PLANT SCIENCE TODAY
Vol 12(sp1): 1-11
https://doi.org/10.14719/pst.9254

eISSN 2348-1900

RESEARCH ARTICLE

Prevalence and transmission potential of tomato yellow leaf curl virus (TYLCV) by whitefly vectors on tomato (*Solanum lycopersicum* L.) in Himachal Pradesh, India

Robin[1*], Rakesh Kumar[1*], Subhash Chander Verma[1], Rohini Sharma[1], Akriti Chauhan[1], Nishant[2] & Amul Yadav[3]

[1]Department of Entomology, Dr YS Parmar University of Horticulture and Forestry, Nauni (Solan) 173 230, Himachal Pradesh, India
[2]Department of Vegetable Science, ICAR-Indian Agriculture Research Institute, New Delhi 110 012, India
[3]Department of Vegetable Science, College of Horticulture and Forestry, Punjab Agricultural University, Ludhiana 141 004, Punjab, India

*Correspondence email - robin-2107008@pau.edu; rakeshd4@gmail.com

Received: 02 May 2025; Accepted: 12 August 2025; Available online: Version 1.0: 07 October 2025

Cite this article: Robin, Rakesh K, Subhash CV, Rohini S, Akriti C, Nishant, Amul Y. Prevalence and transmission potential of tomato yellow leaf curl virus (TYLCV) by whitefly vectors on tomato (*Solanum lycopersicum* L.) in Himachal Pradesh, India. Plant Science Today. 2025; 12(sp1): 1-11. https://doi.org/10.14719/pst.9254

Abstract

Tomato yellow leaf curl virus (TYLCV) is a harmful plant pathogen that severely hampers tomato cultivation by reducing yield and overall plant vigor. The virus is mainly transmitted by the whitefly (*Bemisia tabaci*), although other whitefly species, such as *Trialeurodes vaporariorum,* have also been suspected as possible vectors. This study aimed at assessing the prevalence of TYLCV and its association with *B. tabaci* and *T. vaporariorum* across three key tomato-growing districts, namely Una, Solan and Sirmaur. Field surveys were conducted to assess whitefly populations, plant and insect samples were collected for virus detection. Double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) was employed to detect TYLCV in both tomato plants and whitefly vectors. The results indicate that among the three districts, Una showed the highest TYLCV incidence (66.03 %), severity (MSR 3.78) and ELISA-based infection rate (81.13 %), followed by Solan (31.24 %, MSR 2.45, 63.83 %) and Sirmaur (18.08 %, MSR 1.26, 42.37 %). A strong positive correlation was observed between whitefly density and disease incidence in Solan ($r = 0.75$, $p < 0.00001$), Una ($r = 0.689$, $p < 0.000001$) and Sirmaur ($r = 0.46$, $p = 0.0052$), confirming vector-mediated spread. *B. tabaci* is the main vector of TYLCV, with a much higher incidence in the warmer regions of Una and Sirmaur, where viral infections were most severe. In contrast, *T. vaporariorum* was more prevalent in the relatively cooler region of Solan but showed negligible vector competence, suggesting it plays a limited role in TYLCV transmission. The correlation between abundance and prevalence of TYLCV underscore the impact of climatic conditions on vector dynamics and disease spread. These findings emphasize the need for climate-specific IPM (Integrated Pest Management) strategies, with careful considerations for vector control, host resistance and cultural practices aligned with regional environmental conditions. This study highlights key epidemiological insights into TYLCV and vector ecology, aiding targeted disease management strategies. Controlling vector population dynamics in relation to climatic conditions will be essential to reduce losses and ensure sustainable tomato production in affected areas.

Keywords: DAS-ELISA; disease management; TYLCV; vector ecology: virus; whitefly

Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetables in the production chain worldwide in terms of food security, nutrition and economic contribution. Tomatoes are a reservoir of vitamins and minerals and major antioxidants, enriching the human diet, while supporting the livelihood of millions of farmer families and commercial agribusinesses (1). The economic importance of the crop is reflected in its wide cultivation in various agro-climatic regions, from temperate to tropical zones. According to FAO, some of the top tomato-producing countries are China (69.20 mt), India (20.56 mt), Turkiye (13.15 mt), USA (11.30 mt) and Egypt (6.29 mt). However, tomato production is often faced with a variety of biotic and abiotic stresses that can severely affect yield and fruit quality. Among these, viral diseases pose a major threat, causing heavy economic losses and reduced crop productivity. In India, TYLCV is known to cause yield losses of up to 90 %, resulting in an estimated annual economic loss of over ₹1200 crores in tomato production.

One of the most devastating viral diseases is tomato yellow leaf curl virus (TYLCV). TYLCV belongs to the genus *Begomovirus* within the family Geminiviridae and is primarily transmitted by the whitefly vector *B. tabaci* (2). It was first identified in the Mediterranean region but has spread to multiple tomato-growing areas around the world, including Asia, Africa, the United States of America and Europe. The global expansion of TYLCV is closely associated with the increasing distribution and adaptability of *B. tabaci*, which facilitates rapid virus transmission across vast agricultural landscapes.

The symptoms of TYLCV are distinct and include upward curling and yellowing of leaves or chlorosis, severe stunting, small leaves, flower abortion and significant yield losses (3). In

the infected plants, there is a marked decrease in fruiting and even total yield loss may be possible, especially when high whitefly populations maintain intense virus transmission over a large area. Reports indicate that yield losses from TYLCV can be as high as 100 % in heavily infested fields and the disease is a major concern for tomato producers worldwide (4). The increased virulence of TYLCV strains and the high adaptability of *B. tabaci* have further complicated the management of the disease, resulting in increased economic burdens on farmers and agricultural industries (5).

The epidemiology of TYLCV is influenced by several factors such as environmental conditions, vector population dynamics, the presence of alternative virus-hosting plant species and the genetic diversity of both the virus and its vector (6). Persistence of TYLCV in weed species and non-cultivated host plants offers a reservoir for continued disease outbreaks, thus worsening the impact on tomato production. Effective control of TYLCV requires an integrated disease management approach that includes vector control strategies, the development and deployment of resistant or tolerant tomato cultivars and cultural practices aimed at minimizing virus reservoirs and transmission sources (7). Insecticide -based management of white fly commonly involves the use of neonicotinoids such as imidacloprid and thiamethoxam, as well as pyrethroids like bifenthrin. However, frequent and indiscriminate use of these insecticides has led to the development of resistance in whitefly populations, thereby reducing their effectiveness and complicating disease management strategies. This has led researchers to explore alternative and sustainable management options.

Serological detection methods, especially double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA), are very common for the rapid, sensitive and cost-effective identification of TYLCV in infected tomato plants. DAS-ELISA depends on specific antibodies that recognize viral coat proteins, thereby allowing the detection of TYLCV in plant tissues with high specificity (8). This method is generally applied in epidemiological surveys for the incidence and distribution of TYLCV on field-grown tomatoes. In comparison, DAS-ELISA is much simpler compared to molecular methods such as PCR and does not require sophisticated equipment. It is more suitable for large-scale diagnostic applications in resource-limited regions (9). The sensitivity of the test may, however, be relatively low compared to PCR-based techniques, especially for detecting low titers of the virus in symptomless plants.

