Vol. 24(8), pp.160-169, August 2025
DOI: 10.5897/AJB2025.17756
Article Number: C7D4A1B73572
ISSN: 1684-5315

http://www.academicjournals.org/AJB

ACADEMIC JOURNALS
expand your knowledge

African Journal of Biotechnology

Full Length Research Paper

Cashew phyllosphere metagenomics reveals fungal phytopathogens of cashew trees from Kenya

Dennis Wamalabe Mukhebi[1], Colletah Rhoda Musangi[1], Everlyne Moraa Isoe[1], Edith Muwawa[2], Johnstone Omukhulu Neondo[3] and Wilton Mwema Mbinda[1,4]*

[1]Department of Biochemistry and Biotechnology, Pwani University, Kilifi, Kenya.
[2]Department of Biological Sciences, Pwani University, Kilifi, Kenya.
[3]Institute for Biotechnology Research, Jomo Kenyatta University of Agriculture and Technology, Juja, Kenya.
[4]Pwani University Biosciences Research Centre (PUBReC), Pwani University, Kilifi, Kenya.

Received 8 May, 2025; Accepted 7 July, 2025

Cashew is an important cash crop in coastal Kenya. However, the emergence of phytopathogens poses a growing threat, leading to substantial losses that reduce both the quality and quantity of cashew products. The present study aimed to identify potential fungal pathogens associated with environmental samples from the cashew phyllosphere in coastal Kenya using an amplicon sequencing approach. The cashew samples were collected from Kwale, Kilifi and Lamu counties using a complete randomized stratified approach. An amplicon metagenomic approach targeting the ITS and 28S rRNA regions of fungal rDNA was employed to characterize the composition of potential fungal pathogens associated with different parts of the cashew phyllosphere, including leaves, flowers, and fruits. The ITS and 28S rRNA sequences clustered into 267 and 108 operational taxonomic units (OTUs), respectively, with *Ascomycota* being the dominant phylum, followed by *Basidiomycota*. The cashew phyllosphere harbors a diverse community of potential fungal pathogens, with *Aspergillus, Erysiphe,* and *Lasiodiplodia* being the most abundant genera. Beta diversity analysis revealed distinct differences in fungal community composition between epiphytic and endophytic populations. In contrast, the alpha diversity analysis within each group indicated notable richness and evenness of the fungal communities. These findings underscore the presence of potential fungal pathogens within the fungal communities inhabiting environmental samples from the cashew phyllosphere and highlight the need for further studies targeting functional genes in specific fungal groups to accurately identify pathogenic species to inform the development of appropriate management strategies for cashew production.

Key words: Cashew, fungal pathogens, metagenomics, phyllosphere.

INTRODUCTION

Cashew (*Anacardium occidentale*) is a tropical evergreen tree belonging to the family Anacardiaceae, valued for its production of both cashew apples and nuts. Currently, cashew nuts rank as the second most important tree nuts

*Corresponding author. E-mail: wilton.mbinda@gmail.com or w.mbinda@pu.ac.ke.

in global trade after almonds (Savadi et al., 2020), with an estimated annual global production of approximately 3.85 million metric tons (Olaitan and Oluwayemisi, 2023). This drought-resistant cash crop, originally from Central Brazil, is now widely cultivated across the world, having been introduced to Africa by the Portuguese in the 16th century (de Brito et al., 2018). Despite its economic and nutritional significance to thousands of small-scale farmers worldwide, cashew cultivation is threatened by various pathological factors, including pests and a wide range of bacterial and fungal phytopathogens, which pose substantial risks to this vital tree crop (Muntala et al., 2021).

Diseases caused by fungal pathogens have been reported to result in substantial yield loses in all cashew-growing regions (Wonni et al., 2017; Majune et al., 2018; Pinto et al., 2018; Muntala et al., 2021). The diseases associated with fungal pathogens include anthracnose, leaf spot, gummosis, and dieback, which are primarily linked to the *Colletotrichum gloeosporioides* species complex and other pathogens (Muntala et al., 2020). Pathogens such as *Lasiodiplodia theobromae* (Coutinho et al., 2017) and *Pestalotia* species (Kharwar et al., 2010) play prominent roles in the colonization of plant aerial parts, with associated economic impacts (Muntala et al., 2020; Pinto et al., 2018). To prevent massive economic losses due to fungal pathogens, the application of fungicides has been utilized. However, the application of fungicides raises environmental and health concerns, highlighting the need for a robust integrated pest management system.

Few studies on the fungal or bacterial phytopathogen communities of cashew have been conducted that rely solely on isolation and culture techniques (Wonni et al., 2017). With the evolution of current metagenomic approaches and next-generation sequencing, promising strategies for identifying microbial communities associated with or within plant tissues can now be (Akinsanya et al., 2015; Alahapperuma, 2016). These emerging technologies offer significant advantages over culture-dependent methods in plant microbial ecology and plant pathology due to their ability to capture microbial communities in depth. This enables the detection of less fastidious microbial pathogens, which represent approximately 99% of the total estimated microbial diversity (Pylro and Roesch, 2017). The use of universal fungal barcodes, such as ITS and 28S rRNA, is fundamental for gaining a comprehensive understanding of associated fungal communities.

However, universal marker genes, like those used in this study, have limited interspecies resolution compared to functional genes targeting specific fungal communities. The present study aims to identify potential fungal pathogens from environmental samples of different parts of the cashew phyllosphere (leaf, flower, and fruit) collected along the Kenyan coast.

MATERIALS AND METHODS

Plant

This research was conducted among farmers in open cashew orchards from three counties in conjunction with relevant agricultural authorities through the expertise of the County Agricultural Extension Officers. The cashew phyllosphere was collected from the fields using non-lethal collection methods and shipped to the laboratory for further processing.

The cashew phyllosphere sample materials (leaf, flower and fruit) were collected from Kwale, Kilifi and Lamu counties in coastal Kenya.

