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## S&amp;T REVIEW

# CITRUS GREENING: FROM THREATS TO SOLUTIONS – A STRATEGIC APPROACH FOR NEPAL

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## ABSTRACT

Citrus, a valuable horticultural crop belonging to the Rutaceae family, constitutes 27.27% of Nepal's total fruit cultivation area and holds significant importance in the country's agricultural trade. Huanglongbing (HLB), commonly referred to as citrus greening, is a highly destructive, rapidly expanding, and highly incurable disease of citrus that has caused significant damage to citrus orchards and is the leading factor contributing to the global decline of citrus. Citrus psylla, the major insect vector responsible for spreading citrus greening globally. Despite the lack of effective strategies to combat the disease, comprehensive pest management approaches may yield significant benefits. These include using tolerant rootstocks, the establishment of pathogen-free nurseries, intercropping with guava, nutrient management practices, appropriate quarantine measures, the implementation of yellow sticky traps, and vector control strategies to mitigate this disease in citrus orchards. This review article highlights the characteristics of Huanglongbing encompassing its causes, symptoms, diagnostic methods, and essential management practices for citrus producers.

## KEYWORDS

Citrus decline, Huanglongbing, Psylla, Incurable.

## 1. INTRODUCTION

Citrus, a globally significant horticultural crop, with an annual yield averaging 124,246 thousand tons (FAOSTAT, 2016). It is cultivated in over 140 countries worldwide (Zhong and Nicolosi, 2020) and thrives in diverse agro-ecological zones, ranging from arid to semi-arid regions (Chandrasekaran et al., 2021). In Nepal, citrus occupies about 26.99% (49,306 ha) of the total fruit cultivation area, playing a crucial role in uplifting the socio-economic status of citrus growers and contributing to 3% of the country's total fruit exports by volume (Dahal et al., 2020; MoALD, 2023).

Among the various factors contributing to citrus decline, Huanglongbing is identified as an especially devastating disease, posing a significant threat to citrus growers globally (Singerman and Rogers, 2020). In the 19th century, Southern China recognized citrus greening by the name "Yellow Dragon Disease" (Bove, 2006). Citrus greening primarily impacts the Rutaceae family, encompassing all citrus varieties. Among these, sweet oranges and mandarins exhibit high susceptibility, while limes, lemons, and trifoliolate oranges show lower susceptibility (Knapp et al., 2004). Akhtar and Ahmed (1999) documented significant losses caused by the disease: 22% for Kinnow, 25% to 40% for sweet oranges, 15% for grapefruits, 10% for sweet limes, and 2% for lemons. Currently, it affects almost 69 citrus-producing districts in Nepal. In lower altitude regions of Nepal, the insect vector of the disease, the Asian Citrus Psyllid (ACP), is active due to favorable environmental conditions (Acharya and Adhikari, 2022).

## 2. HISTORY

Citrus greening disease, believed to have originated in China as "Yellow Shoot Disease" in the 1980s, was initially identified under epidemic conditions in 1925 in the Chaoshan and Yuenchung districts of Fujian Province (Graca, 1991; Lin, 1956). In Nepal, citrus decline was first reported in the Pokhara Valley (Thrower, 1968). Researchers suspected that the disease may have been introduced into Nepal through

propagation materials brought in from Saharanpur, India (Knorr et al., 1970). PCR tests revealed the presence of citrus greening in pocket areas of Nepal, specifically in the districts of Kaski, Syangja, Tanahun, Lamjung and Dhading (Bove, 2006; Regmi and Yadav, 2007; Regmi et al., 2010).

## 3. VARIETY SUSCEPTIBILITY TO CITRUS GREENING

The susceptibility of citrus species to greening is classified as follows:

- Mandarin, Sweet Orange, and Tangelo are highly susceptible;
- Grapefruit, Sour Orange, and Lemon exhibit moderate susceptibility;
- Lime, Trifoliolate Orange, and Pummelo are considered tolerant (Maniconi and Van Vuuren, 1990)
- Greening is a significant disease affecting Sweet Orange (*Citrus sinensis*), with Valencia exhibiting more pronounced leaf symptoms compared to Washington Navel (Obergolzer et al., 1965).
- Greening incidence is greater on Trifoliolate orange rootstock compared to Empress Mandarin and Troyer Citrange, due to the prolonged flushing period, which increases citrus psylla feeding time (Van Vuuren and Moll, 1985).

