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Environmental Factors, Insect Vectors Dynamics of Viral Disease Burden in Chilli (*Capsicum annuum* L.) under Field Conditions

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Abstract

Viral diseases and insect vectors are major limitations for chilli (*Capsicum annuum* L.) production. Disease progression, genotypic variations, vectors counts and interactions with environmental factors were studied in 36 chilli genotypes under field conditions in Multan during 2025 and 2026 growing seasons. Weakly percentage disease index (PDI) and whitefly, aphid and thrips counts were recorded. Cumulative disease and vector burdens were determined by the area under the disease progress curve (AUDPC) and area under the vector population curve (AUVPC). Associations were assessed using Spearman correlation and linear regression, while hierarchical clustering characterized genotypic response patterns. Percentage disease index and vector counts increased over both seasons. Genotype AUDPC ranking were highly consistent across years ($r = 0.942$). Advanta 509 F1 and SV 7864 HM F1 had the lowest, whereas HP-264, Ghotki chilli, Longi and Bakhtawar Longi were among the highest disease burden. AUDPC was most strongly associated with whitefly AUVPC ($r = 0.942$) followed by aphids ($r = 0.747$) and thrips ($r = 0.579$). Minimum temperature was positively associated with PDI and vector abundance (correlation up to 0.72), while relative humidity showed contrasting seasonal associations (down to -0.50). Rainfall was positively correlated in 2025 with disease and vector (R^2 up to 0.82), whereas in 2026, it was not correlated with PDI ($R^2 = 0.00$). Disease based clustering identify five groups, and AUDPC tertiles distinguished three relative disease burden categories. These findings identify promising low burden genotypes for further evaluation and highlight seasonal association among disease, vectors and environment.

Keywords: *Capsicum*; Viruses; AUDPC; AUVPC; disease progression; genotype screening

1. Introduction

Chilli (*Capsicum annuum* L.) is one of the most economically important vegetables and spice crops in terms of both production and trade. About 25% of the world's population consumes it in either fresh or processed form on a daily basis (Mandal et al. 2023). Asia leads the way in production, contributing 65% to the global yield, India leads with 1.70 million tonnes, roughly 39% of world output followed by China, Bangladesh and Pakistan. Pakistan ranked eighth globally in chilli production in 2024, contributing approximately 2.2% of global production (FAOSTAT, 2024). According to national estimates chilli production subsequently reached 236,000 tonnes from 57,700 ha during 2025-26 (Pakistan Bureau of Statistics, 2026). The crop holds particular strategic importance in Punjab province, which provides the single largest share of national production and supports the livelihood of a substantial proportion of smallholder farming households. Beyond its economic role chilli is nutritionally dense rich in vitamins A, B complex, C, E, minerals, antioxidant carotenoids and capsaicinoids with documented analgesic and anti-inflammatory properties (Olatunji and Afolayan 2018). Yet it is its commercial value that makes the protection of production against biotic stresses a matter of national agricultural priority. Chilli productivity is still low (2.3 tonnes/ha) as compared to China (6 tonnes/ha) due to various viral diseases which have been a constant threat in Pakistan's chilli growing region despite favorable agro-climatic conditions (Rais et al. 2021).

To date, 75 viruses have been reported to infect chilli worldwide, of which 37 are formally recognized by the International Committee on Taxonomy of Viruses (Shingote et al. 2022). Among these *Begomoviruses*, *Potyviruses*, *Cucumoviruses* and *Orthotospoviruses* are the most economically important damaging disease complexes in chilli growing regions of South Asia (Devi et al. 2022). *Chilli veinal mottle virus* (*Potyvirus*) and *Cucumber mosaic virus* (*Cucumovirus*) are predominantly aphid transmitted, causing characteristic mosaic, mottling and vein banding symptoms that reduce photosynthetic area and impair reproductive development (Zohoungbogbo et al. 2024). The whitefly transmitted *chilli leaf curl Begomovirus* complex, induces severe upward leaf curling, stunting and floral abortion, while *Orthotospovirus* infections manifest as chlorotic ring spots and systemic necrosis. vectored by thrips. Once systemic infection is established, no curative treatment is available, and management relies entirely on preventive strategies, principally the use of resistant germplasm and the reduction of vector populations (Nalla et al. 2023). So, understanding the environmental/epidemiological factors that promote disease outbreak is a necessary step before designing timely/targeted interventions.

Viral disease outbreaks in chilli are strongly affected by the severity and temporal patterns of the disease outbreaks, which in turn are influenced by the prevailing environmental conditions, which also influence host-plant physiology, vector population growth rates and the efficiency of virus acquisition and inoculation (Mangang et al. 2024). Maximum and minimum temperatures influence vector proliferation, survival and dispersal. Relative humidity affects insect activity and survival. On the hand, rainfall may either suppress vector populations through wash off effects or favor disease development by promoting new vegetative growth and creating favorable microclimatic conditions (Jatoth et al. 2022). Consequently, these variables interact simultaneously, integrated evaluation of weather, vector populations and disease incidence provides a more reliable understanding of viral epidemiology than studying individual factors in isolation (Jeger et al. 2023).

Although previous studies have independently examined chilli viruses, insect vectors, and individual climatic variables. Integrated studies linking weather conditions, vector dynamics, viral disease development, and germplasm response remain scarce, particularly in Southern Punjab, Pakistan. Environmental variation is expected to differentially influence vector abundance and viral disease development, leading to distinct disease responses among chilli genotypes (Kushwaha and Kumar 2021). To address this knowledge gap, the present study investigated the relationships of maximum and minimum temperature, relative humidity, and rainfall with viral disease incidence and the abundance of whiteflies, aphids, and thrips, while evaluating the field response of 36 chilli genotypes over two consecutive growing seasons (2025-2026). This integrated assessment provides a basis for identifying resistant germplasm and improving vector and disease management in chilli.

2. Materials and Methods

2.1. Study site and plant material

Field experiments were conducted at the research field of Dept. of Plant Pathology, Muhammad Nawaz Shareef University of Agriculture, Multan, Punjab, Pakistan (30°08'33.3"N 71°26'40.5"E; Figure 1) to evaluate the effects of environmental factors on the development of major viral diseases of chilli during the 2025 and 2026 growing season. A total of 36 chilli genotypes were used in the present study, among them 10 hybrid hot pepper genotypes were obtained from the Vegetable Research Institute (Ayub Agricultural Research Institute, AARI), Faisalabad, and 26 genotypes were collected from the local market, comprising 18 hybrids hot pepper, 4 hybrids sweet pepper, and 4 desi type hot pepper genotypes (Table 1**)**. The collected genotypes represented diverse genetic backgrounds and were screened under open-field conditions for their response against major viral diseases of chilli.

