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Unravelling The Role of *Aspergillus Japonicus* in Post-Harvest Decay and Quality Loss of Papaya (*Carica Papaya* L.) C.V. Coorg Honey Dew

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Abstract
The pod rot caused by post-harvest fruit rot reduces the shelf life and quality of papayas (Carica papaya L) under tropical conditions. The objective of this study was to isolate, identify, and characterize the fungal pathogen related to the post-harvest fruit rot of the papaya cv. Coorg Honey Dew as well as to assess its effect on the quality of the fruits from Eastern Bihar. The pathogen was isolated on potato dextrose agar (PDA), and was identified using morphological characteristics as an Aspergillus species. Molecular confirmation was done by performing PCR amplification of the internal transcribed spacer (ITS) region of rDNA (~500 bp), and sequencing and performing BLAST analysis of the pure fungal pathogen causing papaya fruit rot, Aspergillus japonicus. Pathogenicity tests showed that infected fruits displayed soft rot-type symptoms, and that infected fruits had greater lesion development and sporulation than uninfected fruits. In addition, infection resulted in 96% weight loss of fruit during storage after 30 days of storage and significant declines in physicochemical parameters such as the amount of ascorbic acid, total soluble solids, and titratable acidity. These results indicated rapid metabolic degradation and severe post-harvest quality loss due to fungal infection; therefore, Aspergillus japonicus was determined to be an important post-harvest pathogen of papayas found in Eastern Bihar, highlighting the need for enhanced postharvest production management strategies.

Keywords
Papaya (*Carica papaya* L. cv. Coorg Honey Dew) post-harvest fruit rot pure fungal pathogen *Aspergillus japonicus*; ITS region of rDNA; molecular identification; BLAST analysis; Phylogenetic tree; pathogenicity test; fruit quality deterioration; physicochemical changes in Eastern Bihar

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1. INTRODUCTION
1. Introduction Papaya (Carica papaya L.) is a valuable tropical fruit grown in developing countries for its economic importance, high nutrition value, quick growing ability, and value to industry. Papaya has high levels of vitamins, minerals, antioxidants, and enzymes such as papain, making it useful for food and industrial use. However, because papaya has a soft texture and moistness, it is very perishable and subject to post-harvest deterioration. This results in loss of large amounts of money during use, storage, transpiration and sale (Alara et al., 2020; Perez-Cueto, 2019). Fruit rot, tissue deterioration, and quick senescence due to Microbial disease, mainly fungal pathogens, are the main cause for the vast amount of post-harvest losses in fruit. According

to Zhang et al. (2019); Jiang et al. (2025), fungal pathogens enter the fruit through wounds or natural openings and multiply in favourable environmental conditions such as high humidity and temperature. Research has shown that fungal pathogens cause significant losses in quantity and quality in all countries that produce papaya (Crop Protection, 2023).

Aspergillus species are the most frequently identified fungus to be associated with disease and post-harvest deterioration of crops. These fungi exist naturally in the environment and can proliferate and case decay and degrade the quality of agricultural products by colonizing on a wide range of substrates including fruits, grains, and stored food (Zakaria, 2024; Franco-Duarte et al., 2019). In addition to causing spoiling, some Aspergillus species produce mycotoxin substances posing an additional risk to the health of humans and food safety (Gong et al., 2024; Kumawat et al., 2025).

There is ample evidence that suggests the involvement of several species of Aspergillus (e.g., A. Niger, A. flavus, and A. japonicus) in fruit rot diseases among numerous crops (Wang et al., 2025; Bustmante et al., 2024; Zhang et al., 2026). The adaptability and pathogenic nature of these pathogens allow them to persist in a wide range of environmental conditions and at a very rapid rate colonize the plant host tissue.

