cause symptoms in papaya roots and stems in India (Gupta et al., 2019) and Mexico (Vega-Gutiérrez et al., 2019), like our study's findings. *F. falciforme* was also the most

common species isolated in our study, it's a common pathogen in Brazil causing fruit and root rot in *Cucumis melo* (Araújo et al., 2021, Silva et al., 2023), fruit rot in watermelon (Nascimento et al., 2025), and root rot in *Phaseolus lunatus* (Sousa et al., 2017), *Psidium guajava* (Veloso, Câmara, Souza, 2021), *Manihot esculenta* (Silva et al., 2022), and *Solanum lycopersicum* (Severo et al., 2024), supporting the wide range of hosts of this pathogen. *F. petroliphilum* on the other hand, has never been reported to cause problems on plants in Brazil, but has already been reported to cause fruit and root rot in cucurbits in New Zealand, Spain, and the United States (Costa et al., 2016; González, Armeganol, Garces-Claver, 2018; Brown et al., 2022).

In both experiments, *F. falciforme* and *F. petroliphilum* at the end of our pathogenicity tests contained less aggressive isolates on the plants than other species. Usually, species of FSSC are known to cause root rot and have a hemibiotrophic lifestyle, which means that the fungi behave biotrophiccaly up to a certain point of their cycle of life, where they change to a necrotrophic behavior (de Medeiros et al., 2024). It indicates that the fungi follow the host life cycle, which is common in the behavior of these pathogen species, causing soft symptoms when the plants are young, and leading them to death at the production stage.

Another species complex found in our sampling was FIESC, within this complex, two species (*F. pernambucanum* and *F. sulawesiense*) were isolated from the sampled areas. However, FIESC is more often associated with fruit rot (Lima et al., 2021), cause leaf blight (Lu et al., 2022), and disease in some insects (Santos et al., 2019). In the pathogenicity test, the species *F. pernambucanum* and *F. sulawesiense* showed different levels of aggressiveness. Despite these two species belonging to the same complex, *F. pernambucanum* was more frequent than *F. sulawesiense* in our isolation attempts and caused a higher severity when compared to other species in this work. These results

indicate that *F. sulawesiense* has less capacity or preference to colonize the radicular system of papaya, possibly due to their preference for other plant organs, like fruits. This species has been reported to cause papaya internal fruit rot (Yi et al. 2022) and melon fruit rot and it is one of the most aggressive species to cause this disease in melons (Lima et al., 2021, Araújo et al., 2021, Nogueira et al., 2023), and other fruits like muskmelon (Liu et al., 2023) and chili (Wang et al., 2023).

FDSC is a group of species usually related to humans as saprophytes (Gajjar et al., 2013; Gourav et al., 2024). There are few studies investigating the potential of FDSC to cause diseases in plants (Kulkarni, Sajjan, Karegoudar, 2011; Kulkarni et al., 2013; Yue et al., 2024). However, in our work, we observed that *F. delphinoides* was one of the most aggressive species, causing high severity to papaya plants and leading them to death. Although this species was detected in low frequency in our isolation attempts, their high aggressiveness toward papaya plants can’t be ignored.

It's important to highlight that some big melon producers in the main production areas of Northeast Brazil are replacing the melon plants with papaya. This is concerning because those fields had long periods of melon monoculture with serious problems with soil-borne pathogens, among them *Fusarium* spp. (Nascimento et al., 2018, Figueiredo et al. 2023, Borges et al., 2024). Also, it is common in these regions for growers to make a consortium of watermelon and papaya. These management strategies adopted by local growers may be contributing to the increase of these pathogens inoculum in the soil, since some *Fusarium* species found causing damage to papaya trees in the Northeastern Region of Brazil have already been reported causing damage to melons and watermelons in the same region (Araújo et al., 2021, Silva et al., 2023, Nascimento et al, 2025). Furthermore, there are reports of an increase in problems caused by *Fusarium* in cucurbit production areas in the Northeastern Region of Brazil (Dias &

Terão, 2006; Lima et al., 2021; Araújo et al., 2021, Silva et al., 2023). This reaffirms that planting cucurbits without proper crop rotation increases fusariosis problems, strongly reflected in host successor crops, such as papaya, normally cultivated in the abovementioned production areas.

