RESEARCH ARTICLE

LETHAL YELLOWING DISEASE IN *BORASSUS FLABELLIFER* IN JAFFNA PENINSULA

S. Vinujan[1], C. J. Emmanuel[2], S. J. Arasakesary[3], S. Srivijeindran[1]*

[1]Palmyrah Research Institute, Palmyrah Development Board, Jaffna, Sri Lanka
[2]Department of Botany, Faculty of Science, University of Jaffna, Sri Lanka
[3]Regional Agriculture Research & Development Centre, Kilinochchi, Sri Lanka

ABSTRACT

Palmyrah palm (*Borassus flabellifer* Linn.) is a highly versatile tree mainly distributed in the North and Eastern provinces of Sri Lanka. Even though the plant is rarely affected by pathogens due to its extremely woody nature, some significant abnormalities in plants growing in the Pungudutivu area in Jaffna Peninsula have been noted recently. The present study aimed to characterize the abnormality, identify the disease and confirm the possible causative agent. In this study, the progression and incidence of disease were determined through field visits. A literature search was conducted to compare the symptoms with other palm varieties. Additionally, the possibilities for phytoplasma infection were tested with phytoplasma-specific universal primers using PCR. Initially, the plants showed progressive leaf yellowing and drying from the leaf tip towards the petiole followed by collapsed spear leaf hanging downward in the crown. Finally, the crown falls off the trunk. The symptoms are characteristics of the lethal yellowing disease. The PCR result confirmed the association of phytoplasma with the diseased plants. To our knowledge, this is the first report from Sri Lanka regarding the lethal yellowing disease in Palmyrah palm. Since the disease severely affects the plant and spreads rapidly, there is an urgent need to study the epidemiology of the disease and to find out suitable control measures.

Keywords: *Borassus flabellifer*, *Lethal yellowing*, *Phytoplasma*, *Polymerase Chain Reaction*

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1. INTRODUCTION

Borassus flabellifer Linn. (Palmyrah palm) is a woody perennial plant with an unbranched sturdy trunk. It is distributed throughout the plains of South and Southeast Asia [1]. In Sri Lanka, it is present in eleven districts while prominent in the North and East provinces of Sri Lanka [1]. A survey conducted between 1997 and 2000 had estimated approximately nine million palmyrah trees in the North and East areas like Kilinochchi, Mullaitivu, Vavuniya, Trincomalee, Batticaloa,

* Corresponding author: jeyaseelan.ec@univ.jfn.ac.lk ORCID: 0000-0003-1767-1356

Ampara, and Mannar in Sri Lanka. In these areas, the palm grows in the wild and has been established as natural vegetation for centuries. Other than the above regions, palmyrah palms are extensively distributed in Puttalam and Hambantota districts as well [2].

The erect, straight, unbranched, almost black trunk grows up to 30m. The trunk consists of numerous circular leaf scars evenly arranged as parallel lines. The leaves are fan-shaped and have a long petiole (90-120 cm long). Approximately 30 to 40 leaves form a rosette shape crown at the apex. This slow-growing dioecious plant starts flowering only after 12 to 15 years. It produces flowers and fruits vigorously and continuously throughout its life. The male and female plants are apparently similar except in the morphology of inflorescences. Therefore, male and female plant differentiation is impossible until the first bloom [3, 4].

The parts of the palm tree, which include leaves, flowers, fruits, young tubers, and the tree trunk, provide commercially important raw materials and food. The leaves can be used for roofing and making baskets, umbrellas, mats, fans and hats. The hardy fiber extracted from the plant is used to make utility items such as ropes and brooms. Palm timber is heavy, hard and durable, and is widely used in the construction industry, especially to make roofing beams and wharf pilings. The inflorescence sap is used to produce palm sugar and alcoholic beverages. Palmyrah tubers, the long cylindrical edible sprouts of germinating seeds, can be consumed by munching. It is a good source of carbohydrates; it can be made into a variety of nutritious food products. Furthermore, the extracts from various parts of this palm have exhibited different pharmaceutical benefits such as antioxidant [5], anti-inflammatory [6], anticancer [7], and anti-diabetic [8]. The rural people residing in the palmyrah growing areas are primarily dependent on these products. Therefore, a substantial economic value is aided throughout its parts to the local population. The Palmyrah Development Board (PDB) was established in 1978 to develop the industry, from the cultivation stage to the consumption of palmyrah-based products [2].

