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**Review Article**

# Biological Management of Major Citrus Diseases in Central India–A Review

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**A B S T R A C T**

Citrus occupies an important place in the horticultural wealth and economy of India as the third largest fruit industry after mango and banana. Citrus production in the country is 8.7 million tonnes at present with world ranking at sixth position after China, Brazil, USA, Spain and Mexico. Many fungal, bacterial and viral diseases that threaten citrus crops but some fungal disease caused by *Phytophthora* spp. and bacterial citrus canker caused by *Xanthomonas axonopodis* pv. *citri* are the most devastating in central India. The *Phytophthora* spp. includes *P. parasitica*, *P. citrophthora* and *P. palmivora* are responsible for root rot, gummosis and collar rot in citrus of central India. At least 3 distinct forms or types of citrus canker are recognized among these, Asiatic form (Canker A) is the most destructive and affects most of the major citrus cultivars in our region. Some potential biological agents investigated for control of these dangerous diseases. *Trichoderma* spp., *Pseudomonas fluorescens* and *Bacillus subtilis* were found effective in management of these diseases.

**Keywords**

Citrus, *Phytophthora* spp., Diseases, Canker, Biological agents.

## Introduction

The genus Citrus is one of the most important group of fruit crops worldwide, belongs to the family Rutaceae comprising 140 genera and 1300 species distributed throughout the world (Saunt, 1990; Savita *et al.*, 2012). It is a long-lived perennial crop and is grown in more than 100 countries across the world. Among the various fungal, bacterial and viral diseases few diseases in any citrus growing area cause significant damage and require due attention for their effective management. In consideration of all fungal diseases of citrus *Phytophthora* spp. are the causal agents of several serious diseases of citrus in India. *Phytophthora parasitica*, *P. citrophthora* and *P. palmivora* have been mostly involved in causing damping off, collar rot and root rot in citrus (Naqvi, 1988; Bowman *et al.*, 2007; Shekari *et al.*, 2012; Gade 2012). *Phytophthora* spp. causes foot rot, root rot, crown rot, gummosis, leaf fall, and brown rot diseases in Nagpur mandarin (Gade *et al.*, 2006). However, *Phytophthora parasitica* and *P. palmivora* are the most prevalent species in citrus orchards of Vidarbha region (Das *et al.*, 2011). Citrus canker is one of the most feared of citrus diseases, affecting all types of important citrus crops. The disease causes extensive damage to citrus and severity of this infection varies with different species and varieties and the prevailing climatic conditions. The disease is endemic in India, Japan and other South- East Asian countries, from where it has spread to all other citrus producing continents except Europe. Generally canker does not occur in arid citrus growing areas and has been eradicated from some areas. However, widespread occurrence of the disease in many areas is a continuous threat to citriculture especially in canker free areas. Intensive research on citrus canker is being carried out throughout the world which has been reviewed by Rossetti (1977), Civerolo (1981, 1984), Chand and Pal (1982), Schoulties *et al.*, (1987), Stall and Civerolo (1991) and Goto (1992). However, all these reviews are either brief, restricted to one country, or by now out of date. This review aims to present an overview of citrus canker worldwide with special reference to India.

The several effective chemical management practices are available for management of these epidemic diseases but in view of organic farming and sustainable agriculture several potential biological control agents have been investigated for control of *Phytophthora* diseases and citrus canker. Several studies found that integration of biological agents with fungicides was effective for management of *Phytophthora* spp. *Trichoderma harzianum, T. virens, Pseudomonas fluorescens, Chaetomium globosum, Gliocladium virens* and *Bacillus* spp. were found to be effective against *Phytophthora* in *Citrus* sp. (Heller and Hedtrich, 1994; Abraha 2005; Gade, 2012). Whereas, studies on biological control of citrus canker are still in a preliminary stage. Study on biological control using a bacterial inhibitor *viz*. *Bacillus subtilis* has been quite successful (Das *et al.*, 2014). Some strains of bacteria *viz.*, *Pseudomonas syringae*, *Erwinia herbicola*, *Bacillus subtilis* and *Pseudomonas fluorescence* isolated from citrus phylloplane were reported to be antagonistic *in vitro* to the canker pathogen (Goto *et al.*, 1979; Ota, 1983; Unnimalai and Gnanamanickam, 1984; Kalita *et al.*, 1996).

