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Review Article

Understanding Citrus Greening Disease and Its Possible Management Strategies in Nepal

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Abstract

Huanglongbing (HLB), also known as citrus greening, is a devastating disease of citrus that has decimated several citrus orchards throughout the world. The disease is associated with three species of unculturable and phloem-limited bacteria, *Candidatus* Liberibacter asiaticus, *Candidatus* Liberibacter africanus and *Candidatus* Liberibacter americanus. The most common species of the bacteria found in Nepal is *Candidatus* Liberibacter asiaticus which is transmitted by an insect vector, Asian citrus psyllid (*Diaphorina citri*). This disease has been detected in several economically important citrus production areas of Nepal, which resulted in heavy yield loss. No cure for the disease has been discovered yet and it is essential to practice proper management strategies to maintain citrus health and sustain citrus production under HLB pressure. Several disease-management approaches such as pathogen free nursery establishment, use of disease tolerant rootstock cultivars, proper irrigation and nutrient supply, removal of HLB affected trees and control of psyllid with frequent insecticide application are widely practiced throughout the world. This review article highlights the characteristics of citrus greening disease and its insect vector and gives insights about their management techniques. Several technologically advanced options available to minimize the HLB infection might not be feasible currently in Nepal due to economic and topographic constraints. This article also aims to bring into focus the cost-effective methods that growers in Nepal can practice to mitigate the impact of HLB disease in their citrus orchards.

Keywords: citrus production; HLB in Nepal; citrus greening management

Introduction

Nepal produced 273,000 tonnes of citrus in 2019 and ranked 43rd in global citrus production (Fig. 1). About 177,000 tons of Tangerines, Mandarins, Clementines and Satsumas, and 44,000 tons of oranges were produced in the same year (Fig. 2) (Source: www.fao.org).

The citrus production areas in the world are under the threat of Huanglongbing (HLB) or citrus greening disease (Bove, 2006; McCollum and Baldwin, 2017). The disease has reached most of the commercial citrus production regions and reported in about 50 countries worldwide (CABI, 2020). The detrimental effect of citrus greening has been observed in various countries in the world.

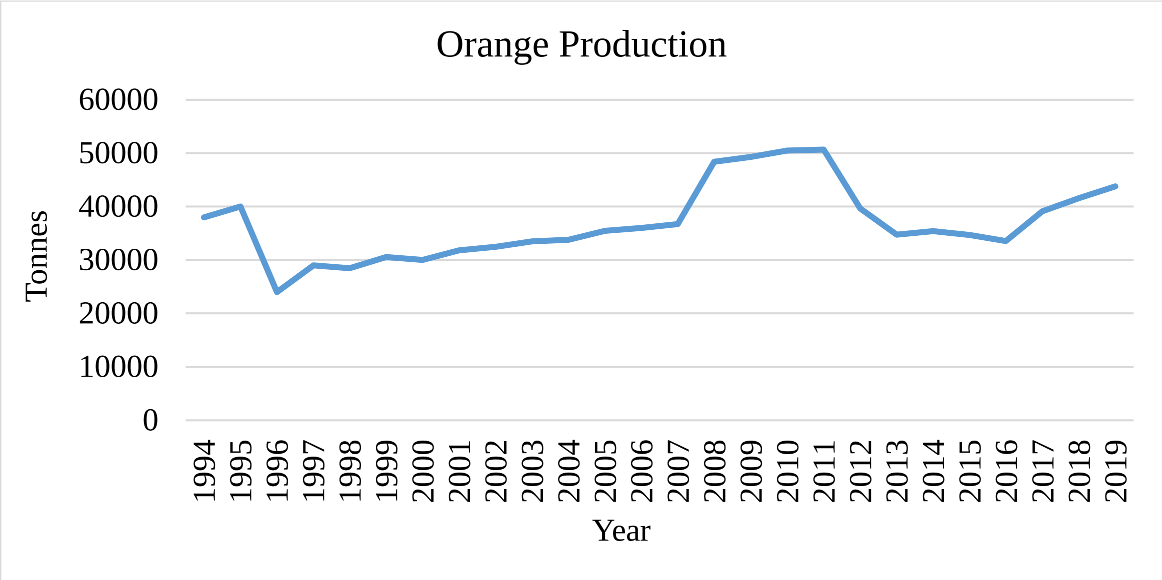

Fig 1: Orange production trend in Nepal (www.fao.org)

