SURVEY AND MONITORING OF CITRUS GREENING DISEASE AND ITS VECTOR, ASIAN CITRUS PSYLLID (*Diaphorina citri* KUWAYAMA) IN SWEET ORANGE ORCHARDS OF SINDHULI, NEPAL

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ABSTRACT

This study was conducted to evaluate farmers' perceptions of citrus greening disease or Huanglongbing (HLB) and its vector, *Diaphorina citri* Kuwayama (Asian Citrus Psyllid (ACP)) in the major sweet orange growing regions of Sindhuli in 2022. The study consisted of two major parts, farmer's survey of HLB and monitoring of the vector. As the study revealed, 73.77% of the orchards exhibited symptoms indicative of citrus greening disease, 83.3% of the farmers were found to be aware of the disease, and 66.67% were aware of the symptoms it produced; however, only 43.14% of farmers were aware of the vector. Cutting off the diseased tree (priority index(I) = 0.63) was the most followed strategy after the incidence of the disease. The use of chemical pesticides (I = 0.85) was ranked first among the strategies being applied to control the vector. The total ACP count was found to be the highest in July ($\bar{x}$ = 33.2). Its appearance in the orchard became evident after the end of winter (March), and gradually increased its population in spring (April-May), attained maximum population in the rainy season (June-July), and started declining again in fall (August).

Key words : Citrus, HLB, monitoring, psyllid, sweet orange

INTRODUCTION

Sweet orange (*Citrus sinensis* L. Osbeck) is commonly referred as ‘Junar’ in Nepal (Adhikari & G.C., 2020). Citrus greening also known as Huanglongbing (HLB) is one of the most serious plant diseases in the world and, currently, there is no cure. The disease is caused by the pathogen, *Candidatus* Liberibacter asiaticus, a fastidious, phloem-restricted, gram-negative bacterium (Garnier et al., 1984). The psyllids, as well as graft transfer, are the medium for the transmission of this disease (Bové, 2006). Biological indexing, PCR (Polymerase Chain Reaction) test, visual symptoms (pen test), scratch test, etc. are the various methods for the detection of citrus greening (Li et al., 2006; Taba et al., 2006; Vashisth et al., 2020). Diagnosing citrus greening poses a formidable challenge, given that its symptoms mirror those of other citrus diseases and nutritional deficiencies (Tipu et al., 2021). In the field, HLB-infected trees typically develop yellow shoots alongside healthy foliage, with leaves showing a blotchy mottle pattern of yellow and green patches, creating distinctive asymmetrical patterns on both halves of the leaf (Bové, 2006). Fruit becomes small, misshapen, bitter due to increased acidity, and often prematurely dies; severely affected fruit may contain undeveloped seeds as well (McClean et al., 1970). Additionally, Acharya & Adhikari (2022) observed atypical symptoms in Nepal, where fruits stayed green at full ripeness and some began to ripen starting from the stem end.

Asian Citrus Psyllid (ACP), *Diaphorina citri* Kuwayama, (Hemiptera: Sternorrhyncha: Liviidae) is the effective vector of citrus greening and this insect can become the most devastating pest of citrus worldwide if it carries the pathogens responsible for citrus greening, as indicated by Halbert & Manjunath, (2004), otherwise, it remains a minor pest. Psyllids are small insects that range in size from 3 to 4 mm in length consisting of a life cycle that progress from egg through 5 nymphal instars to the adult stage (Grafton-Cardwell et al., 2006). The presence of citrus greening disease has been reported in Nepal since mid-1960s along with the presence of ACP (Catling, 1970; Knorr & Shah, 1972; Thrower, 1968).

Planting of certified clean planting materials, effective control of the psyllid populations, and removal of affected trees that serve as an inoculum source for psyllid acquisition are three management methods that are widely agreed upon across the world (Bové, 2006). The main management method has been to eliminate all HLB sources in a region before replanting with HLB-free trees produced from clean budwood. It is critical to prevent transporting HLB-infected propagation materials to non-infected locations (Abdullah et al., 2009). Paudyal (2015) has also emphasized that the disease management is quite difficult if inoculum sources are widespread and the psyllid vector is well established. Trees bearing fruits should undergo careful pruning, while infected trees with a 50-70% infection rate should be subject to eradication (Baniqued, 1998). In mature trees, symptoms of HLB may not appear until over two years after initial infection, yet infected trees can transmit the bacterium to psyllids much earlier, thereby indicating that symptom-free trees in HLB-affected areas may still harbor the infection (Grafton-Cardwell & Daugherty, 2018). Although economic and geographic constraints render many advanced approaches for reducing HLB infection, effective in other regions, quite impractical for Nepal, the concept of a clean plant center for producing disease-free planting material and the implementing of quarantine measures to prevent its spread have been strongly advocated by various government stakeholders in horticulture (Acharya & Adhikari,2022).

