

## ORIGINAL ARTICLE

# Survey on *Citrus tristeza virus* and Haunglongbing (Citrus greening) Associated with Citrus decline of Khasi mandarin in Meghalaya

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### ABSTRACT

*Citrus* is most important fruit crop that can be grown after mango and banana in our country, citrus decline is a serious problem in all the citrus growing tracts. The *Citrus tristeza virus* (CTV) and haunglongbing (HLB) are most lethal, dreadful diseases to the citrus and are the major biotic contributors to the citrus decline of khasi mandarin. The little information is known about the occurrence, incidence, major pathogen that associated with the citrus decline in North Eastern Region (NEH) particularly in Meghalaya. The diagnostic survey was conducted during November, 2015 to April, 2016 in order to find out the status of disease occurrence in various locations of three different altitudes viz., (low, medium, high) of Meghalaya. The locations selected were Dawki and Nongpoh (low altitude, < 500m), Amlarem and Umiam (mid altitude, 500-1000m) and Mawrykneng and Sohra (high altitude, > 1000m). The survey is based on the symptomatology on the host plants, during the survey, viral and fastidious bacterial incidence in affected plants were recorded as 30-70 % and 50-80 % respectively based on symptomatology. The presence of psyllid vector (*Diaphorina citri*) for HLB was more prevalent in mid altitude conditions compared to the low and high altitude conditions. But the citrus brown aphid (*Toxoptera citricidus*) vector for CTV was not noticed during the survey time.

**Key words:** Citrus decline, survey, Khasi mandarin, CTV, HLB, Psyllid, Citrus brown aphid.

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### INTRODUCTION

Citrus is an important fruit crop belongs to the family Rutaceae and grown widely in tropical and sub-tropical regions. Among the fruit crops in India, it occupies the 3<sup>rd</sup> place next to mango and banana [10]. Citrus decline is a serious threat to the citriculture industry, which is due to the several biotic and abiotic causes. Among biotic causes, the diseases are the major contributors to the citrus decline. Among them, citrus crop suffers more extensively due to damage caused by virus and virus like pathogens and their association leading to the citrus decline [1, 2]. Among the virus and virus like pathogens the Tristeza (*Citrus tristeza virus*, CTV) [15] and Huanglongbing (*Candidatus Liberibacter asiaticus*) are the most dreadful diseases of citrus [5] which causes significant losses and leading to the death of millions of citrus trees [1,6].

*Citrus tristeza virus* is the most important viral pathogen under the genus *Closterovirus* in the family *Closteroviridae* and the members of this family are characterized by the long, flexuous and thread like particles with 10-12nm x 2000nm, with single stranded positive sense RNA as genome (monopartite), with 12 ORF'S [12] encoding at least 19 proteins and about 95% of the genome of CTV is encapsidated 25 kDa major CP [1] and transmitted by citrus brown aphid (*Toxoptera citricidus*) in a semi-persistent manner [16, 8, 14]. The virus produces the various symptoms like yellowing, vein clearing, stem pitting etc. depending upon the type of cultivar, environment, the virus strain.

The causal agent for haunglongbing (citrus greening) is '*Candidatus Liberibacter asiaticus*' (*Ca Las*) which is a heat tolerant form and most destructive pathogen to citrus. *Ca Las* is a fastidious, unculturable, phloem limited gram negative bacterium [18] belongs to the  $\alpha$ -subdivision of the phylum Proteobacteria [11], transmitted by the vector asian citrus psyllid (*Diaphorina citri*) [4, 6, 9]. The greening symptoms in infected leaves appear as small, upright and frequently shows a various types of symptoms like chlorotic mottling and zinc deficiency like symptoms. Among them chlorotic mottling in young leaves is a typical

symptom of the CGD [1]. In severe cases leaves, show almost chlorotic with scattered green spots (green islands) on the leaves. Symptoms are also observed on the entire canopy or the part of the canopy. The infected trees may have sparse foliage due to the extensive die-back of the twigs. Infected fruits are small, lopsided and bitter in taste. Many of the fruits fall before they reaching maturity (pre mature falling) or if they still remain in the tree did not color well, remaining green in color and seeds of such fruits are abortive [6], bearing the off-season flowers, fruits produced from that tree has thick peel and pale green in color. Besides that the plants also showing the sparsely foliated, affected by extensive twig die-back [1, 7]. Previous studies were more concerning about study of single pathogen i.e. either CTV or HLB. The present study the attempts were made to study about the two pathogens and their incidence and occurrence, assessment of the major pathogen associated with citrus decline of khasi mandarin in different altitudes of Meghalaya.

## MATERIALS AND METHODS

For present study the citrus orchards were selected based on the altitude (low, medium, high) and on each altitude two locations were selected for the survey. The locations selected were Dawki and Nongpoh in low altitude (< 500m), Amlarem and Umiam in mid altitude (500-1000 m) and Mawryngkneng and Sohra in high altitude (> 1000 m). The diagnostic field survey was conducted during the November-2015 to April-2016 in selected areas or localities in order to find out the status of disease incidence and occurrence of citrus decline of khasi mandarin on various altitudes of the Meghalaya. The survey was done in the orchards by moving in a zig-zag manner. Nearly 10 plants per orchard were selected randomly and the observations were made as, age of tree, severity of symptoms viz., (mild, moderate, severe) and type of the symptoms of CTV and CGB, presence of the insect vectors etc. The GPS locations were noted down on the each surveyed location (Table. 1). The photographic documentation of symptoms (Fig.1, 2, 3a, 3b, 4) and also insect vectors (Fig. 5) was done during the survey.

