

Role of Cell Wall–Degrading Enzymes of *Paradendryphiella arenariae*, a Novel Pathogen in Fruit Rot Disease of Pomegranate

Research Article

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Abstract

Pomegranate (*Punica granatum* L.) is highly susceptible to fungal fruit rot, resulting in substantial yield and postharvest losses worldwide. Disease progression is largely mediated by extracellular cell wall–degrading enzymes (CWDEs), which facilitate host tissue maceration and pathogen invasion. In the present study, *Paradendryphiella arenariae*, isolated from naturally infected pomegranate fruits, was investigated for its CWDE production potential and represents the first report of this fungus associated with pomegranate fruit rot. Enzyme production was carried out under submerged fermentation using a pomegranate peel–based medium to simulate host-derived substrates. Qualitative plate assays confirmed the secretion of cellulase, xylanase, pectinase, lipase, laccase and proteases. Quantitative analysis revealed peak enzyme activities at day 14, with cellulase (11.8 U/mL), β -glucosidase (14.6 U/mL), xylanase (10.0 U/mL), and pectinase (9.6 U/mL). SDS–PAGE profiling demonstrated multiple extracellular proteins in the range of 17–75 kDa, indicating a complex enzyme system. The predominance of pectinolytic, cellulolytic, and hemicellulolytic activities suggests a coordinated mechanism for degradation of fruit cell wall polysaccharides. These findings provide mechanistic insight into CWDE-mediated tissue disintegration during infection and a basis for developing targeted management strategies to mitigate fungal fruit rot and reduce pre- and postharvest losses in pomegranate.

Keywords: Cell Wall Degrading Enzymes; *Paradendryphiella arenariae*; Cellulase; B-Glucosidase; Xylanase; Pectinase; Hemicellulose; Laccase

Introduction

Fruit rot diseases caused by fungal pathogens represent a major constraint on pomegranate (*Punica granatum* L.) production, resulting in significant pre- and post-harvest losses that diminish market value. These infections are caused by a host of fungal species belonging to *Alternaria*, *Aspergillus*, *Beltaraniella*, *Cercospora*, *Cladosporium*, *Colletotrichum*, *Curvularia*, *Fusarium*, *Phomopsis*, *Phytophthora*, *Rhizopus*, etc. The infection process in fruit rot pathogens largely depends on their ability to breach host structural barriers, particularly the plant cell wall, which serves as the first line of defence against microbial invasion. Successful colonization of fruit

tissues is strongly associated with the secretion of fungal cell wall-degrading enzymes (CWDEs), which facilitate tissue maceration [1,2], nutrient acquisition, and pathogen spread within the host.

The pomegranate fruit pericarp and aril tissues are rich in complex polysaccharides such as cellulose, hemicellulose, and pectin, along with phenolic compounds that contribute to host resistance. The fruit-rot-causing fungi secrete a coordinated set of hydrolytic enzymes, including cellulases, β -glucosidase, xylanases, pectinases and proteases, which degrade the structural polysaccharides of the host cell wall and overcome the barriers. Among these, pectinases play a particularly critical role in fruit rot development by depolymerizing

pectin in the middle lamella, leading to cell separation, tissue softening [3,4] and rapid fruit decay.

In addition to polysaccharide-degrading enzymes, oxidative enzymes such as laccases contribute to the pathogenic process by modifying lignin-like components, detoxifying host-derived phenolic compounds [5,6] and facilitating fungal survival in hostile host environments. The combined action of hydrolytic and oxidative CWDEs enhances the virulence of fruit rot pathogens and determines the severity and progression of disease symptoms.

Despite extensive reports on fungal fruit rot pathogens affecting pomegranate, information regarding the enzymatic mechanism employed by *P. arenariae* during host infection is scarce. This dematiaceous fungus has been previously reported from marine and terrestrial environments, with limited evidence of its pathogenicity in economically important fruit crops. In the present study, *P. arenariae* is reported for the first time as a causal agent of natural pomegranate fruit rot. Understanding the enzymatic profile of *P. arenariae* and its role in pomegranate fruit rot provides valuable insights into the mechanisms of host-pathogen interaction and disease development. Such information is essential for elucidating the virulence attributes of newly emerging fruit rot pathogens and may contribute to the development of effective disease management and postharvest control strategies. Hence, in this report we characterize the key CWDEs and their role in pathogenesis in fruit rot disease.

