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Incidence and Management of *Colletotrichum gloeosporioides* Canker on *Jatropha curcas* L. in Ibadan, Southwestern Nigeria

[1]NWOGWUGWU, JO; [2*]BATCHO, AA; [3]TIME, I

[1]*Department of Forest Conservation and Protection, Forestry Research Institute of Nigeria, Ibadan, Nigeria*
[2]*Faculty of Agriculture and Environmental Sciences, Catholic University of the West Africa, Cotonou, Benin*
[3]*Department of Crop & Environmental Protection, University of Agriculture, Makurdi Nigeria*
**Corresponding author*: anicetbatcho1@gmail.com

ABSTRACT: The Impact of fungal pathogens on man is enormous, stretching from infecting humans to destroying a third of all food crops annually thereby causing economic losses and impacting global poverty. As a result, fungal disease incidence was assessed under natural field infection and data were collected randomly from 12 months old jatropha seedlings with cankers. Colletotrichum spp. isolated from the diseased sampled were interacted with known fungi and bacteria biocontrol agents. The experiment was carried out on completely randomized design (CRD) in the laboratory. Data were analyzed using ANOVA and statistically different means were separated using LSD ($p \leq 0.05$). The dual culture interaction proved that all the BCA used, possessed antagonistic potential, and could serve best for prophylactic protection. Plant diseases may be suppressed by the activities of one or more plant-associated microbes hence keeping them in check below threshold levels. This will help to maintain the quality and abundance of food, thereby mitigating hunger. Negative environmental consequences caused by excessive use and misuse of agrochemicals, requires that healthier and cost-effective approaches to disease management should be adopted.

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Colletotrichum gloeosporioides (Penz.) Penz. & Sacc. (teleomorph: *Glomerella cingulate*) is among the most important plant pathogens and it is an economic constraint to crop production worldwide (Ajay, 2014). This fungal organism has been reported to infect a wide range of commercially important crops worldwide. It is a wound causing (canker) and anthracnose pathogen of many plants including wild plant species. *Jatropha curcas* L. (physic nut) is a plant with potential for biodiesel production. It is a species of flowering plant in the spurge family, Euphobiaceae and native of America. The seed contain average 34.4% oil that can be processed to produce a high-quality biodiesel fuel (Achten *et al*., 2008). It has multipurpose uses in folks medicine and other industrial purposes and is believed to have promise to offer solution for the menace of climate changes, energy insecurity and rural poverty in developing countries. Plant diseases are one of the most important components that affects agriculture. They are the result of interaction between susceptible host, virulent pathogen, and favorable environmental conditions (Klopfensten *et al.,* 2009). Ecosystems vulnerability in sub Saharan Africa due to climate change will encourage new strains of emerging pest and diseases thereby increasing disease severity and crop losses (Elad and Pertot, 2014). Strange and Scott (2005), estimated loss of at least 10% in global food production due to diseases. Synthetic chemicals used in disease control do not only result in huge cost but also increase environmental threats and health hazards. Plant disease need to be properly diagnosed and managed sustainably to avoid the huge losses incurred by agroforests to secure global food security and raw materials. Savary *et al*., (2012), fungi genera cause more plant diseases than any other group of plant pathogens with over 8000 species known to cause diseases on different plants species and exhibiting diverse symptoms. Statistics from the 2009–2010 world harvest (www.fao.org) reports that crop losses induced by fungi on five of the most important crops globally (rice, wheat, maize, potatoes, and soybean) could feed 8.5% of the seven billion populations in 2011. Many tropical based crops and trees are affected by fungi without proper biodefense and means of tackling the diseases resulting in disastrous food shortages and economic global consequences. Therefore, the objective of this study is to investigate the incidence of *C. gloeosporioides* canker on *J. curcas* and to employ the application of microbial antagonists as biological control agents for the sustainable disease management of this fungal

**Corresponding author: anicetbatcho1@gmail.com*

pathogen on *Jatropha curcas* thereby decreasing the use of agrochemicals in crop cultivation.

MATERIALS AND METHODS

Disease incidence: Disease incidence was monitored from a year old jatropha plot earlier established from four jatropha accessions at the experimental plot of Plant pathology section of Forestry Research Institute of Nigeria.

