Indian Journal of Ecology (2025) 52(2): 394-398
DOI: https://doi.org/10.55362/IJE/2025/4509

Manuscript Number: 4509
NAAS Rating: 5.38

Prevalence of Citrus Greening Disease and Its Integrated Management under Punjab Conditions

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***Abstract*:** Citrus greening or Huanglongbing (HLB) is the most destructive disease limiting the quality of fruit production in India associated with *Candidatus* Liberibacter asiaticus in Asia. The disease produces typical symptoms of leaf mottling, yellowing and green islands on leaves of citrus plants. During the surveillance (2019-2021), the disease was present in various citrus growing areas of Punjab viz., Hoshiarpur, Fazilka, Faridkot and Ludhiana with incidence varied from 7.99 percent to 16.60 percent in the districts of Faridkot and Hoshiarpur, respectively. The disease index (PDI) was highest in Ludhiana and lowest in Faridkot. Disease management generally depends on the early detection of symptoms and chemical control of insect vector. Ten treatments were selected which includes the different combinations of nutrients, antibiotics, growth regulator and insecticide. Among the different treatments, zinc sulphate + manganese sulphate + boric acid, tetracycline hydrochloride and 2,4-dichlorophenoxyacetic acid (2,4-D sodium salt) was proved significantly superior to other treatments in reducing the percent disease index (13.99) and providing 59.29 percent disease control with significant increase in yield (54.87 kg/tree) as compared to control (38.32 kg/tree).

***Keywords*:** Citrus greening, *Candidatus* Liberibacter asiaticus, Prevalence, Integrated management, Chemicals, Antibiotics

Citrus, a member of family Rutaceae, is grown worldwide including India and occupies a place of prime importance among major fruits in the country. In India, citrus is the third largest fruit crop after mango and banana with estimated production of 1,40,71 thousand MT from a cultivated area of 1064 thousand ha (Anonymous 2023). The important commercial citrus fruits in India are mandarins (*Citrus reticulata* Blanco), sweet orange (*C. sinensis* Osbeck) and acid lime (*C. aurantifolia* Swingle) (Jagtap et al., 2013). Punjab is the largest producer of citrus especially Kinnow with 47,862 ha area and production of 12,84,211 MT (Anonymous 2024). Kinnow is mostly grown in five districts of Punjab viz., Fazilka, Hoshiarpur, Mansa, Muktsar and Bathinda which contribute for 88% of total Kinnow production in Punjab. There are number of biotic and abiotic factors that are responsible for low productivity and widespread problem of citrus decline in India. Among them, Huanglongbing (HLB) or citrus greening (CG) has proven to be the most problematic due to the fastidious nature of its non-culturable Gram-negative bacteria *Candidatus* Liberibacter (Jagoueix et al., 1994). Due to the long latency period, uneven distribution of pathogen and confusing disease symptoms produced in the host, the pathosystem becomes even more complicated (Manjunath et al., 2008). Citrus greening disease can cause nutrient deficiency in citrus plants which affects the plant canopy and finally the yield of plant. Keeping in view the importance and devastating potential of the disease, the present investigation was undertaken to study the prevalence and integrated management of the disease under Punjab conditions.

MATERIAL AND METHODS

Prevalence of greening in citrus orchards of Punjab: The prevalence of citrus greening disease was observed in the citrus growing districts of Punjab namely Hoshiarpur, Fazilka, Faridkot and Ludhiana during the year 2019-2021. The surveys were conducted during the months of September-November which are considered optimum for the development of characteristic symptoms of the disease. Fifty trees of the cultivar Kinnow mandarin were randomly selected per site in each district. The canopy of the tree from each geographical direction was observed for greening symptoms. The Percent Disease Index (PDI) was recorded using 0-4 disease rating scale given by Gottwald et al., (2007) (Table 1). PDI and Percent disease incidence were calculated using the following formula:

$$PDI = \frac{\text{Sum of individual ratings}}{\text{Number of plants examined} \times \text{Maximum disease rating}} \times 100$$

$$\text{Per cent disease incidence} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

