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Pomegranate under threat: An overview of major diseases, their causal agents, and integrated management

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Abstract
Pomegranate (*Punica granatum* L.) is affected by several diseases caused by fungal and bacterial pathogens, resulting in significant losses in yield and fruit quality, ultimately reducing its market value. Common fungal pathogens include *Botrytis cinerea* (gray mold), *Alternaria alternata* (heart rot), *Aspergillus niger*, and *Coniella granati*. Bacterial diseases, particularly those caused by *Xanthomonas axonopodis* pv. *punicae* (bacterial blight), also pose major challenges. Additionally, *Neofusicoccum parvum* and *Lasiodiplodia theobromae* are associated with stem canker and shoot blight, further threatening pomegranate production. Cell wall-degrading enzymes (CWDEs) play a critical role in the pathogenesis of fungal and bacterial infections in pomegranate, particularly during fruiting. Key CWDEs involved include pectinases (such as polygalacturonase, pectin lyase, and pectate lyase), cellulases, and hemicellulases. This review provides a comprehensive overview of the pathogenic microorganisms and cell wall degrading enzymes which affect pomegranate and examines current trends favouring environmentally friendly and cost-efficient disease management practices over chemical interventions.

Keywords: Pomegranate, fungal rots, bacterial blights, cell wall degrading enzymes

Introduction
Pomegranate (*Punica granatum L.*), an ancient fruit of profound historical, cultural, and medicinal importance, has garnered significant global attention due to its high nutritional and therapeutic value. Renowned for its rich composition of polyphenols, flavonoids, vitamins, and minerals, pomegranate is consumed in various forms, including fresh fruit, juice, and processed products such as syrups, jams, and nutraceuticals. Its notable health benefits, including antioxidant, anti-inflammatory, antimicrobial, and anticancer properties, have elevated its status as a prized commodity in local and international markets.

Thriving in tropical and subtropical climates, pomegranate is particularly well-suited to arid and semi-arid regions owing to its drought-tolerant nature. India leads global pomegranate production, followed by Iran, Turkey, and Mediterranean countries. Despite its adaptability, pomegranate cultivation faces significant challenges from various phytopathogen fungi and bacteria that severely impact yield, fruit quality, and marketability.

Among the fungal pathogens, *Botrytis cinerea, Alternaria alternata, Aspergillus niger,* and *Coniella granati* are predominant culprits, causing pre- and postharvest fruit rots. In addition, bacterial blight caused by *Xanthomonas axonopodis pv. punicae* poses a critical threat to both foliage and fruit. Furthermore, wood-infecting pathogens such as *Neofusicoccum parvum* and *Lasiodiplodia theobromae* are responsible for stem canker and shoot blight, contributing to chronic yield decline (Table 1).

Table 1: Major Diseases of Pomegranate and Their Causal Agents

Disease	Causal Agent(s)	Symptoms	Reference
Bacterial Blight	*Xanthomonas axonopodis* pv. *Punicae*	Water-soaked black lesions on leaves, stems, and fruits; cracking; premature defoliation	Kumar *et al*., 2020 [1]
Anthracnose / Fruit Rot	*Colletotrichum gloeosporioides, C. truncatum*	Sunken black lesions with pinkish spores; fruit drop	Ramesh *et al*., 2018 [2]
Alternaria Fruit Spot	*Alternaria alternate*	Small, dark spots that expand; internal rot of arils	Thakur & Singh, 2021 [3]
Aspergillus Rot	*Aspergillus niger, A. flavus*	Dry rot with black or yellow mold on cracked fruits	Reddy & Lakshmi, 2022 [4]
Botrytis Gray Mold	*Botrytis cinerea*	Gray fuzzy mold on surface of stored fruits	Thakur & Singh, 2021 [3]
Heart Rot / Internal Breakdown	*Alternaria, Aspergillus, Fusarium* spp.	Internally decayed arils with no external symptoms	NHB, 2023 [5]
Cercospora Fruit Spot	*Cercospora punicae*	Circular brown necrotic lesions on leaves and fruits	Kumar *et al*., 2020 [1]
Dry Fruit Rot / Charcoal Rot	*Macrophomina phaseolina*	Dry black sunken lesions on fruits and stems	Reddy & Lakshmi, 2022 [4]
Wilt Complex	*Fusarium, Macrophomina,* nematodes	Sudden plant wilting, vascular browning, root decay	NHB, 2023 [5]

