

1 **First report of *Colletotrichum cliviicola* causing anthracnose disease of cowpea (*Vigna unguiculata* L.**  
2 ***Walp*) in Nigeria**

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8 Cowpea (*Vigna unguiculata* L. Walp) is a staple crop for millions of people in sub-Saharan Africa. However,  
9 its production is challenged by various abiotic and biotic constraints, including fungal diseases. In February  
10 2020, around 10% of cowpea plants in IITA-Ibadan research plots (N7°29'49" E3°53'49") had symptoms of  
11 cowpea anthracnose disease (CAD). Symptoms included reddish brown spots, necrotic lesions, and vein  
12 streaks (Fig. 1). Diseased leaves were collected and taken to the laboratory, cut into small discs (3 mm in  
13 diameter) at advancing edges of lesions, and surface disinfected. Dry leaf discs were plated on PDA and  
14 incubated at 28°C for 5 days and sub-cultured in PDA for another 7 days. Isolates yielded phenotypes similar  
15 to *Colletotrichum* spp. (Fig. 2). DNA templates of four isolates (CC17 NG, CC19 NG, CC21 NG, and CC24 NG)  
16 were amplified using primers of the actin (ACT; ACT512F and ACT783R) (Carbone and Kohn, 1999) and  
17 glyceraldehyde-3-phosphate dehydrogenase (GAPDH; GDF and GFR) (Templeton *et al.*, 1992) genes and  
18 sequenced. The sequences were deposited in GenBank (accession numbers OP716557 to OP716560 for ACT  
19 and OP716561 to OP716564 for GADPH). BLASTn results on NCBI showed 98-100% identity of the four  
20 isolates with *C. cliviicola*. A bi-locus phylogenetic tree revealed that the isolates belong to the species *C.*  
21 *cliviicola* (Fig. 3) when compared with existing sequences in the GenBank (Table 1). To fulfill Koch's  
22 postulates, pathogenicity of each of the four *C. cliviicola* isolates was confirmed on 2-week-old cowpea  
23 plants cv. Ife Brown in screenhouse assays. Inocula were prepared from 7-d-old cultures washed with sterile  
24 water containing 0.1% TWEEN®20. Fungal suspensions were adjusted to 10<sup>6</sup> conidia/ml. Inoculations were  
25 carried out using the brush method. Leaves inoculated with sterile water containing 0.1% TWEEN®20 served  
26 as negative controls. Plants were kept in the screenhouse at room temperature for 21 days. All four *C.*  
27 *cliviicola* isolates produced CAD symptoms on inoculated leaves, while control leaves remained  
28 asymptomatic (Fig. 4). Each inoculated isolate was successfully re-isolated from symptomatic tissues and

29 their identity confirmed. The fungus *C. cliviicola* is distributed in tropical and subtropical regions and has a  
30 wide host range, including several legumes (Damm *et al.* 2018). To our knowledge, this is the first report of  
31 *C. cliviicola* causing CAD in Nigeria and the world. There is the need to conduct a comprehensive distribution  
32 survey and develop appropriate control strategies in Nigeria. In addition, breeding for resistance to CAD in  
33 Nigeria should gear the efforts to all causal agents of the disease that occur across the country because  
34 historically CAD has been attributed to *C. lindemuthianum* and *C. destructivum*.

## 35 **References**

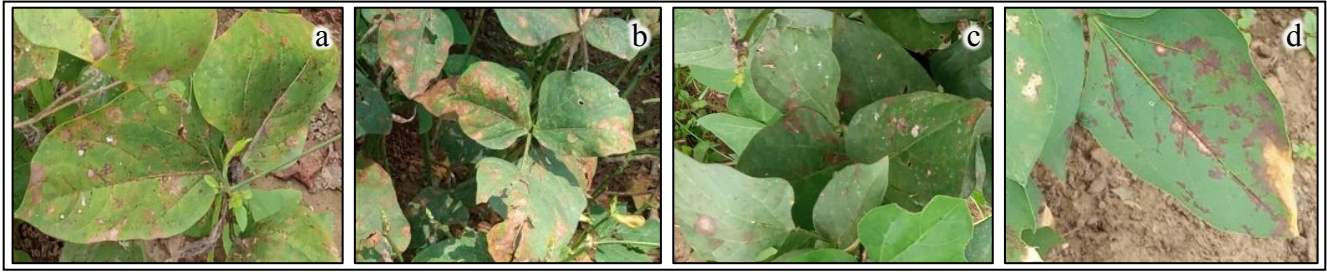
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39 Carbone, I., and Kohn, L. M. 1999. A method for designing primer sets for speciation studies in filamentous  
40 ascomycetes. *Mycologia* 91:553–556.

41 Damm, U., Sato, T., Alizadeh, A., Groenewald, J. Z., and Crous, P. W. 2018. The *Colletotrichum*  
42 *dracaenophilum*, *C. magnum* and *C. orchidearum* species complexes. *Stud. Mycol.* 90:71–118.

Table 1. GenBank information of reference sequences of actin and GADPH genes of the various *Colletotrichum* spp. and *Monilochaetes infuscans* used for phylogenic tree construction.

