Original Research Article

Isolation and characterization of pathogen causing fruit rot diseases from Jackfruit

ABSTRACT

Symptoms of characteristic rot was observed on jackfruits (*Artocarpus heterophyllus*) of Thrissur district in Kerala. This study investigates, the symptomatology, pathogenicity and identification of the causal organism for fruit rot disease. The isolation of fungal sample was obtained from Thrissur district during the month of November 2023. The isolated fungus was successfully grown on potato dextrose agar medium and was characterized. Based on its cultural, morphological traits and molecular characterization, the fungus was identified as *Agroathelia rolfsii* (teleomorph: *Athelia rolfsii,* anamorph: *Sclerotium rolfsii*). In the molecular characterization by amplifying internal transcribed spacer (ITS) region using the ITS1 and ITS4 primer pair, the isolate TK2 showed high per cent identity of 99.35 per cent and query coverage 100 per cent with *Agroathelia rolfsii* in blastN search. Phylogenetic analysis of the internal transcribed spacer (ITS) and large subunit (LSU) genes played a key role in confirming the species identity. *Athelia rolfsii* has been reported for the first time to cause fruit rot disease on jackfruit, from Thiruvananthapuram by Sajeena *et al.* [1]. This study reports the infection of *Agroathelia rolfsii*, on jackfruit from Thrissur district. These soil-borne pathogen can spread easily and stay dormant in soil in the form of sclerotium for long time, hence it is essential to improve the management strategies and prevent its spread to other crops, for that further studies need to be conducted. This study was conducted at Department of Plant Pathology, College of Agriculture, Vellanikkara, Thrissur, Kerala between November 2023 and June 2024.

Keywords: Agroathelia rolfsii, Athelia rolfsii, Characterization, Fruit-rot, Isolation, Jackfruit, Pathogenicity, Sclerotium rolfsii

1. INTRODUCTION

Jackfruit *(Artocarpus heterophyllus Lam., family Moraceae)* is a valuable fruit crop, both economically and nutritionally. However, its cultivation is significantly affected by various fungal diseases, which necessitates effective disease management practices. The fruit rot diseases in jackfruits and other plant parts are a serious disease prevalent these days. This study aims to isolate, and characterize the pathogen causing fruit rot disease in jackfruits, along with their symptom development. There are also fruit rot causing fungal pathogens which can cause diseases on jackfruit flowers and fruits, particularly in regions with high rainfall [2]. Among the soilborne plant pathogens, *Athelia rolfsiii* is a fungus commonly found in warm temperate and subtropical regions [3,4]. *Athelia rolfsii*, was reported to infect over 600

plant species across temperate, tropical, and subtropical regions [5], leading to significant economic losses in various agricultural sectors [3,6,7,8].

The initial symptoms of the disease were found to appear with sudden elevation in humidity and the temperature around 28-33°C. Due to its wide host range and adaptability to diverse environmental conditions, this pathogen exhibits considerable genetic, morphological, and pathogenic diversity [9,10]. According to Aycock *et al*. [3] *Sclerotium rolfsii* is pathogen that has a host range of more than 500 plant species, comprising of dicotyledonous and monocotyledonous species. The characterization and symptom development of *Agroathelia rolfsii* provides critical insights essential for designing effective biological control. By understanding the pathogen's specific traits and growth conditions, potential biocontrol agents can be recognized and developed to mitigate its harmful effects in commercial jackfruit cultivation.

2. MATERIAL AND METHODS

2.1 Sample collection, isolation of pathogen and pathogenicity studies

The pathogen was isolated from the diseased jack fruit collected from Kodungallore, Thrissur District (10.588089^{O}/ 76.215150^{O}). The pathogen was isolated with the standard protocol of tissue segmentation method [11]. Diseased and healthy tissue sections were cut into small pieces ranging from 1.0 to 1.5 cm using sterile blades. These pieces were surface sterilized by immersing them in one per cent sodium hypochlorite solution for one minute. After being rinsed three times with sterile water, the excess moisture was removed using sterile blotting paper. The tissue bits were then placed in sterile Petri dishes containing solidified potato dextrose agar (PDA) medium amended with antibiotics to prevent bacterial contamination. Four bits were placed on a single Petri dish, and incubated for 1-2 days at room temperature (24± 2 °C). Fungal growth was monitored daily, and the hyphal tips were aseptically transferred to fresh PDA and maintained in slants.