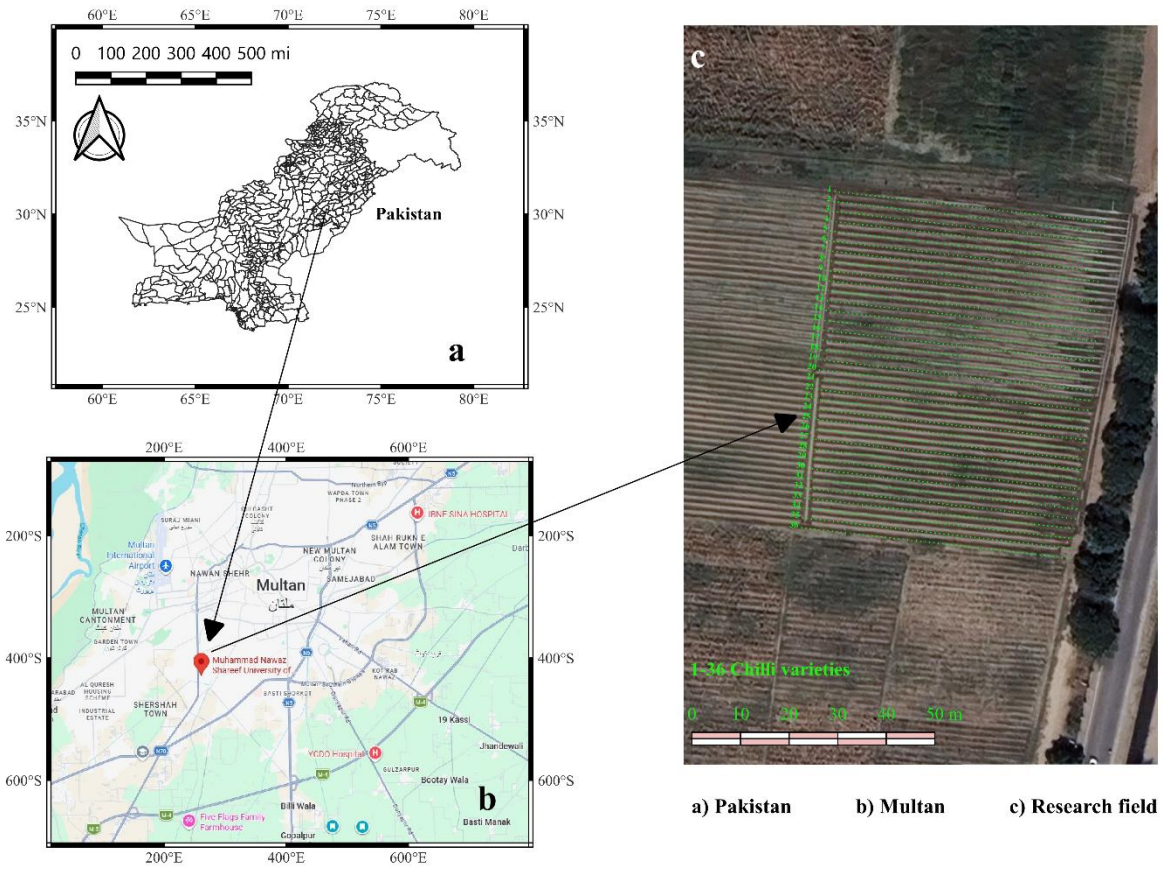


Fig. 1: Location and field layout of the experimental site used for screening chilli genotypes against viral diseases during the 2025 and 2026 growing seasons. (a) Map of Pakistan, (b) Location of the Muhammad Nawaz Shareef University of Agriculture, Multan, Punjab, Pakistan, and (c) Geographic location of research field and layout showing 36 chilli genotypes established under open-field conditions.

Table 1: Chilli genotypes used in this study and their respective sources.

Sr. No.	Genotypes Name	Types	Source/Company
1.	HP-1 (V2)	Lines (Hot Pepper)	AARI Faisalabad
2.	HP-2 (V4)	Lines (Hot Pepper)	
3.	HP-3 (V15)	Lines (Hot Pepper)	
4.	HP-4 (V7)	Lines (Hot Pepper)	
5.	HP-5 (V9)	Lines (Hot Pepper)	
6.	HP-6 (V16)	Lines (Hot Pepper)	
7.	HP-7 (V17)	Lines (Hot Pepper)	
8.	HP-8 (V26)	Lines (Hot Pepper)	
9.	HP-9 (V3)	Lines (Hot Pepper)	
10.	HP-10 (V5)	Lines (Hot Pepper)	
11.	MAS-2482 (V30)	Hybrid (Hot Pepper)	Kalash Seeds
12.	BSS-307 (V27)	Hybrid (Hot Pepper)	
13.	HP-042 F1 (V32)	Hybrid (Hot Pepper)	
14.	Advanta 5018 F1 (V29)	Hybrid (Hot Pepper)	ICI Pakistan (LCI)
15.	Advanta 509 F1 (V11)	Hybrid (Hot Pepper)	

16.	Sky Line 3 (V1)	Hybrid (Hot Pepper)	Seminis Vegetable Seeds
17.	Sky Red (V31)	Hybrid (Hot Pepper)	
18.	SV 7864 HM F1 (V25)	Hybrid (Hot Pepper)	
19.	HHP-082 (V6)	Hybrid (Hot Pepper)	Rasheed Seeds
20.	Sky star 5 F1 (V13)	Hybrid (Hot Pepper)	AgroTech Seeds
21.	HP-075 (V35)	Hybrid (Hot Pepper)	
22.	Sky star 4 F1 (V14)	Hybrid (Hot Pepper)	Green Gold Agri Seed (Pvt) Ltd.
23.	Golden Hero (V33)	Hybrid (Hot Pepper)	Royal Seeds
24.	Eagle F1 (V21)	Hybrid (Hot Pepper)	
25.	HP-264 (V36)	Hybrid (Hot Pepper)	National Seed Company
26.	Golden Hot F1 (V22)	Hybrid (Hot Pepper)	East West Seed International
27.	Shandar-F1 (V10)	Hybrid (Hot Pepper)	Rachna Seeds
28.	Shehzadi (V28)	Hybrid (Hot Pepper)	
29.	Bakhtawar Longi (V20)	Desi (Hot Pepper)	Greenlet International (Suncrop group)
30.	Ghotki Chilli (V18)	Desi (Hot Pepper)	Local Varieties
31.	Tatypuri (V24)	Desi (Hot Pepper)	
32.	Longi (V12)	Desi (Hot Pepper)	
33.	Super Globe-F1 (V23)	Hybrid (Sweet Pepper)	Rachna Seeds
34.	Ziniya (181) (V19)	Hybrid (Sweet Pepper)	Birkel Seeds
35.	Mahraja F1 (V8)	Hybrid (Sweet Pepper)	Mehr Muhammad Din & Sons Seeds, Pakistan
36.	HS-042 (V34)	Hybrid (Sweet Pepper)	

2.2. Experimental design and crop management

The nursery of chilli genotypes was raised following recommended agronomic practices, and healthy 40 days old seedlings were transplanted into the field on 15 January 2025 and 5 January 2026, following an augmented design (Naz et al. 2006; Mahardika and Waluyo 2025). All recommended cultural and agronomic practices including land preparation, fertilizer application, irrigation, weed management, rouging and field sanitation were carried out throughout the experimental period (Glodjinon et al. 2021).

2.3. Disease assessment

After the appearance of typical viral symptoms such as mosaic, leaf curling, chlorosis, mottling and stunted growth, the percentage disease index (PDI) of virus(es) infection was recorded from symptomatic plants. PDI was calculated using the below given formula. PDI data was recorded using the disease rating scale described in Table 2 (Jamir et al. 2020; Loona et al. 2025).

$$\text{Percentage Disease Index (\%)} = \frac{(\text{Severity score} \times \text{Number of infected plants})}{(\text{Total no. of plants observed} \times \text{Maximum rating scale number})} \times 100$$

Table 2: Percentage Disease Index Rating Scale (0-5)

Scale	Symptoms	Remarks
0	No infection	Highly resistant
1	0-10% infection	Resistant
2	11-20% infection	Moderately resistant
3	21-30% infection	Moderately susceptible
4	31-50% infection	Susceptible
5	>50 infection	Highly susceptible

(Ashfaq et al. 2007)

2.4. Insect vector monitoring

Populations of the three principal insect vectors, i.e. whitefly, aphids and thrips, were assessed weekly on a per-plant basis at each observation date according to Mario's method (Nazir et al. 2017). For each genotype, insects count per plant were recorded concurrently with disease severity to facilitate temporal correlation between vector and disease progression. Assessments were conducted in the early morning to minimize disturbance-induced dispersal and maintain consistent sampling conditions across observation dates (Kumar et al. 2022).

2.5. Weather data collection

Weekly meteorological data for the experimental site were obtained from the NASA Prediction of Worldwide Energy Resources (POWER) database using the Data Access Viewer (DAV), covering the assessment periods from 27 March to 15 May 2025 and from 8 March to 3 May 2026. (https://power.larc.nasa.gov/data-access-viewer/). The weather data recorded were the maximum temperature (°C), minimum temperature (°C), relative humidity (%) and rainfall (mm). The daily observations were aggregated following the respective disease and vector observation periods and weekly mean values were determined for each meteorological variable. These weekly mean weather data were then employed to evaluate the association of these with PDI and insect vector count in chilli under open field condition.

2.6. Statistical analysis

2.6.1. Data Preparation

All the data sets were checked for completeness and consistency before statistical analysis. In 2025 and 2026, PDI values were summarized at the plot level for each of eight assessment weeks, as were numbers of whitefly, aphid and thrips. The AUDPC and the AUVPC were used to quantify the disease progression and the abundance of the vectors, respectively. Correlations among environmental variables, disease severity and insect population were made for each week and genotype specific linear regression models were fitted for each growing season. Repeated plant and leaf observations were considered subsamples and not replicates (Subhavyuktha et al. 2024) (Singh et al. 2024).

