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First Report of *Cophinforma tumefaciens* Pathogenic to the Invasive Tree *Neltuma juliflora* in Brazil

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Neltuma juliflora is a medium-sized, fast-growing species with strong nitrogen-fixing capacity and high tolerance to semi-arid and arid conditions (Gebrehiwot and Steger 2024). Native to the Americas, it was introduced to northeastern Brazil in 1942 to enhance drought resilience and restore degraded areas (Damasceno et al. 2020). Its rapid spread in non-native environments has led to its classification as an invasive species that threatens local biodiversity (Lima 2005). Today, it is regarded as one of the world's most aggressive invasive plants.

In September 2023, pods of *N. juliflora* (Fabaceae) with dark superficial lesions and internal rot were observed in Petrolândia, Pernambuco, Brazil (Figure 1). Ten plants were examined, and four showed pod rot symptoms. Tissue fragments from lesion margins were surface-disinfected, plated on potato dextrose agar (PDA) and incubated at 28°C. Emerging colonies were transferred to water agar for hyphal-tip isolation and maintained on PDA. Colonies were olive green to dark grey on the obverse and olive green to black on the reverse (Figure 2). Conidia were hyaline, aseptate, ellipsoid to obovoid, measuring 17.4–30.2 × 6.5–10 µm (average 24.3 × 7.4 µm; $n = 50$) (Figure 3). Three isolates were identified as *Cophinforma* spp. and deposited in the Coleção de Fitopatógenos da Universidade Federal de Alagoas (COUFAL) as COUFAL0602, COUFAL0603, and COUFAL0604.

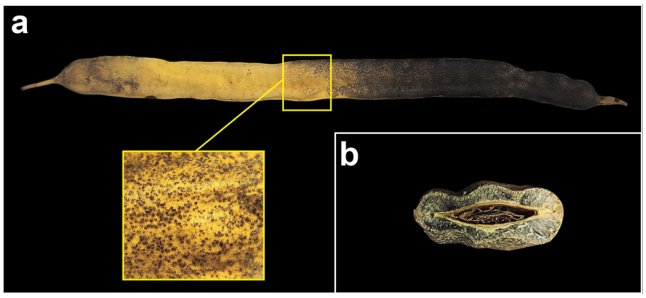


FIGURE 1 | (a, b) Symptoms observed on pods of *Neltuma juliflora* in the field, including dark lesions on the pod surface and dry internal rot.

Genomic DNA was extracted from seven-day-old cultures following the CTAB protocol (Doyle and Doyle 1987). ITS, *tef1-α*, and *tub2* loci were amplified for multilocus analysis using standard primers. Sequences were deposited in GenBank (ITS: Accession Nos. PV544873–PV544875; *tef1-α*: PV553489–PV553491; *tub2*: PV553492–PV553494). Bayesian inference based on the concatenated dataset placed all isolates within *Cophinforma tumefaciens*, with a posterior probability of 1.00 (Figure 4).

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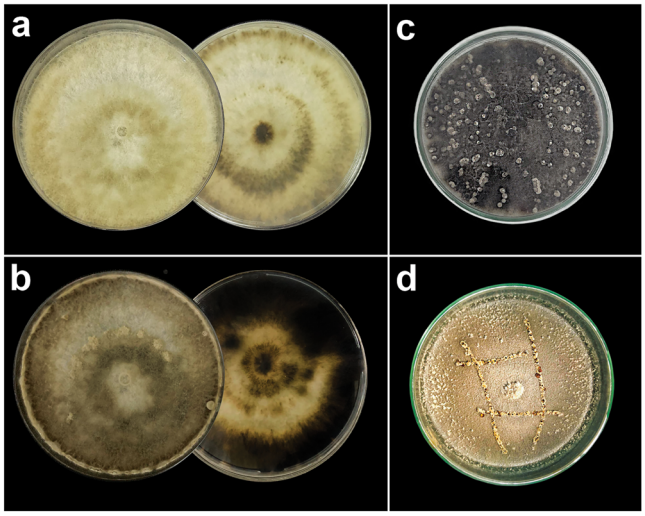


FIGURE 2 | Colony morphology of *Cophinforma tumefaciens* on potato dextrose agar (PDA) after 4 (a) and 14 days (b) incubation. Pycnidia formed on PDA after 20 days (c). PDA supplemented with pine needles to induce pycnidial formation (d).



FIGURE 3 | Hyaline, aseptate, unicellular conidia of *Cophinforma tumefaciens*, ellipsoid to obovoid in shape. Bar = 10 μm.

Although molecular and phylogenetic data generally enable reliable species delimitation in *Cophinforma*, the absence of *tef1-α* sequences for the type species *C. mamane* limits robust comparisons (Phillips et al. 2013).