The presence of the five species of *Fusarium* observed in Baraúna in the state of Rio Grande do Norte is possibly due to the large volume of papaya production in this area (63.000 tons per year) (IBGE, 2023), which is considered one of the largest papaya producing areas of Brazil. In addition, this is a region that also produces melon, in a monoculture regime, which likely fosters the fungi to remain in the soil and increase inoculum pressure.

9. Concluding Remarks

Root and stem rots caused by *Fusarium* spp. are a problem in many countries, and it's becoming an increased issue in the Northeast Region of Brazil. Five species were found in this study causing these disease complexes, and their pathogenicity was confirmed by Koch's postulates. We also investigated the severity and aggressiveness of these isolates on papaya plants to foster more efficient disease management. The findings from this study will help papaya growers and plant pathologists to better understand the causal agents of root and stem rot of papaya in this region. Our results strongly indicate that new crop cultivation strategies need to be developed to substitute the current strategy, consortium of cucurbits in papaya fields, since this option of management, likely increases the inoculum pressure of *Fusarium* spp. over time.

10. Acknowledgments

The authors thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Grant/Award Number: 88887.820470/2023-00, and the Conselho Nacional de Desenvolvimento Científico e Tecnológico CNPq, Grant/Award Number:

307860/2022–1 for the scholarships to support the students involved in this research. This research was partially funded by the USDA Specialty Crop Block Grant Program (SCBGP) – Grant Award #23DAG0087AA and the USDA-NIFA Hatch Grant – Project #1017940.

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12. Attachments

Supplementary Table 1: GenBank access of strains obtained in this study. EF-1α = translation elongation factor 1 alpha, RPB2 = second largest subunit of RNA polymerase.

Isolate	Origin[1]	Specie	Species complex/ Lineage[2]	GenBank code	
				EF-1a	**RPB2**
ARA05	Aracati - CE	*F. falciforme*	FSSC 3+4	PQ672137	PQ672152
APO03	Apodi - RN			PQ672139	PQ672154
APO04				PQ672140	PQ672155
APO05				PQ672141	PQ672156
APO07				PQ672142	PQ672157
APO08				PQ672143	PQ672158
BAR07	Baraúna - RN			PQ672147	PQ672162
BAR08				PQ672148	PQ672163
BAR09				PQ672149	PQ672164
APO02	Apodi - RN	*F. petroliphilum*	FSSC 1	PQ672138	PQ672153
BAR05	Baraúna - RN			PQ672145	PQ672160
BAR06				PQ672146	PQ672161
ARA04	Aracati - CE	*F. pernambucanum*	FIESC 17	PQ672136	PQ672151
ARA01	Aracati - CE	*F. sulawesiense*	FIESC 16	PQ672135	PQ672150
BAR03	Baraúna - RN	*F. delphinoides*	FDSC	PQ672144	PQ672159

[1] CE = Ceará, RN = Rio Grande do Norte.

[2] FSSC = *Fusarium solani* species complex, FIESC = *Fusarium incarnatum-equiseti* species complex, FDSC = *Fusarium dimerum* species complex.

Supplementary Table 2: GenBank access of strains used to build phylogenetic three in this study. EF-1α = translation elongation factor 1 alpha, RPB2 = second largest subunit of RNA polymerase.

