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Short Communication

First Report of *Cophinforma atrovirens* and *Trichoderma ghanense* Associated with Inflorescence Dieback of Cashew and Soil in Nigeria

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Cashew tree (*Anacardium occidentale* L.) is a plant of great economic value not only to growers but other players in the value chain. The crop is mostly cultivated for the nut and can thrive in many agro-ecologies of Nigeria. A case study survey of major cashew plantations and soils of major growing areas in North Central, Nigeria comprising of Oro (Kwara State) and Ejule (Kogi States) showed typical symptom of dieback on cashew panicles recorded in around 35% and 45% of established cashew trees in the plantations respectively. This disease situation typically referred to as inflorescence dieback of cashew in Nigeria, and this also occur on cashew seedlings both in the nursery and newly established fields. This disease condition and incidence has earlier been associated with *Lasiodiplodia* species, while the isolated fungi from the soil have priorly been classify as *Trichoderma harzianum.* The molecular study in this situation depicts the organisms as *Cophinforma atrovirens* and *Trichoderma ghanense* respectively in which very close morphological resemblances were observed.

The isolation from infected floral tissues of cashew inflorescences revealed a fungal colony showing cottony whitish colour at initial and later turned gray on Potato Dextrose Agar (PDA). The isolate did not sporulate after 35 days of incubation on half strength PDA. Mycelial colony were aseptically transferred into a sterile Petri Dish, the mycelial mat was macerated in with sterile distilled water and the volume made up to one litre. The solution was sprayed on healthy cashew inflorescences in replicates and incubated at field temperature (37°C ± 2).

The colony growth rate of 11.95 mm and 14.09 mm were recorded in isolates from Oro and Ejule respectively, while the colony texture and colour comprised of fluffy mouse grey (obverse) and greyish blue (reverse) were the same in both ecologies (Figure 1).

Genomic DNA was extracted from the mycelia of the fungi in pure culture. The fragments of the rDNA internal transcribed spacers (ITS), β-tubulin (TUB2) and the translation elongation factor 1-α (EF-1α) genomic regions were amplified by polymerase chain reaction (PCR) using primers ITS4/ITS5, βT2a/βT2b and EF1-688F/EF1-1251R, respectively following the procedure described by Coutinho., *et al* [1]. The PCR products were sequenced and the sequences obtained were deposited in GenBank. Multiple alignments of the combined data set of the genomic regions and representative sequences obtained from GenBank (NCBI; www.ncbi.gov/) were used for phylogenetic analysis by maximum parsimony (MP) and maximum likelihood (ML) models. The tree topology was tested by the bootstrap method with 1,000 replicates [2,3].

Figure 1: Mycelial mat of *Cophinforma atrovirens* in Kogi (A) and Kwara (B) states.

The pathogenicity of *Cophinforma atrovirens* was evaluated on cashew inflorescences by spraying a prepared suspension onto the cashew trees at pre and full bloom stages of flower development. Twenty (20) inoculated inflorescences and twenty non-inoculated ones which serve as control were tagged with colour codes. The inflorescences were made airtight for 7 days under natural field infection condition with temperature range of 27°C - 34°C.

The evolutionary history was inferred by using the Maximum Likelihood method based on Tamura and Nei [4]. The tree with the highest log likelihood (-1432.6144) is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The analysis involved 4 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total

of 438 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 [5].

The typical symptom of dieback disease was observed for fifteen days after artificial inoculation and evolved to completely cover the entire flower panicles within a week (Figure 2). The re-isolation from infected flora tissues following the procedure of Koch's postulates showed the presence of a fungus with the same morphological characteristics as the originally isolated culture and substantiated by molecular characterization. *Cophinforma atrovirens* belong to Botyosphaeriaceae, and an important genus in the family [3].

Figure 2: Cashew inflorescence showing healthy panicles (A), partial dieback (B) and total dieback (C).

The fungus has also been previously reported by Dissanayake., *et al*. [2] to be associated with dieback and cankers in woody plants. The complete rot and decaying death of the shoots in cashew seedling caused by *C. atrovirens* have also been reported by Cardoso., *et al* [6]. Li., *et al*. [7] also reported the presence of *C. atrovirens* established by molecular tools as a pathogen in a survey of disease symptoms; stem canker, shoot and twig blight in plantations of *Dimocarpus longan* adjacent to *Eucalyptus* to in China, while Mehl., *et al*. [8] reported *C. atrovirens* as pathogen of *Pterocarpus angolensis* in South Africa. The pathogenicity study, morphological descriptions and molecular information confirmed that *Cophinforma atrovirens* is associated with cashew dieback disease in Nigeria.

However, in earlier studies related to this course in Nigeria, only *Lasiodiplodia theobromae* which has a close resemblance with *C. atrovirens* in colony colour, texture and morphology have been reported to be associated with inflorescence and shoot dieback disease in cashew [9-14]. But the association and pathogenicity study on *C. atrovirens* cultured from cashew inflorescences dieback has been established by molecular characterization of the isolate in the study areas (Figure 3). This pathogen poses a threat to cashew production across growing ecologies of Nigeria as it affects many plantations by hindering nut initiation. This study is the first report of *C. atrovirens* on cashew trees in Nigeria and information on existence of *Trichoderma ghanense* in cashew soil is also scarce and likewise were information on *Aspergillus allahabadii* strain NN046949 in Nigeria (Figure 3). This have been recorded in two major cashew growing states, Kogi and Kwara. Further studies which will involve survey of many growing ecologies of Nigeria will be required to ascertain the epidemiology, possible diversity of the pathogen and associated ones and establish their virulence status.

Figure 3: Molecular and evolutionary relationship by maximum likelihood method.

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