CITRUS CANKER DISEASE: STATUS AND SEVERITY IN DIFFERENT GENOTYPES

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SUMMARY

Xanthomonas citri sub sp*. citri* (*Xcc*) causes citrus bacterial canker (CBC), a highly destructive disease of citrus plants globally. A comprehensive survey commenced in seven locations of District Sargodha, Pakistan, to determine the incidence of CBC on Kinnow, Feutrell's early (*Citrus reticulata* Blanco), and Musambi (*Citrus sinensis*) cultivars. Additionally, 47 citrus cultivars reached assessment for their response to *Xcc* for disease severity. The highest incidence of CBC emerged in Kinnow at Shahpur (57.3%), Bhera (40.5%), and Sargodha (35.0%). Feutrell's early showed a higher incidence rate at Shahpur (35.0%), Bhalwal (35.0%), and Sargodha (30.0%). Meanwhile, Musambi plants exhibited an incidence of 15.0% at Shahpur and Bhera and 13.3% at Bhalwal. Among the 47 cultivars, Jaffa, Valencia Late, Sanguinello, and Musambi (*Citrus sinensis*) demonstrated resistance to CBC, as their severity levels were <4.0%. Tracco N (20.3%) and rough lemon (22.0%) proved susceptible cultivars. However, a high susceptibility to canker disease was notable in Shamber grapefruit (31.0%) and Chakotra (Pommelo) (34.6%). Understanding the susceptibility of diverse citrus cultivars to CBC provides valuable insights for breeding programs and integrated management of the CBC disease.

Keywords: Citrus cultivars, disease management, incidence, *Xanthomonas citri*

Key findings: In the District of Sargodha, Pakistan, Kinnow exhibited the highest incidence of CBC, notably at Shahpur, Bhera, and Sargodha. Feutrell's early displayed increased incidence rates at Shahpur, Bhalwal, and Sargodha, while Musambi plants showed a lower incidence of CBC. Among the 47 evaluated cultivars, Jaffa, Valencia Late, Sanginello, and Musambi showed resistance to CBC, with disease severity levels below 4.0%. Conversely, Tracco N and Rough lemon were markedly susceptible cultivars. Higher susceptibility was evident in Shamber grapefruit and Chakotra (Pommelo).

INTRODUCTION

Invasive and emerging plant pathogens and insect pests threaten food security and crop sustainability (Vurro *et al*., 2010). One such perilous threat to the citrus industry is the citrus bacterial canker (CBC), an immensely damaging disease affecting citrus plants caused by *Xanthomonas citri* sub sp. c*itri* (*Xcc*), which is an aerobic, gram-negative, and rod-shaped bacterium (Jalan *et al*., 2013). CBC originated in Southeast Asia or India in the 1800s, with a later introduction to almost all citrus-growing countries, primarily spreading during the transportation of citrus germplasm (Singh *et al*., 2014).

All above-ground parts of citrus plants, such as leaves, twigs, young stems, and fruits, are susceptible to CBC disease. Initially, small blister-like lesions appear on leaves, gradually transitioning from tan to light tan and finally taking on a brown hue with water-soaked borders and yellow halos (Stover *et al*., 2021). As lesions mature, the halos vanish, leaving behind flat, necrotic lesions with dark purplish margins. Similar symptoms manifest on fruits and twigs, displaying a corky appearance with water-soaked margins on both; however, chlorosis often surrounds lesions on fruits, but those on twigs are not (Zhang and Meng, 2011). Advanced stages of the disease can result in twigs dieback and fruit dropping (Das, 2003; Ali *et al.,* 2023). Optimal disease development occurs within a temperature range of 25 °C–35 °C, while temperature below 20 °C or exceeding 40 °C has detrimental impacts on the disease progression (DallaPria *et al*., 2006).

Three different pathotypes (A, B, and C) of *Xcc* have shown distinction (Fonseca *et al*., 2019). Type-A canker, also known as ACC (Asiatic citrus canker), is widely spread and has a broader host range (Bansal *et al.,* 2020).

