OCCURRENCE AND IDENTIFICATION OF DIEBACK FUNGAL PATHOGENS OF *JATROPHA CURCAS* IN SELECTED PLANTATIONS IN NORTH-WEST NIGERIA.

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SUMMARY

***Jatropha curcas* L. (Physic nut) is a drought-tolerant plant used in erosion control, biofuel production, and curing ailments traditionally. Fungal diseases were some of the prevalent diseases recorded on the plant in Nigeria and thus, a survey to determine the incidence, severity and causal organisms of dieback on Jatropha was conducted on three farms at Tsanni (Katsina State), Minjibir (Kano State), and Samaru (Kaduna State). Five quadrants measuring 6 X 6 m^2 were marked per hectare randomly and plants within each quadrant were used to assess disease incidence and severity. Three fungi isolated from collected diseased samples were identified by CABI, London as *Fusarium* clade VII, *Fusarium solani,* and *Gibberella fujikuroi.* These fungal organisms were confirmed as the causal organisms for Jatropha dieback at the three locations upon Koch's postulation. The disease incidence and severity at Samaru, Tsanni, and Minjibir were 100 and 34.0; 78.1 and 24.9; and 31.3 and 22.7 %, respectively. Therefore, it was concluded that Jatropha dieback which is associated with *Fusarium* species and its anamorph *Gibberella fujikuroi* was most prevalent in Samaru.**

Keywords: Incidence, Severity, *Jatropha curcas*, and Dieback

PHYSIC NUT (*Jatropha curcas* L.) belongs to the family *Euphorbiaceae* and is believed to be native of Central America, particularly Mexico and Brazil from where it was spread to Africa and Asia in the 15th and 16th centuries, respectively (Martin and Mayeux, 1984). The word Jatropha was derived from the Greek words '*Jatros'* (doctor) and '*trophe'* (food) which indicates its medicinal uses (Verma and Gaur, 2009). The crop is adapted to tropical and sub-tropical conditions due to its ability to grow even fast on marginal soils and tolerates drought (Ovando-Medina *et al.*, 2011). Jatropha has a wide range of uses such as a cure for various ailments in the ancient days, water conservation and erosion control; seed cakes as organic fertilizer for soil improvement, as a

raw material in the cosmetic industry such as soap making and biofuel extraction (Jones and Miller, 1991; Belewu *et al*., 2010; (Mbewe, 2018).

Initially, Jatropha was believed to be resistant to pests due to its medicinal attributes. However, when it was grown under monoculture various insect pests such as *Ootheca mutabilis, Achaea Janata,* and *Leptoglossus zonatus* have been reported as some of the key pests of the crop (Grimm, 1999; Shanker and Dyani, 2006). Also, fungal pathogens such as *Alternaria alternata, Colletotrichum acutatum, Lasiodiplodia theobromae, Botryosphaeria dothidea,* and *Phoma sp.* were reported to have caused various diseases on the crop (Anitha *et al*., 2005). In Nigeria, some diseases recorded in Samaru were dieback and *Jatropha Nigeria Mosaic Virus* (Zarafi *et al*., 2012; Kashina *et al*., 2013) while insect pests recorded were *Paracoccus marginatum, Tetranynchus* spp. and *Stompastis thraustica* (Alamu *et al*., 2016). *Scutellonema, Meloidogyne,* and *Rotylenchus* sp. were also reported as the most prominent nematodes associated with Jatropha in Sabon Gari Local Government Area (Olatunji, 2016).

Dieback is the drying of plant organs such as stem or branches that starts from the tip and progresses gradually towards the main stem or trunk (Bateman *et al.,* 2006). Symptoms of Jatropha dieback disease include; leaf wilting, defoliation, flower abortion, bark, and vascular tissue discoloration, twigs necrosis, bark splitting, and infected stem/branch breakage (Zarafi *et al*., 2012).

A serious problem of wilting and death of Jatropha stems was reported at the Institute for Agricultural Research (I.A.R.) plantations in Samaru, Zaria, in 2010 which led the Institute to initiate research on the identification, pathogenicity test, and management of diseases on Jatropha field (Alabi *et al*., 2011). Research on isolation and identification of putative causative organism(s), host range using some arable crops, and assessment of incidence and severity was conducted on Jatropha plantations at I.A.R. and National Research Institute for Chemical

Technology (NARICT) Zaria (45). Research conducted in Samaru and Basawa of Sabon Gari Local Government (Kaduna State) identified *Fusarium* sp. as the causal organism of Jatropha dieback (Zarafi *et al*., 2012). However, the identification of the causal organism was based on cultural, morphological, and microscopic characteristics. Therefore, there is the need to expand the research to other parts of North-west Nigeria, as well as the application of molecular techniques for the identification of the pathogen(s).

MATERIALS AND METHODS

A survey was carried out in three agro-ecological zones of North-Western Nigeria. Tsanni Village, Batagarawa Local Government Area (L.G.A.) of Katsina State (Sahel Savannah Zone); Minjibir, Minjibir L.G.A. of Kano State (Sudan Savannah) and Samaru, Sabon Gari L.G.A. of Kaduna State (Northern Guinea Savannah) were the locations visited (Fig. 1). These sites which cut across three different agro-ecological zones were selected based on available information from the Institute for Agricultural Research (I.A.R.) and Green Shield Nigeria. During the survey, a plantation in each agro-ecological zone was visited and five (6 x 6 m^2) quadrats per hectare (Ha) were marked in a systematic pattern (four at the corners and one at the center) as replications. Each quadrat contained 2 to 9 plants depending on the plant spacing adopted.

Information on crop history such as plantation establishment date, source of seeds, propagation method (cuttings or seeds), and pest management strategies adopted (if any) were obtained through an administered checklist. The location (coordinates) and the size of each plantation was determined using a handheld Global Positioning System (GPS). Data on weather parameters such as temperature, rainfall, and relative humidity for the year 2016 were obtained from the Nigerian Meteorological Agency (NIMET), Katsina, and I.A.R. Zaria. Infected twigs and/or stems were recorded and samples were taken to the Myco-pathology Laboratory of the Department of Crop Protection, A. B. U. Zaria for fungal isolation.

