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Combating the Ravaging Huanglongbing Disease by Controlling ACP to Secure the Future of Citrus Growers in Nepal: A Review

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Abstract

The second largest fruit crop of the world, citrus is a high prospect sector in Nepalese hills. Citrus greening (Huanglongbing) is the most complex, challenging, incalcitrant and devastating disease that has threatened the citrus industry worldwide. Citrus Decline in Nepal is claimed to be because of HLB coupled with other diseases and grove management practices. This disease is spread by a sucking insect, the Asian Citrus Psyllid (ACP); by controlling its population the spread of HLB to new groves could be prevented. This review article comprises the best method for rapid detection of HLB and chemical, biological and cultural management practices for controlling ACP to prevent the spread of HLB in Nepal. Iodine based test can be used to screen the samples infested with HLB which could be confirmed after molecular HLB testing i.e. PCR. Chemically, ACP could be best managed by intensive chemical spray during the critical flushing periods and winter. Biologically, ACP could be best managed by the use of parasitoids, predators and pathogen that consumes and destroy ACP nymphs/or adults. Culturally, the spread of ACP could be best managed by protective cultivation, reflective mulch and growing nurseries to produce saplings at high altitudes in Nepal for preventingthe spread of HLBin new groves.

Keywords: Asian Citrus Psyllid; Citrus;Huanglongbing;Identification; Management

Introduction

Citrus, the genus composed of several species, includes oranges, tangerines, mandarins, grapefruits, lemons and lime; is one of the most important popular fruit commodities in the world because of its refreshing flavor and nutritional values. It is the second largest fruit crop of the world [1]. With efficient harvesting and processing technologies, the citrus industry has developed in more than 140 countries across the world, with an annual production more than 146 million tons [2]. The impact of citrus agro-industry in the international economy is huge. Beside their values as commodities, they also provide employment in many segments involved in its production cycle: harvesting, handling, transportation and storage [3].

Citrus is a high prospect sector in Nepalese mid-hills; ranging between 800 to 1400 m.asl because of its contribution for enhancing economy, supplementing nutrition maintenance of ecosystem. Nepalese citrus is a NRs 2,470 million per year industry [4]. In Nepal, the statistics shows that the area and production under citrus fruit crops are increasing during the last 14 years with the area 40,554 ha producing 118,447 metric tons with productivity 8.82 mt/ha; but the productivity is in declining trend [5]. Still, there lies a huge scope of increasing the production and productivity through innovative approaches of citrus rejuvenation and improved technologies. Several barriers have been reported to impede citrus production and quality from different regions of the world; including diseases, insects, pests, availability of quality irrigation water, seasonal calamities/climate change, nature of available farm management practices and quality of farm supplies particularly in developing countries. The most contributing factor for the decline in citrus production is the diseases that reduce production, quality and life of the tree [6]. Citrus decline is becoming a major barrier to the Nepalese citrus enterprises because of the above mentioned attributes. Citrus industries in different parts of the world including Asia, North America, South America and Africa are in threat due to the most complex, challenging and devastating disease citrus greening disease which is also called huanglongbing (HLB). HLB, first observed more than a hundred years ago in Asia, is the most serious disease threat to the citrus-growing industry worldwide due to its complexity, destructiveness, and incalcitrance to management [7].

The citrus associated HLB disease is spread by a sucking insect, the Asian Citrus Psyllid (ACP); *DiphorinaCitri*. Three bacteria are known to be associated with HLB, *Candidatus*Liberi bacterasiaticus(CLas), *Ca.* Liberibacterafricanus(CLaf), and *Ca.*L iberibacteramericanus(CLam). They are the phloem-restricted in *planta*; because they are nonculturable. Within citrus sieve tubes *Ca.* Liberibacters exploit cellular processes for nutrient acquisition, encoding transporters, and enzymes for metabolizing host-derived nutrients [7]. After the tree gets infected, they have latency period of six months to two years before visible symptoms of the disease appear. Since, there are no practical methods available to diagnose asymptomatic trees, they serve as hidden bacterial reservoir for feeding ACP and spread the pathogen rapidly [8]. The possible cause contributing to the decline syndrome may be citrus greening which is one of the serious problems of citrus in Nepal. Citrus decline due to presence of greening disease was first reported in Pokhara valley of Nepal [9]. It is reported that citrus decline in many areas of Nepal was caused by this disease. Up to 39-95% mandarin trees were found infected with greening disease in Pokhara valley [10].

