ISSN: 2347-4688, Vol. 10, No.(3) 2022, pg. 327-339

Current Agriculture Research Journal

www.agriculturejournal.org

Bio-Control of Citrus Canker: An Alternative to Chemical Based Treatment

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Abstract

The importance of the citrus crops is recognized throughout the world. Despite tremendous advancements in agricultural technology, citrus fruit production is still considered a significant challenge. Various biotic factors have trampled down the production rate of Citrus in many places. Among them, canker caused by *Xanthomonas axonopodis pv.* citri *(Xac)* and *Xanthomonas citri pv.* citri *(Xcc)* is of great importance. Infection caused by *Xac* leads to lesion development in leaves, fruits, and stem. Defoliation and early fruit drop can occur as a result of severe infection, resulting in a loss of fruit output. Preliminary management techniques involve quarantining and sanitizing. Chemical copper-based bactericides are frequently employed in places with large-scale production. However, the continued use of chemicals, on the other hand, has led to the evolution of resistant microorganisms and increased the rate of soil pollution. Various alternative strategies have been suggested to address this issue. This review will go over the advances in biocontrol agents that have been used to combat the disease.

Article History

Received: 26 October 2022

Accepted: 19 December 2022

Keywords

canker;
Contamination; Copper;
Defoliation; Disease;
Quarantining;
Xac; Xcc.

Introduction

Citrus economic value has risen dramatically in recent years. Its prominence stems from its demand in the processed juice market, as well as its application in medicine and the pharmaceutical industry. The presence of a diverse variety of secondary metabolites and essential bioactive compounds has eventually widened the scope of its production. According to the Food and Agricultural Organisation of the United Nations (FAO), 143755.6 thousand tonnes of citrus were produced worldwide in 2019. Asia is leading the market with production of over 28920.0 thousand tonnes of citrus fruits, followed by South American countries with an expected output of 20189.8 thousand tonnes.[1]

Citrus production still needs to be improved to meet the present and future demands around the world.

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Doi: http://dx.doi.org/10.12944/CARJ.10.3.16

Unfortunately, certain environmental and biological constraints are posing a threat to citrus cultivation. Citrus crops are plagued with viral, bacterial, and fungal diseases. These natural agents have become a severe headache for farmers as their management is strenuous and can be expensive in the worst-case scenario. Bacterial infections such as Citrus bacterial spot (*Xanthomonas euvesicatoria*), Citrus greening (*Candidatus liberibacter*), Citrus Black pit (*Pseudomonas syringae*), Citrus canker (*Xanthomonas spp*.) are some of the economically significant diseases. Among all of them, citrus canker caused by *Xanthomonasaxonopodis* is known to be the most feared one. It is endemic to Asian and South-East Asian countries and infects various citrus species.[2] *Citrus paradisi* (Grapefruit), *Citrus limon* (Lemons), *Citrus aurantifolia* (Mexican lime) are all particularly susceptible to Citrus canker disease. When conditions are ideal for bacterial growth and development, infection causes lesions on the leaves, fruits and stems, as well as defoliation and fruit drop.[3]

Although Citrus canker is associated mostly with the genus citrus, it has managed to infect many plants belonging to the family Rutaceae. Eradication and management of the disease rely on quarantining the healthy plants from the infected ones and planting disease-resistant varieties of crops. These techniques are helpful during initial exposure of the bacteria but are ineffective in the long run. Copper based bactericides are extensively used throughout the world as an effective technique to minimise infection.[4] Copper treatment reduces canker formation in leaves, fruits and increases yield by reducing premature loss of fruits. However, the excessive use of copper containing chemicals to treat diseases has created a new problem. At higher concentrations, copper is severely toxic to living organisms. Moreover, copper resistant microbes isolated from treated areas show higher resistance to multiple antibiotics.[5]

With recent technological advancements, numerous alternative techniques for reducing the use of copper as an antibacterial agent have been developed. Pesticide containing zinc provide comparable protection against canker even at lower metal concentration.[6] In field trials, nano-formulated zinc oxides have demonstrated superior performance to cuprous oxide.[7] Additionally, it has been discovered that hexanoic acid has low phytotoxicity and can effectively suppress the growth of *Xanthomonas*.[8] Besides using chemical toxins to regulate the growth and transmission of bacterial diseases in plants, many bio-formulations have been developed that can effectively deal with such problems. Bio control agents generally include microbial antagonists that has the ability to control plant pathogens either directly by producing metabolites or indirectly by eliciting plant defence response. Compared to the chemical bactericides used in the field, bio-control agents are more suitable because they are non-toxic by nature.[9] The detection of the canker is the first step in the overall process, followed by the application of biocontrol agents to various plant part. The current review will discuss about the bio-control agents used in the treatment of canker in Citrus plants.

