Tanzania Journal of Science 47(3): 1102-1113, 2021
ISSN 0856-1761, e-ISSN 2507-7961

Antimicrobial Activities of Endophytic Fungal Crude Extracts Isolated from Cashew Tree (*Anacardium occidentale)*

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Received 14 Aug 2020, Revised 6 Jul 2021, Accepted 20 Jul 2021, Published Aug 2021
DOI: https://dx.doi.org/10.4314/tjs.v47i3.20

Abstract

This study presents the antimicrobial activities of endophytic fungal crude extracts isolated from leaves, stems and roots of cashew trees (*Anacardium occidentale)* which showed disease symptoms and those resistant from Fusarium wilt in Mtwara region. Sections of the cashew trees were made and cultured to isolate endophytes. Eight endophytes were isolated and characterized by using morphological and molecular markers. *Neopestalotiopsis, Penicillium, Lasidioplodia* and *Daldinia* sp isolated from resistant tree and *Auxarthron* and *Aspergillus* sp from diseased tree. Antimicrobial activities of the isolated endophytic fungal crude extracts were done against pathogenic *Fusarium oxysporum* and three human pathogens namely, *Staphylococcus aureus, Escherichia coli,* and *Candida tropicalis.* Results showed that all endophytic fungal crude extracts isolated from resistant plants and one from diseased plants exhibited positive antimicrobial activities against bacteria and fungus *Candida tropicalis* but they had no antifungal activity against *Fusarium oxysporum*. This study could contribute to the discovery and innovation of therapeutics. This study also implied that the resistance against Fusarium wilt disease might be caused by other unknown factors. More research work is recommended for establishing other possible factors including endophytic bacteria, biotic and abiotic factors that could contribute to resistance of cashew plants to *Fusarium oxysporum.*

Keywords: Antifungal, Phytopathology, Endophyte and Cashew nut disease.

Introduction

Endophyte refers to a bacterium or fungus that lives within the plant's parts such as stem, roots, leaves and branches and exhibits no harm to that particular plant. Endophytes have been studied in most plants and found to live asymptomatically (Schulz et al. 1993). It has been observed that, they have capacity to affect the physiology, ecology and distribution of the host plants (Sridhar and Raviraja 1995). They can influence the host plant to resist diseases, nematodes, insects and other stresses. Endophytes are also known to produce natural bioactive compounds which are mainly applied in agriculture, food industry and medicine (Verma et al. 2009).

The occurrence of the unknown cashew wilt diseases in Mtwara region, Tanzania, which is among the cashew grower's regions, has brought up another challenge to most growers. The unknown infections resulted in low yields thus increasing poverty and general frustrations which forced them to abandon the crop. That wilting disease widespread within few weeks of its occurrence which led to complete loss of cashew trees at the field. Laboratory analyses and pathogenicity test revealed the pathogen-causing cashew wilting

disease to be *Fusarium oxysporum* (Mbasa et al. 2020, Tibuhwa and Shomari 2016). Recently, it has been observed that there are some cashew trees that do not wilt at all although they grow in the vicinity of wilting ones. This triggered an interest to investigate the possibility of the presence of endophytes that might be promoting their growth and prevent them from pathogen's attacks. The present study aimed at establishing the possibility of the presence of endophytes that might be enhancing immunity in cashew trees and protecting them from *Fusarium* wilt.

Materials and Methods

Sample collection

Samples were collected from Mtwara region in Tandahimba District and Newala District in August 2019. Purposive random sampling was used to choose disease and resistant cashew trees and from those trees, simple random sampling was used. Three symptomatic (diseased) cashew trees per site and three asymptomatic (resistant) cashew trees were selected within the infested cashew fields. Five leaves samples were collected from each diseased and resistant tree, making a total of 30 leaves samples. The same method was applied in collecting stems and roots samples, also resulting in 30 samples. The samples were collected using knife and hand hoe, kept in plastic bags and then transported to the Department of Molecular Biology and Biotechnology laboratories of the University of Dar es Salaam for further analysis.