Vector control serves as a temporary solution while disease-resistant traits or tools for interrupting transmission are developed (Grafton-Cardwell et al., 2013). Applying broad-spectrum insecticides at critical flushing periods and during winter can greatly reduce populations of ACP (Grafton-Cardwell et al., 2013; Rogers, 2008). Nepal Agricultural Research Council (NARC), Nepal has recommended chemical insecticides viz. bifenthrin, imidacloprid, and thiamethoxam for the control of ACP (NARC, 2021). Junar Superzone recommended the sweet orange farmers in Sindhuli to monitor ACP in their orchards, perform PCR tests for confirming greening, and cut off and remove infected trees (PMAMP PIU Sindhuli, 2021b). This study seeks to understand farmers' perceptions of citrus greening disease, examine their management practices, identify key challenges they face in disease management, and monitor the vector over different months in major sweet orange-producing regions of Golanjor Rural Municipality, Sweet orange Superzone, Sindhuli, Nepal.

MATERIALS AND METHODS

Survey

Golanjor Rural Municipality under the command area of the Prime Minister Agriculture Modernization Project (PMAMP), Project Implementation Unit (PIU), Sindhuli was purposively selected for the survey based on the significant production area and suspected presence of citrus greening disease. RAOSOFT software was used to obtain the optimum sample size. Keeping a 10% margin of error and 90% confidence level sample size of 61 was obtained out of a total population of

607 growers in the region (PMAMP PIU Sindhuli, 2021a). The households were selected around the site by the method of simple random sampling. Focus Group Discussion (FGD) was organized at the study area after completing the survey with the help of the checklist to verify the results obtained from the field survey. The necessary information on citrus greening and ACP were collected from the various research articles, websites, blogs, books, magazines and other materials related to citrus in the country and abroad. The collected information was analyzed and visualized using MS Excel (Office 360). Farmers' perceptions of various problems and strategies were recorded and analyzed using a five-point Likert scale. The scale values of 1, 0.8, 0.6, 0.4, and 0.2 were employed to indicate "most severe/very high preference", "severe/high preference", "moderate/moderate preference", "mild/low preference", and "most mild/very low preference" for different problems faced by the farmers and strategies used to address them, respectively. The problems and strategies were ranked using the following formula constructed based on the Likert scale, offering insight into respondents' inclination and intensity towards the given issue (Miẙa, 1993).

$$I_{imp} = \sum \frac{Sifi}{N}$$

Where,

I_{imp} = index of importance
S_i = Ith scale value (1, 0.8, 0.6, 0.4, 0.2)
f_i = frequency of ith importance given by the respondents
N = total number of respondents

Monitoring of ACP

For this study, an orchard that has shown prominent visual symptoms of HLB was selected randomly out of many in Golanjor-05, Nayakharka, Sindhuli located at an altitude of 1083 meters above sea level. The samples of leaves taken from this orchard also manifested positive result in PCR test for HLB. This altitude was particularly selected as altitudes ranging from 1000 to 1200 m were reportedly hotspots for sweet orange production in Sindhuli. For monitoring of the ACP, the published protocol by the National Plant Protection Organization (NPPO), Nepal, “Survey Protocol for Asian Citrus Psyllid (ACP), *Diaphorina Citri* in Citrus Trees” was followed. Citrus trees with spring and rainy flushes were selected for ACP observations typically from March to August. For sampling, the field in each location was divided into five areas each of 10 m x 2 m in dimension. At weekly intervals, one shoot (about 6-10 cm long) was selected at random from each square meter area by throwing a 2-pointed object. Numbers of citrus psyllid adults per shoot were counted and recorded. Two trees were observed in one location, with ten shoots on each tree, totaling 20 shoots per location. Overall, 10 trees and 100 shoots were observed for ACPs in the orchard across five different locations. Sample shoots were observed, and adults were aspirated using a mouth aspirator for 5 minutes into the glass container built with an aspirator. The number of ACPs was counted and printed on a data record sheet. All the data of monitoring were analyzed using MS Excel (NPPO, 2019).