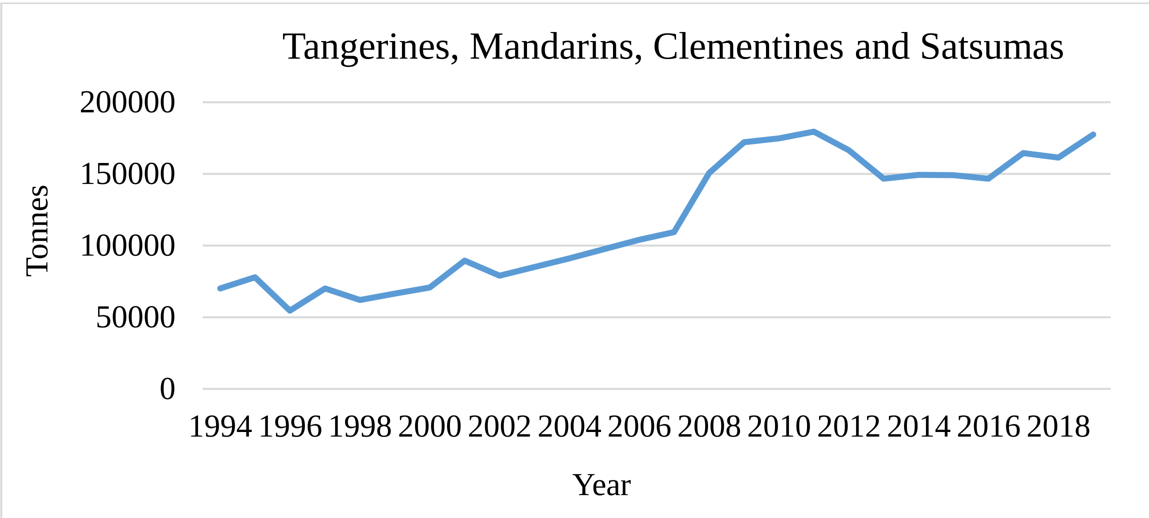

Fig. 2: Tangerines, Mandarins, Clementines and Satsumas production trend in Nepal (www.fao.org)

Citrus greening is associated with three species of gram-negative, unculturable, and phloem-restricted bacteria: *Candidatus* Liberibacter asiaticus, *Candidatus* Liberibacter africanus, and *Candidatus* Liberibacter americanus. *Candidatus* Liberibacter asiaticus is the most common species distributed all over Asia including Nepal (Paudyal, 2015). The disease is transmitted by an insect vector, Asian Citrus Psyllid (*Diaphorina citri*).

Symptoms of HLB Disease

Blotchy Mottle and Yellow Shoots

Blotchy mottling is one of the characteristic symptoms of HLB. The mottling of leaves in HLB infected plants differs from other nutrient deficiency symptoms in that the yellowing is not symmetric across the leaf midrib (see Fig. 3A) and usually crosses leaf veins (Bove, 2006; Halbert and Manjunath, 2004). Besides blotchy mottling, infected leaves may also become thicker, leathery, upright along with the development of interveinal chlorosis. Midribs and lateral veins may sometimes become enlarged, swollen, and corky. In advanced stages of infection, it can eventually be accompanied by zinc deficiency making the leaves have an upright growth with a close angle with the shoot (Bove, 2006). Yellowing can occur in one or several shoots which with time, grows into a larger yellow branch later spreading throughout the canopy (Weinert *et al*., 2004). Off-season bloom and twig dieback are additional symptoms exhibited on the shoots (Halbert and Manjunath, 2004).

Symptoms on Fruit and Juice Quality

Affected fruits look small, asymmetrical, lopsided, and with thicker albedo at the peduncular end. The peduncular end turns yellow/orange but the stylar end may remain green and small, brownish/black aborted seeds could also be observed. Premature fruit drop is very common in HLB affected fruits (McCollum and Baldwin, 2017). The off-flavor of juice from HLB affected fruit is a result of lower sugars, higher bitter limonoids, flavonoids, and terpenoid volatiles (Baldwin *et al*., 2018). The symptomatic fruit also shows lower juice content, lower Total Soluble Solids (TSS) and TSS/Titratable Acidity (Bassanezi *et al*., 2009; Montesino and Stuchi, 2009).

Symptoms on Roots

Candidatus Liberibacter asiaticus is found to severely affect the fibrous root system. This bacterium first colonizes in roots before showing symptoms infecting leaves (Johnson *et al*., 2014). The damage on the root system, especially the loss of fibrous roots can be severe before any symptoms are seen in the canopy. The roots could also be starved led by the accumulation of food resources in the form of stored carbohydrate in the aerial parts inhibiting nutrient partitioning (Dala-Paula *et al*., 2019; Zheng *et al*., 2018). The symptoms of HLB are considered to be more apparent during cooler seasons compared to warmer seasons (Dala-Paula *et al*., 2019, McCollum and Baldwin, 2017).

