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RESEARCH COMMUNICATION

Plant Pathology

First report of rubber circular leaf spot disease pathogen *Colletotrichum siamense* Prihastuti, L. Cai & K.D. Hyde in *Cinnamomum zeylanicum* blume in Sri Lanka

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Abstract: *Cinnamomum zeylanicum,* a member of the family Lauraceae, is commonly grown as an intercrop in Sri Lankan rubber plantations to provide additional income for growers. However, diseases affecting intercrops pose a potential threat to rubber, as pathogens may spread between host species and increase economic losses. This study aimed to identify potential pathogens associated with diseased cinnamon leaves. Leaf samples collected from lesion margins were surface sterilized and cultured on potato dextrose agar (PDA). Distinct fungal colonies were sub-cultured, and ten morphologically different isolates were purified through single-spore isolation. Pathogenicity tests were conducted according to standard procedures to establish Koch's postulates. The most virulent isolate was selected for morphological characterization and molecular identification using bi-directional sequencing of the ITS gene region. The pathogen was identified as *Colletotrichum siamense* (WDCM accession number RRISL87), which showed high pathogenicity on both cinnamon and rubber leaves. This study represents the first report of *C. siamense* infecting cinnamon and highlights the importance of further pathogen identification to strengthen quarantine measures and improve disease management strategies.

Keywords: *Cinnamomum zeylanicum*, circular leaf spot disease, *Colletotrichum siamense, Hevea brasiliensis.*

INTRODUCTION

The genus *Colletotrichum* comprises some of the most successful plant pathogenic species, causing significant economic damage to crops in tropical, subtropical, and temperate regions (Batureine, 2009). They can cause a wide range of disease symptoms including die-back, leaf spots, seedling blight, and leaf blight in almost all parts of a variety of economically important crops, which include vegetables, cereals, legumes, fruits, and even ornamental plants (Cai *et al.*, 2009). *Cinnamomum zeylanicum*, a member of the Lauraceae family is the hardiest spice tree. The inner bark of plants is used as a spice. It has been used for cooking, where it improves the flavour of foods. They are evergreen trees, which are mainly cultivated for their dried inner bark (Ravindran, 2003).

Because of its significant economic returns and proven health-promoting properties, cinnamon has become a strategically important intercrop that is rapidly expanding within Sri Lankan rubber plantations. The leaf spots/blight caused by a fungus, *Colletotrichum* spp., is found in almost all cinnamon growing areas in Sri Lanka mainly affecting the foliage (Rajapakse *et al.*, 2007). The pathogens that infect cinnamon plants may similarly affect rubber plantations and vice versa. Hence, the pathogens in the alternative hosts can cause cross-infections in rubber plantations, leading to economic drawbacks.

Natural rubber plays a significant role in the Sri Lankan economy. Due to the numerous uses natural

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rubber products, humans have enhanced their mobility and daily activities through the utilization of these products in automotive and aircraft tires, water transportation, medical equipment, footwear, adhesives, and toys.

Latex production in rubber plantations can be reduced due to biotic and abiotic stresses. Among them, pathogenic diseases play a significant role and more than 60 pathogens have been recorded, which are capable of causing rubber plant diseases in Sri Lanka (Jayasinghe, 2010). Some pathogens can attack more than one host plant. When the pathogens attack the inter-crops of rubber plantations, the severity of the disease increases. Hence, identification of alternative hosts is important in order to safeguard the inter-crop as well as the main crop. The current study mainly focuses on the isolation and identification of the pathogens from cinnamon plants grown in rubber plantations as an inter-crop. This study aimed to identify *Cinnamomum zeylanicum* as an alternative host for *Colletrotrichum siamense* the main cause of circular leaf spot disease of rubber in Sri Lanka.

METHODOLOGY

Collection of leaf samples

Symptomatic leaves with brown colour lesions from cinnamon plants grown within the rubber plantations were identified. They were collected following a completely randomized design (CRD), and brought to the Plant Pathology and Microbiology laboratory in the Rubber Research Institute of Sri Lanka (RRISL) for pathogen isolation.

