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Study of Pectinase Production of Post-Harvest Fungi in *Carica Papaya* L.

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Abstract

The current study investigated the production and activity of pectinase enzymes by post-harvest fungi isolated from infected *Carica papaya* fruits. Samples were collected from local fruit markets in Chhatrapati Sambhajinagar and Dharashiv across different seasons and ripening stages. Pathogenic fungi were isolated using standard mycological techniques and identified based on morphological characteristics. Pectinase production was evaluated using Oswald's viscometer, with enzyme activity measured as the percentage loss in viscosity of a pectin solution. The influence of multiple physicochemical factors carbon and nitrogen sources, phosphorus and sulfur compounds, trace elements, amino acids, antibiotics, fungicides, and environmental parameters on pectinase activity was systematically examined. Among the isolates, *Aspergillus niger, Aspergillus flavus,* and *Penicillium digitatum* exhibited the highest pectinase activity under optimized conditions. Pectin-rich media significantly enhanced enzyme production compared to non-substrate controls. Sucrose and starch emerged as the most effective carbon sources, while peptone and sodium nitrate supported strong enzyme synthesis. Magnesium sulfate and potassium dihydrogen phosphate proved favorable among sulfur and phosphorus sources, respectively. Manganese and amino acids like aspartic acid and methionine stimulated enzyme production, whereas zinc and certain antibiotics and fungicides suppressed it. Optimal enzyme activity was achieved at 30 °C, pH 6.0–6.5, and with continuous light exposure for 10–15 days. The findings highlighted the critical role of environmental and nutritional factors in modulating pectinase production and offered insights for managing post-harvest fungal spoilage in papaya.

Keywords

Carica papaya, fungi, Oswald's viscometer, pectinase, post-harvest.

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1. INTRODUCTION

Post-harvest losses in papaya (*Carica papaya* L.) are a significant concern, with fungal pathogens being primary contributors to fruit spoilage (Tan *et al.*, 2022). These fungi secrete cell wall-degrading enzymes, notably pectinases, which break down pectin, a major component of the plant cell wall, leading to tissue maceration and decay (Kubicek *et al.*, 2014). Understanding the production and activity of pectinases by these pathogens is crucial for developing effective post-harvest disease management strategies.

Pectinases, including polygalacturonases, pectin lyases, and pectin methylesterases, facilitate the invasion and colonization of host tissues by degrading pectic substances (Prade *et al.*, 1999). Their activity is influenced by various factors, including the type of carbon and nitrogen sources, pH, temperature, and the presence of metal ions and other nutrients. For instance, studies have shown that carbon sources like sucrose and starch can enhance pectinase production in certain fungal species, while others may respond differently depending on the substrate availability (Solís-Pereira *et al.*, 1993).

Existing control measures for post-harvest fungal diseases in papaya include chemical fungicides, refrigeration, and modified atmosphere storage (Singh, 2010). However, these methods have limitations, such as the development of fungicide resistance, environmental concerns, and cost implications. Biological control and the use of natural antifungal compounds are emerging as sustainable alternatives, but their efficacy can be inconsistent due to the complex interactions between pathogens and host tissues.

The main limitation in current management practices is the lack of targeted approaches that consider the specific enzymatic activities of the pathogens involved (El-Baky & Amara, 2021). By focusing on the conditions that favor pectinase production, it is possible to develop strategies that inhibit these enzymes, thereby reducing the pathogenicity of the fungi.

The present study aimed to investigate the pectinase activity and production by pathogenic post-harvest fungi isolated from papaya fruits. By analyzing the influence of various physicochemical factors on enzyme production, the research seeks to identify optimal conditions that promote or inhibit pectinase activity. The findings are expected to contribute to the development of targeted post-harvest treatments that mitigate fruit spoilage by interfering with the enzymatic mechanisms of fungal pathogens.

