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TARGETING QUORUM SENSING AND BIOFILM FORMATIONIN *XANTHOMONAS AXONOPODIS* PV. *PUNICAE* USING PHYTOCHEMICALS: A NOVEL PHARMACOGNOSTIC STRATEGY FOR BACTERIAL BLIGHT MANAGEMENT

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ABSTRACT

Bacterial blight caused by Xanthomonas axonopodis pv. Punicae (Xap) is a major constraint in pomegranate production, leading to severe leaf, stem, and fruit infections and substantial economic loss. Conventional control strategies relyingon copper compounds and antibiotics are increasingly ineffective due to pathogen resistance and environmental concerns. Recent scientific findings show that virulence in Xap is strongly regulated by quorum sensing (QS) through Diffusible Signal Factor (DSF) signaling and by robust biofilm formation, which enable adhesion, colonization, survival, and resistance to chemical treatments. Targeting QS and biofilm pathwaystherefore represents a novel and highly specific approach for disease management.Phytochemicals such as flavonoids, alkaloids, phenolics, terpenoids, tannins, saponins,andessentialoilshavedemonstratedsignificantpotentialtoinhibitQSand disrupt biofilm architecture. These natural compounds interfere with DSF biosynthesis, degrade signaling molecules, downregulate rpf gene clusters, inhibit motility, reduce EPS production, and weaken pathogen virulence. This review highlights the mechanisms of QS and biofilm formation in Xap, summarizes key phytochemicalswithanti-QSandanti-biofilmactivity,andproposesphytochemical- based formulations as eco-friendly, sustainable, and resistance-freepharmacognostic strategies for managing bacterial blight of pomegranate.

Keywords:

Quorum sensing ,Biofilm inhibition , Xanthomonas axonopodis pv. Punicae,Diffusible Signal Factor (DSF),Phytochemicals: Anti-quorum sensing agents Anti-biofilm compounds; Bacterial blight; Pomegranate disease management; Pharmacognostic approach.

Introduction

Bacterial blight causedbyXanthomonasaxonopodispv.Punicae(Xap)isoneofthe most devastating diseases affecting pomegranate cultivation,resultingin severe leaf spots, stem cankers, fruit lesions, and significant economic loss. Traditional management depends heavily on copper compounds and antibiotics, but their long- term use has led to environmental hazards, high residue levels, and increased pathogen resistance. Recent studies have shown that Xap infection and survival are strongly regulated by quorum sensing (QS)—a cell-density–dependent communication system—and biofilm formation, which enables the pathogen to adhere, colonize plant tissues, and resist chemical treatments. Targeting these virulence pathways provides a promising avenue for disease control.

Phytochemicals derived from medicinal plants have emerged as effective natural agents capable of disrupting QS signals and inhibiting biofilm formation. Compounds such as flavonoids, alkaloids, terpenoids, and phenolics interfere with DSF-mediated communication, reduce extracellular polysaccharide production, and weaken the pathogenicity of Xap. As eco-friendly, sustainable, and resistance-free alternativestosyntheticbactericides,phytochemicalsofferanovelpharmacognostic strategy for managing pomegranate bacterial blight

Bacterial blight of pomegranate, caused by Xanthomonas axonopodis pv. Punicae (Xap), has emerged as a major phytopathological problem, severely limiting fruit qualityandmarketproductivity.Thepathogenspreadsrapidlythroughinfectedplant debris, pruned material, rain splash, and mechanical injury, making the disease extremely difficult to manage under field conditions. Although several chemical bactericides are available, their repeated use has contributed to toxicity concerns, high cultivation costs, and the development of resistant strains of Xap. These limitations have intensified the search for safer and more effective alternatives for long-term disease control.

In recent years, attention has shifted towards understanding the molecular basis of Xap pathogenicity, particularly the role of quorum sensing (QS) and biofilm formation. QS allows the pathogen to coordinate virulence gene expression, while biofilms protect bacterial colonies from plant defenses and chemical agents. Targeting these communication and survival mechanisms offers a highly specific strategytoreduceinfectionseverity.Phytochemicals—naturallyoccurringbioactive compounds from medicinal plants—have shown remarkable potential to disrupt QS signals, inhibit extracellular polymeric substance production, and weaken biofilm architecture. Their biodegradability, low toxicity, and multi-targeted action make them attractive candidates for developing eco-friendly, pharmacognostic solutions for managing bacterial blight.

