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Comparative Studies on the Different Management Strategies of Citrus Greening Disease in Mandarin (*Citrus reticulata Blanco.*) at Dailekh, Nepal

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Abstract

Citrus greening (*Candidatus Liberibacter asiaticus*) is an important disease of different Citrus species, including Mandarin, globally. There is a growing need for the sustainable management of this disease since the chemical approaches have led to pathogenic resistance and health concerns. A research study was conducted at Dailekh, Nepal to evaluate the field efficacy of different chemicals and biopesticides against greening disease in Mandarin (*Citrus reticulata* Blanco) following a biological survey on disease prevalence. The iodine-based starch test showed the disease prevalence to be 28.07 % and the disease severity to be 0.184 in the Dailekh district. The field experiment was laid out in Randomized Complete Block Design (RCBD) with 3 replications and 10 treatments of botanicals, biocontrol agents, and chemical consortia. Results revealed that the highest percentage reduction over control was found in the plot treated with (Imidacloprid + Copper oxychloride), (Neem oil + *Bacillus amyloliquefaciens*) and (*Bacillus thuringiensis* + *Bacillus amyloliquefaciens*.) Hence, based on the result of the study, foliar application of Imidacloprid @ 0.02% + Copper oxychloride @ 0.025%, Neem oil @ 5 ml/l + *Bacillus amyloliquefaciens* @ 5 ml/l and *Bacillus thuringiensis* @ 2 ml/l + *Bacillus amyloliquefacians* @ 5 ml/l at monthly interval were found effective in reducing the severity of citrus greening disease in Mandarin.

Keywords: Bacillus, Biocontrol, Hunaglongbing, Pseudomonas, Streptocycline, Trichoderma

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Introduction

Citrus is the second major fruit produced globally in around 10.2 million hectares, amounting to 161.8 million tons in 2021 (Gonzatto and Santos, 2023). The annual citrus production of Nepal is around 314,844 tons (MOALD, 2021). Mandarin is an important group of citrus fruits consisting of 12% carbohydrates, 0.7% protein, 5% fat, and 1.8% fiber (Riaz et al., 2023). Citrus has several nutraceutical benefits, including antioxidant, antidiabetic, anti-cancerous, antimicrobial, antihyperglycemic, and antihypertensive properties (Riaz et al., 2023). They are rich in dietary fibers capable of preventing gastrointestinal diseases and cholesterol complications (Liu et al., 2012). Vitamins C and E are physiological antioxidants present in Mandarin.

Low productivity of Mandarin can be attributed to diseases such as Huanglongbing (Citrus greening), Citrus tresteza, greasy spot, Alternaria brown spot, Phytophthora root rot, scab, canker, powdery mildew, woody gall, and gummosis, and pests such as Asian citrus psyllid, scale insects, aphids, and mites (Teixeira et al., 2005). Among these, Huanglongbing (HLB) is considered the most important disease of Citrus (Iftikhar et al., 2016). Total revenue loss due to HLB in the US alone was around \$3.63 billion from 2006 to 2017 (Munir et al., 2018). In Nepal, Citrus greening disease (CGD) was reported for the first time in Pokhara in 1968 (Pandey, 2022). *Candidatus Liberibacter* sp., which causes Huanglongbing in Citrus, interferes with various physiological pathways and anatomical structures, leading to the yellowing of shoots, blotchy mottled leaves, branch dieback, and the lopsided and uneven coloration of fruits with heavily reduced yield (Graça et al., 2016). *C. Liberibacter* is a phloem-limited (uncultivable) gram-negative bacterium transmitted by Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) (Hall et al., 2013). ACP has five instars. Its eggs are yellowish orange with an oval shape, having a length of 0.3 mm. They are laid on the tips of growing shoots or cracks of stems. ACP feeds directly on newly emerging tender leaves with its piercing-sucking mouthparts and also injects toxic saliva into host plants. It causes the deformation of shoots and the progressive death of new leaves. Honeydew produced by nymphs and adults of ACP enhances the fungal growth (sooty mold), which reduces photosynthesis by covering the leaf surface (Ghosh et al., 2022).

