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Studies of Curvularia and Colletotrichum Foliar Diseases of *jatropha curcas l.* In Some North-West States of Nigeria

[1]A. M. Nasiru [2] O. O. Banwo [1]A. D. Isah and [3]S. Muhammad

[1]Department of Forestry and Environmental studies, Usmanu Danfodiyo University, Sokoto, Nigeria.
[2]Department of Crop Protection, Ahmadu Bello University, Zaria, Nigeria.
[3]Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria

*Corresponding author: A. M. Nasiru, Tel: +234(0)8034566086, E-mail: amnafad597@gmail.com

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ABSTRACT

A study on foliage disease of Jatropha curcas was conducted in Semi–Arid region of North-Western States (Sokoto, Kebbi and Zamfara) of Nigeria to determine the incidence and severity of fungal foliar diseases. Leaves with disease symptoms were collected from the selected locations in each of the states studied and taken to pathology laboratory at Department of Crop Protection (ABU, Zaria) for identification. *Curvularia* and *Colletotrichum* species were the fungal pathogens identified and found to be responsible for leaf blight disease on the leaves of *Jatropha curcas* in the study area. A spore count of the isolates was made with the aid of haemocytometer and used as inocula in the pathogenicity trial in glasshouse to prove Kochs' postulate. Results from the farmers' field revealed that, highest incidence of Curvularia leaf blight was recorded in Tsaki of Sokoto State with 53.33% and a severity of 33.33%. Colletotrichum leaf blight on *Jatropha curcas* was observed to have higher incidence value of 75.67% in Janbaki of Kebbi State when compared with other States. The survey conducted showed that, *Jatropha curcas* planted in lowland areas tend to be more prone to the fungal leaf blight infection particularly those close to water source like streams. In the pathogenicity trial, results indicated that, there was no significant difference with respect to the methods of inoculation (smear, spray and soil inoculation). Number of days after inoculation in relation to fungal count (appearance) on leaves of the seedlings showed no statistical difference. As part of the recommendations, second field survey should be conducted in the study areas to further determine the incidence and severity of fungal leaf blight on *Jatropha curcas*, similarly, to enable the selection of appropriate fungicides for the control of leaf blight and pathogens responsible for the disease should be identified to species level. In vitro and In vivo evaluations of the selected fungicides should also be conducted to ascertain the most effective among them.

Key words: Foliar diseases, Jatropha, Curvularia and Colletotrichum

INTRODUCTION

The name Jatropha is derived from the Greek words (jatros), meaning "physician", and (trophe) meaning "nutrition", hence the common name physic nut [1]. *Jatropha* is a genus of approximately 175 succulent plants, shrubs and trees (some are deciduous, like *Jatropha curcas*) from the family *Euphorbiaceae*. There are several different species of *Jatropha* which are commonly grown in gardens or as ornamental plants. Many of these *Jatropha* species produce very colourful flowers and are huge butterfly and bee attractants. Examples include *Jatropha integerrina, J. cardiophylla, J. catharlica, J. cinerea, J. cuneata, J. podagrica* etc. It is a species of flowery plant and native to the American tropics, most likely Mexico and Central America [2]. The specific name, *curcas* was first used by Portuguese doctor Garcia de Orta more than 400 years ago and is of uncertain origin [1]. It is cultivated in tropical and subtropical regions around the world, becoming naturalized in some areas. 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 [3].

phytopathometry is the most important aspect in any crop loss programme because it is the process that generates data to quantify disease progress, three parameters used for the assessment or measurement of plant diseases are; disease incidence, disease severity and crop yield loss [4]. Disease incidence is the simplest method of assessment which basically involves the counting of the number of diseased plants and expressed as a percentage, while disease severity is assessed by estimating the proportion of total photosynthetic area that is diseased. In other words, disease severity is a quantitative trait which measures the amount of disease on a plant in terms of intensity of symptoms or damage. Among the most important challenges facing man and his environment today is global warming which is attributed to excessive exhausts from combustions in vehicles, companies, industries, households etc. Among the effects of global warming are drought, flood, erratic rainfall, uncondusive atmosphere to man and other living organisms due to high rate of radiation etc. The full potential of *Jatropha curcas* is far from being realized particularly in Nigeria because of several reasons as pests and diseases, technical, economic, cultural and institutional etc which need further discussion and examination. The growing and

management of *Jatropha curcas*, either on private, public or community land is poorly documented and there is little field experience that is being shared especially in Nigeria where it is still grown wild.

