International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 22-05-2026, Accepted: 22-06-2026, Published: 07-07-2026
Volume 11, Issue 3, 2026, Page No. 45-52

Integrative molecular and pathogenic characterization of *Aspergillus flavus* driving post-harvest fruit rot and quality loss in Papaya (*Carica papaya* L. cv. Pusa Nanha)

Rajesh Ranjan[1], Dr. Ravi Shankar Singh[2], H K Chourasia[3]
[1] Research Scholar, Department of Botany, TMBU, Bhagalpur, Bihar, India
[2] Senior Scientist, Crop Improvement Division, ICAR - VPKAS, Hawalbagh, Almora, Uttarakhand, India
[3] Department of Botany, TMBU, Bhagalpur, Bihar, India
DOI: https://doi.org/10.66856/ijbs.2026.11.3.11170

Abstract
Papaya (Carica papaya L.) has very short shelf life, particularly in the tropics, and suffers from post-harvest fruit rot. The objective of this study was to isolate, identify and molecularly characterise the fungal pathogen associated with post-harvest fruit rot of papaya cv. 'Pusa Nanha', in Eastern Bihar, and assess the impact of infection on fruit quality. The pathogen was isolated on potato dextrose agar and subsequently identified, using morphological criteria, as an Aspergillus species. Molecular confirmation of the pathogen was achieved by amplification of the internally transcribed spacer region (ITS) of rDNA (~500 bp) of the fugus, followed by sequence analysis and subsequent BLAST comparison which identified it as Aspergillus flavus. Pathogenicity testing clearly demonstrated that injured fruit show a significantly higher level of lesion development (26.2 mm at 10 days) than non-injured fruit (17.8 mm) at the same period, and conidia produced on injured fruit were significantly greater (68.4 × 10^5 spores/mL in injured fruit) than on non-injured fruit. The infection resulted in severe weight loss of the fruit during the storage period; by 30 days, weight loss had reached 97.8% of the initial weight. Throughout the storage period, infectious fruit underwent significant deterioration in physiochemical parameters; ascorbic acid of 74.2 mg/100g in healthy fruit decreased to 20.2 mg/100g in infected fruit; total soluble solids levels of 8.4 °Brix in healthy fruit dropped to 2.0 °Brix; and titratable acidity of 0.64% level decreased to 0.19% in infected fruit. The results indicated a rapid rate of metabolic degradation and high severity of quality loss due to infection with A. flavus. The study has demonstrated that A. flavus is a significant post-harvest pathogen of papaya cv. 'Pusa Nanha' in Eastern Bihar, causing both quantitative and qualitative losses, highlighting the need for improved post-harvest management practices.
Bihar, along with its impact on disease development and associated physicochemical quality deterioration during storage.

Keywords: *Papaya* (*Carica papaya* L. cv. Pusa Nanha), *Aspergillus flavus* molecular characterization, BLAST analysis, Phylogenetic tree, pathogenicity test, physiochemical changes, frit quality deterioration in Eastern Bihar

Introduction

The papaya (*Carica papaya* L.) is an increasingly significant tropical fruit throughout the world, particularly in less-developed countries because of its nutritional, growth, and financial value. Papayas contain vitamins A & C, minerals, antioxidants, dietary fibre, and proteolytic enzymes (such as papain), which make them economically and nutritionally important in food and industrial applications. The papaya's high moisture content and soft texture cause it to have a rapid response to environmental stress resulting in considerable post-harvest loses during transportation and storage. The post-harvest loss of all produce (including fruits and vegetables) in developing nations has been estimated between 20% to 50% due to poor handling of products (Kader, 2005; Yahia, 2019; FAO, 2023).

One of the most important contributors to the loss of post-harvest quality and economics is to the crop. Fungal pathogens represent a significant contributor to the loss of quality and the resulting economic loss. Fungi are responsible for rot, tissue maceration, discolouration, and accelerated senescence of the fruit. Many fungi will infect fruit through wounds or bruises, or from natural openings on the outside of fruit. Fungi thrive in warm temperatures and high humidity, which make them very successful in growing and reproducing on papaya. Pathogens that infect fruit will cause a number of significant biochemical and physiological changes inside the fruit, including the degradation of sugar (sucrose in particular), organic acids, and colour pigments (carotenoids). Additionally, as fungi grow and reproduce within the fruit, they will use many water-soluble vitamins. This deterioration in nutritional quality and storage life of the fruit occurs because of the rapid ability of pathogenic fungi to grow on papaya. A number of new research studies have focused on using environmentally friendly management methods to reduce the presence of post-harvest fungal diseases while maintaining the quality of many tropical fruits lke papaya (Bautista-Baños, 2013; Sharma *et al*., 2009; Romanazzi *et al*., 2016) [20].

