



Available Online at EScience Press

# Journal of Plant and Environment

ISSN: 2710-1665 (Online), 2710-1657 (Print)

<https://esciencepress.net/journals/JPE>

## Incidence and Molecular Characterization of Citrus Bacterial Canker Disease in Pakistan

Fasiha Fayyaz Khan<sup>1</sup>, Rafia Ahsan<sup>2</sup>, Atif Jamal<sup>3</sup>, Laila Jafri<sup>4</sup>, Rehana Rani<sup>4</sup>, Samreen Saleem<sup>5</sup>, Muhammad Adeel Javed<sup>6</sup>, Kashif Sarfraz Abbasi<sup>6</sup>, Samea Khan<sup>7</sup>, Muhammad Zakria<sup>3\*</sup>

<sup>1</sup>Department of Genetics, Virtual University of Pakistan.

<sup>2</sup>PARC Institute of Advanced Studies in Agriculture, National Agriculture Research Center, Islamabad, Pakistan.

<sup>3</sup>Crop Diseases Research Institute, National Agriculture Research Center, Islamabad, Pakistan.

<sup>4</sup>Department of Life Sciences, Abasyn University Islamabad campus Islamabad, Pakistan.

<sup>5</sup>Health Services Academy, Islamabad, Pakistan.

<sup>6</sup>Institute of Food & Nutritional Sciences PMAS-Arid Agriculture University, Rawalpindi, Pakistan.

<sup>7</sup>Department of Biochemistry and Molecular Biology, Virtual University, Islamabad, Pakistan.

### ARTICLE INFO

#### Article History

Received: July 13, 2023

Revised: September 03, 2023

Accepted: September 05, 2023

#### Keywords

Citrus bacterial canker  
*Xanthomonas citri* pv. *Citri*  
Incidence  
Severity  
PCR

### ABSTRACT

The prevalence of Citrus Bacterial Canker (CBC) in the Indo-Pak subcontinent, particularly in Pakistan, poses a significant economic threat due to its quarantine implications. This research aimed to assess the current status of CBC in major citrus-growing regions of Punjab and KPK, shedding light on the incidence and severity of the disease. In Punjab, varying degrees of CBC incidence (20-81 %) were observed across different districts, with Sargodha, Toba Tek Singh, Faisalabad, Chiniot, and Mianwali showing higher rates. Conversely, Sialkot and Sahiwal were found to be free from the disease. The severity of CBC was most pronounced in Mianwali (26.2 %), highlighting the region's vulnerability. Similarly, in KPK, varying incidence rates (3-7 %) were recorded, with Haripur, Nowshera, Lower Dir, and Swat reporting minimal or no occurrences. A total number of ninety-six were confirmed as *Xanthomonas citri* pv. *citri* through PCR and pathogenicity tests. Despite these findings, further research is imperative to unravel the pathogenic diversity of *X. citri* in Pakistan, paving the way for more targeted and effective management strategies. This research underscores the importance of ongoing monitoring and in-depth investigations to mitigate the economic impact of CBC on the citrus industry in Pakistan. Identifying and understanding the pathogenic variations within *X. citri* will be crucial for devising sustainable and region-specific control measures.

Corresponding Author: Muhammad Zakria

Email: rmzakria@hotmail.com

© The Author(s) 2023.

### INTRODUCTION

Citrus belongs to the family *Rutaceae* and southern Himalaya, Northeast of India and China is believed its origin (Gmitter and Hu, 1990). Citrus is well established in sub-tropical and tropical climatic condition of the world, and it has significant contribution in terms of fruits and juices (Shah, 2004). Pakistan is placed among the top

ten citrus producer countries of the world with cultivated area (192832 hectares) and production (2395550 tonnes). Punjab plays a significant role in citrus production (182558 hectares with 2328090 tonnes) followed by Khyber Pakhtoon Khawa (3840 hectares 30871 tonnes) (GOP, 2016). The average yield of citrus in Pakistan is less than half (11 t/ha) as compared to Turkey

