baked and estimated contribution to vitamin A requirements. *Food Chemistry* 228:85-90.


Colletotrichum gloeosporioides: A BIOTIC CONSTRAINT TO Jatropha curcas L. CULTIVATION IN SOUTH-WESTERN NIGERIA

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Abstract
Jatropha curcas (Physic nut) is a potential plant source for production of biofuel. The growing concern over global warming and climate change due to CO2 emissions from fossil fuel has raised global attention towards renewable, more environmentally friendly alternative sources of energy. Anthracnose disease caused by Colletotrichum species is one of the most widespread plant diseases in the tropics. We investigated the causal pathogen of Jatropha curcas anthracnose in South-western Nigeria. Samples were collected from seeds and diseased seedlings of four J. curcas accessions raised at the Forestry Research Institute of Nigeria nursery under natural infection. Culturing and isolations from the samples were carried out at the Plant Pathology laboratory of Crop Protection and Environmental Biology. Cultural and morphological characters of the fungi were observed. Pathogenicity of the isolate was confirmed on J. curcas seedlings. The experiment was carried out in a Randomized Complete Block Design (RCBD) with five replicates. Data were collected on disease intensity (incidence and severity). Evaluation of disease incidence (%) and severity (1-5, ranging from no disease to highly susceptible) were calculated and data were analysed using Analysis of Variance (ANOVA) and significantly different means were separated using LSD at 5% (p ≤ 0.05) level of significance. Colletotrichum species, was identified to cause anthracnose on four Jatropha accessions, causing symptoms with high disease intensity on the plants; anthracnose (29.0%), cankers (41.4%), shoot dieback (79.4%). The susceptibility of Jatropha accessions to anthracnose caused by the fungus, Colletotrichum gloeosporioides (Penze.) Penze & Sacc was confirmed. To maximize yield of this oil plant and sustain the new hype in its cultivation for biodiesel production, anthracnose disease ought to be effectively managed.

Key words: Accessions, Biodiesel, Cankers, Global warming, Pathogens.

Introduction
Fungi from the genus Colletotrichum are mainly pathogens of plants having worldwide importance as causing diseases of wide range of economic crops and ornamental plants (Agrios, 2004; Liu et al., 2016). This genus includes many ubiquitous plant pathogens that can cause major production losses on many plant crops and threaten food security (Cannon et al., 2012; Hyde et al., 2014). They cause diseases on almost all parts of the plant host tissues they attack, on the field and at the nurseries.

This Ascomycetous fungi, with over a hundred recorded species, are reported to cause diseases on over 3,000 plant species with symptoms such as anthracnose, foliar blight and rot of fruits and stem (Liang et al., 2018; Liu et al., 2014). Colletotrichum species are known to cause a typical disease symptom known as anthracnose on more than 30 plant genera (Diao et al., 2017; Weir et al., 2012). They produce pinkish acervuli, in a concentric pattern around the necrotic tissues, which release spores at very high moisture content. The fungus is favored by high relative humidity and high temperature which in turn promotes spore germination, growth and host colonization (Ashutosh Pandey et al., 2012).

Jatropha curcas is a perennial drought resistant species belonging to the Euphorbiaceae family. It is a multipurpose crop of significant economic importance as a biofuel. Moreover, all parts of the shrub are used in traditional medicine and as raw material for pharmaceutical and cosmetic industries and is useful to control land degradation (Paramathma et al., 2006). It originated from North and Central America but has spread widely to other parts of the tropics and subtropics such as Africa, India, Asia and China due to its several benefits. There is a new quest for alternative renewable energy sources due to the overdependence on fossil fuel and its negative consequences on the environment. Biodiesel is an energy source that is environmentally friendly and has tendency of meeting the world's energy needs.
demand (Qian et al., 2010; Can, 2014; Guan et al., 2017). *Jatropha curcas* L. has emerged a preferred alternative for biodiesel production as non-edible oil with no competing food uses (Kamel et al., 2016; Dena et al. 2018). The *Jatropha* seeds oil content is estimated at about 300–400 g/kg (Reddy et al., 2017; Kamel et al., 2018). The seeds contain 35-40% oil; 50-55% oil on kernel basis (Kausik et al., 2006), similar to palm oil and animal fat, and has multipurpose industrial uses (Kumar and Sharma, 2008). The plant is considered a suitable crop for the production of biodiesel and sustainable development for its ecological, environmental and socio-economic benefits (Karavina et al., 2011; Datimon et al., 2013; Ali et al., 2016). FAO, (2008) reported that production of biodiesel is an alternative to fossil fuels will not only help to mitigate the effect of global warming and climate change but enhance energy security.