## 4. CAUSES

Citrus greening has led to the severe citrus decline in Nepal, significantly impacting the citrus industry (Roistacher, 1996). Before visible symptoms appear on the surface, affected trees may experience a loss of 30-50% of their fibrous roots (Johnson et al., 2014). The primary mode of disease transmission is through insect vectors, notably the Asian Citrus Psyllid (ACP), *Diaphorina citri*, being resistant to heat, while the African Citrus Psyllid (*Trioza erytreae*) is sensitive to heat but resistant to rain and humidity (Bove, 2006). ACP is particularly concerning as it transmits the



Additional symptoms on the shoots include off-season blooming and twig dieback (Halbert and Manjunath, 2004).

### 7.3 Fruit Symptoms

The fruit exhibits greening symptoms with the peduncle end turning yellowish-orange while the stylus end remains green (Bové, 2014). In some instances, the green coloration appears at the peduncular end instead of the stylus end, a condition referred to as "color inversion" or "red nose" (Akhtar and Ahmad, 1999).

Affected fruits are typically asymmetrical, small, lopsided, underdeveloped, and contain aborted seeds. When pressed, a grayish-white waxy mark may appear on the rind surface (Batool et al., 2007). Compared to healthy fruits, those impacted by HLB are more prone to storage decay and less responsive to postharvest degreening treatments (Shahzad et al., 2022). Premature fruit drop is a common occurrence in greening-infected fruits (McCullum and Baldwin, 2017).

### 7.4 Juice quality

HLB-affected fruit exhibits off-flavors due to reduced sugar levels and increased concentrations of bitter compounds such as limonoids, flavonoids, and terpenoid volatiles (Baldwin et al., 2018). Symptomatic fruit also shows reduced juice content, lower Total Soluble Solids (TSS), and a decreased TSS/Titratable Acidity (TA) ratio (Bassanezi et al., 2009; Montesino and Stuchi, 2009). An alternative approach to lessen these bitter compounds in affected citrus fruits is the use of resins (Dala-Paula et al., 2019).

### 7.5 Root symptoms

*Candidatus Liberibacter asiaticus* significantly impacts the fibrous root system, resulting in poorly developed root systems with relatively few fibrous roots due to root starvation (Salibe and Cortez, 1968; McClean et al., 1970; Aubert, 1987). The bacterium initially infects the root system before any visible signs appear on the leaves (Johnson et al., 2014). Root starvation can also occur when excess carbohydrates accumulate in the above-ground parts of the plant, hindering proper nutrient distribution (Dala-Zheng et al., 2018; Paula et al., 2019).

Infected citrus plants exhibit diminished root volume, decreased dry weight of roots, and significant deterioration of feeder, lateral, and fibrous roots (Batool et al., 2007). Additionally, the growth of new roots is inhibited, and decay often begins at the rootlets (Zhao, 1981).

## 8. MANAGEMENT

Here are some management strategies aimed at controlling vectors and reducing the persistence of HLB disease:

### 8.1 Quarantine and regulation

The geographic spread of Asian citrus psyllid and citrus greening continues to widen (Hall et al., 2013), with most management interventions proving largely ineffective (Taylor et al., 2019). In Nepal, 30 km is considered sufficient to manage the spread (Halbert and Manjunath, 2004). Citrus-growing areas across Nepal are affected by HLB. To combat citrus decline, Nepal has implemented several management strategies including detailed surveys of HLB and virus prevalence, alternate host assessments, and the identification of disease-free citrus zones. The Government of Nepal has recognized the absence of internal quarantine as a major problem, contributing to the movement of uncertified propagation materials both within and outside the country. The primary approach for managing HLB involves removing infected plants, which has a direct impact on the grower's finances. Therefore, effective collaboration among growers is essential (Singerman and Rogers, 2020).

Legislative actions have also been implemented to control the spread of pathogens.

The following controls need to be established (Kawano, 1998)

- Regulated plants: Rutaceae family, *Poncirus trifoliata*, *Fortunella species*, and live citrus plants
- Regulated pathogens and insects: Citrus greening bacteria and citrus psylla.
- Protection of mother stock

### 8.2 Inoculum Reduction and Vector Control

The spread of psyllid in Nepal is reported from the eastern to the western citrus-growing regions (Regmi et al., 2010), with the vector favoring hot, dry, and lower-altitude environments (Hall et al., 2007).

Three key strategies for effective citrus greening management (Stuchi and Girardi, 2011) are:

- Planting certified and pathogen-free propagation materials
- Reducing psyllid populations
- Eliminating infected trees that act as inoculum sources for the psyllid vector.

