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SYSTEMIC FUNGAL INFECTION ON *Jatropha Curcas* L. ACCESSIONS CAUSED BY *Lasidioplodia theobromae* (Pat.) GRIFFON AND MAUBL IN SOUTH-WEST NIGERIA

Nwogwugwu, J.O.

Plant Pathology Section, Department of Forest Conservation and Protection,
Forestry Research Institute of Nigeria, PMB 5054, Jericho Hills, Idinshin, Ibadan, Oyo State
Corresponding Authors' email: joyoluchi70@gmail.com

Abstract
Jatropha curcas L. (Physic nut) is a species grown around the world for its great potential for the production of biodiesel and other useful industrial and medicinal purposes. *Lasiodiplodia theobromae* was implicated in systemic infection of the oil-bearing plant, physic nut causing high disease incidences and severities of leaf chlorosis, collar and root rots, wilting and eventual death of plants.Investigation and isolations were made from observed symptoms on four jatropha accessions under field and screen house conditions. Pathogenicity test using Koch's postulate was completed by re-isolating *L. theobromae* fungus from the developed symptoms after artificial inoculations on four weeks old seedlings raised in the screenhouse. Results show high incidence of symptomatic and systemic infection on *Jatropha curcas* caused by the pathogen, *L. theobramea*. The most observed symptom was root rots with least disease incidence recorded on Ex-Misau accession (38.5%). Disease severity was not statistically different in all the accessions tested. Vascular damage on the seedlings with evident symptoms of rots and foliar chlorosis led to eventual death. This study infers that sustainable disease management strategies should be intensified to compliment the efforts made to maximize the production and yield of this biodiesel species; without which growing this plant may serve a threat to other food crops grown as companion crops with the species.

Keywords: Biodiesel, Disease incidence, Pathogenicity, Screenhouse, and Physic nut

Introduction
Jatropha curcas L. is a perennial shrub of the Euphorbiaceae family; believed to be native to Central America (Achten *et al*. 2010). It is currently widely dispersed throughout the world due to its potential benefits. Physic nut is a drought resistant shrub, a multipurpose crop of significant economic importance as a biofuel. Moreover, parts of the shrub are used in traditional medicine and as raw material for pharmaceutical and cosmetic industries (Paramathma *et al*. 2006). Concern over the consequences of global warming has resulted in intensified search for potential plants that could supply raw materials for producing renewable fuels. Physic nut has gained attention as a perennial plant with high oil seeds and excellent properties (Wani *et al.,* 2012). The projection of world energy outlook (IEA, 2013) estimates a 56% rise in global market and energy consumption from 2016 to 2040. Climate change is expected to impact on plant health due to changes in temperature and precipitation regimes, which will in turn alter the growth and development rate, pathogenicity of infectious agents, physiology and the host plant resistance (Cooke, 2006). Though many studies have described physic nut as a plant resistant to pests and diseases, however, intensive cultivation in recent years has proved the plant as being susceptible to many pests and pathogens requiring management in order to optimize yield. *L. theobromae* (Pat.) Griffon & Maubl., Syn. and *B. theobromae* Pat. are pervasive Ascomycetous fungal pathogens across the tropics. They have been reported on a variety of host plants causing great economic losses on cultivated crops, as a rot and wound causing organism especially on staples and fruits such as cassava, yam, cacao, citrus and mango (Twumasi *et al.*, 2014).

It is estimated that this species attacks over 280 plant species and with different pathological symptoms on its hosts (Pha *et al*., 2010; Domsch *et al*., 2007 and Twumasi *et al*., 2014). It has been reported to cause canker and dieback diseases on various hosts (Tri Rapani *et al.,* 2019). Jatropha is not resistant to pest and pathogens. Different studies have reported various fungi and many other pests viz:-, *Fusarium* spp., *Rhizoctonia* spp., *Alternaria, Sclerotium rolfsii,* bacteria, nematodes and insect infestations capable of causing high seedling mortality, and low yield on this species in areas where the jatropha plant is cultivated intensively (Kumar *et al*.,

2011; Neves *et al*. (2009); Sharma and Kumar, 2009; Hedge *et al*. (2009) and Worang (2008). Disease symptoms such as Anthracnose, mildew, canker, dieback, flower abortion, fruit and root rots caused by *Colletotrichum*, *Lasiodiplodia, Alternaria alternata, Curvularia, Fusarium, Rhizopus, Phythophthora and Oidum* species were recorded on *J. curcas* in South-West Nigeria (Nwogwugwu and Ikotun, 2015). These are serious pathogens of several crops and trees species, making *J. curcas* a threat to other plants and to food security. There is need therefore, for disease identification, documentation and management as we adopt intensive cultivation of this species. This paper investigates the incidence of a systemic disease on *Jatropha curcas* and the causal organism, *L. theobromae* in sub-humid tropical zone of Ibadan, Oyo State, with mean average annual temperature and precipitation of about 26.5°C and about 1311mm respectively.

