Nig. J. Biotech. Vol. 37(2): 103-112 (Dec. 2020)
ISSN: 0189 1731
Available online at
http://www.ajol.info/index.php/njb/index
and www.biotechsocietynigeria.org
DOI: https://dx.doi.org/10.4314/njb.v37i2.10

NIGERIAN JOURNAL OF BIOTECHNOLOGY

Isolation and Molecular Identification of some Fungi Associated with *Jatropha curcas* (L.)

[1,2]*Iyanyi, N. G. and [1,2]Ataga, A. E.

[1]Department of Plant Science and Biotechnology, University of Port Harcourt, Port Harcourt, Nigeria.
[2]Regional Center for Biotechnology and Bio-resources Research, University of Port Harcourt, Port Harcourt, Nigeria.

Abstract

***Jatropha curcas* is a plant of great economic importance that experiences high incidence of fungal attack. Misidentification of the fungal species is bound to occur with the use of traditional cultural methods where organisms are identified morphologically and/or microscopically. This study was carried out to isolate and identify the fungi associated with *Jatropha curcas* (L.) using both traditional/ cultural techniques and molecular methods. The fungi were isolated from diseased leaves and stems of *J. curcas* using both Standard Blotter and Potato Dextrose Agar (PDA) methods. DNA was extracted from the fungal isolates using Zymo Fungal/Bacteria DNA MiniPrep Kit. Amplification of the Internal Transcribed Spacer (ITS) regions of the fungal isolates was carried out using fungi universal primer pairs for ITS4 and ITS5. The amplicons were sequenced and the isolates were identified as *Penicillium brevicompactum*, *Aspergillus* sp., *Botryosphaeria rhodina*, *Aspergillus nomius*, *Aspergillus tamarii*, *Rhizopus oryzae*, *Penicillium citrinum* and *Fusarium solani*. Phylogenetic analysis was carried out to know the relationship between the isolates and other closely-related species in GenBank. *Jatropha curcas* is colonized by many fungal species some of which may be pathogenic to the plant, and molecular techniques pose the best alternative for accurate identification of these organisms.**

Keywords: *Jatrophacurcas*, fungi, polymerase chain reaction, phylogeny, sequencing.
Corresponding author's e-mail: nkechi.iyanyi@gmail.com,Tel: +2347037829855

Introduction

Jatropha curcas (Linn.), commonly called "Jatropha" or "physic nut", is a drought-resistant multipurpose shrub that originated from Mexico and Central America (Salvador-Figueroa *et al*., 2015). Today, the plant is cultivated world-wide as a hedge crop and for traditional purposes in both tropical and subtropical regions (Agbogidi*et al*., 2013).

Jatropha is a plant with many economic values. The unique oil extracted from the seeds can be used as a feed stock for biodiesel (Maghuly*et al*., 2013). The seed cake serves as inorganic manure and when derived from a non-toxic variety of *Jatropha curcas*, it may be used as animal feed. The leaves, bark, shoot latex, roots and seed oil are used for medicine and veterinary purposes. In the rural areas in Nigeria such as Aluu, Omuigwe, in Ikwere LGA; and Rumuji, Oduoha in Emohua LGA both in Rivers State, Jatropha leaf is believed to aid in fermentation of cassava and is used for this purpose. After peeling and soaking the cassava, Jatropha leaves are placed on top of the cassava and this reduces the number of days required for the cassava to ferment. The fruit exocarp (coat), seed shell and the press cake of the seed are rich in

nitrogen, phosphorous and potassium and can be used as an inorganic fertilizer to improve plants growth (Islam *et al*., 2011).

Plant diseases can be as a result of nutrient deficiency or invasion by pathogenic microorganisms. Most diseases of plants are caused by fungal organisms. *Jatropha curcas* was previously believed to have a strong resistance against diseases because of the presence of many disease-fighting compounds such as palmitic, palmitoleic, stearic and linoleic acid methyl esters present in the plant (Rahman*et al*., 2014). Many studies have shown that *J. curcas* is susceptible to fungal attacks (Jayaraman*et al*., 2011; Nwaukwu *et al*., 2014; Lateef*et al*. 2019).

Isolation and/or characterization of microorganisms can be carried out using traditional cultural techniques or molecular methods such as culture-dependent [Polymerase Chain reaction (PCR)] and culture-independent (metagenomics) methods. Traditional cultural techniques are based on macroscopic and microscopic examination of the isolates. Standard approaches for identification of microorganisms such as the use of culture media and biochemical tests are known to be very time-consuming and laborious (Franco-Duarte *et al*., 2019). One of the efficient strategies used to reduce the time required for the identification of microorganisms is the use of molecular biology techniques which may be used alongside several molecular fingerprinting techniques (Castro-Escarpulli*et al*., 2016). Fast and accurate detection and identification of microorganisms is very critical in today's sphere of life science. Molecular techniques can be quick and cost effective in classifying fungal and bacterial species , especially if the microorganisms under study are better understood (Franco-Duarte *et al*., 2019). This study was carried out to isolate and identify fungal organisms associated with *Jatropha curcas* using molecular techniques.