Accurate identification of fungal pathogens is essential for understanding the epidemiology of disease and for developing methods for effective management. Traditional morphological identification methods are not completely reliable; particularly for species with very similar features and those exhibiting phenotypic variation. fungal identification has gained result. In recent years, molecular technology has become the preferred method of identification for post-harvest pathogens and many researchers continue to obtain widespread use of molecular methods, such as sequencing the internal transcribed spacer (ITS) region of ribosomal DNA (Franco-Duarte et al., 2019; Hibbett et al., 2016). Identification by ITS provides the highest accuracy and resolution and enables the identification of species by comparison to WHO-ICTR and other national databases such as the International Nucleotide Sequence Database Association (INSD).

The development of molecular diagnostic and rapid detection technologies has improved our ability to detect and characterise post-harvest pathogens through the use of PCR amplification, DNA sequencing, and biosensor-based detection; these technologies provide faster and more accurate identification than traditional methods (Crop Protection, 2023; Liu et al., 2025). These methods also allow us to detect and manage fungal pathogens earlier in the production lifecycle of perishable crops like papaya.

Fungal pathogens produce visible deterioration of fruits, but they also cause biochemical and physicochemical changes in fruits that result in the breakdown of sugars, organic acids and vitamins. Thus, there is a decrease in the market value and changes in flavour and nutritional quality (Zhou et al., 2021; Lv et al., 2024). The degree of deteriorative changes is also dependent on the virulence of the pathogen, the environmental conditions and storage conditions.

While the economic value of papaya is known to be high, there has been limited research on the molecular characterisation of Aspergillus japonicus and post-harvest fruit-rotting pathogens

in particular on papaya in Eastern Bihar and other areas where environmental conditions are conducive to fungal pathogen growth; furthermore, no studies have been conducted that integrate the identification of the pathogen with the quantitative assessment of the quality declines experienced by fruit. The aim of this research was to identify and obtain the fungal pathogen Aspergillus japonicus (de Vries et al., 2021) responsible for post-harvest decay of papaya (Carica papaya L. cv Coorg Honey Dew) using rDNA ITS sequencing and monitor disease progression and associated physicochemical parameters of papaya stored under ambient conditions.

2. MATERIALS AND METHODS

2.1 Study area and sample collection

Samples were obtained from major fruit markets in Eastern Bihar at Bhagalpur, Banka, Purnia, Katihar and Munger Districts, in addition to local markets, from the same storage facility where the sample was obtained. The samples were obtained from Lalpur in Katoria block and Kotwali in Rajoun block, and from Chapper Diyara in Rangra Block and from Sahkund Block, Banka District, Bhagalpur District, Bihar, India. Fruits have been selected that were displaying all classification features of post-harvest decay (e.g. water-soaked lesions, softening, discolouration) and alongside healthy fruits used as controls. All samples were placed into sterile polyethylene bags and transported to the laboratory for analysis under aseptic conditions within 24 hours (Sharma et al., 2021; Kader, 2018).

2.2 Isolation & purification of the pathogen

A 5 × 5 mm section of infected fruit tissue was taken from the advancing margin of diseased fruit and

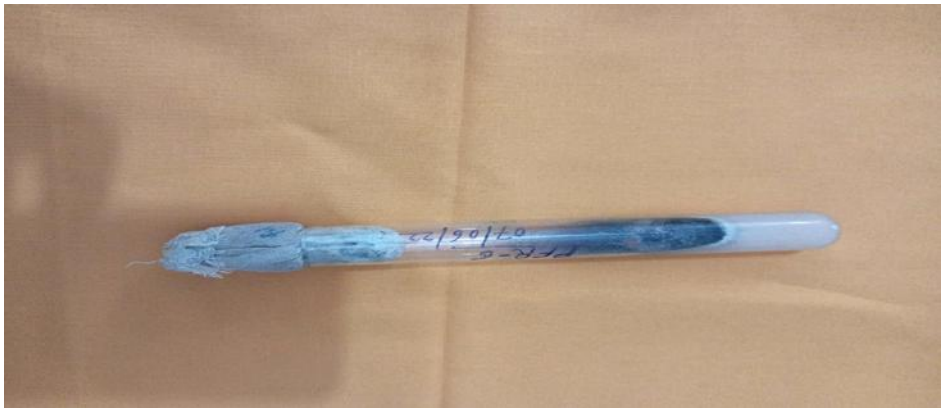
placed in a sterile Petri dish. The issue sample was surface-sterilised with 1% sodium hypochlorite solution for 1 minute, rinsed 3 times in sterile distilled water, and then dried on sterile filter paper. The surface-sterilised tissue was then plated onto Potato Dextrose Agar (PDA) media containing 50 mg/L streptomycin and incubated at 28 ± 2 for 5 to 7 days. Fungal colonies on the surface of agar plates were repeatedly sub-cultured to enable the production of pure cultures (Pitt and Miller, 2017; Bautista-Banos et al., 2016).