(27 t/ha) United States of America (26 t/ha) and Brazil (22 t/ha) (Khan *et al.*, 2016). Different citrus cultivars/varieties are produced in the country and provide the cheapest source of vitamins and minerals. The low yield might be attributed to different biotic and abiotic factors and among abiotic factors harsh environmental condition and manual practices are important. An investigation was conducted about how the growth, yield, and quality of Kinnow citrus crop respond to varying levels of pruning (Hayat *et al.*, 2019). The impact of peel coloration on internal quality of kinnow mandarin (*Citrus nobilis* Lour x *Citrus deliciosa tenora*) at early ripening stage was also confirmed (Nawaz *et al.*, 2019). Among biotic factors different insect pests, fungal, virus, nematodes and bacterial diseases has been reported in major citrus growing areas of Pakistan (Atta *et al.*, 2017, Anjum and Javaid, 2005, Mahmood *et al.*, 2010).

During the year 2015, an uncultured bacterium associated with infection in Mandarin (*Citrus reticulata*) was recorded (Mushtaq *et al.*, 2018a) in Pakistan. Among all these, bacterial canker (*Xanthomonas citri* pv. *citri*) has caused significant yield losses in citrus growing areas of the world. Southeastern Asia is considered its origin (Civerolo, 1984) but it was first reported in Florida in 1910 (Dopson, 1964). Grapefruit (*Citrus paradise* Macf.), sweet oranges (*C. sinensis* (L.) Osbeck), Key lime (*C. aurantifolia* Swingle), and lemons (*C. limon* (L.) Burm. F.) are susceptible to bacterial canker (Sena-Vélez *et al.*, 2016).

It has been reported in citrus growing areas of Asia, Japan, Africa, Middle East, Australia, New Zealand, Pacific Islands, South America, and the southeastern United States (Abubaker *et al.*, 2016). Biotechnology plays a vital role for the confirmation of diseases in agriculture (Khan *et al.*, 2017). The effect of bacterial endophytes isolated from the citrus on the physical parameter of bitter melon (*Momordica charantia* L.) was recorded (Mushtaq *et al.*, 2018b). Keeping in view the importance of citrus and bacterial canker, the current study was designed to determine the current status of bacterial canker in citrus growing areas of Punjab and KPK.

## MATERIAL AND METHODS

### Survey and sample collection

A comprehensive survey was conducted across citrus orchards and commercial nurseries in key citrus-growing regions of Punjab province (including Sargodha, Sahiwal,

Multan, Jhang, Faisalabad, Toba Tek Singh, Gujranwala, Lahore, Sialkot, Mianwali, Layyah, and Bhakkar) and Khyber Pakhtunkhwa (encompassing Mardan, Peshawar, Noshera, Swat, and Hazara), assessing the prevalence and incidence of citrus-related issues. The disease severity of citrus canker using the following formulas.

$$\text{Prevalence (\%)} = \frac{\text{Number of infected orchards}}{\text{Total number of surveyed orchards}} \times 100$$

$$\text{Incidence (\%)} = \frac{\text{Infected citrus tree}}{\text{Total trees}} \times 100$$

$$\text{Average severity} = \frac{\text{Sum of severity of all locations in a District}}{\text{Total number of locations}}$$

### Isolation

In total 303 orchards were surveyed for CBC and other citrus diseases. The diseased samples were collected from different citrus varieties during 2016 and 2017. Diseased tissues were surface-sterilized and crushed in 1 ml of sterile tap H<sub>2</sub>O (dH<sub>2</sub>O) in a test tube using a glass rod. Samples were spread on nutrient agar (NA) plates and incubated at 28°C for 3 days.

### Morpho-molecular confirmation

Yellowish colonies of bacteria were consistently isolated. Purified isolates from single colonies were verified by PCR amplification using *X. citri* pv. *citri* specific primers *J-pth1* and *J-pth2* (Li *et al.*, 2011).

### Pathogenicity test

Isolates were stored at -80 °C in fresh nutrient broth containing 25% glycerol and subjected to pathogenicity by infiltrating the bacterial suspension (10<sup>8</sup> CFU/ml of dH<sub>2</sub>O) into tobacco (*Nicotiana benthamiana*) leaves and host (grapefruit and lime). The strains were cultured for 2 days on NA plates at 28 °C and then resuspended in sterile distilled water. For inoculation bacterial suspension (10<sup>8</sup> CFU/ml) was injected into the intercellular spaces of fully expanded, immature leaves with needleless syringes. The inoculated plants were kept in greenhouse under a temperature regimen of 28 °C. Fourteen days after inoculation, the aggressiveness of each strain was evaluated. Isolates producing an HR and typical disease symptoms were considered pathogenic.

