Research Article

Inspection of Citrus Orchards in Multiple Populations for its Health Assessment Against Greening Disease

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Abstract | An extensive survey of District Layyah was conducted for quantification, mapping and analysis of spatial distribution and temporal dynamics of citrus greening disease-CGD (Huanglongbing-HLB) and patterns of disease prevalence, incidence and severity. Overall, 16 citrus orchards were randomly surveyed from 4 villages of 2 Tehsils of District Layyah. Data of HLB disease prevalence was recorded from Tehsil, village and field scales while of incidence at field scale that was narrowed to tree level for disease severity. There was strong correlation between all disease intensity scales i.e. prevalence, incidence and severity. A higher value of disease prevalence was backed by similar trend in incidence and severity at all locations. Spatial analyses showed that HLB incidence and severity was spatially clustered at all levels of survey according to Moran's Index analysis. Disease intensity levels increased with the passage of time i.e. low at initial DOY and high at the final. The highest disease intensity was recorded at 165 DOY in Layyah and 167 DOY in Karor Lal Esan. The regression model between disease intensity and time of data recording indicated a strongly positive interaction. This study infers about novel quantitative tools and facts for enhanced understanding of spatio-temporal dispersal of HLB disease.

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Introduction

Citrus reticulata belongs to the family Rutaceae and is one of the most important fruit tree crops of the tropical and subtropical areas of the world (Mubeen *et al*., 2024). The citrus production in Pakistan is estimated lower attributed to many biotic and abiotic factors (Mubeen *et al*., 2015a; Mubeen *et al*., 2015b). Citrus Greening Disease (CGD) is the most destructive disease of citrus that extends to all citrus producing regions. It has many alternative names among which Huanglongbing (HLB) is most commonly used (Iftikhar *et al*., 2024; Limayem *et al*., 2024). A fastidious phloem limited bacterium *Candidatus liberibacter* with Asian, African and American strains cause this disease globally. In Asian region *Candidatus liberibacter asiaticus* is the most prevalent causal organism of HLB that enters into the host plant by an insect vector Asian citrus psyllid (*Diaphorina citri*) (Mishra and Ghanim, 2022; Mubeen *et al*., 2024). The major morphological identification of HLB is the yellowing of leaves, misshapen and bitter fruit, with uneven ripening and low sugar content, making them unfit for sale (Sagouti *et al*., 2022; Bashir *et al*., 2023). With the temporal progression of disease, trees lose vigor, roots deteriorate, and fruit production declines (Chuang *et al*., 2024; Usman *et al*., 2024). This decline in fruit quality has significantly impacted both fresh fruit and juice production, raising prices and reducing availability for consumers (da Cruz *et al*., 2023; Shafique *et al*., 2024). HLB has no cure and is therefore a great menace to citrus production. It has led to staggering losses within major citrus producing areas, such as Florida, Brazil and several parts of Asia; which have been severely affected by the disease (Villar-Luna *et al*., 2024). It has caused significant economic losses for farmers by increasing production costs due to enhanced usage of pesticides, tree removal, and replanting (Alquézar *et al*., 2022). Many small-scale farmers of developing countries of Asia, have been forced out of business due to limited resources for managing this disease, leading to job losses and economic hardships in rural communities (Ghosh *et al*., 2022). HLB threatens 25% of global citrus production, with millions of trees lost in key regions like Florida and Brazil (Kwakye and Kadyampakeni, 2022). There is a significant variation in spatial distribution of Citrus Greening Disease-CGD (HLB) at varied landscapes, orchards, or regions (Rehberg *et al*., 2021). This variation is attributed to environmental conditions, agricultural practices, tree density, and the abundance of insect vectors (Garcia-Figuera *et al*., 2021). Within orchards, some areas may have high disease prevalence while others remain unaffected, creating a patchy distribution (Xu *et al*., 2021). Geographic regions also vary in disease incidence due to differences in climate, soil, and farming practices (Zhang *et al*., 2021). These variations are mainly due to weather variables as high temperature favors vector population thus increasing disease intensity; on the other hand poor soil conditions predispose the plant against HLB (Antolinez *et al*., 2021). Marking the specified regions with high HLB disease intensity provide insights for efficient management options and reducing the input costs (Rehberg *et al*., 2021). Spatial analysis helps identify regions with high HLB prevalence, enabling growers and researchers to focus disease management efforts on hotspots and allocate resources effectively (Iftikhar *et al*., 2024). Collaboration among growers, researchers, and policymakers fosters the development of region-specific strategies, promoting effective disease management and resource allocation across citrus-growing areas (Pazolini *et al*., 2021). The geographic coordinates of the surveyed units for mapping the HLB disease intensity indicate the factors affecting HLB disease risk (Galvañ *et al*., 2023). Therefore, the objectives of the current experiment were to (i) quantify the HLB disease intensity at district, tehsil, village and field levels (ii) testing the presence of spatial dependence (clustering) of HLB at the field and village level (iii) identify plant survivorship over time.