Materials and Methods

Study Area and Sample Collection

The study was carried out at the Regional Centre for Biotechnology and Bio-resources Research, University of Port Harcourt, Rivers State, Nigeria. Sequencing was carried out at the International Institute of Tropical Agriculture (IITA), Ibadan.

Diseased leaves and stems of *Jatropha curcas* showing symptoms such as chlorosis, leaf spots, necrosis, leaf dropping, stunted growth, wilting, leaf crumple, blight etc were collected from the three senatorial zones in Rivers State : Obio-Apor, Ikwere, Port Harcourt and Emuoha Local Government Areas (LGAs) for River East; Ahoada-East, Ahoada-West, Ogba/Egbema/Ndonni and Degema LGAs for Rivers West; and Tai, Eleme and Oyigbo LGAs for Rivers South-east senatorial zones.

Isolation of Fungi from Jatropha curcas

The isolation of fungi associated with *Jatropha curcas* was carried out using the Standard Blotter recommended by the International Rules for Seed Testing Association (ISTA, 2016) and Potato Dextrose Agar (PDA) methods. Three layers of Whatman's filter papers were soaked in sterile distilled water and placed in sterilized glass Petri-dishes of 9cm in diameter. The Jatropha leaves and stems were surface sterilized by soaking in 70% ethanol for 5 minutes, rinsed with sterile distilled water for three consecutive times before plating the plant tissues on the Petri-dishes and then incubated for 7 days at room temperature ($28\pm2^{o}C$). The frequency of occurrence of each fungus was determined. After the incubation period, the observed fungal colonies on the plates were transferred into sterilized Potato Dextrose Agar (PDA) medium in Petri dishes to obtain pure cultures. Petri dishes containing fungal cultures were incubated for 7 days at room temperature ($28\pm2^{o}C$). Pure cultures of the isolates were preserved in a refrigerator at $4^{o}C$ until when needed.

Fungal DNA Extraction, DNA Quantification and Quality Check

Genomic DNA of fungal isolates was extracted following the protocol of Quick-DNA Fungal/Bacterial MiniPrep Kit (Zymo Research Group, California, USA) as described by the manufacturer, with slight modifications as obtainable at the Regional Centre for Biotechnology and Bio-resources Research Laboratory, University of Port Harcourt, Rivers State, Nigeria. A sterilized surgical blade was used to scrape off the fungal mycelium. This

was homogenized in a sterilized mortar using 750µl of Bashing Bead Buffer and liquid nitrogen.
The concentration and purity of the DNA were determined using Nanodrop 2000c Spectrophotometer (Thermo Fisher Scientific Inc. Wilmington, Delaware, USA). DNA quality was determined by gel electrophoresis using 1% agarose gel.

PCR Amplification and Sequencing
Fungal universal primer pair ITS4, forward: (5´-TCCTCCGCTTATTGATATGS-3´) and ITS5, reverse: (5´-GGAAGTAAAAGTCGTAACAAGG-3´), were used to amplify the ITS1-2 region of the isolates. The PCR cocktail consisted of 3µL of genomic DNA (10ng/µL), 0.1µL of Taq polymerase, 2.5µL of 10X PCR buffer, 1.0 µL of DMSO, 1.0µL of 2.5mM DNTPs, 1.0µL of 25mM $MgCl_2$(Promega), 1.0µL of each primer (concentration of 5µM) and 13.4µL of Nuclease-free water, making a total volume of 25µL. Amplifications were performed in a thermal cycler (GeneAmp® 9700 PCR System, Applied Biosystems, California, USA) using an initial denaturation step of 94°C for 5 minutes, followed by 36 cycles of denaturation at 94°C for 30 seconds, annealing at 54°C for 30 seconds and elongation at 72°C for 45 seconds with a final extension at 72°C for 7 minutes. The amplicons were stored at 10°C. Amplified products were sequenced on ABI 3500 Genetic Analyzer (Thermo Fisher Scientific, Massachusetts, U.S.A).

Data Analysis and Phylogeny
The generated raw sequences of the isolates were edited to remove PCR artifacts and sequencing noise using Molecular Evolutionary Genetics Analysis (MEGA) software, version 10.0.1 (MEGA X) (Kumar *et al*., 2018). A BLAST n search was conducted using the edited sequences against the GenBank database at the National Centre for Biotechnology Information (NCBI) database for identification of the isolates. A multiple sequence alignment of the sequences obtained from the search query of the isolates was carried out using Clustal W program and a neighbour-joining phylogenetic tree was constructed using maximum composite likelihood method. Other data obtained from the study were analyzed using One-way Analysis of Variance (ANOVA) ($P<0.05$) with the aid of Statistical Package for Social Science (SPSS) software version 23.0

