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## MONITORING OF THE CITRUS GREENING DISEASE (HUANGLONGBING) IN RELATION TO ENVIRONMENTAL FACTORS IN PAKISTAN

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The environment plays a crucial role in disease development and their outbreaks. Understanding the pattern of disease progression requires close examination of environmental factors, which are strongly associated with disease severity. This study aimed to investigate the relationship between severity of the citrus greening disease, also known as Huanglongbing, and ecological variables specifically maximum and minimum temperatures, rainfall, and relative humidity using correlation and regression analysis. The analysis showed a significant correlation between the disease severity and these environmental factors. Correlation and regression equations were employed to develop a predictive model for disease progression. Results indicated that environmental conditions significantly influenced symptom expression, contributing to disease severity. The coefficient of determination ( $R^2$ ) values in regard to temperature dependence for the three citrus varieties i.e. Kinnow, Feutrell's Early, and Mosambi were 75%, 82%, and 83%, respectively. These findings confirm that environmental factors play a substantial role in the progression of Huanglongbing. The developed model can be applied to guide effective disease management strategies.

**Keywords:** Huanglongbing, environmental factors, correlation, regression

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### Introduction

The remarkable role of the citrus greening disease, also known as Huanglongbing (HLB), in citrus decline was noticed in major citrus-growing states of the USA in recent years where HLB has significantly damaged citrus plantations, leading to substantial losses in production (Mubeen et al., 2024). HLB is caused by a non-culturable, gram-negative bacterium that is transmitted through citrus psyllids as vectors and vegetative propagation. Environmental conditions influence the prevalence of various isolates of the HLB-associated bacterium and the corresponding strains of citrus psyllids in citrus-growing regions worldwide. The disease has caused considerable losses in both fruit quality and yield (Iftikhar et al., 2024, Hassan et al., 2025) and the average lifespan of citrus plantations has been reduced to 7–10 years (Jia et al., 2017). HLB continues to be a major concern for citrus production in key citrus-producing countries. In the USA, Florida the largest citrus-producing state has suffered economic losses exceeding \$5 billion due to HLB (Li et al., 2020). Once established in orchards, disease outbreaks are largely driven by abiotic factors; since 2005, the Florida citrus industry has experienced a 74% decline in production due to HLB outbreaks. Predicting the progression of disease incidence and severity is crucial for forecasting conditions conducive to outbreaks. A case

study in Florida showed that, after a slow initial progression, the disease spread at twice the rate and reached nearly 100% infection within 5 years (Singerman, Useche, 2016). This highlights the need to consider environmental factors when developing management strategies for HLB. The correlation between environmental factors and HLB incidence and severity has also been reported under Pakistani conditions (Iftikhar et al., 2017). Environmental factors significantly influence the development and transmission efficiency of citrus psyllid populations (Manjunath et al., 2008). Spatial and temporal assessments have been used to track HLB spread (Bashir et al., 2023). Understanding the relationship between environmental conditions and both disease progression and vector populations is essential for designing effective management strategies (Li et al., 2020). Several studies have highlighted these correlations in Sargodha (Punjab), Pakistan (Sajid, 2022). However, no published literature exists on HLB severity progression in newly established citrus plantations in southern Punjab, particularly in Layyah district. Therefore, this study offers novel insights into disease progression under the prevailing environmental conditions of Layyah, with the aim of developing a disease predictive model.

**Materials and Methods**

**Field Surveys**

A field survey was conducted in District Layyah to monitor severity of HLB and associated environmental factors in three citrus varieties: Kinnow, Feutrell’s Early, and Mosambi, referred to as variety 1, 2, and 3, respectively. The geographical coordinates of Layyah are 30.964750° N latitude and 70.939934° E longitude. The city of Layyah is located approximately 120 km northwest of Multan and 125 Km north of Dera Ghazi Khan. Disease incidence and disease severity were recorded and affected citrus plants were marked for future observations (Bashir et al., 2025).

**Disease Severity Index**

Disease severity on selected citrus trees in District Layyah was assessed over 2 years (2021–2022) using the rating

scale (Table 1) proposed by Akhtar and Ahmed (1999). The observations were made from January 2021 to December 2022 at 30 days interval, recording both disease severity and environmental conditions.

**Data analysis**

Environmental data, including maximum and minimum temperatures, rainfall and relative humidity were obtained from the Meteorological Section of Agriculture Department in District Layyah. Disease severity and environmental factors were analyzed using correlation and regression analysis to develop a disease predictive model for HLB progression. The analysis was performed by using Statistix 8.1 and SPSS software.