Morphological identifications of *B. tabaci*, the primary vector of TYLCV, are essential for efficient monitoring of whitefly populations and effective vector management. *B. tabaci* is a small, soft-bodied insect having a bright yellowish body with two pairs of white wings covered in powdery wax, besides possessing distinct morphological differences from its puparial stage (10). Puparial shape, antennal segmentation, size of operculum and structure of vasiform orifice have to be examined meticulously (11). The traditional morphological identification remains essential for field entomologists but molecular techniques such as mitochondrial DNA (mtCOI) sequencing are increasingly applied to differentiate among cryptic species within the *B. tabaci* complex. Understanding the diversity and distribution of *B. tabaci* populations is crucial for developing targeted control measures and predicting the spread of TYLCV across tomato-growing regions.

This study aims to assess the prevalence, incidence and severity of TYLCV in several counties. Analyzing its association with whitefly populations and evaluating the sensitivity of diagnostic techniques such as DAS-ELISA. Providing a comprehensive epidemiological analysis of this research would enhance the understanding of TYLCV transmission dynamics and contribute to more effective and sustainable management strategies in mitigating the impact of this disease on global tomato production.

Materials and Methods

Study area and sampling

Surveys were conducted across 120 tomato growing locations in Una, Solan and Sirmaur districts during 2019-2021, which are among the high tomato producing regions of Himachal Pradesh. Districts surveyed and locations covered during study are listed in Table 1. A “W-shaped” sampling approach was followed to collect virus infected plants and associated whitefly species present on the respective plant. The W-shaped sampling approach involves collecting samples from five points along a W-shaped path in the field to ensure representative and unbiased data. It was chosen in this study to effectively capture the distribution of TYLCV infection and whitefly vectors across different parts of tomato fields in Himachal Pradesh.

Samples comprising both adult and nymph stages of whiteflies were preserved in an ice box and transported to the laboratory for analysis. Morphological identification of whitefly species present at each location was carried out using standard taxonomic keys under a Carl Zeiss Axioscope 5 phase contrast microscope with a magnification capacity of up to 2000X.

Table 1. Districts surveyed and locations covered during study.

Districts	Locations covered
Una	Santoshgarh, Khadd, Haroli, Shiv Bari, Ghangret, Nehrian, Chururu, Takarla, Mehre, Bani, Baduhi, Badhsali, Bharwaien, Chalali, Charratgarh, Dangoh, Nehrian, Tiudi, Pirthipur, Jorbar, Mawa Kaholan, Saghanai, Ghanari, Deoli, Pandoga, Badhera, Thana Kalan, Ladhiani,Jankaur Har, Sehjowal, Bathu, Thathal, Nangal Jariala, Amlehar, Talmera, Dhundla, Nanwain, Gondpur, Rakkar, Rajpura, Manjhar
Solan	Kandaghat, Chail, Subathu, Kathaon, Durgapur, Banoh, Barhog, Bhojnagar, Chaplah, Charjerah, Majra, Dharowa, Bariyan, Batoli, Kuthar, Gadhog, Radyana, Ramshehar, Darwa, Panjoa, Thar, Kundla, Dannai, Bharti, Sujni, Jagota, Kimta, Delgi, Rangah, Uncha Gaon, Khanol, Samot, Manjhiat, Khokhari, JangalManjhu, Nanun, Basti, Laliana, Bangar Rehan, Sairi
Sirmaur	Baru Sarera, Lana Bhalta, Lana Machher, Paprana, Panyali, Parari, Kaba, BecharKabag, Kandal, Nar Noti, Kamlar, Kataha, ChuliDadahu, Birla, Panar Kalyana, Nahog, Bogdhar, Chokkar, Bharari, Lana Chaita, Rihana, Thanoga, Bhuira, Bhat Ka Saina, Rajgarh,KotiMangwa, Bhanat, Dhar Baghera, Neri Kotli, Palu, Haban, Rana Ghat, Sanwara, Traie, Dhaniya Ser, Ritab Pal.

TYLCV prevalence, incidence and severity

Disease prevalence was assessed by determining the number of fields where TYLCV was recorded in relation to the number of fields sampled in different districts. The disease incidence was visually determined as the percentage ratio of the number of symptomatic plants against the total plant population in the area assessed (Eqn. 1) (12). On average, 50 plants per field were assessed to ensure representative sampling. Disease severity on the other hand, was determined by evaluating the percentage of leaf areas infected against a five-point scale, such that 1 = 1-20 % (very mild), 2 = 21- 40 % (mild), 3 = 41-60 % (severe), 4 = 61-80 % (very severe) and 5 = 81-100 % (almost dead) as described (13).

$$\text{Disease incidence (\%)} = \frac{\text{Number of plants infected}}{\text{Total number of plants}} \times 100 \quad \text{(Eqn. 1)}$$

$$\text{Insect incidence (\%)} = \frac{\text{Number of infested plants}}{\text{Total number of plants}} \times 100 \quad \text{(Eqn. 2)}$$

Serological detection of virus

Leaf samples exhibiting typical symptoms of TYLCV, such as upward leaf curling, yellowing or interveinal chlorosis, leaf thickening and reduced size and stunted growth were drawn from the marked plants in the selected fields and brought to the laboratory in separate polythene bags in an ice box so as to keep the leaf samples fresh for serological detection of tomato yellow leaf curl virus through alkaline phosphatase (ALP) based DAS (double antibody sandwich) forms of ELISA.

DAS-ELISA

Serological detection through DAS-ELISA alkaline phosphatase based direct DAS-ELISA was used to detect the virus as per the protocol with slight modifications (8). The detailed procedure used for DAS-ELISA based serological detection of TYLCV. In DAS-ELISA, wells of the microtitre plate except those of the top and bottom rows on the extreme left and right were first filled with 200 µL coating antibody. The plate was incubated in humid box for 4 hr at 30 °C. The coating antibody suspension was removed by vigorously shaking out the plate. The wells were filled with 1X phosphate-buffered saline (PBS)-Tween and kept for 2 min with gentle shaking, emptied the plate and filled again with PBS-Tween. The washing was repeated three times or by washing in ELISA plate washer.

The leaf extract from the test samples was prepared in PBS buffer. All coated wells were filled with 200 µL aliquots of test samples (each sample at least in duplicate) besides positive control and negative control wells. The plates were incubated in humid box overnight at 4 ± 10 °C. The washing step was repeated as mentioned above. The specific ALP based conjugated antibodies were filled in each well with 200 µL aliquots. The plate was incubated in humid box for 5 hr at 30 °C. The washing was done as mentioned previously. The p-nitrophenyl phosphate (pNPP) substrate was dissolved in 1X substrate buffer by dissolving 5 mg pNPP tablet in 5 mL of 1X substrate buffer under the dark conditions.