Results

Fungal Species Isolated from Jatropha curcas

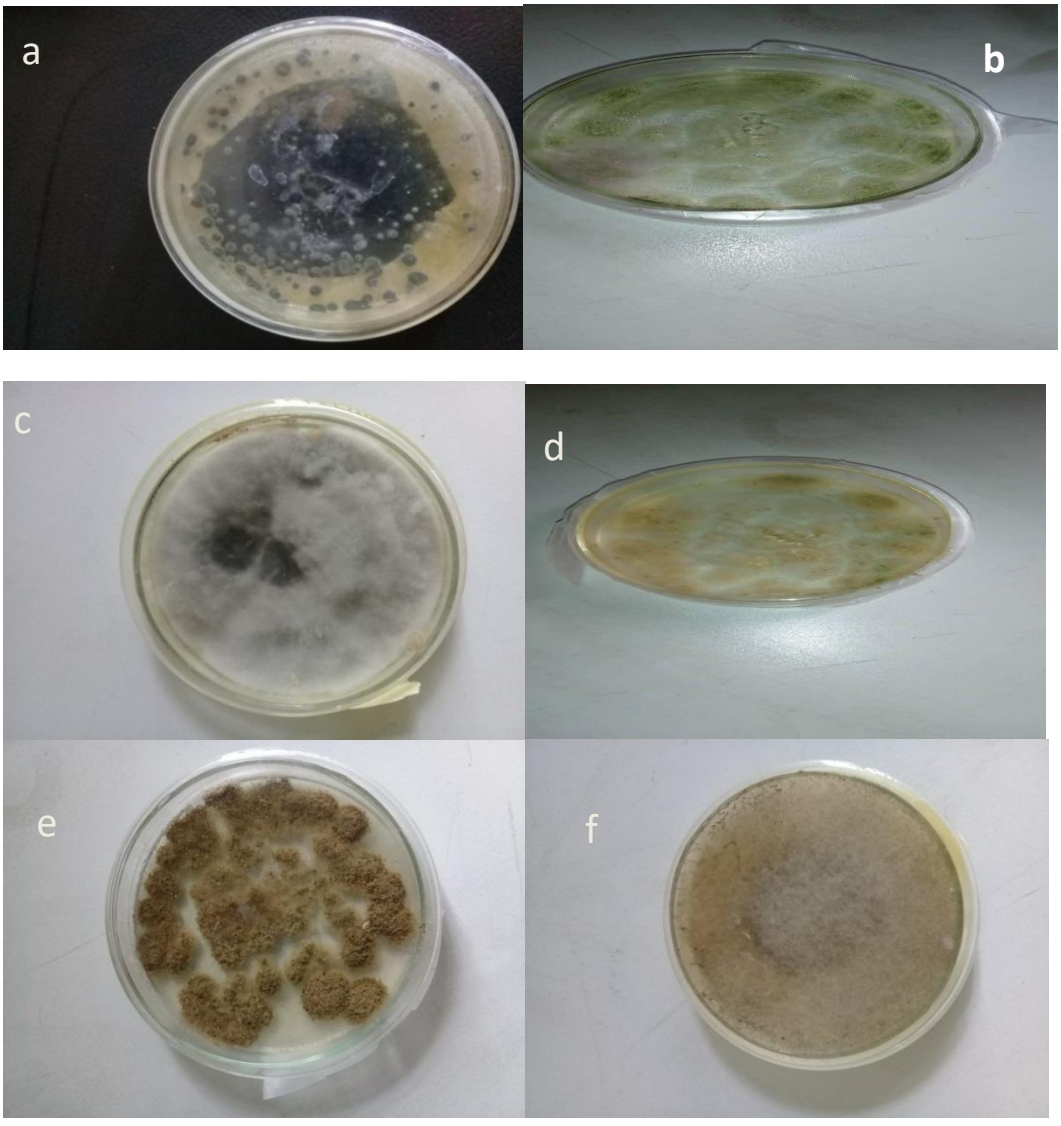
Based on our morphological characterization on PDA plates (Figure 1 and Table 1), eight fungal species were isolated from the leaves and stems of *Jatropha curcas*. The frequency of occurrence and the morphological characteristics of the isolates are presented in Table 1.
For the organisms isolated from the leaves, sample 2 had the highest mean occurrence (42.00), followed by sample 8 (37.00), sample 7 (28.66), sample 3 (25.00), sample 5 (21.00), sample 6 (18.00), sample 4 (12.00) and sample 1 (10.33). For organisms isolated from the stem, sample 8 had the highest mean occurrence (50.00) followed by sample 2 (41.00), sample 7 (31.00), sample 3 (26.66), sample 5 (21.33) and lastly sample 6 (18.00). Sample 1 and 4 were not found in the stems. More fungal isolates were obtained from the leaves than from the stems of the *J. curcas* trees sampled. Pure cultures of the fungal isolates are presented in Figure 1. The Zymo Fungal /Bacteria Miniprep kit effectively extracted the fungal DNA and yielded good quality DNA with 260/280nm ratio between 1.78 and 2.13.

Table1: Mean occurrence of fungal isolates from *Jatrophacurcas* leaves and stem.

