

REVIEW ARTICLE

CITRUS GREENING: A MAJOR THREAT TO CITRUS GROWERS IN NEPAL

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ABSTRACT

Citrus is a high-value horticultural crop belonging to the family Rutaceae, contributing 27.27% of the total fruit area in Nepal. In recent years, citrus decline has been a multifaceted issue for most of the citrus growers, with abated production and productivity. Among the various causes of citrus decline, citrus greening is considered the most devastating, rapidly expanding, and highly incurable disease, which has wreaked havoc on the citrus industry across the globe. Citrus psylla is the major insect vector to spread this malady worldwide. There are still no proper strategies to cope with the impact of citrus greening. However, integrated pest management strategies could be adopted in Nepal like the use of tolerant rootstocks, a proper quarantine system, guava intercropping, nutrient management, the use of yellow sticky traps, and vector control methods to reduce the greening infestation in citrus trees. This study aims to review all the relevant information regarding citrus greening, including its causes, symptoms, diagnosis, and management practices that are pivotal for citrus growers.

KEYWORDS

Decline, Incurable, Malady, Psylla, Quarantine

1. INTRODUCTION

Citrus is the most important and traded horticultural high-value crop, which is grown in multifarious agro-ecological zones like arid to semi-arid zones (Chandrasekaran et al., 2021). It is believed to have originated in Southeast Asia and belongs to the Rutaceae family. It contributes about 27.27% (46,715 ha) of the total area among the fruit areas in Nepal, regarding a pivotal crop to uplift the socioeconomic standards of the farmers, and shares 3% of total fruit exports by volume in Nepal (MoALD, 2021; Dahal et al., 2020). In the last 12 years, the productive orchard size has declined from 69.56% to 58.52% (MoALD, 2021). In 2019 A.D., it is cultivated in 46,412-hectare area with 28,406 hectare of productive area having 2, 71,908 metric tons as a total production and 9.57 metric tons per hectare as productivity (MoALD, 2019).

Traditionally, in general citrus and mandarin (*Citrus reticulata*) is grown as one of the paramount fruit crops in Nepal. However, in recent times; improper cultivation practices have undermined both production and productivity of citrus (MoAC, 2011). Low-quality planting materials, poor orchard management, citrus greening, citrus tristeza virus (transmitted by the aphid *Toxoptera citridus*), incompatibility of rootstock, etc. are the major causes of the citrus decline. Among the diverse causes of citrus decline, citrus greening is regarded as a destructive disease of citrus, which is ubiquitous producing a major threat to the citrus growers around the whole globe (Singerman and Rogers, 2020).

Citrus greening is also referred as "Huanglongbing" (HLB) disease, wreaks havoc to the citrus industry of world because once trees got affected, there is no chance of the panacea (Joshi and Khanal, 2020). During 19th century, in Southern china, HLB is also known as "yellow dragon disease" (Bove, 2006). This disease is widely recorded and is the most devastating and rapidly expanding disease in most citrus species (Regmi and Yadav, 2007). At present, the disease seems prevalent in almost 69 citrus-producing districts in Nepal. Some of the citrus nurseries in the country are located below 1000 masl altitude under an open-sapling production system. In

lower altitude areas of Nepal, the insect vector of greening disease (Asian Citrus Psyllid—ACP) is considered active because of the availability of a favorable environment (Acharya and Adhikari, 2022).

On December 6–7, 2021; the interaction program was organized by the National Fruit Development Centre, Kritipur on fruit nursery certification under the Department of Agriculture (DOA), Ministry of Agriculture and Livestock Development (MoALD), Nepal related to the concept of a clean plant center for the disease-free citrus planting material production (Acharya and Adhikari, 2022).

2. VARIETY SUSCEPTIBILITY TO CITRUS GREENING

- In South Africa, greening is the major malady of Sweet Orange (*Citrus sinensis*), with Valencia showing vigorous symptoms in leaves than Washington navel (Obergolzer et al., 1965).
- According to the common citrus cultivars susceptible to greening disease are categorized as: Severe (mandarin, sweet orange, tangelo), Moderate (grapefruit, sour orange, lemon), and Tolerant (lime, trifoliate orange, pummelo) (Maniconi and Van Vuuren, 1990).
- In South Africa, the citrus greening percentages was found higher on Trifoliate orange rootstock than on Empress Mandarin and Troyer citrange because the trifoliate rootstock causes an extension of the period of flush and thus distend the feeding time of the citrus psylla (Van Vuuren and Moll, 1985).