Each well was filled with 200 µL aliquots of substrate. The plates were kept in humid box in the dark condition at room temperature after giving a brief incubation of 15 min at 30 °C. The plates were incubated until a yellow colour was clearly visible in the positive controls (usually between 30 and 90 min). If desired, the reaction was stopped by adding 50 µL of 3M NaOH to each well. The results were assessed either by measurement of the absorbance value of the hydrolyzed substrate (p-nitrophenyl) at 405 nm wavelength in a microtitre ELISA plate reader (Micro Scan MS5605A, Electronic Corporation of India Limited) or through visual screening.

Flow chart for DAS-ELISA is given in Appendix I. Leaf samples from symptomatic plants were collected for further serological detection through DAS-ELISA from selected fields surveyed in Himachal Pradesh. For all serological tests of ELISA, immune reagents, buffers, positive and negative control supplied by BIOREBA AG (Switzerland) were used as per the instructions issued by the supplier. The results of the ELISA for detection were interpreted as suggested (14, 15). Samples were considered infected if their OD values at 405 nm exceeded two times the mean values of respective healthy and negative control samples.

Morphological identification of whiteflies

Field collected adults were brought to lab for microscopic examination with Carl Zeiss Axioscope 5 phase contrast microscope at 100X magnification. Infested leaves were examined under high magnification to observe different stages of pest and morphological characters like size, shape, presence or absence of setaeon nymphs and adults. Morphology of eggs, nymphs and adults was taken into consideration for identification of whitefly species as per keys illustrated in literature (16, 17).

Data analysis

Disease incidence was recorded as a percentage of symptomatic plants in each surveyed location per district. While whitefly populations were categorized into *B. tabaci* and *T. vaporariorum* and their prevalence was documented. Correlation between vector populations, TYLCV prevalence and climatic condition were analyzed. Mean, median, standard deviation and minimum -maximum were calculated. To find the correlation Pearson's coefficient was calculated. All data were analyzed using R software (version 4.4.1) and Microsoft Excel 2019.

Results

Disease and pest incidence

Tomato yellow leaf curl prevalence, incidence and severity in Una, Solan and Sirmaur are given in Table 2-4. The disease prevalence in Una District had a mean of 66.03 % and a median of 63.0 %. The values ranged from a minimum of 41.0 to a maximum of 96.0, with a standard deviation of 15.51, reflecting significant variation in disease prevalence. Whitefly density had a mean of 36.36 and a median of 31.75, with values ranging from 13.0 to 80.1 per plant. The standard deviation of 17.31 reflects significant variation in whitefly infestation levels. The Pearson correlation coefficient between whitefly density and disease prevalence was 0.689, with a highly significant *p*-value of 8.97×10^{-7}. This highly significant positive correlation implies that an increase in whitefly infestation is strongly linked to an increase in disease prevalence in area.

Table 2. Disease incidence, whitefly incidence, disease severity and severity level in Una.

S. No.	Locations	Disease (%)	Whitefly (%)	Disease severity	Severity level
1.	Ajauli	41.0	26.0	Severe	3
2.	Ajnoli	72.0	36.6	Very severe	4
3.	Amb	80.0	42.5	Very severe	4
4.	Ambota	60.0	32.0	Severe	3
5.	Anandpur Sahib	61.0	46.5	Very severe	4
6.	Baduh	41.5	15.0	Severe	3
7.	Bangrh	89.0	40.5	Almost dead	5
8.	Basal	53.0	22.0	Severe	3
9.	Basoli	58.0	30.0	Severe	3
10.	Basrara	64.0	18.0	Very severe	4
11.	Batuhi	78.0	41.0	Very severe	4
12.	Bhadsali	65.5	30.1	Very severe	4
13.	Bharwain	52.0	25.0	Severe	3
14.	Chalola	76.5	36.0	Very severe	4
15.	Chharatgarh	96.0	64.7	Almost dead	5
16.	Chatra Khas	76.0	60.0	Very severe	4
17.	Chintpurni	54.5	26.3	Severe	3
18.	Dangoli	46.0	15.4	Severe	3
19.	Daulatpur	56.0	24.5	Severe	3
20.	Fatehwal	93.0	65.1	Almost dead	5
21.	Fatehpur	80.0	16.7	Very severe	4
22.	Gagret	81.0	29.0	Almost dead	5
23.	Galua	53.0	27.5	Severe	3
24.	Gugaroo	61.0	43.0	Very severe	4
25.	Haroli	91.0	75.5	Almost dead	5
26.	Jhalera	66.5	45.0	Very severe	4
27.	Kasba	62.0	27.5	Very severe	4
28.	Majara	53.0	40.0	Severe	3
29.	Mehatpur	78.0	64.1	Very severe	4
30.	Nagnuli Har	58.5	20.0	Severe	3
31.	Nandpur	72.0	50.0	Very severe	4
32.	Nangal	82.0	50.0	Almost dead	5
33.	Rajpura	86.0	31.5	Almost dead	5
34.	Tibba	42.0	13.0	Severe	3
35.	Salangari	60.0	15.2	Severe	3
36.	Sanjhot	86.0	80.1	Almost dead	5
37.	Sasan	69.0	30.0	Very severe	4
38.	Surjehra	42.0	15.0	Severe	3
39.	Rampur Bela	55.0	50.6	Severe	3
40.	Udheypur	50.0	33.5	Severe	3

Table 3. Disease, whitefly incidence, disease severity and severity level in Solan.

S. No.	Locations	Disease (%)	Whitefly (%)	Disease severity	Severity level
1.	Albora	22.0	20.0	Mild	2
2.	Anun	24.5	16.0	Mild	2
3.	Arki	43.5	31.5	Severe	3
4.	Patta	75.0	65.5	Very severe	4
5.	Badkhor	39.0	22.0	Severe	3
6.	Bagor	36.0	32.5	Severe	3
7.	Barog	20.0	12.0	Very mild	1
8.	Bhoj Nagar	38.5	25.5	Severe	3
9.	Chapla	20.5	12.0	Very mild	1
10.	Charjera	26.0	11.3	Mild	2
11.	Chilri	32.0	36.0	Severe	3
12.	Deothal	40.0	20.0	Mild	2
13.	Deothi	41.0	15.7	Severe	3
14.	Dharampur	11.0	10.5	Very mild	1
15.	Dhilon	42.5	18.5	Severe	3
16.	Galanag	31.0	40.1	Severe	3
17.	Ghori	20.8	18.8	Mild	2
18.	Ramshahr	36.0	10.0	Severe	3
19.	Haripur	30.0	15.2	Severe	3
20.	Jarai	18.0	10.1	Very mild	1
21.	Jatoli	28.0	20.3	Severe	3
22.	Kalaghat	26.0	10.5	Severe	3
23.	Khalwa	30.0	20.5	Severe	3
24.	Lakharanji	25.0	12.5	Severe	3
25.	Mahlog	21.0	20.0	Severe	3
26.	Majhgaon	40.0	50.0	Severe	3
27.	Manjhar	17.0	20.7	Very mild	1
28.	Nalagarh	61.0	29.0	Very severe	4
29.	Nauni	19.0	15.1	Very mild	1
30.	Nehr	19.0	30.0	Very mild	1
31.	Ranga	46.0	45.0	Severe	3
32.	Sabathu	38.0	20.6	Severe	3
33.	Dhobghat	76.0	81.5	Very severe	4
34.	Sehal	11.0	10.0	Very mild	1
35.	Shamror	36.0	32.0	Severe	3
36.	Shamti	25.0	20.0	Severe	3
37.	Shili	9.0	10.0	Very mild	1
38.	Thapo	18.0	16.4	Very mild	1
39.	Tikar	32.0	17.0	Severe	3
40.	Tiwakri	25.5	42.0	Severe	3

Table 4. Disease incidence, whitefly incidence, disease severity and severity level in Sirmaur.