Asian Citrus Psyllid

Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) is a small plant feeding insect with the size of an adult ranging from 2.7-3.3mm long and identified by distinctive mottled brown wings and abdomen with three color ranges: gray/brown, blue/green and orange/yellow (Fig.4). The insect has five instars with docile early instars and mobile older nymphs and adults. The eggs are oval with light yellow color when freshly deposited and distinctive red eye spots when mature (Hall *et al*., 2013) Citrus psyllid (*Diaphorina citri*) damages the plant by sucking the sap from the foliage and excreting sugary substance that covers the leaf with honeydew which then gets covered by sooty mold. As there is no remedial therapy available for the HLB disease, it is important to reduce the transmission of the disease by controlling its vector (Yan *et al*., 2015).

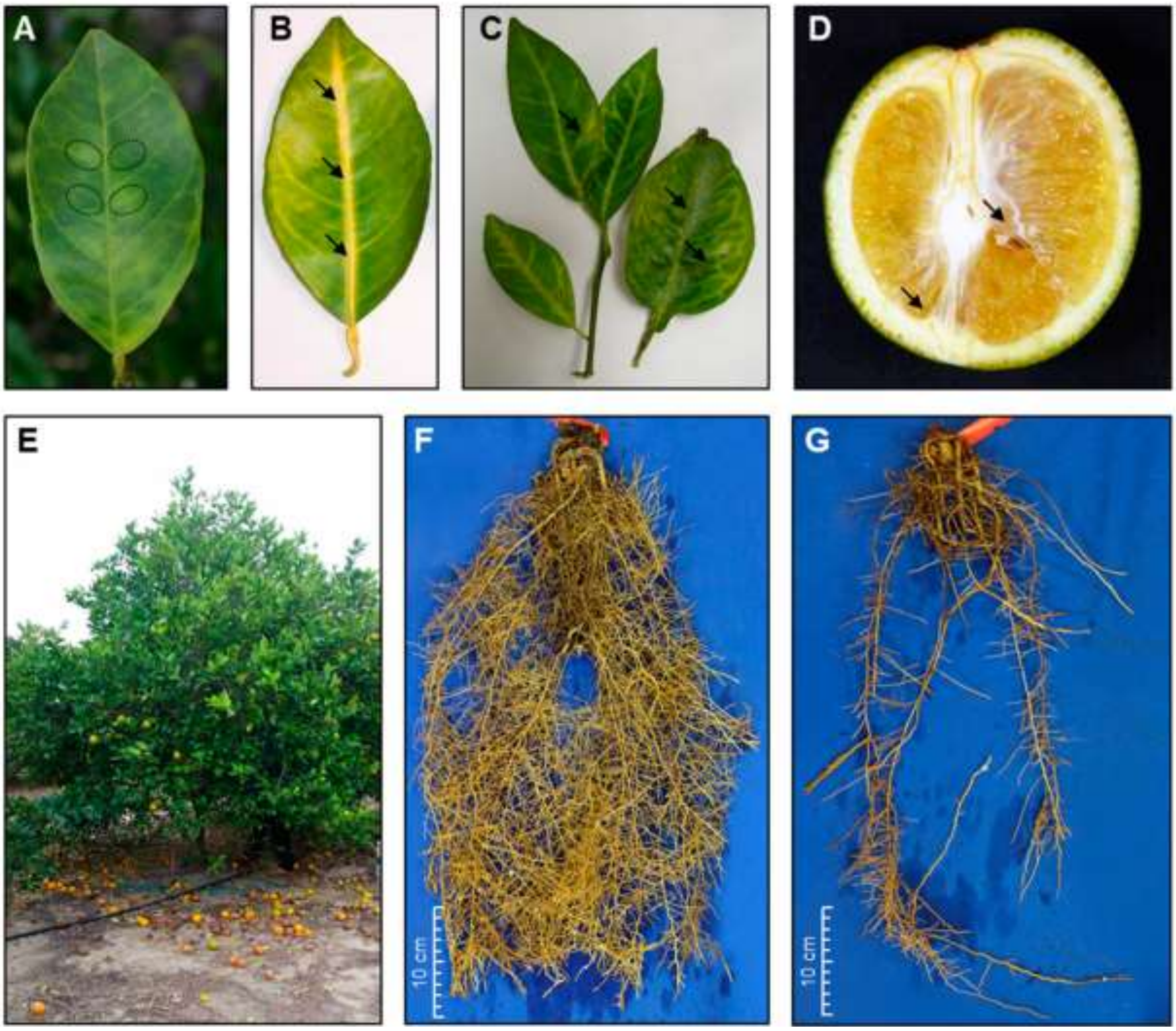

Fig. 3: Symptoms related to HLB disease: (A) Asymmetrical blotchy mottle in leaves (B) Yellow coloration in leaf vein (C) Leathery leaves and corky veins (D) Lopsided and asymmetrical fruits with aborted seeds (E) Severe pre-harvest fruit drop (F) Healthy roots and (G) severe fibrous root loss due to HLB disease. *(Source: Nehela and Killiny,2020)*

