

# Present Status of Huanglongbing in some Western Districts of Nepal

Chiranjivi Regmi and Bishwanath Pd. Yadav<sup>1</sup>

## Abstract

Huanglongbing (Citrus greening disease), the most destructive disease of citrus, is spreading in different citrus growing areas of Nepal. The research was carried out in December 2006 in three locations of the country namely Bimalnagar of Tanahun, Udipur of Lamjung and Syadul of Dhading districts. Field survey and sample collection was carried out by a team of experts including Prof. J.M.Bove and Dr. Nuria. Samples were analysed by PCR technique in the laboratory of NAST. Results show that HLB is present in all the location under study. It has totally destroyed citrus orchard of two hectare with in 10 years of time. It is spreading very fast in new areas of Udipur causing severe damage to citrus cultivation. Vector *Diaphorina citri* is contributing to its spread in Bimalnagar and Udipur. Introduction of planting material from low-land is the main source of infection in Syadul, where only few newly planted plants were confirmed HLB positive. Syadul is very potential pocket area of citrus, where more than 100,000 citrus trees are being grown in a cluster. There is an urgent need to rescue citrus from HLB in Syadul.

**Keywords:** Huanglongbing, Vector, Survey, Diagnosis, PCR-test, Rescue

## Introduction

Huanglongbing is the most destructive disease of citrus. It has been a serious threat to citrus industry in Asian countries like China, Thailand, Indonesia, India, Nepal, Pakistan and Bhutan. South Africa is another country facing the problem since many years. Recently, the disease is found in Brazil and USA also. The disease has been reported by different names; 'Likubin' in Taiwan, 'Leaf-mottling' in the Philippines, Huanglongbing in China, Citrus-greening-disease in South Africa, India, Pakistan and Nepal and Citrus-vein-phloem-degeneration (CVPD) in Indonesia. . The 13th Conference of International Organization of Citrus Virologists (IOCV) held in Fuzhou (China) in 1995 has recommended to name this disease as HUANGLONGBING in honour of the Chinese scientist Prof. Lin, who first described the disease by this name in 1920. Therefore, it has to be called as Huanglongbing (HLB) worldwide.

Although the pathogen of HLB was supposed to be a virus and, sometimes, Mycoplasma in the past, it has been now proved to be a gram negative bacterium. The bacterium is pleomorphic, phloem restricted, and sensitive to tetracycline. As the bacterium can not be cultured in artificial media, prefix *Candidatus* has to be added to the name of the species. Slight differences among HLB pathogens in Asia, Africa and America have been found; accordingly, they have been considered as different species of the same genus *Liberibacter*. The pathogen of Asian HLB is *Candidatus Liberibacter asiaticus*, that of African HLB is the *Candidatus Liberibacter africanus*, and American one is the *Candidatus Liberibacter americanus* ( Bove, 2006). HLB is spreading in different citrus growing areas of Nepal. For example HLB has destroyed all the citrus at Horticulture Research Station Pokhara and Dailekh. There are unreported similar cases of private

<sup>1</sup> Nepal Academy of Science and Technology (NAST), Khumaltar, P.O.Box-3323 Kathmandu, Nepal

citrus orchards in other locations. Dhading, Tanahun and Lamjung are major citrus fruit producing districts, where the disease has been reported. The study was carried out focused in the major citrus growing pocket-areas of these districts.

### Objectives

The objective of this study is to reveal the present status of HLB in selected citrus orchards so that necessary action be undertaken by concerned organizations for its control by carrying out surveys of selected citrus orchards to find out the trees with HLB symptoms and polymeric chain reaction (PCR) tests to confirm HLB presence in the suspected samples.

### Materials and Methods

The research was carried out in some orchards of Syadul (Dhading), Udipur (Lamjung) and Dumre (Tanahun). All species of citrus grown in the selected orchards were under study. A team of experts comprising of Prof. Bove, Dr. Nuria, Dr. Regmi, Dr. K.P. Paudel, Mr. L.N.Deoju and Mr. R.P Devkota carried general survey of selected orchards during the first half of December 2006. Leaf samples were collected from symptomatic trees, and analyzed immediately in the PCR Laboratory of Nepal Academy of Science and Technology following the procedure described by Shrestha et al 2003.

### Results and discussions

Quite a large number of trees were observed with HLB symptoms during the survey in Udipur and Bimalnagar. Excepting 15-29 years old trees, most of the newly planted trees in Syadul were also showing HLB symptoms. It might be explained by the fact, according to local farmers, that the planting materials for new plantations were brought to Syadul from lower altitude- Charaudi or Mugling, where the disease and the vector *Diaphorina citri* prevailed. And the older trees were of seedling origin locally produced. Results of PCR tests are presented in tables 1, 2 and 3. The results comply with the observations during survey.