Isolation: Fungal pathogen was isolated from canker infected seedlings. The isolated pathogen was purified and maintained by sub-culturing on acidified PDA until pure cultures were obtained. Potato Dextrose Agar medium was used for this study.

Identification: The purity of the cultures was confirmed by the colony characteristics and microscopic identification by Barnett and Hunter (2001) identification key. Stock cultures of three potential biological control agents (BCA) or antagonists viz. bacteria (*Bacillus subtilis*, *B. cereus*, and fungi (*Trichoderma pseudokoningii*) were collected from the plant pathology laboratory, Crop Protection and Environmental Biology, (CPEB), University of Ibadan. The antagonists were resuscitated and maintained by sub-culturing on freshly prepared sterile Potato Dextrose Agar (PDA) medium. The accession with least record of disease incidence was adopted for the biological control experiment.

Dual culture interaction: A dual culture using the dixenic technique was used to study antagonism and mode of parasitism. Five-millimeter (5mm) culture disc of the actively growing test fungus was inoculated at four equidistant points of the 9cm petri plate and the agar disc of the antagonist was inoculated at the center of the plate, inoculated plates with fungus alone served as the control. The experiment was carried out in three stages: Both the antagonist and target pathogen inoculated in petri plate same day simultaneously; Inoculating target pathogen 24hrs and 48hrs before the antagonist and inoculating the antagonists 24hrs and 48hrs before the target pathogen. They were incubated after inoculation at 28-30°C. Observations and measurements were taken from 24hours intervals to 72 hours after inoculation. The growth diameter of the target organism and BCA were measured using transparent measuring rule in an axenic and dixenic cultures.

Data collection and analysis: The field experiment was carried out on Randomized Complete Block Design (RCBD) with four replicates while the invitro experiment was designed in a Completely Randomized Design (CRD) and all the treatments were replicated three times. Data were collected on percentage disease incidence in the field which was calculated as the percentage of the plants infected in a random sample of 25 plants. Data for the biological control assessment: radial growth of the fungal mycelia both for the target organism (pathogen) and the antagonists were measured at 24 hours interval and percentage growth inhibition at dual culture interaction was recorded. The data obtained were subjected to analysis of variance (ANOVA). Mean values of different treatments in each experiment were compared at 5% (p= 0.05) level of significance. Where they were significantly different the means were separated using least significant difference (LSD) test.

RESULTS AND DISCUSSION

Jatropha accessions were susceptible to canker disease caused by *C. gloeosporioides* under natural infection. The observed symptom on the infected seedlings were round to irregular sunken, flattened cracked dead areas on the shoot and twig of infected seedlings (Plate 1a, b & c). This pathogen caused lesions on the twig and deep necrosis on the shoot. The disease girdled the shoots and led to defoliation and death of the affected part. The internal section of the infected twigs when sliced open, showed browning and death of the vascular bundle which was the cause of death as the nutrient and water could not be conducted to the growing part. Isolations made from the infected samples consistently yielded *C. gloeosporioides* on cultural and microscopic examinations Plate 1c & d). There were significant differences among the accessions in canker disease incidences with a marked diseased progression recorded between intervals at 12 months (July) and 15 months (October). The accession, Ex-Misau (Tab. 1.) recorded lowest incidences (5.8/ 6.2) and was therefore used for the second experiment of test of BCAs. The test of antagonism showed that there was parasitism by the mycoparasite *T. pseudokoningii* on the pathogen thereby covering the 9cm plate after 72hrs of simultaneous interaction.

The Bacterial antagonist also performed well by inhibiting mycelia growth and density (Plate. 1. d, i & ii) and (Tab. 2,3 & 4.). The results recorded showed that when the antagonists were paired simultaneously with the target pathogen, there inhibition (Table. 2) better than when the 24 hrs. Old pathogen was paired with the antagonist (Table.3). Best antagonism was recorded with 24 and 48hrs. Antagonists were paired with fresh inoculum of the pathogen (Tab. 4). In all the interactions there were significant statistical difference between the mycelia growth of the control (axenic) and the diaxenic/ paired cultures.