Sample collection

The environmental sample of cashew phyllosphere materials were collected using complete randomized stratified approach (Lance and Hattori, 2016). The farmers were grouped into strata that were equivalent to the sub-counties, from which 75 samples (25 for leaf, flowers, and fruit, respectively) were collected: Kwale (21), Kilifi (42) and Lamu (12). The number of samples collected was proportional to the number of farmers and the density of cashew trees in each subcounty. Sampling was conducted randomly across the study sites and included both symptomatic and asymptomatic plant material. The samples were preserved in DESS buffer (20% dimethyl sulfoxide, ethylenediamine tetra acetic acid, and saturated salt) as described by Pavlovska et al. (2021) and transported to the Pwani University Bioscience Research Centre (PUBReC), Kilifi, Kenya, following standard operating procedures for sample storage.

Isolation of fungal epiphytes and endophytes

The samples were retrieved from PUBReC biobank, containing 50 mL of the DESS buffer transferred into a 100 mL conical flask, and placed on a rotary shaker at 180 revolutions per minute (rpm) for two hours to facilitate the dislodgment of fungal spores for epiphyte isolation. A sterile 0.2 µm filter membrane fitted onto a micro funnel was used to capture fungal spores by filtering the tissue storage buffer. The filter membrane was then transferred into a 2 mL microcentrifuge tube using sterile forceps and immediately flash-frozen in liquid nitrogen. The plant tissues were subsequently rinsed three times with double-distilled water to remove any residual buffer. Surface sterilization of plant tissues for endophyte isolation was performed by sequential incubation: 1 min in sterile distilled water, 1 min in 75% (v/v) ethanol, 3 min in 3.25% sodium hypochlorite, and 30 s in 75% (v/v) ethanol (Gomes et al., 2018). The sterilized tissues were then transferred into 2 mL microcentrifuge tubes and flash-frozen in liquid nitrogen.

Total fungal DNA extraction and sequencing

Genomic DNA (gDNA) was obtained from crushed samples (epiphytes and endophytes) under aseptic cold conditions in liquid nitrogen using a chilled mortar and pestle in a laminar airflow to minimize cross and external contamination. The gDNA was isolated from 1 g of sample via the Qiagen DNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's prescribed manual. The DNA was quantified using a NanoDrop 2000 spectrophotometer, and its integrity was assessed by 1% agarose gel electrophoresis. The gDNA from epiphytes and endophytes collected within the same geographical coordinates was pooled, resulting in a total of 18 composite samples. Universal primers targeting the ITS and 28S

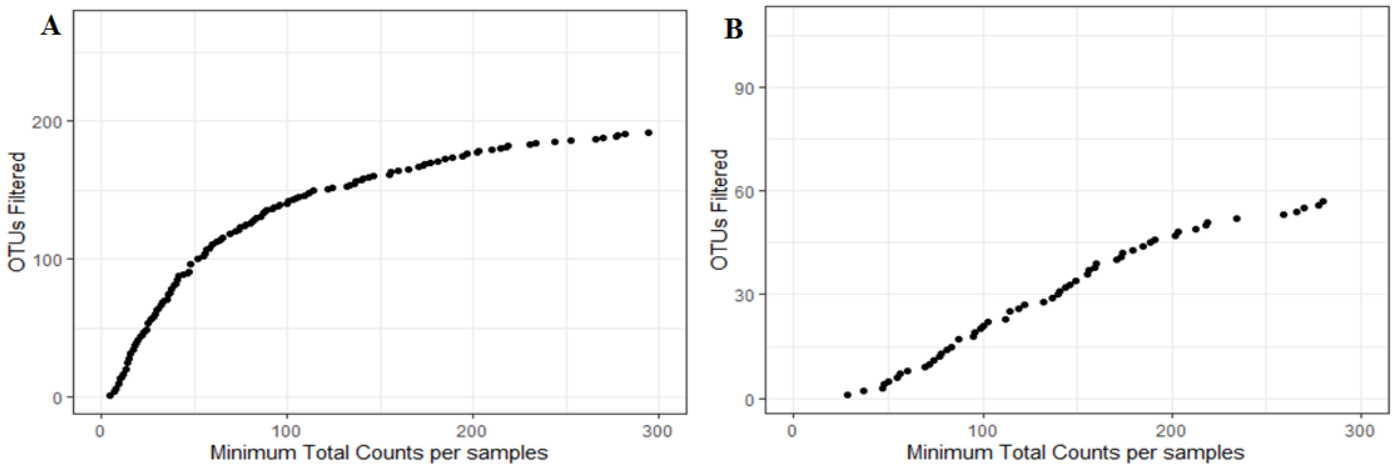


Figure 1. Sequence analysis results: A) Rarefaction curve for ITS reads, B) Rarefaction curve for 28S rRNA.

rRNA regions were used for amplification: ITS1 (5'-TCCGTCGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') for the ITS region, and NL1 (5'-GCATATCAATAAGCGGAGGAAAAG-3') and NL4 (5'-GGTCCGTGTTTCAAGACGG-3') for the 28S rRNA region. The genomic DNA was submitted for paired-end 2 × 300 bp sequencing using the Illumina MiSeq platform at Macrogen, Inc., Republic of Korea.

