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The Incidence and Severity of Jatropha Dieback Disease in Zaria, Nigeria

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Abstract: A survey was conducted on Jatropha plantations within Zaria to assess disease incidence and severity on 2 fields along Shika road and near the dam (Irrigation site) of the Institute for Agricultural Research (IAR) at Samaru and a plantation of the National Research Institute for Chemical Technology (NARICT) at Basawa. An assessment of incidence and severity of the disease was done on six accessions collected from four local government areas (Giwa, B/Gwari, Soba, Lere) of Kaduna State and grown in one of the IAR fields along Bomo road at Samaru. The accessions were collected from Gangara, Maidaro, Farin Gida (Giwa LGA), Birnin Gwari (B/Gwari LGA), Kayarda (Lere LGA) and Soba (Soba LGA). The causal organism of dieback on *Jaropha curcas* L. was isolated and its pathogenicity determined. The isolated *Fusarium* sp., incited die back/stem rot disease when inoculated on healthy Jatropha seedlings. The disease incidence was significantly higher on NARICT plantation compared to the 2 IAR fields which were not significantly different from each other. The accessions from Gangara, Kayarda, Birnin Gwari and Soba did not significantly differed from each other but had significantly higher disease incidence than those from Maidaro and Farin Gida. Accession from Soba had significantly higher disease severity compared to the accessions from all the other LGAs which did not differ statistically from each other.

Key words: Physic nut (*Jatropha curcas*), die back/stem rot disease, *Fusarium* sp., disease incidence, disease severity

INTRODUCTION

Jatropha is a drought resistant perennial plant, a native of Central America that can be grown in marginal soils. It grows relatively quick as fence or sole or intercropped plantation and can live for up to 50 years producing seeds. Jatropha a wonder plant is a deciduous shrub which belongs to the family Euphorbiaceae (Arbonnier, 2004).

The term Jatropha was derived from the Greek words (doctor) and trophe (food) which indicates its medicinal use (Heller, 1996). Recently cited *Jatropha curcas* was cited as one of the best candidate for future bio-fuel production whose oil can be combusted unrefined (Grimm, 1996; Heller, 1996; Gubitz *et al*., 1999; Goldman, 2007). Jatropha oil is also an efficient biofuel for the home (Takeda, 1982; Ishii and Takeuchi, 1987; Rockefeller Foundation, 1998). Jatropha seeds are made up of 18, 38, 17, 16 and 5% protein, fat, carbohydrates, fibre and ash, respectively and 50-60% of its oil is contained in the kernel (Salimon and Abdullah, 2008). Jatropha oil has been used commercially as a raw material for soap manufacturing for decades, both by large and small industrial producers (Rockefeller Foundation, 1998). Its rural-industrial usage and development include lubrication, soap, candle, furniture varnish and dyeing assistants making (Ochse, 1980; Tigere *et al*., 2006) and adulteration of olive and castor oil (Henning, 1997).

The oil is also widely used for skin diseases; to soothe rheumatism and to prevent constipation (List and Horhammer, 1979; Duke and Wain, 1981). The leaves extract is said to be curative against cough and an antiseptic after birth (Heller, 1996). The aqueous fruit extracts controlled schistosome vector snails (Rug *et al*., 1997). Twigs are used as a chewing stick in Nigeria (Isawumi, 1978). The sap flowing from the stem is used to arrest bleeding of wounds. Nath and Dutta (1992) demonstrated the wound-healing properties of curcain, a proteolytic enzyme isolated from its latex. The latex has antimicrobial properties against many micro organisms (Thomas, 1989). It has also been used in the treatment of high blood pressure, herpes, dermatitis; cancer and snake bite (Gill, 1992; Heller, 1996; Arbonnier, 2004). Its oil has been reported to control many insect pests on crops (Heller, 1996; Grainge and Ahmed, 1988); aqueous leaf extracts were found to be effective against *Sclerotium* sp., and *Azolla* sp., fungal plant pathogens (Garcia and Lawas, 1990). Pounded leaves are applied near horses' eyes to repel flies India (www.purdue.edu) and also used as tea against many diseases such as malaria in Nigeria (Yammama, 2010). The nuts are sometimes roasted and