Materials and Methods

Fungal isolate

The fungal isolate *Paradendryphiella arenariae* used in the present study was previously isolated from naturally infected pomegranate fruits collected from Srinivaspura Taluk, Karnataka, and identified based on detailed morphological and molecular characterisation in our earlier report [7]. The culture was maintained on Potato Dextrose Agar (PDA) slants at 4 °C and subcultured periodically to ensure viability. The previously characterised isolate was used for subsequent submerged fermentation, qualitative and quantitative enzyme production studies.

Qualitative Enzyme profile

Cellulase: Cellulase activity was screened using carboxymethyl cellulose (CMC) agar following the Congo red plate assay method [8,9]. The medium contained (g L⁻¹): NaNO₃, 2.0; KH₂PO₄, 1.0; KCl, 0.5; MgSO₄·7H₂O, 0.5; FeSO₄·7H₂O, 0.01; agar, 15.0; and carboxymethyl cellulose (CMC), 10.0 as the sole carbon source. Plates were inoculated with the fungal culture and incubated at 28 °C for 7 days. After incubation, plates were flooded with 0.1% Congo red for 15 min and destained with 1 M NaCl for 10 min. Formation of clear hydrolysis zones around the colony indicated cellulase activity. The cellulolytic index (CI) was calculated as the ratio of hydrolysis zone diameter to colony diameter.

Xylanase: Xylanase activity was determined using xylan agar media [10]. The medium contained (g L⁻¹): NaCl (0.5), KH₂PO₄ (1.0), NH₄NO₃ (0.3), MgSO₄·7H₂O (0.5), FeSO₄·7H₂O (0.01), MnSO₄·H₂O (0.01), agar (20.0), and beechwood xylan (10.0) as the sole carbon source. Plates were inoculated with fungal cultures and incubated

at 28 °C for 7 days. After incubation, plates were flooded with 0.1% Congo red solution for 15 min and destained with 1 M NaCl for 10–15 min. The appearance of clear hydrolysis zones surrounding fungal colonies indicated xylanase activity.

Pectinase: Pectinase activity was qualitatively screened on pectin agar [11,12] media. The medium contained (g L⁻¹): NaNO₃ (1.0), KCl (1.0), K₂HPO₄ (1.0), MgSO₄·7H₂O (0.5), yeast extract (0.5), citrus pectin (10.0), and agar (20.0), with the pH adjusted to 7.0 before sterilisation. Plates were inoculated with 5 mm diameter fungal mycelial discs and incubated at 28 °C for 7 days. After incubation, plates were flooded with 0.1% Gram's iodine solution for 5 min and rinsed gently with distilled water. The formation of clear hydrolysis zones surrounding the fungal colonies indicated pectinolytic activity.

Lipase: Lipase activity was qualitatively screened using tributyrin agar media [13]. The medium contained (g L⁻¹): peptone (5.0), yeast extract (3.0), NaCl (5.0), agar (15.0), and tributyrin (10 mL L⁻¹; 1% v/v). The pH of the medium was adjusted to 7.0 before sterilisation. Plates were centrally inoculated with 5 mm diameter fungal mycelial discs and incubated at 28 °C for 5–7 days. The formation of clear hydrolysis zones surrounding fungal colonies was recorded as a positive indication of extracellular lipase activity.

Amylase: Amylolytic activity was qualitatively screened on starch agar media [14]. The medium contained (g L⁻¹): peptone (5.0), yeast extract (3.0), NaCl (5.0), soluble starch (10.0), and agar (15.0). The pH of the medium was adjusted to 7.0 before sterilisation. Plates were centrally inoculated with 5 mm diameter fungal mycelial discs and incubated at 28 °C for 7 days. After incubation, the plates were flooded with Gram's iodine solution for 5 min and rinsed gently with distilled water. The formation of clear hydrolysis zones surrounding the fungal colonies against a dark blue background was recorded as a positive indication of extracellular amylase activity.