The pathogenicity of the fungal isolate TK2 was carried out and the symptoms of the diseases were studied in detail under natural and artificial conditions by conducting pathogenicity test and proving Koch's postulates. The study involved inoculating fungal mycelial bits onto detached healthy jack fruits. The fungal isolate's ability to infect jackfruits were evaluated using the Mycelial Bit Inoculation Method (MBIM) as described by Rocha *et al*. [12] under artificial conditions. Mycelial bits, of 8mm diameter, were taken from the actively growing edges of four-day-old fungal cultures. These mycelial bits were placed in an inverted position on the surface of healthy jackfruits with pin prick [13] made using a sterile dissection needle. A pad of sterile cotton moistened with sterile water was placed above the inoculated portion and again covered with polythene bags to provide humid condition. The cotton was watered frequently to prevent it from losing moisture until the symptom appears. A fruit without inoculating with the pathogen was kept as control. Then the observations were taken until the symptom occurs. Different stages of symptom expression, including mycelial growth, characteristics colour and, the time required for symptom development, were documented.

2.2 Cultural and Morphological characterization

Cultural and morphological characterization of the isolate TK2 were studied. An eight mm mycelial disc from five days old fungal culture was transferred onto a Petri dish containing PDA medium. The plates were incubated at room temperature (24± 2 °C), and diameter of the colony was measured every 24 hours for 3 days. To observe sclerotia formation, the plates were incubated for 12 days at 24± 2 °C. The number of days taken for the sclerotia production were noted and their size was measured using micrometry in 40X. The slide culture technique was carried out to observe the mycelial growth and the presence of clamp connections. For

that Whatmann No. 1 filter paper was sterilized and placed in a sterile petri dish. A sterile bent glass rod was positioned at the bottom of the dish, and 2 ml of sterile distilled water was added. A sterile glass slide was then placed on top of the bent rod. Using a sterile needle, a small square (around 1 x 1 cm) of plain agar (PA) was carefully cut and placed at the center of the slide. The agar block was inoculated with a small quantity of fungus under study on all four sides using a sterile inoculation needle. A heat-sterilized coverslip was then gently placed over the agar block. These inoculated plates were incubated at room temperature for 3 to 5 days. Once the desired growth was observed, the slides were prepared for permanent mounting by adding a few drops of lactophenol cotton blue. This was observed under a microscope, and characters were observed.

2.3 Molecular characterization and phylogenetic analysis

The selected fungal pathogen, TK2 causing fruit rot, isolated from the diseased jackfruit was selected for molecular characterization. For the species level identification, the ITS region was amplified using the universal primers ITS1/ITS4.

3. RESULTS AND DISCUSSION

3.1 Symptoms under natural condition and artificial conditions

The samples were obtained from jack fruits with characteristic rotting symptoms along with numerous sclerotia production from house premises of Kodungallore, at Thrissur district (Fig. 1). The isolation of the pathogens was successfully accomplished with the mycelial growth in the isolation plate after two days of incubation (Fig. 2). Symptoms of the isolate TK2 was observed under natural conditions with fruit rot accompanied with pure white coloured fluffy mycelium, in fan-shape, along with abundant production of brown to blackish sclerotia. Similar to this result, Sajeena *et al*. [1], reported the first report of fruit rot infection caused by *Athelia rolfsii* in jackfruit in India, Kerala, Thiruvananthapuram. Elahi *et al*. [14] on 2021 had reported the occurrence of fruit rot disease on jackfruit (*Artocarpus heterophyllus)* caused by *Athelia rolfsii* (*Sclerotium rolfsii*) for the first time in the world. In July 2022, jackfruit rot was first detected at the base of the trees in an orchard located in Zhanjiang, Guangdong (21°9′27″N, 110°17′54″E) China [15].