Fungal*Jatrophacurcas*			
Isolate ID	**Morphological Characteristics**	**Leaves**	**Stem**
1	Dark green with white edges and powdery surface	10.33^{a}	0.00^{b}
2	Green colony with a powdery surface	42.00^{f}	41.00^{b}

3	Dark grey cottony colonies	25.00^{c}	26.66^{a}
4	Velvety colonies, brownish green in colour	12.00^{a}	0.00^{b}
5	Bronze to dark-brown velvety colonies with conspicuously roughened thick walls	21.00^{b}	21.33^{b}
6	Copious cottony colony with black globules	18.00^{b}	18.00^{a}
7	Dark green granular colony with powdery surface	28.66^{a}	31.00^{b}
8	Snow white colony that changes to pink as the culture becomes old	37.00^{a}	50.00^{a}
Total		24.25	23.62
ANOVA) F-statistics		241.188	601.289
p-value 0.000	0.000		

Each value is a mean of three test replicates at 95% confidence limit
Row mean $\pm$ standard deviation with different alphabet is significant at 5%.
The ANOVA result showed that for leaves and stem fungi isolates are significant at p-value (0.000) < 5% significant



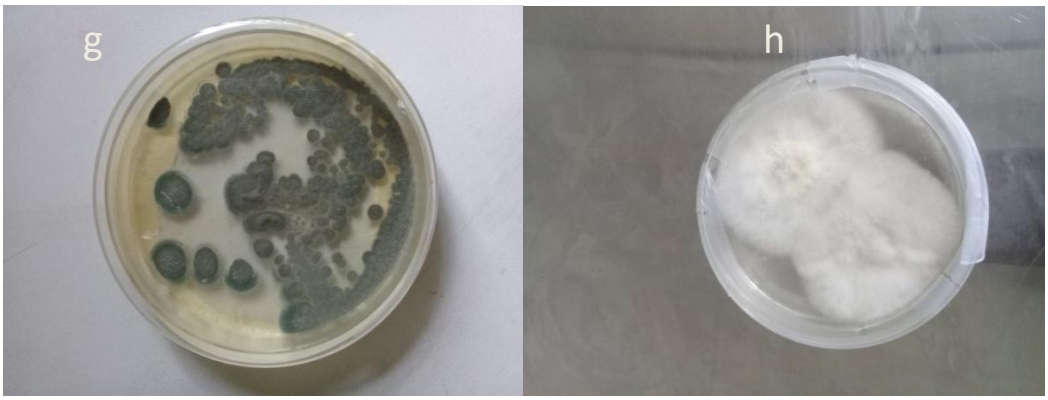


Figure 1: Pure cultures of the fungi isolated from *Jatropha curcas* leaves and stems, grown on Potato Dextrose Agar at 27±2°C.
The alphabets (a to h) represent the fungal isolates (1 to 8)

Polymerase Chain Reaction (PCR)
The quantity and quality of the extracted DNA were able to yield a good result with PCR. Gel bands of the amplicons from the PCR, as viewed under UV light, are shown in Figure 2.

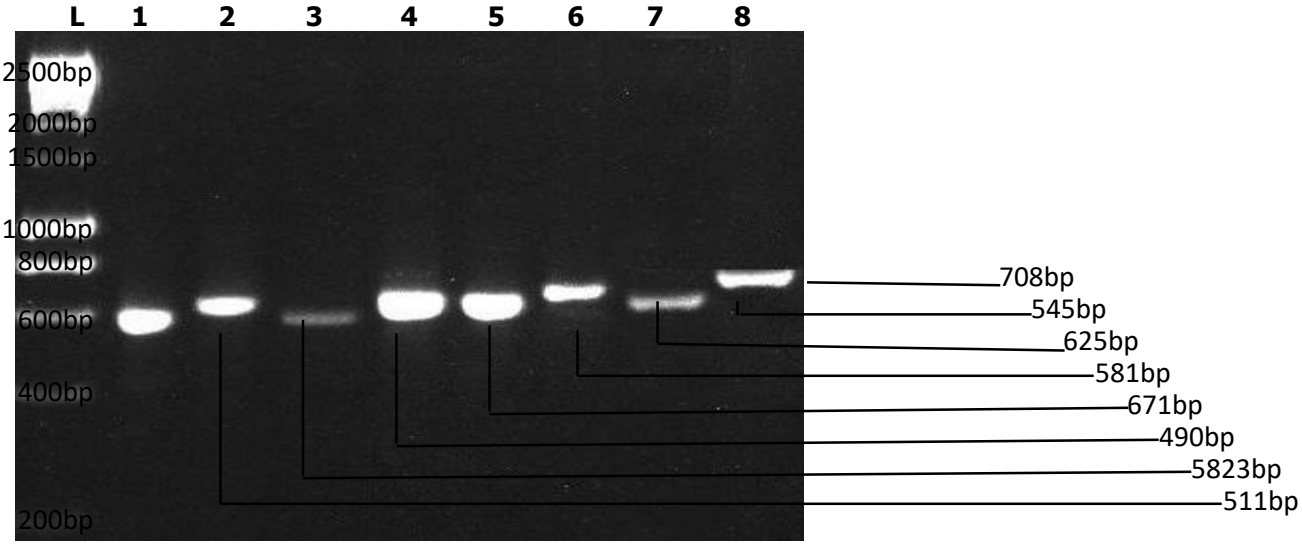


Figure 2: Gel electropherogram showing the results of PCR amplification generated from the ITS1-2 region of the fungal isolates.
L - 1kb DNA Ladder; the numbers (1 to 8) represent the fungal isolates

Identification of Fungal Isolates
The nucleotide lengths of each ITS sequence obtained from the fungal isolates of *Jatropha curcas* was determined to be 511, 582, 490, 671, 581, 625, 545 and 708 base pairs for the fungal isolates 1 to 8, respectively. The sequence alignments of all the samples were enough for identification of isolates with a high confidence limit.