Quantitative enzyme production

The quantitative enzyme production was carried out using submerged fermentation in an agro-residue-based medium [15] with slight modifications. One hundred millilitres of distilled water was dispensed into 250 mL Erlenmeyer flasks and sterilised by autoclaving at 121 °C for 15 min. After cooling to room temperature under aseptic conditions, healthy pomegranate peels (10%, w/v), thoroughly washed with double-distilled water and surface sterilised with 70% ethanol, were aseptically added to each flask to serve as the sole carbon source. The pH of the medium was adjusted to 7.0. Mycelial discs (5 mm diameter) obtained from a 7-day-old potato dextrose agar culture of *P. arenariae* were used as inoculum. The inoculated flasks were incubated at 28 °C on an orbital shaker at 120 rpm. Uninoculated flasks served as controls. Samples were collected at 7, 14, 21, and 28 days to evaluate enzyme production dynamics.

Preparation of crude enzyme extract

At each sampling interval, cultures were filtered through Whatman No. 1 filter paper to remove mycelial biomass. The filtrate was centrifuged at 10,000 × g for 15 min at 4 °C, and the resulting supernatant was used as the crude enzyme extract for subsequent assays.

Protein determination

Total extracellular protein concentration was determined by the Lowry protein assay using bovine serum albumin as a standard. 1.0 ml of appropriately diluted crude supernatant and 5ml of alkaline copper reagent were incubated for 10 min, followed by the addition of diluted Folin–Ciocalteu reagent. The absorbance was measured at 660 nm using a UV–VIS spectrophotometer after incubation in the dark for 20 min at room temperature. Protein concentration was calculated from a BSA standard curve and expressed as mg/100 ml [16].

Enzyme assays

The crude enzyme extract was used for the quantitative estimation of cellulase, β -glucosidase, pectinase, xylanase, protease, and laccase activities. The production of these enzymes is associated with the degradation of plant cell wall components, particularly cellulose, hemicellulose, and pectin [17,18].

Cellulase: Cellulase activity was determined using 1% (w/v) carboxymethyl cellulose (HiMedia Laboratories, Mumbai, India) in 50 mM citrate buffer (pH 5.0). The reaction mixture (1 mL substrate + 1 mL enzyme) was incubated at 50 °C for 30 min. The reaction was terminated by adding 2 mL dinitrosalicylic acid (DNS) reagent prepared according to Miller (1959), followed by boiling for 5 min. Absorbance was measured at 540 nm using a UV–VIS spectrophotometer. Reducing sugars were quantified using a glucose standard curve. Appropriate enzyme and substrate blanks were included. One unit (U) of cellulase activity was defined as the amount of enzyme liberating 1 μ mol glucose min^{-1} , expressed as U mL^{-1} [19–20].

β -Glucosidase: β -Glucosidase activity was assayed using 5 mM p-nitrophenyl- β -D-glucopyranoside in 50 mM phosphate–citrate buffer (pH 5.0). The reaction mixture (1.5 mL substrate + 0.5 mL enzyme) was incubated at 35 °C for 30 min and terminated by adding 1 mL of 1 M Na_2CO_3 . Absorbance was measured at 405 nm. The released p-nitro phenol was quantified using a standard curve. Blanks were included for correction. One unit (U) was defined as 1 μ mol pNP released min^{-1} and expressed as U mL^{-1} [21].

Xylanase: Xylanase activity was measured using 1% (w/v) beechwood xylan (TCI Chemicals, Tokyo, Japan) in 50 mM sodium phosphate buffer (pH 5.0). The reaction mixture (1.5 mL substrate + 0.5 mL enzyme) was incubated at 45 °C for 15 min. The reaction was stopped with 2 mL DNS reagent (Miller, 1959) and boiled for 5 min. Absorbance was recorded at 540 nm. Reducing sugars were quantified using xylose standard. One unit (U) corresponded to 1 μ mol xylose released min^{-1} and was expressed as U mL^{-1} [22, 23].