## RESULTS AND DISCUSSION

In total 303 orchards were visited during four surveys of citrus orchards in districts Sargodha, Faisalabad, Chinniot and Toba Tek Singh, Mianwali, Bhakkar, Layyah and Multan, Gujrat, Mandi Bahuddin, Sialkot, Lahore, Sahiwal, Chichawatni and Faisalabad, Attock, Swabi, Mardan, Nowshera, Lower Dir, Swat and Haripur to record the current status of citrus canker (Figure 1) and the survey

revealed that the disease was prevailing in both provinces with different incidence. A total number of 72 different varieties/cultivars of citrus are being cultivated in major citrus growing areas of Pakistan (Table 1). Erumpent, callus-like water-soaked lesions with oily, tan colored margins were recorded on leaves and fruits in both provinces (Figure 2). In Punjab, maximum 81 % disease incidence was recorded in Sargodha, followed by 64% in Chinniot, 60 % in Toba Tek Singh, Faisalabad and Mianwali, 54 % in Bhakkar, 51 % in Layyah, 32 % in

Lahore, 29 % in Jhang, 27 % in Mandi Bahauddin, 20 % in Multan, Gujrat and Attock and 2 % in Chichawatni. No disease was recorded in Sialkot and Sahiwal. The maximum disease incidence in Sargodha was due to dispersal of infected leaves and fruits debris while proper sanitation measures were recorded in Sialkot and Sahiwal. Maximum 7 % disease incidence was recorded in Swabi while only 3 % was observed in Mardan. No disease was recorded in Haripur, Nowshera, Lower Dir and Swat (Figure 3).

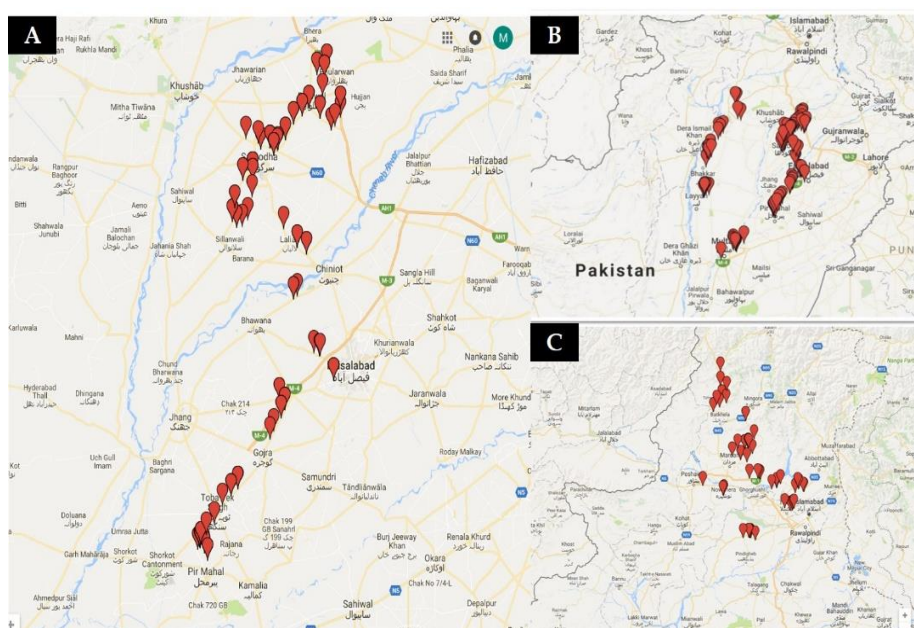


Figure 1. Map showing the GPS based surveyed locations/citrus orchards in district Sargodha, Faisalabad, Chinniot and Toba Tek Singh (A), district Mianwali, Bhakkar, Layyah and Multan (B) and district Attock, Haripur, Swabi, Mardan, Nowshera, Lower Dir and Swat (C).



Figure 2. The symptoms of citrus canker on leaves (A) and grapefruits (B).

