

# New Record of Stem Anthracnose and Dieback Disease in Jack Fruit Trees (*Artocarpus heterophyllus*) from Bengaluru, Karnataka, India

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## Abstract

At this time the whole world food security is considered, so the study of a highly valuable Jackfruit trees showing stem Anthracnose and dieback diseases is a very urgent problem. A survey was done from summer months of 2020 to the summer months of 2022 in Jack fruit trees growing areas of Bengaluru like Arrupe Nivas and Ashirvad campus to identify different fungal diseases and pathogens causing that disease in it. Different stem and leaf samples were collected from study areas for isolation of fungal pathogens by the latest research methods like Blotter and Culture Potato Dextrose Agar (PDA) medium. Arrupe Nivas, jack fruit leaf and stem samples shown the *Colletotrichum gloeosporioides* (Penz.) Sacc., *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl., *Aspergillus niger* Tieghem and *Trichoderma* sp; whereas Ashirvad campus, Jack fruit leaf and stem samples showed the *C. gloeosporioides*, *Fusarium oxysporum* Schlecht. and *Epicoccum nigrum* Link. The frequently isolated fungal pathogens from Arrupe Nivas jack fruit trees were *C. gloeosporioides* and *L. theobromae*, whereas the frequently isolated fungal pathogen from Ashirvad campus jackfruit trees was *C. gloeosporioides*. The pathogenicity test confirms that the anthracnose and dieback diseases are responsible for the death of Jackfruit trees in Arrupe Nivas and dieback disease in Ashirvad campus. The similar type of diseases symptoms was also observed in the Jackfruit growing areas of Bengaluru, Karnataka, India. For the first time the Anthracnose in stem and dieback in trees of jackfruit growing areas of Bengaluru of Karnataka was reported.

**Keywords:** Anthracnose; Dieback disease; Jack fruit; *Colletotrichum gloeosporioides*; *Lasiodiplodia theobromae*

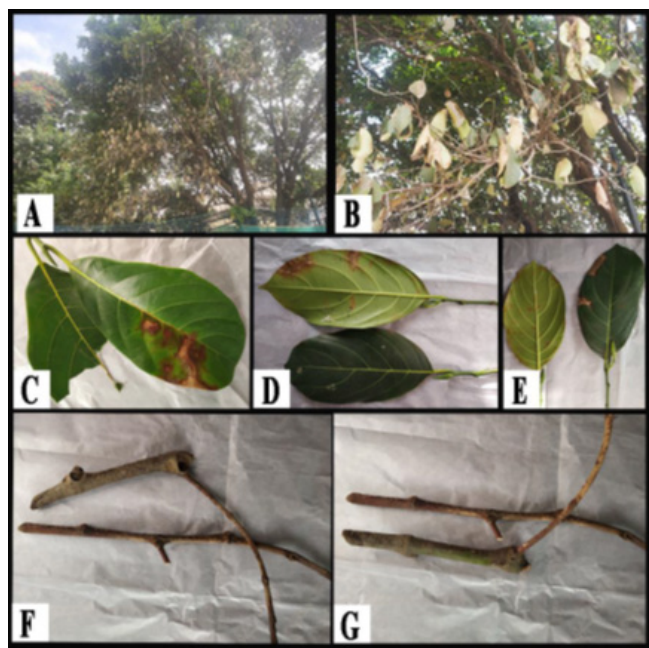
## Introduction

Jackfruit is one of the commonly consumed fruits in the tropical regions from the ancient times. Scientifically it is known as *A. heterophyllus* which is a medium-sized, evergreen tree species, growing up to 8-25m in height, belonging to the family of Moraceae. The tree stem usually grows straight and of diameter 30-80cm [1]. The tree is commonly known as 'Jackfruit tree' in Bangladesh-Kathal, in Brazil, Jaqueira, in Germany Jackfruchtbaum, in Indonesia Nangka or Nongko etc. In India, it is called by unique vernacular names like, halasu (Kannada), kathal (Hindi), phanas (Marathi), pila or pilavu or chakka (Tamil & Malayalam) and so on [2]. It is a tropical, climacteric fruit, native to Western Ghats of India, Asia, Africa and some regions of South America.

The bark of the tree is rough, dark grey to brownish colour and scaly. Almost all the living parts of the tree exudes gum-like white latex when injured. The tree leaves are dark green on the adaxial side and dull green on the abaxial side, glabrous, leathery and obovate to



(Figure 1). It was noted that the unique fungal disease symptoms in *A. heterophyllum* was observed in many other urban areas of Bengaluru city also. The age of both the *A. heterophyllum* trees plantation seems to be approximately 15-20 years (Figure 2). Different stem and leaf samples were collected from study sites and is used for isolation of fungal pathogens by the latest research methods like Blotter and Culture Potato Dextrose Agar (PDA) medium.