Pectinase (polygalacturonase): Pectinase activity was determined using 0.5% (w/v) citrus pectin (HiMedia Laboratories, Mumbai, India) in 50 mM sodium acetate buffer (pH 5.5). The reaction mixture (1 mL substrate + 1 mL enzyme) was incubated at 50 °C for 30 min. The reaction was terminated with 2 mL DNS reagent (Miller, 1959) and boiled for 10 min. Absorbance was measured at 540 nm. Galacturonic acid was used as a standard. One unit (U) was defined as 1 μ mol product released min^{-1} and expressed as U mL^{-1} [23,24].

Protease: Protease activity was assayed using 0.65% casein as substrate. The reaction mixture was incubated at 37 °C for 30 min and terminated with trichloroacetic acid. The soluble peptides were quantified using the Folin–Ciocalteu method, and absorbance was measured at 660 nm. Tyrosine was used as standard. One unit (U) of protease activity was defined as 1 μ mol tyrosine released min^{-1} and expressed as U mL^{-1} [25].

Laccase: Laccase activity was determined using 1 mM ABTS (Sigma-Aldrich, St. Louis, USA) in 100 mM sodium acetate buffer (pH 4.5). The reaction mixture (0.9 mL substrate + 0.1 mL enzyme) was monitored at 420 nm. Activity was calculated using an extinction coefficient ($\epsilon_{420} = 36,000 \text{ M}^{-1} \text{ cm}^{-1}$) assuming a 1 cm path length. One unit (U) was defined as the amount of enzyme oxidising 1 μ mol ABTS min^{-1} and expressed as U mL^{-1} [26].

Fractionation and dialysis of extracellular proteins

Extracellular proteins were partially purified from the culture filtrate obtained at day 14 of incubation, corresponding to peak enzyme production. The culture broth was filtered through Whatman No. 1 filter paper and centrifuged at 10,000 \times g for 15 min at 4 °C to obtain a clear supernatant. Protein precipitation [27] was carried out by gradual addition of solid ammonium sulfate to the supernatant under continuous stirring at 4 °C to achieve 80% saturation, following standard salting-out procedures for protein. The mixture was stirred for 3 h and further incubated overnight at 4 °C to ensure complete precipitation of extracellular proteins. The precipitated proteins were recovered by centrifugation at 10,000 \times g for 20 min at 4 °C. The resulting pellet was resuspended in a minimal volume of 50 mM sodium phosphate buffer (pH 7.0) and subjected to dialysis against the same buffer at 4 °C for 24 h with periodic buffer changes to remove residual ammonium sulfate, as described in standard protein purification protocols [28]. The dialysed protein extract was used for SDS–PAGE analysis.

SDS–PAGE analysis of extracellular proteins

Extracellular proteins were analysed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) using a Mini-PROTEAN Tetra Cell, 2-Gel System, vertical electrophoresis system (Bio-Rad Laboratories, China), following the method of Ulrich K. Laemmli (1970) with minor modifications [29]. A discontinuous gel system consisting of 12% resolving gel and 4% stacking gel was employed, as described in standard protein electrophoresis protocols [29,30]. The 12% resolving gel (10 mL) was prepared by mixing 4.0 mL of 30% acrylamide solution, 2.5 mL of 1.5 M Tris–HCl buffer (pH 8.8), 0.1 mL of 10% (w/v) SDS, and 3.35 mL of distilled water. Polymerisation was initiated by adding 50 μ L of 10% ammonium persulfate (APS) and 15 μ L of N, N, N', N'-tetramethylethylenediamine (TEMED). The 4% stacking gel (5 mL) was prepared using 0.665 mL of 30% acrylamide/bis-acrylamide solution, 1.25 mL of 0.5 M Tris–HCl buffer (pH 6.8), 50 μ L of 10% (w/v) SDS, and 3.0 mL distilled water, followed by the addition of 25 μ L APS and 10 μ L TEMED to initiate polymerisation. The resolving gel was cast between glass plates and overlaid with n-butanol to ensure a uniform surface. After polymerisation, the overlay was removed, and the stacking gel was poured over the resolving gel. A comb

was inserted to form wells, and the gel was allowed to polymerise completely. Following polymerisation, the comb was removed and the wells were rinsed with running buffer. Dialysed protein extract was loaded, and electrophoresis was carried out at a constant voltage of 150 V for approximately 50 min. A pre-stained protein molecular weight marker (MBT092, HiMedia Laboratories, India) was used as a standard. The apparent molecular weights of protein bands were estimated by comparing their relative mobility (Rf values) with those of the marker proteins (Ulrich K. Laemmli, 1970).

