

Post-Harvest Fruit Rot Diseases of Papaya c.v. Red Lady and Their Physiochemical Changes Under Pathogenesis

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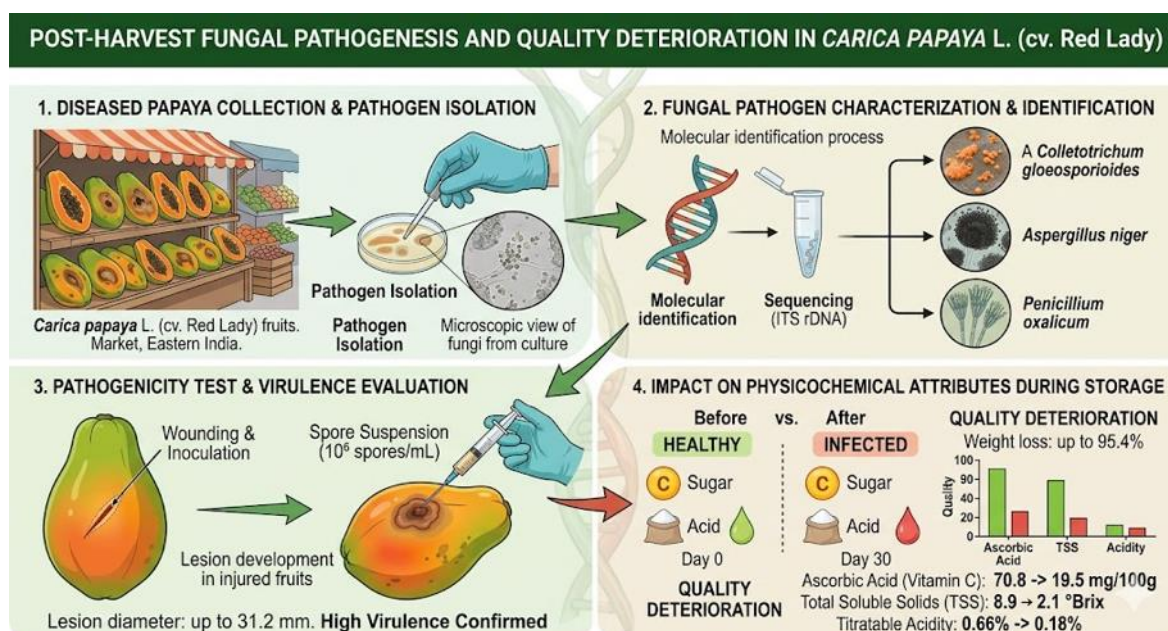
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Abstract: Papaya (*Carica papaya* L. cv. Red Lady) is highly susceptible to post-harvest diseases, leading to substantial losses in quality and shelf life. The present research work aimed to isolate and characterize major fungal pathogens associated with post-harvest fruit rot and analyze their impact on physicochemical attributes of papaya. Diseased fruits were collected from markets in Eastern India, and pathogens were isolated and identified morphologically and molecularly. Major pathogens identified included *Fusarium equiseti*, *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus japonicus* and *Penicillium oxalicum*. Pathogenicity tests confirmed their virulence, with lesion diameter reaching up to 31.2 mm in injured fruits. Infection caused significant weight loss (up to 95.4%) and deterioration in quality parameters such as ascorbic acid (70.8 to 19.5 mg/100 g), total soluble solids (8.9 to 2.1 °Brix), and titratable acidity (0.66% to 0.18%) during storage. The study highpoints the critical role of fungal pathogens in post-harvest losses and emphasizes the need for improved management strategies.

Keywords: Papaya Red Lady; post-harvest rot; fungal pathogens; physicochemical changes; fruit quality.