Materials and Methods

Experimental location and source of planting materials

This study was carried out at the Department of Crop Protection and Environmental Biology (CPEB), University of Ibadan, Latitude 7° 27' 01" N and 3° 53' 43″E. Four *J. curcas* accessions were used for this study. Seeds of these four accessions were collected from seed bank of Forestry Research Institute of Nigeria (FRIN) Ibadan.

Sterilization of laboratory materials

Media preparation followed standard laboratory procedures; distilled water and Potato Dextrose Agar (PDA) were autoclaved at 121°C and 1.05 kg/cm^2 pressure for 15 minutes. Glass wares were washed and sterilized at 160°C for 3 hours in a hot air oven. The inoculating needles were flame sterilized on spirit lamp. The inoculating chamber was disinfected by swabbing with cotton wool soaked in 70% ethanol. All isolations and inoculations were carried out under sterile laminar flow. PDA used for fungal isolations was supplemented with 1.4ml lactic acid per litre of medium for this study.

Nursery and Field establishment

One hundred seeds per accession were raised on germination trays, and at one month after germination. Fifty healthy, uniform and randomly selected seedlings per accession were transplanted to the teaching and research farm of the Department of Crop Protection and Environmental Biology Ibadan (CPEB) at 10 stands per row and five rows per accession (4 accession: five rows:10stands: 4x5x10 = 200 seedlings) population size. They were observed for six months for disease development.

Disease monitoring and measurement

The primary disease investigation was carried out on symptomatic seedlings of *J. curcas* in the field under natural infection by fungal pathogen at the experimental field. Assessment of disease was based on the symptoms observed on jatropha seedlings: chlorosis, defoliation, collar and fruit rot and wilting. Disease incidence was estimated by taking random samples of five infected plants per row from the ten stands at 2 months sampling intervals. Twenty plant samples were collected per time from the accessions.

Disease incidence was measured and calculated thus:

Pest or disease incidence (I)

$$I = \frac{\textbf{Number of plants affected (n)}}{\textbf{Number of plants observed (N)}} \text{ X } 100\%$$

Disease severity (S)

$$S = \frac{\Sigma(ni.vi)}{N \text{ X } V} \text{X } 100\%$$

Where,

ni = Number of plants affected at category level i

vi = Damage category level i

N = Number of plants observed

V = Highest damage category value

Preparation of Fungal culture and isolation

The infected seedlings were carefully uprooted from the soil, fruits showing rot symptom were also harvested and taken to the Plant Pathology Laboratory of CPEB. The samples were washed under running tap water. Segments (2cm) from the necrotic lesions of the infected root and collar regions were excised together with the healthy-looking parts. They were placed in 250ml beakers and were surface sterilized with 5% (Is this not too concentrated?) commercial sodium hypochlorite for three minutes and thereafter rinsed with three changes of sterile distilled water. They were then dried for a minute on sterile filter papers. The samples were cultured on freshly prepared sterile Potato Dextrose Agar (PDA) at three samples per plate for fungal development and growth. The cultures were incubated at room temperature (30±2°C) for seven days.

Preparation of pure inoculum

The (fungal) cultures were purified through sub-culturing on PDA for separation into individual isolates and pure culture was maintained on PDA amended with chloramphenicol (1g/L) to supress bacterial growth. Pure cultures of the isolates were finally maintained on PDA slants preserved in an incubator at room temperature. The isolated organisms were identified up to species level after reference to Barnett and Hunter (2001); Dugan, 2006 and Domsch *et al*. (1980) using both colony and conidial morphology. The isolates from infected roots, collar and fruits of *J. curcas* accessions were subjected to pathogenicity test.

Pathogenicity test

Pure inoculum preparation and Inoculation of seedlings with L. theobromae

Forty seedlings were raised from seeds of the four accessions at 10 seedlings per accession in trays. Forty polythene pots were filled each with one kilogram of sterilized top soil in the screenhouse. At four weeks old, seedlings were artificially inoculated using pure culture of *L. Theobromae* that was earlier isolated from infected jatropha plant. Inoculum suspension of the isolate was

prepared from culture in a seven-day old PDA broth; the concentration was adjusted to 10^6 conidia/ml using a hemocytometer. The inoculation method used for the seedlings was the soil infection method. Five seedlings were inoculated per accession while five served as control/check, giving a total of 40 seedlings for the experiment. For each polythene pot, 500ml spore suspension was introduced in the potted soil and mixed thoroughly. Then the seedlings were transplanted immediately to the infected soil at one plant per pot. The control plants were inoculated with only sterile distilled water (SDW). They were incubated in the screenhouse by covering them with transparent polythene sheets for 24 hours to maintain high humidity. They were then monitored during which observations were made daily for disease symptoms for up to 12 weeks after inoculation (WAI). Koch's postulate was completed by re-isolating *L. theobromae* fungus from the developed symptoms. The experiment was conducted in a complete randomized design in five replicates. Data on disease incidence and severity were collected at natural infection in the field and in screen house artificial inoculation. Data collected were analyzed using ANOVA, and means separated using FLSD ($p \leq 0.05$).

