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REVIEW ARTICLE

A REVIEW ON GREENING DISEASE AND INTEGRATED MANAGEMENT OF CANDIDATUS LIBERIBACTER SPECIES IN CITRUS

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ABSTRACT

Citrus greening disease, recently known as huanglongbing disease, is known to wreak havoc not only in Nepal but across the globe as it is non-curable once trees are affected. In 1919, this disease was reported first time in China, most likely originating from Taiwan. It then spread to more than a hundred countries and was recorded in Nepal for the first time from Pokhara Valley in 1968. Till now, there is no established solution for this century-old and yet, newly evolving disease. Being a vector-borne bacterial disease, the use of antibiotics had been a practice on a large scale that had led to the development of resistance. This disease has been more challenging to the developing and least- developed countries like Nepal. Thus, this paper aims to solve the problems of all the farmers engaged in citriculture by making them aware of the best-integrated management approaches that would help them increase their productivity and ultimately contribute to food security and sustainability. Detailed information about the disease, together with various techniques for integrated management and control of this disease is in this review. Thus, this paper will help farmers, traders, and planners.

KEYWORDS

Bacteria, Huanglongbing, Host, Liberibacter, Vector.

1. INTRODUCTION

Citrus is suspected to have emerged from the region within Northeast India, South China, Indonesia, and Peninsular Malaysia (Kamble et al., 2017). The genus Citrus contains a wide number of species and belonging to the Rutaceae family, it contains flowering plants that generally have a strong scent. Plants within the genus "Citrus" produces citrus fruits, including important crops such as sweet oranges, lemons, grapefruits, pomelos, and limes(Wikipedia, n.d.). It is one of the most popular commodities around the world because of its high nutrient value, strong aroma, unique taste, and high commercial value. The total global production of tangerines, mandarins, and clementines was estimated at 34,393,430 tonnes, oranges were estimated 75,413,374 tonnes whereas, 19,368,838 tonnes of lemons and limes were produced at an area of 3,639,084 hectares, 4,469,719 hectares, and 1,267,401 hectares respectively in the year 2018 (FAOSTAT, 2020). Citrus cultivation is a high prospect sector in the mid-hills of Nepal; ranging between 800 to 1400 meters above sea level because of its contribution to enhancing the economy, uplifting the living standard of poor farmers, and supplementing the nutrition maintenance of the ecosystem (Timilsina, 2019b). Despite this, the yield of citrus is in decreasing trend in Nepal; it was 11.3 tonnes per hectare in the year 2008/09 and decreased to 9.57 in the year 2018/19 (MoALD, 2020).

Shrink in the yield from citrus groves is the result of different diseases caused by different plant pathogens such as fungi, prokaryotes, nematodes, viroids, and viruses. Citrus greening, most recently known as huanglongbing disease (HLB), a vector-borne bacterial disease, is one of the most usual reasons for the citrus decline in citrus orchards across the globe (Batool et al., 2007). Three bacteria are found to be associated with this disease, *Candidatus* Liberibacter *asiaticus* (CLas), *Ca.* Liberibacter *africanus* (CLaf), and *Candidatis* Liberibacter *americanus* (CLam) (Timilsina, 2019b). The main vector of this disease is a sucking insect, the Asian Citrus Psyllid (ACP); *Diphorina Citri,* which transmits Asian and American species; *Trioza erytreae* is also suspected to be the vector transmitting African species (Bove, 2006). This disease was reported for the first time in China in 1919, probably originating from Taiwan in the 1870s (Bove, 2006). Subsequently, the disease was then reported from several different countries under different names such as citrus die-back from India, mottle leaf disease from Philipinnes, yellow branch, or greening from South Africa (Bove and Sagilo, 1974). In Nepal, it was first observed in the citrus orchard of the Horticulture Research Station located in Pokhara valley, in the mid-sixties (Regmi and Lama, 1988). Ancient crop growers used supernatural practices to cope with diseases of plants, as the appearance of diseases of a plant is as primeval as the plant itself; however, with the development of contemporary plant pathology, various scientific approaches have been used to curtail the losses that are occurring due to plant diseases (Martinelli et al., 2015). Still, farmers are facing many challenges to manage citrus greening cost-effectively. That is why, it is a century-old and yet, newly emerging disease (Bove, 2006).