A BLAST search using the sequences of the fungal ITS1-2 was conducted at the NCBI database and the identity of the isolates was revealed to be *Penicillium brevicompactum* Dierckx, *Aspergillus* sp. Micheli, *Botryosphaeria rhodina* (Berk and Curtis) Arx, *Aspergillus nomius* Kurtzman, B. W. Horn & Hesseltine, *Aspergillus tamarii* Kita, *Rhizopus oryzae* Went and Prins. Geerl., *Penicillium citrinum* Thom, C. and *Fusarium solani* (Mart.) Sacc., for the fungal isolates 1 to 8, respectively. Table 2 shows the taxonomic affinities of the isolates with searches on the nucleotides database of

GenBank using Basic Local Alignment Search Tool (BLAST).

Table 2: Taxonomic affinities of sequence types inferred from BLAST searches of ITS sequences obtained from fungal isolates of *Jatropha curcas*.

Fungal Isolate ID	Taxonomic affinity (Gene bank no.)	Similarity (%)
1	*Penicillium brevicompactum* (MH634497.1)	89
2	*Aspergillus* sp. (HM560042.1)	87
3	*Botryosphaeria rhodina* (HM156070.1)	91
4	*Aspergillus nomius* (MG575481.1)	85
5	*Aspergillus tamarii* (JN419193.1)	95
6	*Rhizopus oryzae* (MG554240.1)	95
7	*Penicillium citrinum* (MG554246.1)	98
8	*Fusarium solani* (KJ620369.1)	98

The DNA sequences obtained were submitted to the GenBank and each sequence was assigned an accession number (in parenthesis) as follows:
Penicillium brevicompactum(MK447615) strain RCBBR_AEANK1
Aspergillus sp. (MK881171) strain RCBBR_AEANK2
Botryosphaeria rhodina (MK447616) strain RCBBR_AEANK3
Aspergillus nomius (MK989664) strain RCBBR_AEANK4
Aspergillus tamarii (MK447617) strain RCBBR_AEANK5
Rhizopus oryzae (MK447618) strain RCBBR_AEANK6
Penicillium citrinum (MK447619) strain RCBBR_AEANK7
Fusarium solani (MK447620) strain RCBBR_AEANK8

Phylogenetic Analysis

Phylogenetic tree was constructed to establish the relationship between the fungal species isolated from *Jatropha curcas* and other known species on GenBank. The sequence analysis showed genetic diversity among the isolates with three other isolates belonging to the genus *Aspergillus*. *Aspergillus caelatus, Aspergillus toxicarius, Penicillium sp., Lasiodiplodia theobromae, Rhizopus delemar* and *Fusarium oxyspor um* were most closely related to the fungal isolates from this study (Figure 3). The tree enriches our understanding of how the fungal species evolved; which organisms evolved before the other. The greater the length of the vertical lines on the tree, the more the difference between the organisms.

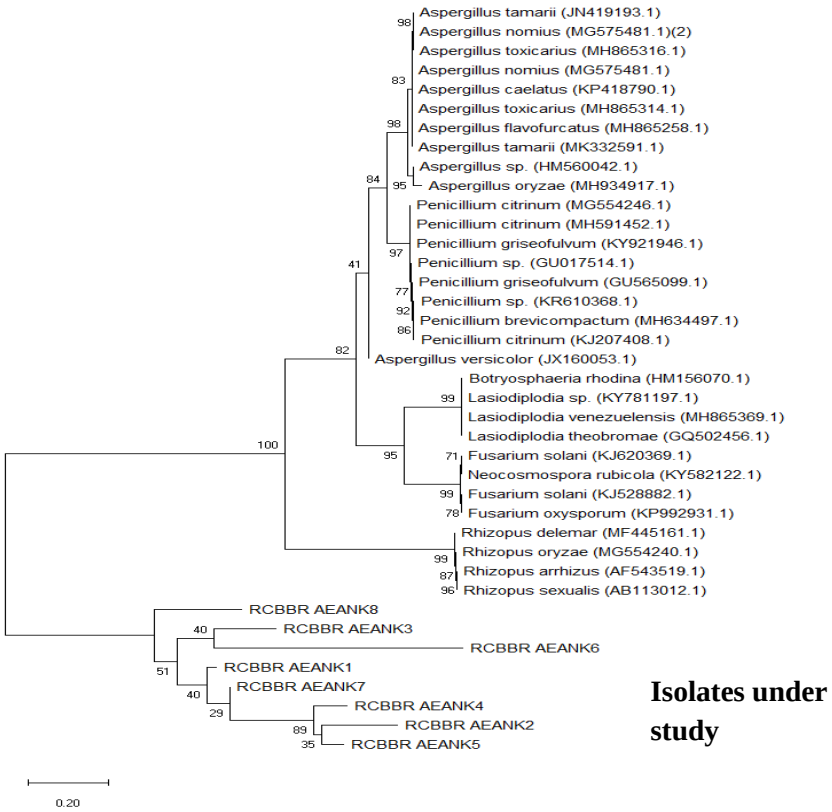


Figure 3: Phylogenetic tree showing the relationship between fungal species isolated from *Jatropha curcas* and other closely related species on GenBank.