Isolation of endophytic fungi

Leaves, stems and roots from the collected samples were cleaned under tap running water for 30 min and cut into 1 cm segments. Surface sterilizations were done using 70% ethanol for 2 min followed by sodium hypochlorite solution (2% available chlorine) for 3 min and 70% ethanol for 30 s followed by three washes in sterile distilled water. Leaf, stem and root segments of about 2 cm each were placed on Petri plates containing Potato Dextrose Agar (PDA) medium amended with streptomycin (250 mg/l) to slow down bacterial growth from the tissue (Schulz et al. 1993). PDA plates with samples and controls (without samples) were incubated at 30 °C for 5-6 days. Fungi that grew out from the plant tissues were transferred onto fresh PDA medium. After purifying the isolates three times, final pure cultures were transferred into PDA slants in test tubes and stored at 4 °C for further examinations.

Morphological characterization

Macro morphological and micro-morphological characterizations were used to characterize the pure culture isolates. From a pure culture colony, various parameters such as mycelia types, colony appearance and colony colour were determined. Staining of the colony were done with lactophenol cotton blue stain and microscopic features were studied under a compound microscope (Leica 2500, Leica Microsystems CMS GmbH, and Wetzler, Germany). The observed fungi were identified to the genus level using standard identification keys as reported by Masumi et al. (2015). Further characterization for ascertaining the identified isolated were done using molecular markers.

Molecular characterization

Genomic DNA was extracted by CTAB extraction method as described by Umesha et al. (2016), with some modification. Briefly, 100 mg of fungi isolates were placed in eppendorf tube followed by addition of glass beads, 200 µL CTAB and grinding. CTAB (300 µL) was added followed by incubation in water bath at 65 °C for 45 min followed by centrifugation for 5 min at 8000 rpm. 400 µL of the aqueous phase obtained were placed in a new collection tubes then 500 µL of chloroform: isoamyl alcohol (24:1) were added, mixed gently followed by centrifugation for 5 min at 8000 rpm. In the aqueous phase, 500 µL of isopropanol were added, mixed gently followed by incubation at –20 °C for 1 hour to precipitate DNA. Following incubation, the solution was centrifuged for 10 min at 14000 rpm and supernatant discarded.

Subsequently, 100 µL of 70% ethanol were added to wash the DNA, vortex and allowed to dry for 30 min. The DNAs obtained were stored in 30 µL of DNAse free water for further analysis. DNA concentrations were determined using the Nanodrop spectrophotometer (Thermo Scientific Nanodrop 2000) at absorbance of 260/280 nm.

PCR amplification of the fungal rDNA internal transcribed spacers (ITS) regions of all the isolates were done using the primer pairs ITS1 and ITS4. PCR amplifications conditions included 1 cycle of 94 °C for 3 min, followed by 35 cycles of 94 °C for 30 sec, 55 °C for 1 min, and 72 °C for 1 min and a final extension cycle of 72 °C for 10 min (Ramesh et al. 2017). The PCR products were checked for quality using gel electrophoresis. The PCR products were then directly sequenced at INQABA South Africa. The nucleotide sequences attained from INQABA were checked first for quality control, and analyzed using Basic Alignment Search Tool (BLAST) against the GenBank sequence database available in the National Center for Biotechnology Information (NCBI) website at: http://blast.ncbi.nlm.nih.gov (Ronoh et al. 2013)

Phylogenetic analysis

The obtained nucleotide sequences were deposited in public database the GenBank and were assigned the following accession numbers: SUB9369567 seq1 MW840081, SUB9369567 seq2 MW840082, SUB9369567 seq3 MW840083, SUB9369567 seq4 MW840084, SUB9369567 seq5 MW840085, SUB9369567 seq6 MW840086, SUB9369567 seq7 MW840087 and SUB9369567 seq8 MW840088.

The sequences were further aligned using the CLUSTAL W program against the nearest neighbours. The phylogenetic analyses were done using Maximum likelihood and Bayesian analysis while the optimal substitution model for ITS was designated using Akaike's Information Criterion (AIC) with MrModeltest 2.3 (Nylander 2004). Phylogenetic analysis was performed in a Bayesian Inference conducted using MrBayes v3.2.6 (Ronquist and Huelsenbeck 2003). Two independent runs of four Monte Carlo Markov Chains (MCMC) were performed; a run length of 10,000,000 generations sampled every 1,000th, and a GTR+I substitution model. A burn-in of 25% was removed from each run. Maximum likelihood estimates were carried out by RAxML v.8.2.10 using the GTR + G + I model of site substitution (Stamatakis 2014). The branch support was obtained by maximum likelihood bootstrapping (MLbs) of 1000 replicates (Hillis and Bull 1993). The evolutionary history was inferred using the Maximum likelihood method. The bootstrap consensus trees inferred from 1000 replicates were taken to represent the evolutionary history of the taxa analyzed. The evolutionary distances were computed using the Maximum Composite Likelihood method (Ronoh et al. 2013).

