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Postharvest stem-end browning (SEB) disease in ripe mango (*Mangifera indica* L.) cultivar TomEJC

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Abstract Browning of the fruit peel around the stem-end region of ripe mangoes, cultivar TomEJC, was observed in a commercial mango plantation in Dambulla, Sri Lanka (Central Province) in 2016. The condition was recognized as a fungal disease, new to mango and named stem-end browning (SEB). The disease commenced as a diffused, yellowish-brown ring around the pedicel and expanded with ripening, covering the upper one-third of ripe fruit. The lenticels within the affected area darkened. A 2–3 cm diameter peel, immediately surrounding the pedicel and the pulp underneath, turned necrotic. The disease did not lead to any softening of the fruit tissues. Ten fungi, isolated from the peel of symptomatic or health fruit, were identified by multi-gene phylogenetic analysis as nine species, *Curvularia dactyloctenicola, Diaporthe endophytica, D. eugeniae, D. pseudophoenicicola, Fusarium mangiferae, Neocosmospora* sp., *Neofusicoccum brasiliense, Neopestalotiopsis rhizophorae* and *Pestalotiopsis adusta.* Most of these fungi could also be isolated from the pedicel or the peel of younger, developing fruits, 2, 4, 6, 8, 10 or 12 weeks after anthesis. Pathogenicity of isolates was tested and confirmed. All nine species produced lesions, slightly variable in size, shape or colour from each other, 8–10 days after inoculation. Different colour forms of the SEB were observed in certain fruit harvesting seasons, probably depending on differences in pathogen combination and dominance. SEB substantially reduced fruit quality, incurring 12–27% postharvest fruit losses. The study describes symptomatology, inoculum source, mode of fruit infection, the identity, phylogeny and pathogenicity of fungi isolated.

Keywords Phylogenetic analysis · Pathogenicity · *Diaporthe* spp. · *Neofusicoccum brasiliense* · *Fusarium mangiferae* · *Pestalotiopsis adusta*

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Introduction

Mango (*Mangifera indica* L., family Anacardiaceae) is native to South Asia viz. Eastern India, Burma and, the Malay Archipelago (Singh & Singh, 2012). Mango and cashew (*Anacardium occidentale* L.) are among the species of highest economic and social importance belonging to the Family Anacardiaceae that consists of eight genera and 22 species in Sri Lanka. Mango fruit is a large, fleshy drupe with edible mesocarp, and usually one seed (Meijer, 1983), the size and shape vary depending upon the cultivar.

Many postharvest diseases of tropical and subtropical fruits develop from the stem-end and most of them lead

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to stem-end rots (SER). SER occurs in a variety of fruits including, mango, avocado (*Persea americana* Mill.), papaya (*Carica papaya* L.*)* and citrus. Fungal pathogens known to be causing the SER in mango are *Dothiorella dominicana*, *Dinoderus mangiferae*, *Lasiodiplodia theobromae*, *Phomopsis mangiferae*, *Cytosphaera mangiferae, Pestalotiopsis* sp. (Johnson et al., 1991, 1992), *Alternaria alternata* and *Colletotrichum gloeosporioides* (Johnson et al., 1992; Prusky et al., 2009), *Pseudofusicoccum kimberleyyense* or *Neoscytalidium dimidiatum* (Li et al., 2021). The major SER pathogens in mango are botryosphaeriaceous species.

All fungi that are known as causing mango SER occur endophytically in the stem tissue of mango prior to inflorescence emergence (Johnson et al., 1992). Endophytic colonization of inflorescence and pedicel tissue was the primary route of infection for fruit which develop SER during ripening. During the endophytic stage, the fungus colonizes the phloem and xylem of the fruit at stem-end (Galsurker et al., 2018; Johnson et al., 1992). Moreover, systemic colonization of extracambial tissues of stem, inflorescence and fruit pedicel tissue occurs (Galsurker et al., 2018). *Dothiorella* spp., *Phomopsis mangiferae*, *Pestalotiopsis* sp. and *Cytosphaera mangiferae* gradually colonize the inflorescence, reaching the pedicel tissue of fruit, 8 weeks after flowering. The earliest indicator of SER incidence at harvest was the infection level in peduncle tissue sampled 11 weeks after flowering (Johnson et al., 1991). Some of the fungi in the stem-end make transition from endophytic to necrotrophic and become pathogenic during fruit ripening. In ripe mangoes, watery decay develops around the stem-end which rapidly expands through the pulp tissues, softening the entire storage parenchyma tissues in the upper half of the fruit.

SER symptoms produced in mango also vary with the pathogens involved in causing the disease. Fast moving dark lesions are due to infection by botryosphaeriaceous fungi, including *L. theobromae*. Steel grey mycelium is developed by *Neofusicoccum* species over the surface on fruits affected. Pycnidia may develop around the stem-end particularly with *N. parvum* infections. Symptom development is slower with *Diaporthe mangiferae* (synonym: *Phomopsis mangiferae*) and a dark lesion of more uniform radius develops at the stem-end. SER caused by *D. mangiferae* occurs more frequently in fruit from dry areas (Johnson et al., 1990). Past studies have revealed that the fungi causing SER in different regions of the world may vary, *Neofusicoccum parvum* in Australia (Johnson et al., 1990), *L. theobromae* in Tropical Asia, *Pseudofusicoccum kimberleyense* and *Neoscytalidium dimidiatum* in Malaysia (Li et al., 2021). From the mango stem-end rot, either *Lasiodiplodia theobromae* or *Neofusicoccum parvum* was found in Okinawa Prefecture, Japan but *Diaporthe* species were consistently isolated from the SER in mango in Japan (Ajitomi et al., 2020).

TomEJC is a unique cultivar of mango, the fruits are golden coloured, deliciously succulent and substantial in size, with an average fruit weight from 650 to 1400 g. The fruits are resistant to the anthracnose disease and partially to the SER. Field diseases such as the scab (*Denticularia mangiferae*) and sooty moulds (*Capnodium* sp.) occur in TomEJC mangoes. Over-ripened fruits develop dendritic spots caused by *Colletotrichum* spp. and *Botryosphaeria* sp. and the SER occasionally (Adikaram, unpublished data).

The stem-end browning (SEB) was first encountered in ripe mangoes of cultivar TomEJC, in a commercial plantation at the Rajarata Farm, Dambewatana, Galkiriagama, Dambulla, Central Province of Sri Lanka in 2016. The disease was found to have spread within a matter of 6 months to many TomEJC plantations throughout the dry zone of Sri Lanka, covering the North Central Province (NCP), Central Province (the drier areas bordering the NCP), North Western Province (NWP), Northern Province (NP) and Southern Province (SP). SEB was, however, confined to the cultivar TomEJC and not observed in any other mango cultivar within Sri Lanka. Stem-end browning substantially lowered the quality of fruits, posing a considerable threat to marketing of TomEJC mangoes.

Generally, the incidence of SEB was below 10%, during the first month of each fruit harvesting season, June to August and October to January. However, the incidence of SEB increases to 10–15% during the second month. SEB incidence was also higher when fruits were harvested under wet weather conditions especially prevailing from October to January in the dry zone of Sri Lanka.

Being a new disease, very little is known about the SEB of TomEJC mangoes, such as the causal agents, the process of infection and disease development. In this backdrop, the present investigations were undertaken to understand the disease, mostly plant pathological aspects. The major research questions to be answered include what are the causal agents of the SEB and identity, whether they are pathogenic or not on TomEJC

mangoes, source of inoculum, the mode of infection and, factors, if any, that influence the disease. It is important to find out whether the fungi that infect mangoes causing SEB are endophytic in the mango stem, similar to those that cause mango SER. The main objectives were to accurately identify the fungi associated with SEB, test and confirm their pathogenicity, and determine the stage of maturity and the process of fruit infection. A proper understanding of the disease is essential to develop effective and sustainable disease management strategies.

Materials and methods

Plant material

Plant material, including ripe or ripening fruits showing SEB symptoms, and healthy fruits at the same stage of maturity, younger fruits at different stages of development, 2, 4, 6, 8, 10 and 12 weeks after anthesis and the pedicel or the stalk of ripe/ripening fruits with or without SEB symptoms were collected from the Rajarata Farm, Ellawala Horticulture (Pvt.) Ltd., Dambewatana, Galkiriyagama, Dambulla (7°58′11.7”N 80°34′10.7″E) during the study period of three years from 2016. Plant materials collected were delivered in cardboard boxes or sealed polythene bags to the laboratory at the National Institute of Fundamental Studies (NIFS), Hantana road, Kandy for investigations.

Isolation of fungi

Isolation from SEB-affected and healthy fruits

Ripe fruits showing SEB symptoms at different stages of disease development were used for isolation of fungi. Thin (1–2 mm thick) tissue segments (0.5 × 0.5 cm^2) were cut from the symptomatic peel in the stem-end region, along two radial axes, crossing each other the pedicel at 90^o, and ending 4 cm away from the pedicel-end. Tissue segments were surface sterilized in 1% sodium hypochlorite (Clorox©) for 1–3 min. and, after placing on sterilized filter papers to remove excess liquid, the segments were transferred aseptically on to Potato Dextrose Agar (PDA) medium, supplemented with 50 μg/ml tetracycline to suppress bacterial growth. Ten PDA plates, each consisting 4 tissue segments, were prepared for each isolation. Similarly, isolations were made from the peel of healthy fruits cut out from the stem-end.