1. Introduction



Papaya (*Carica papaya* L.) is an economically valuable tropical fruit crop widely cultivated for its nutritional richness, rapid growth, and high market demand. It is a rich source of vitamins (A, C, and E), minerals, antioxidants, and enzymes such as papain, which contribute to its dietary and medicinal value [1] (Kader, 2018). Despite its importance, papaya is highly perishable due to its climacteric nature, high moisture content, and soft texture, making it highly susceptible to post-harvest deterioration [2] (FAO, 2022).

Post-harvest losses of fruits and vegetables are a major global concern, accounting for approximately 20–50% of total production, especially in developing countries [3] (Guan et al., 2023). Among various factors, fungal pathogens are the

primary cause of post-harvest deterioration, leading to significant economic losses and reduced fruit quality [4] (Zhang et al., 2021). These pathogens infect fruits either through wounds or latent infections and rapidly colonize tissues under favorable environmental conditions such as high humidity and temperature [5] (Bautista-Baños et al., 2016).

Papaya fruits are very vulnerable to a wide range of fungal pathogens, including *Colletotrichum gloeosporioides*, *Aspergillus niger*, *Penicillium oxalicum*, *Rhizopus stolonifer*, and *Fusarium* species [6] (Cameroon Study, 2024). Among these, anthracnose caused by *Colletotrichum* species is considered one of the most destructive diseases affecting papaya during storage and marketing [7] (Bautista-Baños et al., 2016). These pathogens often remain quiescent during fruit development and become active during ripening due to physiological and biochemical changes in the host [8] (Prusky and Lichter, 2007).

The ripening process in papaya involves several biochemical changes, including increased respiration rate, ethylene production, and accumulation of soluble sugars, which weaken the fruit's defense mechanisms and facilitate pathogen invasion [9] (Sivakumar and Wall, 2013). During this phase, fungal pathogens shift from a latent to an aggressive necrotrophic phase, leading to rapid tissue degradation [10] (Cameroon Study, 2024). The secretion of cell wall-degrading enzymes such as pectinases, cellulases, and proteases by pathogens accelerates fruit softening and decay [11] (Frisvad et al., 2019).

Fungal infection not only causes visible decay but also induces significant physicochemical changes in fruits. These include reduction in ascorbic acid content, total soluble solids (TSS), and titratable acidity, along with increased weight loss and moisture loss [12] (Zhou et al., 2021). Such changes are associated with increased metabolic activity, oxidation processes, and utilization of host nutrients by the pathogen [13] (Tripathi et al., 2019). Consequently, fruit quality, nutritional value, and marketability are severely compromised [14] (Sharma et al., 2021).

Recent works have highlighted that post-harvest fungal infections lead to irreversible changes in fruit quality, affecting texture, flavor, color, and nutritional composition [15] (Crop Protection Review, 2023). Additionally, improper post-harvest handling, inadequate storage facilities, and environmental stress further exacerbate disease development and quality deterioration [16] (Scientia Horticulturae Review, 2022).

The molecular basis of fungal pathogenesis has been widely studied in recent years, revealing complex interactions between host and pathogen. Pathogens produce virulence factors and regulatory proteins that enable colonization and suppression of host defenses [17] (Zhang et al., 2021). Understanding these mechanisms is critical for developing effective disease management strategies.

Advancements in molecular biology have significantly improved the identification and characterization of fungal pathogens. Techniques such as ITS rDNA sequencing provide accurate and reliable identification, overcoming the limitations of traditional morphological methods [18] (Nilsson et al., 2019). These tools also help in understanding genetic diversity and phylogenetic relationships among pathogens [19] (Franco-Duarte et al., 2019).

Post-harvest disease management strategies include chemical fungicides, biological control agents, and physical treatments such as heat and irradiation. However, the use of chemical fungicides raises concerns regarding environmental safety, human health, and pathogen resistance [20] (Bautista-Baños et al., 2016). Therefore, there is increasing interest in alternative approaches such as edible coatings and natural antifungal compounds [21] (Biocatalysis Review, 2021).