Materials and Methods

Survey for HLB disease

A wide HLB disease survey was conducted in 2 Tehsils of District Layyah during the seasons of 2022 and 2023. In each Tehsil i.e. Layyah and Karor Lal Esan; 4 villages were selected by using stratified random sampling for inclusion of maximum area and data was recorded fortnightly for 4 times. In each village 4 orchards were selected and thus a total of 16 orchards were marked for sampling in each tehsil. All the orchards were considered for sampling and selection was made from each corner of the village so a variation can be maintained. During survey and data recording procedure; every time a varying geographic coordinate was selected by using GPS location so that maximum representative units of the selected locations could be included in the survey. Data of disease intensity (prevalence, incidence and severity) was recorded from citrus orchards. A systemic sampling design was used i.e. W and X to select 30 citrus plants from each citrus field. Each time data was recorded from 5 plants in each GPS location randomly in such a way that a particular GPS point was marked and 5 plants were observed for data recording in that surrounding radius for a comprehensive representative data. The sample size kept 5 for efficient time utilization and resource management.

HLB disease data recording regarding disease prevalence, incidence and severity

The data of HLB disease prevalence was recorded at

Tehsil level that means if a particular field was found with diseased unit by using the following formula: Disease prevalence was recorded on a broader level i.e. Tehsil/Field; disease incidence on plants/field and severity on individual plants.

$$\frac{\text{Number of villages in which disease was found}}{\text{Number of villages surveyed for HLB disease}} \times 100$$

It was further splitted on field level:

$$\frac{\text{Number of fields where disease symptoms were present in a particular village}}{\text{Total number of fields surveyed in that village}} \times 100$$

The data of disease incidence and severity was proceeded in only those villages and fields where disease prevailed. The data of disease incidence was recorded more precisely in such a way that number of symptomatic plants taken as a main indicator from the prevalent fields and following formula:

$$\frac{\text{Number of symptomatic plants}}{\text{Total number of plants in the field}} \times 100$$

The fields having more disease incidence were marked for recording of disease severity data; trees were selected randomly while walking in "W" and "X" patterns to get maximum representative samples across the field and minimizing biasness in samples; and number of leaves were averaged from all 4 directions and severity was calculated as follows:

$$\frac{\text{Number of symptomatic leaves}}{\text{Total number of leaves}} \times 100$$

Spatial variations in HLB prevalence, incidence and severity

During data recording at fortnight interval, day of year (DOY) was mentioned to clarify the temporal disease progression at different locations. Location wise HLB disease incidence and prevalence data was plotted against survey dates to obtain temporal disease progression for prevalence, incidence and severity (Mastin *et al*., 2022).

Analyses of spatial data and temporal distributions

The data of HLB disease incidence and severity at Tehsil, village and field levels were analyzed using R software for spatial autocorrelation and comparison was made by analysis of variance (ANOVA). Summary of the analysis was presented by using least significant difference (LSD) test followed by presenting he confidence interval. Spatial distribution was assessed by using Moran's index analysis for description of clustering with Z score and P values determined the significance of clustering. Temporal pattern of HLB disease intensity was assessed by comparing disease values on all the dates of survey and data recording (Galvañ *et al*., 2023). Regression analysis was used to assess the relationship of disease intensity an environmental factors where goodness of fit was assessed using R^2 values and residual analysis.

