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Research Article

Colletotrichum Leaf Blight of Jatropha curcas in Sokoto State, Nigeria

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Research on occurrence, incidence and identification of Colletotrichum blight of *Jatropha curcas* was conducted in Sokoto State of Nigeria. In the survey, diseased leaves were collected from the selected locations in each of the selected Agricultural zones of Sokoto state. Fungal pathogen identified and found to be responsible for the leaf blight in the study areas was *Colletotrichum* spp. Spores count was made (52328125spores/ml) and pathogenicity trial conducted in the glass house to confirm Kochs postulate. Results from the farmers' field revealed that Tambuwal Agricultural Zone had the highest incidence and severity of Colletotrichum leaf blight (75.67%). The survey also showed that *J. curcas* planted in Fadama areas tend to be more prone to the leaf blight particularly those close to water source like streams. It is recommended that; further investigation should be conducted to identify effective fungicides for the management of Colletotrichum foliar blight of *Jatropha curcas.*

Key words: - Incidence, Severity, Colletotrichum, Leaf blight and *Jatropha curcas*

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INTRODUCTION

Jatropha curcas is a species of flowering plant in the spurge family, Euphorbiaceae, that is native to the Americantropics, most likely Mexico and Central America (Janick and Robert, 2008). It is cultivated in tropical and subtropical regions around the world, becoming naturalized in some areas. Common names include Barbados nut, purging nut, physic nut, or JCL (abbreviation of *Jatropha curcas* Linnaeus). *J. curcas* is a poisonous, semi-evergreenshrub or small tree, reaching a height of 6 m (20 ft), it is resistant to a high degree of aridity, allowing it to be grown in deserts (Janick and Robert, 2008).

The seeds contain 27-40% oil (average 34.4%) that can be processed to produce a high-quality biodiesel fuel, usable in a standard diesel engine (Joshua, 2010). When jatropha seeds are crushed, the resulting jatropha oil can be processed to produce a high-quality biofuel or biodiesel tested successfully as fuel for simple diesel engine that can be used in a standard diesel car or further processed into jet fuel (Achten *et al*., 2008). Dar (2007) added that, oil can be combusted as fuel without being refined, it burns with clear smoke-free flame, while the residue (press cake) can also be used as biomass feedstock to power electricity plants, used as fertilizer (it contains nitrogen, phosphorus and potassium), or as animal fodder, also cake can be used as feed in digesters and gasifiers to produce biogas.

Estimates of *Jatropha* seed yield vary widely, due to a lack of research data, the genetic diversity of the crop, the range of environments in which it is grown, and *Jatropha*'s perennial life cycle. Seed yields under cultivation can range from 1,500 to 2,000 kilograms per hectare, corresponding to extractable oil yields of 540 to 680 litres per hectare (58 to 73 US gallons per acre). In 2009 *Time* magazine cited the potential for as much as 1,600 gallons of diesel fuel per acre per year. A hectare of jatropha has been claimed to produce 1,892 litres of fuel (Janick and Robert, 2008).

McGraw (2003) stated that, it is cultivated in tropical and subtropical regions around the world, *Jatropha curcas* grows almost anywhere, even on gravelly, sandy and saline soils, it can thrive on the poorest stony soil, it can also grow even in the crevices of rocks. The leaves shed during the winter months form mulch around the base of the plant. The organic matter from shed leaves enhances earth-worm activity in the soil around the root-zone of the plants, which improves the fertility of the soil. It grows in a number of climatic zones including areas of rainfall and problem sites. *Jatropha curcas* is easy to establish, grows relatively quickly and is hardy. Being drought tolerant, it can be used to reclaim eroded areas, be grown as a boundary fence or live hedge in the arid/semi arid areas (Anon. 1998).

Jatropha is not browsed, for its leaves and stems are toxic to animals, but after treatment, the seeds or seed cake could be used as an animal feed. Being rich in nitrogen, the seed cake is an excellent source of plant nutrients. Various parts of the plant are of medicinal value; its bark contains tannin, the flowers attract bees and thus the plant has honey production potential (McGraw, 2003).

Leaf blight, a disease caused by fungal pathogens results in the destruction of leaf tissue of which photosynthesis is reduced, this affects the growth and development of the Jatropha plant and subsequently its potentials, other diseases like root rot, caused by *Fusarium moniliforme,* causes wilt and death of Jatropha in waterlogged condition, and diseases like leaf spot, rusts, collar rot can also damage and kill *Jatropha curcas*.