## Citrus diseases caused by *Phytophthora* spp.

*Phytophthora* spp. causes the most serious and economically important soil borne diseases of Citrus crops. *Phytophthora parasitica*, *P. citrophthora* and *P. palmivora* have been mostly involved in causing damping off, collar rot and root rot in citrus (Naqvi, 1988; Gade, 2012).

## History and distribution

In 1836, large scale destruction of citrus plants because of *Phytophthora* infection was recorded in Azore Island, much before the potato famine of Ireland in 1845, and after 31 years of famine Anton de Bary discovered the fungus as *Phytophthora* means “plant destroyer”. The first appearance of *Phytophthora* epidemic of citrus was reported in Azore Island during 1832-1836, (Bonawia, 1988). An extensive survey of citrus orchards and citrus nurseries in India has been conducted under National Network Project on *Phytophthora* disease of Horticulture Crops. Citrus cultivation belt of Vidarbha and Marathwada region of Maharashtra, Punjab, Madhya Pradesh, Andhra Pradesh and North Eastern States of India were surveyed to assess the impact of *Phytophthora* diseases. Two mating type (A1 and A2) of *Phytophthora parasitica* (*nicotianae*) have been recorded from the orchards of Nagpur mandarin in Nagpur district, (Naqvi, 2000; Naqvi, 2002). In Madhya Pradesh adjoining to Vidharbha region of Maharashtra, India, 20-50% Nagpur mandarin plants were found to be affected resulting in severe decline due to *P. parasitica*, *P. citrophthora* along with *P. palmivora* (Naqvi, 2000; Gade, 2012).

## Symptomatology

*Phytophthora* spp. cause foot rot, root rot, crown rot, gummosis, leaf fall, and brown rot diseases in Nagpur mandarin (Gade *et al.*, 2006; Savita *et al.*, 2012). *Phytophthora* causes the following common diseases in citrus cultivars.

## Damping-off

Damping-off of *Citrus* seedlings is caused by *Phytophthora parasitica*, *P. citrophthora* and *P. palmivora.* Typical symptoms of damping-off result when the soil-borne fungus penetrates the stem just above the soil line and causes the seedling to topple. *Phytophthora* spp. also causes seed rot or pre-emergence rot. Infected seedlings are killed rapidly when moisture is abundant and temperatures are favorable for fungal growth (Naqvi, 2000).

## Foot rot and gummosis

Foot rot results from an infection of the scion near the ground level, producing lesions which extend down to the bud union on resistant rootstocks (Fawcett, 1936). When susceptible rootstocks are used scaffold root rot or crown rot below ground may occur. Infected bark remains firm with small cracks through which abundant gum exudation occurs. Citrus gum disappears after heavy rains but remain persistent on the trunk under dry conditions. Lesions spread around the circumference of the trunk, slowly girdling the tree (Timmer and Menge, 1993).

## Root rot

*Phytophthora* spp. infects to the root cortex which starts decaying of fibrous roots. The cortex turns soft, becomes somewhat discolored and appears water soaked. After severe infection cortex of fibrous roots get destroyed leaving only the white thread-like stele, which gives the root system a stringy appearance. This results in the reduction of fruit size and production, loss of leaves and twig dieback of the canopy (Timmer and Menge, 1993).

## Brown fruit rot

When fruits are infected by Phytophthora, it produces a decay in which the affected area is light brown, leathery and not sunken compared to the adjacent rind. Under humid conditions, white mycelium grows on the rind surface.

In the orchard, fruits near the ground become infected when splashed with soil containing the fungus. In storage, infected fruit have a characteristic pungent, rancid odour. Brown rot epidemics are usually restricted to areas where rainfall coincides with the early stages of fruit maturity (Timmer and Menge, 1993).

## Disease cycle and epidemiology

The fungus primarily lives and reproduces in soil and usually attacks susceptible plants at or below the soil line. Sometimes, however spores of the fungus may be splashed into injured above the ground bark of low lying leaves and fruits and cause subsequent infections at these points.

The fungus overwinters in infected tissues as chlamydospores. Chlamydospores germinate in response to nutrients from roots to form sporangium that liberates zoospores, which finally infects the host plant. *Phytophthora citrophthora* grows best at 24-28$^0$ C while *P. nicotianae* 28-32$^0$ C. Prolonged humid weather favorable temperature results in disease outbreak (Naqvi, 1999; Anadraj *et al.*, 2012).