Due to the woody nature of palmyrah palm, they are generally resistant to pests, pathogens, and other stress conditions caused by environmental factors. The palmyrah palm exhibits the fewest symptoms in response to biotic and abiotic stress, similar to the majority of monocots. Some of the common diseases recorded in *B. flabellifer* are leaf spot, leaf blight, bud rot, and tuber rot [1]. In recent days, some visible abnormalities in the appearance and growth habit have been observed in the palmyrah plants growing in several areas of the Jaffna Peninsula. The severely affected plants lose their crown and eventually die. The present study aimed to characterize the symptoms that associated with this disease and determine the possible causes.

2. MATERIAL AND METHODS

2.1 Study site

The study was carried out in Pungudutivu, a small island located west of the Jaffna Peninsula in Sri Lanka (Figure 1). The identified location is close to Madathuveli Murugan temple and close to the Valukkairaru – Pungudutivu – Kurikadduwan road, approximately 50 km away from Jaffna town, geologically located at 9° 35’ North and 79° 51’ East. This site belongs to the South Divisional Secretariat division of the Island with an area of 22.5 km^2.

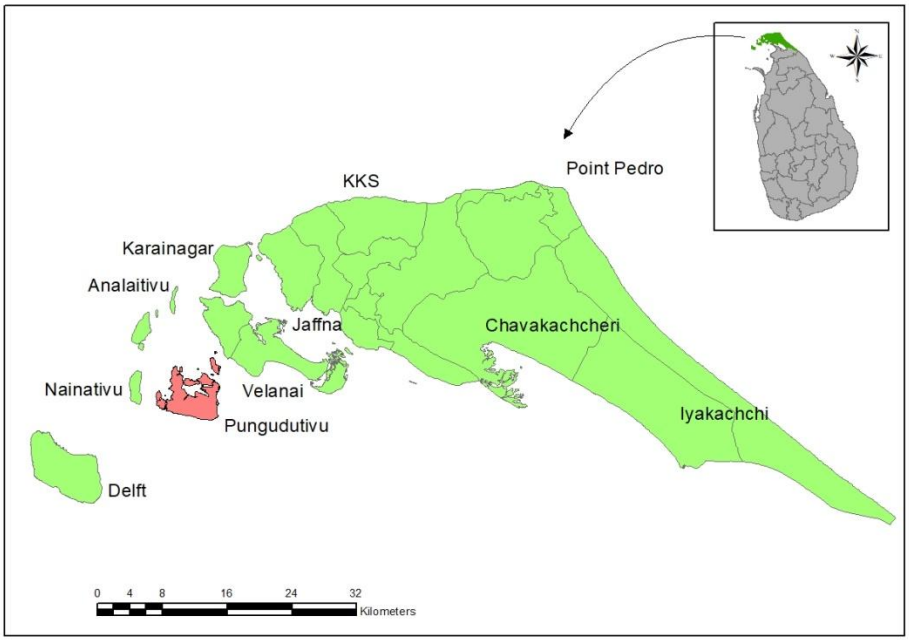

Figure 1: The geographical location of the study area. The Pungudutivu Island (highlighted in red) is located just west of the Jaffna Peninsula

2.2 Characterization of Disease Symptoms

During the field visit, randomly selected trees were observed for the abnormal symptoms (e.g. collapsed leaves and shoot, the crown separated from the trunk). Prominent variations were noted in leaves, reproductive structures, and the crown of the trees. All the abnormal characters were listed, and they were compared with already reported characteristic symptoms of diseases in palms. A literature review was performed using the Web of Science, Scopus, and Google Scholar to find the appropriate published work.