MATERIALS AND METHOD

Study Area

The research was conducted in three Agricultural zones, (Tambuwal Agricultural Zone, Sokoto Agricultural Zone and Isa Agricultural Zone) identified to have high population of *Jatropha curcas* growing in the wild. The areas exhibit tropical dry climate where rainfall is 550 to 650mm recorded for only three to four months (June – September) while the rest of the year is dry and hot (Anon. 2010; UNO, 2004). According to Nigerian Meteorological Service Report (2009), mean maximum temperature ranges from 25^{o}C to 43^{o}C and mean minimum temperature is between 19^{o}C and 24^{o}C. The area falls within the Sudan savanna characterized by short grasses and scattered trees (Anon. 2011).

Sampling Technique

Based on the list of accessions obtained from Institute for Agricultural Research (IAR) Zaria, a Multi-stage Purposive sampling was employed to select village locations with high population of *Jatropha curcas* in three Agricultural zones of Sokoto states, Nigeria. These were Tambuwal, Sokoto and Isa Agricultural Zones. Each zone has 6-7 local government Areas. Bodinga, Yabo, Shagari, Tambuwal and Kebbe were the local government selected in Tambuwal Agricultural Zone, Wamakko, Sokoto North, Kware, Binji and Silame was also selected from Sokoto Agricultural Zone, while Rabah, Wurna, Isa, Sabon-Birni and Goronyo were the local government selected from Isa Agricultural Zone.

Disease Sample Collection

Occurrence of foliage diseases of *Jatropha curcas* was examined in the selected areas, and disease incidence and severity were determined. Diseased leaves were randomly collected and labelled from the sampled areas in line with Timothy *et al.* (1999) procedure.

Assessment of Incidence and Severity of Diseases of *Jatropha curcas*

Disease incidence is the number of plant units infected, expressed as a percentage of the total number of units assessed as follows;

$$\text{Disease incidence (I)} = \frac{\text{Number of infected plant units}}{\text{Total number (healthy and infected) of units assessed}} \times 100$$

Disease severity scores, on the other hand were obtained using a scale of 1-5 adapted from Zelalem *et al.* (2012), Ghose *et al.* (2010), Ghosh *et al.* (2009) and Tarr (1981) where:

1. No symptoms on leaves
2. 1-25% number of leaves diseased
3. 26-50% number of leaves diseased
4. 51-75% number of leaves diseased
5. 76% or more number of leaves diseased

Disease severity percentage will be determined using the formula (Ghosh *et al.,* 2009) thus;

$$\text{Disease severity index} = \frac{\text{Sum of all leaves disease rating}}{\text{Total number of leaves examined x maximum rating}} \times 100$$

Preparation of Media (Potato Dextrose Ager)

Two hundred grams of peeled slices of Irish potato tubers were boiled in one litre of water. Solution was filtered through doubled layer muslin cloth. Dextrose (20g) and agar-agar powder (15g) were added to the filtrate; volume was made to one litre and reboiled to dissolve

agar-agar (homogenized) through stirring. The solution (media) was poured into conical flask and autoclaved at pressure 15psi. Streptomycin sulphate was prepared by diluting 5g with 10ml sterile distilled water and added into the media when it has cooled. The media was allowed to cool in the pouring room.

Isolation and Identification of Fungal Pathogens

Diseased *J. curcas* leaves collected from the study area were sorted based on symptoms and taken to the pathology laboratory (Department of Crop Protection, I.A.R., Zaria) for isolation. The infected leaves were washed with distilled water, cut into pieces and sterilized for five minutes using 0.5% sodium hypochlorite and rinsed thrice with sterile distilled water and blotted dry with a sterile filter paper (Rizvi and Yang, 1996). The pieces were then placed onto a 90 mm (diameter) Petri dishes containing freshly prepared Potato Dextrose Ager with streptomycin (PDAs) and labelled. The plates were incubated at 27^{o}C and observed daily (Ndiaye, 2007). Fungal mycelia of the isolated organisms were sub-cultured on fresh PDAs to obtain pure cultures. The cultural characteristics of the isolates were noted and detailed microscopic characteristics (morphological) were observed. The fungi were identified using Identification Manual of Barnett and Hunter (2006), further identification to species level was made by CAB International (United Kingdom). The pure cultures of the isolated fungal pathogens were preserved in McCartney bottles containing PDAs in a slanting position for inoculation.

Pathogenicity Trial in the Screen house

Certifiedseeds of *J. curcas*were obtained from Institute for Agricultural Research (IAR), ABU Zaria. The seeds were soaked in Sodium hypochlorite for five minutes then washed with sterile water; again, they were washed with 20 ml of alcohol and rinsed with sterile water to ensure safety against dust and other pathogens that may be present in the surface. Thirty-nine clay pots with diameter and depth of 25 cm and 24 cm respectively were washed, filled with heat sterilized soil and watered. Two seeds were sown in each pot and watered for 28 days under aseptic condition to prevent contamination. The seedlings were later thinned to one.

