

Citrus greening (HLB) disease survey protocol for Nepal

Pathogen of HLB- *Candidatus Liberibacter asiaticus*

SURVEY PROTOCOL

1. TIME OF SURVEY-

October-December

SELECTION OF SURVEY AREA-

As per the requirement of NPPO

- (To begin with -Citrus pockets of Syanja and Sindhuli)

2. NUMBER OF TREES FOR OBSERVATION –

All the trees of the orchard to find out the suspected symptoms of CGD (HLB) . Blitz survey or full sampling survey

Observe for the presence of any of the following symptoms-

General aspect of the tree

At the beginning leaf mottling (yellowing) is seen on single branch which gradually spreads out to other branches

Thus, slightly infected tree becomes severely affected with symptoms like open growth, stunting, twig die back , sparse foliage and severe leaf and fruit drop

The whole orchard declines with 2-3 years

Symptoms on Leaves

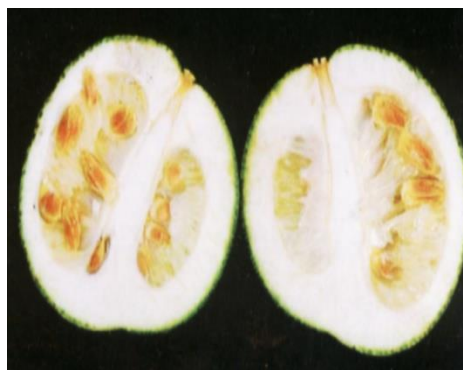
- Leaves are reduced in shape and size
- Leaf mottling and specific mosaic symptoms are common
- Matured leaves often show irregular patches between the main veins
- Sometimes vein corking is also observed
- The veins are often prominent and yellow



- Excessive leaf drop and unseasonal flushing with very small but erect type of leaves are developed

Symptoms on fruits

- Fruits are under developed and reduced in size with lopsided shape
- Most of them remain green or poorly coloured even after maturity specially at the rind part
- The side exposed to sun light shows normal colour and the remaining part shows dull olive colour (This symptom is rare in Nepal)



3. PARTS TO BE OBSERVED-

Leaves, twigs and fruits

4. SAMPLE COLLECTION PROCEDURE AND PRESERVATION-

- Identify moderately or severely declined trees
- Tag the tree with the serial number of your sample and write the same number on the standard sample collection poly bag
- Look at the part of the tree with leaves having HLB Symptoms (mottling, asymmetric mosaic)
- Collect 15-20 leaves with HLB symptoms but without insect damage from the 5-6 month old twigs.
- Put all the leaves in the plastic bag , be sure that there is no wet leaf, if there are wet leaves dry them with tissue paper
- Arrange all the leaves inside the poly bag in such a way that leaves do not overlap to each other
- Close the bag properly
- Put the bag with sample in ice box immediately, if possible
- If it is not possible put them in shaded and cool place
- Prepare packs of 20 such bags
- Prepare the list of samples with sample number, date of sample collection , name and address of owner, citrus species, age of the tree and type of origin (grafted or seedling) and any special feature of the tree and other details specified by the laboratory
- Prepare a covering letter to the laboratory requesting for HLB testing the list of samples prepared as above
- Dispatch the packs of samples to the laboratory by faster means of transport so that it reaches the laboratory within the seven days of sample collection
- Maintain your diary writing all these operations with dates and all other details
- Mark the trees with HLB positive immediately after receiving the test results.

5. DIAGNOSTIC LABORATORY-

The facility for Confirmative diagnosis of CGD (HLB) by PCR technique is available only in the Biotechnology Laboratory of Nepal Academy of Science and Technology (NAST), Khumaltar.

6. Sampling Procedure Flow Chart for HLB detection

SN	Sample type	Sample size and methods
1	Suspected prominent visual symptoms on tree	<ul style="list-style-type: none"> - All samples 100% Iodine Scratch test (IST) - ALL 100% IST positive samples for PCR testing - 5 % negative samples of IST test for PCR testing
2	Suspected but less visual symptoms of HLB	<ul style="list-style-type: none"> - All samples for IST - All 100 IST positive samples to PCR test - 2% of negative samples for PCR test
3	No visual symptoms	<ul style="list-style-type: none"> - 1% of tree IST test - All positive samples for PCR test - 0.01% of negative samples for PCR test

7. DATA COLLECTION FORM

Field Datasheet		
1. Orchard General Information		
A Name of Field/Site visited:		
B. Citrus pocket		
C. Age of the orchard		
D. Kind of orchard :	Seedling / Grafted	
E. Relief of the orchard:	Valley / Terrace / Slope	
F. Aspect/Facing of orchard	North / South / East / West	
G. Total number of trees planted in the orchard :		
H. Total number of trees in the orchard present		
I. Trees died in last five years		
J. Saplings supplied by:	DAO / NGO / Local market / produced by own	
K. Management practices applied by the farmers :		
I. Pruning and cutting of dry or diseased branches :	Yes / No	
II. Application of plant protection chemicals :	Yes / No <i>If yes:</i> <i>What:.....</i> <i>Dose:.....</i> <i>Time:.....</i>	