## Mode of spread of *Phytophthora* spp.

The primary means by which *Phytophthora* spp. are spread through citrus orchards is by use of infected nursery stock, (Naqvi, 2000). Mechanisms of dispersal of *Phytophthora* species were reported (Ristaino and Gimpertz, 2000) *viz.* (i) dispersal from root to root in soil involves either root growth to inoculums, inoculum movement to roots or root to root contacts. (ii) Inoculum dispersal in surface water. (iii) Splash dispersal from soil to aerial parts of the plant. (iv) Aerial dispersal from sporulating lesions on leaves, stem, fruits or other aerial parts of the plant and (v) dispersal by human or invertebrate activities including movement of soil, plants or propagules.

## Biological management of *Phytophthora* spp.

Several studies reported some potential biological agents for *Phytophthora* management in citrus. Gade (2012) reported that, *in vitro* antagonism showed by *Trichoderma harzianum* and *T. virens* (84.96%) suppress *P. parasitica* significantly. Intensity of antagonism was different as per medium but, there was a continuous reduction in pathogen population from 41 to 8 propagules/g soil with reduction in root rot /collar rot in *Citurs jambhiri*. All Thirty seven native isolates of *Pseudomonas* spp. were found positive for production of IAA, HCN and Siderophore. Pf XXVI (16.80%) and Pf IV (24.10%) were found effective to manage the disease in addition to increased growth response under glass house condition. Biochemically efficient strain of *Pseudomonas fluroescens* Pf IV was found effective to arrest the percent mycelial growth (55.20%) of *P. parasitica* (Gade and Armarkar, 2011). Gade and Koche (2012) reported that, *Pseudomonas fluroescens* in combination with fungicides and organic amendments (module IV) was found effective in management of root rot and gummosis in Nagpur mandarin. There was consistent reduction in population density from 24.33 cfu/cc soil in May 2008 to 6.20 cfu/cc soil in May 2010 of *Phytophthora* spp. in module IV. Significant decrease in intensity of root rot and gummosis was recorded where integrated management module (module IV). Root rot intensity was reduced in this module from 36.18 % initially to 16.70 % at the end of the experiment. Similarly, significant reduction in gummosis up to 54.76 per cent was recorded in the same module. *Trichoderma viride* inhibits highest mycelial growth of *Phytophthora paracitica* (75.33%) *in vitro*, whereas, under glass house experiment combined application of *Trichoderma viride* @ 4g/Kg +Garlic clove extract @ 5% significantly reduced percent root rot incidence (11.32%) as compared to untreated control (44.99%) (Pente *et al.*, 2015). Lende *et al.*, (2015) found significant increase in shoot length (23.55 cm) and canopy volume of tree (10.89%) in Nagpur mandarin when bioagent *T. harzianum* incorporated in combination with chemicals and organic amendments and also found significant reduction in root rot intensity. *In vitro* studies of Heller and Hedtrich (1994) suggested that, *Chaetomium globosum, Gliocladium virens* and *Trichoderma viride* are effective inhibitors of all the *Phytophthora* spp. tested including *P. nicotianae*. Greenhouse studies conducted by Abraha (2005) showed that, the *Bacillus* isolates and some *Trichoderma* isolates suppressed *Phytophthora* root rot disease.

## Citrus canker

Citrus canker is one of the major citrus disease which affects all types of important citrus crops. The disease is endemic in India, Japan and other South-East Asian countries, where it has spread to all other citrus producing continents except Europe. However, widespread occurrence of the disease in many areas is a continuous threat to citriculture especially in canker free areas.

## Forms

There are three different forms of citrus canker disease caused by various pathovars and variants of the bacterium *Xanthomonas axonopodis* Starr. and Garces emend. Vauterin *et al.,* (1995). Differentiation of these forms is mainly based on geographical distribution and host range of the pathogen (Stall and Seymour, 1983). The Asiatic form of canker (canker *A. cancrosis* A or true canker), caused by *X. axonopodis* pv, citri (Hasse) Vauterin *(Xac)* is the most common, widespread and severe form of disease (Gopal *et al.,* 2012).