Current methods to reduce the infection of trees by HLB rely primarily on monitoring, identification and removal symptomatic citrus trees, in combination with vector population control [11]. The current short-term strategies to manage HLB is not limited to early detection and clearing infected trees, improving insecticide-based management of ACP, biological method to control ACP, conventional citrus breeding for resistance, giving therapies to protect existing trees in groves. The long-term strategies for its control may include development of transgenic HLB-resistant and ACP-resistant citrus, development of bactericides and therapeutics, sustainable ACP management strategies through evaluation of other bacteria to trigger ACP immune response, Citrus tristeza virus delivery of RNAi to target ACP, assessment of glycolytic activity of CLas to use carbohydrates as bait to halt the infection through root etc.

In Nepal, at present we need immediate short-term solutions to rejuvenate the citrus industry to retain the dream of golden revolution for citrus rejuvenation before it's become too late to act. There are various researches going around the globe to combat this ravaging disease. There are rare collections of all those probable most effective methods developed for short-term and long-term management of devastating HLB disease. This paper examines the research from multiple citrus systems to distill the methodologies that could be applied for saving citrus from HLB, to provide cohesive guidance for citrus growers in Nepal and other developing nations on HLB management.

Identification of citrus huanglongbing: In the places, where the incidence of HLB has been occurred, its symptoms can be clearly seen. The early and characteristic symptom of the disease in infected trees is referred as a blotchy mottle condition of leaves that results in the development of yellow shoots. Trees are stunted, declining and bear a few, small sized, and deformed (lop sided) fruits, normally poorly colored (greening) with coloration starting at the peduncular end [1]. There is no effective treatment found till date for huanglongbing. It is necessary to detect infection prior to symptom appearance. After the infection, the tree eventually dies. The chance of production loss in the majority of Florida countries was found to be over 90%. As there is no existence of efficient management measures, it is likely for the failure of current HLB disease control and management procedures.

Development of a quick and reliable method of diagnosis of CGD has got a significant importance because of similar symptoms may be caused by various biotic and abiotic factors. They may vary depending upon the season, environment strain of pathogen etc. Moreover, the visual symptoms of HLB appear only at the fruit bearing stage of plant. Some common methods of diagnosis being used for the detection of CGD are plant indicator method, histochemical method, thin layer chromatography test, monoclonal antibody technique, serological method, electron microscope method and in addition scratch method which uses the technique involved in iodine starch test (IST) is a quick, presumptive field test for HLB. It is based on the accumulation of starch in the infected leaves and has given some good correlation to molecular HLB testing i.e. polymerase chain reaction (PCR). The amylase in the starch stains blue with iodine while amylopectin stains purple with iodine [12-14].Scratch method, which is efficient, quick and simple diagnostic method for HLB, indicated 73.9% results agreed with LAMP. Thus it can be used widely and can hasten the detection of HLB throughout the citrus growing areas of country like Nepal.The loop mediated amplification technology (LAMP) employed for developing the detection kit for field testing of psyllids for CLas that associates with HLB can be used as an alternative to polymerase chain reaction (PCR) for testing both psyllids and both DNA extractions. This method takes about 30 minutes to test six samples as well as negative and positive control which is not repaid comparing to the real time methods. The SE-Quant Tube Scanner can also be used to develop an improved real-time fluorescence loop-mediated isothermal amplification (Real Amp) for the purpose of quantitative and rapid detection of CLas.

In the large scale multispectral satellite image could be deployed for the identification of HLB in citrus trees. From the analysis of multispectral satellite image acquired by WorldView-2 was used for assessing the feasibility of HLB detection in the large scale with an achievement of overall accuracy of 81% using Mahalanobis distance classifier. Symptomatically, HLB is often confused with Zn deficiency; the combination of machine learning technique and fluorescence imaging spectroscopy (FIS) can be used to identify two citrus diseases, HLB, and citrus canker and differentiate them from other conditions such as Zn deficiency and citrus scab. This showed 95% of accuracy [11].There is no exact estimation about how early HLB disease can be diagnosed after bacterial transfer to healthy plant because of which at the incubation period of the disease we cannot evaluate the efficiency of an HLB diagnosis method. The hyper accumulation of starch at an early stage is the indication of HLB disease on citrus leaves which could be detected by a polarized imaging system to acquire time-lapse images of citrus leaves.