Data processing and statistical analysis

The raw fastq reads from the sequencer were processed using Fastqc (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/) for pre- and post-quality checks and trimmed via the Trimmomatic tool (Bolger et al., 2014). Sequence analysis was conducted using VSEARCH (Rognes et al., 2016). ITS and 28S rRNA reads were merged and concatenated into separate single files. The merged reads were then quality-filtered, dereplicated, denoised, and screened for chimeras. Operational taxonomic units (OTUs) were clustered at 97% sequence similarity using the USEARCH UPARSE pipeline. A greedy clustering approach was used for read mapping and out creation. A BLASTn local database was created via the Makeblastdb function for sequences from the UNITE (Abarenkov et al., 2023) and LSU 28S rRNA (https://www.arb-silva.de/browser/lsu/) databases to generate indices. BLASTn was employed for OTU classification and taxonomic assignment to generate a taxon file from the corresponding database. The OTU's table were rarefied to an even depth to reduce the compositional nature of microbiome sequence data. Shannon's diversity index (Maniruzzaman, 2006) and Chaol's estimate were calculated to determine the alpha fungal diversity. The species abundance among the environmental samples of cashew phyllosphere was evaluated using heatmap which depicted distribution of relatively abundant fungal OTU. The impact of distinct microenvironments on OTU richness was evaluated via the Kruskal–Wallis test (Frey, 2023) and one-way analysis of variance (ANOVA). The weighted and unweighted Unifrac distance matrices were applied for nonmetric multidimensional scaling (NMDS) ordination, and a two-dimensional plane was used to determine whether communities with similar characteristics tended to cluster together. The dissimilarity was confirmed via the analysis of similarity test (Somerfield et al., 2021).

RESULTS

Sequence statistics

Quality validation of the demultiplexed fastq sequence for both ITS and 28S rRNA yielded 389456 and 299998 high quality sequence reads. The reads were clustered into 267 and 108 OTUs with undetected singletons and doubletons. The rarefaction curves for both the ITS and 28S rRNA regions were developed (Figure 1). These rarefaction curves for both the ITS and 28S rRNA regions reveal adequate coverage of the fungal community through metagenomic profiling.

Fungal diversity analysis among epiphytes and endophytes of the cashew phyllosphere

Alpha diversity metrics for both epiphytic and endophytic fungal communities, assessed using ITS and 28S rRNA markers (Figure 2), revealed moderate species richness and evenness, as indicated by Shannon index values. The lack of statistically significant differences between groups suggests comparable community complexity and sufficient within-sample sequencing depth. NMDS analysis, combined with a distance-based redundancy analysis (db-RDA) using unweighted UniFrac metrics, revealed significant differences in fungal community composition between epiphytes and endophytes based on clustering (Figure 3).

Relative abundance of associated fungal pathogens in cashew phyllosphere

Analysis of the complete ITS and 28S rRNA datasets revealed that the phylum *Ascomycota* was the most

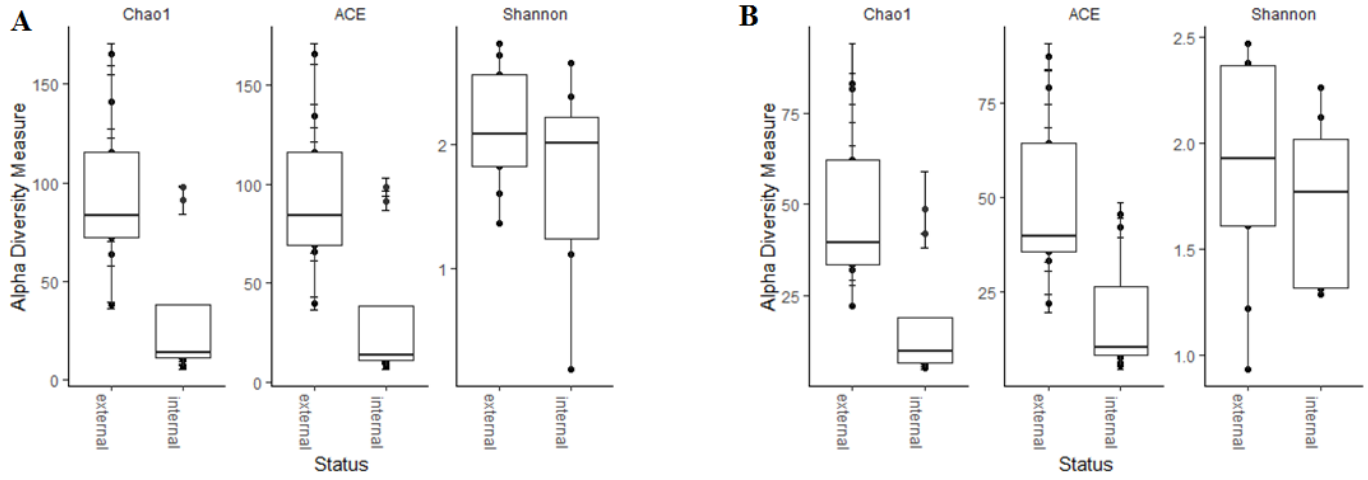


Figure 2. Box plot visualizing results of alpha diversity: comparison of alpha diversity in epiphytes and endophytes: A) ITS and B) 28S rRNA.

abundant, followed by *Basidiomycota*, unclassified fungi (*Fungi incertae sedis*), and *Glomeromycota* (Figure 4). The microbial composition based on ITS sequences included members of the Aspergillaceae family, notably the potentially pathogenic species *Aspergillus tubingensis* and *Aspergillus stromatoides*, with *A. tubingensis* being more abundant than *A. stromatoides* in epiphytic fruit samples (Figure 5A).

The family Erysiphaceae has two commonly known species, *Blumeria graminis* and *Erysiphe quercicola* (Figure 5B). *B. graminis* was notably more abundant than *E. quercicola* in endophytic leaf and fruit samples. In contrast to this endophytic dominance, the epiphytic abundances of *B. graminis* and *E. quercicola* were relatively similar (Figure 5B). The family Botryosphaeriaceae includes two prominent genera: *Lasiodiplodia* and *Neofusicoccum* (Figure 5C). Among fruit samples, *Lasiodiplodia pseudotheobromae* was more abundant than *Neofusicoccum parvum* in both epiphytic and endophytic communities. However, *N. parvum* showed relatively higher abundance in epiphytic fruit samples (Figure 5C). The family Pleosporaceae, represented by *Alternaria* species, was more abundant in epiphytic leaf and flower samples than in fruit samples (Figure 5D). Similarly, the family Mycosphaerellaceae, represented by *Pseudocercospora luzardii*, was more prevalent in epiphytic leaf samples compared to flower and fruit samples (Figure 5E).

