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State of Knowledge Regarding the Recent Outbreak of Coconut Root Disease (Wilt)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The Integrated Disease Management (IDM) approach consolidates these various strategies into a unified framework aimed at reducing disease burden while enhancing crop productivity. IDM promotes minimal reliance on chemical inputs and favours the adoption of biostimulants, neem-based formulations, and microbial inoculants. The paper aims to explore the Recent outbreak of coconut root (wilt) disease in Tamil Nadu. Serological detection methods for phytoplasma detection were initiated in 1980's involving the production of polyclonal, monoclonal antibodies, followed by antisera and utilising them for ELISA and immunofluorescent assay. Plant diseases with yellowing,

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little leaf, white leaf, witches broom, stunting symptoms erroneously misidentified as viral menace were later classified as Mycoplasma-like organisms (MLOs) based on their morphology and ultrastructural similarity. A multiplex qPCR assay was developed for the detection of phytoplasma in sesame and *Rubus sp.* Hence, it would serve as a myriad, speed, simplicity and reproducibility. Thus, the new era of CRWD management is marked by eco-friendly innovation, biological synergy, and sustainable agricultural practices. Looking ahead, advanced tools such as RNA-based biopesticides and CRISPR-mediated genome editing offer promising avenues for long-term disease resistance.

Keywords: Integrated disease management; mycoplasma; biostimulants; plant diseases; coconut.

1. INTRODUCTION

The coconut, or *Cocos nucifera* L., is a major crop cultivated across 12,257 million hectares of land globally with an annual yield of 66 metric tonnes of nuts (FAOSTAT, 2023). The coconut seems to have spread from South East Asia, including Australasia, eastward through the Pacific Ocean and further into America, into the West. Over the calm tropical waters, it made its way to Madagascar and India (Narmadha & Karunakaran, 2022; Surya *et al*., 2024). “Root (wilt) disease (RWD), which is caused by phytoplasma and can, in extreme circumstances, can drastically reduce its yield ranging from 35% to as high as 80%, - crop productivity” (Ramjegathesh *et al.,* 2019). “Root wilt disease (RWD) caused by phytoplasma is the most important one and was first observed in Kerala in 1882. It causes a 35% yield reduction, and the losses may extend up to 80% in severe cases” (Balasubramaniam, 2022). Consequently, Root wilt is regarded as a risk factor globally, across allthe coconut plantations in Kerala, as well as the adjacent regions of Tamil Nadu. A wide number of palms exhibit characteristic symptoms exhibit typical flaccidity ribbing of the leaves, yellowing and necrosis of the leaflets. A significant RWD outbreak became apparent in 2019. Although Root (wilt) disease has been prevalent in the state of Kerala for nearly 130 years, it is believed to have made its emergence after the great floods of 1882. It has now established itself almost contiguously in eight southern districts of Kerala, viz. Thiruvananthapuram, Alappuzha, Kollam, Kottayam, Pathanamthitta, Idukki, Ernakulam and Thrissur. sporadic incidence has been observed in the districts of Malapuram, Palakkad, Kozhikode, Wayanad and Kannur and in some groves in the neighbouring states of Tamil Nadu, Karnataka, and Goa. “The primary signs of illness are Wilting, drooping, and loss of leaf turgidity. Other common associated symptoms of foliar diseases include ribbing, paling or yellowing, and necrosis of leaflets” (Ramaswamy et al., 2016). Despite decades of intensive research, there are no defined remedies for phytoplasma. However, various steps have been implemented to curb its proliferation.

1.1 Nature of Disease

Plant diseases with yellowing, little leaf, white leaf, witches broom, stunting symptoms erroneously misidentified as viral menace were later classified as Mycoplasma-like organisms (MLOs) based on their morphology and ultrastructural similarity. These etiological agents are pleomorphic, wall-less less possess a 680-1530 KB small genome size as demonstrated by Japanese scientists in 1957 (Bertaccini, 2018). The term "root disease" was coined by Butler, likely due to the observation of decayed roots in the affected palms. McRae (1916) later referred it as leaf rot disease. The disease is not lethal but rather chronic progressing slowly, spreading and gradually debilitating reducing itsyield; the management practices can alleviate the symptoms and improve the yieldof wilt in the leaves or roots, proving that the name "root" (wilt) is misleading and misleads readers about the disease's symptoms. Considering these factors, Dr. M.S. Swaminathan proposed the moniker COCONUT DECLINE during his 1976 keynote speech at the International Symposium on Coconut Research and Development held at CPCRI Kasaragod, Kerala.