3. CAUSES

Citrus greening cause severe citrus decline in Nepal and had an intimidate effect on the citrus industry (Roistacher, 1996). Before symptoms appeared above ground, a loss of 30-50% tree's fibrous roots had been accommodated (Johnson et al., 2014). Sweet orange and Mandarin are prone to HLB while lime and lemon are less prone to HLB (Shokrollah et

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al., 2011). It is mainly transmitted by insect vectors: Asian Citrus Psyllid (ACP) with scientific name *Diaphorina citri* (Hemiptera: Liviidae) is heat resistant and African citrus psyllid (*Trioza erytreae*) which is sensitive to heat but resistant to rainfall and humidity (Bove, 2006). ACP has become immensely important as it can carry gram negative bacterium *Candidatus liberibacter asiaticus* (CLas) bacterium causes HLB disease which is particularly restricted to vascular bundle i.e. phloem vessels (Chandrasekaran et al., 2021). Citrus psyllid (*Diaphorina citri*) affects the plant leaves by sucking sap from it. This vector excretes honeydew that spread throughout the whole leaf and further by sooty mold (Yan et al., 2015). HLB symptoms in cooler months are more apparent than warmer months. Generally, bacteria are associated with three different species:

- Asian form ; *Candidatus liberibacter asiaticus* (heat tolerant)
- African form; *Candidatus liberibacter africanus* (heat sensitive)
- American form; *Candidatus liberibacter americanus* (heat sensitive)

Source: (Tipu et al., 2020)

ACP can transmit both forms of greening bacteria (Asian and African) contemporarily; the major differences between Asian greening and African greening are given below:

Asian Greening	African Greening
It develops greening symptoms up to temperature (30-35 degree celsius)	It develops greening symptoms up to temperature (20-25 degree celsius)
It can cause more extensive dieback	It can cause less extensive dieback
It is less difficult to transmit through grafting	It is more difficult to transmit through grafting

Source: (Batool, et al., 2007)

3.1 Host plants of Asian Citrus Psyllid

The most common host for Asian Citrus Psyllid is in the genera *Citropsis*, *Citrus*, *Murraya paniculata*. Due to its restricted host range, monitoring and evaluation efforts should be conducted on varieties of citrus and closely related plants.

3.2 Life cycle of Asian Citrus Psyllid

ACP female lays eggs which are yellow-orange in color and these eggs tucked into the top portion of new leaves. Eggs are difficult to discern by naked eyes as they are minuscule in nature. Adult females lay up to 800 eggs during their lifetime and hatching of eggs occurs after 3-4 days at 77°F (25°C) and enter into the nymphal stage. Nymphs are green or dull orange and feed on young leaves and stems. In one generation, there are up to five nymphal stages that look similar but increases in size after each molt. The final instars have large wing pads that develop into an adult after 10-15 days by feeding on new shoots and making the leaves, stems yellow and even roots are affected severely which hinders root growth. Adults are infinitesimal (2.7-3.3 mm long) having brown wings mottled. They have relatively three distinct colors of abdomen: orange/yellow, blue/green, grey/brown (Grafton-Cardwell and E, 2006).

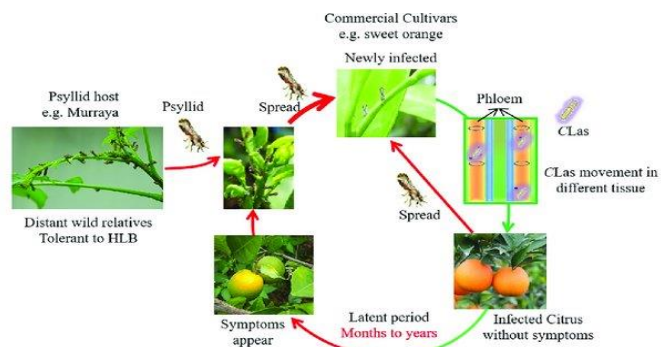


Figure 1: Disease cycle of HLB (Source: Aglave, 2018)

4. DIAGNOSIS

For the diagnosis of biotic factors like Huanglongbing (HLB), there are two widely used molecular techniques as given below:

- Conventional Polymerase Chain Reaction (PCR)
- Real Time-PCR (RT-PCR), also known as quantitative PCR (qPCR)