The microbial composition based on 28S rRNA data included fungal orders with potential pathogenic species across all cashew phyllosphere samples. The order Eurotiales comprised two species, *Aspergillus niger* and *Thermomyces lanuginosus* (Figure 6). Compared to endophytes, *A. niger* was more abundant in epiphytic samples, while *T. lanuginosus* was more prevalent in epiphytic fruit samples (Figure 6A).

The microbial composition within the fungal order Erysiphales, analyzed up to the family level, included two potential fungal pathogens: *Erysiphe necator*, known to cause grapevine powdery mildew, and *E. quericola*, associated with powdery mildew in *Mangifera indica* (Figure 6B). *E. quericola* was more abundant in both epiphytic and endophytic flower samples compared to other sample types, whereas *E. necator* was detected at lower abundance (Figure 6B).

Notably, *E. quericola* exhibited a higher relative abundance in endophytic flower samples than in epiphytic ones. Within the order Botryosphaeriales, *N. parvum* strain UCRNP2 was detected exclusively in epiphytic fruit samples (Figure 6C). The distribution of relatively abundant fungal OTUs across ITS and 28S rRNA datasets revealed the dominance of several fungal orders (Supplementary material 1)

DISCUSSION

The results of this study indicate a wide diversity of potential fungal pathogens present in environmental samples from the cashew phyllosphere. Notably, many of these prospective fungal pathogens detected in coastal Kenya have also been reported in cashew-growing regions of West Africa (Monteiro et al., 2015), causing economic losses and affecting other horticultural crops, such as tomato (Dong et al., 2021). The fungal families Aspergillaceae, Botryosphaeriaceae, Erysiphaceae, Pleosporaceae, and Mycosphaerellaceae are well-documented for containing phytopathogenic species that

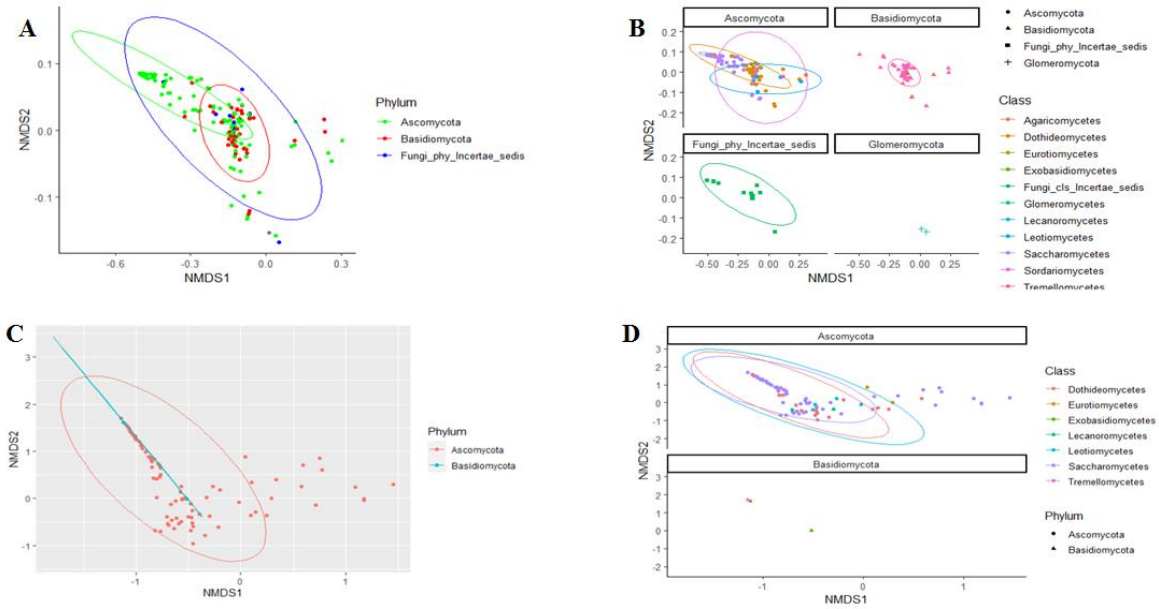


Figure 3. Nonmetric multidimensional scaling (NMDS) showing the results of beta diversity analysis via unweighted UniFrac metrics. A) Clustering among fungal phyla for ITSs, B) Clustering among different classes of fungal phyla for ITSs, C) Clustering among fungal phyla for 28S rRNA, D) Clustering among different classes of fungal phyla for 28S rRNA.