HLB is graft-transmissible, vector-transmissible (by psyllids), and dodder-transmissible (experimentally). The disease is a result of a complex interaction between the pathogen, vector, host, and the environment, i.e., weather, soil, plant nutrition, and presence of pest and pathogen (Graça et al., 2016). HLB reduces root abundance by 40-50% before the appearance of symptoms in the tree canopy (Johnson et al., 2014). The incubation period of the HLB pathogen is very long, ranging between 6 and 12 months (Bové, 2006). Due to a long latent period, the pathogen spreads widely before its detection (Grafton-Cardwell et al., 2012). HLB is sometimes confused with boron and zinc deficiencies due to vein corking and chlorotic patterns on leaves. However, the random chlorotic mottles on the leaf surface indicate HLB infection, which occurs due to the disruption of the chloroplast and accumulation of starch(Vashisth and Kadyampakeni, 2020). A pen test can be used to distinguish HLB from nutrient deficiencies, in which a circle is drawn on both sides of the leaf blade. If the pattern of chlorosis is identical on both sides of the circle drawn, it is a nutritional deficiency. And if the pattern of chlorosis is random, it is HLB. An iodine test can be performed to detect higher starch content seen during HLB infestation, which is rapid, easy, and economical for the diagnosis of Citrus greening disease (Taba et al., 2001). The accumulation of starch in HLB-infected leaves was six times that of healthy leaves (Etxeberria et al., 2007). The iodine scratch test was in 90% agreement with PCR analysis(Irey et al., 2006). Identification of HLB disease is important under field conditions for its timely management. The detection of Liberibacter (the pathogen of HLB) through polymerase chain reaction using specific primers in 16s rDNA or ribosomal protein genes is preferred due to its accuracy (Ghosh et al., 2022). Other techniques include nucleic acid hybridization using different probes for the detection of pathogenic bacteria in plants as well as the insect vector (Ghosh et al., 2022). One of the important factors responsible for the devastating effect of HLB disease is the lack of natural resistance. No resistant citrus seedlings or scion-rootstock combination is available that could resist the Citrus greening disease (da Graça et al., 2016). Efforts to transfer resistant genes into cultivable germplasms are underway.

HLB can be managed by controlling Citrus psyllids (vectors of pathogenic bacteria) using insecticides like Dimethoate, regular inspection of citrus orchards, and removal of infected trees to reduce the inoculum (Bové, 2006). *Murraya paniculata* and *Severinia buxifolia*, being the alternate hosts of ACP, should be removed from the citrus groves (Ghosh et al., 2022). Systemic insecticides such as imidacloprid, thiamethoxam, and clothianidin, which are applied in soil, can control the psyllid population (Cocco and Hoy, 2008). The citrus orchards can be sprayed with effective insecticides before and after the initiation of the new flush to prevent the psyllid attack. Several other strategies, such as the use of parasitoids, sterile male technique, and RNA interference (RNAi), are being explored for controlling ACP (Ghosh et al., 2022). Foliar application of antibiotics such as chrimycin and ledermycin on the branches was effective in suppressing the HLB symptoms, while oxytetracycline and penicillin reduced the disease severity (Shokrollah et al., 2011). However, there is always a risk of bacteria developing resistance against antibiotics and fruits getting contaminated with the chemicals (Ghosh et al., 2022). Zhang et al. (2012) reported that the combination of penicillin and streptomycin as effective management strategy against *C. liberibacter*. An integrated disease management system is necessary, which includes phytosanitary approaches based on pathogen detection, control of vectors, cultural practices, and chemical treatments (Iftikhar et al., 2016). This study intended to evaluate the field efficacy of different chemicals and biopesticides against Citrus greening disease in Mandarin. The prevalence of Citrus greening disease in the Dailekh district (the research site) was also revealed based on an Iodine starch test.

Methodology

Experimental site

The research site was Dullu Municipality of Dailekh district, Nepal. The district lies in the sub-tropical region, between 28°35’ N to 29°88’ N latitude and 81°25’ E to 81°55’ E longitudes. Mandarin accounts for 76% of the total Citrus production area of the Dailekh district (MOALD, 2020). The iodine-based starch test was performed by the scratch method at major citrus growing areas of Dullu Municipality, Dailekh, from wards no. 1 to 13, before the experimental treatment. These wards were the major Mandarin-producing areas and had been designated as the Citrus Zone by the Prime Minister Agriculture Modernization Project (PMAMP), Nepal. A citrus grove with a local cultivar of Mandarin (*Sunatala*), infected with HLB disease, was selected for the experimental setup under open field conditions, which lies between 28.850586^0N latitude and 81.612917^0E longitude.

