Short communication

ASIAN CITRUS PSYLLID *Diaphorina citri* (Kuwayama) (Hemiptera: Liviidae) AND ITS DETECTION SURVEY IN CITRUS ORCHARDS OF SINDHULI, NEPAL

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ABSTRACT

Citrus greening, also known as Huanglongbing (HLB), is one of the world's most devastating citrus diseases. In Asia, including Nepal, the disease is vectored by Asian citrus psyllid, *Diaphorina citri* (Kuwayama) that transmits the pathogen *Candidatus* Liberibacter asiaticus, which is a fastidious, phloem-limited bacterium. Recently, the Sindhuli district of Nepal, well-known for sweet orange production, has been suspected of this destructive disease. Therefore, a detection survey on the vector was conducted by Plant Quarantine and Pesticide Management Centre, Lalitpur, in coordination with Prime Minister Agriculture Modernization Project, Project Implementation Unit, Sindhuli, during the second week of April 2021. The survey carried out on six major citrus growing pockets in the Sindhuli district revealed the presence of *D. citri* in the citrus orchards of Golanjor rural municipality ward no. 3, Sindhuli. Also, it was found that 2.96 percent and 20 percent of the surveyed citrus orchards of Sindhuli and Golanjor-3 were infested by *D. citri*, respectively. The existence of the vector in the orchards has increased the chances of citrus greening occurrence; however, tests like Polymerase Chain Reaction (PCR) can be helpful for the further confirmation of the disease in the region. In addition, the government should organize campaigns to make the citrus growers familiar with its vector insect-citrus psyllid, HLB, and its preventive and curative management measures as soon as possible to minimize the losses from the disease.

Keywords: *Asian citrus psyllid, citrus, citrus greening, fastidious, vector insect*

INTRODUCTION

Citrus greening, also known as Huanglongbing (HLB) disease, is one of the devastating diseases of citrus fruits worldwide that causes reduced fruit yield, quality, and ultimately death of the infected trees. It was first reported in Southern China in 1919 and is now known

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to occur in more than 40 countries, including the three major citrus-producing areas of the world; China, Brazil, and Florida (Bové, 2014). HLB is caused by a fastidious phloem-restricted gram-negative bacterium, *Candidatus* Liberibacter. Three species of the bacterium, i.e., *Candidatus* Liberibacter africanus*, Ca.* Liberibacter asiaticus*, and, Ca.* Liberibacter americanus, known to date, are termed according to the continent in which they are prominent (Bové, 2006). This disease is distinguished by the common symptoms of yellowing of the veins and adjacent tissues followed by splotchy molting of the entire leaf defoliation, dieback of twigs, decay of feeder rootlets as well as lateral roots, decline in vigor, and ultimately death of the entire plant.

Citrus greening in Nepal was reported for the first time from Pokhara valley in 1967 by Thrower (DoA and FAO, 2011). Similarly, the disease has been confirmed in many citrus-growing pockets of Kaski, Tanahu, Syangja, Lamjung, and Dhading districts through PCR testing. Hence, HLB has developed as one of the serious issues for citrus fruit production in the country. In Nepal, HLB is caused by motile bacteria *Ca.* Liberibacter asiaticus, vectored by Asian citrus psyllid (ACP), *Diaphorina citri* (Kuwayama). The psyllids are usually the minor pest of citrus; however, in the presence of the pathogen that causes HLB, they may be the most serious pest of citrus in the world (Halbert and Manjunath, 2004). It is challenging to detect HLB through visual symptoms as the symptoms observed on leaves and fruits vary and resemble other disorders like micronutrient deficiencies, mainly zinc, iron, and manganese (Etxeberria *et al.*, 2008). Although, the PCR test is one of the most reliable methods to confirm the occurrence of the disease, ACP's presence in the citrus growing area suspects the prevalence of citrus greening in that locale.

Sindhuli district of Nepal, renowned for sweet orange production, is an important sweet orange producing district with the sweet orange production and productivity of 9850 mt and 13.48 mt/ha, respectively, in 2019/20 A.D. (Adhikari and Rayamajhi, 2012; MoALD, 2021). After the agreement between Nepal and China in 2012 to export citrus fruit from Nepal to China, orchard management requirements basically to fruit flies and addressing quarantine issues are crucial concerns. Some initiatives have been executed in Nepal (Syangja and Sindhuli districts for Mandarin and sweet orange, respectively) in this regard (Adhikari and GC, 2020). However, lately, the citrus orchards of the district were suspected of the devastating citrus greening disease; hence, a survey was conducted to detect the presence of the vector *D. citri* in the orchards.