2.3 Morphological Characterisation

Purified fungal isolates were morphologically characterised based on the characteristics of colonies found on PDA media and included characteristics such as colour, texture, and colony growth rate. The microscopic morphology of each isolate was examined for the arrangement of conidiophores and vesicles, and conidia of each isolate using a compound microscope. Identification was confirmed by comparison with published taxonomic keys for Aspergillus (Samson et al., 2014; Hibbett et al., 2016).

2.4 Pathogenicity Tests

Pathogenicity testing using Koch's postulates demonstrated the pathogenicity of the isolated fungi on healthy papaya fruit. Fruits were surface sterilized with 70% ethanol and divided into groups, with half representing injured fruits and half representing uninjured fruits. With the use of a 7-day old culture of fungal spores, a concentration of approximately one hundred spores per millilitre was created and introduced to the papaya fruits. Control objects were injected with sterile, distilled water. Once introduced to the fruits, all papayas were kept at ambient temperatures (25-28°C) and monitored regularly for the appearance of fungal symptoms. Pathogenicity of isolated fungal pathogens was verified by re-isolating the fungal pathogen from each infected fruit (Agrios, 2005; Prusky and Lichter, 2007).



2.5 Molecular Identification of the Pathgen

2.5.1 Extracting DNA

Genomic DNA extracted from newly cultivated fungal mycelia using a method of fungal DNA extraction is CTAB/Tissue Mizer or a commercial kit. Quality and quantity were evaluated using agarose gel electrophoresis and spectrophotometric assessment (Sambrook and Russell, 2001).

2.5.2 PCR Amplification of ITS Region

Using Universal Primers Convert; ITS1 5' – TCCGTAGGTGAACCTGCGG– 3' and ITS4 5' – TCCTCCGCTTATTGATATGC-3'. PCR Amplification; 25µL Reaction volume standard cycling conditions. Initial denaturation step 95°C 3 minutes; 35 denaturation steps 95°C 30 seconds, annealing 55°C 30 seconds, extension 72°C 45 seconds and one additional extension 72°C for 7 minutes (White et al., 1990; Schoch et al., 2012).

2.5.3 Gel Electrophoresis

Molecular weights of expected amplicon size of 500 bp were verified by gel electrophoresis using a 2% Agarose gel stained with ethidium bromide and viewed using ultraviolet (UV) transillumination (Lee et al., 2012).

2.5.4 Sequencing and BLAST Analysis –

PCR products were purified and undergone bidirectional sequencing. Acquired sequences were aligned together to create a consensus sequence then compared to the NCBI GenBank database using the basic local alignment search tool (BLAST) to identify species. The best possible matching sequences were identified using maximum identity, query coverage, and minimum E-value as selection criteria (Altschul et al., 1997; Nilsson et al., 2019).

2.6 Measurement of Weght Loss

The initial weight of the fruits was recorded before storage. The subsequent weights of the fruits were recorded periodically at 10, 20 and 30 days. The percentage of weight loss was calculated using the formula:

$$\textbf{Weight Loss (\%)} = \frac{\textbf{Initial Weight} - \textbf{Final Weight}}{\textbf{Initial Weight}} \times \mathbf{100}$$

The post-harvest deterioration of produce is evaluated using this method by a number of authors (Kader, 2018; Teka et al., 2025).

2.7 Physicochemical analyses

Measurements for physicochemical parameters of fruits were performed on the samples taken from the different storage intervals.

