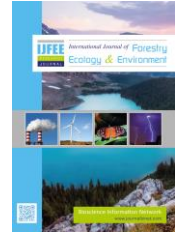


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Fungal pathogens associated with wilting of *Neolitsea cassia* trees

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ABSTRACT

Neolitsea cassia, a tree species, belongs to family Lauraceae, is endemic to Sri Lanka. Few planted trees of *Neolitsea cassia* were severely affected by wilt disease. Two fungal pathogens were consistently isolated from collar region and roots of symptomatic trees. These two fungal species were identified as *Colletotrichum* and *Pestalotiopsis* by microscopic observations of conidia and culture characters of isolates on PDA. This is the first report of *Colletotrichum* and *Pestalotiopsis* species causing tree wilting of *Neolitsea cassia* in Sri Lanka. Application of 25 l of fungicide, Thiram + Thiophanate Methyl, 50+30% WP (Homai) solution (1g/l) around the base of each infected plant as a soil drenching was effectively controlled wilting of *Neolitsea cassia* caused by both pathogenic fungi.

Key Words: *Colletotrichum* and *Pestalotiopsis* species, *Neolitsea cassia* and Tree wilting.

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I. Introduction

Neolitsea cassia (L.) Kosterm is a tree species in the family Lauraceae and known as "Dawulukurundu" or "Kudu dawula" in Sinhala and "Katttukaruwa" in Tamil languages. It is endemic to Sri Lanka and native in India, Bhutan, Bangladesh, Sumatra and Java and also reported in the Philippines and northern Australia. Synonyms of the *Neolitsea cassia* are *Litsea zeylanica* C. G & Fr. Nees and *Neolitsea Zeylanica*. *Neolitsea cassia* (L.) is a common plant found in mountain understory evergreen rainforests at 700-1400 m altitude in Sri Lanka. Trees are small or medium sized, height up to 18 m, flowering and fruiting usually occurs between October and July. This tree has smooth thick grayish bark and wood is hard, lightweight and pale orange. The wood is used in paneling and mucilaginous extract of leaves to prepare local sweets. None of the parts are edible and bark and leaves are used to treat fractures in Ayurveda medicine. Further, Terpenes linalool and B caryophyllene are extracted from essential oil of 54 leaves and water soluble Arabinoxylan is obtained from leaves (Padmakumari and Narayanan, 1992).

Wilting of *Neolissea* cassia trees have been reported from various Sri Lanka parts (Personal communications with forest extension officials). However, recently yellowing and wilting of trees was reported in *Neolissea* cassia established at Kurunegala, in the North-western province of Sri Lanka. Yellowing of leaves was the initial symptom followed by wilting of plants and, finally, death of plants. It was observed discoloration on the surface of bark of the collar region and progressed up to hardwood (cambium) in most of the areas, when bark was peeled off using a knife. Venial necrosis and intra-venial chlorosis were also observed in infected trees. The disease has not been reported globally and no research information can be found (Jamaluddin, 1997; Poonam, 2015). Therefore, it would be noted that this is the first study on wilting of *Neolitsea cassia*. The study's objective was to identify the causal factors and find out suitable fungicides for develop proper control measures for tree death of *Neolitsea cassia*.



Figure 01. Initial symptoms- wilting of some branches of *Neolitsea cassia* tree



Figure 02. Discoloration on the surface of bark of the collar region and disease progressed up to hardwood.

II. Materials and Methods

Site description

Fifty five trees of *Neolitsea cassia* have been planted in 2' x 2' x 2' size pit for ornamental purpose grown very well and well nourished. These trees have been established in a single row (Coordinates of 2 endpoints of the row are E 00154042, N00253066 and E 00154078, N 0025102) in 3 m intervals. However, this incident was reported in February 2020 and research work carried for about eight months.

Sampling procedure

Roots, stems and leaves of samples of wilted and healthy plants were taken and dispatched to Horticultural Crops Research and Development Institute (HORDI) of the Department of Agriculture for laboratory analysis to confirm the causal organism/s.

Pathogen/s identification

Microscopic observations were made in wilted and healthy tissues of stem and roots to detect fungal infection. It was observed that fungal mycelium and two types of morphologically different conidia by microscopy. Pieces of rotten tissues were kept on Potato Dextrose Agar (PDA) medium and incubated 10 days under room temperature. The mycelia and conidia development on PDA was observed. Then single spore inoculants of fungi isolates were made and each isolate was subcultured on PDA and incubated ten days at room temperature in continuous light to inspect the culture characters of isolates and morphological features of conidia by microscopy. Four isolates suspected as *Colletotrichum* and *Pestalotiopsis* species and derived from single spores were grown on PDA. Spore size of each isolate was determined by measuring the length and width of 20 conidia from a conidial suspension of each isolate prepared in sterile distilled water.

Identification of effective fungicides

Two fungicides *i.e.*, Tebuconazole 250g EW and Thiram + Thiophanate Methyl, 50+30% WP (Homai) were evaluated to study the efficacy as soil drenching of fungicides in controlling associated fungi of symptomatic trees. Approximately 25 liters of the solution of Tebuconazole (10ml/10 lit) and Homai (10g/10 lit) per plant basis were applied around the collar region of symptomatic plants.