Statistical analysis

All experiments were performed in triplicate, and the results are expressed as mean ± standard deviation (SD).

Results

Qualitative profiling of extracellular enzyme activities

The fungal isolate was screened for extracellular enzyme production using substrate-specific plate assays. The isolate exhibited positive activity for cellulase, pectinase, xylanase, amylase, and lipase, as indicated by the formation of distinct clear halo zones around the colonies on respective agar media (Figure 1). The appearance of these zones confirmed the extracellular hydrolysis of cellulose, pectin, xylan, starch, and lipid substrates. However, no distinct qualitative halo zones were observed for protease and laccase, suggesting low extracellular expression under plate assay conditions; however, low but measurable activities were detected in submerged fermentation assays. Overall, the fungal isolate demonstrated strong qualitative production of multiple extracellular hydrolytic enzymes, except protease and laccase, during preliminary screening.

Time-course quantitative production of extracellular cell wall-degrading enzymes by *Paradendryphiella arenariae*

The temporal production of extracellular enzymes by *P. arenariae* was evaluated at 7-day intervals up to 28 days under submerged fermentation (Table 1). Enzyme activities increased from day 7 to day 14, followed by a gradual decline. Maximum production of all enzymes was recorded on day 14 (Figure 2), indicating this as the optimal incubation period. β-Glucosidase showed the highest activity (14.6 U/mL), followed by cellulase (11.8 U/mL), xylanase (10.0 U/mL), and pectinase (9.6 U/mL), demonstrating the pronounced lignocellulolytic potential of the isolate. The concurrent peak of these enzymes suggests a coordinated role in lignocellulose degradation, where cellulases and xylanases hydrolyse structural polysaccharides

Table 1: Extracellular enzyme activity profile of *Paradendryphiella arenariae* at different incubation periods during submerged fermentation using pomegranate peel as substrate.

ENZYME	Day 7 (U/ml)	Day 14 (U/mL)	Day 21 (U/mL)	Day 28 (U/mL)
Cellulase	4	11.8	5.5	2.5
β-Glucosidase	6.8	14.6	7.3	4.5
Pectinase	3.5	9.6	4.0	1.2
Xylanase	4	10	6	2.9
Protease	1.05	1.85	0.97	0.17
Laccase	0.12	0.22	0.09	0.0076

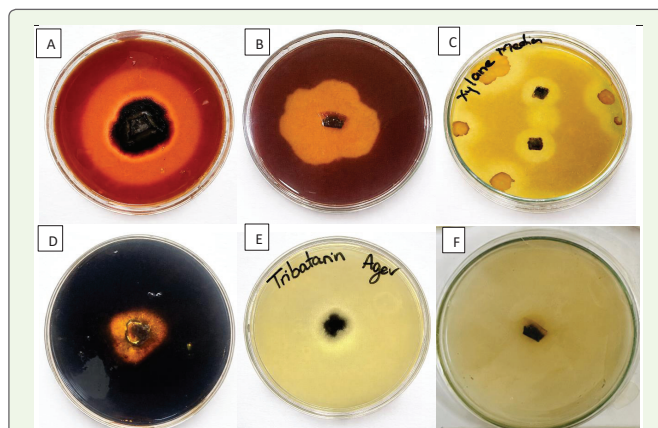


Figure 1: Plate assay-based qualitative detection of extracellular enzymes produced by *Paradendryphiella arenariae*. Representative substrate-specific agar plates showing hydrolysis zones for (A) cellulase, (B) pectinase, (C) xylanase, (D) amylase, and (E) lipase (F) laccase. The formation of distinct clearance zones around the colonies confirms extracellular enzyme production.