**Figure 2:** Sample collection from two different *Artocarpus heterophyllum* trees.

(A, C, D, F): Samples of leaf and stem from diseased *Artocarpus heterophyllum* tree of St. Joseph's College (Autonomous) campus, Shantinagar, Bengaluru- 27.

(B, E, G): Samples of leaf and stem from diseased *Artocarpus heterophyllum* tree of Ashirvad campus, near St. Mark's Road, Bengaluru.

### Blotter method of isolating fungi

10 samples of infected leaves and stems of the tree were collected from both the study areas the diseased *A. heterophyllum* trees for isolation of fungal pathogens in it. The twigs samples were cleaned under slow running tap water and air dried on the blotting papers. The symptoms of Anthracnose and dieback disease were noted and photographed. The infected areas on leaf and stem were cut 1mm size bits with the help of scalpel. The surface sterilization of the leaf and stem bits was done by treating them with Sodium hypochlorite ( $\text{Na}_2\text{HCl}_2$ ) and 70% ethanol. The sample bits were kept for 15 minutes and immediately transferred into the distilled water. The Petri plates containing required size of blotting papers is wetted and autoclaved. Under sterile conditions the three bits of leaf and stem were transfer into the Petri plates (3 replicates each) and incubated at 27 °C temperature with 90% relative humidity for seven days. The isolated fungal colonies were sub culture in PDA slants for furthers identification of fungal pathogen [28].

### Isolation of fungi by using PDA medium

200g of peeled potato is taken. Cut into small pieces and boiled in 500ml of  $\text{H}_2\text{O}$  till it is easily squashed by the glass rod. The solution is then filtered using what man no.1 filter paper and poured it into the beaker which contains 20g of dextrose, 15g of Agar with 1000ml of distilled water. It is then stirred well and heated in the micro-Owen until the solution gets transparent. The nutrient media is poured into the 250ml conical flask and corked tightly with the sterile cotton plug. Twelve petri-plates were washed with distilled water and both medium containing conical flasks and petri-plates were autoclaved for the complete sterilization. The laminar air flow is kept ready after sterilizing the floor with 70% ethanol and keeping the UV light on for 15 minutes. The surface sterilization of samples was done as described above Under the sterile conditions the PDA medium was poured into the petri-plate and waited for solidification. After solidification, the infected leaf and stem cut pieces were placed in PDA medium by using the forceps. The petri-plates were categorized into two different sets based on the different diseased tree samples. One of the sets included leaf and stem samples of St. Joseph's college and the other sets included leaf and stem samples of Ashirvad, Bengaluru. The plates were labelled using the marker. Finally, the petri-plates were kept in the incubation chamber for 7 days under  $27 \pm 2$  °C. The isolated fungal colonies were sub cultured and used for identification of fungal pathogens [29,30].

### Identification of the fungi

After placing the petri-plates in the incubation chamber for five days, the fungal growth started appearing. A small portion of each of the fungal colonies grown in the petri-plates taken using the inoculation loop, under sterile conditions and slides were prepared. The fungal sample was teased nicely so that the fungal colonies are not lumped together. Cotton blue stain was used to stain the fungus. The prepared slide was observed in the Onix Vision Trinocular microscope EX30, which has camera and the computer screen attached for the visualization of fungi. The identification of the fungus was done based on the morphological characters of the fungal pathogen with the help of reference manuals. They were compared with the standard works of manual of soil fungi [31], Hyphomycetes [32], A Manual of *Penicillium* [33], Manual of *Aspergillus* [34] and Soil fungi [35].