**Table 1.** Rating scale of Huanglongbing severity (Akhtar, Ahmed,1999)

Rating scale	Description
0	No symptoms
1	Up to 25 % of twigs and leaves with blotchy mottle symptoms
2	25 to 50 % of canopy with color inversion and lopsided fruit symptoms
3	51 to 75 % tree dieback
4	Over 75 % tree dieback

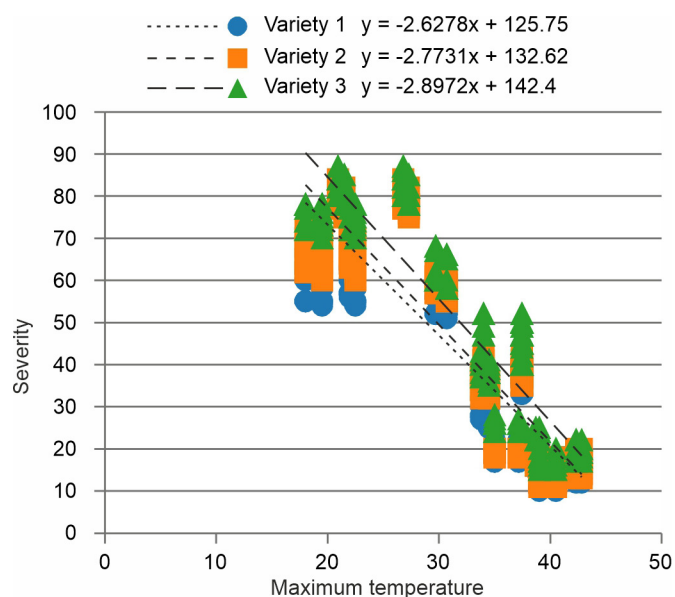
**Таблица 1.** Шкала оценки степени поражения цитруса позеленением цитрусовых (Akhtar, Ahmed, 1999)

Балл оценки	Описание
0	Нет симптомов
1	До 25% листьев и побегов с симптомами крапчатости
2	От 25 до 50% посадок с симптомами инверсии цвета и однобокого плода
3	От 51 до 75% отмирания деревьев
4	Более 75% отмирания деревьев

**Results**

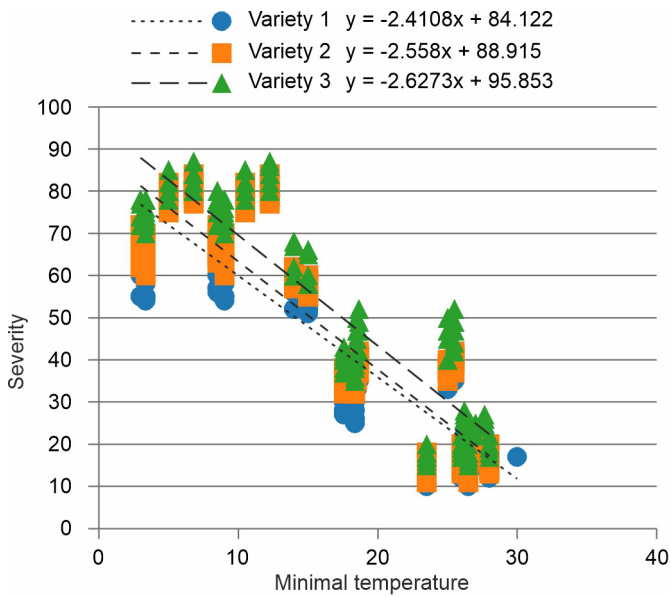
The effect of weather variables on HLB development in citrus plantations was assessed using regression analysis, which quantified the influence of temperature, rainfall and relative humidity on disease. The analysis compared the responses of 3 citrus varieties to different environmental factors associated with HLB. According to the regression results, all 3 cultivars showed a significantly negative relationship between maximum temperature and HLB (Figure 1).

The regression graphs clearly showed that HLB severity tends to decrease with an increase in maximum temperature and the varietal response followed the same trend. The slopes of regression lines indicate the relative response of varieties to maximum temperature; variety 3 exhibited strongest response, with the steepest slope (-2.93) and coefficient of determination (R<sup>2</sup>) of 83%. A strong relationship was also observed in varieties 2 and 1, with slope values of (-2.77 and -2.63) and R<sup>2</sup> values (80 and 77%), respectively. The steep slope of variety 3 is indicates a pronounced decrease in HLB as temperature increases. Overall trend of increasing temperature was similar for declining in HLB severity either for maximum and minimum temperature. There was consistency in all 3 cultivars with steep, steeper and steepest slopes, respectively showing strongly negative relationship for disease development with increase in minimum temperatures (Figure 2).