These techniques are based on the use of primers of PCR that amplify sequences of *Liberibacter* DNA associated with HLB. PCR test for diagnosis of HLB is unsuitable for higher numbers of samples, expensive method and requirement of labour is also high but considered as a reliable method (Etxeberria et al., 2007). To overcome this problem, field diagnostic iodine test (rapid, simple) has been developed to pre-screen samples intended for PCR analysis (Taba et al., 2006). In past few years, USA has initiated more efficient and cost-effective HLB detection techniques using spectroscopy especially after detection of HLB in Brazil and Florida. Visible-near infrared spectroscopy technique has feasibility evaluation of 92% accuracy for field detection of HLB in citrus trees using spectral reflectance data from the wavelength range of 350–2500 nm (Sankaran, 2010). Fourier transform infrared-attenuated total reflection (FT-IR-ATR) spectroscopy was found as a potentially suitable and inexpensive technique for rapid and early detection of HLB (Hawkins et al., 2010). The mid-infrared region of the spectrum reveals dramatic changes that take place in the infected leaves when compared to healthy non-infected leaves (Sankaran, 2010).

5. SYMPTOMS

HLB bacteria enter into the citrus trees through leaves. But the disease attacks roots long before the leaves show symptoms. When bacteria enter through psyllid sucks into leaves, then the bacteria move down to the roots for replication, and destroy the entire root system and diffuse to the rest part of citrus tree (Johnson et al., 2014). In comparison to healthy fruit, HLB-affected fruit are prone to higher storage decay incidence and do not respond well to postharvest degreening (Shahzad et al., 2022). Mottling of leaves, premature falling of leaves, and bitter tasted fruits which are due to high amounts of acidity and low amount of sugar, poor root growth are the major common symptoms of greening disease leading to the death of entire plant (Bist & Bista, 2020). Sometimes, HLB disease can be mistaken with the leaves with micro-nutritional deficiencies like Zn. In the case of Zn deficiency, chlorosis are seen as symmetrical on both sides of leaves whereas greening leads to an irregular, mottled (asymmetrical) appearance without forming uniform pattern in the leaves of citrus species (Mattos-Jr et al., 2020).

5.1 Yellow Shoots Symptoms

The 'yellow shoots' is the common symptom of greening disease in which leaves have the several shades of yellow, pale green, dark green blending into each other. This symptom is also known as "blotchy mottle" which means the discoloration spreads away from the veins (McClellan and Schwarz, 1970). At first, the diminutive leaves are pale and at mature stage; later they develop the secondary chlorotic patterns (yellow and green). Small terminal leaves are mostly green and only a few of them showing a slight interveinal chlorosis. Yellowing first occurs in several shoots, and with respect to time, it grows into a larger yellow branch which later promulgate throughout the canopy (Weinert et al., 2004). The seasonal change in temperature seems to cause an "ebb and flow" in the severity of symptoms (McClellan and Schwarz, 1970).

5.2 Fruit Symptoms

The causative bacteria *Candidatus liberibacter* spp does severe effect on the quality of citrus fruits. Infected orange fruits can be either symptomatic or asymptomatic. High level of titratable acidity (TA), monoterpenes, increase in secondary metabolites like hydroxycinnamic acid, limonin, nomilin, hesperidin, the polymethoxylated flavones (PMFs), nobiletin and tangeretin causing bitterness to the fruits, lower level of total soluble solids (TSS), TSS/TA ratio, malic acid and total sugars and premature dropping of fruits are shown by symptomatic orange fruits. These symptomatic orange juices tastes distinctly bitter, sour, salty, astringent, tingling which is more marked in the early part of the season. Use of resins could be the alternative way to reduce the bitter compounds in symptomatic citrus fruits. (Dala-Paula et al., 2019). The peduncle end is yellow-orange and the stylus end is still green showing greening symptoms (Bové, 2014). Fruits are lopsided, poorly developed, and have aborted seeds. After exerting the pressure, a grayish-white waxy mark occurs in the surface of rind (Batool et al., 2007).

5.3 Root Symptoms

Definitely, the root system is hindered by HLB infected trees. Due to nutrient starvation, the root systems of the HLB-symptomatic trees are poorly developed. Symptoms showing citrus plants have fewer roots,

lower root density and lower dry weight of roots and show paramount decay of feeder, lateral roots and fibrous roots (Batool et al., 2007). The roots acts as the phloem sink and were found to act as a reservoir for '*Candidatus liberibacter asiaticus*' and preceded foliar symptoms. Due to an unidentified bacterial effector or toxin, the fibrous root mass loss was observed but this loss was not due to phloem collapse or carbohydrate starvation (Johnson et al., 2014). The pathogen probably moves bidirectionally in the phloem with the flow of photo assimilate from leaves to sugar-consuming plant organs, including roots, and from roots to shoots when leaves and fruit are produced (Graham et al., 2013). Due to accumulation of food resources in the form of stored carbohydrate in the aerial parts, the roots could be starved by inhibiting nutrient partitioning in roots (Dala-Paula, et al., 2019).