2.6.2. Statistical Software and Data Visualization

Data analysis was accomplished with the R v 4.5.0 software (R Core Team 2025). Base R functions were used for calculating Spearman's correlation coefficients and the corrplot package (He 2024) was used to generate the

correlation heatmaps. Linear regression analyses were computed using the lm () function in the stats package and scatter plots with fitted linear regression were created using ggplot2 (Tollefson 2021). Standardized variables, Euclidean distances, and Ward.D2 linkage were used to determine standardized variables for hierarchical clustering.

3. Results

3.1. Variation in environmental factors during the study period

The environmental conditions during the 8 weeks' assessment period were quite different in both study years. In 2025, maximum and minimum temperatures ranged from 35.83 to 45.34℃ and 16.29 to 27.96℃, respectively, while relative humidity varied from 11.41 to 33.69%. Rainfall remained generally low, ranging from 0.00 to 0.93 mm. In 2026, maximum temperature ranged from 30.33 to 42.12℃ and minimum temperature from 15.86 to 28.75℃, whereas relative humidity ranged from 22.53 to 45.08%. Rainfall varied from 0.02 to 2.23 mm during the assessment period. Overall, the recorded environmental conditions showed distinct inter-annual variation, providing a variable climatic background for assessing temporal changes in disease severity and insects count.

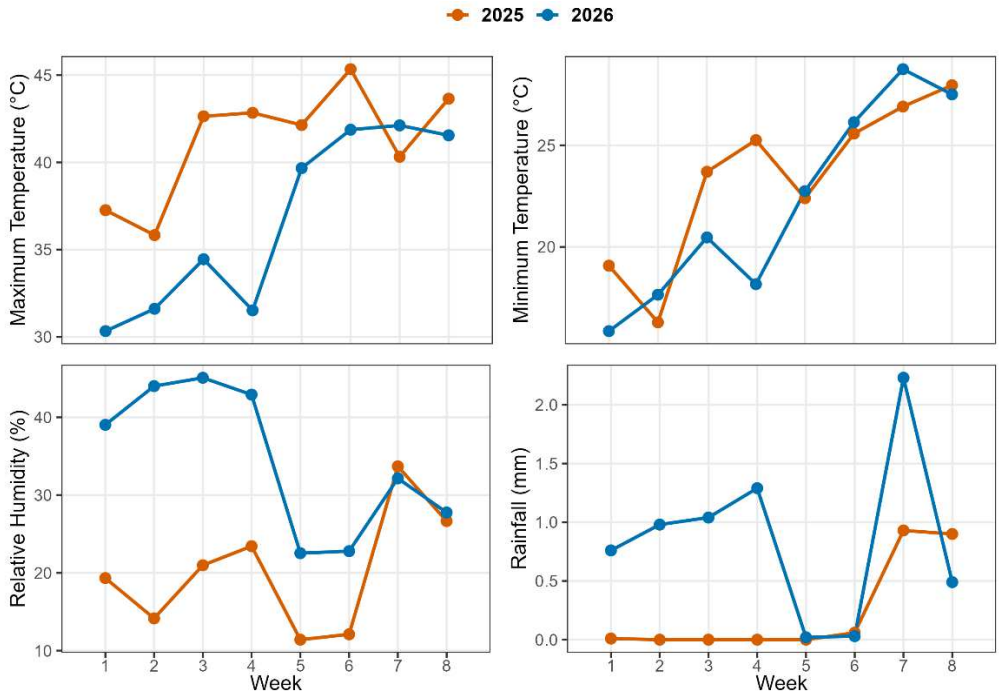


Fig. 2: Weekly comparison of environmental factors during the 2025 (27 March to 15 May) and 2026 (8 March to 3 May) cropping seasons.

3.2. Effect of Temperature on PDI and Vector Counts

3.2.1. Maximum temperature

Maximum temperature was positively correlated with PDI (r = 0.41 and 0.54), whitefly (r = 0.52 and 0.61), and aphid counts (r = 0.49 and 0.58) in 2025 and 2026, respectively, whereas associations with thrips were weak (r = 0.21-0.22; Figure 3). Genotype specific regressions showed positive PDI responses in HP-1 (R^2 = 0.63-0.81) and HP-6 (R^2 = 0.46-0.83), compared with Sky-star 4 F1 (R^2 = 0.22-0.62). Whitefly associations were evident in Sky-star 4 F1, HHP-

082, and Shehzadi (R^2 = 0.28-0.85), while aphid responses varied among HP-5, BSS-307, and Sky-star 4 F1 (R^2 = 0.09-0.80). Thrips generally showed negligible relationships, except in Bakhtawar Longi and HHP-082 (R^2 = 0.28-0.93). Detailed regression results are presented in Supplementary Table S1.

3.2.2. Minimum temperature

Minimum temperature showed strong positive correlations than maximum temperature with PDI (r = 0.55 and 0.60), whitefly (r = 0.72 and 0.67), and aphids (r = 0.64 and 0.63) in 2025 and 2026, respectively, while correlations with thrips remained weak (r = 0.35 and 0.22; Figure 3). Regression analysis revealed substantial PDI associations in Shehzadi (R^2 = 0.80-0.87) and HP-7 (R^2 = 0.70-0.90), compared with Sky-star 4 F1 (R^2 = 0.46-0.73). Whitefly responses were pronounced in HP-042 F1 and BSS-307 (R^2 = 0.70-0.91), whereas aphid associations were strong in MAS-2482 (R^2 = 0.81-0.85). Thrips responses were generally limited, except in Bakhtawar Longi and HP-042 F1 (R^2 = 0.50-0.81). Detailed regression results are presented in Supplementary Table S1.

3.3. Effect of Relative Humidity on PDI and Vector Counts

Relative humidity showed a weak positive correlation with PDI in 2025 (r = 0.19), which reversed in 2026 (r = -0.40). Similar reversals occurred for whitefly (r = 0.28 to -0.45) and aphids (r = 0.22 to -0.50), whereas thrips remained weakly associated (Figure 3). Genotype specific regressions confirmed contrasting annual patterns, particularly for PDI in HP-8, Tatypuri, and Super Globe-F1 (R^2 = 0.07-0.50), whitefly in HP-8, Longi, and HP-2 (R^2 = 0.08-0.57), and aphids in MAS-2482, HP-10, and HP-9 (R^2 = 0.00-0.87). Thrips relationships were generally negligible, although isolated genotypes exhibited higher R^2 values (up to 0.87). Detailed regression results are presented in Supplementary Table S2.

3.4. Effect of Rainfall on PDI and Vector Counts

Rainfall was positively correlated with PDI (r = 0.36), whitefly (r = 0.49), aphids (r = 0.44), and thrips (r = 0.31) in 2025, whereas correlations were negligible in 2026 (r = -0.14 to -0.04; Figure 3). Regression analysis showed strong associations in 2025 for PDI in HP-10 and MAS-2482 (R^2 = 0.54-0.69), whitefly in Ghotki Chilli and MAS-2482 (R^2 = 0.62-0.82), and aphids in Ghotki Chilli and Bakhtawar Longi (R^2 = 0.62-0.73). These relationships weakened considerably in 2026. Thrips generally exhibited weak associations, except for HP-10 and Sky star 5 F1 in 2025 (R^2 = 0.60–1.00). Detailed regression results are presented in Supplementary Table S2.