The aims of this study is to isolate and identify the foliar diseases of *Jatropha curcas*, Determine incidence and severity of fungal foliar diseases on *Jatropha curcas* in farmers' field and pathogenicity of the isolated organisms

MATERIALS AND METHOD

Study Area

The study was conducted in some parts of Sokoto, Kebbi and Zamfara identified to have high population of *Jatropha curcas* growing in the wild. The area lies in north-west part of Nigeria between latitudes of $10^0 2^1$ N and $13^o 9^1$N and longitudes $3^o 7^1$ E and $7^o 2^1$ E (UNO, 2004). The areas exhibit tropical dry climate where rainfall is 550-650mm recorded for only three to four months (June – September) while the rest of the year is dry and hot (Anon. 2010). According to Nigerian Metrological Service Report (2009), mean maximum temperature ranges from 25°C to 43°C and mean minimum temperature is between 19°C and 24°C. The area falls within the Sudan savanna characterized by short grasses and scattered thorny short trees [5].

Sampling Technique

A purposive and Multi-stage sampling was employed to identify the Local Governments Areas and villages in North-West States of Nigeria with high population of *Jatropha curcas* from the list of assertions obtained from the Institute of Agricultural Research (IAR) ABU, Zaria. Tsaki, Kajiji and Barkeji in Kware, Shagari and Tambuwal Local Government Areas respectively were selected from Sokoto State, while in Zamfara State, Wanke, Nasarawar wanke in Gusau Local Government Area and Yartukunya in Bungudu Local Government Area were selected. In Kebbi State were Basaura (Janbaki), Jega Birni (Gadar Ruwan Kanwa) in Jega Local Government Area and Gangije in Aliero Local Government Area were selected.

Disease Sample Collection

Occurrence of foliage diseases of *Jatropha curcas* was determined in the selected areas, and disease incidence and severity were determined. Ten diseased leaf samples of *Jatropha curcas* were randomly collected and labelled from three different sites in each of the locations in line with procedure and preserved for isolation [6] in the Pathology Laboratory, Department of Crop Protection, ABU, Zaria

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;

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

While, Disease severity scores were obtained using 1-5 scale adapted [7,8,9] where;

- ❖ No symptoms on leaves
- ❖ 1-25% number of leaves diseased
- ❖ 26-50% number of leaves diseased
- ❖ 51-75% number of leaves diseased
- ❖ 76% or more number of leaves diseased

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

Isolation and Identification of Pathogens

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. The pieces were then placed in 90 mm (diameter) petridishes containing Potato Dextrose Ager with streptomycin (PDAs) and labelled. The plates were incubated at 28°C for 7 – 10 days. Fungal mycelia of the isolated organisms were sub-cultured on fresh PDAs to get pure culture. The cultural characteristics were noted and detailed microscopic characteristics (morphological) were observed. The fungi were identified using Identification Manual [10]. The isolated fungi were preserved in Marcoteny bottles containing PDAs in a slanting position before the time of inoculation.

Pathogenicity Trial in the Screenhouse

Certified seeds of *Jatropha 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

The preserved pure cultures of the isolated pathogens were grown on PDAs in the laboratory until they sporulated. 10 ml of sterilized distilled water was added to each petridish and grown mycelia mat from the culture was harvested using a sterile scalpel. The mycelia were blended in an electric blender for five minutes, 200 ml of sterile distilled water was added in 500 ml conical flask and filtered using a double layer muslin cloth. Spores count was made using haemocytometer and compound microscope using the equation;

$$\text{Spores/ml} = (n) \times 10^4$$

Where: n = the average cell count per square of the four corner squares counted.