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eaten (Watt and Breyer-Brandwijk, 1962). It has been used as a contraceptive in South Sudan (List and Horhammer, 1979). Seed husks can be used as domestic fuel generators. The root ashes are used as a salt substitute (Morton, 1981). Jatropha can be planted to serve as fields and homesteads boundary demarcations as well as erosion and desertification control (Yammama, 2010). The sap and bark of the plant produces a dark blue dye which is used for colouring cloth, fishing nets, lines and making of markers (Mitchell and Rook, 1979; Singh *et al*., 2007). It can also be used as a fish poison the latex strongly inhibits the watermelon mosaic virus (Tewari and Shukla, 1982). According to Agaceta *et al.* (1981), the latex can be used as a remedy for alopecia, anasorca, burns, dropsy, eczema, inflammation, paralysis and yellow fever as well as for wound healing in Nigeria. It also is used to treat various skin diseases and rheumatism (Heller, 1996). The emulsified oil has been found to be an effective insecticide against weevil pests and houseflies and an oil extract has been found to control cotton bollworm and sorghum stem borers (Shanker and Dhyani, 2006). Thomas (1989), Achten *et al.* (2008) and Oluma *et al.* (2008) reported the usage of jatropha extracts as an insecticide, molluscicide, fungicide and nematicide. Jatropha oil cake was reported to be used as domestic fuel, animal feed and organic manure (Makkar *et al*., 2001). In Nigeria, Jatropha is planted in the form of hedges around gardens or fields to protect the crops against roaming animals like cattle or goats as well as used as a shade for coffee, tomato, pepper, etc., nurseries, demarcate the boundaries of farmlands and house stead.

Currently, the whole world is faced with critical fuel shortages accompanied with high prices as well as the global warming issue. This has prompted governmental and Non-Governmental Organizations (NGOs) to search for alternative sources of energy which are renewable, safe and non-polluting and renewable vegetable fuels have assumed top-priority with physic nut whose fuel is the best being the most promising option (Grimm, 1996; Heller, 1996). In view of the importance of the crop and its potential role in world economy generally, Nigerian government embarked on a massive campaign to promote its production and utilization. The crop improvement research mandate of Jatropha was therefore given to Institute for Agricultural Research (IAR), Zaria while the oil extraction and processing research mandate was given to chemical technology research (NARICT), Zaria. As part of their mandate research, the two institutes established Jatropha plantations each. Also, due to the effect of fungal diseases on yield of crops, the incidence and severity of dieback disease commonly observed in these fields was assessed on both IAR and NARICT plantation and its causal agent isolated and identified.

MATERIALS AND METHODS

Pathogen isolation and pathogenicity test: Infected stems/branches of *Jatropha carcus* were collected from Institute for Agricultural Research (IAR) field. In the laboratory, stem portions with die back symptoms were cut into 2 cm pieces and surface sterilized for 5 min in 0.5% solution of sodium hypochlorite. The sterilized pieces were plated in 9 cm petri dishes containing Potato Dextrose Agar with Streptomycin (PDAS) after rinsing twice with sterile water. The plates were incubated at room temperature (28±2°C). Fungal outgrowth was sub-cultured in fresh media to obtain pure culture. The fungus isolated consistently was identified using identification manuals (Barnett and Hunter, 2006; Leslie and Summerell, 2006). Cultural and microscopic examinations of the isolated fungus were also carried out. The pathogenicity test of the fungus was also conducted on Jatropha. In the pathogenicity test, the Jatropha seeds were preconditioned before sowing as follows: The seeds were surface sterilized by soaking in 1% sodium hyphochloride for 5 min, rinsed thrice with Sterile Distilled Water (SDW), placed in folded jute bag which was earlier autoclaved autoclaved at 120°C and 15 bars for 3 h and allowed to cool. The sterile jute bag containing Jatropha seeds was watered daily with SDW to encourage germination aseptically. On the 8th day after conditioning, germinated seeds were transplanted in eight 20 cm diameter clay pots filled with heat sterilized soil in a glass house bench at a rate of 2 seedlings per pot and watered regularly with tap water. About 14 days old, pure culture grown on 3 petri dishes with PDAS was harvested, blended, filtered through a double layer muslin cloth and spore concentration determined using a heamocytomer. The spore suspension, adjusted to a concentration of Ca 30×10^3 mL^{-1} was spray inoculated on the leaf surfaces of Jatropha seedlings at 14 days after transplanting. Another set of seedlings were soil inoculated. The check seedlings (2 each) were spray and soil inoculated with Sterile Distilled Water (SDW), respectively. Humidity around the plants was maintained by covering the potted seedlings with polyethylene bags during the first 3 days with daily aeration. The plants were observed daily for symptom appearance.