Species	Strain code	Species complex/ Lineage [1]	Origin	GenBank access number	
				EF-1α	RPB2
F. petroliphilum	NRRL 22141	FSSC 1	New Zealand	AF178329.1	EU329491.1
F. falciforme	NRRL 43441	FSSC 3+4	USA	DQ790478.1	DQ790566.1
F. solani	MRC 2565	FSSC 5	USA	MH582410.1	MH582420.1
F. cucurbiticola	NRRL 22153	FSSC 10	USA	AF178346.1	EU329492.1
F. ambrosium	NRRL 22346	FSSC 19	India	FJ240350.1	EU329503.1
F. suttonianum	NRRL 32858	FSSC 20	USA	DQ247163.1	EU329630.1
F. bataticola	NRRL 22402	FSSC 23	USA	AF178344.1	FJ240381.1
F. euwallaceae	NRRL 54722	FSSC 36	Israel	JQ038007.1	JQ038028.1
F. equiseti	NRRL 36466	FIESC 14	USA	GQ505653.1	GQ505831.1
F. sulawesiense	NRRL 34004	FIESC 16	USA	GQ505628.1	GQ505806.1
F. pernambucanum	NRRL 32864	FIESC 17	USA	GQ505613.1	GQ505791.1
F. caatingaense	NRRL 34003	FIESC 20	USA	GQ505627.1	GQ505805.1
F. incarnatum	CBS 132.73	FIESC 23	Malawi	MN170476.1	MN170409.1
F. coffeatum	CBS 635.76	FIESC 28	South Africa	MN120755.1	MN120736.1
F. aberrans	CBS 131385	FIESC	Australia	MN170445.1	MN170378.1
F. monophialidicum	NRRL 54973	FIESC	USA	MN170483.1	MN170416.1
F. delphinoides	JZB3630001	FDSC	China	OQ122081.1	OQ124043.1
F. biseptatum	CBS 110311	FDSC	France	MW811086.1	MW811071.1
F. dimerum	NRRL 36140	FDSC	USA	HM347133.1	HM347218.1
F. penzigii	NRRL 20711	FDSC	USA	HM347132.1	HM347217.1
F. staphylae	NRRL 22316	FSTSC	USA	AF178361.1	EU329502.1
F. cicatricum	CBS 125740	FSTSC	Nethherlands	HM626646.1	HM626680.1

[1] FSSC = *Fusarium solani* species complex, FIESC = *Fusarium incarnatum-equiseti* species complex, FDSC = *Fusarium dimerum species complex*, FSTSC = *Fusarium staphylae* species complex.

Supplementary Table 3: GenBank access of strains used in the pathogenicity test from the previous study. EF-1α = translation elongation factor 1 alpha, RPB2 = second largest subunit of RNA polymerase.

Isolate	Origin[1]	Specie	Species complex/ Lineage[2]	GenBank code	
				EF-1a	RPB2
ARA03	Aracati - CE	*F. falciforme*	FSSC 3+4	PP723773.1	PP723783.1
CAR01	Caraúbas - RN			PP723774.1	PP723784.1
CAR03				PP723775.1	PP723785.1
CAR04				PP723776.1	PP723786.1
CAR05				PP723777.1	PP723787.1
APO06				PP723779.1	PP723789.1
BAR04	Baraúna - RN			PP723778.1	PP723788.1
APO01	Apodi - RN	*F. pernambucanum*	FIESC 17	PP723780.1	PP723790.1
BAR01	Baraúna - RN			PP723781.1	PP723791.1
BAR10				PP723782.1	PP723792.1

[1] CE = Ceará, RN = Rio Grande do Norte.

[2] FSSC = *Fusarium solani* species complex, FIESC = *Fusarium incarnatum-equiseti* species complex.

Supplementary Table 4: Severity of root rot and stem rot in papaya in experiments 1 and 2.

