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MAPPING THE GEOSPATIAL DISTRIBUTION OF CITRUS GREENING DISEASE IN DISTRICT LAYYAH, PAKISTAN

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ABSTRACT

Citrus greening disease is one of the established causes of citrus decline in tropical and subtropical areas of the world, including Pakistan. Citrus greening disease is being recognized based on its characteristic symptoms in the field. Symptomatology and Iodo-starch test are the parameters for rapid disease indexing in the field and laboratory and are also helpful for disease mapping. A study was carried out to record the incidence and severity of citrus greening disease within the area of new citrus plantations of Layyah district, including the tehsils viz., Layyah and Karror. The Layyah tehsil showed an incidence of 18%, while Karror showed an incidence of 5.5%. Among the cultivars, the highest disease incidence was attributed to Kinnow (*Citrus nobilis x C. deliciosa*) at 33% followed by Mosambi (*C. sinensis*) at 16% and Feutrall's early at 5%. The highest disease severity was recorded at Layyah with 22.2%. The lowest was observed at Karror with 13.3%. Among the cultivars, Kinnow showed the highest severity, ranging from 27.22% to 36.6% and the least disease severity was recorded in Feutrall's early of 8.3% at ± 0.05 level of significance. The present study not only helped establish the relationship between the symptomology and quick indexing of disease through iodo-starch test but also provided information through disease mapping in the new citrus plantation of Layyah district for the first time.

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INTRODUCTION

Kinnow (*Citrus nobilis x C. deliciosa*) is an important fruit in citrus industry and economy of Pakistan (Bukhari *et al.*, 2022). Pakistan is ranked 15th in citrus production with 2.32 million metric tons in 2021 (https:/www.reportlinker). Citrus greening disease (CGD), alternatively known as Huanglongbing (HLB), affecting citrus trees (Xiao *et al.*, 2022). The citrus greening disease is widely spread in citrus-producing areas worldwide, causing a significant impact on the production of citrus (Taylor *et al.*, 2019). HLB, a century-old disease, probably originated at the end of the 19th century in China (Batool *et al.*, 2007; Bové, 2006). The greening disease is caused by a gram-negative unculturable phloem limited bacterium (Murray and Stackebrandt, 1995) named *Candidatus Liberibacter with three strains Asian* (*C*Las), African (*C*Laf) and Americanum (*C*Lam) (Jagoueix *et al.*, 1996; Iftikhar *et al.*, 2017). Among them, *Candidatus* Liberibacter asiaticus (*C*Las), is the most important and devastating which is transmitted in a persistent circulative manner through Asian citrus psyllid (ACP, *Diaphorina citri Kuwayma*) (Mishra *et al.*, 2023). The affected plants' symptoms are characterized by chlorosis, yellowing of the leaves, mottling resembling symptoms of zinc deficiency, fruit with aborted seeds and lopsided fruits. An exciting symptom that has been noted is the "color inversion" or "red nose" phenomenon where citrus fruits may exhibit green coloration at the styler or peduncular (Sajid *et al.*, 2022). Previously, the severity of this disease ranged

from 30-100% has been reported in the Faisalabad region of Punjab, Pakistan (Yaqub *et al.*, 2017). During previous surveys in different years and areas, the disease incidence was 8.2-90% and vector-disease relationship has been established (Akhtar and Ahmad, 1999; Saifullah *et al.*, 2015). Similarly, in previous surveys, the highest disease incidence was recorded in Mosambi (*C. sinensis*) and Kinnow (*Citrus nobilis x C. deliciosa*), 26% in Kot Momin and 23% in Bhalwal, respectively (Sajid *et al.*, 2021). Substantial prevalence of CGD in Punjab (16-66%) and KPK (90%) has been observed previously (Ashraf *et al.*, 2014). The primary mode of disease transmission is facilitated by an insect vector called the citrus psyllid (Martini *et al.*, 2020). Another means of disease spread is vegetative propagation techniques (Tran *et al.*, 2020). The present study was conducted to monitor the disease incidence and severity of HLB in citrus growing in Thal areas like Layyah of Punjab, Pakistan. In the Layyah district of Punjab, no work has been reported on this disease. This is the novelty of this research work: areas of study differ from already published data. We visited different areas of Layyah, and the data was collected for disease incidence and severity.