Cultivation of citrus fruits has been the source of bread for the majority of people residing in the mid-hills for ages. Mandarin occupies the greatest of 64 percent and 68 percent of the total citrus growing area and production, respectively, in Nepal (Timilsina, 2019a). But the Nepali citrus industry is struggling with the most serious threat: a vector-borne bacterial disease with no cure has infected most of the citrus growing hubs and is yet to receive any national attention. An exponential decrease in

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citrus production, as a result of citrus greening, needs to be managed as soon as possible. Thus, this paper aims to provide necessary knowledge about the pathogen, vector, host range, symptoms, easy diagnosis, and effective management strategies with their advantages as well as disadvantages for farmers of least developed countries like Nepal. It also discusses the future outlook of this disease which will help farmers, traders, and planners.

2. Materials and Methods

Information on citrus greening disease and its management strategies were collected via more than six dozen pieces of literature that include journal articles, dissertations, websites, and other reports. Moreover, we used various governmental publications for additional data. Hence, a conclusive outline of the disease management was drawn by summarizing these data, facts, and figures.

3. Results and Discussions

3.1 Causative agent

The main causative agents of this disease are three species of Liberibacter, among several species of Liberibacter. They are namely, "*Candidatus* Liberibacter *asiaticus"*(CLas), "*Candidatus* Liberibacter *africanus"*(CLaf) and "*Candidatus* Liberibacter *americanus"*(CLam) (Timilsina, 2019b; Villechanoux et al., 1992; Villechanoux et al., 1993; Jagoueix et al., 1997). These bacteria are non-cultured, gram-negative, fastidious, phloem-limited bacteria (Paudyal, 2015; Halbert and Manjunath, 2004). These affect the transport of phloem, cause root dysfunction, reduce foliar density in the tree canopy, and eventually halts citrus production (Gottwald, 2010; Johnson et al., 2014). The 16s RNA sequence was used as a basis to study its taxonomical classification instead of using growth, morphology, and enzymatic activity, as this bacterial pathogen was not cultured on an artificial medium (Jagoueix et al., 1997). According to a study, the pathogen was the member of the alpha subdivision of proteobacteria with the genus *Candidatus* Liberibacter in the family Rhizobiaceae (Jagoueix et al., 1994).

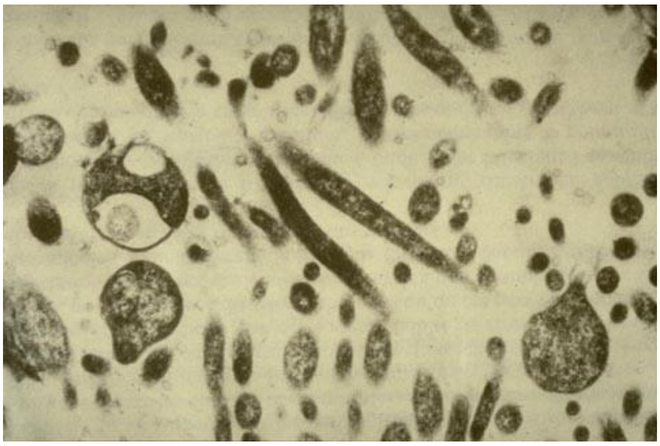

Figure 1: Transmission electron microscopy (TEM) micrograph of *Candidatus* Liberibacter *asiaticus* bacteria, the causal agent of HLB, inside phloem sieve tube of citrus.

3.2 Vector

As this disease is a vector-borne bacterial disease, insects are the carrier of this disease. Citrus psylla is the most puissant insect vector for the transmission of this disease (Iktikhar et al., 2016). The Asian and the American species of Liberibacter could get transmitted by the psyllid *Diaphornia* Kuwayama *citri*, usually called Asian citrus psyllid (ACP); whereas the African species by the vector *Trioza erytreae* (Bove, 2006). ACP was first detected in Florida in 1998, spreading to 31 Florida countries by 2000 (Halbert and Manjunath, 2004). ACP is accountable for the transmission of CLas in America, Brazil, and Asia and CLam in Brazil, whereas *Trioza erytreae for* Lam in the Middle East, Reunion, and Africa (Halbert and Manjunath, 2004).