Integrated management of greening disease under field conditions: Ten treatments of different nutrients, antibiotics,

growth regulators and insecticide were selected in different combinations to evaluate their efficacy in managing the disease in a Randomized Block Design (RBD) at Punjab Agricultural University, Ludhiana during the year 2020-2022 on the cultivar Kinnow (Table 2). Ten treatments were kept and each treatment was replicated thrice. The treatments, T_1 (zinc sulphate + manganese sulphate), T_2 (boric acid), T_3 (T_1+T_2) and T_6 (2,4-dichlorophenoxyacetic acid (2,4-D) sodium salt) were applied as foliar spray during the end of April and mid of August. Two sprays of treatments T_4 (tetracycline hydrochloride) and T_5 (streptocycline) were given during August-September at 45 days interval. Recommended insecticide T_9 (imidacloprid) was sprayed in each treatment during March and again in first week of September. PDI based on the visual symptoms was recorded and percent disease control was calculated. The yield of citrus trees per treatment was also recorded. Canopy volume was calculated by using the following formula:

Canopy Volume = 0.5236 × H × (N-S) × (E-W) (William 1983)

where, H= Height of tree, N-S and E-W= Spread of plant in North-South and East-West directions

RESULTS AND DISCUSSION

Disease survey: During the surveillance, the trees showing typical greening symptoms viz., leaf mottling, yellowing and green islands were rated as infected (Fig. 1). Different locations of Punjab were surveyed to record the prevalence, incidence and PDI and it was observed that the disease was widespread in different locations of Punjab (Table 3 and 4). Maximum mean disease prevalence (33.33%) was in Ludhiana district while minimum was in Faridkot (19.31%). However, number of sites in Ludhiana (9) was comparatively lesser than the two major citrus growing areas of Hoshiarpur (33) and Fazilka (36). In the current assessment, the disease was present at more than thirty percent citrus orchards i.e. 32.60 % and 33.33% at Hoshiarpur and Ludhiana, respectively.

The disease incidence and PDI were further recorded during all three years (2019 to 2021) in these four districts of Punjab (Table 4). The maximum mean disease incidence (16.60 %) was in Hoshiarpur district in each year followed by Ludhiana (15.37%) and Fazilka (9.21%) whereas; the PDI was at par in Ludhiana district (11.98%) as well as Hoshiarpur district (11.49%). The minimum disease incidence of 7.98% and PDI of 8.76% was in Faridkot.

These findings are in conformity with those of Das (2008) who observed 30-40 percent disease incidence in Malta Sweet orange and 3-15 percent in Kinnow mandarin from

Table 1. Disease score used for assessment of PDI given by Gottwald et al (2007)

Disease score	Disease rating	Description
0	No symptoms	No greening symptoms observed on the plant canopy
1	Mild	Greening symptoms up to 25% of the canopy
2	Moderate	26-50% canopy symptoms
3	Severe	51-75% canopy symptoms
4	Very severe	76-100% canopy symptoms

Table 3. Prevalence of citrus greening in different districts of Punjab on the basis of visual symptoms during 2019-2021

District	Prevalence (%)			Mean
	2019	2020	2021	
Fazilka (36)	25.00	27.77	30.00	27.59
Faridkot (30)	16.66	18.18	23.08	19.31
Hoshiarpur (33)	28.57	30.77	38.46	32.60
Ludhiana (9)	33.33	33.33	33.33	33.33
Total (108)/Mean	25.89	27.51	31.22	28.21

Figure in parenthesis include number of sites visited in each district

Table 2. Chemicals evaluated for management of citrus greening disease

Treatment	Description	Time of application
T_1	Zinc sulphate (4.7 g/litre) + Manganese sulphate (3.3g/litre)	April and mid August
T_2	Boric acid (0.05 g/litre)	April and mid August
T_3	T_1 + T_2	April and mid August
T_4	Tetracycline hydrochloride (0.6g/litre)	August-September
T_5	Streptocycline (0.025g/litre)	August-September
T_6	2,4-Dichlorophenoxyacetic acid (2,4-D sodium salt) (0.01g/litre)	April and mid August
T_7	T3+T4+T6	April and mid; August August-September
T_8	T3+T5+T6	April and mid August; August-September
T_9	Imidacloprid (17.8 SL) (0.4ml/litre)	March and September
T_{10}	Control	-