Isolation of pathogen

Under the cleaned laminar flow cabinet diseased leaf samples were cut into small pieces of 2 x 2 mm, from the advancing margin of lesions. Leaf pieces were surface sterilized with 70% alcohol for 2 minutes and rinsed three times with a sterile distilled water series. Finally, they were dried out using a sterilized filter paper. Surface sterilized leaf pieces were placed on the solidified PDA and incubated at 27 ^{0}C for 3 days (Hunupolagama *et al*., 2017). The different mycelia arising from the diseased tissues were sub-cultured into a new series using PDA and 10 morphologically different cultures were selected.

Single spore isolation

Single spore isolation was performed to obtain pure cultures from the sub-cultures. One sporulated mycelial plug from a 7 day old culture was cut by a 5 mm cork-borer and added to 10 mL of sterilized distilled water in a sterilized McCartney bottle. It was mixed well and 50 µL of each culture was added to a separate PDA plate. The suspension was spread throughout the PDA surface using a glass spreader. The plates were incubated for 3 days at 27 ^{0}C in the culture room. Germinating spores were transferred to new PDA plates and incubated for 3 days at 27 ^{0}C (Choi *et al*., 1999) to obtain pure cultures.

Proving Koch's postulates

The cinnamon plants were artificially inoculated with a $1x10^5$ conidia/mL spore suspension of 7-day old *C. siamense* cultures. The plants were kept in a moist chamber lined with moistened papers and the spraying of the spore suspension was undertaken every other day. The pathogen was successfully re-isolated from the symptomatic inoculated plants and subsequently identified, confirming the fulfillment of Koch's postulates.

Comparison of pathogenicity

Each conidial ($1x10^5$ conidia/mL) suspension was prepared from ten-day-old isolate cultures by re-suspending the conidia with 10 mL of sterilized distilled water per culture and conidial concentration was adjusted using a haemocytometer. Then, six rubber leaves belonging to the RRIC 121 clone, at the maturity stage between copper brown colour and apple green colour, were inoculated by placing 06 drops of the conidial suspension per leaf (20 µL per drop) of each culture. They were applied to the upper surface of the detached rubber leaves without wounding. Another six leaves were maintained as the control by placing drops of sterilized distilled water. The same experiment was repeated three times. The inoculated leaves were incubated in a moist chamber at 28 °C and the symptoms were observed after 2 days from the spore inoculation (Lin *et al*., 2017). Disease severity was assessed using a 0–4 scale, based on a modified version of the method described by Ma & Michailides (2002).

0 - No visible infection
1 - Light lesion around inoculation point lacking spores
2 - Large dark lesion without sporulation
3 - Large lesion and weak sporulation
4 - Very strong sporulation on the upper and lower surfaces of the leaf

The results were expressed as the disease index (DI) for each leaf which was calculated using the following formula (Ma & Michialides, 2002).

$$DI = \left(\sum_{i=0}^{4} N \times i\right) / \sum_{i=0}^{4} N$$

Where,
i is the severity (0–4), and
N is the number of leaves with the severity of i.

The *DI* for each leaf in each isolate was calculated using a modified method described in Ngobisa et al., (2013). The most pathogenic culture was selected for further studies.

Morphological analysis

Observation of reproductive characteristics

Reproductive characteristics such as conidia size, shape, and mycelial characters were observed under an Olympus light microscope with the addition of lacto phenol-cotton blue as the stain.

Observation of cultural characteristics

A mycelial plug was cut from the growing edge of each 7 day old colony using a 5 mm cork-borer and transferred on to the middle of a pure PDA plate. Cultural characters such as shape, elevation, nature, margin and pigmentation were recorded after 10 days of incubation under normal light and dark conditions at room temperature (27±2 ^{o}C).

Molecular level identification

Pure cultures of the selected isolate were prepared and submitted to Macrogen (Korea) via Genetech (Sri Lanka) for bi-directional Sanger sequencing of the internal transcribed spacer (ITS) gene region. Genomic DNA was extracted using the cetyltrimethylammonium bromide (CTAB) method, and PCR amplification of the ITS region was carried out using the primers ITS1 and ITS4.