- Ascorbic acid was measured using 2,6-dichlorophenolindophenol titration method
- Total soluble solids (TSS) were measured using the a99 digital refractometer (°Brix)
- Titratable acidity was determined through titration using 0.1N NaOH

All three measures were repeated three times so that an accurate determination was made (AOAC, 2019; Ranganna, 2010).

2.8 Statistical analysis

The data from each experiment will be analysed using Analysis of Variance (ANOVA) and the Tukey HSD test at the 0.05 level will be used to test for significant differences among treatment groups. All experimental data will be expressed as mean ± standard deviation (Gomez and Gomez, 1984).

Table 1 shows a summary of the experimental workflow.

Step	Procedure	Description
1	Sample Collection	Collection of healthy and infected papaya fruits.
2	Isolation	PDA (Potato Dextrose Agar) plating of sterilized tissues.
3	Morphology	Colony and microscopic observation (spore shape, color, etc.).
4	Pathogenicity	Koch's Postulates: Inoculation in injured/uninjured fruits.
5	DNA Etraction	Isolation of fungal genomic DNA from pure culture.
6	PCR	Amplification of the **ITS** (Internal Transcribed Spacer) region.
7	Electrophoresis	Visual confirmation of the expected $~500$ bp DNA band.
8	Sequencing	DNA sequencing and BLAST-based species identification.

Figure 1. Experimental Workflow Diagram

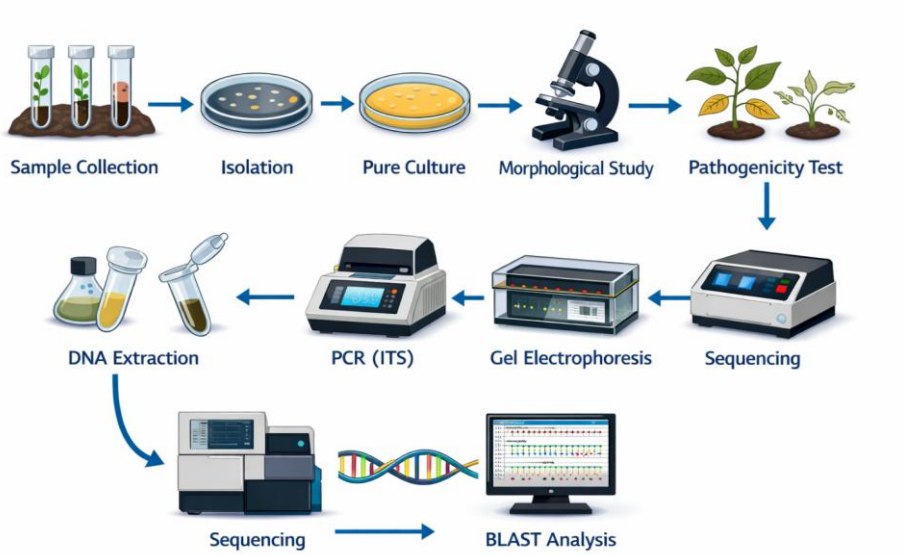


Figure:1 Workflow diagram illustrating the sequential steps involved in fungal identification and molecular characterization: Sample Collection → Isolation → Pure Culture → Morphological Study → Pathogenicity Test → DNA Extraction → PCR (ITS region amplification) → Gel Electrophoresis → Sequencing → BLAST Analysis.

3. RESULTS AND DISCUSSION

3.1 Symptom Expression and Development of the Disease

Inoculated with the isolated fungus, papaya (Carica papaya L. cv. Coorg Honey) fruits produced classic symptoms of post-harvest rotting. The first sign of disease is small, fluid-soaked spots that eventually become soft, sunken areas. In later stages of infection, the affected area shows excessive maceration, discolouration, and abundant black sporulation, leading to the fruit being unsuitable for sale (Snowdon, 2010; Sharma et al., 2022).

Injured or wounded fruits develop the disease much more quickly than uninjured or unwounded fruits; therefore, wounded or injured fruit provides easier access to the pathogen for both infection and

growth. These findings agree with other studies demonstrating that many post-harvest fungal pathogens can be accelerated in tropical storge conditions (Prusky et al., 2016; Teka et al., 2025). Symptoms develop on papaya fruit and are shown in Figure 2.