## History and distribution

The geographical origin of citrus canker is a matter of controversy. Lee (1918) reported that, it may have arisen in Southern China, and he assumed *Fortunella hindsii* to be the wild host plant. However, Fawcett and Jenkins (1933) reported that citrus canker originated in India and Java, rather than in other regions of the Orient, because they detected canker lesions on the oldest citrus herbaria kept at the Herbaria of the Royal Botanic Gardens in Kew, England (*i.e.*, *Citrus medica* collected from India in 1827-1831 and *C. aurantifolia* from Indonesia in 1842-1844). These findings suggest the origin of disease in the tropical areas of Asia, such as South China, Indonesia, and India, where Citrus species are presumed to have originated and to have been distributed to other citrus- growing areas in the form of bud wood. In India, citrus occupies third position among fruits after mango and banana and canker is one of the major constraints of its cultivation. Citrus canker was first reported from Punjab (Luthra and Sattar, 1942; Bedi, 1961). Its occurrence was further recorded in Tamil Nadu (Ramakrishnan, 1954). Several others have reported the incidence of canker on the acid lime and other varieties of citrus. Further, the disease appear as a serious problem where ever acid lime (*C. aurantifolia*) is grown on a large and commercial scale (*e.g.*, Akola region in central India, Nellore and Periyakulum regions in southern India and Khera region of western India) and has become a permanent major problem to the citrus growers of this country. Recently canker has been detected in kinnow mandarin nursery in the state of Punjab (Anonymous, 2000).

## Symptomatology

In India, citrus canker is reported to be relatively more on acid lime and less commonly on mandarin and sweet orange (Ramakrishnan, 1954). The diseased plants are characterized by the occurrence of conspicuous raised necrotic lesions that develop on leaves, twigs and fruits. Lesions can be detected by drawing the fingers over the surface of infected tissues. On leaves, first appearance is as oily looking, 2-10 mm circular spots, usually on the abaxial surface (reflecting stomatal entry following rain dispersal).

Lesions are often similarly sized. Later, both epidermal surfaces may become ruptured by tissue hyperplasia induced by the pathogen. On leaves, stems, thorns and fruit, circular lesions become raised and blister-like, growing into white or yellow spongy pustules. These pustules then darken and thicken into a light tan to brown corky canker, which is rough to the touch. Often a water- soaked margin develops around the necrotic tissue and is easily viewed with transmitted light. On stems, pustules may coalesce to split the epidermis along the stem length, and occasionally girdling of young stems may occur. Older lesions on leaves and fruit tend to have more elevated margins and are at times surrounded by a yellow chlorotic halo (that may disappear as canker lesions age) and a sunken center. Sunken centers are especially noticeable on fruits, but the lesions do not penetrate far into the rind thereby not affecting internal quality.

Severe infection results in defoliation, die-back, deformation of fruit and premature fruit drop (Rossetti, 1977; Stall and Seymour, 1983). Canker causes fruit losses ranging from premature fruit drop due to abscission to non-marketable quality due to lesions. Disease of the fruit is probably the most economically important damage since fruits with canker lesion are not acceptable for fresh market and fetch very little price. An essential diagnostic symptom of the disease is citrus tissue hyperplasia (excessive mitotic cell divisions), resulting in cankers (Gabriel *et al.,* 2000; Gade and lad, 2016).

## Disease cycle and epidemiology

### Survival

*Xac* survives primarily in naturally occurring lesions. Cankerous leaves, twigs and branches constitute the main source of inoculum. Since affected leaves drop early, they may not serve as the main source of inoculum (Nirvan, 1963), but Rao and Hingorani (1963) found that the bacterium survives upto 6 months in the infected leaves. The disease is carried from season to season mainly in the cankers on twigs and branches. The pathogen can survive in diseased twigs upto 76 months (Chakravarti *et al.,* 1966).

### Infection

Bacterial cells ooze from existing lesions during wet weather to provide inoculum for further disease development. Infection by *Xac* occurs, like many other bacterial diseases, primarily through stomatas, and wounds produced during strong winds and by insects. Presence of free moisture on the host surface for 20 min. is essential for successful infection (Ramakrishnan, 1954).