Symptoms and Mode of Transmission

Citrus canker is highly prevalent in areas with warm temperatures and heavy rainfall. Symptoms can be generally observed in leaves, fruits and stems. At first, tiny blister like lesions can be visible, but as the infection progresses, 2 to 10 mm in diameter lesions start appearing within 7 to 10 days of initial infection.[3] Lesion in the leaf is visible from both sides. Young lesions appear yellow in colour, but as they progress, they become brown and corky. Yellow or brown blister-like lesions form on stems and twigs, becoming elevated and spongy over time. These spongy pustules grow into brown corky cankers and gradually darken and thicken. Under wet conditions, the lesion generally has a water-soaked appearance.[2]

Transmission of citrus canker occurs mostly through wind and rain. Heavy wind or rain can transmit the disease to nearby plants. After exposure, the bacterium can easily penetrate the plant through stomatal openings and wounds created by unfavourable environmental circumstances. Spread of the disease over long distances is also observed during storms, hurricanes and tornadoes.[4] Human activities such as transportation of infected fruit, leaves or twigs have resulted in the transmission of the disease to uninfected areas.

Effective Biocontrol Agents

Bacillus Spp.

The genus *Bacillus* is included in the group of bacteria that encourage Plant Growth (PGPB). They are known for producing a wide range of secondary metabolites that prevent infections caused by numerous bacterial and fungal strains. *Bacillus* strains such as *Bacillus subtilis*, B. amyloliquifaciens, B. *licheniformes* and B. *velezensis* have been extensively used by researchers to treat variety of plant diseases. Against canker-causing *Xanthomonas*, *Bacillus subtilis*, *Bacillus velezensis* and *Bacillus amyloliquifaciens* are the strains that have been widely used as a biocontrol agent.

Bacillus amyloliquefaciens for example have shown efficient suppression of canker when tested in navel orange leaves. The frequency and size of canker lesions decreased by about 77% in B. *amyloliquefaciens*- treated leaves compared to the untreated one.[10] Another study conducted by Daungfu11 also reported the effectiveness of crude and cell suspension extract of B.*amyloliquefaciens* in controlling canker in *Citrus aurantifolia* leaves. The study suggested that treatment with B. *amyloliquefaciens* can completely control the disease incidence on lime plants in greenhouse condition. Additionally, because *Bacillus* can create endospores, it can endure extreme environments for an extended period of time.

Many locations where streptomycin is continuously applied to treat canker have produced isolated strains of *Xcc* that are resistant to the antibiotics. Streptomycin works by preventing bacterial ribosomal subunit from doing their job. However, it has been demonstrated that point mutations in the bacterial *strB* gene and rpsL gene change the ribosomal proteins normal structure, conferring resistance to antibiotic streptomycin.[12] Nurul Islam[13] studied the impact of endophytic bacteria against wild and streptomycin resistant *Xanthomonas* strains. Two isolated bacteria TbL-22 and TbL-26 later found to be *Bacillus thuringiensis* inhibited both wild and streptomycin resistant strains of *Xanthomonas*. Ethyl acetate extract of Tbl-22 inhibited XccW3 (wild) and XccM6 (streptomycin resistant) strains with the highest zone of inhibition of 20.64± 0.96 and 19.91± 0.87 mm, respectively.