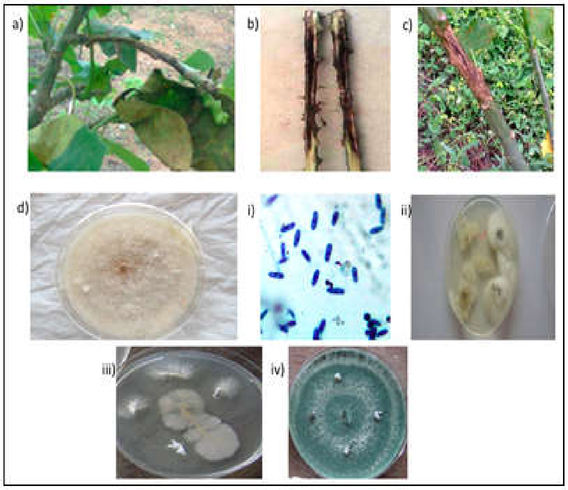

Plate 1. Showing disease symptoms caused by *Colletotrichum gloeosporioides*:twig necrosis (a); longitidunal section of infected twig (b); shoot canker; pure culture of *C. gloeosporioides* (c); photomicrograph of the isolate (d). *C. gloeosporioides* paired with different antagonists: (i) with *B. subtilis*; (ii) with *B. cereus*; (iii) with *T. viride* after 7days of simultaneous pairing.

Table 1. Incidence of canker on shoots of *J. curcas* accessions under natural infection at 12 and 15 months (July and October) after planting (MAP)

	% incidence	
Accession	1st	2nd
Ex – Basirika	9.8	17.8
Ex-Mbatdiya	10.3	17.5
Ex – Misau	5.6	6.2
Ex – Kano	11.5	18.3
$LSD_{0.05}$	1.68	0.45

Table 2. Mycelia growth inhibition on target organism paired with antagonists simultaneously

Pathogen	antagonists	Mycelia growth (cm)
	Control	2.97
	T. pseudokoningii	1.27
Colletotrichum sp.	*B. subtilis*	1.17
	B. cereus	1.57
	LSD	1.30

Table 3. Mycelia growth inhibition on 24 and 48 hours-old target organisms paired with antagonists

Pathogen	Antagonists	Time of inoculation/ mycelia growth (cm)	
		24h	48h
	Control	2.90	2.26
Colletotrichum sp.	*T. pseudokoningii*	2.78	2.13
	B. subtilis	2.82	1.71
	B. cereus	2.72	2.02
	LSD	2.10	1.87

All the tested antagonists reduced the mycelial density of the test pathogen, *C. gloeosporioides*. The two Bacillus spp. inhibited the growth of the fungal pathogen and this was seen in the typical inhibition zones seen on the cultures thereby causing a fungistatic effect on the test pathogen. The test pathogen was outcompeted in all the dual cultures with the Trichoderma spp. at all stages of inoculations. In all the different stages of dual culture experiment, introducing the biocontrol in the culture days before pathogen gave best result followed by simultaneous pairing.

Table 4. Mycelia growth inhibition on target organism paired with 24 & 48hr-old antagonists before the pathogen

Pathogen	Antagonists	Time of inoculation/ mycelia growth (cm)	
		24h	48h
	Control	2.97	2.97
Colletotrichum sp.	*T. pseudokoningii*	0.00	0.00
	B. subtilis	1.48	0.55
	B. cereus	1.32	0.34
	LSD	0.01	1.2

This may give indication for the best application time and potential of these BCAs. This suggests that these organisms will function best as prophylactic treatment rather than in therapeutic application, such as in preservation/ seed dressing (protectants) and soil application treatment before planting for effective management before the pathogenic inoculum. It can also be effective when applied during planting or ploughing rather than when the disease inoculum has already been established. In this study, shoot canker on jatropha was caused by soil borne fungal pathogen, *C. gloeosporioides* which was in line with the findings that *C. gloeosporioides* cause perennial canker disease on apple (Malus spp) and dieback on many plant species (Nouri *et al.,* 2019). Cankers are areas of necrotic bark and outer sapwood that result from the invasion of stems by a range of fungal pathogens (Old *et al*., 2000). The genus, Colletotrichum is a known pathogen causing anthracnose which is observed as necrotic areas bounded by brown, black or discoloured margins thereby predisposing the plant other secondary infections such as cankers. The Cassava anthracnose disease (CAD) caused by *Colletotrichum gloeosporioides* Penz f.sp manihotis Chev is the most important fungal disease of cassava in the field causing the most outstanding effect of severe stem damage, canker on stem, wilting of leaves and diebacks in Nigeria. Latunde (2007) reported the antagonistic efficacy of *Trichoderma koningii* on *Sclerotium rolfsii* causing damping off, blight and wilting on tomato.

