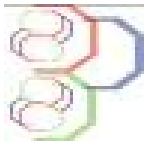

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First report of *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl causing root rot and collar rot disease of *Jatropha curcas* L. in Benin

SHORT COMMUNICATION

[*1]Appolinaire Adandonon; [2]Binjamin Datinon, [3]Hugues Baimey and [2]Joelle Toffa

[*1]School of Crop and Seed Production and Management (EGPVS), University of Agriculture of Ketou (UAK), 08 BP 1055 Cotonou, Rep. Benin; email: adanappo@yahoo.fr

[2]International Institute of Tropical Agriculture, 08 BP 0932, Cotonou, Rep. Benin

[3]National Higher School of Agricultural and technical Sciences of Djougou (ENSTA – Djougou), University of Parakou, Parakou, Rep. Benin.

*Corresponding author email: adanappo@yahoo.fr

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ABSTRACT

Objective: Jatropha curcas L. is an important biofuel crop grown worldwide. In Benin, however, the plant is attacked by rot diseases resulting in rotting, wilting, yellowing, dropping of leaves, blackening, decaying of affected areas and later death of the plant. The objective of the current study was to identify the causal agents of the disease.

Methodology and results: Diseased samples were collected from field, surface sterilized and plated on PDA medium. Pure cultures were observed on plate and under microscope. *Lasiodiplodia theobromae* was consistently isolated from diseased plants collected from the field. Inoculation of *L. theobromae* to 1-year-old jatropha plants resulted in typical symptoms of the disease, confirming the fungus pathogenicity on *Jatropha*.

Conclusion and application of results: As conclusion, of *L. theobromae* is the causal agent of the disease and this is the first report of *L. theobromae* causing rot diseases on *J. curcas* in the Republic of Benin. Identification of the pathogen of the disease is of paramount importance and the direct application is that this causal agent identification will be taken into account for adequate control programme of the disease in Benin.

INTRODUCTION

Jatropha curcas L. (Euphorbiaceae) is a multipurpose crop. In fact, *J. curcas* is such a promising species because many products from the plant can be made useful. Primarily the oil from the seeds, but other products must not be forgotten to make the whole use as profitable as possible. Oil from the seeds are very important for instance as biofuel and feedstock for soap production, When the nuts are pressed to oil, the press

cake is first for energy purposes and then as a fertilizer, briquettes for fuel, insecticides. (Rijssenbeek, 2008**)**. A good application for the jatropha seed cake is to use it as organic manure, replacing chemical fertilizer. It has a nitrogen content similar to that from cake of castor bean or chicken manure. The nitrogen content ranges from 3.2 to 3.8 %. (Juillet *et al* 1955; Moreira 1979; Vohringer 1987). Briefly, to produce biofuel from jatropha (briefly): (1) first dissolve the lye into the methanol. Shake or swirl until all the lye has dissolved. This may take 10 minutes. It is normal that temperature rises. This mixture is called sodium methoxide. (2) Now make sure the pure plant oil (PPO) from jatropha seeds is in a vessel large enough (at least 150% of its volume), preferably with a valve at the bottom, and heat it to about 60 °C, then stop heating. Then add the methoxide mixture and make sure it is mixed well for at least 10 minutes. Leave the vessel and let the different constituents separate by sedimentation. The glycerine will settle out at the bottom. After 8 to 24 hours the sedimentation is complete and the glycerine can be drained off. What remains is raw biodiesel. If the reaction went well and the biodiesel is clear, it may be used straight, although its quality may be inferior because of impurities. Water washing will remove most of these impurities. Though *Jatropha* is reported as resistant to most bioconstraints as insects and diseases (Chitra & Dhyani, 2006), diseases, such as die back, caused by fungi resulting in great yield losses have been recorded in different countries including Malaisia in 2008 (Sulaiman & Thamarajoo, 2012). Projects done in *Jatropha* fields in South Benin, where GERES NGO is promoting the production of the crop for biofuel, showed *Jatropha* plants suffering from general yellowing and wilting with drops of leaves and subsequent death of the plants. *Jatropha* disease samples were collected in the field to identify the pathogens associated with the diseases. *Jatropha* diseased tissue was cut into small segments (± 2 mm^2), surface disinfected with 0.5 % sodium hypochlorite (NaOCl) for 15 s, rinsed twice in SDW, blotted dry on sterile tissue paper and plated onto potato dextrose agar (PDA) amended with 0.025 % chloramphenicol. After 4-days incubation at 25±1 °C, pure cultures were obtained by transferring fungal colonies to new PDA plates and incubating at 25±1 °C under fluorescent light for 10 days. The pure fungal cultures were maintained on PDA slants at 4 °C. In plates, the fungus initially produced white colonies (Photo1a), which later (8–10 days) turned black (Photo1b).