It is prevalent across various citrus-growing regions, including Asia, the USA, South America, and Oceania (Shahbaz *et al*., 2023). Type A infects major citrus cultivars, such as *Citrus reticulata*, *C. paradisi*, *C. sinensis,* and *C. aurantifolia* (da Silva *et al.,* 2002). Type-B canker is present in Argentina, Paraguay, and Uruguay. It displays a slower symptom development due to its sluggish growth. It has a limited host range, primarily infecting *C. limon*, *C. sinensis,* and *C. paradise* (Brunings and Gabriel, 2003). Type-C canker has confinements in Brazil and only infects *C. aurantifolia* (Ibrahim *et al*., 2019). Additionally, two variants of canker-A, A* and A^w, gained detection. The discovery of Type-A* first occurred in the 1990s in Southeast Asia, infecting *C. aurantifolia,* and later, emerging in Ethiopia, infecting Alemow (*C. macrophylla*), Tahiti lime (*C. latifolia*), and Mexican lime (*C. aurantifolia*) but not grapefruit (*C. paradisi*) (Sun *et al.,* 2004). The type-A^w variant of *Xcc-A* first appeared in the USA in 2003, infecting Alemow and Mexican lime (Patané *et al*., 2019).

The increase in the pathogen spread across different regions in the last century closely correlated to international trade (Bebber *et al*., 2014). CBC exhibits a wide range of hosts, including Mexican lime (*Citrus x aurantiifolia*), sour orange (*C. aurantium*), and alemow (*C. macrophylla*) (Sun *et al*., 2004). The impact of CBC disease varies based on the host and environment, and the spread of this pathogen is mostly due to transporting disease-nursery plants and extreme weather events (Vernière *et al*., 2014).

CBC poses a significant threat due to its high infectivity, leading to huge economic losses of up to USD 1 billion annually for low fruit quality and productivity, alongside the costs of managing this disease (Behlau *et al*., 2016). Citrus growers heavily rely on chemical

pesticides to combat citrus canker despite the recognized adverse effects on human health and the environment (Carvalho and Sette, 2015). Knowledge about the susceptibility or resistance level of various citrus cultivars could be helpful in a successful breeding program. Incorporating resistant or tolerant cultivars into disease management strategies could offer advantages, potentially reducing the cost of control measures (Pitino *et al.*, 2015). A comprehensive study on the status of CBC incidence and severity in Sargodha citrus was lacking. Therefore, this study aimed to determine CBC incidence on the three most grown cultivars, Kinnow, Feutrell's early, and Musambi in various locations of Sargodha, Punjab, Pakistan. Additionally, the objective was to assess the response of diverse citrus cultivars to CBC, aiding in identifying the different potential tolerant cultivars.

## MATERIALS AND METHODS

Study sites

Orchards from seven different locations—Silanwali, Sargodha, Kotmomin, Shahpur, Bhalwal, Bhera, and Sahiwal—selected from the district of Sargodha, Punjab, Pakistan, helped determine the incidence of citrus canker

disease (Figure 1). The total citrus-cultivated area in Pakistan is 183,149 hectares (GOP 2019–2020). During 2019–2020, three citrus cultivars belonging to the mandarin group, Kinnow, Feutrell's early, and Musambi, were options from each location. These cultivars hold significant economic value, with an extensive cultivation (about 90.6% of the total citrus growing area) compared with other citrus cultivars throughout the country. Ten orchards (each approximately 1 acre or 0.40 ha in size) were random choices for each cultivar per location. From each orchard, 20 plants of each cultivar became samples to record the data on disease incidence based on the observed symptoms (Figure 2). The age range of the selected plants was between 12–20 years.

Incidence of citrus canker disease

The recorded data on CBC incidence came from 20 plants, four from each corner and four from the center of the orchard to cover the whole field (Derso *et al.*, 2007). The disease incidence measurement in each orchard used the following formula (Honger *et al.*, 2016):

$$CBC\ Incidence\ (\%) = \frac{Number\ of\ Plants\ having\ disease\ symptoms}{Total\ number\ of\ Plants} \times 100$$
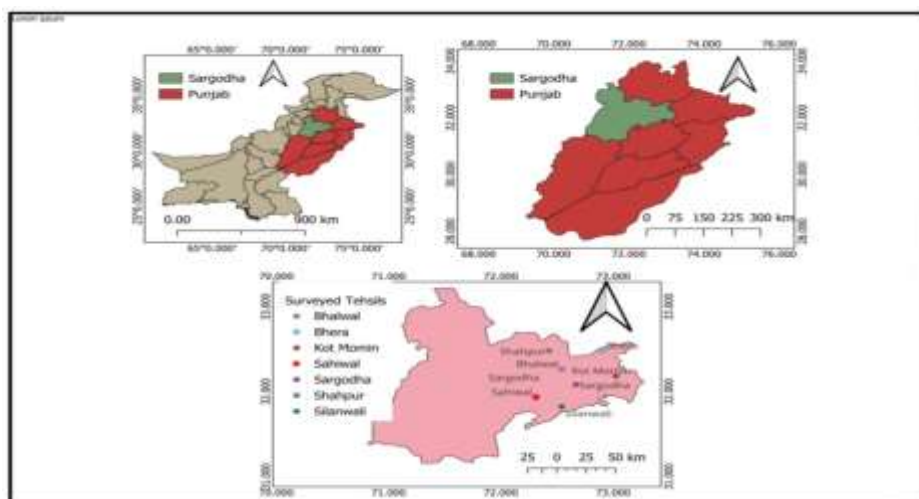
Figure 1. Map showing study sites, including seven different tehsils of Sargodha district of Punjab, Pakistan.