The most concerning pathogens related to post-harvest degradation caused by fungi are *Aspergillus flavus* due to its wide geographic distribution and ability to produce aflatoxins. These are among the most potent naturally occurring carcinogens and thus pose a great concern for food safety, public health, and international trade. The presence of *A. flavus* can be found in a variety of commodities such as fruits, vegetables, grains, and other agricultural products; in addition, many of these commodities are in warm and humid climates which are highly conducive to the growth and production of aflatoxins by fungi. Recent molecular and genomic studies have revealed a high degree of genetic variability and ecological flexibility within a given population of *A. flavus* allowing for the colonization of multiple substrates, as well as

persistence in the environment after the harvest. In addition, there are studies that indicate that many *Aspergillus* species are the major mycotoxin-producing fungi found in stored agricultural items and food products throughout the world (Amaike & Keller, 2011; Kumar *et al*., 2017; Frisvad *et al*., 2019) [9, 16].

Identifying fungi is critically important because this understanding plays a significant role in effective management practices; the development of disease management practices relies much on studying how the disease affects its distribution. Therefore, the use of molecular methods for fungal identification has become an important tool; for example, the identification of fungi by their morphology and culture can be difficult due to the extensive variety in morphology, environmental factors, and the similarity between closely related species of fungi. Thus, many molecular tools have been introduced for use in the classification of fungi and in the diagnosis of fungi. One molecular method, the sequencing of internal transcribed spacer (ITS) regions of ribosomal DNA is established as a universal DNA barcode for the identification of fungi. By using its molecular characterization, fungi are able to be identified and used for phylogenetic analysis due to their high specificity, reproducibility, and conformity to international databases (Schoch *et al*., 2012; Raja *et al*., 2017; Nilsson *et al*., 2019) [7].

There is a need for research characterizing the *Aspergillus flavus* species of fungi that causes post-harvest papaya (Carica papaya L. var. Pusa Nanha) fruit to rot; there is a limited understanding of how *A. flavus* aflatoxins affect papaya fruit quality due to studies that have been conducted in areas of Eastern Bihar, India, with climate conditions conducive to the growth of *A. flavus* and development of post-harvest diseases. Finally, only a limited number of studies have been conducted that assess the degree to which fungal infections cause the deterioration or change in physicochemical properties of various quality measurements (i.e. vitamin C, total soluble solids, pH, and titratable acidity) that help us understand the impact of disease and to develop effective post-harvest management methods for minimizing the impact of post-harvest diseases on quality (Paull & Duarte, 2011; Singh & Rao, 2015; Yahia & Carrillo-López, 2018).

The goal of the current research was to isolate and molecularly characterize *A. flavus* associated with post-harvest papaya fruit rot (C. papaya L. var. Pusa Nanha) through ITS rDNA sequencing. Secondly, the study will measure the progression of the disease and its respective physicochemical changes in disease-affected cotton fruit during normal ambient storage conditions to provide insight into the effects of fungal infection on papaya fruit quality as well as post-harvest shelf life.

Materials and Methods

1. Sample Collection and Disease Assessment

Papaya (Carica papaya L. cv. Pusa Nanha) fruits that were naturally infected and showed signs of post-harvest rot were gathered from many districts in Eastern Bihar, including local markets and storage facilities in Bhagalpur, Banka, Purnia, katihar and Munger district and Lalpur area located at Katoria block and Kotwali area located at Rajoun Block at Banka district and Chapper Diyara area located at Rangra block and Shahkund block area at Bhagalpur district of Bihar, India. Fruits with a range of infection stages were chosen, from early water-soaked lesions to more severe soft rot. As controls, healthy fruits of comparable size and maturity were gathered. Sterile plastic bags were used to transfer the samples to the lab, where they were processed within 24 hours after being collected.

2. Isolation and Purification of the Pathogen

Using a sterile scalpel, pieces of infected (5 mm × 5 mm) tissue were excised from the edge of each of the infected lesions. After excising each section of infected tissue, the piece was surface sterilized by immersing it in a 1% Sodium Hypochlorite solution for 1 minute and then rinsing in sterile distilled water three times. After rinsing, the section was dried on sterile filter paper. The sterilized tissue was then placed onto Potato Dextrose Agar (PDA) with the addition of Streptomycin (50 mg/L) to control bacterial contamination. The inoculated plates were then incubated at 28–30°C for 5–7 days. Pure fungal cultures were obtained by repeated sub-culturing of emerging fungal colonies (Dhingra and Sinclair, 1995; Leslie and Summerell, 2006; Watanabe, 2010).

3. Morphological Characterization

Microscopic characteristics and colony shape were used to characterize the pure fungal isolate. The colour, texture, and growth rate of the colonies were noted. Conidiophores, vesicles, and conidial structures were observed using a compound microscope and compared with conventional taxonomic descriptions.

4. Pathogenicity Test

Healthy papaya fruits were used to confirm pathogenicity. Fruits were injected with a spore suspension (~1×10^2 spores mL^{-1}) made from 7-day-old culture after being surface sterilized with 70% ethanol. There were two treatments applied:

- Fruits that have been pin-picked
- Unharmed fruits

Fruits were cultured at room temperature (25–28°C) and checked every day for sporulation, lesion growth, and symptom development.