Pathogenicity was confirmed through assays on mature pods and healthy 90-day-old seedlings of *N. juliflora*. For pod inoculation, mycelial plugs were taken from 7-day-old PDA cultures; for seedlings, colonised parboiled rice grains served as inoculum. Inoculated pods and seedlings were kept in a humid chamber at 28°C for 2 days. Pods developed dark surface lesions and dry internal rot four days post inoculation (dpi) (Figure 5). Seedlings also showed symptoms from four dpi, including cankers on woody tissues (Figure 5), followed by resinous exudation and branch dieback seven dpi. Controls remained symptomless. Fungi reisolated from symptomatic tissues matched the original isolates, fulfilling Koch's postulates.

C. tumefaciens is globally distributed and recorded on various hosts. In Brazil, it has been reported to cause stem rot and dieback in cashew (Cardoso et al. 2019). To our knowledge, this is the first report of rot symptoms caused by *C. tumefaciens* on *N. juliflora* worldwide.

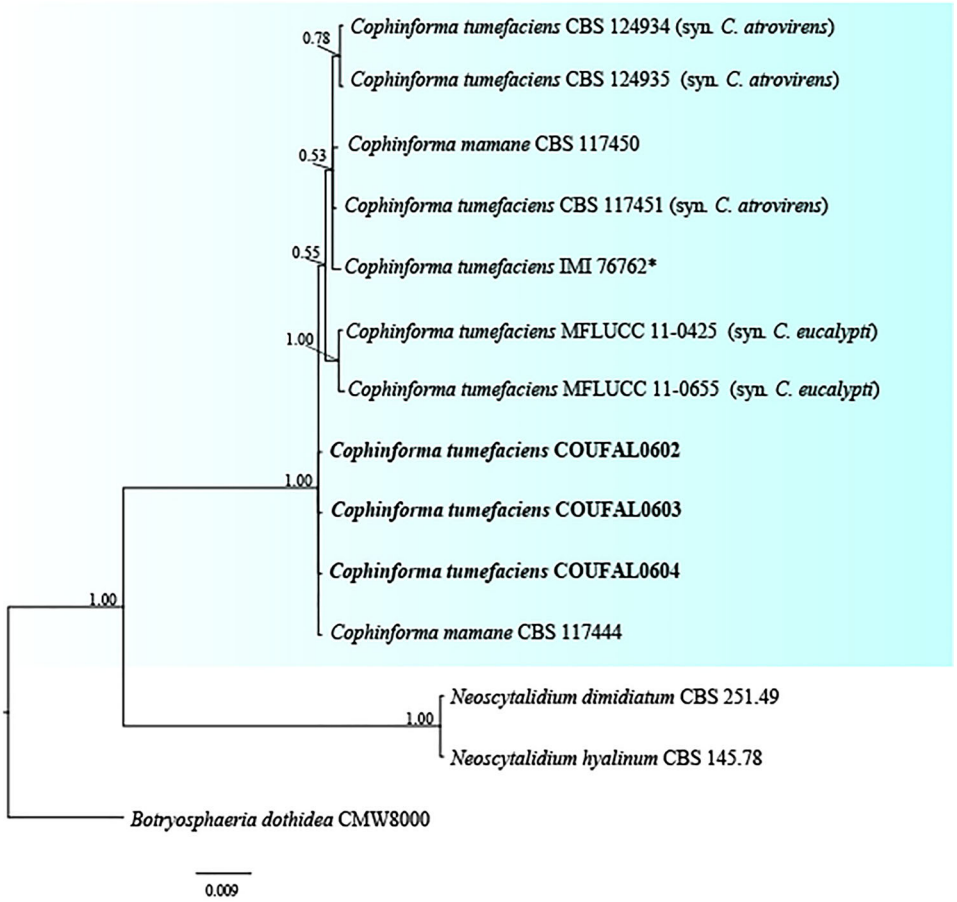


FIGURE 4 | Bayesian phylogenetic tree constructed utilising MrBayes version 3.2.6. of *Cophinforma* isolates from *Neltuma juliflora*. The tree was built using concatenated sequences of the genes ITS, *tef-1α*, and *tub2*, bootstrap with 1000 replications. Bayesian posterior probability values are indicated above nodes. The sequences generated in this study are highlighted in bold. The type-strains are marked with an asterisk. This tree is rooted in *Botryosphaeria dothidea*.



FIGURE 5 | Pathogenicity test conducted on mature pods and seedlings of *Neltuma juliflora*: (a) symptoms induced on inoculated pod by isolates COUFAL0602, COUFAL0603, and COUFAL0604; (b) asymptomatic pod (negative control); and (c) dieback symptoms induced in the seedling by isolate COUFAL0604.

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