2. MATERIAL AND METHODS

2.1. Collection samples

Infected *Carica papaya* fruits were collected primarily from fruit markets in Chhatrapati Sambhajinagar and Dharashiv during different seasons and ripening stages. Due to difficulty in identifying infections before fruiting in commercial fields, visibly infected fruits at harvest and market stages were prioritized. These samples were used for both fungal and bacterial isolation, as well as for studying fungal occurrence.

2.2. Isolation and identification of fungi

Infected tissue sections (2 mm^2) from *Carica papaya* fruits were excised using a sterile scalpel and transferred onto potato dextrose agar (PDA) plates. These were incubated at 28 °C for seven days, and emerging fungal colonies were observed daily. Pure isolates were obtained using the single spore technique (Leyronas *et al.*, 2012) and maintained on PDA slants. The isolates were identified based on morphological basis like colony characters, spore formation, mycelial and conidial structures.

2.3. Production of pectinase and physicochemical factors affecting its activity

Pectinase production was carried out by cultivating fungal isolates in a liquid medium containing 1% pectin, 0.25% KNO_3, 0.1% KH_2PO_4, and 0.05% $MgSO_4 \cdot 7H_2O$ at pH 5.0, following the method described by Kamble *et al.* (2019). A total of 25 mL of this medium was dispensed into 100 mL conical flasks, autoclaved, and inoculated with 1 mL of a standardized fungal spore or mycelial suspension derived from 7-day-old PDA cultures. The flasks were incubated for six days at 25 ± 1 °C under diurnal light conditions. On the seventh day, the cultures were filtered, and the filtrate was collected as the crude enzyme extract. Pectinase activity was assayed using Oswald's viscometer by measuring the reduction in viscosity of a pectin solution over time at 25 °C (Papdiwal, 1982).

Pectinase activity was estimated by measuring the decrease in viscosity of a pectin solution using an Oswald's viscometer. A reaction mixture containing 6 mL of 1% pectin dissolved in 2 mL of 0.2 M acetate buffer (pH 5.2) and 4 mL of the crude enzyme extract was incubated at 25 °C. The efflux time was recorded at regular intervals (0, 5, 10, 20, 30, and 40 minutes) using a stopwatch. The percentage loss of viscosity was calculated using the following formula:

$$\text{Percent loss of viscosity} = \left(\frac{\text{To} - \text{Tx}}{\text{To} - \text{Tw}}\right) * 100$$

Where, To - Flow time in seconds at zero time

Tx - Flow time of the reaction mixture at the time 'T"

Tw, - Flow time of distilled water.

To evaluate the influence of physicochemical factors on pectinase production, various parameters were tested individually by modifying the basal pectin medium. The effects of different carbon sources (glucose, fructose, xylose, sucrose, maltose, and starch) were compared to CMC as control, while nitrogen sources including inorganic (e.g., potassium nitrate, sodium nitrate, sodium nitrite, ammonium salts), organic (peptone, casein, gelatin), and amide

forms (urea) were tested with potassium nitrate as control. Similarly, the impact of various phosphorus sources (e.g., sodium and potassium phosphate salts) and sulphur sources (e.g., ammonium, magnesium, ferrous, and zinc sulphates) on enzyme production was analyzed. Trace elements (100 ppm), including iron, manganese, copper, barium, and zinc, were tested, using a trace element-free medium as control. The influence of amino acids (e.g., alanine, threonine, methionine, arginine, and aspartic acid), antibiotics (e.g., ampicillin, streptomycin, doxycycline), and fungicides (e.g., captan, benomyl, dithane-M45) was also evaluated. Additionally, environmental conditions such as light regimes (light, dark, alternating), varying incubation periods (5–20 days), temperatures (10–40 °C), and pH ranges (3.0–8.5) were assessed to determine optimal conditions for maximum pectinase production. The statistical significance between the treatments was analysed by one way ANOVA Tukey's HSD post hoc analysis at 0.05 level of significance.