MATERIALS AND METHODS

Symptomology and Iodo-starch Test

A thorough investigation was conducted to monitor the prevalence of HLB disease in Layyah District. The survey involved the observation of 432 plants in each tehsil. The samples were obtained from trees showing characteristic symptoms suspected to be a sign of infection. Sample collection also involved recording symptoms on fruits, twigs, and leaves (Sajid *et al.*, 2021). The assessment of the disease severity was recorded by utilizing the rating scale of (Akhtar and Ahmad, 1999) as follows;

Table 1: Disease rating scale of citrus greening.

Score	Severity
0	No disease
1	Blotchy mottles on leaves are observed, with symptoms affecting up to 25% of the leaves.
2	Lopsided fruit and color inversion are observed in up to 26-50% of the canopy, indicating a potential issue with the fruit development process.
3	A tree that has suffered damage ranging from 51% to 75% has partially declined.
4	A tree that has experienced a significant decrease in its health and vitality, with a decline rate exceeding 75%.

The disease incidence was calculated as follows;

$$\% \text{ Incidence } = \frac{\text{No. of Infected Trees}}{\text{Total No. of trees examined}} \text{x } 100$$

The iodo-starch test was performed for quick indexing using the method of Etxeberria *et al.* (2016) and described by Akhtar and Ahmad (1999) .

Data Analysis

One-way ANOVA was performed to check the significance of disease incidence and severity among locations. Means were compared using LSD in all pairwise comparison test at 5% level of significance. All the analyses were performed using Statistics 8.1 software.

RESULTS

Disease Mapping of Citrus Greening in District Layyah

Extensive surveys were conducted in various villages within District Layyah, including Tehsil Layyah and Karror. The visible symptoms of Citrus Greening were observed, and it was found that six villages in Tehsil Layyah showed a higher susceptibility to the disease. These villages, Chak 146, Chak 151, Chak 156, Chak 153, Chak 228, and Chak 232 were highlighted on maps using red to indicate their infection status. The other two villages in Karror (Chak 105 TDA, Chak 99 ML) showed no or minimal incidence and severity of Citrus Greening. A blue color on the District Layyah Map denoted these uninfected villages. The map was made by QGIS Software (http://qgis.org) (Figure 1).

Symptomology

During the survey, characteristic symptoms of Citrus Greening disease (CGD) were observed (Figure 2). Blotchy mottling and chlorosis were observed in leaf samples. In contrast, lopsided fruits and full or half-green fruits were observed (Figure 2). Our results regarding symptomology were by Sajid *et al.* (2021). They reviewed the characteristic symptoms like yellowing of leaves, Lopsided fruits, blotchy mottle and unripe fruits (Fruit remains green). Similar results have also been reported by (Saifullah *et al.*, 2015; Inoue *et al.*, 2020), including yellowing of leaves, blotchy mottling,

upright growth and lopsided fruits, chlorosis (yellowing), mottling resembling zinc deficiency symptoms, interesting symptom mentioned is the "color inversion" or "red nose" phenomenon, where citrus fruits may remain green at the styler end or sometimes at the peduncular end when they should have reached maturity (Sajid *et al.*, 2022). These results highlight the importance of identifying and monitoring the characteristic symptoms of CGD for effective disease management and control.