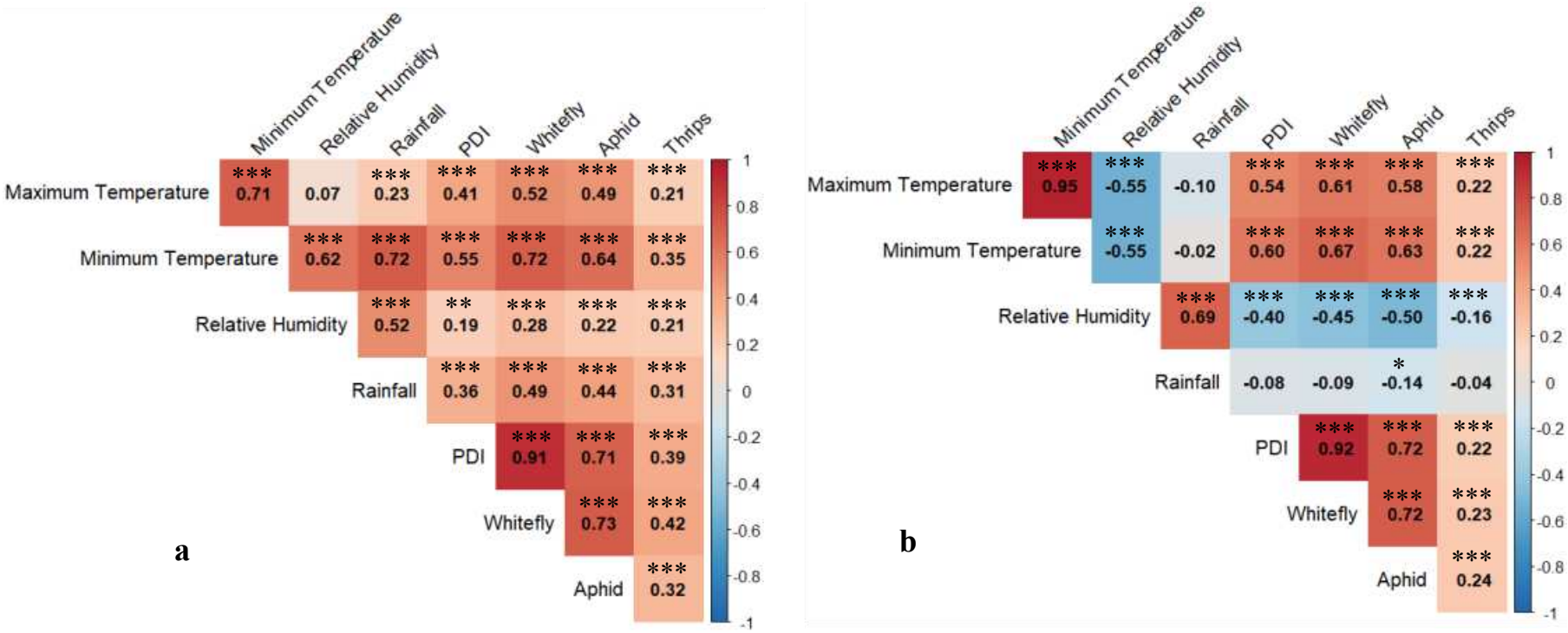


Fig. 3: Spearman's correlation heatmaps illustrating the relationships among environmental variables, percentage disease index (PDI), and insect vector counts during 2025 (a) and 2026 (b). The matrix depicts pairwise correlation coefficients between maximum, minimum temperature, relative humidity, rainfall, percentage disease index (PDI), whitefly, aphid and thrips counts. Warmer colors indicate stronger positive correlations, whereas cooler colors represent negative correlations. ρ values are shown within cells. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

3.5. Temporal Progression of Percentage Disease Index and Insect Vector counts

PDI increased progressively across the eight weekly assessments in both seasons (Figure 4 (a); Table 3). In 2025, mean observed PDI increased from 6.29% in week 1 to 43.93% in week 8, compared with 3.50% and 36.79%, respectively, in 2026. A repeated measures model with first order autoregressive correlation identified a significant effect of assessment week ($F_{(7,490)} = 187.92$, $p < 0.001$). However, neither the overall year effect ($F_{(1,70)} = 2.03$, $p = 0.159$) nor the year × week interaction ($F_{(7,490)} = 0.97$, $p = 0.455$) was significant. Thus, despite numerically higher observed disease levels in 2025, the analysis did not establish a significant difference between years. Plot cluster bootstrap 95% intervals for the weekly estimates are presented in Table 1. Whitefly, aphid, and thrips counts also increased steadily in eight weeks of evaluation for both growing seasons (Figure 4 (b); Supplementary Table S3). Whitefly increased from 2.214 to 24.094 insects per plant in 2025 and from 1.081 to 20.422 in 2026. Corresponding increases were recorded for aphids (0.586-4.162 and 0.244-3.044) and thrips (0.093-1.224 and 0.047-0.989), respectively. Whiteflies were always most prevalent followed by aphids and thrips. The AUVPC was used to quantify cumulative (total) vector counts. Count data models were not well suited to the diagnostic assumptions and hence were interpreted descriptively.

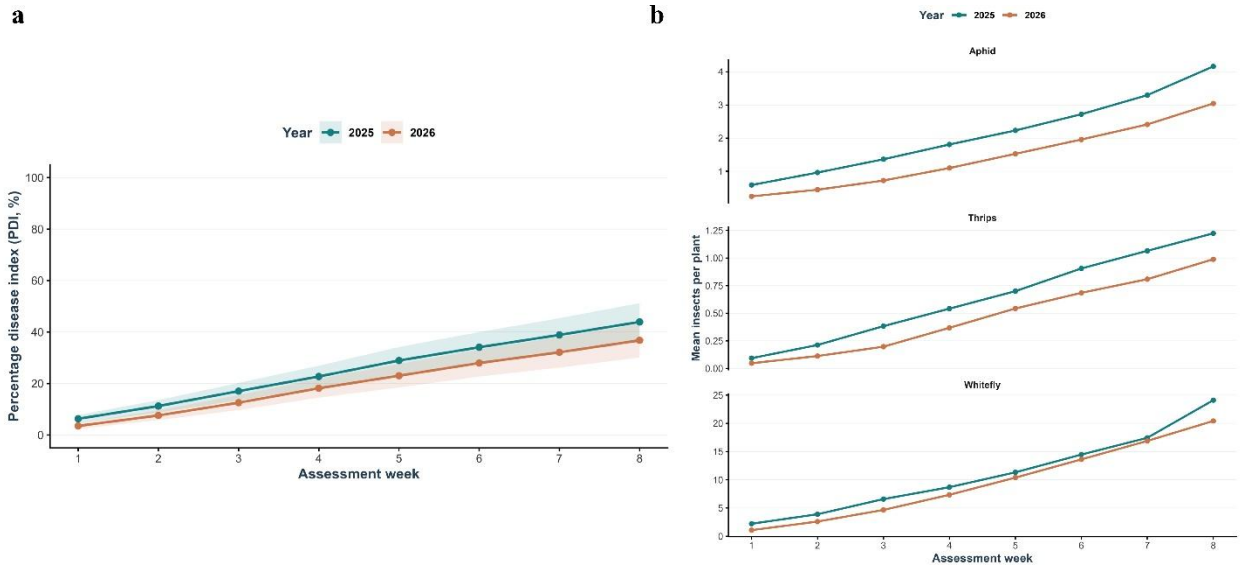


Fig. 4: Temporal dynamics of disease severity and insect vector populations across the 2025 and 2026 growing seasons. **(a)** Overall observed percentage disease index (PDI) by assessment week and year across 36 chilli genotype plots per year. Shaded ribbons represent 95% percentile confidence intervals estimated from 2,000 plot-cluster bootstrap resamples, preserving the eight-week trajectory of each plot. **(b)** Mean weekly populations of whiteflies, aphids, and thrips per plant during the 2025 and 2026 growing seasons. Each insect vector panel is presented with an independent y-axis scale.

Table 3: Overall PDI by year and assessment week

Week	2025 PDI (%)	95% CI	2026 PDI (%)	95% CI
1	6.29	4.72–7.73	3.50	2.34–4.68
2	11.24	8.71–13.54	7.59	5.79–9.63
3	17.03	13.50–20.22	12.55	9.68–15.49

4	22.72	18.13–26.88	18.16	14.51–21.84
5	28.93	23.51–34.16	23.03	18.48–27.57
6	34.09	27.90–39.97	27.95	22.71–33.18
7	38.87	31.82–45.37	32.12	26.20–38.00
8	43.93	35.95–51.21	36.79	30.12–43.07

3.6. Genotypic Variation in Disease Burden

Genotype level disease burden, expressed as the two year mean AUDPC, varied substantially among the 36 chilli genotypes (Figure 5 (a); Table 4; Supplementary Table S4). The lowest observed AUDPC values were recorded for Advanta 509 F1 (V11; 0.0) and SV 7864 HM F1 (V25; 0.0), followed by Golden Hot F1 (V22; 6.2), HP-8 (V26; 11.7), and Sky Star 4 F1 (V14; 15.2). Conversely, the highest AUDPC values were observed in HP-264 (V36; 303.1), Ghotki Chilli (V18; 298.2), HS-042 (V32; 294.0), Longi (V12; 288.8), and Bakhtawar Longi (V20; 273.2). The corresponding final PDI and cumulative insect abundance profiles are presented in Table 2. These differences reflect observed screening performance rather than statistically tested genotype contrasts, as each genotype was represented by a single field plot per year. Comparison of raw and common check adjusted AUDPC indicated that within year genotypes rankings shifted by no more than three positions (Supplementary Table S5), suggesting that the rankings were relatively insensitive to this adjustment. Nevertheless, raw AUDPC was retained as the primary outcome because the common check also served as a spreader.