1.2 Spread of Coconut Root (Wilt) from Kottayam to Tamil Nadu

Coconut is cultivated on more than 89,927 hectares (CDB, 2023) in Coimbatore district, mainly in the blocks of Pollachi, Kinathukadavu, Sulthanpettai, and Anaimalai.

Durgadevi et al. (2024) indicated that the disease was prevalent in Pollachi South, Pollachi North, and Anaimalai blocks of Coimbatore, Tamil Nadu, which led to a reduction in the average productivity from 13,637 nuts per hectare in 2017-18 to 9,430 nuts per hectare in 2022-23 (Fig. 1).

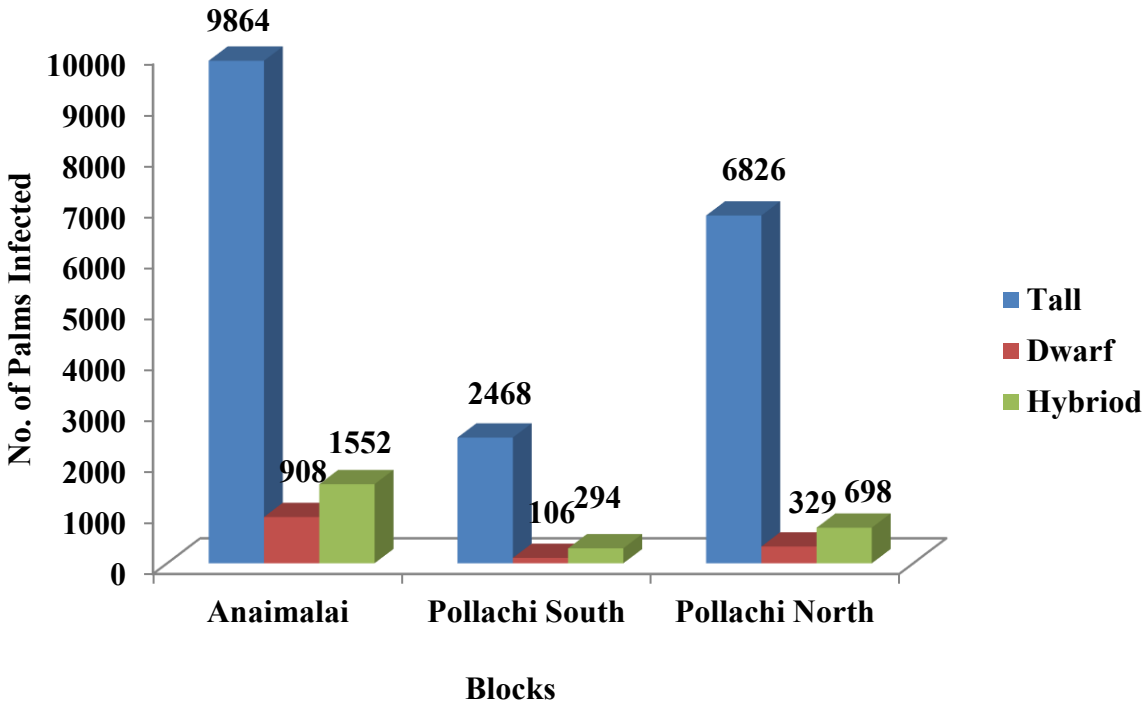

Fig. 1. Block-wise disease spread

1.3 Molecular Detection of Phytoplasma

The genome of phytoplasma was found to contain all the essential genes for replication, transcription and translation, along with that it also has sec transporter system, maltose ABC transporter system. Immunodominant membrane proteins are the major cell membrane proteins of phytoplasma. They also contain jumping genes and potential mobile units helping phytoplasma to adapt to the host environment.

“These pathogens were found to infect both plant and insect species and are vulnerable to more than 700 species” (Hogenhout et al., 2008) and are vectored by various phloem feeders like plant hoppers, leaf hoppers, as well as by few parasitic plants. The infected plants are symptomatised by little leaf, yellowing, greening of flower petals, loss of apical dominance of the shoot, loss of vitality, and root growth leading to eventual mortality of the plant. As the pathogens proliferate in the phloem tissue, it poses a significant challenge in in vitro culturing, making it difficult to characterise (Contaldo et al., 2012).