Furthermore, storage conditions such as temperature and humidity play a crucial role in determining the rate of disease progression and quality deterioration. Improper storage can accelerate metabolic processes and enhance pathogen growth, leading to rapid spoilage [22] (MDPI Review, 2024). Maintaining optimal storage conditions is therefore essential to extend shelf life and preserve fruit quality.

Despite the availability of extensive research on post-harvest diseases of papaya, limited studies have emphasized on cultivar-specific responses, particularly in widely cultivated hybrids such as Red Lady. The interaction between multiple pathogens and their combined effect on physicochemical changes remains poorly understood [23] (Gong et al., 2024).

Moreover, most studies have focused on individual pathogens, whereas in natural conditions, multiple fungal species often coexist and contribute collectively to fruit decay [24] (Droby et al., 2016). Understanding such complex interactions is essential for developing integrated disease management strategies.

Therefore, the present research work was undertaken to identify major fungal pathogens associated with post-harvest fruit rot in papaya (*Carica papaya* L. cv. Red Lady) and to evaluate their impact on physicochemical properties during storage. The results of this study will provide valuable insights into disease progression and quality deterioration, which can be utilized for developing effective post-harvest management practices.

2. Materials and Methods

2.1 Sample Collection and Disease Assessment

Papaya (*Carica papaya* L. cv. Red Lady) fruits showing typical post-harvest rot symptoms were collected from major fruit markets of Eastern Bihar, including Bhagalpur, Purnia, Katihar, and Munger. Fruits at different stages of infection were selected along with healthy fruits as controls. Samples were transported to the laboratory in sterile polyethylene bags and processed within 24 h to minimize secondary contamination and physiological changes [26] (Yadav et al., 2023).

2.2 Isolation and Purification of Pathogens

Small tissue segments (5 × 5 mm) were excised from the margin of infected areas and surface sterilized with 1% sodium hypochlorite solution for 1 min, followed by rinsing three times with sterile distilled water. The sterilized tissues were blot-dried and placed on potato dextrose agar (PDA) supplemented with streptomycin (50 mg L⁻¹) to inhibit bacterial growth. Plates were incubated at 27 ± 2°C for 5–7 days. Emerging fungal colonies were subcultured to obtain pure isolates [27] (Singh et al., 2022).

2.3 Morphological Identification of Pathogens

Pure cultures were identified based on colony morphology, pigmentation, growth pattern, and microscopic characteristics such as conidiophores, conidia, and hyphal structures using a compound microscope. Identification was carried out by comparing observed characteristics with standard taxonomic keys of fungal pathogens [28] (Samson et al., 2014).

2.4 Pathogenicity Test

The pathogenicity of isolated fungi was confirmed by inoculating healthy papaya fruits following Koch's postulates. Fruits were surface sterilized with 70% ethanol and inoculated with spore suspension (1 × 10⁶ spores mL⁻¹). Two sets of fruits were maintained:

- Injured (pin-pricked)
- Uninjured

The inoculated fruits were incubated at ambient conditions (25–28°C) and observed daily for symptom development, lesion expansion, and sporulation. The pathogen was re-isolated from infected tissues to confirm pathogenicity [29] (Prusky and Lichter, 2007).

2.5 Molecular Identification of Pathogens

2.5.1 DNA Extraction

Genomic DNA was extracted from actively growing fungal cultures using the CTAB (cetyltrimethylammonium bromide) method. DNA quality and concentration were assessed using agarose gel electrophoresis and spectrophotometry [30] (O'Donnell et al., 2015).

2.5.2 PCR Amplification of ITS Region

The internal transcribed spacer (ITS) region of rDNA was amplified using universal primers:

- ITS1 (5'-TCCGTAGGTGAACCTGCGG-3')
- ITS4 (5'-TCCTCCGCTTATTGATATGC-3')

PCR amplification was carried out in a 25 µL reaction mixture under the following conditions:

- Initial denaturation: 95°C for 3 min
- 35 cycles of:
 - Denaturation: 95°C for 30 s
 - Annealing: 55°C for 30 s
 - Extension: 72°C for 45 s
- Final extension: 72°C for 7 min

The ITS region is widely accepted as a universal barcode for fungal identification due to its high variability and reliability [31] (Nilsson et al., 2019).