Despite all the promising benefits of this oil plant, there are still so many unresolved constraints before its benefits could be fully actualized. Though this species was earlier reported to be pest and disease free, many field trials have classified *Jatropha* as a plant that is susceptible to the attack of many diseases that are caused by bacteria, fungi, nematodes and insect pests (Heller, 1996). Yield losses of up to 56.9% have been reported due to attack by different pests on its leaves and fruits (Singh et al., 2007). Datimon et al. (2013) listed many pathogens that are potential threat to large scale production of *Jatropha* in Benin Republic. It has been reported that the plant can survive on marginal lands with low-nutrient or low-moisture, but maximizing fruit and oil yield requires detailed sufficient light, water and nutrients (Spinelli et al., 2010; Reis et al., 2015).

Plant diseases are posing serious and continuing threat to crop security, food safety, agriculture and forestry worldwide especially in the tropics (Dilami et al: 2017). In this work, we examined the threats of the fungi, *Colletotrichum gloeosporioides* to *J. curcas* production in Nigeria.

**Materials and Methods**

**Location of Experiment and Source of Seeds and Seedlings**

The studies were conducted on the field and screen house of the Department of Crop Protection and Environmental Biology (CPEB), University of Ibadan and the laboratory of Forestry Research Institute of Nigeria (FRIN) Ibadan.

Four *J.curcas* accessions (Ex-Basirika, Ex-Mbatdiya , Ex-Misu , Ex-Kano) used for this study were obtained from the Genebank of Forestry Research Institute of Nigeria (FRIN) Ibadan, Nigeria.

Mycological analyses were carried out on 400 seeds from the Genebank using Potato dextrose Agar (PDA) and blotter techniques. They were incubated at 28±2°C in the dark for seven days. Fungi isolated from the seeds were identified through colony appearance and sporulation morphology.

Two hundred seedlings of *Jatropha* obtained from FRIN Nursery at 4 weeks after planting were transplanted into CPEB research farm and were investigated to determine the pathogenic fungi causing symptoms of necrosis, chlorosis and blight. Fifty seedlings were raised per accession and arranged: 10 seedlings per row plot replicated five times. Anthracnose disease investigations was carried out by observing and recording disease incidences and severities on two-year-old *Jatropha* seedlings at the CPEB Teaching and Research farm. Each diseased plant was assessed for the occurrence of anthracnose. Five randomly selected plants were used as sampling units for disease assessment. The same were subjected to laboratory examinations for isolation and identification of the pathogen, by culturing and microscopy.

**Isolation**

Isolations of pathogens were done from the symptomatic tissues of *Jatropha*. The tissues of the shoots and roots were washed under running water; thereafter 5cm tissues were cut-off with a sterile scalpel and subject to surface disinfection using 5% sodium hypochlorite solution (NaOCl) for three minutes. They were rinsed in three changes of sterile distilled water (SDW). Thereafter, the tissues were dried on sterile filter paper and were transferred to freshly prepared sterile PDA plates, and incubated at 28±2°C for 7days.

**Morphological and Cultural Characters of Isolate**

Purification of the isolates was done by subsequent sub-culturing on acidified PDA until pure cultures were maintained. The Fungi isolates were identified using standard procedures. The identification was confirmed under the microscope by the Commonwealth Mycological Institute, England and Barnett and Hunter (2001) identification keys using the morphological and cultural characters of the isolates (colony morphology, colour in culture, hyphae and spore/conidia structure and morphology).

**Inoculation Techniques and Pathogenicity Tests**

Pathogenicity of the isolates was performed using standard procedure of Koch's postulate at the (CPEB) screen house. The isolate was inoculated on the leaves of 20 young seedlings (2weeks old) of each accession by spraying a spore suspension with an atomizer. Ten seedlings were sprayed with sterile-distilled water as control. Spore suspensions were prepared by flooding a week-old culture with sterile saline containing 0.01% volume per volume (√v/v) Tween 80 and dislodging the spores by scraping the fungal culture and straining the suspension through a clean cheese cloth to remove fragments of agar and hyphae with a sterile glass. The inoculum suspension was adjusted by the aid of a haemocytometer and the suspension was diluted to 10° conidia/mL concentration. Throughout the experimental period, seedlings were maintained in a
moist chamber (temperature of 28-30°C and 78-88% Relative humidity. For the artificial inoculation studies in the field, foliar spray technique was used and the conidia concentration of 10^6 conidia/mL was maintained.