Punjab. The incidence varied from 12.5-30 percent in Sikkim mandarin, 10-20 percent in Darjeeling mandarin, 5-8.3 percent in Khasi mandarin and 12.5 percent on Sweet orange cultivars in Northeastern region of India (Das et al., 2007). Gupta et al. (2012) observed 40 percent of disease incidence in Kinnow mandarin at Hoshiarpur district of Punjab. Ghosh et al. (2013) also observed greening like symptoms among Cleopatra, Musambi, Rangpur Lime, Acid Lime and Rough Lemon and reported incidence between 8.2-41.5 percent in Ahmednagar and 7.8-28.3 percent in Pune districts of Maharastra. Sonavane and Venkataravanappa (2017) observed the incidence of 35-64.2 percent of greening disease in Coorg mandarin in Kodagu and Hassan districts of Karnataka. Similarly, Arora (2017) reported that citrus greening was more prevalent in the Central and Sub-mountainous regions, particularly on older trees.

Integrated management of greening disease: The treatment T_7 (zinc suphate + manganese sulphate + boric acid+tetracycline hydrochloride and 2,4-

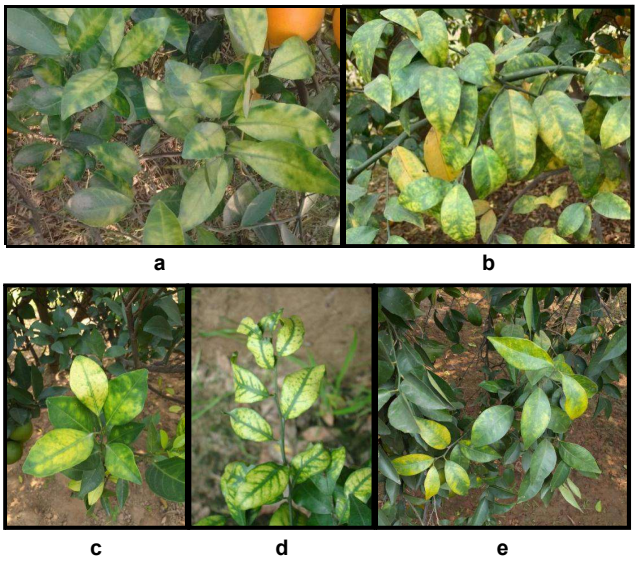

Fig. 1. Leaf mottling (a, b), green island (c, d, e) type of symptoms on citrus variety Kinnow naturally infected with citrus greening bacterium

Table 4. Disease incidence and percent disease index of citrus greening in different districts of Punjab during 2019-2021

Districts	2019		2020		2021		Mean disease incidence (%)	Mean PDI (%)
	Disease incidence (%)	PDI (%)	Disease incidence (%)	PDI (%)	Disease incidence (%)	PDI (%)		
Fazilka (36)	9.09	7.72	9.17	8.69	9.37	11.63	9.21	9.35
Faridkot (30)	7.14	7.15	8.00	8.33	8.82	10.81	7.98	8.76
Hoshiarpur (33)	15.38	9.40	16.66	12.06	17.77	13.01	16.60	11.49
Ludhiana (9)	13.33	11.07	15.83	11.29	16.66	13.58	15.27	11.98
Mean	11.24	8.84	12.41	10.09	13.15	12.26		

Figure in parenthesis include number of sites vis ited in each district

Table 5. Effect of different treatments on percent index of greening disease in Kinnow mandarin during 2020-21 at PAU, Ludhiana

Treatment	Percent disease index				Mean percent disease index		Disease control (%)
	2020		2021		Before treatment	After treatment	
	Before treatment	After treatment	Before treatment	After treatment			
T1	22.8	20.3	23.7	21.9	23.24	21.12	38.55
T2	23.1	22.1	23.9	21.0	23.50	21.53	37.36
T3	25.8	19.7	21.5	18.3	23.65	18.96	44.83
T4	24.1	22.7	24.0	21.0	24.07	21.86	36.40
T5	22.5	22.0	24.3	22.7	23.41	22.30	35.12
T6	24.6	23.2	25.0	24.2	24.79	23.67	31.13
T7	23.4	15.6	16.9	12.4	20.15	13.99	59.29
T8	25.0	18.7	19.1	16.3	22.02	17.49	49.11
T9	22.7	21.9	24.5	22.9	23.57	22.40	34.82
T10	25.6	30.5	34.0	38.9	29.80	34.37	-
CD (p=0.05)	1.27	1.78	1.24	1.70	1.26	1.74	