Isolation of endophytic fungi for antimicrobial activities

Endophytic fungal metabolites were screened for antimicrobial activities. By using sterile cork borer, mycelial agar from each endophyte was cut from the edge of an actively grown pure culture and inoculated into a 500 mL conical flask containing 250 mL of sterile potato dextrose broth media by using sterilized spatula and then followed by incubation at room temperature for three weeks without shaking as described by Campos et al. (2015).

Extraction of metabolites of the endophytic fungi

After three weeks incubations, cultures of endophytic fungi were filtered using cotton gauze to remove mycelial mats. Fungal metabolites were extracted by solvent extraction procedures using ethyl acetate as organic solvent (Mwanga et al. 2019). Equal volumes of metabolites filtrate and ethyl acetate were measured and placed into separating funnel; the mixture was shaken vigorously for 10 min and then left to stand to

allow cell masses to get separated from the solution. The aqueous solution was discarded and the organic solution was collected and kept in a sterilized flask (with known mass) and metabolites were extracted by removing excess solvent using rotary vacuum evaporator (BUCHI Rota vapor Model R-210) under reduced pressure at 35 °C. The fungal crude extracts obtained were weighed, dissolved in dimethyl sulfoxide (DMSO) and stored at 4 °C for antimicrobial assays.

Inoculum preparation

Gram-positive bacteria, *Staphylococcus aureus (ATCC-25923)*, gram negative bacteria *Escherichia coli* (ATCC-25922) and a yeast *Candida tropicalis* used were obtained from the Department of Molecular Biology and Biotechnology, University of Dar es Salaam. The bacteria and yeast were grown in nutrient agar (NA) and potato dextrose agar (PDA) and incubated at 37 °C and 30 °C, respectively for 24 hours. By using sterile inoculating loop, cultures of test organisms were placed into sterile test tubes each containing 1 mL of sterile saline water, and the suspension was adjusted to match with 0.5 McFarland standards approximately 1.5×10^8 CFU/mL (prepared by adding 0.05 mL of 1.175% $BaCl_2$ to 9.95 mL of 1% H_2SO_4). The suspensions of tested organisms and saline water were then kept in the test tubes and stored at 4 °C until used following the procedure described by Jahiri (2013) and Mwanga et al. (2019).

Screening for antimicrobial activity

Only eight (8) crude extracts of the isolated and ascertained endophytic fungi were screened for their antibacterial and antifungal activities against the *Escherichia coli*, *Staphylococcus aureus*, *Fusarium oxysporum* and *Candida tropicalis*. About 20 µL/disc solvent extract with concentrations of 200 mg/mL were soaked to each sterile Whatman disc with a diameter of 6 mm and allowed to dry and then placed on the inoculated media. Mueller-Hinton sterile agar plates were seeded with indicator bacterial strains (10^8 CFU), while Sabouraud dextrose agar media were seeded with indicator fungus. Control experiments were carried out under similar conditions by using chloromphenical for antibacterial activities and fluconazole for antifungal activities (positive control) as standard drugs while dimethyl sulfoxide (DMSO) was used as a negative control. The zones of growth inhibition around the disks were measured after 18 to 24 hours of incubation at 37 °C for bacteria and 48 to 96 hours for fungi at 28 °C. The sensitivities of the microorganisms to the fungal extracts were determined by measuring the sizes of inhibitory zones in millimeters (including the diameters of disks) on the agar surface around the disks, and those showing no zones were termed as negative (Bhalodia and Shukla 2011). The treatments were performed in triplicates and the results presented as mean value (± standard deviation). The fungal crude extracts that showed high zones of inhibition against the test microorganisms were tested for Minimum Inhibitory Concentrations (MIC).

Minimum inhibitory concentrations

Determination of minimum inhibitory concentrations of fungal crude extracts which showed higher zones of inhibition against test organisms were performed using the 96-well microtiter plate with lid, having 12 columns and 8 rows (A-H). The fungal extracts and the standard drugs were prepared in a concentrations that were twice the desired final concentration (200 µL) as were diluted with an equal amount of bacteria in broth. Two fold serial dilutions using a micropipette were done systematically down the columns 1–9. 100 µL was removed from the starting concentrations (columns 1–9 in row A) and transferred to the next row with the 100 µL broth, properly mixed, and the procedure was repeated up to the last row (H) where the last 100 µL was discarded. This brought the final volume in all the test wells with the extracts and the standard drugs to 100 µL, except the 10^{th} column which had 200 µL of the broth that served as growth control (drug free). Each fungal extract was set

with its blank which contained fungal extracts and broth without microorganisms. Columns 11 and 12 served as positive control and negative control, respectively. Microtiter plates were incubated at 37 °C and the MIC values were determined by Spectrostar analyser (Dharajiya et al. 2017).