Figure 2. Healthy citrus leaves of Kinnow (Left) and infected leaves with CBC (Right).

Table 1. Rating scale used for CBC severity

Rating	Disease Severity %	Response of cultivar
0	00-00	Immune
1	01-05	Resistant
3	06-10	Moderately Resistant
5	11-15	Moderately Susceptible
7	16-25	Susceptible
9	>26	Highly Susceptible

Citrus screening against citrus canker

Recording the CBC severity transpired on 47 varied citrus cultivars at the Citrus Research Institute (CRI) in Sargodha, Pakistan. For each cultivar, randomly selecting five plants ensued, with 80 leaves from a single tree observed (20 from each side). The severity assessment of CBC used the rating scale (Table 1) by Fatima *et al*. (2019). The severity calculation of CBC employed the formula:

$$\text{Disease severity \%} = \frac{\text{Infected leaves}}{\text{Total no. of leaves}} \times 100$$

Data analysis

The disease-incidence data assessment used a one-way analysis of variance (ANOVA) by keeping locations as the main factor. However, cultivar consideration was the main factor for the disease severity data. Means separation engaged Tukey HSD all pairwise comparison tests at p = 0.05. All analyses ran in the SPSS 20.0 software (IBM, Armonk, NY).


RESULTS AND DISCUSSION

The disease incidence varied significantly (F = 4.23, $P < 0.001$) in Kinnow plants at different locations of Sargodha. The highest disease incidence in Kinnow mandarins was dominant in Shahpur (57.3%), followed by Bhera (40.5%) and Sargodha (35.0%). Meanwhile, the lowest disease incidence on Kinnow mandarins emerged in Silanwali (25.5%). Similarly, in Feutrell's early plants, a significant (F = 8.16, $P < 0.001$) difference in disease incidence was prevalent across different locations. Disease incidence appeared higher on Feutrell's early plants at Shahpur (35.0%), Bhalwal (35.0%), and Sargodha (30.0%). The lowest incidence of CBC resulted in Bhera (16.6%) and Sahiwal (05.0%) on Feutrell's early. Musambi orchard displayed a significant

(F = 2.85, *P* < 0.05) difference in CBC incidence across different locations of Sargodha. The CBC incidence was higher (15.0%) on Musambi plants at Shahpur and Bhera, followed by Bhalwal (13.3%). The lowest incidence showed in Silanwali (5.0%) and Kotmomin (3.3%) on Musambi plants (Figure 3).

Among various citrus cultivars, a significant (F = 5.50, *P* < 0.001) difference in CBC severity arose. Of the 47 citrus cultivars, four (Jaffa, Valencia late, Sanginello, and Musambi) proved resistant, exhibiting a severity of disease below 4.0%. About 29 cultivars demonstrated moderate resistance (6%–10%), while 10 were moderately susceptible (11%–15%). Tracco N (20.3%) and rough lemon (22.0%) manifested as susceptible cultivars for CBC. Shamber grapefruit and Chakotra (Pommelo) were highly susceptible cultivars to CBC, with 31.0% and 34.6% severity levels, respectively (Figure 4).