3. RESULTS

3.1. Identification of fungi

The identification of pathogenic fungal isolates was initially carried out based on morphological characteristics, including colony morphology, pigmentation, hyphal structure, and spore morphology. Distinct conidial and hyphal features enabled the preliminary identification of isolates as *Alternaria alternata, Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Colletotrichum gloeosporioides, Curvularia lunata, Fusarium equiseti, Fusarium moniliforme, Fusarium oxysporum, Penicillium digitatum, Penicillium islandicum,* and *Rhizopus stolonifer*.

3.2. Production of pectinase and its activity

3.2.1. Effect of substrate and non-substrate

Among the tested isolates, *A. niger* showed the highest pectinase activity on pectin substrate with 58 ± 0.33% viscosity loss, followed closely by *A. flavus* and *A. alternata*. The lowest activity on substrate media was observed in *R. stolonifer* and *F. equiseti* (43 ± 0.33%). On non-substrate GN media, *P. digitatum* exhibited the highest activity (45 ± 0%), while *A. niger*, *C. lunata*, and *R. stolonifer* showed the lowest (41 ± 0–0.33%) (Figure 1).

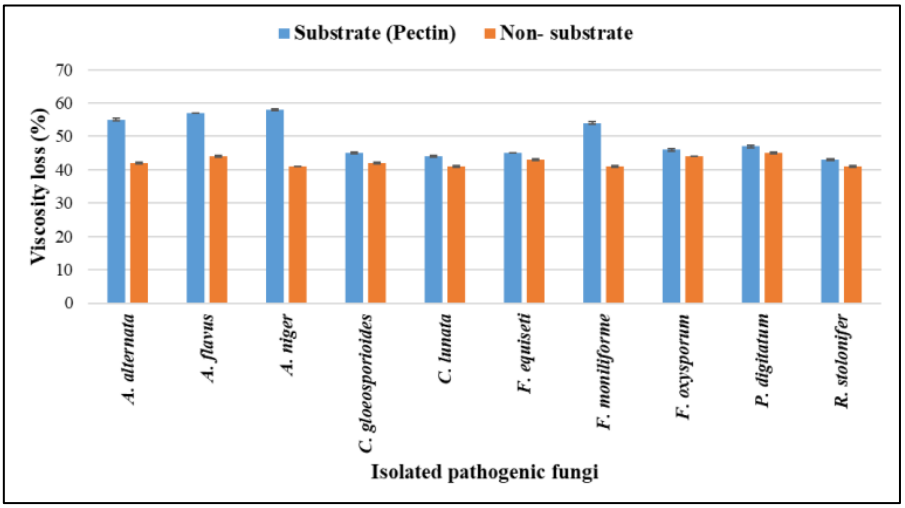

Figure 1 Effect of substrate and non-substrate on the pectinase activity of post-harvest fungi in papaya

3.2.2. Effect of nutritional parameters

a. Carbohydrate source

Among the carbohydrate sources, starch supported the highest pectinase activity, with *C. gloeosporioides, F. oxysporum,* and *P. digitatum* showing 61 ± 0.33% activity. *A. niger* also showed high activity on sucrose (60.33 ± 0%). In contrast, *A. alternata* exhibited the lowest activity on starch (35 ± 0.33%) and CMC. Overall, starch, sucrose, and glucose were the most favorable carbohydrates for pectinase production across fungi (Figure 2-A).

b. Nitrogen source

Among all nitrogen sources, peptone supported the highest pectinase production, with *P. digitatum* showing 70 ± 0.33% viscosity loss, the highest across all treatments. *A. niger* showed maximum activity on sodium nitrate (69 ± 0.33%), while urea resulted in the lowest mean activity (44.4%), with *P. digitatum* performing poorly (19 ± 0.33%). Gelatin also showed low activity, with *R. stolonifer* being the best performer (58 ± 0.33%) and *P. digitatum* again showing the least. Potassium nitrate and ammonium

sulphate produced statistically similar pectinase levels (Figure 2-B).