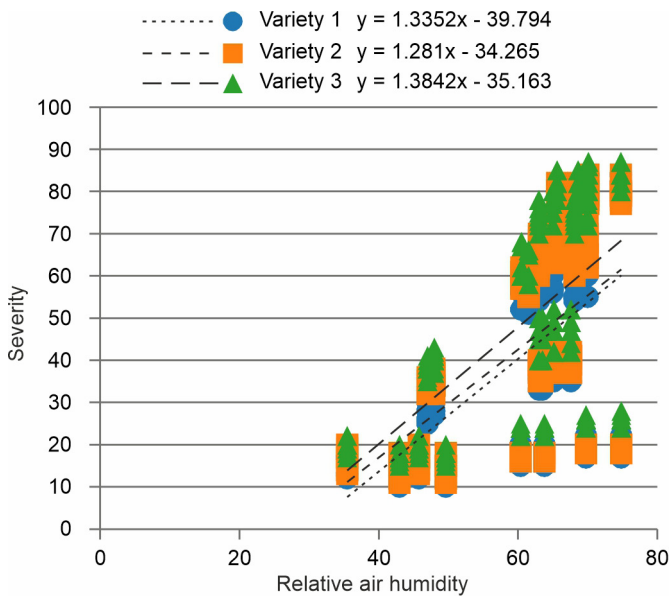
**Figure 1.** Relationship between maximum temperature and Huanglongbing severity in three citrus varieties

**Рисунок 1.** Связь между максимальной температурой и степенью поражения трёх сортов цитруса позеленением цитрусовых



**Figure 2.** Relationship between minimal temperature and Huanglongbing severity in three citrus varieties

**Рисунок 2.** Связь между минимальной температурой и степенью поражения трёх сортов цитруса позеленением цитрусовых



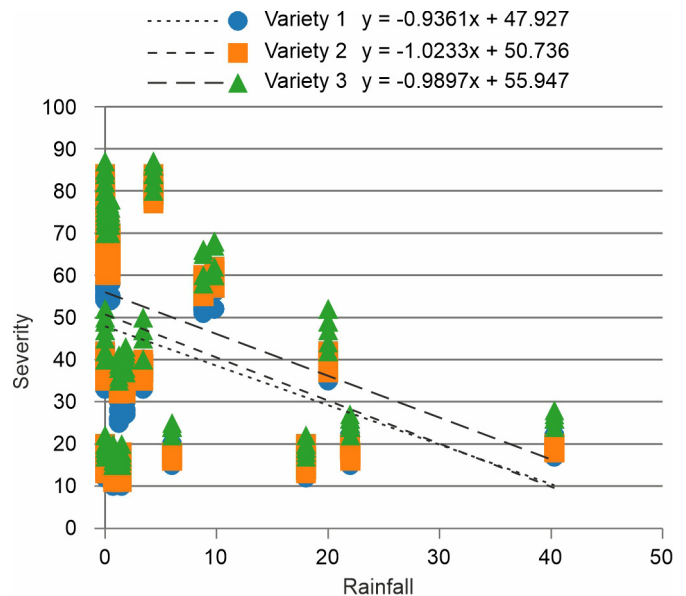
**Figure 3.** Relationship between relative air humidity and Huanlongbing severity in three citrus varieties

**Рисунок 3.** Связь между относительной влажностью воздуха и степенью поражения трёх сортов цитруса позеленением цитрусовых

The respective values for  $R^2$  indicate the fitness of the relationship with 75, 82 and 83 %, respectively for varieties 1, 2 and 3.

The relationship between HLB and relative air humidity (RH) was illustrated using regression trend lines for 3 cultivars. A positive relationship was observed between RH & HLB; with the disease severity elevating with the increase in RH. The more responsive variety was 3 followed by 1 and 2, respectively. The fitness of the regression model was moderate for RH as  $R^2$  values ranged from 0.31–0.35, for 3 varieties. The steepness values were positive for all varieties; with the steepest slope depicted by variety 3. The slope values indicate the relative increase in HLB severity with 1 unit increase in RH. The correlation coefficient ( $r$ ) values range from 0.56–0.59, indicating increasing trend with observations in red color with rise in relative humidity (Figure 3).

Rainfall negatively influenced HLB severity, as evident from the regression trend lines that a relative increase in rainfall leads to a decrease in severity of the disease. The fitness of the model was weak showing  $R^2$  values 0.16, 0.17 and 0.19, respectively for 3 varieties. The strongest varietal response was given by variety, 3 followed by 2 and 1, respectively. There were negative values of coefficient correlation for rainfall and HLB severity (-0.40, -0.44 and -.042) (Figure 4).