3.3 Host range

There is the involvement of two types of hosts for the greening disease. Citrus host, one in which the pathogen multiplies, and the vectors feed and proliferate, and another alternate host where infected vectors get nourished (Gottwald, 2010; Halbert and Manjunath, 2004). Alternative hosts include *Microcitrus australasica* (Finger lime), *Swinglea glutinosa* (Tabog), *Limonia acidisimma* (Wood apple), *Severinia buxifolia* (Chinese box-orange), *Murraya paniculata* (Orange jessamine), *Aeglopsis chevalieria, Atalantia missionis*, etc. (Knapp et al., 2004). *M. paniculata* is the most favored alternative host for Asian citrus psyllid (Ghosh et al., 2018). Reported non-Rutaceous hosts are *Catharanthus roseus* (Periwinkle) and *Nicotiana xanthii* (Tobacco). All three pathogens get colonized and divide inside the dodder (Cuscuta spp.), and infected dodder could transmit the pathogen to citrus, periwinkle plant, etc. (Garnier and Bove, 1983; Hartung et al., 2010). The study of both types of host plants is very crucial for disease management.

3.4 Disease Cycle and Epidemiology

This disease is not seed-transmissible as infected plants contain a high number of aborted seeds (Ghosh et al., 2018). The pathogen survives in infected bud woods and is also transmissible by grafting. The pathogen population residing inner to the host tree is found to liberate specific volatile chemical methyl salicylate, which lures the vector population to feed on the infected tree (Mann et al., 2012). Fourth and fifth instars of *D. citri* nymphs that are growing on infected trees can acquire the pathogen which the emerging adult can transmit in a persistent manner (Xu et al., 1988). Also, *T. erytreae* nymphs can acquire the pathogen, and transovarial transmission occurs (Gottwald, 2010). Thus, vector becomes a source of inoculum and helps in the spread of pathogens. On dormant seasons, these vectors survive on their alternate host and get nourished there. After acquiring the pathogen, adults remain infective throughout their life, and the pathogen multiplies in the vector (Hung et al., 2004; Xu et al., 1988). The pathogen divides in the nymphs only but not in the adults of *D. citri* (Inoue et al., 2009). Reports on acquisition times vary widely from 15 to 30 minutes or 5 hours minimum in the case of *D. citri* to 24 hours for *T. erytreae* (Xu et al., 1988). The insect vector carries the for 12 weeks (Hung et al., 2004). ACP is heat tolerant but sensitive to high rainfall and humidity, whereas *T. erytreae* is heat sensitive and cannot stay alive above 32 degrees celsius (Inoue et al., 2009; Gottwald et al., 2007).

3.5 Symptomatology

Fully matured leaves show the most indicative type of symptoms of leaf mottling *viz.,* yellowing along the midrib and leaves veins that extend in the adjoining tissues to produce a mottled effect (Kamble et al., 2017). Mottling bears a resemblance to zinc deficiency symptoms, which are characteristic of the disease (Elizbeth et al., 2005). Leaf cupping and vein cupping are diagnostic symptoms. In the case of Zn deficiency, chlorosis is symmetrical on both sides, but in the case of greening, there is an irregular, mottled appearance. They don't have a uniform pattern (Vashisth and Kadyampakeni, 2020).

Figure 2: Leaf showing differences in symptoms caused by Zn deficiency and HLB. (Source: Pourreza, 2016)

Chronically infected trees are scantly foliated with substantial twig dieback and do not fruit or produce deformed fruits (Kamble et al., 2017). Fruits are under-developed, lopsided, poorly colored, and have aborted seeds. When pressure exerts with a finger, grayish-white waxy marks appear on the rind surface (Batool et al., 2007). Attacks of beetle and *Phytophthora* fungi on trees are some of the problems associated with greening disease (Halbert and Manjunath, 2004). Also, the root systems develop poorly. Further, citrus is often attacked by more than one pathogen at a time and exhibits symptoms similar to the Tristeza virus (Graca, 1991).