S. No.	Locations	Disease	Whitefly	Severity	Severity grade
1.	Amboa	20.0	35.0	Very mild	1
2.	Anji	16.0	24.0	Very mild	1
3.	Badiana	23.0	10.0	Mild	1
4.	Batol	15.0	26.0	Very mild	1
5.	Bhalana	10.5	22.0	Very mild	1
6.	Bhutli	16.0	40.5	Very mild	1
7.	Bias	24.0	57.0	Mild	2
8.	Chandol	14.0	27.2	Very mild	1
9.	Chhog Tali	29.0	21.5	Mild	2
10.	Dahan	18.0	17.3	Very mild	1
11.	Darena	6.0	2.4	Very mild	1
12.	Dhamandar	18.0	13.0	Very mild	1
13.	Dhamla	21.0	10.0	Mild	2
14.	Dharja	18.0	19.0	Very mild	1
15.	Gawahi	13.0	15.0	Very mild	1
16.	Giripul	26.5	32.0	Mild	1
17.	Jola	21.5	10.0	Mild	1
18.	Karoli	18.0	10.0	Very mild	1
19.	Kafota	0.8	0.6	Very mild	1
20.	Kulath	20.0	32.0	Very mild	1
21.	Lanaru	4.0	1.6	Very mild	1
22.	Dhaula Kuan	34.5	20.0	Mild	2
23.	Nihar Pab	3.5	0.3	Very mild	1
24.	Pabiana	28.0	63.0	Very mild	1
25.	Palu	3.0	4.0	Very mild	1
26.	Phagu	10.0	15.0	Very mild	1
27.	Rajgarh	25.0	20.1	Mild	2
28.	Rana Ghat	19.0	10.4	Very mild	1
29.	Dadahu	47.0	18.2	Severe	3
30.	Rihana	15.0	6.0	Very mild	1
31.	Sail	11.0	4.6	Very mild	1
32.	Sanora	26.0	31.0	Mild	2
33.	Ser	12.5	13.1	Very mild	1
34.	Tharu	21.0	36.0	Mild	2
35.	Tikri Jijah	25.0	10.5	Mild	2

In Solan District, the disease incidence had a mean of 31.24 with a median value of 29.0. The values of incidence ranged from a minimum of 9.0 to a maximum of 76.0 with a standard deviation of 14.95 and represent a moderate degree of variability in the incidence of the disease. Similarly, the infestation by whiteflies had a mean value of 24.16 with a median value of 20.0, with values ranging from a minimum of 10.0 to a maximum of 81.5. The standard deviation of 15.55 represents a similar degree of variability. Notably, the Pearson correlation coefficient for disease incidence and whitefly infestation was estimated at 0.75 with a *p*-value of 2.16×10^{-8}, which indicates a highly significant association. The high positive correlation suggests that any rise in the infestation by whiteflies goes hand in hand with an increased disease incidence in the region. This confirms a strong positive correlation between disease occurrence and whitefly presence. The *p*-value is very small, indicating that the correlation is statistically significant.

In Sirmaur District, the disease prevalence was 18.08 % with median 18.0 %. Values ranged from a minimum of 0.8 % to a maximum of 47.0 % with a standard deviation of 9.40 %, reflecting moderate variation in disease occurrence. Whitefly infestation revealed a mean of 19.38 % and median of 17.3 % with values ranging from 0.3 % to 63.0 %. The high standard deviation of 14.82 % reflects wide variation in infestation levels. Pearson correlation between whitefly infestation and disease prevalence was found to be 0.46 with a statistically significant *p*-value of 0.0052 ($p < 0.05$). The correlation reflects moderate positive correlation where higher numbers of whiteflies are partly accountable for higher disease prevalence. The correlation is, however, weaker than that observed for Solan District. Number of fields surveyed for TYLCV, mean incidence, prevalence, severity rating and severity level are given in Table 5.

Disease prevalence and severity

A total of 50 fields were surveyed in each district and the number of fields with TYLCV varied. Incidence, prevalence and severity ratings were analyzed and there were differences in the patterns for Una, Solan and Sirmaur districts. In Una, 40 out of 50 surveyed fields were affected by TYLCV, with a mean incidence of 66.03 % based on field observations and 82 % in ELISA-based detection. The disease prevalence was recorded at 80 %, with a mean severity rating (MSR) of 3.775, classifying the severity as severe. In Solan, 40 out of 50 surveyed fields had TYLCV infection. The mean incidence was 31.24 %, while ELISA results had a higher incidence of 63.83 %. The prevalence remained at 80 %, though the MSR was lower at 2.45, which classified the disease severity as mild. In Sirmaur, TYLCV infections were found in 35 of the 50 surveyed fields, with the lowest mean incidence recorded in field surveys at 18.08 % and 42.37 % through ELISA tests. The prevalence of the disease was 70 %, while the MSR of 1.26 fell

Table 5. Number of fields surveyed for TYLCV, mean incidence, prevalence, severity rating and severity level.

Name of district	Num of fields surveyed	Fields with TYLCV	Mean incidence	Mean incidence ELISA	Prevalence	Mean severity rating	Severity
Una	50 (40)	40	66.03	82	80	3.775	Severe
Solan	50 (40)	40	31.24	63.829	80	2.45	Mild
Sirmaur	50 (35)	35	18.08	42.37	70	1.2647	Very mild

into the category of very mild severity. Overall, TYLCV severity was highest in Una, moderate in Solan and lowest in Sirmaur. Symptoms of TYLCV on tomato plants observed during surveys are shown in Fig. 1. Scatter plot graphs were prepared for Solan, Sirmaur and Una districts to show the correlation of TYLCV with whitefly and shown in Fig. 2-4.

Serological detection in plants

A DAS-ELISA test was conducted to determine the incidence of infection in symptomatic plants in three districts, namely Una, Solan and Sirmaur. Una, 53 symptomatic plants were tested, of which 43 tested positive and an infection rate of 81.13 % were recorded. Solan, 47 symptomatic plants were tested, of which 30 were tested positive and an infection rate of 63.83 % was recorded. Sirmaur, 59 symptomatic plants were tested and 25 tested positive, which is equivalent to an infection rate of 42.37 %. The findings indicate high variability in infection prevalence among districts, with the highest incidence in Una, followed by Solan and Sirmaur. The differences noted in infection rates can be due to environmental factors, host susceptibility or vector dynamics influencing the transmission of pathogens. Optical density values were analyzed and presence of individual specific symptoms (Table 6).