Table 6. Effect of different treatments on canopy volume and fruit yield in Kinnow mandarin during 2020-21 at PAU, Ludhiana

Treatment	Canopy volume (m^3)		Mean canopy volume (m^3)	Fruit yield (kg/tree)		Mean fruit yield (kg/tree)
	2020	2021		2020	2021	
T1	22.3	23.1	22.70	43.74	46.24	44.99
T2	21.3	23.8	22.55	42.48	46.10	44.29
T3	22.9	24.7	23.82	47.74	51.86	49.80
T4	21.8	22.5	22.15	43.67	45.50	44.58
T5	21.9	22.5	22.20	43.10	45.27	44.18
T6	22.0	23.5	22.75	43.36	45.62	44.49
T7	23.9	27.3	25.60	53.25	56.50	54.87
T8	23.4	25.0	24.20	51.75	55.00	53.37
T9	20.5	22.4	21.45	42.50	43.83	43.16
T10	21.2	21.0	21.10	40.37	36.28	38.32
CD (p= 0.05)	0.04	1.49	0.77	3.29	2.63	2.96

dichlorophenoxyacetic acid (2,4-D sodium salt) proved significantly superior to other treatments by reducing the percent disease index (13.99) (Table 5). The percent disease control was highest with T_7 (59.29) treatment followed by T_8 (49.11) and T_3 (44.83). The maximum canopy volume was also highest in T_7 (25.57 m^3). Foliar spray of 2,4-dichlorophenoxyacetic acid (2,4-D sodium salt) (T_6) was, however, the least effective. The plants sprayed with treatment T_7 yielded 54.87 kg/ tree followed by treatment T_8 with 53.37 kg/tree (Table 6). Zhang et al. (2011) also reported that the combination of penicillin and streptomycin (PS) was effective in suppressing the Las bacterium but due to the short term effect of antibiotics, it is not considered as sustainable approach in HLB control (Bove 2006).

Foliar spray of systemic insecticides such as imidacloprid, fenpropathrin, chlorpyrifos and dimethoate is an effective approach to manage citrus psylla (Tiwari et al., 2011). Arora et al., (2020) also reported that the best management programme for citrus greening is integrated approach including use of disease free nursery plants, management of zinc and manganese deficiency and citrus psylla vector. Integrated management approaches consisting of use of disease free nursery planting material, foliar spray of tetracycline hydrochloride, application of recommended doses of both micro and macro nutrients and foliar spray of insecticides at the time of new flush emergence can help in reducing losses due to citrus greening disease, psylla vector and prolonging lifespan of citrus (Anonymous 2022).

CONCLUSION

From survey of the citrus orchards during the three consecutive years, it was evident that the maximum greening disease incidence was in Hoshiarpur district in each year followed by Ludhiana and Fazilka. The intensity of citrus greening disease can be reduced by foliar spray of micro nutrients like zinc sulphate, manganese sulphate, boric acid, growth regulator 2,4-D sodium salt in the months of April and mid August and two sprays of tetracycline hydrochloride at an interval of 45 days during the months of August-September. Application of micronutrients reduces the nutrient deficiency symptoms caused by citrus greening disease and regain the vigor of plant whereas antibiotics slow down the progress of disease in the plant.

AUTHOR'S CONTRIBUTION

Conceptualization of research work and designing of experiments (AA, AK, MSH); Survey for disease incidence (SK, AA, AK MG); Execution of field experiments and data collection (SK, AA, AK, MSH); Analysis of data and interpretation (AA, SK, MSH, GSS); Preparation of manuscript (AA, AK, SK).

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Received 14 January, 2025; Accepted 28 March, 2025