Jatropha 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 four isolated pathogens were labelled and arranged in completely randomized design. After the inoculation, seedlings were covered with wet polythene bags to increase humidity. 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. Numbers of days as it affects blight symptoms appearance on the leaves of Jatropha seedlings with respect to the isolates were determined through counting the number of infected leaves at seven days. Effect of the methods of inoculation as it affects the symptoms appearance was also evaluated.

Leaves showing blight symptoms were collected and taken to laboratory for re-isolation and subculture to obtain pure culture of the organism and then 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 in the treatment means were separated using Duncan Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Incidence and Severity of Fungal Blight on Leaves of *Jatropha curcas*

Table 1 shows the incidence and severity of Curvularia and Colletotrichum leaf blights on the leaves of *Jatropha curcas* in the study area. Tsaki in Sokoto State recorded the highest incidence of Curvularia blight (53.33%). This was followed by Janbaki in Kebbi State with 43.33 and Kajiji in Sokoto State with 41.67%, Barkeji in Sokoto State (33.33%) which were statistically at par with each other but significantly higher than what was obtained from Gadar Ruwan Kanwa (11.67%) in Kebbi State. Similarly, Tsaki recorded the highest severity (33.33%) of Curvularia blight. This was higher than what was obtained from Kajiji (26.67%), Barkeji (20.00%), Yartukunya (20.00%), Janbaki (20.00%), Gangije (13.33%) and Wanke (13.33%) which were all statistically similar. Gadar Ruwan Kanwa recorded statistically the lowest value (6.67%) in terms of Curvularia blight severity on the leaves of *Jatropha curcas*. Leaves turn yellow and then become brown from leaf tip downwards. This could be because the pathogen is favoured by high temperatures and adverse growing conditions [11], they added that, *Curvalaria* is primarily a stress pathogen that attacks low fertility and heat and drought stressed plants [12] however, [13] had different opinion that, *Curvularia* species cause a leaf spot on leaves of *Jatropha* in form of tan to dark brown, showing on both sides of the leaf bordered with a brown spring slightly depressed and with a narrow yellowish region between the spot and normal green of the leaf, this mostly occurs in areas that experienced prolonged leaf wetness for several consecutive days and temperatures between 25-30^{0}C. By and large, [5] concluded that, *Curvularia* is a mold fungus which is a facultative pathogen of many plants species and of the soil, mostly found in tropical regions.

The incidence of Colletotrichum leaf blight in Table 1 indicates significantly higher in Janbaki of Kebbi State (75.67%). This was followed by Gadar Ruwan Kanwa (Kebbi State), Tsaki (Sokoto State) and Gangije (in Kebbi State) that recorded statistically similar values of 70.67%, 67.67% and 65.00% respectively which were significantly higher than those obtained from Barkeji, Wanke, Nasarawa Wanke and Yar Tukunya in Sokoto and Zamfara States that recorded zero values.

The severity of Colletotrichum leaf blight on *Jatropha* plants was significantly highest in Gadar Ruwan Kanwa in Kebbi State (60.00%). This was closely followed by Tsaki in Sokoto State and Janbaki in Kebbi with 53.33% each. Kajiji in Sokoto State recorded 40.00% of severity with respect to the blight, while Barkeji in Sokoto, Wanke and Nasarawa Wanke and Yartukunya in Zamfara States recorded zero values. Highest incidence and severity of *Colletotrichum* blight on leaves of *Jatropha curcas* was obtained in the study areas close to streams or Fadama lands in Kebbi and Sokoto States. None was recorded in study areas of Zamfara State, probably because they were located in the uplands; this shows that, water and humidity plays an important role in the life cycle of *Colletotrichum* species. These tallies with the one finding [14] 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, tools and so on [15] stressed that, conidia germinate only in the presence of water and penetrate the host tissues directly.