### 8.3 Use of tolerant rootstocks

The tolerant rootstocks are:

- US-897 (*Citrus reticulata* Blanco × *Poncirus trifoliata* L. Ra.)
- US-802 (*Citrus maxima* × *Poncirus trifoliata*) and
- US-812 ('Sunki' mandarin × 'Bencke' trifoliolate orange) (Albrecht and Bowman, 2012)
- US-942 (*C. reticulata* × *Poncirus trifoliata*) (Bowman et al., 2016)

### 8.4 Irrigation, nutrition management and hormones

Various commercial citrus varieties require an adequate supply of macronutrients to mitigate the effects of citrus greening disease. For mandarin trees, it is recommended to apply 475 g of nitrogen (N), 320 g of phosphorus (P<sub>2</sub>O<sub>5</sub>), and 355 g of potassium (K<sub>2</sub>O) per tree annually (Koseoglu et al., 1995). In addition, micronutrient supplementation with copper sulphate, iron sulphate, and zinc sulphate is advised at a rate of 50 g per tree, either through soil application or via foliar spray at a 0.5% concentration (Shrivastav and Singh, 2009). Fertigation and controlled-release fertilizers have been identified as effective nutrient delivery methods (Morgan et al., 2016; Phuyal et al., 2020).

Brassinosteroids, a novel group of phytohormones, have shown potential in managing HLB by reducing symptoms during early bloom, reducing fruit drop, and enhancing yields (Alferez et al., 2019). Moreover, the use of strigolactones to HLB-affected trees has been found to promote more flowering branches, improve fruit set, and encourage additional flushes during the summer season (Zheng et al., 2018).

### 8.5 Biological method

Increasing insecticide use to control *D. citri* can negatively impact natural enemies, including parasitoids, and predators. An alternative to chemical control is the implementation of biological control methods.

In Asia, native parasitoids such as *Tamarixia radiata* and *Diaphorencyrtus aligarhensis* have been proven effective in managing ACP populations (Hall et al., 2013). Entomopathogenic fungi such as *Beauveria bassiana*, *Hirsutella citriformis*, and *Isaria fumosorosea* have also been found effective (Ghosh et al., 2018). Predators of *D. citri* include lacewings, syrphids, lady beetles, and spiders (Grafton-Cardwell et al., 2013). Research in 2016 revealed that both adult and larval *Adalia bipunctata* feed on *D. citri* nymphs (Khan et al., 2016).

Additionally, predatory mites like *Amblyseius swirskii* reduce *D. citri* populations by preying on their eggs and neonates (Juan-Blasco et al., 2012). Pathogenic fungi such as *Capnodium citri*, *Cladosporium sp.*, and *Hirsutella citriformis* have been utilized for biocontrol purposes for an extended period (Halbert and Manjunath, 2004; Hall et al., 2013).

### 8.6 Chemical Method

Applying dimethoate 30% E.C. to the leaves at a concentration of 1 ml / liter of water before the blossoming period effectively reduces psyllid populations (AITC, 2020). As HLB is highly contagious by insect vectors like psyllid, systemic insecticides like Malathion should be used for control. 50% Malathion EC, 44% Dimethoate, as well as 40.64% carbofuran FP revealed potent control of the psylla (Chen, 1998). Penicillin carbendazim also have effective control of psylla (Cheema et al., 1986). Administering tetracycline hydrochloride injections in the spring season has proven to be the most successful approach for controlling HLB disease (Schwarz and Van Vureen, 1970).

Intensive insecticide application during peak citrus flushes effectively reduces ACP populations (Hall et al., 2013). Solutions such as 0.05% dimethoate, 0.02% chlorpyrifos, or imidacloprid applied at the bud burst stage are effective for controlling psyllids and are also easily accessible in the Nepalese market (Bhusal et al., 2019; Diwakar et al., 2008)

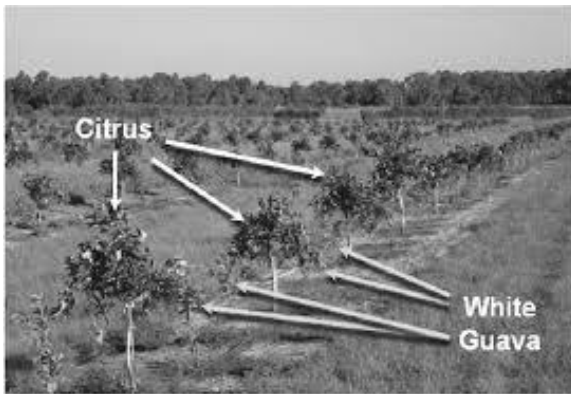
Horticultural oils and Insect Growth Regulators (IGRs) have proven more effective in targeting psyllid eggs and nymphs than adult psyllids (Grafton-Cardwell et al., 2013). Although the essential oil of *Artemisia absinthium* exhibits toxicity to psyllids, it is less potent than synthetic insecticides (Rizvi et al., 2018). To manage psyllid populations, it is recommended to use insecticides 10 to 13 times annually during flush periods. Even healthy plants should be treated and young trees should be sprayed at weekly intervals in the rainy season (Tolley 1990; Roistacher 1996).