2.3 Disease Incidence Assessment

In the study site (approximate land area of 50,125 m^2), three 100m x 100m size quadrats were placed. The disease incidence was determined by calculating the percentage of palmyrah trees showing abnormal symptoms out of the total number of plants encountered in each quadrat.

$$Disease\ incidence = \left(\frac{Number\ of\ trees\ with\ abnormal\ symptoms}{Total\ number\ of\ trees\ assessed}\right) \times 100$$

2.4 PCR Mediated Detection of Phytoplasma

Newly developed leaf samples were collected from six symptomatic and six non-symptomatic plants. Samples were immediately brought to the laboratory in sterile sealed bags and kept at – 20°C until DNA extraction.

The DNA was extracted by a method described by Marcone (2019) [9]. The method has been specially recommended for extracting DNA from phytoplasma-infected plants for PCR assays. In this Phytoplasma enrichment procedure, 1 g of the tissue was ground in ice-cold phytoplasma

grinding buffer. Then the pellet was suspended in 1 mL of warm extraction buffer and kept at 60 °C for 30 min. After the heat treatment, chloroform/isoamyl alcohol (24:1, v/v) was added to the above suspension and mixed well until it forms an emulsion. The mixture was centrifuged (Z 206 A, Hermle, Germany) at 7600 x g for 10 min and the upper aqueous phase was transferred to a 2 mL tube. One volume of ice-cold isopropanol was added and mixed thoroughly and centrifuged at 15,000 x g for 10 min. The pellet was dissolved in 70% (v/v) cold ethanol and centrifuged at 15,000 x g for 5 min. Finally, the pellet was dissolved in TE buffer.

The selection of PCR primers and amplification conditions were adapted from Bertaccini *et al*., (2019) [10]. A universal primer pair R16F2n/R2 which targets a small part of the 23S rRNA gene, intergenic 16S–23S and 16S rRNA, was used with the following amplification conditions: Initial denaturation at 95 °C for 3 min; followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 2 min and extension at 72 °C for 3 min; final extension at 72 °C for 10 min. To visualize the PCR product, 1% agarose gel incorporated with ethidium bromide was prepared, and gel-electrophoresis was performed with 50V for 30 min. Finally, the gel was kept on a transilluminator to visualize the DNA bands under UV light.

3. RESULTS AND DISCUSSION

The plants in this site showed different stages of disease development: from mild symptoms like foliage yellowing or browning to severely affected dead plants. The diseased plants were situated randomly in the visited study site (Figure 2). The above two observations showed that the disease might be caused by a biotic factor rather than an abiotic factor, which causes relatively, uniform disease development in plants.

Figure 2: A view of the wild-growing palmyrah palms in the study area. Healthy plants (a), plants with different stages of disease development (b), and completely affected (without crown) plants (c) are in the image