Isolate	Specie	Severity		Normal score	
		Experiment 1[1]	Experiment 2[1]	Experiment 1	Experiment 2
Control		1 g	1 h	-1,92683657	-1,99539331
ARA03	***F. falciforme***	4 cdef	4.2 bcdefg	-0,16516935	-0,08418358
ARA05		5 abc	4.6 abcde	0,53568669	0,19461186
CAR01		3.2 def	3.2 g	-0,57217963	-0,87698848
CAR03		3 f	3.2 fg	-0,87076014	-0,78940249
CAR04		4.6 bcd	5 abc	0,13448936	0,75290966
CAR05		3.8 def	4.2 bcdefg	-0,40115922	-0,08271728
APO03		3.2 ef	3.8 defg	-0,70728119	-0,3197773
APO04		5 abc	5.2 a	0,46490429	0,79767363
APO05		3 f	3.6 efg	-0,87248865	-0,63992847
APO06		5 abc	4.8 abcd	0,46490429	0,37986015
APO07		4 cdef	4.6 abcde	-0,23422324	0,33509619
APO08		5.6 a	4.2 cdefg	1,17287389	-0,13003416
BAR04		3.8 def	4 defg	-0,40115922	-0,31528245
BAR07		5 abc	5 abc	0,53568669	0,56510845
BAR08		4.6 bcd	4 defg	0,20527176	-0,31528245
BAR09		4.4 bcde	4.4 abcdef	-0,03071811	0,05668044
APO02	***F. petroliphilum***	3.2 ef	4.2 cdefg	-0,70728119	-0,17588474
BAR05		3.8 def	4 defg	-0,46374986	-0,31528245
BAR06		5 abc	5 abc	0,46490429	0,56510845
ARA04	***F. pernambucanum***	5 abc	4.8 abcd	0,46490429	0,37986015
APO01		5.2 ab	4.8 abcd	0,70089416	0,37986015
BAR01		5 abc	5 abc	0,46490429	0,56510845
BAR10		5.2 ab	5 abc	0,70089416	0,56510845
ARA01	***F. sulawesiense***	3.8 cdef	3.4 fg	-0,14616662	-0,69174019
BAR03	***F. delphinoides***	5.2 ab	5.2 a	0,70089416	0,79767363

[1] Means followed by the same letter indicate no significant difference with the Wan der Waerden test $p \leq 0.05$.

First report of *Fusarium falciforme* **and** *Fusarium pernambucanum* **causing root and stem rot on papaya plants in Brazil**

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Papaya (*Carica papaya* L.) is one of the major fruit crops of northeast Brazil, with an average annual production of 571,693 tons (IBGE, 2022). In August 2023, papaya plants (hybrid Tainung 01) in the production stage showed dark brown symptoms on roots and stems, wilt progression, and collapse of the plants (disease incidence of 20 - 50 % in sampled fields). Samples were collected from six production farms located in Apodi, Baraúna, and Caraúbas municipalities in Rio Grande do Norte state and Aracati in Ceará state. Small fragments of symptomatic tissues were surface sterilized sequentially in ethanol 70 % (1 sec), sodium hypochlorite 2.5 % (60 secs), and sterilized water. The fragments were placed in potato dextrose agar (PDA), supplemented with 0.05 % tetracycline, and incubated at 28° C with 12 hours of photoperiod for five days. Monosporic cultures were obtained from 10 isolates of *Fusarium*, characterized by morphology. The translation elongation factor 1 alpha (EF-1α) and the second largest subunit of RNA polymerase (RPB2) genes were partially amplified by PCR and sequenced from all isolates - the sequences were deposited in GenBank (PP723773.1 - PP723792.1). Maximum-parsimony tree was built in the Software MEGA (Version 11.0.10) (Tamura et al. 2021) with the concatenated partial sequences. The species were morphologically characterized in PDA (10 days), Spezieller Nährstoffarmer agar (SNA) (10 days), and carnation leaf agar (CLA) (30 days) (Leslie & Summerell, 2006). Phylogenetic analysis revealed that seven isolates are most closely related to *F. falciforme* (99 % bootstrap), and three isolates are most closely related to *F. pernambucanum* (99 % bootstrap). The morphological characters of the isolates correlated with the original descriptions of each species (Leslie & Summerell, 2006). Pathogenicity tests were performed on 45-day-old papaya seedlings (hybrid Tainung 01) using the infested soil method (Lefèvre & Souza, 1993). Autoclaved substrate was

infested with fragments of PDA from each isolate colony and incubated for seven days to create the inoculum. Then, 36 g L^{-1} of inoculum was added to each pot, in which a papaya seedling was planted, and grown for 60 days under greenhouse conditions (33°C ± 5°). The experiment was conducted twice, each time five plants were inoculated with each isolate, and five plants were left uninoculated (mock). Symptoms on stems appeared 30 days after inoculation, while on roots it took 60 days. *F. falciforme* and *F. pernambucanum* caused identical symptoms in the field and in our pathogenicity test. No symptoms were observed on plants from the mock treatment. The pathogens were re-isolated from the necrotic tissue and re-identified, morphologically and through Sanger sequencing as described above, to fulfill Koch's postulates. Correia et al. (2013) reported the *Fusarium solani* species complex (FSSC) as the etiologic agent of stem rot in *C. papaya* in Brazil; however, they weren't able to identify the isolates at the species level. In our studies, we identified *F. falciforme* (FSSC 3 + 4) and *F. pernambucanum* (*Fusarium incarnatum-equiseti* species complex 17) as the pathogens causing this disease in northeast Brazil. To the best of our knowledge, this is the first report of *F. falciforme* and *F. pernambucanum* causing root and stem rot on papaya plants in Brazil. This study will help papaya growers and plant pathologists to better understand the causal agents of this disease complex in Brazil to develop disease management strategies.