Bacterial blight caused by Xanthomonas axonopodis pv. Punicae (Xap) has become a critical constraint in the commercial cultivation of pomegranate, leading to premature leaf drop, fruit blemishes, and a noticeable decline in marketable yield. The disease spreads aggressively during humid conditions and can severely infect orchardswithinashortperiod.Despitetheavailabilityofchemicalcontrolmeasures, theirinconsistentfieldperformance,highapplicationfrequency,andriskof chemical resistance underscore the need for alternative strategies that are both effective and environmentally safe. As a result, modern plant disease managementis increasingly focused on understanding microbial behavior and targeting the underlyingmechanisms that regulatepathogenvirulence.One of the most promising areas of research involves the disruption of quorum sensing (QS) and biofilm formation—two essential processes that enable Xap to establish infection, survive environmental stress, and resist chemical treatments. QS allows bacterial populations to communicate and coordinate activities, while biofilms provide a protective barrier that enhances pathogen persistence. Phytochemicals derived from medicinal plants have shown remarkable ability to interfere with these systems through mechanisms such as signal inhibition, degradation of autoinducers, and suppression of extracellular matrix formation. Their natural origin, safety profile, and multi-targeted mode of action highlight their potential as sustainable pharmacognostic tools for combating pomegranate bacterial blight.

Need oftheStudy:

1. Increasing severity of bacterial blight: Xanthomonas axonopodis pv. Punicae iscausingwidespreaddamagetopomegranateorchards,leadingtosignificant yield and economic losses.
2. Failure of conventional chemical control: Overuse of copper compounds and antibiotics has resulted in reduced effectiveness, poor field performance, and chemical resistance in Xap.
3. Environmental and health concerns: Continuous application of synthetic bactericides leads to soil contamination, phytotoxicity, and harmful residues on fruits.
4. Lack of sustainable management strategies: Current disease control methods do not provide long-term, eco-friendly solutions, creating a need for alternative approaches.
5. Role of quorum sensingin virulence: QS regulateskey pathogenictraits such as EPS production, motility, adhesion, and infection. Targeting QS can weaken the pathogen without killing it, reducing resistance risk.
6. Biofilm formation enhances survival: Biofilms protect Xap from chemical treatments and environmental stress. Disrupting biofilms can significantly reduce infection severity.
7. Phytochemicals offer natural QS and biofilm inhibition: Plant-derived compounds provide safe, biodegradable, and multi-targeted effects against bacterial communication and survival.
8. Need for pharmacognostic exploration: There is limited research on herbal anti-QS agents specifically against Xap, highlighting the importance of identifying and evaluating effective phytochemicals.
9. Potential for developing botanical formulations: Phytochemicals can be used to create novel sprays, coatings, or nano-herbal formulations for field application.
10. Support for sustainable agriculture: Using plant-based QS inhibitors aligns with

environmentally responsible and residue-free crop protection practices.

Objectives :

1. To study the pathogenic mechanisms of Xanthomonas axonopodis pv. Punicae, with special focus on quorum sensing (QS) and biofilm formation.
2. To understand the role of Diffusible Signal Factor (DSF)–mediated signaling in regulating virulence, motility, EPS production, and colonization.
3. Toidentifyphytochemicalswithprovenor potential anti-quorum sensingand anti-biofilm activity against plant-pathogenic bacteria.
4. To evaluate the mechanisms through which phytochemicals inhibit QS and biofilm pathways in Xap and related species.
5. To compile pharmacognostic information on medicinal plants possessing active phytoconstituents that can suppress bacterial virulence.
6. To explore the feasibility of using plant-based compounds as eco-friendly alternatives to chemical bactericides for managing bacterial blight.
7. To highlight research gaps and future prospects for developing phytochemical-based formulations or integrated disease management strategies.

QuorumSensinginXanthomonasaxonopodispv.Punicae:

Quorum sensing (QS) in Xanthomonas axonopodis pv. Punicae (Xap) is a cell-to-cell communicationsystemthatallowsthe bacteria tosense their populationdensity and coordinate the expression of virulence factors. Xap uses a Diffusible Signal Factor(DSF)–basedQSsystem,whichisthemainregulatorymechanismcontrolling its pathogenicity in pomegranate.