MATERIALS AND METHODS

The necessary information on Asian citrus psyllid was extracted from the various research articles, websites, reviews, and materials from reliable sources. In addition, the detection survey on ACP was conducted by Plant Quarantine and Pesticide Management Centre (PQPMC), Hariharbhawan, Lalitpur, in coordination with Prime Minister Agriculture Modernization Project (PMAMP), Project Implementation Unit (PIU), Sindhuli, during the second week of April 2021. For the survey, 15 surveyors were involved in the purposefully

selected six major citrus fruit (mainly sweet orange) grown pocket areas of Sindhuli district where the citrus decline problem (suspected citrus greening disease) was observed. Before the survey, an orientation program was organized by PMAMP, PIU, Sindhuli, for the surveyors. They were made familiar with the life cycle, morphology, and the habit of the vector for its proper identification and collection using the hand lenses, plastic vials, aspirators, and transparent polybags. From each selected citrus orchard, a minimum of ten trees were considered during the survey. Details of detection survey location, citrus orchards, and citrus trees are presented in Table 1.

Table 1. The number of orchards and trees sampled during the survey of Asian citrus psyllid in the Sindhuli district during 2021

S.N.	Location of survey	No. of orchards	No. of orchard owners	No. of citrus trees observed
1	Golanjor-3 (Bhadaure, Sanney, Okhle)	20	10	100
			10	100
2	Golanjor-4 (Tallo dude, Tadhi, Khokling, Majhkubhinde, Talloaalegaun, Tamaure)	30	10	100
			10	100
			10	100
3	Golanjor-5 (Nayakharka, Chisapani, Ratanchura, Khaniyakharka, Bijayachhap)	30	10	100
			10	100
			10	100
4	Golanjor-6 (Nakajoli, Haibar, Chhetpa)	20	10	100
			10	100
5	Kamalamai-2 (Besare, Lekhark, Dujling, Maiware, Kusumtaar)	15	5	50
			5	50
			5	50
6	Kamalamai-3 (Jalakanya, Batonigale, Chapauli)	20	10	100
			10	100
	Total	**135**	**135**	**1350**

RESULTS AND DISCUSSION

Life cycle of Asian citrus psyllid, *D. citri*

Asian citrus psyllid, *D. citri* is a sap-sucking, hemipteran bug in the family Liviidae. Eggs are (0.3 mm long, elongate, almond-shaped) deposited exclusively on "feather flush"; which hatches within 2 to 4 days. The vector goes through five nymphal instars and develops into adults within 11 to 15 days (Chavan *et al.*, 1993). ACP requires around 15 to 47 days to

complete its lifecycle, depending upon the season (Mead, 1977). Copulation occurs on new leaf flush during photophase for approximately 20 to 100 minutes, and the female starts to lay eggs one day after mating (Wenninger and Hall, 2007). ACP readily jumps when approached; thus, collected by using aspirators, bagging the entire shoot, or placing an inverted empty test tube above an infested shoot (Halbert and Manjunath, 2004). The vector is most active during June-July in Brazil (Yamamoto *et al.*, 2001), May-June in the Philippines (Catling, 1970), and March-April in India (Pande, 1971).

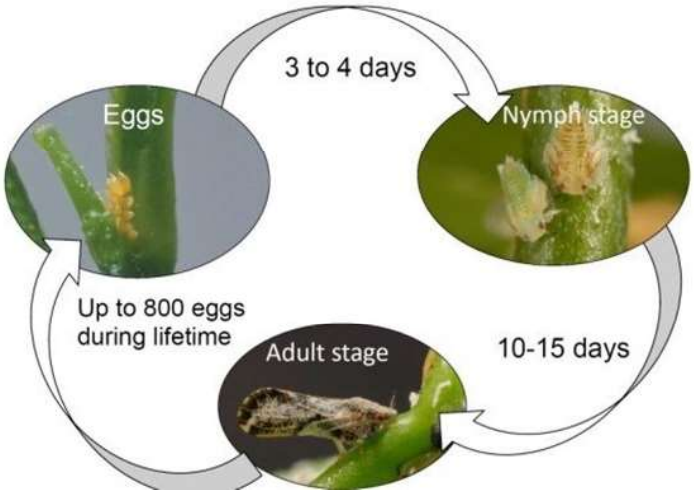

Fig. 1. Life cycle of *Diaphorina citri*
Source: **Velprabakaran (2020**)

Host

Asian citrus psyllid is mainly confined to the Rutaceae plant, residin g both on wild hosts as well as on *Citrus* species. *Murraya paniculata* (Jasmine orange or Kamini), an ornamental rutaceous tree or shrub often used for hedges, represents the preferred host of the vector, *D. citri* (Bové, 2006). Due to the more continuous flushing pattern of the *M. paniculata*, the plant serves as an alternate host for *D. citri* when citrus is not in the flush. Also, *Murraya exotica* (Kadipatti/Asare/Bokejamun), another