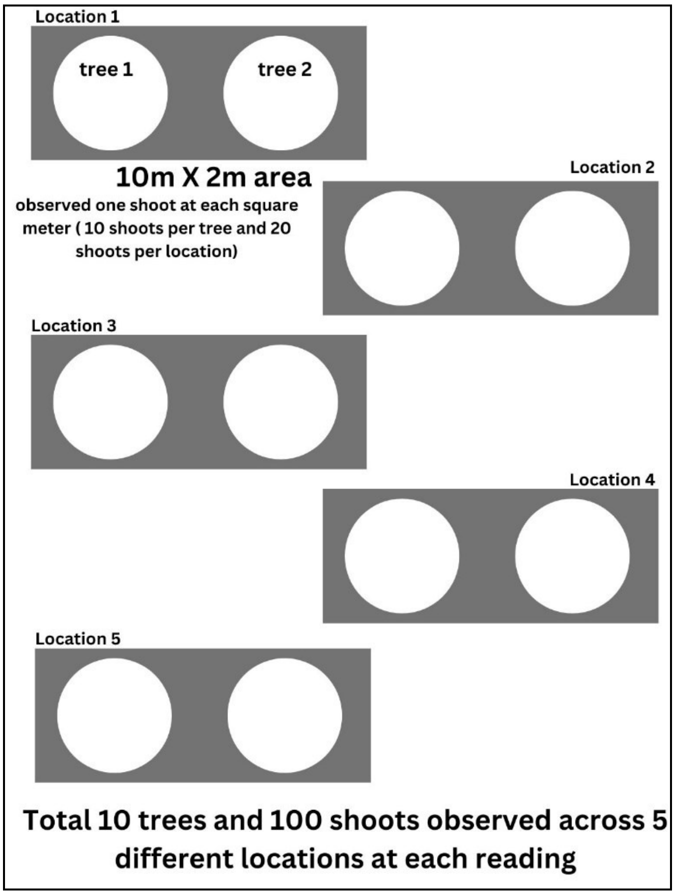

Fig. 1. Diagrammatic representation of monitoring of ACP

RESULTS AND DISCUSSION

Prevalence of citrus greening disease in the orchards (based on symptoms)

Prevalence of citrus greening was found in 73.77% (n = 45) of the orchards as they were observed with similar symptoms to the trees found to be affected with HLB (n = number of respondents), as presented in Fig. 2. The findings indicated that most orchards were likely afflicted with citrus greening, and those that remained uninfected were in a precarious situation. Pokhrel et al. (2021) also reported similar findings in their study, indicating that this disease was detected in numerous economically significant citrus-producing areas in Nepal and has led to substantial yield losses. This finding differs from the research conducted by Adhikari et al. (2012), who performed field-level scratch tests on 68 samples of suspected leaves from various orchards in major sweet orange-growing regions of Sindhuli. Their results indicated that all orchards were free from citrus greening back in 2012. The current manifestation of citrus greening in the area may be attributed to ongoing changes in weather patterns, which have created a new environment conducive to the spread of ACP in new areas and the expression of the disease in citrus trees. Variations in rainfall distribution, such as prolonged periods of drought coupled with high heat followed by heavy rain, adversely affect the root health and cause stress to the citrus trees cultivated on mountain slopes in Nepal, where soils are also prone to runoff and acidification, rendering them susceptible to HLB (Rogers, 2023). The shifting of psyllid vectors to higher altitudes, where they were not previously present, could be another contributing factor to the spread of the disease in these regions. Visual symptom observation alone is insufficient for confirming the disease; further studies utilizing PCR tests are necessary to establish the reliability of the disease prevalence.

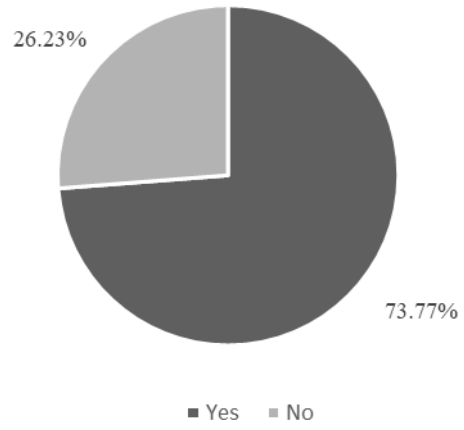

Fig. 2. Presence of HLB symptoms in the farmers' orchards

Respondent's awareness about citrus greening disease and its symptoms

Majority of the respondents i.e. 83.61% (n = 51) were aware of the citrus greening while only 16.39% (n = 10) had no idea of the prevalent disease (n = number of respondents), as presented in Fig. 3. Some of the unaware respondents had confused greening bacterial disease with virus. The study revealed that, out of respondents who were aware of the citrus greening, 66.67% (n = 34) of the respondents were aware of the symptoms the disease produces but 33.33% (n = 17) were not fully aware (n = number of respondents), as presented in Fig. 3.