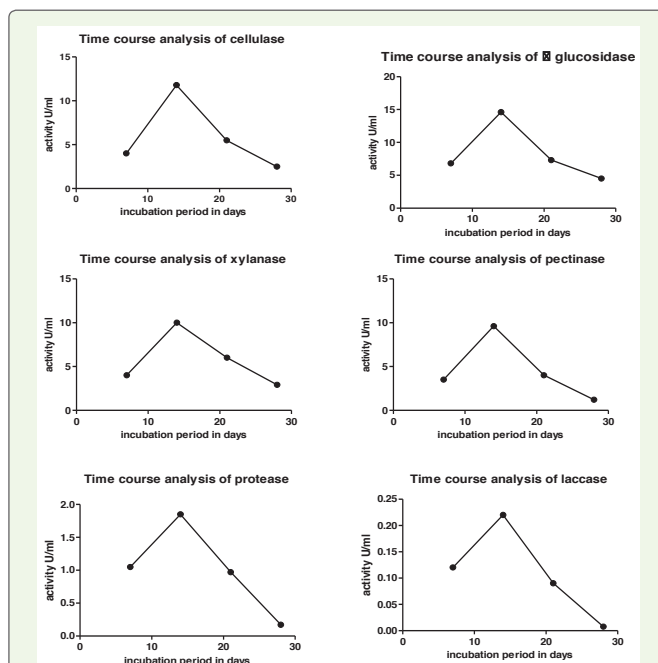


Figure 2: Time-course of enzyme production by *Paradendryphiella arenariae* during submerged fermentation. Activities of cellulase, β-glucosidase, xylanase, pectinase, protease, and laccase were measured at 7-day intervals over a 28-day incubation period.

and β-glucosidase facilitates glucose release, enhancing saccharification efficiency. Such elevated hydrolytic enzyme activity is also indicative of the organism’s capacity to degrade plant cell wall components, thereby contributing to tissue maceration and fruit rot during spoilage. In contrast, protease and laccase were produced at lower levels, with maximum activities of 1.85 U/mL and 0.22 U/mL, respectively. A progressive decline in enzyme activities was observed after day 14, with substantial reductions by day 28, particularly for laccase (0.0076 U/mL) and protease (0.17 U/mL). Overall, the

integrated CWDE profile supports the pathogenic competence of *P. arenariae*, demonstrating its ability to produce a spectrum of hydrolytic enzymes essential for host cell wall degradation.

Quantification of enzymes

The total extracellular protein concentration of the culture filtrate at day 14 was estimated to be 1.61 mg mL⁻¹. Specific activities of extracellular enzymes were calculated by normalising enzyme activity against total protein concentration (Figure 3). Among the enzymes, β -glucosidase exhibited the highest specific activity (9.07 U mg⁻¹ protein), followed by cellulase (7.33 U mg⁻¹), xylanase (6.21 U mg⁻¹), and pectinase (5.96 U mg⁻¹). Protease and laccase showed comparatively lower specific activities of 1.15 and 0.14 U mg⁻¹ protein, respectively.

SDS-PAGE analysis of extracellular proteins

SDS-PAGE analysis of dialysed crude extracellular enzyme extracts revealed multiple protein bands ranging from approximately 17 to 75 kDa (Fig 4). The crude sample (lane 4) showed prominent bands at ~35–48 kDa, along with additional bands at ~25 kDa and ~17 kDa. The observed banding pattern indicates the presence of a complex mixture of cell wall-degrading enzymes, with major proteins corresponding to typical molecular weights of cellulases, pectinases, β -glucosidases, and xylanases. The intensity of bands in the mid molecular weight range suggests high enzyme expression at the selected incubation period (day 14). However, these assignments are tentative and based on molecular weight comparison.

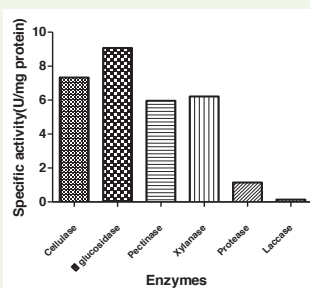


Figure 3: Specific activities of extracellular enzymes secreted by *Paradendryphiella arenariae* at day 14 of incubation.

