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First report of *Phytophthora colocasiae* associated with stem and leaf-rot of jackfruit (*Artocarpus heterophyllus*), in Kerala

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Phytophthora species is a destructive plant pathogenic oomycete that has caused lethal diseases in a wide range of hosts like black pepper, rubber, cocoa etc. and serious threat to crop yield at global level. These hemi-biotrophic pathogens are now infecting tropical fruit crops also. This study reports the first occurrence of *Phytophthora* sp. in jackfruit (*Artocarpus heterophyllus*), where plant parts are infected with water-soaked lesions and sporulating hyphae. Affected plant parts also exhibited symptoms like abnormal leaf-fall, and stem-rot leading to significant mortality in early stages of growth. Based on the morphological and molecular characterisation, the fungus was identified as *Phytophthora colocasiae*. This is the first report of *Phytophthora* rot (*Phytophthora colocasiae*) on jackfruit (*Artocarpus heterophyllus*) in Kerala.

Key words: Characterisation, jackfruit, pathogenicity, *Phytophthora colocasiae*, rot

INTRODUCTION

Jackfruit (*Artocarpus heterophyllus* Lam., family Moraceae) is an important multi-purpose fruit crop found in Kerala and other tropical and subtropical regions. Traditionally grown trees are commonly found in the home gardens, that provide food, timber, fuel, fodder, medicinal and industrial products. They are native to South-Western rain forests of India, and is now grown widely in many Asian countries, especially Bangladesh, Myanmar, Nepal, Sri Lanka, Thailand, Vietnam, Malaysia, Indonesia, India and Philippines (Elevitch and Manner, 2006). Nowadays,the crop is affected with different fungal and bacterial diseases. Compared to the traditional varieties, the newly introduced jackfruits are more prone to diseases.

The commonly occurring diseases are leaf spot (*Gloeosporium* sp./ *Phyllosticta* sp.), fruit soft-rot (*Rhizopus artocarpi*), root-rot (*Pythium splendens*, *Phytophthora* sp., *Fusarium* sp., *Rhizoctonia* sp.), decline disease (*Phytophthora palmivora*) (Borines *et al*. 2014), die-back (*Lasiodiplodia theobromae*),anthracnose (*Colletotrichum gloeosporioides*) (Srivastava and Mehra, 2004), Corynespora leafspot (*Corynespora cassiicola*) (Sangchote *et al*. 2003). In the case of *Phytophthora* infection, the wet condition during monsoon season favours a perfect condition for its development and spread. According to Anandaraj (2012), the infections were expressed in late season on crops like apples, citrus and black pepper. So, it is necessary to understand about the development, spread, and control of soil-borne pathogens like *Phytophthora,* to maintain productivity under changing environmental conditions. This investigation is the first report of *Phytophthora* infection in jackfruit that aims to isolate, identify and characterise the pathogen based on the cultural, morphological and molecular characters.

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MATERIALS AND METHODS

Sample collection

During the North-East monsoon (October, 2023), jackfruit plants showing symptoms of stem-rot and abnormal leaf-fall were observed, in Ernakulam district (Kottappady, 10.103616^{0}C/ 76.588608^{0}C)which were collected, kept separately and placed in polythene bags. After few hours, it was brought to laboratory of Plant Pathology department, College of Agriculture, Vellanikkara for the isolation of the pathogen.

Isolation of the pathogen

Isolation of the pathogen regarding the present study was collected from severely diseased jackfruit plants infected with stem-rot and leaf-fall (Fig.1 A & B). Samples collected were isolated separately under *in vitro* conditions with tissue segmentation method (Rangaswamy, 1958). Plant parts such as leaf and stem, expressing the typical symptoms of *Phytophthora* rot were washed thoroughly and cut into small pieces having both healthy and diseased portions. Surface sterilization of the plant tissues was done using sodium hypochlorite solution (1 per cent) for one minute, followed by three consecutive washes with sterile distilled water. Washed pieces were dried and the bits were placed onto the sterile Petri plates containing a 15 ml solidified sterile potato dextrose agar (PDA) medium impregnated with streptomycin and soon Petri plates were sealed. These plates were kept for incubation at 26^{0}C± 2^{0}C for one day. The fungal mycelium was observed one day after incubation. The growing tips of the fungus was sub-cultured and purified by single hyphal tip method for further studies.