### Pathogenicity test

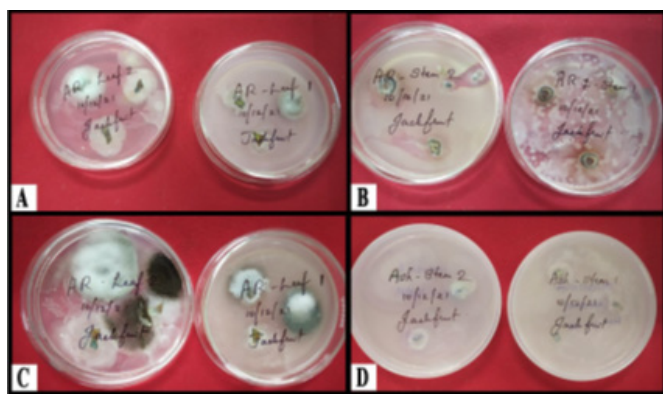
Fresh healthy *A. heterophyllum* twigs consisting of a minimum number of 15-20 leaves were used for the pathogenicity test. The surface sterilization of the healthy twig was done using Sodium hypochlorite and 70% ethanol in excess to clear the possible contaminants from the healthy twigs. A small portion of the epidermal tissue of the abaxial and adaxial side of the leaves as well as of the bark on stem was removed using a sterilized blade. The isolated fungal pathogen was identified as pathogenic from infected samples was isolated in pure form. The fungal pathogen was applied on the damaged parts of the leaves and the stem of



fresh samples. A small piece of sterilized cotton was wetted with the distilled water and placed on the injured parts, so that the pathogens receive necessary moisture for the growth. The twig is then placed carefully inside the polythene cover with moist cotton plug and sealed. The polythene cover is labelled accordingly and placed in the incubation chamber for 7 days, at  $27\pm 2$  °C temperature. After seven to ten days the symptoms were developed in artificially inoculated fresh samples. From these artificially infected samples the fungal pathogen was re-isolated and identified [30]. This pathogenicity test was used for confirmation of diseases like Anthracnose in stem and Dieback disease in trees of Jack fruit by fungal pathogens isolated.

## Result

### Isolation of Pathogen



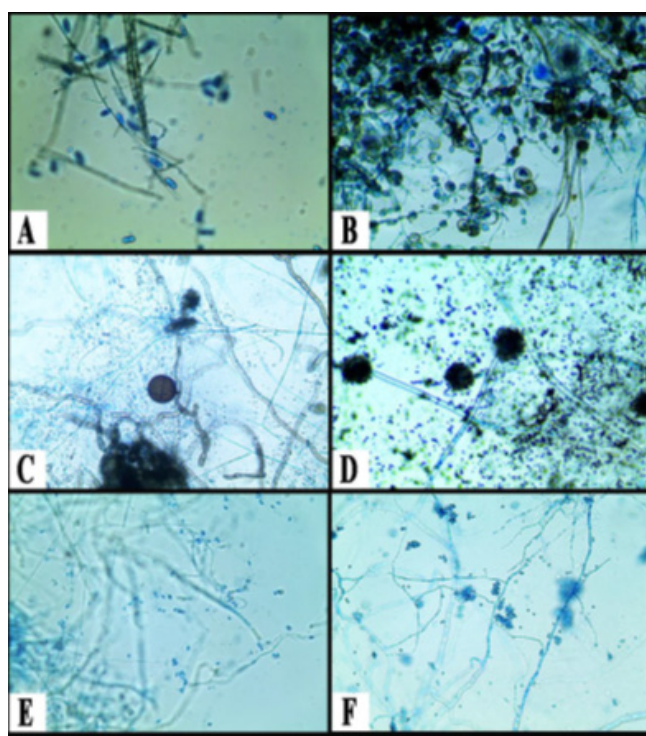
**Figure 3:** Potato dextrose media based Fungal culture plates.

(A, B): Fungal growth from leaf and stem samples of St. Joseph's College (Autonomous) campus, Shantinagar, Bengaluru- 27.

(C, D): Fungal growth from leaf and stem samples of Ashirvad campus, near St. Mark's Road, Bengaluru.

From Arrupe Nivas, the leaf and stem Jackfruit samples, *C. gloeosporioides*, *L. theobromae*, *A. niger* and *Trichoderma sp.* were isolated. Whereas from Ashirvad campus, the leaf and stem Jackfruit samples, *C. gloeosporioides*, *F. oxysporum* and *E. nigrum* were isolated. The frequently isolated fungal pathogens from Arrupe Nivas jack fruit trees were *C. gloeosporioides* and *L. theobromae*; whereas the frequently isolated fungal pathogen from Ashirvad campus jackfruit trees was *C. gloeosporioides* (Figure 3, 4). The death of Jackfruit trees in Arrupae nivas campus was due to the anthracnose on stem and dieback disease caused by both *C. gloeosporioides* and *L. theobromae* respectively. The death of jackfruit trees in Ashirvad campus was due to the dieback disease caused by *C. gloeosporioides*. The sunken, water-soaked, black to dark brown lesions begins to appear on stem and leaf of Jackfruit plants. Conidial masses were produced on the infected stem and leaf during the favourable conditions like the rainy seasons and humid weather. The necrosis of infected stem and leaves appears due to the asexual bodies 'acervuli' produced. A small flask shaped

structure with a short mass of conidiophores is formed on the surface of necrotic spots on stem and leaves of jackfruit. The conidia escape through an opening of acervuli through necrotic spots. There will be also long brownish coloured projections emerged from acervuli called setae which also comes out through necrotic spots. Based on the above symptoms in infected stem and leaves of jackfruit the disease was identified as anthracnose disease caused by *C. gloeosporioides* (Figure 2).