Discussion

Eight fungal organisms, *Penicillium brevicompactum*, *Aspergillus* sp., *Botryosphaeria rhodina*, *Aspergillus nomius*, *Aspergillus tamarii*, *Rhizopus oryzae, Penicillium citrinum* and *Fusarium solani* were isolated from *Jatropha curcas* using the traditional cultural method and identified using molecular techniques.

Fungal organisms associated with *Jatropha curcas* have been reported by some authors. Nwaukwu *et al*. (2014) reported the occurrence of *Alternaria alternata*, *Botryodi plodia theobromae* , *Cercospora beticola*, and *Macrophomina phaseolina* on diseased leaves of *Jatropha curcas*. Jayaraman*et al*. (2011) isolated *Aspergillus glaucus*, *A. flavus*, *A. niger* and *Penicillium citrinum* from seeds of *Jatropha curcas*. Worang*et al*. (2008) reported the occurrence of *Aspergillus ochraceus*, *A. penicillioides*, *A. tamarii*, *Fusarium moniliforme*, and *Penicillium citrinum* on *Jatropha curcas* seeds. All these fungi were isolated using Standard Blotter and Potato Dextrose Agar methods and identified by microscopy.

Lateef *et al*., (2019) isolated *Curvularia geniculata* and *Phyllosticta capitalensis* from *Jatropha curcas* leaves using morphological and molecular methods. These organisms are endophytes and establish symbiotic- to-pathogenic relationships with plants. These fungi can assume a pathogenic condition when the host plant is under stress. Santos *et al*., (2013) isolated *Colletotrichum capsici* and *Colletotrichum gloeosporioides* from seeds of *Jatropha curcas* through morphological, cultural, and molecular analyses. Leaves and fruits of the physic nut are susceptible to anthracnose caused by species of *Colletotrichum capsici* and *Colletotrichum gloeosporioides*.

Most of the fungal isolates obtained from this study are plant pathogens. Leaf dropping and

leaf crumple which were among the symptoms observed on the sampled *J. curcas* leaves are symptoms associated with fungal diseases of plants. Destruction of succulent plant tissues (blight) such as leaves and shoots were also obtained from the *J. curcas* plants sampled. Leaf spots which are localized lesions produced on plant leaves due to fungal invasion was also observed on the sampled leaves. *Fusarium* species are known to cause wilting of plants; some of the sampled *J. curcas* plants showed this symptom. The genera *Aspergillus* and *Penicillium* produce several mycotoxins which cause diseases in plants and health complications in man and animals. *Aspergillus nomius* and *Aspergillus tamarii* are usually misinterpreted as *Aspergillus flavus* based on phenotype; but using molecular methods such as ITS, Calmodulin Gene and β-Tubulin sequencing, these organisms are identified as distinct species (Tam *et al*., 2014).

Aspergillus tamarii is an endophytic fungus that could be a potential source of antibacterial agents. Ogbole*et al*., (2017) reported the antibacterial activity of *Aspergillus tamarii* against *Salmonella typhi*, *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli*. *A. tamarii* has also been reported to produce the enzyme, tannase which is used in the food industry (Costa *et al*., 2008). *Aspergillus tamarii* has been found to be pathogenic to man. The production of aflatoxin B1 and G1 by *A. nomius* strain ASR3 has also been reported by Da Silva-Junior *et al.* (2017).

Penicillium citrinum is known to produce a mycotoxin called citrinin. *P. citrinum* has been recorded as one of the seed-borne pathogenic fungi in common bean (*Phaseolus vulgaris*) (Marcenaro and Valkonen, 2016). *P. citrinum* has also been connected with the promotion of plant growth and mitigation of the adverse effects of stem rot (Muhammad *et al*., 2015). *Penicillium brevicompactum* and. *P. solitum* were reported as pathogenic organisms on pear (Louw and Korsten, 2014). Wang *et al*., (2011) reported *Botryosphaeria rhodina* as one of the pathogens responsible for gummosis on Peach (*Prunuspersica*) trees in China. Symptoms include dark lesions on twigs and branches and in some trees, gum exudation occurred from diseased parts. *Rhizopus oryzae* has been reported to be responsible for soft rot on banana, apple, avocado and guava (Jin-Hyeuk*et al*., 2011; Jin-Hyeuk*et al*., 2012; Kurniawati and Sardjono, 2014). *R. oryzae* has also been reported to produce lactic acid which is widely used in food and food-related products (Kurniawati and Sardjono, 2014).