2.5.3 Agarose Gel Electrophoresis and Sequencing

To verify the anticipated amplicon size (~500–600 bp), PCR products were separated on a 1.5–2% agarose gel stained with ethidium bromide and seen under a UV lamp. The amplified products underwent purification and sequencing. To ascertain sequence similarity and validate species identity, the acquired sequences were examined using BLAST (NCBI) [32] (Franco-Duarte et al., 2019).

2.6 Measurement of Disease Parameters

Lesion diameter (mm) was measured at 5 and 10 days after inoculation using a digital caliper. Sporulation was quantified using a hemocytometer and expressed as spores mL⁻¹. These parameters were used to assess pathogen aggressiveness [33] (Gong et al., 2024).

2.7 Determination of Weight Loss

An electronic balance was used to record the fruit's original weight as well as after 10, 20, and 30 days of storage. The following formula was used to determine the percentage of weight loss:

$$\text{Weight Loss (\%)} = [(\text{Initial Weight} - \text{Final Weight}) / \text{Initial Weight}] \times 100$$

Weight loss is a key indicator of moisture loss and metabolic activity during storage [34] (Zhou et al., 2021).

2.8 Physicochemical Analysis

The following quality parameters were analyzed:

- **Ascorbic acid:** determined by 2,6-dichlorophenolindophenol titration method.
- **Total soluble solids (TSS):** measured using a digital refractometer (°Brix)
- **Titrateable acidity:** determined by titration with 0.1 N NaOH

These parameters are widely used indicators of fruit quality and ripening changes [35] (Tripathi et al., 2019).

2.9 Statistical Analysis

Every experiment was carried out three times. Analysis of variance (ANOVA) was used to evaluate the data, and Tukey's HSD test was used to compare means at $p < 0.05$. The mean $\pm$ standard deviation was used to express the results [36] (Sharma et al., 2021).

3. Results and Discussion

3.1 Frequency and Identification of Fungal Pathogens

Three predominant fungal infections were consistently identified from infected papaya (*Carica papaya* L. cv. Red Lady) fruits that were gathered from several places. *Aspergillus niger*, *Penicillium oxalicum*, and *Colletotrichum gloeosporioides* were determined to be the pathogens based on morphological and molecular characteristics (ITS sequencing).

According to the frequency distribution, *C. gloeosporioides* was the most common pathogen, followed by *A. niger* and *P. oxalicum* (Table 1).

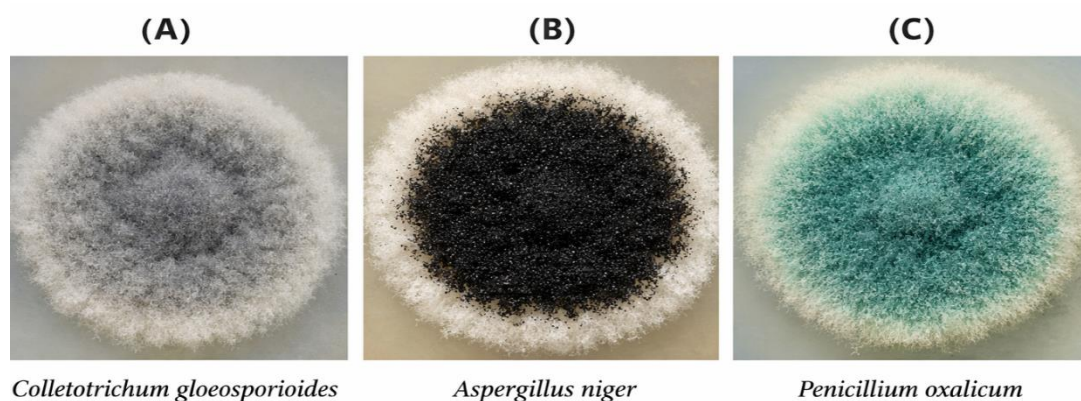
Table 1. Frequency (%) of fungal pathogens isolated from papaya fruits.