### Dispersal

Since Xanthomonads have mucilaginous coat, they easily suspend in water and are dispersed in droplets. Spread of canker bacteria by wind and rain is mostly over short distances, *i.e.,* within trees or to neighbouring trees. Cankers develop more severely on the side of the tree exposed to wind-driven rain. Rainwater collected from foliage with lesions contains bacterial population between 105-108 cfu/ml (Goto, 1962; Stall *et al.,* 1980). If the average wind speed during rains exceeds 8 m/sec (18 mph), the disease may be very severe (Kuhara, 1978). Wind-blown inoculum was detected upto 32 meters from infected trees in Argentina (Stall *et al.,* 1982). Spread over longer distances, up to 7 miles, can occur during severe tropical storms, hurricanes, and tornadoes (Gottwald *et al.,* 2001).

Temperature between $20^0$C to $30^0$C with evenly distributed rains are most suitable for the disease (Ramakrishnan, 1954).

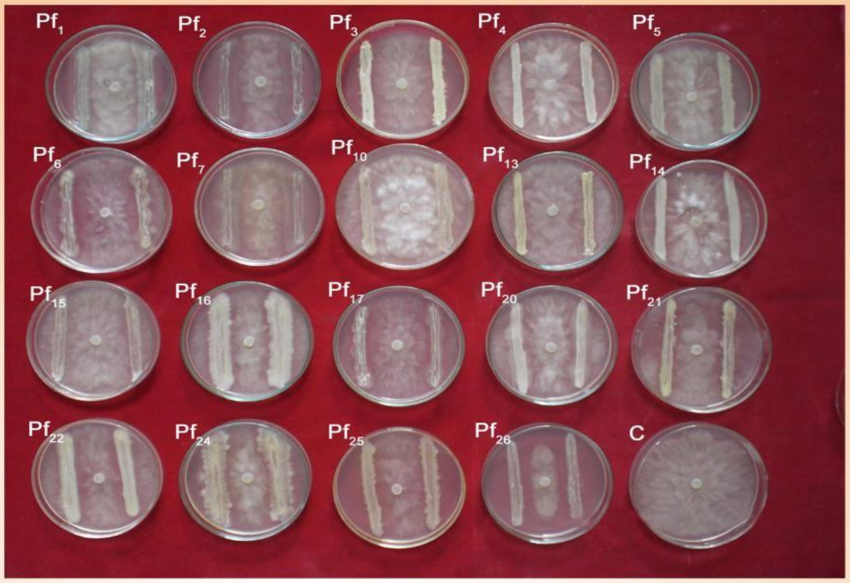
### Leaf miner interaction

The Asian leaf miner, *Phyllocnistis citrella* Stainton, can infest leaves, stems, and fruit and greatly increase the number of individual lesions which quickly coalesce and form large irregular shaped lesions that follow the outlines of the feeding galleries.

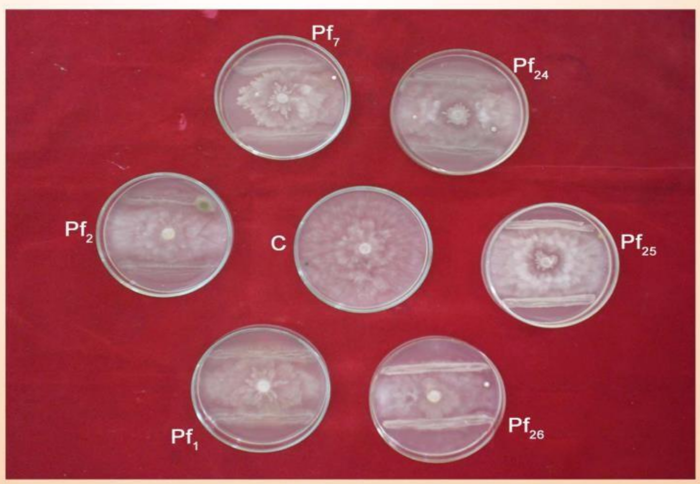
**Gummosis**

**Yellowing of leaves and drying of branches due to root rot**

**Plate1. Symptoms of Gummosis and Root rot of citrus caused by *Phytophthora* spp.**




Simultaneous inoculation of *Pseudomonas fluorescens* and *Phytophthora parasitica*




Inoculation of *Phytophthora parasitica* 3 days prior to *Pseudomonas fluorescens*

**Plate2. Efficacy of *Pseudomonas fluorescens* against *Phytophthora parasitica* by dual culture technique (Gade and Armarkar, 2011).**