Field Datasheet		
III. Use of manures and chemical fertilizers:	Yes / No <i>If yes,</i> <i>FYM or</i> <i>compost:.....Urea:</i> <i>..... / tree, DAP:</i> <i>...../ tree, Potash</i> <i>:...../ tree</i>	
IV. Intercrop grown :	Maize / Millet / vegetables / Other crops like...../ no intercropping	
V. Mulching :	Practiced / Not practiced	
VI. Irrigation :	Applied / Not applied	
L. General appearance of the orchard and tree health :		
I. Number of plants showing declined stage :	(<i>Early stage of decline :</i> <i><25 % , Medium decline:</i> <i>25-50 % , Severe decline :</i> <i>> 50 %</i>	
II. No of plants that are in bearing stage		
III. Fruit yield / tree :		
2. Date/Time of Visit:		
3. GPS Reference Point	Latitude:	
	Longitude:	
	Altitude:	
4. Locality:	Village & ward:	
	VDC:	
	District:	
5. Climate Data of Locality:	Average Min. temp (in °C):	
	Average Max. temp (in °C):	
	Rainfall (in mm)	
6. Survey/Field plot No.		
7. Host Plant species/Variety inspected		
7.1 Description of habitat (e.g., vegetation, soil type and depth	Sandy.... toclay, 1 meter / 1-3 M / more than 3 M	
7.2 Alternate Host Plant species/Variety inspected		
8. Phenological Stage of the plant	Flushing, fruiting, flowering	
8.1 Main host		
8.2 Alternate host including vectors		
9. Sampling method	Full Sampling	
10. Contact detail of local people involved in the survey	11. Details of pest recorded	

S N	Scientific Name	Common Name	Category	Order	Family	Life Stages	Time	Plant parts affected	Symptom & Sign)	Behavioural notes	Intensity
1	CLA	HLB	Bacteria					
10. Any additional information (including collection of specimens for investigation):											
11. Name/Signature of surveyor with Date:											

Format for specimen forward

Specimen Forwarded for identification by Diagnostic/Referral Laboratory	
1. Collection Number:	2. Date of Collection:
3. Submitting Organization:	
4. Name/Address of the Sender:	
5. Locality of Collection (District/VDC/village):	
6. Reasons for identification:	
7. Name of the host plant species (Common/Scientific)/variety and or/ commodity:	
8. Origin of host/commodity (where applicable):	
9. Plant Parts affected:	leaves; [] fruits; [] seeds [] others (_____) *tick out in appropriate box
10. Category of pest specimen/organism submitted	bacteria; [] others (_____)
12. Type of pest specimen/organism submitted	* [] preserved specimen; [] dry specimen with host; [] culture; [] disease specimen (fresh); [] disease specimen (partially dry);
14. Number of specimens submitted per each collection:	
15. Signature/stamp/office seal of the Sender with date:	
For Identifier Use	
16. Name & Address of Diagnostic/Referral Laboratory:	
17. Remarks of identifier (condition of receipt of	
18. Pest Identification (Common/Scientific Name/Taxon):	
19. Description Notes, if any:	
Place: _____	
Date: _____	
Note: This form should be prepared in duplicate by the sender and forwarded to the identifier/referral laboratory along with each collection of specimen. The identifier should return the original copy after entering the particulars of the pest identified along with description notes and remarks if the identifier will retain any to the sender of the specimen and duplicate copy.	

Pest record datasheet.

Pest Record	
Reference Number	Name of laboratory, address, catalogue reference number of the specimen in the pest library etc.
Scientific name of pest	
Common Name:	
Growth habit:	
Habitat characters:	
Species Name:	
Family	
Order:	
Life stage of pest	Actively dividing or dormant spore, etc
Scientific Name of host	
Variety	
Common Name:	
Species Name	
Family	
Plant parts affected:	<input type="checkbox"/> Leaves; <input type="checkbox"/> Buds, <input type="checkbox"/> Fruits; <input type="checkbox"/> Seed ; and <input type="checkbox"/> Whole plant
Stage of plant:	<input type="checkbox"/> Seedling stage; <input type="checkbox"/> Vegetative Growth stage; <input type="checkbox"/> Flowering stage; and <input type="checkbox"/> Fruiting stage
Locality	
Village	
VDC	
District	

Pest Record	
Province/State:	
Date , time and GPS location of pest	
Method of collection	
Name of the Collector	
Species accumulation curve	
Method of Identification	
Name of the Identifier	
Date of Verification	
Method of verification	
Name of the Verifier	
Method of pest preservation (detail)	
Suitable for:	Taxonomic analysis or DNA analysis
Life stage of pest affecting alternate host	Actively dividing or dormant spore, etc
Scientific Name of alt. host Common Name: Species Name Family	
Alt. host. Plant parts affected:	<input type="checkbox"/> Leaves; <input type="checkbox"/> Stem; <input type="checkbox"/> Roots; <input type="checkbox"/> Buds/Flowers; <input type="checkbox"/> Fruits; <input type="checkbox"/> Seed ; and <input type="checkbox"/> Whole plant
Stage of alt. host plant:	<input type="checkbox"/> Seedling stage; <input type="checkbox"/> Vegetative Growth stage; <input type="checkbox"/> Flowering stage; and
Locality, Village, VDC, District, Province/State:	