Isolations were also made from fruits showing different morphological forms of SEB symptoms, from light brown or greyish brown, dark brown to steel grey, observed during different fruit harvesting seasons. Fungi were also isolated from mature fruits showing no stem-end browning symptoms. The plates were incubated at 25 °C for 7–10 days. Fungal colonies that emerged from tissue segments were sub-cultured by transferring mycelial discs on to fresh PDA plates and the plates were incubated at 25 °C.

Isolation from fruit pedicel

Fruit pedicel (stalk) is often recognized as a source of fungi for infections at the stem-end region of fruits. Isolations were made from the pedicel of ripe fruits, healthy or showing browning symptoms. Pedicels (2.5 cm long) obtained from diseased or healthy fruits, were sliced in to 1–2 mm thick discs (8–10). The discs were surface sterilised as described previously and transferred aseptically on to PDA medium (4 discs per petri dish) and the plates were incubated at 25 °C.

Isolation from younger fruits

Fruits at different stages of development, 2, 4, 6, 8, 10 and 12 weeks after anthesis, were collected in four replicates. Fungi were isolated from the peel around the stem-end region and from the pedicel following surface sterilization, as described previously. The mycelium grown out from tissue segments were sub-cultured the plates were incubated at 25 °C.

All isolations of pathogens from healthy and SEB-affected mangoes, the pedicel and younger fruits that commenced in 2016, continued over the two peak fruit harvesting seasons, October to January and June to August, annually for three years.

Preparation of mono-conidial cultures

A suspension of conidia, prepared by scraping mycelia from two weeks old cultures and suspending in sterile distilled water (SDW), was filtered through sterile glass wool, and the concentration was adjusted to 1 × 10^6 conidia ml^{-1}. A loopful of the suspension was streaked on Tap Water Agar and the plates were incubated at 26–28 °C for 18 h. A small piece of agar with a single

germinated conidium, located by moving the objective lens (× 25) of a light microscope along the streak line on inverted agar plate, was cut and transferred on to fresh PDA plates. The plates were incubated for 7 days at 28 °C and the isolates were sub-cultured on PDA to be used for morphological and molecular studies.

Pathogenicity test

Nine sets of fruits, each consisting of four freshly harvested TomEJC mangoes uniform in size and maturity, without any visible signs of disease or blemishes, were inoculated separately with the nine (9) species of fungi (Table 2), isolated from fruits showing SEB symptoms. Suspensions of conidia (5×10^4 ml^{-1}) were prepared from two weeks old cultures of each isolate, as described previously. One drop (20 μl) of conidia each was separately applied on to three equally distanced points at the stem-end region, 2 cm away from the pedicel, using a micro-pipette. Control fruits were similarly treated with 20 μl drops of SDW. Where the isolates did not sporulate, vegetative mycelium was scraped, fragmented and suspended in SDW, and mycelial drops were applied on to the fruits as described previously. The inoculated and control fruits, arranged in plastic boxes lined with moistened tissues to provide moist conditions, were incubated at RT. The fruits were examined daily, and the symptoms of lesions developed were described. The fungi were re-isolated from diseased tissues of symptomatic fruits on PDA, following surface sterilization. Morphological characteristics of the colonies and reproductive structures, if any, were compared with those of the original isolates.

Morphological identification

Colony and reproductive morphology of fungi isolated from healthy, SEB-affected fruits, the pedicel and young developing fruits were studied using 10-day old monoconidial cultures, grown on PDA. Sporulating cultures of each isolate were photographed and examined for colony characteristics and the observations were recorded. Suspensions of conidia were prepared by scraping the mycelium and suspending in SDW as described previously. The concentration of conidia was adjusted to 10×10^4 ml^{-1}. Drops of conidia of each isolate were mounted on microscopic slides and examined under light microscope and photographed (LM, Olympus BX53 with Olympus DP72 digital camera Olympus cellSens software). Morphological characteristics and dimensions etc. of asexual and/or sexual structures, if any, were recorded and photographed. Morphological characters were used for possible identification of the fungi isolated from the fruit peel of both healthy, and SEB-affected fruits, the pedicel and younger fruits to genus level.

Molecular identification of fungi

DNA purification

Mycelium, scraped from fresh 10-day old colonies grown on PDA with a sterile pipette tip, was used for the DNA purification which was performed using Wizard® genomic DNA purification kit (Promega Corporation, USA), according to the manufacturer's protocol with modifications (addition of proteinase K). The quality and quantity of DNA were estimated visually by 2% agarose gel electrophoresis stained with ethidium bromide.

PCR amplification

Based on the initial morphological characteristics, the fungi isolated were identified to seven genera viz. *Curvularia*, *Diaporthe*, *Fusarium*, *Neocosmospora*, *Neofusicoccum*, *Neopestalotiopsis* and *Pestalotiopsis*. The DNA of these fungal isolates were amplified using appropriate primers and identified using multi-locus sequences data. PCR reactions were performed using Applied Biosystems Veriti 96-well thermal cycler (9902, Singapore). Amplification reactions had a total reaction volume of 40 μL which consisted of 1× PCR buffer (Promega Corporation, USA), 5.6% DMSO (v/v), 40 μM dNTPs mixed (Promega Corporation, USA), 1.5 mM $MgCl_2$, 0.2 μM of each forward and reverse primers, 0.25 U of GoTaq™ *Taq* DNA polymerase (Promega Corporation, USA), sterilized water and genomic DNA.

The gene regions used for amplification of loci of seven different genera of fungi are given in the Table 1. Primer details of each locus are, internal transcribed spacer of the ribosomal DNA (ITS) (ITS-1F 5′-CTTGGTCATTTAGAGGAA GTA A-3′) (Gardes & Bruns, 1993), (ITS-4 5'-TCCTCCGCTTATTG ATATGC- 3′) (White et al., 1990); translation elongation factor (EF1-α) (EF1-728F 5'-CATCGAGA AGTTCGAGAAGG-3′), (EF1-986R 5'-TACTTGAA

Table 1 The gene regions used for PCR amplification of DNA loci of fungi isolated from stem-end browning

Isolate	Genus	Primers used
N1	*Curvularia*	ITS
N2	*Curvularia*	ITS
PHI	*Diaporthe*	ITS, *TEF*
PH2	*Diaporthe*	ITS, *TEF*
AV7	*Diaporthe*	ITS, *TEF*
F	*Fusarium*	*TUB, TEF*
A	*Neocosmospora*	ITS, *TEF*
AV3	*Neofusicoccum*	ITS, *TUB*
Pe1	*Neopestalotiopsis*	ITS, *TUB, TEF*
Pes2	*Pestalotiopsis*	ITS, *TUB, TEF*

GGAACCCTTA-CC-3′) (Carbone & Kohn, 1999) and β-tubulin2 (*TUB2*) (BT2a 5'-GGTAACCAA ATCGGTGCTTTC-3′), (BT2b 5'-ACCCTCAG TGTAGTGACCCTTGC3') (Glass & Donaldson, 1995).

The thermal programme used to amplify the gene regions are as follows, except the annealing temperature the other conditions remained same for all regions; 4 min at 95 °C, followed by 35 cycles of denaturation (94 °C for 30 s), annealing (30 s at 52 °C for ITS; 54 °C for *TEF* and 55 °C for *TUB2*), elongation (72 °C for 90 s), and a final 7 min extension at 72 °C and cooling down step to 4 °C. PCR products were examined by electrophoresis on a 2% agarose gel stained with ethidium bromide. Amplicons were sequenced for both directions (Applied Biosystems, 3500 genetic analyser) at the Department of Molecular Biology and Biotechnology, Faculty of Science, University of Peradeniya, Sri Lanka.

Phylogenetic analysis

Complimentary sequences for each isolate were assembled with DNASTAR Lasergene SeqMan Pro v. 8.1.3 and deposited at GenBank under the accession numbers listed in the Tables 2. Seven different datasets were used to estimate seven phylogenies: *Diaporthe* and *Neocosmospora* trees based on combined ITS and *TEF* loci, *Neopestalotiopsis* and *Pestalotiopsis* trees based on combined ITS, *TUB* and *TEF* loci, *Fusarium* tree based on combined *TUB* and *TEF* loci, *Neofusicoccum* tree based on combined ITS and *TUB* loci and *Curvularia* trees based on ITS region. Multiple sequence alignments were generated in MEGA v.7.0.26 (Kumar et al., 2016) and edited manually. Phylogenetic analyses were carried out using maximum likelihood (ML). The RAxML analyses were run on the CIPRES Science Gateway portal (Miller et al., 2012). ML analyses for the datasets were performed with RAxML-HPC2 on XSEDE v. 8.2.10 (Stamatakis, 2014) using a GTR + GAMMA substitution model with 1000 bootstrap iterations.

Results

Symptoms of stem-end browning

The symptoms occurred in the peel of ripe or ripening fruit at the stem-end region, initially as a yellowish-brown, narrow, defused ring around the pedicel which expanded with the advancement of further ripening, covering the upper one-third of the fruit peel (Fig. 1). Lenticels in the region of affected peel were darkened and visible as darker dots. Stem-end browning was first observed at the stem-end as dark brown to steel grey in colour. The colour of diseased area slightly changed subsequently to light brown or greyish brown in certain fruit harvesting seasons (Fig. 1). The advancing margin of the SEB was irregularly wavy and light brown. A smaller ring of about 2–3 cm diameter area in the peel, immediately surrounding the pedicel, became necrotic. Also, the pulp tissue at a depth of 2–3 cm, underneath the ring of affected peel, also turned darker (Fig. 1). There were no fungal structures or mycelium visible on the SEB-affected fruit peel. Occasionally in some fruits, the browning started from the pedicel and developed towards only one direction of the stem-end (Fig. 1).