c. Phosphorous source

Among the phosphorus sources tested, *F. oxysporum* exhibited the highest pectinase activity on ammonium phosphate (59 ± 0.33%) and ammonium bisphosphate (57 ± 0.33%). *P. digitatum* consistently showed strong activity across sources, with the highest on potassium dihydrogen phosphate (59 ± 0.33%). In contrast, the lowest pectinase activity was observed in *C. lunata* (41 ± 0.33%) on potassium dihydrogen phosphate and *F. equiseti* on ammonium bisphosphate. All phosphorus sources significantly influenced pectinase production, as indicated by Tukey's HSD test (Figure 2-C).

d. Sulphur source

Among the sulphur sources tested, *R. stolonifer* exhibited the highest pectinase activity with sodium thiosulphate (59 ± 0%) even higher than the control (magnesium sulphate, 54 ± 0.33%). In contrast, *A. alternata* showed the lowest activity with sodium sulphate (39 ± 0.33%). *A. niger* performed best on sodium sulphate (54 ± 0.33%), while zinc sulphate generally delayed enzyme activity across fungi. Sodium thiosulphate, ammonium sulphate, and potassium sulphate showed comparable activity to the control, whereas zinc, sodium, and ferrous sulphate resulted in reduced pectinase production across most isolates (Figure 2-D).

e. Trace elements

Among the trace elements tested, manganese supported the highest mean pectinase activity (48.5%), with *C. lunata* showing the peak value of 57 ± 0.33%. In contrast, zinc resulted in the lowest overall activity (mean 37.6%), with *C. gloeosporioides* showing the least (27 ± 0.33%). Copper and barium had similar mean activities (40.5%), with *A. alternata* showing the highest value on barium (54 ± 0.33%) and *P. digitatum* showing the lowest on copper (29 ± 0.33%). The control group exhibited consistently higher pectinase activity across all fungi (49 ± 0.33% to 57 ± 0.33%), indicating that the addition of trace elements did not enhance enzyme production beyond baseline levels (Figure 2-E).

f. Amino acids

Among the amino acids tested, aspartic acid and methionine supported the highest pectinase production with a mean activity of 54.1%, where *P. digitatum* showed peak activity (57 ± 0–0.33%) in both treatments. In contrast, alanine resulted in the lowest activity, with *C. lunata* showing just 44 ± 0.33%. Arginine monohydrochloride led to uniformly low pectinase levels (47–49 ± 0.33%) across all fungi. Threonine showed moderate activity with *C. gloeosporioides*, *F. oxysporum,* and *P. digitatum* at 53 ± 0.33%, while *R. stolonifer* had the lowest (47 ± 0.33%). Overall, aspartic acid and methionine enhanced pectinase production significantly more than the control (Figure 2-F).