3.7. Cross Year Consistency in AUDPC Based Genotype Rankings

The genotype ranking based on observed AUDPC was highly consistent between 2025 and 2026 (Spearman r = 0.942, n = 36, $p < 0.001$; Figure 5 (b)). This strong positive association does not imply stability of relative disease burden patterns across untested sites or conditions, but does suggest similar patterns at the study site in the two seasons.

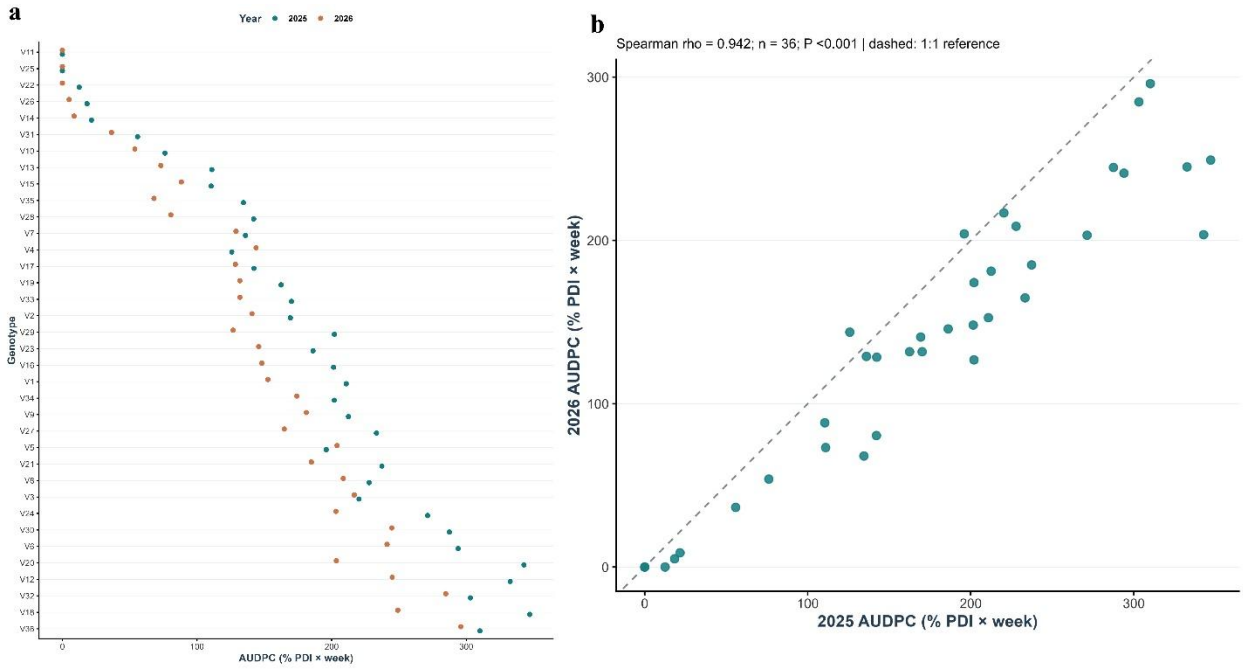


Fig. 5: AUDPC of the 36 chilli genotypes measured for two consecutive years (2025 and 2026). One field plot per genotype per year **(a)**. The line of equality (1:1) is shown as a dashed line **(b)**.

Table 4: Selected low and high AUDPC genotype profiles

Genotype	AUDPC	Final PDI (%)	Whitefly AUVPC	Aphid AUVPC	Thrips AUVPC
V11	0.0	0.0	20.3	6.4	1.7
V25	0.0	0.0	14.7	4.3	0.0
V22	6.2	2.5	11.9	5.6	0.0
V26	11.7	6.7	15.7	5.1	0.5
V14	15.2	6.0	23.9	5.3	0.0
V36	303.1	70.7	130.0	23.3	6.1
V18	298.2	68.3	156.6	19.7	5.5
V32	294.0	71.5	99.8	5.6	4.1
V12	288.8	69.8	114.0	19.4	3.0
V20	273.2	69.7	119.9	24.5	11.6

3.8. Associations Between Disease Burden and Insect Vector Abundance

Across 36 two-year genotype mean profiles, AUDPC showed strong positive correlations with whitefly AUVPC (r = 0.942, BH-adjusted $p < 0.001$), followed by aphid (r = 0.747, $p < 0.001$) and thrips AUVPC (r = 0.579, $p < 0.001$; Figure 6). Final PDI exhibited similar associations with whitefly (r = 0.916), aphid (r = 0.709), and thrips AUVPC (r = 0.549). AUDPC was also strongly correlated with final PDI (r = 0.986), as both metrics were derived from the same disease assessments. Year specific correlations are presented in Supplementary Table S4.

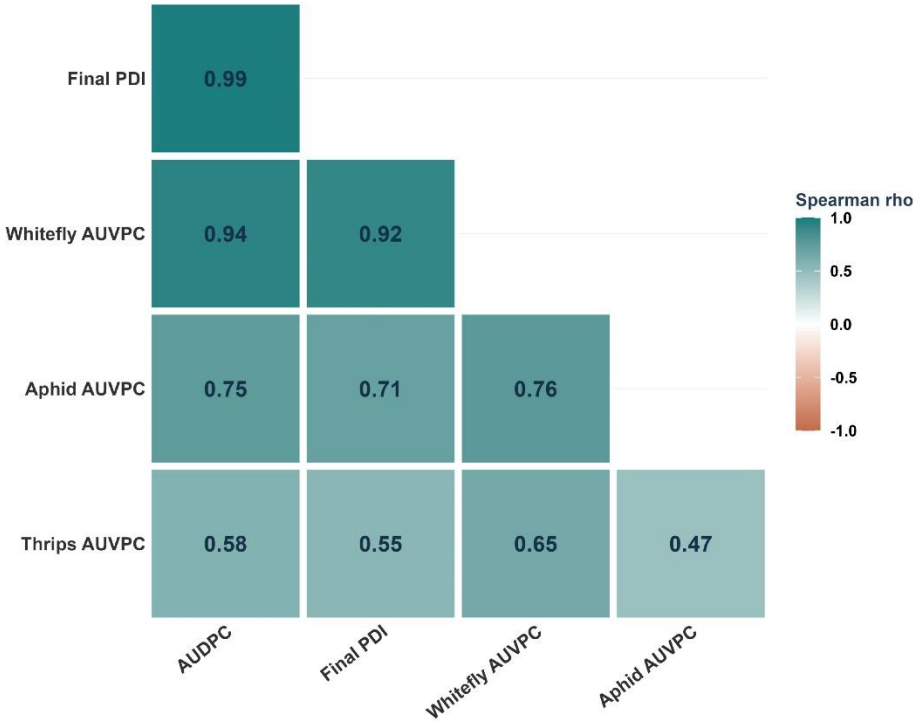


Fig. 6: Spearman correlation matrix of AUDPC with final PDI and cumulative insect count (whitefly, aphid and thrips AUVPC with final PDI) of 36 chilli genotypes based on the mean over two years.

3.9. Multivariate Clustering of Disease and Insect Vector Burdens

The AUDPC and whitefly/aphid/thrips AUVPC were standardized for each respective species and the resulting data were used to create two disease vector groups for hierarchical clustering (Figures 7 (a)). The two cluster solution had the largest mean silhouette width (MSW: 0.395) compared to three clusters (MSW: 0.389) (Supplementary Table S5). Eight genotypes were included in C2 with higher mean AUDPC (269.09) and final PDI (66.65%) while 28 genotypes were assigned to C1 with lower values of mean AUDPC (128.53) and final PDI (32.85%) (Table 5). C2 also had higher cumulative abundances for all three groups of insects. The standardized trait heatmap (Figure 7(b)) further illustrated the separation between clusters, with C2 characterized by relatively higher disease and insect burden profiles and C1 showing comparatively lower values across the four clustering variables. These clusters represent descriptive disease vector profiles rather than independently validated resistance categories.