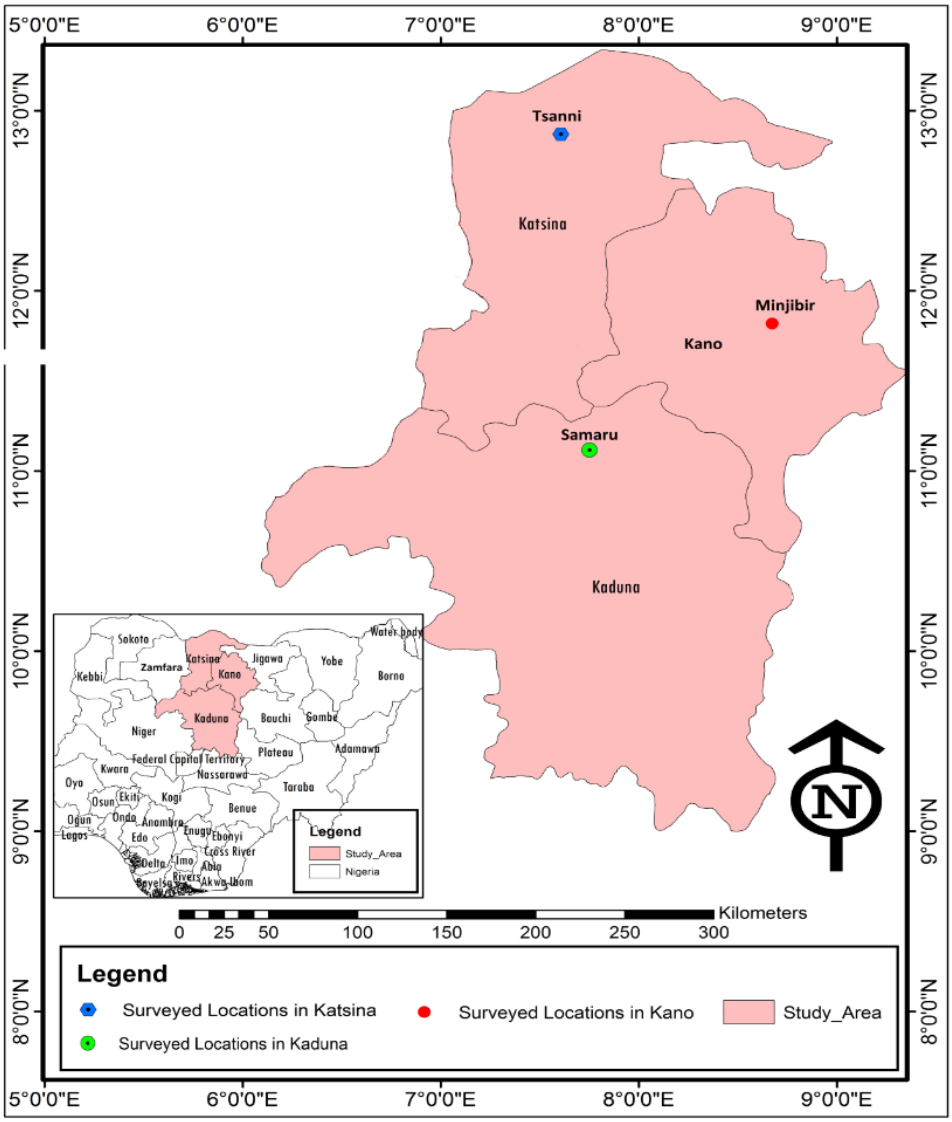

Figure 1: Surveyed locations in Katsina, Kano, and Kaduna States.

Assessment of Disease Incidence and Severity

Jatropha dieback incidence and severity assessment was conducted in August 2016. The incidence (DI) was obtained by expressing diseased plants as a percentage of the total number of plants in each quadrat.

$$DI\ (\%) = \frac{\text{Number of infected plants}}{\text{The total number of plants sampled}} \times 100$$

On the other hand, the severity was assessed on individual plants using a modified scale of 1-5 developed by Cardoso *et al.* (1998). where:

1 = no visible symptom on leaves and twigs

2 = 1- 15 % leaves and twigs wilted

3 = 16 – 30 % wilt, yellow leaves, and brown vascular tissues

4 = 31 – 45 % dry branches, brown/dark twigs

5 = > 45 % bark split, twigs/stems necrosis and breaking.

Disease Severity (DS) was calculated for each quadrat using the following formula (Marley, 2013)

$$DS\ (\%) = \frac{\Sigma \text{ of disease ratings}}{\text{Total number of plants X Maximum grade}} \times 100$$

During the survey, thirty-six (36), twenty (20), and four (4) quadrats were marked in Samaru, Tsanni, and Minjibir respectively. The quadrats were grouped into four (4) replications by taking the average in each location for data analysis. Data collected were subjected to Analysis of Variance (ANOVA) and means separated using Least Significant Difference (LSD) at 5 % level of significance using SAS software version 9. Bar charts were drawn using the Microsoft Office Excel software package.

Isolation and Identification of Fungi

A 2-cm piece of infected stem tissue was surface-sterilized for 2 min in 0.5% solution of sodium hypochlorite (NaOCl), rinsed in three changes of sterile distilled water (SDW), placed in a 9 cm plastic petri-dish lined with moistened filter paper, and incubated in a moist chamber for two days to induce mycelial growth which was transferred aseptically to freshly prepared Potato Dextrose Agar (PDAs) amended with streptomycin sulfate at 0.6 µg/L and incubated at 28 ± 2^0 C. Cultural and microscopic characteristics of the isolates were studied and organisms were identified to genus

level using a standard identification monograph (Barnett and Hunter, 2006). Isolates obtained were coded and sent to the Centre for Biosciences and Agriculture International (CABI), Egham, Surrey, the United Kingdom for species identification.

Pathogenicity Test

Fourteen-day-old fungal culture was harvested in 15 ml SDW per plate, macerated, filtered through a double-layer muslin cloth, and standardized to 30 x 10^3 spores per ml. Jatropha seeds ("Soba" accession) obtained from Legumes and Oil Seeds Research Program of I.A.R., Samaru were surface sterilized in 1 % NaOCl for 2 minutes, rinsed in three changes of SDW, blotted on sterile filter paper, and sown at five seeds per 15 X 22 cm pot in heat sterilized soil in the screen house which were thinned to two at 2 weeks after sowing (WAS).

Twenty-one-day-old seedlings were inoculated with the conidia suspension of fourteen-day-old PDAs grown cultures of the isolated organisms. The smear inoculation method was used by gently spreading the inoculum on the adaxial and abaxial surfaces of leaves. Stems were also smear inoculated gently with a paintbrush. Another set of seedlings were inoculated with SDW which served as a control treatment. Inoculated seedlings were covered with polyethylene bags for 24 hours, arranged in a Complete Randomized Design (CRD), and observed daily for the development of symptoms. Inoculated stands developing symptoms as the initial specimen was re-isolated on PDAs and re-examined under the microscope.

