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Research Review

Various Biocontrol Techniques of Citrus Canker: A Brief Review

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Abstract

Citrus plants are one of the most economically significant plants, which often get suffered from a wide range of diseases. Among them citrus canker is the most contagious, and pervasive bacterial disease which is caused by some pathogenic species of *Xanthomonas*. Previous researches revealed that three pathotypes belonging to two species of *Xanthomonas* i.e., *Xanthomonas citri and Xanthomonas fuscans are responsible for developing the disease.* In citrus canker, brown and corky necrotic lesions appear mainly in the fruits, leaves and twigs due to hyperplasia of host tissues induced by the pathogens. The disease causes severe crop loss every year throughout the world and significant visual impairment of fruits that reduce its value in fresh markets. Although chemical controlling approaches are common ways for management of any plant disease, use of chemicals may cause environmental pollution and several health hazards in human being. As an alternative, biological control has become a popular and safer means of disease management where any organism or its products are utilized. This article focuses on various biocontrol techniques that are proved to be good enough for controlling citrus canker.

***Key words*:** Citrus, Disease, Citrus canker, *Xanthomonas*, Biological control

Citrus canker is considered as one of the most significant and devastating Citrus disease which is caused by the pathogenic genus *Xanthomonas*. Over time it has become the most destructive biotic stress to the citrus plants as it affects almost all the varieties along with many other rutaceous plants and thus causes a huge crop loss every year across many countries [1]. Symptoms manifested due to the disease is the prime factor for the reduction of market value. The pathogens caused substantial hyperplasia that leads to the formation of raised necrotic lesions on leaves, branches, and fruits. Besides, several other physiological changes such as premature fruit drop, twigs dieback, defoliation, and stunting can severely affect the overall fruit production and plant longevity [2]. At current scenario the disease is frequently found in major part of the globe encompassing more than 30 Asian countries, South America, and the South eastern United States [3]. In present there are different thoughts regarding the geographical origin of the disease as few studies suggest that it was first emerged in Southeast Asian countries like India and Indonesia [4-7] and some researcher like Lee (1918) and others reported southern China to be its source [8-9]. Based on geographical distribution and host range, three pathotypes of the disease viz., type: A, B, and C are established till date [10] caused by two species of *Xanthomonas*. Most common and widespread type of the disease is the canker A or Asian type of canker that is seen in major citrus plants like *C. paradisi*, *C. aurantifoli*, *C. sinensis* and many more. The other two types are Type B and Type C that manifest similar symptoms as type A canker. Fruits are the most important and usable parts of citrus plants with a huge source of essential micronutrient vitamin C along with several other essential nutrients that must obtained through diet. Generally, fruits are either eaten directly, or may be consumed in the form of fresh juice but citrus is also cultivated for the manufacture of oil and citric acid. Metabolic by-products are often used as animal feeds. Fruit juices are concentrated and used as flavouring agent also in many types of drinks. Since, animals cannot produce vitamin C and must obtain it in sufficient amount through diet, deficiency of vitamin C can cause scurvy in animals. As every plant disease creates adverse impact in our daily life, these serious issues must not be ignored. In present days, citrus canker has become a major threat for us and proper preventive care must be employed to the plants. Many approaches are made till now for the management of this canker disease like quarantine laws, physical, chemical and biological measuring methods. This review discusses on the different researches and efforts regarding the various bio-controlling techniques that are thought or implemented for management or eradication of the disease.

Nature of the pathogen

The causal organisms of the concerned disease are two pathotypes of the genus *Xanthomonas* that belongs to the family Xanthomonadaceae *under Gamma-Proteobacteria.* Type A

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canker or Asian type of canker occurs due the virulent activities of *Xanthomonas citri* subsp. *citri* (Xcc) [11]. On the other hand, type B and C are caused by the two other pathogenic species viz., *Xanthomonas fuscans* subsp. *aurantifolii* type B (*XauB*) and *Xanthomonas fuscans* subsp. *aurantifolii* type C (XauC) [12], respectively. The pathogens are Gram negative, rod-shaped and ranges in size from 1.5–2.0 × 0.5–0.75 µm [13]. Previously, the bacterium was named *Pseudomonas citri*, having rod shaped configuration and rounded ends with a polar flagellum [14-15]. Later, the bacterium was renamed as *Xanthomonas campestris pv. citri*. Furthermore, based on genomic studies it was reclassified as *Xanthomonas axonopodis pv. citri* [16-18]. The pathogen is considered as a quarantine organism in many nations have eliminated the disease successfully [19]. Host pathogenic interactions and attachments depends upon many factors among which presence of pathogenic surface structure adhesins is a key parameter. Usually, they are chemically exo or lipopolysaccharides in nature but some are proteinaceous like type IV pili, two partner secretion system, chaperones and many more [20]. The pathogens are also found to show six protein secretion system [21] among which T3SS, T4SS and T5SS along with their effector molecules play major roles during infection and multiplication of the pathogen inside the host.