Respondents' awareness about the vector of the disease

According to the survey, it was revealed that among 83.61% (n = 51) who were aware of the citrus greening, 43.14% (n = 22) of the respondents were aware of the vector of the disease while 56.86% (n = 29) had no idea of the psyllids vector (n = number of respondents), as presented in Fig. 3. Respondents who were aware of the vector insect were also capable of identifying the insect as the process was assisted by PMAMP, PIU, Sindhuli. Though respondents were aware of the insect vector of the disease, they were totally unaware about the lifecycle of the insect.

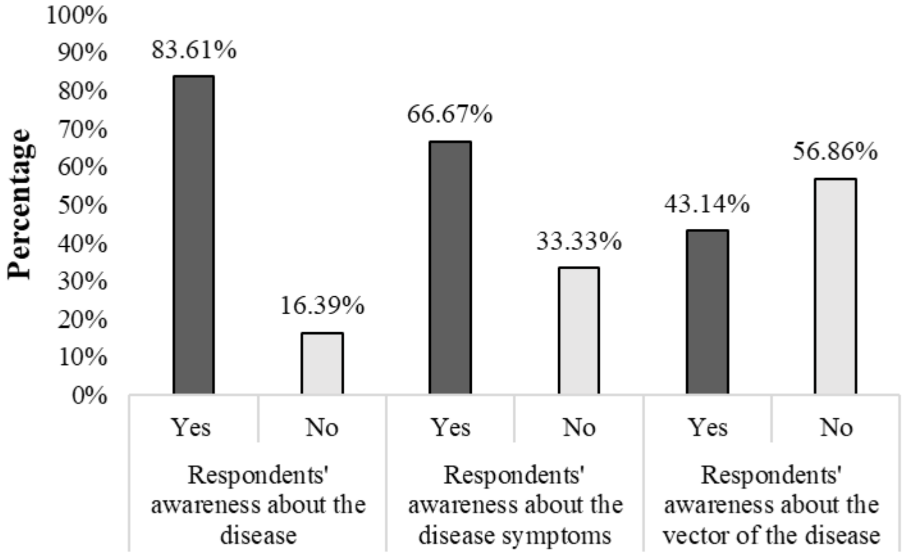

Fig. 3. Respondents' awareness on citrus greening disease, its symptoms, and the vector

Reasons behind lacking the knowledge of the disease

Farmers' main reason for lacking the knowledge of the disease was lack of government-led activities (I = 0.72), followed by lack of technical know-how (I = 0.48), and lack of self-interest (I = 0.2), (I = index of importance), as presented in Table 1.

Table 1. Reason behind lacking the idea of the disease

Symptoms	Index (I)	Ranking
Lack of government-led activities	0.72	I
Lack of technical know-how	0.48	II
Lack of self-interest to know	0.2	III

Management strategies after the incidence of the disease

While ranking the farmers' management strategies after the incidence of the disease, cutting off the diseased tree was first with the index value of 0.63 followed by leaving the tree as it is with the index value of 0.55. Fertilizers, micronutrients, and irrigation optimization in the greening-affected orchards was the least followed strategy with the index value of 0.28. Good irrigation practices and nutrient management is necessary to maintain yield on HLB-affected trees. Research indicates that frequent irrigations with smaller water quantities can benefit HLB-affected trees by enhancing tree canopy density, increasing leaf area, and reducing leaf fall (Morgan et al., 2023). Due to fibrous root loss in HLB-affected trees, maintaining optimal concentrations of macro and micronutrients becomes essential for their health and development (Kadyampakeni et al., 2023).