Determination of relationship between disease incidence and severity

This was determined by using least square regression by keeping incidence on (x) axis and severity on (y) axis for both Tehsil separately. As a whole all types statistical analysis including tables and figures were made in R software.

Risk factors associated with HLB disease

To describe the effect of varied locations within Layyah District, geographic coordinates were plotted against HLB disease incidence at different locations.

Table 1: *Day of year (DOY) when HLB data was first collected, highest prevalence, incidence and severity*

Loca-tions	DOY when 1st data was collected	DOY with highest prevalence	DOY with highest incidence	DOY with highest severity
Layyah	120	165	165	165
Karor lal esan	122	167	167	167

DOY = Day of year (Date) when survey was conducted on temporal basis

Results

HLB data was first recorded on 120th DOY in Tehsil Layyah and 122nd DOY in Tehsil Karor Lal Esan of District Layyah; the DOY for maximum disease prevalence, incidence and severity was almost similar in both Tehsils (Table 1). The data was recorded 4 times on fortnight interval; first data was taken on 30th of April followed by 15th and 30th of May and 14th June in Tehsil Layyah while data in Tehsil Karor Lal Esan was taken 2 days later each time. The maximum disease intensity was recorded on 14th and 16th of June in Tehsil Layyah and Karor Lal Esan, respectively.

The data for disease prevalence was recorded 4 times on Tehsil, village and field level that tends to increase with the passage of time (Table 2). Disease prevalence at Tehsil level was 100% from 1st to 4th data recording but it increased gradually in village and field scales with each date of survey. On first date of survey, half of the villages did not exhibit any prevalence in both Tehsils while 25% fields were observed with no symptoms on the same date. It is evident from the results that disease prevalence is getting stable as the standard error is reducing with temporal progression (frequency increase).

Table 2: *HLB disease prevalence at different scales*

Fre-quency	Teh-sils n	%	Villag-es n	%±SE	Fields n	%±SE
1	2	100	8	50±4.5	16	75±1.7
2	2	100	8	62.5±2.8	16	81.25±1.2
3	2	100	8	87.5±1.2	16	87.5±0.7
4	2	100	8	100	16	100

n is number of surveyed units at tehsil, village and field scales; SE (Standard error)

Average HLB disease incidence was recorded from 16 sampling units i.e. 4 locations in each Tehsil and 4 fields in each location that ranged from 0.65 to 0.83 in Tehsil Layyah and 0.67 to 0.86% in Tehsil Karor Lal Esan (Table 3). The results depicted that highest disease incidence was found in Chak #99 ML of Karor Lal Esan and lowest was in Chak #153 TDA of Tehsil Layyah. The overall trend of HLB disease incidence indicated that there were higher percentages in locations of Tehsil Karor Lal Easn than in Layyah. There was smaller standard error in areas with high disease incidence while it was larger in areas with less disease incidence. The values for each village are the average of HLB disease incidence data from 4 fields with different geographic coordinates.

A representative sample of the marked fields after detailed survey is indicating the disease severity that was taken on an average from almost 10-unit plants with varied geographic coordinates. HLB disease severity was more in Karor Lal Esan as compared to Layyah i.e. maximum value in Karor was 0.52 that was stopped at 0.44% in case of Layyah. In an overview of 2 Tehsils Chak # 151 TDA in Layyah has less severity while Chak # 105 TDA in Karor Lal Esan has more HLB disease severity. In separate comparison, Chak # 151 TDA and Chak # 156 in Layyah; while Chak # 99 ML and Chak # 105 TDA in Karor Lal Esan hasminimum and maximum severity values (Table 3).