5. Molecular Identification of Pathogen

5.1 DNA Extraction

Genomic DNA was extracted from fresh fungal mycelium using the CTAB method. DNA quality and concentration were assessed using agarose gel electrophoresis and spectrophotometry.

5.2 PCR Amplification of ITS Region

scribed spacer (ITS) region of fungal rDNA was amplified using the universally conserved primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′). PCR amplification was performed using a thermal cycler in a 25 µL reaction mixture. The components of the rection mixture were as follows: 5 µL template DNA, 10 µL PCR buffer, 1.5 mM MgCl2, 0.25 mM dNTPs, 0.2 µM primers, 1 unit Taq DNA polymerase, and nuclease-free water to make up the volume to 25 µL. PCR amplification consisted of an initial denaturation (3 minutes at 95°C), followed by 35 cycles consisting of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds and extension at 72°C for 45 seconds; with a final extension at 72°C for 7 minutes. Amplified products were analysed by agarose gel electrophoresis. The ITS region was chosen as the universal

DNA barcode for taxa of fungi and for studies of phylogenetics (White *et al*., 1990; Schoch *et al*., 2012).

5.3 Gel Electrophoresis and Sequencing

PCR products were resolved on 2% agarose gel to confirm the expected ~500 bp amplicon. The amplified products were purified and sequenced. Sequence data were analysed using BLAST to confirm the identity of the pathogen.

6. Weight Loss Measurement

Fruit weight was recorded at 0, 10, 20, and 30 days of storage. Weight loss (%) was calculated using:

Weight Loss (%) = [(Initial Weight − Final Weight) / Initial Weight] × 100

7. Physiochemical Analysis

The following parameters were evaluated:

Ascorbic acid: Determined using 2,6-dichlorphenol/indophenol titration method

Total soluble solids (TSS): Measured using a digital refractometer (°Brix)

Titratable acidity: Determined by titration with 0.1 N NaOH All analysis were conducted in triplicate.

8. Statistical Analysis

Experimental data were analysed using analysis of variance (ANOVA). Treatment means were compared using Tukey's HSD test at $p < 0.05$. Results were expressed as mean ± standard deviation.

Table 1: Summary of Experimental Procedures

Step	Procedure	Description
1. Collection	Field Sampling	Healthy and infected Papaya (cv. Pusa Nanha) collected.
2. Isolation	PDA Plating	Surface-sterilized tissues cultured on agar.
3. Morphology	Microscopy	Examination oF yellow-green colonies and spores.
4. Pathogenicity	Inoculation	Test on injured vs. uninjured fruits to confirm rot.
5. DNA Extraction	CTAB Method	Isolation of genomic DNA from the fungal culture.
6. PCR	ITS Primers	Amplification of the ITS region (ITS1/ITS4).
7. Analysis	Electrophoresis	Confirmation of the ~500 bp band on agarose gel.
8. Sequencing	BLAST	DNA sequence comparison to identify *A. flavus*.

Results and Discussion

1. Symptom Expression and Disease Development

Papaya (*Carica papaya* L. cv. Pusa Nanha) fruits inoculated with *Aspergillus flavus* developed characteristic post-harvest fruit rot symptoms. Initial infection appeared as small, water-soaked lesions, which progressively enlarged into soft, sunken rot during storage. In advanced stages, extensive tissue maceration, discoloration, and fungal sporulation were observed, rendering the fruits unmarketable.

Disease development was significantly faster in injured fruits compared to uninjured ones, indicating that mechanical injuries play a crucial role in facilitating pathogen entry and colonization. Similar observations have been reported for post-harvest fungal pathogens under tropical storage conditions (Teka *et al*., 2025) [2]

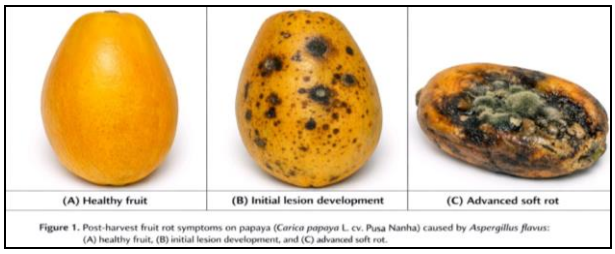

Figure 1. Post-harvest fruit rot symptoms on papaya (*Carica papaya* L. cv. Pusa Nanha) caused by *Aspergillus flavus*: (A) healthy fruit, (B) initial lesion development, and (C) advanced soft rot.

2. Lesion Development and Sporulation

Injured and unharmed fruit have quantitative differences (Table 2) in the diameter of lesions produced and the ability to produce conidia (spores from fungal growth). The infected fruit produced exponentially larger diameter lesions. The concentration of conidia was also much greater on injured fruit indicating that injury provided excellent opportunities for fungal growth.