Pathogenicity test

The pathogenicity was proved by artificial inoculation on live as well as detached plant parts such as leaves and stem. Mycelial Bit Inoculation Method (MBIM), by Rocha *et al*. (1998) was followed, for pathogenicity studies. For this healthy growing one year old jackfruit plant (Muttom varikka) was inoculated on the stem and leaves with mycelial discs of 8 mm diameter. Leaves along with stem of jackfruit trees were also inoculated similarly for performing inoculation on detached plant parts. The discs of seven-day old culture of *Phytophthora* isolates grown on PDA medium were taken and placed with and without pinpricks in respective parts mentioned above. This was then covered with sterile cotton moistened with sterilized water. The inoculated plant and detached plant parts were incubated in polythene bags to provide humid condition. Cotton was watered frequently to prevent it from drying, until the symptom appears.

Cultural, morphological and molecular characterisation

The fungal colony characteristics, including colony colour, reverse side pigmentation of the culture plate, mycelial texture, growth pattern, and growth rate, were carefully observed and documented. Spore morphology and hyphal septation were examined and recorded for detailed characterisation. Molecular characterisation was done using the fungal specific primers ITS 1 and 4.

RESULTS AND DISCUSSION

The fungal mycelium growing from the bits were observed one day after incubation. In the artificial inoculation on live as well as detached plant parts, characteristics symptom was observed seven-days- after inoculation. Pathogen was reisolated, and Koch's postulates were proved.

Symptomatology

The symptom development of the isolated pathogen was studied under both natural conditions and artificial inoculation on healthy plant parts. Symptoms on natural conditions include stem rot accompanied with water-soaked lesions, abnormal leaf fall and sporulating hyphae near the broken edge (Fig. 1 A & B). The symptom was observed seven days after artificial inoculation at the point of mycelial bit, which was spreading towards all direction in dark black colour. Later turned to black colour lesions on inoculated leaves (Fig. 2 A & B). In the inoculated stem white colour mycelial development and sporulation was observed.

Fig.1: (A) Stem rot with sporulating hyphae near the broken edge (B) leaf with water-soaked lesions

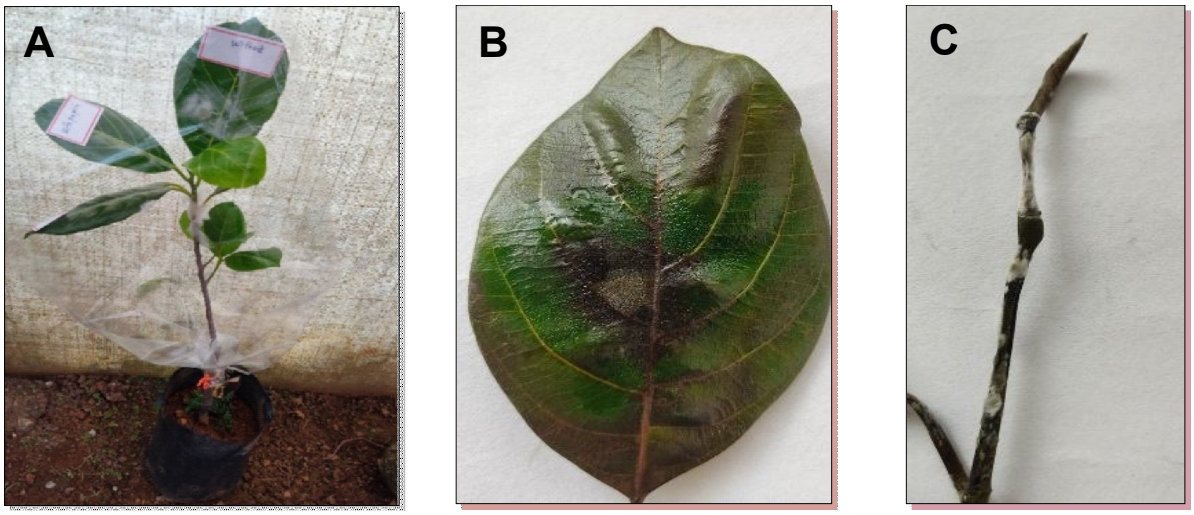

Fig.2: (A) Artificial inoculation on 1 year old jackfruit plant (Muttam varikka variety) (B) Symptoms developed on leaf and (C) Symptom developed on stem after artificial inoculation