**Table 1. Description of CTV and HLB symptoms observed in different locations of Meghalaya**

Altitude	District	Area	Location		Place of collection	Cultivar / Variety	Age (Yrs)	Symptoms observed		Prevalence Insect vectors	
			Latitude	Longitude				CTV	HLB	Psyllids	Aphids
Low	W. Jaintia Hills	Dawki	25.1116	92.0486	Orchard	Khasi mandarin	5-10	Ylw, Vcl, Vf	Ylw, Chl.sp	Less	Not found
	Ri-Bhoi	Nongpoh	25.5445	91.5641	-do-	-do-	< 5	-do-	-do-	-do-	-do-
Mid	W. Jaintia Hills	Amlarem	25.1271	92.6292	-do-	-do-	5-10	Ylw, Vcl, Vf, dcln	GI, S.chl.sp, Df, Ylw, P.dck	More	-do-
	Ri-Bhoi	Umiam	25.4101	91.5446	-do-	-do-	5-10	-do-	-do-	-do-	-do-
High	E. khasi Hills	Mawryngkneng	25.3335	92.3503	-do-	-do-	> 5	Ylw, Vcl, Vf, sev. dcln	Mod.GI, ylw, P.dck	Less	-do-
	E. khasi Hills	Sohra	25.1381	91.3857	-do-	-do-	> 10	Ylw, vcl, mod dcln.	Sev ylw, GI, Sev.dck	-do-	-do-

\*\* Ylw- yellowing, Sev ylw- severe yellowing, Vcl- Vein clearing, Vf- vein flecking, mod. dcln- moderate declining, sev. dcln- severe declining, Chl.sp- Chlorotic patches, S.Chl.sp- severe chlorotic patches GI- green islands, Mod.GI- moderate green islands, P.dck- partial die-back, Sev.dck- severe die-back.

W. Jaintia Hills - West Jaintia Hills, E. khasi Hills- East khasi. Hills.

## RESULTS AND DISCUSSION

### Survey on CTV and HLB in various altitudes of Meghalaya.

Citrus decline is a serious threat to the citriculture and citrus industry throughout the world including India. Therefore the present study was done to know about the status and occurrence of CTV & HLB and their association leading to citrus decline of khasi mandarin in Meghalaya. A field survey was done during the November, 2015 to April, 2016 based on altitude wise in order to know the status of disease occurrence in different altitudes of Meghalaya. During the survey the CTV symptoms were noticed as vein clearing, vein flecking, yellowing etc (Fig. 2). but the stem-pitting a typical symptom of CTV, were not noticed on khasi mandarin [3] but it is present in the other citrus species like Pummelo. The citrus brown aphids (*Toxoptera citricidus*) acts as carriers or vector for the CTV was not noticed during the survey time. The HLB symptoms were noticed as the small upright leaves, yellowing, green islands, chlorotic or scattered yellow patches on the leaves (Fig. 1), partial die-back and complete die-back etc (Fig. 3a, 3b). The psyllid insect vector (*Diaphorina citri*) (Fig. 5) was most prevalent in the mid altitude regions compared to the low and higher altitudes during survey. At least 5-6 plants with typical CTV and HLB symptoms as yellowing, vein clearing (CTV), small upright leaves and chlorotic patches or green islands (HLB) were majorly observed during survey. The HLB symptoms were observed more in mid altitude conditions. The disease incidence was found to be 30-70% in case of CTV [1, 17, 13] and 50-80% in case of the HLB [2, 6] based on symptomatology. Earlier researchers were also found the similar findings that we noticed in this study. Recently, a survey was made during October, 2011 to February, 2012 in Assam, Meghalaya, Arunachal Pradesh and Nagaland in North Eastern states, and 39.13% to 58.93 % incidence of CTV was reported based on the symptomatology and RT-PCR results [13].



**Fig.1 Suspected samples of HLB**

**Fig.2 Suspected samples of CTV**



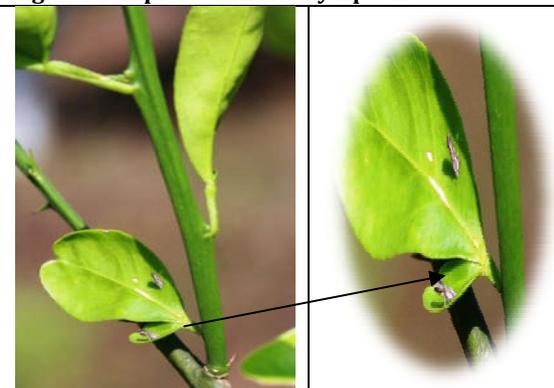
**Fig. 3a. Partial die-back symptom**



**Fig. 3b. Complete die-back symptom**



**Fig. 4 Completely declined Citrus plant.**



**Fig. 5 Psyllid vectors (*Diaphorina citri*) present on the plant.**

Similarly, another survey was made by Das *et al.* [6] during February, 2006 in Assam, Meghalaya, West Bengal and Sikkim, They recorded 12.5% to 30.0 % incidence of HLB based on the symptomatology and PCR results and also incidence of psyllid population was also observed in these region by them. In the present study we noticed more HLB symptoms compared to the CTV and also observed the psyllid (*Diaphorina citri*) insect vectors (Fig.2) on the different altitudes but we did not noticed the aphids (*Toxoptera citricidus*). The severity of the symptoms (CTV & HLB) were found more on the mid altitude and then followed by the high and low altitudes (Table. 1). Based on the symptomatology it may not possible to assess the major pathogen associated with the disease, further molecular detection has to be done for correct assessment and also to find the major pathogen associated with the declining of citrus trees. More over the older plants of age > 5-10 years showing the more symptoms compared to the younger age plants that may due to the latent nature of pathogen in the host plant.

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