Identification of fungi isolated from fruits

Ten fungi were isolated from the peel tissues of fruits that showed SEB symptoms, first in 2016 and isolations continued for three years. All isolates from SEB, produced abundant conidia on PDA except for one isolate which was found sterile, and later identified as *Diaporthe eugeniae*. Morphology of colonies and conidia of each isolate was described and photographed (Figs. 2 & 3). Using both colony and conidial morphology, the isolates were tentatively identified to genus level.

Table 2 Molecular identification of fungi isolated from peel tissue healthy or stem-end browning-affected fruits

Taxon	Isolate	GenBank Accession numbers		
		ITS	*TUB2*	*TEF*
Curvularia dactyloctenicola	N1	OL813872	–	–
Curvularia dactyloctenicola	N2	OL813871	–	–
Diaporthe endophytica	PH1	OL813873	–	OL840860
Diaporthe eugeniae	PH2	OL813874	–	OL840861
Diaporthe pseudophoenicicola	AV7	OL813875	–	–
Fusarium mangiferae	F	–	OL840866	OL840862
Neocosmospora sp.	A	OL813876	–	OL840863
Neofusicoccum brasiliense	AV3	OL813877	OL840868	–
Neopestalotiopsis rhizophorae	Pe1	OL813878	OL840869	OL840864
Pestalotiopsis adusta	Pes2	OL813879	OL840867	OL840865

The ten isolates were subsequently subjected to multigene DNA sequence analysis and identified to species level as *Curvularia dactyloctenicola* (N1 and N2; Fig. 4), *Diaporthe endophytica* (Fig. 5) *D. eugeniae*

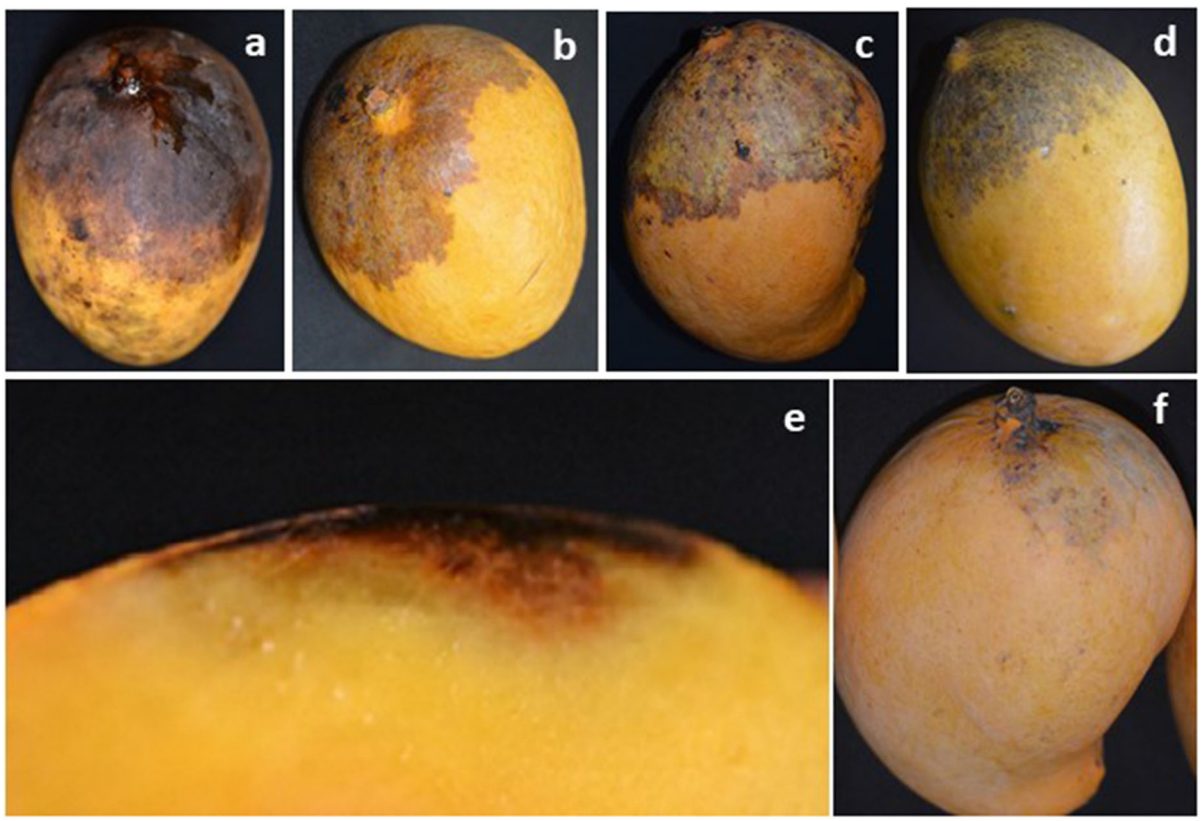

Fig. 1 Ripe mango fruits cultivar TomEJC showing symptoms of stem-end browning (SEB) disease. Three colour forms of SEB were recognised in fruits, (**a**) Dark brown to steal grey, (**b**) Light brown, and (**c** & **d**) greyish brown. (**e**) Browning of 4–6 cm diameter area of the peel surrounding the pedicel and heavy darkening of the inner pulp just beneath the peel and blackish smaller spots of darkened lenticels in between, (**f**) Occasional development of SEB symptoms towards only one half of the fruit where browning developed only towards one side of the pedicel

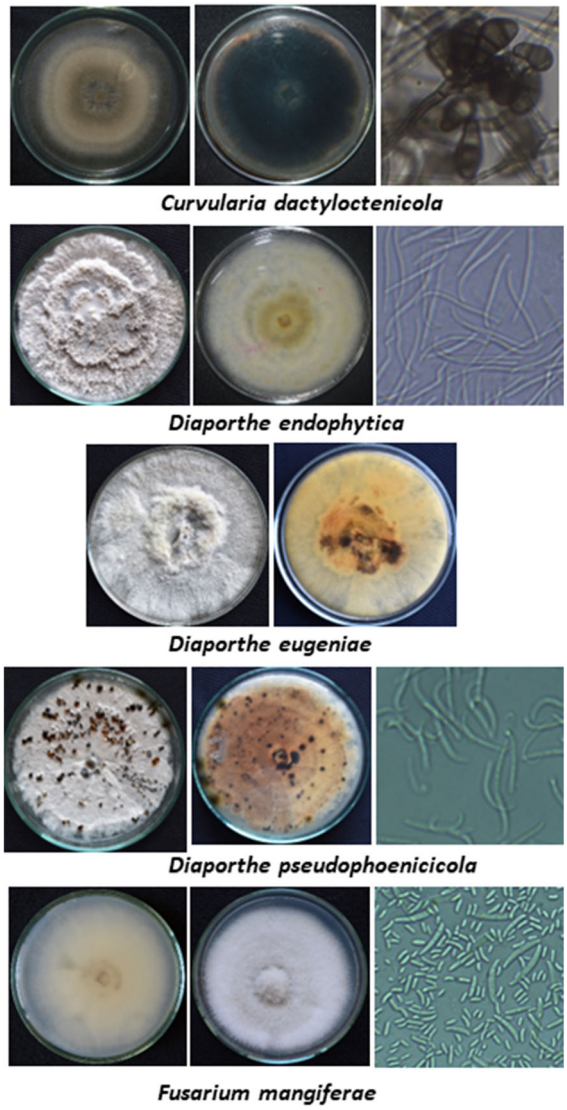

Fig. 2 Colony and conidia morphology of *Curvularia dactyloctenicola* (N1), *Diaporthe endophytica*, *D. eugeniae*, *D. pseudophoenicicola*, *Fusarium mangiferae* and *Neocosmospora* sp. grown on PDA, (**a**) Upper, and (**b**) Lower surface appearance, (**c**) Conidia

(Fig. 5), *D. pseudophoenicicola* (Fig. 5), *Fusarium mangiferae* (Fig. 6), *Neocosmospora* sp. (Fig. 7), *Neofusicoccum brasiliense* (Fig. 8), *Neopestalotiopsis rhizophorae* (Fig. 9) and *Pestalotiopsis adusta* (Fig. 10) and listed in Table 2. *Neofusicoccum brasiliense* and *D. endophytica* were also isolated from the stem-end region of healthy fruits that did not show SEB symptoms, indicating that the fruits had been infected by these fungi early in the field but remained asymptomatic.