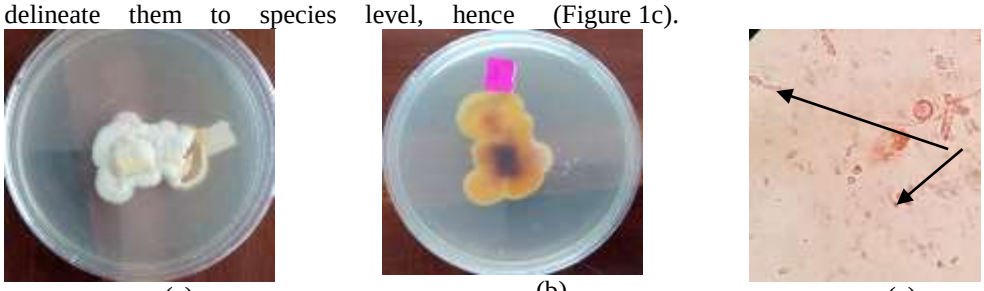
Results

Macro-morphological characterization and sequencing

In this study, twenty three (23) endophytic fungi were successfully isolated from healthy and diseased plants. Both macro-morphological and micro-morphological characterizations were carried out which categorized the isolated endophytes into six genera corresponding to *Neopestalotiopsis, Penicillium, Daldinia, Auxarthron, Aspergillus* and *Lasidioplodia.* However, morphological characters failed to delineate them to species level, hence molecular based techniques were deployed for further delimitations. Out of the 23 isolated endophytes, DNA extraction, amplification and purification for sequencing were successfully done to only thirteen (13) endophytes. Out of the 13 sequenced, only eight (8) were clean sequences for phylogenetic analysis, thus clearly ascertained their identity. The eight clean sequences were from health leaves (3), diseased roots (2), while the resistant roots and diseased stems each had one (1) clean sequence.

Neopestalotiopsis sp

After 7 days of incubation, at the upper side of the medium, white colonies, cottony like, circular in shape were observed, while at the reverse side of the medium, light-yellow colouration portrayed (Figure 1a & b). Conidia were fusiform to ellipsoid under microscope (Figure 1c).

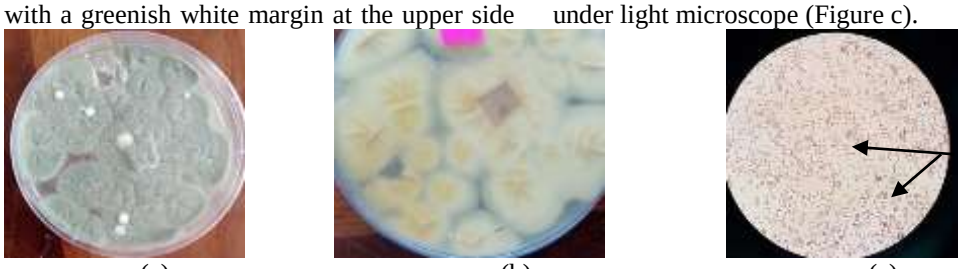


(a) (b) (c)

Figure 1: *Neopestalotiopsis* sp isolated from (a) healthy leaves (b) reverse side (c) conidia (arrows) observed under light microscope with 40X magnification.

Penicillium sp

After 7 days of incubation, colonies grew rapidly, and were predominantly pale green with a greenish white margin at the upper side of the medium, while the reverse side of the medium was pale yellow (Figure 2a & 2b). Conidia were spherical and closely packed under light microscope (Figure c).