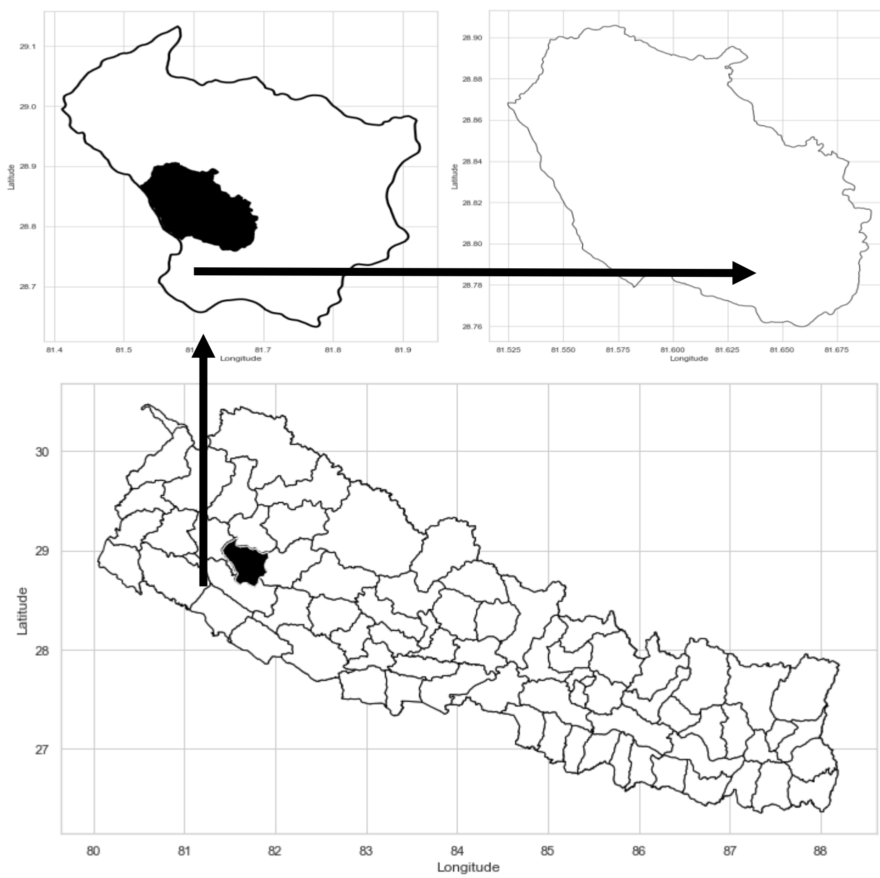

Fig. 1. Map of Nepal showing the experimental location

The study was carried out from March 2021 to July 2021.

Weather and soil

The average minimum temperature during the study period was between 20° to 24 °C. The average maximum temperature was between 25° to 36 °C. The soil of the experimental site was sandy loam. The data related to rainfall, relative humidity, and temperature during the study period are presented below in Figure 2.

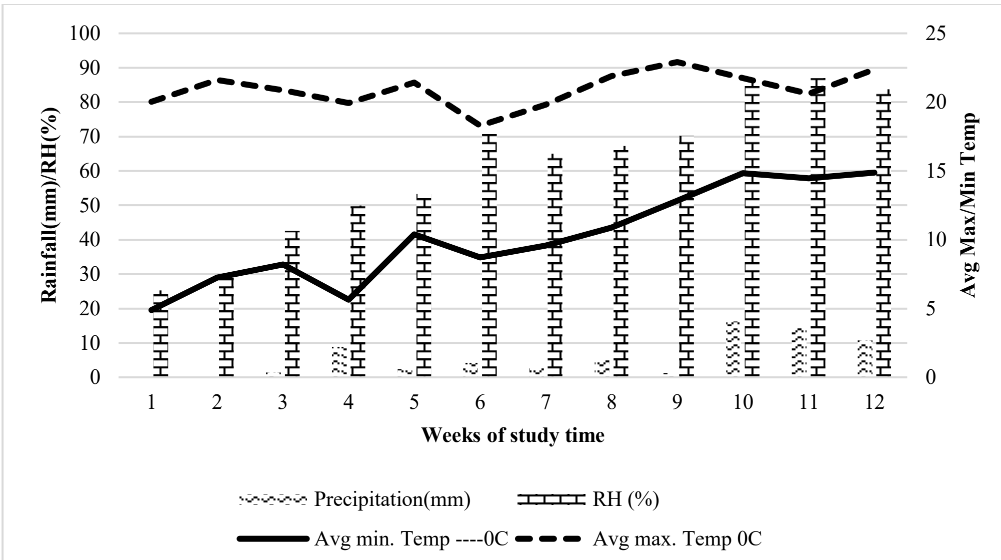

Fig. 2. Meteorological data of the study site during the period of study (Last week of March to the last week of June) (Source: Regional Department of Hydrology and Meteorological Station, Dailekh)

Experimental design

A citrus grove with a local cultivar of Mandarin (*Suntala*), infected with HLB disease, was selected for the experimental setup under open field conditions. The experiment consisted of ten treatments with three replications under a Randomized Complete Block Design (RCBD). Different concentrations of insecticides and pesticides were applied to study their efficacy against HLB disease in Mandarin. Table 1 below shows the details of the experimental layout in the field.