CBC is one of the most threatening diseases of citrus crops. Across the surveyed regions of the District Sargodha, Pakistan, the incidence of CBC showed variations in Kinnow, Feutrell's early, and Musambi plants. CBC incidence in Kinnow plants was dominant in all selected locations; however, the incidence was lower in Silanwali. In Feutrell's early orchards, CBC incidence was higher in Shahpur, Bhalwal, and Sargodha, and in Musambi orchards, the incidence was higher in Shahpur, Bhalwal, and Bhera locations. Kinnow orchards experienced a high incidence of CBC, followed by Feutrell's early and Musambi. These fluctuations in disease incidence could refer to environmental factors, notably, temperature and rainfall, which favor the rapid development and spread of *Xcc* (DallaPria *et al.*, 2006; Tahir*et al.*, 2023). Plant age and cultivar might be other factors involved in the sensitivity of canker disease; young citrus plants and leaves tend to be more susceptible to CBC (Canteros *et al.*, 2017; Fatonah *et al.*, 2018).
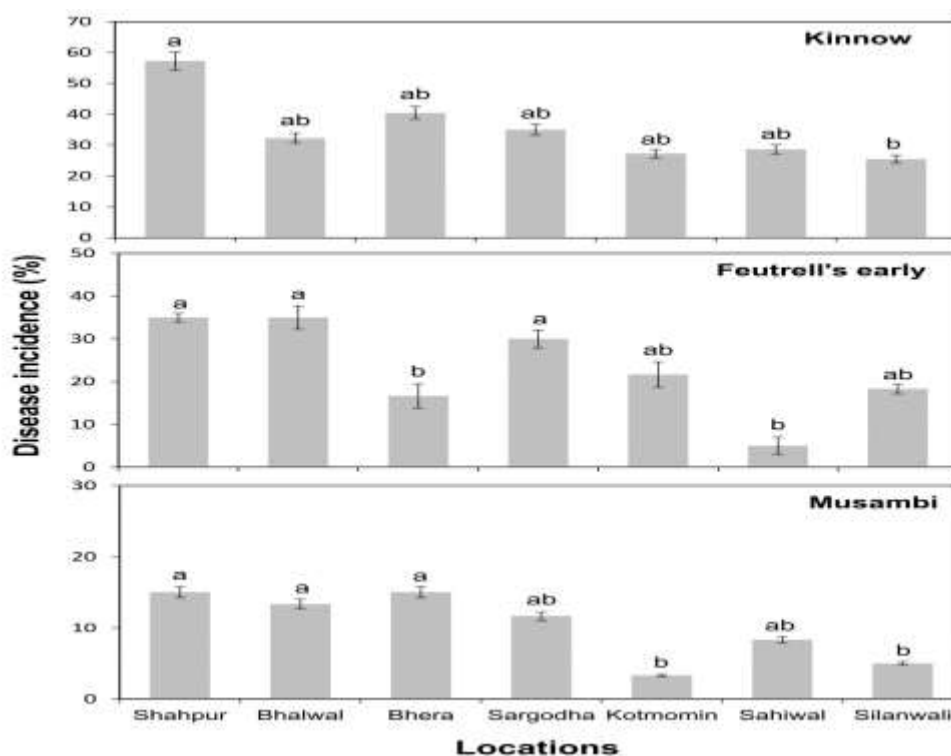
Figure 3. Disease incidence (%) of citrus canker on Kinnow, Feutrell's early, and Musambi orchards at different locations of district Sargodha; means sharing similar letters are not significantly (*P* > 0.05) different.
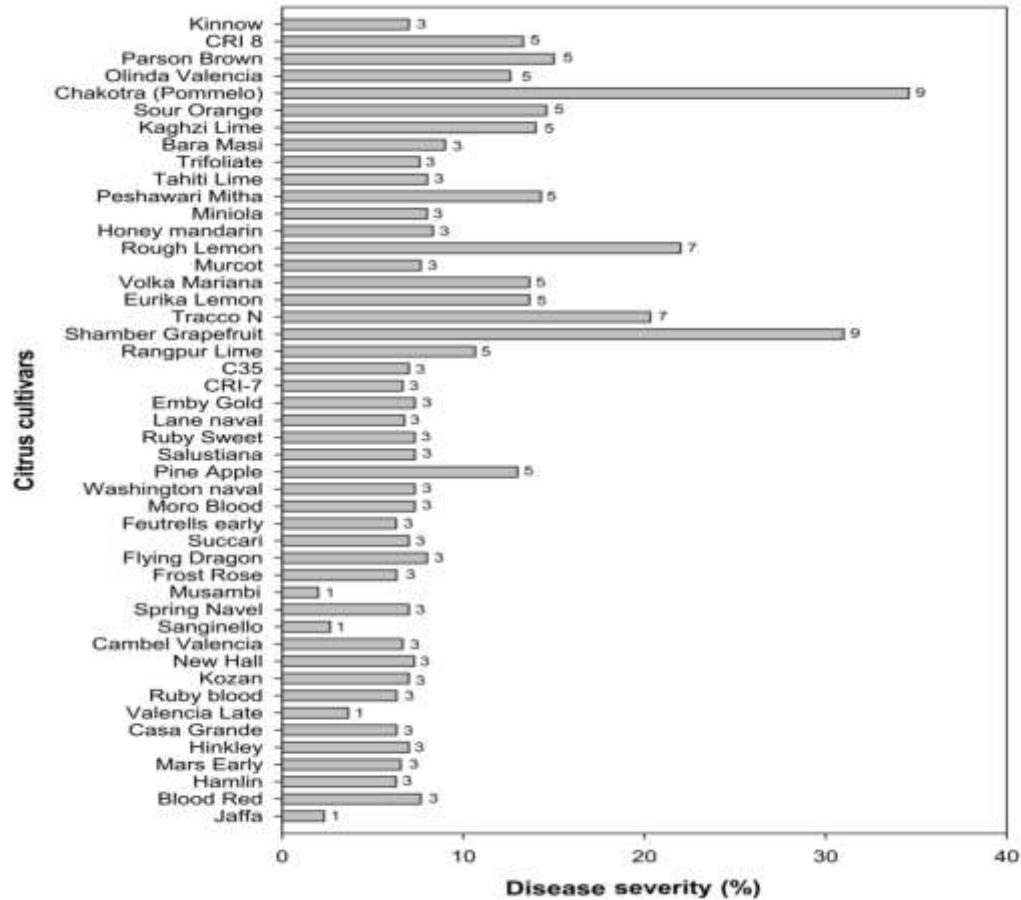
Figure 4. Disease severity (%) (with a rating scale) of citrus canker on various citrus cultivars; numbers on bars show the rating of cultivars in response to citrus canker disease, 1 = resistant, 3 = moderately resistant, 5 = moderately susceptible, 7 = susceptible, and 9 = highly susceptible.