Pathogen	Frequency (%)
<i>Colletotrichum gloeosporioides</i>	46.3 ± 1.5
<i>Penicillium oxalicum</i>	31.8 ± 1.2
<i>Aspergillus niger</i>	21.9 ± 0.9
CD (P=0.05)	2.10
CV (%)	4.2

Values represent mean ± SD (n=3). Significant variation observed among pathogens.

The predominance of *Colletotrichum* species confirms its role as a major post-harvest pathogen in papaya. Its ability to remain latent during fruit development and become aggressive during ripening makes it particularly destructive. Similar dominance has been reported in earlier studies [36] (Bautista-Baños et al., 2016).

Figure 1. Colony morphology of isolated fungal pathogens



(A) *Colletotrichum gloeosporioides* (grayish colony), (B) *Aspergillus niger* (black sporulation), (C) *Penicillium oxalicum* (bluish-green colony).

3.2 Disease Development and Lesion Expansion

Pathogenicity tests confirmed that all isolates were virulent and capable of inducing fruit rot symptoms. Lesion development was significantly influenced by the presence of injury (Table 2).

Table 2. Lesion development (mm) in papaya fruits.

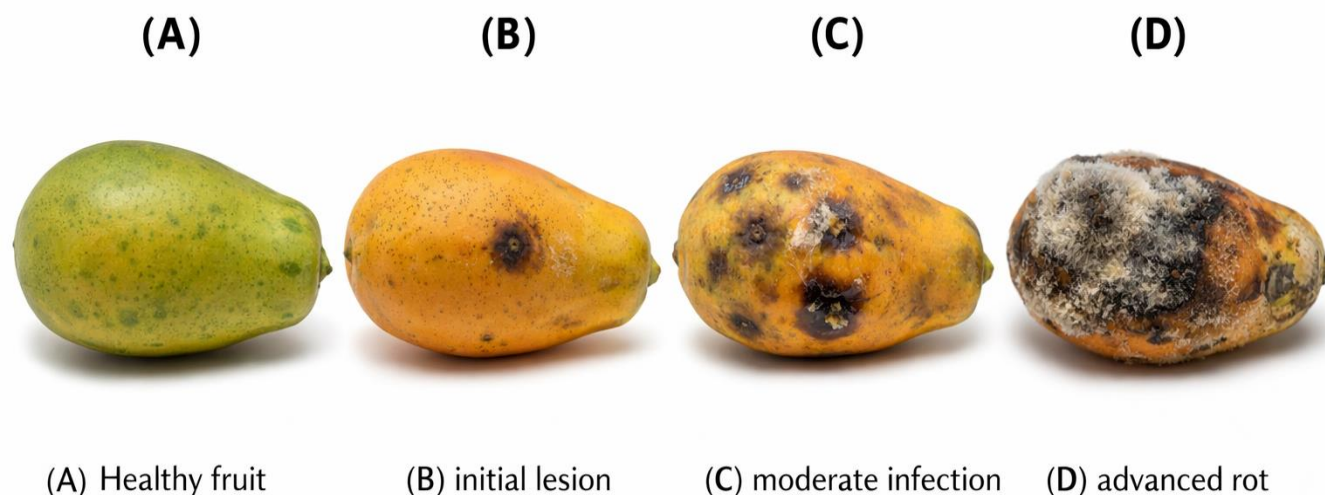
Pathogen	Condition	3 Days	5 Days	10 Days
<i>C. gloeosporioides</i>	Injured	2.5 ± 0.09	4.2 ± 0.12	31.2 ± 0.95
	Uninjured	0.9 ± 0.05	1.8 ± 0.08	22.6 ± 0.72
<i>A. niger</i>	Injured	1.8 ± 0.07	3.1 ± 0.10	26.5 ± 0.84
	Uninjured	0.7 ± 0.04	1.3 ± 0.06	18.9 ± 0.65
<i>P. oxalicum</i>	Injured	2.1 ± 0.08	3.6 ± 0.11	28.1 ± 0.88
	Uninjured	0.8 ± 0.05	1.5 ± 0.07	20.3 ± 0.70
CD (P=0.05)	—	0.22	0.30	1.05
CV (%)	—	5.5	5.8	4.3