### 8.6.1 Antibiotics

Antibiotics such as carbenicillin, ampicillin, cephalexin, oxytetracycline (OTC), penicillin, rifampicin, streptomycin sulfate, and sulfadimethoxine have demonstrated significant effectiveness in reducing pathogen populations in affected trees (Zhang et al., 2014).

### 8.7 Intercropping with guava

Studies conducted by researchers from Vietnam, Japan, and Australia suggest that inter-planting citrus with guava helps decrease Asian citrus psyllid infestations, thereby reducing the incidence of HLB (Beattie, 2006). This phenomenon is believed to result from the volatile compounds in guava that deter psyllids or hinder their ability to find and infest citrus trees (Yang et al., 2006). Specifically, terpenoids in guava are responsible for repelling psyllids (Silva et al., 2016).



**Figure 4:** A newly interplanted Valencia sweet orange and white guava in an experimental plot at USDA-ARS in Fort Pierce, Florida.

(Source: Gottwald et al., 2010)

### 8.8 Physical and chemical repellent

Physical repellents such as kaolin clay particle film and metalized polyethylene mulch, which repel citrus psyllids (Hall et al., 2007; Grafton-Cardwell et al., 2013; Croxton and Stansly, 2014). Additionally, pest exclusion nets are effective in preventing psyllids from accessing citrus trees and crucial to keep trees HLB-free in the early growth stages (Alferez, 2019).

Volatile compounds found in garlic chives such as disulfides and trisulfides, along with volatile oils from non-host plants like *Lantana camara*, *Mikania micrantha* and *Eupatorium catarium*, have been shown to effectively reduce psyllid populations (Yijing et al., 2005; Mann et al., 2011)

### 8.9 Thermotherapy and Chemotherapy

In India, a two-hour bud treatment at 47°C significantly reduces the incidence of citrus greening and the pathogen was eliminated by longer duration treatment (Narani and Bhagabati, 1980; Cheema et al., 1982).

Infected young seedlings budded with infected tissue can be treated within 3 to 4 weeks at temperatures between 38-40°C, effectively killing the pathogen (Haug, 1978; Narani and Bhagabati, 1980). Similarly, leaf symptoms of citrus greening can be controlled using tetracycline hydrochloride and penicillin carbendazim (Cheema et al., 1986).

### 8.10 Eradication and replacement

Fruit-bearing trees should undergo regular pruning, and trees with an infection rate of 50-70% should be removed (Baniqued, 1998).

### 8.11 Other Management Practices

- Effective management of citrus psyllids requires timely pesticide application, which involves pest monitoring through scouting and the use of yellow sticky traps to determine the optimal timing for control actions (Halbert and Manjunath, 2004; Hall et al., 2013).
- Research has shown that trees treated with oak extract showed improved stomatal conductance, increased chlorophyll content, and nitrogen uptake (Pitino et al., 2020). Several oak species, including *Quercus glauca* (found in Pokhara), *Quercus semecarpifolia* (in Lumle and Phulchowki, Kathmandu), and *Quercus incana* (in Hattiban, Kathmandu), are present in Nepal (Adhikari, 2014).
- Exposing seedlings to temperatures of 45 or 48 degrees Celsius for four hours once a week over three consecutive weeks resulted in a 30% reduction in HLB incidence in leaves (Fan et al., 2016).
- Strategies for managing HLB include foliar spraying, root drenching, and trunk injection of antibiotics (Zhang et al., 2011; Puttamuk et al., 2014).
- Additionally, peptide-conjugated morpholinos (PPMOs) have been utilized to inhibit bacterial growth (Wesolowski et al., 2013; Hegarty and Stewart, 2018).

## 9. CONCLUSION

Citrus greening has severely impacted the global citrus industry. The disease manifests through symptoms such as mottling of leaves, irregular fruit shape, premature defoliation, and ultimately, the death of the entire plant. Despite extensive research, no cure for HLB has been found. However, various management strategies, including strict quarantine measures, integrated pest management (IPM), biological control, and proper nutrition management, have shown potential in mitigating the disease's impact. These strategies, though sometimes costly, are essential for sustaining citrus production, especially in HLB-affected regions. In Nepal, where citrus greening has severely impacted production, adopting these techniques and focusing on vector control is crucial to revitalizing the citrus industry and ensuring orchard health and productivity.

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