**Figure 4.** Relationship between rainfall and Huanglongbing severity in three citrus varieties

**Рисунок 4.** Связь между осадками и степенью поражения цитруса трёх сортов позеленением цитрусовых

**Discussion**

The outcomes of the present experiment signify the influence of weather variables i.e. temperature (maximum and minimum), relative humidity, rainfall and wind speed on Huanglongbing (HLB) development and severity. Both maximum and minimum temperature had a significantly negative impact on HLB severity. The strongly negative correlation indicated decrease in disease severity with increase in temperature. These findings are in line with earlier studies depicting that weather conditions influence activities of the pathogen and its vector. Godefroid (2023) found the highest

psyllid population infestation and subsequent incidence of HLB during winter, early and late summer seasons which have moderate temperatures. Temperature plays a vital role in symptom expression of citrus greening disease which is more obvious with decreasing temperature (Tipu et al., 2021). The areas with elevated temperatures exhibited more disease intensity of HLB because of high transmission efficiency of the vector and pathogen colonization ability in the host (Li et al., 2021). Hussain et al. (2022) recorded the psyllid population from HLB affected orchards, pointing out that there

was a reduced number of insects at temperature extremes due to increase in mortality. Due to a limited number of insect vector, the transmission efficiency also decreased; the ability of the pathogen *Candidatus liberibacter asiaticus* (CLAs) to multiply also decreases inside the host plant at high temperature (Hosseinzadeh and Heck, 2023). Huanglongbing was decreased due to increase in temperature because it has significant impact on CLAs, vector and plant behavior (Thakuria et al., 2023). The causal organism of HLB is affected at raised temperature as the bacterial ability to multiply seems to be inhibited above 35 °C; consequently, interruption with plant physiology decreases that results in less disease incidence (Zheng et al., 2024). Prolonged exposure at higher temperature for longer time causes loss in bacterial viability and its capacity for colonization in phloem of the host plant (Mishra and Ghanim, 2022). It was noted from the phloem studies that movement of photosynthates in phloem portion of vascular bundle increases result in low persistence of the bacterial pathogen inside the phloem that ultimately reduces the incidence of HLB (Raiol-Junior et al., 2021). High temperature boosts the underlying resistance mechanism of host plant and restricts the CLAs movement by triggering the heat-shock proteins and enhancing quantity of secondary metabolites (Shelake et al., 2024). The metabolism of host plants gets faster with increase in temperature that helps in healing of the phloem tissues destructed due to pathogen invasion that leads to mitigation of HLB (Limayem et al., 2024). CLAs latent period inside the vector is reduced at high temperature which disrupts its lifecycle and extent of transmission (Ammar et al., 2020). Layyah has hot dry summer where high temperature not only reduces the bacterial multiplication but also increasing insect mortality. Both factors; low insect population and limited multiplication of the pathogen resulted in decreased HLB severity with increasing in temperature. The optimum temperature for CLAs multiplication is almost 25 °C while Layyah exceeds 40 °C during peak summer months. The relationship between relative humidity and HLB determines directly proportional trend in all varieties. There was a significantly positive relationship i.e. HLB increased with high relative humidity. Relative humidity facilitates CLAs for its interaction with citrus plant and insect vector. It increases the movement of the pathogen and its ability to survive and colonize within host plant resulting in enhanced disease severity (Duan et al., 2021). Being an obligate pathogen, CLAs depends on the insect vector for entry into citrus plants. The elevated levels of humidity pave the easiness for its survival and growth inside and above the host (Andrade et al., 2020). The increase in HLB severity at high humidity can be attributed to the enhanced chances for bacterial persistence in saliva of insect vector (Ma et al., 2022). Orbović et al., (2023) described the more active states of psyllid in humid conditions and probed the improved reproducibility of the insect with enhancement in humidity. The increased population infestation results in a more successful transmission and higher disease severity. The increment in humidity has significant impact on plant defense response, i.e. plants experience stress conditions and predispose towards stresses. Such conditions may favor the bacterial invasion and contribute to the increased disease severity (Chowdhury et al., 2021). In humid environment stomata remain open for extended period