Table 1. Experimental design adopted in Mandarin orchard

S. No.	Particulars	Trial details
1	Design	Randomized Complete Block Design (RCBD)
2	Replication (Blocks)	3
3	Treatments	10
4	Total number of plots	30
5	Number of plants per plot	2
6	Spacing of the plant	5m * 5m (row to row and plant to plant respectively)
7	Sample plant	2
8	Variety used	Local

The details of the treatments are as follows:

T_1: Imidacloprid 0.02% + Streptocycline 250 ppm

T_2: Imidacloprid 0.02% + Copper Oxychloride 0.025%

T_3: Neem oil 5 ml/ l + Lentinan 2 ml/ l

T_4: Neem oil 5 ml/ l + *Pseudomonas* 5 ml/ l

T_5: Neem oil 5 ml/ l + *Trichoderma* 5 ml/ l

T_6: Neem Oil 5 ml/ l + *Bacillus amyloliquefacians* 5 ml/ l

T_7: *Bacillus thuringiensis* 2 ml/ l + *Pseudomonas* 5 ml/ l

T_8: *Bacillus thuringiensis* 2 ml/ l + *Trichoderma* 5 ml/ l

T_9: *Bacillus thuringiensis* 2 ml/ l + *Bacillus amyloliquefacians* 5 ml/ l

T_{10}: Control

Pesticides were sprayed thrice at monthly intervals, starting on the last week of March 2021. The Citrus greening scale of the individual plot was recorded before treatment application. Consecutive data was recorded at 1-month intervals three times.

Preparation of pesticides for spray

The pesticides for the spray were prepared as follows:

- **Imidacloprid 0.02%:** 1 ml of imidacloprid was dissolved in 5 liters of water to make a 200-ppm solution.
- **Streptomycin 250 ppm:** 1 ml of streptomycin was dissolved in 4 liters of water to make a 250-ppm solution.
- **Copper oxychloride 0.025%:** 1 gm of copper oxychloride was dissolved in 4 liters of water to make a 250-ppm solution.

- **Neem oil:** 5 ml of neem oil was dissolved in 1 liter of water.
- **Lentinan:** 2 ml of lentinan was dissolved in 1 liter of water.
- ***Pseudomonas*****:** 5ml of *Pseudomonas* was dissolved in 1 liter of water.
- ***Trichoderma*****:** 5ml *Trichoderma* was dissolved in 1 liter of water.
- ***Bacillus amyloliquefaciens*****:** 5ml of *Bacillus amyloliquefaciens* was dissolved in 1 litre of water.
- ***Bacillus thuringiensis*****:** 2ml of *Bacillus thuringiensis* was dissolved in 1 litre of water.

Application of pesticides was done in the evening to reduce evaporation loss. Stickers and adhesives were used with pesticide solutions to prevent the washing-off and for better attachment to the plant parts.

Calculation and data analysis

Data analysis was performed using R Studio. Analysis of variance (ANOVA) was performed for the percentage of disease severity. The means were compared by using Duncan's Multiple Range Test (DMRT) at a 5% level of significance.

- **Percentage of disease prevalence**

The percentage of disease prevalence was calculated based on the number of observed symptomatic plants over the total number of assessed plants. The formula below was used to determine the percentage of disease prevalence (Ahmad et al., 2011).

$$\text{Percentage disease incidence} = \frac{Total\ infected\ citrus\ orchard}{total\ number\ of\ citrus\ orchard\ surveyed} \text{ x } 100$$

- **Percentage of disease severity**

Citrus greening severity was evaluated on a scale of 0 to 4 based on the method used by Ahmad et al. (2011). The scale value of 0 indicates the absence of disease symptoms, 1 indicates up to 25% symptoms in leaves and twigs, 2 indicates 26-50% canopy symptoms, 3 indicates 51-75% canopy symptoms, and 4 indicates 75-100% canopy symptoms with dieback. Trees with a severity scale of 1 and 2 were considered slightly damaged, whereas those assigned a scale of 3 were considered moderately damaged, and those on a scale of 4 were considered severely damaged. The percentage of disease severity was calculated based on the formula used by Ahmad et al. (2011).

Mathematically,

$$\text{Percentage disease severity} = \frac{X1 + X2 + \ldots\ldots\ldots + Xn}{Y * maximum\ rating\ scale}$$

Where,

X= Sum score of disease severity of individual citrus plant

Y= Total number of plants in the same experiment

Results and discussions

Prevalence of Citrus greening Disease

Out of 57 Mandarin orchards in the Dailekh district, 16 were found to be affected by the Citrus greening disease as indicated by the Iodine-based starch test. The disease severity in those sites ranged from 0 to 2, with an average value of 0.184. Table 2 presents the Longitude and Latitude of the Citrus orchard and the result of the Starch-iodine test with the disease severity index.