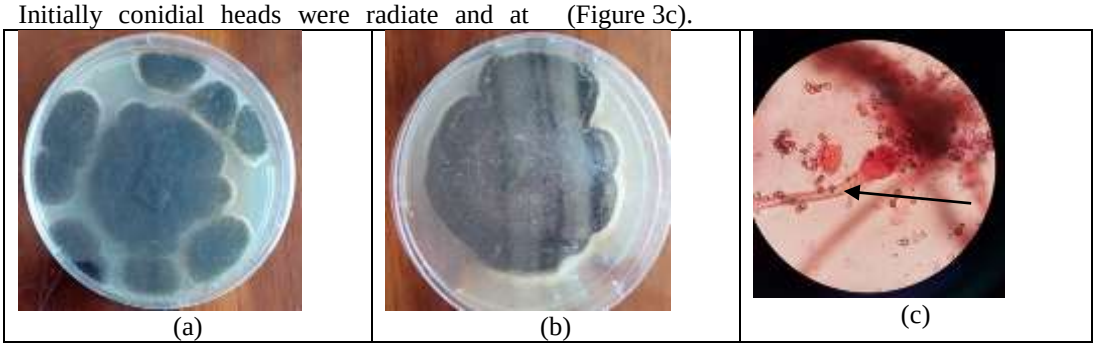


(a) (b) (c)

Figure 2: *Penicillium* sp isolated from (a) healthy leaves (b) reverse side (c) conidia (arrows). observed under light microscope with 40X magnification.

***Aspergillus* sp**

After 7 days of incubation, white colonies appeared first then becoming black with the production of conidiophore (Figure 3a and b). Initially conidial heads were radiate and at maturity they split into two columns. Under microscope, the conidiophores were long, radiate, smooth and hyaline, becoming darker at the apex, and terminate in a globose vesicle (Figure 3c).



(a) (b) (c)

Figure 3: *Aspergillus* sp isolated from (a) diseased leaf (b) diseased root (c) conidiophore (arrow) observed under light microscope with 40X magnification

Molecular characterization of endophytic fungi

Molecular characterization of identified endophytic fungi isolated from leaves, stems and roots of cashew trees were done using rDNA sequences. The internal transcribed spacer (ITS) sequences of the specimen were compared with sequences in the GenBank, using Blast to determine identity levels. Analysis showed that all the sequences of the endophytic fungi were greater than 90% and were similar when compared with the sequences from the database, and hence confirmed them to belong to the genera *Neopestalotiopsis, Penicillium, Daldinia, Lasidioplodia, Auxarthron, Aspergillus,* and *Lasidioplodia* with the percentage identities of 100%, 99.61%, 100%, 100%, 99.45%, 100%, 99.81% and 100%, respectively (Table 1).

Table 1: Identified fungi and the percentage identity of isolated endophytic fungi from different parts of cashew trees and that of the taxa found in the NCBI.

Code number	Name of species	Accession number	Maximum % identity	References
Isolate 3(C)	*Neopestalotiopsis mesopotamica*	NR_145244	100	Maharachchikumbura et al. (2014)
	Neopestalotiopsis javaensis	MH855207	100	Vu et al. (2019)
	Neopestalotiopsis acrostichi	MK764272	100	Norphanphoun et al. (2019)
	Neopestalotiopsis surinamensis	NR_145240	100	Maharachchikumbura et al. (2014)
	Neopestalotiopsis javaensis	NR_145241	100	Maharachchikumbura et al. (2014) Norphanphoun et al. (2019)
	Neopestalotiopsis australis	KM199348	100	Maharachchikumbura et al. (2014)
	Neopestalotiopsis rosae	NR_145243	100	
Isolate 4(D)	*Penicillium wotroi Penicillium*	NR_119813	99.61	Schoch et al. (2014)