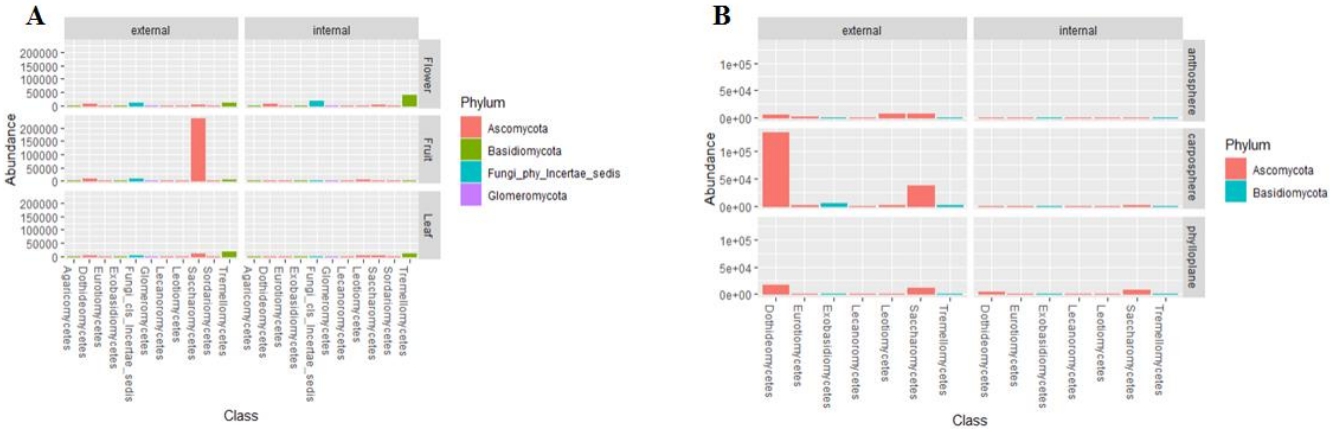


Figure 4. Fungal relative abundance and composition of epiphytes and endophytes in the cashew phyllosphere: A) Phylum abundance and composition via the ITS sequence and B) Phylum abundance and composition via the 28S rRNA sequence.

inhabit the cashew phyllosphere. In this study, species such as *A. niger*, *L. pseudotheobromae*, *A. tubingensis*, *E. quercicola*, and *P. luzardii*, which belong to these families, were detected across various parts of the environmental samples from the cashew phyllosphere. Therefore, their presence suggests on their ecological role as causative agents of nut contamination, gummosis, branch dieback, inflorescences resulting into decline in nut production and nutrition. These findings together with (Wonni et al., 2017) synergistically communicate on the need for tailored study targeting fungal disease across Africa. The pathogen-associated genera identified in both studies include *Aspergillus*, *Erysiphe*, and *Cladosporium* species in cashew plants (Wonni et al., 2017).

The genus *Aspergillus* was highly prevalent among epiphytic fruit samples, reflecting its ecological roles in fruit spoilage, decomposition, and mycotoxin production. In this study, *A. tubingensis* was prominent in epiphytic

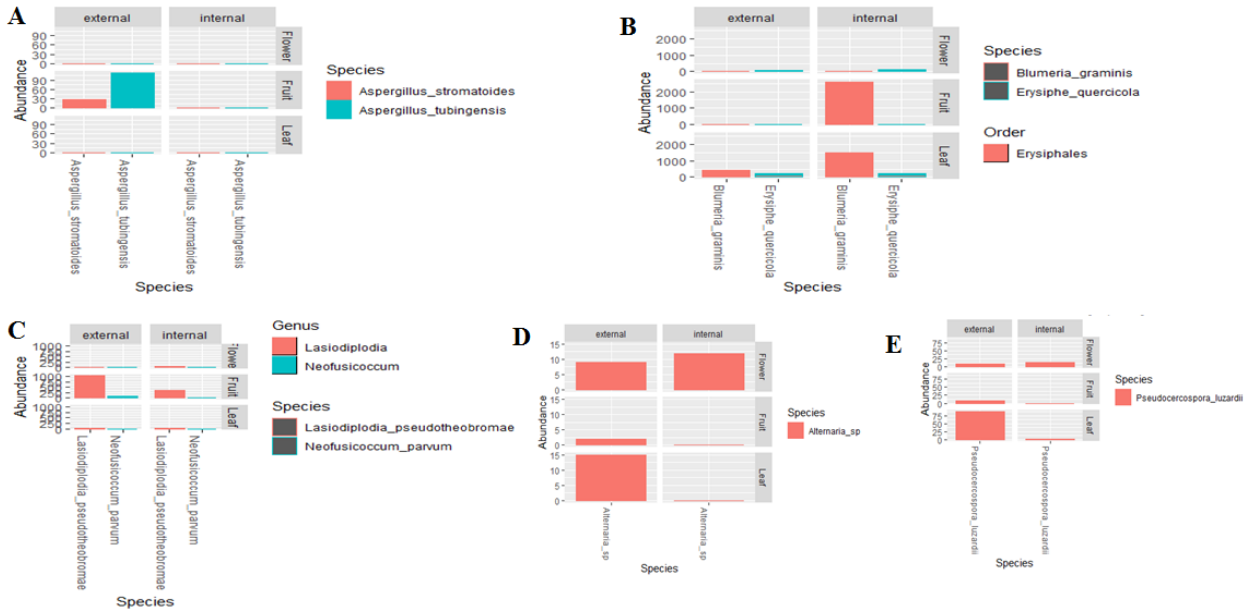


Figure 5. Potential fungal pathogens of the phylum *Ascomycota* of fungal families and *Capnodiales* detected among the cashew phyllosphere for ITS sequences: A) Aspergillaceae, B) Botryosphaeriaceae, C) Erysiphaceae, D) Pleosporaceae, E) Mycosphaerellaceae.