DNA Extraction and Polymerase Chain Reaction

The genomic DNA of the isolated fungal organisms was extracted using ZR Fungal/Bacterial DNA miniprep™ kit (Zymo Research Corporation, USA) according to protocols. The PCR was conducted using AB28 (5'-ATATGCTTAAGTTCAGCGGGT-3') and TW1 (5'-GTTTCCGTAGGTGAACCTGC-3') primers for the amplification of the Internal Transcribed Spacer (ITS) region. The 20µl PCR mixture consisted of 1µl of DNA template, 1µl of forward and

reverse primers each, 10µl of the master mix, and 7µl of SDW. The PCR cycling conditions were preincubation for 5 minutes at 95°C followed by 35 cycles of denaturation for 30 seconds at 95°C, annealing for 30 sec at 52°C and extension for 1 minute at 72°C and a final extension at 72°C for 10 minutes. A 1% agarose gel electrophoresis was conducted for 30 minutes at 100 volts. The PCR product was purified using the MicroClean® (Microzone Limited, England). Sanger DNA sequencing was conducted at CABI Biosciences, UK. Sequence data was BLAST on the European Molecular Biology Laboratory (EMBL) database and ClustalW in MEGA 7 software was used to generate consensus sequences for alignment (Kumar *et al*., 2016), construct maximum likelihood analysis of taxa, and estimate evolutionary divergence between sequences.

RESULTS

Description of plantations

The plantation in Tsanni, Katsina State established in 2007 with a spacing of 3 x 4m^2 is located at 12^{0}48'06.7'' N, 7^{0}36'50.6''E and covers an area of 7.66 ha with some mango trees sparsely distributed in it. The plantation manager explained that wilting of leaves and death of branches were observed in 2013, but decreased by 2015. The information obtained showed that there has never been any pesticide application on the plantation for pest and disease control. Cow dung and poultry droppings organic manure was applied once or twice a year with no specific quantification. From four years (2011) after establishment, the plantation produces an average of two tonnes per hectare (2 t/ha) of seeds annually as shown in Table 1. The plantation in Minjibir, Kano State is located at 12^{0}14'67.2'' N, 8^0 66'46.2" E and covers an area of about 0.6 ha. The plantation was also established in 2007 at a spacing of 5 x 7 m^2 using "matsaya" accession locally sourced from Katsina State. Seedlings were raised at I. A. R. Samaru nursery and transplanted at Minjibir plantation. There was little information on management practices about the plantation. The plantation in Zaria, Kaduna State is located at 11^{0}17'42.75" N, 7^{0}61'29.31" E and covers an area of 4.174 ha. The plantation was established in 2006 at a spacing of 2 x 2 m^2 as a demonstration plantation. NPK fertilizer 15-15-15 is applied at the rate of 120 kg/ha annually at the onset of the

rainy season for optimum seed production. The plantation produces an average yield of 11.5 t/ha while the total output of the plantation was forty-eight tonnes per hectare (48 tonnes) as shown in Table 1.

Disease Incidence and Severity

Samaru recorded the highest disease incidence (100.0 %) and it significantly differed from Tsanni (78.1 %) and Minjibir (31.3 %) at a 5 % level of significance (P≥0.05) as shown in Fig. 2. Disease severity also followed a similar pattern where Samaru recorded the highest (34.0 %) and significantly differed from Tsanni (24.9 %) and Minjibir (22.7 %) at (P≥0.05). The average temperature recorded in the year of the survey (2016) was 32.9^0 C, 33.8^0 C, and 34.0^0 C for Samaru, Tsanni, and Minjibir respectively. Average relative humidity of 51.1 %, 45.0 %, and 52.8 % were recorded while the annual rainfall obtained was 1073.4 mm, 540.9 mm, 800.1 mm in Samaru, Tsanni, and Minjibir respectively as obtained from NIMET and I.A.R. The different symptoms observed in the plantations were leaf wilting, defoliation, flower abortion, bark, and vascular tissue discoloration, twigs necrosis, and stem breaking as shown on Plates 1.

Pathogen identification

The identification of fungi isolated from the diseased Jatropha stems/twigs was confirmed by CABI, United Kingdom as *Fusarium solani, Gibberella fujikuroi, Fusarium* Clade VII. Cultures of *Fusarium* clade VII on PDAs were initially white to peach in color, but finally, turned to buff-brown with brown background coloration. *Fusarium* clade VII took 7 to 10 days to cover a 9 cm diameter petri dish at 28 ± 2^0 C. Conidia were cylindrical with 2 to 5 septations as shown in Plate 2. Cultures of *Fusarium solani* took 6 to 8 days to cover the 9 cm diameter petri dish at 28 ± 2^0 C, appear as cottony-like grayish-white on PDAs, and produced light to dark brown coloration on the background. Under the microscope, conidia were cylindrical to canoe-shaped with 4-5 septations and tapered at the apex as shown on Plate 3. Cultures of *Gibberella fujikuroi* covered 9 cm diameter Petri-dish at 28 ± 2^0 C in 10 to 12 days and produced a white cottony growth on PDAs. It also

produced a milk-colored background and the conidia were slender with three septations as shown on plate 4.