Inocula Preparation and Inoculation of Seedlings in Glasshouse for Pathogenicity Trial

Spore suspensions of the fungal pathogen used as inocula for *in vivo* bioassay were prepared as follows;

At 14 days after inoculation, cultures of the isolates on PDAs from control were harvested through scraping the Mycelia mat using sterile scalpel to determine the spore concentration. The harvested Mycelia was placed in a 250ml beaker, blended in 80ml distilled water, using Binatone 5 speed Turbo Blender (model number; HM - 350S), it was then filtered through double layer muslin cloth. The remaining 20ml of the distilled water was used to rinse the blender and beaker used. A sterile pipette was used to collect 0.1ml of the suspension and placed on the surface of the counting chamber of haemocytometer and covered with a cover slip. The suspension was left for 15 – 20 seconds to allow conidia to settle. Numbers of conidia were counted from square grids in the counting unit of the haemocytometer under electrically powered binocular microscope at x400 magnification. Same procedure was repeated four times per treatment. Spore concentration was calculated using the formula adopted by Marley (2013);

$$C = \frac{n}{256} \; 4 \times 10^{6}$$

Where:
C = number of conidia per millilitre
n = number of conidia counted in the chamber
256 = constant volume obtained from 16 x 16 square grids
4×10^{6} = constant

Spores Count = 52328125spores/ml

Seedlings were inoculated outside the glass house to avoid contamination using three methods viz; smear, spray and soil inoculations. Smearing was conducted through direct application of harvested mat on leaves using finger tips fitted with gloves. The soil inoculation was made by pouring 20 ml of inocula suspension at the base of stem (root collar) of each plant. Inoculation through spray was carried out using hand atomizer to spray on the leaves. Seedlings for control were treated with sterile distilled water only. One seedling per pot and three pots for each inoculation method for the isolated pathogen were labelled and arranged in completely randomized design. After the inoculation, seedlings were covered with wet polythene bags to increase humidity around the plants. After 24 hours, the polythene bags were removed for 20 minutes to aerate the seedlings and were re-wet before replacement for another 24 hours and finally removed. Seedlings were watered in the morning and evening. Leaves showing disease symptoms were collected and taken to laboratory for re-isolation of the organism and compared with original preserved culture to confirm Koch's postulates.

Statistical Analysis

The data collected were subjected to analysis of variance (ANOVA) procedure using SAS (2012) software. Significant difference among the treatment means were separated using Duncan Multiple Range Test (DMRT).

RESULTS

Incidence and Severity of Colletotrichum Blight on Leaves of *Jatropha curcas* according to the Zones surveyed.

Incidence and Severity of Colletotrichum Blight on the Leaves of *Jatropha curcas* as Influenced by Zones is presented in Table 1. Colletotrichum blight incidence was found to be significant across the Zones with Tambuwal Agricultural Zone having the highest value (70.44%) which was followed by Sokoto Agricultural Zone (42.89%). Isa Agricultural Zone recorded zero value.
The severity of Colletotrichum blight was highest in Tambuwal Agricultural Zone (53.33%) followed by that of Sokoto Agricultural Zone (31.11%) while Isa Agricultural Zone recorded zero value.

Table 1. Incidence and Severity of Colletotrichum Blight on Leaves of *Jatropha curcas* in Three Agricultural Zones.

States	Incidence (%)	Severity (%)
Sokoto Agricultural Zone	42.89±32.98b	31.11±24.72b
Tambuwal Agricultural Zone	70.44±9.33a	53.33±10.00a
Isa Agricultural Zone	0.00±0.00c	0.00±0.00c
Significance	*	*

Means followed by the same letter(s) do not differ significantly according to Duncan Multiple Range Test (DMRT) at 5 % level of significance.

Morphological and Microscopic Features of *Colletotrichum spp* Isolate Associated with Leave Blight of *Jatropha curcas*

The acervuli are disc shaped or moon shaped, single celled, waxy with dark underground colour, with setae that carries the spores, the spore is hyaline with milky white fluffy mycelia growth with ring shape on it (Plate 1).

Plate 1: An Isolate of *Colletotrichum* species
Determination of the Pathogenicity of the Isolated Organisms
Note: Arrows in each of the plates indicate Colletotrichum leaf blight symptom