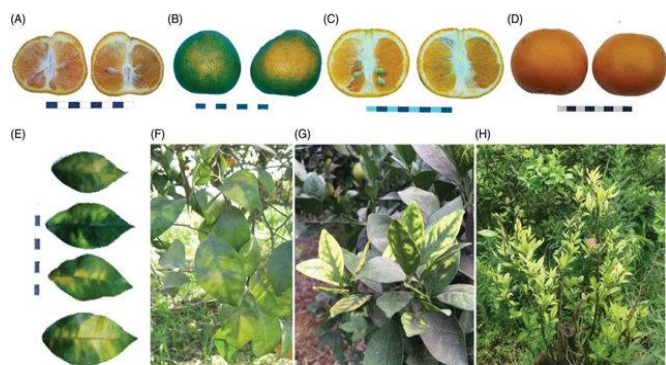


Figure 2: HLB symptoms on infected Kinnow Mandarins (a hybrid between *Citrus nobilis* and *Citrus deliciosa*) (Source: Rao et al., 2018)



Figure 3: Showing symptoms of preharvest fruit drop and root system of healthy citrus plant, HLB-infected plant respectively (Source: Rao et al., 2018)

6. MANAGEMENT

Some of the management practices to control vector and reduce the persistence of HLB diseases are given below:

6.1 Inoculum Reduction and Vector Control

- Planting of certified and pathogen free planting materials
- Reduction of psyllid populations
- Removal of infected trees which act as a source of inoculum for psyllid vector

These are three general management practices that must be used effectively for a successful citrus greening management programmes (Stuchi and Girardi, 2011). Establishment of the psyllid vector and dissemination of inoculum sources will make HLB management too difficult. At present, there is no HLB tolerant citrus species for commercial purpose (Bove, 2006). The general strategy is to eradicate all existing inoculum of HLB within area and then pathogen free plants are replanted which are grown from clean nursery. Insect vectors like citrus psyllid populations need to be reduced as much as possible.

6.2 Chemical Method

In Nepal, spraying of dimethoate 30% E.C. in the foliage at the rate of 1 ml per liter of water before flowering period has been found effective method to reduce the psyllid population in citrus species (AITC, 2020). Since HLB is highly transmissible by insect vector like psyllid, control of this insect with application of systemic insecticides like Malathion should be done.

Malathion sprayed on citrus trees from March to May at 10–12-day intervals was not effective against HLB disease. 44% Dimethoate EC, 50% Malathion EC and 40.64% carbofuran FP showed effective control of the psylla (Chen, 1998). For the control of bacterium, tetracycline hydrochloride as an antibiotic showed effective in reducing leaf symptoms (Martinez et al., 1970). In the same way, Penicillin carbendazim also showed good control of psylla (Cheema et al., 1986). Injection of tetracycline hydrochloride during spring season showed the best result against the disease (Schwarz and Van Vureen, 1970).

6.3 Biological method

- Parasitoids-*Tamarixia radiata* (Hymenoptera: Eulophidae)
- Entomo-pathogens fungi - *Beauveria bassiana*, *Hirsutella citrififormis*, *Isaria (Paecilomyces) fumosorosea*, etc (Ghosh et al., 2018)
- Lacewings, syrphids, lady beetles, and spiders are the predators of *D. citri* (Grafton-Cardwell et al., 2013)

6.4 Nutrition management

For different commercial citrus cultivars, the moderate amount of macronutrients is required to control greening disease. So, for mandarin recommended amount is 475 g N, 320 g P₂O₅, 355 g K₂O per tree every year (Koseoglu et al., 1995). For the supplement of micronutrients copper sulphate, Iron sulphate and Zinc sulphate need to be carried out through soil application at 50g per tree or foliar application of 0.5% concentration (Shrivastav and Singh, 2009). In China, there is the practice of adding extra the micronutrients expecting to distend the life span of HLB-infected trees (Spann et al., 2011).