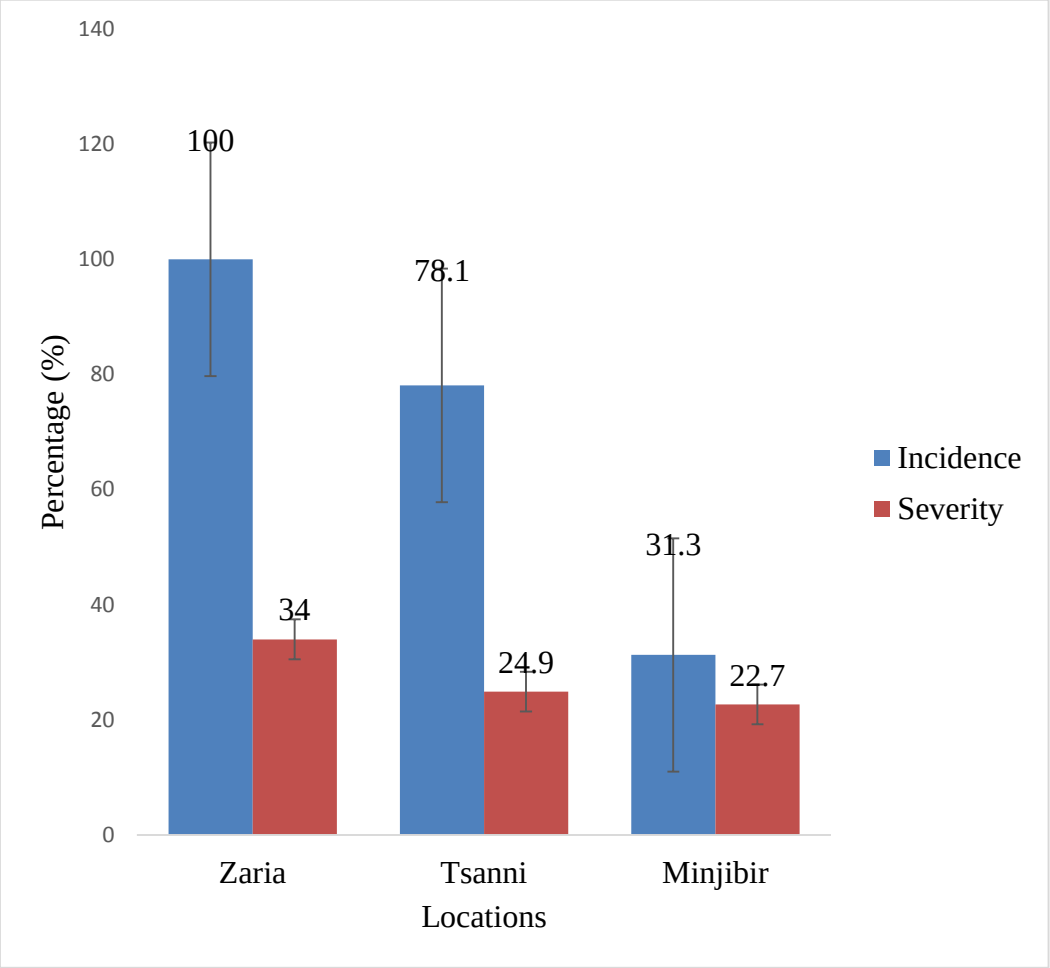

Figure 2: Incidence and Severity of Jatropha Dieback in Samaru, Tsanni, and Minjibir, August 2016.

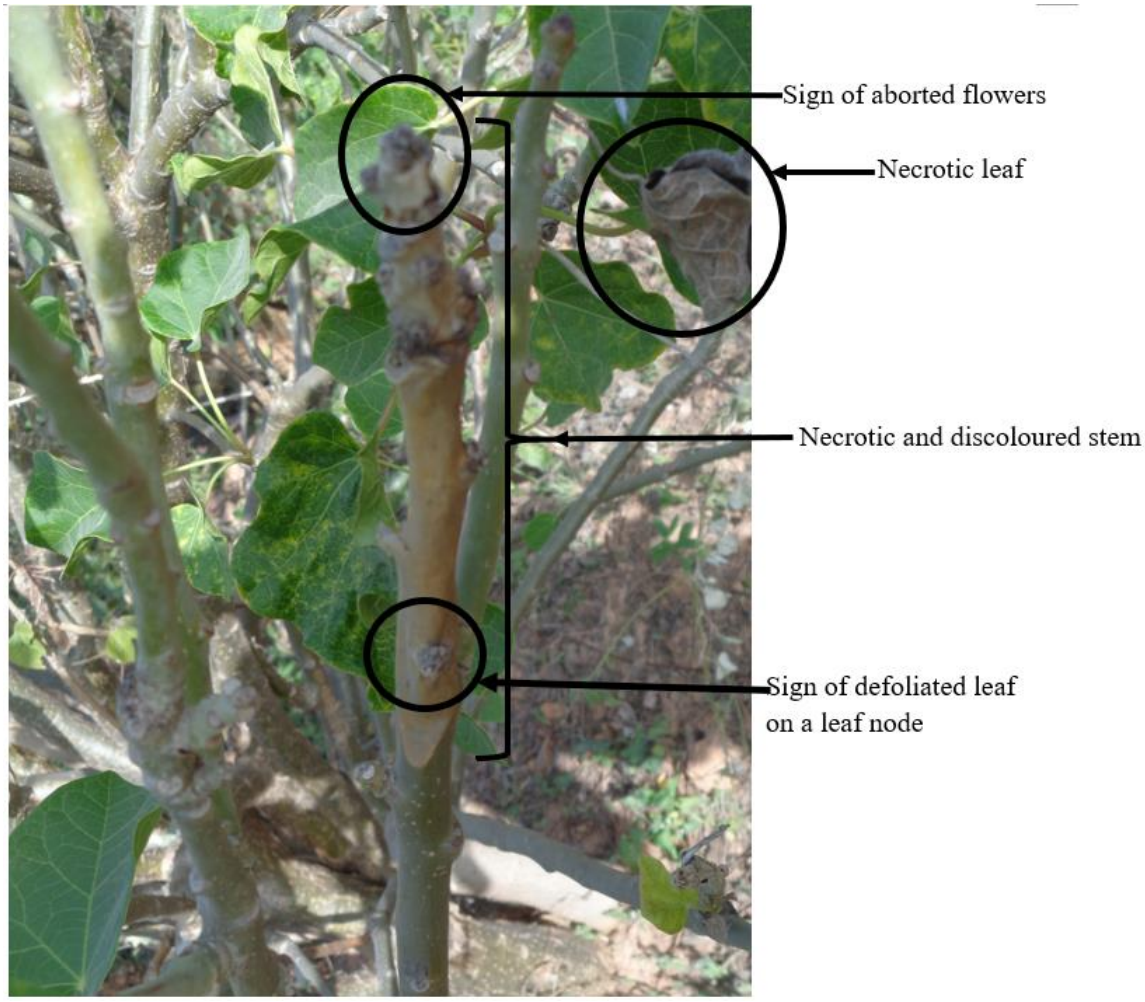

Plate 1: Jatropha branch showing discolored stem, necrotic leaf, and nodes of aborted leaves and flowers.