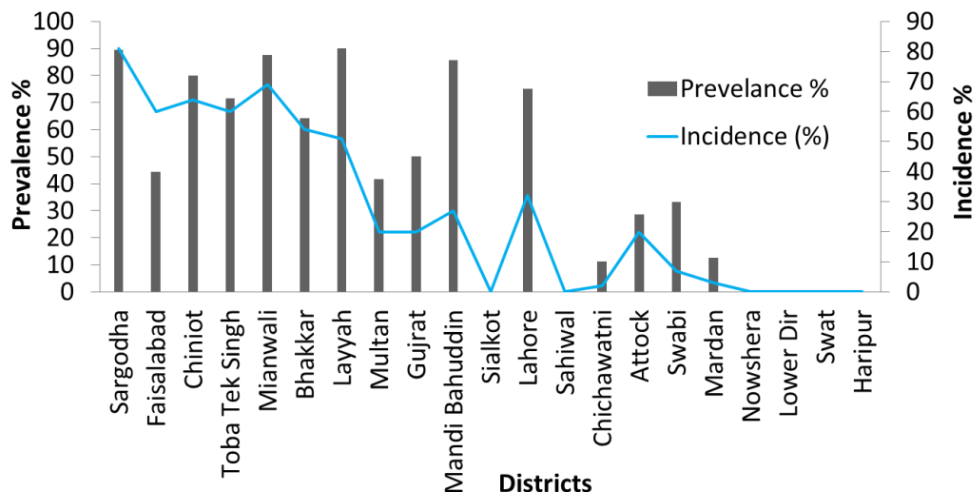


Figure 3. Prevalence and incidence of citrus canker in different districts of Pakistan.

The average severity of each district was computed from Punjab and KPK. In Punjab, the maximum disease severity was recorded in Mianwali (26.2) followed by Bhakar (22.6), Chiniot (21.2), Faisalabad (18.3), Mundi Bhaudin (17.8), Lahore (16.2), Gujrat (15), Layah (14.7), Multan (7.5), Attock (6.4), Sargoda (3.6), Chichawatni (2.8) and Toba Take Singh (2.2). No severity was recorded in Sahiwal and Sialkot. In KPK, only 1.7 and 0.1 average severity was recorded in Swabi and Mardan (0.1) while Noshera, Lower Dir, Swat and Haripur were free from this disease (Figure 4). Citrus nurseries at Citrus Research Institute, Sargodha, Agriculture

Research Institute Tarnab, Peshawar and Haripur were also visited. Citrus nurseries of various varieties were found infected with citrus canker. Diseased samples from the surveyed orchards, Institutes (CRI, Sargodha, NARC and Tarnab farms) and commercial nurseries were collected during the surveys. Ninety-six isolates were confirmed as *X. citripv. Citri* based on specific primers (J-pth-1 & J-pth-2) generating a specific band of 197bp (Figure 5) and preserved. All the isolates confirmed by PCR showed HR on tobacco and disease symptoms on susceptible hosts (grapefruit and Sweet Lime) (Figure 6).