3.6 Easy HLB diagnosis

3.6.1 Pentest

Pentest is the rapid diagnosis method that can be practiced even in the field at no cost. It is the most common and easy way to distinguish between zinc deficiency and HLB symptoms. We circle both sides of the leaf blade with the help of a pen in the same position. If the chlorosis pattern in the area occupied in the circle is uniform, then most likely it is a nutrient deficiency (zinc deficiency); if the chlorosis pattern is random, then it can be HLB (Vashisth and Kadyampakeni, 2020). It is necessary to take into consideration that HLB-affected trees often exhibit nutrient deficiencies as well; therefore, it is imperative to distinguish nutritional deficiencies from HLB symptoms and sort them in the right way (Vashisth and Kadyampakeni, 2020).

Figure 3: Pentest to distinguish nutrient deficiency from an HLB-caused blotchy mottle in leaf. (Source: Vashisth and Kadyampakeni, 2020)

3.6.2 Scratch Method Using Abrasive Paper for HLB diagnosis

Another cheap and commonly practiced diagnosis method of HLB in Nepal is the "Iodine-Starch" method. It is for laboratory conditions. In this method, we rub the surface of a citrus leaf gently for at least 20 times with abrasive paper. And the abrasive paper is placed into 1 ml water in a vinyl pack added to about 25 µl iodine solution 50 mM for dyeing starch is the common method of the iodine-starch reaction. The iodine-reacted mixture shows mostly dark brown or black and yellow or orange that are HLB-positive and negative, respectively (Adhikari et al., 2012). The scratch method indicated 73.9% of results agreed with LAMP (loop-mediated amplification technology), showing appropriateness for countries like Nepal (Timilsina, 2019b). Besides these, different advanced techniques like electron microscopy, monoclonal antibodies, polymerase chain reaction (PCR) assays, immunofluorescence, LAMP, ELISA, DNA probes, etc. are used for the diagnosis of HLB in developed countries. These techniques are not developed in commercial scale in countries like Nepal.

3.7 Disease management

Commercial citrus cultivars and varieties comprise of species of the genus *Citrus* and various other related genera. And an abundance of intergeneric and interspecific crosses, where diversity of pathogens frequently attacks (Gottwald, 2010). Therefore, it is necessary to manage these pathogens to optimize the yield and ultimately contribute food security as well as sustainability.

3.7.1 Use of disease-free planting materials

It is one of the practices to be followed by citriculture farmers to prevent inoculum and is imperative. Bud woods obtained from horticulturally superior mother plants, which are free from any disease-pest, are employed to produce greening free plantlets by grafting them on nursery-grown healthy rootstock (Gottwald, 2010). Usually, we should discard saplings raised below 1300 masl (AITC, 2020). The nursery and greenhouse should be free from any insect-pest, as an infected psyllid can act as a good source. Moreover, instead of replanting one-year-old seedlings, two- or three-year-old seedlings would be more secure, preferable, and economical (Schumann and Singerman, 2016; Serikawa et al., 2012).

3.7.2 Vector population control

Vector is most lured to infected trees until it fed on them afterward, prefers feeding on uninfected trees to infected ones (Timilsina, 2019b). Thus, helping in the spread of disease to all nearby areas. If stopped at an appropriate time, they would not create a disaster. We must be able to eliminate these vectors to prevent citrus trees. For this purpose, we can use either of the following methods depending upon our suitability and profitability.