Table 2: Lesion development and sporulation of *Aspergillus flavus*

Fruit Condition	Lesin Size (mm) 5 Days	Lesin Size (mm) 10 Days	Sporulation (×105 spores/mL)
Uninjured	0.9 ± 0.05	17.8 ± 0.62	28.5 ± 1.2
Injured	3.1 ± 0.08	26.2 ± 0.75	68.4 ± 1.8
CD (*P* =0.05)	0.21	0.88	2.35
CV (%)	6.8	4.5	5.2

These results confirm the aggressive pathogenic nature of *A. flavus*, consistent with previous reports on fungal virulence and colonization efficiency (Gong *et al*., 2024) [3].

3. Molecular Confirmation of the Pathogen

Through the PCR method, the ITS rDNA region produced an amplicon length of approximately 500 bp, which indicates successful amplification of the fungal genomic DNA. Sequencing of the amplified product with the BLAST method identified the sample to be very closely related at the nucleotide level with Aspergillus flavus based on nucleotide similarity to another sequence. Both the sequencing chromatogram and the BLAST alignment results are shown in Fig 2 and 3, respectively. The results from phylogenetic analysis placed this isolate together with reference strains of *A. flavus*, confirming its taxonomic status. The above results also demonstrate that the use of ITS rDNA sequencing provides a reliable method for the accurate identification and classification of fungi (White *et al*., 1990; Schoch *et al*., 2012; Altschul *et al*., 1997).

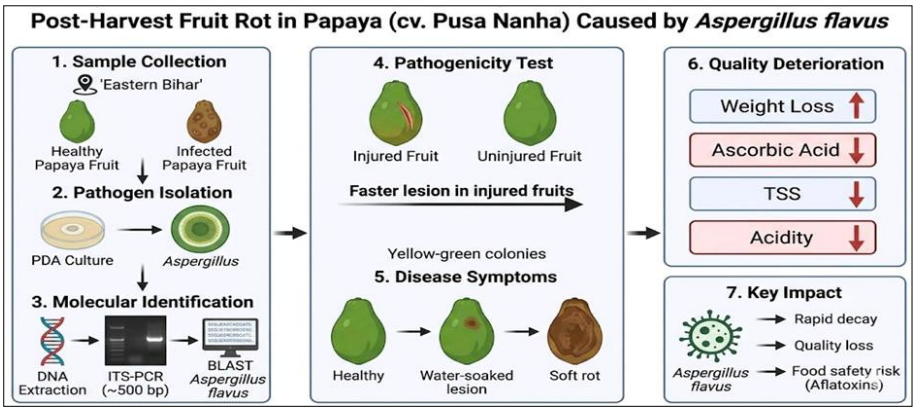

Fig 1: Graphical representation of the isolation, molecular identification, and pathogenic characterization of *Aspergillus flavus* causing post-harvest fruit rot in papaya (*Carica papaya* L. cv. Pusa Nanha) from Eastern Bihar, along with its impact on disease development and associated physicochemical quality deterioration during storage.

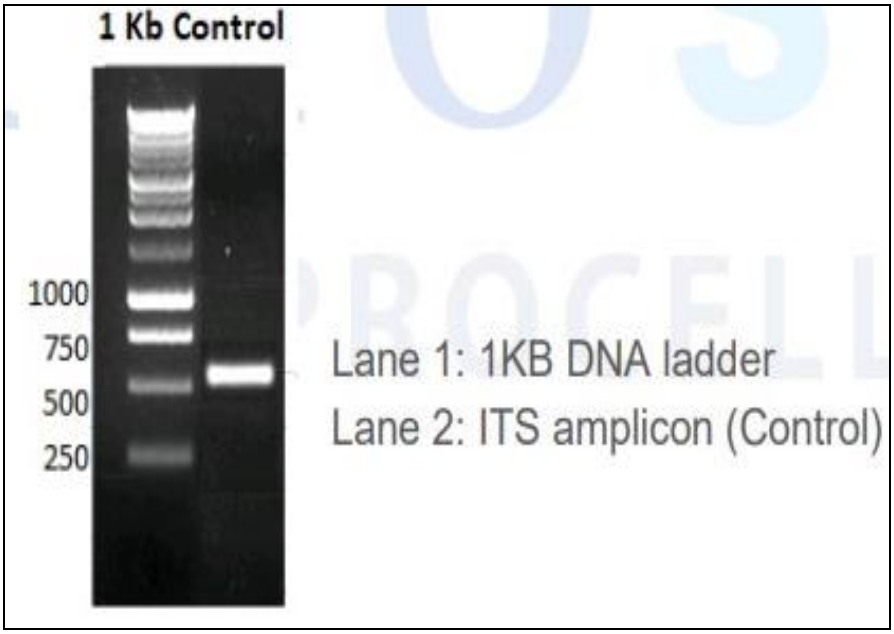

Fig 2: Agarose gel electrophoresis showing ITS rDNA amplification (~500 bp) of Aspergillus flavus