Funding: The authors thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Grant/Award Number: 88887.820470/2023-00, and the Conselho Nacional de Desenvolvimento Científico e Tecnológico CNPq, rant/Award Number:

307860/2022–1 for the granting of scholarships. This research was partially funded by the USDA Special Specialty Crop Block Grant Program (SCBGP) - Grant Award #23DAG0087AA and by the USDA-NIFA Hatch Grant - Project #1017940.

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FIGURES LEGENDS:

Fig. Supplemental S1. Maximun-parsimony tree built with concatenate EF-1α + RPB2 gene set, from 10 isolates obtained in this study (bold letter), and reference sequences from GenBank (strain in parenthesis) of *Fusarium solani* species complex (FSSC), *Fusarium incarnatum-equiseti species complex* (FIESC), and *Fusarium staphylae species complex* (FSTSC). Isolates ARA03, CAR01, CAR03, CAR04, CAR05, BAR04, and APO06 are grouped in *F. falciforme* clade (FSSC 3+4) with 99 % of Bootstrap support (1000 replicants). Isolates APO01, BAR01, and BAR10 are grouped in the *F. pernambucanum* clade (FIESC 17), with 99 % Bootstrap support (1000 replicants).

Fig. supplemental S2. Morphology of *Fusarium falciforme* (FSSC 3 + 4) (A - G). The isolates ARA03, CAR01, CAR03, CAR04, CAR05, APO06, and BAR04 showed white-fluffed aerial mycelium and a diffusible dark pigment in the media (A). Sphorodochia were white to cream (B). In SNA, microconidia were ovoid-shaped, hyaline, with 0-2 septa, and produced in monophyialides with well-developed foot cells and false heads (C - D). Macroconidia were falcate-shaped, hyaline, with 3-5 septa (E). Chlamydospores were abundant, globose, located terminally or interspersed in hyphae, single or in chains (F - G). Morphology of *Fusarium pernambucanum* (FIESC 17) (H - N). The APO01, BAR01, and BAR10 isolates showed orange-fluffed aerial mycelium and white edges (H). Sphorodochia were pale orange (I). In SNA, microconidia and macroconidia were falcate-shaped, hyaline, with 2-5 septa, produced in branched phialides (J - M). Chlamydospores were rare, globose, located interspersed in hyphae, single or in chains (N). Symptoms of stem rot in papaya plant in the field (O). The collapse of papaya plants in the production stage due to root rot in the field (P). Necrotic root tissues of papaya plants from commercial fields (Q). Papaya plant with root rot after 60 days inoculated with *F. falciforme* (R), *F. pernambucanum* (S), and non-inoculated control without symptoms (T). Petri dishes with re-isolations of *F. falciforme* (U) and *F. pernambucanum* (V) after the pathogenicity test.