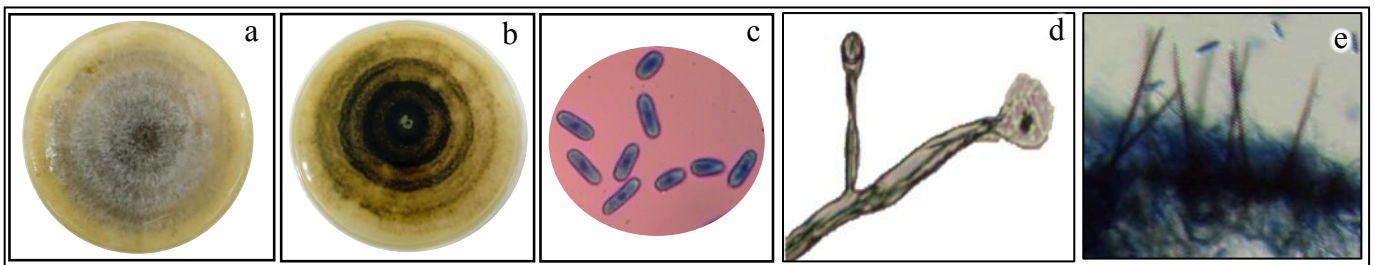
Reference species	Isolate	Accession number		Country of origin	Crop
		Actin	GADPH		
<i>C. lindemuthianum</i>	CBS 130841	JX546627	JX546723	China	-
	CG2	MT424581	MT411505	India	<i>Phaseolus vulgaris</i>
<i>C. cliviicola</i>	I5H	MF940416	MG001923	Brazil	<i>Glycine max</i>
	YN8-2	MH622565	MH681366	China	<i>Mangifera indica</i>
	HN18	MW971912	MW971916	China	Rubber tree
	CMM3366	KY498353	KY498351	Brazil	<i>P. lunatus</i>
<i>C. destructivum</i>	AH1A2	KU251679	KU251946	China	<i>Camellia sinensis</i>
	CBS 119187	KM105430	KM105575	-	-
	IMI 387103	KM105431	KM105576	-	-
<i>Monilochaetes infuscans</i>	CBS 869.96	JQ005843	JX546612	-	-

1 **Supplementary files.**

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3 Fig. 1. Plants of cowpea cultivar Ife Brown showing symptoms of anthracnose disease (a-d).

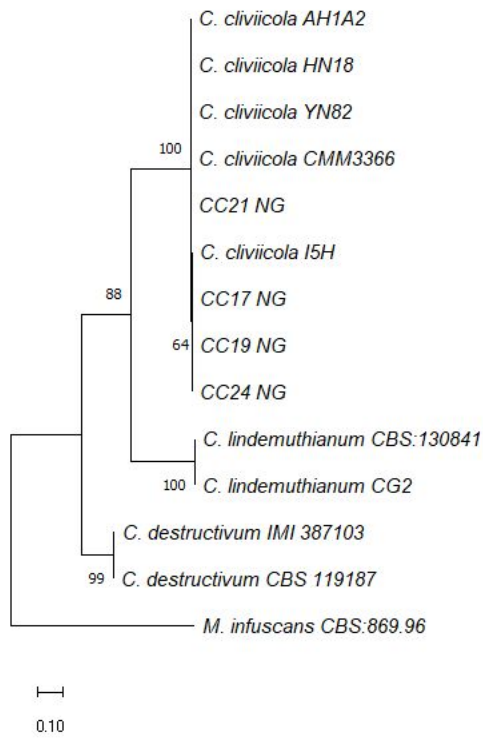
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6 Fig. 2. Morphology of *Colletotrichum cliviicola* sp. Front (a) and back (b) view of the culture on PDA; conidia  
7 (c); appressorium (d); and setae (e).

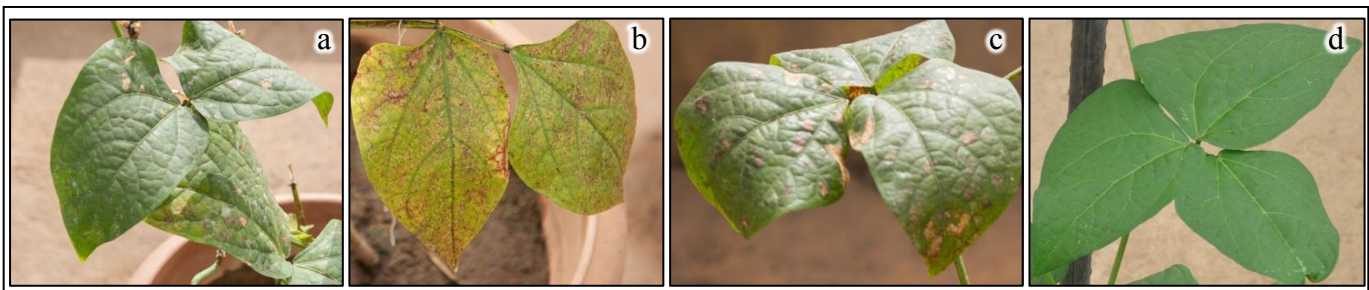
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10 Fig. 3. Phylogenetic tree showing *Colletotrichum* spp. associated with cowpea plants in Nigeria and related  
 11 spp. based on the Bayesian inference of combined sequences of portions of the glyceraldehyde-3-phosphate  
 12 dehydrogenase (GAPDH) and actin (ACT) genes. The posterior probability is indicated near the branch nodes.  
 13 *Monilochaetes infuscans* CBS 869.96 was used as an outgroup. Isolates with a NG legend represent those  
 14 obtained from the cowpea plants from Nigeria.

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17 Fig. 4. Symptoms of anthracnose disease in artificially inoculated plants of Ife Brown cowpea cultivar (a-c)  
 18 and water-uninoculated control plant (d).

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