The artificial symptoms were observed 4 days after inoculation (DAI) and mycelia was spread on all directions on the fruit surface on six-days- after inoculation (Fig. 3. A, B). On ten days after inoculation, the mycelia spread on the fruits completely and numerous numbers of sclerotia bodies on the rind and fruit peduncle was observed (Fig. 3.C). Fruits started to rot and bad odour was produced. There were no symptoms on the fruit kept as control. Finally, the pathogen was reisolated from the fruit with symptom, and Koch's postulates were proved. Similar pathogenicity studies were conducted by Sajeena *et al*. [1] by inoculating pathogen on five mature jackfruits, with 5 mm mycelial discs of 5-day-old culture on the rind. The incubated fruits were observed with the signs of rotting on the fruits by the 10th day of incubation.

Figure 1. Natural symptoms of isolate, TK2 from Kodungallore, Thrissur district

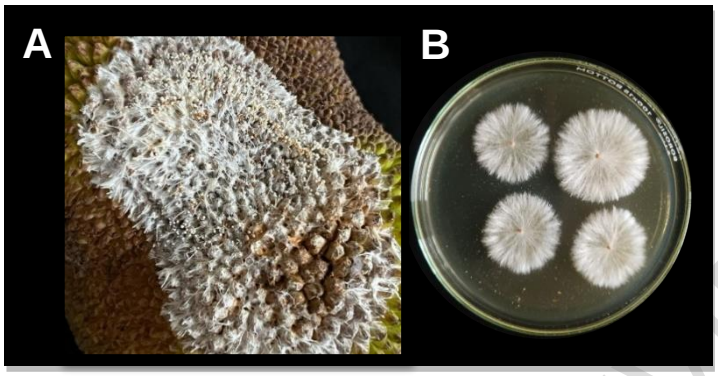


Figure 2. A) Natural symptoms of selected isolate with sclerotia B) Isolation plate of pathogen

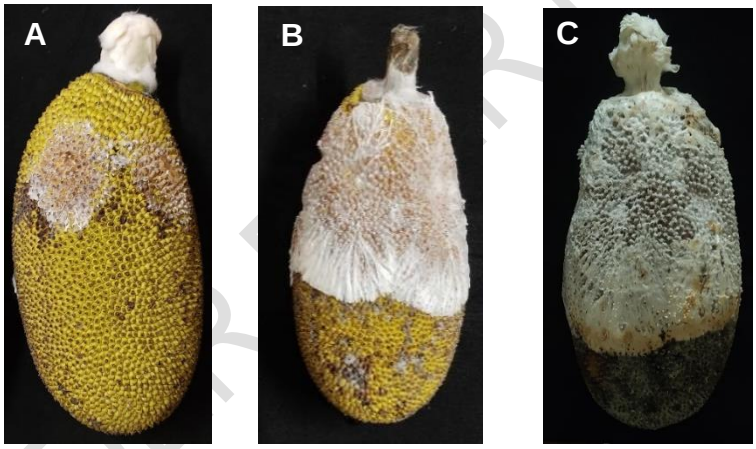
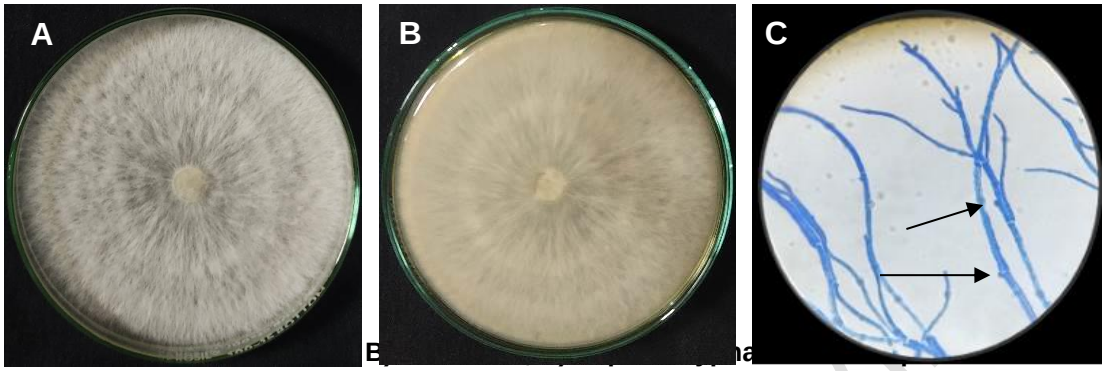