Fig. 1. Symptoms of TYLCV on tomato plants observed during surveys.

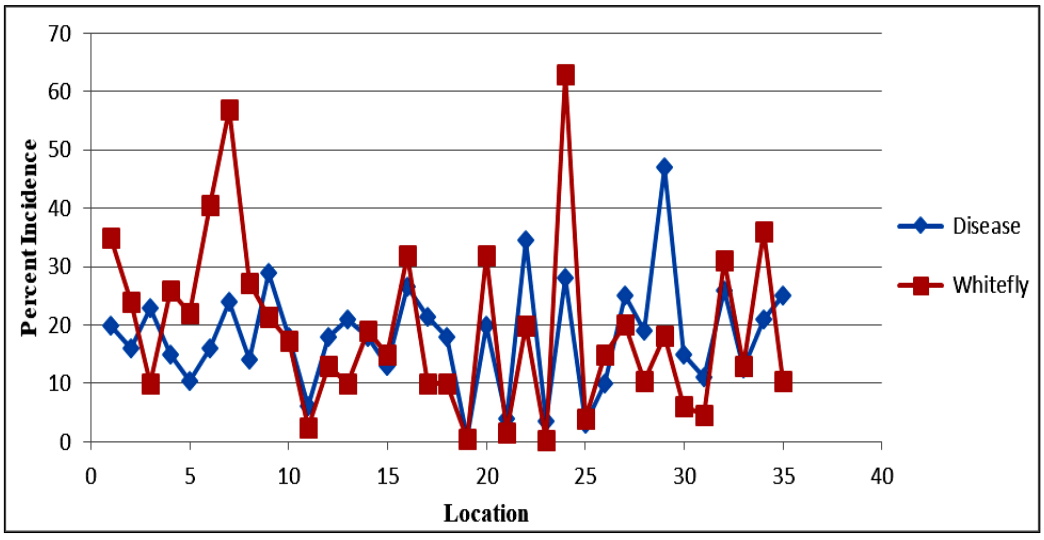

Fig. 2. Correlation of whitefly and disease incidence in Sirmaur district.

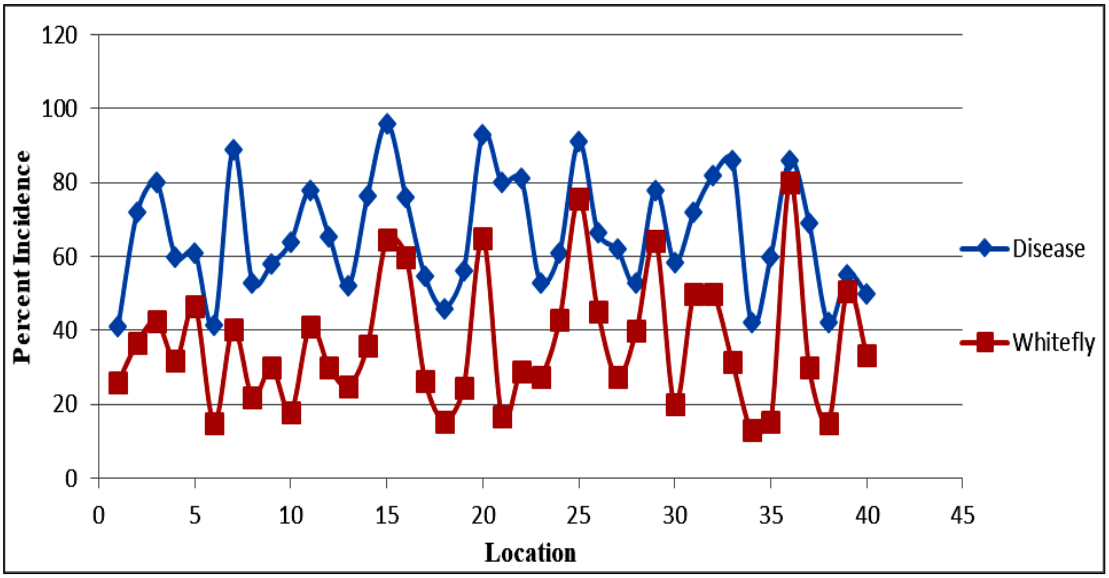

Fig. 3. Correlation of whitefly and disease incidence in Una district.

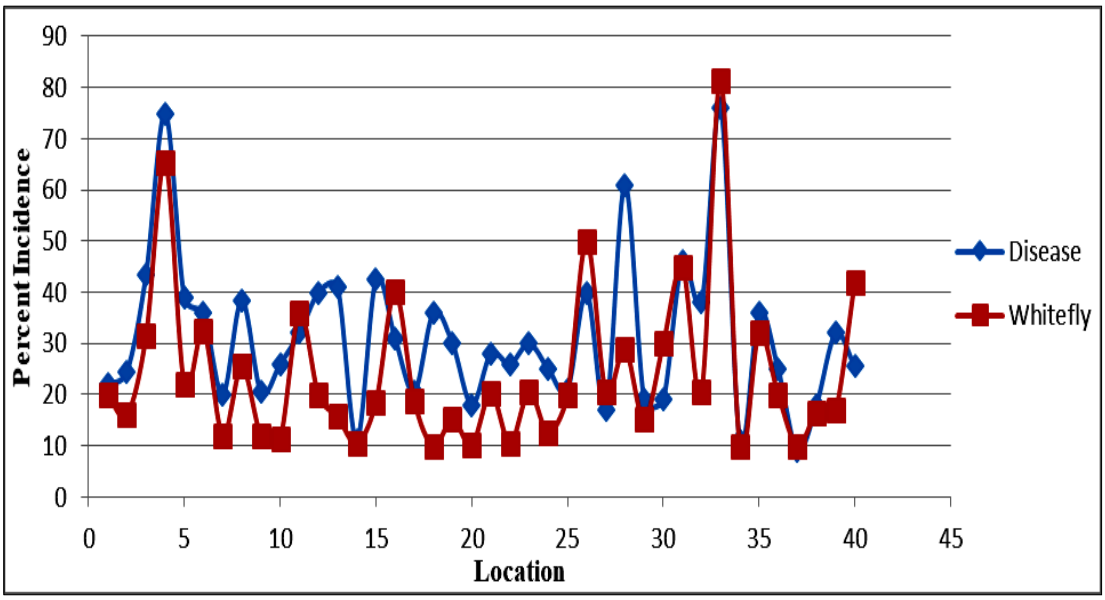

Fig. 4. Correlation of whitefly and disease incidence in Solan district.

Table 6. Calculation of percentage infection on basis of DAS-ELISA.