Fusarium solani has been reported as a rot pathogen by many authors. *Fusarium* species including *F. solani* are the most predominant soil-borne fungal pathogens on plants causing severe economic damages especially in wheat, potato, pea, bean, rice and corn in Iran (Saremi*et al*., 2011). *Fusarium solani* f. sp. *cu curbitae* has been reported to cause crown and root rot on *Cu curbitapepo* (zucchini squash) in Spain, and experiments conducted to determine the ability of the pathogen to survive in bags filled with food products showed that the pathogen survived for 20 months (Perez-Hernandez *et al*., 2017). *F. solani* has been reported to be associated with crown disease of oil palm (Hafizi*et al*., 2014) and as the causal agent of crown and root rot of strawberry crops in Southwestern Spain (Pastrana *et al*., 2014).

Conclusion

In this study, eight fungal organisms associated with *Jatropha curcas* were isolated using traditional cultural techniques and identified using molecular methods. The use of traditional cultural techniques in the identification of microorganisms is not reliable as misinterpretation and misidentification of organisms abounds. The cultural techniques used in this study only aided in the processes that led to the correct identification of the isolates. The morphological characteristics were only able to suggest the suspected organisms but cannot be used to successfully characterize the isolates to the species level. Identification of the fungal isolates by the use of molecular techniques such as Polymerase Chain Reaction amplification and sequencing of the ITS regions of the fungal genome yielded a good result. Some of the organisms isolated in this study exist as endophytes, having a symbiotic relationship with plants. Under stress or unfavourable conditions, endophytic organisms become pathogenic causing deterioration on plants. These fungi

cause deterioration of the seeds, leaves and bark (or stem) of *Jatropha curcas* plants leading to a decrease in the quantity and quality of the plant products obtained. This study has provided information on some of the fungal organisms associated with *Jatropha curcas* which will help in proffering prevention and control measures to reduce the incidence of some of these pathogenic organisms on *J. curcas*.

Refrences

Agbogidi, O. M., Akparobi, S. O. and Eruotor, P. G. (2013). Health and Environmental Benefits of *Jatrophacurcas* (Linn.). *Appl. Sci. Rep.,* 1(2): 36-39.

Castro-Escarpulli, G., Alonso-Aguilar, N. M., Rivera, G., Bocanegra-Garcia, V., Guo, X., Jurez-Enrquez, S. R., Luna-Herrera, J., Martnez, C. M., Guadalupe, A. M. (2016). Identification and Typing Methods for the Study of Bacterial Infections: A Brief Review and Mycobacterial as Case of Study. *Arch. Clin. Microbiol.*, 7: 1–10.

Costa, A. M., Ribeiro, W. X., Kato, E., Monteiro, A. G.and Peralta, R. M. (2008). Production of Tannase by *Aspergillus tamari i*n Submerged Cultures. *Braz. Arch. Biol. Technol.*, 51(2): 399-404.

Da Silva-Junior, E. A., Paludo, C. R., Valadares, L., Lopes, L. P., Do Nascimento, F. S. and Pupo, M. T. (2017). Aflatoxins produced by *Aspergillusnomius* ASR3, a Pathogen Isolated from the Leaf-cutter Ant *Atta sexdensrubropilosa*. *Braz. J. Pharmacog.*, 27: 529–532.

Franco-Duarte, R., Cernáková, L., Kadam, S., Kaushik, K. S., Salehi, B., Bevilacqua, A., Corbo, M. R., Antolak, H., Dybka-St¸epie, K., Leszczewicz, M., Tintino, S. R., Alexandrino de Souza, V. C., Sharifi-Rad, J., Coutinho, H. D., Martins, N. and Rodrigues, C. F. Advances in Chemical and Biological Methods to Identify Microorganisms—From Past to Present. *Microorg.*, 7: 130-161.

Hafizi, R., Salleh, B. and Latiffah, Z (2014). Morphological and Molecular Characterization of *Fusarium solani* and *F. oxysporum* Associated with Crown Disease of Oil Palm. *Braz. J. Microbiol.*, 4(3): 959-968.

Islam, A., Yaakob, Z. and Anuar, N. (2011). Jatropha: A Multipurpose Plant with Considerable Potential for the Tropics. *Sci. Res. Essays*, 6(13): 2597-2605.

ISTA (International Seed Testing Association) (2016). International Rules for Seed Testing. Rules Ammendments. *Seed Sci. Technol.*, 29: 1-127.

Jayaraman, P., NesaPriya, S., Parameshwari, S., ShyamalaPriya, S., Jawahar, N. andSekarBabu, H. (2011). Occurrence of Storage Fungi in Jatropha (*Jatrophacurcas* L.) Seeds. *African J. Microbiol. Res.*, 5(5): 475-480.

Jin-Hyeuk, K., Jae-San, R., Tran, T. and Okhee, C. (2012). Soft Rot of *Rhizopusoryzae* as a Postharvest Pathogen of Banana Fruit in Korea. *Mycobiol.*, 40(3):214-216.

Jin-Hyeuk, K., Jinwoo, K., and Won-Il, K. (2011). First Report of *Rhizopusoryzae* as a Postharvest Pathogen of Apple in Korea. *Mycobiol.*, 39(2): 140–142.

Kumar, S., Stecher, G., Li, M., Knyaz, C. and Tamura K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across Computing platforms. *Mol. Biol. Evol.* 35:1547-1549.

Kurniawati, T. and Sardjono, R. I. (2014). Isolation of*Rhizopusoryzae*from Rotten Fruit and its Potency for Lactic Acid Production in Glucose Medium with and without Addition of Calcium Carbonate. *Agritech*, 34(2): 170-176.