Disease cycle and symptomatology

The pathogen enters the hosts through stomata and wounds in the plant tissues and infects various plant parts like stems, leaves, and fruits. The pathogen colonizes the intercellular spaces of leaves and propagate that results in to rupture of epidermis due to hyperplastic reactions. These changes lead to the formation of elevated necrotic that are dark, thick. These lesions harbour the site of propagation in moist conditions [22]. Pathogenic transmission to other plants takes place from those characteristic lesions where adequate amount of rain and wind plays major roles. It is reported that, several infective pathways are used by the pathogen during attacking the Citrus plants [23]. After entry, the pathogen Xcc infects young leaves, twigs, and fruits where noticeable necrotic lesions develop within 4-7 days. Presence of water film and temperatures ranging 20–30 °C are key conditions for disease development [24]. But symptoms appear later if the conditions are not suitable [25]. More the vulnerable plant parts become mature, more they become harder to be infected [26]. Tissue hyperplasia is the major sign of citrus canker which induces the formation of canker lesions [27-28]. In leaves, initially the symptoms appear as circular patches on the lower epidermis [27] and lesions appear on both sides of the leaf. Round spots rise and develop white or yellow coloured soft, spongy eruptions that become rigid later. Then corky, hard lesions are formed frequently with water-soaked margin [2]. Finally, the infected leaves exhibit formation of depression at the centre of the lesions and often they fall out. Symptoms are almost the same for affected twigs and fruits, where elevated lesions are found having oil or a water-soaked margin. The most adverse effect is the discoloration of fruits which results to huge financial loss and market acceptability.

Management of the disease

As citrus represents a very useful and economically important cluster of plants, so many attempts for preventing and curing of citrus canker are made through a huge number of researches. In early days, chemical control was established as potent management system for saving plants. Chemicals especially copper-based compounds [29-31], zinc-based compounds [32-33] and antibiotics were given priority for controlling citrus canker. But at present, use of chemicals for crop protection are thought to be harmful and avoided [34-36] as they accumulate in the plant systems and causes several health hazards [37-38]. Another disadvantage of chemical control is that prolonged and multiple application of the pesticides can develop resistance in pathogens which has become a serious issue [39]. For example, application of copper-based compounds for controlling citrus canker was a very common and famous approach over the past few decades but several reports suggested that continue exposure of the copper compounds develop resistant strains of *Xanthomonas* [40]. Same issue also arises regarding the use of antibiotics as resistant pathogenic strains may develop [41-42]. Thus, many nations are continuously taking initiatives and introducing rules and regulations regarding banning or lowering the use of synthetic pesticides [43-44]. Therefore, alternatives like cultural, physical and biological controlling measures are encouraged and considered safe for both human health as well as in sustainable agriculture.

Biological control

Nowadays, biological control has become a very popular topic in crop protection and many researches has been caried out successfully regarding controlling of various plant diseases. In biological control or biocontrol methos the pathogens are killed, or their activities are reduced by using some other organisms or substances having biological sources. The entity used in this concern are call biocontrol agent (BCA). Initially, crop saving mechanism of the biocontrol agents are realized carefully and then they are applied in proper conditions for having successful results [45]. The eco-friendly nature of biocontrol techniques is the key parameter regarding their superior acceptance and use over chemical controls. For preventing diseases usually, a single BCA is used but it has been also reported that in some cases consortium of BCAs is more effective in controlling the plant diseases. Although a number of bio-control techniques are reported in many studies, application of the suitable and best method must be done after gathering proper knowledges about the disease triangle. BCAs can be applied on the host plant directly or indirectly for reducing the disease severity [46]. Till now quite a few BCAs has been commercialized by certain agricultural companies as their marketing products. Till date citrus canker has been controlled biologically in many ways and significant methods are discussed next in this article.

Use of endophytic bacteria

Endophytes are the organisms which colonize the plant's internal tissues but never show any adverse effect to the hosts [47]. In modern crop protective researches many works have been carried out regarding the utilization of endophytes as biocontrol agents [48]. It is believed that endophytic bacteria can produce huge number of secondary metabolites in their host plants which is responsible for the antibiosis of certain numbers of pathogens [49]. Previous researches revealed that several endophytic bacterial strains viz., *Burkholderia cepacia*, *Bacillus velezensis*, *Bacillus amyloliquefaciens*, *Kosakonia cowanii*, *Bacillus subtilis*, *Staphylococcus pasteuri*, *Staphylococcus warneri* and *Bacillus thuringiensis* exhibit promising anti canker activities. In a study, *Pseudomonas fluorescens* and salicylic acid were used to control the disease both individually as well as combinedly and more disease reduction was noticed when both the biocontrol agents were used in consortium [50].