Plate 2: Fourteen-day old culture of *Fusarium* clade VII and conidia grown on PDAs

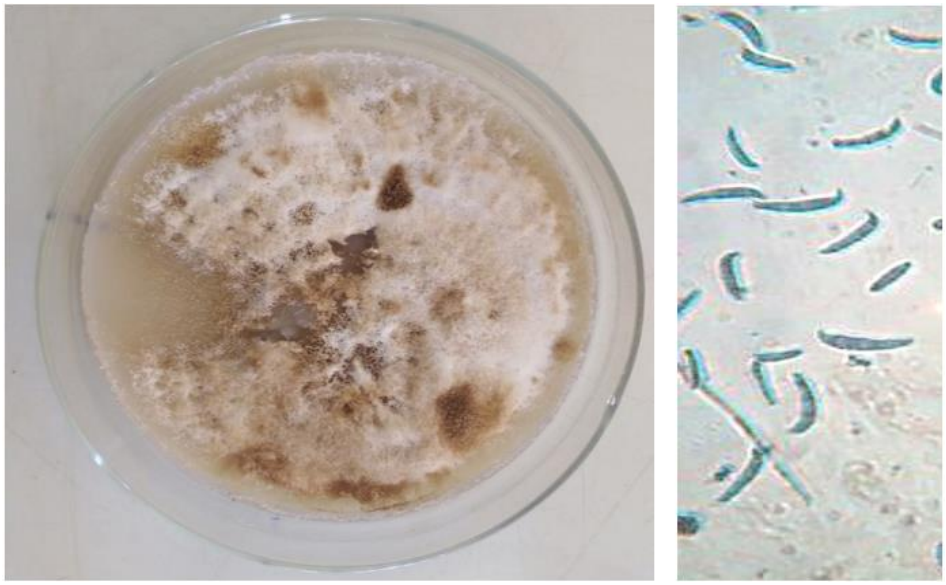

Plate 3: Fourteen-day old culture of *Fusarium solani* and conidia grown on PDAs

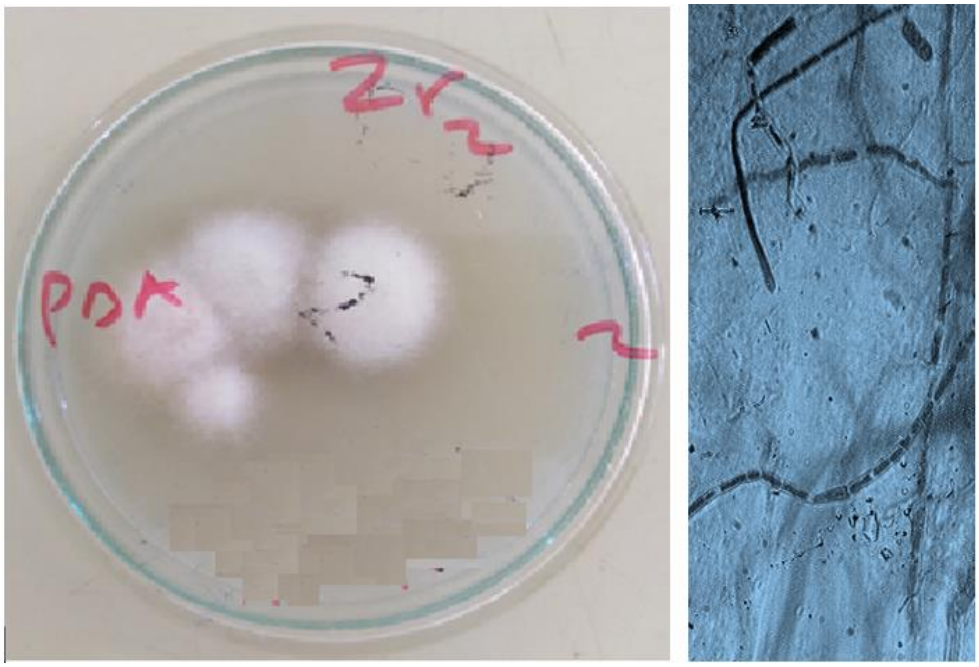

Plate 4: Seven-day old culture of *Gibberella fujikuroi*, mycelium, and conidium grown on PDAs

Table 1: Jatropha production practices adopted in surveyed fields

Plantation Location	Accession	Source of Seed	Spacing (m^2)	Est. Date	Plantation Size (ha)	Fertilizer Type	Fertilizer Time	Fertilizer Amount	Cropping Pattern	Pest management strategy adopted	Weed Control	Pesticide Used	Yield (t/ha)
Tsanni	Unknown	Abuja	3 x 4	2007	7.7	Poultry and cow dung manure	Once or twice a year	No quantification	Intercropped with some tree crops	None	Hoeing	None	2
Minjibir	Matsaya	I.A.R	5 x 7	2007	0.6	None	None	None	Sole cropping	None	None	None	Not collected
Samaru	Unknown	I.A.R	2 x 2	2006	4.2	N.P.K. 15-15-15	June-July	120 kg/ha	Sole cropping	Yes	Plowing	Fungicide	11.5

Table 2: CABI identification of fungi isolates inducing Jatropha dieback

S/No.	Code	Source of Sample	CABI No.	Identified Organism	Species Complex	EMBL/ Genebank Accession No.
1.	KN 1	Minjibir	505726	*Fusarium* clade VII	*Fusarium incarnatum-equiseti*	JX162358
2.	KT 1	Tsanni	505727	*Fusarium* clade VII	*Fusarium incarnatum-equiseti*	GQ505677
3.	KD 1	Zaria	505728	*Fusarium solani*	*Fusarium solani*	JX435199
4.	KD 2	Zaria	505729	*Gibberella fujikuroi*	*Gibberella fujikuroi*	AF160263
5.	KT 2	Tsanni	505730	*Fusarium* clade VII	*Fusarium incarnatum-equiseti*	GQ505677

Key:

KN 1: Kano Isolate 1

KT 1: Katsina Isolate 1

KD 1: Kaduna Isolate 1

KD 2: Kaduna Isolate 2

KT2:KatsinaIsolate2

Pathogenicity Test

All the seedlings inoculated with the three isolates from the three locations showed brown to black rot typical of dieback after 8 to 12 days of inoculation and took another 8 to 10 days to cover 50 % of the young seedling stem as shown in Plate 5 when compared with healthy seedling on Plate 6. It was also observed that *Fusarium solani* expressed disease symptoms 8 days after inoculation (DAI), *Fusarium* clade VI expressed symptoms 9 DAI, and *Gibberella fujikuroi* expressed symptoms 12 DAI. Disease symptom expression started with leaf yellowing, wilting, browning of stems, defoliation, stem rot, and split. Fungi re-isolated from the inoculated diseased tissues were culturally and morphologically similar to those described in 3.3 and thus, confirmed Koch postulation.