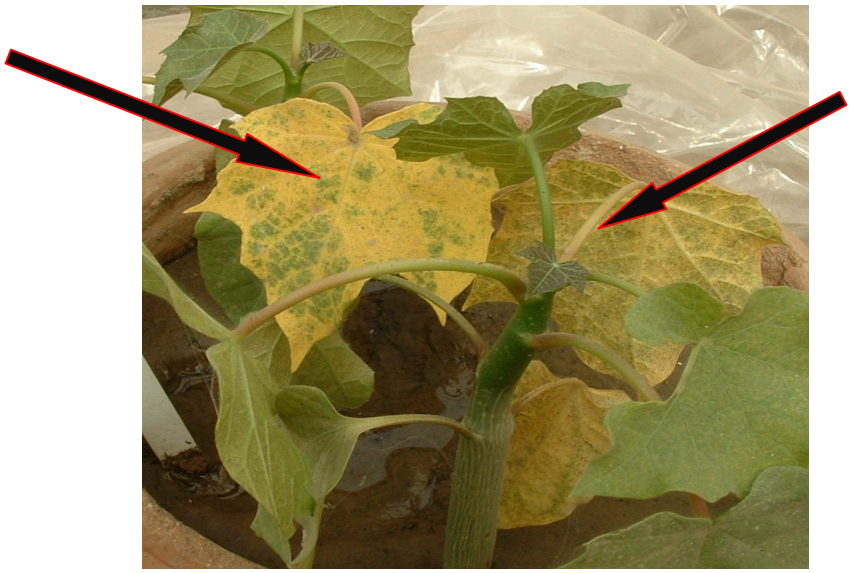

Plate 2: Colletotrichum Leaf Blight on Seedling of *Jatropha curcas* at 12 days after Inoculation through Spray

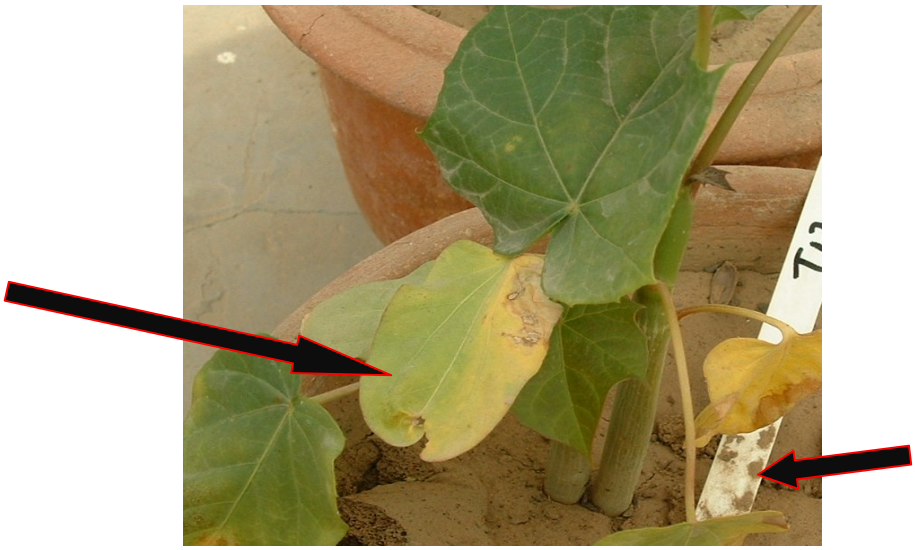

Plate 3: Colletotrichum Leaf Blight on Seedling of *Jatropha curcas* at 13 days after Inoculation through Soil

DISCUSSION

Most of the leaves of *J.* curcas in the study area were found to be plagued with one form of pathogenic fungi or the other. This is in line with the opinion of Robert *et al.,* (2006) that the largest group of plant pathogens is the fungi, which could be because fungi are disseminated primarily in the form of spores. Spore dissemination in almost all fungi is passive, although the initial discharge of spores in some fungi is forcible. Agrios (2004) stressed that wind is probably the most important disseminating agent of spores of most fungi and may carry spores over great distances. Also, for specific fungi, other agents such as water or insects may play a much more important role than wind in the dissemination of their spores.

Highest incidence and severity of Colletotrichum blight on leaves of *Jatropha curcas* was obtained in study areas close to stream or Fadama in Tambuwal and Sokoto zones and none was recorded in study areas of Isa Zone, probably because they were located upland, this confirms that water plays an important role in life cycle of *Colletotrichum* spp. This tally with the findings of Prusky *et al.* (2000) who reported that fungus is favoured by high temperature and humid or moist weather, they added that conidia are released and spread only when the acervuli are wet and are generally spread by splashing and blowing rain or by coming in contact with insects, other animals, and tools. Agrios (2004) stressed that, conidia germinate only in the presence of water and penetrate the host tissues directly.

CONCLUSION

Most of the leaves of *Jatropha curcas* studied were infected with fungal leaf blight. Tambuwal Agricultural Zone had the highest incidence and severity of fungal folia blight caused by Colletotrichum. The survey also showed that *J. curcas* planted in Fadama areas tend to be more prone to the leaf blight particularly those close to water source like streams. It is recommended that; further investigation should be conducted to identify effective fungicides for the management of Colletotrichum foliar blight of *Jatropha curcas.*

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