**Experimental Lay-out**

On the field and the screenhouse, the experiment was laid out on Randomized Complete Block Design (RCBD) of five replications. Ten plants were raised in a row plot per accession to which the fungus (Colletotrichum spp.) was administered. The experimental layout for the laboratory investigation was carried out on Completely Randomized Design (CRD) with five replications. The results of the radial growth of the cultures were expressed as mean values of all measurements in the five replications.

**Method of Data Analyses**

Data collected on the disease incidence and severity on Jatropha accessions were analyzed using Analysis of Variance (ANOVA) and the treatment means were separated using Least significance difference at (LSD) at 5%. The disease intensity expressed as disease incidence and disease severity were determined by using the formula of Seem (1984):

\[
\text{DS} = \frac{\Sigma (n_i \times v_i) \times 100\%}{N \times V}
\]

Where: \( N_i \) = Number of plants affected at category level i
\( v_i \) = Damage category level i
\( N \) = Number of plants observed
\( V \) = Highest damage category value

**Results**

There was high incidence of seed to seedling infection on jatropha accession used in this work. Seed health test revealed very high incidence of seed borne mycoflora, Colletotrichum spp. from a two-month stored seeds (Plate 1). Anthracnose (foliar infection): leaf chlorosis, leaf spot, leaf blight and leaf defoliation were observed at very early stages after germination. Isolations made from the lesions yielded similar fungal pathogen as that from the cultured seeds, indicating that the infection may be a carried-over from the seeds and that Colletotrichum spp. could be a seedborne mycoflora (Table 1) and serving as a potent inoculum for early infection on jatropha seedlings.

**Colletotrichum gloeosporioides** isolated from the lesions (Plates 1 & 2), infected leaves, shoots, fruits and flowers and were pathogenic on jatropha accessions satisfying Koch postulate. Anthracnose symptoms were observed at all the stages of the plant development. On artificial inoculation (Table 2), there was progressive rise in percentage disease incidences with weeks after inoculation (WAI) except for Ex-Misau accession which decreased progressively with weeks after inoculation for all the foliar symptoms observed. For Ex-Misau accession, leaf blight incidence dropped from (11.6a%) at 4 WAI to (9.6a%) at 12 WAI. For the other three accessions, there were significant differences (p< 0.05) and rise in disease incidences from 4WAI and 12WAI. For example, the highest observed symptom (leaf spot) percentage for Ex-Basirika (23.6a /25.8a%); Ex-Mbatidiya (21.6a/24.8b%); Ex-Kano (25.2a/26.5a%) at 4 and 12WAI respectively.
Plate 1. (A) Colletotrichum gloeosporioides rotting fruit of *J. curcas*; (B) Healthy fruit of *Jatropha* (C) Pure culture of *C. gloeosporioides* on PDA isolated from *Jatropha* samples; (D) culture of *C. gloeosporioides* showing orange conidial pustules at 7 days of incubation (E) photomicrograph of *C. gloeosporioides* from *J. curcas* fruit.

Plate 2: *Jatropha* plants under attack, showing foliar necrosis caused by *C. gloeosporioides*
Table 2. Percentage incidence (%) of anthracnose on *J. curcas* accessions weeks after inoculation with *Colletotrichum* sp.,

<table>
<thead>
<tr>
<th>Accession</th>
<th>WAI</th>
<th>Ex-Basirika</th>
<th>LC</th>
<th>LS</th>
<th>LB</th>
<th>Ex-Mbatdiya</th>
<th>LC</th>
<th>LS</th>
<th>LB</th>
<th>Ex-Misu</th>
<th>LS</th>
<th>LB</th>
<th>Ex-Kano</th>
<th>LC</th>
<th>LS</th>
<th>LB</th>
</tr>
</thead>
<tbody>
<tr>
<td>4WAI</td>
<td></td>
<td>20.6 a</td>
<td>23.6 a</td>
<td>23.0 a</td>
<td>24.4</td>
<td>19.9 a</td>
<td>21.6 a</td>
<td>23.3 a</td>
<td>10.8 a</td>
<td>10.3 a</td>
<td>11.6 a</td>
<td>21.6 a</td>
<td>25.2 a</td>
<td>23.4 a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8WAI</td>
<td></td>
<td>22.5 b</td>
<td>25.2 a b</td>
<td>24.2</td>
<td>22.1 b</td>
<td>24.4 b</td>
<td>25.2 b</td>
<td>11.2 a</td>
<td>10.3 a</td>
<td>9.3 a</td>
<td>23.1 b</td>
<td>25.9 a</td>
<td>25.3 a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12WAI</td>
<td></td>
<td>22.5 b</td>
<td>25.8 a b</td>
<td>24.2</td>
<td>22.2 b</td>
<td>24.8 b</td>
<td>25.5 b</td>
<td>9.0 c</td>
<td>9.9 a</td>
<td>9.6 a</td>
<td>23.1 b</td>
<td>26.5 a</td>
<td>25.5 a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LSD&lt;sub&gt;0.05&lt;/sub&gt;</td>
<td></td>
<td>1.30</td>
<td>1.49</td>
<td>0.85</td>
<td>1.76</td>
<td>2.27</td>
<td>1.72</td>
<td>1.71</td>
<td>2.15</td>
<td>3.13</td>
<td>1.40</td>
<td>1.24</td>
<td>2.10</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