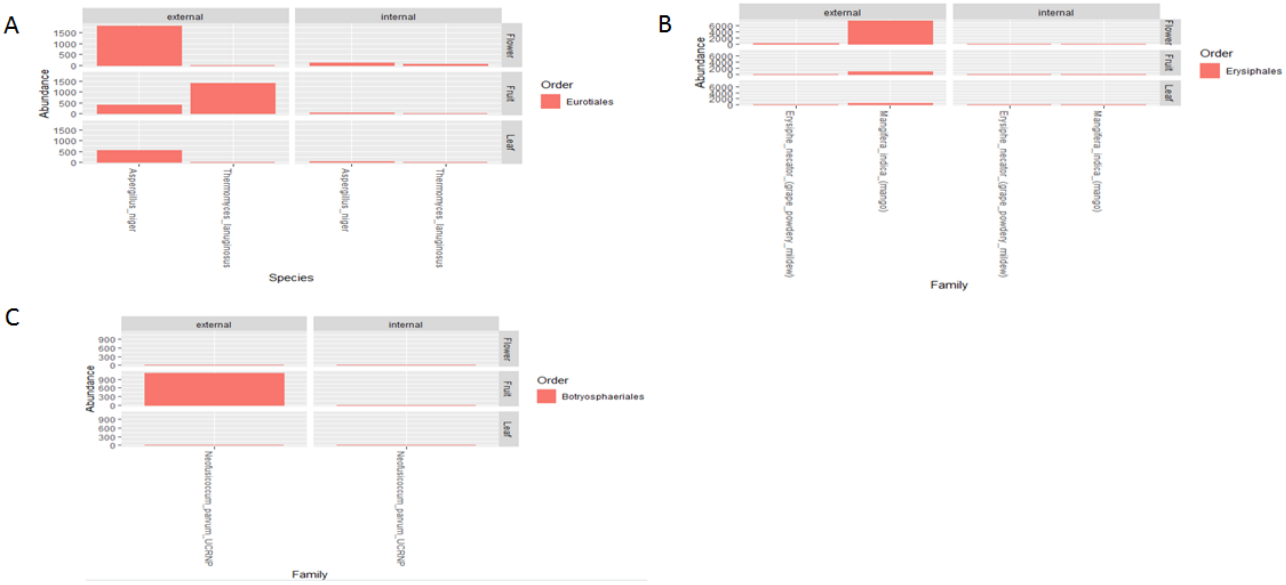


Figure 6. Potential fungal pathogens of the phylum *Ascomycota* were detected among the cashew phyllospheres from Kwale, Kilifi and Lamu via 28S rRNA sequences: A) *Eurotiales,* B) *Erysiphales,* C) *Botryosphaeriale.*

fruit samples, suggesting its potential ecological role in fruit spoilage and decomposition (Guo et al., 2021). Although *A. tubingensis* is recognized as an endophyte and has been employed as a biocontrol agent against gray mold in tomato plants (Zhao et al., 2018), its pathogenicity is influenced by plant health due to the endophyte–pathogen continuum (Guo et al., 2021). These findings, not only reflects on pathogenicity of *A. tubingensis* but also the need for appropriate farm practices among cashew farmers and agricultural training

centers (ATC). This is due to *A. tubingensis* potential to threaten seed germination and viability, hence affecting nut quality and cashew germplasm integrity.

A. niger is a ubiquitous fungus that poses a pathogenic threat to stressed plants. Strains of *Aspergillus* are known to produce allergenic spores and mycotoxins, including aflatoxins, which can contaminate fruits and reduce their value due to associated health risks (Lamboni et al., 2016; Musangi et al., 2024). The production of these toxins by *A. niger*, often referred to as black mold, more often it causes pre and post-harvest losses among cashew nuts. This is a major concern in cashew cultivation, compromising both the quality and quantity of nuts, thereby affecting the income of smallholder farmers in Kenya (Musangi et al., 2024). The presence of *A. niger* in cashew plants has also been documented in Tanzania (Majune et al., 2018), Burkina Faso (Wonni et al., 2017) and Brazil (Pinto et al., 2018). Its detection across various cashew plant parts including leaves, twigs, branches, flowers, nuts, and apples, as reported by Wonni et al. (2017), corroborates the findings and highlights its potential role in the decline of cashew production and nutritional value. This is particularly critical given that kernel rot, which affects both immature and mature nuts, can significantly impair nut integrity and marketability (Muntala et al., 2021).

L. pseudotheobromae, a notorious phytopathogen known to affect trees, crops, and ornamental plants (Biosci et al., 2017). *L. pseudotheobromae* infests host plants and is associated with symptoms such as gummosis, twig dieback, and fruit rot (Monteiro et al., 2015). It has a broad host range, including fruit-bearing trees like cashew (Coutinho et al., 2017). The pathogen penetrates internal plant tissues through wounds and spreads via the vascular system, leading to photosynthetic tissue loss and, in severe cases, plant death. This study reports the first detection of *L. pseudotheobromae* in the cashew phyllosphere in coastal Kenya, offering valuable insights for further investigation into its role in local cashew production systems.

N. parvum, a phytopathogen in the genus *Neofusicoccum*, affects woody plants and is associated with symptoms such as fruit rot, leaf spots, twig dieback, and floral blight (Abdelfattah et al., 2015). Its detection in environmental samples from epiphytic cashew fruit suggests potential rotting impacts on the health and quality of the cashew carposphere. The identification of *N. parvum* in cashew fruit samples represents a noteworthy finding that warrants further investigation into its pathogenicity, epidemiology, and potential threat to cashew production in Kenya.

E. quericola (Pinto et al., 2018), *E. nectator* (Gadoury et al., 2012) and *B. graminis (E. graminis)* (Liu et al., 2021) of the genus *Erysiphe* are phytopathogens associated with powdery mildew disease in tree species, grasses, and cereal crops. Pinto et al. (2018) identified *E. quericola* as a causal agent of powdery mildew in cashew plants in northeastern Brazil, following a study on the disease response of commercial cashew clones. Therefore, the detection of *E. quericola* in environmental samples from the Kenyan cashew phyllosphere suggests its role as a pathogenic factor with the potential to cause economic losses in cashew production. Fonseca et al. (2019) further demonstrated that *E. nectator* and *E. quericola* colonize distinct tissues. *E. quericola* infects young and immature tissues such as shiny leaves, flowers, and developing fruits, while *E. necator* is typically restricted to mature leaves. The present study detected diverse *Erysiphe* spp. in the cashew phyllosphere in Kenya, confirming the presence of potential fungal pathogens previously associated with powdery mildew disease. Powdery mildew disease has been a significant constraint in cashew-producing regions, with economic losses reported in both West Africa (Muntala et al., 2020; Wonni et al., 2017) and East Africa, including Kenya (Muniu et al., 2019). The disease manifests as grayish-white powdery patches on the upper leaf surface and floral parts (Muniu et al., 2019; Pinto et al., 2018). While *E. quericola* and *E. graminis* are typically associated with oak trees and cereal crops, respectively (Fonseca et al., 2019), their presence in the cashew phyllosphere may be attributed to their asexual reproductive strategies and wind-dispersed spores, facilitating cross-host transmission (Gadoury et al., 2012). These findings highlight the need for monitoring and managing powdery mildew pathogens in local cashew agroecosystems.