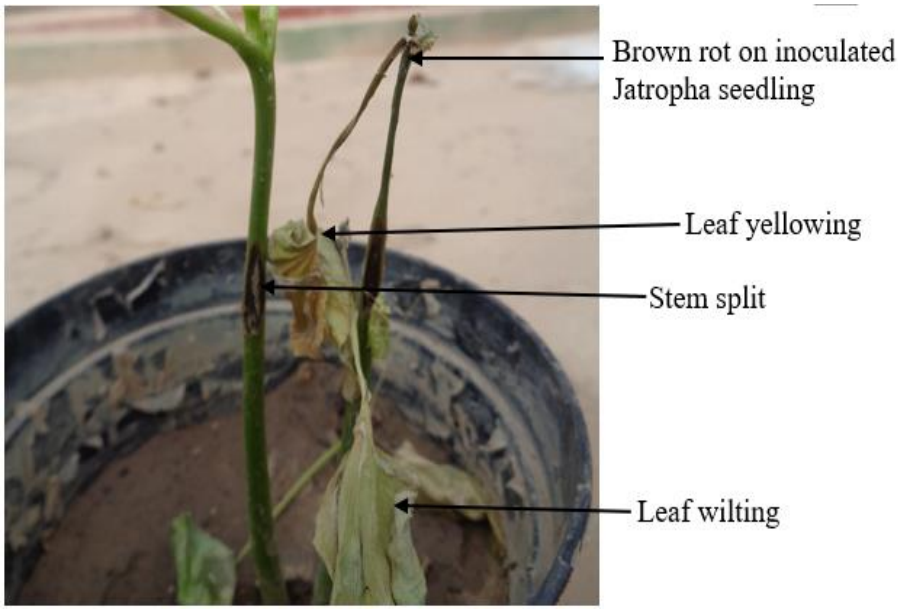

Plate 5: Jatropha seedling inoculated with *Fusarium solani* showing typical dieback symptoms at 17 days after inoculation

Plate 6: Un-inoculated healthy seedling of Jatropha (Control)

Polymerase Chain Reaction and DNA sequencing

The PCR result showed that the ITS region of fungal organisms isolated on Jatropha from Tsanni, Samaru, and Minjibir was amplified at ~550 bp. However, the TEF region was not amplified. The sequences of organisms such as *Fusarium verticillioides*, *Fusarium diversisporum*, *Fusarium chlamydosporum*, *Fusarium incarnatum*, *Fusarium equiseti*, *Fusarium lacertarum,* and *Fusarium fujikuroi* had homology with the sequences of fungi causing dieback on Jatropha (*Fusarium* clade VII) identified in Tsanni and Minjibir. *Fusarium solani* and *Gibberella fujikuroi* identified in Samaru had homology with *Fusarium solani* and *Fusarium pseudonygamai* respectively upon Blast search at NCBI database. A phylogenetic tree constructed grouped organisms with common homology in the same group as shown in Figure 3.

Gene sequence of the causal fungal pathogens of *Jatropha curcas* dieback was divided into various clades of *Fusarium* species when a phylogenetic tree was constructed using maximum likelihood. *Fusarium incarnatum-equiseti* isolated in Minjibir, Kano and Tsanni, Katsina States were under clade VII while *Fusarium solani* and *Gibberella fujikuroi* isolated in Samaru, Kaduna State was under clade III and V respectively when compared with gene sequences of *Fusarium* species in various clades (I-VII) as obtained from the NCBI database (Fig. 4).

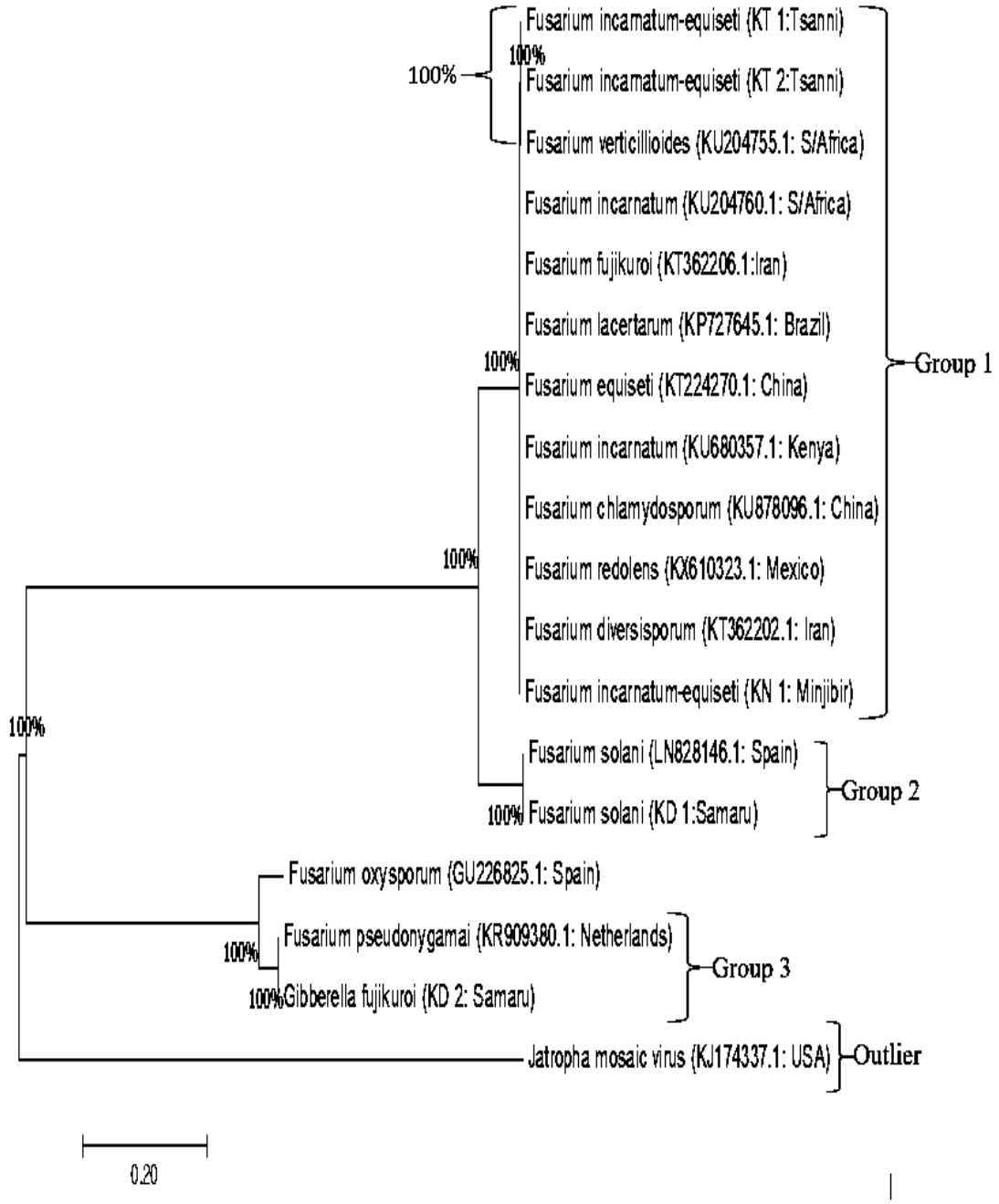

Figure 3: Phylogenetic tree of fungi that had homology with the identified causal organisms of Jatropha dieback in North West Nigeria upon BLAST search at NCBI database.