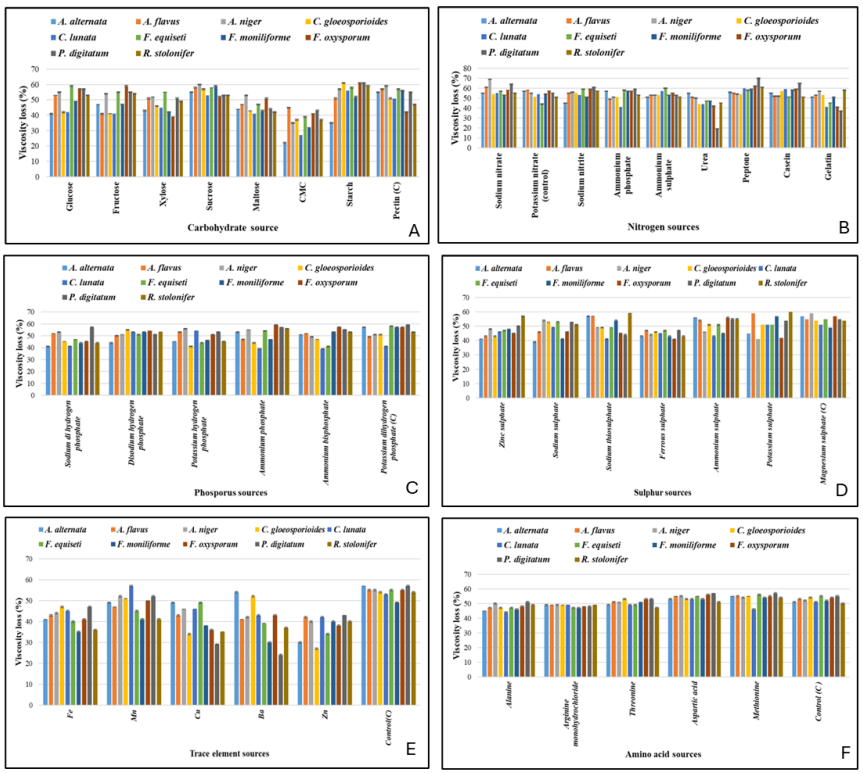

Figure 2 Effect of different nutritional parameters on the pectinase activity of post-harvest fungi in papaya

3.2.3. Effect of antibiotics and fungicides

Among the antibiotics tested, *A. flavus* and *C. lunata* showed the highest pectinase activity (57 ± 0.33%) with streptomycin, while ampicillin severely inhibited activity across all fungi, with *A. alternata* showing the lowest (41 ± 0.33%). Overall, antibiotic treatments reduced pectinase activity compared to the control (mean 54.3%) (Figure 3-A). In fungicide treatments, all compounds significantly suppressed pectinase production, with *C. gloeosporioides* showing complete inhibition under all fungicides. Captan allowed limited activity in a few fungi, while Dithane Z-78 caused the most suppression, reducing activity to as low as 22 ± 0.33% (Figure 3-B). Controls in both sets showed the highest pectinase levels, confirming the inhibitory effect of these agents.

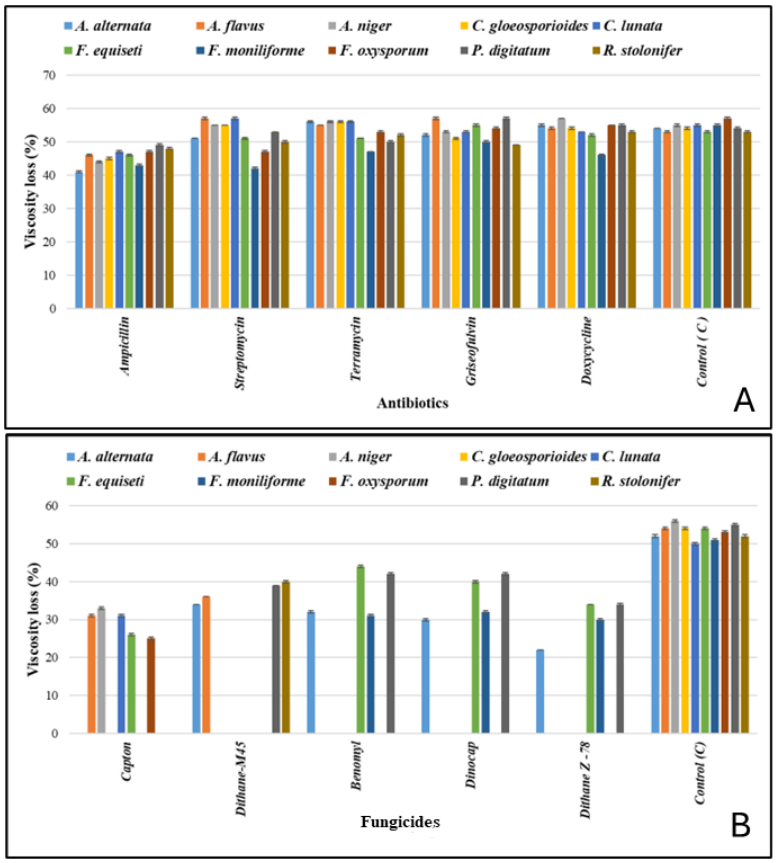

Figure 3 Effect of antibiotics and fungicides on the pectinase activity of post-harvest fungi in papaya