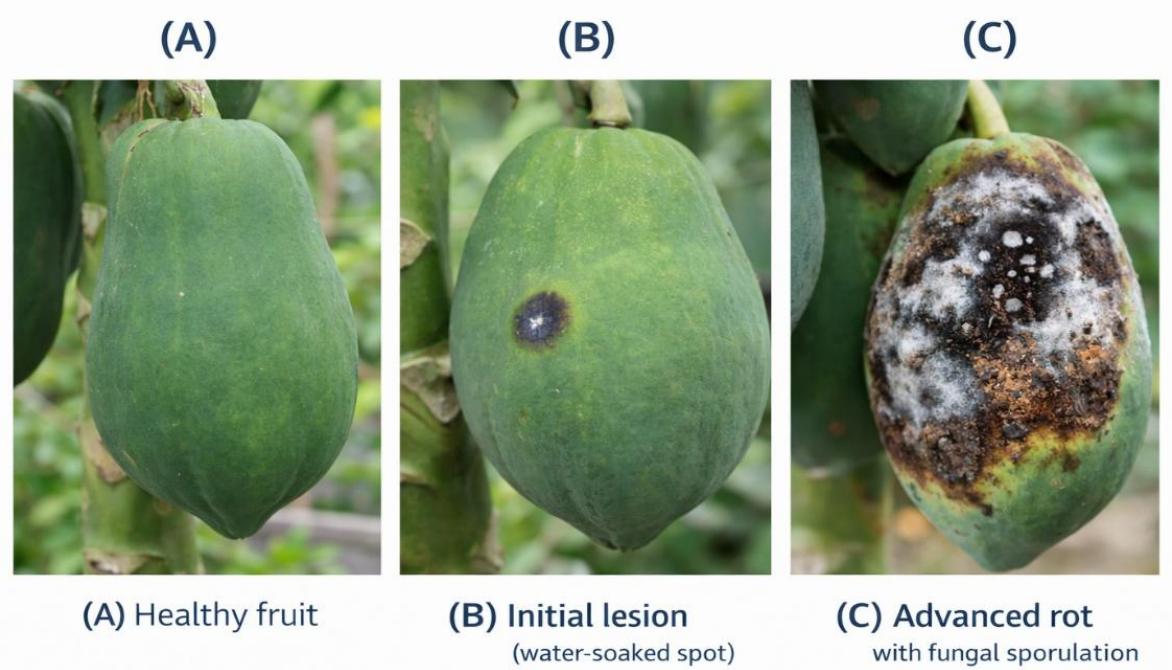


Figure 2. Symptom development in papaya fruits illustrating disease progression: (A) Healthy fruit with no visible defects; (B) Initial lesion showing a characteristic water-soaked spot; (C) Advanced rot stage with extensive tissue degradation and visible fungal sporulation.

3.2 Lesion Development and Sporulation

Quantitative analyses showed significant differences in lesion size and both expansion and sporulation with respect to treatments (Table 2). The lesion size of all samples expanded over time; however, the non-injured fruit had a significantly larger lesion diameter than did injured fruit for both injurious treatments with respect to lesion size. Injured fruit demonstrated a much greater amount of spores produced by the fungus, suggesting an increased likelihood of fungal growth and reproduction in these injuries.

Table 2. Lesion Development and Sporulation of *Aspergillus japonicus*

Fruit Condition	Lesion Size (5 Days, mm)	Lesion Size (10 Days, mm)	Sporulation (×105 spores/mL)
Uninjured	1.1 ± 0.06	18.6 ±0.65	30.4 ± 1.3
Injured	3.4±.0.09	27.4± 0.82	70.2 ±1.9
CD (P=0.05)	0.25	0.92	2.45
CV (%)	6.2	4.8	5.5

The data collected confirm that Aspergillus japonicus is an aggressive pathogen, as there are significantly more lesions on injured fruit than on non-injured fruit, indicating that mechanical injury in fruit significantly increases the likelihood of infection, as previously reported (Gong et al., 2024; Bautista-Baños, 2016).