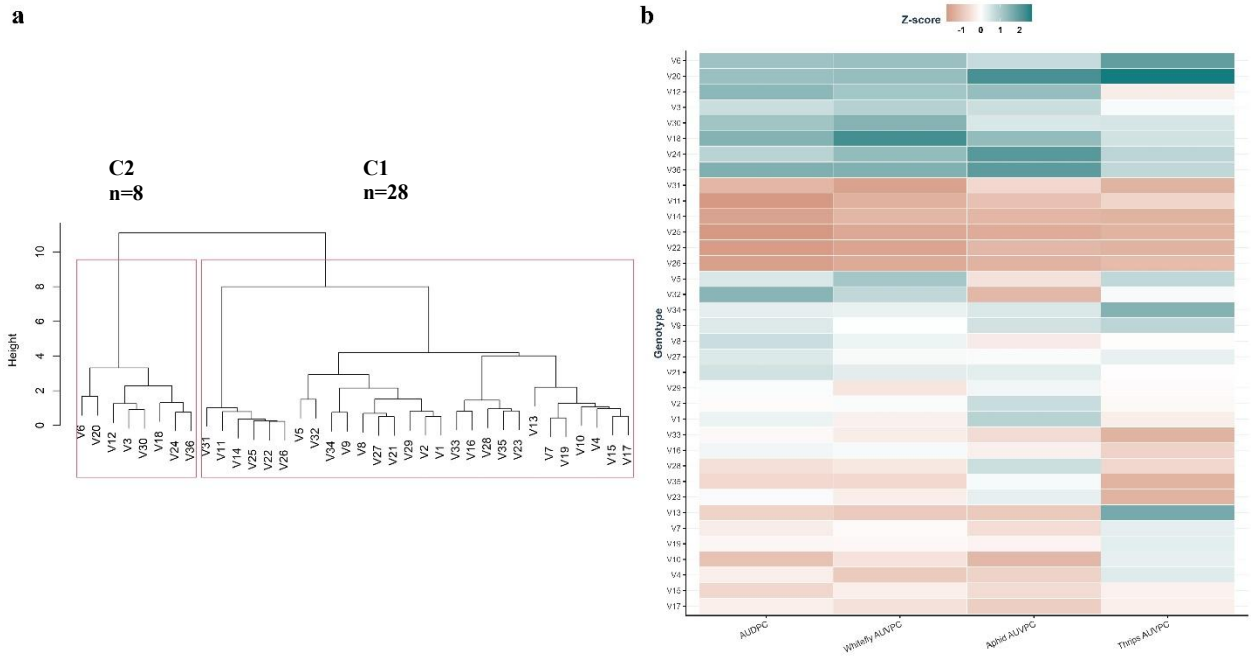


Fig. 7: Hierarchical clustering and standardized trait profiles of 36 chilli genotypes. **(a)** Ward.D2 hierarchical clustering based on standardized AUDPC and whitefly, aphid, and thrips AUVPC using Euclidean distance, with the optimal number of clusters determined by mean silhouette width. **(b)** Heatmap showing standardized percentage disease index and insect vector counts across 36 chilli genotypes arranged according to hierarchical clustering. Colors represent trait-specific Z-scores.

Table 5: Multivariate cluster profiles

Cluster	n	AUDPC	Final PDI (%)	Whitefly AUVPC	Aphid AUVPC	Thrips AUVPC
C1	28	128.5	32.85	55.7	10.2	3.1
C2	8	269.1	66.65	124.2	19.7	6.4

3.10. Disease Based Clustering and Relative Disease Burden Classification

Disease based hierarchical clustering using standardized AUDPC and final PDI identified five descriptive groups among the 36 chilli genotypes (Figure 8 (b)). The five clusters solution yielded the highest mean silhouette width (0.579), although comparable values were obtained for four (0.572) and six clusters (0.575), indicating limited differences in clustering quality among these solutions (Supplementary Table S5). Comparison with the combined disease vector clustering revealed differences in genotype grouping, with the distribution of assignments presented in Supplementary Table S6. Independently, classification based on two year mean AUDPC divided the genotypes into three equally sized categories representing lower, intermediate, and higher disease burden (n = 12 per category; Figure 8 (a)). These categories reflect relative differences in observed disease burden, whereas the hierarchical clusters describe similarities in combined disease metrics. Neither classification represents independently validated resistance or susceptibility categories.

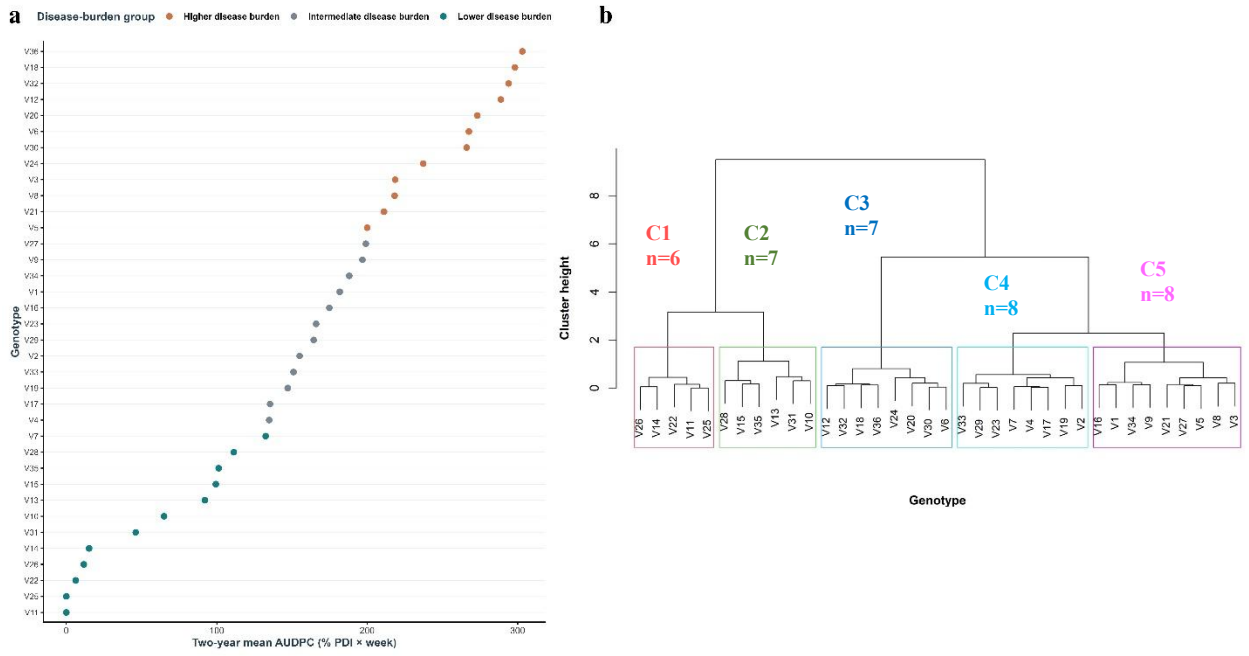


Fig. 8: Relative disease burden classification and disease-based hierarchical clustering of 36 chilli genotypes. **(a)** Classification of genotypes into low, intermediate, and high relative disease burden groups based on tertiles of the two-year mean AUDPC, with genotypes ranked in ascending order of AUDPC. **(b)** Ward.D2 hierarchical clustering using Euclidean distances calculated from standardized two-year mean AUDPC and final PDI. Five clusters were identified based on the highest mean silhouette width among the evaluated solutions (k = 2–6). Colored rectangles indicate cluster membership, while dendrogram height represents dissimilarity among genotype profiles. Both classifications are descriptive and do not represent independently validated resistance or susceptibility categories.