of times facilitating the establishment of more moisture in phloem of the plants that provides favorable environment for the bacterial residence (Maciag et al., 2024). Naz et al. (2024) unveiled the mechanism of plant defense, disclosing that the humidity levels affect the plant genes encoding for expression of salicylic acid biosynthesis upon the invasion of the biotic stress. These genes are down regulated during high humidity conditions that reduce the formation of pathogenesis-related (PR) proteins leading to decreased defense and increased severity of the disease. The increased disease severity at high relative humidity was observed because it suppresses the callose formation in phloem that is used by the host plant to block the movement of the pathogen (Gričar et al., 2022). Vayabari et al., (2023) concluded that plants availing ample quantity of water prefer the promotion of growth factors rather than production of lignin and suberin which are defense substances against stresses. This deficiency pushes the plants in a situation with reduced production of defense substances causing increased HLB severity. A decreased production of volatile organic compounds (VOCs) was observed under high humidity conditions. These VOCs attract natural predators towards plants that check the insect vector population within limits. Because of high infestation of insect vectors, more transmission and disease severity are recorded during humid conditions (Midzi et al., 2022). There was negative effect of rainfall (RF) on HLB severity despite rainfall contributes the moisture development. RF removes the insect vectors from host surface thus minimizing their infestation in plant microclimate reducing the chances for CLAs transmission and minimum disease severity (Leong et al., 2022). Rainfall reduces the activities of the insects by restricting the chances for feeding and reproduction rate (Gaire et al., 2022). Although rainfall causes a temporary increase in relative humidity but it indirectly reduces the HLB severity by washing off insects from the plant surface, reducing insect population and interrupting with transmission of CLAs. Rainfall events reduce the chances for insect landing on plants, its feeding capacity and reproduction ultimately lowering the severity. The lower R<sup>2</sup> values for rainfall and RH determine that HLB prediction is dependent upon multiple factors rather than these. Both rainfall and RH influence HLB disease but these are not sole determining factors. Prediction can be improved by considering multiple factors of host and pathogen along with weather conditions as a whole. This predictive model was developed by considering the local conditions of District Layyah, which is necessary for understanding of disease dynamics in specific environmental conditions. It provides a baseline to devise area wise management strategies particularly for new plantations. However, future models would be developed by incorporating disease scenario from varied areas and over longer time for better applicability. The overall relationship of HLB with environmental conditions was well explained by using linear models and the residuals fall within acceptable limits. As far as the multicollinearity is concerned, we have used separate regression analysis for each environmental variable so multicollinearity is not applicable. However, for a predictive model covering more spatio-temporal data; non-linear models would be more beneficial to explain the relationship between weather and HLB.

### Conclusions

The study focused on the complex interaction of environmental conditions on citrus Huanglongbing disease. The effect of maximum temperature, minimum temperature, relative humidity and rainfall was studied on the development of HLB. Elevated temperature (both maximum and minimum) inhibits CLas bacteria growth, reduce psyllid population, limit bacterial colonization within phloem and enhance plant defense mechanisms, thereby slowing disease progression. The

effect of humidity on HLB disease was positive and enhanced disease development with every unit increase in RH. Rainfall, although having direct relation with moisture suppressed the disease development through insect disruption and reducing its reproducibility. Acquaintance with local weather conditions would be beneficial in devising sustainable management decisions.

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Полнотекстовая статья

## МОНИТОРИНГ ПОЗЕЛЕНЕНИЯ ЦИТРУСОВЫХ С УЧЁТОМ ВЛИЯНИЯ ФАКТОРОВ ОКРУЖАЮЩЕЙ СРЕДЫ В ПАКИСТАНЕ

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Окружающая среда играет решающую роль в развитии заболеваний и их вспышек. Для понимания закономерностей этого процесса необходимо тщательное изучение факторов окружающей среды, которые тесно связаны со степенью поражения. Целью данного исследования было изучение взаимосвязи между степенью поражения цитруса бактериальным заболеванием – позеленением цитрусовых, и экологическими переменными, в частности, максимальной и минимальной температурами, количеством осадков и относительной влажностью, с использованием корреляционного и регрессионного анализа. Анализ показал значительную корреляцию между степенью поражения растений и этими факторами окружающей среды. Для разработки прогностической модели развития заболевания были использованы уравнения корреляции и регрессии. Результаты показали, что условия окружающей среды значительно влияют на проявление симптомов, усиливая степень поражения. Значения коэффициента детерминации ( $R^2$ ) в отношении температурной зависимости для трех сортов цитруса, а именно Кинноу, Фейтреллс Эрли и Мосамби, составили 75 %, 82 % и 83 %, соответственно. Эти результаты подтверждают, что факторы окружающей среды играют существенную роль в развитии позеленения цитрусовых. Созданная модель может быть использована для разработки эффективных стратегий борьбы с заболеванием.

**Ключевые слова:** позеленение цитрусовых, факторы окружающей среды, корреляция, регрессия

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