Districts	Locations covered for DAS-ELISA	Number of plants with symptoms	Number of plants tested positive	Percentage infection
Una	Ajauli, Ajnoli, Amb, Ambota, Anandpur Sahib, Baduh, Bangarh, Basal, Basoli, Basrara, Batuhi, Bhadsali, Bharwain, Chalola, Chharatgarh, Chatra Khas, Chintpurni, Dangoli, Daulatpur, Fatehwal, Fatehpur, Gagret, Galua, Gugaroo, Haroli, Jhalera, Kasba, Majara, Mehatpur, Nagnuli Har, Nandpur, Nangal, Rajpura, Tibba, Salangari, Sanjhot, Sasan, Surjehra, Rampur Bela, Udheypur	53	43	81.13
Solan	Albora, Anun, Arki, Patta, Badkhor, Bagor, Barog, Bhoj Nagar, Chapla, Charjera, Chilri, Deothal, Deothi, Dharampur, Dhilon, Galanag, Ghori, Ramshahr, Haripur, Jarai, Jatoli, Kalaghat, Khalwa, Lakharanji, Mahlog, Majhgaon, Manjhar, Nalagarh, Nauni, Nehr, Ranga, Sabathu, Dhobghat, Sehal, Shamror, Shamti, Shili, Thapo, Tikar, Tiwakri	47	30	63.829
Sirmaur	Amboa, Anji, Badiana, Batol, Bhalana, Bhutli, Bias, Chandol, Chhog Tali, Dahan, Darena, Dhamandar, Dhamla, Dharja, Gawahi, Giripul, Jola, Karoli, Kafota, Kulath, Lanaru, Dhaula Kuan, Nihar Pab, Pabiana, Palu, Phagu, Rajgarh, Rana Ghat, Dadahu, Rihana, Sail, Sanora, Ser, Tharu, Tikri Jijah	59	25	42.37

A descriptive analysis of diseased plants has been done and infection prevalence according to different kinds of symptoms of the disease that have been distinguished by DAS-ELISA is given in Table 7. Among the symptoms that were observed, puckering had the highest infection rate of 60.00 %, followed by shoe stringing of 56.52 %, dwarfing of 52.17 % and mosaic of 47.15 %. Curling and leaf deformation also presented relatively high infection rates of 46.15 % and 43.29 %, respectively. Yellowing had a relatively low infection rate of 40.74 %, mottle at 36.11 %, cupping at 30.99 % and vein clearing at 30.00 %. In total, 505 symptomatic plants were tested for infection and 225 of them tested positive, while the remaining 270 tested negative. This brought the overall infection rate to 44.55 %. The results indicate that the symptoms of puckering and shoe stringing seem to be indicative of infection but not as significant as some of the other symptoms. Further work is needed to determine if such correlations exist. Percentage infection in individual symptomatic plants is shown in Fig. 5.

Serological detection in whitefly

Whitefly samples collected from various locations were tested using a DAS-ELISA assay to measure their respective OD values. The results determined the presence of viruses between the two whitefly species, namely *B. tabaci* and *T. vaporariorum* and shown in Table 8. Among the stations where *B. tabaci* was present, the highest OD value was observed in Chharatgarh (0.964), followed by Sai (0.925), Fatehpur (0.921) and Ambota (0.869), while minimum OD value for *B. tabaci* was reported in Daulatpur (0.711). The results indicated all the *B. tabaci* samples tested positive with respect to viral presence. In contrast, samples of *T. vaporariorum* contained lower OD values. Samples from Khanog (0.473) were found positive, that from Dharja (0.211), Prada (0.060) and Karganun (0.176) were negative. The positive control gave an OD value of 1.201, while the negative control had 0.184.

Table 8. DAS-ELISA based detection of TYLCV in whitefly vectors collected from severely infected tomato fields.

S. No.	Location	Whitefly	OD Value (A_{405nm})
1.	Chharatgarh	*B. tabaci*	0.964(+)
2.	Fatehpur	*B. tabaci*	0.921(+)
3.	Khanpur	*B. tabaci*	0.800(+)
4.	Sai	*B. tabaci*	0.925(+)
5.	Daulatpur	*B. tabaci*	0.711(+)
6.	Ambota	*B. tabaci*	0.869(+)
7.	Khanog	*T. vaporariorum*	0.473(+)
8.	Dharja	*T. vaporariorum*	0.211(-)
9.	Prada	*T. vaporariorum*	0.060(-)
10.	Karganun	*T. vaporariorum*	0.176(-)
	Positive control		1.201
	Negative control		0.184

A total of 10 samples were tested from three districts, Una, Solan and Sirmaur, to assess the presence of infection (Table 9). Una district, samples collected from Bangarh, Chharatgarh, Haroli and Sanjhot showed the highest infection rate, with 3 out of 4 samples testing positive (75 %). Solan district, samples from Arki, Dhobghat and Shamti were found to be less infected. Only 1 out of 3 samples tested positive (33.33 %).

Table 7. Individual symptom contributing to OD value of TYLCV infected plants.

S. No.	Individual symptoms	Total	Positive	Negative	Percentage infection
1.	Vein clearing	10	3	7	30
2.	Cupping	55	17	38	30.99
3.	Mottle	36	13	23	36.11
4.	Yellowing	54	22	32	40.74
5.	Leaf deformation	97	42	55	43.29
6.	Curling	26	12	14	46.15
7.	Mosaic	123	58	65	47.15
8.	Dwarfing	46	24	12	52.17
9.	Shoe stringing	23	13	10	56.52
10.	Puckering	35	21	14	60
Total	10	505	225	270	

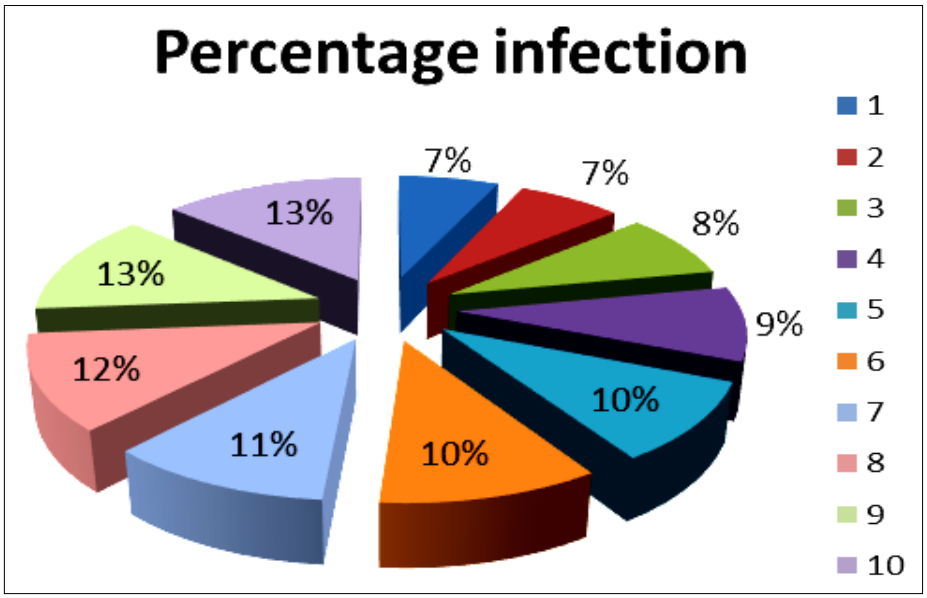

Fig. 5. Percentage of infected plants showing respective individual symptom on basis of OD values. **1**. vein clearing, **2**. cupping, mottle, **3**. yellowing, **4**. leaf deformation, **5**. curling, **6**. mosaic, **7**. dwarfing, **8**. shoe stringing and **9**. puckering.