Fig. 4: Images of Asian Citrus Psyllid. Source: www.aphis.usda.gov

HLB and Economic Loss

HLB spreads rapidly throughout the tree canopy and in the orchard, reducing the economic life of affected trees (Bove, 2014). As no cure for the disease has been found, growers need to rely on various management techniques in order to maintain the tree's health and longevity. Those management strategies often have very high costs associated with them (Li *et al*., 2020).

Since the advent of HLB disease in Florida in 2005, the citrus production in the USA has dropped from 16 million tons in 2003-04 to 8 million tons in 2019-2020 (www.nass.usda.gov). In the same time period, the reduction in citrus production in Florida was from about 13 million tons to 3 million tons. It has been estimated that the cultural cost to manage HLB in citrus orchards in Florida has been increased by 67% compared to pre-HLB period (Singerman, 2019).

HLB damaged millions of hectares of citrus orchards in China (Li *et al*., 2020), which is believed to be one the places of origin of the disease. Citrus production in Guangdong, Guangxi and Fujian provinces has been severely affected and a recent HLB outbreak in 2013 in Ganzhou, Jiangxi province resulted in the destruction of about 50 million trees. In previous years, HLB had decreased the productivity of citrus up to 80% in different areas of India (Singh, 1977). It was suspected that more than 40-70% trees were infected in Thailand and Nepal in past years (Regmi, 1982).

HLB History

There is no definite evidence of the origin of HLB disease. It was observed as early as in 18th century in India, which was first thought to be associated with Citrus tristeza virus but later suggested to be citrus greening (da Graca, 2008). The HLB disease has been known to be existing in China for more than a century (Zhao, 1981). The clear symptoms of disease were observed in 1938 (Chen,1943) and in 1927 the insect vector *Diaphorina citri* was first described (Husain and Nath,1927). In South Africa, symptoms for the disease were observed in 1937 (van der Merwe and Anderson, 1937). The disease was mostly detected and was rapidly spreading during the 19th century. It has reached over 50 citrus producing countries of Africa, Asia, Europe, North America and South America (https://www.cabi.org/isc/datasheet/16567).

China and India, the two neighboring countries of Nepal were among the first countries to report the symptoms of HLB disease in citrus (da Graça, 2008; Bove, 2006). HLB is believed to be transmitted in Nepal while importing the rootstocks from Uttar Pradesh, India. The disease symptoms of tree decline were reported for the first time in Nepal in Pokhara valley in 1968 (Thrower, 1968). Catling (1968) visited that area and considered that the citrus decline was associated with HLB disease. It was later confirmed that 54% among 132 trees sampled from the location (Pokhara to Ranigaun) were infected by citrus greening disease (Knorr *et al*., 1970). The same study also suggested the HLB incidence rate of 53% in west Nepal, 43% in Kathmandu valley and 59% in east Nepal. By 1982, 55 % of trees in Pokhara valley and 100 % of trees in Horticulture research station were found symptomatic of citrus greening disease (Regmi, 1982). Recently, the disease has been detected on several important citrus production regions of Nepal as evident by the presence of citrus psyllid, visual disease symptoms and other confirmatory tests (Manandhar *et al*., 2004; Regmi *et al*., 2010; Regmi and Yadav, 2007).

Host Range and Ecology for Disease Progression

Although all citrus species could be affected by HLB (Bové, 2006), those species had shown variable responses. Sweet orange, mandarin and tangelo trees were severely affected while other species had displayed more or less pronounced symptoms. Even though Mexican lime (*Citrus aurantifolia*) is a preferred host of the vector *Diaphorina citri* (Roastcher, 1996), it was less susceptible than sweet orange and mandarin. However, the mandarin hybrid 'Sugar Bell' was found tolerant to HLB disease (Killiny *et al*., 2017). Besides those cultivated citrus species, other species of Rutaceae family such as cape chestnut (*Calodendrum capense*), Orange Jasmine (*Murraya paniculata*), and Kumquats (*Fortunella*) are also the hosts of Huanglongbing disease (www.cabi.org/isc/).

HLB transmission is feasible between 16 to 33°C with peak transmission at around 25°C (Taylor *et al*., 2019). The disease progression is highly favored near the equator's large citrus-producing regions such as Brazil and South-East Asia. The Asian Citrus psyllid is tolerant to heat and can withstand 30-35 °C but the optimum range for its development is 25-28 °C (da Graca and Korsten, 2004).