Alternaria spp., are cosmopolitan phytopathogens that infect a wide range of plant hosts (Abdelfattah et al., 2015). The fungi are associated with blight diseases in leaves and fruits, typically presenting as dark lesions with concentric rings. These symptoms impair photosynthesis and significantly reduce plant productivity. In pear fruits, *Alternaria* spp. have been reported to pose both economic and health risks due to their production of mycotoxins, including tetramic acid derivatives and tenuazonic acid (Patriarca and Pinto, 2017). The detection of *Alternaria* spp. in environmental samples of cashew leaves and flowers highlights their potential as phytopathogens capable of producing host-specific mycotoxins that contribute to pathogenicity and virulence. These toxins accelerate the spoilage of cashew apples and nuts, thereby diminishing the nutritional quality and economic value of cashew products (Patriarca and Pinto, 2017).

P. luzardii is a highly diverse phytopathogen commonly found in tropical and subtropical regions. The Symptoms include leaf spots on both the upper and lower surfaces of leaves. In our study, a high prevalence of *P. luzardii* in environmental cashew epiphytic leaf samples highlights its potential role as a phytopathogen in cashew production.

These findings offer important insights into the

presence of prospective phytopathogens in the cashew phyllosphere and provide a foundation for addressing the economic implications of such pathogens in Kenyan cashew orchards. The warm and humid conditions characteristic of Kenya's coastal lowlands likely favor the establishment and spread of these pathogens, further emphasizing the need for effective surveillance and management strategies.

Conclusion

Cashew phytopathogens represent a major threat to both the quality and quantity of cashew production worldwide. The findings of this study provide a critical foundation for identifying and understanding the potential fungal pathogens inhabiting the cashew phyllosphere, which contribute to disease development in cashew plants. Although many cashew producers have traditionally relied on cultural and biological management practices, these methods have often proven insufficient in effectively controlling disease outbreaks. The detection of potential fungal phytopathogens in environmental samples from Kenyan cashew plants is therefore vital for advancing our understanding of their pathogenic mechanisms and for developing effective containment strategies to improve cashew productivity in the region. In our study, we employed the ITS and 28S rRNA gene regions, which offer strong utility due to their high variability, broad database coverage, and ability to differentiate among higher taxonomic levels. However, limitations such as low interspecies resolution, the potential for horizontal gene transfer, and a relatively slow rate of evolution pose challenges for precise species-level identification. To overcome these issues, we recommend adopting a multilocus sequencing approach that incorporates protein-coding genes such as TEF1-α, RPB1, RPB2, and β-tubulin, in combination with universal markers, to enhance phylogenetic resolution and taxonomic accuracy.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors are grateful to Pwani University for providing the laboratory space at the Pwani University Biosciences Research Centre (PUBReC) to perform this work. This research was supported by the National Research Fund, Kenya (NRF/2/MMC/158), and The World Academy of Sciences (RGA No. 21--302 RG/BIO/AF/AC_G), with funds from the Swedish International Development Cooperation Agency (Sida).

REFERENCES

Abdelfattah A, Li Destri Nicosia MG, Cacciola SO, Droby S, Schena L (2015). Metabarcoding analysis of fungal diversity in the phyllosphere and carposphere of olive (*Olea europaea*). PLoS ONE 10(7):0131069

Akinsanya MA, Goh JK, Lim SP, Ting ASY (2015). Metagenomics study of endophytic bacteria in Aloe vera using next-generation technology. Genomics Data 6:159-163.

Alahapperuma YB (2016). Development of a Bioinformatics Pipeline and Identification of Plant Pathogens using Next Generation Sequencing (NGS) Data.

Biosci IJ, Khatoon A, Mohapatra A, Satapathy KB (2017). Major diseases of cashew (A*nacardium occidentale* L.) Caused by fungi and their control in Odisha, India. International Journal of Biosciences 11(1):68-74.

Coutinho IBL, Freire FCO, Lima CS, Lima JS, Gonçalves FJT, Machado AR, Silva AMS, Cardoso JE (2017). Diversity of genus Lasiodiplodia associated with perennial tropical fruit plants in northeastern Brazil. Plant Pathology 66(1):90-104.

de Brito ES, de Oliveira Silva E, Rodrigues S (2018). Caju—Anacardium occidentale. In Exotic Fruits. Academic Press. pp. 85-89

Dong C, Wang L, Li Q, Shang Q (2021). Epiphytic and Endophytic Fungal Communities of Tomato Plants. Horticultural Plant Journal 7(1):38-48.

Gadoury DM, Cadle-Davidson L, Wilcox WF, Dry IB, Seem RC, Milgroom MG (2012). Grapevine powdery mildew (*Erysiphe necator*): A fascinating system for the study of the biology, ecology and epidemiology of an obligate biotroph. Molecular Plant Pathology 13(1):1-16.

Guo MJ, Wang QT, Cheng YH, Hou CL (2021). Identification of Aspergillus tubingensis causing pomegranate fruit rot in China. Australasian Plant Pathology 50(2):233-240.

Kharwar RN, Gond SK, Kumar A, Mishra A (2010). A comparative study of endophytic and epiphytic fungal association with leaf of Eucalyptus citriodora Hook and their antimicrobial activity. World Journal of Microbiology and Biotechnology 26(11):1941-1948.

Lamboni Y, Frisvad JC, Hell K, Linnemann AR, Nout RM, Tamo M, Smid EJ (2016). Occurrence of Aspergillus section Flavi and section Nigri and aflatoxins in raw cashew kernels (*Anacardium occidentale* L.) from Benin. LWT 70:71-77.