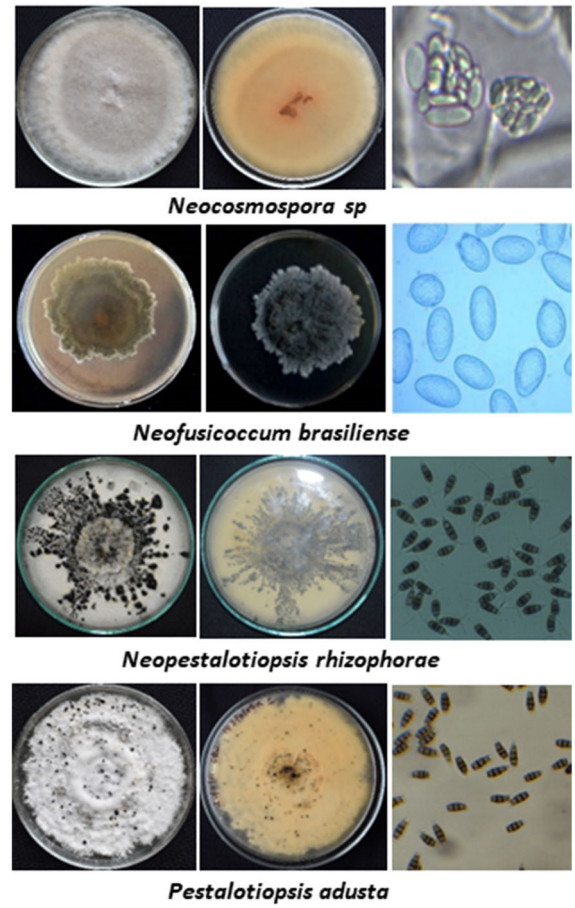

Fig. 3 Colony and conidia morphology of *Neofusicoccum brasiliense*, *Neopestalotiopsis rhizophorae*, *Neopestalotiopsis rhizophorae* and *Pestalotiopsis adusta* grown on PDA, (**a**) Upper, and (**b**) Lower surface appearance; (**c**) Conidia

Varied colour forms of stem-end browning

Varied colour forms of SEB were observed in ripe mangoes (Fig. 1) in different peak fruit harvesting periods or seasons. The fungal species identified from different colour forms of the SEB, and their composition slightly varied. *Neofusicoccum brasiliense* was isolated from fruits showing dark brown to steal grey colour SEB (Fig. 1) at a somewhat relatively larger frequency of 45.5%, showing its dominance. The other fungi isolated were *F. mangiferae* (18. 5%), *D. endophytica* (26%) and *P. adusta* (5%). On the other hand, *Neofusicoccum brasiliense* was isolated at a lower frequency (17.71%) from the fruits that showed light brown colouration of SEB. *Diaporthe eugeniae* (44.45%), *F. mangiferae* (8.33%), *D. endophytica*

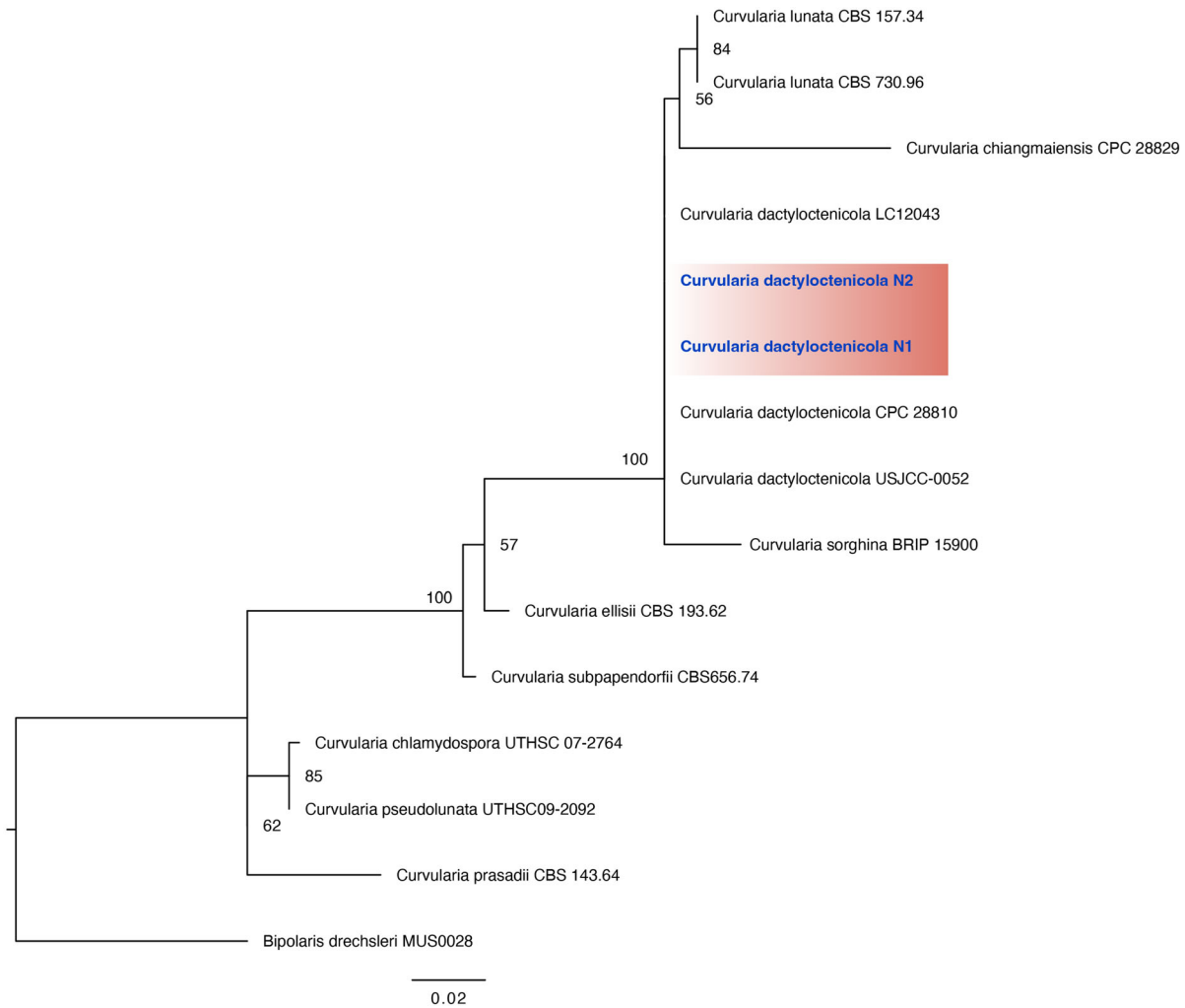

Fig. 4 Phylogram generated from ML analysis based on ITS sequence data for the analysed *Curvularia* species. The bootstrap support values above 50% are given at the nodes. The species name is followed by the strain accession numbers, and the strain from this study are in blue bold. *Bipolaris drechsleri* (MUS0028) is used as the outgroup taxon

(8.33%) and *P. adusta* (18.05%) were also isolated from the peel with light browning form of SEB.

Identification of fungi from pedicel and younger, developing fruits

Fungi were isolated from the pedicel of fruits that showed SEB as well as healthy fruits without disease. *Fusarium mangiferae*, *N. brasiliense* and *P. adusta* were isolated from the pedicel obtained from diseased fruits. While *C. dactyloctenicola* was isolated only from the pedicel excised from healthy fruits, showing that diverse species of fungi were present in the pedicel attached to healthy fruits. *Pestalotiopsis adusta* was isolated at a greater frequency (67%) than all other fungal species found in pedicels obtained from both sources (Table 3). *Fusarium mangiferae*, *C. dactyloctenicola*, *D. endophytica*, *N. brasiliense* and *P. adusta* were frequently isolated from the peel of developing fruits 2, 4, 6, 8. 10 and 12 weeks after anthesis. *Pestalotiopsis adusta* was the most dominant and frequently isolated in almost every isolation.

Pathogenicity test

TomEJC mangoes, artificially inoculated with individual species of fungi isolated from SEB tissues, initiated lesions within 8 to 10 days in ripe fruits. The lesions expanded gradually in size to form brownish to blackish