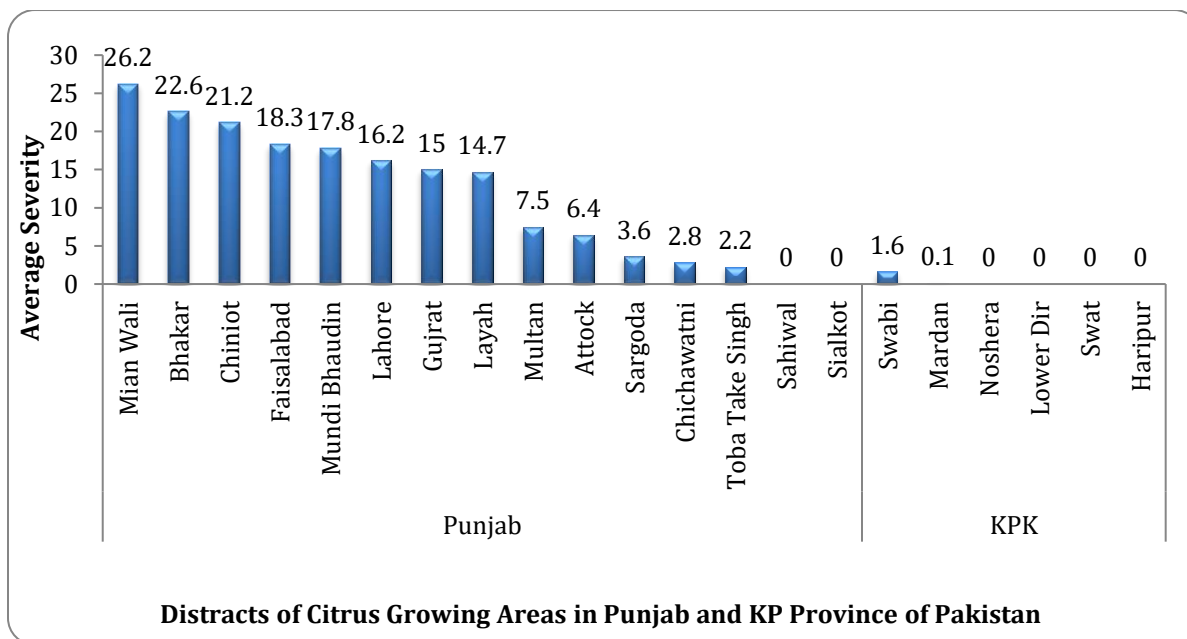


Figure 4. Severity of citrus canker in surveyed districts of Punjab and KPK.



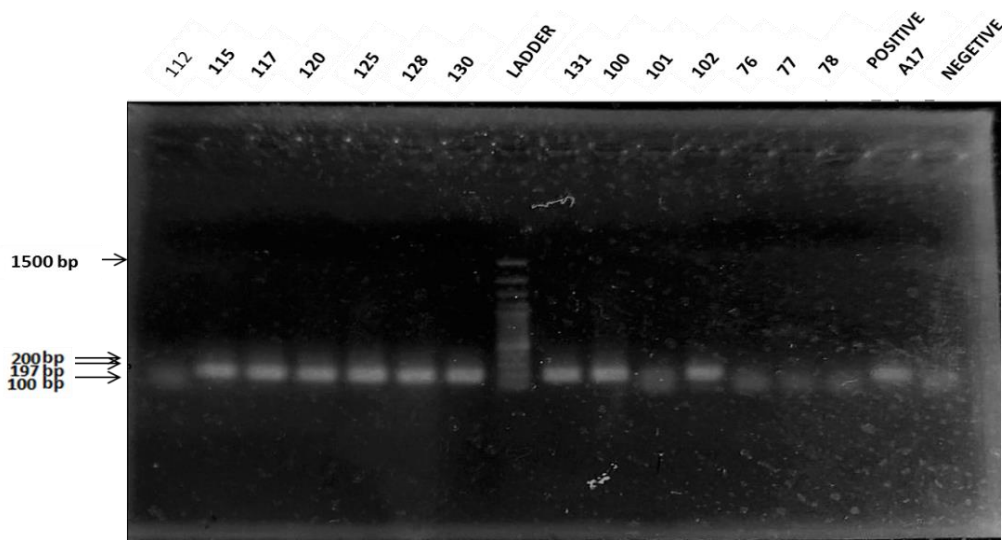


Figure 5. Gel showing PCR product of 197bp in positive samples.