Table 2. Iodine-based starch test by the scratch method for Citrus greening disease in the study area

S.No.	Longitude	Latitude	Starch-iodine test	Disease Scale
1.	81.609301^{0}E	28.852343^{0}N	Negative	0
2.	81.612917^{0}E	28.850586^{0}N	Positive	1
3.	81.598149^{0}E	28.804898^{0}N	Negative	0
4.	81.613317^{0}E	28.846461^{0}N	Positive	1
5.	81.655959^{0}E	28.812627^{0}N	Negative	0
6.	81.61462^{0}E	28.846186^{0}N	Positive	2
7.	81.614265^{0}E	28.845541^{0}N	Negative	0
8.	81.615033^{0}E	28.844359^{0}N	Negative	0
9.	81.616106^{0}E	28.843867^{0}N	Negative	0
10.	81.611953^{0}E	28.8506^{0}N	Negative	0
11.	81.612099^{0}E	28.850064^{0}N	Negative	0
12.	81.612886^{0}E	28.850764^{0}N	Negative	0
13.	81.605121^{0}E	28.859388^{0}N	Positive	1
14.	81.60254^{0}E	28.858584^{0}N	Positive	1
15.	81.602311^{0}E	28.857454^{0}N	Negative	0
16.	81.614896^{0}E	28.853323^{0}N	Negative	0
17.	81.61344^{0}E	28.852965^{0}N	Negative	0
18.	81.605449^{0}E	28.857613^{0}N	Negative	0
19.	81.604649^{0}E	28.860854^{0}N	Negative	0
20.	81.613803^{0}E	28.811749^{0}N	Negative	0
21.	81.626369^{0}E	28.841401^{0}N	Negative	0
22.	81.591993^{0}E	28.850323^{0}N	Positive	1
23.	81.615774^{0}E	28.854986^{0}N	Positive	1
24.	81.594433^{0}E	28.851122^{0}N	Negative	0

S.No.	Longitude	Latitude	Starch-iodine test	Disease Scale
25.	81.62839^{0}E	28.841436^{0}N	Negative	0
26.	81.62136^{0}E	28.814662^{0}N	Negative	0
27.	81.618039^{0}E	28.854979^{0}N	Negative	0
28.	81.614168^{0}E	28.861535^{0}N	Negative	0
29.	81.607863^{0}E	28.864925^{0}N	Negative	0
30.	81.605558^{0}E	28.854821^{0}N	Negative	0
31.	81.610642^{0}E	28.849538^{0}N	Negative	0
32.	81.66585^{0}E	28.805139^{0}N	Positive	2
33.	81.665883^{0}E	28.805133^{0}N	Positive	1
34.	81.662113^{0}E	28.803311^{0}N	Positive	1
35.	81.66263^{0}E	28.816226^{0}N	Negative	0
36.	81.660704^{0}E	28.815002^{0}N	Negative	0
37.	81.660282^{0}E	28.802015^{0}N	Negative	0
38.	81.663744^{0}E	28.804324^{0}N	Negative	0
39.	81.658775^{0}E	28.803016^{0}N	Negative	0
40.	81.656251^{0}E	28.805401^{0}N	Negative	0
41.	81.609741^{0}E	28.854811^{0}N	Positive	2
42.	81.609722^{0}E	28.8553^{0}N	Positive	2
43.	81.610074^{0}E	28.8549^{0}N	Negative	0
44.	81.610209^{0}E	28.855317^{0}N	Negative	0
45.	81.608003^{0}E	28.860244^{0}N	Negative	0
46.	81.609026^{0}E	28.858901^{0}N	Negative	0
47.	81.610508^{0}E	28.854921^{0}N	Positive	1
48.	81.619747^{0}E	28.818331^{0}N	Negative	0
49.	81.605227^{0}E	28.856151^{0}N	Positive	2
50.	81.61997^{0}E	28.820367^{0}N	Negative	0
51.	$81.631734E^{0}$E	28.825602^{0}N	Negative	0
52.	$81.629223E^{0}$E	28.825057^{0}N	Positive	1
53.	81.594994^{0}E	28.852205^{0}N	Negative	0
54.	$81.652773\ ^{0}$E	28.842962^{0}N	Positive	1
55.	$81.656433\ ^{0}$E	$28.84563\ ^{0}$N	Negative	0
56.	81.621324^{0}E	28.814663^{0}N	Negative	0
57.	81.624318^{0}E	28.815337^{0}N	Negative	0