3.7.2.1 Chemical method-

Based on the threat level of pathogen and insect vector epidemiology, the easiest and less-time consuming control strategy is the use of chemical pesticides when the insect and pathogen of HLB are present conceivably (Abdullah et al., 2009). Foliar spraying of dimethoate 30% E.C. at the rate of 1 milliliter per liter of water before flowering has been found effective against citrus psyllid and is recommended in Nepal (AITC, 2020). Very little information on insecticidal control against ACP is there in the literature before the arrival of HLB disease (Grafton-Cardwell et al., 2013). Insecticides in the pyrethroid, organophosphate, and neonicotinoid classes carry greater potency against D. citri (especially adults). Oils and insect growth regulators are more effective against eggs and nymphs than adults (Boina and Bloomquist, 2015). Moreover**,** insecticides such as imidacloprid, fenpropathrin, chlorpyrifos, abamectin, diflubenzuron, and dimethoate are effective and are registered for major citrus growing areas in the world (Rogers, as cited in Timilsina, 2019b). Though, using this strategy depends upon the economy of the individual grower as it is expensive. The annual cost for the management of ACP in citrus groves could range from $US 240 to $US 1000, depending upon the type of insecticide used, and method and frequency of application (Belasque et al., 2010). An even persisting challenge is the large number of HLB positive residential citrus groves, where chemical control is not an option (Abdullah et al., 2009). Also, insecticide resistance is the utmost problem for managing citrus psyllid; therefore, insecticides with different chemistries and modes of action need to be used alternatively (Boina and Bloomquist, 2015).

3.7.2.2 Biological method-

The biological measures involve the use of parasitoids, predators, fungal entomo-pathogens, etc. for controlling the vector population. These measures are eco-friendly. The only effectual primary parasitoids of *D. citri* nymphs identified till now are *Tamarixiaradiata* (Hymenoptera: Eulophidae); an ectoparasitiod & *Diaphorencyrtus aligarhensis* (Hemiptera: Encyrtidae); an endoparasitoid (Waterson, 1922; Shafee et al., 1975). Multiple attempts were unsuccessful in Florida regarding the successful introduction of *D. aligarhensis* (Rohrig et al., 2012). In contrast, given the speed with which *T. radiata* has established and spread, it is an obvious choice for effective biological control of *D. citri* (Chen and Stansly, 2014). Different from earlier studies, the parasitoid complexes of *T. erytreae* include three species of primary parasitoids: *Tamarixia dryi*, *Psyllaephagus pulvinatus,* and another parasitoid of the genus *Tamarixia*, whose molecular analysis showed that it is a new species closely related to *T. dryi* (Perez-Rodriguez et al., 2019). Several species of parasitoid complexes parasitize *Trioza erytreae* (Perez-Rodriguez et al., 2019). Lady beetles, lacewings, syrphids, and spiders are the predators of *D. citri* (Grafton-cardwell et al., 2013). Another best way to manage ACP populations biologically is by the use of entomopathogenic fungi (Timilsina, 2019b). Several pathogens infect *D. citri* in highly humid conditions, for example, *Isaria (Paecilomyces) fumosorosea, Lecanicillium lecanii, Beauveria bassiana, Hirsutella citriformis*, etc. (Ghosh et al., 2018). *I. fumosorosea* has the most impact among various fungal entomopathogens, but there is no published report of its successful use against *D. citri* in the field (Subandiyah et al., 2000; Meyer and Hoy, 2008; Casique-Valdes et al., 2011).

3.7.3 Legislative control

This disease entered and spread in different countries because of the weak quarantine regulations. One of the most popular assumptions is that this disease was originated from Africa, likely in an asymptomatic host such as *Verpris lanceolata* (Beattie et al., 2005). Then disseminated to citrus tree through insect in European settlement on the east coast of Africa and further traveled to the Indian subcontinent in infected plants or bud woods 300 to 500 years ago, and then into China later (Beattie et al., 2005). Planting materials and citrus psyllids are supposed to be the media of transmission of HLB to other Asian and south-east Asian countries. The incidence of HLB infections within the surrounding area affects the probability and efficacy of slowing the epidemic in large amounts. Thus, mother plants should be periodically checked thoroughly for citrus greening and should be left under isolation to prevent the plants from insects and diseases (Kawano, 1998 as cited in Batool et al., 2007).