RESULTS AND DISCUSSION

Brown circular lesions were observed on the cinnamon leaves. The lesion enlarges with time making a silvery area in the middle. Out of the 10 isolates, only *Colletrotrichum* sp. showed the pathogenicity for cinnamon (Koch's postulates proven) and on rubber leaves under the detached leaf assay. All the resulting *Colletotrichum* sp. isolated were morphologically determined as the same species. Based on the sequencing results, the isolate was identified as *Colletrotrichum siamense* (WDCM accession number RRISL87). The colony had a circular shape, flat elevation, smooth and cloudy nature, entire margin, and opaque nature. However, both upper and lower surfaces were pale white in colour with orange colour conidiomata arranged in rings. Conidia were aseptate with a single cell, green colour, smooth-walled, cylindrical, with both ends rounded, and granular. The conidia size was (13-16) x (4-6) µm. Conidiophores were directly formed from hyphae which were septate and branched. The mycelium of the culture was septate and branched with a width of 4-6 µm.

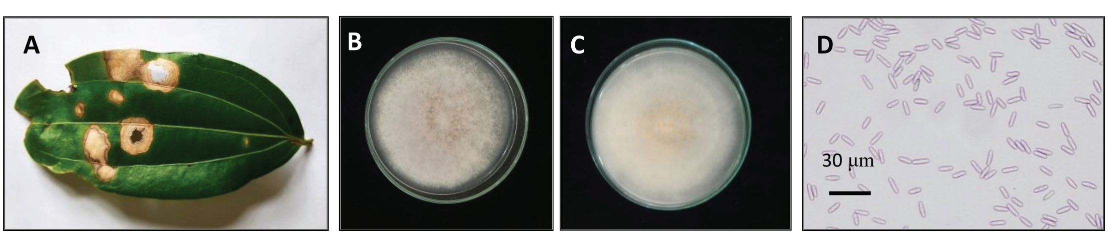

Figure 1: A: Symptomatic Cinnamon leaf; B: Upper surface of 7-day old culture grown on PDA medium; C: Lower surface of 7-day old pathogenic culture grown on PDA medium; D: Cylindrical conidia with rounded ends

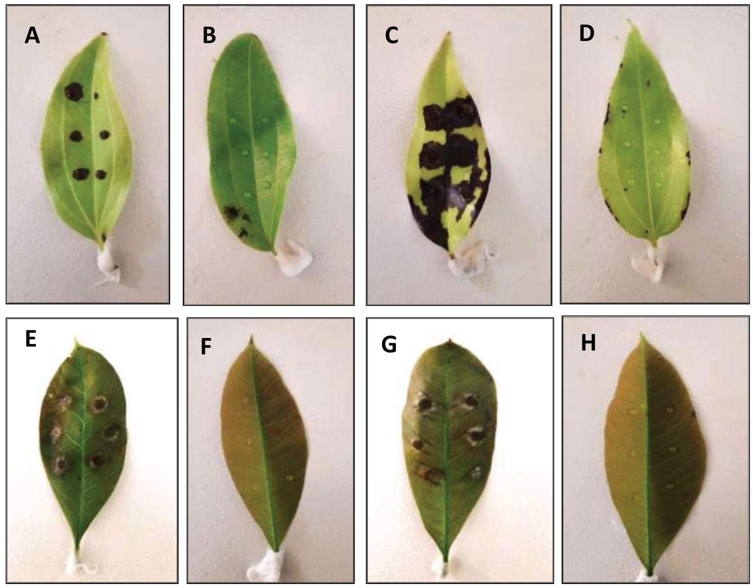

Figure 2: A: Non-wounded cinnamon leaf inoculated with *Colletotrichum siamense*; B: Non wounded cinnamon leaf inoculated with sterilized distilled water (non-wounded control); C: wounded cinnamon leaves inoculated with *C. siamense* D: wounded cinnamon leaves inoculated with sterilized distilled water (wounded control); E: Non-wounded rubber leaves inoculated with *C. siamense*; F: Non wounded rubber leaves inoculated with sterilized distilled water (non-wounded control); G: wounded rubber leaves inoculated with *C. siamense*; H: wounded rubber leaves inoculated with sterilized distilled water (wounded control).