TCATTACCGAGTGTAGGGTTCCTAGCGAGCCCAACCTCCCACCCGTGTTTACTGTACC
TTAGTTGCTTCGGCGGGCCCGCCATTCATGGCCGCCGGGGGCTCTCAGCCCCGGGC
CCGCGCCCGCCGGAGACACCACGAACTCTGTCTGATCTAGTGAAGTCTGAGTTGATTG
TATCGCAATCAGTTAAAACTTTCAACAATGGATCTCTTGGTTCCGGCATCGATGAAGAA
CGCAGCGAAATGCGATAACTAGTGTGAATTGCAGAATTCCGTGAATCATCGAGTCTTTG
AACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTGCT
GCCCATCAAGCACGGCTTGTGTGTTGGGTCGTCGTCCCCTCTCCGGGGGGGGACGGGC
CCCAAAGGCAGCGGCGGCACCGCGTCCGATCCTCGAGCGTATGGGGCTTTGTCACCC
GCTCTGTAGGCCCGGCCGGCGCTTGCCGAACGCAAATCAATCTTTTTCCAGGTTGACC
TCGGATCAGGTAGGGATACCCGCTGAACTTAA

Fig 3: Consensus sequence of Aspergillus flavus

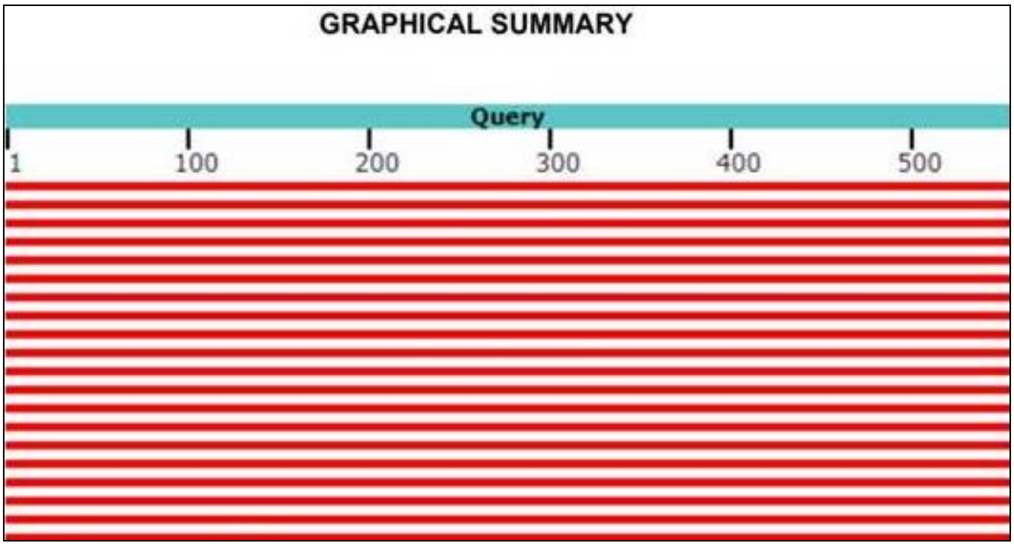

Fig 4: BLAST analysis Graphical Summary

The consensus sequence obtained (~500 bp) exhibited high query coverage and identity with *A. flavus* sequences in the NCBI database, confirming the taxonomic identity of the pathogen.

The phylogenetic analysis further supported the close relationship of the isolate with reference *A. flavus* strains. These findings validate the use of ITS rDNA sequencing as a reliable tool for fungal identification, as also reported in recent molecular studies (Katati *et al*., 2024 [5]; Franco Duarte *et al*., 2019).

Description	Max Score	Total Score	Query Cover	E value	Per. Ident
Aspergillus tamarii strain M24 small subunit ribosomal RNA gene, partial sequence; internal transcribed space...	1018	1579	100%	0.0	100.00%
Aspergillus flavus genomic DNA sequence contains 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2, 28S rRNA ...	1018	1018	100%	0.0	100.00%
Aspergillus flavus genomic DNA sequence contains 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2, 28S rRNA ...	1018	1018	100%	0.0	100.00%
Aspergillus oryzae genomic DNA sequence contains 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2, 28S rRNA ...	1018	1018	100%	0.0	100.00%
Aspergillus flavus genomic DNA sequence contains 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2, 28S rRNA ...	1018	1018	100%	0.0	100.00%
Aspergillus flavus genomic DNA sequence contains 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2, 28S rRNA ...	1018	1018	100%	0.0	100.00%
Aspergillus flavus genomic DNA sequence contains 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2, 28S rRNA ...	1018	1018	100%	0.0	100.00%
Aspergillus flavus genomic DNA sequence contains 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2, 28S rRNA ...	1018	1018	100%	0.0	100.00%
Aspergillus flavus genomic DNA sequence contains 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2, 28S rRNA ...	1018	1018	100%	0.0	100.00%
Aspergillus flavus genomic DNA sequence contains 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2, 28S rRNA ...	1018	1018	100%	0.0	100.00%