Potential of *Bacillus thuringiensis* in controlling canker has not been utilised to its fullest extent. In one study a microbial bioformulation composed of Pseudomonas, *Bacillus thuringiensis*, *Bauveria* and *Trichoderma* were applied on canker incidence. The bio formulation was found to be effective in field trials, although such trials cannot determine the precise part played by B. *thuringiensis* in suppressing the canker.[14]

Bacillus velezensis, another endophytic bacterium from the genus Bacillus, has demonstrated antimicrobial activity against both wild and streptomycin resistant *Xcc* strains. The MIC for streptomycin resistant (33.88 ±1.3mm) *Xcc* was found to be greater than that for wild strains (29.28±0.6 mm). The *Xcc* cell was distorted and lysed by the endophytic bacteria, thanks to the secretion of certain unknown antimicrobial chemicals.[15]

Despite the fact that copper-based bactericides are no longer favoured due to the emergence of copper-resistant microbes.[5] A number of recent studies have demonstrated the efficacy of using copper and microbes together to treat canker. Experiments have shown that combining *B.velezensis* cell free extract with 0.01% $CuCl_2$ provide better results in controlling citrus canker.[16]

Combination of *Bacillus subtilis* and copper to treat canker has also been reported. YE Ibrahim[17] reported decrease in *Xanthomonas* infection in citrus seedlings when treated with a formulation of Serenade® MAX containing 10^7 cells/ml^{-1} *Bacillus subtilis* QST 713. Combination of copper with Serenade® MAX showed 87.79% reduction in disease compared to 85.55% against Copper. Bioactive copper has the ability to induce SAR (Systemic acquired resistance) in plants by activating genes that encode the production of β-1,3 glucanase proteins, making it a preferred treatment option for Canker when combined with *Bacillus subtilis.*[18]

Even in direct *in-vivo* field trials, administration of *Bacillus subtilis* aqueous suspension (2.7× 10^9 cells/ml) on *Citrus aurantifolia* trees demonstrated a significant reduction in the presence of canker in the leaves. The quick colonisation of the leaf area

within 20 days of spraying leaves little chance for the *Xcc* to induce infection in the plant.[19]

Pseudomonas Spp

Pseudomonas species are gram negative rhizospheric bacteria that are well known for assisting plant growth and development. Pseudomonas is frequently used as a biocontrol agent throughout the world (Table 1). As an opponent to chemotherapy, different strains of Pseudomonas have already been used to kill plant pathogenic bacteria such as *Phytopthora infestans*, *Rhizoctonia solani*, *Botyris cinerea* and *Ralstonia solanacearum*.[20,21,22,23] Additionally, Pseudomonas increases plants ability to with stand biotic and abiotic stress and controls plants growth by producing Indole acetic acid, Hydrocyanic acid and siderophore.[21] These attributes of Pseudomonas make it a perfect choice to be used as a biocontrol agent against various plant diseases. Numerous earlier investigations have supported Pseudomonas spp. effectiveness against *Xanthomonas* strains. Ota[24] observed the potential antagonistic activity of Pseudomonas against citrus canker bacterium *Xanthomonas campestris* pv. *citri* both *in-vivo* and *in-vitro*. Two inhibitory substance CLP-3 and CLP-5 later isolated by TLC from the same experiment were assumed to be Phytoalexins.[25] When tested by the Agar plug method *Pseudomonas fluoresecence* successfully inhibited the growth of *Xanthomonas in-vitro*. Out of the four species of bacteria, the second largest zone of inhibition (14.77 mm) was observed for P. *fluorescens*.[26]

Table 1: Microbes used as Bio-control agents against *Citrus* Canker.

Sl. No	GENUS	SPECIES	CONDITION	PLANT	XANTHOMONAS strain	REFERANCE
1.	*Bacillus*	*Bacillus amyloliquefaciens* QC-Y	*In-vitro*	Navel orange leaves	*Xac*	Qian *et al.* 2021
		Bacillus amyloliquefaciens LE109	Greenhouse	*Citrus aurantifolia* leaves	*Xcc*	Daungfu *et al.* 2019
		Bacillus thuringiensis TbL-22,TbL-26	*In-vitro*, MIC	*Citrus* spp.	*Xcc*^{str}	Islam *et al.* 2019
		Bacillus velezensis	Greenhouse, MIC	*Citrus* spp.	*Xcc*^{str}	Rabbee *et al.* 2019
		Bacillus velezensis	*In-Vitro*	*Citrus aurantifolia* seeding and tree	*Xcc*	Sudyong *et al.* 2019
		Bacillus subtilis	*In-vivo*	*Citrus aurantifolia*	*Xcc*	Das *et al.* 2014
2.	*Pseudomonas*	*Pseudomonas*spp	*In-vitro*/ *in-vivo*	*Citraus* spp. leaves	*Xcc**	Ota, 1983a
		Pseudomonas fluorescence	*In-vitro*/ *in-vivo*	*Citrus limon* *Citrus aurantifolia*	*Xcc**	Kalita *et al.* 1996; Patel *et al.* 2020
		*Pseudomonas protegens*CS1 *Pseudomonas aeuroginosa*	*In-vitro*/in vivo	*Citrus limon* leaves	*Xcc*	Michavila *et al.* 2017 Rajesh *et al.* 2015