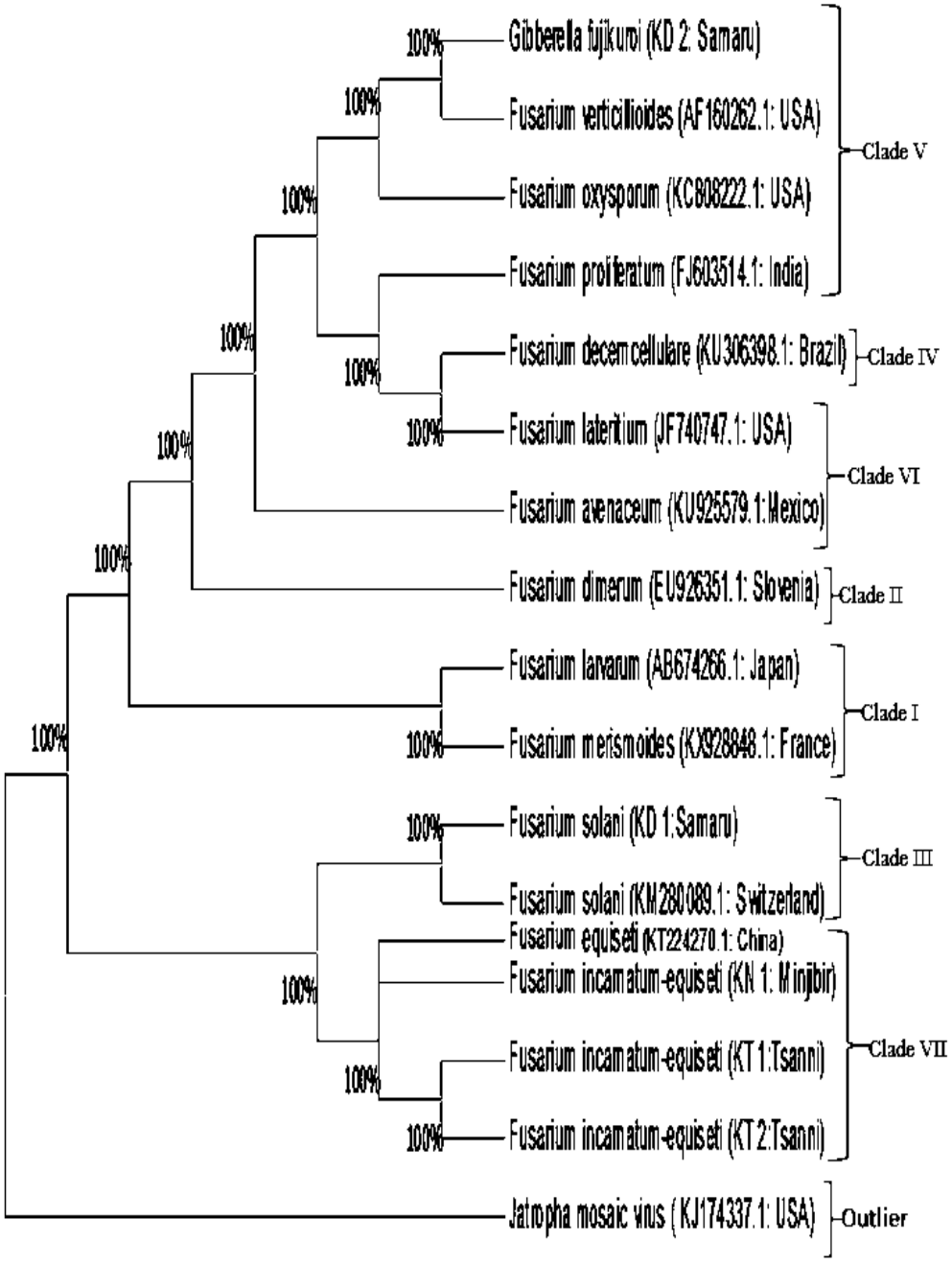

Figure 4: Phylogenetic tree of fungi causing Jatropha dieback when compared with members of various *Fusarium species* clades.

DISCUSSION

Jatropha dieback symptoms such as leaf wilting, defoliation, flower abortion, bark, and vascular tissue discoloration, twigs necrosis, and bark split recorded in all the study area were similar to those recorded by (Zarafi *et al*., 2012) who identified *Fusarium* as the causal organism of the Jatropha dieback disease in Samaru. Stem canker and dieback caused by *Lasiodiplodia theobromae* showed symptoms such as canker, vascular tissue discoloration, defoliation, leaf yellowing, and wilting and these are also similar to the ones recorded in the study area as cited by Machado and Pereira (2013). Ellison and others (Ellison *et al*., 2015) reported similar symptoms such as necrosis, leaf chlorosis, wilting, branch death, lodging, and cankers but were caused by *Colletotrichum truncatum* on *Jatropha curcas* in Burkina Faso. The first report of dieback disease at I.A.R. Samaru plantation in 2010 coincided with the report of a disease (Black rot of Jatropha) caused by *Botryosphaeria dothidea* that had similar symptoms at ICRISAT plantations in India (Rao *et al*., 2011). This implies that different fungi incite diseases manifested by very close symptoms on Jatropha across the globe. The reason for the differences in the causal organisms might be attributed to the differences in environmental factors, the virulence of the pathogens, susceptibility of the host, time taken for host-pathogen interaction, and the influence of man through cultural activities as evident in the disease tetrahedron (Mohan, 1996; Agrios, 2005; Burrows, 2013).