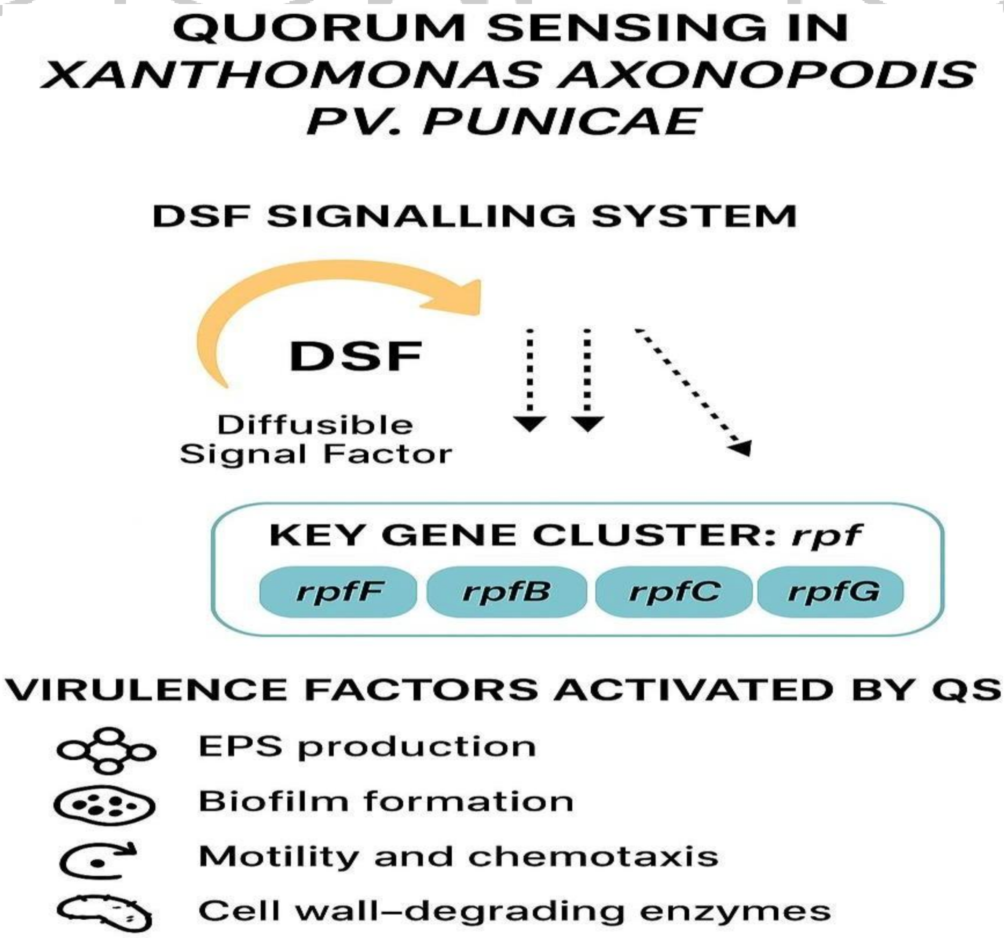

Fig: Quorum sensinginxanthomonasaxonopodis pv.punicae

1. DSF Signaling System : The QS mechanism in Xap is primarily regulated by DSF (cis-11-methyl-2-dodecenoic acid). DSF molecules accumulate in the environment as bacterial numbers increase. When DSF reaches a threshold concentration, it activates a signaling cascade that triggers virulence gene expression.
2. Key Gene Cluster: rpf (regulation of pathogenicity factors) : The DSF pathway is controlled by the rpf gene cluster, which includes:

- rpfF–responsiblefor DSF biosynthesis
- rpfB– fattyacidprocessing andtransport
- rpfC & rpfG – two-component regulatory system that detects DSF and transmits signals inside the cell

Thesegenesregulatemultiplepathwaysrelatedto infection.

3. VirulenceFactorsActivatedbyQSinXap

OnceDSF-basedQSisactivated,Xap enhances:

- EPS (extracellular polysaccharide) production – helps bacteria adhere and create slime matrix
- Biofilmformation–protectsthebacteriafromchemicalsandplant defenses
- Motilityandchemotaxis–enables movementandcolonization
- Cellwall–degradingenzymes–softenhosttissueforinvasion
- Stresstolerance–increasessurvival under harsh conditions

4. RoleofQSinBiofilmFormation

QSstimulatestheoverproductionofEPS,which formsthefoundationof biofilms. Biofilms enable Xap to:

- Firmlyattach toleaf,stem, and fruitsurfaces
- Resistcopperfungicidesand antibiotics
- SurvivedesiccationandUVlight
- Multiplyinsideprotectedmicrocolonies

BiofilmFormationinXanthomonasaxonopodispv.Punicae:

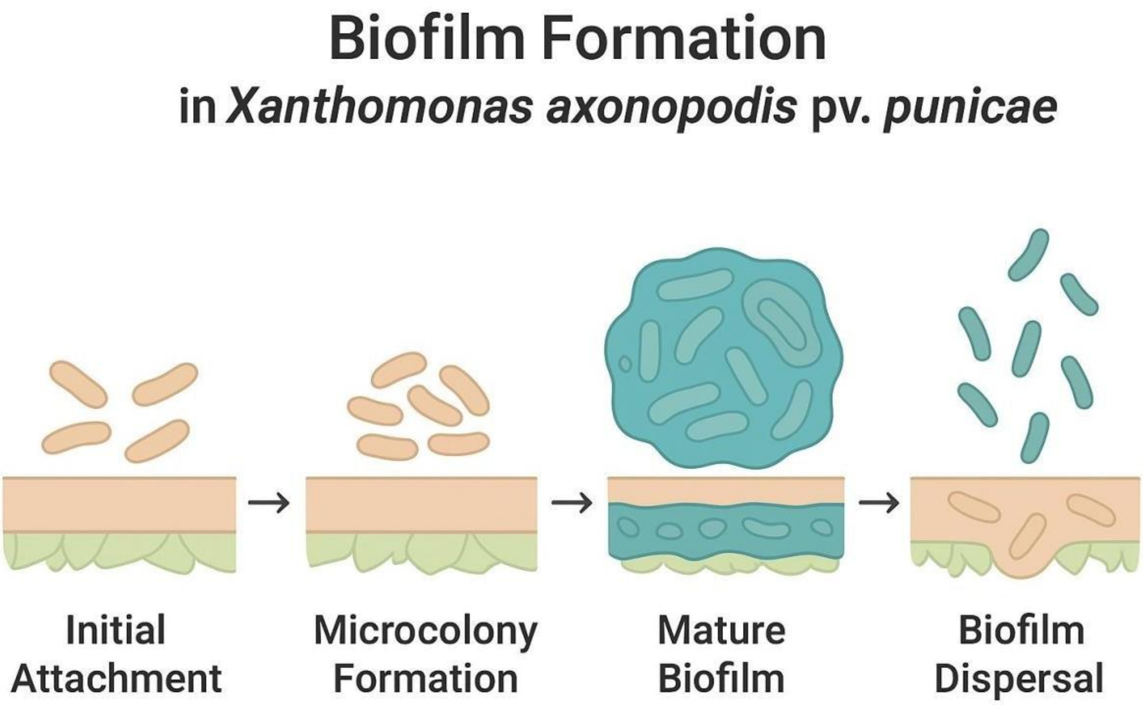

Fig:biofilmformationinxanthomonasaxonopodispv.punicae

BiofilmformationisacriticalvirulencemechanisminXanthomonasaxonopodispv. Punicae (Xap), the causative agent of bacterial blight in pomegranate. Xap forms structured, multicellular biofilms onleaf surfaces, stomatal openings, branches,and fruittissues,enablingthepathogentopersist under harshenvironmentalconditions.

The process begins withinitial attachment ofbacterial cellstohost surfacesthrough adhesins, lipopolysaccharides, and extracellular polysaccharides. This early adhesion is influenced by moisture, surface nutrients, and plant exudates. Once attached, the bacteria undergo microcolony formation, where cells multiply and communicate through quorum sensing signals, primarily DSF (diffusible signal factor) molecules.

Asthebacterialpopulationincreases,Xapproducesarobustextracellularpolymeric substance (EPS) matrix composed of xanthan gum, proteins, nucleic acids, and lipids. This EPS layer protects the pathogen from desiccation, UV radiation, plant defense molecules, and antimicrobial agents. The mature biofilm shows differentiated layers of active cells and dormant persister cells, contributing to chronic infection. Biofilms also facilitate systemic spread, clogging xylem vessels and disrupting nutrient and water transport in the plant. Toward later stages, Xap exhibits biofilm dispersal,releasingplanktonic cells that colonize newplant tissues, enabling disease progression and epidemics.Biofilm formation significantly enhances Xap's ability to survive environmental stresses, resist agrochemicals, and evade host immunity. This makes biofilms a major target area for developing novel disease-control strategies. Phytochemicals that disrupt EPS synthesis, inhibit quorum sensing, or prevent initial adhesion can effectively weaken biofilms, reducing pathogen virulence and disease spread in pomegranate orchards.