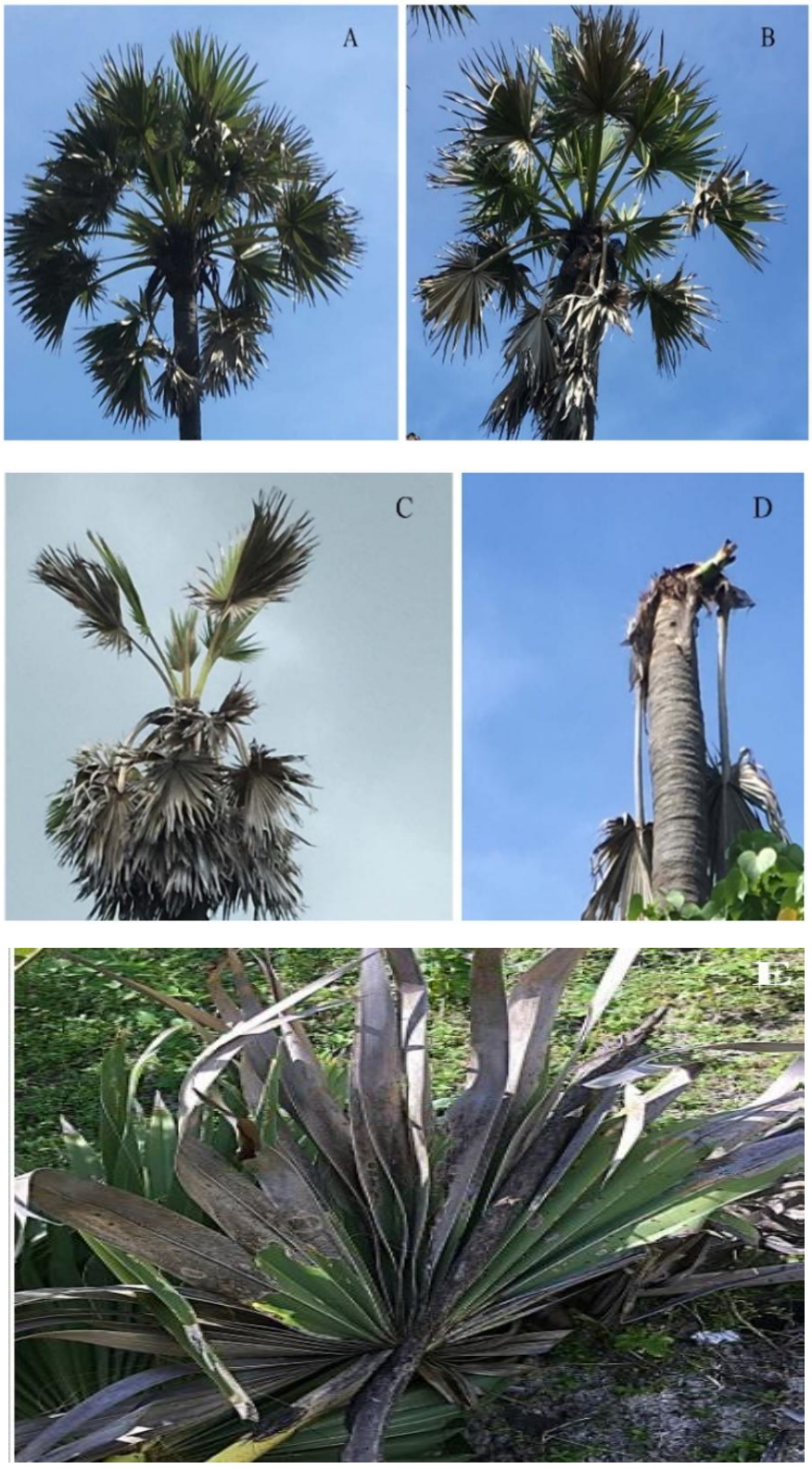

Figure 3: Different stages of disease development. (A) Healthy plant (B) Progressive tissue death in older leaves (C) Brown, dry leaves hung around the trunk and failed to produce reproductive structures and progressive leaf death in the plants and D) Trunk without crown F) A leaf with tissue death.

The first noticeable symptom was foliage yellowing or browning. The yellowing starts in the oldest leaves and progress towards the younger leaves. The yellow leaves finally turned brown, dry, and hung on the trunk for a few weeks before dropping (Figure 3). Plants failed to produce reproductive structures (flowering structures) or had poorly developed reproductive structures. In some cases, the developed young flowering structures showed burning or necrotized symptoms without producing fruit.

While the symptom development progressed towards the crown, the youngest leaf collapsed and dropped from the crown due to the tissue death in the apical meristem. In severely affected plants, all leaves collapsed (including spear) and hung down in the crown. Finally, the crown separated from the trunk. The disease incidence determination showed that the percentage of infected plants varied between 38.5% and 55.8% in the selected three quadrats (Table 1). These disease incidence values exhibit the prevalence of the disease in the selected location, and proper care should be given to surrounding uninfected areas.