A key virulence strategy utilized by plant pathogens involves the secretion of cell wall-degrading enzymes (CWDEs), which play a pivotal role in host invasion and tissue degradation. These enzymes, including polygalacturonases, pectin lyases, cellulases, and hemicellulases target the structural polysaccharides of the plant cell wall, undermining its integrity. The disruption facilitates tissue maceration and invasion, particularly during the fruiting stage, where the susceptibility of plant tissues is heightened.

Integrated disease management (IDM), which combines cultural, biological, chemical, and physical methods in a coordinated strategy, has emerged as a sustainable solution to manage pomegranate diseases (Table 2). In recent years, research has focused on environmentally friendly and residue-free approaches such as the use of biocontrol agents, resistant varieties, and improved pre - postharvest technologies.

Given the environmental concerns and limitations associated with chemical control measures, there is an urgent need for sustainable, cost-effective, and eco-friendly disease management strategies. This review aims to provide a comprehensive overview of the major fungal and bacterial pathogens affecting pomegranate, elucidate the role of cell wall-degrading enzymes (CWDEs) in disease progression, and explore emerging trends in integrated and sustainable disease management. It also highlights the need for continued research and field-level implementation of IDM practices to ensure the economic viability and sustainability of pomegranate cultivation

Table 2: Innovative and Integrated Control Measures for Pomegranate Diseases

Control Type	Innovative Strategy	Application/Impact	Reference
Biological Control	Use of *Trichoderma harzianum*, *Pseudomonas fluorescens*, *Bacillus subtilis*	Suppress soil- and air-borne fungal pathogens; improve plant immunity	Thakur & Singh, 2021 [3]
Cultural Practices	Pruning, sanitation, wide spacing, drip irrigation, crop rotation	Reduces inoculum load, improves airflow and soil health	Kumar *et al*., 2020 [1]
Chemical Control (Eco-safe)	Minimal & targeted use of copper-based fungicides, systemic fungicides (azoxystrobin, difenoconazole)	Control of foliar and fruit-borne pathogens; part of IDM strategy	Reddy & Lakshmi, 2022 [4]
Nutrient Management	Foliar sprays of boron, calcium, and potassium	Prevents fruit cracking and improves fruit firmness	NHB, 2023 [5]
Postharvest Treatment	Hot water dip (45–50°C for 10 minutes), fungicide + wax coating, cold storage (5–7°C)	Reduces postharvest rot and extends shelf life	Ramesh *et al*., 2018 [2]
Resistance Breeding	Development and deployment of bacterial blight-resistant and wilt-tolerant cultivars	Long-term sustainable solution to major pomegranate diseases	Reddy & Lakshmi, 2022 [4]
Digital Tools & Monitoring	Disease prediction models, mobile apps for disease diagnosis, remote sensing for crop health monitoring	Early detection and timely management decisions	Thakur & Singh, 2021 [3]
Organic Amendments	Neem cake, compost, vermicompost, biochar	Enhances soil microbial health and indirectly suppresses soil pathogens	NHB, 2023 [5]

Cell wall degrading enzymes

The intensity of CWDE activity is directly correlated with the severity of rotting and the rate of fruit deterioration. This enzymatic activity not only facilitates pathogen spread but also contributes significantly to the economic losses associated with fruit spoilage. Understanding the mechanisms of CWDE action provides a foundation for developing targeted strategies to mitigate fungal infections and improve post-harvest fruit management. This narrative sets the stage for a deeper exploration of CWDEs in the context of host-pathogen interactions and their implications for plant health and agricultural sustainability.

Role of Fungal CWDEs in Pomegranate Diseases Enzymatic Mechanisms of Pathogenesis in Plant-Bacterial Interactions

Fungal pathogens rely heavily on enzymatic strategies to breach plant cell walls, overcome host defences, and establish infections. These enzymes primarily target structural components of the plant cell wall, enabling fungal penetration and colonization.

1. Pectinases

Pectinases are a group of enzymes critical for degrading pectin, a major polysaccharide found in the primary plant cell wall's middle lamella, facilitating cell separation and tissue maceration.