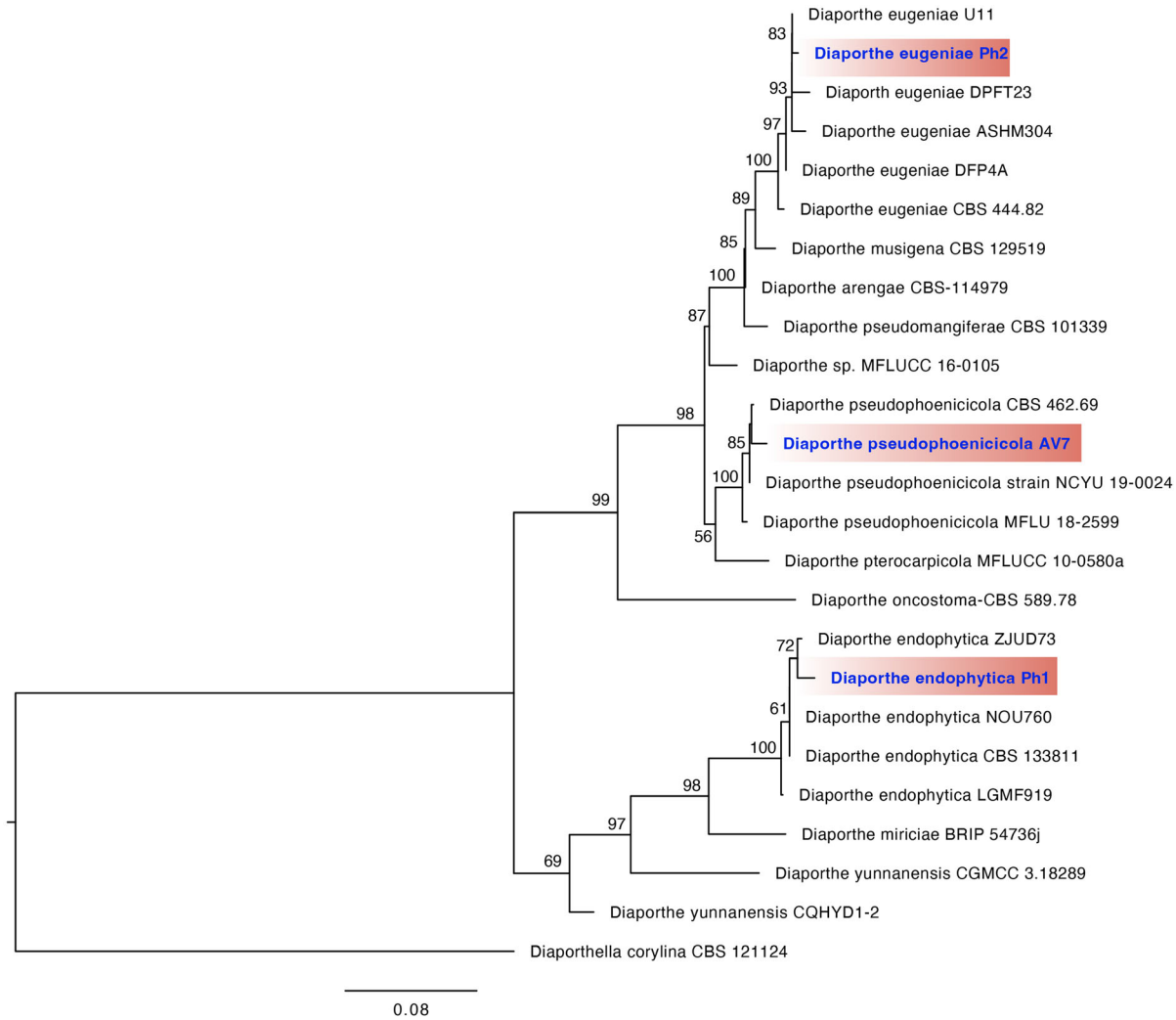

Fig. 5 Phylogram generated from ML analysis based on combined ITS and *TEF* sequence data for the analysed *Diaporthe* species. The bootstrap support values above 50% are given at the nodes. The species name is followed by the strain accession numbers, and the strain from this study are in blue bold. *Diaporthella corylina* (CBS 121124) is used as the outgroup taxon

lesions (Fig. 11). All nine species did not, however, expand lesions at a similar rate. The fungi were re-isolated on PDA and the morphology of colony and conidia produced in cultures was found to be identical to the isolates of each species used originally for fruit inoculation. *Neofusicoccum brasiliense* developed medium-sized, round, dark brown to blackish lesions with slightly irregular margins (Fig. 11a). The isolate penetrated deep in to the pulp beneath the peel tissues, showing its comparatively higher pathogenicity. The lesion produced by *Fusarium mangiferae* was slightly smaller, round to oval in shape, depressed slightly and greenish brown with irregular margins (Fig. 11b). *Curvularia dactyloctenicola* produced somewhat larger, pale brown lesions of 3.5–5.0 cm in diameter, with an extended periphery and blackish smaller island in each lesion towards the pedicel end (Fig. 11c). A mixed inoculum of *N. brasiliense* and *F. mangiferae* formed 3–4 cm wide and 3–9 cm long, brownish lesions, intermingled with darkened lenticels (Fig. 11d). Lesions formed by *Neocosmospora* sp. were blackish, irregularly spread covering a larger area away from the pedicel end with a diffused margin (Fig. 11e), displaying a reasonably higher pathogenicity. *Pestalotiopsis adusta* lesions were darker but much smaller, oval shaped with entire margins (Fig. 11f). *Diaporthe endophytica*, *D. eugeniae* and *D. pseudophoenicicola* developed medium-sized, dark grey individual lesions with irregular margins, around the stem-end region.

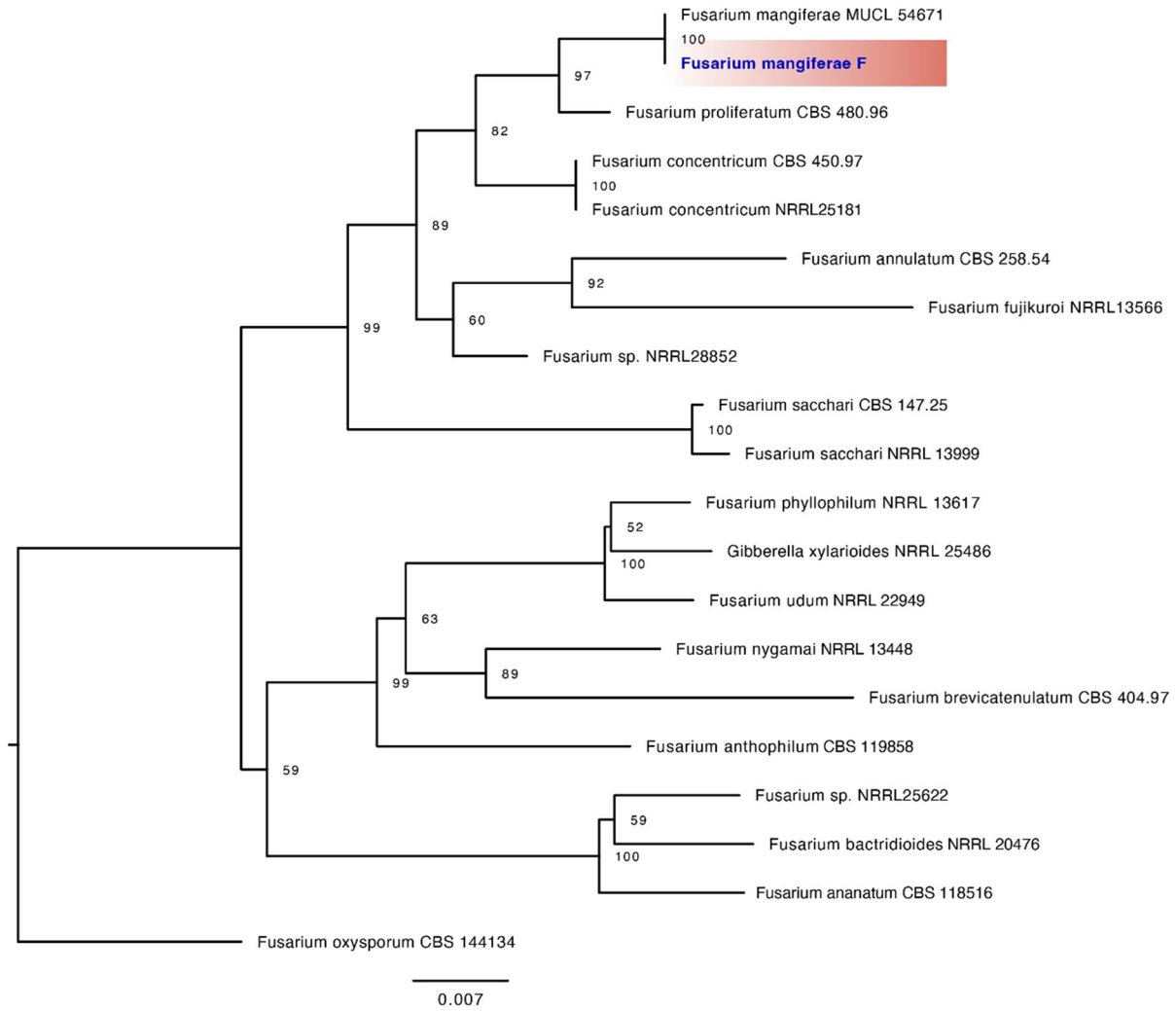

Fig. 6 Phylogram generated from ML analysis based on combined *TUB* and *TEF* sequence data for the analysed *Fusarium* species. The bootstrap support values above 50% are given at the nodes. The species name is followed by the strain accession numbers, and the strain from this study are in blue bold. *Fusarium oxysporum* (CBS 144134) is used as the outgroup taxon

Discussion

The present study reports a new fungal disease in ripe mangoes of the cultivar TomEJC, named the stem-end browning (SEB). The disease can be recognized externally from browning symptoms at the stem-end of the peel around the fruit pedicel. Exhaustive literature searches conducted using several keywords, could not find any disease, similar in symptomatically or pathogen composition to the SEB in mango, reported previously. The only postharvest disease that has some closeness to the SEB was the stem-end rot of mango. SER causes excessive disintegration of pulp tissues of ripe mangoes, converting the pulp at the stem-end half of the fruit, a soft and watery rot. The damage that the SER incurs on ripe mangoes is many times heavier than that of the SEB.