The disease incidence of 100 and 78.1 % recorded in Samaru and Tsanni is similar to that of Zarafi and Abdulkadir (2013) who recorded an incidence of between 60.8 % to 84.2 % in Samaru and Basawa. The result for incidence also agrees with the findings of Nasiru *et al.* (2015) who reported an incidence of between 67.6 % and 81.6 % as a result of leaf blight caused by *Phomopsis* and *Fusarium* species. The low incidence of 31.3 % recorded in Minjibir is similar to that of Nasiru *et al.* (2015) who reported an incidence of 33.3 % in Gangije (Kebbi) when leaf blight was assessed. The similarities in the incidence at these two locations (Minjibir and Gangije) might be as a result of similarities in ecology since they are all located in Sudan Savannah.

The high incidence and severity recorded in Samaru may be attributed to the fact that this plantation is located in the Northern Guinea Savannah (NGS) where there is high relative humidity, temperature, and rainfall which favor the development of fungal organisms. This agrees with the report of Marin *et al.* (1996) who reported that the germination of *Fusarium* species was influenced by water availability, temperature, and warm humid condition (Popovski and Celar, 2013).

The close spacing (2 x 2 m^2) also probably made the spread of the disease easier due to rain and wind splash that can easily move the spores to the neighboring plants. This corroborates the findings of Legard *et al.* (2000) who reported that close spacing in a strawberry plantation planted with Camarosa cultivar had the highest disease incidence of Botrytis fruit rot. The plantation in Tsanni is located in the Sahel Savannah (SS) and this location is characterized by low rainfall and relative humidity and high temperature. Even though the plants were widely spaced (3 x 4 m^2) but prolong drought (with an average rainfall of four months in a year) and poor fertilization may be attributed to the relatively high incidence of the disease. This corroborates the finding of Machado and Pereira (2013) who reported that the stem canker and dieback disease on Jatropha can be controlled by balanced fertilization and sufficient irrigation in areas with a prolonged drought.

The low disease incidence and severity recorded in Minjibir might be due to poor management, wide spacing (5 x 7 m^2), and poor establishment of Jatropha stands. This is contrary to the view that good management practices such as crop nutrition, use of clean seeds, field sanitation, and crop rotation as plant disease management options (Stephen, 1998).

Fusarium clade VII identified as the causal organism of Jatropha dieback agrees with the findings of Zarafi and Abdulkadir (2013) who reported that *Fusarium* sp. was the causal organism of Jatropha dieback in Samaru. Rao *et al.* (2011) also reported *F. oxysporum* as one of the organisms isolated on black rot infected stem even though *Botryosphaeria dothidea* was identified as the

causal organism of the disease and suggested that further investigation should be carried out to determine the role of the *F. oxysporum* in the disease. This is also similar to a report that identified *Fusarium species* as the causal organism of *Fusarium* wilt in Tanjung Raya Subdistrict, Tulang Bawang District of Indonesia (Ginting and Maryono, 2009). *F. oxysporum* also caused root rot on Jatropha (Hu *et al*., 2009), however, the research was only conducted on the stems, not the roots. A report showed that *F. oxysporum* was one of the pathogens that recorded the highest occurrence of 54 % when Jatropha diseased tissues were collected at Owerri (Nigeria) and fungal pathogens isolated, but they did not describe the disease symptoms (Ihejirika *et al*., 2014).

Fusarium solani was isolated in Samaru and members of the *Fusarium solani* species complex (FSSC) have been reported to cause many diseases on important crops. *Fusarium solani* and its teleomorph *Nectria haematococca were identified as the causal organisms of Jatropha root rot in China (Yue-Kai et al., 2011).* This finding agrees with the report that *Fusarium solani* was the causal organism of dieback on tea, mango, and red date, respectively (Khanzada *et al*., 2005; Mirzaee *et al.,* 2011; Kumhar *et al*., 2015). It was reported that *Fusarium solani and Septoria apii* were isolated on Jatropha leaves though they did not describe the disease and symptoms associated with the samples from which the pathogens were isolated (Ihejirika *et al*., 2014). Tiwari *et al.* (2012) also reported that *Fusarium solani was isolated on Jatropha seeds and therefore referred to it as a seed-borne fungus on the crop. Gibberella fujikuroi* was also isolated in Samaru and the anamorphic stage of *Gibberella fujikuroi* (*Fusarium moniliforme) has been associated with root rot of Jatropha (Sharma et al., 2001; Jongshaap et al., 2007).*

Based on the CABI report, a recent multigene reappraisal of the genus *Fusarium* by Watanabe *et al.* (42) recorded seven major clades that were robust across several genetic loci, including the rDNA gene cluster (incorporating the Internal Transcribed Spacer [ITS] regions) and translation elongation factor 1 alpha (TEF) whilst ITS is considered to be the DNA "barcode" of choice for fungi, in *Fusarium* the species delimitation with ITS is not ideal owing mainly to the presence of

non-orthologous copies of the ITS which can confound analyses. TEF offers better resolution and is used widely within the genus for species identification. However, Watanabe *et al.* (2011) showed that the composition of clade 7 was consistent across the gene trees even if the topology within the clade varied according to the locus used. This clade includes the *incarnatum-equiseti* species complex, *F. graminearum, F. culmorum,* and several other species.

Based on the phylogenetic tree generated in this study, KD 1 (*F. solani*) was categorized under clade III and this is in agreement with Watanabe *et al.* (2011) Classification of *Fusarium* species. KD 2 (*G. fujikuroi*) falls under clade V with *F. proliferatum*, *F. oxysporum,* and *F. verticillioides* as classified by Watanabe *et al.* (2011). Isolates from Tsanni and Minjibir (KT 1, KT 2, and KN 1) were categorized under clade VII as classified by Watanabe *et al.* (2011). The use of multi loci phylogenetic methods used in this study agrees with the findings that multilocus phylogenetic methods allow for the objective identification of species boundaries in the fungi and a great deal of species diversity when compared with morphological methods earlier used (Taylor *et al*., 2000; Ward *et al*., 2002; Geiser *et al.*, 2004).

It can be concluded that Jatropha dieback occurred in all the three locations surveyed in North West Nigeria and the high incidence and severity were recorded in Samaru which shows the need for urgent control measures. *Fusarium* and its anamorph *Gibberella fujikuroi* were identified and characterized as the causal organisms of Jatropha dieback in the surveyed locations. However, the TEF region for *Fusarium* clade VII was not found and that would have provided more detailed information about the fungus. Therefore, it is recommended that further studies should be extended to all parts of the country to ascertain the status of the disease, epidemiological studies on the infection cycle of the disease should be conducted and research on the influence of cropping pattern on incidence and severity of dieback be carried out.

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