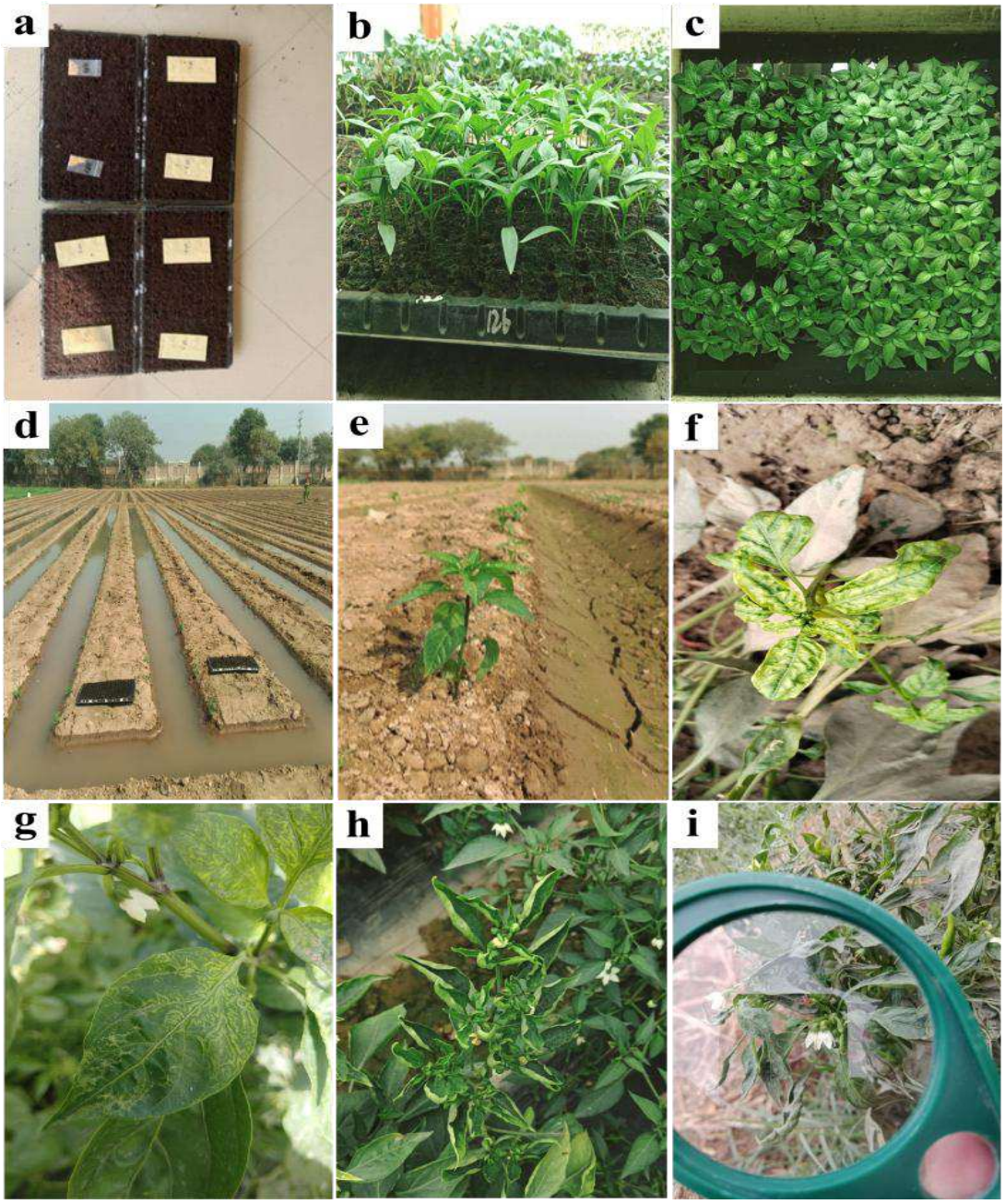


Fig. 9: Sequential representation of chilli cultivation and disease and their vector assessment under experimental field conditions. **(a-c)** Nursery establishment and production of chilli seedlings; **(d)** field preparation and irrigation; **(e)** transplanting of chilli seedlings; **(f)** leaf mosaic and yellowing **(g)** Vein clearing and chlorosis, **(h)** leaf curling and distortion, expressed on chilli plants under field conditions; **(i)** magnified observation of insect vectors for field diagnosis.

4. Discussion

significant differences in disease incidence and insect vector counts among the 36 chilli genotypes tested under cultivation in 2025 and 2026 growing seasons. In both seasons, PDI continued to build up and there was a very consistent rank order of the genotypes. Whitefly was the species that was most strongly associated with cumulative disease burden, followed by aphids and thrips. The results are a reminder of the need to consider disease development, genotype specific responses, and vector counts when assessing chilli germplasm under field conditions.

4.1. Environmental Influences on Disease and Vector counts

Environmental conditions may contribute to the observed disease and vector dynamics, although their relationships can vary among seasons and insect groups. In the present study, maximum temperature was positively associated with PDI (r = 0.41 in 2025 and 0.54 in 2026), whitefly abundance (r = 0.52-0.61), and aphid abundance (r = 0.49-0.58), whereas associations with thrips were weaker (r = 0.21-0.22). Genotype specific regressions indicated increases in PDI of 12.04 and 8.55 units per 1°C in 2025 and 2026, respectively, with corresponding R^2 values of 0.63 and 0.81. These observations are broadly consistent with Sujatha et al. (2025); Kumari et al. (2027); Misal et al. (2016); and Iftikhar et al. (2017) who reported positive relationships between maximum temperature and whitefly abundance in chilli. For aphids, (Bhatt and Karnatak 2020) reported a positive temperature association, whereas Havanoor and Rafee (2018) and Khatun et al. (2023) observed comparatively weak relationships. Similarly, positive associations between maximum temperature and thrips were reported by Jayewar et al. (2018) and Patel et al. (2009), while Borgohain et al. (2024) observed a negative relationship. These contrasting findings suggest that temperature responses depend on local conditions and vector biology.

Minimum temperature showed stronger positive associations with PDI (r = 0.55-0.60), whiteflies (r = 0.67-0.72), and aphids (r = 0.63-0.64), whereas relationships with thrips remained relatively weak (r = 0.22-0.35). Genotype-specific regression slopes indicated increases in PDI of 12.72 and 9.05 units per 1°C in 2025 and 2026, respectively, with R^2 values of 0.80 and 0.87. Positive relationships between minimum temperature and whitefly populations have also been documented by Misal et al. (2016), Iftikhar et al. (2017) and Kumari et al. (2027). For aphids, the present relationships were comparable to those reported by Bhatt and Karnatak (2020), although Khatun et al. (2023) observed a weaker association. Thrips responses have likewise varied among studies, with positive relationships reported by Jayewar et al. (2018) and Kumari et al. (2027), but negative relationships reported by (Deepak et al. 2019). Satar et al. (2008) demonstrated that aphid developmental time declined from 13 days at 15°C to 4 days at 30°C, while the intrinsic rate of increase rose from 0.16 to 0.50 before declining at 32.5°C. Thus, positive field correlations should not be interpreted as evidence of unlimited benefits of increasing temperature for insect development. Disease associations reported by Iftikhar et al. (2017) and Haider et al. (2017) further suggest that temperature may influence disease development through vector activity and host pathogen interactions.

Relative humidity exhibited contrasting seasonal relationships. Its association with PDI changed from r = 0.19 in 2025 to r = -0.40 in 2026, while corresponding whitefly and aphid correlations shifted from 0.28 to -0.45 and from 0.22 to -0.50, respectively. The correlations with thrips relationships were low (r = 0.21, -0.16). Sujatha et al. (2025) and Misal et al. (2016) reported negative association while Iftikhar et al. (2017) and Saini et al. (2024) reported positive association between relative humidity and whitefly abundance. In the same way, Borgohain et al. (2024), Patel et al.

(2009) and Deepak et al. (2019) reported negative association of humidity with thrips while Khatun et al. (2023) found positive association with thrips. The differences indicate that relative humidity may have a non-uniform effect on the populations of the various vectors, and that the effect may be influenced by the local microclimatic conditions, as well as by temperature and crop phenology.

There was also significant year to year variability in rainfall. The correlation of PDI with rainfall was positive in 2025 ($r = 0.36$) while it was negligible in 2026 ($r = -0.08$). Correlations between the two corresponding dates changed from 0.49 to -0.09 for whiteflies, from 0.44 to 0.14 for aphids, and from 0.31 to -0.04 for thrips. The genotype-specific regressions showed that selected genotypes had an increase of 20.35 and 23.65 unit's rainfall in 2025 with R^2 of 0.69 and 0.54, respectively. The results are contrary to the mostly negative rainfall association reported by Sujatha et al. (2025), Iftikhar et al. (2017) and Haider et al. (2017). But, there is evidence from (Kumari et al. 2027) that the whitefly and rainfall relationship was positive in one season and negative in another, suggesting some seasonality. The same goes for Khatun et al. (2023) who found weak positive correlations for both aphids and thrips, Borgohain et al. (2024) and Deepak et al. (2019) who found negative relationships. The inconsistencies may be attributed to the variations in the time, intensity and accompanying microclimatic changes of the precipitation.