3.2.4. Effect of physical factors

Among the illumination regimes, continuous light significantly enhanced pectinase production in all fungi, with *R. stolonifer* showing the highest activity (55 ± 0.33%), while continuous darkness resulted in the lowest activity, particularly in *A. alternata* and *C. lunata* (39 ± 0.33%) (

Figure 4-A). Alternating light and dark conditions produced moderate enzyme levels. Regarding incubation period, maximum pectinase activity was observed at 10 days (mean 48.3%), with *A. flavus* reaching 55 ± 0.33%, and this activity remained stable through day 15. A decline was noted at 20 days (mean 41.2%), indicating the onset of enzyme degradation or nutrient limitation (

Figure 4-D).

Figure 4-B).

Pectinase production by post-harvest fungi was significantly influenced by temperature and pH. Optimal enzyme activity was observed at 30 °C, with *A. flavus* and *A. alternata* showing the highest viscosity loss (61 ± 0.33%). Activity declined at 35 °C and was lowest at 10 °C, where most fungi were inactive (

Figure 4-C). Regarding pH, maximum pectinase production occurred at pH 6.0–6.5, where *A. niger, F. oxysporum,* and *P. digitatum* achieved peak activity (60 ± 0.33%). Extreme acidic (pH 3.5–4.0) and basic conditions (pH 8.0–8.5) suppressed enzyme synthesis, with most fungi exhibiting significantly reduced activity (

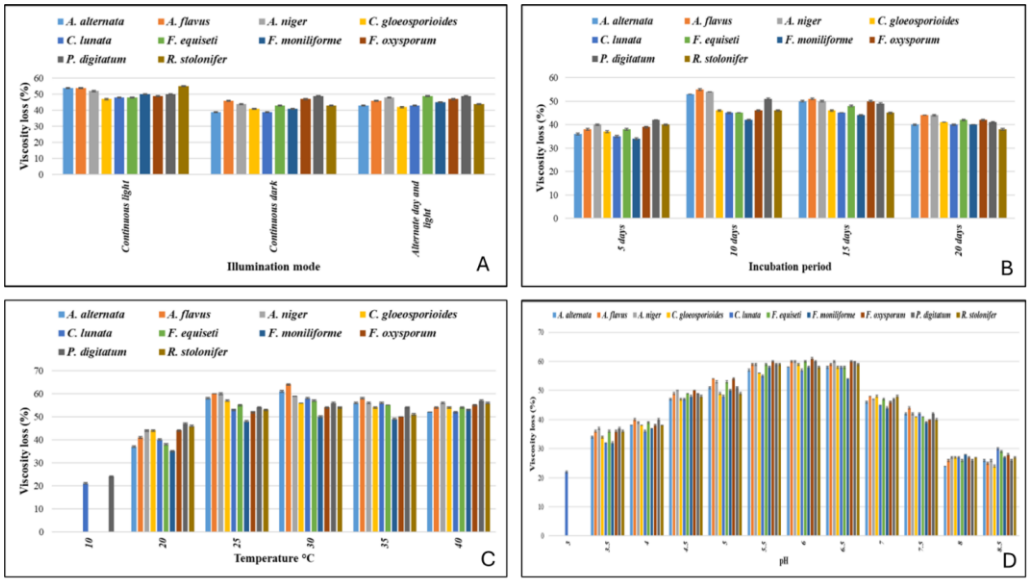

Figure 4 Effect of different physical parameters on the pectinase activity of post-harvest fungi in papaya