Through the image analysis algorithm HLB could be identified at pre-symptomatic stage. Timely detection and removal of HLB-affected trees are substantially necessary for managing the trees at present to prevent the spread of disease to other trees and groves. It is essential to improve the understanding of the movement of the pathogen in or between trees, and to elucidate the nature of signals from the pathogen to develop next generation early diagnostics for saving our citrus groves [11].

Controlling HLB vector:HLB disease is transmitted by an insect Asian Citrus Psyllid (ACP), has devastated the citrus growers wiping out entire groves and significantly affecting trees, quality and yield. It causes both direct and indirect damage to citrus trees by heavy ingestion of phloem sap by adults and nymphs and by heavy ingestion of toxins through saliva respectively [15]. The transmission cycle of HLB generally consists of ACP acquiring the pathogens after feeding the diseased plant, which then retains after brief incubation period and transfers the pathogen to other trees. Efficient ACP control programs can be obtained by deep understanding of certain vector-pathogen interactions involved in the acquisition, persistence and transmission of CLas by ACP [11]. ACPs are most attracted to infected trees until they fed on them; then they prefer feeding on uninfected trees. The uninfected ACP can acquire the CLas bacteria in less than an hour of feeding and after the pathogen complete certain incubation period inside it; it is able to transmit it to other non-infected trees. The continued research in the area of ACP chemical ecology should be beneficial as it relates to ACP flight, host finding, feeding, mate finding, ovipositional, and other biological parameters [16].

Chemical methods: In order to combat HLB by growers in areas where we have not still stepped for ACP control like in Nepal, intensive chemical control programs have been deemed essential for citrus growers. The application of insecticides is the most widely followed options for reducing ACP populations. The perfect insecticide application periods are during the critical flushing periods and winter for effectively reducing the populations of ACP. Young trees need continuous protection from ACP feeding and pathogen transmission owing to their frequent flushing pattern and ACP colonization. Foliar applied broad-spectrum insecticides are most widely used for controlling both adults and immatures of ACP which kill the insect vector upon contact/feeding. Soil application of systemic insecticides offers continuous protection and is widely followed practice [15]. Insecticides such as imidaclorpid, fenpropathrin, chloropyrifos, abamectin, diflubenzuron, dimethoateand are considered effective and registered for major citrus regimes in this globe [17]. The chemistry, mode of action and IRAC classification of insecticides used in citrus for controlling ACP are given in **(Table 1)**.The per hectare annual cost of ACP management in citrus could range from $US 240 to $US 1000, depending on the type of insecticide used, application frequency and method of application [18]. The new citrus plants are however proven ineffective to prevent the introduction and spread of HLB intensively from the experiences substantiated by research in different geographical regimes and in Florida; the populations of ACP are becoming less susceptible to some insecticides [19,20]. The management of ACP in conventional citrus is based on intensive use of synthetic insecticides, and it has developed high propensity for its population to become resistant reducing the effectiveness of insecticides. For preventing the insecticide resistant problem in the sustainable management of ACP, rotation/alternation of insecticides with different chemistries and modes of action needs to be followed [15]. The understanding of the insect, from the behavior to biochemistry, becomes vital, and more we know in advance of detecting a problem, the better [21].