In the presented study, the screening of 47 citrus cultivars assessed their susceptibility to CBC. Study findings showed Shamber, Grapefruit, and Chakotra Pommelo were highly susceptible to canker pathogens. Moreover, Tracco N and Rough lemon proved to be susceptible cultivars to CBC. Ten cultivars—Pine apple, Rangpur lime, Eureka lemon, Volkamariana, Peshawari mitha, Kaghzi lime, Sour orange, Olinda Valencia, Parson brown, and CRI 8—demonstrated moderate susceptibility to CBC. However, Jaffa, Valencia late, Sanginello, and Musambi emerged as resistant cultivars to the canker pathogen. Previously, Gottwald *et al*. (2002) reported Pera, Valencia, and Natal as moderately susceptible, and Hamlin as a susceptible cultivar to CBC. Burhan *et al*. (2007) screened

26 citrus cultivars against CBC and reported Jaffa and Tracco as resistant cultivars. Similarly, Abid *et al*. (2008) reported Jaffa as a resistant cultivar to CBC among 15 cultivars. Although lesions of CBC were prominent on all citrus cultivars, varying degrees of susceptibility showed. Previous studies highlighted grapefruit as highly susceptible, mandarins as moderately resistant, and sour and sweet oranges as less susceptible to CBC (Gottwald *et al*., 2002; Stover and McCollum, 2011).

The *Xanthomonas* pathogen can infect almost all parts of the plants, including leaves, stems, and fruits, resulting in defoliation, dieback, and premature fruit drop. The greater severity of the CBC seems to correlate with a higher number of lesions on the plants

(DallaPria *et al.*, 2006) and the effective establishment and spread of CBC occurs in the presence of free water and vulnerable citrus tissues, with disease dispersal often facilitated by wind. In the presence of free moisture, bacterial cells ooze from lesions initiating new infections through stomata or wounds/injuries caused by insects, or equipment (Schubert *et al.*, 2001). Spread of disease can also occur via contaminated equipment and individuals, while long dispersal occurs by infected plant materials across geographical regions (Schubert *et al.*, 2001; Al-Karboli and Al-Janabi, 2024).

The feeding behavior of insects like citrus leafminer (*Phyllocnistis citrella* Stainton) on new leaves enhances the severity of CBC (Gottwald *et al.*, 2007). The substantial economic damages caused by severe CBC on citrus cultivars in Sargodha remain a significant concern. Government authorities should implement effective measures to eradicate the disease and prevent its spread to other parts of the country. The ineffective control methods are leading to significant economic losses for the citrus industry. Therefore, a dire need to develop an effective and eco-friendly management strategy for CBC requires implementation.

CONCLUSIONS

The CBC disease has persisted in almost all citrus-growing areas of District Sargodha, Pakistan. This study provides valuable and reliable field-based information on the susceptibility of Kinnow and Feutrell's early. Based on the findings of this study, four cultivars (Musambi, Sangenillo, Jaffa, and Valencia late) proved as resistant to CBC. These cultivars hold potential in resistant breeding programs to develop new resilient cultivars against CBC.

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