3.3 Molecular Confirmation of Pathogen

A successful amplification of fungal DNA was achieved by regularly using PCR to produce a clean band of approximately 500 bp in length for the ITS rDNA region of the pathogen. In addition, sequencing and subsequent BLAST search for sample ID PFR-6 indicated that A. japonicus has a high degree of similarity based on nucleotide sequence homology and phylogenetic characteristics with A. japonicus (Altschul et al., 1997; Nilsson et al., 2019). A graphical representation of sample PFR-6 is found below (figure 3).

Figure 3. Agarose Gel Electrophoresis of ITS rDNA (~500 bp)

Lane 1: DNA ladder
Lane 2: ITS amplicon of fungal isolate

CCCGTGCTTACCGTACCCTGTTGCTTCGGCGGGCCCGCCTTCGGGCGGCCCGGGGC
CTGCCCCCGGGACCGCGCCCGCCGGAGACCCCAATGGAACACTGTCTGAAAGCGTG
CAGTCTGAGTTGATTGATACCAATCAGTTAAAACTTTCAACAATGGATCTCTTGGTTCCG
GCATCGATGAAGAACGCAGCGAAATGCGATAACTAATGTGAATTGCAGAATTCAGTGA
ATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTC
CGAGCGTCATTTCTCCCCTCCAGCCCCGCTGGTTGTTGGGCCGCGCCCCCCCGGGGG
CGGGCCTCGAGAGAAACGGCGGCACCGTCCGGTCCTCGAGCGTATGGGGCTCTGTC
ACCCGCTCTATGGGCCCGGCCGGGGCTTGCCTCGACCCCCAATCTTCTCAGATTGAC
CTCGGATCAGGTAGGGATACCCGCTGAA
Consensus sequence of Aspergillus japonicus

BLAST analysis

GRAPHICAL SUMMARY

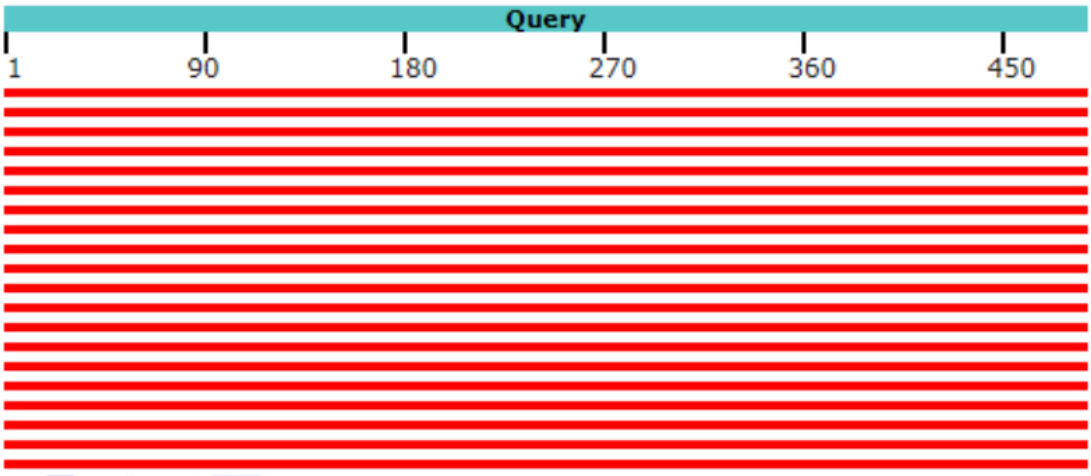
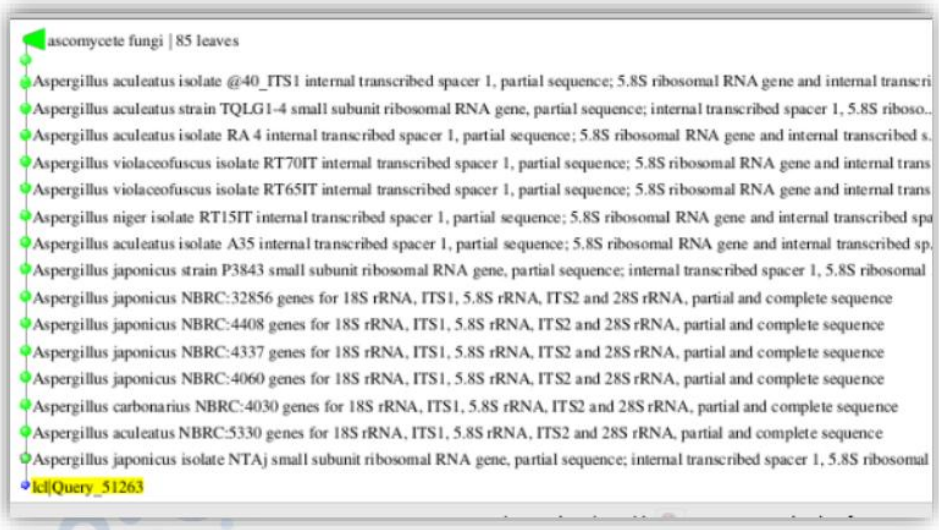