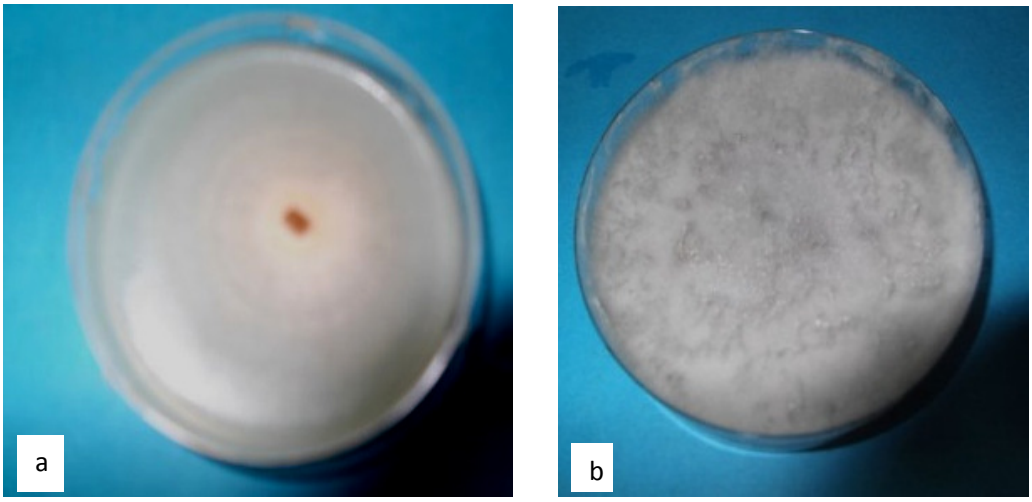

Photo 1 Aspects of *L. theonromae* in PDA culture at 2 to 4 days (a) and at 8 to 10 days (b) after plating

The mycelium was fast spreading, immersed, branched and septate. Shiny black pycnidia were produced on the medium surface after 5 to 10 days. Conidia were initially unicellular, ellipsoidal, hyaline, and thick-walled with granular content. Mature conidia were one-septate, dark brown with longitudinal striations. Based on its morphology the fungus was identified as *Lasiodiplodia theobromae* (Punithalingam 1976; Latha *et al*., 2009). Pathogenicity of the isolated fungi was tested in