Note: Based on the percentage identity and E-Value [E-values in blast results represent the probability of the alignment occurring by chance], the identified species is being reported. An E value close to zero means that you would practically expect no unrelated sequence to score as high to your query sequence

Fig 5: Sequence Producing Significant Alignments in NCBI Database

The fungal isolate showing successful amplification of fungal rDNA genomic material has a PCR product (~500 bp) that confirms the successful amplification of genomic DNA (rDNA). The PCR product was sequenced and performed a BAST search against NCBI (GenBank) databases. The BLAST results indicated 100% coverage of query, 100% sequence identity, and an E-value of 0.0 relative to many deposited sequences of Aspergillus flavus (Fig 5). The highest alignment scores further confirmed that the isolate is closely related genetically to other reference A. flavus strains found in GenBank.

Phylogenetic analysis of the ITS rDNA sequences placed the fungal isolate within the Aspergillus flavus clade, thus confirming the isolate's taxonomic identity. In addition, the results of the molecular characterization were consistent with the morphological features observed during the fungal isolation and culture studies. The results of this study provide evidence that sequencing of the ITS rDNA and subsequent analysis by BLAST are accurate methods of identifying fungal pathogens that case post-harvest fruit rot (White *et al*. 1990; Schoch *et al*. 2012; Samson *et al*. 2014)

Phylogeny Analysis

A phylogenetic tree (fig 6) is a diagram representative of the evolution of organisms based on genetic, morphological or molecular data. The termina or outside branches of the phylogenetic tree represent the descendant taxa while the internal nodes of the phylogenetic tee represent the most recent ancestor of their descendant taxa. descendant taxa are called sister groups if they share a more recent common ancestor with each other than they do to any other taxa in the phylogenetic tree (Hall, 2011, Kumar *et al*., 2018).

Phylogenetic trees usually include outgroups, which are known to be outside the evolutionary relationship of the taxa being studied. Outgroups are the reference for rooting and the direction of the phylogenetic tree and determining the direction of the evolutionary process. Therefore, all the taxa of interest (in group) are more closely related to each other

than to the outgroup; hence, the outgroup branches from the base of the phylogenetic tree (Felsenstein, 2004).

The length of the horizontal branches of a phylogenetic tree can be related to how far apart the two taxa are from one another (fundamentally meaning how many incremental evolutionary steps there have been between two taxa); the larger the branch length representing two taxa, the more evolutionary steps fund between the two taxa than would be found between taxa with larger branch lengths. The length of each branch is generally measured in the number of nucleotides on a per-site basis (the number of times there was a change in the sequence of the nucleotides divided by the total number of nucleotides for which data was collected). Phylogenetic tees do contain scale bars, which inform how large the distance between the two taxa represented on the given length or branch is for the estimated amount of genetic change (Hall, 2011; Kumar *et al*., 2018).

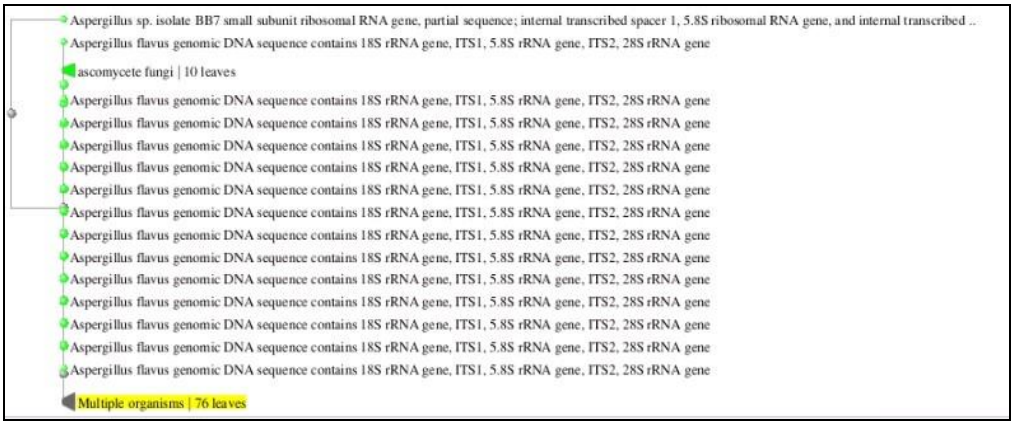

Fig 6: Phytogenic Tree

4. Effect of Infection on Fruit Weight Loss

Infection by *A. flavus* resulted in significant weight loss in papaya fruits during storage (Table 3). Weight loss increased progressively with storage duration, reaching maximum levels at 30 days.