Code number	Name of species	Accession number	Maximum % identity	References
	pedernalence	NR_146250	98.88	Laich and Andrade (2016) Visagie et al. (2016)
	Penicillium infrabuccalum	KT887849	98.86	
Isolate 6 (F)	*Lasidioplodia iranensis*	NR_147327	100	Abdollahzadeh et al. (2010)
	Lasidioplodia sterculiae	NR_147365	100	Yang et al. (2017)
	Lasidioplodia jastrophicola	NR_147348	100	Machado et al. (2014)
	Lasidioplodia chinensis	NR_152983	100	Dou et al. (2017)
	Lasidioplodia hyalina	NR_152982	100	Dou et al. (2017)
	Lasidioplodia pseudotheobromae	NR_111264	100	Schoch et al. (2014)
Isolate 7(G)	*Daldinia starbaeckii*	NR_147519	100	Stadler et al. (2014)
	Daldinia bambusicola	KU683755	100	U'Ren et al. (2016)
	Daldinia bambusicola	NR_152464	100	U'Ren et al. (2016)
Isolate 9(H)	*Auxarnthron conjugatum*	NR_121475	99.44	Schoch et al. (2014)
	Auxarnthron conjugatum	MH857772	99.45	Vu et al. (2019)
	Auxarnthron zuffianum	MH869293	91.44	Vu et al. (2019)
	Auxarnthron compactum	NR_103574	91.17	Schoch et al. (2014)
	Auxarthron alboluteum	NR_111137	90.25	Schoch et al. (2014)
Isolate 10(I)	*Aspergillus tubingensis*	EF661193	100	Peterson (2008)
	Aspergillus tubingensis	NR_131293	100	Peterson (2008)
	Aspergillus piperis	NR_077191	100	Schoch et al. (2014)
	Aspergillus costaricaensis	NR_103604	100	Schoch et al. (2014)
Isolate 11(J)	*Aspergillus aculeatus*	NR_111412	99.81	Schoch et al. (2014)
	Aspergillus uvarum	NR_135330	100	Perrone et al. (2008)
	Aspergillus japonicus	NR_131268	100	Parenicova et al. (2015)
Isolate 13(L)	*Lasidioplodia parva*	MH861166	100	Vu et al. (2019)
	Diplodia cajani	NR_163672	100	Vu et al. (2019)
	Lasidioplodia brasiliensis	NR_147338	100	Marques et al. (2013)
	Lasidioplodia parva	NR_111265	100	Schoch et al. (2014)
	Diplodia cajani	MH856592	100	Vu et al. (2019)
	Lasidioplodia marypalmiae	MH856592	100	Vu et al. (2019)

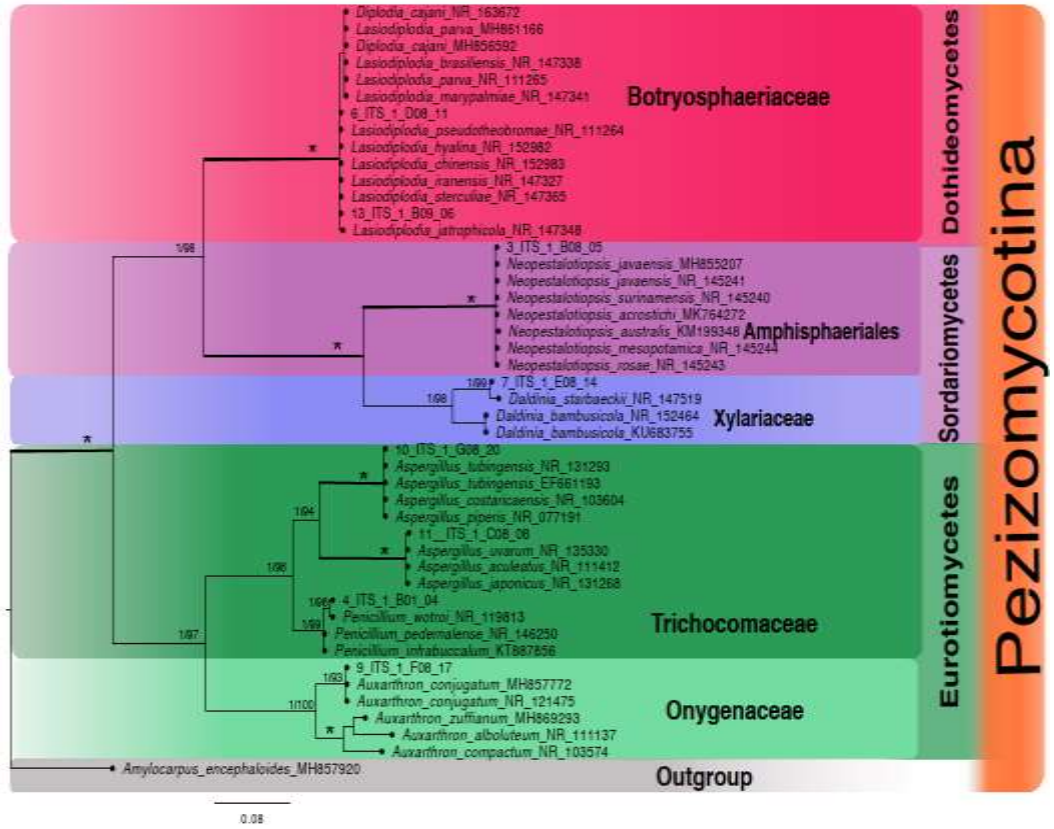


Figure 4 : Phylogenetic relationships of isolates from cashew trees based on Bayesian and ML analyses of rDNA ITS datasets. The tree was rooted with *Amylocarpus encephaloides*. The two support values associated with each internal branch correspond to PPs and MLbs proportions, respectively. Branches in bold indicate a support of PP ≥ 0.95 and MLbs ≥ 70%. An asterisk on a bold branch indicates that this node has a support of PP = 1.0 and MLbs = 100.