6.5 Use of Tolerant Rootstocks

In Brazil, twelve different rootstocks have been tested for HLB reaction. After a first 7-year evaluation cycle for HLB infection the average cumulative incidence (CI) of HLB was 72.1%, with CI values varying from 10 to 100%. Evaluation of the tolerance of some *Citrus* × *Poncirus* hybrids to infection of *Candidatus liberibacter asiaticus* (Las) was carried out under protected and field conditions where the results revealed that the citrus rootstock US-897 (*Citrus reticulata* Blanco × *Poncirus trifoliata* L. Raf.) was found to be tolerant to greening disease (Albrecht and Bowman, 2012).

6.6 Intercropping with Guava Plant

Guava intercropping with citrus tree was started by farmers of Vietnam. From collaborative scientific research conducted by Vietnamese, Japanese and Australian scientists shows that inter-planting citrus with guava reduce the infestations of Asian citrus psyllid and consequently HLB (Beattie, 2006). The reason behind the reduction in psylla infestation while interplanting citrus with guava may be due to volatiles present in guava that repel psyllid or impede with ability of psyllid to find and infest citrus (Yang et al., 2006). In choice test, adult psyllid preferred not to settle on leaves treated with extracts of guava leaves (hexone, acetone). The researchers speculated that terpenoids present in guava were responsible to repel psyllids (Silva et al., 2016). A team of American scientists who also made an observation tour to Vietnam in April 2000 noted that the citrus interplanted with white guava were surviving up to 15 years whereas normal life of only citrus plantings in Mekon region of Vietnam was 2 to 4 years (Gottwald, 2010).



Figure 4: A new Valencia sweet orange/white guava interplanted in a USDA-ARS experimental plot in Fort Pierce, Florida. (Source: Gottwald et al., 2010)

6.7 Other Management Practices

Seedlings were exposed to 45 or 48 degree celsius for 4 hr on 1 day per week for 3 consecutive weeks, the incidence of HLB within leaves declined by 30% (FAN et al., 2016). The combination of treatments such as nutrients and antibiotics, insecticides and foliar nutrients, and biological with chemicals have been conducted in recent times and were found to a better performance to control ACP (Tansey et al., 2017; Kumar et al., 2017; Zhang et al., 2019). The use of neonicotinoids like Imidacloprid and Neem extract do not let to feed psyllid which abruptly carry bacterial pathogens and hence reduce HLB disease. Recently, researchers use two genes of spinach inserted into citrus to get a resistant variety towards greening disease. The use of antibiotics and thermotherapy may reduce greening disease to some extent in developed countries. Nowadays, researchers have revealed that use of reflective metalized polyethylene film as mulch beneath citrus trees (newly planted) may impair the ability of psyllid vector to detect and infect citrus trees. This experiment has shown successful in specific vegetable crops and prevent greening disease in new planted citrus trees. HLB-infected trees are heated in the sun and encased them in plastic "tents" reduce the psyllid number, which may ultimately enhance the productivity of citrus trees. Treatment with steam is also promising to extend the lifespan of tress and has more ability to produce fruit.

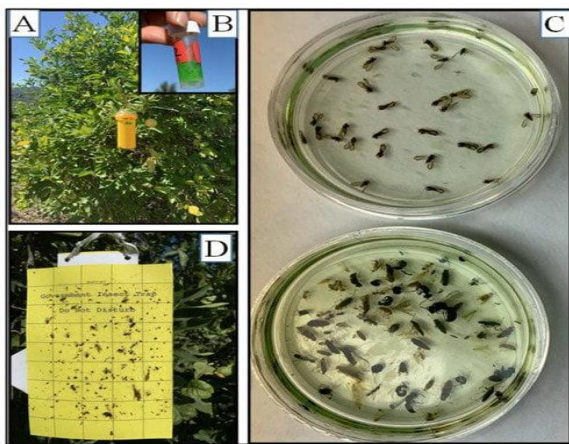


Figure 5: Cylinder traps 1 deployed in a citrus grove in Temecula, CA. (Source: Snyder et al., 2022)

7. CONCLUSION

Researchers and citrus growers are solicitous for strategies to mitigate HLB, but there is no cure to date. It has been observed in different field conditions and protected structure studies that the performance of citrus greening-affected trees can be improvised with good nutrition management which is beneficial for managing good tree health and productivity. Strict quarantine system and integrated management approach should be practiced to overcome the threat of citrus industry due to citrus greening disease.

AUTHOR'S CONTRIBUTION

S. Pandey reviewed the literature, wrote the review article and Dr. A.K. Shrestha guided and revised the article for publication

AUTHORS DECLARATION

The authors declare that there is no conflict of interest regarding the publication of this paper.

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