SER is a common disease, known to be caused by about 12 species of fungi or more, in different mango growing regions in the world (Galsurker et al., 2020). SEB is also caused by a relatively larger assemblage of nine species of fungi which is a similarity between the two diseases, SEB and SER. The fungal species known to be causing the two diseases are therefore distinctly different from each other. *Lasiodiplodia theobromae*, a major pathogen causing SER disease in mango, mostly in the dry and warmer regions of the world (Karunanayake & Adikaram, 2020; Karunanayake et al., 2015), was never

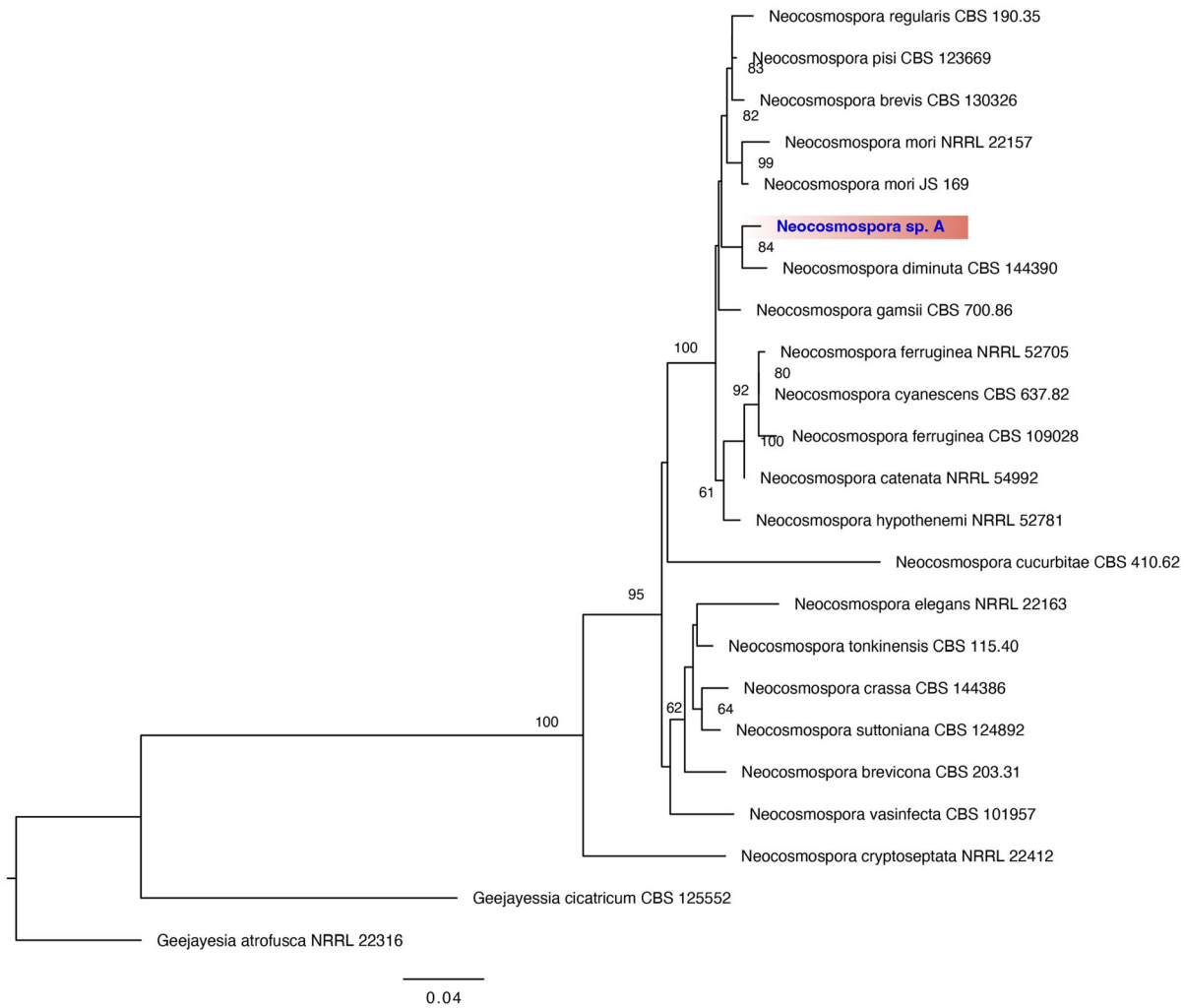

Fig. 7 Phylogram generated from ML analysis based on combined ITS and *TEF* sequence data for the analysed *Neocosmospora* species. The bootstrap support values above 50% are given at the nodes. The species name is followed by the strain accession numbers, and the strain from this study are in blue bold. *Geejayesia atrofusca* (NRRL 22316) and *Geejayessia cicatricum* (CBS 125552) are used as the outgroup taxa

encountered in SEB in the isolations made over three consecutive years since 2016. None of the other species reported as causing the SER, *Dothiorella dominicana*, *Phomopsis mangiferae*, *Pestalotiopsis mangiferae*, *Cytosphaera mangiferae* (Johnson et al., 1991, 1992), *Colletotrichum gloeosporioides* (Prusky et al., 2009), *Pseudofusicoccum kimberleyyense*, *Neoscytalidium dimidiatum* etc. (Li et al., 2021), were among the fungi isolated from SEB.

Six out of nine fungal species involved in causing the SEB in TomEJC mango were also reported from mango elsewhere. Of the six species, only *D. eugeniae* (Lim et al., 2019) had been isolated from the SER of mango and the rest were isolated from other parts than the fruits of mango. *Diaporthe eugeniae* was also not pathogenic on the fruit of all mango cultivars tested in the study (Lim et al., 2019). *Diaporthe eugeniae* may possibly be endophytic as mango stem is known to be populated with various species of non-pathogenic microorganisms, including fungi, yeasts and bacteria (Diskin et al., 2017). *Pestalotiopsis adusta*, another SEB fungus, was also isolated from a leaf spot of mango in Southern China (Shu et al., 2020).

The sharp differences in the combination of fungal species involved in causing the two diseases, together with the totally contrasting symptoms expressed by them, strongly support the suggestion that the SEB and SER are two separate diseases. One difference in species

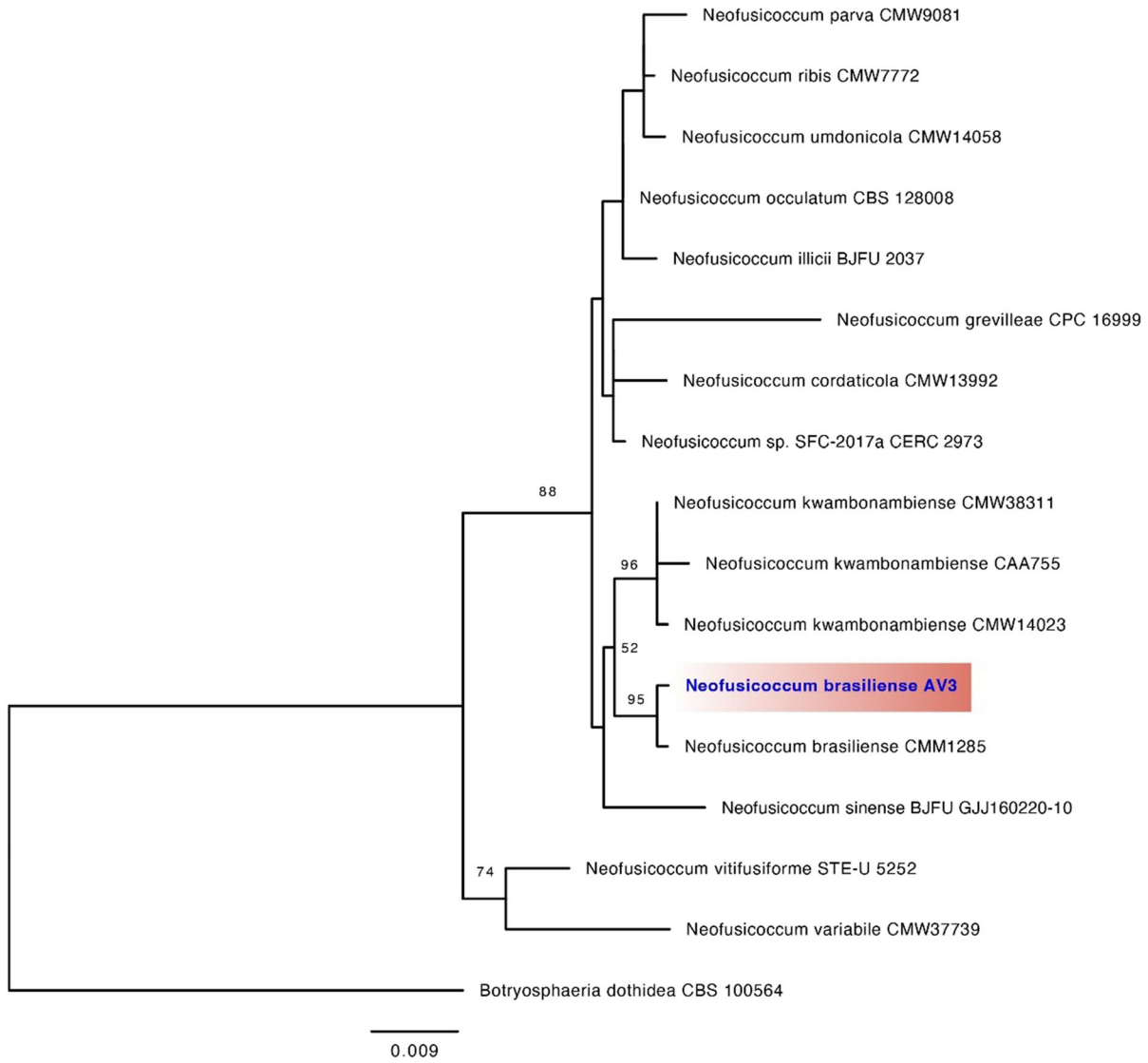

Fig. 8 Phylogram generated from ML analysis based on combined ITS and *TUB* sequence data for the analysed *Neofusicoccum* species. The bootstrap support values above 50% are given at the nodes. The species name is followed by the strain accession numbers, and the strain from this study are in blue bold. *Botryosphaeria dothidea* (CBS 100564) is used as the outgroup taxon

level identification was that all SEB pathogens were identified by using molecular tools whereas some of the SER pathogens had been identified using morphological characters. Symptoms and the causal agent/s are main criteria that distinguish plant diseases, even the closely related diseases, from each other.