Chemistry group	Mode(s) of action	IRAC Classificationb	Insecticides
Cyclodiene organochlorines	Antagonist of γ-aminobutyric-acid-gated chloride channels	2A	Endosulfan
Organophosphates	Acetylcholineesterase inhibition	1A	Aldicarb, fenobucarb, methomyl and oxamyl
Synthetic pyrethroid	Sodium channel modulation	3A	α-Cyhalothrin, cypermethrin, fenpropathrin, λ-cyhalothrin and ζ-cypermethrin
Avermectin	Chloride channel activation	6	Abamectin
Neonicotinoids and sufoximines	Agonist of nicotinic acetylcholine (nACh) receptors	4A	Acetamiprid, clothianidin, dinotefuran, imidacloprid and thiamethoxam
		4C	Sulfoxaflor

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Butenolides	Agonist of nACh receptors	4D	Flupyradifurone
Spinosyns	nACh receptor allosteric modulators and antagonists of GABA-gated chloride channels	5	Spinosad and spinetoram
Insect growth regulators	Juvenile hormone mimic	7C	Pyriproxyfen
-	Chitin biosynthesis inhibition (benzoylphenylureas)	15 type 0	Diflubenzuron, flufenoxuron, lufenuron, novaluron and teflubenzuron
-	-	16 type 1	Buprofezin
Selective homopteran feeding blockers	Paralysis of cibarium or mouthparts used for ingesting plant sap	9B	Pymetrozine
METI insecticides	Mitochondrial complex I electron transport inhibition	21A	Pyridaben, fenpyroximate
Tetronic and tetramic acid derivatives	Acetyl CoA carboxylase inhibition	23	Spirodiclofen and spirotetramat
Anthrallicdiamides	Ryanodine receptor modulation	28	Chlorantraniliprole, cyantraniliprole
Compounds of unknown or uncertain mode of action	Chitin synthesis inhibition, feeding and oviposition deterrence, suffocation and alterations in cuticle composition	UN	Azadirachtin, sucrose octanoate, Silwet L-77, Kinetic, petroleum spray oil, horticultural spray oil, nC24 horticultural mineral oil and oil

Table 1: Chemistry, mode of action and IRAC classification of insecticides used in citrus for controlling thrACP[a]

[a]Includes insecticides that are under experimental use, as well as insecticides that were labeled for use on citrus but are now banned.

[b]Based on the mode of action classification of the Insecticide Resistance Action Committee (IRAC), version 7.3, February 2014.

Biological methods: The biological control has become an advantageous development direction for the prevention and control of agricultural pests because of the undesirable effects of chemical pesticides. Biological control of ACP includes the use of parasitoids, predators and pathogens that consume and destroy ACP nymphs and/or adults thus reducing their populations.Conservation of wild predators as well as augmentation with commercially available biological control agents will have a synergistic effect for growers looking to utilize predation and parasitoidism as a control measure for ACP [11]. The predaceous arthropods like lady beetles, lacewings, and spiders have been effective to reduce the populations of ACP significantly [22]. There are over 6,000 species of lady beetles worldwide that are accessible to growers from the wild, though all do not prey upon the ACP [23]. Lady beetles are strictly carnivorous predators at the larval stage and actively feast on ACP nymphs; when they become adults they are omnivorous and they feed on nectar and honey dew as well as on ACP.A study in central Florida showed that when predators such as lady beetles were excluded from citrus branches infested with ACP, the duration of infestation was doubled and the chance of ACP reaching adulthood increased by 120 folds [11].

Tamarixiaradiata, a parasitoid tiny wasp has co-evolved with ACP in China and surrounding regions [24]. The female wasp lays her eggs under a fourth or fifth instar ACP nymph. Once hatched, the wasp larva consumes the nymph, attach the shell to the plant tissue and pupate inside. There is the evidence of female wasps feeding on nymphs and destroying up to 500 ACP [24]. The population of *T. radiata* are well established in citrus growing regimes but the mass release of the, require a sugar source to fulfill the nutritional requirement of the wasps. This provision could be made by intercropping citrus groves with plants that provide extra floral nectaries, to support the establishment of population of wasps. The legumes like cowpea and snap beans are best suggested to intercrop with citrus; helps for nitrogen fixation, ground cover and could be utilized as green manure[25].Entomopathogenic fungi are another best tool to biologically manage ACP populations. *Isariaflumosorosea*Wize (Ifr) and associated species are available in commercially formulated products such as PFR-97 20% WDG® (Certis USA) [11]. It is mandatory to consider the destruction of natural enemies in addition to the target ACP when planning an application. The fungus targets the specific species of insects and do not attack plants [26]. The biological control strategies using both T. radiata and *Ifr* could be a great promising tool for suppressing the populations of ACP. Another entomopathogenic fungus with similar mode of action to *Ifr* is *Beauveriabassiana*, the active ingredient available in the commercially available product

Mycotrol ESO® (BioWorks, Inc). This product is admired by most of the commercial citrus growers and considered to be effective than *Chromobacteriumsubtsugae* strain a microbial insecticide product containing bacteria that control ACP [11]. Also, guava extract from the guava leaves repels ACP, volatiles from guava extract mask citrus emissions or change volatile emitted from citrus and affects psyllids host finding volatile cues [27].