4. DISCUSSION

Pectinase plays a crucial role in the pathogenicity of post-harvest fungi, as it facilitates the degradation of plant cell wall pectin, enabling tissue invasion and spoilage. Understanding the nutritional and environmental factors influencing pectinase production is essential for developing targeted strategies to manage fungal infections in stored fruits. The current study showed that pectin-containing substrate media significantly enhanced pectinase production in most fungi, especially *A. niger*, compared to non-substrate GN media. These results align with findings by Osagie and Obuekwe (1991) and Kalra and Tandon (1995), who also reported increased hydrolytic enzyme production in substrate-rich environments. Among carbon sources, sucrose proved to be the most effective for enzyme induction, followed closely by starch, while CMC showed the least activity, consistent with observations by Begum and Munjam (2021) and Rajmane and Korekar (2012). Nitrogen sources such as peptone supported high pectinase production in *P. digitatum*, while urea was the least effective, in agreement with Batool *et al.* (2013) and Purnachandra Reddy and Saritha (2015).

Inorganic phosphorus and sulfur sources also influenced enzyme activity. Potassium dihydrogen phosphate and magnesium sulfate supported higher pectinase activity, while ferrous and zinc sulfates suppressed it, in accordance with the findings of Gadgile and Chavan (2009). Trace elements such as Mn enhanced enzyme production, but others like Cu and Ba inhibited it, possibly due to supra-optimal concentrations, a concern also highlighted by Mukherjee and Majumdar (1974). Among amino acids, aspartic acid and methionine stimulated enzyme synthesis, while alanine and arginine were inhibitory, supporting observations by Shrestha *et al.* (2023). The study also revealed that antibiotics like ampicillin significantly suppressed pectinase activity, while doxycycline and griseofulvin had minimal inhibitory effects, consistent with reports by Mehta *et al.* (1993) and Rathod and Chavan (2010).

Physical factors played a pivotal role in optimizing enzyme production. Continuous light exposure enhanced pectinase synthesis in all fungi, while continuous darkness and alternating light-dark regimes reduced activity. The optimal incubation period was between 10–15 days, beyond which enzyme activity plateaued or declined. The most favorable temperature for maximum enzyme production was 30 °C, with reduced activity at 10 °C and 40 °C. These results corroborate earlier studies by Rathod and Chavan (2013), which demonstrated that light, incubation duration, temperature, and pH significantly influence hydrolytic enzyme production in post-harvest fungi. Overall, the findings highlight the need for tailored media formulations and controlled environmental conditions to regulate fungal enzyme activity in post-harvest disease management.

5. CONCLUSION

This study investigated the pectinase activity of post-harvest fungi isolated from *Carica papaya* fruits collected in Chhatrapati Sambhajinagar and Dharashiv. The research aimed to understand how various nutritional and environmental factors influence pectinase production, which is crucial for the pathogenicity of these fungi. Optimal pectinase

activity was observed at 30 °C and pH 6.0–6.5, with *A. flavus, A. alternata,* and *P. digitatum* showing the highest enzyme activity. Pectin-containing media significantly enhanced pectinase production compared to non-substrate media. Sucrose and starch were the most effective carbon sources, while peptone and sodium nitrate served as favorable nitrogen sources. Among phosphorus sources, potassium dihydrogen phosphate supported higher enzyme activity, and magnesium sulfate was the most effective sulfur source. Manganese as a trace element enhanced pectinase production, whereas copper and barium were inhibitory. Aspartic acid and methionine stimulated enzyme synthesis, while alanine and arginine were less effective. Continuous light exposure and an incubation period of 10–15 days were optimal for enzyme production. Antibiotics like ampicillin and fungicides such as Dithane Z-78 significantly suppressed pectinase activity. These results provided insights into the conditions that favor or inhibit pectinase production in post-harvest fungi, which is essential for developing strategies to manage post-harvest diseases in papaya fruits.

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