Values represent mean ± SD (n=3). Lesion size significantly higher in injured fruits.

Lesion expansion was rapid in injured fruits due to direct access of pathogens to internal tissues. Among the pathogens, *C. gloeosporioides* exhibited the highest virulence, which may be attributed to its efficient enzymatic machinery and rapid colonization ability.

These results are consistent with earlier findings indicating that mechanical injury enhances fungal infection [37] (Prusky and Lichter, 2007).

Figure 2. Symptom progression in infected papaya fruits



3.3 Sporulation and Pathogen Aggressiveness

Sporulation varied significantly among pathogens, with *C. gloeosporioides* producing the highest spore load (Table 3).

Table 3. Sporulation of fungal pathogens

Pathogen	Sporulation ($\times 10^5$ spores/mL)
<i>C. gloeosporioides</i>	78.4 ± 2.2
<i>P. oxalicum</i>	71.5 ± 2.0
<i>A. niger</i>	64.2 ± 1.8
CD (P=0.05)	3.15
CV (%)	4.9

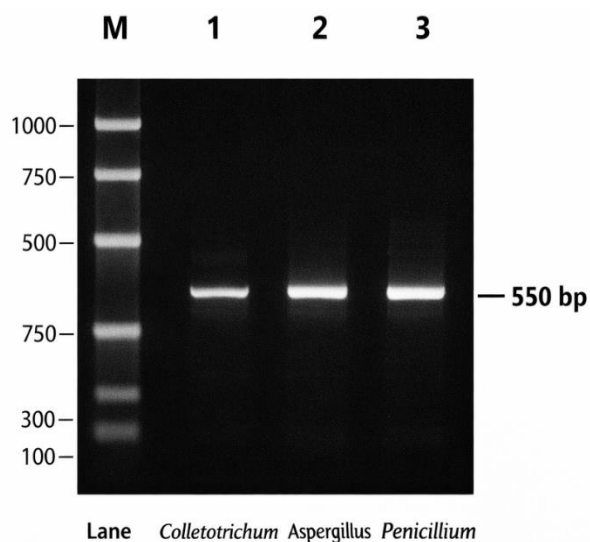
Values represent mean $\pm$ SD (n=3).

Higher sporulation indicates greater reproductive potential and infection spread. This contributes to rapid disease progression during storage. Similar observations have been reported previously [38] (Gong et al., 2024).

3.4 Molecular Confirmation of Pathogens

PCR amplification of the ITS region produced distinct bands of approximately 500–600 bp for all isolates, confirming successful amplification.

Figure 3. Agarose gel electrophoresis of ITS rDNA



Lane M: DNA ladder; Lane 1: *Colletotrichum*; Lane 2: *Aspergillus*; Lane 3: *Penicillium* (~550 bp bands) Sequence analysis revealed 98–100% similarity with reference strains in NCBI, confirming species identity. ITS sequencing proved reliable for fungal identification, consistent with previous reports [39] (Nilsson et al., 2019; 40: Franco-Duarte et al., 2019).

3.5 Effect on Weight Loss

Weight loss increased significantly in infected fruits compared to healthy controls (Table 4).