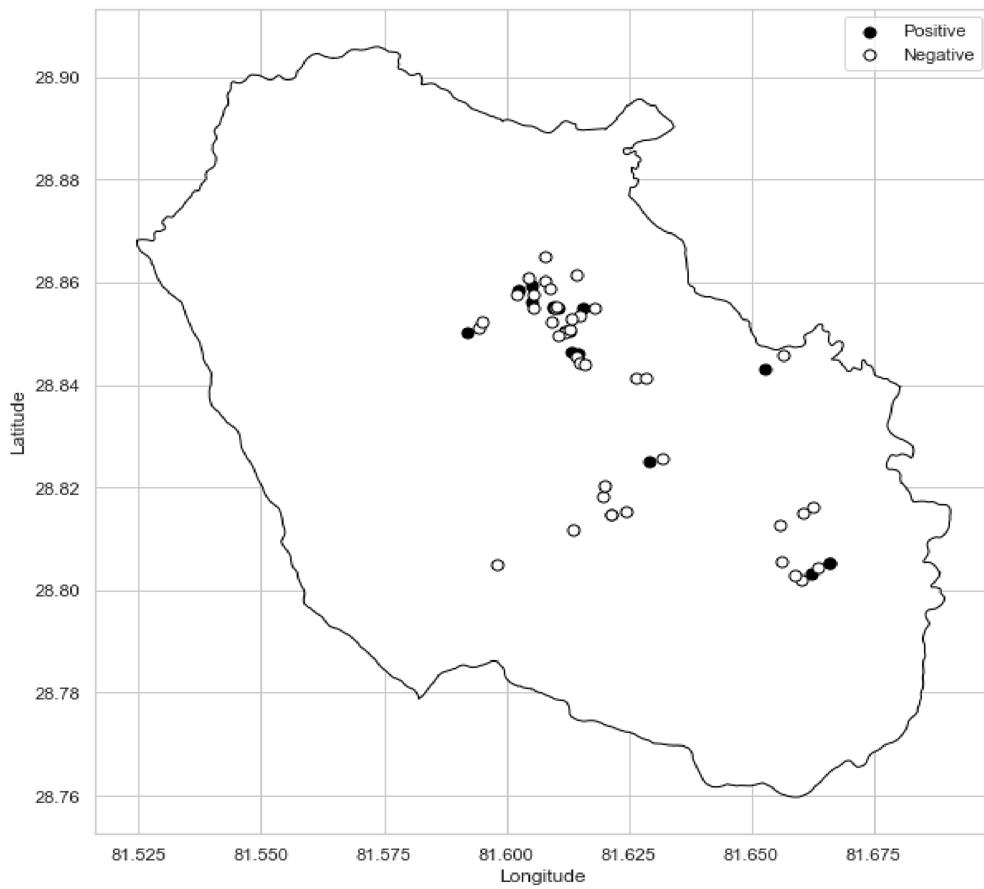

Fig. 3. Prevalence of Citrus greening in Citrus orchards of the study area

Figure 3 shows the map of the Dailekh district with all 57 Mandarin orchards. The iodine-based starch test was performed by a scratch method for the detection of Citrus greening disease, of which 16 showed a positive reaction, denoted by a black dot, whereas 41 showed a negative reaction, denoted by white dots in the map. The disease prevalence percentage was found to be 28.07 %, whereas disease severity was found to be 0.184.

The first report of Citrus greening disease in Nepal was from Pokhara Valley in 1968 (Pokhrel et al., 2021). It might have entered Nepal along with the planting materials introduced to the Horticultural Research Station from India (Knorr and Shah, 1971). Later, more surveys and studies were carried out in other parts of the country to explore the distribution of greening disease and its vector. The PCR test showed that HLB was found to be widespread in many citrus pockets of Kaski, Syangja, Kathmandu, Tanahun, Lamjung, Dhankuta, and Myagdi districts (Lama et al., 1988; Pokhrel et al., 2021; Poudel et al., 2022). Chhetri et al. (2019) reported the HLB disease from the Salyan district of Nepal based on the PCR diagnosis. This study showed the presence of HLB in the Dailekh district, indicating the quick spread of HLB disease towards the western region of Nepal.