Lance PM, Hattori A (2016). Sampling and evaluation, a guide to sampling for program impact evaluation." Chapel Hill, North Carolina: MEASURE Evaluation, University of North Carolina.

Majune DJ, Masawe PA, Mbega ER (2018). Status and Management of Cashew Disease in Tanzania. International Journal of Environment, Agriculture and Biotechnology 3(5):1590-1597.

Monteiro F, Romeiras MM, Figueiredo A, Sebastiana M, Baldé A, Catarino L, Batista D (2015). Tracking cashew economically important diseases in the West African region using metagenomics. Frontiers in Plant Science 6:482.

Muntala A, Gyasi SK, Norshie PM, Larbi-Koranteng S, Ackah FK, Ntiamoah DA, Mohamed MA (2021). Diseases and insect pests associated with cashew (*Anacardium occidentale* L.) orchards in Ghana. European Journal of Agriculture and Food Sciences 3(5):23-32.

Muntala A, Norshie PM, Santo KG, Saba CKS (2020). Colletotrichum Gloeosporioides Species Complex: Pathogen Causing Anthracnose, Gummosis and Die-Back Diseases of Cashew (*Anacardium Occidentale* L.) In Ghana. European Journal of Agriculture and Food Sciences 2(6):1-10.

Musangi CR, Juma BS, Mukhebi DW, Isoe EM, Kibiti CM, Mbinda WM (2024). Aspergillus population diversity and its role in aflatoxin contamination of cashew nuts from coastal Kenya. Plos one 19(1):e0292519.

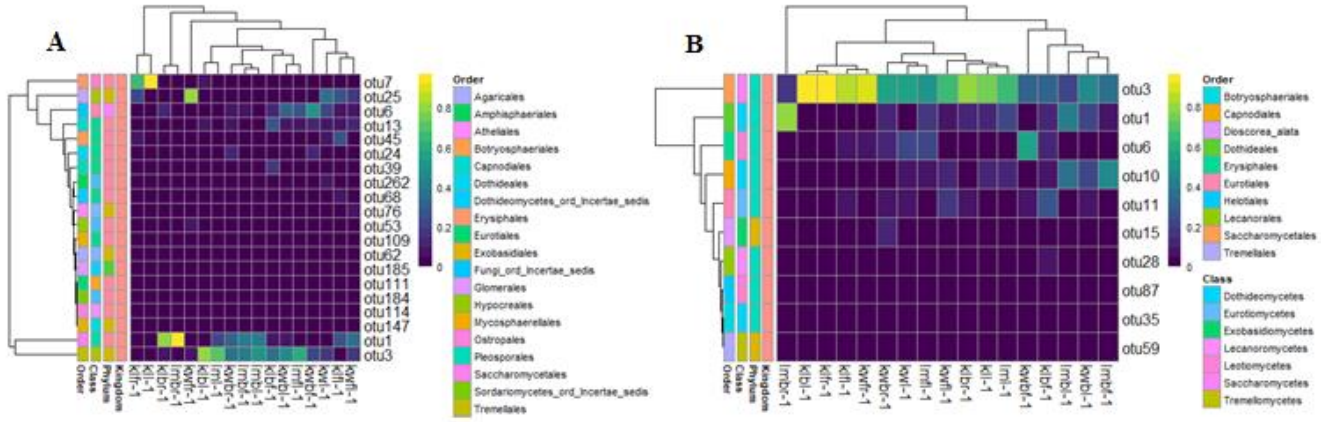
Olaitan P, Oluwayemisi O (2023). Cashew Production and Breeding in 5 West African Countries. Journal of Scientific Research and Reports, 29(5):28-39.

Patriarca A, Pinto VF (2017). Prevalence of mycotoxins in foods and decontamination. Current Opinion in Food Science 14:50-60.

Pavlovska M, Prekrasna I, Parnikoza I, Dykyi E (2021). Soil sample

preservation strategy affects the microbial community structure. Microbes and Environments 36(1):17.
Pinto OR, Cardoso JE, Maia AH, Pinto CM, Lima JS, Viana FMP, Martins MV (2018). Reaction of commercial clones of cashew to powdery mildew in northeastern Brazil. Crop Protection 112:282-287.
Pylro V, Roesch L (2017). The Brazilian microbiome: Current status and perspectives. The Brazilian Microbiome: Current Status and Perspectives.
Savadi S, Muralidhara BM, Preethi P (2020). Advances in genomics of cashew tree: molecular tools and strategies for accelerated breeding. Tree Genetics & Genomes 16(5):61.
Somerfield PJ, Clarke KR, Gorley RN (2021). Analysis of similarities (ANOSIM) for 2-way layouts using a generalised ANOSIM statistic, with comparative notes on Permutational Multivariate Analysis of Variance (PERMANOVA). Austral Ecology 46(6):911-926.
Liu M, Braun U, Takamatsu S, Hambleton S, Shoukouhi P, Bisson KR, Hubbard K (2021). Taxonomic revision of Blumeria based on multi-gene DNA sequences, host preferences and morphology. Mycoscience 62(3):143-165.
Wonni I, Sereme D, Ouédraogo I, Kassankagno AI, Dao I, Ouedraogo L, Nacro S (2017). Diseases of cashew nut plants (*Anacardium occidentale* L.) in Burkina Faso. Advances in Plants & Agriculture Research 6(3):00216.
Zhao J, Liu W, Liu D, Lu C, Zhang D, Wu H, Dong D, Meng L (2018). Identification and evaluation of Aspergillus tubingensis as a potential biocontrol agent against grey mould on tomato. Journal of General Plant Pathology 84(2):148-159.

APPENDIX



Supplementary material 1. Heatmap depicting the distribution of relatively abundant fungal operational taxonomic units: A) ITS, B) 28S rRNA sequence.