Figure 3. Artificial symptoms observed A) 3 DAI C) 6 DAI D) 11 DAI, along with sclerotia production

3.2 Cultural and morphological characters

The cultural characters were observed in the PDA media with abundantly growing white colored uniform mycelia, with growth rate of 2.25 cm per day. The coarse hyphal strands with ropy appearance were observed in the culture plate with distinctive fan-shaped pattern (Fig. 4). After 10-12 days of incubation on room temperature (24± 2°C), spherical or irregularly shaped sclerotium bodies with mix of white and brown colour was formed on the culture plate. The morphological characters of the pathogen include hyaline and septate hyphae, with profuse branching and presence of clamp connections. Sclerotia produced were measured

and average diameter of a sclerotium was found to be 0.6 mm. Sajeena *et al*. [1] reported circular, tan to brown sclerotia (0.3 to 2.0 mm) on after 5 days after incubation. Similar results were also reported by Yi *et al.,* [15] where the hyphae of the isolates were transparent, branched, and exhibited clamp connections at the septa.



3.3 Molecular characterization and phylogenetic results

In the molecular characterization the isolate TK2 showed 99.35 per cent identity and 100 per cent query coverage with *Agroathelia rolfsii* isolate SRYLB (MT 560347) in BLASTn program. The amplicon size of the sample was found to be 660 bp. The sequence of the isolate TK2 (Fig. 5) was deposited in the GenBank with accession number PQ650119. The Phylogenetic analysis of the ITS sequences showed close identity of the fungus, with the ITS sequences of other *Agroathelia rolfsii* isolates also. TK2 had showed 100 per cent query coverage with other isolates with accession number *viz*., MT 560347, MN 872304, and MN 696630. Comparing the cultural, morphological and molecular data, the fungus was identified as *Agroathelia rolfsii.* The sequences were aligned using the ClustalW program, and a phylogenetic tree was constructed using MEGA 11 software. The Maximum Composite Likelihood (MCL) method was applied, and the phylogenetic tree was validated by bootstrap analysis with 1000 replicates (Fig. 6). Similarly, in the study conducted by Sajeena *et al*. BLASTn analysis of the ITS region showed a 99.62% identity with multiple isolates of *Athelia rolfsii* and phylogenetic analysis based on ITS sequences confirmed its identity, with the ITS sequences of *A. rolfsii* isolates (MT 126471, MT 308987, and MK 300726).

In the evidence of first report of fruit rot in jackfruit (*Artocarpus heterophyllus*) in China, Yi *et al*. [15] obtained *Athelia rolfsii* (*Sclerotium rolfsii*), in which the precise identification of the fungus, was done using the primer pairs ITS1/ITS4 and V9G/LR5 by amplifying the internal transcribed spacer (ITS) gene and large subunit rRNA (LSU) gene of the isolate CASS-BLM-1.