Antimicrobial activities of fungal crude extracts

The results were analysed by using the protocol of Quinto and Santos (2005) whereby the activities are presented as > 19 mm ZOI (very active), 14-19 mm ZOI (active), 10-13 mm ZOI (partially active), and < 10 mm ZOI (inactive). They showed that the endophytic fungal crude extracts isolated from resistant cashew trees exhibited antimicrobial activities with inhibition zones ranging from 9 to 13 mm against *S. aureus*, 13-23 mm against *E. coli* and 6 to 10 mm against *C. tropicalis* but had no activity against *F.oxysporum* (Table 2). Only one fungal species isolated from diseased cashew tree exhibited antimicrobial activities against the tested microorganisms (*S. aureus*, *E. coli* and *C. tropicalis*) but had no activity on *F.oxysporum.*

Table 2: Antimicrobial activities of the crude extracts of endophytic fungi isolated from resistant and diseased cashew trees against *Fusarium oxysporum* and pathogens of medical importance

Source	Endophytic fungi	Inhibition zone (mm) concentration 20 µL/disc			
		Fusarium oxysporum	*Staphylococcus aureus*	*Escherichi a coli*	*Candida tropicalis*
R_1 Leaves (n = 5)	*Neopestalotiopsis*	–	13.0 ± 0.47	23.3 ± 0.47	10.7 ± 0.47
R_2 Leaves (n = 5)	*Penicillium*	–	12.0 ± 0.47	20.3 ± 0.47	6.67 ± 0
R_1 Roots (n = 5)	*Lasidioplodia*	–	10.0 ± 0.47	17.0 ± 0.47	9.67 ± 0.47
R_3 Leaves (n = 5)	*Daldinia*	–	9.0 ± 0	13.0 ± 0	–
D_1 Roots (n = 5)	*Aspergillus*	–	–	–	–
D_2 Roots (n = 5)	*Auxarthron*	–	–	–	–
D_1Leaves (n = 5)	*Aspergillus*	–	12.3 ± 0.47	20.3 ± 0.47	8.3 ± 0.47
D_1 Stems (n = 5)	*Lasidioplodia*	–	–	–	–
	+ve		18.3 ± 0.47	25.3	10.3
C	–ve	–	–	–	–

Note R_1 = Resistant tree 1, R_2 = Resistant tree 2, R_3 = Resistant tree 3, D_1 = Diseased tree 1, D_2 = Diseased tree 2, +ve = positive control, –ve = negative control (DMSO), Inhibition zones expressed as mean ± standard deviation and – = no zone of inhibition.

Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentrations (MICs) of the crude extracts of the endophytic fungi isolated from healthy cashew trees against pathogen of medical importance are presented in Table 3. The concentrations are presented as mean ± standard deviation. *Aspergillus* species portrayed the strongest activity against fungal pathogens with the strongest MIC value of 3.125 mg/ml, followed by *Neopestalotiopsis* and *Penicillium* species both portraying strong antimicrobial activity against the tested bacteria with MIC value of 6.25 mg/ml, while *Daldinia* species portrayed the weakest antimicrobial activity against the bacteria with MIC value of 12.5 mg/ml.

Table 3: Minimum inhibitory concentrations (mg/mL) of the crude extracts of endophytic fungi isolated from disease and resistant cashew trees against pathogens of medical importance.

Source	Endophytes	Tested pathogen		
		Staphylococcus aureus	*Escherichia coli*	*Candida tropicalis*
R_1 Leaves	*Neopestalotiopsis*	50	6.25	6.25
R_2 Leaves	*Penicillium*	6.25	50	25
R_1 Roots	*Lasidioplodia*	100	50	3.125
R_3 Leaves	*Daldinia*	100	12.5	6.25
D_1 Leaves	*Aspergillus*	50	100	3.125

Note: R_1 = Resistant tree 1, R_2 = Resistant tree 2, R_3 = Resistant tree 3, D_1 = Diseased tree 1. Concentration presented as mean ± standard deviation.