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Table 2: Known predators, pathogen, and parasitoids of Asian citrus psyllid.

Cultural/Mechanical Control of ACP: Alteration in the growing ecosystem to cause disruptions of feeding or breeding of the ACP by changing the certain properties of soil or peripheral plants are included in cultural control methods. The physically destructive activities such as soil discing or vacuuming greenhouse plants are included in mechanical methods to control the population of ACP.CLas is transferred by a single psyllid during single feeding. So by preventing a single ACP from attacking a citrus tree might save a citrus from

HLB.Citrus under Protective Screen (CUPS) Production Systems have been developed to prevent ACP from assessing the citrus tree in order to prevent it from HLB. The combination use of some insecticides with CUPS system has been found very effective [28]. CUPS facilitates normal tree growth, encourage higher yields of premium-quality fruit and reduces fruit drops with the allowance of uncomplicated irrigation and fertilizer application in high density planting. This is an effective but complicated and expensive method that may allow the entry of mites and thrips through the screen and prevents some of the beneficial insects; as well as greasy spot and other fungal diseases may may establish more easily in the more humid atmosphere [28]. In Nepal, this method will be applicable for raising nurseries.As most of our citrus grown land have hill topography and farmer will not be able to afford the cost for installing CUPS. Growing citrus in completely enclosed screen houses has the benefit of mitigating the frequency of insecticide sprays and excessive nutrition to combat HLB [29].

The highly reflective film applicable to planting beds, Metallized Reflective Mulch (MRM), can provide better trees growth due to increased soil water retention, reduced weed pressure and control of ACP populations to control the incidence of HLB [30]. The light reflected by mulch deters the ACP from colonizing young trees. In addition to this, the mulch facilitates amplified growth because of the provision of amplified photosynthetic light, weed control and sustainable supply of fertigation. Photonic Fence (PF) is a laser system that identifies, tracks and kill the flying insects, which is still in the experimental phase is the another promising technology being developed to control the ACP attack to new citrus groves. The PF is an optical system able to monitor a boundary for motion, evaluate and identify specific species of insect by wing beat frequency and, if the movement matches the identification profile in the database, destroy the insect with a single laser pulse [31].In Nepal, citrus is found at high altitude than 1300m.asl. The citrus at high altitude are relatively of low quality in terms of physical and chemical attributes [32-33].The high altitude can be best utilized to raise nursery of most of the local varieties grafting with some quality citrus scions and distribute the plants to the citrus growers. In Nepal, the locally produced seedling trees of sweet orange and mandarin have the advantage of being free of HLB like diseases but affected by Phytoptora gummosis.Citrus in Nepal can be greatly improved not only by trying to avoid HLB but also by improved nursery and horticulture practices [34].

Conclusion

The citrus production and consumption have been growing strongly around the globe and huanglongbing disease is ravaging citrus groves of the world putting the threat to citrus industry. In Nepal, since last six years though the area under citrus cultivation is increasing; the productivity has decreased by more than 4 Mt/ha because of poor orchard management practices coupled with HLB. The immediate action and research is essential in Nepal to promote the hilly economics. The world has spent hundreds of millions of dollars for searching the treatment for HLB and till date has come up with the conclusion that the cure techniques for this ravaging disease are not available yet. HLB could be best managed by controlling its vector Asian Citrus Psyllid from attacking the new citrus groves for which different chemical, biological and cultural mechanism can be best employed for saving the new groves from HLB. In Nepal, high altitude above 1400 m.asl could be best utilized to raise the nursery to produce healthycitrus saplings free from HLB.

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