The presence of cultivated and wild host plants for HLB disease progression and the favorable climate for HLB transmission and psyllid development in citrus production areas of Nepal renders Nepalese citrus orchards vulnerable to citrus greening disease.

HLB Detection

Besides the visual symptoms as mentioned previously in this article, there are several other methods to detect the HLB disease in *Citrus sp*. One of the simple and faster diagnostic methods is by using the Iodine test. (Taba *et al*., 2006). Citrus greening leads to starch accumulation in the leaves (Etxeberria *et al*., 2007). The starch reacts with iodine to give a dark gray to black stain, which can be a convenient way to identify HLB disease. The correct selection of the infected leaves and the concentration and quality of the iodine solution used in the test determines the accuracy of the scratch test (Adhikari *et al*., 2012).

HLB symptoms are often mistaken for leaf nutrient deficiencies. Pen test is a simple tool that helps to distinguish the asymmetrical pattern of chlorosis due to HLB as compared to the symmetrical chlorosis because of mineral deficiencies (Vashisth and Kadyampakeni, 2020). In this test, a circle needs to be drawn on each side of midvein of a leaf. If the same pattern of Chlorosis is observed within two circles, it indicates the nutrient deficiency symptom while different pattern indicates the possibility of HLB disease.

A real time PCR (RT-PCR) has been widely used for the detection of the citrus greening bacteria. It is based on the use of appropriate primers and probes that amplify DNA sequences of Liberibacter associated with HLB (Li *et al*., 2006). HLB can also be detected using spectroscopy technique. Canopy reflectance spectra can be measured on both infected and healthy trees using a spectroradiometer. using three common classification algorithms to distinguish the infected trees from the healthy trees. By using multiple spectral measurements from the canopy of a single tree, the classification accuracy can be increased (Mishra *et al*., 2012).

HLB Management Strategies

HLB-affected trees, if not removed and managed in time, serve as the inoculum source. A minimum of 15 minutes of feeding by the psyllid is sufficient to transmit the causal agent of citrus greening to the healthy plants (Hall *et al*., 2007). Therefore, early scouting when the number of infected trees is low and more frequent scouting if the disease has been present on the field or nearby will help in managing HLB disease. It is important to use the propagation material from disease free nursery to reduce the damage of HLB infection during early growth stage. Once the majority of trees in the orchards are infected by the citrus greening disease, it is hard to control the disease. Following options are commonly practiced in order to maintain tree health and sustain the tree yield despite the presence of the disease in the field.

Use of HLB-Tolerant Citrus Rootstocks

Citrus rootstocks have played an important role in the management of diseases such as gummosis, Phytophthora root rot, and citrus tristeza virus during the 19th and 20th century (Bowman and Joubert, 2020). The necessity of grafting desirable citrus varieties with the disease free and high-quality rootstocks was realized several years ago in Nepal (Regmi *et al*., 2010).

There are several rootstocks that are tolerant to HLB disease (Albrecht and Bowman, 2011; Ramadugu *et al*., 2016; Kunwar *et al*., 2020). Grafting the desired scion on the HLB tolerant rootstocks can be an effective HLB management strategy without addition of extra cost. Rootstock cultivars such as 'US-897' ('Cleopatra' mandarin [*Citrus reticulata*] × 'Flying Dragon' trifoliate orange [*Poncirus trifoliata*] (Albrecht and Bowman, 2011), 'US-942' (*C. reticulata* 'Sunki' × *Poncirus trifoliata* 'Flying Dragon') (Bowman *et al*., 2016), and Carrizo citrange (*C. sinensis* × *Poncirus trifoliata*) (Albrecht and Bowman, 2012) were found tolerant to HLB disease. Similarly, the rootstocks 'US-802' ('Siamese' pummelo [*Citrus maxima*] × 'Gotha Road' trifoliate orange [*Poncirus trifoliata*]), and 'US-812' ('Sunki' mandarin × 'Benecke' trifoliate orange) were found moderately tolerant to citrus greening (Albrecht and Bowman, 2012).

The altitude for citrus production in Nepal ranges from 450 masl to 1300 masl (Manandhar *et al*., 2004). Mandarin orange is an indigenous fruit of Nepal (Bonvia, 1989) and several desirable varieties are locally available (Gautam *et al*., 2004). Several trifoliate and other rootstocks are also already in use in the country (Lama, 2004). Conducting research on HLB tolerant rootstocks and scion cultivars in various ecological zones of the nation facilitates the exploration for the best performing rootstocks and scion partners under HLB pressure.