Polygalacturonases (PGs)

- PGs hydrolyzes alpha 1,4- galacturonic acid residues, a component of pectin, leading to tissue softening and rot.
- *Botrytis cinerea* and *Alternaria alternata* produce high levels of polygalacturonases, correlating with rapid tissue decay in infected pomegranate fruits (Prusky *et al*., 2013) [7].

Pectate Lyases and Pectin Lyases

- These enzymes cleave de-esterified pectin and esterified pectin, respectively.
- *Aspergillus niger* utilizes pectate lyases to establish infections, especially in overripe or mechanically damaged fruits (Reddy *et al*., 2020) [11].

2. Cellulases

Cellulases are the group of enzymes that break down cellulose, made up of by alpha D-glucose units with β-1,4 and β-1,6-glycosidic linkages. Cellulose is a key structural component of the plant cell wall.

Endoglucanases and Exoglucanases

- These enzymes hydrolyze internal and terminal β-1,4-glycosidic linkages in cellulose.
- In *Alternaria alternata*, cellulase activity has been shown to facilitate rapid tissue colonization and symptom development, particularly under humid storage conditions (Thakur *et al*., 2021) [3].

3. Hemicellulases

Hemicellulases target hemicellulose, an important component of the cell wall matrix.

Xylanases

- These enzymes degrade xylan, a major hemicellulosic polysaccharide.
- Studies on *Coniella granati* reveal xylanase as a key virulence factor, with higher enzymatic activity during initial infection stages (Kumar *et al*., 2022) [1].

4. Proteases

Fungal proteases degrade structural and defensive proteins, weakening the cell wall and suppressing plant immunity.

Aspartic and Serine Proteases

- *Botrytis cinerea* secretes proteases to degrade pathogenesis-related (PR) proteins, dampening host defenses and exacerbating infection (Prusky *et al*., 2013) [7].

5. Laccases

Laccases oxidize phenolic compounds, which are crucial for cell wall lignification and defence responses.

- *Aspergillus flavus* produces laccases to overcome lignin barriers, enhancing tissue invasion in pomegranate fruits (Baldrian, 2006) [8].

Pathogenesis in Pomegranate

Fungal pathogens deploy CWDEs in a sequential manner to breach the pomegranate cell wall, colonize tissues, and induce symptoms.

Symptomatology

- Enzymatic degradation of pectin and cellulose leads to softening, rotting, and eventual collapse of fruit tissues.
- High levels of pectinase and xylanase activities are associated with severe symptoms, such as internal fruit breakdown and aril decay (Ramesh *et al*., 2018) [2].

Research Insights and Advances in Fungal CWDE Studies in Pomegranate

Quantitative Studies

- Studies on *Colletotrichum raphigera* reported pectinase, cellulase, and xylanase activities of 71, 13.8, and 54.3 U/ml, respectively, in liquid cultures, highlighting their role in tissue maceration during pathogenesis (Kumar *et al*., 2020) [1].

Genomic and Transcriptomic Insights

- Advances in fungal genomics have identified gene clusters encoding CWDEs, providing targets for molecular interventions (Prusky *et al*., 2013) [7].

Biocontrol Strategies

- Biological control agents such as *Trichoderma harzianum* and *Pseudomonas fluorescens* suppress CWDE activity in pathogenic fungi, reducing disease severity (Thakur *et al*., 2021) [3].

Enzymatic Mechanisms of Pathogenesis in Plant-Bacterial Interactions

Plant-bacterial interactions are complex processes often mediated by an arsenal of enzymes secreted by pathogenic bacteria. These enzymes play a pivotal role in facilitating host colonization, disrupting plant defense mechanisms, and degrading structural components of the plant cell wall.

Role of Enzymes in Bacterial Pathogenesis

1. Cell Wall-Degrading Enzymes (CWDEs)

Bacterial pathogens deploy CWDEs to breach the plant cell wall, a formidable barrier that protects the plant from external threats.

Pectinases

- Pectin-degrading enzymes, such as polygalacturonases, pectin lyases, and pectate lyases, dissolve the pectic polysaccharides in the middle lamella, leading to tissue maceration.
- In *Xanthomonas axonopodis* pv. *punicae*, pectinase activity correlates with the formation of water-soaked lesions and fruit cracking (Kumar *et al*., 2020) [1].