**Table 1: HLB in mandarin orchard in Udipur, Lamjung**

S.N	Owner/ Address	Symptom	PCR test
1	Ganesh Pant, Udipur-2,	Yellowing of whole tree	Positive
2	Ganesh Pant, Udipur-2,	Yellowing of whole tree	Positive
3	Ramesh Paudel, Udipur-2	Yellow shoot and blotchy mottle	Positive
4	Ramesh Paudel, Udipur-2	Yellow shoot and blotchy mottle	Positive
5	Ramesh Paudel, Udipur-2	Yellow shoot	Negative
6	Ramesh Paudel, Udipur-2	Yellow shoot	Positive
7	Hari Kumar Shrestha , Udipur- 2	Yellow shoot and blotchy mottle	Positive
8	Hari Kumar Shrestha , Udipur- 2	Yellow shoot and blotchy mottle	Positive
9	Hari Kumar Shrestha , Udipur- 2	Yellow shoot	Negative
10	Indra Bahdur Khadka, Udipur-2	Yellow shoot	Positive
11	Indra Bahdur Khadka, Udipur-2	Yellow shoot	Positive

The results also show that HLB is spreading very fast in new areas. For example not a single tree was found with HLB symptoms in Bimalnagar in 1992 and 1994, when Prof. Bove, Dr. Garnier and Dr. Regmi visited the 2-hectare citrus orchard of Chij Kaji Shrestha ( Regmi et al 1996), while the recent survey in 2006 revealed that most of the trees in the orchard were cutdown following infection. HLB has totally destroyed the orchard causing serious set back to citrus cultivation in the area within last 12-years. Similar situation is

seen in Udipur (Lamjung), where about 100 trees were seen severely damaged. The trees were unproductive, and the farmers were ready to cutdown. The disease is spreading very fast and posing a great threat to other citrus orchards nearby. Both of the locations are at low altitude, and the presence of HLB vector *D. Citri* is accelerating the spread of the disease.

**Table 2: HLB in mandarin orchard in Bimalnagar, Tanahun**

S.N	Owner/ Address	Symptom	PCR test
1	Chij kaji Shrestha, Bimalnagar	Yellowing of whole tree	Positive
2	Chij kaji Shrestha, Bimalnagar	Yellowing of whole tree	Positive
3	Chij kaji Shrestha, Bimalnagar	Yellow shoot and blotchy mottle	Positive
4	Chij kaji Shrestha, Bimalnagar	Yellow shoot and blotchy mottle	Positive
5	Chij kaji Shrestha, Bimalnagar	Yellow shoot	Positive
6	Annonymus, Dumre	Yellow shoot	Positive
7	Annonymus, Dumre	Yellow shoot and blotchy mottle	Positive
8	Annonymus, Dumre	Yellow shoot and blotchy mottle	Positive

**Table 3: HLB in mandarin orchard in Syadul, Dhading**

S.N	Owner/ Address	Symptom	PCR test
1	Dilli Narayan Shrestha, Dhusha-8	Yellowing of whole tree	Negative
2	Bal Bahdur Thapa, Dhusha-8	Yellowing of whole tree	Negative
3	Bal Bahdur Thapa, Dhusha-8	Yellow shoot and blotchy mottle	Negative
4	Chandra Bahadur Baral, Dhusha-9	Yellow shoot and blotchy mottle	Positive
5	Chandra Bahadur Baral, Dhusha-9		Positive
6	Kula Devi Silwal, Dhusha-9	Yellow shoot	Negative

Syadul was supposed to be free of HLB, but our results showed that the disease has already entered the area. Though the disease is limited to newly planted trees brought from low-land nurseries, there is no doubt that all the citrus trees (about 100,000) in the pocket will totally be destroyed within few years.

### Recommendations

Following recommendations can be drawn from our research.

- 1 Rescue of citrus in Syadul from being destroyed by HLB. There is an urgent need of developing and implementing special program in the pocket
- 2 Spatial distribution of vector has to be monitored in all the locations.
- 3 Infected trees have to be replaced in all locations.
- 4 Measures have to be applied for vector control in all the locations

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### *[Queries from the participants of the seminar:*

*Dr. K.P. Poudel suggested that serious decline in citrus is also observed in some areas of Far Western Region, where nurseries have been established, and saplings are distributed therefrom. If such has to continue, citrus in the country can be finished in 10 years. In this connection, Mr. G.P. Shrestha also raised a question how the nurseries could be in better way controlled or managed. Mr. S.P. Sharma gave an example of Gunja Bahadur, a farmer in Sindhuli, who cultivated junar in seven ropani land successfully and has been reaping good income. However, other grooves nearby have already been destroyed. Therefore, the problem is easily manageable at farmers' field provided an appropriate package of cultivation practice. With his consent on the paper, Mr. L.N. Dewaju mentioned that though attempts were being made to manage huanglongbing, he observed the problem being serious while inspecting citrus orchards and nurseries including those in Central Horticulture Center (Kirtipur). He proposed that control on seedling distribution from such orchards and nurseries would be impossible unless a Nursery Act is enacted. "It has been almost forty years since the problem was noticed in the country", Dr. M. Ranjit said. However, he commented, none of the technical recommendations and action plans made earlier had been implemented. As a result, the problem was expanding as 20-30% of the collected samples had turned out huanglongbing positive in PCR test.*

*The presenter responded to the queries with some remarks such as only agronomic management of the crop would not work nicely once the disease had penetrated the groove - even though DOA, NARC and other organizations such as NAST and NARDEF had been working, they lacked good collaboration in dealing with the problem - compared to huanglongbing CTV had not been much destructive in Nepal - cautions were to be taken regarding the activities by private/ donor agencies since the planting materials distributed/ imported by them bore the disease inoculums - (on such ground) we had to look for donor agencies' support as well. Upon concluding remarks, Mr. S.B. Nepali, the chair-person of the session, warned to monitor the disease that could have spread to the higher altitude due to global warming though it is in general believed to confine below 1000 m in Nepal .He added the problem called for necessary addresses bt the concerned agencies such as Citrus Development Program, Fruit Development Directorate, other GOs and even Nepalese Horticulture Society in the country.]*