Among the fungi isolated and identified from SEB, *Diaporthe* was the most dominant with three species, *Diaporthe endophytica, D. eugeniae* and *D. pseudophoenicicola*. *Diaporthe* is one of the most frequently encountered genus of endophytic fungi in plant species (Botella & Diez, 2011; Murali et al., 2006) and are responsible for causing diseases in a wider range of host plants, including root and fruit rots, dieback, cankers, leaf spots, blights, decay and wilt (Gomes et al., 2013). *Diaporthe endophytica* was first isolated as an endophyte from the leaf of *Schinus terebinthifolius* (Anacardiaceae) in Brazil. *Diaporthe eugeniae,* isolated from SEB in the present study, was found to be sterile. Coincidently, the original isolate of *D. eugeniae*, described from *Eugenia aromatica* in West Sumatra was also sterile (Gomes et al., 2013).

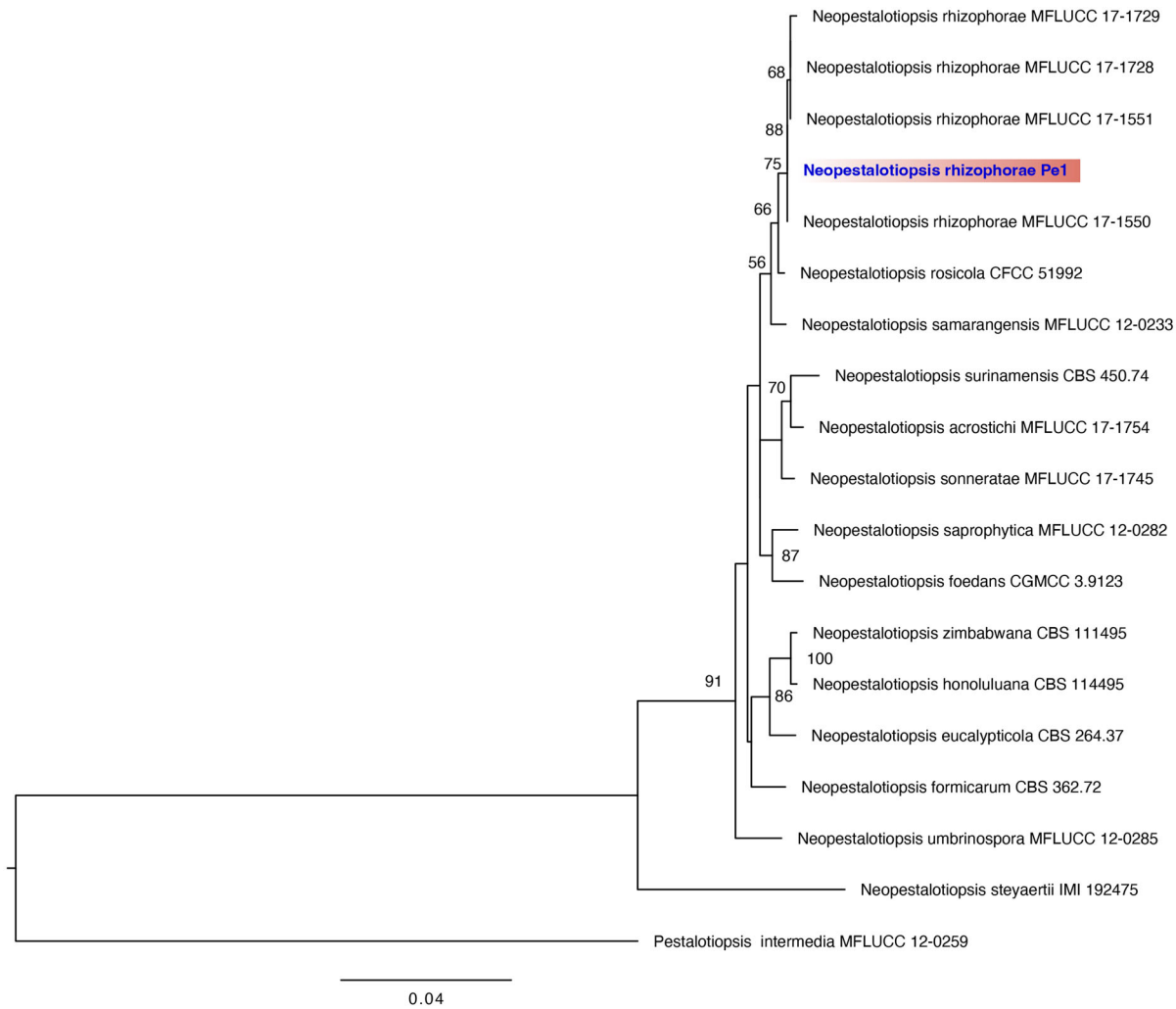

Fig. 9 Phylogram generated from ML analysis based on combined ITS, *TUB* and *TEF* sequence data for the analysed *Neopestalotiopsis* species. The bootstrap support values above 50% are given at the nodes. The species name is followed by the strain accession numbers, and the strain from this study are in blue bold. *Pestalotiopsis intermedia* (MFLUCC 12–0259) is used as the outgroup taxon

Neofusicoccum brasiliense was consistently isolated from the SEB in TomEJC mango, commencing from 2016 when the disease was first encountered. The fungus, on inoculation, produced a medium-sized lesion on the peel and invaded across the peel, in to the pulp tissues, displaying its relatively greater virulence. *Neofusicoccum brasiliense* was first recorded in Brazil from the stem of mango tree showing die-back symptoms and recognized as the third most prevalent fungal species on mango (Coutinho et al., 2018; Marques et al., 2013).

Pestalotiopsis adusta was among the most frequently isolated fungi in the present study from healthy and the SEB-affected fruits and the pedicels. The species, however, appears a relatively weaker pathogen contributing only slightly to SEB development. *Neocosmospora* sp., on inoculation, developed relatively larger lesions in ripe mango, displaying its higher degree of pathogenicity. *Neocosmospora* spp. are well known as plant pathogens. *Neocosmospora perseae* causes trunk canker in avocado (*P. americana*) in Sicily, Italy (Guarnaccia et al., 2018). *Fusarium mangiferae* was identified as causing the mango malformation in Sri Lanka (Sinniah et al., 2013). *Neopestalotiopsis rhizophorae*, first isolated from leaf spots of *Rhizophora mucronata* in Thailand (Norphanphoun et al., 2020), was among the nine species identified as causing the stem-end browning in mango.

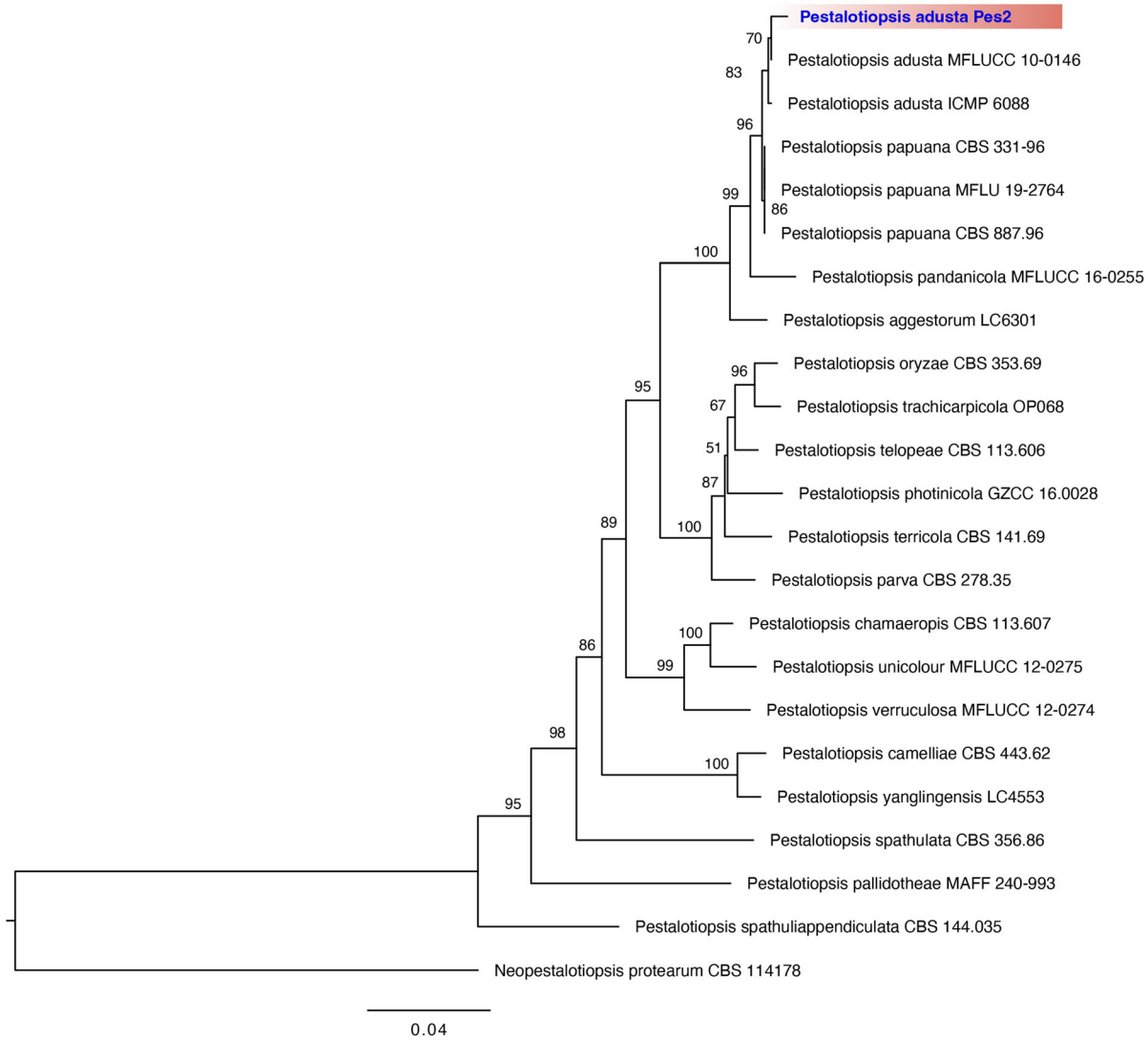

Fig. 10 Phylogram generated from ML analysis based on combined ITS, *TUB* and *TEF* sequence data for the analysed *Pestalotiopsis* species. The bootstrap support values above 50% are given at the nodes. The species name is followed by the strain accession numbers, and the strain from this study are in blue bold. *Neopestalotiopsis protearum* (CBS 114178) is used as the outgroup taxon