		Pseudomonas entomophila	*In-vivo*	*Citrus limon*	*Xcc*	Villamizar *et al.* 2020
		Pseudomonas putida, P.fluorescence	*In-vitro*	-	*Xac*	Badiger *et al.* 2016
		Pseudomonas geniculata	*In-vivo*	*Duncan grapefruit*	*Xcc*	Riera *et al.* 2018
3.	Kosakonia	*Kosakoniacowanii GN223*	*In-vivo*	*Naval orange*	*Xcc*	Jiahao *et al.* 2021
4.	Staphylococcus	Staphylococcus *pasteuri,* S. warnei	*In-vitro*	Rangpur lime	*Xcc*	Nugroho *et al.* 2022
5.	Burkholderia	*Burkholderiate rritorri* A63, B. *metallica* A53	*In-vivo*	*Duncan grapefruit*	*Xcc*	Riera *et al.* 2018
6.	*Bacteriophage*	*Bacteriophage* spp	*In-vivo*/ Field trials	*Citrus aurantifolia*	*Xcc*^{co}	Ibrahim *et al.* 2017
		Podophage, *Siphophage, T4 phage*	*In-vitro*	*Hamlin sweet orange*	*Xac*	Le (2019)
		Filamentous *phage XacF1*	*In-vitro*	-	*Xac*	Ahmad *et al.* 2014

Note: *Xac*: *Xanthomonas axonopodis* subp.*citri*., *Xcc*: *Xanthomonascitri su*bp. citri.,*Xcc**: *Xanthomonas campestris subp. citri.*, *Xcc*^{str}: Streptomycin resistant *Xanthomonas citri* subp. *citri*.,*Xcc*^{Co}: Copper resistant *Xanthomonas citri* subp. *citri*.

Recent discoveries of numerous novel Pseudomonas strains may offer fresh information in this area. The use of *Pseudomonas protegens* as a biocontrol agent against *Xcc* is relatively very new. This species CS1 strain generates the pyochelin enantiomer and ROS necessary to block *Xcc* in both *in-vitro* and *in-vivo* conditions.[27] *Pseudomonas entomophila*, a powerful pesticide, inhibits *Xcc* by creating certain secondary metabolites. The species was found to cure canker completely in *in-vivo* conditions within 21 days of inoculation.[28]

Rajesh[29] evaluated the antagonistic activity of six Pseudomonas spp. against *Xanthomonas axonopodispv. citri.* P. *aeruginosa* Rambhas-2 (PaRS) showed the maximum zone of inhibition (18.67mm) followed by P. fluorescens Navs`ari-2 (PfNC) and P. *aeruginosa* Navsari-1 (PaNS). Least inhibition (9mm) was observed in the isolate P. *fluorescens* Rambhas-1 (PfRB). The antagonistic activity was assumed to be due to the production of secondary metabolites or cell wall degrading enzymes.

Recent *in-vitro* experiments, however, revealed that *Pseudomonas fluorescens* efficacy was much inferior to that of *Bacillus subtilis.* Badiger[30] noted that *Bacillus subtilis* had a substantially higher MIC against Xac (16.16mm) then did *Pseudomonas fluorescens* (14.63mm) and *Pseudomonas putida* (7.42mm).