Table 1: Incidence and severity of Curvularia and Colletotrichum Leaf blights

Locations	Curvularia Incidence (%)	Severity (%)	Colletotrichum Incidence (%)	Severity (%)
Kajiji	41.67±16.07ab	26.67±11.55ab	61.00±2.65b	40.00±0.00c
Tsaki	53.33±20.20a	33.33±11.55a	67.67±13.05ab	53.33±11.55ab
Barkeji	33.33±35.12ab	20.00±20.00ab	0.00±0.00c	0.00±0.00d
Wanke	18.33±16.07ab	13.33±11.55ab	0.00±0.00c	0.00±0.00d
N/ Wanke	21.67±18.93ab	13.33±11.55ab	0.00±0.00c	0.00±0.00d
Yartukunya	31.67±2.89ab	20.00±0.00ab	0.00±0.00c	0.00±0.00d
Janbaki	43.33±5.73ab	20.00±0.00ab	75.67±15.04a	53.33±11.55ab
Gangije	21.67±18.93ab	13.33±11.55ab	65.00±0.00ab	46.67±11.55bc

G/Ruwan kanwa	11.67±20.21b	6.67±11.55b	70.67±6.03ab	60.00±0.00a
Significance	*	*	*	*

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

Pathogenicity of the Isolated Organisms

Spores count

The values of spores count obtained from each of the fungal isolates used for inoculation were as follows;

- *Colletotrichum* species = 5447 x 10^4 spores/ml
- *Curvularia* species = 13.2 x 10^4 spores/ml

Results on leaf blight symptoms appearance on the leaves of *Jatropha curcas* seedlings at 7 days after inoculation is presented in Table 2. The results revealed that, *Colletotrichum* species (plate1) require fewer days (0.22) to appear on the leaves after inoculation. This could be attributed to the continuous wetting of incubated seedlings through watering which favours the germination and penetration of conidia. *Curvularia* species (plates 2) require longer time (0.67) days to manifest their symptoms on the leaves of seedlings [15] in which it starts inflicting from the roots through the stem to the leaves, while *Curvularia* species is favoured by high temperature and prefers low fertility and drought stressed plants while on the other hand, pathogenicity trial was conducted when the temperature was low at IAR, Zaria and seedlings were under regular watering. Control seedlings did not produce any disease symptoms during the period under observation.

Plate 1: Colletotrichum leaf blight on seedling of *Jatropha curcas* planted in g lasshouse at IAR, Zaria.

Plate 8: Curvularia leaf blight on seedling of *Jatropha curcas* planted in glasshouse at IAR, Zaria.

Table 2: Leaf blight symptoms appearance on the leaves of Jatropha curcas seedlings in glasshouse at 7 Days After Inoculation

S/No.	Pathogens	Occurrence (%)
1.	*Curvularia* species	0.67± 0.69a
2.	Colletotrichum species	0.22±0.65ab
3.	Control	0.00±0.00b
	Significance	*

Means followed by the same letter(s) do not differ significantly according to Duncan

Multiple Range Test (DMRT) at 5 % level of significance.

Table 3: Effects of inoculation methods on fungal blight appearance on the leaves of Jatropha curcas seedlings at IAR, Zaria.

SNO.	Inoculation Methods	Occurrence (%)
1.	Soil inoculation	0.47±0.90
2.	Spray	0.37±0.62
3.	Smear	0.33±0.71
	Significance	Ns

Table 4: Results on the general symptoms appearance of fungal pathogens on the leaves of *Jatropha curcas* seedlings as influenced by number of Days After Inoculation (DAI)

Days After Inoculation (DAI)	Occurrence (%)
7 DAI	0.27±0.60
9 DAI	0.20±0.41
11 DAI	0.53±0.92
13 DAI	0.20±0.41
15 DAI	0.40±0.83
17 DAI	0.73±1.03
Significance	Ns

CONCLUSION

Results of the study showed that, most of the leaves of *Jatropha curcas* in the areas studied were infected with fungal leaf blight caused by *Curvularia*, and *Colletotrichum* species. Tsaki in Sokoto State and Janbaki in Kebbi State recorded the highest incidence and severity of curvularia leaf blights. *Jatropha curcas* planted in lowlands were found to be more prone to infection than those planted in uplands, particularly those that are close to streams.

There was no significant effect among the methods of inoculation used during the pathogenicity trial with respect to the appearance of fungal pathogens (symptoms). Also, number of days after inoculation did not had any effect statistically on the appearance of leaf blight on the leaves of the seedlings of *Jatropha curcas* planted in the glasshouse of IAR, Zaria.

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