Discussion

This paper is the first report on isolation of endophytic fungi from diseased and resistant cashew trees intended to isolate endophytes and test their antimicrobial activities against *Fusarium oxysporum* causing cashew wilt disease and pathogens of medical importance namely *S. aureus*, *E. coli* and *C. tropicalis*. Eight (8) endophytic fungal species were isolated, of which four (4) isolates were from diseased plants and four (4) isolates were from resistant plants. The isolates were confirmed to belong to the eight genera using the ITS molecular mark which delineated closely

related taxa. The isolates were well supported to belong to the eight genera compared to the already described sequences with support values of PP ≥ 0.95 and MLbs ≥ 70% (Figure 4). Those isolates from resistant plants are *Daldinia* sp, *Neopestalotiopsis* sp, *Penicillium* sp, and *Lasidioplodia* sp and those from diseased plants are *Auxarthron* sp, *Aspergillus* sp, *Aspergillus* sp, and *Lasidioplodia* sp. The species composition differed between resistant and diseased cashew tree except for one species *Lasidioplodia* sp which appeared in both resistant and diseased cashew trees. The non-specificity of endophytes has been also shown in other studies. For example, Huang et al. (2008) established that some endophytes are host specific which means that they only restricted to single host, thus not occurring in different plants in the same habitat, while others are host recurrence whereby they predominantly occur on a specific host or can be occurring in infrequently on other host plant in the same habitat. All fungal endophytes from resistant trees and only one endophytic fungus *Aspergillus* sp from diseased plants had antimicrobial activities against the tested bacteria and fungus *Candida tropicalis*. *Neopestalotiopsis* sp showed strong antimicrobial activities against all the tested pathogens and this was also reported by Tanapichatsakul et al. (2019) as they did production of eugenol from fungal endophytes *Neopestalotiopsis* sp. *Lasiodiplodia* sp were very active against the fungus *Candida tropicalis*. The findings are similar to those reported by Moron et al. (2018) in their studies on antimicrobial activities of crude culture extracts from mangrove fungal endophytes collected in Luzon Island, Philippines. *Penicillium* sp was active against tested bacteria and had activity against fungi, and this was also reported to produce a range of medicinally important metabolites including antimicrobials (Lucas et al. 2007) and antifungals (Nicoletti et al. 2007). *Daldinia* sp has got partially active antibacterial activities since this plant was reported to possess many therapeutic properties like antimicrobial, cytotoxicity and anti-inflammatory activities (Shylaja et al. 2018). Only one endophytic fungus *Aspergillus* sp from diseased plants showed activities against all the tested microbes.

All endophytic fungal isolates revealed no activity against *Fusarium oxysporum* that causes cashew wilt disease. This implies that endophytes harbored in these resistant plants could not be the ones influencing resistance against pathogenic *Fusarium*. Possibly there might be other factors that contribute to their resistance to cashew wilt diseases. Some literature denotes that mineral contents like zinc, potassium, iron, manganese, calcium and silicon mineral elements contribute to plants protection against pathogens (Orr and Nelson 2018). Other known factors such as availability of organic matter that influence the chemical, physical and biological characteristics of soil, and endophytic bacteria (da Costa Ribeiro Lins et al. 2014) have also been reported to cause disease suppression.

Conclusion

From this study, eight endophytes were isolated from cashew trees and based on phylogenetic analysis, the isolates from resistant plants were grouped together with species of *Neopestalotiopsis, Penicillium, Lasidioplodia* and *Daldinia* and those from infected trees were grouped with species of *Auxarthron and Aspergillus.* Differences in species composition were observed between the infected trees and resistant ones. The isolated endophytic fungal crude extracts had antimicrobial activities against selected human pathogenic bacteria and fungi. This could contribute to the discovery and innovation of therapeutics and products of pharmacological importance. The isolated endophytic fungal crude extracts had no antimicrobial activities against *Fusarium oxysporum*. More research work should be conducted to elucidate factors that could contribute to resistance of cashew plants to *Fusarium oxysporum*. The scope of the studied area could be increased to other infected areas beyond Mtwara cashew

plantations including coastal areas with similar cashew wilting disaster.

Acknowledgment

The authors send their heartfelt gratitude and thanks to the Department of Molecular Biology and Biotechnology, Chemistry Department, Botany Department, TARI-Naliendele-Cashew Research Programme and other people who provided assistance in this research.

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