Disease Severity

The severity of Citrus greening disease in Mandarin was affected by the application of the pesticides. After two months of spraying, the lowest disease severity (1.00) was observed on the plots treated with (Imidacloprid 0.02% + Streptocycline 250 ppm), (Imidacloprid 0.02%+Copper Oxychloride 0.025%), and (*Bacillus thuringiensis* 2 ml/l + *Pseudomonas* 5 ml/l), whereas the highest disease severity was found on the negative control plots. The greater severity in Neem oil-treated plots was due to the early infestation, which was checked at later stages. This suggests the potential of Neem oil-based formulations in controlling citrus greening.

There was no significant difference between treatments at other times of observation (except the second month of spray), most likely due to the timing of the pathogen attack (which is most severe during winter). The pre-monsoon rain in May might have affected the disease severity score of the third month. Similarly, lack of significance in the disease severity in the early months (i.e., March and April) could be due to the new spring flushes (young leaves), which make scoring of the disease difficult.

Though the severity of Citrus greening begins from September through March, the vector of the disease, i.e., the Asian citrus psyllid, shows an increasing population from March through mid-July (Neupane et al., 2024). This could be the reason for the quick spread of the disease in the third month of study (as noted in control plots).

The weak disease progression in the control suggests low disease pressure in general, leading to variable results. However, the significant difference between treatments during the second month shows that there was a quick rise in disease pressure during May. This indicates that frequent treatments and management strategies could be effective to combat the citrus greening disease between May and June.

Table 3. Efficacy of different chemicals and biopesticides against citrus greening in Mandarin

Treatments	Disease Severity Scale			
	Severity scale before spray	Severity scale after 1 month of 1st spray	Severity scale after 1 month of 2nd spray	Severity scale after 1 month of 3rd spray
T1 (Imidacloprid 0.02% + Streptocycline 250 ppm)	1.50	1.50	1.00[b]	1.00
T2 (Imidacloprid 0.02% + Copper Oxychloride 0.025%)	1.00	1.00	1.00[b]	0.83
T3 (Neem oil 5 ml/ l + Lentinan 2 ml/ l)	2.00	1.83	1.67[a]	1.33
T4 (Neem oil 5 ml/ l +*Pseudomonas* 5 ml/ l)	2.33	2.16	1.50[ab]	1.13
T5 (Neem oil 5 ml/ l + *Trichoderma* 5 ml/ l)	2.00	2.00	1.75[a]	1.25
T6 (Neem oil 5 ml/ l + *Bacillus amyloliquefaciens5 ml*/ l)	1.83	1.67	1.17[ab]	0.83
T7 (*Bacillus thuringiensis* 2 ml/ l + *Pseudomonas* 5 ml/ l)	1.83	1.83	1.00[b]	1.00
T8 (*Bacillus thuringiensis* 2 ml/ l + *Trichoderma* 5 ml/ l)	1.16	1.17	1.17[ab]	1.17
T9 (*Bacillus thuringiensis* 2 ml/ l + *Bacillus amyloliquefaciens* 5 ml/ l)	1.50	1.33	1.17[ab]	0.83
T10 (Control)	1.67	1.67	1.67[a]	1.83
LSD (0.05):	NS	NS	0.56*	NS
SEM (±):	0.09	0.09	0.05	0.09
CV (%):	32.13	31.26	24.69	43.97
Grand Mean:	1.68	1.61	1.30	1.14

Note: NS= non-significant, and * represents significant at 5 % level of significance. Treatment means followed by a common letter(s) within a column are not significantly different from each other. The first data recording was made in the last week of March 2021, whereas the final recording was done in the last week of June 2021.

In the table 3, the greater value for the coefficient of variance is likely due to the differential disease pressure. The variable weather conditions, as discussed by

Antolinez et al. (2022), could lead to uneven dispersal of pests and pathogens, which is seen in the first and third months after the spray. Higher precipitation rate, warmer temperatures, and humid conditions, as indicated by meteorological data (Figure 3), could have led to dispersal of the pathogen in the treated orchards. The lesser efficacy of the biological control agent could be due to short-term observation (three months).

The graph below (Figure 4) shows the percentage reduction of the severity index with different treatments over the control. The highest percentage reduction over control was found in the plot treated with (Imidacloprid + Copper oxychloride), (Neem oil + *Bacillus amyloliquefaciens*), and (*Bacillus thuringiensis* + *Bacillus amyloliquefaciens*), followed by the plots treated with (Imidacloprid + Streptocycline) and (*Bacillus thuringiensis* + *Pseudomonas*).