Lateef, A., Garuba, T., Sa'ad, G., Olesin, M., Eperetun, G. and Tiamiyu, B. (2019). Isolation and Molecular Identification of Fungal Endophytes from Green Leaves of Physic Nut (*Jatropha curcas*) from Unilorin Plantation, Ilorin, Nigeria. *Sri Lankan J. Biol.*, 4 (1): 1-13.

Louw, J. P. and Korsten, L. (2014). Pathogenic *Penicillium* spp. on Apple and Pear. *Plant Dis.*, 98:590-598.

Maghuly, F., Jankowicz-Cieslak, J., Till, B. and Laimer, M. (2013). The Use of Ecotilling for the Genetic Improvement of *Jatrophacurcas*. In: *Jatropha, Challenges for a New Energy Crop*. Volume 2: Genetic Improvement and Biotechnology. Bahadur, L., Sujatha, M., Carels, N. (eds). Springer New York Heidelberg Dordrecht London, pp. 335-349.

Marcenaro, D. and Valkonen, J. (2016) Seedborne Pathogenic Fungi in Common Bean (*Phaseolus vulgaris* cv. INTARojo) in Nicaragua. *PLoSONE*, 11(12): 1-18.

Muhammad, W., Abdul, K., Muhammad, H., Raheem, S., Sang-Mo, K., Jong-Guk, K., In-Jung, L. (2015). Endophytic Fungi Promote Plant Growth and Mitigate the Adverse Effects of Stem Rot: An example of *Penicilliumcitrinum* and *Aspergillus terreus*. *J. Plant Inter.*, 10(1): 1-18.

Nwaukwu, I. A., Akinseye, O. F. and Ataga, A. E. (2014). Mycoflora of Physic Nut (*Jatrophacurcas*): A Biofuel Plant. *Nig. J. Bot.*, 27(2): 191 – 198.

Ogbole, O. O, Adebayo-Tayo, B. C., Salawu, K. M. and Okoli, V. C (2017). Molecular Identification and Antimicrobial Activity of Endophytic Fungi *Aspergillustamarii* (Trichomaceae). *Nig. J. Pharm. Sci.*, 16: 41-48.

Pastrana, A. M., Capote, N., De los Santos, B., Romero, F. and Basallote-Ureba, M. J. (2014). First Report of *Fusarium solani*causing Crown and Root Rot on Strawberry Crops in Southwestern Spain. *Plant Dis.*, 98(1): 161.

Perez-Hernandez, A. Porcel-Rodriguez, E. and Gomez-Vazquez, J. (2017). Survival of *Fusarium solani* f. sp. *curcurbitae* and Fungicide Application, Soil Solarization, and Biosolarization for Control of Crown and Foot Rot of Zucchini Squash. *Plant Dis.*, 101(8): 1507-1514.

Rahman, M. M., Ahmad, S. H., Mohamed, M. T. and Abrahman, M. Z. (2014). Antimicrobial Compounds from Leaf Extracts of *Jatrophacurcas*, *Psidiumguajava* and *Andropraphispaniculata*. *Sci. World J.*, 9(2): 1-9.

Salvador-Figueroa, M., Magana-Ramos, J., Vazquez-Ovando, J., Adriano-Anaya, M. andOvando-Medina, I. (2015). Genetic Diversity and Structure of *Jatrophacurcas* (L.) in its Centre of Origin. *Plant Gen. Res.,* 13(1): 9 – 17.

Santos, G. R., TozzeJúnior, H. J., Corrêa de Sa, D. A, Furtado, G. Q. and Massola-Júnior, N. S. (2013). Etiology and Pathogenicity of two Different Isolates of *Colletotrichum*spp. obtained from Physic Nut Seeds. *J. Seed Sci.*, 35(2):139-146.

Saremi, H., Okhovvat, S. M. and Ashrafi, S. J. (2011).*Fusarium* Diseases as the main Soil-borne Fungal Pathogen on Plants and their Control Management with Soil Solarization in Iran. *African J. Biotech.*, 10(80): 18391-18398.

Tam, E. W.,Chen, H. K. Lau, E. C., Ngan, A. H., Fung, K. S., Lee, K., Lam, C., Yuen, K., Lau, S. K. and Woo, P. C. (2014). Misidentification of *Aspergillus nomius*and *Aspergillus tamarii* as *Aspergillus flavus*: Characterization by Internal Transcribed Spacer, β-Tubulin, and Calmodulin Gene Sequencing, Metabolic Fingerprinting, and Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry. *J. Clin. Microbiol.*, 52(4): 1153–1160.

Wang, F., Zhao, L. and Li, G. (2011). Identification and Characterization of *Botryosphaeria* spp. causing Gummosis of Peach Trees in Hubei Province, Central China. *Plant Dis.*, 95(11): 1378-1384.

Worang, R. L., Dharmaputra, O. S. and Syarief, R. M. (2008). The Quality of Physic nut (*Jatrophacurcas*L.) Seeds Packed in Plastic Material During Storage. *Biotropia*, 15(1): 25-36.