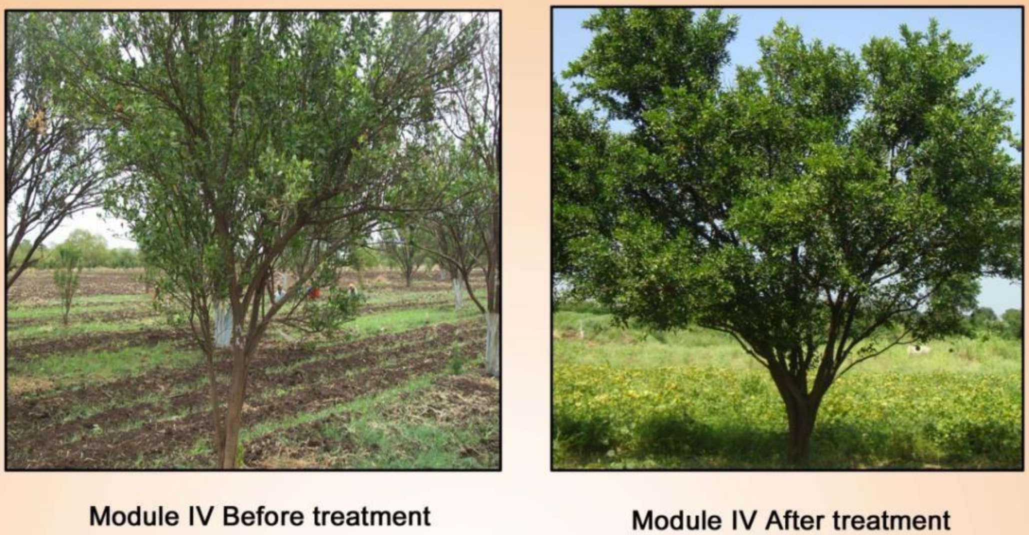



**Plate3. Effective manage ment of root rot and gummosis in Nagpur mandarin with *Pseudomonas fluroescens* in combination with fungicides and organic amendments (module IV) (Gade and Koche, 2012)**

Leaf miners wound leaves when they begin feeding. The feeding galleries are just below the epidermis. When the galleries become contaminated with citrus canker bacteria, numerous infections can occur, resulting in tremendous inoculums production and canker infection (Cook, 1988). Trees with wounds caused by leaf miner remain susceptible for 7-14 days compared to only 24 hours for wounds caused by wind, thorns or pruning (Filho and Hughes, 2000).

## Biological management of citrus canker

Studies on biological control of citrus canker are still in a preliminary stage. Some strains of bacteria *viz.*, *Pseudomonas syringae*, *Erwinia herbicola*, *Bacillus subtilis* and *Pseudomonas fluorescence* isolated from citrus phylloplane were reported to be antagonistic *in vitro* to the canker pathogen (Ota 1983; Goto *et al.*, 1979; Unnimalai and Gnanamanickam, 1984). However, it seems difficult to find antagonistic bacteria that reside stably on smooth surfaces of mature citrus leaves.

An experiment was set up by Das *et al.*, (2014) in a farmer's field (acid lime orchard) using an inhibitory strain of *Bacillus subtilis* (S-12) showed that, single spray of aqueous suspension (2.7 x $10^9$ cells/ml) of bacterial cells was spread on 5 batches (6 numbers of plants/batch) of plants keeping 4 batches unsprayed. Per cent Disease Index (PDI) was recorded throughout the year at every month. Initial PDI was also taken before one week of spraying. A single spray of the bacterial suspension during the peak season for disease that is in July has resulted in a satisfactory decline of the disease. A sharp decline of the disease was recorded at 20 days after treatment indicating that the spore forming bacteria might have taken over on the leaf surfaces of the plants. Kalita *et al*., (1996) reported that, among the tested species of bacteria *viz*. *Bacillus subtilis, B. polymyxa, Pseudomonas fluorescens,* and three species of fungi *viz*. *Aspergillus terreus, Trichoderma viride* and *T. harzianum* isolated from the phylloplane of citrus variety Assam lemon *(Citrus limon)* inhibited the growth of *Xanthomonas compestris* pv. *citri, in vitro.* When the antagonists were tested for their efficacy under field condition by applying them over crop foliage of Assam lemon, they also reduced citrus canker incidence and *Bacillus subtilis* was found to be most effective antagonist exhibiting maximum (14.7 mm) inhibition of the pathogen and reducing the disease incidence to an extent of 61.9 per cent.

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