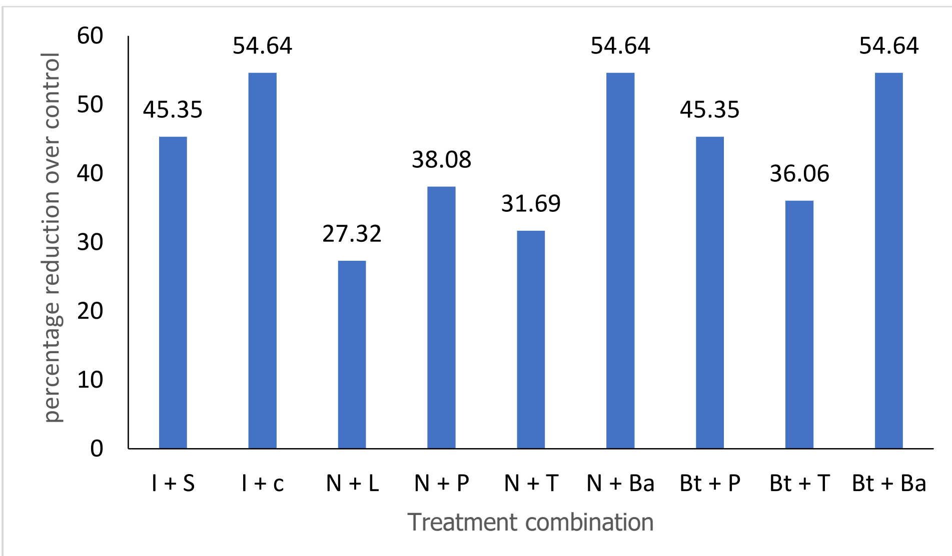

Fig. 4. Percentage reduction of the severity index by different combinations of treatments over the control

Note: I + S: Imidacloprid + Streptocycline., I + C: Imidacloprid + Copper oxychloride., N + L: Neem oil + *Pseudomaonas.*, N + P: Neem oil + *Pseudomonas.*, N + T: Neem oil + *Trichoderma.*, N + Ba: Neem oil + *Bacillus amyloliquefaciens.*, Bt + P: *Bacillus thuringiensis* + *Pseudomonas.*, Bt + T: *Bacillus thuringiensis* + *Trichoderma.*, Bt + Ba: *Bacillus thuringiensis* + *Bacillus amyloliquefaciens*

Imidacloprid acts on the nervous system of insects by attaching to the acetylcholine binding sites and preventing the transmission of information, leading to impairment of the nervous system and eventual death (Amirzade et al., 2014). The foliar application of Imidacloprid was effective in controlling 50 to 90% of the ACP adults, as shown by Ichinose et al. (2010). Entomopathogenic bacteria like *Bacillus thuringiensis* (Bt) have been explored as a potential management tool for ACP, as it results in high mortality of third instars of *D. citri* (Dorta et al., 2020). Fernandez-luna et al., (2019) reported the pesticidal properties of certain proteins derived from *B. thuringiensis* against ACP. Bt lyses the midgut epithelial cells and forms pores in the membrane, leading to the eventual death of the insect larva (Bravo et al., 2007). Earlier studies like these are similar to our current observations. Azadirachtin present in the Neem oils has an insecticidal activity and acts as a feeding inhibitor. It delays the development and growth of larvae and reduces the fecundity and fertility of many insects (Zanuncio et al., 2016). The application of Neem oil might have controlled the population of HLB and consequently the HLB severity.

Similarly, the secondary metabolites produced from *Pseudomonas* were reported to be effective in controlling several plant diseases, including HLB in Citrus (Pistori et al., 2018). Antibiotics like streptomycin were found to be effective against *Candidatus liberibacter asiaticus* (*C*Las) (Zhang et al., 2014). The findings of this study differed from those of Zhang et al. (2011), who reported greater suppression of *C*Las by streptocycline as compared to biopesticides. Further studies are necessary to confirm these results and to explore the new management strategies for HLB disease.

Conclusion and recommendations

The combinations of (Imidacloprid + Copper oxychloride), and (*Bacillus thuringiensis* + *Bacillus amyloliquefaciens*) were effective against HLB disease in general. These treatments showed the greatest reduction in severity percentage. The findings demonstrate the capabilities of biocontrol agents and biopesticides in managing HLB disease. Farmers should be more aware during May and June to lessen the spread of the disease. Frequent spray and orchard management should be done to protect the trees from the devastating pathogen. In order to effectively tackle vector-borne bacterial disease, it is crucial to select the appropriate combination and optimal dosage of insecticides and bactericides. Further studies are necessary to explore the potential of biocontrol, botanicals, and their consortia in controlling HLB disease. Other chemical combinations should be tested that could suppress the HLB pathogen and its vector under diverse environmental conditions using different citrus varieties. Constant surveys and surveillance on disease prevalence are necessary to detect the disease in important citrus-growing regions of the country. Quick management techniques must be deployed following the detection to control the spread of the pathogens.

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