Treatment with bacteriophages

In different literatures, use of bacteriophage is reported for controlling citrus canker that can significantly reduce the virulence of different pathogenic strains. There are several instances regarding the use of bacteriophage in this concern viz., Cp1 and Cp2 bacteriophages were found to inhibit the activity of Xcc strains isolated from Japan. On the other hand, Cp3 bacteriophage was found useful against the XauB strains [51-53]. Another study reveals that the disease may be also controlled by a filamentous phage XacF1 [54]. It has been observed in a study that consortium of phage and copper-mancozeb could not improve the results than treatment with copper-mancozeb alone [55]. On the other hand, significant reduction of Asiatic citrus canker was noticed in a study where it was controlled using bacteriophage along with acibenzolar-S-methyl (ASM) [56]. Application of the bacteriophage may also improve controlling results when applied with Systematic Acquired Resistance (SAR) inducers [57].

Eradication of CLM

Citrus Leafminer (CLM) i.e., *Phyllocnists citrella* is a small, light-coloured moth that feed upon the citrus leaf. They are not responsible for direct transmission of the disease but exposes the mesophill tissues which enhances pathogenic inoculum within the host in significant amount. *Ageniaspis citricola* is a type of wasp that fed upon the CLM and therefore can be used for management of the disease in an indirect controlling machinery [58-59]. Besides, the CLM can also be killed by some other naturally occurring wasps like *Cirrospilus* and *Pnigalio* species. These parasites larva consumes the CLM larva and thus are very important for reducing citrus leafminer levels.

Use of natural products

With advance of knowledges, many researches have been carried out regarding plant disease control and it has been reported that extracts of different plant parts can suppress plant diseases potentially. A number of studies carried out in this context [60] suggest that both crude extracts or purified compounds can be used for controlling plant diseases [61]. It is believed that variety of biomolecules, secondary metabolites present in the plants are the prime compounds for controlling plant pathogens [62] and in recent scenario they are utilized to formulate new environmentally friendly and cost-effective bactericides. Till now extracts of several plants like *Allium cepa* L., *Allium sativum* L., *Azadirachta indica*, *Spondias pinnata* L., *Gardenia florida*, *Tamarinus indica* L., *Punica granatum* L. and many more are proved to be potent BCAs for management of citrus canker [63-64]. In another study, similar result was found using extracts of *Acacia nilotica* and *Datura alba* [65].

Use of resistant varieties

As there are so many citrus cultivars present with different infection rate of citrus canker, use of resistant cultivars can significantly manage the pathogens even when the hosts grow in the region with higher risk of infection. In this approach the hosts are genetically engineered by incorporating certain genes responsible for disease resistance within them. Many resistant varieties of citrus plants against canker are reported till now. Among them mandarin, tangerine of *C. reticulata* Blanco, pummelo of *C. maxima* (Burm.) Merr., tahiti lime of *C. aurantifolia* (Christ.) Swingle are examples of some moderately resistant varieties [27]. On the other hand, kumquat of *Fortunella* spp., citron of *C. medica* L., calamondin of *C. microcarpa* (Bunge) Wijnands and many more represent the highly resistant varieties [27].

Activation of systemic resistance

Often plants get exposed with certain microorganisms that induce systemic resistance [66] and confer protection from pathogen attack. Some works have been carried out regarding the use of plant growth promoting rhizobacteria (PGPR) for the induction of systemic resistance in plants [67]. Generally, PGPR mediated resistance are induced by two plant defence pathways viz., jasmonate/ethylene dependent (JA/ET) [68] and Salicylate (SA) dependent pathway [69-70]. Expression of many defence genes of plants get activated or altered upon the exposure of PGPR. PGPR promoted systemic resistance can also induce several physiological alterations in the hosts as changes in phytohormone content, reactive oxygen species (ROS) levels etc. [71]. Therefore, as an alternative strategy for controlling plant diseases PGPR can be utilized. In a study it was found that several rhizospheric bacterial strains of *Burkholderia* and *Pseudomonas* can induce the defence system against citrus canker.

CONCLUSION

As avoidance of synthetic pesticides are gradually becoming necessary, alternative measures for controlling plant diseases are encouraged in modern agricultural practices. Due to high virulence and fast transmissibility, citrus canker has become a major issue both in the citrus industry as well as consumers. Besides, it has been also seen that the pathogen having high mutation rate that generates new strains. As it causes severe noticeable damage to the plants and lowers the economic value of the fruits in fresh markets, proper controlling management must be employed. Through many studies it has been found that use of BCAs has been established as an excellent option for crop protection for its eco-friendly attributes. Copper based disease management are not encouraged nowadays due to its harmful attributes and many biocontrol approaches are tried and implanted. Many researches revealed that utilization of endophytic bacteria, bacteriophage, natural products and resistant varieties are the common biocontrolling methods for protecting the citrus plants and are good enough for promoting sustainable agriculture. As the agents are harmless, they should be utilized for better agriculture and more researches must be idealised for developing more eco-friendly models for saving the plants.

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