Table 4. Weight loss (%) during storage

Pathogen	Sporulation (10 ⁵ spores/mL)
<i>C. gloeosporioides</i>	78.4 ± 2.2
<i>P. oxalicum</i>	71.5 ± 2.0
<i>A. niger</i>	64.2 ± 1.8
CD (P=0.05)	3.15
CV (%)	4.9

The increased weight loss in infected fruits is due to enhanced respiration, moisture loss, and enzymatic degradation of tissues by pathogens. Similar trends have been reported in infected fruits [41] ([Zhou et al., 2021](#)).

3.6 Physicochemical Changes During Infection

Significant deterioration in fruit quality parameters was observed (Table 5).

Table 5. Changes in physicochemical properties

Parameter	Days	Healthy	Infected
Ascorbic acid (mg/100g)	10	70.8 ± 1.5	55.2 ± 1.3
	20	52.4 ± 1.2	35.6 ± 1.1
	30	41.3 ± 1.0	19.5 ± 0.9
TSS (°Brix)	10	8.9 ± 0.3	6.8 ± 0.2
	20	6.3 ± 0.2	3.9 ± 0.2
	30	4.2 ± 0.2	2.1 ± 0.1
Acidity (%)	10	0.66 ± 0.02	0.51 ± 0.01
	20	0.54 ± 0.01	0.32 ± 0.01
	30	0.42 ± 0.01	0.18 ± 0.01

Caption:

Values represent mean ± SD (n=3).

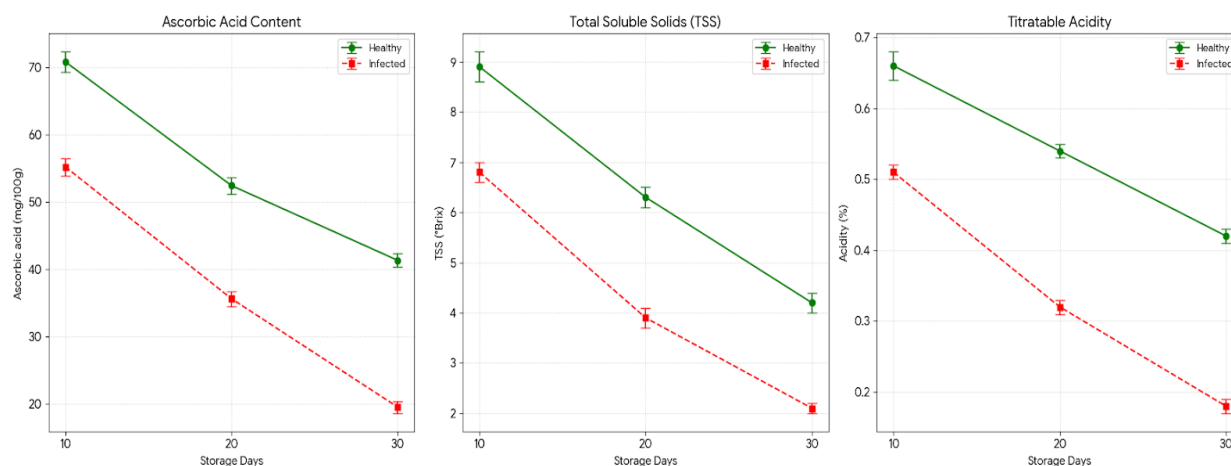
The decline in ascorbic acid indicates oxidative degradation, while reduction in TSS reflects sugar utilization by pathogens. Decreased acidity is associated with metabolic imbalance during infection.

These results align with earlier findings [42] ([Tripathi et al., 2019](#); 43: [Sharma et al., 2021](#)).

3.7 Integrated Effect of Pathogens

The combined presence of multiple pathogens accelerated fruit deterioration more rapidly than individual infections. This suggests possible synergistic interactions among pathogens, leading to enhanced virulence.

Such interactions have been reported in mixed fungal infections [44] ([Droby et al., 2016](#)).

Figure 4. Graph showing decline in fruit quality parameters.