In a different, opposing report, *Pseudomonas fluorescens* administration in an *in-vivo* setting resulted in decrease in disease spots on *Citrus aurantifolia* leaf and fruit. The outcome was found to be comparable to chemical canker control (streptomycin sulphate + copper oxychloride).[31] Application of *Pseudomonas geniculata* strain 95 in Duncan grapefruit root reduces *Xanthomonas* infection by increasing the expression of salicyclic acid genes such as PR1, PR2, PR5 and SAM-SCAM and reactive oxygen species in aerial tissues.[32]

Other Endophytic Bacteria

A gram-negative endophytic bacterium called *Kosakonia cowanii* has recently been identified

as the disease-causing agent in Soybean plants (*Glycine max*). Although, the presence of these bacteria in environment could be dangerous, it is also known that non-pathogenic strains of these bacteria exist in nature.[33] The effectiveness of this bacterial species as a biocontrol agent is less known to the scientific community. When tested against Citrus canker on adult trees, the strain *Kosakonia cowanii* GN223 inhibited *Xcc* growth on seedlings and Navel orange by 40.0% and 50.15 respectively. The effectiveness of GN223 in reducing disease severity was found to be equivalent to copper hydroxide treatment.[34] The endophytic bacteria *Kosakonia cowanii* strain GN223 inhibits citrus canker formation by inducing a host defensive enzyme. GN223 has been shown to enhance the activity of catalase (CAT) and peroxidase (POD), effect of which can significantly decrease the prevalence of Canker in plants.[35]

Staphylococcus species, *Staphylococcus pasteuri* and Staphylococcus warnei are effective in preventing the formation of *Xcc*as revealed by *in-vitro* studies and *in-vivo* studies as well. The bacteria produce certain unknown secondary metabolites that are toxic to *Xcc*. This can be confirmed by cell free supernatant (CFS) treatment, which showed clear MIC of 7.23mm and 6.22mm against *Xcc*. The CS and CFS extracts of the bacterial strains also significantly decreased 50% of the canker infection in leaves within 28 days of inoculation.[36]

When sprayed to the roots, two novel rhizobacterial strain *Burkholderia territorri* and *Burkholderia metallica* enhances plant defence response against Citrus canker.[32] Due to their innate tendency to induce infections like Cystic fibrosis and pneumonia in immuno-compromised humans, the usage of these bacterial strains are restricted.[37]

Bacteriophage Virus

Bacteriophage viruses are natural bacteria killer. In a number of investigations, the bacteriophage has been shown to be effective in managing variety of plant disease in addition to citrus canker. However, the main problem in utilising bacteriophage is their inability to survive long enough in plant surface mostly because of their low tolerance level in UV light. However, it has been demonstrated that microencapsulated bacteriophages exhibit greater tolerance to changing pH, UV and temperature conditions.[38] Formulating the bacteriophage with Riboflavin, ascorbic acid or skimmed milk drastically reduces the effect of UV light on the virus.[39] Formulated bacteriophages have been used extensively to treat Citrus canker with great success. Administration of Bacteriophage in combination with acibenzolar-S-methyl (ASM) reduced the incidence of Asiatic Citrus canker (ACC) on leaves from 75.2% to 18.3% in greenhouse condition. Field trials also showed similar results. Application of formulated phage (to protect them from environmental damage) with ASM showed higher inhibition of Citrus canker by 82-86% 40. However, in a contrasting report published by Balogh[41] it was shown that formulation of phage (with skim milk) compromised the effectiveness in treating Citrus canker. When phagesare administered without skim milk, they significantly reduce disease severity.

A growing trend is phage therapy, which involves using the bacteriophage virus to treat bacterial diseases. Bacteriophages are now a more effective treatment option for citrus canker than chemotheraputic methods thanks to successful *in-vitro* studies of phages against the condition.[42] In his research Le,[43] isolated three bacteriophage that were successful in preventing canker. KMV-Like podophages, siphophage and T4 like phage were identified as the isolated phages. Phage cocktail comprising Podophage and siphophage when applied on Hamlin sweet orange leaves showed reduction in canker lesion. According to the *in-vitro* research, pre-treating leaves with Phage cocktail yielded greater result than post infection therapy, with canker reduction rates of 52.7% and 47.4% respectively.

Filamentous phage XacF1 inhibits canker expansion by drastically reducing extracellular polysaccharide production in host cells. Reduction in motility of XacF1-infected host bacterial cells was also observed.[44]

Plant Based Extracts/oils

People utilise plant extracts and oils extensively because of their medicinal and flavouring benefits. The extracts/ oils of root, leaf, stem, flower and other parts of the plants contain a variety of bioactive compounds such as phenols, terpenes, alkaloids etc.[45] The presence of these bioactive compounds

has revealed the antibacterial, anti-pesticidal and anti-insecticidal potential of plant-based product.[46,47,48] The use of these plant-based solution is more appropriate since, unlike bacterial or fungal bio-control agents, they have no known negative effects on people or the plant itself.