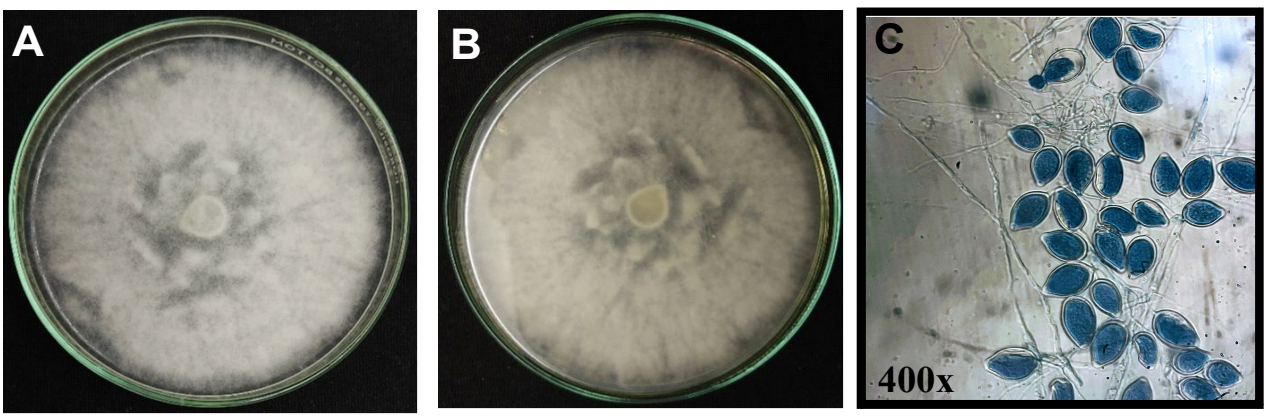

Figure3: (A) Front view of *P.colocasiae*,(B)Rear view of *P.colocasiae,* (C) Microscopic view of semi-papillate, ovoid sporangia of *P.colocasiae*

Characterisation and identification of the pathogen

The initial culture showed cottony white mycelium with a slightly petaloid pattern at the center, which later developed into a dense, white cottony texture without any specific pattern. The reverse side of the Petri plate also appeared white. The growth rate on PDA was observed to be 1.2 cm/day. The mycelium was hyaline, aseptate, sporangiophore irregularly branched, shape of sporangia ovoid, semi-papillate, and moderately caducous. The average length-to-breadth ratio (L/B) of the sporangia was 1.42, and the average diameter of the hyphae was 4.29 µm (Fig.3 A- C).

Molecular identification and phylogenetic analysis

The molecular characterisation was conducted, and the resultant sequence was analysed using the BLASTn program. The amplicon size of the sample was found to be 840 bp. Later, the sequence was deposited in the GenBank with accession number PQ475941.The pathogen showed 100 per cent query cover with *P. colocasiae* strain CBS 358.30 (GenBank accession MH401210.1) and *P. colocasiae* isolate BI3 (GenBank accession JN661139.1). The rDNA sequences were aligned using the ClustalW program, and a phylogenetic tree was generated with MEGA 11 software. Using the Maximum Composite Likelihood (MCL) method, and the phylogeny was validated through bootstrap analysis with 1000 replicates (Fig. 4). The DNA sequence obtained from the infected jackfruit plants were closely grouped with previously published ITS sequences of *P. colocasiae* strain CBS 358.30 (GenBank accession MH401210.1) and *P. colocasiae* isolate BI3 (GenBank accession JN661139.1). Thus, the fungal isolate (PQ475941) was identified as *P. colocasiae*.