Citrus are commonly propagated by seeds as nucellar polyembryony can produce true-to-type plants from seeds (Koltunow *et al*., 1995). Trees can take several years for seed production and some of the rootstocks might not exhibit nucellar polyembryony. In such cases, they can be propagated alternatively by cuttings or tissue culture (Pokhrel *et al*., 2020). This is important in Nepal as the country has diversity in citrus genotypes (Budathoki *et al*., 2004), but there is limited commercialization of many citrus varieties.

Irrigation, Nutrition Management and Hormones

In comparison to healthy trees, HLB-affected trees bear weaker root systems and also have been reported to have lower concentration of nutrients like Potassium, Calcium, Magnesium, Copper, Iron, Zinc, and Manganese (Vashisth, 2016). Frequent application of small doses of fertilizers (in leaves or roots) enabling regular supply of nutrients are more effective in reducing potential nutrient leaching. Fertigation and controlled-release fertilizers are also effective nutrient delivery methods (Phuyal *et al*., 2020; Morgan *et al*., 2016).

In a study by Uthman *et al*. (2020), it was suggested that for up to 224 kg N ha^{-1} supply, the leaf N concentration, canopy volume and yield of HLB-affected trees increased whereas beyond that level no additional advantage was obtained. Atta *et al*. (2021) also found out that split ground application of 224 $kgha^{-1}$ of N and both foliar applied coupled with ground-applied Mn and Zn each at 9 $kgha^{-1}$ sustainably supplied these nutrients within optimum ranges and further improved vegetative growth of HLB-affected trees. Foliar sprays are widely used but more expensive than soil-applied fertilizers and are more affected by regular rainfall, high temperatures, and heavy wind (Phuyal *et al*., 2020).

Brassinosteroids, a relatively new class of phytohormones has shown good results against HLB through reduction in symptoms following early bloom, reduced fruit drop and increased yield (Alferez *et al*., 2019). Foliar application of Epibrassinolide, a type of Brassinosteroid, resulted in no re-emergence of symptoms in new shoots whereas the old leaves retained the symptoms. An application of another plant hormone 'Strigolactone' on HLB-affected trees was found to reverse certain physiological processes responsible for tree decline by restoring tree growth and mobilizing carbohydrate. Strigolactone application on HLB affected tree was found to increase the number of flowers bearing branches, active flowers, fruit setting and additional summer flushes (Zheng *et al*., 2018). Studies have suggested that frequent irrigations with smaller quantities of water benefit HLB-affected trees in terms of improvement in tree canopy density, leaf area, and reduction in leaf fall. Higher content of bicarbonates in irrigation water escalates the problem of higher fibrous root loss due to HLB (Vashisth, 2016).

Other Strategies

The application of aqueous extract from *Quercus hemisphaerica* has been found to reduce the bacterial titer and enhance the overall tree physiology of HLB affected trees. The trees treated with oak extract had better stomatal conductance, chlorophyll content and nitrogen uptake (Pitino *et al*., 2020). Several oak species such as *Quercus glauca* (Pokhara); *Quercus semecarpifolia*: Lumle, Phulchowki (Kathmandu); *Quercus incana*: Hattiban (Kathmandu) are found in Nepal (Adhikari, 2014). This suggests a possibility of evaluating the effect of oak extracts for the management of citrus greening disease in the country.

HLB management strategies also include foliar spray, root drench and trunk injection of antibiotics (Puttamuk *et al*., 2014; Zhang *et al*., 2011). Peptide conjugated morpholinos (PPMOs) have been applied to prevent bacterial growth (Wesolowski *et al*., 2013; Hegarty and Stewart, 2018). The continuous exposure of infected citrus seedlings to 40 to 42 °C heat for at least 48 hours has been reported to be sufficient to reduce pathogen titers (Doud *et al*., 2017). However, all these methods are often debatable and have limited success. Furthermore, these technologies involve very high cost and currently might not be feasible for Nepalese growers.

Strategies to Manage Citrus Psyllid (*Vector Control)*

The psyllid is reported to be spread from eastern to western borders of citrus producing areas of Nepal (Regmi *et al*., 2010). The vector prefers lower altitude and hot and dry conditions (Hall et. al., 2007).