Results and Discussion

Disease detection

There was high incidence of rot leading to high mortality in the first 6 months after planting. The shoots were mummified, and roots decayed when uprooted from the pots. The leaves were chlorotic and at later stages of growth, defoliation and foliar infection were recorded on the infected seedlings. There were no significant differences recorded on disease incidence in all the four accessions except for Ex-Misau with 38.5% rot incidence at natural infection. However, disease severity index was statistically different among the accessions. Ex-Ibadan and Ex-Basirika had the highest mean score (51.4 and 52.7 %); the least value of 32.0 % was recorded on Ex-Misau (Table 1). On artificial inoculation, incidence of root rot was also high with the percentage increasing with weeks after inoculation 4, 8 and 12 WAI. There were no statically observed differences between incidence at 8 and 12 WAI as the infection was stabilized with weeks after inoculation (Table 2.). The accession Ex-Misau was the least susceptible to root rot infection (20.9%), and differed significantly from the other accessions. Disease recognition is paramount to its strategy for management both for prevention and eradication. The accessions were susceptible to collar rot pathogen, there were necrotic lesions observed at the base of the affected plants. The leaves were chlorotic and defoliated. This result is in line with the report by (Pha *et al*., 2010) in India, who reported significant losses on *J. curcas* with disease symptoms ranging from yellowing, drooping and shedding of leaves, blackening and decaying of the collar region of the stem. and rotting of roots and seedling mortality due to infection by *Lasiodiplodia theobromae.* Fu *et al.* (2007) also observed gummosis incidence on *Jatropha podagrica, a* disease caused by *L. theobromae.* It was a systemic infection that affected the absorptive system of infected plants, thereby resulting in insufficient water and nutrients, stunting of shoot system, yellowing and wilting of leaves. It was reported to cause cassava root rot in Nigeria, (Dixon *et al.,* 2005). *L. theobromae* spp. are known to produce cankers and die-backs on several woody hosts. It was isolated from the bark of canker of walnut in Egypt (Haggag, 2007).

Conclusion

This result from this study has negated the belief that jatropha is a specie resistant to pathogens. Prompt monitoring and early disease detection in jatropha nursery and plantations, and the adoption of sustainable disease management practices will be necessary, considering the high susceptibility displayed by this specie to the rot and wound causing pathogen, *L. theobromae*. It is a soil borne pathogen that can overwinter in the soil and litter till favourable condition, causing systemic damage to the host. Soil and seed treatment will be recommended for pathogen avoidance.

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Table 1: Percentage disease intensity under natural infection caused by *L. theobromae*on *J. Curcas* at three months after planting (MAP)

Accession	% root rots		
	Incidence	Severity	Mortality%
Ex – Misau	38.5	32.0	8.9
Ex - Mbatdiya	46.7	38.0	15.3
Ex - Kano	45.7	43.6	14.8
Ex - Basirika	43.2	51.4	14.2
Ex - Ibadan	43.8	52.7	13.2
$LSD_{0.05}$	4.6	3.5	

MAP = Month after planting

Table 2: Root rot incidence on *J. curcas* accessions inoculated with *L. Theobromae* in screen house

WAI	Accession			
	Ex-Basirika	Ex-Kano	Ex-Misau	Ex-Mbatdiya
4 WAI	34.4	40.1	20.9	36.2
8 WAI	39.0	40.8	18.5	38.8
12WAI	39.6	41.4	18.9	38.5
$LSD_{0.05}$	2.13	2.90	2.73	1.67

WAI = Weeks after inoculation
MAI = Months after inoculation. NS = Not significantly different

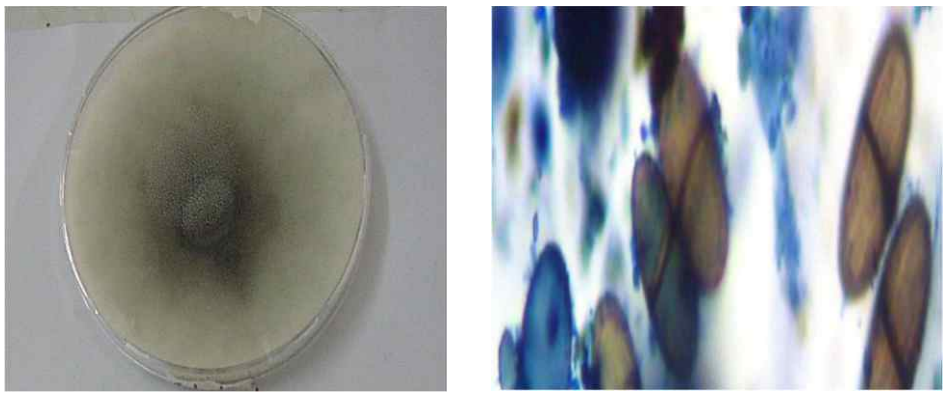

Figure 1: Culture (left) and photomicrograph (right) of *L. theobromae* conidia isolated from infected Jatropha plant