Figure 3. BLAST Analysis Graphical Summary of *Aspergillus japonicus Sequence*

The graphical output in **fig 4** shows high query coverage and maximum identity with *Aspergillus japonicus* sequences in the NCBI database.



3.4 Phylogenetic Tree Representation of Relationship

The phylogenetic tree depicted in figure four shows that the isolate clusters closely with Aspergillus japonicus, which supports the conclusion of its taxonomic identity.

Effect of Aspergillus japonicus Infection on Fruit Weight Loss

Aspergillus japonicas infection resulted in weight losses while in storage as shown in table 3. The weight losses increased progressively through time; with maximum weight loss at 30 days.

Research Component	Key Observation / Data Point	Statistical / Molecular Value
Field Incidence (30 Days)	Highest infection observed in Banka district	96.8 (1.1) %
Pathogenicity (Injured)	Maximum lesion size after 10 days	27.4 (0.82) mm
Pathogenicity (Uninjured)	Minimum lesion size after 10 days	18.6 (0.65) mm
Fungal Reproduction	Spore count on injured papaya tissue	70.2 (1.9) x 10^5 spores/mL
Molecular Marker	PCR amplification of the ITS region	~500 bp (Base Pairs)
Identification	BLAST analysis of DNA sequence	High Similarity Index

The increased weight loss may be attributed to enhanced respiration, moisture loss, and enzymatic degradation of tissues caused by fungal infection (Kader, 2018; Romanazzi et al., 2021).

3.5 Physicochemical Changes in Infected Fruits

Significant deterioration in physicochemical properties of papaya fruits was observed during storage (Table 4).

Table 4. Changes in Physicochemical Parameters

Parameter	Storage Period	Healthy Fruits	Infected Fruits
Ascorbic acid (mg/100g)	10 days	72.5	60.4
	20 days	60.8	38.2
	30 days	44.6	18.6
TSS (°Brix)	10 days	8.2	6.3
	20 days	5.5	3.2
	30 days	3.9	1.8
Titratable acidity (%)	10 days	0.62	0.46
	20 days	0.54	0.30
	30 days	0.39	0.17

Ascorbic acid concentrations drop significantly when ascorbic acid oxidatively degrades due to the sugars being used by A. japonicus. The loss of TSS (total soluble solids) is a reflection of how much sugar has been consumed by a pathogen, while acidity correlates with the alterations in metabolic activity associated with the pathogens (Zhou et al., 2021; Lv et al., 2024).

3.6 Overall Impact of Aspergillus japonicus as a Post-Harvest Pathogen

The data suggest that A. japonicus is a very important, economically important, and detrimental pathogen of post-harvest fruit worldwide because when combined with the previously discussed data, they unequivocally demonstrate that A. japonicas caused rapid deterioration of fruit and also has a very significant impact on the nutritional and sensory value of the fruit. The ability of this pathogen to increase on fruit under ambient conditions exemplifies its significance in tropical fruit post-harvest systems (Gong et al., 2024; Zakaria, 2024).