Table 3: Percentage weight loss of infected papaya fruits

Location	10 Days (%)	20 Days (%)	30 Days (%)
Bhagalpur Mandi	36.4 ± 1.1	69.8 ± 1.5	95.6 ± 1.2
Banka Market	39.2 ± 1.3	72.5 ± 1.8	97.1 ± 0.9
Purnia Market	41.6 ± 1.5	74.3 ± 1.6	97.8 ± 1.0
Munger Market	38.7 ± 1.2	71.2 ± 1.7	96.3 ± 1.1
CD (P =0.05)	2.45	3.12	1.98
CV (%)	5.6	4.8	3.2

The increased weight loss can be attributed to enhanced respiration, moisture loss, and enzymatic degradation of host tissues caused by fungal infection. Similar trends have been observed in post-harvest studies of tropical fruits (Physiol. Mol. Plant Pathol, 2024) [1].

5. Physicochemical Changes in Infected Fruits

Infection by *A. flavus* caused significant deterioration in physicochemical properties of papaya fruits (Table 4).

Table 4: Physicochemical changes in papaya during storage

Parameter	Storage Period	Healthy Fruits	Infected Fruits
Ascorbic acid (mg/100g)	10 d	74.2	62.5
	20 d	61.3	40.8
	30 d	45.6	20.2
TSS (°Brix)	10 d	8.4	6.5
	20 d	5.8	3.4
	30 d	4.1	2.0
Titratable acidity (%)	10 d	0.64	0.48
	20 d	0.56	0.31
	30 d	0.41	0.19

A sharp decline in ascorbic acid content indicates oxidative degradation and increased metabolic activity. Reduction in TSS reflects utilisation of sugars by the pathogen, while decreased acidity is associated with altered organic acid metabolism.

The results of this research are similar to other studies showing that fungal infections cause biochemical changes to fruits (Zhou *et al*. 2021) [4]

6. Overall Impact of *Aspergillus flavus*

Aspergillus flavus is one of the most serious postharvest pathogens. It causes rapid decay, along with severe deterioration of the nutritional value and sensory characteristics of fruit. Furthermore, the potential production of aflatoxin makes Aspergillus flavus a major concern in terms of food safety (Gong *et al*., 2024) [3].

The present study demonstrated that Aerobic flavus is the most significant post-harvest fungal agent associated with fruit rot and deterioration of quality in papaya (Carica papaya L. cv. Pusa Nanha) during storage at room temperature, in Eastern Bihar. The results of molecular identification through ITS rDNA sequencing, confirmed via BLAST, and phylogenetic analysis provided clear confirmation of the pathogen and; thus proved the usefulness of molecular techniques compared to traditional morphological methods of identifying pathogens. The pathogen exhibited extremely high virulence in pathogenicity tests, with higher levels of disease occurring in injured fruit. Mechanical damage to fruit plays an extremely important role in facilitating infection.

Conclusions

The advancement of disease due to Aspergillus flavus infection was characterized by accelerated expansion of lesions, considerable tissue breakdown and high levels of sporulation. In addition, the disease caused enormous amounts of quantitative and qualitative loss of fruit as reflected in the physical weight loss and decrease in chemical quality parameters (ascorbic acid content; total soluble solids; and titratable acidity) during storage. The changes in these measurable parameters indicated

accelerated metabolic degradation and decreased nutrient and sensory quality of the fruit. The significance of improved postharvest management strategies, including minimizing mechanical injury and maintaining sanitary storage conditions, in order to minimize disease incidence and enhance shelf life, is emphasized by the current findings. The potential for aflatoxin contamination associated with Aspergillus flavus underscores the need for development of integrated pest management methods, including biological control and environmentally sound alternatives. Results from this research will also support future investigations of postharvest disease management and the safety and quality of papaya fruits, thus contributing to the scientific base for future work in this area.

Limitation of the study

The limitations of this research should be considered when interpreting its results. 1) This research was done in ambient storage conditions and as such, varying climatic factors could affect relative humidity and temperature in each season, resulting in differing storage conditions. 2) Locational constraints of the data collection limits data to a number of locations within Eastern Bihar, and thus do not provide insight on the full range of pathogen diversity throughout broader areas. 3) The method of detection of the molecular identifications occurred primarily through sequencing of the ITS and rDNA of the organisms, however, while this method is widely accepted for identification the intricacies of the classification tree used may also contribute to the identification of closely adjacent species within the Aspergillus genus Including additional gene markers (like β-tubulin and calmodulin) could help improve the accuracy of identifying A. flavus infections in crops and assessing the quality of those crops. The current research didn't identify any aflatoxins produced by A. flavus; this is important because it is a major factor in determining food safety when an A. flavus infection occurs.
The study mainly compared physical characteristics (total soluble solids, acidity, and ascorbic acid) but did not compare other important aspects of a fruit's quality (like firmness, colour, and sensory attributes). Post-harvest management and control strategies that could be used to prevent an A. flavus-infected fruit from rotting were not evaluated in this research.