Chemical Management

Since the timing of pesticide use is critical for management of citrus psyllids, monitoring of the pest through scouting of the pests and use of yellow sticky traps is necessary to time control action (Halbert and Manjunath, 2004; Hall *et al*., 2013). Reduced population of ACP can be achieved when the intensive insecticides are applied at peak citrus flushing period (Hall *et al*., 2013). The use of foliar and systemic insecticides such as imidacloprid, fenpropathrin, chlorpyrifos and dimethoate are considered an effective approach to reduce the citrus psyllid numbers. The approach of using the aqueous solution of either 0.05% dimethoate or 0.02% chlorpyrifos or imidacloprid at bud burst stage for the control of citrus psyllid is also followed in India and China (Department of Agriculture, 2011) and are also readily available in the Nepalese markets (Bhusal *et al*., 2019; Diwakar *et al*., 2008). It is found that systemic soil applied imidacloprid properties has less impact on natural enemies (Boina *et al*., 2009) and also provides a longer period of protection than many foliar insecticides (Grafton-Cardwell *et al*., 2013) The systemic soil applied imidacloprid were also found to decrease their potential of acquiring and transmitting the HLB pathogen (Boina *et al*., 2009). Systemic insecticides are most useful in the young trees that flush more regularly and require constant protection. Rotation of pesticides with different mode of actions should be considered to prevent psyllids from developing resistance against the used pesticides (Tiwari *et al*., 2011).

Horticultural oils and Insect Growth Regulators (IGRs) are found to be more effective against eggs and nymphs than adults (Grafton-Cardwell *et al.*, 2013). In some studies, essential oil from *Artemisia absinthium* is also found toxic to the psyllids but its toxicity was reported to be weaker than synthetic insecticides (Rizvi *et al.*, 2018). However, the availability of the essential oils in the Nepalese market might render its use in HLB management (Bhusal *et al.*, 2019).

Biological Control

Increased use of insecticides for the control of the *D. citri* may cause negative impact on natural enemies, parasitoids and predators. The alternative to chemical management is the use of biological control. In Asia, the native parasitoids *Tamarixia radiata* and *Diaphorencyrtus aligarhensis* are found to attack and control ACP (Hall *et al.*, 2013). In studies conducted by Chien and Chu (1996) in Taiwan and Michaud (2002) in Phillippines, these two parasitoids were found effective in parasitizing ACP nymphs in both laboratory and field conditions. A study by Khan *et al.* (2016) found that *Adalia bipunctata* adults and larvae fed on the diet of *D. citri* nymphs. *A. bipunctata* (Coleoptera: Coccinellidae) is found in the various crop and forest ecosystem in Asia and was also observed in the field crop survey in Pakistan along with other species of coccinellids that might potentially be predatory to *D. citri* (Khan *et al.*, 2016). In a study conducted by Qureshi and Stansly (2009), the cohorts of *D. citri* that were protected by cages from natural enemies were found to have low mortality compared to the unprotected ones and the significant natural mortality was attributed to lady beetles (Qureshi and Stansly, 2007). The predatory mites *Ambylseius swirskii* were also found useful in reducing *D. citri* through feeding on its eggs and neonates (Juan-Blasco *et al.*, 2012). Pathogenic fungi like *Cladosporium* sp., *Capnodium citri*, *Hirsutella citriformis* have been used for biocontrol purposes since a long time (Halbert and Manjunath, 2004; Hall *et al.*, 2013).

Physical and Chemical Repellents

The use of physical repellants like kaolin clay particle film and metallized polyethylene mulch is also an area of research in citrus psyllid management (Croxton and Stansly, 2014; Grafton-Cardwell *et al.*, 2013; Hall *et al.*, 2007). The effectiveness of kaolin clay particles is accounted to the fact that the clay particle film inhibits the grasping, oviposition ability and movement of the psyllid adults. However, kaolin poses the problem of being easily washed away by the rain or being ruptured by growing and expanding shoots (Hall *et al.*, 2007). Therefore, care should be taken while selecting a proper day and time of kaolin application. Use of pest exclusion nets can also exclude the psyllid from citrus trees and can help to produce HLB free trees during young growth stage (Alferez, 2019).

Intercropping of guava (*Psidium guajava*) with citrus plants was found to decrease psyllid population and increase longevity of citrus trees compared to monocultures in Japan and Australia but the results are not consistent (Hall *et al.*, 2013). Gas chromatography- mass spectrometry (GC-MS) analysis attributed the repellence of psyllids to the plant volatile dimethyl disulfide (DMDs) present in wounded guava leaves (Rouseff *et al.*, 2008; Onagbola *et al.*, 2011). The plant volatiles present in garlic chives (*Allium tuberosum*) disulfides and trisulfides, volatile oils extracted from non-host plants *Mikania micrantha, Lantana camara, Eupatorium catarium*, *Wedelia chinensis* were found to reduce the number of ACP adults (Yijing *et al.*, 2005; Mann *et al.*, 2011).