4.2. Disease Progression and Genotypic Variation

PDI values are continually rising from one assessment to the next, then the disease is continuing to progress throughout the crop's life cycle. The year effect was numerically greater in 2025 than in 2026, but was not statistically significant, nor was the year ^{x} week interaction. This indicates the overall trend of disease development was quite similar from season to season. Studies on chilli leaf curl disease also report similar increases in disease severity in the field. Iftikhar et al. (2017) found correlations between the environmental conditions and chilli leaf curl disease, and Hussain et al. (2017) and Mustafa et al. (2023) reported significant differences in the response to the disease among chilli genotypes in the field. The amount of disease development from one study to another, however, may vary due to variation in virus composition, inoculum pressure, host material, and environment.

However, HP-264 (V36), Ghotki Chilli (V18), HS-042 (V32), Longi (V12) and Bakhtawar Longi (V20) had the highest cumulative disease burden. These differences are similar to earlier research showing significant genetic variation in chilli's reaction to virus associated disease. It was observed that there was significant difference in the reaction of 307 Capsicum genotypes to the infection of pepper leaf curl virus and further controlled inoculation was required to establish resistance in the selected lines (Kumar et al. 2006). Likewise, 60 chilli genotypes were screened over four successive seasons and only three genotypes showed consistent resistance to chilli leaf curl disease (CLCD) (Srivastava et al. 2017). The need for repeated evaluation and confirmatory inoculations was stressed in these studies, and it was confirmed that definitive resistance categories should not be assigned until after repeated evaluation and confirmatory inoculations.

There was little variation in disease performance between 2025 and 2026; the correlation between genotype AUDPC performance in both years was 0.942. In addition, the ranks of the check-adjusted AUDPCs were not more than three positions different from the raw ranks, indicating the stability of the ordering observed. However, there is no independent field plot replication within the years, which restricts formal genotype comparisons. The identified lower disease burden types, therefore, are promising candidates for further screening and not definite sources of resistance.

This is especially relevant, because the presence of the virus does not necessarily mean it will be expressed in symptoms. Previously, it was found that symptomless plants can be virus infected but not symptomatic, which necessitated molecular confirmation and controlled inoculation in the process of chilli resistance evaluation (Mangal et al. 2024).

4.3. Insect Vector Dynamics and Associations with Disease Burden

The counts of whitefly, aphid and thrips increased each week, and the highest numbers of whitefly were always recorded. Positive correlation was strong with AUDPC for whitefly AUVPC (r = 0.942), aphid (r = 0.747) and thrips AUVPC (r = 0.579). Similar relationships were found for final PDI. The patterns suggest that the greater the cumulative vector abundance of a genotype, the more disease burden it had. But these observational relationships are unable to infer the direct effect of increased vector abundance on the observed differences in disease severity.

The strong correlation between whitefly counts and percentage disease index has a biological basis as whitefly is a vector of several important begomoviruses of chilli crop. (Iftikhar et al. 2017) found positive correlation between the environmental factors and whitefly population in the presence of chilli leaf curl disease. Similarly, it has been confirmed in Pakistan that development of chilli leaf curl disease is influenced by whitefly transmission. However, under natural field conditions, symptoms of disease could reflect the combined effect of several virus pathogens and interactions. Thus, an association with the stronger whitefly does not indicate that begomoviruses caused all the disease.

4.4. Multivariate Clustering and Disease-Burden Classification

The higher burden cluster differed from the lower burden cluster in chilli genotypes due to increased AUDPC, final PDI and increased whitefly, aphid and thrips counts at the end of the season. Five clusters were detected based on disease data using hierarchical clustering methods (AUDPC and final PDI) whereas three groups of disease burden were identified by the tertiles of the AUDPC data. The results show the advantages of complementary analytical methods for genotypic characterization in field. Likewise, (Salotti et al. 2022) used AUDPC based hierarchical clustering for the classification of grapevine varieties according to their disease-response pattern, whereas (Hegde et al. 2026) used both AUDPC and PDI to classify cucumber genotypes into four categories for the characterization of their responses to downy mildew. (Abuley and Hansen 2022) also employed hierarchical clustering based on relative AUDPC and other measures of disease progression to classify the resistance patterns of 54 potato varieties to late blight. (Srivastava et al. 2017) have developed the genetic groups of chilli based on SSR based clustering and (Sharma et al. 2018) and (Thakur et al. 2019) have highlighted the significance of the disease severity assessment and controlled inoculation for resistance evaluation. These studies in total show that clustering is a valuable method for initial germplasm characterization. The identified clusters and AUDPC based categories do not, however, represent experimental classes of resistance; replicated field testing, controlled inoculation and molecular determination are necessary to confirm resistance and susceptibility status of evaluated genotypes.

5. Conclusion

This study quantitatively characterized the environmental conditions that favor the development of viral diseases and the buildup of insect vectors in chilli under field conditions in Southern Punjab, Pakistan over two seasons. Climatic drivers for whitefly, aphid and disease dynamics were mostly moderate and improved to very strong across most

susceptible entries with temperature, particularly minimum temperature, being the greatest driver. the findings demonstrate that disease development in chilli is associated with substantial genotype-dependent variation and concurrent changes in insect vector abundance. The consistency of genotype rankings across seasons supports the usefulness of AUDPC for preliminary germplasm evaluation, while the strong disease–vector associations emphasize the relevance of integrated vector monitoring. Based on the PDI rating scale, the tested chilli varieties exhibited a wide range of disease reactions, ranging from highly resistant to highly susceptible. Varieties Advanta-509 F1 and SV-7864 HM F1 showed highly resistant (HR) reactions, with no infection (0% PDI). Sky-star 4 F1, Golden Hot F1 and HP-8 showed low disease levels and were classified as resistant (R), while Sky-Red showed a moderately resistant (MR) reaction. Varieties Shandar-F1, Sky-star 5 F1 and HP-3 were categorized as moderately susceptible (MS). A relatively larger group comprising (Sky-Line 3, HP-1, HP-2, HP-4, HP-5, HP-6, HP-7, Ziniya (181), Super Globe-F1, BSS-307, Shehzadi, Advanta-5018 F1, Golden Hero, HP-042 F1 and HP-075 were classified as susceptible (S). In contrast, HP-9, HP-10, HHP-082, Mahraja F1, Longi, Ghotki Chilli, Bakhtawar Longi, Eagle F1, Tatypuri, MAS-2482, HS-042 and HP-264 were categorized as highly susceptible (HS). Overall, the screening revealed considerable variation in disease response among the tested chilli varieties, with two varieties showing a highly resistant reaction, three as resistant, one as moderately resistant, three as moderately susceptible, fifteen as susceptible, and twelve varieties falling in the highly susceptible category.

These results may be utilized for the development of chilli viral disease risk-forecasting model for Southern Punjab, timing of vector control intervention to thermal permissive periods and integration of resistance breeding program. Future studies could expand the monitoring to other sites and times of year to validate the temperature mediated and humidity reversal patterns reported here. This correlation between climate, vector dynamics and virus identity will be crucial to move the current results into a framework that can be deployed to forecast and manage chilli diseases in Pakistan.

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Statements and Declarations

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Ethical Declaration

We declare that we have no human participants, human data and human tissues

Consent to Participate
The authors have nothing relevant to declare

Consent to Publish
All authors have a consensus regarding the publication of original research work

Competing Interests

The authors declare no known financial, or personal relationship and research conflict of interest that could influence the work reported in this manuscript.

Author Contributions

Muhammad Shan Latif conducted the research, collected and compiled the experimental data, and prepared the original manuscript draft. Muhammad Ashfaq supervised the research and critically reviewed the manuscript. Mirza Abid Mehmood performed the statistical analyses and reviewed the manuscript. Naeem Iqbal assisted with insect data collection. All authors read and approved the final version of the manuscript.

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