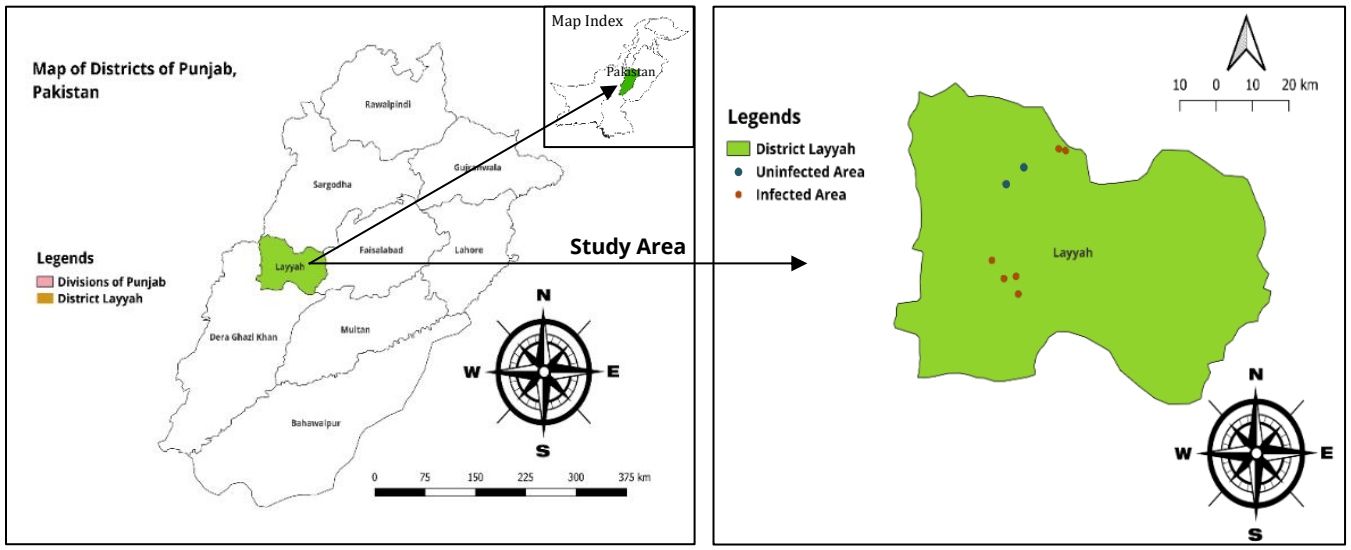

Figure 1. Disease mapping of suspected citrus greening citrus areas.

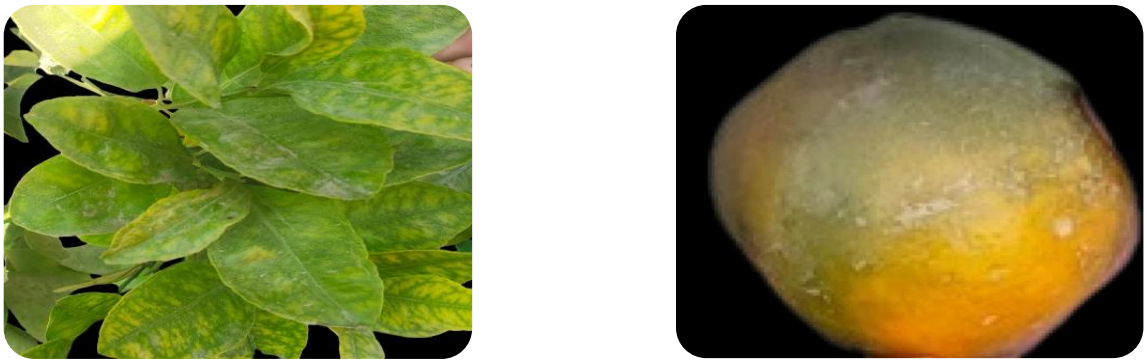

Figure 2. Characteristic HLB symptoms in citrus infected samples.

Starch Accumulation Test through Iodo-starch Test

Citrus greening leaves were treated with an iodine solution, Results of the iodo-starch test indicated that HLB positive leaves exhibiting significant intense dark grey-to-black staining pattern throughout the entire cut surface. The healthy leaves displayed limited or no staining along the edges, which was significantly lower in comparison to the samples of leaves that were suspected positive for HLB (Figure 3). Iodo-starch test was developed by Etxeberria *et al.* (2016) and found its reliability in case of early detection Saifullah *et al.* (2015).