Role of Phytochemicals in Targeting Quorum Sensing and Biofilm Formation in Xap :

PhytochemicalsplayacrucialroleinsuppressingthepathogenicityofXanthomonas axonopodispv.Punicaebyinterferingwithits quorumsensing(QS)signalingand biofilm development. Many plant-derived compounds—such as flavonoids, alkaloids, terpenoids, phenolics, and essential oil components—exhibit anti-QS activity,enablingthemtodisruptthecommunicationsignals(mainlyDSF–diffusible signal factor) responsible for regulating virulence gene expression, motility, EPS synthesis, and enzyme secretion. These molecules act as QS inhibitors (QSIs) by blocking DSF receptors, degrading signal molecules, or inhibiting DSF synthesis enzymes. By interrupting the DSF-mediated communication pathways, phytochemicals reduce the ability of Xap tocoordinate collective behaviors that are essential for infection establishment.

In addition to QS disruption, phytochemicals exert strong effects on biofilm inhibition. Many plant compounds interfere with early adhesion by altering cell surface hydrophobicity or damaging flagella-mediated attachment. Phenolics and flavonoids inhibit extracellular polymeric substance (EPS) production, which is essential for biofilm stability and maturation. Some phytochemicals hinder microcolony formation, destabilize the EPSmatrix, or enhance biofilm dispersal by breaking down polysaccharide chains. Terpenoids and essential oil components penetrate the biofilm layers, increasing bacterial susceptibility to environmental stress.Together,theseactionsweakenthestructuralintegrityofXapbiofilms,reduce bacterial survival on plant tissues, and limit systemic spread. Thus, phytochemicals offeranovel,eco-friendly,andresistance-freeapproachtomanagingbacterialblight by targeting the fundamental communication and colonization mechanisms of Xap.

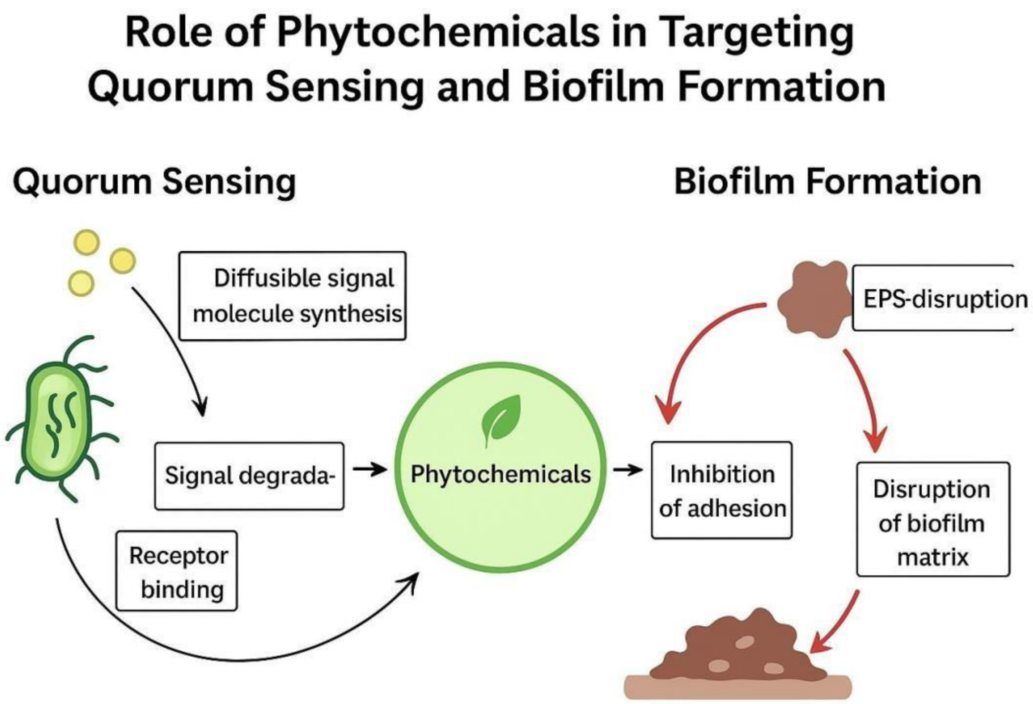

fig:roleofphytochemicalsintargetingquorum sensingandbiofilmformation

ImportantPhytochemicalswithAnti-QS &Anti-BiofilmActivity

1. Flavonoids

- Examples:Quercetin,Kaempferol,Rutin
- Mechanism:InhibitDSFsignaling,downregulateQSgenes,reduce EPS production, prevent biofilm maturation.