GTTGTGCTGGCAATAAATATTGCATGTGCACACTCTGAAGCTATATAACACA
TACACCTGTGAACCAACTGTAGTCTGGAGAAATCCTGACTATGATTACTCTA
TATAACTCTTATTGTATGTTACATAGAACGATCTCATATTGAAGCTTTGTTTTT
TTTTACAAGTTTCTCTTAATTGAAAAATACACAACTTTCAACAACGGATCTCTT
GGCTCTTGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATT
GCAGAATCCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCCCTTTGGTA
TTCCGAGGGGCATGCCTGTTTGAGAGTCATTAAATTCTCAACCTTACAAATT
TTTGTATTTGTCAAGGCTTGGATGTGAGAGTTGCTAGTTAAGAATATCTGACT
GGCTCTCTTTAAAATTATTAGTAGGACATATAGAAATGCCTGCGGTTGGTGT
GATAATATGTCTACGCCTATACCAAAGGGGATTCTAGCTTGTATGCACTACT
TATAAAATCATGCGCATATATCTAGCATATAAGTGCATACATTGACCATTTGA
CCTCAAATCAGGTAGGACTACCCGCTGAACTTAAGCATATCAATAAGGCGG
AGGAAAAGGATCATTATTGAATTCATATATGCAAAGGAGTTGTGCTGGCAAT
AAATATTGCATGTGCACACTCTGAAGCTATATAACATATACACCTGTGAACCA
ACTGTAGTCTGGAGAAATCCTGACTATGATTACTCTATATAACTCTT

Figure 5: Nucleotide sequence of ITS region of *Agroathelia rolfsii,* TK2

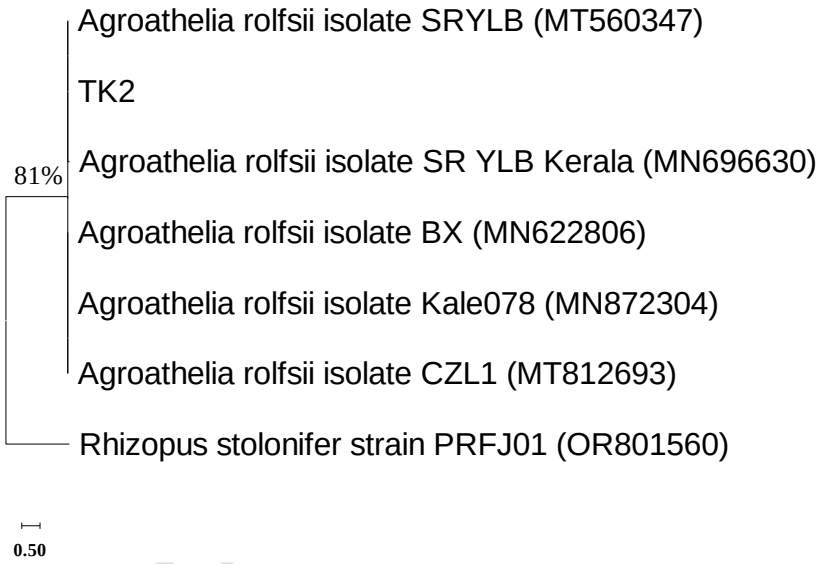


Figure 6: Phylogenetic tree from internal transcribed spacer (ITS) sequences created by Maximum likelihood method

4. CONCLUSION

Kerala, a major jackfruit-producing state in India, is ideal conditions for fungal infection due to its warm temperatures and acidic soil. High temperature and humid environment favours the growth and development of the pathogen *Athelia rolfsii* [4]. In the current research, the pathogen *Agroathelia rolfsii* was identified as the causative agent of rot in jackfruit, through cultural, morphological and molecular characterizations. The pathogen poses significant challenges in terms of control due to the production of sclerotial bodies, which are durable structures capable of surviving in the soil for long periods. These sclerotia allow the pathogen to persist in the environment, even in the absence of suitable hosts. Thus, the pathogen poses a potential threat to jackfruit production in the future, making it crucial to conduct an extensive survey of the disease in Kerala to prevent its spread. Further studies on the efficacy of biocontrol agents and synthetic fungicides can be conducted and sustainable disease management approaches can be developed, thereby ensuring the protection of commercial jackfruit cultivation.