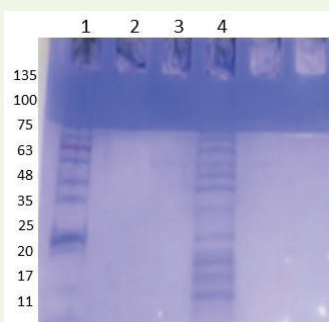


Figure 4: SDS-PAGE profile of extracellular proteins (CWDEs) resolved on a 12% resolving gel with a 4% stacking gel. Lane 1: molecular weight marker (11–135 kDa) Lane 4: crude enzyme extract (day 14) showing multiple bands (~17–75 kDa), with prominent bands at ~35–48 kDa. Molecular weights were estimated relative to the marker.

Discussion

The present study provides the first evidence of extracellular cell wall-degrading enzyme (CWDE) production by *Paradendryphiella arenariae* associated with pomegranate fruit rot, supporting its pathogenic potential and establishing its role as an emerging fungal pathogen of pomegranate under field conditions. The secretion of extracellular hydrolytic enzymes is a critical determinant of fungal pathogenicity, enabling host penetration, tissue maceration, nutrient acquisition, and disease progression through degradation of structural polysaccharides in plant cell walls [1-4, 6,8].

Qualitative plate assays confirmed the secretion of cellulase, xylanase, pectinase, lipase, and amylase, indicating the broad degradative capacity of *P. arenariae*. No distinct halo zones were observed for protease and laccase, suggesting comparatively lower expression under plate assay conditions; However, measurable quantitative activities detected under submerged fermentation indicate that enzyme production is influenced by environmental and nutritional factors [15,18].

Time-course of enzyme analysis revealed coordinated induction of cellulase, β -glucosidase, xylanase, and pectinase, with maximum activities recorded on day 14, followed by gradual decline. Such synchronized enzyme production is characteristic of active substrate colonization during fungal growth and reflects efficient adaptation to host-derived lignocellulosic substrates [2,4,18,20]. Among the enzymes, β -glucosidase exhibited the highest activity and specific activity, suggesting efficient cellulose saccharification and sustained fungal carbon utilization. Elevated cellulase and β -glucosidase activities indicate an effective cellulolytic system that likely contributes to tissue softening and structural collapse during infection [20,21].

Substantial pectinase activity observed highlights its likely role in pathogenesis. Pectinases are well-recognized pathogenicity factors in fruit rot fungi due to their ability to hydrolyze pectin-rich middle lamellae, leading to cell separation and tissue maceration [12,24]. Since pomegranate pericarp is rich in pectic polysaccharides, pectinase secretion by *P. arenariae* likely facilitates early-stage penetration and symptom expansion. Concurrent xylanase production further supports efficient degradation of hemicellulosic cell wall components [10,22].

Protease and laccase activities remained comparatively low throughout incubation, suggesting a secondary role in pathogenicity. Reduced laccase activity may reflect the relatively low lignin content of pomegranate fruit tissues, where extensive oxidative degradation is less critical for host colonization. Similar extracellular enzyme patterns have been reported for fruit-associated necrotrophic fungi, where polysaccharide-degrading enzymes predominate over oxidative enzymes [5,26].

The extracellular protein concentration and corresponding specific activities confirmed active enzyme secretion during peak growth. SDS-PAGE analysis further revealed multiple extracellular proteins ranging from approximately 17–75 kDa, consistent with reported molecular weights of fungal cellulases, xylanases, pectinases, and β -glucosidases [27-30]. The observed protein profile supports the

biochemical evidence for a complex hydrolytic secretome involved in host tissue degradation.

Collectively, these findings establish *P. arenariae* as an emerging pomegranate fruit rot pathogen possessing a robust extracellular hydrolytic arsenal. The coordinated secretion of cellulolytic, pectinolytic, and hemicellulolytic enzymes likely underpins host colonization, symptom development, and rapid fruit decay. These enzymes represent important virulence determinants and potential biochemical targets for developing effective disease management strategies to reduce pre- and postharvest losses in pomegranate [1-4,6,7,18].

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