**Figure 4:** Identification of different fungal pathogens isolated from fungal pure cultures.

(A): *Colletotrichum gloeosporioides* (B): *Lasiodiplodia theobromae* (C): *Epicoccum nigrum* (D): *Aspergillus niger* (E): *Fusarium oxysporum* (F): *Trichoderma spp.*

The discolouration and darkening of the bark from the tip to downwards was observed in infected jackfruit trees in both the study areas. The young twigs of jack fruit tree start withering from the base and the infected leaves turn brown with their margins rolling upwards. The infected leaves, twigs and branches of jackfruit tree showed necrosis, dried and fall off. In severe condition the branches dry one after another and entire plant died. the fungal pathogen producing pycnidia, two-celled, dark brown, striated conidia in infected parts of Jackfruit trees. These conidia were then dispersed by wind or rain-splash which fall on the damaged parts of the tree and develop disease in health parts of jack fruit. Based on the above symptoms in infected Jackfruit trees the disease was identified as dieback disease caused by *L. theobromae* and *C. gloeosporioides* (Figure 2). Both the anthracnose in stem and dieback disease in trees are found to cause stunted tree growth and severe decline in fruit crop in *A. heterophyllum*, which has resulted in significant yield losses to the Jackfruit cultivars.

## Pathogenicity test

The healthy *A. heterophyllum* twigs were artificially induced with the fungal pathogens *C. gloeosporioides* and *L. theobromae* to compare and contrast the disease symptoms of the infected healthy twig with that of the diseased tree. After seven to ten days, it was observed that the infection induced healthy twig developed similar symptoms that of anthracnose and dieback disease of Jackfruit (Figure 5). In case of anthracnose disease, the sunken, water-soaked, black to dark brown lesions begins to appear on stem and leaf of Jackfruit artificially infected plants whereas in case of dieback disease the fungal pathogen producing necrotic spots on artificially infected leaves and stem parts, and the pathogen producing pycnidia, two-celled, dark brown, striated conidia also. The artificially inoculated twigs were carefully removed from the polythene bags and re-isolation was done. The re-isolated fungi were identified as *C. gloeosporioides* and *L. theobromae* so the present pathogenicity study confirms that the anthracnose and dieback diseases are responsible for the death of Jackfruit trees in Arupae nivas and die back disease in Ashirvad campus.



**Figure 5:** Pathogenicity Tests using the healthy twig of *Artocarpus heterophyllum* trees.

(A & D): Healthy twigs of *Artocarpus heterophyllum* trees.

(B & C): Development of anthracnose and dieback disease after infecting the healthy twigs with the fungal samples of the diseased *Artocarpus heterophyllum* tree from St. Joseph's College (Autonomous) campus, Shantinagar, Bengaluru-27.

(E & F): Development of anthracnose and dieback disease after infecting the healthy twigs with the fungal samples of the diseased *Artocarpus heterophyllum* tree from Ashirvad campus, near St. Mark's Road, Bengaluru.

## Discussion

*A. heterophyllum* is a high value fruit, is also marketed as 'flagship commodity', because of its potential to provide sustainable income to the local farming communities. It possesses antibacterial, anti-inflammatory, antidiabetic, antioxidant, antimicrobial and immunomodulatory properties. Consumption of Jackfruit has inevitable positive impact on human health. If such a medically as well as commercially high-valued fruit crop is attacked by the

phytopathogens, then there would obviously be huge economical loss for the country [36]. In the present study, the high economic valued jackfruit was found to be affected by anthracnose and dieback diseases in Bengaluru. Borines et al. [36] reports that many other fungal pathogens also are found in the dieback diseased tree of *A. heterophyllum* like, *Fusarium*, *Pythium* and *Phytophthora species* [36]. The present study confirms that the anthracnose and dieback diseases of *A. heterophyllum* due to the fungal pathogens *C. gloeosporioides* and *L. Theobromae* respectively. The other fungal species isolated along with these disease-causing pathogens in the Jackfruit tree were namely, *Trichoderma sp.*, *F. oxysporum*, *E. nigrum*, and *A. niger*. *C. gloeosporioides* was first identified by Peznieg. The christening of the pathogen was based on the type of a specimen *Vermicularia gloeosporioides* which was collected from Citrus in Italy. In India, this phytopathogen was first observed on *Coffea arabica* and was reported by Butler. McRae identified it as the causal pathogen of anthracnose disease of mango [22]. In the present study, *C. gloeosporioides* was isolated from leaf and stem samples of Jackfruit, which is responsible for anthracnose disease in Bengaluru.