The slope of the model (b_1) for HLB disease incidence infers the significantly positive relationship between disease incidence and time in both Tehsils i.e. HLB

Table 3: *HLB disease mean incidence and severity at different locations*

Location	Villages	Sampling units n	% ± SE	Sampling units n	% ± SE
Layyah	Chak # 146 TDA	16	0.83± 0.19	40	0.38±0.24
	Chak # 151 TDA		0.75 ± 0.22		0.26±0.22
	Chak #153 TDA		0.65 ± 0.24		0.35±0.23
	Chak #156 TDA		0.75 ± 0.22		0.44±0.24
Karor lal esan	Chak # 105 TDA	16	0.85 ± 0.18	40	0.52±0.25
	Chak # 228 TDA		0.67± 0.24		0.35±0.24
	Chak # 232 TDA		0.72 ± 0.22		0.42±0.24
	Chak # 99 ML		0.86 ± 0.17		0.31±0.23

**Calculations used Tehsil, villages, and fields as sampling units (n). SE = standard error.*

Table 4: *Regression between DOY and HLB disease incidence and severity mean*

Parameters	Layyah	t value	Pr (>|t|)	Karor Lal Esan	t value	Pr (>|t|)
b_0 (± SE)	0.12(0.15)	0.76	0.46	0.13(0.193)	0.69	0.49
b_1 (± SE)	0.04(0.01)	4.19	0.009***	0.01(0.0013)	3.33	0.04**
HLB disease severity						
b_0 (± SE)	0.72(0.08)	0.24	0.35	0.45(0.04)	0.16	0.29
b_1 (± SE)	0.05(0.01)	2.86	0.04***	0.05(0.09)	2.24	0.01**

Linear model was fitted to the HLB disease incidence and severity data.
SE = standard error; Pr = Probability of observing any value; b_0 = Intercept; b_1 = Slope

continued to increase with each upcoming day of survey perhaps due to the favorable environmental factors (Table 4). The intercept for both Tehsil is indicating that HLB disease incidence in the start of the year cannot properly predict the amount of disease incidence for the rest of year unless it is modeled with temporal progression. An almost similar trend for slope and intercept was found in case of disease severity in both Tehsils of District Layyah (Table 4).

The variation of HLB disease incidence and time of survey (DOY) after the appearance of symptomatic plants was described by using linear regression model in an effective manner (Figure 1). This describes about the relation of disease incidence and its dependence upon time variation across 2 Tehsils i.e. Layyah and Karor Lal Esan. The trend lines in the graph are depiction of strong relationship between both variables. Both trend lines are indicating all the individual points where orchards were surveyed for the purpose of data recording. Regression coefficients (slopes) were positive for both Tehsils that disease incidence is increasing with increase in day of year. Coefficient of determination values R^2 values explained 44-56% of the variation in HLB disease incidence over time. In Tehsil Layyah 56% of disease variation is dependent upon the passing day of year while 44% other factors are influencing the disease incidence. It is evident from the figure that there is strong correlation between disease incidence and time of survey at all the surveyed points of both Tehsils. Despite individual data points around the trend lines are showing bit variability at some positions but overall trend is positive with respect to time and disease incidence.

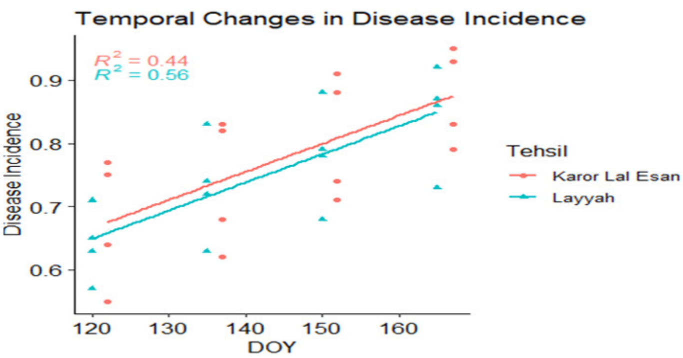

Figure 1: *Linear relationship between day of year (DOY) and disease incidence (%) in tehsil layyah and karor lal esan*

There was positive relationship between time of survey and disease severity in all the fields of both Tehsils (Figure 2). As there is a steep slope of trend lines for both Tehsils which means that disease severity tends to increase very fast with passage of time at all the points of survey. At initial points the trend showing that disease severity was less but on final date of survey it increased significantly. This graph also describes the linear prediction of the disease severity with respect to time. There was a different trend in coefficient of determination from disease incidence as R^2 is higher in HLB disease severity at Karor Lal Esan which was lower in case of incidence. R^2 explains the extent of relation between 2 variables although it was low in current graph but the trend of lines has very steep slope inferring about the effect of temporal dynamics in disease severity. Disease severity was explained by day of year ranged from 26-45% which is weak; it means DOY has influence on disease severity but is more likely being influenced by other factors.