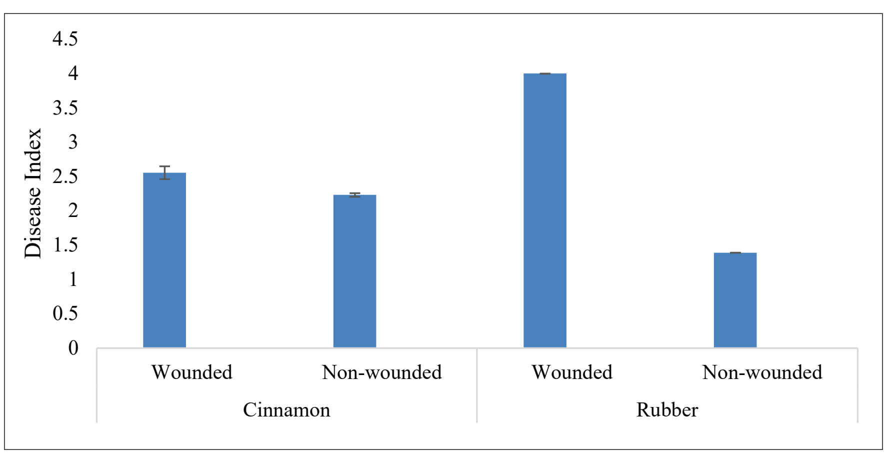

Figure 3: Disease index on the assessment of cross-infection possibility (Error bars indicate variability among replicates)

In rubber, *Colletotrichum gloeosporioides* was identified as the main causative agent of CLD (Petch, 1906) as well as in cinnamon. Later, *C. acutatum* was identified as the main cause of Colletotrichum leaf disease of rubber (Jayasinghe *et al*., 1997). Recently, *C. simmondsii, C. laticiphilum, C. nymphaeae,* and *C. citri* were identified as the pathogens of rubber (Hunupolagama *et al.*, 2017). The leaf spots/blight caused by the fungus *C. gloeosporioides* (Anandaraj & Devashayam, 2004) is found in almost all cinnamon growing areas in Sri Lanka mainly affecting the foliage. Moreover, *C. horii* has been previously identified as a pathogen of *Cinnamomum zeylanicum* (Madhurangi *et al.*, 2022). Hence, the identification of *C. siamense* as a pathogen in cinnamon with 93.14% identity in NCBI BLAST results, becomes the first report in Sri Lanka. The confirmation of *C. siamense* pathogenicity on cinnamon highlights a potential phyto pathological risk within cinnamon–rubber intercropping systems. Since rubber (*Hevea brasiliensis*) and cinnamon (*Cinnamomum zeylanicum*) are grown in close proximity, there is a plausible risk that pathogens affecting cinnamon could serve as alternate hosts or reservoirs, potentially facilitating cross-infection or increasing the inoculum load in the plantation. While *C. siamense* is primarily a cinnamon pathogen, its presence in intercrops may create conditions conducive to disease spread under favorable environmental conditions, such as high humidity (Hyde *et al*., 2009). Consequently, careful monitoring and integrated disease management strategies are recommended to minimize potential transmission and safeguard the health of both cinnamon and rubber crops. It is understood that a detailed study is much needed for accurate characterization of the current causative agents of this disease as the information is important for the establishment of causative pathogens, screening chemicals, and resistance breeding and also to formulate effective management practices.

CONCLUSION

According to the results of this study, *Colletotrichum siamense* was identified as a new pathogen of *Cinnamomum zeylanicum*, one of the most valuable spice crops that can be cultivated as an intercrop with rubber. However, further detailed characterization and identification are essential for the development and implementation of effective management strategies. For future studies, it is recommended to employ a multilocus molecular approach, as ITS sequencing alone may not provide sufficient resolution for accurate species-level identification within the *Colletotrichum* genus. Additionally, comprehensive assessments of disease severity across different seasons and geographical regions are necessary to evaluate the epidemiological risk and to design location-specific management practices. Such studies will provide a robust basis for minimizing potential yield losses and ensuring the sustainability of cinnamon–rubber intercropping systems.