Direct parasitism of fungal plant pathogens has been recorded as mechanism of antagonism for Trichoderma species. Also, the production of hydrolases enzymes such as chitinase and glucanase help them in degrading the cell walls of plant pathogens thereby killing their targets. The antagonistic bacillus species are noted for employing antibiosis in their mode of antagonism by enzymatically digesting the chitinous cell wall of their target enemy. Therefore *B. subtilis* and *B. cereus,* used in this work showed great antagonistic potential in inhibiting the growth of the target pathogen invitro with a clear zone of inhibition in the dixenic culture.

It is critical to sustainably manage plant diseases to maintain the quality and abundance of food, feed, and fiber produced by growers around the world. The hazards of overuse of agrochemicals calls for employing biological controls. This is the use of alternative inputs to synthetic chemicals for controlling pests and diseases. It refers to the purposeful utilization of introduced or resident living organisms, other than disease resistant host plants, to suppress the activities and populations of one or more plant pathogens.

Conclusion: *Jatropha curcas* was susceptible to the canker causing fungal pathogen, *Colletotrichum gloeosporioides* in this study. The wide host range of this pathogen and its ability to infect plants, both on the field and at postharvest, inciting different symptoms on leaves, flowers, root, and fruits makes its management problematic. The use of microbial antagonist is a biological control strategy and a sustainable management approach to managing this pathogen.

REFERENCES

Achten, WM; Verchot, L; Franken, YJ; Mathijs, E; Singh, VP; Aerts, R; & Muys, B (2008) Jatropha bio-diesel production and use. *Biomass and bioenergy*, *32*(12), 1063-1084.

Ajay Kumar, G (2014) *Colletotrichum gloeosporioides*: biology, pathogenicity and management in India. *Journal of Plant Physiology and Pathology*, *2*, 2-11.

Elad, Y; Pertot, I (2014) Climate change impacts on plant pathogens and plant diseases. *Journal of Crop Improvement*, *28*(1), 99-139.

Gautam, J (2014) Plant Physiol Pathol, 2:2 http://dx.doi.org/10.4172/2329-955X.1000125

Klopfenstein, NB (2009) *Approaches to predicting potential impacts of climate change on forest disease: an example with Armillaria root disease*. US Department of Agriculture, Forest Service, Rocky Mountain Research Station.

Latunde-Dada, AO (2007) Biological control of southern blight disease of tomato caused by *Sclerotium rolfsii* with simplified mycelial formulations of *Trichoderma koningii*. Plant Pathology, 42 (4): 522 – 529.

Nouri, MT; Lawrence, DP; Holland, LA; Doll, DA; Kallsen, CE; Culumber, CM; Trouillas, FP (2019) Identification and pathogenicity of fungal species associated with canker diseases of pistachio in California. *Plant Disease*, *103*(9), 2397-2411.

Old, KM; See, LS; Sharma, JK; Yuan, ZQ (2000) *A manual of diseases of tropical acacias in Australia, South-East Asia and India*. CIFOR.

Savary, S; Ficke, A; Aubertot, JN; Hollier, C (2012) Crop losses due to diseases and their implications for global food production losses and food security.

Strange, RN; Scott, PR (2005) Plant disease: a threat to global food security. *Annual review of phytopathology*, *43*.

Vieira, WA; Michereff, SJ; de Morais, MA; Hyde, KD; Câmara, MP (2014) Endophytic species of Colletotrichum associated with mango in northeastern Brazil. *Fungal diversity*, *67*(1), 181-202.