Disease Incidence on Locations and Varieties

The investigation into the incidence of Citrus greening disease in Layyah and Karror tehsils of Punjab, Pakistan, revealed distinct patterns. The Layyah had a significantly higher incidence as compared to Karror tehsil. The Layyah tehsil exhibited a disease incidence of 18.51%, indicating a greater prevalence of symptomatic citrus trees. In contrast, Karror tehsil displayed a lower incidence 5.55% (Figure 4a). Among the studied citrus cultivars in Tehsil Layyah, Kinnow had the highest disease incidence 33%, followed by Mosambi (*C. sinensis*) 16% and Feutrall's early 5%. In contrast, in Tehsil Karror, Kinnow exhibited the highest incidence rate of 11%, whereas Musambi demonstrated an incidence of 5%, with no reported occurrences of Feutrall's early throughout the study (Figure 4b). The Standard error values were 5.0±0.05%.

Figure 3. CGD symptomatic leaf showing the gray stained cutting edge in Iodo-starch test.

Figure 4a: Disease Incidence at different locations.

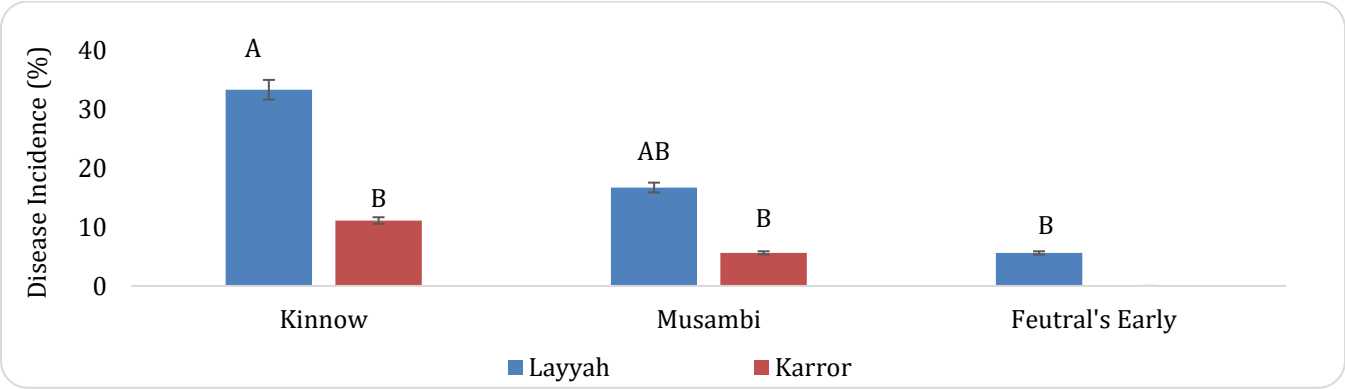

Figure 4b: Disease Incidence in different varieties.

Disease Severity for District Layyah and Varieties

The severity assessment of Citrus greening disease symptoms in Layyah and Karror tehsils of Punjab, Pakistan, revealed distinct patterns. Disease severity depends on the visual symptoms of the disease, as the rating scale provided by Akhtar and Ahmad (1999)

based on the visual symptoms of citrus greening. Layyah district exhibited a significantly higher severity rate at 22.22%. A less than 30% severity will indicate blotchy mottle leaves indicating a greater prevalence of symptomatic citrus trees. Karror tehsil displayed a lower severity value of 13.33%; similarly, for severity less than 25% will show symptoms of blotchy mottle leaves of citrus, suggesting a comparatively lower severity of Citrus Greening symptoms in the area (Figure 5a). Among the studied citrus cultivars in Layyah, Kinnow had the highest disease severity at 36.6%. According to that scale the severity will be higher when the rating scale is more than 30%, in which color inversion and lopsided of citrus fruit were observed, followed by Mosambi (*C. sinensis*) at 21%, and Feutrall's early at 8.3%. In Karror tehsil, Kinnow showed the highest severity rate of 27.22%, while Musambi showed a severity rate of 12.7%. Feutrall's early was not found in the area (Figure 5b). The Standard error values were 5.0±0.05%.