3.7.4 Use of tolerant varieties

The responses of different citrus species to the causative agent are fluctuating (Paudyal, 2015). There is no resistant citrus cultivar known so far. Still, some cultivars are found more tolerant compared to the others. For example, grapefruit is tolerant than the sweet orange (Bove, 2006). Sweet orange, tangelo, and mandarin are the susceptible cultivars, whereas lime, sour orange, and trifoliate orange are least than them (Knapp et al., 2004). Besides, symptoms also differ from cultivar to cultivar / that they are found severe on sweet orange, mandarin, tangelo, and grapefruit whereas less observed on lemon, rough lemon, and sour orange cultivars (Bove, 2006; Liu et al., 2011). Also, infected lemons, grapefruits, and sour oranges remained unproductive for their whole life, while Mexican-limes, trifoliate- oranges, and some trifoliate orange hybrid showed only leaf symptoms (Chung and Brlansky, 2006). Recently, a study reported that among 19 citrus species, percent disease incidence was observed highest for *C. sinensis, C. reticulata,* tangelo*, C. grandis, C. jambhiri, C. paradisi, C. limonia, C. limon and Citrus reshni* in field conditions, indicating their higher susceptibility (Kamble et al., 2017). Based on these, citrus growers must select the best variety for combating this disease.

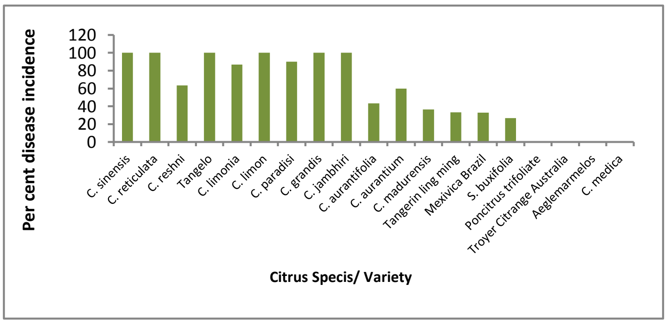

Figure 4: Incidence of greening disease in different citrus species/varieties (Source: Kamble et al., 2017.)

3.7.5 Thermo-therapy

Thermo-therapy is one of the best environmentally friendly measures, does not require a regulatory permit, is suitable for both organic and conventional farming, and can be quickly adopted by growers (Doud et al., 2017). Earlier studies indicated that thermotherapy is best to control HLB effectively (Hoffman et al., 2012). It appears to be somewhat effective at suppressing phytopathogen titer, at least in greenhouse/growth chamber settings reported that field thermotherapy also stimulated growth in diseased trees by reducing pathogen concentration for 18 months of post-therapy (Guo-cheng et al., 2016; Doud et al., 2017). It is effective against a wide range of plant pathogens (Guo-cheng et al., 2016). Continuous exposure to 40 to 42 degrees Celsius heat for at least 48 hours has been reported sufficient to decrease pathogen titers or eliminate it in infected citrus seedlings entirely (Hoffman et al., 2012). A group researchers reported that by the end of eight weeks of post-treatment, pathogen concentration in the new flushes and mature leaves of thermotherapy treated plants were found to be decreased by more than 90 and 56% respectively (Guo-cheng et al., 2016). Although thermotherapy is time consuming and expensive process in long run for poor farmers to adapt quickly, there have been efforts to develop commercial equipment that could make it more feasible at a larger scale for countries like Nepal too.

3.7.6 Cultural Practices

Cultural practices are the oldest management method employed by farmers. Different ways of cultural practices are effective against the incidence of the disease. These methods include the complete elimination of infected symptomatic trees, the use of certified pathogen and pest-free seedlings for replanting and implementing optimized nutritional programs, and sufficient irrigation (Bove, 2006). Binh and Lam introduced the term "intensive farming" that includes pruning, pest protection, and use of fertilizer to improve the growth & production in infected plants (Binh and Lam, 2004). Nguyen and Nguyen reported that continuous foliar application of micronutrients such as zinc sulfate and manganese sulfate in the ratio of 3:1 for seven weeks induce growth, as well as overall yield, in HLB infected trees (Nguyen and Nguyen, 2004). Also, cover crops are supposed to improve water and nutrient retention in soil, promote soil microbial activity, reduce weed growth, eradicate insect-pests, and hastens plant development under investigations in various agricultural cropping systems in HLB endemic citrus-producing regions (Strauss et al., 2019; Johnson et al., 2014). Soil application of zinc sulfate, iron sulfate, and manganese sulfate at the rate of 150-200 gram per tree also improves the general health of the infected citrus groves. The degree of disease growth was found 47.8% in alkaline soil that leads to a decrease in the absorption of micronutrient whereas, disease was undetected in acidic soil (Inoue et al., 2020). Therefore, it is necessary to maintain the appropriate pH of the soil for the eradication of this disease.