Species[1]	Strain code	Species complex/ Lineage[2]	Origin[3]	GenBank access number	
				EF-1α	**RPB2**
F. petroliphilum	NRRL 22141	FSSC 1	New zealand	AF178329.1	EU329491.1
F. keratoplasticum	FRC S-2477	FSSC 2	USA	JN235712.1	JN235897.1
F. falciforme	**ARA03**	**FSSC 3+4**	**Aracati/CE/Brazil**	**PP723773.1**	**PP723783.1**
F. falciforme	**CAR01**	**FSSC 3+4**	**Caraúbas/RN/Brazil**	**PP723774.1**	**PP723784.1**
F. falciforme	**CAR03**	**FSSC 3+4**	**Caraúbas/RN/Brazil**	**PP723775.1**	**PP723785.1**
F. falciforme	**CAR04**	**FSSC 3+4**	**Caraúbas/RN/Brazil**	**PP723776.1**	**PP723786.1**
F. falciforme	**CAR05**	**FSSC 3+4**	**Caraúbas/RN/Brazil**	**PP723777.1**	**PP723787.1**
F. falciforme	**BAR04**	**FSSC 3+4**	**Baraúna/RN/Brazil**	**PP723778.1**	**PP723788.1**
F. falciforme	**APO06**	**FSSC 3+4**	**Apodi/RN/Brazil**	**PP723779.1**	**PP723789.1**
F. falciforme	NRRL 43441	FSSC 3+4	USA	DQ790478.1	DQ790566.1
F. solani	MRC 2565	FSSC 5	USA	MH582410.1	MH582420.1
F. neocosmosporiellum	NRRL 22166	FSSC 8	USA	AF178350.1	EU329497.1
F. cucurbiticola	NRRL 22153	FSSC 10	USA	AF178346.1	EU329492.1
F. vanettenii	LC13837	FSSC 11	USA	MW620183.1	MW474708.1

F. silvicola	LC5482	FSSC 13	China	MW620187.1	MW474712.1
F. lichenicola	NRRL 32434	FSSC 16	Germany	DQ246977.1	EF470161.1
F. mori	NRRL 22230	FSSC 17	Japan	AF178358.1	EU329499.1
F. parceramosum	HT-2D	FSSC 18	China	OP994261.1	OP994263.2
F. ambrosium	NRRL 22346	FSSC 19	India	FJ240350.1	EU329503.1
F. suttonianum	NRRL 32858	FSSC 20	USA	DQ247163.1	EU329630.1
F. bataticola	NRRL 22402	FSSC 23	USA	AF178344.1	FJ240381.1
F. liriodendri	NC21953	FSSC 24	South Korea	OP920672.1	OP920674.1
F. ferrugineum	NC21952	FSSC 28	South Korea	OP784438.1	OP784448.1
F. pseudensiforme	NRRL 46517	FSSC 33	Sri Lanka	KC691555.1	KC691674.1
F. euwallaceae	NRRL 54722	FSSC 36	Israel	JQ038007.1	JQ038028.1
F. illudens	NRRL 22090	FSSC	New Zealand	AF178326.1	JX171601.1
F. plagianthi	NRRL 22632	FSSC	New Zealand	AF178354.1	EU329519.1
F. equiseti	NRRL 36466	FIESC 14	USA	GQ505653.1	GQ505831.1
F. equiseti	Indo138	FIESC 14	Indonesia	LS479443.1	LS479855.1
F. irregulare	LC7188	FIESC 15	China	MK289629.1	MK289783.1
F. sulawesiense	NRRL 34004	FIESC 16	USA	GQ505628.1	GQ505806.1

F. pernambucanum	**APO01**	**FIESC 17**	**Apodi/RN/Brazil**	**PP723780.1**	**PP723790.1**
F. pernambucanum	**BAR01**	**FIESC 17**	**Baraúna/RN/Brazil**	**PP723781.1**	**PP723791.1**
F. pernambucanum	**BAR10**	**FIESC 17**	**Baraúna/RN/Brazil**	**PP723782.1**	**PP723792.1**
F. pernambucanum	NRRL 32864	FIESC 17	USA	GQ505613.1	GQ505791.1
F. caatingaense	NRRL 34003	FIESC 20	USA	GQ505627.1	GQ505805.1
F. guilinense	LC12160	FIESC 21	China	MK289594.1	MK289747.1
F. incarnatum	CBS 132.73	FIESC 23	Malawi	MN170476.1	MN170409.1
F. nanum	LC12168	FIESC 25	China	MK289602.1	MK289755.1
F. hainanense	LC11638	FIESC 26	China	MK289581.1	MK289735.1
F. coffeatum	CBS 635.76	FIESC 28	South Africa	MN120755.1	MN120736.1
F. citri	LC6896	FIESC 29	China	MK289617.1	MK289771.1
F. humuli	CQ1039	FIESC 33	China	MK289570.1	MK289724.1
F. aberrans	CBS 131385	FIESC	Australia	MN170445.1	MN170378.1
F. monophialidicum	NRRL 54973	FIESC	USA	MN170483.1	MN170416.1
F. staphylae	NRRL 22316	FSTSC	USA	AF178361.1	EU329502.1
F. cicatricum	CBS 125740	FSTSC	Netherlands	HM626646.1	HM626680.1