Table 9. Whitefly samples tested positive for TYLCV and percentage infected samples in 3 districts.

Districts	Una	Samples tested	Positive	Negative	Percentage
Una	Bangarh, Chharatgarh, Haroli, Sanjhot	4	3	1	75
Solan	Arki, Dhobghat, Shamti	3	1	2	33.33
Sirmaur	Anji, Batol, Giripul	3	0	3	0
Total		10	4	6	

Sirmaur district, samples from Anji, Batol and Giripul were found entirely negative, thereby showing an infection rate of 0 %. In total, 40 % of the samples (4 out of 10) were positive, which signifies district-wise variation in the presence of pathogens. Therefore, the infection percentage is highest in Una district followed by moderate cases in Solan and no positive cases in Sirmaur districts. Fig. 6 presents the number of positive and negative samples across the three districts.

Discussion

The variation in infection prevalence across Una, Solan and Sirmaur districts suggests that multiple environmental, biological and ecological factors influence virus transmission. The high infection rate in Una (75 %) indicates that conditions in this district are particularly conducive to viral spread, likely due to higher vector density, favourable climatic conditions and increased host plant susceptibility. It is well established that whiteflies (*B. tabaci*) are vectors of major plant viruses, notably TYLCV in tomato and they prefer warm and humid climates that facilitate rapid virus transmission (18). Furthermore, infection rates might be further aggravated in Una by continuous cropping systems, which includes maize, wheat, paddy, mustard, pulses and vegetables like tomato, cauliflower and okra and high host plant availability due to the provision of uninterrupted cycles for vector propagation and pathogen spread (19).

The incidence of mild infection in Solan at 33.33 % may suggest that the virus is being transmitted at a lower rate and this is due to environmental constraints upon vector activity, natural host resistance or effective management of vectors. It has been shown in previous research studies that fluctuations in temperature can affect whitefly survival and reproduction, especially when cooler night temperatures prevail, thereby reducing its capability to transmit viruses efficiently (20). In addition, the farmers in Solan may have implemented IPM practices such as biopesticides, insecticidal nets and resistant cultivars, which may reduce the infection rate than Una (4).

No infection was recorded in Sirmaur district (0 % incidence), indicating that this district may be inherently resistant to viral spread or that environmental conditions are unfavourable for vector survival. High-altitude areas with cooler temperatures and higher rainfall have been demonstrated to limit the reproductive success and dispersal capabilities of *B. tabaci*, which could further limit virus transmission (18, 21). In addition, variation in cropping patterns and plant genotypes in Sirmaur might be responsible for lower infection rates because some varieties of host plants have natural resistance to the transmission of viruses by enhanced production of secondary metabolites and activation of plant defense pathways (22). However, it is also possible that sampling limitations have influenced the results and further large-scale studies are required to confirm the absence of infection across different seasons.

Another key factor influencing the varying infection rates across districts is the vector-pathogen relationship. For instance, studies have shown that certain whitefly populations exhibit preferences, such as differences in feeding behavior or viral acquisition efficiency for specific virus strains, which could explain why some areas experience infections at higher rates than others (5). Moreover, the efficacy of virus transmission depends on the genetic makeup of the viral strain. For example, particular variants have higher virulence than others as well as greater transmission efficiencies (20). In regions where mixed infections occur-where plants are infected by multiple viral strains simultaneously-the seriousness of the disease and the efficiency of transmission may be enhanced, thus leaving higher infection rates (19).

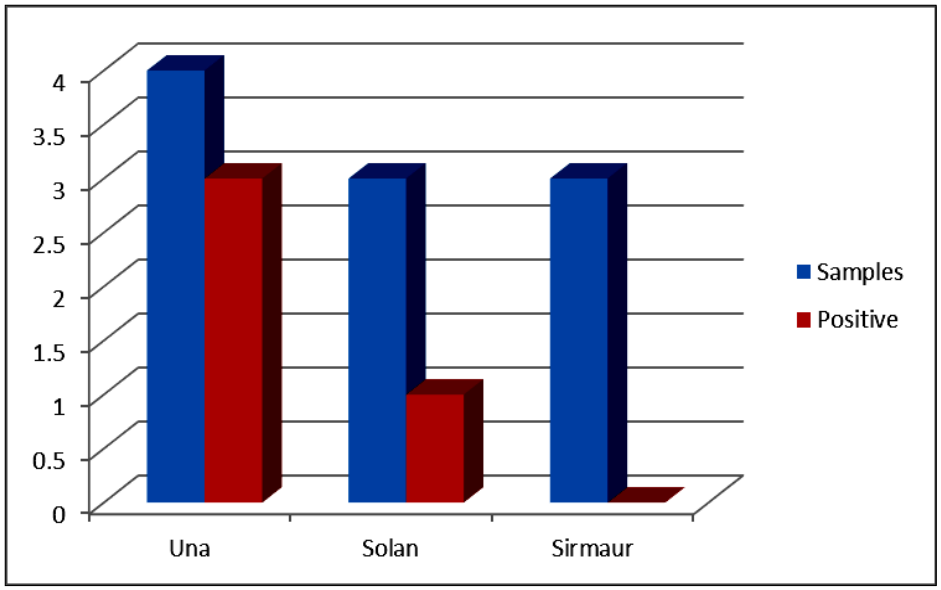

Fig. 6. Bar graph of positive and negative samples variation in 3 districts on basis of DAS-ELISA.

Agricultural practices significantly impact infection patterns. For example, in Una and Solan, continuous tomato cultivation without crop rotation and the use of susceptible cultivars may contribute to higher TYLCV incidence, whereas in Sirmaur, lower infection rates could be linked to more diverse cropping systems and limited whitefly host availability. In monoculture planting areas, the likelihood of disease spread tends to increase with increased availability of host plants and reduced genetic diversity, resulting in rapid proliferation of the pathogen (23). This contrasts with areas where diverse cropping systems, intercropping and crop rotation are practiced, disease transmission tending to be lower due to reduced vector preference and interrupted pathogen life cycles (4).