Incidence and severity survey: A survey was conducted in the month of June, 2011 in order to determine the incidence and severity of die back disease in two Jatropha plantations of the Institute for Agricultural Research (IAR) and one plantation of the National Research Institute for Chemical Technology (NARICT) located in Samaru and Basawa areas of Zaria, respectively. In each

plantation, a total of 6 quadrants (Replicates) of 6×6 m were thrown, 1 each at the four corners of the field and 2 at the middle (Fig. 1) and disease assessment was done on plants within the quadrants. The quadrants (replicates) had a range of 8-9 plants depending on the plant establishment.

Disease incidence was determined by counting the number of diseased plants and expressed as a percentage of the total number of plants in each quadrant. Disease severity was assessed on individual plant by measuring the length of the diseased portion(s) of each diseased stem/branch and height of infected stem/branch and used to calculate the percentage of area covered by the disease as follows:

$$\text{Infected area}(\%) = \frac{\sum \text{of the size of infected area}}{\sum \text{of height of infected branches}} \times 100$$

The plants were categorized based on the percentage of infected area using a 1-5 rating scale where, 1 = No visible symptom; 2 = 1-15; 3 = 16-30; 4 = 31-50 and 5 = 51-100% of the stems/branch(es) area diseased (Tar, 1981).

Decease Severity (DS) was then calculated for each quadrant using the following formula:

$$DS = \frac{\sum \text{of disease rating}}{\text{Total number of plants} \times \text{Maximum grade}} \times 100$$

Similarly, the incidence and severity assessment on Jatropha accessions collected from four local government areas (Giwa, Lere, Soba and Birnin Gwari) within Kaduna state and grown in another field (Along Bomo road) also located in Samaru was conducted. In this field, each accession was grown a 20 m row and for each accession plants within the first 1 m at the beginning, 10 m into the field (Middle) and the last metre of the row were used for data collection representing 3 replicates. Data collected were subjected to ANOVA using SAS program version 9 and means were separated at 5% level of significance using Least Significant Degree of variance (LSD).

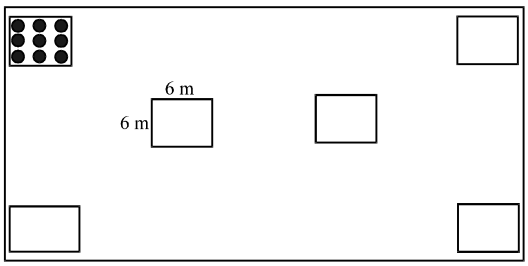

Fig. 1: A sketch showing how the quadrants were made in the plantations

RESULTS AND DISCUSSION

The branches collected for this study showed typical dieback symptoms which included discolouration and darkening of bark, vascular tissues colouration, wilting, defoliation and/or flower abortion, necrosis, bark splitting, gradual killing of stem and stem breakage (Fig. 2-6).

The most commonly organism isolated from Jatropha stems showing dieback symptoms when cultured on PDAs, initially appeared as a white floppy culture which later turned pinkish-brown colour under a whitish growth from the top of the plate and had black-brown background colouration (Fig. 7). It took the organism 10 days to cover a 9 cm petri dish at 28±2°C. The spores under a light microscope were canoe shaped with 3-5 septae

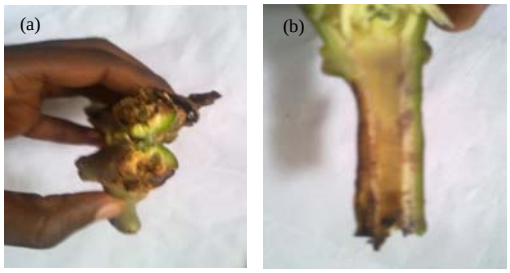

Fig. 2: a) Transverse section; b) Longitudinal section of diseased stem showing discolouration

Fig. 3: Dieback symptoms: a) Bark blackening; b) Gradual top to bottom stem dying; c) Completely killed stems

Fig. 4: Defoliated infected stem

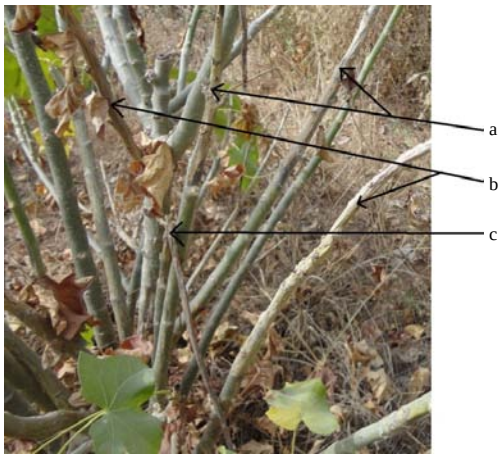

Fig. 5: Severely infected plant: a) Gradual stem drying; b) Completely dead stems; c) Stem breaking

Fig. 6: Bark splitting of diseased stem/branch

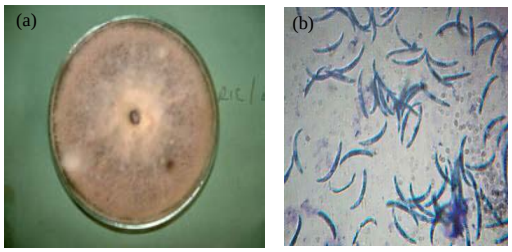

Fig. 7: About 14 days old: a) Pure culture; b) Spores of *Fusarium* sp.