REFERENCES

1. Sajeena, A., Mathew, D., John, J., *et al*. First report of fruit rot of jackfruit caused by *Athelia rolfsii* in India. J. Plant Pathol. 2022; 104:1171. doi: 10.1007/s42161-022-01145-8.

2. Ghosh, G.H. Studies on flowering and prevalence of fruit drop in jackfruit. Annual Report. Hortic. Res. Cent. BARI, Gazipur, Bangladesh. 1994; 4-9.

3. Aycock, R. Stem rot and other diseases caused by *Sclerotium rolfsii* or the status of Rolf's fungus after 70 years. Tech. Bull. 174, N. Carol. Agric. Exp. Stn. 1966; 202 pp.

4. Punja, Z.K. The biology, ecology, and control of *Sclerotium rolfsii*. Annu. Rev. Phytopathol. 1985; 23(1): 97-127.

5. Xie, C.Z., Huang, C.H., Vallad, G.E. Mycelial compatibility and pathogenic diversity among *Sclerotium rolfsii* isolates in the Southern United States. Plant Dis. 2014; 98:1685-1694.

6. Chen, K.R., Ren, L., Xu, L., Chen, W., Liu, F., Fang, X.P. Research progress on peanut southern stem rot caused by *Sclerotium rolfsii*. Chin. J. Oil Crop Sci. 2018; 40:302-308.

7. Cilliers, A.J., Herselman, L., Pretorius, Z.A. Genetic variability within and among mycelial compatibility groups of *Sclerotium rolfsii* in South Africa. Phytopathol. 2018; 90:1026-1031.

8. Thirumalaisamy, P.P., Narendra, K., Radhakrishnan, T., Rathnakumar, A.L., Bera, S.K., Jadon, K.S., Mishra, G.P., Riddhi, R., Binal, J. Phenotyping of groundnut genotypes for resistance to Sclerotium stem rot. J. Mycol. Plant Pathol. 2014; 44:459-462.

9. Paparu, P., Acur, A., Kato, F., Acam, C., Nakibuule, J., Nkuboye, A., Musoke, S., Mukankusi, C. Morphological and pathogenic characterization of *Sclerotium rolfsii*, the causal agent of southern blight disease on common bean in Uganda. Plant Dis. 2020; 104(8):2130-2137.

10. Le, C.N., Mendes, R., Kruijt, M., Raaijmakers, J.M. Genetic and phenotypic diversity of *Sclerotium rolfsii* in groundnut fields in central Vietnam. Plant Dis. 2012; 96:389-397.

11. Rangaswamy, G. Disease of crop plants in India. Prentice Hall of India Pvt. Ltd., New Delhi. 1958; 540.

12. Rocha, J.R.S., Oliveira, N.T., Menezes, M. Comparison of inoculation methods efficiency for evaluation of *Colletotrichum gloeosporoides* isolates pathogenicity on passion fruit (*Passiflora edulis*). Braz. Arch. Biol. Technol. 1998; 41(1):145-153.

13. Tomkins, R.G., Trout, S.A. The use of ammonium salts for the preservation of green mould in citrus. J. Pomol. Hortic. Sci.1931; 9:257-264.

14. Elahi, F.E., Islam, M., Alam, M., Islam, N., Tipu, M.M.H., Khatun, F., *et al.* First report of *Athelia rolfsii* on jackfruit (*Artocarpus heterophyllus*). Australas. Plant Dis. Notes. 2021; 16:1-3.

15. Yi, R.H., Liao, H.Z., Li, K.Y., Feng, F. Fruit rot caused by *Athelia rolfsii* (*Sclerotium rolfsii*) on jackfruit (*Artocarpus heterophyllus*) in China. Plant Dis. 2023; 107(10):3314.