Three different colour forms of the SEB, dark brown to steel grey, light brown or lighter greyish brown, were observed in different peak fruit harvesting seasons at the Rajarata Farm. The colour forms appeared to have been due to the composition of fungi involved in causing the disease in fruits and also depending on the species that are dominant. The dark brown to steel black colouration of SEB could have been due to the dominant *N. brasiliense*. Steel grey mycelium can also develop over the surface on fruit affected by *Neofusicoccum* spp. during SER development (Johnson et al., 1991).

Table 3 Fungi isolated from the stalk obtained from stem-end browning-affected and healthy fruits

Pedicel from SEB affected fruit	Pedicel from healthy fruit
Fusarium mangiferae	*Pestalotiopsis adusta*
Neofusicoccum brasiliense	*Diaporthe endophytica*
Pestalotiopsis adusta	*Fusarium mangiferae*
–	*Curvularia dactyloctenicola*

The study revealed that the harvested mature fruits were already infected in the field. Most of the fungi

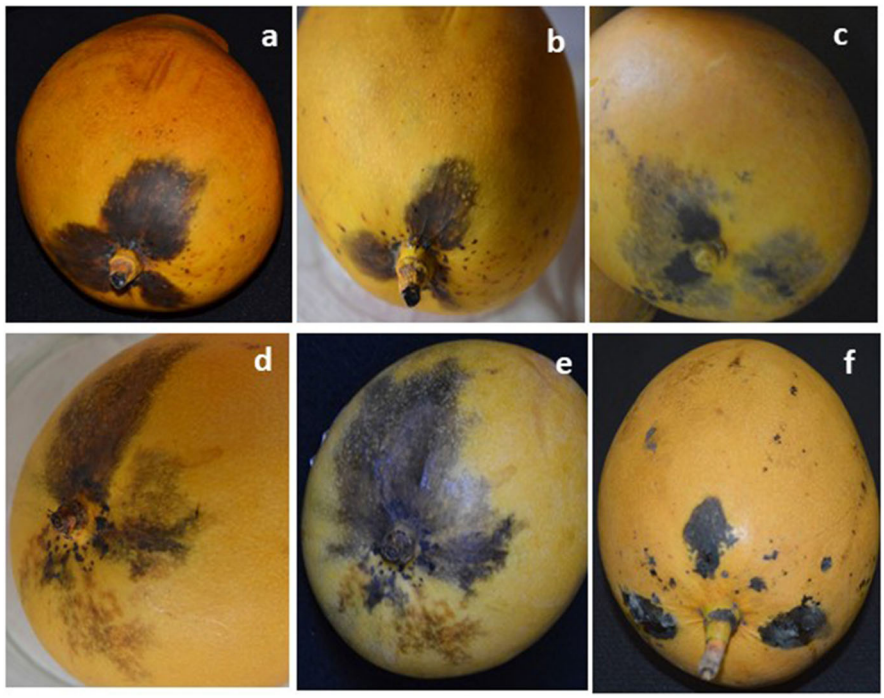

Fig. 11 Symptoms developed in freshly harvested, ripe fruits, artificially inoculated with (**a**) *Neofusicoccum brasiliense* (**b**) *Fusarium mangiferae,* (**c**). *Curvularia dactyloctenicola*, (**d**) Combined inoculum of *Neofusicoccum brasiliense* and *Fusarium mangiferae,* (**e**) *Neocosmospora* sp. and (**f**) *Pestalotiopsis adusta*

causing SEB, were also isolated in the present study from younger fruits at early stages of maturity. The earliest stage of maturity of fruits from which the fungi could be isolated was two (2) weeks after anthesis/flowering. Some of the fungi isolated from SEB-affected tissue were also isolated from the pedicel of developing fruits. SEB fungi were most likely infecting the inflorescence, the stem and the pedicels at inflorescence stage, and enter in to endophytic lifestyle. The endophytic fungi establish within inflorescence tissues and stay endophytic until the fruits are matured. In the present study, fungi were often isolated from the pedicel, not just only in younger and developing fruit, but also of fruits harvested at mature stage. This may indicate that the source of inoculum of SEB is the stem or the pedicel of mango.

Endophytic colonization of inflorescence, and pedicel tissue, was a primary route of infection for fruits which develop SER of mango during ripening (Johnson et al., 2008). They enter the stem tissues through natural openings and wounds, mainly during flowering stages. According to literature, some botryosphaeraceaeous species also can survive as endophytes in symptomless tissue (Twizeyimana et al., 2013) and become pathogenic when the host is subjected to stress conditions (Slippers & Wingfield, 2007). Not all fungi present in the stem-end appeared to make a transition from endophytic to necrotrophic lifestyle and become pathogenic during fruit ripening. Little is, however, known about the endophytic microbiome at the stem-end of fruit (Galsurker et al., 2020).

SEB continues to cause fruit losses of the cultivar TomEJC and the rate of infection escalates especially when fruits are harvested during wet weather conditions. Generally, the disease remains at a low scale at the beginning of a harvesting season. The incidence of the SEB, however, increases in the middle of each fruit season and towards the end of the harvesting season.

Since the cultivar TomEJC is firmly established in export markets, the maintenance of highest quality of fruit in the long run is mandatory. Regular field sanitation, canopy management, fertilizer application and manipulation of flowering to occur during dry weather conditions are field strategies that reduce infection at flowering and fruit development. Orchard sanitation by removal of dead wood and mummified fruits within the canopy, pruning and their removal from the orchard help reduce the field inoculum. These, together with induction of flowering at dry weather may reduce infection of inflorescences and developing fruits and intern

the incidence of stem-end diseases. Control of fungal diseases in commercial orchards depends on multiple field application of fungicides. Governmental regulation of fungicide usage and, increased consumer demand for fruits without pesticide residues, however, restrict fungicide application necessitating the formulation of alternative disease control strategies.

In summary, the present study reports a new disease in TomEJC mangoes, named stem-end browning, caused by ten fungi, isolated and identified to species level using multi-loci phylogenetic analysis, and their pathogenicity was confirmed. Postharvest diseases pose most acute problems of fruit industries, significantly limiting the harvest, marketing, and storage of fruits leading to significant economic losses. It is highly desirable to have an efficient, environmentally friendly and bio-safe approach to the reduction of postharvest food losses.

Conclusions

Browning of the peel around the stem-end of ripe mangoes, cultivar TomEJC, covering the upper one-third of the fruit and necrotic pulp tissues beneath the pedicel area was observed in a commercial mango plantation in Dambulla (Central Province), Sri Lanka. This was recognized as a new fungal disease in mango, and named stem-end browning (SEB).

Ten fungi, isolated from the SEB-affected fruit peel, were identified by multi-gene phylogenetic analysis as nine species, *Curvularia dactyloctenicola*, *Diaporthe endophytica*, *Diaporthe eugeniae*, *Diaporthe pseudophoenicicola*, *Fusarium mangiferae*, *Neocosmospora* sp., *Neofusicoccum brasiliense*, *Neopestalotiopsis rhizophorae* and *Pestalotiopsis adusta*. The pathogenicity of the nine species on mango fruits cultivar TomEJC was confirmed.

Most of these fungi could also be isolated from the pedicel of healthy or diseased fruits of cultivar TomEJC, and also from developing fruits at different stages of maturity, from 2 to 12 weeks after anthesis. This may suggest that the fungi may be infecting the inflorescence during flowering and becoming endophytic.

The disease substantially reduces the fruit quality, incurring up to 27% loss of harvested fruit. SEB is a new postharvest fungal disease in mango.

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Author contribution All authors contributed to the conception and design of the study. Material preparation, data collection and analysis were performed by [NKBA], [DMDY], [SSNM], [LM], [DMSD] and [LJ]. The first draft of the manuscript was written by [NKBA, DMDY, SSNM] and all authors commented on subsequent versions of the manuscript. All authors read and approved the final manuscript.

Declarations

The authors declare that this manuscript had not been published elsewhere. All the authors have read the current version of this manuscript and given consent to submit the manuscript.

Ethical approval No humans or animals were used to conduct the research work presented in this manuscript.

Conflict of interest Authors have no conflicts of interest relevant to the content of this article to declare.

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