(Fig. 7). Using these criteria and identification manuals, the fungus was identified as *Fusarium* sp. (Barnett and Hunter, 2006; Leslie and Summerell, 2006).

The isolated *Fusarium* sp., when inoculated on healthy young Jatropha seedlings induced black lesion on the stem and brown blight on leaf (Typical dieback symptom) (Fig. 8). Symptoms were first observed 10 days after inoculation. From the artificially inoculated infected

Fig. 8: Jatropha leaf and stem artificially inoculated with *Fusarium* sp.

Table1: Disease incidence and severity among plantations in Samaru, Zaria.

Field	Location	Incidence (%)	Severity (%)
NARICT	Basawa	84.2[a]	71.2[a]
IAR Irrigation site	Samaru	70.0[b]	60.2[b]
IAR Shika	Samaru	60.8[b]	52.8[b]

In each column values carrying the same latter(s) are not significantly different at p = 0.05

stems and leaves, the organisms re-isolated had cultural behaviours, conidial shape and septation similar to the original organism used, thus confirming that *Fusarium* sp., is the causal organism of Jatropha dieback according to Koch postulate. The finding that the dieback disease on jatropha was caused by *Fusarium* sp., is similar to a report of Dudley *et al.* (2007) who researched on Koa wilt/dieback and confirmed that the disease was caused by *Fusarium oxysporum* f.sp., *koae.* Rajput *et al.* (2008) also reported that plants inoculated with *Fusarium solani* either alone or in combination with *Rhizoctonia solani* or *Curvularia lunata* showed typical dieback symptoms. Shakya and Lakhey (2006) in a separate research reported that *Fusarium solani* was the causal agent of dieback disease of *Dalbrgia sissoo*. Mango die back which caused discoloration and darkening of bark, leaf shedding, drying of twig from top to bottom, slow drying and complete killing and splitting of diseased twig was reported to be incited by *Fusarium equiesti* (Rockfeller and Foundation, 1998). Both the soil and smear inoculation methods were able to cause infection and a similar result was reported by Rajput *et al.* (2008) who inoculated seedlings with *Fusarium* sp. and such seedlings showed typical dieback symptoms with yellowing of leaves and internal browning of stem. Mamza *et al.* (2008) induced stem rot and leaf blight on castor seedlings with *F. pallidoroseum* using spray, smear and soil inoculation methods.

The disease incidence and severity recorded in NARICT plantation at Basawa were significantly higher compared to the 2 IAR plantations at Samaru (Table 1). The variation on the die back disease incidence and severity obtained in this fields survey could be as a

Table 2: Disease incidence and severity among accessions collected within Kaduna State and grown on field along Bomo road, Samaru, Zaria

Sources	LGA	Incidence (%)	Severity (%)
Gangara	Giwa	83.3[a]	66.7[b]
Birnin Gwari	B/Gwari	83.3[a]	60.0[b]
Kayarda	Lere	83.3[a]	66.7[b]
Soba	Soba	83.3[a]	80.0[a]
Farin Gida	Giwa	66.7[b]	56.7[b]
Maidaro	Giwa	66.7[b]	63.3[b]

In each column values carrying the same latter(s) are not significantly different at p = 0.05

result of genetic differences. The NARICT plantation was established with exotic seeds obtained from Israel (Personal comm with NARICT farm manager) while IAR used indigenous seeds obtained locally from Bomo village near the institute (Personal comm with staff member). Those seedlings used by IAR are likely to have adopted the area since they are locally obtained and the contrary to NARICT field.

The incidence of dieback disease recorded on accessions from Gangara, Birnin Gwari Kayarda and Soba in Giwa, B/Gwari, Lere and Soba LGAs, respectively did not differ from each other but recorded significantly higher disease incidence than accessions from Farin Gida and Maidaro both in Giwa LGA which did not also differ statistically from each other (Table 2).

CONCLUSION

Disease severities recorded on all accessions from all the locations were statistically the same except the accession from Soba which had significantly higher (80.0%) disease severity.

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