Both neem (*Azardiricta indica*) extract and neem oil have numerous uses. The existence of various secondary metabolites in neem extracts can be used to explain their appeal as an antibacterial agent.[49] *In-vitro* tests have suggested that, both aqueous and alcoholic neem leaf extract have potential to be used against various strains of *Xanthomonas* that cause citrus canker,[50,51] When applied externally to plant infected with *Xanthomonas*, the oil extracted from neem significantly reduced canker incidence, demonstrating its *in-vivo* anti canker potential.[52] Streptomycin combined with *A.indica* also effectively combats citrus canker in green house conditions. Reduction of disease can be observed within 45 days of administration.[53]

Both *in-vitro* and *in-vivo* studies on the treatment of canker with alcoholic and aqueous extract of Allium cepa showed a reduction in canker incidence.[50,54] In comparison to numerous chemical alternatives, like streptomycin, copper oxychloride and validamycin, onion extract is found to be superior. Interestingly, applying onion extracts to fruit and leaf surface produces superior outcomes for treating canker than do *Bacillus* species.[52]

Except neem oil as discussed above, oil extracted from Ginger (*Zingiber officinale*), Common yarrow (*Achillea millefolium*), Common sage (*Saliva officinales*), Summer savory (*Satureja hortensis*) and True *car*damom (*Elettaria cardamomum*) are equally effective against *Xcc* patho type A* under greenhouse and laboratory conditions.[55] Essential oil extracted from Citrusaurantium and C. *aurantifolia* also inhibit canker by damaging cell wall of *Xcc*. The presence of secondary metabolites such as α-terpineol, citronellal, geraniol and linalool may be the cause behind its antibacterial effect.[56] Clove essential oil can be recommended as an alternative sanitisation product for decontamination of citrus fruits. The anti-bactericidal effect of clove oil against *Xcc* can be explained by the presence of high concentration of eugenol in it.[57]

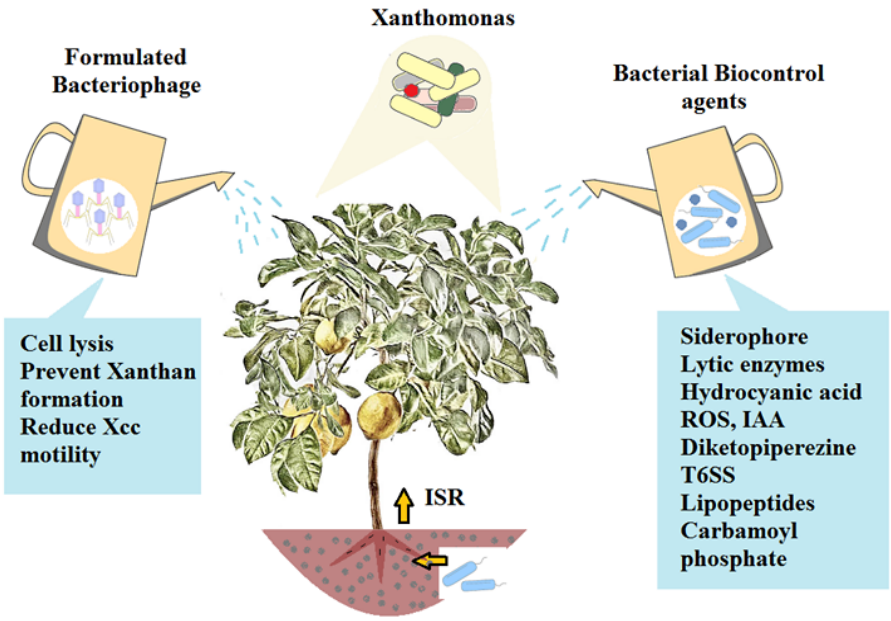

Fig.1: Mechanism of action of biocontrol agents in controlling canker.