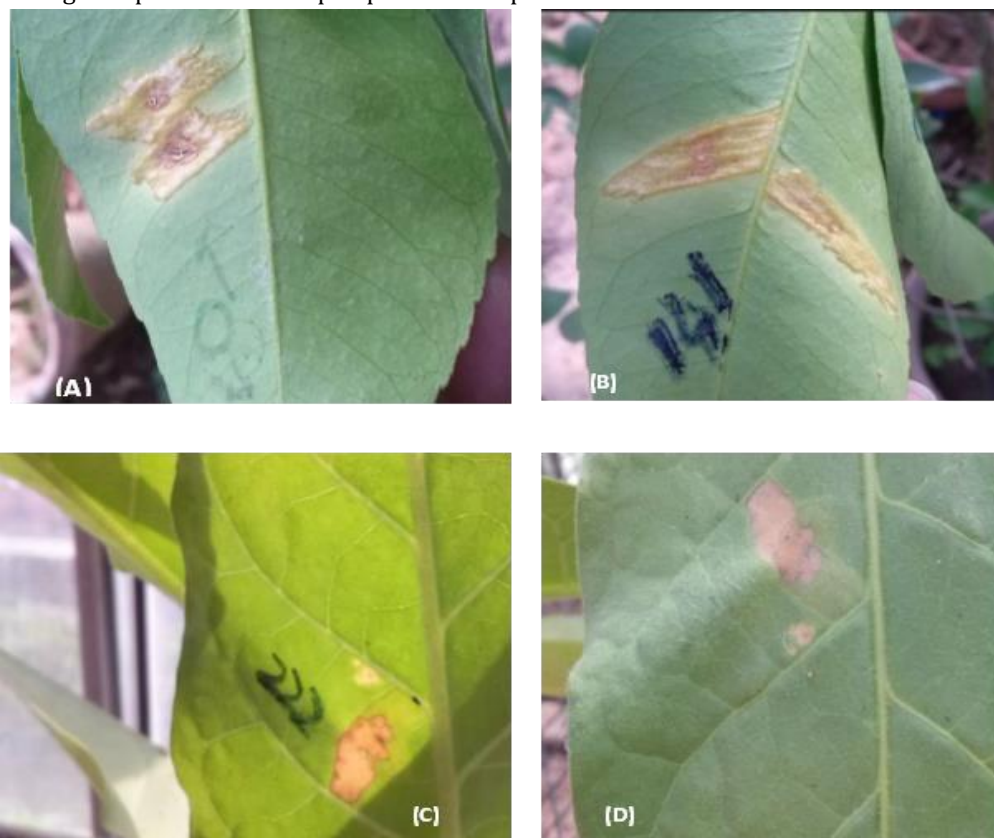


Figure 6. Pathogenicity test on Grapefruit leaf (A) and Sweet lime (B). Inoculated leaves of grapefruit and sweet lime showing typical symptoms of *Xanthomonas citri* pv. *citri* 14 days after inoculation. Tobacco leaves showing HR response 24 hrs after inoculation (C and D).

Kinnow is the most cultivated variety of Pakistan, and it is also the most affected one. Asiatic citrus canker caused by *X. citri* pv. *citri* is on rise and emerging as a potential threat to citrus crops all over the globe and this study was designed to determine the current status of citrus canker

disease in Pakistan. Survey results showed that the citrus canker is increasing in the traditional Kinnow growing areas of the Punjab. The environmental conditions were more favorable for the growth and development of bacterial canker disease in Punjab as compared to KPK

and it is one of the main reasons that the incidence was higher in Punjab. Environmental conditions are the key factor for development of disease (Bock *et al.*, 2010). Previously known resistant citrus cultivars are now becoming susceptible. Under favorable conditions for the pathogen, the management of disease with commercially acceptable methods in disease endemic areas is difficult. Khan, (1992) recorded 10-12.5% incidence of citrus canker in Faisalabad district and it has increased up to 60% in the surveyed year. In three Tehsils Kalurkot, Darya Khan and Bhakkar of the Punjab Province incidence was recorded as 7.5% (Sahi *et al.*, 2007) which has increased up to 54% in Bhakar. However, the disease is also spreading to relatively newer areas such as Layyah, Bakhar and Attock. The main reason of citrus canker spread in newer areas is the use of infected nursery citrus plants. These nurseries are coming mainly from Sargodha, Patoki (kinnow), Tarnab and Khanpur (Musambi and Orange) without any certification. Farmers are also not following the recommended management practices such as sanitation, pruning and chemical sprays to reduce the inoculum load in their infected citrus orchards. As a result, incidence of canker has increased alarmingly, and precaution measures are required to reduce the yield losses in citrus production of Pakistan.

#### ACKNOWLEDGEMENT

The current status of bacterial canker disease was investigated with the financial support of Research for Agricultural Development Program (RADP), Pakistan.

#### CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

#### REFERENCES

- Abubaker, M., O.A. Dibar, S. Elhassan and N. Yousif. 2016. First report of citrus bacterial canker disease in lime (*Citrus Aurantifolia Swingle*) In Gadaref State-Eastern Sudan.
- Anjum, T. and A. Javaid. 2005. Major diseases of citrus in Pakistan: A Review. International Journal Of Biology and Biotechnology (Pakistan).
- Atta S., M. Siddiq, S. Ashiq and A. Hannan. (2017). Citrus Tristeza Virus In Pakistan: A Review. Pakistan Journal of Phytopathology, 29: 273-279.
- Bock, C., J. Graham, T. Gottwald, A. Cook and P. Parker. 2010. Wind speed effects on the quantity of *Xanthomonas Citri* Sub. *citri* dispersed downwind from canopies of grapefruit trees infected with citrus canker. Plant Disease, 94: 725-736.
- Civerolo, E. 1984. Bacterial Canker Disease Of Citrus [*Xanthomonas campestris*]. Journal of the Rio grande Valley Horticultural Society.
- Dopson, R.N. 1964. The Eradication Of Citrus Canker. Plant Disease, 48: 30-31.
- Gmitter, F.G. and X. Hu. 1990. The Possible role of Yunnan, China, In The origin of contemporary citrus species (Rutaceae). Economic Botany, 44, 267-277.
- Gop. 2012. Agricultural Statistics Of Pakistan. Ministry Of Food, Agriculture And Livestock, Economic Affairs Wing, Islamabad.
- <http://www.pbs.gov.pk/content/agriculture-statistics>.
- Hayat, A., M. Asim, N. Anjum, M. Zubair, M. Nawaz and R. Salik. 2019. Response of kinnow crop with respect to growth, yield and quality to different levels of pruning. World Journal of Biology and Biotechnology, 4: 1-5.
- Khan, F.F., K. Ahmad, A. Ahmed and S. Haider. 2017. Applications of Biotechnology In Agriculture-Review Article. World Journal of Biology and Biotechnology, 2: 139-142.
- Khan, N., A. Khan, M. Ahmad, M. Nouman and B. Islam. 2016. Evaluation and screening of sweet orange cultivars for vegetative growth and citrus canker. Sarhad Journal of Agriculture, 32: 121-126.
- Li, Y.R., Y.Z. Che, H.S. Zou, Y.P. Cui, W. Guo, L.F. Zou, E.M. Biddle, C.H. Yang and G.Y. Chen. 2011. Hpa2 Is required by HrpF To translocate *Xanthomonas oryzae* Tal effectors into rice for pathogenicity. Applied and Environmental Microbiology, 77: 3809-3818.
- Mahmood, K., T. Muhammad, M. Akhtar and N. Khan. 2010. Incidence of citrus nematode in the citrus growing areas of NWFP. Sarhad Journal of Agriculture, 26: 271-274.
- Mushtaq, S., M. Shafiq, S. Afzaal, T. Ashraf and M.S. Haider. 2018a. An uncultured bacterium associated with infection in Mandarin (*Citrus Reticulata*) In Pakistan. World Journal of Biology and Biotechnology, 3: 179-181.
- Mushtaq, S., M. Shafiq, F. Khan, T. Ashraf and M.S. Haider. 2018b. Effect of bacterial endophytes isolated from the citrus on the physical parameter of bitter melon (*Momordica charantia* L.). World Journal of Biology and Biotechnology, 3: 193-197.
- Nawaz, R., N.A. Abbasi, I.A. Hafiz, Z. Khan, M.R. Khan and

A. Khalid. 2019. Impact of peel coloration on internal quality of kinnow Mandarin (*Citrus nobilis* Lour X *Citrus Deliciosa tenora*) at early ripening stage. World Journal of Biology and Biotechnology, 4: 7-17.

Sena-Vélez, M., C. Redondo, J.H. Graham and J. Cubero.

2016. Presence of extracellular dna during biofilm formation by *Xanthomonas citri* Subsp. Citri strains with different host range. Plos One, 11: E0156695.

Shah, M.A. Citrus cultivation In NWFP. Proceedings of the 1<sup>st</sup> International Conference on Citriculture, 2004: 36-39.

**Publisher's note:** ESscience Press remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



**Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license and indicate if changes were made. The images or other third-party material in this article are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0/>.