LC = leaf chlorosis, LS = leaf spot, LB = leaf blight
WAI = Weeks after Inoculation

Under natural field infection (Tab. 3.), disease severity scores followed the same trend of progressive increase with months after planting (MAP). Open wounds (cankers) were observed on the shoots as the disease progressed leading to high severity indexes recorded at 9 and 12 MAP. Ex-Misu had least disease severity of 8.9% shoot canker and 33.5% shoot lesion compared to the other three accessions. Ex-Mbatdiya (43.4c%) leaf spot; Ex-Basirika (58.4c%); Ex-Kano (59.5c%). Shoot dieback (Tab. 4.) progressed from first year after planting (YAP) with high infection rate by the fungus *C. gloeosporioides*. The least incidence and severity score were recorded on Ex-Misu at 3<sup>rd</sup> year after planting, 60.0c% and 14.0% respectively while the most susceptible among the accessions to shoot dieback was Ex-Kano with 69.4c% incidence and 32.2a% severity score at 3<sup>rd</sup> year after planting. Fresh plant weight of seedlings inoculated with *Colletotrichum* were significantly lower than the uninoculated control plant in all the accessions tested (Fig. 1).

Table 3. Anthracnose Disease severity on *J. curcas* Accessions Under Natural Infection at 9 and 12 MAP

<table>
<thead>
<tr>
<th>Accession</th>
<th>9 MAP</th>
<th>12 MAP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaf lesion</td>
<td>Shoot lesion</td>
</tr>
<tr>
<td>Ex – Misau</td>
<td>14.7a</td>
<td>11.6a</td>
</tr>
<tr>
<td>Ex-Mbatdiya</td>
<td>17.0b</td>
<td>22.1b</td>
</tr>
<tr>
<td>Ex – Basirika</td>
<td>33.6c</td>
<td>19.6c</td>
</tr>
<tr>
<td>Ex – Kano</td>
<td>35.3d</td>
<td>20.7c</td>
</tr>
<tr>
<td>LSD&lt;sub&gt;0.05&lt;/sub&gt;</td>
<td>1.80</td>
<td>1.2</td>
</tr>
</tbody>
</table>

MAP = Months after planting

Table 4. Incidence and severity of shoot dieback caused by *C. gloeosporioides* on *J. curcas* accessions under natural field infection.

<table>
<thead>
<tr>
<th>Accession</th>
<th>Ex-Basirika</th>
<th>Ex-Mbatdiya</th>
<th>Ex-Kano</th>
<th>Ex-Misu</th>
</tr>
</thead>
<tbody>
<tr>
<td>YAP</td>
<td>DI %</td>
<td>DS</td>
<td>DI %</td>
<td>DS</td>
</tr>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt; yr</td>
<td>26.0a</td>
<td>37.8a</td>
<td>20.8a</td>
<td>42.1a</td>
</tr>
<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt; yr</td>
<td>33.6b</td>
<td>79.4b</td>
<td>30.6b</td>
<td>60.5b</td>
</tr>
<tr>
<td>3&lt;sup&gt;rd&lt;/sup&gt; yr</td>
<td>38.9d</td>
<td>33.6c</td>
<td>73.4c</td>
<td>30.5c</td>
</tr>
<tr>
<td>LSD&lt;sub&gt;0.05&lt;/sub&gt;</td>
<td>4.0</td>
<td>3.3</td>
<td>2.7</td>
<td>3.5</td>
</tr>
</tbody>
</table>