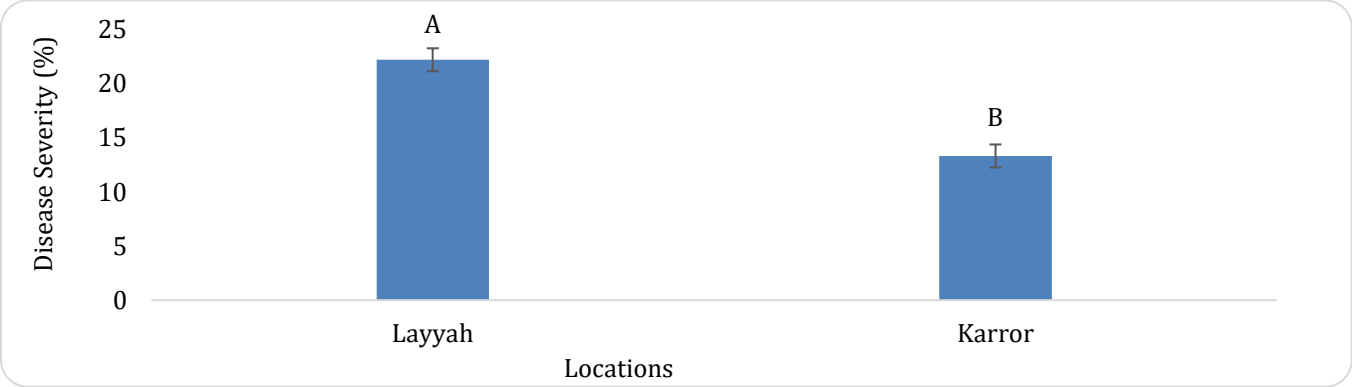

Figure 5a: Disease Severity at different locations.

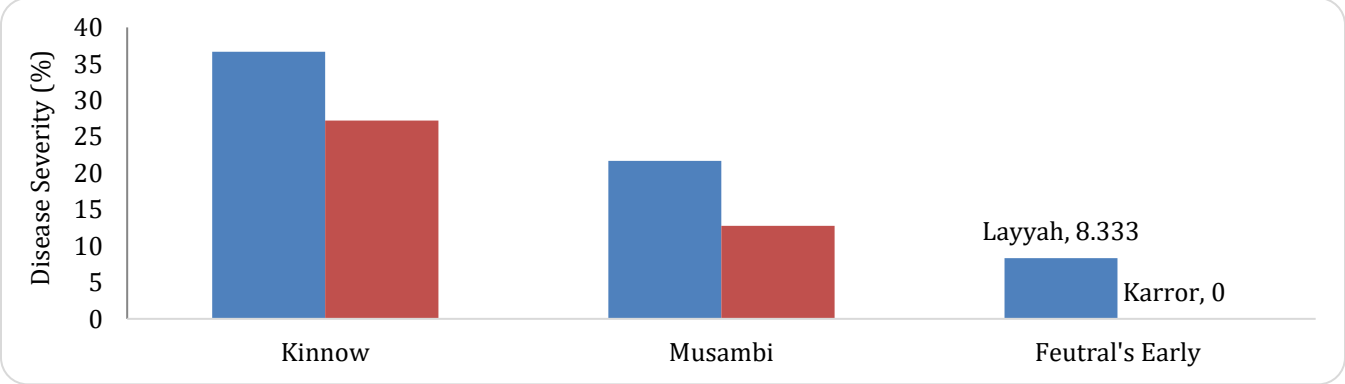

Figure 5b: Disease Severity in different varieties.

DISCUSSION

During the survey, characteristic symptoms of Citrus Greening disease (CGD) were observed. Our results regarding symptomology were in accordance with Sajid *et al.* (2021). They reviewed the characteristic symptoms like yellowing of leaves, Lopsided fruits, blotchy mottle, aborted seeds, and unripe fruits (Fruit remain green). Similar results have also been reported by Inoue *et al.* (2020) and Saifullah *et al.* (2015) including yellowing of leaves, blotchy mottling, upright growth, and lopsided fruits, chlorosis (yellowing), mottling resembling zinc deficiency symptoms, another interesting symptom mentioned is the "color inversion" or "red nose" phenomenon, where citrus fruits may remain green at the styler end or sometimes at the peduncular end when they should have reached maturity (Sajid *et al.*, 2022). These results highlighted the importance of identifying and monitoring the characteristic symptoms of CGD for effective disease management and control. Using UAV hyperspectral remote sensing to efficiently detect Huanglongbing (HLB) on large-scale orchard citrus trees is a promising approach (Deng *et al.*, 2019).