Table eXtra1: GenBank access of strains used to build the phylogenetic three in this study. EF-1α = translation elongation factor 1 alpha, RPB2 = second largest subunit of RNA polymerase.

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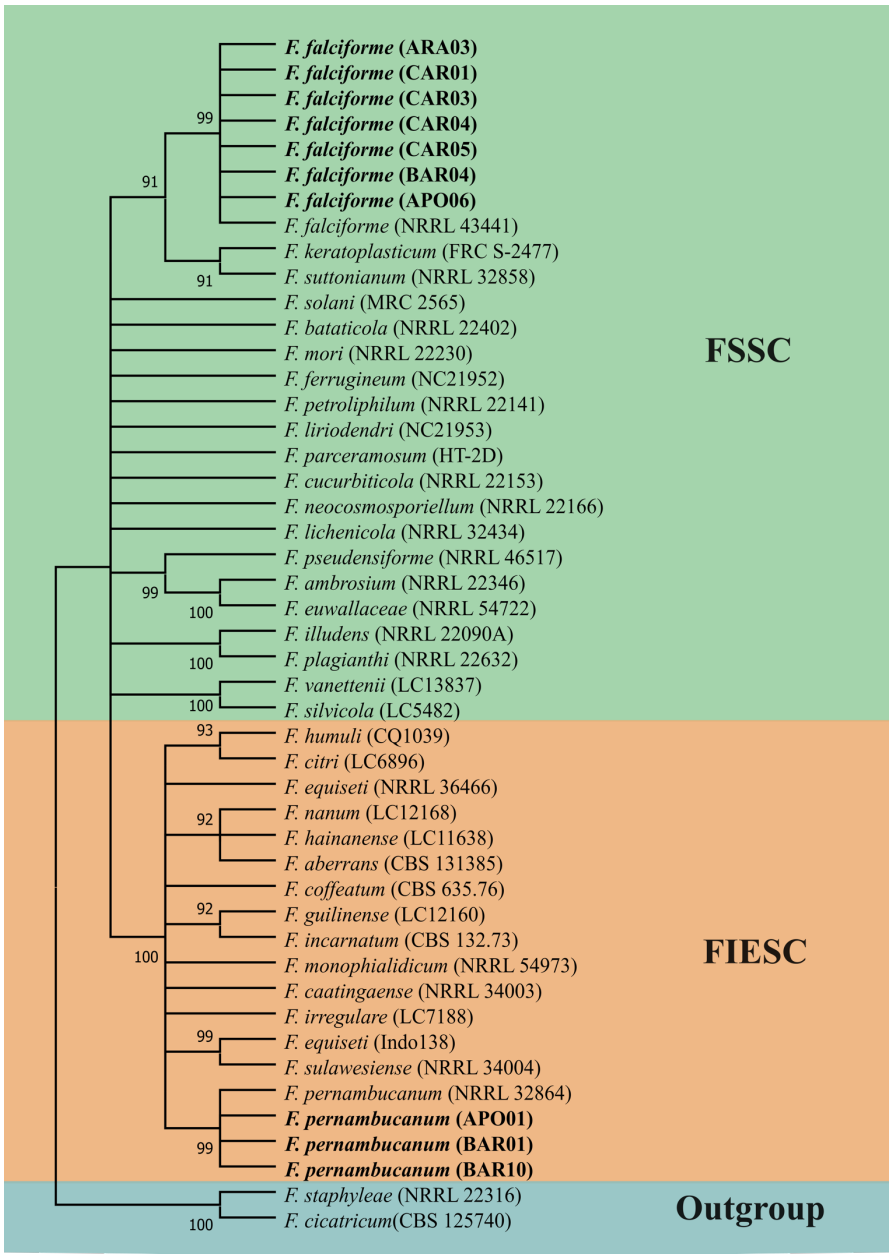