3.7.7 Trap Cropping and guava intercropping

Trap cropping is one of the best economical management strategies used for controlling vectors, can be practiced in least-developed countries like Nepal. Orange jasmine (*Murraya paniculata* (L.), syn*. Murraya exotica* L.) was estimated as a potential trap crop for lately planted as well as well-established orange orchards, in a 4 years study in Brazil (Tomaseto et al., 2019). The results demonstrated that in a lately established (6 months old) orchard of citrus, the trap crop diminished the number of psyllids that settled on citrus trees by 83% and reduced the HLB incidence by 43%, whereas in a well-established (7 years old) orchard, the trap crop did not show a significant effect on psyllid settlement on citrus trees or HLB incidence (Tomaseto et al., 2019).

Guava intercropping is one of the cheapest methods of citrus greening management and can help establish new orchards. Farmers of Vietnam were first to practice guava intercropping with citrus and the attention of scientific researchers and workers of many countries were drawn during the 1990s initiating studies to understand its usefulness (Paudyal, 2015). For a long time, farmers from Mekong Delta of South Vietnam have been practicing guava interplanting in citrus orchards and those orchards planted with guava showed much lower levels of psyllid infestation and also a low incidence of HLB compared to citrus orchards lacking guava (Hall et al., 2007).

3.7.8 Biological measure to control the pathogen

Several previous studies have discovered some plant beneficial bacteria and tested their efficacy against HLB pathogen and HLB under controlled conditions. Although the *in-vitro* selection of bacteria antagonistic to non-cultured HLB pathogen is quite difficult, successfully screened and evaluated antagonistic bacteria by the use of potted plants (Tang et al., 2018). For example, a study reported that the biocontrol agent Bacillusamyloliquefaciens GJ1, when applied through root irrigation in the greenhouse, alleviated the pathogen infection rate of citrus by 50% (Tang et al., 2018). Moreover, the induction of systemic resistance in citrus via plant growth-promoting bacteria such as Azospirillum brasilense could be an alternative for mitigating the harmful effects of greening and slowing the rate of disease progress (Trinidad-Cruz et al., 2019). Under greenhouse conditions, the inoculation of Azospirillum brasilense directly in the soil every 20 days significantly reduced the pathogen titer in Mexican lemon trees in an 8-month experiment (Trinidad-Cruz et al., 2019). This treatment reduced the titer of CLas in citrus by approximately three-folds as compared to the untreated ones (Trinidad-Cruz et al., 2019). In sum, these promising discoveries justify field tests for practical application under field conditions on a large scale. These could also be the best alternatives to chemicals.

3.7.9 Chemical measure to control the pathogen

As it is a vector-borne bacterial disease, different antibiotics are used by farmers and researchers to control the pathogen of this disease. A group researchers found that some antibiotics Ampicillin, Carbenicillin, Cefalexin, Penicillin, Rifampicin, and Sulfadimethoxine were all highly effective in eradicating or suppressing the pathogen despite their several modes of actions (Zhang et al., 2014). Broad-spectrum antibiotics that have differing antibacterial activities had been evaluated for effectiveness against Las under greenhouse and field conditions (Yang et al., 2018; Zhang et al., 2019). Those antibiotics were aminoglycosides (streptomycin and kasugamycin), tetracyclines (oxytetracycline), β-lactams (penicillin), and sulfonamides (sulfadimethoxine and sulfathiazole) (Yang et al., 2018; Zhang et al., 2019)**.** Hu and Wang (2016) reported that recently in Florida, foliar spray of streptomycin sulfate, OTC hydrochloride, and OTC calcium complex have been approved in controlling greening (Hu and and Wang, 2016). Trunk injection of these antibiotics has also been found more efficient but, it leads to damage of the trunk permanently, and scarcity of suitable equipment for injection may limit its use at a commercial scale (Hu and Wang, 2016). Antibiotics injected plants showed short-term disappearance of symptoms though it is unknown that the titer of pathogen gets reduced or not (Hu and Wang, 2016). Various efforts are being made by researchers and scientists to enhance the application of antibiotics as there is a chance that multidrug resistance protein, ABC transporters MsbA1 and MsbA2, may lead to the development of resistance to OTC, streptomycin, or other antibiotics (Li et al., 2012; Liu et al., 2011).