Table 2. Management strategies after the occurrence of the disease

Strategies	Index (I)	Ranking
Cutting off the diseased tree (eradication)	0.63	I
Leaving the tree as it is	0.55	II
Fertilizers, micronutrients, and irrigation optimization	0.28	III

Strategies being applied to prevent the disease

While ranking the strategies that were applied to prevent the disease, planting the disease-free saplings came first with the index value of 0.76, followed by the control of the vector with the index value of 0.65 and strict quarantine procedures came third with the index value of 0.23. Few progressive farmers implemented quarantine methods, like avoiding non-local saplings and restricting their movement. Acharya & Adhikari (2022) suggested that there shouldn't be any previous history of HLB in new plantation areas and that only healthy and identified saplings should be planted in such areas to prevent the disease.

Table 3. Strategies being applied to prevent the disease

Strategies	Index (I)	Ranking
Planting of the disease-free saplings	0.76	I
Control of the psyllids vector	0.65	II
Strict quarantine procedures	0.23	III

Strategies being applied to control the psyllids vector

65.57% (n = 40) of the total respondents apply certain strategies to control the psyllids vector. While ranking the strategies being applied to control the vector, chemical pesticides came first (I = 0.85) followed by physical methods (light traps, sticky traps, etc.) (I = 0.35) and cultural methods (windbreaks, reflective mulches, particle film spray, etc.) (I = 0.27) (I = priority index) but none of the respondents used biological methods (use of natural enemies). *Tamarixa radiata,* a chalcidoid insect that preys on ACP, could serve as an effective biocontrol agent for managing psyllid populations (Chen et al., 2017). The findings from Lama et al., (1988) suggested that *T. radiata* has been successfully reared in Nepal and such biological control holds promising potential, particularly in areas like Pokhara, Syangja, Sindhuli, and Kathmandu, where ACP is currently thriving without any known parasites.

Table 4. Strategies being applied to control the psyllids vector

Strategies	Index (I)	Ranking
Chemical pesticides	0.85	I
Physical methods (light traps, yellow sticky traps, protective covers, etc.)	0.35	II
Cultural methods (windbreaks, reflective mulches, particle film spray, etc.)	0.27	III

Major responsible agents to lead efforts to reduce the threat of citrus greening

Prime Minister Agriculture Modernization Project (PMAMP) (Government) was believed to be the major responsible agent (I = 0.85), followed by the growers themselves (I = 0.64) and local institutions like Junar Cooperatives (I = 0.29) (I = priority index).

Table 5. Major responsible agents to lead efforts to reduce the threat of citrus greening

Major responsibility	Index (I)	Ranking
PMAMP (Government)	0.85	I
Growers themselves	0.64	II
Local institutions like Junar Cooperatives	0.29	III

Monitoring of ACP

The population dynamics of ACP at different time of the year was recorded in the selected field of sweet orange. The average ACP population went on increasing gradually from March to mid-July and gradually subsiding towards August. The first mean population on 25th March was 0.09, the peak mean population on 15th July was 0.36, whereas the last mean population at the final reading on 12th August was 0.2 as presented in Fig. 4. While calculating the average population monthly the least population was encountered in March (n = 9) and the maximum population was recorded in July (n = 33.2) (n = average psyllid population for respective months), as shown in Fig. 5. However, in 2021, a different scenario was reported in Golanjor-05, Nayakharka, Sindhuli, where none of the surveyed orchards were infested with ACP in April (Dhakal et al., 2022).

Regmi and Lama (1988) while studying the population dynamics of ACP in Pokhara valley revealed that the population dynamics of ACP exhibited multiple peaks due to multiple generations per year. According to them populations reached a maximum before the monsoon during hot and dry weather and declined during the rainy season as heavy rainfall reduced eggs and nymphs but adults persisted by taking shelter on lower leaf surfaces. Later on, Manandhar et al. (2004) documented comparable results during surveillance conducted in the Western hills of Nepal. They reported that at low-altitude sites below 1,000 m, the psyllid population peaked in spring, whereas at higher altitudes above 1,000 m, the peak population occurred during the rainy season. Warmer rainy season at high altitudes could explain the peak in the psyllid population during these periods. Leong et al. (2011) observed that the psyllid population peaked three times a year in August-September, February-March, and June-July, coinciding with the time of developing new flushes by the plant. Hall et al. (2008) suggested that the outbreak of ACP can occur at any time of the year given that the environment is suitable and new flushes are available. Further study is needed to explore the year-round population dynamics of ACP, including eggs and nymphs, with a focus on its life cycle, weather patterns, and adaptation to the climate.