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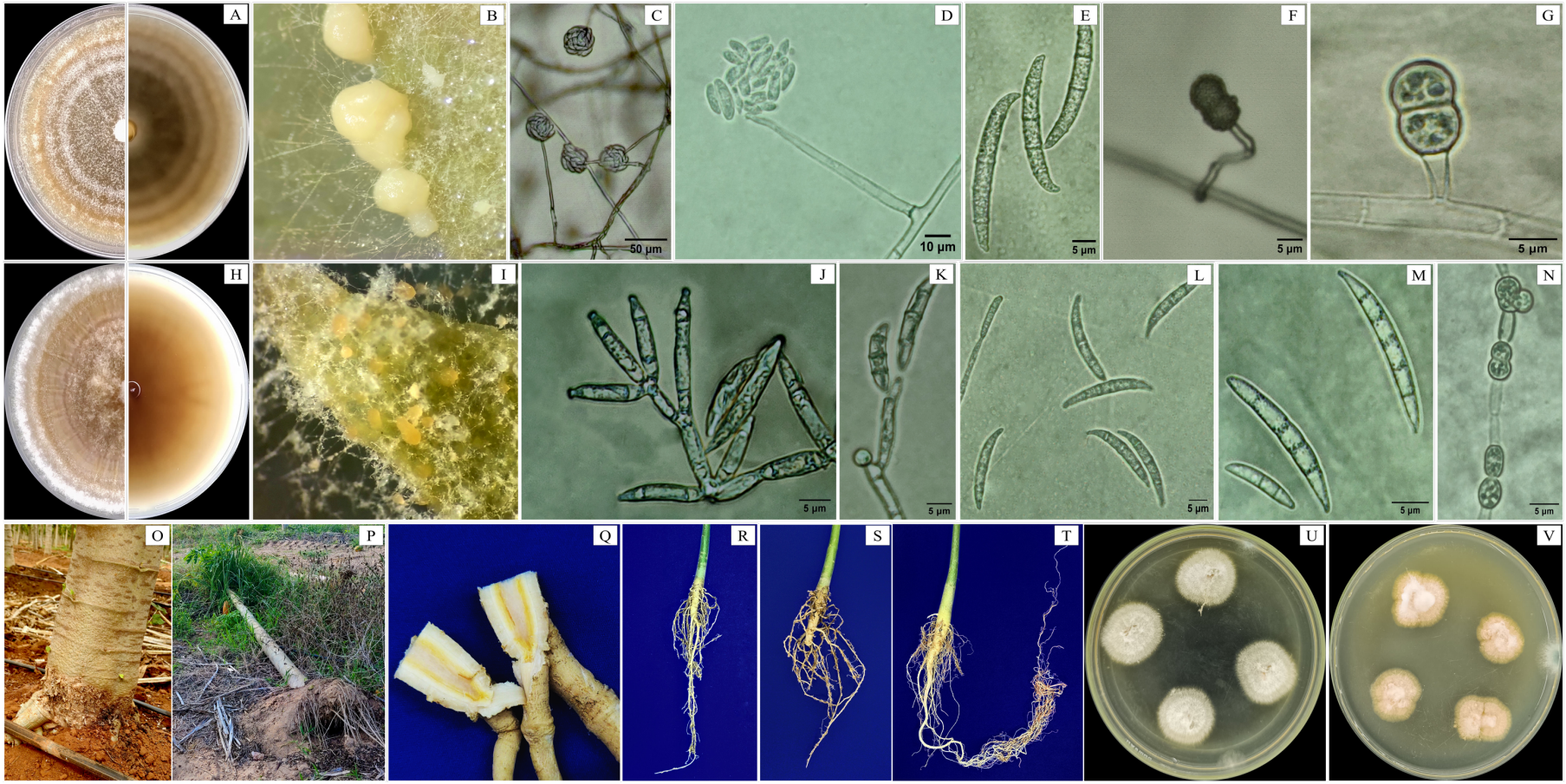


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