Cellulases

- These enzymes hydrolyze cellulose, the primary component of the plant cell wall.
- *Xanthomonas spp.* utilize cellulases to weaken the cell wall, enhancing bacterial entry and tissue invasion (Sundin *et al*., 2001) [13].

Hemicellulases

- Targeting hemicellulose, enzymes like xylanase disrupt the cross-linking matrix in the cell wall, facilitating pathogen spread.
- Hemicellulase activity has been implicated in tissue degradation during advanced stages of bacterial blight (Ramesh *et al*., 2013).

2. Proteases

Proteases degrade plant proteins, particularly those involved in defence signalling and structural integrity.

Serine and Metalloproteases

- These proteases degrade cell wall-associated proteins and disrupt plant immune signalling.
- *Ralstonia solanacearum*, another bacterial pathogen observed in woody plants, has been shown to secrete proteases with a similar mode of action, suggesting a conserved strategy among vascular pathogens (Flores-Cruz & Allen, 2009) [14].

3. Lipases and Phospholipases

Lipases degrade lipid components of the plant cell membrane, while phospholipases hydrolyze phospholipids, aiding bacterial invasion.

- *Xanthomonas spp.* secrete phospholipases that compromise membrane integrity, a critical step in lesion formation in pomegranate fruits (Wagner *et al*., 2008) [15].

Symptomatology

- The enzymatic degradation of the middle lamella by pectinases results in water-soaked lesions, which subsequently turn necrotic.
- Cellulase and xylanase activities contribute to fruit cracking, while proteases suppress plant immune responses, exacerbating disease severity (Thakur *et al*., 2021) [3].

Research Insights and Advances in Bacterial CWDE Studies in Pomegranate

- **Genomic Studies:** Comprehensive genomic analyses of *Xanthomonas axonopodis* pv. *punicae* have identified key genes encoding pectinases, cellulases, and lipases. Their expression levels are directly associated with the severity of bacterial blight in pomegranate (*Kumar et al., 2020*) [1].

- **Structural Insights:** Structural elucidation of bacterial cell wall-degrading enzymes (CWDEs) has pinpointed critical active sites. These findings are instrumental in designing targeted enzyme inhibitors to manage bacterial blight effectively (*Chatterjee et al., 2012*).

- **Biocontrol Approaches:** Application of bacterial antagonists, such as *Pseudomonas fluorescens* and *Bacillus subtilis,* has demonstrated significant potential in reducing enzymatic activity and alleviating disease symptoms in pomegranate (Ramesh *et al.*, 2018) [2].

Conclusion

Pomegranate cultivation holds immense promise due to its nutritional, therapeutic, and economic significance. However, its production faces formidable challenges from a range of fungal and bacterial pathogens that threaten yield, quality, and marketability. These pathogens employ sophisticated mechanisms, such as the secretion of cell wall-degrading enzymes (CWDEs), to invade host tissues and facilitate disease progression. The enzymatic degradation of structural polysaccharides in plant cell walls highlights the critical role of CWDEs in pathogenesis, directly impacting fruit deterioration and postharvest losses.

Given the limitations and environmental concerns associated with chemical-based management, the adoption of integrated disease management (IDM) strategies has emerged as a sustainable approach. Incorporating cultural, biological, chemical, and postharvest measures in a holistic framework is key to mitigating the impact of these diseases. Biological control agents, innovative postharvest treatments, and resistant cultivars represent promising tools in reducing disease severity while ensuring environmental safety. Additionally, digital tools and predictive models have enhanced early detection and decision-making capabilities, underscoring the importance of technology in modern agriculture.

Moving forward, interdisciplinary research is needed to better understand host-pathogen interactions, particularly the molecular and enzymatic basis of virulence, to develop targeted interventions. Field-level implementation of eco-friendly practices, combined with the development of resistant cultivars and improved crop management techniques, will be pivotal in safeguarding pomegranate production. By leveraging scientific advancements and sustainable practices, it is possible to ensure the long-term viability of this valuable crop and maintain its status as a prized agricultural commodity in both domestic and global markets.

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