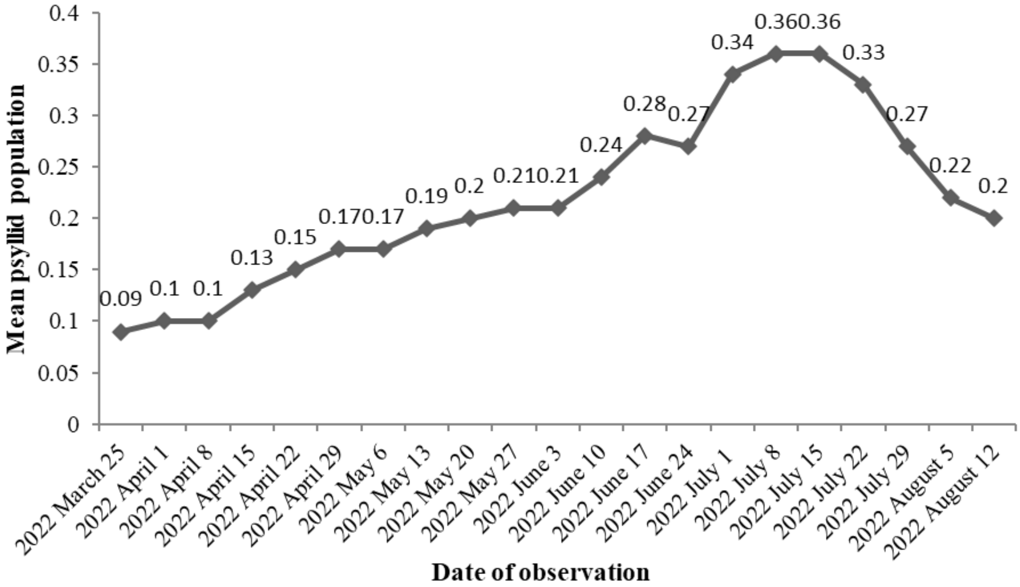

Fig. 4. Average psyllid population at each observation

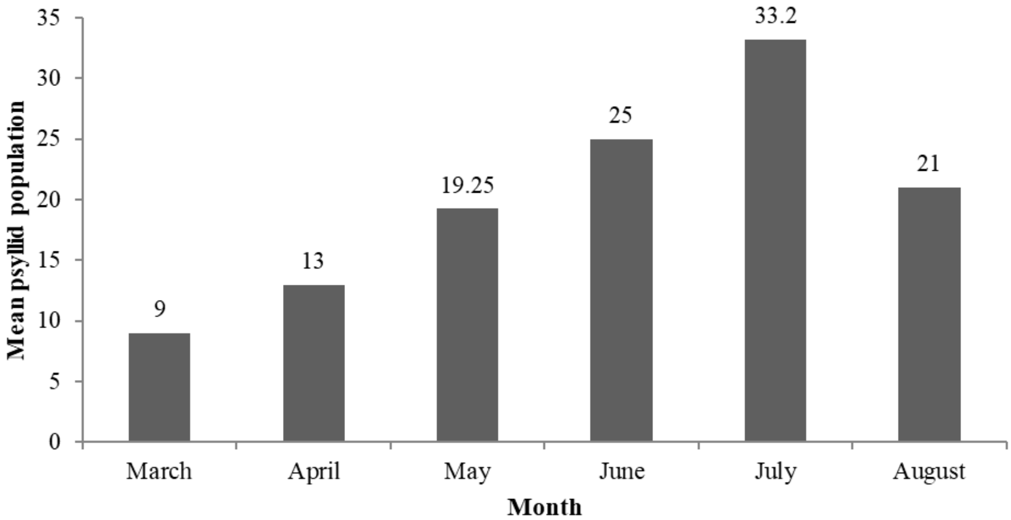

Fig. 5. Mean psyllid population in each month

CONCLUSIONS

Farmer's survey indicated that most of the orchards in the area were suspected with the citrus greening disease. Respondents of the study area demonstrated fair knowledge regarding the disease and the symptoms it produced but lacked knowledge of its vector, ACP. Farmers have not received adequate guidance on preventive measures such as quarantine procedures and management techniques like good irrigation and nutrient management from the relevant authorities. Timely detection of the disease, awareness, vector control, and appropriate management strategies were critical needs of sweet orange farmers in Sindhuli, Nepal. The psyllid population began emerging in the field after winter (March), steadily increased during spring (April), reached its peak during the rainy season (July), and subsequently declined as fall approached (August). Vector control should be initiated in dormant period before the onset of spring (April/May) when trees excessively produce new flush and adult psyllids start laying eggs.

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