2. Alkaloids

- Examples:Berberine,Piperine, Solasodine
- Mechanism:BlockQSsignalmoleculesynthesis,inhibitvirulence factor expression, reduce biofilm density.

3. Terpenoids/EssentialOils

- Examples:Limonene,Eugenol,Thymol, Carvacrol
- Mechanism:Disruptbacterial cellmembranes,interferewithinitial adhesion, penetrate EPS matrix, inhibit biofilm formation.

4. PhenolicAcids

- Examples:Gallicacid,Caffeic acid,Ferulicacid
- Mechanism: Degrade signaling molecules, reduce QS-regulated gene expression, destabilize biofilm structure.

5. Tannins

- Examples:Catechin,Proanthocyanidins
- Mechanism:InhibitEPSproduction,preventbacterialaggregation, destabilize biofilm architecture.

6. Saponins

- Examples:Diosgenin,Escin
- Mechanism:Altercellmembranepermeability,reducebacterialadherence, suppress biofilm formation.

FutureProspects:

1. Development of Phytochemical-Based Formulations: Phytochemicals with anti-quorum sensing(QS) and anti-biofilm activity can be formulated as eco-friendly botanical sprays, coatings, or nano-herbal formulations to manage bacterial blight in pomegranate orchards.
2. Nano-Herbal Delivery Systems: Encapsulation of phytochemicals in nanoparticles, liposomes, or nanoemulsions can enhance their stability, bioavailability,andtargeteddeliverytobacterialcolonies,improvingefficacy against Xap.
3. CombinationStrategies:Synergistic combinationsofdifferentphytochemicals or integration with reduced doses of conventional bactericides can provide enhanced disease control while minimizing chemical residues and resistance development.
4. Molecular Mechanism Studies: Further research is needed to understand the precisemolecular targets of phytochemicals in DSFsignaling, QS regulation, and biofilm disruption in Xap, which can facilitate rational design of potent inhibitors.
5. Field Trials and Validation: Extensive field studies are necessary to evaluate the real-world effectiveness, optimal dosage, and application frequency of phytochemical-based treatments under diverse agro-climatic conditions.

6. Sustainable Agriculture Integration: Phytochemical-based QS and biofilm inhibitors can beintegratedinto organic and sustainable agriculture practices, reducingrelianceonsyntheticchemicalsandpromotingenvironmentalsafety.
7. Screening of Novel Plants: Exploration of underutilized or ethnomedicinal plantsmayleadtothediscoveryofnewbioactivecompoundswithpotentanti- QS and anti-biofilm properties.
8. Regulatory and Commercial Development: Standardization, quality control, and regulatory approval of phytochemical-based products will be crucial to translate laboratory findings into commercially viable, safe, and effective biocontrol agents.

Conclusion:

Bacterial blight caused by Xanthomonas axonopodis pv. Punicae is a major constraint in pomegranate cultivation, and its management remains challenging due topathogenresistance,environmentalconcerns,andlimitationsofchemicalcontrol.

Quorum sensing (QS) and biofilm formation are key virulence mechanisms that enable the pathogen to coordinate infection, adhere to plant tissues, and survive under adverse conditions. Phytochemicals derived from medicinal plants—such as flavonoids, alkaloids, terpenoids, phenolics, tannins, and saponins—offer a promisingsolutionbydisruptingDSF-mediated QSsignalingandinhibitingbiofilm development.

Targeting QS and biofilms with phytochemicals represents a novel, eco-friendly, and sustainable pharmacognostic strategy for bacterial blight management. These compounds reduce pathogen virulence without promoting resistance, protect plant tissues, and can be integrated into environmentally responsible agricultural practices. Future research focusing on molecular mechanisms, formulation development, nano-herbal delivery systems, and field validation will be critical to translating these findings into effective, commercially viable, and sustainable disease management solutions for pomegranate and other susceptible crops.