Mechanism of Action of Biocontrol Agents Against Xanthomonas

Bacillus and Pseudomonas are regarded as plant rhizospheric bacteria. They are known to secret a variety of metabolites and substances that either directly or indirectly aid in plant growth as shown in Fig.1. Both species of bacteria produces Siderophores, IAA, lytic enzymes, organic acid, oxalate oxidase and Hydrocyanic acid.[21,58,16] Particularly crucial are the siderophores made by rhizospheric bacteria because they aid in chelating Fe3+ from environment and make it available to plant cells, rendering them inaccessible to pathogenic bacteria.[44] *Pseudomonas protegens* produces a major siderophore called pyochelinenetiomer and also ROS in plants infected with *Xcc.*[59] *Bacillus velezensis* produces high amount of Siderophore and IAA against *Xcc in-vitro*.[16]

Bacillus and Pseudomonas also increases ISR (Induced systemic resistance) in plants infected with *Xanthomonas* (Fig.1). As discussed earlier, exposure of plant with *Xcc* induces P. *geniculata strain* to increases expression of genes such as PR1, PR2, PR5, SAM-SCAM and Phenylalanine ammonia lyase 1 which are related to Salicylic acid signalling pathway (Riera *et al.* 2018). Pseudomonas spp. also produces different antimicrobial compounds against Bacteria. One such example is the production of diketopiperezine and T6SS (Type 6 secretion system) by *P. entomophila*.[28] The T6SS is an essential tool of gram-negative bacteria to deliver toxic compounds in host cell and subsequently plays important role in inter-bacterial competition in environment.[60]

In addition to ISR, *Bacillus* also produces lipopetides such as surfactins, iturin and fengycin. Lipopeptides mode of action against bacteria includes cell wall disruption and pore formation. These lipopeptides can further be classified into a number of sub classes, and each has a unique mode of action against variety of microbes.[61,11] *Bacillus amyloliquefaciens* for example produces Iturin A/ Mycosubtilin, Iturin B, Surfactin A/B and Fengycin A/B against *Xanthomonas* spp.[62] *Bacillus velezensis* show antagonistic activity against *Xanthomonas* strains by releasing four antimicrobial compound bacillibactin, fengycin, surfactin and bacillomycin D.[63]

Inhibition of *Xanthomonas* quorum sensing by *bacillus* and pseudomonas species have also been reported recently. Quorum sensing is mediated by DSF (diffusible signal factor), which is released by *Xcc* and encoded by the rpfgene cluster. The bacteria need this cell-cell signalling pathway in order to be virulent. *Bacillus* and Pseudomonas species contains genes homologous to *car* A and *car* B that encode DSF degrading enzyme car bamoylphosphate synthase, which allows them to successfully inhibit *Xcc* Quorum sensing.[64]

The mechanism of action of Virus on the other hand is different from the techniques we have discussed earlier. After infecting bacteria, a virus passes through either a lytic or lysogenic cycle depending upon environmental condition. To infect virus uses receptors preset on Gram negative and gram-positive bacteria. Transmembrane protein OmpA, Porins such as OmpC /OmpF, pili, flagella and lypopolysaccharide serve as receptor for the attachment of bacteriophage virus in bacteria.[65] Against *Xanthomonas* spp. Bacteriophage FoX2 and FoX6 recognise specific lipopolysaccharide present on the surface of the bacterial cells wall.[66] In lytic cycle, the bacteriophage initially adheres to the surface of the host bacteria before injecting its genetic material into the cell. Virus after producing new virions by hijacking host cells replicative machinery, brusts out to release the new virions. In lysogenic cycle, the genetic material of bacteriophage after insertion into host cell gets integrated with the host cell chromosome. The bacterial cell reproduces normally along with phage genetic material.

The production of antimicrobial peptide is a quality of bacteria but not virus. Since virus contains no known mechanism to produce proteins by itself so such findings are very rare. However, in one recent study, a novel *Xanthomonascitri* infecting jumbo virus XacN1 has been isolated which encodes some special lytic enzymes such as lipases, chitinase, cell wall hydrolase and M23 family peptidase (ORF118, ORF322, ORF423 and ORF322) that break slimy polysaccharide matrix (xanthan gum) produced by the bacteria.[67] The Filamentous phage XacF1 similarly aims to prevent Xanthan formation. The bacterium needs Xanthan to endure biotic and

abiotic stress. Inability to produce xanthan by Xac leads to the cessation of Citrus canker.[68]

Acknowledgement
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Funding
The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflitct of Interest
Authors have no Conflict of Interest.

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