Bajpai [17], reports the anthracnose disease in the pomaceous fruit, *Malus domestica* (Apple) affecting the fruit, stolon and crowns. The infection of fungal pathogen was thought to be possible when the fungi enzymatically penetrated the cuticle of the fruit [17]. Coffee is a major commercial export crop and the predominant threat to this cash crop is anthracnose disease. After analysing the isolates of the fungal colonies on coffee reported that 17% of 60 colonies corresponded to the six species of *Colletotrichum* [18]. Strawberry crop being infected is due to anthracnose fruit rot disease caused by the *Colletotrichum sp. C. acutatum* seemed to colonize strongly on the foliage parts of these runner plants. The infected runners show lesions on the petiole and roots [19]. Guo et al. [21] reports that in the healthy dragon fruits, small light brown spots started appearing on the surface of the fruits and rapidly developed into sunken soaked lesions having grey to black acervuli of 1cm in diameter [21]. Almonds are commonly affected by anthracnose disease, where the infected fruits are depressed and exhibit round, orange lesions from 5-12mm on the fruit surface [15]. Severely infected fruits become unfit for consumption which results in the dropping of market value for the fruits. Many commercial producers of fruits like *Psidium guajava* think of giving up cultivation of fruit crop due to the great loss by this disease [20]. In the present study also, the above symptoms were observed in Jackfruit leaf and stem samples, which confirms anthracnose disease is caused by *C. gloeosporioides*.

The first report of dieback in Strawberry plants in Kenya reveals that the symptoms of dieback began on runners, causing black rot on roots and necrotic discoloration in the crown of the plants [27]. In *Theobroma cocoa* of Malasia, the isolates of *L. theobromae* were responsible to cause leaf blight, stem canker and pod rot [24]. Shahbaz [26] found that *L. theobromae* was associated with the decline of mango trees. As an aggressive and vigorous pathogen, it caused tip dieback and twig blight in mango crops [26] Khanzada et al., denotes referring to the survey done on the infected fields



of mango groove that twigs die from the tips becoming black and wood forming into a scorched appearance. Leaves on the infected branch turned brown with margins rolled upwards and fall of leaving the dead branches [25]. In the present study also, the above symptoms were shown in roots, stem, branches and leaves of Jackfruit tree which is infected by *L. theobromae*. So, in the Jackfruit growing areas of Bengaluru all the trees in orchards were dying due to dieback disease.

## Conclusion

Jackfruit is a highly valuable fruit, because of its nutrition and health benefits. A study was done in Jack fruit trees growing areas of Bengaluru like Arrupe Nivas and Ashirvad campus from different seasons of 2020 to the summer months of 2022 to identify different fungal diseases and pathogens causing diseases in it. The Blotter and culture PDA medium methods shown the fungal colonies from Arrupe Nivas, jack fruit leaf and stem samples which were identified as *C. gloeosporioides*, *L. theobromae*, *A. niger* and *Trichoderma sp*; whereas Ashirvad campus, Jack fruit leaf and stem samples showed the occurrence of *C. gloeosporioides*, *F. oxysporum* and *E. nigrum*. The frequently isolated fungal pathogens from Arrupe Nivas jack fruit trees was *C. gloeosporioides* and *L. theobromae*; whereas from Ashirvad campus jackfruit trees was *C. gloeosporioides* Based on the symptoms in infected stem and leaves of jackfruit the disease was identified as anthracnose disease caused by *C. gloeosporioides* and dieback disease caused by *L. theobromae* in case of Arrupe Nivas jackfruit trees whereas dieback disease caused by *C. gloeosporioides* in case of Ashirvad campus jackfruit trees. The pathogenicity test also confirms that the anthracnose and dieback diseases are responsible for the death of Jackfruit trees in Arrupe Nivas and dieback disease in Ashirvad campus. The similar fungal disease symptoms were observed in all the jackfruit growing orchards of urban Bengaluru city, Karnataka. For the first time the Anthracnose in stem and dieback in trees of jackfruit growing areas of Bengaluru of Karnataka was reported. The further research is required to control both the diseases to safe guard the Jackfruit trees.

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