Table 1: Comparison of various management strategies.

Management approaches	Advantages	Disadvantages
Use of disease-free planting materials	Very effective in reducing a source of inoculum	Does not assure us of overall disease reduction
Vector population control		
a. Chemical measure	Long-lasting control of psyllids; Less-time consuming	Negative impact on other non-targeted, beneficial insects; Problem of resistance development
b. Biological measure	The best alternative to chemicals; Reduces adult & nymph populations	Dependent on environmental conditions for effectiveness; Takes more time
Legislative control	Eradicates inoculum source completely	Not that efficient for HLB established areas; No use for open boarders like Nepal
Thermotherapy	Environmentally sound; Effective in reducing Clas titers	Multiple treatments are needed; Does not work for root-infected with HLB
Cultural practices	One of the easiest & most commonly practiced method to cope with the disease	Heavy economic loss in replacing bearing trees
Trap cropping & guava intercropping	Helpful in newly established orchards for controlling psyllid populations as well as reducing Clas infection rates	Not that proven for well-established orchards/ HLB infected orchards
Biological measure to control the pathogen	Environment-friendly; Reduces Clas populations & infection rate of citrus seedlings in greenhouse conditions	Long-term effectiveness in the field is still doubtful
Chemical measure to control the pathogen	Effective in reducing Clas populations; less time consuming	Risk of antibiotic resistance; Inconsistent effectiveness; Quite expensive
Use of tolerant varieties	Risk minimization	Still possess chances of disease development

3.8 Future outlook

Transgenic approaches have been used in citrus resistance against the disease, as traditional breeding approaches are not much useful for citrus because greening leaves no citrus cultivars. These modern approaches comprise of overexpression NPR1 (nonexpression of pathogenesis-related genes 1), antimicrobial proteins (e.g., thionins and plant defensins), or antimicrobial peptides (e.g., cecropin B) (Dutt et al., 2016; Stover et al., 2013). But, consumer's acceptance and preference for transgenic citrus varieties are the biggest problems in the commercialization of transgenic citrus. Moreover, different researchers across the globe are exploring clustered regularly interspaced short palindromic repeat (CRISPR)-based targeted genome-editing technologies to create resistant citrus cultivars, which include the CRISPR-Cas9 and CRISPRLbCas12a (Cpf1) genome-editing tools (Jia et al., 2019; Wang, 2019). One of the disease susceptibility gene CsLOB1 that favors citrus bacterial canker disease was modified to develop canker resistant plants (Jia et al., 2016a; Jia et al., 2016b). These studies in this area of research could be a potential remedy in controlling greening and also provide a huge opportunity to accelerate the process of developing HLB-resistant/tolerant citrus varieties, thus providing a long-term solution for citrus HLB.

4. Conclusion

HLB has become a disease of economic importance leading up to 100% damage. Being a vector-borne bacterial disease, the use of antibiotics had been a practice on a large scale that had led to the development of antibiotic resistance. Owing to many other side effects of antibiotic application, scientists and researchers have been seeking alternative management practices, which lower the damage of *Candidatus sp.* along with maintaining environmental sustainability. Thus, alternative management strategies like the use of disease-free planting materials, the use of antagonistic bacteria targeting pathogens, quarantine control, etc. can be adopted for disease management based on the situation. Similarly, the cost-efficacy of these alternative management strategies over chemical control makes the practice easy for adoption for rural marginal farmers of Nepal. Therefore, awareness programs are necessary to carry out for promoting the use of these techniques in different regions of the country. The government should encourage farmers to implement these integrated management strategies by providing subsidies or other measures. The government, along with other stakeholders, should form a task force for implementing the techniques to control and eliminate citrus greening. For effective control, there is a need to establish early disease detection methods and proper quarantine regulation in non-invaded areas. Moreover, modern transgenic breeding approaches also provide a scope for citrus growers in the future to come. So, future researchers should effectively work on that.

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