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REFERENCES

Anandaraj, M., & Devasahayam, S. (2004). Pests and diseases of Cinnamon and Cassia. In: Cinamon and cassia. (Eds. P. N. Ravindran, K. N. Babu and M. Shylajah), *CRC Press, New York,* 239-258.

Batureine, M. (2009). Diversity of *Colletotrichum lindemuthianum* and Reduction of common bean germplasm to anthracnose disease. *MSc thesis submitted to the school of post graduate studies, Makerere University, Uganda.*

Cai, L., Hyde, K., Taylor, P., Weir, B., Waller, J., Abang, M., Zang, J., Yang, Y., Phoulvong, S., Liu, Z., Prihastuti, H., Shivas, R., McKenzie, E., & Johnston, P. (2009). A polyphasic approach for studying *Colletotrichum*. *Fungal Diversity*, *39*, 183–204.

Choi, Y., Hyde, K., & Ho, W. (1999). Single spore isolation of fungi. *Fungal Diversity, 3*, 29-38.

Hunupolagama, D., Chandrasekaran, N., Wijesundera, W., & Kathriarachchi, H. (2017). Unveiling members of *Colletotrichum acutatum* species complex causing Colletotrichum leaf disease of *Hevea brasiliensis. Current Microbiology*. *74*, 747-756. DOI: 10.1007/s00284-017-1238-6

Hyde, K. D., Cai, L., Cannon, P. F., Crouch, J.L. *et al.* (2009). *Colletotrichum* – names in current use. *Fungal Diversity 39*, 147-182.

Jayasinghe, C.K., Fernando, T.H.P.S., & Priyanka, U.M.S. (1997).. *Colletotrichum acutatum* is the main cause of *Colletotrichum* leaf disease of rubber in Sri Lanka. *Mycopathologia 137*, 53–56. DOI: 10.1023/A:1006850119146

Jayasinghe, C. (2010). White root disease of the rubber tree: an overview. *Paper presented at the proceedings of the International workshop on White root disease of* Hevea *rubber*, 1-8. https://doi.org/10.1111/efp.12794

Lin, C., Liu, X., Shi, T., Li, C., & Huang, G. (2017). The *Colletotrichum gloeosporioides* perilipin homologue CAP 20 regulates functional appressorial formation and fungal Virulence. *Journal of Phytopathology*, *166*, 216-225. https://doi.org/10.1111/jph.12678

Ma, Z., & Michialides, T. (2002). Characterization of *Botryosphaeria dothidea* isolates collected from pistachio and other plant host in California. *Phytopathology*, *92*, 519−526. DOI: 10.1094/PHYTO.2002.92.5.519

Madhurangi, H. M. T. T., Wanigatunge, R.P., Jayasinghe, G.G., & Attanayake, R.N. (2022). Identification of the fungal pathogen causing leaf blight of *Cinnamomum zeylanicum* Blume and *in vitro* screening of Tebuconazole sensitivity. *Plant Health- The Second National Symposium of Sri Lanka Association for Mycology and Plant Pathology (SLAMPP),* 18.

Ngobisa, A., Abidin, M., Wong, M., & Noordin, M. (2013). *Neofusicoccum ribis* Associated with Leaf Blight on Rubber (*Hevea brasiliensis*) in Peninsular Malaysia. *Plant Pathology Journal*, *29*(1), 10-16. DOI: 10.5423/PPJ.OA.07.2012.0110

Petch, T. (1906). Description of new Ceylon fungi. *Annual Report of Botanical Gardens*, *Peradeniya*, *3*, 1-10.

Rajapakse, R. H. S., & Wasantha Kumara, K. L. (2007) A Review of Identification and Management of Pests and Diseases of Cinnamon (*Cinnamomum zeylanicum* Blume). *Tropical Agricultural Research & Extension, 10*, 2007. DOI: 10.4038/tare.v10i0.1864

Ravindran, P. N., Shylaja, M., Babu, K. N., & Krishnamoorthy, B. (2003). Botany and crop improvement of cinnamon and cassia. In *Cinnamon and cassia,* CRC Press. 30-95.