DI% = Disease incidence percentage, DS= Disease severity of symptoms on plant; SS = susceptible; MS = moderately susceptible; VS= very susceptible. YAP (Year after planting).
The main aim of this study was to establish the pathogen responsible for the anthracnose disease of *Jatropha curcas* in south-west Nigeria and to determine its pathogenicity by measuring the disease intensity on the plant. No doubt, observation from this study has implicated *C. gloeosporioides* as systemic and major pathogen of *J. curcas* in southwestern Nigeria. Observations from the artificial inoculated seedlings and the field infected plants showed the same symptoms; chlorotic patches on the shoots at the onset of infection which later turned dark brown and necrotic; girdling the whole stem. In severe infections, there were foliar necrosis, wilting, defoliation and twig and shoot diebacks.

The present investigation confirmed the report of Lindy Coates *et al.* (2018) and revealed that *C. gloeosporioides*, an anthracnose causing pathogen, can infect all plant parts, displaying varying symptoms ranging from foliar infections, cankers and flower abortion to fruit and seed rot. Plant infections at mature stages did not inhibit seed setting but infections of the leaves, shoots and the flowers caused severe loss of inflorescence. Shriveling of young fruits were observed at later stages of the disease. The four *Jatropha* accessions were susceptible to the pathogen, *C. gloeosporioides*; though, there were differences in their level of susceptibility to anthracnose expression; both under natural infection and at the artificial inoculation.

Seed borne pathogens caused by *C. gloeosporioides* recorded from this study agree with the findings of Pinto *et al.* (2018) who reported that the yield quality and oil production of this species could be affected by fungi infections. *C. gloeosporioides* attacks on *J. curcas* plant has been widely reported in places where this plant is cultivated intensively; anthracnose outbreaks on this plant in the recent times is spreading widely establishing the pest status of this pathogen as serious threat to biofuel production. Carels (2009) and Suryanarayana (2010) reported that anthracnose disease caused by *C. gloeosporioides* on *J. curcas* is an economic important pest of this species causing substantial damages and yield losses on *Jatropha* production in Brazil and India. Though the fruit extracts of physic nut exhibited in-vitro properties against fungi (Kwon *et al.*, 2012), the versatility of anthracnose disease on this plant suggests that this fungus can overcome the plant resistance. Machado and Pereira (2013) described the genus *C. gloeosporioides* as one of the most common and devastating pathogens of *Jatropha* in all the areas where the species is cultivated thereby confirming our observations in this study. Bhuyan and Boruah (2015) reported that *C. gloeosporioides* Penz. was the causal pathogen of *Jatropha* anthracnose in India.

*C. gloeosporioides* has been implicated as fungal pathogen causing anthracnose and postharvest rots of many crops as has been confirmed by many researchers. Onyeani and Amusa (2015; Dionicio *et al.*, 2018), recorded the postharvest susceptibility of many fruits and vegetables to *Colletotrichum gloeosporioides* causing rot. Buyoye *et al.* (2017) reported on his investigation on the anthracnose-like symptom on *Cutnut, Barringtoniaedulis* caused by the pathogen *C. gloeosporioides* inciting economic yield losses on the plant. Emehute *et al.* (1998) reported that this pathogen one of the most devastating biotic constraints to the worldwide production of *D. alata* (winged yam). Fokunang *et al.* (2001) reported that anthracnose disease of *Manihot esculenta* caused by *C. gloeosporioides*, causes significant yield losses and is rated as an economic disease worldwide.

**Discussion**

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**Conclusion**

This study presents a baseline information on *Jatropha* susceptibility to anthracnose causing fungal pathogen, *Colletotrichum gloeosporioides* in Nigeria. There is no doubt that this fungal pathogen reported to be of worldwide economic importance could pose a constraint to achieving optimum yield of this oil-bearing species. The potential aggressiveness observed in this work, could mean a risk to food security as it could be spread to other crops. Pest and diseases need to be properly identified and managed to ensure sufficiently high *Jatropha* yields and oil production. Yield improvements through the release of disease resistant varieties by proper natural selections and breeding should be encouraged.
References


Plate 2. (A) Colletotrichum gloeosporioides rotted fruits of J. curcas; (B) Healthy fruits of Jatropha © Pure culture of C.
*Colletotrichum gloeosporioides* is a biotic constraint to *Jatropha curcas* L. cultivation. (D) Culture of *C. gloeosporioides* showing orange conidial pustules at 7 days of incubation. (E) Photomicrograph of *C. gloeosporioides* from *J. curcas* fruit.