In addition, the chemical insecticides that are used to control vectors might have both a positive and a negative impact on disease prevalence. Although insecticides are effective for controlling vector populations in the short term, their long-term use may result in resistance of the *B. tabaci* population, making control difficult (24). Alternatives include biological controls, such as the introduction of natural predators such as lady beetles and parasitic wasps, which were found to control whitefly population and reduce rates of viral transmission (25). Perhaps the best control strategy for viruses in affected areas would be successful implementation of an IPM by combining biological controls with cultural and host resistance.

Conclusion

Disease prevalence variations at the district level indicate that environmental conditions and vector presence play a key role in infection rates. Additionally, interactions between vectors and plants, host plant susceptibility and agricultural practices significantly influence the distribution of infection. Una had the highest infection rate, followed by Solan, while Sirmaur recorded no infection. This highlights the significance of vector-pathogen interaction, climatic influence and regional farming practices in disease management. A multi-disciplinary approach involving molecular diagnostics, vector surveillance and climate modeling will be important for the development of effective and region-specific disease control strategies in the future.

Acknowledgements

The authors sincerely acknowledge the Department of Entomology, Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh, India for providing infrastructural, laboratory and farm facilities to conduct the research studies.

Authors' contributions

R carried out the surveys, serological studies and drafted the manuscript. RK and SCV participated in the design of the study. RS and AC performed the statistical analysis. N and AY participated in its design and coordination. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None.

References

1. Hanssen IM, Lapidot M, Thomma BP. Emerging viral diseases of tomato crops. Mol Plant Microbe Interact. 2010;23(5):539-48. https://doi.org/10.1094/MPMI-23-5-0539
2. Moriones E, Navas-Castillo J. Tomato yellow leaf curl virus, an emerging virus complex causing devastating disease in world agriculture. J Plant Pathol. 2000;82(2):123-35. https://doi.org/10.1016/S0168-1702(00)00193-3
3. Czosnek H, Laterrot H. A worldwide survey of tomato yellow leaf curl viruses. Arch Virol. 1997;142:1391-406. https://doi.org/10.1007/s007050050168
4. Lapidot M, Friedmann M. Breeding for resistance to whitefly-transmitted geminiviruses. Ann Appl Biol. 2002;140(2):109-27. https://doi.org/10.1111/j.1744-7348.2002.tb00163.x
5. Navas-Castillo J, Fiallo-Olivé E, Sánchez-Campos S. Emerging virus diseases transmitted by whiteflies. Annu Rev Phytopathol. 2011;49:219-48. https://doi.org/10.1146/annurev-phyto-072910-095235
6. Polston JE, Lapidot M. Management of tomato yellow leaf curl virus: US and Israeli perspectives. Dordrecht, Netherlands: Springer; 2007. p. 251-62.
7. Hanssen IM, Lapidot M. Major tomato viruses in the Mediterranean basin. Adv Virus Res. 2012;84:31-66. https://doi.org/10.1016/B978-0-12-394314-9.00002-6
8. Clark MF, Adams A. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J Gen Virol. 1977;34(3):475-83. https://doi.org/10.1099/0022-1317-34-3-475
9. López C, Soler S, Navas-Castillo J, Moriones E. Comparison of different molecular and serological detection methods for tomato yellow leaf curl virus in tomato and weed hosts. Plant Pathol. 2003;52(6):735-41.
10. Byrne DN, Bellows TS. Whitefly biology. Annu Rev Entomol. 1991;36:431-57. https://doi.org/10.1146/annurev.en.36.010191.002243
11. De Barro PJ, Liu SS, Boykin LM, Dinsdale AB. *Bemisia tabaci*: A statement of species status. Annu Rev Entomol. 2011;56(1):1-9. https://doi.org/10.1146/annurev-ento-112408-085504
12. Nono-Womdim R, Swai I, Chadha MM, Green S. Tomato yellow leaf curl virus and tomato leaf curl-like viruses in Eastern and Southern Africa. Building a Knowledge. 2005:157.
13. Avedi EK, Adediji AO, Kilalo DC, Olubayo FM, Macharia I. Incidence, severity and distribution of yellow leaf curl disease of tomato in Kenya. Afr Crop Sci J. 2022;30(1):1-11.
14. Lemmetty A. Isolation and purification of apple chlorotic leaf spot virus and its occurrence in Finnish orchards. Acta Hortic. 1988;235:177-80. https://doi.org/10.17660/ActaHortic.1989.235.25
15. Dijkstra J, Jager CP. Practical Plant Virology: Protocols and Exercises. New York: Springer-Verlag; 1998. p. 459.
16. Hill BG. A morphological comparison between two species of whitefly, *Trialeurodes vaporariorum* and *Bemisia tabaci* (Homoptera: Aleurodidae) which occur on tobacco in the Transvaal. Phytophylactica. 1969;1(3-4):127-46.
17. Patel C, Srivastava RM, Samraj JM. Comparative study of morphology and developmental biology of two agriculturally important whitefly species *Bemisia tabaci* (Asia II 5) and

Trialeurodes vaporariorum from North-Western Himalayan region of India. Braz Arch Biol Technol. 2022;65:e22210034. https://doi.org/10.1590/1678-4324-2022210034

18. Legg JP, Shirima R, Tajebe LS, Guastella D, Boniface S, Jeremiah S, et al. Biology and management of whitefly vectors of cassava viruses in Africa. Pest Manag Sci. 2014;70(10):1446-53. https://doi.org/10.1002/ps.3793
19. Jones RA. Global plant virus disease pandemics and epidemics. Plants. 2021;10(2):233. https://doi.org/10.3390/plants10020233
20. Polston JE, Capobianco H. Introduction of the exotic tomato yellow leaf curl virus-Israel to Florida and implications for its spread in the US. Viruses. 2013;5(12):3192-211.
21. Rojas MR, Macedo MA, Maliano MR, Soto-Aguilar M, Souza JO, Briddon RW, et al. Resistance to whitefly-transmitted geminiviruses in wild relatives of tomato. Mol Plant Microbe Interact. 2005;18 (2):144-56. https://doi.org/10.1146/annurev-phyto-080615-100327
22. Thompson GA, Goggin FL. Transcriptomics and functional genomics of plant defense induction by phloem-feeding insects. J Exp Bot. 2006;57(4):755-66. https://doi.org/10.1093/jxb/erj135
23. Thresh JM. Control of plant virus diseases in sub-Saharan Africa: The role of vector control. Adv Virus Res. 2006;67:419-67. https://doi.org/10.4314/acsj.v11i3.27571
24. Horowitz AR, Denholm I, Gorman K. Management of whitefly resistance to insecticides: Progress and challenges. Annu Rev Entomol. 2020;65:265-82. https://doi.org/10.1146/annurev-ento-011019-025104
25. Ghanim M, Brumin M, Popov C. A new approach to whitefly and virus management using biological control strategies. Pest Manag Sci. 2001;57(5):370-75.

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information is available at https://horizonepublishing.com/journals/index.php/PST/open_access_policy

Publisher's Note: Horizon e-Publishing Group remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc
See https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting

Publisher information: Plant Science Today is published by HORIZON e-Publishing Group with support from Empirion Publishers Private Limited, Thiruvananthapuram, India.