4. Conclusion

The study confirmed that A. japonicus is a virulent fungal pathogen of post-harvest papaya fruit (Carica papaya L. cv. Coorg Honey) obtained from Eastern Bihar, India. A. japonicus was confirmed to be a consistent fungus based on molecular characterization using ITS rDNA sequencing in association with phylogenetic analysis and BLAST confirmation. Molecular tools were superior to traditional morphology-based methods of identification. Pathogenicity tests provided validation of the virulence of the fungus, while fruit to which mechanical damage had occurred displayed higher levels of disease severity than undamaged fruits, indicating that mechanical injury significantly increases susceptibility to A. japonicus infection.

A. japonicus cause rapid progression of disease, with the A. japonicus-insulated fruit showing lesion enlargement, extensive tissue maceration, and abundant sporulation. The pathogen caused considerable losses in both quantity and quality resulting from a significant reduction in fruit weight and the deterioration of other key physicochemical characteristics, for example, ascorbic acid content, total soluble solids, and titratable acidity, during storage. These results suggest that there was an increase in metabolic damage and decrease in the nutritional and market quality of the fruit.

Overall, this research has shown that Aspergillus japonicus represents a significant postharvest fruit rot pathogen of papaya fruit stored under ambient conditions in tropical regions and highlights the necessity for improvements in postharvest handling practices, including reduced mechanical damage and hygienic storage, as well as improved eco-friendly strategies for disease management to reduce losses and increase the shelf life and fruit quality.

LIMITATION OF THE STUDY

The current study does provide useful insights into the molecular characterization and effect of Aspergillus japonicas as a postharvest fruit rot pathogen of papaya (Carica papaya L. cv. Coorg Honey); however, some limitations to this research exist. The first limitation is that this research has been conducted under ambient storage conditions, which have many variations associated with seasonal and geographical differences. Without genetically controlling for temperature or humidity during storage could impact how quickly the disease progressed and how the quality of the fruit changed. A second limitation of this research is that only selected Districts from the Eastern region of Bihar have been sampled therefore the results may not adequately represent or characterize the diversity and distribution of this pathogen in the Eastern ranks of India. Three different ways to identify Aspergillus species were used: DNA sequencing, traditional identification, and morphological identification. The use of molecular identification was determined by DNA sequencing of the ribosomal DNA region (ITS rDNA) of the fungi. Although this technique is accepted by the scientific community, the ability of this method to differentiate between closely related species of the same genus (in this case, aspergillus) may not be as precise as was believed. Therefore, if additional gene sequences could have been utilized, the taxonomic resolution would have been more accurate. In addition to not using mycotoxin production as an important part of identifying the species of Aspergillus, the researchers only studied some physiochemical parameters and did not obtain other important parameters such as texture and colour. Lastly, no experimental trials were conducted to determine effective post-harvest disease management strategies, limiting the potential applications of the study.

FUTURE PROSPECTIVE

Future directions include expanding the understanding of Aspergillus japonicum's role in the post-harvest spoilage of papaya fruit, specifically through multi-gene molecular characterizations using additional marker genes, such as the β-tubulin and calmodulin genes, to increase the accuracy of species identification. Future studies should also consider conducting detailed studies on myotoxic production and their effect on food safety, particularly with regard to storage conditions. Although the use of biological control agents, plant-derived antimicrobials, and environmentally safe edible coatings should be included in developed integrated disease management strategies, advanced technologies such as nanotechnology-based packaging and antimicrobial coatings could also be useful for controlling post-harvest pathogens. Further, studies on host-pathogen

interactions through enzyme activity and expression of genes during infection will provide a basic understanding of disease mechanisms. Additionally, studies that expand to different areas and varieties of papaya will provide data to help understand the variation of the pathogen and how environmental conditions influence the severity of the disease. Finally, the development of rapid diagnostic methods, such as molecular assays or biosensors, for early detection and management of post-harvest losses will ultimately allow for increased quality and safety of food products.

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