The first report on the incidence of Coconut Phytoplasma in Kerala was in 1874, which became epidemic, followed that it was yellow disease in arecanut in 1914 (Solomon et al., 2001). The phylogenetic analysis placed the pathogen to be belonging to the genus *Candidus* Phytoplasma which was obtained by 16S rRNA sequencing analysis. The molecular data derived from Phytoplasma DNA sequencing, mainly 16s rRNA sequence, have been instrumental in identifying the breadth of the diversity and its genetic relationship underpinning the phylogenetic and taxonomical classification of Phytoplasma. The 16s rRNA, despite being the mainspring in taxonomic frameworking, may not be optimal for species-level differentiation. The advent of whole genome sequencing had overtopped the above limitation, leading to the complete genome sequencing and the first being the Onion Yellow Phytoplasma Mild strain (OY-M) in the year 2004. Till now, 47 phytoplasma genomes with 13 groups and 29 subgroups have been sequenced (Debonneville et al., 2022).

1.4 Detection based on Microscopy

The difficulty in culturing the phytoplasma had hampered the disease detection for a long time, and in the nascent stage, Light microscopy was employed for the localisation and visualisation of pathogens within the infected tissues, utilising an array of dyes. The subsequent staining and semi-thin sectioning yielded more detailed information regarding the infection (Musetti and Favali, 2004). For visualisation, Diene’s and DAPI staining techniques were also utilised, followed by fluorescent microscopy using fluorophores and transmission electron microscopy to achieve high-resolution imaging of Phytoplasma infection (Bertaccini & Lee, 2018). Electron microscopy revealed that the Phytoplasma measured between 250-400 nm. Although various microscopic techniques were implemented, they primarily facilitated the detection and morphological identification of the pathogen, which led to their eventual replacement by advanced molecular diagnostic methods.

1.5 Serology-based Detection

Serological detection methods for phytoplasma detection were initiated in the 1980sinvolving the production of polyclonal, monoclonal antibodies, followed by antisera and utilising them for ELISA and immunofluorescent assay. Enzyme-linked Immunosorbent Assay (ELISA) was used for the detection of phytoplasma in Brinjal and Sugarcane (Viswanathan, 2001). Even though the reaction is considered simple, the process of developing the antisera and monoclonal antibody is time-consuming and difficult, marking the limitation of this method in phytoplasma detection.

1.6 Detection based on Nested PCR

It was in 2000 that the golden standard molecular diagnostic method, Polymerase Chain reaction (PCR), came into the limelight for its application in the identification and characterisation of phytoplasma. PCR is considered to be more sensitive than serological, microscopic and other available hybridisation methods (Schneider et al., 1993). The efficiency of PCR depends on the quality of DNA, the primer and the conditions for the reaction. The 16s rRNA genes, which were found to be highly conserved, were the target of amplification, accompanied by nested PCR followed by RFLP became the routine for molecular diagnosis of Phytoplasma (Rao et al., 2017).

The subgroup responsible for inducing root wilt in Indian coconut trees has been identified and confirmed as the 16sr XI-B Phytoplasma where as the root wilt in the Kalimantan region of Indonesia has been associated with the 16sr XII subgroup (Manimekalai et al., 2014).

Furthermore, the causative agent of the Indian arecanut yellow lethal disease has been determined to be the 16sr X subgroup. The identification of Coconut root wilt and the substantiation of phytoplasma association with Indian Coconut root wilt disease were identified by utilising the 16s rRNA gene with P4/P7 primers (Sharrnlla et al., 2004). A nested PCR approach was implemented for the molecular detection of phytoplasma in coconuts using these primers (Manimekalai et al., 2010). The apex concentration of pathogenic phytoplasma was discerned in the palm trunk, followed by the root, mature inflorescence, spear leaf, and flag leaf. Additionally, "a higher concentration of DNA was extracted from young leaf tissue with the midrib, which was used for the nested and gene-specific PCR. Further nested PCR was developed using the *imp* gene to identify the chickpea phytoplasma" (Reddy & Rao, 2019). Even though nested PCR is considered one of the widely accepted methods for the detection of phytoplasma, it carries its own disadvantage, including risk of contamination with two PCR cycles and an extended time of analysis.