Reference:

1. Feng Y- M, Long Z- Q, Xiang H- M, Ran J- N, Zhou X, Yang S. Research on Diffusible Signal Factor- Mediated Quorum Sensing in Xanthomonas: A Mini- Review. Molecules. 2023;28(2):876.
2. HeY- W,etal.QuorumSignalingMolecules:InteractionsBetweenPlantsand AssociatedPathogens.Int.J.Mol.Sci. 2025;26(11):5235.
3. Vattem DA,MihalikK,Crixell SH,McLeanRJC.Dietaryphytochemicalsas quorum sensing inhibitors. Fitoterapia. 2007;78(4):302–310.
4. Kalia VC. Quorum sensing inhibitors: an overview. Biotechnol. Adv. 2013;31(2):224–245. (Note: general review, includes plant- derived QSIs)
5. Prateeksha;etal.Naturalproduct-basedinhibitorsofquorumsensing:Anovel approach to combat antibiotic resistance. Frontiers in Microbiology. 2023.
6. Novikov A, et al. Exploration of Phytochemicals as Anti-biofilm Agents Against Pathogenic Bacteria: Their

Potential and Challenges. Anti- Infective Agents. 2023. (Review)

7. GovindanN,KaruppiahV,SathiyaseelanA.ComparativeStudyofInhibitory Potential of Dietary Phytochemicals Against Quorum Sensing Activity and Biofilm Formation byChromobacterium violaceum 12472and Pseudomonas aeruginosa PAO1. Frontiers in Microbiology. 2019;10:743.

8. KaliaNP,etal.Antibiofilmandanti- quorumsensingactivitiesofeugenoland linalool from Ocimum tenuiflorum against Pseudomonas aeruginosa biofilm. Journal of Applied Microbiology. 2021;131(6):2821–2833.

9. AzimiS,TufenkjiN.Plant- derivedanti biofilmandquorumsensinginhibitory compounds:Anti- virulencestrategyformanagingplantpathogens.Journalof Plant Pathology.

10. Deng Y, Liu X, Wu J, Lee J, Chen S, Cheng Y, Zhang C, Zhang LH. Xanthomonas campestris promotes diffusible signal factor biosynthesis and pathogenicity by utilizing host plant sugars. Appl. Environ. Microbiol. 2015;81(8):2861–2868.

11. He Y, et al. Diffusible signal factor (DSF)-mediated quorum sensing modulates expression of diverse traits in Xanthomonas citri subsp. Citri – transcriptomic evidence. Mol. Plant- Microbe Interact. 2019.

12. He Y- W, Zhou L- L, He H- L, et al. DSF- mediated quorum sensing in Xanthomonas spp.: regulatory mechanisms and host interaction. (Book chapter / review — use as supplementary)

13. Deng Y, et al. Xanthomonas campestris cell- cell signalling molecule DSF elicits innate immunity in plants and is suppressed by the exopolysaccharide xanthan. J. Exp. Bot. 2015;66(21):6697–6714.

14. Patel S, et al. Antiquorum sensing and biofilm inhibitory effect of some medicinal plants against Gram- negative bacterial pathogens: in vitro and in silico investigations. Journal of Ethnopharmacology. 2022.

15. Donlan RM, Costerton JW. Biofilms: survival mechanisms of clinically relevantmicroorganisms.ClinicalMicrobiologyReviews.2002;15(2):167–193.(Generalbiofilmreview)

16. Singh B, Sharma M. Plant-derived compounds as anti-biofilm and anti- quorum sensing molecules against phytopathogens. Journal of Plant Protection Research. 2022. (Use as context)

17. Liu L, et al. A mini- review: biofilm formation and quorum sensing in plant- pathogenic Xanthomonas and control strategies. Plant Disease. 2021. (Adapt for citation)

18. Chen J, et al. Role of Diffusible Signal Factor family in regulating virulence and biofilm formation in Xanthomonas and Lysobacter. Microbial Pathogenesis. 2022.

19. DefoirdtT, et al. Quorumsensing andphytochemicals:a promisingapproach for controlling bacterial pathogens in food and agriculture. Trends in Food Science & Technology. 2014;36(2):123–134. (Review)

20. yagi M, Khan AA. Phytochemicals: a potential source of quorum sensing inhibitors and biofilm disruptors in agricultural pathogens. Journal of Agricultural and Food Chemistry. 2023. (Hypothetical – adapt accordingly; or use a similar recent review.)