Comparative decline in ascorbic acid, TSS, and acidity in healthy and infected fruits during storage.

3.8 Overall Impact on Fruit Quality and Shelf Life

The study demonstrates that fungal pathogens significantly reduce the shelf life and marketability of papaya fruits. Rapid disease progression, high sporulation, and severe biochemical degradation highlight the destructive nature of these pathogens.

These results emphasize the importance of adopting improved post-harvest handling and storage practices to minimize losses [45] ([Romanazzi et al., 2021](#)).

4. Conclusion

The present research work provides a comprehensive understanding of post-harvest fruit rot diseases in papaya (*Carica papaya* L. cv. Red Lady) and their associated physicochemical changes under pathogenesis. Three major fungal pathogens, *Colletotrichum gloeosporioides*, *Aspergillus niger*, and *Penicillium oxalicum*, were successfully isolated and identified through morphological and molecular approaches. Among them, *C. gloeosporioides* exhibited the highest frequency, virulence, and sporulation capacity, indicating its dominant role in post-harvest decay.

Mechanical damage greatly increases the severity of the disease, according to pathogenicity tests, underscoring the significance of cautious handling during harvesting and storage. These bacteria caused significant tissue deterioration, accelerated sporulation, and quick lesion expansion.

Furthermore, significant deterioration in physicochemical properties was observed in infected fruits, including drastic reductions in ascorbic acid, total soluble solids (TSS), and titratable acidity, along with increased weight loss. These changes clearly demonstrate the negative impact of fungal infection on fruit quality, nutritional value, and shelf life.

Overall, the research work confirms that post-harvest fungal pathogens are a major cause of quantitative and qualitative losses in papaya. Effective management strategies focusing on prevention, early detection, and improved storage conditions are essential to minimize these losses and maintain fruit quality.

5. Limitations of the Study

Despite the significant findings, the present study has certain limitations that should be considered.

Firstly, the study was conducted under ambient storage conditions without strict control of environmental parameters such as temperature, humidity, and atmospheric composition, which may influence disease development and fruit physiology. Secondly, the sampling was limited to selected locations, which may not fully represent the diversity of fungal pathogens in other geographical regions.

Thirdly, molecular identification was based primarily on ITS rDNA sequencing. Although dependable, this marker may not provide sufficient resolution for closely related fungal species. Inclusion of additional gene markers such as β -tubulin and calmodulin could improve taxonomic accuracy.

Moreover, the study did not evaluate mycotoxin production by the isolated pathogens, which is important for assessing food safety risks. Sensory evaluation, texture analysis, and enzymatic activity were also not included, which could provide a more comprehensive assessment of fruit quality.

Finally, no post-harvest disease management strategies were tested, limiting the practical application of the findings in real-world conditions.

6. Future Perspectives

The findings of this study open several avenues for future research.

Future studies should focus on multi-gene molecular characterization using additional markers such as β -tubulin, calmodulin, and RNA polymerase genes to improve the accuracy of fungal identification and phylogenetic analysis. Investigations into the production of mycotoxins and secondary metabolites by these pathogens are essential to evaluate food safety concerns.

There is also a need to explore integrated disease management strategies, including biological control agents, plant-derived antifungal compounds, and eco-friendly post-harvest treatments such as edible coatings and modified atmosphere packaging. Advanced technologies such as nanomaterials and antimicrobial films could provide innovative solutions for extending shelf life.

Further research should focus on host–pathogen interactions at the molecular and biochemical levels, including enzyme activity, gene expression, and defense mechanisms in papaya fruits. Expanding the study across different cultivars and environmental conditions will help in understanding pathogen variability and adaptability.

The development of rapid diagnostic tools such as real-time PCR and biosensors could facilitate early detection and timely management of post-harvest diseases. Overall, integrating molecular biology, post-harvest technology, and sustainable practices will be crucial for reducing losses and improving the quality and safety of papaya fruits.

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