1.7 Gene-specific PCR/qPCR-based Detection

Recent studies suggested the use of mild conserved genes coding secretory proteins (SEC A, SEC Y), gyrase (gyr A), ABC transporter gene, etc., along with the 16 S rRNA gene. Conventional PCR holds a major disadvantage as it is less sensitive to detect the low concentration of phytoplasma pathogen in the infected plant and the insects. Secondly, the significant advancement of the PCR assay, which is qPCR (quantitative PCR) helped in accurate disease detection in less time with reduced risk of cross-contamination (Satta et al., 2017). "It allows the detection of phytoplasma at a lower titer in both plants and insects, which supports the early detection, in turn, a faster management of disease" (Monis & Giglio 2006). A multiplex qPCR assay was developed for the detection of phytoplasma in sesame and *Rubus sp.* Hence, it would serve as a myriad, speed, simplicity and reproducibility (Ikten et al., 2016). Even though they are sensitive and accurate, they employ a series of costly equipment with expertise for analysis, and can be performed only in the laboratory.

1.8 Point-of-Care Detection Methods

The limitations of conventional and modern diagnostic techniques necessitate the development of simple, cost-effective bedside diagnostic methods that enable rapid detection and circumvent the logistical challenges associated with handling infected samples. Recent advancements in nanotechnology and biosensor-based diagnostics, combined with portable systems integrating IoT, have revolutionised the diagnostic field. The rapid, sensitive, cost-effective diagnostic methods for detection of plant pathogens have been developed in recent years, and the key techniques include lateral flow assay, loop-mediated isothermal amplification assay for visual detection of pathogens and diseases (Kumar et al., 2021). These techniques help the farmers to detect the infection at the field level without the need for expertise, rapidly and economically, which in turn helps them to reduce the crop loss by following the management and control strategies at the initial stage of infection. "A molecular method ie., loop mediated isothermal amplification assay (LAMP) found to be more specific, sensitive, efficient and works extensively at the field level without the need for any costly equipments, which was employed for the detection of phytoplasma causing napier stunt in Africa followed by the detection of phytoplasma infection in coconut and sugarcane" (Obura et al. , 2010). To date, there are no effective treatment methods available for the control of phytoplasma infecting various plant and insect species. Since they are unculturable creatures, the study of host-pathogen interaction and the metabolic pathway of the pathogen is at the initial stage of research. A strong and precise diagnostic method for the precise detection of the development of a management scheme is the need of the hour.

1.9 Insect Transmission of RWD in Coconut

"The disease was transmitted by planthopper *Proutista moesta* (Westwood) [Derbidae: Hemiptera] and lacewing bug *Stephanitis typica* (Distant) [Tingidae: Heteroptera]. Typically, these insect vectors' salivary glands and phloem sieve tubes contain phytoplasmas" (Ramjegathesh et al., 2012).

"The tingid *S. typica* transmits coconut root (wilt) disease in Southeast Asia. This lacewing bug breeds on coconut foliage and feeds from the undersurface of the mature leaves in a destructive manner. Stylet course studies revealed that the lace bug inserts its stylet through stomata and ruptures the walls of the

cells traversed in the course to reach the vascular bundles. The stylet tip in such cases terminates in phloem, thereby suggesting the ability of the bug to acquire the phloem-bound phytoplasmas" (Solomon and Geetha, 2004).

"The planthopper *P. moesta* breeds in decaying organic matter in the soil, and only adult insects are seen feeding on the leaves of coconut palms. The planthopper, *P. moesta,* is the phloem feeder in a non-destructive manner. The feeding of this species in coconut is confined to the leaflets of middle and outer whorls" (Rajan, 2011). "They suck the sap from the abaxial surface of leaflets, and no feeding marks were observed in the leaflets due to their feeding. The absence of honeydew, presence of stylet sheath and permeable midgut for traversing phytoplasma attribute good vector ability" (Edwin and Mohankumar, 2007).

The symptoms of root (wilt) disease can be effectively reduced by applying fertilisers at the correct dosage and treating the roots with oxytetracycline at a rate of 100 mg/100 ml water per palm monthly. However, because of the increased incidence of phytoplasma, symptoms would reappear after three years if root therapy and other management methods were stopped (Rajendran et al., 2018). Furthermore, no documented vector control technique for controlling the vectors of the root (wilt) disease in coconuts has been discovered to yet.