Studies also suggest the attraction of citrus psyllids to the chemical methyl salicylate which is produced by infested pear trees (Molleman *et al.*, 1997) as well as ACP infested citrus trees (Mann *et al.*, 2012). The presence of host plants of preferred family, Rutaceae, such as *Murraya paniculata* and *Citrus paradisii* were found to be determinant in increasing psyllid densities (Tsai and Liu, 2000). The odor from the host plants infected by *Candidatus* liberibacter were found to be significantly attractive to psyllid oviposition and adult population (Zhao *et al.*, 2013). They were also found to react to olfactory and visual cues like bright yellow and green of the flushing shoots for the detection, location and evaluation of potential hosts (Wenninger *et al.*, 2009). This information can be useful in understanding the management strategies for the control of the psyllid population by getting rid of psyllid host plants (Department of Agriculture, 2011) or developing an attract and kill approach by trap cropping, intercropping or using chemicals (Yan *et al.*, 2015). It is found that extremely low temperatures limit the development and dispersal of *D. citri* (Wang *et al.*, 2019). In Asia, *Diaphorina citri* was not found above the elevation of 1300-1500m which could be due to its low frost tolerance and weak flight ability making them unable to sustain long distance flights (Halbert and Manjunath, 2004; Hall *et al.*, 2013). It opens the possibility of establishing citrus nurseries in the high hills of the country to observe whether those nurseries would be free from HLB infection. However, it is also equally essential to consider the physiology of citrus under the chilling temperature of those regions.

Quarantine and Regulation

The geographical range of Asian citrus psyllid and HLB is ever expanding (Hall *et al.*, 2013) and the intervention for its management is mostly ineffective (Taylor *et al.*, 2019). Citrus production is a great contributor to the economy of the United States and Brazil, but these countries are facing severe decline of citrus due to citrus greening (Olexa and Benn, 2018). The first line of defense for the management of ACP and HLB is restricting the movement of infested plants and quarantining areas where the pests are abundant (Hall *et al.*, 2013). The estimation of maximum flight distance could be a good measure of determining proper quarantine strategies for HLB management. In Nepal, 30 km of separation was found to be sufficient (Halbert and Manjunath, 2004). However, more research is necessary to support the claim. Citrus growing regions all over Nepal are impacted by HLB (Department of Agriculture, 2011). Nepal has developed various strategies and survey protocols to combat the problem of citrus decline. Some of these strategies include detailed survey of citrus greening and virus incidence, survey of alternate hosts, and identification of disease-free citrus growing areas. Government of Nepal has identified lack of internal quarantine as a problem that has been causing the flow of uncertified plant materials in and out of the country. The major problem lies within the small land holding (<0.5ha) of citrus cultivating growers and small production volume as well as open border with India that makes implementation of management strategies more difficult (Department of Agriculture, 2011).

The same problem is faced by the USA and Brazil, both the countries have been working together through information dissemination and knowledge sharing to understand the pathogen and the disease best possible (Olexa and Benn, 2018). The collaborations of the government agencies with the agriculture universities in the US has helped research on the problem and find potential solutions like the USDA funded projects in University of Florida Institute of Agriculture and Food Sciences. Local grower group (Citrus Health Management Areas) has been formed in Florida that coordinates the rotation of pesticides amongst themselves to ensure pest resistance doesn't pose a problem. The Florida legislature has also passed the Greening Removal or Vector Elimination (GROVE) program that helps growers that wish to remove and replant infected plants by cost sharing. The Federal government of Brazil has also placed policies and rules to citrus growers to inspect and report the citrus groves quarterly. The Agriculture Defense Coordination of Sao Paulo, Brazil is also responsible for enforcing laws for nursery management, providing resources to growers to mitigate the citrus greening problem and also regulating the rootstocks used by the growers. In Brazil, growers have started working together to remove and replant the citrus trees which has been effective in managing citrus greening.

Since, the major strategy for the management of HLB is eradication of the infected plants which directly impacts the grower's economy, a proper cooperation of growers will be crucial (Singerman and Rogers, 2020). Nepalese government can mitigate the loss to the growers by providing compensation to the growers who endure economic loss of certain percentage taking example from the initiative by USDA's Tree Assistance Program in the US (Olexa and Benn, 2018) and various other initiatives from these countries and implementing as much is feasible.

Conclusion

Citrus greening disease has challenged the citrus production in various areas of the world. It is imperative to rely on management strategies to mitigate the impact of HLB in citrus as there is no cure available for the disease. Several management options are practiced throughout the world in order to sustain citrus health and production under HLB-endemic condition. Although some management tools require heavy economic investment and are unsuitable for small farm holder, several other options are economically feasible and environmentally sound. To rejuvenate citrus production in Nepal, it is essential to look for proper HLB management techniques and suitable HLB-vector control strategies.

Authors' Contribution

All authors contributed equally in all stages of this work. Final form of manuscript was approved by all authors.

Conflict of Interest

The authors declare that there is no conflict of interest with present publication.

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