This study confirms the pathogen *P. colocasiae* as the causal agent for leaf and stem-rot infection in jackfruit plants. The pathogen was isolated from the stem as well as leaves showing stem and leaf-rot infection. Drenth and Guest (2004) have reported that *Phytophthora* diseases rank among the most economically impactful diseases affecting tropical fruit tree crops. In 1994, Tsao *et al*. identified *P. palmivora* on jackfruit plants in the Philippines; however, its role in producing the syndrome now recognized as "jackfruit decline" remained unconfirmed since re-inoculation was not performed to verify the connection. Later the symptoms of *Phytophthora* infection in jackfruit plant parts were observed in

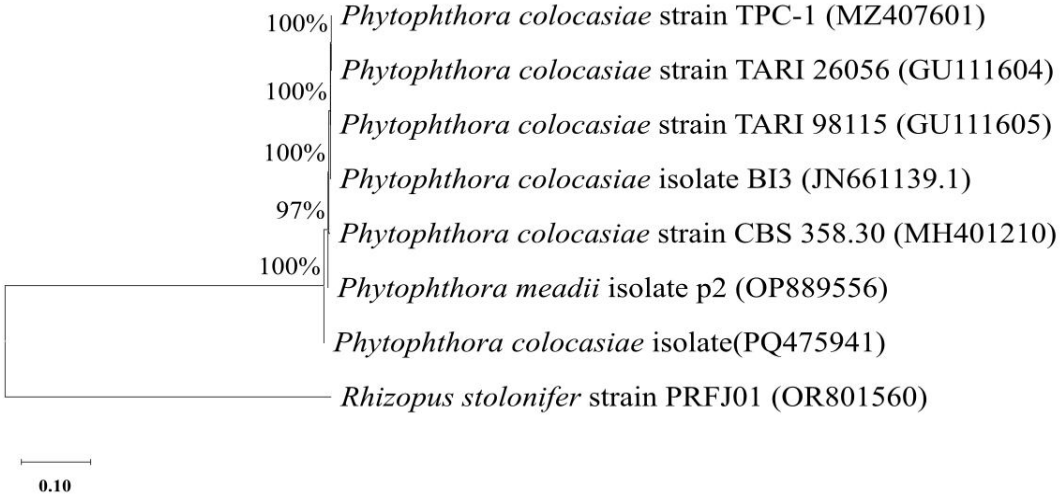

Fig. 4: Phylogenetic tree from internal transcribed spacer (ITS) sequences created by Maximum likehood method, the sequence of *Phytophthora colocasiae* isolate (PQ475941) obtained from Ernakulam, five other *Phytophthora colocasiae*s equences (MH401210.1, JN661139.1, GU111605.1, MZ407601.1, GU111604.1), one *Phytophthora meadii* isolate (OP889556.1)and one sequence of *Rhizopus Stolonifer* strain (OR801560.1) an out group were retrieved from GenBank. Accession numbers are given in parentheses.

similar species, such as breadfruit (*A. integer*) and chempedak (*A. altilis*), also (Sangchote *et al*. 2003). Initial symptoms on infected breadfruit and chempedak include small, water-soaked lesions that gradually expand, developing light brown centres with edges marked by white mycelium and sporulation. All these findings underscore the aggressive impact of *Phytophthora* sp. in causing stem and leaf rot infections and there by economic loss.

Borines *et al*. (2014) reported that jackfruit decline affects different parts of the tree, including its roots, trunk, branches, leaves, and fruit, significantly diminishing the tree's overall health and productivity. These comparative observations are valuable for developing management strategies for *Phytophthora* in jackfruit, where targeted treatments for stem, root, and leaf infections can help prevent severe crop loss.

CONCLUSION

Kerala is a major jackfruit growing state in India. The weather is hot and humid during the growing season of jackfruit, which are highly favourable for the pathogen's development and spread in Kerala. In the future, *P. colocasiae* may pose a threat to jackfruit production. According to the available literature, there are no reports of *P. colocasiae* causing stem and leaf rot in jackfruit under Kerala conditions. The present study provides the first evidence of *P. colocasiae* causing stem and leaf rot in jackfruit in Kerala. Therefore, an extensive survey of this disease is necessary in Kerala to prevent its spread.

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DECLARATIONS

Conflict of interest: Author declares no conflict of interest.

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