1.10 To Study the Microbiome in Healthy and Root-Wilt-Affected Crops

Dynamic changes in climatic patterns, plants face continuous exposure to various biotic stressors. However, plants possess their own defence mechanisms against invaders. Recent studies have highlighted the crucial role of plant microbiomes in disease tolerance and resilience. Barbieri et al. (2023) reported a shift in the citrus endophytic microbial profile after infestation with Liberibacter asiaticus, while Bulgari et al. (2012) found that grapevines infested with phytoplasmas exhibited lower microbial diversity compared to healthy plants.

Moreover, More et al. (2022) observed that inoculation with *Dyella* spp. and *Pseudomonas migulae* reduced the severity of grapevine infestations with phytoplasmas. Additionally, the endophytic diversity of infected coconut palms was significantly reduced compared to healthy plants (Morales-Lizcano et al., 2017). In addition to endophytic microbes, rhizosphere microbes also play a crucial role in nutrient acquisition, providing disease tolerance, and recruiting antagonistic microbes against pathogens.

Gopal et al. (2005) determined the rhizosphere microbial community in both phytoplasma-infested and tolerant plants, revealing an increased population of bacteria and fungi in disease-infested palms compared to healthy ones. However, actinobacterial populations were significantly higher in healthy palms than in infested plants. Although the total population of the microbial community is higher in diseased plants, specific functional microbes such as nitrogen fixers, phosphate, and silicate solubilisers are more abundant in disease-tolerant plants.

The presence of silicate microbes indicates increased silica uptake, enhancing the tensile strength of leaves to resist vector feeding and prevent phytoplasma infection (Li et al., 2024). In this context, determining the microbial community in both root wilt-affected and healthy coconut palms reveals the importance of microbes in sustainable crop management strategies.

1.11 Management of Coconut Root Wilt Disease: A New Era of Integrated Approaches

Although extensive research has been conducted over the years, successful control of phytoplasma has yet to be achieved. Nevertheless, strategies to mitigate its spread include implementing quarantine measures, maintaining sanitation, applying pesticides, using resistant plant varieties, employing biological control techniques and practising integrated nutrient management to manage coconut root (wilt) disease (Maheswarappa et al., 2005).

The management of Coconut Root Wilt Disease (CRWD), caused by phytoplasma, has undergone significant transformation in recent years. Rather than relying on single-method treatments, the focus has shifted toward integrated strategies that combine cultural, chemical, biological, and genetic interventions. Among them, the first line of defence is the removal of severely affected palms and their replacement with disease-free seedlings. Ensuring proper irrigation, improving soil health, and incorporating leguminous intercrops and organic manures help to improve palm vigour

and resilience. These cultural practices not only suppress disease progression but also contribute to overall farm sustainability.

A key component of modern management is phytosanitation, which involves early identification and eradication of infected trees to minimise the source of infection. Planting resistant or tolerant coconut varieties, such as Kalpa Raksha and Kalpa Haritha, has demonstrated promising results in minimising yield loss in endemic zones. These improved cultivars are now promoted through national coconut development initiatives. Maintaining soil fertility through green manuring, mulching, and the application of compost has also been recognised as vital in promoting plant defence mechanisms and reducing disease incidence.

Chemical interventions, mainly the application of tetracycline through root feeding or stem injection, have provided short-term suppression of phytoplasma but are no longer preferred due to concerns about environmental toxicity and resistance development. Consequently, researchers are now exploring safer, plant-based compounds and nanoformulations that are both environmentally friendly and effective. In parallel, biological control strategies have seen increasing use. Microbial agents such as *Pseudomonas fluorescens*, *Trichoderma harzianum*, and *Bacillus subtilis* have shown potential in inducing systemic resistance and promoting plant health when used as seedling root dips or soil amendments.

2. CONCLUSION

The Integrated Disease Management (IDM) approach consolidates these various strategies into a unified framework aimed at reducing disease burden while enhancing crop productivity. IDM promotes minimal reliance on chemical inputs and favours the adoption of biostimulants, neem-based formulations, and microbial inoculants. In addition to managing existing infections, these measures enhance the long-term sustainability of coconut plantations. The integration of tolerant varieties, consistent monitoring, vector control, and improved cultural practices forms the backbone of IDM. Looking ahead, advanced tools such as RNA-based biopesticides and CRISPR-mediated genome editing offer promising avenues for long-term disease resistance. Thus, the new era of CRWD management is marked by eco-friendly innovation, biological synergy, and sustainable agricultural practices.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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