III. Results and Discussion

The isolates were initially identified by comparing their morphological and cultural characteristics on PDA with published data (Figure: 3 & 4). Two fungal genera were observed and identified as *Colletotrichum* and *Pestalotiopsis* by the morphology of conidia (Table 01 Figure: 5 & 6).

Table 01. Morphological and culture characters of fungal genera isolated from rotten tissues in the *Neolitsea cassia* trees region.

Characters of fungi isolates	Morphology and culture characters of isolated fungal genera	
	<i>Colletotrichum</i>	<i>Pestalotiopsis</i>
Colony colour on PDA	initially grey then turned brown	First developed grayish to white zonate colonies later became darker with age of the fungal colonies.
Reverse colony colour on PDA	Brown	Cream
Acervuli	Appeared as brown colour masses, conidia present inside and setae was absent.	Did not produce visible acervuli within 12 days of growth on PDA.
Conidia	Round end shaped, aseptate, fat globules present inside, 12-18 µm in length and 2-4 µm in width.	Five celled conidia showing appendages at the edge of apical and basal cells. Apical and basal cells of conidia were hyaline and three median cells. Measurements of conidia were revealed few variations in size among isolates. Conidia varied from 20 to 29 µm mean length and 6 to 8 µm mean width. Numbers of apical and basal appendages were two and one respectively.

Research findings showed that variation between isolates is typical for many genus *Colletotrichum* species (Pring et al., 1993; Sutton, 1992). However, characteristics of tested fungi species were within the published range of *Colletotrichum gloeosporioides*. *Pestalotiopsis* species were identified by conidial shape, size, septation, pigmentation and apical and basal appendages and culture characters on PDA to those described in published data *Pestalotiopsis* (Lisa et al., 2006; Mordue, 1980).

Pestalotiopsis species was generally regarded as weak, opportunistic pathogens that caused little damage to tree crops. It had evident that *Pestalotiopsis* caused minor diseases on mango and guava trees in Sri Lanka. However, many research reports revealed that *Pestalotiopsis* were causing widespread damage to some hardy trees like Rhododendron, conifers, Eucalyptus and *Canthium dicoccumin* trees in some countries (Hopkins and McQuilken, 2000; Mahadevakumar and Janardhana, 2014).

Colletotrichum species has been reported on several tree species, mainly mango and Jack tree and it cause fruit anthracnose as well as tree and seedling wilt of many fruit crops in Sri Lanka. *Colletotrichum* invades jack trees from the roots or collar region of the stem tissues or tip of the trees and subsequently, it advanced upward or downward, causing defoliation and eventual sudden wilting and drying of trees within a few days (Technical Leaflets of DOA).

Two fungicides *i.e.* Tebuconazole 250g EW and Homai (Thiram+Thiophanate Methyl, 50+30% WP) were tested as soil drenching (25 l/plant) to control disease of symptomatic trees. Results revealed that application of Homai was completely controlled wilt disease of affected plants. However, spreading disease was continued for few more trees even after two weeks of application of Tebuconazole.

Many environmental factors such as drought, high rainfall and stem and root damages and overcrowding can cause a tree to be stressed and invite infection by creating entry points for pathogens. The site inspections where *Neolitsea cassia* had grown, disease symptoms, laboratory tests and basic information analysis made us believe that the tree wilt of *Neolitsea cassia* might be a fungal infestation due to prevailed high rainfall and waterlogging condition in the site.

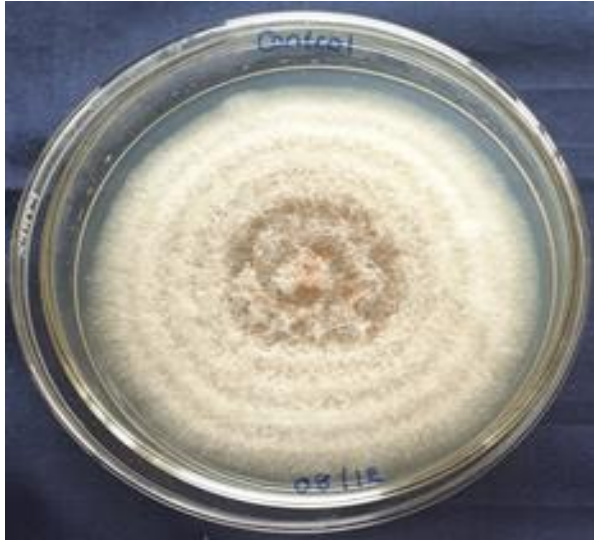


Figure 03. Colony of *Colletotrichum gloeosporioides* on PDA



Figure 04. Colony of *Pestalotiopsis* PDA



Figure 05. Microscopic view of *Colletotrichum gloeosporioides*



Figure 06. Microscopic view of *Pestalotiopsis*

IV. Conclusion

Two fungal pathogens *Colletotrichum gloeosporioides* and *Pestalotiopsis* species, were identified as associated with wilt of *Neolitsea cassia* trees. Homai (Thiophanate methyl 50WG + Thiram 30WG) 1g/1lit can be recommended as an effective fungicide for controlling wilting *Neolitsea cassia*. To our knowledge, this is the first report to provide evidence that *Pestalotiopsis* and *Colletotrichum gloeosporioides* can use wilting and stem rotting of *Neolitsea cassia* trees.

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