Fig. 2. Citrus psyllids in Pumelo fruit tree.
Photo : D.B. Tiwari, 2013, Kathmandu

host plant of the vector, growing at a low altitude in Tanahun, Dhading, and Lamjung districts in the wild, is causing a severe problem of citrus greening in those areas (DoA and FAO, 2011). It was observed in the pumelo fruit tree in Kathmandu in 2013 April in mass. A pest survey jointly carried out by the Department of Plant Resources Thapathali, National Plant Quarantine Programme Hariharbhawan, Nepal Herbs and Herbal Product Association with the financial assistance of GIZ reported citrus psyllids from Rim 6 and Rim 7 of Salyan district in *Zanthoxylum arnatum* (timur tree) in 2015 (DPR *et al.*, 2015). Similarly, nymphal and adult stages of ACP were observed in the Kamini plant at Tuber and Vegetable Development Centre, Sindhulimadi.

Transmission of pathogen

Although the citrus greening disease also gets transmitted by grafting and anthropogenic factors, *D. citri* is the important vector of the disease transmission. Asian citrus psyllids at the 3rd, 4th, and 5th instar stages or their adults can transmit the greening disease. Since the transmission type is persistent, once the vector gets infected by the HLB bacteria, it can transmit the disease lifelong as the bacterium gets multiplied within its body (DoA and FAO, 2011).

Findings of the survey

D. citri were observed in one (Golanjor-3) out of the six surveyed areas of the Sindhuli district as presented in Table 2. Morphology of the collected *D. citri* was further studied in the lab at PMAMP PIU, Sindhuli, and confirmed the presence of the pest.

Table 2. Status of *D. citri* in citrus orchards of Sindhuli, Nepal during April 2022

S.N.	Surveyed Locality	Observed numbers of orchards	Status	
			Presence/ Absence	Occurrence (%)
1	Golanjor-3, Sindhuli	20	**Presence** (4 orchards)	20
2	Golanjor-4, Sindhuli	30	Absence	0
3	Golanjor-5, Sindhuli	30	Absence	0
4	Golanjor-6, Sindhuli	20	Absence	0
5	Kamalamai-2, Sindhuli	15	Absence	0
6	Kamalamai-3, Sindhuli	20	Absence	0
	Total	135		2.96

Out of 135 surveyed orchards in the Sindhuli district, 2.96% (Table 2) of the orchards were found to be infested by citrus psyllids. In Golanjor-3, the psyllids were detected in four out of twenty citrus orchards. Among 10 trees surveyed in each of the four orchards, 5, 6, 8, and 10 trees, respectively, from each orchard were found to be infested.

The presence of Asian citrus psyllid has increased the chances of HLB occurrence in the citrus orchards of Sindhuli, Nepal. Additionally, PCR tests in the future might be helpful for the further confirmation of the disease in those suspected areas.

Management approaches

Management of HLB disease includes inspection for early diagnosis of the disease, removal of infected trees, planting with citrus greening-free rootstock, and aggressive control of the vector, ACP (Alvarez *et al.*, 2016).The vector has created difficulty in the management of the vector as well as a disease due to its high reproductive potential, fairly wide temperature tolerance, rapid growth and development of population, persistent type of pathogen transmission, and alternative hosts that flush frequently. Despite these challenges, integrated pest management programs, including yellow sticky cards to monitor ACP, maintenance of effectiveness of natural enemies, broad-spectrum insecticides during the dormant period, and selective insecticides in season in the rotation, are helpful to suppress *D. citri*. The reduced use of broad-spectrum insecticide and higher dependence on more diverse and selective chemistries and focusing on the biological control measures (using parasitoid wasp, *Tamarixia radiata)* are necessary to manage pesticide resistance and maintain a sustainable equilibrium between pests and natural enemies (Grafton-Cardwell *et al.*, 2013). Similarly, intercropping citrus with guava can be encouraged in the orchards, as in Vietnam; it was reported to control the infestation of ACP and consequently HLB incidence (Beattie *et al*., 2006).The use of various rootstocks of citrus was observed to be tolerant to HLB in field plantings in Brazil (Albrecht and Bowman 2012).

CONCLUSION AND RECOMMENDATION

Asian citrus psyllid, a vector of citrus greening disease, can be considered a major pest when the pathogen causing the disease is present in that environment. According to the survey findings, one out of the six surveyed citrus orchards of Sindhuli is detected with ACP. The result of the survey reveals 20% infested with citrus psyllids in the Golanjor-surveyed citrus orchards while, in totality, it is only 2.96% orchards infested in Sindhuli district. The detection survey result has increased the chance of citrus greening disease occurrence in Sindhuli due to the presence of ACP. Further confirmation of the disease can be made through reliable methods like PCR testing in the future. In addition, the government must be more concerned and start campaigns to make citrus growers aware of the diseases, their prevention, and integrated management measures.

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