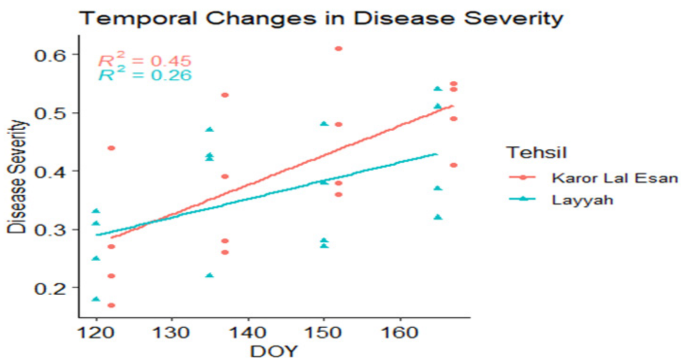

Figure 2: *Linear relationship between DOY and disease severity (%) in tehsil layyah and karor lal esan*

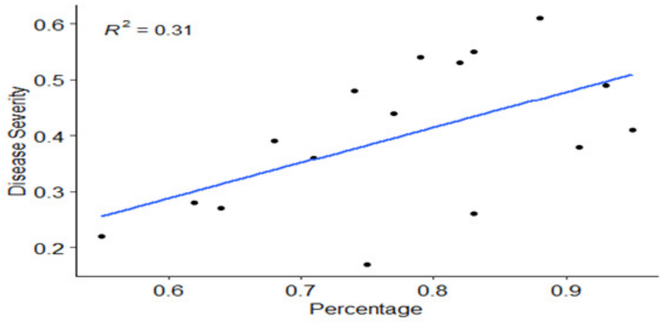

Figure 3: *Association between disease incidence and severity in tehsil layyah*

Avery strongly positive and significant interaction was recorded between disease incidence and severity. As the number of plants infected with pathogen is increased with time progression the extent of damage on individual plants has also been increased in both Tehsils (Figure 3, Figure 4). These figures are describing that as the pathogen load is increased within a population of plants i.e. orchards it got more aggressive on the individual unit plants as well. Overall a directly proportional correlation was noted that describes the intensity of disease at larger scale and its aggression at small scale tend to increase accordingly. The R^2 values indicated that there were 22 and 31% disease severity and incidence relationship in Karor Lal Esan and Layyah which was weak but statistically significant correlation. There was non-significant difference for HLB disease severity and incidence at Tehsil level while it has significant difference at some locations (Table 5, Figure 5, Figure 6). The lower values of standard errors in case of severity and incidence in both Tehsils are indicating the accuracy in overall disease intensity predictions. The figures depicting the data of individual locations are providing clearer trend of HLB disease incidence and severity distribution at field scales. The graphs in (Figure 6) are highlighting the areas with more attention for disease management decisions. Although there was almost similar trend at Tehsil level but surveyed points are indicating some hotspots.

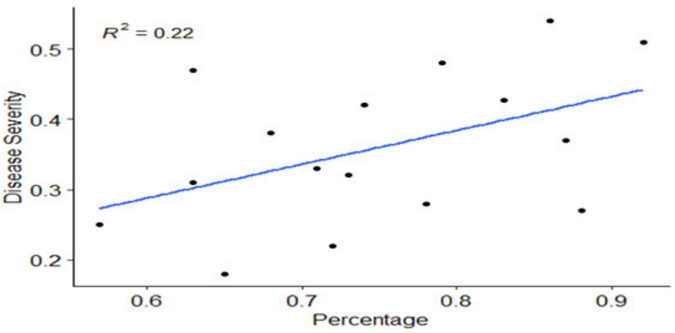

Figure 4: *Association between disease incidence and severity in tehsil karor lal esan*