According to the current study incidence and severity of the disease in different tehsils of Layyah district were observed. Our study found that Layyah district had the highest incidence rate of the disease while Karror tehsil showed the lowest disease incidence rate. Among the studied citrus cultivars in Tehsil Layyah, Kinnow had the highest disease incidence, followed by Mosambi (*C. sinensis*) and Feutrall's early. In contrast, in tehsil Karror, Kinnow exhibited the highest disease incidence. Musambi demonstrated a disease incidence of 5%, with no reported occurrences of Feutrall's early throughout the study. Our investigation also recorded a high disease severity in Layyah, while the lowest severity was observed in Karror. Kinnow displayed the highest severity among the studied citrus cultivars, followed by Mosambi and Feutrall's early. These findings are effective interventions to adopt the management strategies regarding the spread and impact of Citrus Greening disease on citrus plantations in areas where the disease was not reported previously.

Our work was in accordance with already published research, but the difference between our work and already published data is the place of citrus plantation. Similar studies on Citrus greening disease incidence have been conducted in different central Punjab tehsils of the Sargodha district, where the highest incidence was recorded in Kot Momin (24%) followed by Bhalwal (22%), while Sahiwal tehsil showed the least incidence at 4.6% (Sajid *et al.*, 2021). The initial incidence of Citrus greening disease in Pakistan was reported by (Catara *et al.*, 1991) and (Akhtar and Ahmad, 1999) in KPK and Punjab. They documented high incidence rates of CGD in Kinnow (22%) and sweet orange (25-40%) in Punjab. (Ashraf *et al.*, 2014) reported similar findings showing a high prevalence of CGD in Punjab (16-66%) and KPK (90%), indicating the widespread impact of the disease in these regions. The disease incidence in different varieties was 22% in Kinnow, 25-40% in sweet orange, 15% in grapefruit, 10% in sweet lime, and 2% in lemon (Ashraf *et al.*, 2014).

Our study also found similar symptoms during the sample collections, which are consistent with their observations. These results emphasize the need for continued research and efforts to manage and control the spread of CGD in affected areas. The CGD was confirmed in 41% of citrus samples collected from different orchards in the Punjab during a survey (Razi *et al.*, 2011). CGD incidence of 42% in kinnow and sweet orange was also recorded by (Zafarullah and Saleem, 2016). Our results are also in accordance with the previous studies. We also observed the same type of symptoms and trend in CGD incidence. (Razi *et al.*, 2014) collected the number of samples in different months of a year and found positive for CGD detection. High incidences were found in February, April, and May. Therefore, symptomology plays a vital role in the early detection of CGD in the field. Iodo-starch test also found its reliability in case of early detection (Sajid *et al.*, 2021). Our findings were in accordance with previous results. Our results also revealed the efficacy of the Iodo-starch test. In conclusion, this research paper sheds light on the destructive nature of HLB and its impact on the global citrus industry. The study focused on monitoring the occurrence and severity of HLB disease in the Layyah district. Two parameters, symptomatology, and the Iodo-starch test, were utilized for rapid disease indexing in the field. The survey findings revealed variations in the incidence and severity of HLB across different geographical regions and citrus plant varieties. According to findings, the disease is migrating from old established citrus plantation areas of the Punjab to the new areas of citrus plantations. The continuous monitoring of citrus greening disease in areas where the disease has not been reported is highly recommended.

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NOVELTY STATEMENT

The disease mapping of HLB in Layyah, Pakistan, will strengthen the knowledge about the diversity of this disease throughout Punjab, Pakistan.

AUTHOR'S CONTRIBUTION

Sonum Bashir conducted the research and wrote the initial draft of the manuscript. Yasir Iftikhar designed and supervised the research work. Muhammad Ahmad Zeshan helped in data analysis. Mustansar Mubeen helped in graphical representation, revision, editing and finalized the manuscript. Muhammad Usman Ghazanfar proofread the final draft.

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