the greenhouse at the International Institute of Tropical Agriculture (IITA), Cotonou Station, Republic of Benin. The pure culture of the isolated fungus was inoculated to 6-month old *Jatropha* healthy plants. One 1 x 1 cm portion was cut from the fungal pure culture on PDA as inoculum and placed on a 1 cm x 2 cm wounds cut from the stem of healthy plant. Another segment of unioculated PDA was also deposited on wounds of healthy plant to serve as uninoculated control plants. The deposited PDA portions with or without inoculum were coverd with moist cotton and wrapped with parafilm to maintain humidity during the first weeks. The treated *Jatropha* plants were regularly watered and followed in the greenhouse for six months. The fungus was consistently reisolated from all inoculated plants showing symptoms similar to the origin ones observed in the field. Uninoculated control plants did not show any similar of the disease. Similar symptoms of the disease on *J. curcas* were reported in many countries including Brazil (Pereira *et al*., 2009) Malaysia (Sulaiman & Thanarajoo, 2011) India (Rao *et al.,* 2011), Senegal (Terren *et al.,* 2012), and Nigeria (Zarafi & Abdulkadir, 2013), with *L. theobromae* identified as causal agent of the disease in all these above countries. Zarafi & Abdulkadir (2013) called the disease in Nigeria as die back « die back ». In India, other fungi were also asociated with the disease and included *Fusarium moniliiforme* Shel. (Kaushik *et al.,* 2001) and *Botryosphaeria dothidea* (Mougeot: E.M. Fries) Cesati & De Notaris (Rao *et al*., 2011). *Lasiodiplodia theobromae* is a ubiquitous pathogen of tropical woody trees, causing shoot blight and dieback of many plant species (Mohali *et al*. 2005; Latha *et al.,* 2009) including: dieback and gummosis of mango (Khanzada *et al*., 2004); black branch and dieback disease of cashew in Brazil (Cardoso *et al*. 2002); and collar rot of peanut in Virginia and North Carolina, USA (Phipps & Porter 1998). To our knowledge, this is the first report of *Lasiodiplodia theobromae* causing disease to *Jatropha* in Benin. The fungal culture is being sent for confirmation and collection in the National Collection of Fungi, Biosystematics Division, Plant Protection Research Institute, Pretoria.

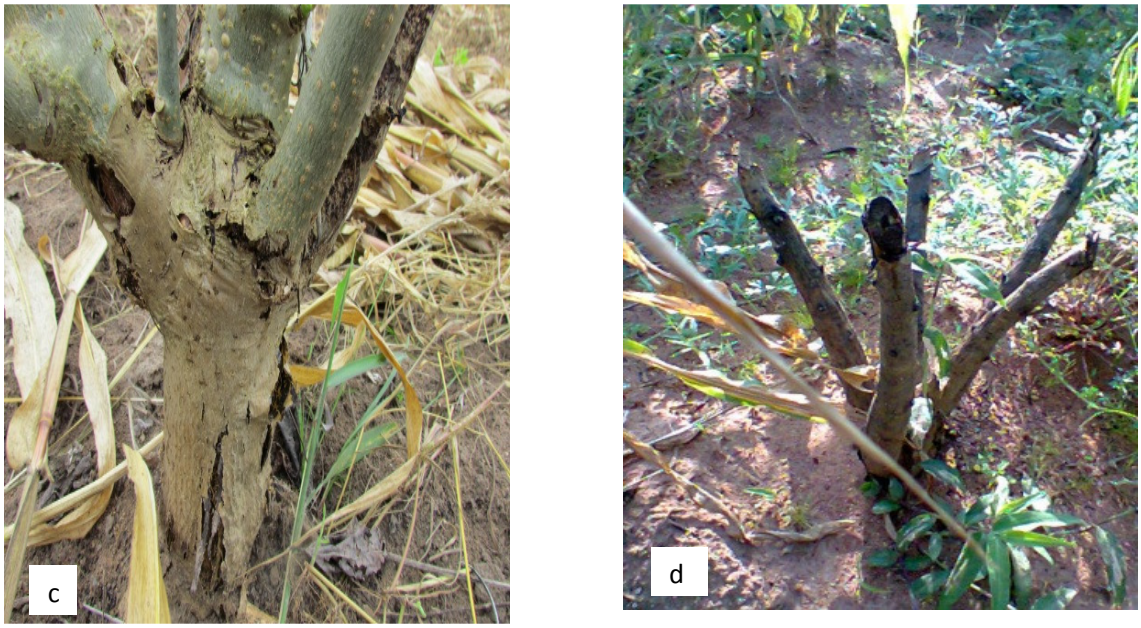

Photo 2 Plants of *Jatropha* showing symptoms of general wilting or « die back » with yellowing (a); leaves dropping (b) and collar rotting (c). *Jatropha* plants cut showed stem end blacking (d)

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