Table 5: *Summary of disease intensity at tehsil level*

Tehsil	Severity	Standard error	LCL	UCL
Karor lal esan	0.39 a	0.13	0.35	0.46
Layyah	0.36 a	0.11	0.31	0.41
Incidence				
Karor lal esan	0.78 a	0.12	0.73	0.82
Layyah	0.75 a	0.13	0.74	0.75

**Similar Letters are indicating non-significance between 2 means. LCL= lower confidence limit, UCL= Upper confidence limit. LCL and UCL are indicating confidence interval.*

Moran's Index inferred that average HLB disease prevalence, incidence and severity at all scales of survey showed spatial dependence when prevalence was high at a larger scale i.e. Tehsil level; it was at the same time elevated at village and field level (Table 6). These 3 levels of survey were clustered i.e. almost similar trend of disease distribution was recorded. A similar trend was found for disease incidence and severity at all locations; points with disease intensity values closer to others were clustered.

Table 6: *Spatial dependence of 3 disease intensity scales by Moran's Index analysis*

Frequency of survey	Moran's Index	Z score	P value	Spatial Pattern
Prevalence				
1	0.23	3.05	0.01	Clustered
2	0.24	3.11	0.01	Clustered
3	0.27	3.23	0.01	Clustered
4	0.28	3.29	0.01	Clustered
Incidence				
1	0.43	3.73	0.01	Clustered
2	0.46	3.89	0.01	Clustered
3	0.49	3.92	0.01	Clustered
4	0.53	4.21	0.01	Clustered
Severity				
1	0.65	4.67	0.01	Clustered
2	0.68	5.03	0.01	Clustered
3	0.69	5.65	0.01	Clustered
4	0.71	5.97	0.01	Clustered

**Z score higher than 1.96 indicates the significant clustering. Moran Index values in positive indicates significant spatial clustering. Clustering indicates close values (dependence) at a particular point.*

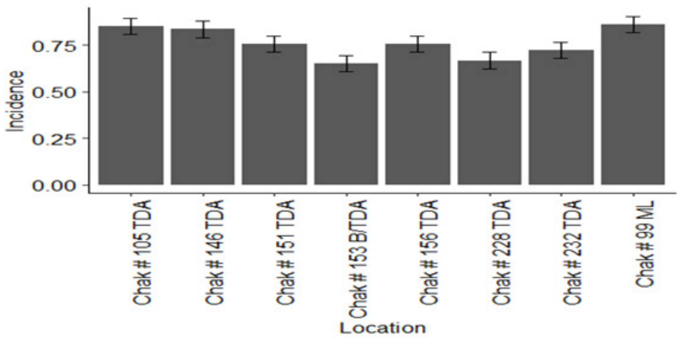

Figure 5: *Location wise comparison of HLB disease severity in both Tehsils*

The assessment of plants surviving ability was checked through Kaplan-Meier analysis; a marked difference of survived plants can be assessed after each day of observation (Figure 7). It explained that number of plants remain free from disease onset over time. At point 1 along Y axis it is indicating that 100% plants were free from disease and each down step showing the increasing in diseased plants. There was significant reduction in survival of plants after each observation; at the same time risk for disease initiation tends to be decreased after each observation as more plants are incited with disease symptoms. According to Kaplan-Meier analysis, it can also be concluded that died ratio increased with progress of time while censored units decreased at the same time.