Table 1. Disease incidence for lethal yellowing disease in three different sites

Sampling area	Total number of trees	Number of infected trees	Disease incidence (%)
Quadrat 01	128	52	40.6
Quadrat 02	86	48	55.8
Quadrat 03	109	42	38.5

A rapidly spreading non-lethal disease in coconut palm caused a severe outbreak in Sri Lanka in 2006. The disease was later named Weligama coconut leaf wilt disease. The diseased plants showed extreme yellowing of fronds and marginal necrosis on the flaccid leaflets. Later, the crown turned smaller, the trunk became tapered, and finally, the plant failed to produce flowering structures and fruits [11], [12]. In Weligama coconut leaf wilt disease, the symptom first appears in young leaves [13]. Nevertheless, in the present study, the symptoms were initially noted in mature leaves, progressing to young leaves (Figure 3 A-C). Furthermore, the disease noted in the palmyrah palm is lethal to the plant, in severe disease conditions as, the plants lose their crown entirely. The defect in reproductive structures was the prominent similarity between these two diseases.

Lethal yellowing is another severe problem reported in the coconut palm, palmyrah palm, and 35 additional palm species in Florida, Caribbean countries, Southeast Asia, and East and West Africa [14]. This disease is a rapidly spreading and highly devastating pandemic disease. The symptoms of lethal yellowing had the most resemblance to those seen in palmyrah palms in the current investigation. The symptom includes the progressive yellowing or browning of leaves, the decay of the youngest leaf and apical meristem, the collapsing of the crown, and finally leaving a trunk without a crown [15]. According to previous reports, a cell wall-less bacterium, known as phytoplasma, is the causative agent of the lethal yellowing disease. The planthopper transmits the pathogen, and in host plants, it spread systemically with in the phloem tissue [16].

Based on the above symptomatology and literature search, the disease reported in this study might be the lethal yellowing disease and it might be caused by phytoplasma. Since *in vitro* culture is not feasible for phytoplasma, the pathogen is commonly detected based on molecular diagnostic tests. A PCR test for the DNA extracted from six diseased and six healthy palmyrah palm was conducted using phytoplasma-specific universal primer. The PCR reactions yielded the expected PCR product of 1400 bp in size (Figure 4). The phytoplasma infected Churai (*Ziziphus oenoplia*) sample was used as a positive control. There was no amplification noted in DNA samples collected from healthy plants (Figure 4). Since the coconut palm is also highly susceptible to the disease [16], it is essential to take precautions to manage the disease spread among the palmyrah palms and spread from palmyrah palms to coconut palms.

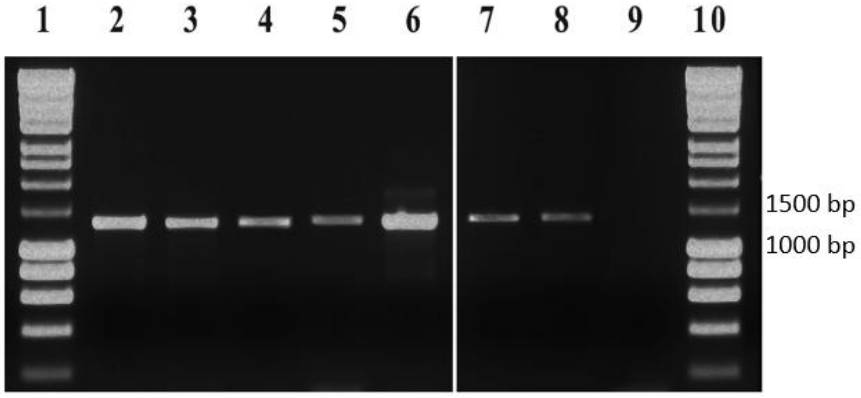

Figure 4: Gel image of PCR products after electrophoresis. The size of the PCR products was about 1400 bp. 1 - DNA ladder, 2 - Symptomatic Churai (*Ziziphus oenoplia*), 3 – 8 Symptomatic palmyrah plant samples collected from the sampling area, 9 - Non-symptomatic palmyrah plant, 10 - DNA ladder.

CONCLUSION

The present study has revealed that the palmyrah palm present in the Pungudutivu area in Jaffna Peninsula is possibly affected by lethal yellowing disease, and the infected plants seem to be associated with phytoplasma. Since the disease spreads rapidly and causes severe losses, it is urgent to take precautions to prevent the spread of the disease to other parts of the Jaffna Peninsula.

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