Future perspectives

Future studies should have used several genes to identify A. flavus infections in papayas; collected and analysed toxin production levels from infected papaya fruits; investigated control strategies; and included several geographical locations and cultivars of papaya fruits to determine what characteristics lead to A. flavus infections and therefore determine the most effective way to prevent A. flavus from infecting papaya fruit.
The current study serves as an important starting point for understanding the role of A. flavus in post-harvest rotting of papaya, so future studies can help researchers understand A. flavus better and create ways to prevent it from infecting food. Future research should focus on multi-gene molecular characterization (including other markers—e.g., β-tubulin and calmodulin genes). Detailed studies are needed on how aflatoxins are produced by A. flavus in infected papayas, so that food safety risks can be determined along with what are the appropriate aflatoxin limits for storage of fruits.
There are also future research opportunities in developing integrated management strategies for diseases of fruits after harvest that use biological controls, plant-based antifungal agents, and environmentally friendly coatings to minimize A. flavus infections and improve fruit quality. Using advanced technologies such as nanotechnology-based coatings and antimicrobial packaging should provide additional avenues for controlling A. flavus after crops are harvested. Studies that look at the interaction between host and pathogen by studying enzyme activity and gene expression during the infection of hosts will also provide valuable information to help control pathogens.
Continuing to perform research in different geographical areas, as well as using different cultivars of papaya or other fruit, will provide researchers with additional potential sources of genetic variability to help them understand how the environment may influence disease severity in fruit. Rapid Molecular Diagnostics and biosensors could also be used to assist growers with identifying A. flavus infections in their crops early and therefore, minimize the potential for post-harvest loss from A. flavus. Finally, utilizing molecular biology, post-harvest technology, and sustainable management will be critical for improving the overall quality, safety, and market value of fruit products.

References

1. Exploring the non-chemical alternatives for management of post-harvest fungal diseases of tropical fruits. Physiological and Molecular Plant Pathology, 2024.
2. Teka T, *et al*. Control of postharvest diseases in papaya fruit. Postharvest Biology and Technology,2025:221:113304.
3. Gong A, Song M, Zhang J. Current strategies in controlling *Aspergillus flavus* and aflatoxins. Sustainability,2024:16:3171.
4. Zhou Y, Xu J, Zhu Y, Duan X, Liu W. Effects of fungal infection on postharvest fruit quality. Food Chemistry,2021:339:127847.
5. Katati B, *et al*. Diversity of *Aspergillus* section *Flavi* and aflatoxin contamination. Mycotoxin Research,2024:40:351-367.
6. Franco-Duarte R, *et al*. Advances in chemical and biological methods to identify microorganisms—from past to present. Microorganisms,2019:7(5):130.
7. Nilsson RH, *et al*. Mycobiome diversity: High-throughput sequencing and identification of fungi. Nature Reviews Microbiology,2019:17:95-109.
8. Romanazzi G, Feliziani E, Smilanick JL. Integrated management of postharvest decay of fruits. Postharvest Biology and Technology,2021:178:111564.
9. Pitt JI, Miller JD. A concise history of mycotoxin research. Journal of Agricultural and Food Chemistry,2017:65(33):7021-7033.
10. Kader AA. Increasing food availability by reducing postharvest losses of fresh produce. Acta Horticulturae,2018:1221:45-54.
11. Sharma RR, Singh D, Singh R. Advances in post-harvest management of fruits. Journal of Horticultural Science,2021:16(2):101-115.
12. Food and Agriculture Organization of the United Nations. FAOSTAT statistical database. Food and Agriculture Organization of the United Nations, 2022.

13. Prusky D, Lichter A. Activation of quiescent infections by postharvest pathogens. Annual Review of Phytopathology,2007:45:395-417.
14. Droby S, Wisniewski M, Teixidó N, Spadaro D, Jijakli MH. The science behind postharvest biocontrol. Postharvest Biology and Technology,2016:122:22-29.
15. Valencia-Chamorro SA, Palou L, del Río MA, Pérez-Gago MB. Antimicrobial edible coatings for fresh fruits. Critical Reviews in Food Science and Nutrition,2017:57(16):3440-3470.
16. Tripathi P, Dubey NK, Shukla AK. Use of plant-based antimicrobials for postharvest disease management. Journal of Food Science and Technology,2019:56:435-447.
17. Sundelin T, Castelin M, Wahlström M, Rosenberg O. Postharvest pathogens and control strategies. Plant Pathology,2019:68(4):635-648.
18. O'Donnell K, Ward TJ, Geiser DM, Kistler HC. DNA sequence based identification of fungal pathogens. Studies in Mycology,2015:80:53-68.
19. Hibbett DS, *et al*. Sequence-based classification and identification of fungi. Mycologia,2016:108(6):1049-1068.
20. Bautista-Baños S, *et al*. A review of postharvest fungal diseases of fruits and vegetables. Crop Protection,2016:65:26-41.