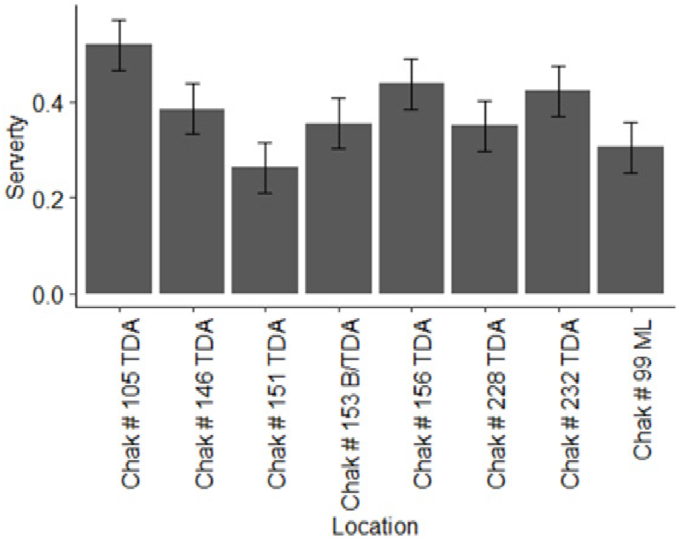

Figure 6: *Location wise comparison of HLB disease incidence in both Tehsils*

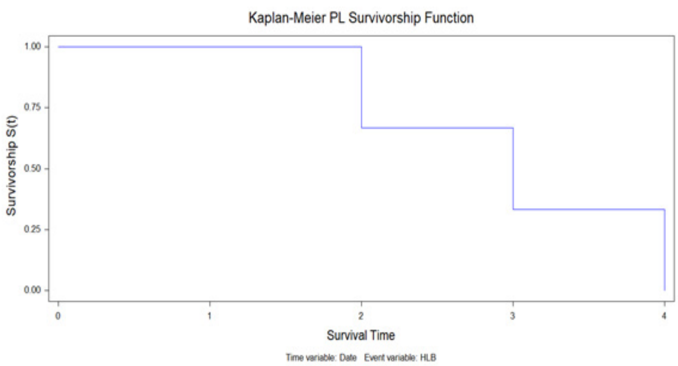

Figure 7: *Kaplan-Meier Survival for monitoring HLB disease progress*

Discussion

Citrus greening is prevalent worldwide having a vast range of nomenclature in different citrus growing areas incited by a fastidious bacterium. Mapping spatial distribution infers about the disease hotspots helps in efficient resource management (Mubeen *et al*., 2024). HLB disease variations are the consequence of changing weather conditions, insect infestation and management tactics used at each orchard. The variation in different locations could be attributed to the presence of insect vector influenced by the weather variables (Ali *et al*., 2023). HLB disease mapping and quantification was done by combining geographic coordinates with disease intensity from district to field levels (Guo *et al*., 2024). The results of current study has significant decisive role about time, location, and quantity of the management treatments; and to where and when conduct the disease risk survey (Wu *et al*., 2024). Disease intensity was increased with the passage of time and it was more in Tehsil Karor than Tehsil Layyah. It suggests the timely applications of insecticides to get rid from psyllid as the increasing intensity may be attributed to the elevated psyllid infestation. These results guide the growers about finding the more critical time period of the year when management options can be intervened. The variation in spatial distribution of disease especially in Karor necessitates the extensive monitoring of psyllid population. According to previous studies HLB disease disperses within a radius of 300-560m in a particular location (Olvera-Vargas *et al*., 2020). The variation in disease intensity at various locations is ascertained to be the effect of weather variables on the insect vector (Leong *et al*., 2022). The areas with high disease intensity have more vector population as compared with areas of low disease intensity. The abundance of insect vector population guarantees the more smooth entry of CLas into the host plants (Espinosa-Zaragoza *et al*., 2021). Remote sensing uses satellite images of and analyze by machine algorithms to infers about the presence or onset of a HLB disease (Menger *et al*., 2022). As the major management option for the HLB disease is the use of insecticides for the control of insect vector; the methods of application also affect the distribution of HLB disease within locations (Li and Nangong, 2022). Spatial and temporal disease variation is attributed to the factors influencing the host pathogen interaction. This finding has been previously supported by the literature as spatio-temporal progress of HLB was significantly affected by rainfall in different areas of Florida (Alves *et al*., 2022). Spatial distribution of HLB disease was assessed in various geographic regions of Brazil with the conclusion that central region has more disease intensity than southern. A detailed analysis indicated that there are variations in soil factors and weather variables due to which variations were recorded (Alves *et al*., 2020). Spatio-temporal studies for HLB disease distribution indicated that citrus greening disease was patchily distributed and followed a clustered pattern at the orchard, village, tehsil and District level scales (Pleydell *et al*., 2018; Bajwa *et al*., 2024). Several factors may be responsible for these hotspots which include the vector population density especially the Asian citrus psyllid, environmental factors and the level of management that is being practiced in the areas (Alonso-Hernández *et al.*, 2024). Local clustering of infections is more actionable than at state level for disease management (Fourie *et al.*, 2021). In this study, temporal analysis was conducted through which it was described that HLB disease outbreaks affected some seasons of the year much more seriously than others. This may be related to the life cycle of the Asian citrus psyllid (ASP) which is the principal vehicle for transmitting the disease (Richard *et al.*, 2018). The rate of HLB transmission depends on the timing of psyllid activity that means during the activity of higher numbers of psyllids at a certain period, the disease propagated more intensively (Alonso-Hernández *et al.*, 2024). This study showed that spatial distribution of HLB was influenced by climatic factors such as latitude and longitude, and other factors like type of soil, and practices adopted in the orchards (Martini *et al.*, 2020). The control measures for HLB comprised of insecticides against ASP, biological control agents, and disease resistant citrus varieties (Ghosh *et al.*, 2022). The chemical control has the disadvantage of development of resistance by pests and its effect on the environment with increased frequency and distribution density of the psyllid (Molina *et al.*, 2022). The fact that disease hotspots formed clusters indicates that present management approaches could be enhanced by using location data to deliver interventions that were more efficient, less costly and less invasive to the environment. The results showed that HLB disease prevalence, incidence and severity was spatially dependent in Tehsils. This cluster has significant role in HLB disease management which are opted at Tehsil level. The data from different locations within Tehsil indicated that some points are more vulnerable as compared to others. HLB disease mapping is beneficial for targeted disease management especially for high risk areas through early intervention (Luo *et al.*, 2023). Integrated disease management (IDM) minimizes pesticide usage, prevent pathogen resistance and reduce environmental deterioration (Sun *et al.*, 2024). The current study has also provided the base for conducting new field experiments in a wider range of locations for targeted and site specific management options for disease mitigation and sustainable citrus production (Chuang *et al.*, 2024). The study has marked the areas with high disease intensity and the timing of their appearance which would pave for early disease warning system to implement efficient action plans for sustainable citrus production.

Conclusions and Recommendations

The dynamics of citrus greening disease reflect spatial and temporal distribution of different factors such as the environment, vectors and management practices. It could be concluded that spatial disposition and temporal variation should be taken into account when designing disease control measures. Thus, we will be in a position to come up with more focused and location specific controls in expectation of improving the fight against HLB attacks and subsequently reducing the impacts on the citrus industries around the world. Plan the pesticide application when pest population is at peak during fall and spring. Use insecticide a 50-meter radius of the affected plants against psyllid. Map the disease hotspots by using GPS for a focused disease scouting and management. The spray application should be accomplished in a coordinated way to eradicate the vectors from whole area.

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Novelty Statement

The novelty integrates multiple population data, providing a more holistic view of the disease's impact and the potential for disease mitigation strategies. The application of novel diagnostic tools or techniques for early detection, enabling more precise and scalable management approaches against this devastating citrus disease.

Author's Contribution

Sonum Bashir: Conducted the research trial, writing-review and editing.
Yasir Iftikhar: Conceptualized and supervised the trial.
Mustansar Mubeen: Formal analysis, finalization, software, writing the original draft, writing-review and editing.
Muhammad Ahmad Zeeshan: Co-supervised the trial.
Talha Shafique: Visualization and statistical analysis
Muhammad Shah Jahan: Validation.
Ashara Sajid: Helped technical assistance for lab

analysis.

Data Svailability

Data sharing available on request.

Generative AI or AI assisted technology statement

The authors declare that no generative AI or AI-assisted technologies were used in the writing, analysis, or preparation of this manuscript.

Conflict of interest

The authors declare no conflict of interest.

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